STUDIES ON THE REPRODUCTION AND CULTURE OF THE ROACH (*RUTILUS RUTILUS*)

by

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DECLARATION

Whilst registered as a CNAA candidate for the degree of PhD, the author has not been a registered candidate for another award from CNAA or a university.

No work from this thesis has been submitted, nor will be submitted in candidature for any other degree.

Due acknowledgement has been made to those who assisted during the study.

During the programme of research, courses were undertaken in biostatistics and computing techniques, and library information. The author took part in a post-graduate seminar programme. Oral and poster papers were presented at conferences and symposia. Instructional courses organised by the Institute of Fisheries Management and the Regional Water Authorities were also attended.

Signed .....
(Candidate)

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(Director of Studies)

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ABSTRACT

Studies on the reproduction and culture of the roach (Rutilus rutilus)

by A D Worthington

Aspects of roach culture have been investigated with the aim of providing information applicable to the commercial farming of this species.

Laboratory-maintained adult roach captured from the wild developed a fatal bacterial disease thought to be caused by host stress. This was investigated by determining cortisol titres from serial plasma samples taken from fish maintained in various environments. Plasma cortisol rose and remained elevated for over 60 days from capture in survivors. The cortisol stress-response to such treatments is probably sufficient to induce manifestation of the disease.

The manipulation of reproduction is facilitated by knowledge of natural cycles. Annual patterns were established: in females for gonosomatic index (GSI), hepatosomatic index (HSI) and plasma calcium as an index of vitellogenesis; and in males for GSI and plasma cortisol.

Induced spawning of roach with carp pituitary extract (CPE) and the antioestrogens tamoxifen and clomiphene citrate was studied. The effectiveness of CPE was confirmed. Tamoxifen and, to a lesser extent, clomiphene were also effective.

To extend the growing season of fry, methods of early spawning by hormonal and environmental manipulations were investigated. Salmon GtH, clomiphene and tamoxifen treatments did not accelerate gametogenesis. However, the early provision of spring-like light and temperature regimes advanced spawning by 13 to 16 weeks compared with wild fish.

The rearing of larvae in manured ponds enclosed by greenhouses was studied as an alternative technique to intensive hatchery methods. Roach larvae grew very rapidly in greenhouse ponds, with a high percentage surviving to 50 mm.

The production of fry stocked as larvae at 10, 15, 20 and 30 m^{-2} in outside ponds was examined. Rapid growth, high survival and yield were observed at 20 m^{-2}.

The results were discussed and integrated to form a series of recommended procedures for the culture of roach.

CONTENTS

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Abbreviations

ACTH	Adrenocorticotrophic hormone
CPE	Carp pituitary extract
GAS	General adaptation syndrome
GnRH	Gonadotrophin releasing hormone
GRIF	Gonadotrophin release - inhibitory factor
GSI	Gonosomatic index
GtH	Gonadotrophic hormone
HCG	Human chorionic gonadotrophin
HSI	Hepatosomatic index
IP	Intraperitoneal
LH-RH	Luteinising hormone - releasing hormone
MAFF	Ministry of Agriculture Fisheries and Food
NLT	Nucleus lateralis tuberis
NPO	Nucleus preopticus
RIA	Radioimmunoassay
STWA	Severn-Trent Water Authority
UV	Ultra-violet

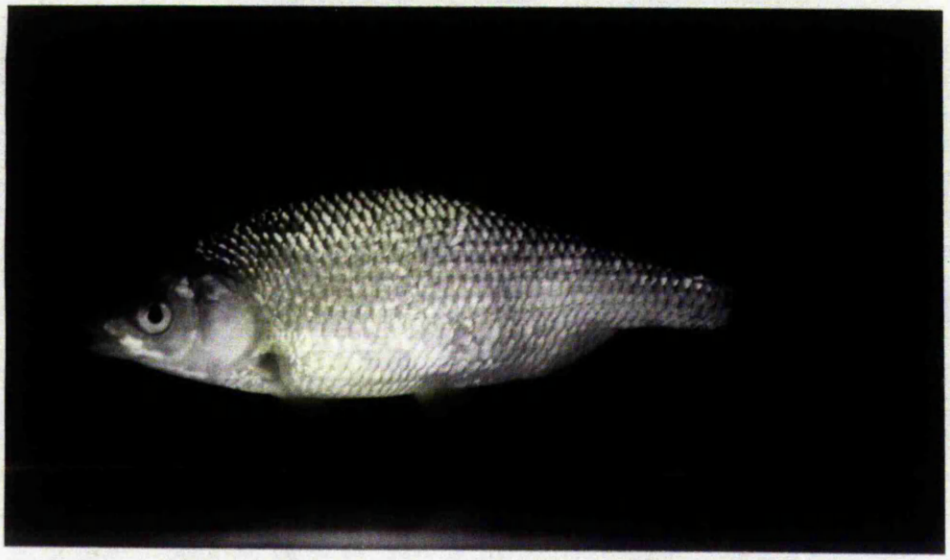


Plate 1 The roach, Rutilus rutilus, Linnaeus

1 INTRODUCTION

The culture of fish is thought to have been practised for several thousand years. Archaeological evidence reveals that the Ancient Egyptians and Romans both tended fish in ponds. The Chinese are thought to have initiated the culture of fish some 3,000 years ago. In 460 BC, the culture of common carp, Cyprinus carpio, in China was very popular and prompted Fan Li to write the first handbook of fish culture entitled "Yang Yu Ching" (Lin, 1982).

Currently, fish are cultured mainly for food, but also for ornamental purposes or angling (for restocking or bait). The farming of food fishes is very important in the Indo-Pacific regions, China, Eastern Europe and, increasingly, in Africa and the Middle East. The culture of fish for food is less important in other regions including Western Europe and the United Kingdom (UK). Although angling, and hence the demand for fish for restocking or bait, is a relatively new phenomenon, ornamental fish have been kept for centuries. For example, the Ancient Romans used to keep red mullet for their aesthetic qualities (Taylor, 1901).

The species chosen for culture obviously depends on the demand, but is principally determined by the climate. Fish have been arbitrarily divided into either "warm" or "cold" water species, which respectively thrive and breed in water mainly above, or below 20°C (Huet, 1970). Thus, fish culture in Northern Europe has been restricted historically to salmonids, and to a lesser extent, common carp and other indigenous cyprinids, all species which can grow in this temperate climate (Huet, 1970). On the other hand, in warmer climates, an important contribution to food production is made by a wide variety of fish including milkfish, Chanos chanos; mullets, Mugilidae; and yellowtail, Seriola quinqueradiata (Pullin and Kuo, 1980). In China, a great deal of food is derived from the culture of cyprinids, including grass carp, Ctenopharyngodon idella; silver carp,

Hypophthalmichthys molitrix; and bighead carp, Aristichthys nobilis, (Lin, 1982). The latter species are also important in the Middle East, with the notable addition of the tilapias (Hepher and Pruginin, 1981).

Fish culture has advanced considerably in the last century. Earlier, "extensive" methods of culture were often practised, that is, there were no additional energy contributions (manure or supplementary food) made to the ponds. Consequently, the yield corresponded to the natural productivity only. This excludes the Chinese, who have practised pond manuring for centuries (Wohlfarth and Schroeder, 1979). The transition from extensive to semi-intensive or intensive culture came when the potential benefits of supplementary feeding, manuring and general control of fish production began to be fully appreciated. Progress beyond this level was severely restricted because many species would not reproduce in captivity. Thus, eggs, larvae or fry still had to be collected from the wild with the associated risk of introducing predators or disease organisms. In 1934, von Ihering in Brazil made a discovery which was to have a profound impact on fish culture. He induced gravid fish to spawn in captivity by injecting them with extracts of fish pituitary glands (von Ihering, 1937). Indeed this technique is still used routinely to spawn fish. However, some species such as mullet, milk fish and eels are largely refractory to this and more-refined techniques. Consequently, fry of these species are still collected from the wild (Harvey and Hoar, 1979; Pullin and Kuo, 1980).

The past twenty years have seen a proliferation of research into fish farming with commensurate increases in capability. The application of genetic techniques has greatly improved some aspects of aquaculture. Moav, Soller and Hulata (1976) have considered the benefits of artificial selection, that is, controlled breeding, in the culture of carp. Traditional, wild-type breeds of fish have high tolerances to disease but often do not respond well to

improved management. On the other hand, modern, selected breeds have reduced disease tolerance but benefit more from modern, high-input culture. Crosses between these strains show a degree of heterosis ("hybrid-vigour") and Moav et al. suggest that when new management techniques are implemented, new, more-efficient strains should be sought by selective breeding. Genetics has found many applications in the culture of species where one sex is undesirable or inefficient. For example, once sexually mature, the rate of growth of female tilapia is drastically reduced. By feeding androgens to larvae before the genotypic sex is expressed in the phenotype, an all-male population develops (Tayamen and Shelton, 1978). In salmonids, the male is unwanted, so by feeding oestrogens to young rainbow trout (Salmo gairdneri) and atlantic salmon (Salmo salar) functional females are produced (Johnstone, Simpson and Youngson, 1978). This can be taken further by androgenising salmonids. The resultant phenotypic males with female genotype (XX) may be crossed with normal females (XX) to give all-female offspring. Another potentially useful technique is inducing polyploidy (having more than the usual two sets of chromosomes) by shocking (eg, thermal) the embryos before fertilisation (Purdom, 1976). The sterile progeny show superior growth when compared to fish showing normal gametogenesis.

In addition to various genetic techniques, the reproductive process itself has become much more controllable as knowledge increases of the natural mechanisms which regulate reproduction. Thus, an appreciation of the roles of photoperiod and temperature in synchronising reproduction in many teleosts has allowed spawning to be advanced by 12 weeks in the rainbow trout (Whitehead, Bromage, Forster and Matty, 1978), 13 - 16 weeks in the roach (Worthington, Macfarlane and Easton, 1982), and 8 weeks in the catfish, Heteropneustes fossilis (Sundararaj and Vasal, 1976). By controlling spermiation and ovulation, the gametes may be stripped from the fish which enables fertilisation to take place under hygienic, artificial conditions. Furthermore, the technique of cryopreservation currently enables sperm to

be stored for extended periods until required (review: Stoss and Donaldson, 1982). This enables sperm to be utilised more efficiently, and could reduce the number of males required in a fish farm operation.

The techniques of larval rearing have improved considerably in the last 50 years. A significant contribution was made around 1940 by a Norwegian, Rollefson. He discovered that newly-hatched nauplii of the crustacean Artemia salina formed an acceptable diet during the larval phase of the plaice, Pleuronectes platessa. Although it is expensive, Artemia is still widely used for this purpose. The culture of other invertebrates, particularly the rotifer Brachionus plicatilis, has extended larval rearing capabilities to include species with small larvae, such as turbot, Scophthalmus maximus (Purdom, 1977).

It is clear from the foregoing that the scientific rationalisation of fish culture has enabled it to be developed to the high degree seen today. However, many species new to fish culture sometimes pose problems which have never previously been encountered. Thus, there is a need to perpetuate the rationalisation process.

According to statistics given by the Ministry of Agriculture Fisheries and Food (MAFF), fish farming in the UK in 1980 produced 10,000 tonnes of fish. This comprised 8,000 tonnes of rainbow trout, 1,200 tonnes of salmon and approximately 800 tonnes of other fish including carp, eels and marine flatfish. The majority of this was for human consumption.

The culture of coarse fish in the UK has increased rapidly in the last ten years, particularly after 1974, when the Regional Water Authorities were established. The Water Authorities identified a demand for coarse fish stemming from their obligation under the Salmon and Freshwater Fisheries Act, 1969, to "maintain, improve and develop fisheries". Since 1974, some of the Water Authorities (particularly Severn-Trent (STWA), Yorkshire, North-West and

Thames) have been developing culture techniques for coarse fish, mainly carp, tench (Tinca tinca) and roach. In addition, culture of grass carp, a potential remedy for weed-choked waters, has been investigated by MAFF since 1964 (B.Stott, pers. comm.). Since the techniques used in the culture of carp and tench are both relatively straightforward and well-established (Huet, 1970), "new" species such as roach were investigated by STWA in a programme beginning in 1976. Roach was chosen as the main subject for study because it is the most popular species with coarse anglers in the UK, and demand for it exceeds that of all other species. Initial work entailed monitoring the growth in ponds of fry which were collected from the wild as fry or eggs. This early work was encouraging, and after ripe roach were induced to spawn by hypophysation in 1978, further research was deemed worthwhile. Thus, a programme was formulated to investigate the husbandry of brood fish, the control of reproduction, the viability of hatchery techniques for rearing larvae, and the production of fry in ponds. The following is an account of this research which was conducted by the author at Trent Polytechnic in conjunction with STWA between July 1979 and September 1982.

2 BROOD FISH HUSBANDRY

2.1 Introduction

Maintaining the good health of brood stock is fundamental to fish culture. Species which have been cultured over several generations have often been selected unwittingly in favour of those fish which are able to tolerate the stresses encountered in artificial environments that is, they tend to become "domesticated". In contrast, fish newly-captured from the wild will be stressed during capture and introduction to an artificial environment such as a hatchery.

In the following text, the term "stressor" is used to describe the environmental alteration affecting the animal. "Stress" is used as defined by Selye (1950): "the sum of all the physiological responses by which an animal tries to maintain or re-establish a normal metabolism in the face of a physical or chemical force". Collectively, the responses are known as the General Adaptation Syndrome (GAS) and may be temporally divided into phases of alarm, resistance and exhaustion.

The physiological responses of the GAS are usually classified as primary, secondary or tertiary. The primary responses are the release of adrenocorticotrophic hormone (ACTH) from the pituitary, and the release of the "stress hormones" cortico steroids (under ACTH regulation) and catecholamines (under neural control). The secondary responses are the changes in the physiology including blood chemistry, carbohydrate metabolism and respiration. The tertiary effects may include reduced resistance to disease, and impaired growth, both of which may be detrimental to the individual but beneficial to the population, by reducing intraspecific competition. An example of such tertiary effects was reported by de Montalambert, Bry and Billard (1978) who demonstrated that the pike (Esox lucius) after handling in captivity failed to ovulate in response to exogenous GtH, indeed the ovaries showed follicular atresia.

In addition, Scott (1979) reported ovarian atresia in minnows (Phoxinus phoxinus) introduced to an aquarium from the wild. Thus, traditional culture practices may fail in undomesticated species. In this study, initial attempts to maintain wild brood roach in artificial spawning flumes did not succeed. Within two to three weeks of introduction, most fish developed areas of raised scales with underlying dermal lesions and haemorrhage. These lesions sometimes allowed drastic erosion of tissue, exposing the peritoneal cavity and gut. This condition has become known as "roach ulcer disease". Despite chemotherapy with, for example: formalin, malachite green, hyamine, proflavine and the antibiotics oxytetracycline and tribrissen, all afflicted fish died. Pathological examination of affected fish by the STWA fish pathologist and staff at the MAFF Fish Diseases Laboratory suggest the causative agent belonged to the Aeromonas genus of bacteria, probably A. hydrophila. This conclusion has also been drawn by Thames Water Authority about roach with ulcer disease in the River Lee system.

Since the Aeromonas genus is a stress-opportunistic, facultative pathogen to fish (Wedemeyer and McLeay, 1981), the present study prompted the hypothesis that roach ulcer disease was precipitated by the stress caused by capture and the introduction to an artificial environment. To test this hypothesis, an experiment was designed to identify those aspects of the artificial environment which were most stressful to the fish. In this way, it might be possible to refine holding conditions. The available facilities restricted the investigation to the three major factors: photoperiod, water quality and temperature. Ideally, the study might have included rate of change in temperature, stocking density, oxygen/metabolite concentrations etc. Donaldson (1981) reviewed many studies where stress responses of salmonids to various culture procedures have been investigated, and emphasised that: "To date investigations have only scratched the surface in terms of the number of cultural stresses which have been studied".

The present study demanded serial monitoring of the fish, which restricted the methods of detecting stress (excluding, for example, changes in histopathology). It was decided to measure a blood parameter, either a catecholamine or corticosteroid. Since corticosteroids have been shown to indicate both acute and chronic stresses, cortisol, the most abundant in fish plasma (eg, Singley and Chavin, 1971; Campbell, Walsh and Idler, 1976), was chosen as the indicator of stress. Cortisol is generally considered to be the most convenient measure of the primary stress response in teleosts, although in some circumstances, such as exposure to lethal concentrations of cadmium, where cortisol is unaffected (Schreck and Lorz, 1978), it is inappropriate.

2.2 Materials and Methods

2.2.1 Animals

On 25 November 1980, 47 adult roach (50 - 300 g) were captured by electrofishing from the River Sence, Leicestershire. The fish were transported in approximately 250 l of river water and introduced to the flumes (F1 - F4; Fig 1) of STWA Colwick laboratory on the north bank of the River Trent.

2.2.2 Experimental regimes

During the two to three weeks after capture, the fish were acclimated to the regimes shown in Table 1. Each flume was constructed of concrete blocks, sealed with the waterproof paint "Aquaseal". F1 and F2 were supplied with water pumped from the River Trent in flow-through systems. F3 and F4 were provided with water recirculating through a settling tank and a biological filter. F3 and F4 were served by two 30 W ultraviolet water sterilisers (Coast Air Ltd). Water temperature in F2, F3 and F4 was regulated thermostatically by 3 kW domestic immersion heaters. Temperatures were measured at one to three day intervals. F3 was enclosed in a light-proof box, and the fluorescent light source

Table 1 Experimental regimes

Flume	Volume (l)	Number of fish	Water Source	Temperature (°C)	Photoperiod (h)
F1	700	11	Pumped River Trent Water	Prevailing river temp.	Natural
F2	1200	12	Pumped River Trent water	13-16	Natural
F3*	1000	12	Recirculated mains water	†14-16	†16L:8D
F4*	1000	12	Recirculated mains water	†14-16	Natural

* Settling tank and biological filter water volume = 2,400 l.

† These photoperiod and temperature regimes occur naturally in late spring, and might be expected to accelerate gametogenesis.

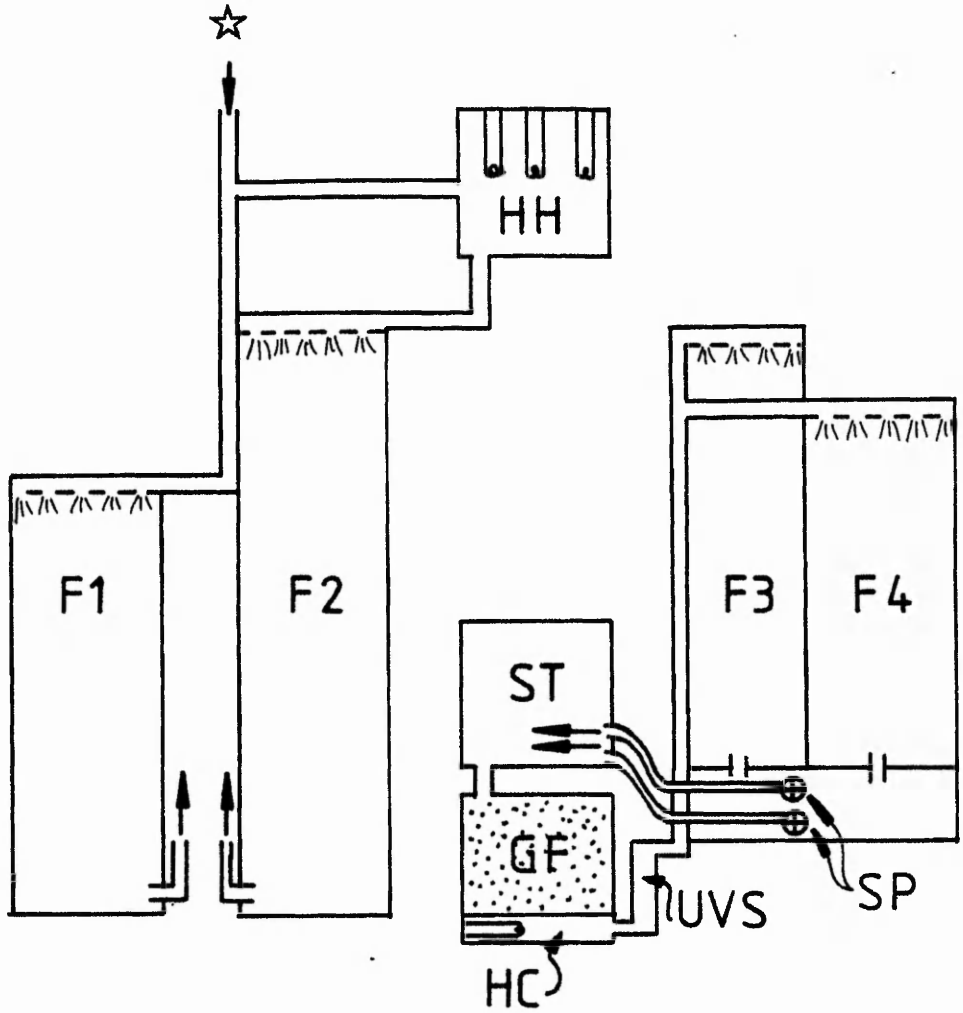


Figure 1. Aerial plan of the flumes (F1 to F4) used in the investigation of the effects of various artificial environments on plasma cortisol in roach. HH = heated header tank; ☆ = water pumped from River Trent; ST = settling tank; GF = gravel filter; SP = submersible pumps; HC = heating chamber; UVS = ultraviolet sterilisers.

controlled by an automatic time switch. The fish were fed with "Omega" trout food (Edward Baker Ltd) daily between 8.30 and 11.00 h.

2.2.3 Blood sampling

Blood samples were taken from all fish after they had been anaesthetised with benzocaine. Each group of fish was captured and placed in the anaesthetic as a whole, excluding those caught directly from the River Sence, which were anaesthetised and bled individually. 0.3 to 0.4 ml blood was withdrawn by caudal venipuncture using disposable 1 ml syringes and 31 x 0.6 mm needles treated with sodium heparin. Blood samples were centrifuged for five minutes at approximately 1,500 g and the plasma withdrawn and stored at -20°C prior to cortisol analysis. Bleeding was conducted at the instant of capture, the following day, and then at three-weekly intervals. On the second day, five fish from each group of eleven or twelve were bled excluding those sampled the previous day. Subsequently, blood samples were taken from each member of a group. Blood sampling took place between 10.00 and 13.00 h.

2.2.4 Cortisol radioimmunoassay

25 µl samples of plasma were assayed in duplicate for cortisol with an "Amerlex" cortisol radioimmunoassay (RIA) kit (Amersham International). This method employs specific antibody-coated particles, and has low cross-reactivity with steroids other than cortisol. To check the reliability of the results, cortisol control sera were assayed in the same way as the unknown samples. Typical mean values and the acceptable ranges of the control sera used were: 139 (109 - 169) and 328 (266 - 390) ng ml^{-1}, as determined by the Amersham Quality Control laboratories. A typical standard curve is shown in Appendix 1.

2.2.5 Statistics

The values of plasma cortisol for each sampling time were analysed separately first by analysis of variance for unequal sample numbers, then by Student's t-test. In this way, the number of t-tests was reduced, and consequently the risk of error.

2.3 Results

Plasma cortisol and water temperature data from each group are illustrated in Figures 2 and 3 respectively, with detailed information on blood sampling given in Table 2. During the course of the experiment, many fish became diseased, and these events are presented in Table 3.

A maximum of twelve minutes elapsed from capture to the taking of the last blood sample for each group of eleven or twelve fish. Plasma cortisol had increased the day following capture ($p < 0.01$), being highest in F4 and lowest in F1 groups. In the latter group, cortisol titre reached a peak after 22 days, whereas this occurred after 43 days in the other three groups (F2, 3 and 4). The F2 group became heavily infested with white spot, Ichthyophthirius multifilis, between 22 and 43 days. This went undetected until blood samples were taken on day 43 due to the turbidity of the river water supply. These fish had a mean plasma cortisol level of 721 ± 59 ng ml^{-1}, significantly higher than the nearest mean value in F4 (281 ± 51 ng ml^{-1}; $p < 0.001$). At this point, cortisol of the F1 group (98 ± 23 ng ml^{-1}) was considerably lower than the nearest group, F3 (247 ± 34 ng ml^{-1}; $p < 0.01$). The F2 group all died within 24 hours of blood sampling on day 43. During the following 21 days, plasma cortisol approached the concentration of day 0 in the survivors of F1, F3 and F4.

On day 18, an ulcerative lesion was observed on a fish from the F4 group. Plates 2 - 4 show examples of such lesions and other manifestations of roach ulcer disease. A fish

Table 2 (see text) Data on blood sampling and plasma cortisol concentrations

Date	Day	Number of fish	Group	Plasma cortisol ($ng\ ml^{-1}$) $\bar{x} \pm$ SEM	Total bleeding time (min)
24.11.80	0	9	IC*	12 ± 5	4 per fish
25.11	1	5	F1	52 ± 9	10
		"	F2	90 ± 14	9
		"	F3	76 ± 16	11
		"	F4	147 ± 30	7
12.12	18	1	F4#	320	3
16.12	22	11	F1	130 ± 31	7
		12	F2	81 ± 28	8
		12	F3	103 ± 14	12
		12	F4	197 ± 48	13
30.12	30	1	F3#	120	2
6.1.81	43	9	F1	98 ± 49	11
		11	F2	721 ± 123	11
		10	F3	247 ± 72	9
		10	F4	281 ± 198	10
27.1	64	10	F1	28 ± 7	12
		10	F3	42 ± 9	9
		4	F4	16 ± 16	8

* IC = Immediately post-capture

fish with ulcerative lesions

Table 3 The incidence of disease during the investigation of brood fish husbandry

Date	Day	Group	Total number of fish affected	Symptom	Pathogen	Mortality
24.11.80	0					
10.12	16	F4	1*	Furuncles	Suspected *Aeromonas*	0
22.12	28	F4	5	Furuncles	" "	0
30.12	36	F4 F3	7 1	Furuncles "	" " " "	0 1
2.1.81	39	F4	8	Furuncles	" "	1*
6.1	43	F2	12	White Spot	*Ichthyophthirius multifilis*	
7.1	44	F2		White Spot	" "	12
12.1	49	F4	9	Furuncles	Suspected *Aeromonas*	1
14.1	51	F4	9	Furuncles	" "	2
18.1	55	F4	7	Furuncles	" "	2
20.1	57	F4	5	Furuncles	" "	1
10.2	78	F1	3	Furuncles	" "	3

* The same fish

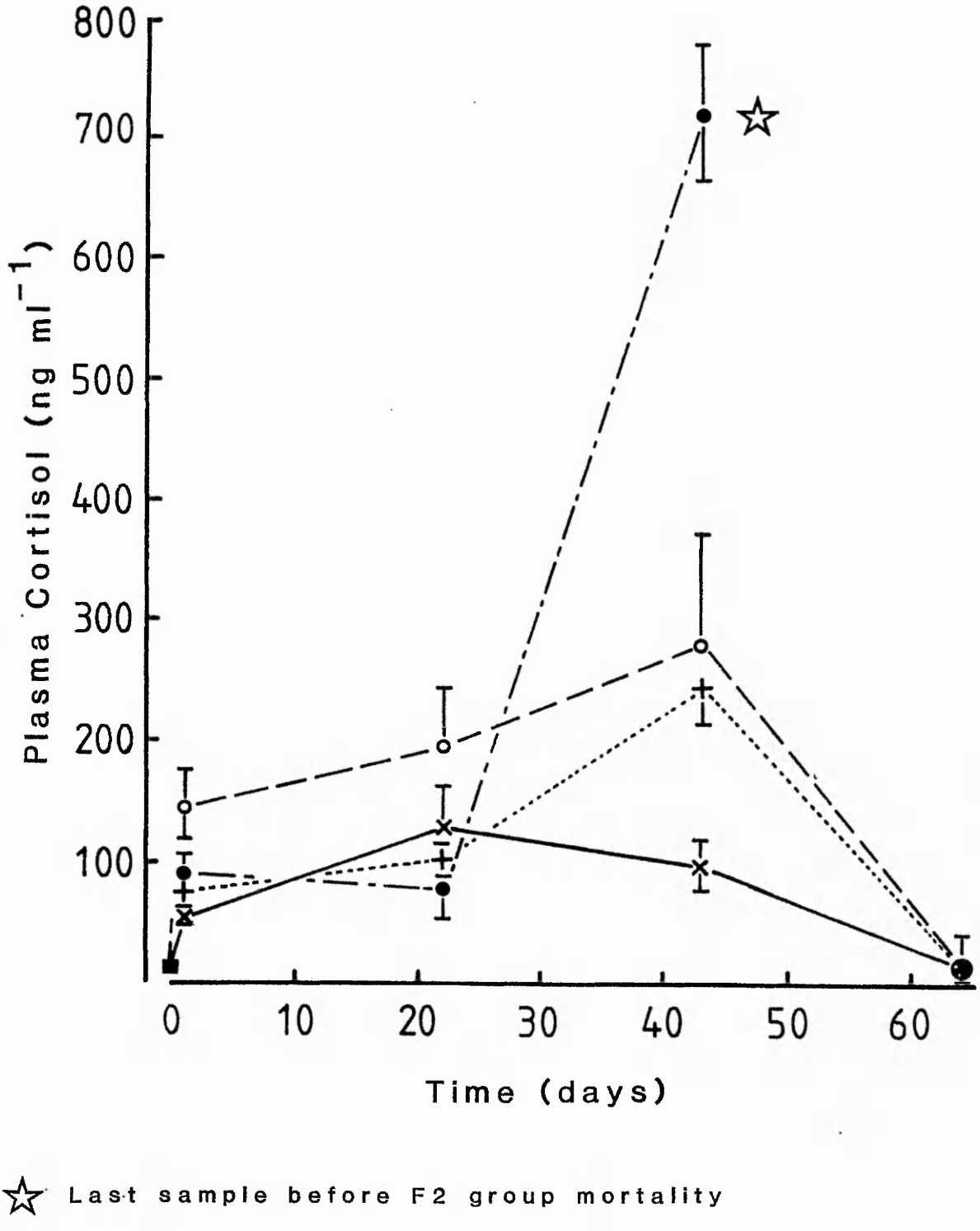


Figure 2. Changes in plasma cortisol ($\bar{x} \pm$ SEM) in four groups of roach maintained under different regimes: F1, R. Trent water, natural temp. and photoperiod (×——×); F2, R. Trent water, 13 to 16°C, natural photoperiod (●·—·●); F3, recirculated mains water, 14 to 16°C, 16L : 8D photoperiod (+····+); F4, recirculated mains water, 14 to 16°C, natural photoperiod (o——o). F1, n = 11; F2, F3, F4, n = 12.

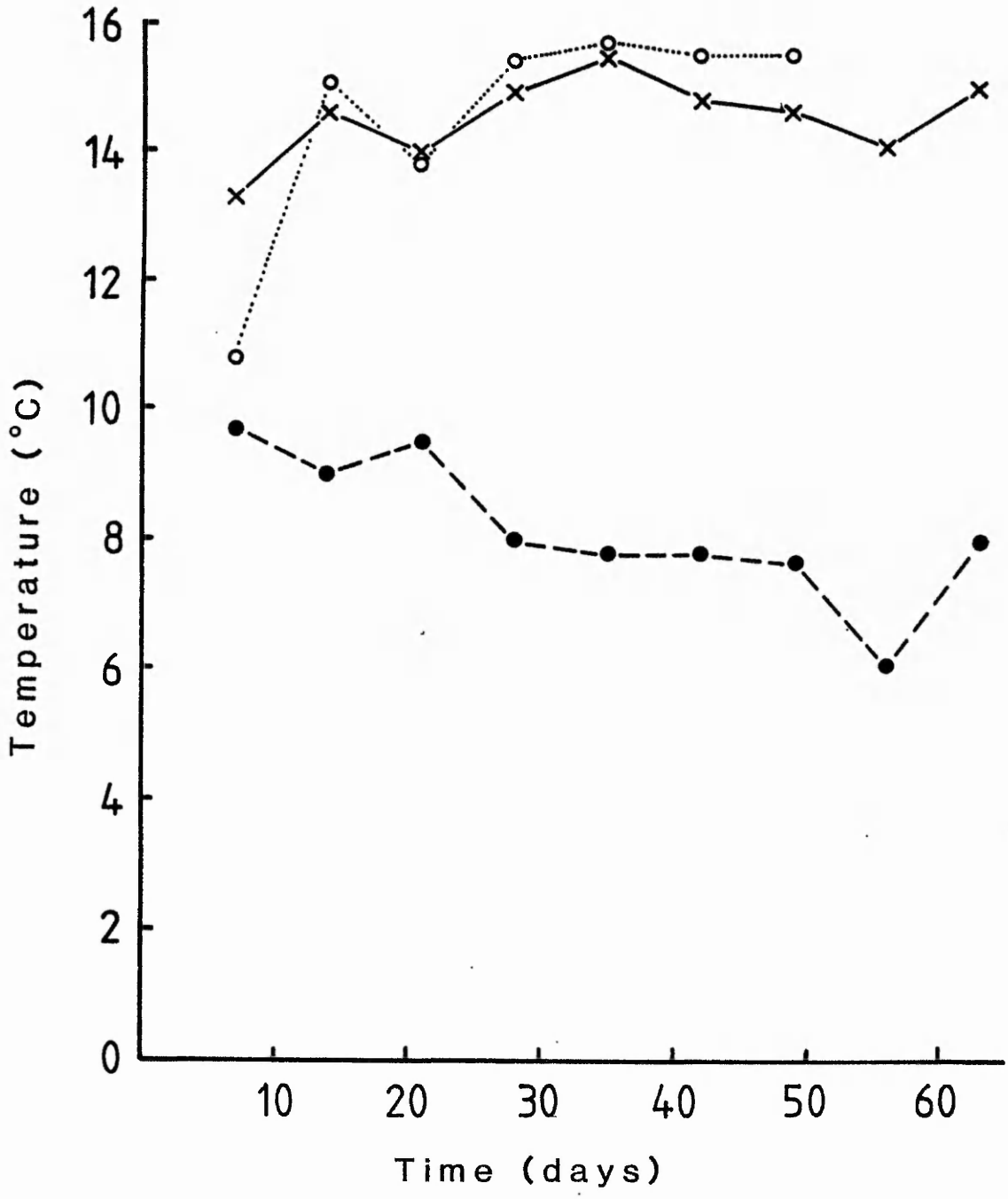
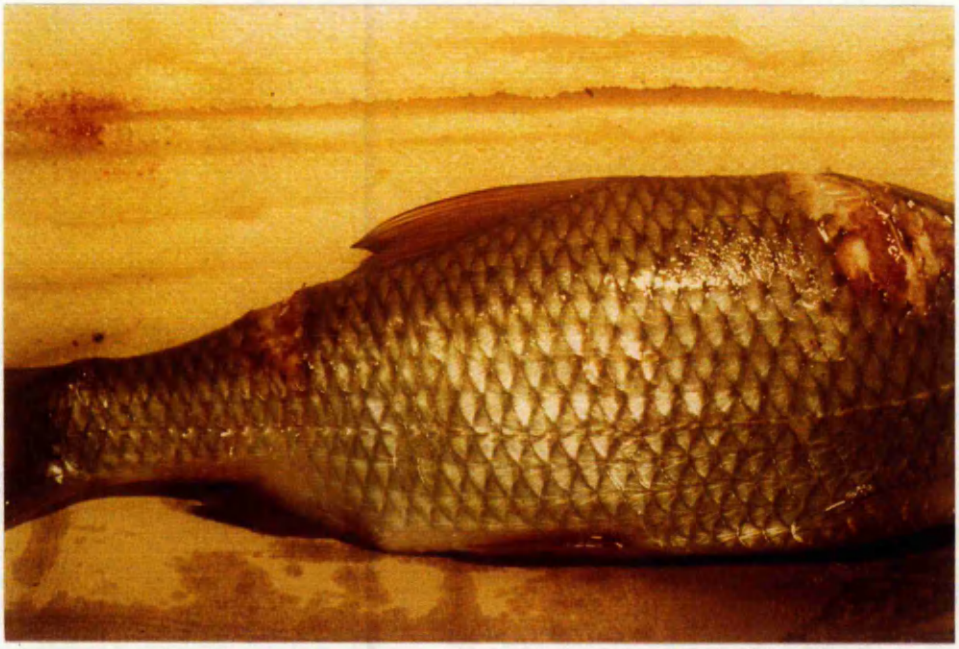
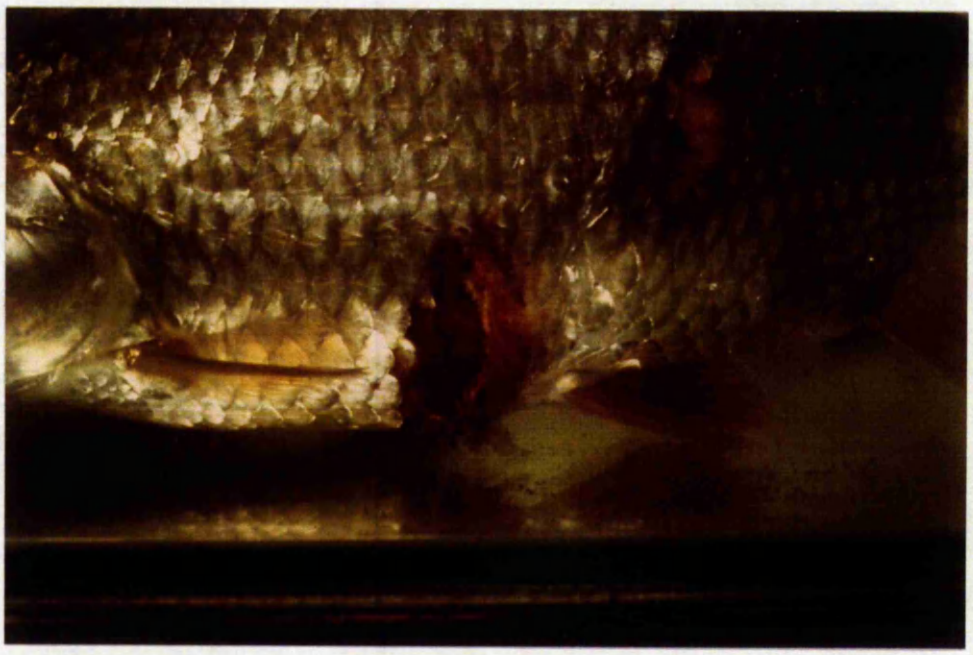


Figure 3. The weekly mean water temperatures of flumes F1 to F4 during the experiment to monitor the cortisol stress response to different environmental conditions (described in Figure 2). F1 = (●——●); F2 = (o·····o); F3, F4 = (×——×).





Plates 2 and 3. Two roach affected by Aeromonas hydrophila

Severe erosion of the epidermal and dermal layers has exposed the underlying musculature. These lesions render affected fish vulnerable to loss of blood, osmotic disruption and secondary attack from fungi.

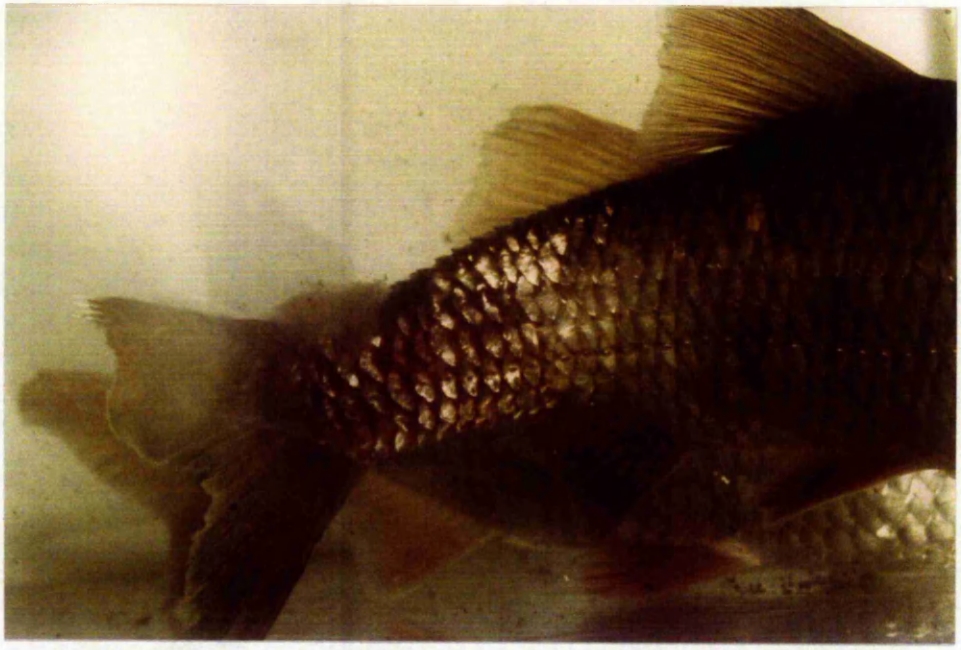


Plate 4. A roach showing another common manifestation of roach ulcer disease. The caudal peduncle characteristically becomes swollen, haemorrhages and becomes secondarily attacked by the fungus Saprolegnia

from the F3 group had developed similar symptoms by day 30. Blood samples were taken from each fish on days 18 and 30 respectively, as soon as the lesions were detected. The fish from the F3 group died soon after bleeding through excessive loss of blood. The fish from the F4 group died as a result of the disease on day 39. During the following 39 days there were nine more mortalities due to the ulcer disease, six from F4 and three from F1. Disease lesions which developed on fish in the river water flumes (F1 and F2) became secondarily infected with the fungus Saprolegnia. It was observed that the slight damage caused by blood sampling healed between samples in the warm water environments (F2 to F4) in contrast to the fish in F1.

The fish kept under simulated spring conditions (F3) displayed preliminary courtship (pursuit swimming) from December 16. To determine whether gametogenesis had been completed under these environmental conditions, each fish was given priming doses of carp pituitary extract, 0.5, 1 and 1 mg kg^{-1}, on 6, 17 and 18 February respectively. A spawning dose (5 mg kg^{-1}) was given on 3 March. The hormone vehicle was 100 mM NaCl, and the injection volume was 0.1 ml per 100 g body weight. Spawning did not take place, and three fish developed ulcers from 1 March. The survivors developed similar symptoms. Spawning seemed unlikely, so the fish were killed to determine the developmental stage of the gonads. As suspected, the eggs of the females had been resorbed, and the gonads of both sexes had regressed.

All water temperatures were affected somewhat by the ambient temperature. Hence, the temperatures varied between the heated flumes F2, 3 and 4 by several degrees. The temperature of the unheated flume F1, fluctuated by the greatest amount. After acclimation, the temperatures of each flume fluctuated thus: F1, 5 - 13°C; F2, 13 - 16°C; F3 and F4, 14 - 17°C.

2.4 Discussion

The maximum sampling duration of ten to twelve minutes for any group is before any stress-induced elevation of plasma cortisol in the goldfish (10 - 22 min: Spieler, 1974), but it was not possible to investigate further whether or not the stress induced by the sampling procedure exaggerated the cortisol values obtained. Studies with other teleosts are not consistent with the findings of Spieler. For example, Redgate (1974) reported a rapid cortisol response to a handling stressor in carp (within ten minutes) and Strange, Schreck and Golden (1977) found a significant increase in plasma corticoid concentration of cutthroat trout, Salmo clarki clarki, also within ten minutes of handling. On the other hand, the roach in the present study were all placed in anaesthetic immediately on capture, a procedure which moderates the cortisol response in chinook salmon, Oncorhyncus tshawytscha (Strange and Schreck, 1978). Barton and Peter (1982) however, found no evidence for suppression of the cortisol stress response of rainbow trout to the anaesthetics MS222 (tricaine methanesulphonate) and 2-phenoxyethanol. Thus these aspects of the experiment require further study. In addition, it is possible that plasma cortisol has a diurnal rhythm as in the rainbow trout (Rance, Baker and Webley, 1982) which may have interfered with the results. However, these effects would have been reduced by the practice of sampling around the same time of day.

The mean "resting" cortisol concentration of 12 ng ml^{-1} of wild roach immediately post-capture is lower than that reported by Broughton (1978) of 81.8 ng ml^{-1}, although he sampled fish in the laboratory. In addition a competitive protein binding assay was employed in the latter study, which may have been less specific than the RIA used in this study. The basal level is comparable to that found in other species: carp, 10 ng ml^{-1} (Redgate, 1974); goldfish, 58 ng ml^{-1} (Spieler, 1974); catfish, Ictalurus punctatus, 50 ng ml^{-1} (Strange, 1980); rainbow trout, 2, 0 ng ml^{-1}

(Barton, Peter and Paulencu, 1980 ; Barton and Peter, 1982, respectively); and brown trout, Salmo trutta, 30 ng ml^{-1} (Pickering and Christie, 1981).

The elevation of plasma cortisol on day one, was probably due to capture, handling, transport and the imposition of the artificial environments. Thus, the roach in this study were subjected to acute (capture, handling and transport) and thereafter chronic (artificial environments) stressors. Electrofishing has been shown to elicit an acute cortisol response (up to 172 ng ml^{-1}) in rainbow trout of at least six hours duration (Schreck, Whaley, Bass, Maughan and Solazzi, 1976). Many investigations have shown acute cortisol stress responses to transport, and particularly handling in salmonids (eg, Strange et al., 1977; Barton et al., 1980; Pickering, Pottinger and Christie, 1982; review, Donaldson, 1981). Despite the fact that the latter studies involved the use of "domesticated" species, the effects of acute stress may persist for a considerable time. For example, Pickering et al. (1982) found that it took at least ten days for plasma cortisol to return to resting levels in brown trout exposed to a single experience of handling for two minutes. In the light of the evidence, the cortisol response of roach the day following capture is considered to be very modest, taking into account the severity of the stressors experienced.

The relatively mild stress due to the artificial environments clearly compounded the after-effects of the initial acute stress, as plasma cortisol continued to rise until at least 22 days (F1) and even 44 days (F2, 3 and 4) post-capture. Chronic stress has been shown to have similar effects in other species. For example, Redgate (1974) found that concentrations of plasma cortisol in carp were similar 2 and 42 days after being transferred from a natural to an artificial environment. This is similar to the finding of the present study, where plasma cortisol began to decline 43 days after capture in the surviving groups F1, F3 and F4. Plasma cortisol values of these groups on day 64 indicated

that perfect compensation ie, acclimation had taken place by this time. Despite this apparently favourable situation, the F2 group had died, and nearly all surviving fish from F1, 3 and 4 were suffering from ulcer disease. The infestation of the F2 group with Ichthyophthirius was clearly very severe on day 43 as evidenced by the extremely high levels of cortisol. In terms of the GAS, these fish presumably entered the exhaustion phase after blood sampling. During such an advanced stage of Ichthyophthirius infestation, gill function and hence respiration are often impaired (I.Pocock, STWA fish pathologist, pers. comm.). Light infestation of brown trout with various ectoparasites, including Ichthyophthirius, failed to affect significantly circulating cortisol levels (Pickering and Christie, 1981). The saprophytic fungus Saprolegnia on the other hand, caused plasma cortisol to rise to 650 ng ml^{-1}, similar to the cortisol response of roach to Ichthyophthirius (720 ng ml^{-1}). Various parasitic infections have been frequently associated with marked elevation of plasma cortisol in other teleosts, including bacterial kidney disease in pink salmon, Oncorhyncus gorbuscha, (Donaldson and Dye, 1981). Nevertheless, the relationship between Aeromonas infection and plasma cortisol in roach is unclear. The fish afflicted with ulcer disease in F4 on day 18 did have a relatively high cortisol level but that of a similar fish from F3 on day 30 was not markedly different from fellow group members of the same period. Despite this, the incidence of ulcer disease and elevated plasma cortisol concentration was greater in the study period in F4 than any other group (except F4, day 43).

Overall, it seems likely that the stressors outlined earlier, precipitated the outbreak of the Aeromonas infection by weakening the fish (they only resumed feeding several weeks after capture) and causing elevated plasma cortisol which is known to reduce resistance to disease (for review of mechanisms, see Ellis, 1981). A similar situation exists with American largemouth bass, Micropterus salmoides. This fish is prone to "red-sore" disease, known

to be caused by Aeromonas hydrophila. Evidence from studies on the epizootiology of this bacterium implicates host stress as the initiator of such disease outbreaks in largemouth bass (Esch and Hazen, 1980). The latter study showed a significant correlation between poor condition and both leucopenia (paucity of white blood cells) and elevated plasma cortisol, implying increased susceptibility to red-sore disease. Indeed, many other diseases are associated with stress-opportunistic organisms eg furunculosis, Aeromonas salmonicida; bacterial gill disease, Myxobacteria spp.; and various parasite infestations eg, Costia, Trichodina, Hexomita (Wedemeyer and McLeay, 1981).

In conclusion, of the environments investigated, heated river water appears to be the most unsuitable for holding brood roach, particularly over winter when the water is often turbid. Constant water turnover may allow continuous pathogen challenges to the fish's immune system, already weakened by the stressors mentioned previously. These conditions are particularly favourable to parasites with a direct life cycle such as Ichthyophthirius, which multiply rapidly and thrive where their hosts are crowded. Plasma cortisol profiles suggest that raising the temperature and purifying the water (as in the F3, F4 groups) stresses the fish more than pumped river water at natural temperature (the F1 group). This is probably due to the fact that the latter environment most closely resembles the natural one. Despite this, all members of the F1 group, together with the F3 and F4 groups, eventually succumbed to the Aeromonas infection. Thus, this experiment gave an insight into some of the problems of brood roach husbandry, and confirmed suspicions that this would be a difficult species to maintain under laboratory conditions.

The failure of the roach to spawn after inducement with CPE may mean that stress blocked gametogenesis. It is more likely however, that long photoperiod/raised temperature

(F3) and natural photoperiod/raised temperature (F4) were non-stimulatory to reproduction.

3 THE REPRODUCTIVE CYCLE

3.1 Introduction

Investigation of the artificial manipulation of reproduction of any animal requires prior knowledge of the natural cycle of reproduction. The basic pattern of events during the course of a reproductive cycle has already been established for many teleosts. However, each species is unique, and thus reproductive cycles have evolved to fit the particular environmental niche.

Characteristically, the gonads have pronounced cycles of activity, with one year periodicity in temperate species for example. The production of eggs and sperm, gametogenesis, comprises a multiplicity of integrated physiological processes. These, under neuroendocrine (and ultimately environmental) control, result in the production of mature gametes at the time most favouring the subsequent survival of larvae and fry.

Investigations of reproductive cycles have largely been confined to economically important species, both marine, for example the plaice (Wingfield and Grimm, 1977), and freshwater (mainly salmonids), for example the brown trout (Crim and Idler, 1978). The investigations themselves vary in sophistication, but usually involve monitoring of changes in anatomy (for example, gonad weight), histology (frequently, gonad structure), or blood parameters (hormones or electrolytes). Generally speaking, there has been a trend towards the measurement of blood parameters with the advent of modern methods of analysis, such as RIA.

Among the crudest indicators of reproductive status, but the most easily measured, are the condition factors, indices of the "well-being" of a fish. The one used most frequently, K, is equal to $\frac{100W}{L^3}$ (Hile, 1936), where W is the weight (g) and L the length (cm) (Le Cren, 1951, for derivation). This

has been related to the reproductive cycle in the powan, Coregonus lavaretus, for example (Scott, 1979).

More useful than condition factors are the indices relating the weight of a particular organ to the total body weight, for example, gonosomatic index (GSI; gonad : body weight), and hepatosomatic index (HSI; liver : body weight). Despite its disadvantages, the use of GSI is very popular and has been measured over the reproductive cycle of different species in numerous studies (Le Cren, 1951; Campbell, Walsh and Idler, 1976; Wingfield and Grimm, 1977; Billard, Breton, Fostier, Jalabert and Weil, 1978; Scott, 1979; Eliassen and Vahl, 1982). The seasonal changes are most pronounced in the species with group-synchronous gonads (after Marza, 1938), that is, gametes are brought to maturity in "batches". Such fish include the roach, which spawns once annually. In these species, post-spawning, GSI characteristically increases at an accelerating rate, to reach a peak prior to the shedding of the gametes when GSI falls to a minimum. The unqualified use of GSI is justifiably open to criticism. For example, teleosts which spawn many times during a year may show no change in GSI, as in Tilapia leucosticta (Siddiqui, 1977). In addition, GSI is subject to distortion due to depletion of energy reserves of the soma (eg, liver and muscle) which, in temperate species, commonly occurs over the winter period.

The aspect of ovogenesis which requires the largest investment of energy by the parent is the production of yolk, vitellogenesis. There are two forms of yolk, each of which originates at a distinct site. Endogenous yolk is synthesised within the developing oocytes themselves, whereas exogenous yolk is synthesised in the liver under oestrogen stimulation (Wallace, 1978), released into the blood stream, and subsequently sequestered by the developing oocytes (Wallace, 1978; Le Menn, 1979). Thus, HSI is sometimes used as an indicator of vitellogenesis (de Vlaming, Vodicnik, Bauer, Murphy and Evans, 1977; Wingfield and Grimm, 1977; Olivereau and Olivereau, 1979). One

disadvantage of using HSI for this purpose is that the hepatic carbohydrate stores may be utilised for other purposes such as maintaining the somatic tissues during starvation.

The measurement of blood parameters can be a convenient way to assess certain aspects of the reproductive cycle. For example, it is known that in teleosts, the vitellogenin produced by the liver actively binds calcium (Dacke, 1979). In rainbow trout, binding occurs in a 1 : 1 ratio (Elliot, Bromage and Whitehead, 1979). Thus, blood calcium is a useful indicator of circulating vitellogenin and has been monitored in several studies of reproductive cycles (eg, Yaron, Cocos and Salzer, 1980; Bromage, Whitehead and Breton, 1982).

In recent years, there has been a proliferation of studies of the circulating reproductive hormones. Despite modern RIA techniques, the determination of some hormones still poses problems. For example, gonadotrophin being a glycoprotein, has been shown to be species-specific (Fontaine, Salmon, Fontaine-Bertrand, Burzawa-Gerard and Donaldson, 1972). Thus, development of a specific immunoassay for a particular fish GtH would require isolation and purification of the natural hormone. This is necessary to raise specific antibodies to that hormone, and also for radiolabelling in the RIA. This is an expensive and time-consuming process. On the other hand, there are commercially available antibodies for the major steroids associated with reproduction in teleosts. These have been developed primarily for medical use, but since sex steriods are not species-specific, they can also be used in the radioimmunoassay of fish plasma. There are also RIA kits now available, which require a minimum of specialised knowledge and equipment. Sex steriods have been monitored over the reproductive cycles of several fish, for example the goldfish (Schreck and Hopwood, 1974), the winter flounder, Pseudopleuronectes americanus (Campbell et al., 1976), and the rainbow trout (Bromage, et al., 1982).

However, RIA of sex steroids generally requires relatively large volumes of plasma, and roach do not usually yield much blood (Broughton, 1978). On the other hand, cortisol has been implicated in gametogenesis and consequently has been studied over the reproductive cycle of several species (eg, Wingfield and Grimm, 1977; Peter, Hontela, Cook and Paulencu, 1980; Terkatin-Shimony, Ilan, Yaron and Johnson, 1980). The role of cortisol in gametogenesis is still incompletely understood, but it has been variously associated with vitellogenesis (Peter et al., 1978), oocyte maturation (Sundararaj and Goswami, 1977) and ovulation (Cook, Stacey and Peter, 1980).

Published information on roach reproductive physiology is very scarce, although Jafri (1980) did conduct some laboratory studies on this species. He made a study of the reproductive cycle, which provided valuable data on the histology of the gonads and on the GSI of both sexes. However, difficulties with obtaining fish precluded completion of the cycle, and data from the crucial winter period were not acquired. The present study was intended to extend knowledge of the reproductive cycle of wild roach by examining GSI, HSI, condition factor K, and plasma calcium and cortisol. The results might then be used to aid the interpretation of laboratory-based experiments concerning controlled reproduction and also, in the case of the cortisol data, the study of brood fish husbandry (Chapter 2).

3.2 Materials and Methods

3.2.1 Animals

Samples of approximately twenty roach were collected monthly (where possible) from a wild population in Highfields Lake, two miles west of Nottingham. STWA use this venue as a stock pond, which holds fish collected on fish rescues. No fish were introduced during the year of the study.

3.2.2 Capture of fish and blood sampling

Sampling usually took place between 10.00 and 14.00 h. Fish were captured by pulsed DC electrofishing from a boat, allowing their rapid retrieval, and killed with a sharp blow to the head. Blood samples (0.3 to 1.0 ml) were taken by caudal venipuncture. The time between capture and the end of bleeding was noted for each individual, and was usually less than three minutes. Blood samples were packed in ice, transported to the laboratory, along with the fish, and centrifuged at 1,500 g for approximately five minutes. Plasma was withdrawn and stored at -20°C (within five hours of bleeding) prior to analysis. The fish were kept at 4°C before dissection which took place within 18 hours.

3.2.3 Anatomical measurements

Fork length was measured to the nearest millimetre for each fish, and intact body, gonad and liver weights recorded in grammes. Weighing was carried out on a top-pan balance to two decimal places. From the latter measurements, GSI, HSI, and condition factor (K) were calculated:

$$GSI = \frac{\text{Weight of gonad (g)}}{\text{Total body weight (g)}} \times 100\ \%$$

$$HSI = \frac{\text{Weight of liver (g)}}{\text{Total body weight (g)}} \times 100\ \%$$

$$K = \frac{\text{Weight (g)}}{\text{Length (cm)}^3} \times 100\ \%$$

3.2.4 Analysis of plasma calcium

Total plasma calcium was determined by fluorimetric titration using a Corning 940 instrument, which operates on the following principle. The dye calcein (a fluoroscein derivative) forms an intensely fluorescent, non-dissociated complex with calcium ions in an alkaline medium. The technique is based on a quenching of the fluorescence by

chelating calcium ions with EGTA, and detecting this change with a colourimeter. 25 µl plasma samples were dispensed with a Hamilton microsyringe, and analysed in triplicate. The mean value was calculated in mM. Precision and reproducibility were ensured by analysing "Wellcomtrol" (human serum) and carp plasma standards. The reproducibility of the calcium analysis technique was less than ± 4 %for 25 µl plasma samples.

3.2.5 Cortisol radioimmunoassay

Plasma cortisol was determined by the technique described in 2.2.4.

3.2.6 Statistical analysis

Where appropriate, differences between sets of data were analysed with Student's t-test. The correlation coefficient 'r' was calculated between GSI and HSI for groups of individual females sampled before or from February.

3.3 Results

Data on GSI, HSI, condition factor, plasma calcium and cortisol are presented in Figures 4 to 9, and are also tabulated in Appendices 2 to 4.

3.3.1 Sampling

It is notable that excepting the sample taken on 19 May, males number four or less per sample. Consistent failure to capture more males prompted an increase in total sample number to thirty fish, where possible. However, this did not increase the number of males captured until May, when they developed external distinguishing features (tubercles) and could be selected.

The lake was frozen frequently between November 1981 and February 1982, which severely hampered sampling. During the

winter months, total sample numbers were low. This was due to the fish being inaccessible in deeper water. In addition, the water was turbid and highly conductive, so reducing the efficiency of the electrofishing technique.

Blood sampling was always completed within five minutes of retrieval of the fish from the water.

3.3.2 Seasonal changes in anatomy (Figures 4 to 7)

From August (post-spawning), female GSI rose steadily until the following May (pre-spawning) then decreased abruptly immediately after spawning in June. Spawning was not actually witnessed, therefore the precise time of spawning in 1982 is unknown, but it may be deduced from the data that it took place between 19 May and 9 June. During the same period, male GSI followed a similar pattern but this was reduced in amplitude. Unfortunately, the data from males is not conclusive because sample numbers were low. Female HSI increased consistently from August to February, then decreased until May. There appears to be no such pattern in male HSI, although low sample numbers prevent conclusive interpretation. In females, HSI and GSI were positively correlated up to January ($r = 0.29$, $p < 0.02$). From February, HSI and GSI were negatively correlated ($r = -.30$, $p < 0.02$). Condition factor did not show a substantial pattern throughout the study period in both sexes.

3.3.3 Seasonal changes in plasma calcium and cortisol (Figures 8 and 9)

Apart from a brief fall in February, plasma calcium appeared to rise steadily in females from September to May (increasing by 38%, $p < 0.05$), then decline immediately after spawning by 22% ($p < 0.001$). There was a further fall between July and August ($p < 0.001$). In males, plasma calcium did not appear to change, and never approached the highest values of the females.

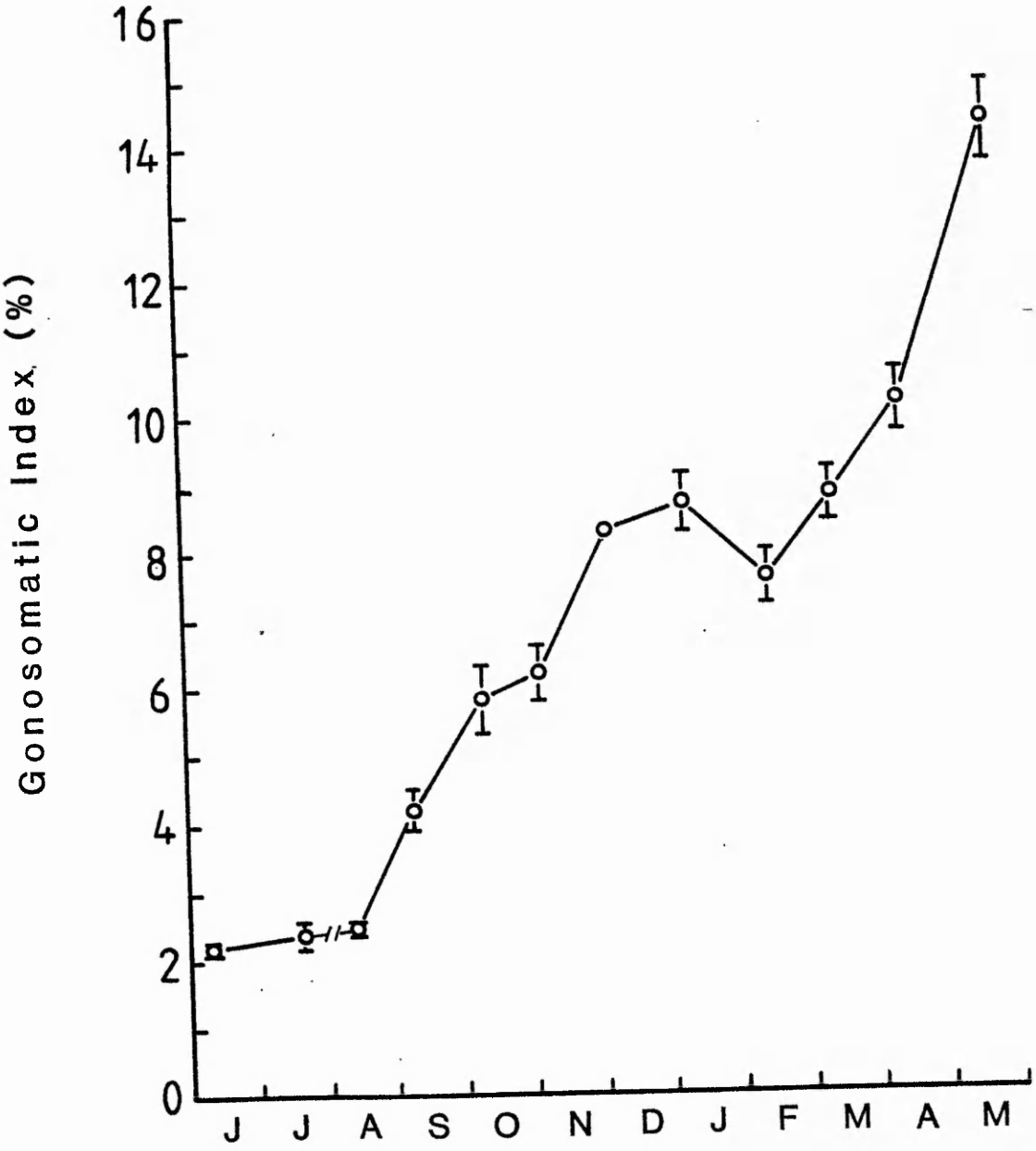


Figure 4. The mean gonosomatic index ($\bar{x}$ ± SEM) recorded over an annual reproductive cycle of females from a wild population. Sample numbers are given in Appendix 2.

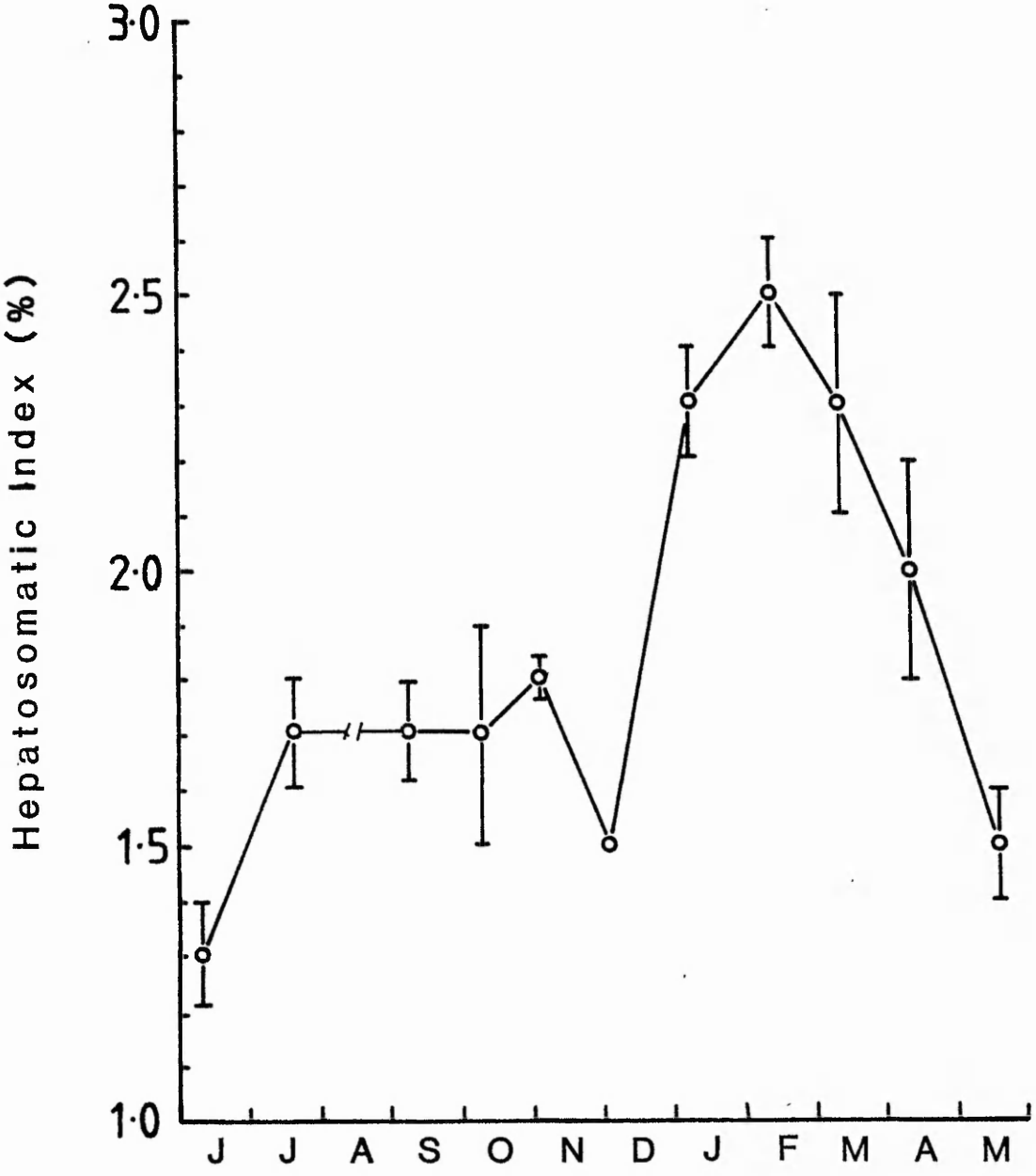


Figure 5. The mean hepatosomatic index ($\bar{x}$ + SEM) recorded over a reproductive cycle of females from a wild population. Sample numbers are given in Appendix 2.

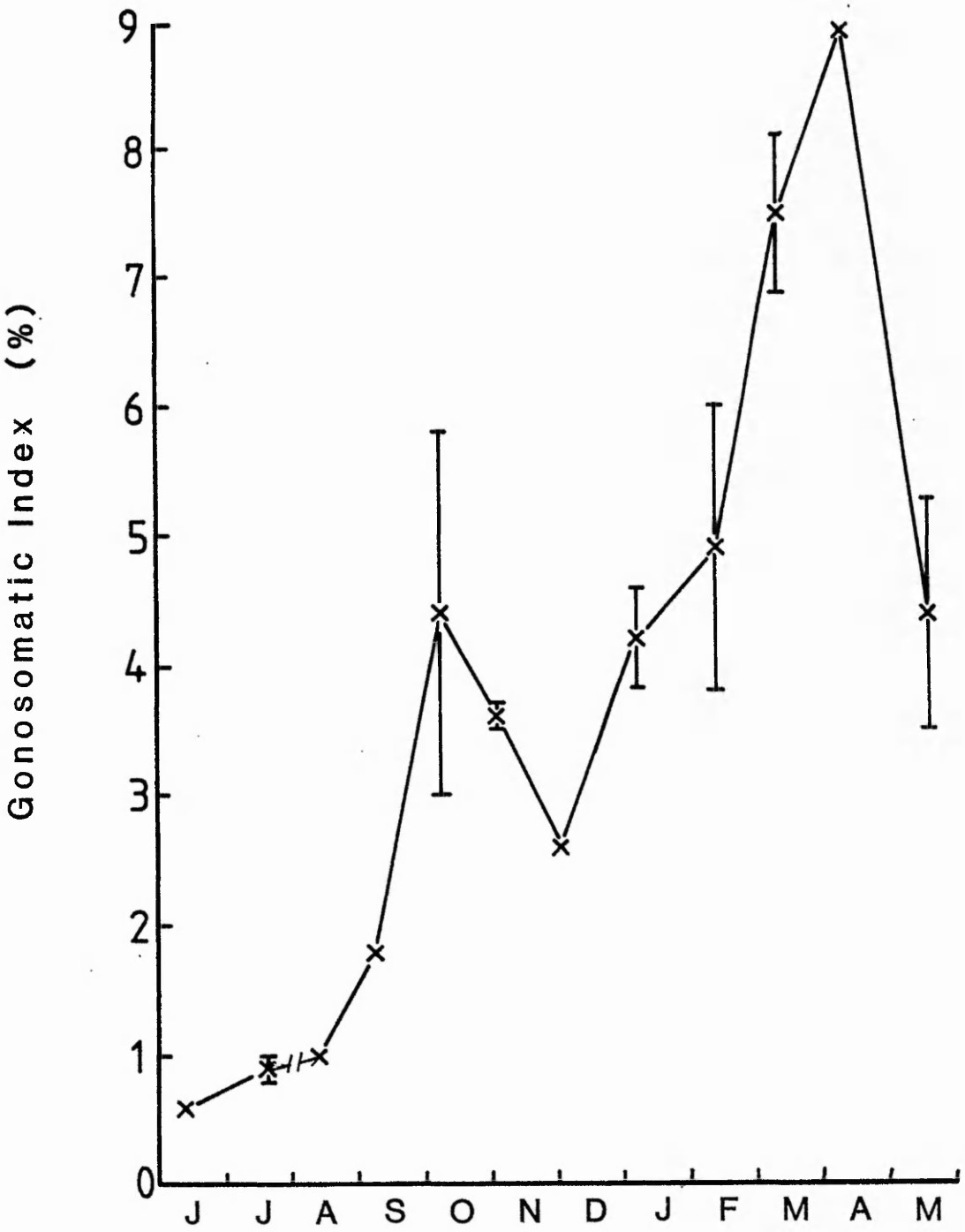


Figure 6. The mean gonosomatic index ($\bar{x} \pm$ SEM) recorded over a reproductive cycle of males from a wild population. Sample numbers are given in Appendix 3.

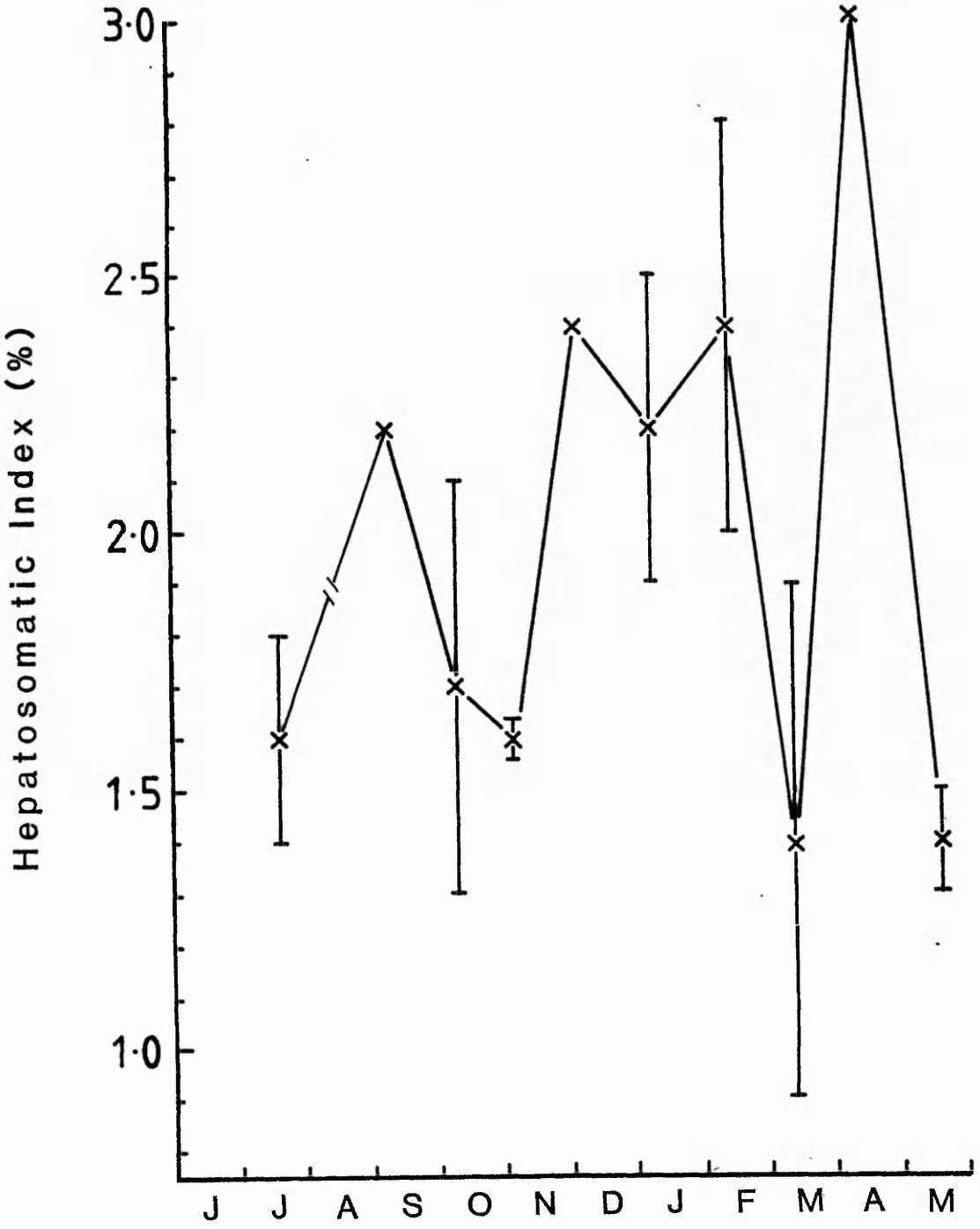


Figure 7. The mean hepatosomatic index ($\bar{x}$ + SEM) recorded over a reproductive cycle of males from a wild population. Sample numbers are given in Appendix 3.

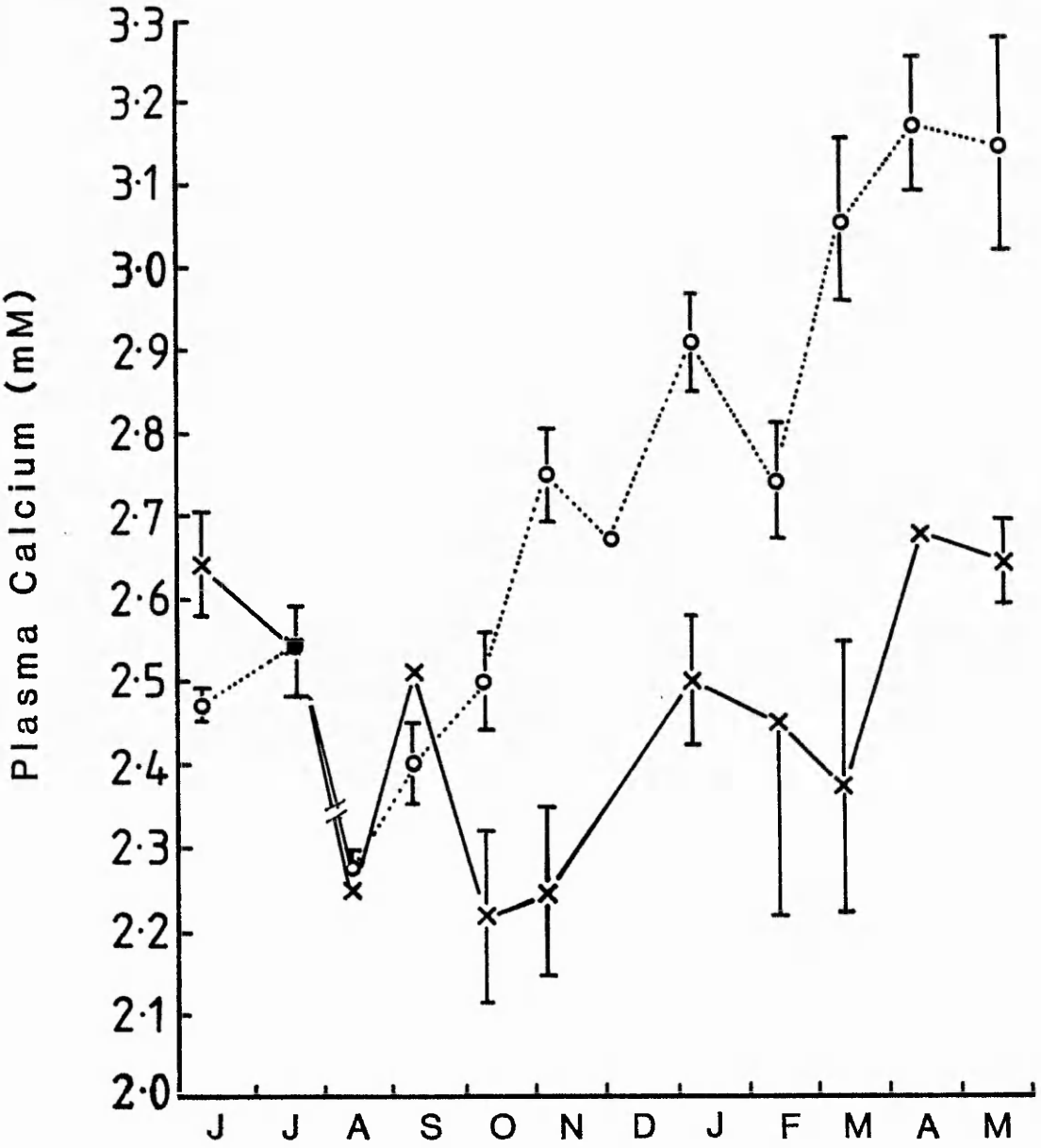


Figure 8. The changes in plasma calcium ($\bar{x} \pm$ SEM) recorded over a reproductive cycle of males (×——×) and females (o·····o) from a wild population. Sample numbers are given in Appendix 3.

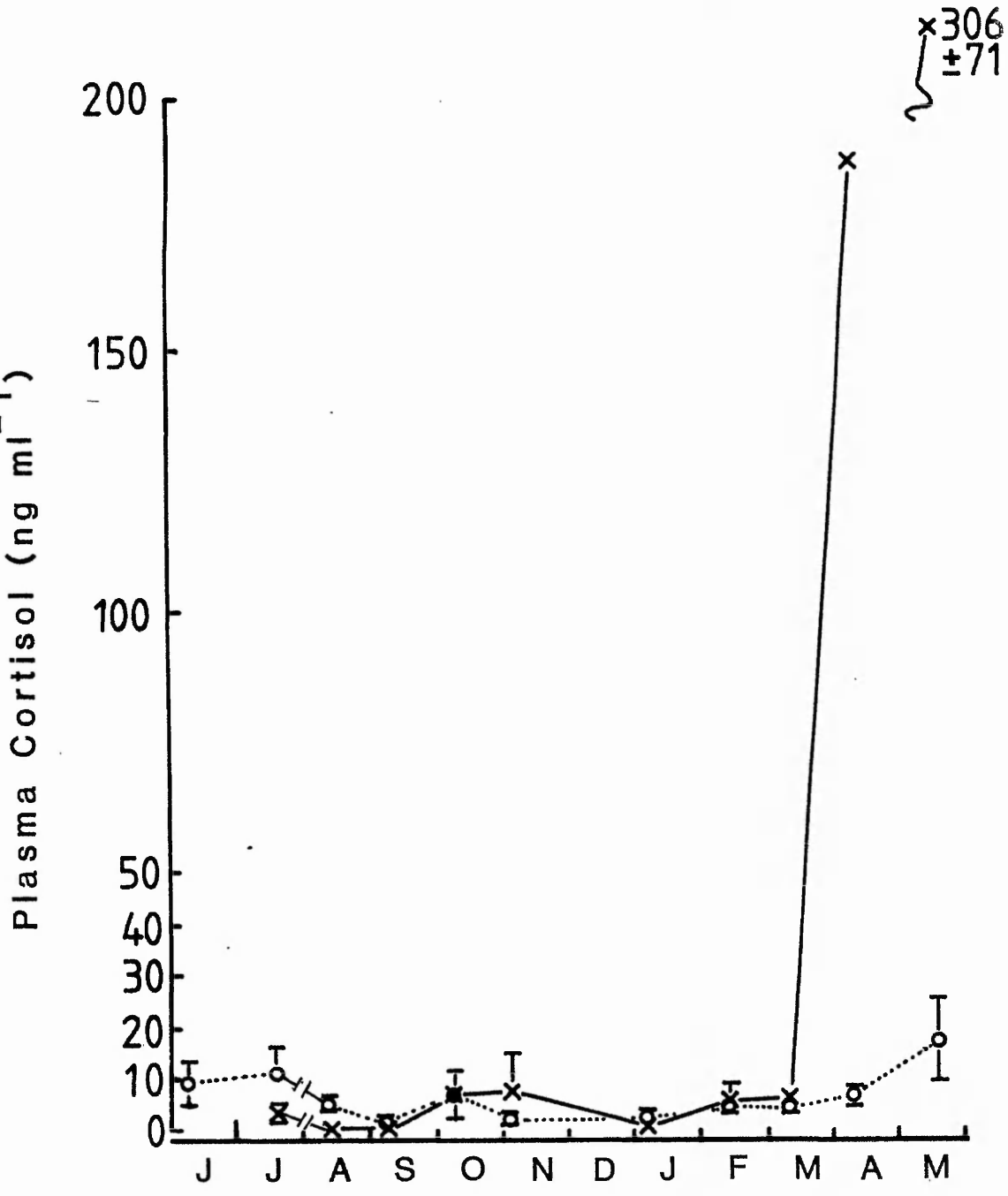


Figure 9. Mean cortisol ($\bar{x} \pm$ SEM) measured from plasma samples collected over a reproductive cycle of males (×——×) and females (o·····o) from a wild population. Sample numbers are given in Appendix 4.

In females, plasma cortisol did not change significantly during the whole year, and generally remained below the peak mean of 17 $\pm$ 8 ng ml^{-1} (May). Concentrations of plasma cortisol in males were similarly low during the period July to March. However, the large sample of males taken on the shallow spawning grounds in May was shown to have a mean cortisol titre of 306 $\pm$ 71 ng ml^{-1}. The one male captured in the previous (March) sample had a plasma cortisol concentration of 187 ng ml^{-1}. Unfortunately, no males at all were captured during June.

3.4 Discussion

The roach population of Highfields Lake spawned during the same period as the wild population monitored by Jafri (1980) between late May and early June. Ripe males had already gathered in the spawning areas by May 19, as indicated by the simultaneous electroshocking of groups of males around reed beds. The females were located around the periphery of the spawning areas at this time.

An interesting feature of the samples collected was the rarity of males (excluding the May 19 sample). Consequently, only cautious interpretation of the data from males is possible. It is not known why so few males were caught. The origin of fish is impossible to determine. Many fish from a variety of different populations have been introduced to Highfields during recent years. Other evidence suggests that it is unlikely that natural sex ratios of roach favour females to the extent suggested by the data. For example, the sex ratio of roach derived from artificial spawnings by STWA, and subsequently used as brood fish in this study (see Chapter 5, Table 10) was found to be approximately 2 : 1. Jafri (1980) made no mention of sex ratios, but generally samples contained more males than samples from Highfields. The sampling technique may have selected females preferentially, although it is difficult to see why this should be so. It is noteworthy that the

capture of male plaice was found to be equally difficult by Wingfield and Grimm (1977), and they too gave no explanation.

GSI of both sexes follows the pattern characteristic of annually-spawning species, being highest immediately before, and lowest immediately after spawning. Over the winter period, Jafri (1980) found higher GSI values than in this study for both sexes. However, the source of fish was not stated, and they may have been maintained in the laboratory at elevated temperatures which would probably have accelerated gametogenesis (see Chapter 5).

The relationship between HSI and GSI strongly implicates the liver in ovogenesis, as demonstrated in many other studies (eg, de Vlaming et al., 1977; Olivereau and Olivereau, 1979). There seem to be two phases to this relationship: predominantly anabolic, and catabolic. It is likely that up until February, the liver accumulated glycogen and lipid, and possibly synthesised yolk lipoglycophosphoproteins from these precursors and circulating amino acids (Wallace, 1978). Elevated plasma calcium indicates the presence of circulating vitellogenin from September, which is supported by the finding of Jafri (1980), that primary yolk oocytes first appear in the ovary during the same month. All of these changes have been induced artificially with oestradiol-17β in the eel, Anguilla anguilla, by Olivereau and Olivereau (1979). This also suggests that plasma concentrations of this hormone increase during this phase. Since vitellogenesis begins in September, it might be expected that the liver would fall in weight from then. However, feeding continues through the winter (evidence from dissection), thus there is continued nutritional input to the liver. Furthermore, oocytes are small at this stage (Jafri, 1980) which would suggest a low demand for vitellogenin. Vitellogenesis, and hence depletion of hepatic lipid and glycogen, appears to begin in earnest from February, evidenced by the inverse correlation of GSI and HSI from this point until spawning. Declining liver weight from February is explained by the fact that the liver is

considered to be the most important source of lipid for vitellogenesis (Takahashi, 1974). However, a seasonal study (Htun-han, 1978a) of the dab, Limanda limanda, which spawns in winter, suggests that it may be possible that the correlation between HSI and GSI is simply coincidental. Htun-han found no differences in HSI between males and females, nor any relationship of HSI and GSI during the pre-spawning period of females when vitellogenesis begins in the dab (Htun-Han, 1978b). He suggests that vitellogenesis does not seriously deplete liver reserves. Thus, feeding during this period must be sufficient to sustain gametogenesis. However, HSI in the dab falls drastically only during spawning, whereas HSI falls more gradually during the pre-spawning period in the roach over a period of four months. In addition, plasma calcium reaches a plateau which is maintained from March to May, indicating high plasma vitellogenin levels during this period. The general pattern of plasma calcium of females is in broad agreement with the plasma profile of vitellogenin for other species. Crim and Idler (1978) found that plasma vitellogenin (determined by RIA) increased significantly four months before the GSI peak prior to spawning. Furthermore, Bromage et al. (1982) demonstrated elevated vitellogenin levels (as indicated by increases in both plasma phosphoprotein-phosphorus and calcium) four months before spawning, in rainbow trout under similar conditions. Vitellogenin remains in circulation after oviposition as indicated by elevated plasma calcium on two sampling occasions following spawning. This phenomenon was also observed in the studies of Crim and Idler (1978), Bromage et al., (1982) and also le Menn (1979). The latter author suggested that during post-spawning ovarian atresia, yolk proteins are returned to the blood for eventual catabolism in the liver.

Few firm conclusions can be drawn from the data of males. Plasma calcium does not appear to change throughout the year. The HSI data is equivocal, but there does seem to be glycogen depletion from February to spawning, as shown by falling HSI. It is likely that the GSI value recorded in

May (before spawning) is an underestimate because all of the males captured were "running" profusely and inevitably lost semen before the gonads were weighed. This is supported by the fact that Jafri (1980) recorded male roach GSI of 8.08 ± 0.8% and 10.2 ± 0.12% in the two months prior to spawning.

Condition factor is evidently not sensitive enough to register distinctly the processes of reproduction in roach, although it does appear to increase slightly just prior to spawning. Indeed, K fell by very little in females immediately after spawning, despite the fact that the mean GSI fell by more than 12%. A change of K of greater magnitude was reported for powan by Scott (1979), despite the fact that GSI fell by an amount (13%) similar to female roach.

It is likely that blood sampling of each fish was completed before plasma cortisol was stimulated by capture, as discussed in 2.4. It is interesting to note that there are no detectable annual changes in plasma cortisol in females, because cycles have been demonstrated in several other species, and in many cases, associated with reproduction. For example, Peter et al., (1978) showed that maturing female goldfish had higher levels of plasma cortisol than mature fish which had completed vitellogenesis. In addition, Sundararaj, Nath and Jeet (1978) demonstrated that plasma cortisol rises during vitellogenesis in the channel catfish. These elevations in plasma cortisol may be involved with mobilisation of hepatic glycogen reserves, since cortisol and cortisone modify enzyme activity associated with protein catabolism in the liver of rainbow and brook trout, Salvelinus fontinalis, (Freeman and Idler, 1973). However, there may be an undetected short-term elevation of plasma cortisol in roach around maturation and ovulation which occurred between two samples. Raised activity of the interrenal system and elevation of blood cortisol titres have been associated with spawning time in several other species, eg, sockeye salmon, Oncorhyncus nerka (Donaldson and Fagerlund, 1968); powan (Fuller, Scott and

Fraser, 1976); catfish (Sundararaj, Nath and Jeet, 1978); goldfish (Cook et al., 1980); brown trout (Pickering and Christie, 1981) and plaice (Wingfield and Grimm, 1977). The latter authors attribute the plasma cortisol rise around spawning time to mobilisation of energy reserves during the season of poor feeding. This highlights a problem; elevated plasma cortisol may be due to processes other than gametogenesis, including stress responses, starvation and migration, and should therefore be interpreted cautiously.

Male roach did show a cycle of plasma cortisol, with increases around spawning time, reaching a peak immediately prior to spawning. This is almost certainly due to increased activity around spawning time. From observations of courting male roach, where they actively seek and chase females, attempting to drive them to the spawning grounds (Plates 7 to 10), it is clear that a great deal of energy must be expended during the spawning season. This is probably mobilised from somatic stores by cortisol.

Since the interrenal system appears to be relatively inactive between November and February, it is unlikely that the experiment described in Chapter 1 would have been confounded by underlying, seasonal changes in plasma cortisol.

4 INDUCED SPAWNING

4.1 Introduction

The ability to control reproduction is essential for the effective and efficient culture of many fish species. Among the advantages of this capability are the possibilities of the temporal control of the production of larvae, the artificial incubation of fertilised eggs, the genetic improvement of stock and the control of disease. The term "induced spawning" is used to refer to the artificially controlled spawning of ripe fish (having completed gametogenesis except for gamete maturation). Natural control mechanisms have evolved to ensure that spawning takes place when environmental conditions are most suitable for the development and survival of the progeny. Thus, environmental cues (such as changes in water temperature or flow) are used by the fish to synchronise reproduction. Traditionally, the spawning of captive fish was often controlled by simulating these natural cues by, for example, water changes or thermal shock. Though these techniques do undoubtedly work for some, they cannot be applied to all species, and they may not be effective for all the individuals of a group simultaneously. Prospects for the artificial control of reproduction were greatly improved when Houssay (1931) demonstrated that the injection of a fish pituitary extract induced premature parturition in a viviparous fish. A few years later, ovulation was induced successfully in an oviparous species using the same hypophysation technique, by von Ihering (1937). This pioneering work was perfected in subsequent years as knowledge of the various endocrine mechanisms controlling reproduction in fish increased.

The control of reproduction in fishes has been reviewed several times recently (for example, Chaudhuri, 1976; Fontaine, 1976; Harvey and Hoar, 1979; Lam, 1982; Peter, 1982), but a brief account will be given here. The hypothalamo-hypophysial unit is at the centre of control of

reproduction. The adenohypopysis of the pituitary contains cyanophilic cells (the gonadotrophs) which synthesise and store gonadotrophic hormone, GtH, (Atz and Pickford, 1959; Peute, Goos, de Bruyn and van Oordt, 1978). There is evidence (Peter, 1982) that the nucleus preopticus (NPO) and nucleus lateralis tuberis (NLT) regions of the hypothalamus have neurosecretory activity and control the gonadotrophs. The NPO and the NLT respectively, serve to inhibit (via GtH release - inhibitory factor, GRIF), or to stimulate (via GtH - releasing hormone; GnRH) GtH release from the pituitary (reviews: Ball, 1981; Peter, 1970, 1982). In the few species which have been investigated, many show annual (Billard, Breton, Fostier, Jalabert and Weil, 1978) and daily cycles (Breton, Billard, Jalabert and Kann, 1972; Hontela and Peter, 1978), of circulating GtH. During gonad recrudescence in the rainbow trout for example, under the influence of steadily rising blood GtH, the testes produce androgens (Ng and Idler, 1980) and the ovaries oestrogens (Bromage, Whitehead and Breton, 1982). These sex steroids, among other actions, feedback to the NLT pituitary and (Billard and Peter, 1977), both positively: inducing the synthesis and accumulation of GtH (Crim, Peter and Billard, 1981), and negatively: curtailing further GtH release (Billard, 1978). The final stages of spermatogenesis (the migration of non-motile spermatazoa from seminiferous tubules to the vas deferens), and the initiation of spermiation, have been associated with elevated, then slowly declining plasma GtH in rainbow trout. GtH is thought to act indirectly by stimulating, and changing the balance of plasma androgens (Fostier, Billard, Breton, Legendre and Marlot, 1982; Billard, Fostier, Weil and Breton, 1982). In females, a more pronounced GtH surge usually occurs around the time of ovulation, as demonstrated in the goldfish (Stacey, Cook and Peter, 1979) and the rainbow trout (Fostier, Weil, Terqui, Breton and Jalabert, 1978; Bromage et al., 1982). Prior to ovulation, oocytes must undergo maturation (the resumption of meiosis) involving migration of the germinal vesicle from a central position, and its breakdown at the periphery. GtH brings about maturation

indirectly by stimulating plasma levels of maturation-inducing steroids, most likely progestogens or corticosteroids. The evidence suggests that the most commonly occurring maturation-inducing steroid is 17α-hydroxy-20β-dihydroprogesterone. This is synthesised by the ovarian follicles under GtH stimulation in, for example, the rainbow trout, northern pike and the goldfish (Jalabert, 1976). However, in the catfish, it appears that maturation is induced by C21 steroids produced by the interrenal gland under GtH control (Sundararaj and Goswami, 1977). There is strong evidence that ovulation sensu stricto is induced by prostaglandins (reviews: Lam, 1982; Stacey and Goetz, 1982). Consequently, where maturation is induced in vitro with progestogens, ovulation only takes place after treatment with prostaglandins (Jalabert, Breton and Fostier, 1978).

Thus, knowledge of the various endocrine mechanisms controlling reproduction in teleosts enables it to be manipulated with the use of appropriate exogenous hormone preparations. The majority of induced spawning practice involves the injection of GtH of varying purity, usually a pituitary preparation. However, cheaper mammalian hormones such as human chorionic gonadotrophin (HCG) are also used. Despite their effectiveness, there are several disadvantages to the use of pituitary gonadotrophins. Since the GtH content of pituitaries varies seasonally (Peute et al., 1978) and diurnally (de Vlaming and Vodicnik, 1977) it is impossible to standardise dosages without performing an assay of pituitary GtH beforehand. In addition, correct dosing is rendered difficult due to species-specificity (Fontaine et al., 1972) and deterioration of the potency of pituitaries during storage (Yaron, Bogomolnaya and Hilge, 1982). Thus, synthetic compounds have been investigated increasingly. Spawning has been induced successfully with luteinising hormone - releasing hormone (LH - RH) and superactive analogues, the antioestrogen clomiphene citrate, corticosteroids, and progestogens (review: Lam, 1982).

The objective of the present study was to refine the techniques of induced spawning of roach. Limited success had been achieved previously using the hypophysation technique with carp pituitary extract (Easton and Dolben, 1980). This method was employed in the present study after slight modification in order to produce larvae for the pond rearing experiments described in Chapter 7. Subsequently, the antioestrogen tamoxifen (ICI 46474), used in medical practice for the treatment of breast cancer (Ward, 1973), was evaluated along with clomiphene citrate as potential spawning agents for roach. Although pituitary implantation of tamoxifen has been shown to stimulate GtH release from the pituitary (Billard and Peter, 1977), it had not previously been used successfully to induce spawning in fish. However, tamoxifen was effective (when used in conjunction with salmon gonadotrophin) in inducing ovulation in coho salmon, Oncorhyncus kisutch (Donaldson, Hunter and Dye, 1982).

4.2 Materials and Methods

4.2.1 Carp pituitary extract

Induced spawning procedures using carp pituitary extract (CPE), took place between 22 April and 21 May 1980 (Table 5).

4.2.1.1 Animals

Brood roach were captured from a number of sources, mainly the Rivers Meden, Poulter and Greet, with additional fish coming from Foston and Highfields reservoirs, and a Rotherham pit. All the venues were within a 65 km radius of Nottingham. The fish, ranging from 30 to 700 g, were captured by pulsed DC electrofishing from the rivers and seine netting from the still waters. The sex ratios were approximately 1:1.

4.2.1.2 Holding conditions

The fish were introduced to the two 1,000 l recirculating flumes described in 2.2.2. Each flume was provided with running water, diffused aeration and "kakaban" spawning substrate, the latter being a necessary spawning stimulus to the goldfish (Stacey et al., 1979). The kakabans were made from rectangular pieces of green plastic netting incompletely cut across the width into strips, resembling a comb. These were rolled-up and bound at the uncut end with lead strip to counteract buoyancy. These could be unwound for the purposes of egg counting for example. The water temperature varied between 16 and 20°C, and natural daylight provided the illumination.

4.2.1.3 Hormone administration

The acetone-dried pituitary extract ("Stoller Fisheries", USA) was prepared as a 2.5 mg ml^{-1} suspension in 100 mM NaCl. This was thoroughly mixed, centrifuged and the supernatant used for injection. The injection volume was 0.1 ml 100 g^{-1} body weight. Two or three intraperitoneal injections were given, with 1 ml syringes and 16 x 0.5 mm needles, according to the regime in Table 4.

Table 4 The dosage regime of CPE used to spawn roach

		Day 1		Day 2
Time (h)		09.00 - 11.00	16.00 - 18.00	(last doses repeated 10.00 - 11.00 for unspawned fish)
Dose CPE	♀	2.5	5	
(mg kg^{-1})	♂	2.5	5	
Spermiated	♂	-	2.5	

The kakabans were inspected daily for viable eggs.

4.2.2 Antioestrogens

4.2.2.1 Animals

64 brood roach (30 - 100 g) were captured by seine netting from Highfields Lake, Nottinghamshire. The sex ratios of each group could not be determined at the time of capture. This was because the tubercles of the males, the main distinguishing secondary sexual character, had not developed.

4.2.2.2 Holding conditions

On 29 April 1981, groups of eight fish were introduced to the eight aquaria (70 l) of two similar recirculating systems. Each system consisted of four polypropylene tanks (each of 150 l capacity), 18 W UV water sterilisers, and settling, biological filter and heater/header tanks, the latter fitted with a 1 kW electric heater and thermostat. Aeration was by means of diffused compressed air. Water was recirculated by means of centrifugal pumps. Photoperiod was controlled by automatic time switches. Acclimation from the lake temperature (11°C) to 16°C took place over a period of three days. 16°C is a temperature favourable to spawning in roach (Easton and Dolben, 1980). A summer photoperiod (16L:8D) was maintained, in contrast to the natural period of slightly less than 15L:9D.

4.2.2.3 Drug administration

Clomiphene citrate (Richardson Merrell) was dissolved in distilled water with the addition of Tween 80 at a rate of two drops per 3 ml to enhance solution. This 5 mg ml^{-1} stock solution was serially diluted for the lower dosages. Tamoxifen base (ICI) is very insoluble in water (like the citrate form) and initially required absolute ethanol to dissolve it. After ten-fold dilution with distilled water, precipitation occurs, so the suspension was vortex-mixed to ensure homogeneity for serial dilution. NaCl was added to make a 100 mM vehicle for both compounds. Groups of fish

were injected intraperitoneally at 17.30 and 16.00 h on 1 and 5 May (days 0 and 4 respectively), according to the following regime:

Tamoxifen (TAM, mg kg^{-1}) : 0.1, 1 or 10. Saline control.
Clomiphene (CLOM, mg kg^{-1}) : 0.1, 1 or 10. Saline control.

Injection volumes were 1 $ml kg^{-1}$. Each group was provided with kakabans. Each morning, the fish were inspected and gentle finger pressure applied to the abdomen. The occurrence of spermiation, the development of tubercles (males), or the release of ovulated eggs (females) were noted. All fish were killed and dissected on day 7 to determine sex and gonad condition.

4.3 Results

4.3.1 Carp pituitary extract

203 roach in seven batches (Table 5) were hypophysised with CPE and approximately 70 % of these spawned, producing an estimated minimum of 150,000 viable eggs. In the majority of the females, ovulation and spawning began the morning of day 2, after two injections (Table 5). The males which did not initially release semen, usually did so after the first CPE injection. Spawning appeared to be compulsive and often continued through day 2. Eggs often completely covered the kakabans (Plate 5), the surrounding walls and bed of the flume. A high proportion of the eggs were viable and the larvae which hatched 7 - 10 days after spawning (Plate 6) were transported to growing-on ponds at Calverton Fish Farm.

Spawning behaviour in roach begins with congregation of males around the spawning substrate, where they constantly search for females. Unovulated females meanwhile, are less active than the males and avoid them. Males periodically swim amongst the females and attempt to "round" them up. Ovulated females cooperate with this behaviour, and are

Table 5 The results of CPE treatment of brood fish from various origins

Origin of groups	No. of fish	Date of intro"	Hormone treatment date / time	Date of spawning
R. Poulter	13	22.4	24.4/11.30,17.00 25.4/11.00	 26.4
R. Meden	17	30.4	3.5/10.00,17.00 4.5/11.00	 4.5,5.5
R. Meden	31	30.4	7.5/11.00,15.00	8.5
Highfields Lake R. Meden	22 12	12.5 13.5	14.5/08.30,11.30	15.5
" + Foston	40	15.5	17.5/12.30,16.30	18.5
Highfields Lake R. Meden Foston	28	Various	19.5/12.00,17.00	20.5
Rotherham coalpit	40	21.5	22.5/10.00,14.00 23.5/10.30	23.5 24.5

Table 6 The responses of ripe roach to tamoxifen or clomiphene given at various dose levels

TREATMENT		TIME (days)							
TAMOXIFEN	n sex	0+	1	2	3	4+	5	6	7
mg kg^{-1}	6 ♀								
0.1	2 ♂	**	**	**	**	**	**	**	**
1	6 ♀					XXXXX S	XXXX S	S	
	2 ♂	**	**	**	**	**	**	**	**
10	7 ♀								
	1 ♂	*	*	*	*	*	*	*	*
SALINE	7 ♀								
	1 ♂	*	*	*	*	*	*	*	*
CLOMIPHENE	n sex	0+	1	2	3	4+	5	6	7
mg kg^{-1}	6 ♀								
0.1	2 ♂	**	**	**	**	*	*	*	*
1	8 ♀							X	X S
10	6 ♀							X S	
	2 ♂	*	*	*	*	**	**	**	**
SALINE	6 ♀								
	2 ♂								**

KEY + = Injection ; x = Number of females releasing ovulated eggs ; * = Number of males releasing semen Day 0 = 1.5.81 S = spawning.

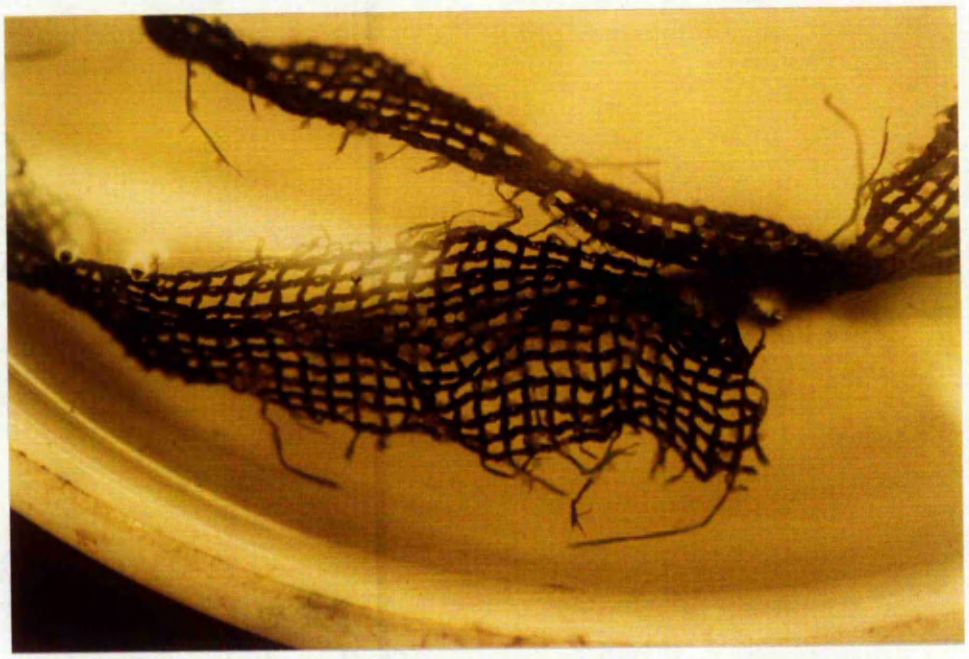


Plate 5. Roach eggs adhering to "kakaban" spawning substrate. Slightly less than natural size.

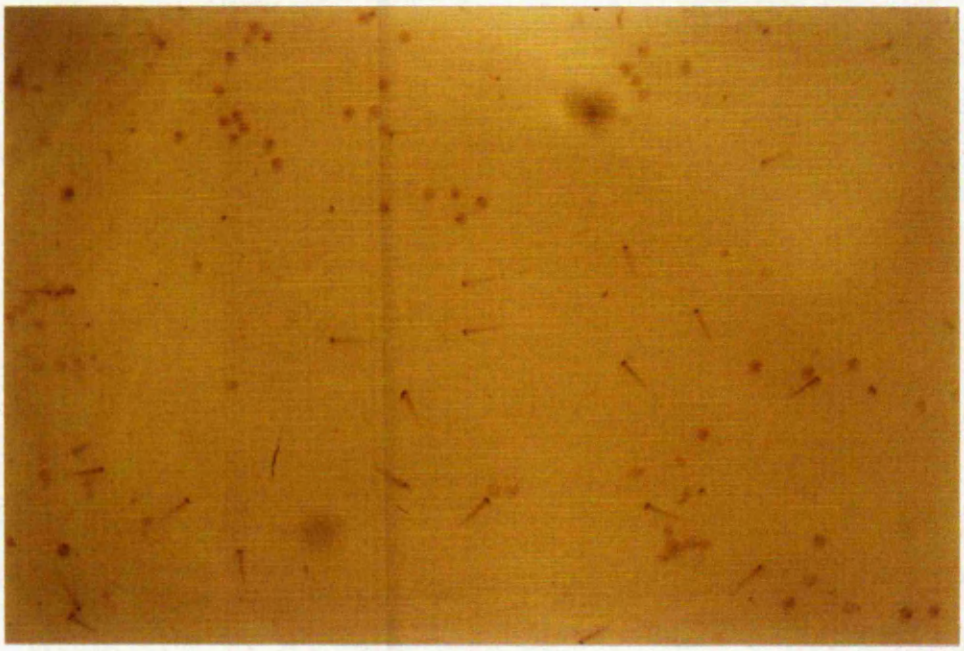


Plate 6. Free-swimming roach larvae approximately 3 days after hatching. Also shown are some "eyed" eggs from a subsequent spawning. Slightly less than natural size.

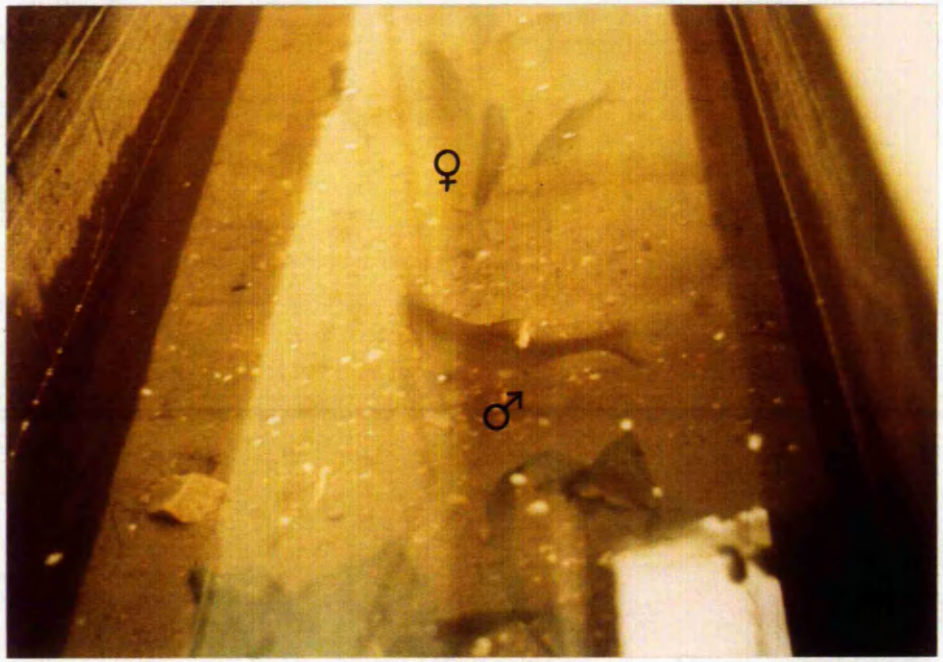


Plate 7

Plate 7 shows an unsuccessful attempt of the male in the centre of the picture to drive a female (upper centre) into the kakabans at the bottom of the picture to spawn.

Plate 8. The male in the centre of the picture has coaxed a female (lower centre) towards the kakabans where spawning will take place.

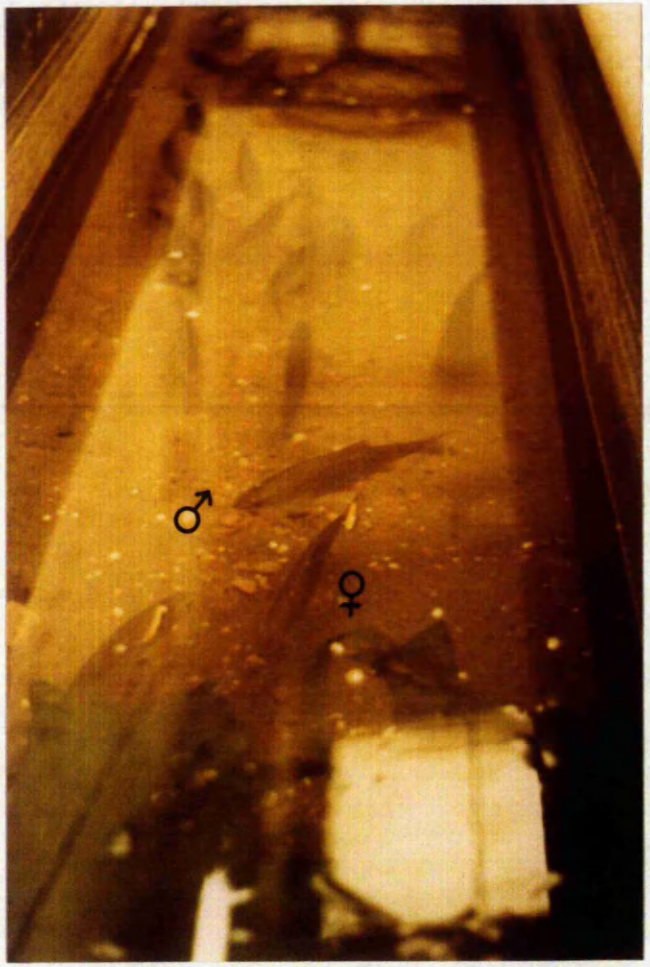


Plate 8

Plate 9

The male, covered in white tubercles, has guided the female into the kakabans which have been liberally covered with eggs from previous spawning.

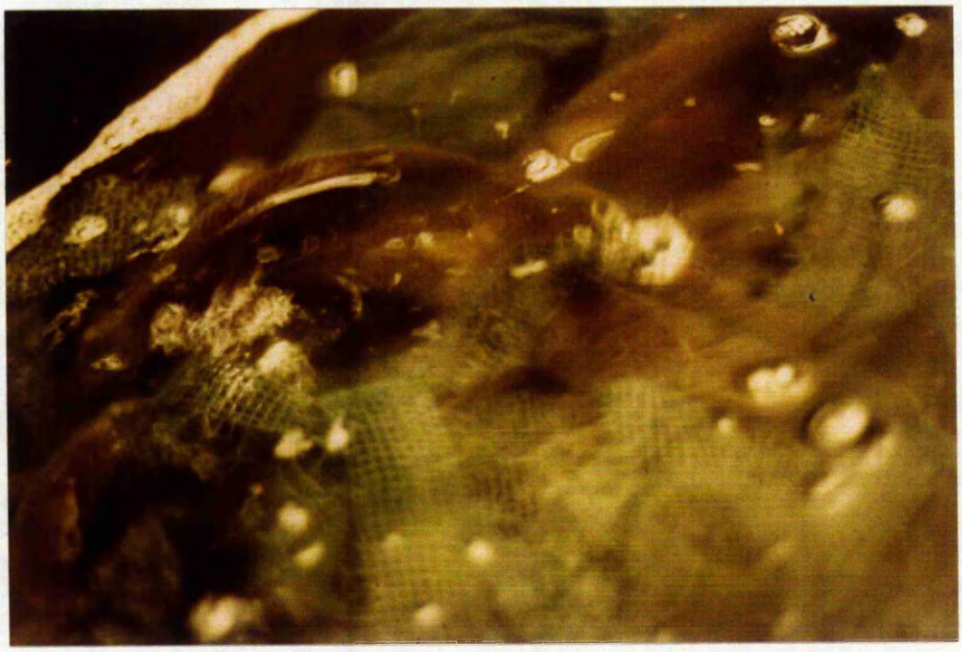


Plate 10

Spawning takes place as both sexes swim rapidly, side-by-side, through the entangled kakabans. This activity may be so vigorous that the fish momentarily leave the water, as in this example.

driven through the kakabans where oviposition takes place (Plates 7 to 10).

4.3.2 Antioestrogens

Sex ratio data, along with spermiation, ovulation and oviposition events are presented in Table 6. Excepting the 1 mg kg^{-1} CLOM group, all included at least one functional male.

When examined on the morning of day 4 (after one injection), five of the six females in the 1 mg kg^{-1} TAM group released ovulated eggs. Some spawning had taken place, and this continued until day 6 when the fish were "spent". This was the only TAM group which ovulated or spawned, whereas both of these events occurred in two CLOM groups. Two females in the 1 mg kg^{-1} CLOM group ovulated and spawned by day 7, despite the absence of males. In the 10 mg kg^{-1} CLOM group, one female ovulated and spawned on day 6. Spawning took place in the latter two groups two days after the second injection. The eggs produced from these spawnings developed normally and produced viable larvae, with the exception of 1 mg kg^{-1} CLOM group which contained no males. All of the saline treated fish failed to ovulate or spawn.

4.4 Discussion

The induced spawning with carp pituitary extract was employed as a means of obtaining larvae for pond rearing experiments (Chapter 7). Thus, without experimental controls, only limited interpretation of the results is possible. However, the regime used is evidently very effective with ripe roach, with a high proportion of each group spawning after treatment. Easton and Dolben (1980) reported successful spawning on 50 % of occasions after injecting groups of roach with CPE using either the same regime, or another involving a single injection (male, 2.5 mg kg^{-1}; female, 10 mg kg^{-1}). The reduced

effectiveness of hypophysation reported in the earlier study may have been due to a number of factors, for example the presentation of the hormone preparation, which was intramuscular. A significant drawback to intramuscular delivery is leakage of injectant. The peritoneal cavity is considered to be more capable of retaining and rapidly absorbing injectant, hence intraperitoneal delivery was used in this study. However, absorption of GtH into the bloodstream is more rapid after intramuscular rather than intraperitoneal injection in the catfish (BI Sundararaj, pers comm). One method to reduce leakage is to increase the viscosity of the injectant by adding glycerine, in the ratio 7:3 (Woynarovich, 1975).

Procedures for hypophysation in current use vary a great deal, but it is generally considered that a "priming" followed by a "resolving" dose is most effective (reviews: Donaldson, 1975; Chaudhuri, 1976; Fontaine, 1976; Harvey and Hoar, 1979; Pullin and Kuo, 1980; Lam, 1982). For example, Jungwirth (1979) found that two injections were better than one. Thus, in the work of Easton and Dolben (1980), where more than half of the trials involved the use of a single dose of CPE, greater success may have been achieved by dividing the dose between two injections.

It is noteworthy that four of the seven groups spawned soon after capture by electroshocking, in the case of group four, two days after capture. Indeed, running males were observed courting females within an hour of their introduction to the laboratory. Therefore, electrofishing does not necessarily impair the reproductive function of ripe fish.

Both clomiphene citrate and tamoxifen base are clearly effective in inducing ovulation and spawning in ripe roach. Clomiphene has been used successfully to induce ovulation in other studies with a variety of species, notably the goldfish (Pandey and Hoar, 1972; Pandey, Stacey and Hoar, 1973), the carp (Kapur and Toor, 1979), the freshwater catfish (Singh and Singh, 1976), and to a limited extent,

the roach (Jafri, 1980). Tamoxifen alone has not previously been used to induce ovulation and spawning in fish. However, trials by Donaldson, Hunter and Dye (1978, 1982) with coho salmon demonstrated an ovulation-inducing effect of tamoxifen used in conjunction with salmon GtH (SG-G100), but no significant effect of tamoxifen alone. Since males were not included in the latter study, the spermiation response was not investigated.

To date, the mode of action of both drugs in inducing ovulation has not been determined unequivocally. However, there is a great deal of evidence to suggest an indirect mechanism. Both clomiphene and tamoxifen induced substantial increases in plasma GtH when implanted into the pituitary and (to a lesser extent) the NLT of the goldfish (Billard and Peter, 1977). This suggests that, firstly, both drugs have an indirect action, and secondly, the pituitary, and to a lesser extent the NLT, are both sites for negative feedback of sex steroids. In addition, after clomiphene treatment, hypophysectomy blocks ovulation in the goldfish (Pandey et al., 1973) and freshwater catfish (Singh and Singh, 1976). In contrast, sham-operated controls ovulated. Thus, since the pituitary is necessary for the ovulation-inducing effects, the drugs probably act via GtH secretion. Kapur and Toor (1979) suggested that the ovulation-inducing effects of clomiphene may be mediated by prostaglandins. By treating carp with indomethacin (an inhibitor of prostaglandin synthesis) ovulation was blocked. These effects were superseded when clomiphene was given simultaneously, although the response was subdued. However, this experiment does not definitively demonstrate prostaglandin mediation in the mode of action of clomiphene. The prostaglandins necessary for ovulation are synthesised by the ovarian follicles (Jalabert, 1976). Thus, it is likely that, in the experiment by Kapur and Toor (1979), the indomethacin, by inhibiting prostaglandin synthesis, blocked the action of GtH and not clomiphene, since circulating GtH is known to increase after treatment with clomiphene (Breton, Jalabert and Fostier, 1975; Billard

and Peter, 1977). To inhibit GtH secretion, indomethacin would have to interfere with binding of clomiphene with sex-steroid receptors in the hypothalamo-hypophysial unit, and there is no evidence for this.

In this study, the observed delay between first injections and spawning (3 days for 1 mg kg^{-1} TAM, 6 days for 10 mg kg^{-1} CLOM and 7 days for 1 mg kg^{-1} CLOM) also suggests an indirect mechanism, via the hypothalamo-hypophysial unit, rather than direct action on the gonad. Similar latency of response has been demonstrated in other studies. Peaks of plasma GtH occurred 12 - 60 hours after injecting carp with 0.1, 1 or 10 mg kg^{-1} clomiphene (Breton et al., 1975). After treatment with clomiphene, most ovulations/spawnings occurred within 2 to 4 days in the goldfish (Pandey and Hoar, 1972), 7 days in the catfish (Singh and Singh, 1976) and 4 days in the carp (Kapur and Toor, 1979).

Failure of roach to ovulate when given the lowest dose of tamoxifen or clomiphene (0.1 mg kg^{-1}) suggests that any elevation in plasma GtH did not surpass the threshold for ovulation. There are no reports of spawning success with dosages as low as 0.1 mg kg^{-1}. In other studies, effective dosages (mg kg^{-1}) of clomiphene were: 1, 10 (Pandey and Hoar, 1972); approximately 3 (Singh and Singh, 1976); and 5 to 20 (Kapur and Toor, 1979). In the present study, the failure of the 10 mg kg^{-1} TAM group to ovulate and spawn may be a result of oestrogen-agonistic properties of a high dosage. Such effects have been observed in mammals, where tamoxifen may have agonistic or antagonistic properties, depending on the species and dosage (review: Patterson, 1981). Thus, there may be a critical distinction betwen dosages of tamoxifen having oestrogen-antagonistic (1 mg kg^{-1}) or agonistic properties (10 mg kg^{-1}) in the roach.

In conclusion, although the hypophysation procedure employed was very effective, the substitution of tamoxifen in induced

spawning practice may be beneficial on the grounds of cost alone. Furthermore, because tamoxifen is administered orally in clinical medicine (marketed as "Nolvadex") this form of presentation might be applied to fish culture. This would be very convenient if effective, and would minimise stress and physical damage to brood stock. Tamoxifen is likely to be more useful than clomiphene because it was found to be the most potent antioestrogen in the trials of Miller and Huang (1981). The latter study involved measuring the antagonistic effects of antioestrogens on oestradiol-induced GtH secretion from ovine pituitary gonadotrophs in vitro.

5 THE ADVANCEMENT OF GAMETOGENESIS AND SPAWNING

5.1 Introduction

The mechanisms which control the reproductive cycles of teleosts have evolved to ensure that the progeny emerge during the time of year most conducive to their survival. Thus, most species rely on regular predictable changes in the environment, frequently temperature and photoperiod, to cue the endocrine processes which control gametogenesis. As stated by Lam (1982), the majority of artificially controlled reproduction involves induced spawning of ripe brood fish obtained during the spawning season. Many fish, particularly cyprinids, complete gametogenesis at around the same time each year, and await an environmental signal, such as a rise in temperature, which induces ovulation or spermiation, and spawning. The timing of this signal varies slightly from year to year. Thus, after the completion of gametogenesis (excluding maturation), further gonad development may be held in abeyance for several weeks. By recognising the time of completed gametogenesis, man can advance spawning by providing the appropriate environmental cue or administering hormones. However, in order to advance spawning further, it is necessary to accelerate gametogenesis itself. There are two main strategies adopted in this cause. Firstly, appropriate hormones may be administered on a long term basis, thereby manipulating endogenous control mechanisms. Secondly, the natural changes in the environment which induce gametogenesis may be simulated and advanced.

Hormonal manipulation of gametogenesis, like induced spawning, requires some prior knowledge of natural endocrine control. As outlined in 4.1, gonad recrudescence usually takes place under steadily rising blood GtH (review: Peter, 1981). In the female, this GtH stimulates the synthesis and release of ovarian oestrogens (Billard, et al., 1978), which in turn stimulates the production and release of vitellogenin (Elliot, et al., 1979). This is subsequently

taken-up by the ovary by pinocytosis, and deposited as yolk in the oocytes (Wallace, 1978). Gonadotrophin appears to stimulate both hepatic synthesis and release of vitellogenin (indirectly via oestrogens), and also incorporation of this as yolk into oocytes in hypophysectomised catfish Heteropneustes fossilis (Nath and Sundararaj, 1981). However, in the rainbow trout, salmon pituitary extract, but not purified S-GtH alone, stimulated such complete vitellogenesis (Upadhyay, Breton and Billard, 1978) indicating that some other pituitary hormone(s) is involved in the incorporation of yolk into oocytes. Thus, GtH plays a major role in vitellogenesis, but may act in conjunction with another pituitary hormone(s).

The endocrine control of spermatogenesis has been reviewed extensively by Billard et al. (1982). As in the female, GtH rises towards the end of gonad recrudescence in cyprinids and salmonids. Hypophysectomy causes testicular regression in most cases, for example, in H. fossilis (Sundararaj and Nayyar, 1967) and the goldfish (Billard et al., 1982). Experiments involving hormone replacement therapy of hypophysectomised fish have given unclear results. Although GtH is able to restore spermatogenesis to hypophysectomised fish, regeneration of the testis is often limited (Billard, Burzawa-Gerard and Breton, 1970). It has been suggested that this incomplete response may be due to post-operative impairment of function (Billard et al., 1982). Unlike the female, rising GtH apparently does not stimulate an increase of plasma sex steroid concentrations, and total androgen levels remain constant throughout the cycle, as in the brown trout (Billard et al., 1978) and the carp (Weil, 1981). Despite this, there is some evidence that the action of GtH on spermatogenesis is mediated by androgens: spermatogenesis has been restored to hypophysectomised fish after treatment with androgens, although high doses are often required (Billard, 1974).

The foregoing is a very brief summary and, for example, takes little account of species differences. However,

gametogenesis has been artificially controlled successfully with techniques based on this knowledge. This subject has been reviewed many times, most recently by Lam (1982), but a basic account of some successful methods will be given here. According to Lam (1982), the inducement of spermatogenesis is not a problem in general, and HCG, SG-G100 and androgens have all been successful. However, the acceleration of ovogenesis is more difficult. As previously outlined, GtH is at the hub of ovogenesis and most strategies to accelerate the process involve raising circulating GtH. For example, accelerated ovogenesis has been achieved using pituitary GtH (rainbow trout: Upadhyay et al., 1978), purified GtH (H. fossilis: Nath and Sundararaj, 1981), clomiphene citrate (goldfish: Ueda and Takahashi, 1977) and LH-RH (goldfish: Lam, 1982). In the rainbow trout, oestradiol-17β used in conjunction with S-GtH was, like S-GtH alone, only partially effective, and oestradiol-17β alone was ineffective in inducing vitellogenesis (Upadhyay et al., 1978). Due to the obvious complexity of control mechanisms, acceleration of gametogenesis has evidently proved more difficult than the inducement of spawning by similar methods.

The main alternative strategy to advance gametogenesis is to manipulate the environmental cues which naturally synchronise reproduction. In recent years, a great deal of progress has been made, particularly with salmonids, in our understanding of the physiological bases of environmental cues. The subject has been reviewed many times (de Vlaming, 1972; Htun-Han, 1977; Peter and Hontela, 1978; Peter and Crim, 1979; Scott, 1979; Peter, 1981; Crim, 1982). These environmental cues stimulate the appropriate response via exteroreceptors or transducers (review: Scott, 1979). Light, hence photoperiod, is detected by the eyes and the pineal organ, as evidenced by numerous experiments where gonad regression resulted from pinealectomy and/or blinding (for example, Delahunty, Schreck, Specker, Olcese, Vodicnik and de Vlaming, 1979; review: Peter, 1981). The pineal is thought to act by modulating GtH secretion (Hontela and

Peter, 1980). Environmental temperature affects reproduction both directly, by influencing the rates of GtH secretion and clearance (Cook and Peter, 1980), and indirectly, via temperature receptors and the hypothalamo-hypophysial axis. Thus, reproduction may be temporally influenced by the environment in regions where regular, predictable cycles of daylength and temperature exist. Species such as H. fossilis, which are found near the equator where there is little seasonal change in temperature and daylength, rely mainly on endogenous rhythms (Sundararaj, Nath and Jeet, 1978). Since seasonal changes in daylength and temperature are very pronounced in temperate climes, it is not surprising that the reproductive cycles of teleosts which inhabit these regions are strongly influenced by these factors. For example, daylength plays the major role in synchronising reproduction in salmonids, whereas both daylength and temperature may be important in many cyprinids (de Vlaming, 1972). Thus, spawning has been advanced considerably by manipulating photoperiod in the rainbow trout (Whitehead, et al., 1978), and temperature in the tench (Breton, Horoszewicz, Billard and Bieniarz, 1980 a, b). The influence of environmental factors on reproduction has been reviewed by Crim, 1982.

The aim of the present experiments is to investigate the possibilities of accelerating gametogenesis and thereby to advance spawning in the roach, by two different routes; hormonal and environmental. Thus, the effects of long-term administration of purified gonadotrophin, and the antioestrogens clomiphene citrate and tamoxifen were evaluated. Since both photoperiod and temperature have a considerable influence on reproduction in the roach (Jafri, 1980), these factors were manipulated in an attempt to advance spawning.

5.2 Materials and methods

5.2.1 Effects of hormones

5.2.1.1 Animals

On 27 February 1981, 48 roach (38 - 93 g) were obtained from the STWA fish farm at Calverton, Nottinghamshire. Each group of six fish was individually identified with a high-pressure sub-cutaneous injection of alcian blue dye along the ventral surface (Fig 10) with a "Panjet".

5.2.1.2 Holding conditions

The fish were introduced to the recirculating system described in 4.2.2.2 and provided with kakaban spawning substrate. The natural rate of change in daylength (based on Greenwich Mean Time sunrise/setting times) was doubled, 15L:9D being scheduled for early May. Temperature was raised from 10 to 15°C with daily increments of 1°C.

5.2.1.3 Hormone administration

The fish were divided into four groups and were injected twice per week for ten weeks with salmon gonadotrophin (S-GtH; Windsor Laboratories), clomiphene citrate (Richardson-Merrell), tamoxifen base (ICI 46474), or 100 mM NaCl in water. 1 mg of S-GtH is equivalent to 2.15 mg of SG-G100, as measured on a salmonid ovary cyclic AMP assay (Windsor Laboratories). The dosages of consecutive injections of the hormone preparations were increased as follows:

S-GtH, 20, 25, 30.....65 $\mu g\ kg^{-1}$; clomiphene, tamoxifen, 10, 20, 30..... 100 $\mu g\ kg^{-1}$. 100 mM NaCl was used as the injection vehicle. The solution of clomiphene was aided by the addition of two drops of absolute alcohol and made up with 100 mM NaCl. Fish were injected IP just above the pelvic fins, using 1 ml syringes and 16 x 0.5 mm needles.

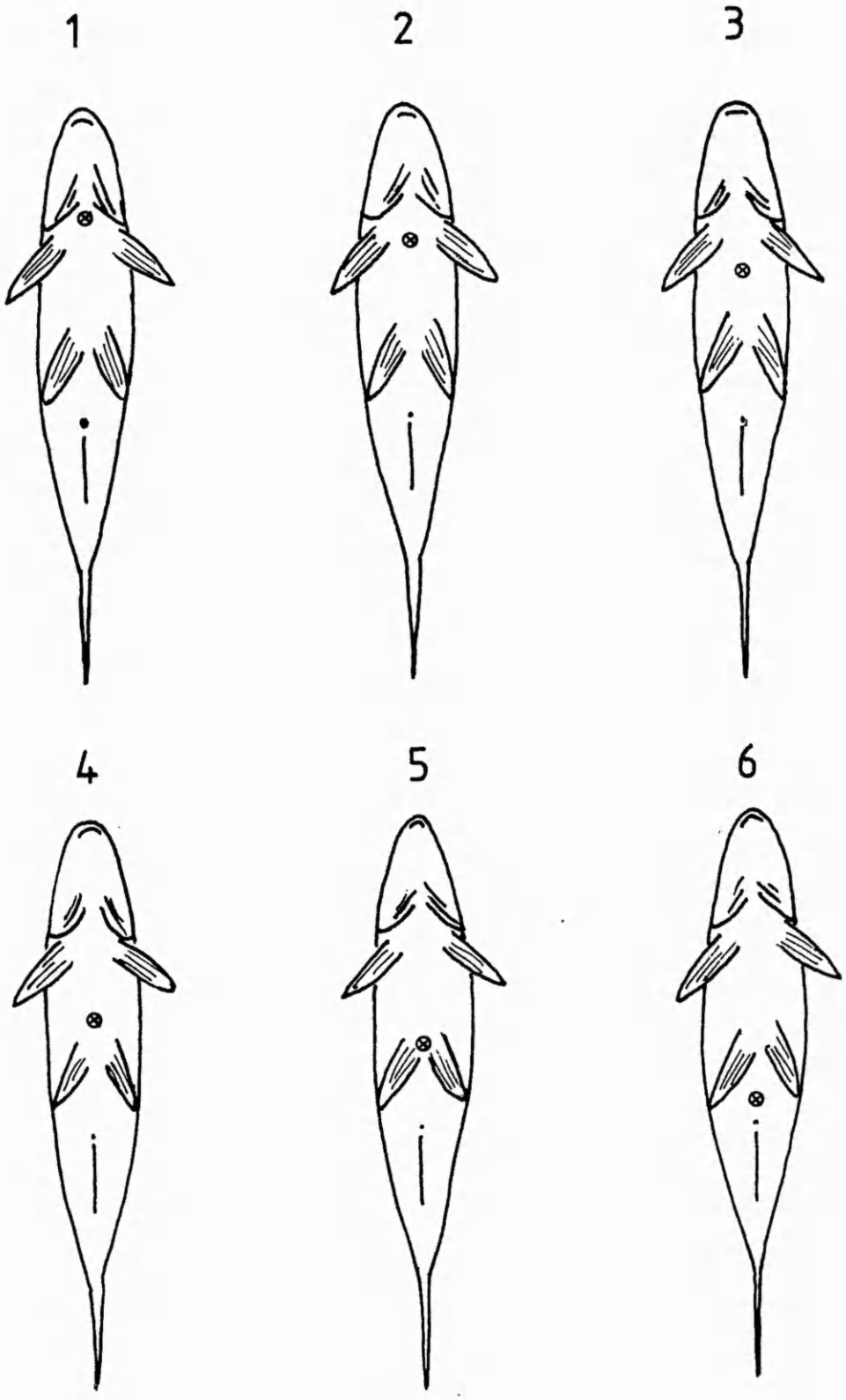


Figure 10. Representation of the approximate position of the "panjet" marks (⊗) which identified the individuals of groups of six fish in the advancement of spawning experiments.

5.2.1.4 Blood sampling and analysis

All fish were bled 4 days before and 3, 17 and 24 days after the first injection on 13 March 1981 according to the method described in 2.2.3. Fork length and weight were measured weekly, and the condition factor (K, see 3.2.3), calculated for each fish. Plasma calcium was determined as in 3.2.4.

To determine whether any fish were capable of spawning before the experiment was terminated, they were injected with CPE in 100 mM NaCl: three injections, 2.5, 5 and 5 μg g^{-1}, given over a 36 hour period.

5.2.2 Effects of light and temperature

5.2.2.1 Animals

On 23 September 1981, 48 roach (59 - 145 g) from Calverton Fish Farm (15°C) were introduced to the recirculating system described in 4.2.2.2 at 16.5°C. On 30 September another 24 were introduced to four 100 l tanks supplied with mains water in a flow-through arrangement. Each group was provided with kakabans. Each group of six fish was identified individually as in 5.2.1.1.

5.2.2.2 Environmental regimes

The fish were maintained under the following regimes:

Table 7 Maintenance regimes

	Group 1	Group 2	Group 3
Number	24	24	24
Temperature °C	10	10	Mains water (5-15)
Photoperiod	8L:16D to 16L:8D 2 x Natural rate of change	Natural	Natural
Water supply	Recirculated	Recirculated	Flow-through mains

Group 1 was acclimated to the eight hour photoperiod at a rate of one hour per day.

Unfortunately, as a consequence of mechanical failure of the time switch controlling the photoperiod of group 1, these fish were exposed to continuous light for an unknown period. Consequently, on 20 November 1981 group 1 fish were substituted with those of group 3 which were acclimated to an eight hour photoperiod maintained for 16 days. Thereafter, the natural rate of increase was doubled with 14L:10D scheduled for late February. Photoperiod was changed once per week, and temperatures were recorded daily.

5.2.2.3 Blood sampling and analysis

Blood samples were taken as in 2.2.3 at four, and later three-weekly intervals. Calcium was analysed from plasma samples as in 3.2.4. During blood sampling, fish were examined for the development of tubercles, spermiation, or ovulation. Before the experiment was terminated, the fish were hypophysised with CPE according to the regime given in Table 4.

5.3 Results

5.3.1 Effects of hormones

Tables 8 and 9 show the final gonosomatic indices, and the changes in the values of plasma calcium and condition factor for both sexes.

Student's t-test showed that both plasma calcium and condition factor decreased significantly in all groups during the course of the experiment ($p < 0.02$). Analysis of variance determined that at each sampling time, there was no difference in plasma calcium between the males or females of any group. All of the hormone treatments were ineffective in advancing gonad maturation or spawning. However, the control fish in one control group tank spawned spontaneously

Table 8 Changes in condition factor (K, as $\bar{x} \pm$ SEM) and GSI ($\bar{x} \pm$ SEM) in roach treated long-term with various hormone preparations

Condition factor (K)

Date	CLOM ♀ n = 8	CLOM ♂ n = 4	TAM ♀ n = 9	TAM ♂ n = 3	S-GtH ♀ n = 8	S-GtH ♂ n = 4	CONTROL ♀ n = 10	CONTROL ♂ n = 2
9.3.81	1.5 ± 0.03	1.4 ± 0.02	1.5 ± 0.02	1.3 ± 0.10	1.5 ± 0.02	1.4 ± 0.10	1.5 ± 0.03	1.4 ± 0.02
16.3	1.4 ± 0.03	1.4 ± 0.02	1.5 ± 0.02	1.3 ± 0.03	1.5 ± 0.03	1.3 ± 0.04	1.4 ± 0.10	1.3 ± 0.01
23.3	1.4 ± 0.02	1.4 ± 0.01	1.4 ± 0.03	1.3 ± 0.04	1.4 ± 0.03	1.3 ± 0.10	1.4 ± 0.03	1.3 ± 0.00
30.3	1.4 ± 0.03	1.3 ± 0.01	1.4 ± 0.02	1.4 ± 0.02	1.4 ± 0.04	1.4 ± 0.10	1.4 ± 0.03	1.3 ± 0.04
6.4	1.3 ± 0.03	1.3 ± 0.04	1.4 ± 0.03	1.3 ± 0.02	1.4 ± 0.02	1.3 ± 0.04	1.3 ± 0.03	1.3 ± 0.00
13.4	1.3 ± 0.01	1.3 ± 0.04	1.4 ± 0.02	1.3 ± 0.00	1.4 ± 0.02	1.3 ± 0.01	1.3 ± 0.02	1.3 ± 0.03

GSI %

13.4	3.5 ± 1.1	1.8 ± 0.1	3.4 ± 0.6	1.5 ± 0.1	3.5 ± 0.8	1.2 ± 0.1	3.3 ± 0.9	1.2 ± 0.1

Table 9 Changes in plasma calcium ($\bar{x} \pm$ SEM) in roach treated long-term with various hormone preparations

Plasma calcium mM $\bar{x} \pm$ SEM

Date	CLOM		TAM		S-GtH		CONTROL	
	♀ n = 8	♂ n = 4	♀ n = 9	♂ n = 3	♀ n = 8	♂ n = 4	♀ n = 10	♂ n = 2
9.3.81	3.51 ± 0.12	3.23 ± 0.08	3.72 ± 0.21	3.27 ± 0.27	4.03 ± 0.24	3.34 ± 0.14	3.92 ± 0.22	3.97 ± 0.29
16.3	3.03 ± 0.23	2.65 ± 0.12	3.32 ± 0.24	2.64 ± 0.15	3.47 ± 0.25	2.96 ± 0.09	3.25 ± 0.01	3.07 ± 0.01
30.3	2.86 ± 0.21	2.50 ± 0.07	2.78 ± 0.13	2.88 ± 0.13	2.96 ± 0.15	2.74 ± 0.10	2.85 ± 0.03	2.92 ± 0.04
6.4	2.68 ± 0.12	2.50 ± 0.03	2.66 ± 0.07	2.68 ± 0.11	2.55 ± 0.07	2.63 ± 0.07	2.61 ± 0.08	2.81 ± 0.16

Table 10 Reproductive events recorded from roach maintained under different photoperiod and temperature regimes

Date	Advanced Photoperiod Group 3 ♀ (total n = 16) Number releasing ovulated eggs	♂ (total n = 8) Number with tubercles	♂ (total n = 8) Number releasing semen	Spawning	Natural Photoperiod Group 2 ♀ (total n = 15) Number releasing ovulated eggs	♂ (total n = 9) Number with tubercles	♂ (total n = 9) Number releasing semen	Spawning
5.2.82	0	7	3		0	7	0	
7.2	2	8	4	✓	0	7	0	
8.2	0	8	4		0	7	0	
10.2	1	8	7	✓	0	7	2	
11.2	1	8	7	✓	0	7	2	
14.2	1	6	6	✓	0	7	0	
17.2	2R	4	2		0	3	1	
19.2*								
20.2	0	4	6		0	3	3	
21.2	1	4	6	✓	1	3	3	✓+

KEY R = resorbing female
* = administered CPE (regime as in table 7)
+ = poor spawning - few eggs produced

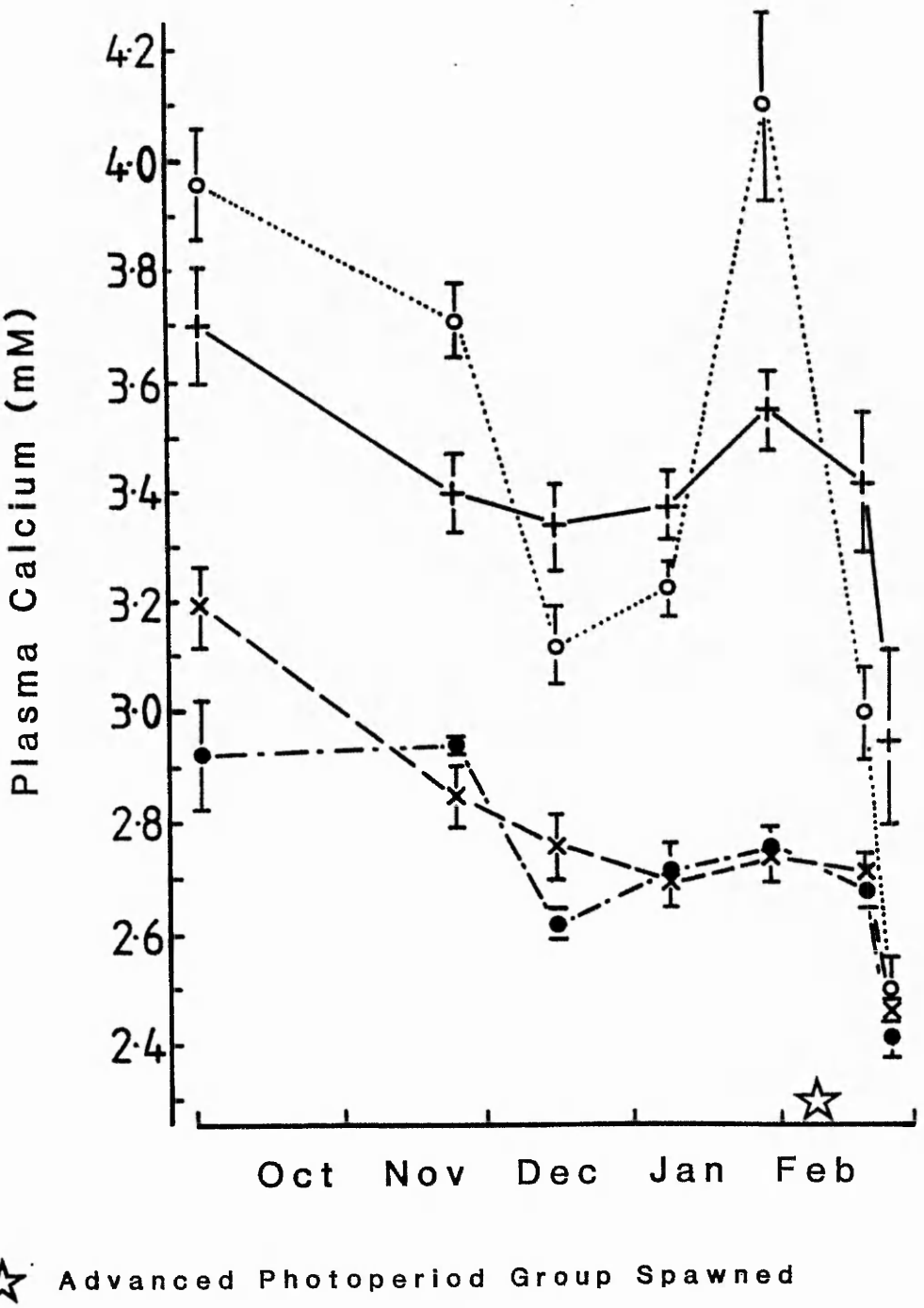


Figure 11. Mean plasma calcium ($\bar{x} \pm$ SEM) of males and females maintained under regimes of accelerating light and increasing temperature (males ●·—·●, n = 8; females o·····o, n = 16) or natural light and increasing temperature (males ×—×, n = 9; females +—+, n = 15).

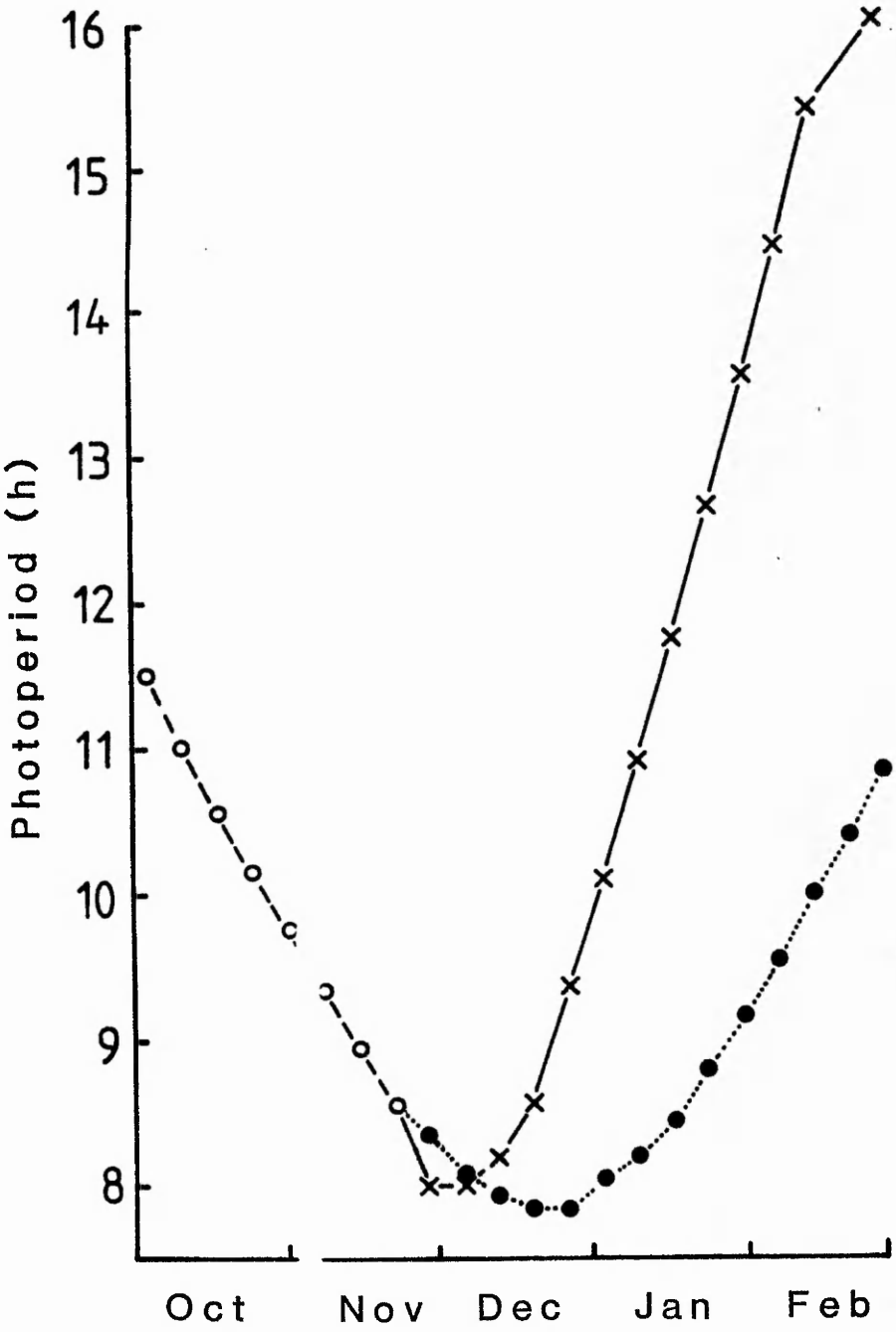


Figure 12. Photoperiod regimes during the study of environmental advancement of spawning ×—× = advanced photoperiod; ●·····● = natural photoperiod.

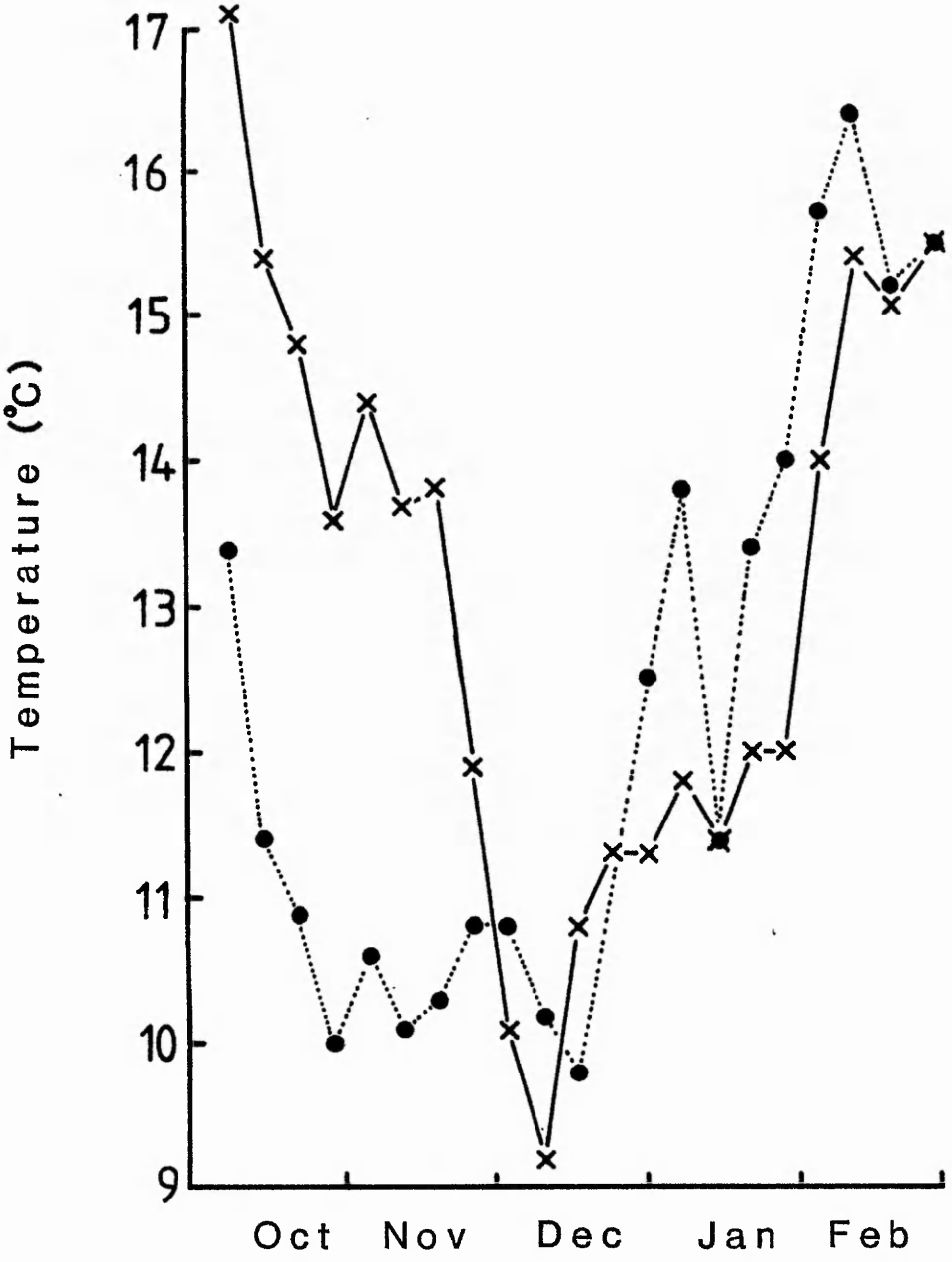


Figure 13. Weekly mean temperatures recorded during the experiment to advance spawning of roach by manipulation of environmental conditions. ●·····● = advanced photoperiod; ×—× = natural photoperiod.

on March 20 and 26. The eggs were viable though poorly fertilised and a few larvae hatched. The GSIs of the spawned control group were: females, 2.43 ± 0.11 %(n = 4); males, 1.18 - 0.11 %(n = 2). In addition, one female from the other control group ovulated on 2 April, but the group contained no males, so spawning did not take place.

Before the experiment was terminated, all groups were treated with spawning doses of CPE (Table 4, Chapter 4) in order to determine whether any fish were capable of spawning. No fish ovulated or spermiated as a result of this procedure.

5.3.2 Effects of light and temperature

Plasma calcium data are shown in Figure 11 and Appendix 5. Temperature and photoperiod regimes are shown in Figures 12 and 13 respectively. It will be noted that the temperature of both groups sometimes varied by several degrees. This was due to the black-outs of the advanced photoperiod room offering a greater degree of insulation against low ambient temperatures.

It was noticed during blood sampling on 29 January that the females in group 3 (advanced photoperiod) were very rotund and appeared to be near spawning. Thereafter, the fish from both groups were examined on a regular basis for spawning, ovulation, spermiation or the development of tubercles. These events are presented in Table 10. The fish under an advanced photoperiod spawned spontaneously on four occasions beginning February 7. One female in each experimental and control group ovulated and spawned after treatment with CPE. Both of these spawnings produced viable eggs although the proportion of these was much lower in the control group (c.30 - 40 % compared with c.70 - 80%). A large number of larvae were maintained for several weeks by feeding "Liquifry", which includes yeast and cooked egg yolk.

Calcium titres showed a slight decline for females of both groups between 1 October and 15 December ($p < 0.05$). However, marked changes were confined to females of the experimental group, which showed a large increase between 8 and 29 January ($p < 0.001$). During the following three weeks during spawning, this fell equally sharply ($p < 0.001$). No such changes were observed in control fish. All of the fish from both groups showed a significant decline between 18 and 23 February ($p < 0.05$).

5.4 Discussion

5.4.1 Effects of hormones

Despite the fact that the hormone treatments did not apparently accelerate gametogenesis, the early provision of a long photoperiod did, as indicated by the ovulations and spawnings seen in the controls. Indeed, since the photoperiod regime had a positive effect on the controls and no hormone-treated fish ovulated or spawned, the hormone treatments may have inhibited gametogenesis. The steady decline in plasma calcium indicates that either vitellogenesis was inhibited or had been completed. The former seems more likely, especially when plasma calcium is compared to the normal female pattern (3.3) where a plateau is reached two months before spawning. In addition, the hatching/survival rate of larvae was poor in the successful spawnings, but this may have been due to poor fertilisation, rather than egg quality. It is difficult to interpret the effects of the hormones, particularly since the oestrogen antagonists may have opposite effects in high dosages (Miller and Huang, 1981; Patterson, 1981). The problem of dose-response effects can only be evaluated with further experiments, impossible at the time of this study. Upadhyay et al. (1978) induced incomplete vitellogenesis (which excludes the incorporation of yolk into oocytes) in the rainbow trout injected three times per week with 100 or 500 $\mu g\ kg^{-1}$ S-GtH (activity unspecified). In comparison, the dosages used in roach were probably too low to have an

effect. In addition, there would probably have been species-specific effects, that is, reduced efficacy of heterogeneous GtH (Fontaine et al., 1972). Lam (1982) used a high dosage (10 µg g^{-1} on alternate days) of SG-G100, and induced vitellogenesis in female goldfish and spermatogenesis in some males.

Tamoxifen and clomiphene appeared to have no positive effects on reproduction. This again may be due to the use of inadequate dosages. However, a relatively high dosage of clomiphene (10 µg g^{-1} on alternate days; Pandey and Stacey, 1975) retained antagonistic properties, inhibiting vitellogenesis in hypophysectomised goldfish. In contrast, a lower dosage (1 µg g^{-1}, every third day) induced vitellogenesis in intact goldfish (Ueda and Takahashi, 1977). Since both clomiphene and tamoxifen are weakly agonistic at high dosages, this is the reverse of the expected result. However, intact fish were used in the second study. Thus, the expected rise in blood GtH (induced by blocking feedback-inhibition of the pituitary by sex steroids: Billard and Peter, 1977) after clomiphene treatment must have been sufficient to supersede the antioestrogenic effects on vitellogenesis in the liver. Moreover, the effectiveness of the antioestrogens in raising blood GtH must depend on the pituitary content of GtH which is synthesised under the positive feedback influences of androgens or oestrogens (Peute, Goos, de Bruyn and van Oordt, 1978). Hence, because the blood concentrations of either sex steroids or blood GtH are unknown in this study, it is impossible to determine whether the antioestrogens exerted any effect.

5.4.2 Effects of light and temperature

In contrast to the hormone treatments, the group maintained under compressed light and temperature cycles underwent accelerated gametogenesis. As a consequence, spontaneous spawning took place 13 to 16 weeks in advance of the wild population of Highfields Lake (see 3.3). Previous studies

have also shown that gametogenesis may be accelerated in teleosts by manipulation of the environment. Henderson (1963) advanced gonadal maturity in brook trout by accelerating natural light cycles. Spawning has been advanced 11 weeks in coho salmon (Macquarrie, Markert and Vanstone, 1978), and 12 weeks in rainbow trout (Whitehead et al., 1978; Bromage et al., 1982) by compressing natural light cycles over periods of 33 months and 6 months respectively. By raising water temperature by up to 6°C above ambient, spawning took place recurringly two months early in the tench (Breton et al., 1980a, b). In addition, a combination of long photoperiod (14L:10D) and warm temperature (25°C) for six weeks during the preparatory period induces precocious gonadal recrudescence in Heteropneustes fossilis (Sundararaj and Vasal, 1976). However, it was necessary to induce spawning with mammalian luteinising hormone in the latter study. Furthermore, the gonads of this catfish, like the tench, have a potentially short gametogenic cycle and several spawnings may take place annually, unlike the roach and rainbow trout. Thus, it is not surprising that a shorter period of environmental manipulation can advance spawning successfully in tench and catfish. In temperate cyprinids like the roach, it is likely that the preparatory/prespawning periods offer the greatest potential for advancing spawning because gonad activity is greatly retarded over winter (Billard and Breton, 1979).

The environmental requirements for complete gametogenesis and spawning were clearly fulfilled in the experimental group, as evidenced by the spontaneous nature of spawning and high hatching/survival rate of eggs and larvae. Despite the fact that this experiment was designed more to advance spawning than to discover the factors controlling gametogenesis in roach, it is possible to draw a few tentative conclusions in this respect. The control group did not spawn spontaneously despite the temperature threshold for spawning (14°C, K.Easton, pers. comm.) being exceeded. However, one control female did ovulate and spawn

sparingly after hypophysation, but few of these eggs hatched and survived for long. This may have been due to poor egg quality or fertilisation. Therefore, it seems that although temperature may induce limited precocious gonad development, an appropriate photoperiod is essential for spontaneous maturation and spawning. Jafri (1980) also found that a long photoperiod was necessary for spontaneous spawning to take place in the roach. Moreover, a short-day photoperiod (8L:16D) during this period not only blocked spawning, but caused the oocytes to become atretic. However, it is considered that a period of short daylength and low temperature is probably necessary during gametogenesis, since gonad regression was observed in roach kept under 16L:8D, 16°C during the preparatory/prespawning phases (Worthington, unpublished data). The fish may respond to this situation as if spawning had been missed, particularly since roach appear to spawn under 14.5 hours light (from this experiment and the Highfields population). Furthermore, it has been demonstrated, with the cyprinid Mirogrex terrae-sanctae, that low temperatures are necessary for vitellogenesis, although this species does spawn in winter (Yaron, Cocos and Salzer, 1980). With the present evidence, it is impossible to say whether the absolute daylength or the rate of change cues reproduction in the roach. In view of their sensitive nature and poor adjustment to captivity (see Chapter 2) it was considered appropriate to alter light and temperature gradually. This aspect has been investigated in only a few species, including the rainbow trout. However, these experiments have yielded conflicting results. Breton and Billard (1977) found that the spermatogenic response of males was much stronger in fish exposed to a gradually decreasing photoperiod than fish exposed to either constant long or short ones. However, more recently, it has been suggested that it is the absolute amount of light received during a day, and not its rate of change during a cycle which controls gonad development in this species (Bromage et al., 1982). The work of Baggerman (1972), with sticklebacks (Gasterosteus aculeatus), suggests that there are daily

phases of photosensitivity, the threshold for which alters seasonally. Thus, longer photoperiods are necessary to overcome this threshold at certain times of the reproductive cycle than others. In any case, it is clear from the literature that each species can differ in respect of which environmental cues control reproduction. In each case the cue will be appropriate to the ecological niche occupied by that species.

It is remarkable that all the fish under the accelerated light cycle matured within one week of each other, when the photoperiod was 14.5L:9.5D. This corresponds to the natural daylength during mid-May, when the fish in Highfields Lake spawned. This precise group synchrony is essential to shoal-spawning species which reproduce only once per year. However, it is possible that this synchrony of maturation was brought about by communication between fish by way of pheromones, which have been shown to be important in the spawning behaviour of goldfish (Partridge, Liley and Stacey, 1976). This is possible, since the water was recirculated in a common circuit.

The absolute values of plasma calcium characteristic of females are lower than those of salmonids, presumably because more yolk is deposited in the proportionally larger salmonid eggs. Despite this, the pattern leading up to spawning is similar to that observed in the rainbow trout (Whitehead et al., 1978; Bromage et al., 1982), but is more acute though less pronounced. Control females showed no elevation of plasma calcium during the course of the experiment. The calcium peak seen in female roach under a compressed light cycle is of shorter duration than the wild population of Highfields Lake (see 3.3) where elevated plasma calcium persists for approximately three months prior to spawning. This indicates that vitellogenesis was accelerated under the conditions of increasing daylength and temperature.

From the foregoing it is clear that the acceleration of gametogenesis and consequent advancement of spawning is more readily achieved by manipulating the environment of roach rather than the endogenous endocrine processes controlling reproduction. If the hormonal approach were to be pursued, gonadotrophin treatment is considered more likely to be effective than anti-oestrogens. This is mainly because of the likelihood of secondary effects of antioestrogens, particularly in respect of vitellogenesis. The gonadotrophin preparation would most conveniently (and cheaply) be a pituitary extract, which is more likely to induce full ovogenesis than purified GtH (Upadhyay et al., 1978).

6 LARVAL REARING

6.1 Introduction

The egg and larval phases of the life cycle of fish are the most vulnerable. The larvae which survive hatching are very delicate, and susceptible to predation and environmental change. Furthermore, starvation is distinctly possible during the transition from yolk sac to exogenous feeding, particularly if suitable food is scarce. Hence, many teleost species which do not show parental care have evolved high fecundity, to "anticipate" high mortality of offspring. This ensures that some fish will reach sexual maturity and reproduce, though the proportion may be extremely small. For example, in the bream (Abramis brama) which is closely related to the roach, only 0.006 to 0.002 per cent of larvae survive to maturity (Nikol'skii, 1963). Fish culture usually attempts to capitalise on the high fecundity by minimising losses of larvae through mortality and predation. To this end, traditional, semi-intensive culture frequently involves segregation of brood fish and larvae, removal or elimination of predators, and pond fertilisation (Huet, 1970). The latter is probably the best contributor towards larval survival because it increases the production of natural food (phyto- and zooplankton). However, semi-intensive larval rearing is rather speculative because plankton blooms are difficult to predict. Larval rearing, being dependent on these plankton blooms, is therefore restricted to the spring and summer months in temperate climes. For these reasons, intensive rearing of larvae in hatcheries has become very popular, enabling a great degree of control over the environment. Consequently, many potential causes of death such as oxygen deficiency or super-saturation, sudden temperature changes or high suspended solids, can be eliminated. Nevertheless, fulfilling the nutritional requirements of larvae may still pose problems. Many salmonids can be reared from larva to adult on artificial food alone, but in many other cultured teleosts, shortage of live food of the correct size is

probably responsible for most larval mortalities. Food requirements are considerable in the first few days of exogenous feeding. For example, Huisman (1979) calculated that the larvae of grass carp need a daily food ration of at least 400 per cent of the body weight in the post-hatching period. Furthermore, Hunter (1977) calculated that newly-hatched anchovy (Engraulis mordax) must consume approximately 230 food items of 50 µm or less per day. A breakthrough in the capabilities of larval rearing was the use of the newly-hatched nauplii of the crustacean Artemia salina as a source of food. These can be produced in quantities sufficient to sustain large numbers of fish larvae, but the eggs are expensive and the nauplii are too large for the larvae of many species of fish, apparently including the roach (Dolben, 1979). So, for larvae which require small food items, hatchery culture requires that natural food is either collected from the wild (again restricting operations to spring and summer) or cultured in the laboratory. The latter has been achieved very successfully with the marine rotifer Brachionus plicatilis (Pullin and Kuo, 1980), but it seems that there have been few attempts to culture a freshwater equivalent.

Since rotifers form a major part of the natural diet of roach larvae (Grigorash, Spanovskaya and Lebedeva, 1973), an attempt was made to culture a Brachionid isolated from pond water. The method employed was that of Gilbert (1970) who cultured Brachionus calyciflorus in association with the flagellate Euglena gracilis. Unfortunately, all attempts were unsuccessful, so a more easily cultured live food, Paramecium caudatum, was grown in crude straw-infusion cultures of the bacterium Bacillus subtilis. Roach larvae which were given Paramecium daily, failed to thrive, and began to die after 10 - 14 days. This was probably because there were insufficient Paramecium to sustain the large numbers of larvae. It is possible that Paramecium is unacceptable, but this is unlikely because food is usually accepted by larvae if it is small enough to be ingested (Pullin and Kuo, 1980).

The difficulties which were experienced maintaining roach larvae in a hatchery prompted experimentation with pond culture. The objective was to assess semi-intensive rearing in well-manured ponds enclosed in greenhouses (which conserve heat and raise water temperature) as substitutes for intensive hatchery rearing methods. The experiment was designed to determine survival and growth throughout the first growing season. Greenhouse ponds had been used successfully by STWA for the culture and growing-on of carp and other species for several years previously.

6.2 Materials and Methods

6.2.1 Stocking of rearing ponds

The following experiments were performed at the STWA fish farm at Calverton, Nottinghamshire. The ponds used for this study (designated A17 and A23) were of earth construction, and 35 x 3 m wide. They had triangular cross-sections, and volumes of approximately 30 m^3. Each was enclosed by a polythene horticultural tunnel, which maintains the water temperature above ambient for most of the year. Each pond was supplied with diffused aeration in order to reduce the risk of de-oxygenation. On 9 May 1980, two such ponds (A17 and A23) were each stocked with 3,000 eggs at the "eyed" stage, adhering to kakabans. From personal experience, eggs at the eyed stage (approximately two to three days prior to hatching) are quite robust, and can be handled carefully without causing appreciable deformities or premature hatching. The eggs were counted individually and were derived from spawnings on 4 May.

6.2.2 Sampling of rearing ponds

Any sampling of the larvae in the weeks immediately following hatching was necessarily destructive, and was therefore largely confined to one pond, A17. This group was sampled at 3, 4, 7, 8, 16, 22, 25 and 28 days post-hatching in order to monitor early growth. During the same period,

only three samples were taken from the A23 group. Subsequently, samples were taken weekly from both ponds. Up until 9 June (28 days post-hatching), samples were caught in a hand-net, killed with benzocaine and stored at -20°C prior to measurement. After larvae reach 3 cm in length they are robust enough to be measured and returned alive. The fish were caught in perspex "bottle-neck" type traps and narcotised with benzocaine. The fork-length was measured to the nearest millimetre. Batch weights were recorded to the nearest gramme by placing the fish in a mesh bag, allowing the water to drain away, and suspending from the appropriate 10, 50 or 100 g "Pesola" spring balance. The net was weighed and subtracted from the total. Sampling was continued until the experiment was terminated on 19 November 1980, when the ponds were drained and the fish counted.

6.2.3 Supplementary feeding

In addition to natural food, the fish were fed ad libitum from 8 June with size 0, 1, 2 and 3 "Omega" trout food (Edward Baker Ltd). This was dispensed automatically during daylight hours by means of a clockwork belt-type feeder.

6.2.4 Fertilisation of ponds

Before the ponds were stocked on March 6, they were treated with calf manure at a rate of 1.5 kg m^{-2} to enhance primary production. After this initial addition of organic fertiliser, from June 4, inorganic sources of the major phytoplankton nutrients (nitrogen, phosphorus, potassium and silicon) were added. The respective sources of these nutrients used were ammonium nitrate ("Nitram", NH_4NO_3) phosphorus pentoxide ("Triple superphosphate", P_2O_5) potassium nitrate ("salt petre", KNO_3) and sodium silicate (Na_2SiO_3). The objective was to achieve final concentrations in excess of the likely demands of a phytoplankton bloom (nitrogen, potassium: 10 mg l^{-1}; phosphorus, silicon: 5 mg l^{-1}). The major nutrient levels were monitored in the field with a "La Motte" water quality

test kit, which uses a colourimeter in analysis procedures. This was carried out daily for ten days following June 4, thereafter weekly. Chlorophyll A, indicative of primary production (Moss, 1980), was measured by absorption spectrophotometry from water samples taken weekly from May to October. The method for the determination of chlorophyll A first entailed filtering the phytoplankton from the water with a fibre glass filter paper, and extraction of the pigment in 90% boiling methanol (at 75° - 90°C). The sample was centrifuged at 3 - 4,000 rpm for five minutes. The supernatant (with the extracted pigment) was withdrawn, and the absorbance read at 665 and 750 nm on a spectrophotometer using 90% methanol as a blank. The concentration of chlorophyll A was calculated from the following formula:

$$\text{chlorophyll A } \mu g\ l^{-1} = \frac{V_e . E . OD_{750}^{665}}{V_f . 1}$$

Where:

V_e = Volume of extract in ml

E = Extinction coefficient of chlorophll A in 90% methanol = 13.9 from the work of Talling and Driver (1963)

OD_{750}^{665} = Absorbance of the extract at 665 less the absorbance at 750 nm

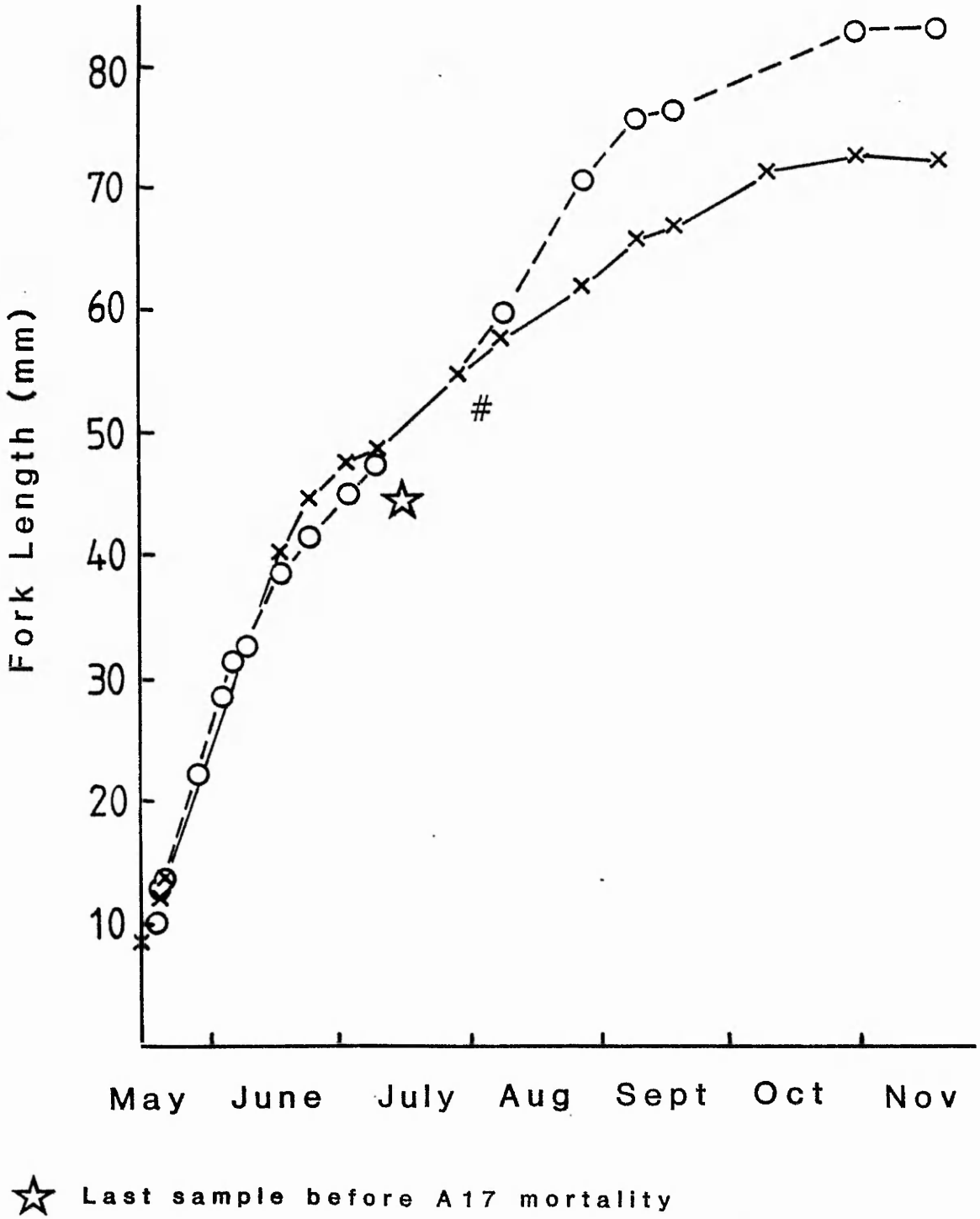
V_f = Volume of water filtered in litres

6.3 Results

Growth in terms of length and weight for A17 and A23 groups is illustrated in Figures 14 and 15, and Appendices 6 and 7. Temperature data are illustrated in Figure 16. Final values for mean size, survival and yield are given in Table 11. Growth rate (percentage mean daily weight increase, MDWI: Figure 17) was calculated according to Grigorash, Spanovskaya and Lebedeva, 1973:

Table 11 Growth, survival and yield data after one growing season of 0+ roach larvae in greenhouse ponds A17 and A23

Pond	Weight $\bar{x}$ (g)	Length $\bar{x} \pm$ SEM (mm)	Survival (from 55 mm)	Yield, kg ha^{-1}
A17	7.5	82.7 ± 1.0	100	757
A23	5.0	72.0 ± 0.7	100	504



☆ Last sample before A17 mortality

A23 divided

Figure 14. Growth interms of mean fork length of fry stocked as eggs in greenhouse ponds A17 (o—o) and A23 (×—×). Although not plotted, all SEM's<1mm (appendices 6 and 7).

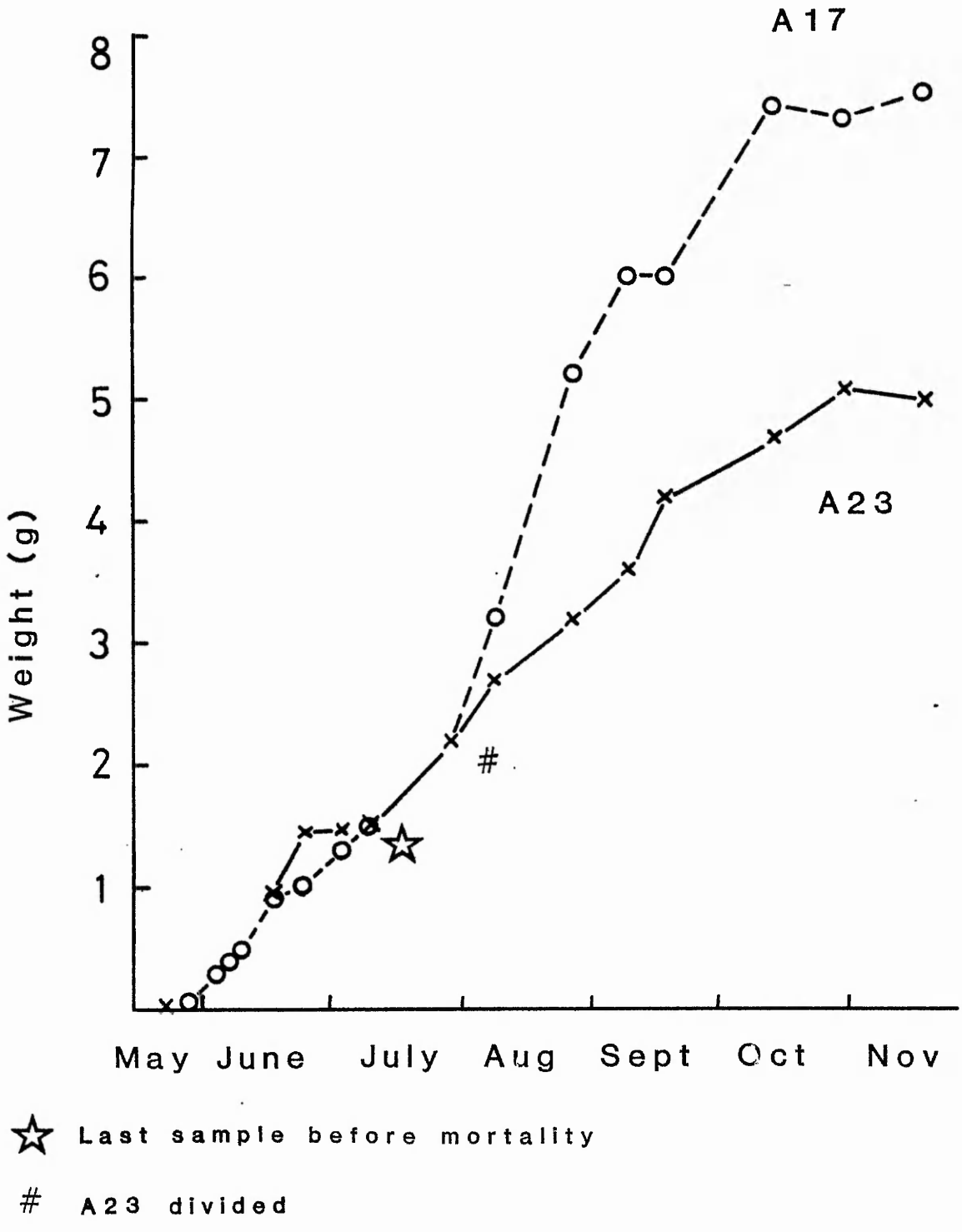


Figure 15. Growth in terms of mean weight (from batch weights) of fry stocked as eggs in greenhouse ponds A17 (o—o) and A23 (×—×). Data are also given in Appendices 6 and 7.

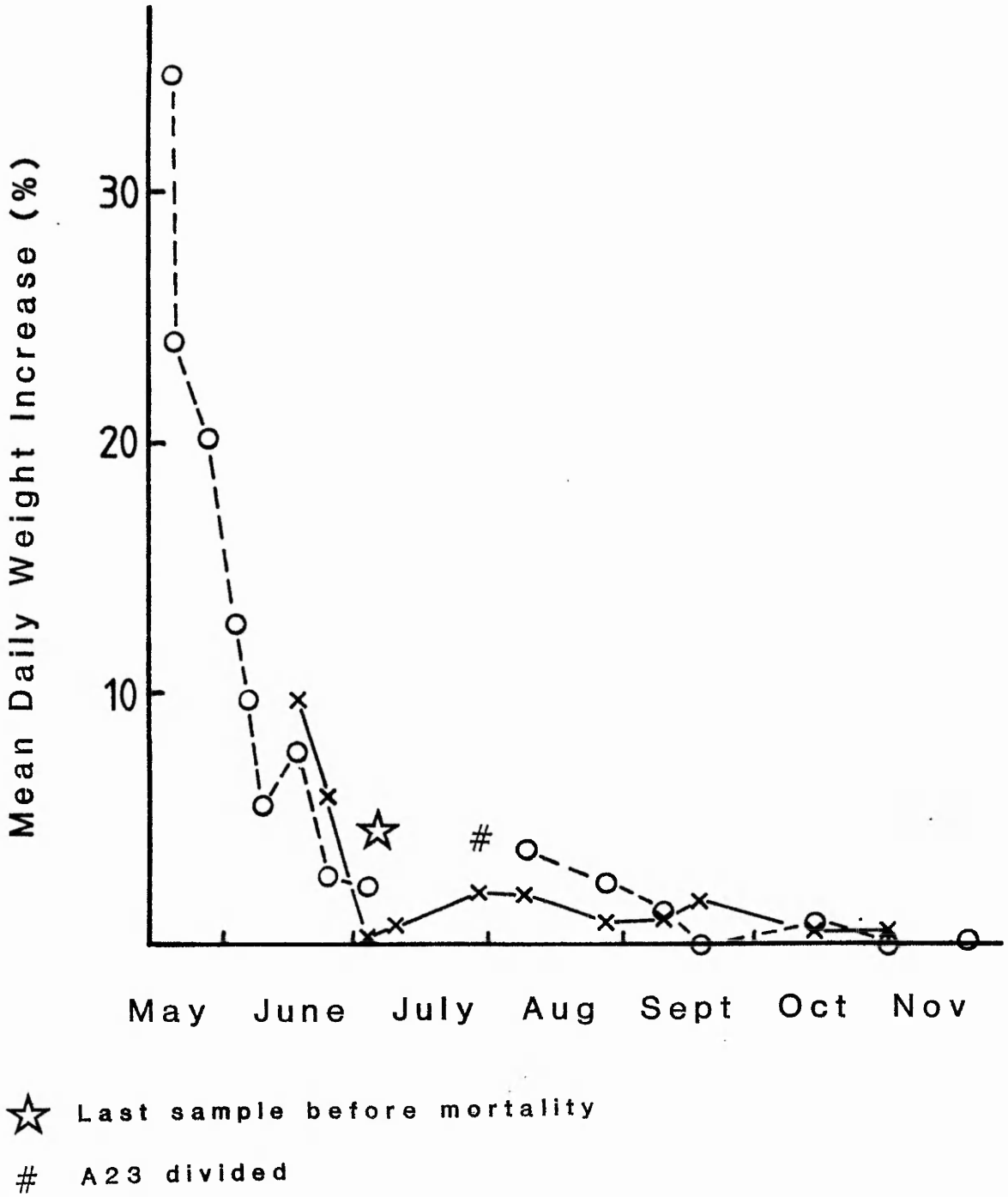


Figure 16. Growth rate (MDWI) of fry stocked as eggs in greenhouse ponds A17 (o·····o) and A23 (x—x).

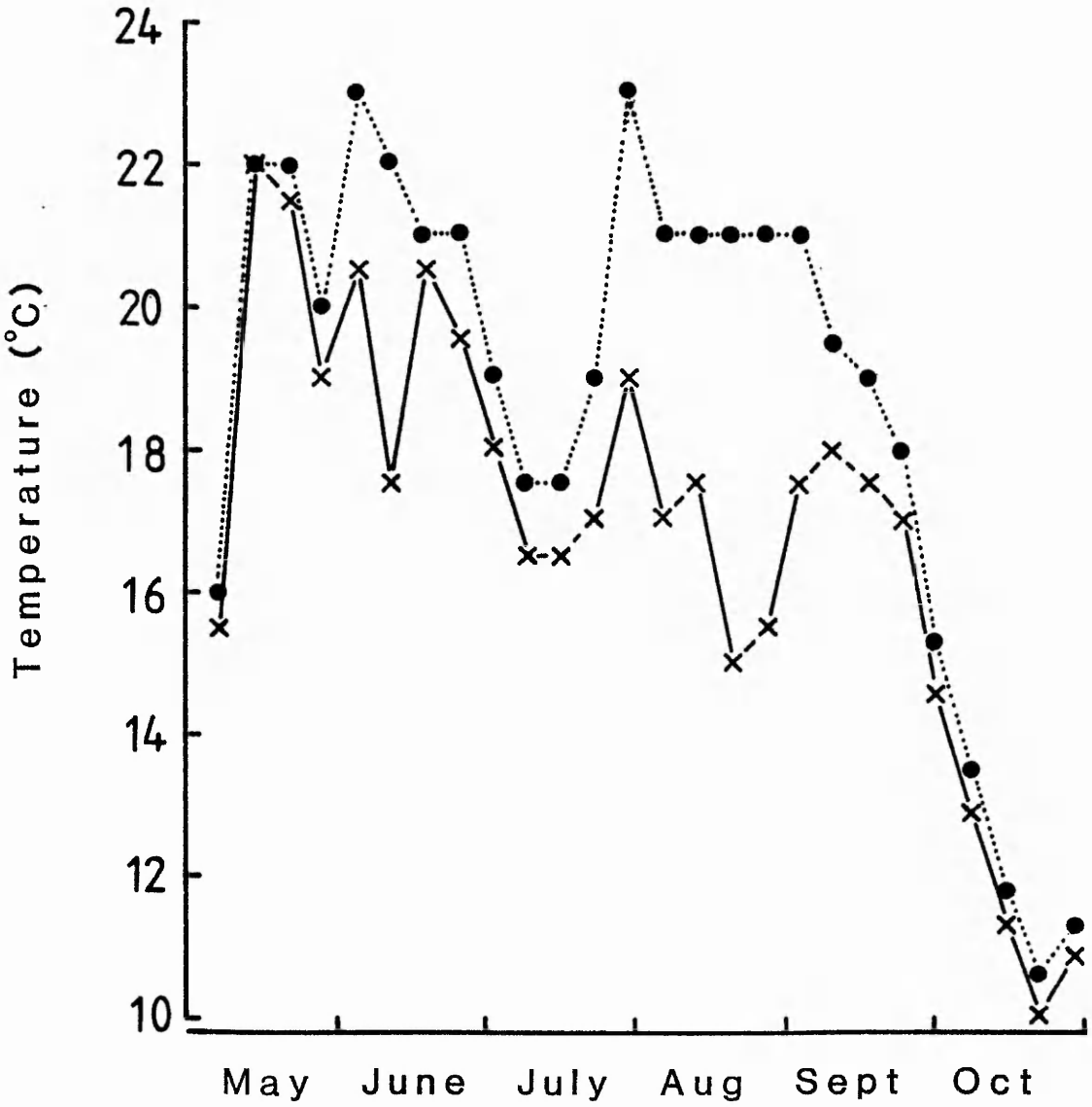


Figure 17. Weekly mean temperatures of greenhouse ponds A17 (●·····●) and A23 (×—×) recorded during the larval rearing experiment (results plotted in Figures 16 and 17).

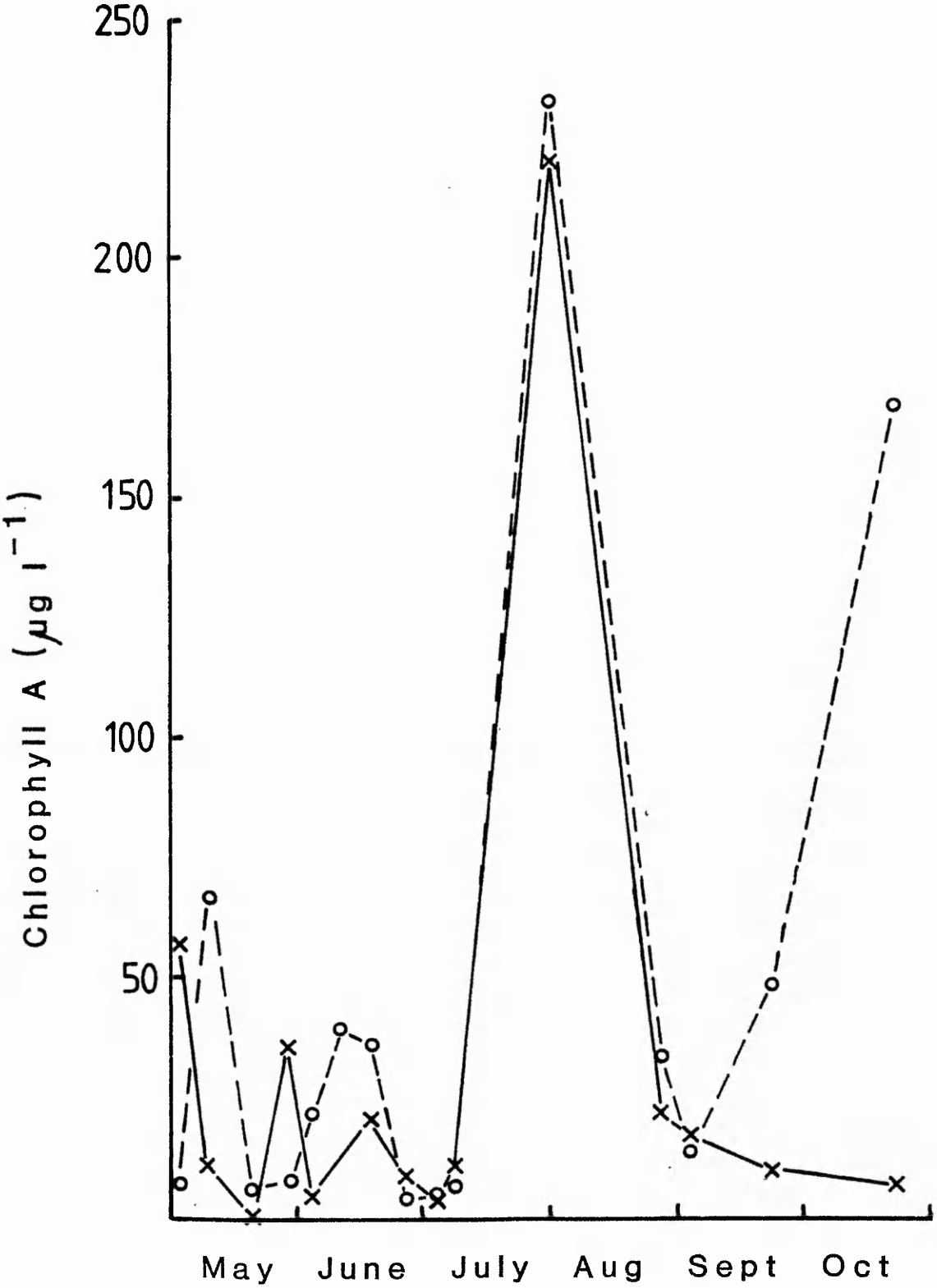


Figure 18. Chlorophyll A concentrations of water samples taken from greenhouse ponds A17 (o—o) and A23 (x—x) during the larval rearing experiment (results plotted in Figures 16 and 17).

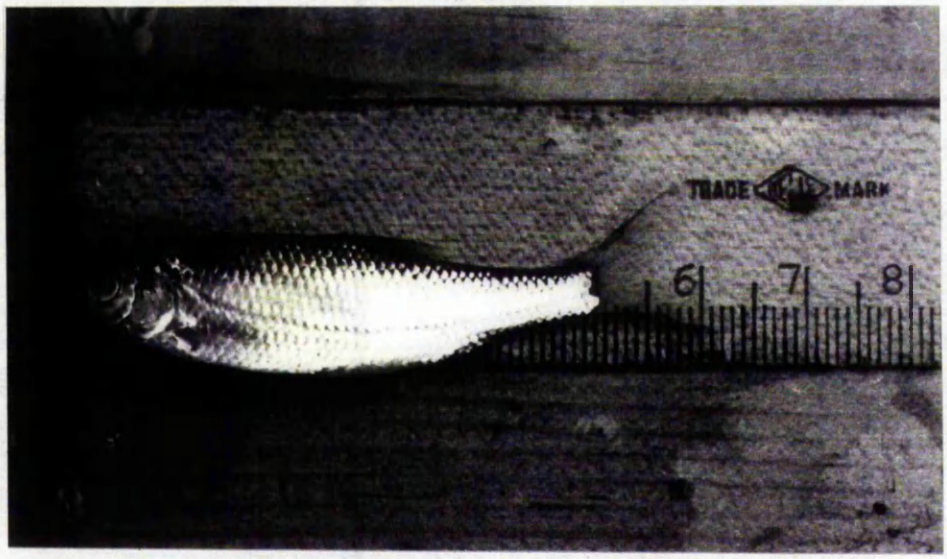


Plate 11

Plate 12

Specimens of roach fry grown in a greenhouse pond (A17), sampled less than 13 weeks after hatching. The mean length of fish from A17 at day 88 was 59.9 $\pm$ 0.4 mm, mean weight 3.2g.

$$\% \text{ MDWI} = \frac{2\ (WT - wt)}{(T - t)(WT - wt)} 100$$

Where WT is fish weight in grammes after T days, wt after t days; T being larger than t.

At the prevailing temperature (19°C) the eggs hatched seven days from spawning (three days after they were introduced). Fish in both ponds grew at a similarly rapid rate, reaching approximately 48 mm and 1.5 g at 59 days of age. During the early stages of this period, MDWI of the A17 group reached more than 34%. However, in the latter part of July, the fish in A17 were subject to an acute infestation of the protozoan "white spot", Ichthyophthirius multifilis, and all died whilst being transferred to another pond for treatment. At that time, the number of survivors from the A23 group was greater than expected (2,131: 71%; = 20 m^{-2}, 68 m^{-3}) and it was considered wise to divide these equally between the two ponds. There were nine mortalities during the transfer so 1,061 were put into each pond (10 m^{-2}, 34 m^{-3}). These fish measured 55 mm mean length and 2.2 g mean weight (Plates 11 and 12). During the remainder of the growing season (until late October) the growth rate of the A17 group was considerably superior to the A23 group. During the same period, the polythene cover over A23 was damaged, and had the effect of reducing the water temperature.

Chlorophyll A data from both ponds is plotted in Figure 18. Since the ponds were managed in favour of unicellular phytoplankton, at the base of the food chain of roach, other plants were regularly removed and allowed to decompose on the pond banks. These included macrophytes e.g., Elodea canadensis and Potamogeton crispus, and microphytes e.g., Lemna spp and various species of filamentous algae. These management techniques did not appear to sustain high levels of primary production, as indicated by the chlorophyll A measurements.

6.4 Discussion

The use of greenhouse ponds for larval rearing is clearly very successful. It would be difficult to match the growth and survival obtained using conventional hatchery techniques. Growth was greater than that characteristic of many wild populations. For example, the length attained after four or five weeks (40 mm) is greater than that recorded (32 mm) after a complete growing season for roach from the River Hull (Worthington, unpublished). At the end of their respective growing seasons, A17 fish were more than twenty times heavier and two and a half times longer than the River Hull fish.

The observed pattern of growth rate is typical of animal growth in that growth rate is inversely related to the time elapsed since the onset of growth (Balinsky, 1970). The maximum value of MDWI (34%) is very high for roach. Grigorash et al. (1973) studied young roach in the Mozhaysk reservoir in Russia and found that in the first few weeks of life MDWI was approximately 13 - 20% in "good" years and 5 - 9% in "poor" years. Moreover, young roach from the River Hull had a MDWI of 16% in 1978 (Worthington, unpublished). On the other hand, some other species are capable of much higher rates of growth. For example, specific growth rates of 60 - 100% per day were reported during the first week of life of the grass carp (Huisman, 1979). Superior growth of roach kept in greenhouse ponds, compared to that of wild fish, is probably attributable to richer food supplies (due to pond fertilisation and supplementary feeding) and higher temperatures. The optimum growth temperature for 0+ roach is not known, but is probably around 20°C. The optimum temperature range referred to by Elliot (1981) was 8 to 25°C, but neither the processes measured (growth, respiration, food conversion, etc,) nor the age of the fish in question were stated. It is known that the minimum temperature for appreciable growth is approximately 12 to 14°C (Broughton and Jones, 1978), so that fish in greenhouse ponds would be kept above 14°C for longer, that is, have an

extended growing season. The growth of both A17 and A23 groups was similar for most of the season but it did diverge after A23 was divided. This can probably be explained by reference to the temperature data (Fig 16) which illustrate that the temperature was considerably higher in A17 from early August to mid-September.

Survival in the first two months was much better than anticipated and both A17 and A23 became overstocked. A probable consequence of this was the fatal epidemic caused by Ichthyophthirius multifilis in the A17 group. This parasite thrives in conditions of host crowding, high temperatures and still water, and for these reasons fish grown in greenhouse ponds are particularly vulnerable to infestation. The percentage (71%) of the A23 group surviving to 55 mm compares very well with data for other species. Pullin and Kuo (1980) reviewed successful hatchery rearing of various marine and brackish water fish. In only two of fourteen species referred to did larval survival exceed 40%, indeed it was less than 20% in most cases.

There is insufficient evidence to draw firm conclusions about possible benefits of pond fertilisation, although this was not one of the original objectives. The addition of fertiliser was, like the supplementary feeding and removal of macrophytes, part of a policy calculated to provide what were considered to be the best conditions for growth. However, observations do indicate that it is difficult to alter the process of biological succession of the flora. During a season, this usually climaxes with macrophytes and filamentous algae, both being primary producers which are unavailable to 0+ roach. The pond fertilisation policy employed did not perpetuate the initial bloom of phytoplankton, suggesting that factor(s) other than nutrient insufficiency control phytoplankton growth. It has been suggested that phytoplankton produces substances which, above certain concentrations, act as auto-inhibitors which induce the demise of the population. Perhaps this explains the observation that a second phytoplankton bloom in one

season only occurs after emptying and refilling a pond with freshwater. Thus, if primary production in the form of phytoplankton is required, then it may be necessary to change the water periodically.

However, this is considered to be unnecessary once the fish are past the larval stage, when the fish are particularly dependent on plankton. Further plankton blooms may not occur, but other forms of natural food (eg, insect larvae) will be produced. In addition, water changes will waste valuable heat, and consequently reduce fish growth.

In conclusion, it is evident that rearing larvae in greenhouse ponds gives very satisfactory results. Such semi-intensive pond culture is considered to provide a more stable environment for larvae than a typical hatchery, and requires less equipment and labour. Unfortunately though, larval rearing by this technique is confined to the warmer summer months.

7 POND PRODUCTION OF FRY

7.1 Introduction

Cyprinids have been cultured in Europe for many centuries. Traditionally, culture was extensive, that is, no artificial contribution was made to natural production, and parents and progeny were often kept together (Huet, 1970). In the past hundred years, a rationalisation of cyprinid culture in general and fry production in particular, has led to great increases in efficiency. Hence, the use of modern techniques has enabled ponds to be exploited more effectively, giving greater yields of fish per unit area. The yield is governed by various biological constraints which usually depend on the number of fish per unit area (the "density"), more specifically, the biomass. The nature of the biological constraints depends on the species being cultured. For example, salmonids are usually fed artificial diets (at cost effective rations), so growth is seldom limited to a great extent by food supply. In these species, yield depends more frequently on the quantity of water flowing through the ponds, which serves to provide oxygen and dilute metabolic waste products. Cyprinid culture, on the other hand, is commonly semi-intensive and relies heavily on natural food production. Considerable improvement in fish yields accompanied the advent of pond enrichment or fertilisation practices (Huet, 1970). This may entail the addition of organic manures (Wohlfarth and Schroeder, 1979) or inorganic fertilisers (von Lucowicz, 1971), or a combination of both. However, both methods act by increasing the productivity of the pond. When fish are introduced to a pond, they will grow according to the environmental constraints imposed on them. Obviously, the more fish there are to exploit a resource, such as food for example, the smaller their individual share will be. This phenomenon has been demonstrated by, for example, Hepher (1967). He showed that the growth rate of carp fry was negatively correlated with density. Thus, successful

culture of cyprinids in ponds depends, among other factors, on the stocking density and the productivity of the pond.

Since the roach has been rarely cultured in the UK, there has been little research on growth of fry in ponds. The aim of this two-year study was to evaluate the growth of fry at various stocking densities. This information could then be used predictively by fish culturists to maximise the production of first year fish of the desired size. In the first year, the strategy was to evaluate the effects of what were considered to be high and low stocking densities on growth and yield. On the basis of these results, intermediate stocking densities were used in the second year, in order to define more closely the maximum stocking density which has a negligible depressive effect on growth.

7.2 Materials and Methods

7.2.1 Stocking densities 10 and 30 m^{-2} in 1980

7.2.1.1 Animals and growing-on ponds

Larvae from spawnings induced artificially between 4 and 20 May (see 4.2.1), were grown in two stock ponds before being introduced to the pairs of growing-on ponds on 13 and 16 June, 1980. These were designated B7, B8, C5 and C6 with the following characteristics:

	B7, B8	C5, C6
Length (m)	26	29
Width (m)	13	25
Depth (m)	0.9	1.5
Area (m^{-2})	338	720
Volume (m^{-3})	320	1,080

Larvae were counted into the ponds by slowly decanting plastic cups filled with larvae. Each pair of ponds was stocked at a rate of 10 or 30 m^{-2} (Table 12). The facilities available dictated that each group could not be

replicated. However, the productivity of each pond was considered to be similar, from the evidence of previous experiments (Easton, pers. comm.).

7.2.1.2 Sampling of growing-on ponds

A sample from each stock pond was taken at the time of transfer of the larvae, and batch-weighed in the laboratory on a top-pan balance to the nearest milligramme. Fork-length was measured by examining through a low-power binocular microscope (x 20 magnification) larvae placed on a microscope slide on 1 mm graph paper. Fish at each density were sampled at intervals of approximately three weeks. Samples of at least 30 fish, where possible, were captured with a 20 metre seine net. The measuring techniques employed in this experiment were the same used in 6.2.2. The fish were retained after October, which is the normal limit of the growing season, to determine the additional growth to the following May/June. After November, the frequency of sampling was reduced to once every six weeks. The experiment was terminated between 22 May and 6 June 1981, when the ponds were drained, the fish counted and final samples taken.

7.2.1.3 Statistics

Differences between data were analysed, where appropriate, using Student's t-test.

7.2.1.4 Fertilisation of ponds

Pond enrichment procedure was identical to that described in 6.2.4, with the exception of the addition of the sodium silicate. Chlorophyll A was measured (as described in 6.2.4) from water samples taken once per week from May to October. Unwanted plants were removed as in 6.2.

7.2.2 Stocking densities 10, 15 and 20 m^{-2} in 1981

7.2.2.1 Animals and ponds

Three ponds of equal area (B7, 8 and 9: 33 m^2) were stocked with roach larvae at rates of 10, 15 or 20 m^{-2} (Table 12) on 4 and 5 June, 1981. These fish were derived from spawnings on 19 and 20 April at Calverton Fish Farm.

7.2.2.2 Sampling

A sample was taken at the time of stocking and the fry measured in the laboratory. Subsequently, samples were taken every three weeks. The methods of capture and measurement were the same as in 7.2.1.2. Final samples were taken, and the survivors counted, when the ponds were drained between 2 and 6 November.

7.2.2.3 Statistics

Where appropriate, differences between data were analysed using Student's t-test.

7.2.2.4 Fertilisation of ponds

Prior to the introduction of fish, the ponds were treated with inorganic fertilisers as in 6.2.4. No organic fertilisers were added. Chlorophyll A was not determined in this experiment.

7.3 Results

7.3.1 Stocking densities 10 m^{-2} (B7, C5) and 30 m^{-2} (B8, C6) in 1980

Growth is represented for each group in terms of length and weight in Figures 19 and 20 respectively, and also Appendix 8. Temperature data during the growing season is shown in Figure 21. Figure 22 shows changes in primary production of

phytoplankton, as indicated by chlorophyll A measurements, for each pond. Final growth, survival and yield data are summarised in Table 12.

Growth (mean fork length) of fish in the four ponds diverges from 1 August, such that C5 > B7 > C6 > B8 ($p < 0.01$). From this point, the size differences between groups are more pronounced between B7 and C5. In these two ponds, C5 fish were larger than B7 fish ($p < 0.05$) until April, when there was no significant difference between them. Significant differences existed sporadically between fish stocked at 30 m^{-2}. When the experiment was terminated, C6 fish were larger than those from B8 ($p < 0.02$).

Survival (and therefore yield) figures were reduced by problems with pond drainage in B8 and a disease epidemic in C6. A number of fish were lost in the excessive weed and mud in B8, but their exact number was not known. Fish from C6 contracted an infestation of Ichthyophthirius multifilis during the period 1 January to 25 March. The water volume was reduced and 25 mg l^{-1} formalin (40% formaldehyde in water) added. This treatment was efficacious, and it was estimated that only a few hundred fish died, Thus, mortalities were probably less than 2% of the total figure. Growth (in terms of weight) during the period November to April was minimal, except for the B7 and C6 groups which increased by 23% and 17% respectively.

Chlorophyll A levels suggest that primary production of phytoplankton was similar in each pond except B8, which appeared to be poor in phytoplankton. Some plant species other than phytoplankton became very abundant. C6 became covered with Lemna, whilst B8 and B7 developed large populations of Lemna and filamentous algae in addition to macrophytes such as Potamogeton crispus and Elodea canadensis. As described in 6.3, excessive weed growth was removed and decomposed on the bank of the pond.

7.3.2 Stocking densities 10, 15 and 20 m^{-2} (B7, 8 and 9) in 1981

Growth at each density in terms of length and weight is given in Figures 23 and 24, and Appendix 9. In 1981, growth was similar at each stocking density. However, the fish at 15 and 20 m^{-2} grew to be 18°/o heavier than those at 10 m^{-2} at the end of the growing season, in November. Growth in length of fish stocked at 15 and 20 m^{-2} was similar to that of fish stocked at 10 m^{-2} in the 1980 experiments. Growth of fish stocked at 10, 15 and 20 m^{-2} in 1981 was much greater than fish stocked at 30 m^{-2} in B8 and C6 in 1980. The 1981 yield figures were much higher than 1980 (Table 12) excepting C6 stocked at 30 m^{-2} (371 kg ha^{-1}) which yielded more fish than B8 at 10 m^{-2} (391 kg ha^{-1}).

Discrepancies between counts "in" and "out" suggest that the numbers of fish introduced to B8, B9 and probably B7 were underestimated. This is one of the problems associated with the counting of larvae, and the final count is considered to be more accurate. This makes the density of the fish coming out of each pond: B7, 13.6; B8, 11.8; B9, 20.9 m^{-2} the number of fish removed from B7 is an underestimate because during pond drainage, large quantities of residual weed and mud prevented complete recovery of the fish (Plates 13 to 15). This falsely reduces the yield from this group. B8 supported particularly luxuriant populations of macrophytes. B9 was almost devoid of these because it was occupied the previous year by carp which cleared them. In terms of macrophyte populations, B7 resembled B8 more closely than B9.

Table 12 Stocking density, growth and yield data for 0+ roach stocked at various densities in outside ponds

Pond	Date	Stocking Density m^{-2}	Stocking Density m^{-3}	Number in	Number out	*Adjusted Stocking Density m^{-2}	*Adjusted Stocking Density m^{-3}	Survival %	Final Weight $\bar{x}$ (g)	Size Length $\bar{x} \pm$ SEM (mm)	Yield $kg\ ha^{-1}$
B7	22.4.81	10	10.6	3,380	2,945	-	-	87	3.2	62.0 ± 0.49	279
B8	24.4.81	30	31.7	10,140	7,867	-	-	78	1.2	45.8 ± 0.54	279
C5	24.4.81	10	6.7	7,200	6,680	-	-	93	3.1	62.9 ± 0.60	292
C6	6.5.81	30	20.0	21,600	19,089	-	-	88	1.4	47.5 ± 0.44	371
B7	6.11.81	15	15.9	5,070	4,608	13.6	14.4	91	3.2	63.9 ± 0.5	480
B8	2.11.81	10	10.6	3,380	3,998	11.8	12.5	100	2.7	60.7 ± 0.4	319
B9	3.11.81	20	21.2	6,760	7,080	20.9	22.1	100	3.3	64.6 ± 0.4	691

* Stocking density figures adjusted to the number of survivors (which is likely to be more accurate than the figures for the number of fish stocked - see 7.3.2).

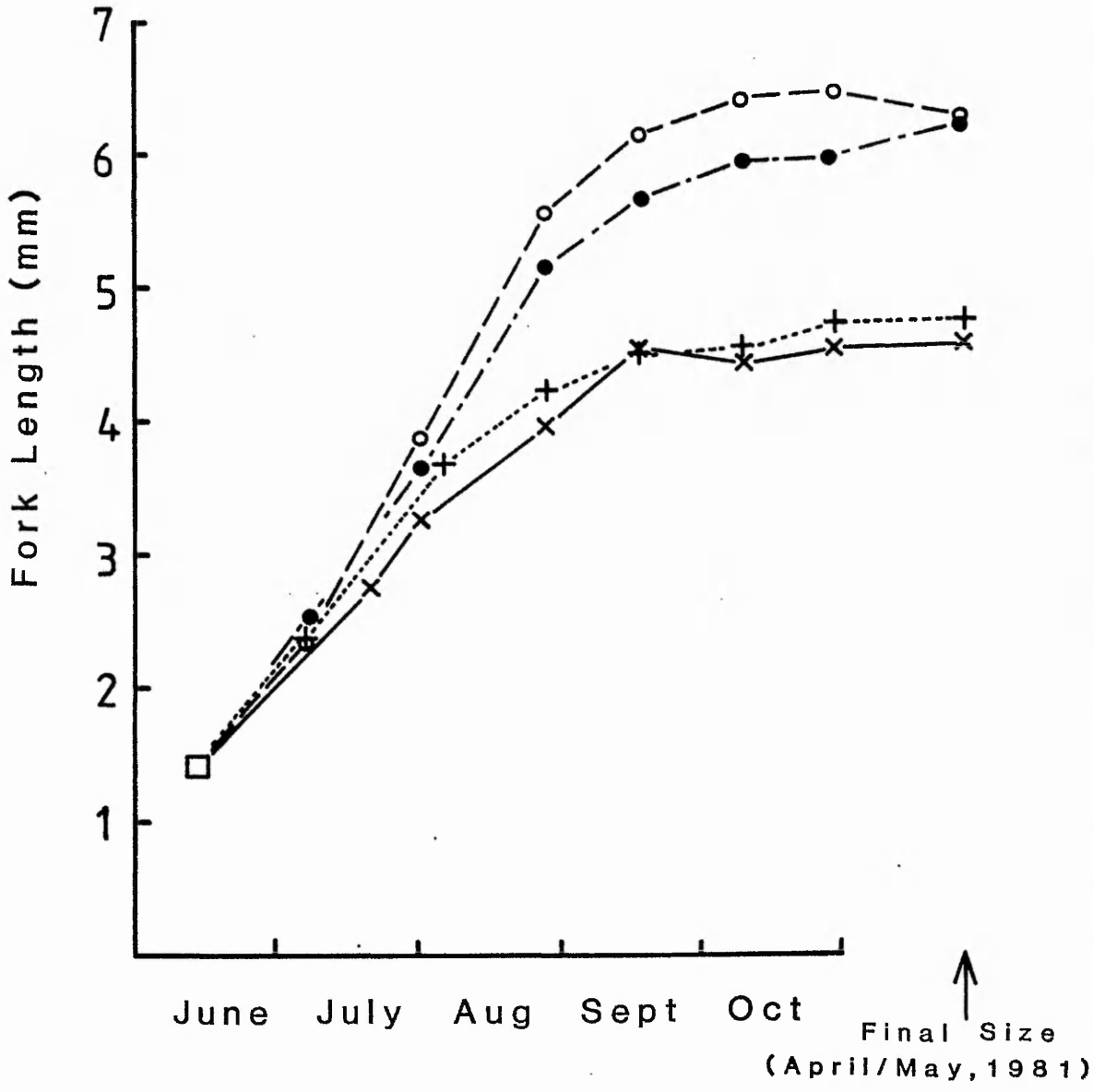


Figure 19. Growth, in terms of mean fork length, of fry stocked as larvae at two stocking densities in outside ponds of two sizes: B7 (●—·—●), 10 m^{-2}, pond area 338 m^{-2}; B8 (×—×), 30 m^{-2}, pond area 338 m^2; C5 (o——o), 10 m^{-2}, pond area 720 m^2; C6 (+····+), 30 m^2, pond area 720 m^2. Although not plotted, all SEM's<1mm (Appendix 9). The fish remained in the ponds over winter until the following spring.

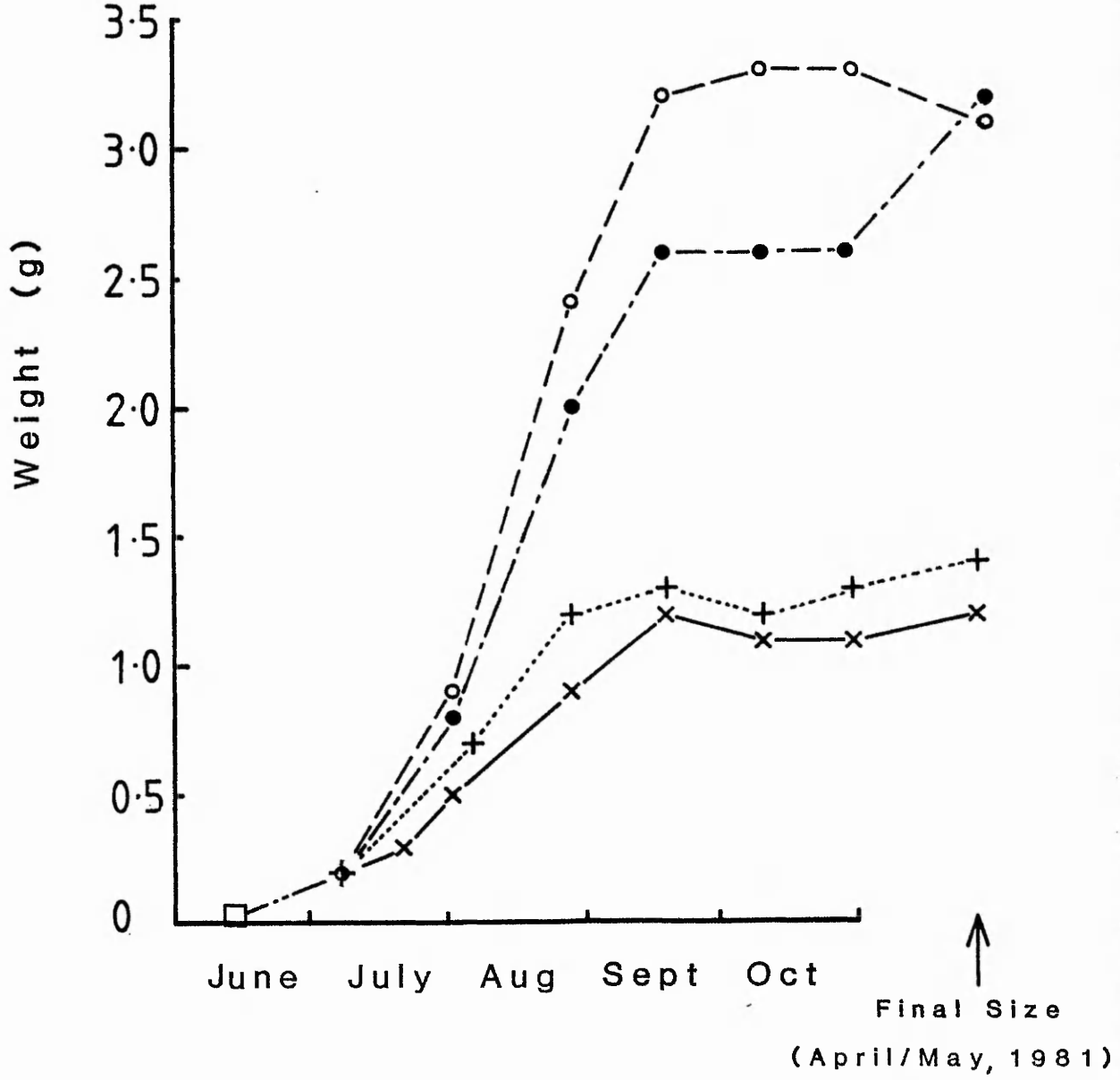


Figure 20. Growth in terms of mean weight (from batch weights) of fry stocked as larvae at two stocking densities in outside ponds of two sizes: B7 (●—·—●), 10 m^{-2}, pond area 338 m^2; B8 (×——×), 30 m^{-2}, pond area 338 m^2; C5 (o——o), 10 m^{-2}, pond area 720 m^{-2}; C6 (+·····+), 30 m^{-2}, pond area 720 m^2. The fish remained in the ponds over winter until the following spring.

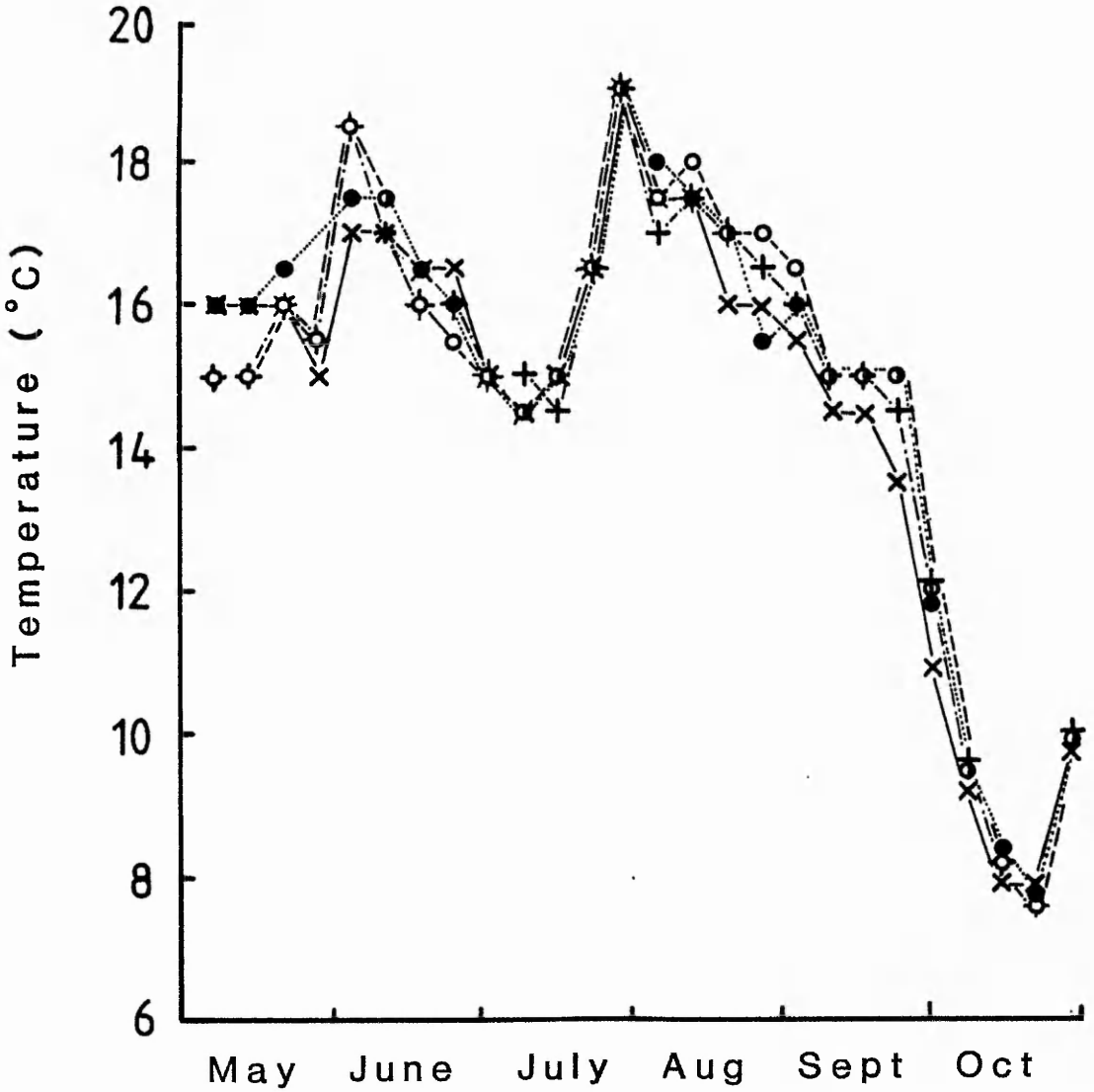


Figure 21. Weekly mean temperatures recorded from the outside ponds B7 (●·····●), B8 (×—×), C5 (o--o) and C6 (+-·-+) during the fry rearing experiment (results plotted in Figures 19 and 20).

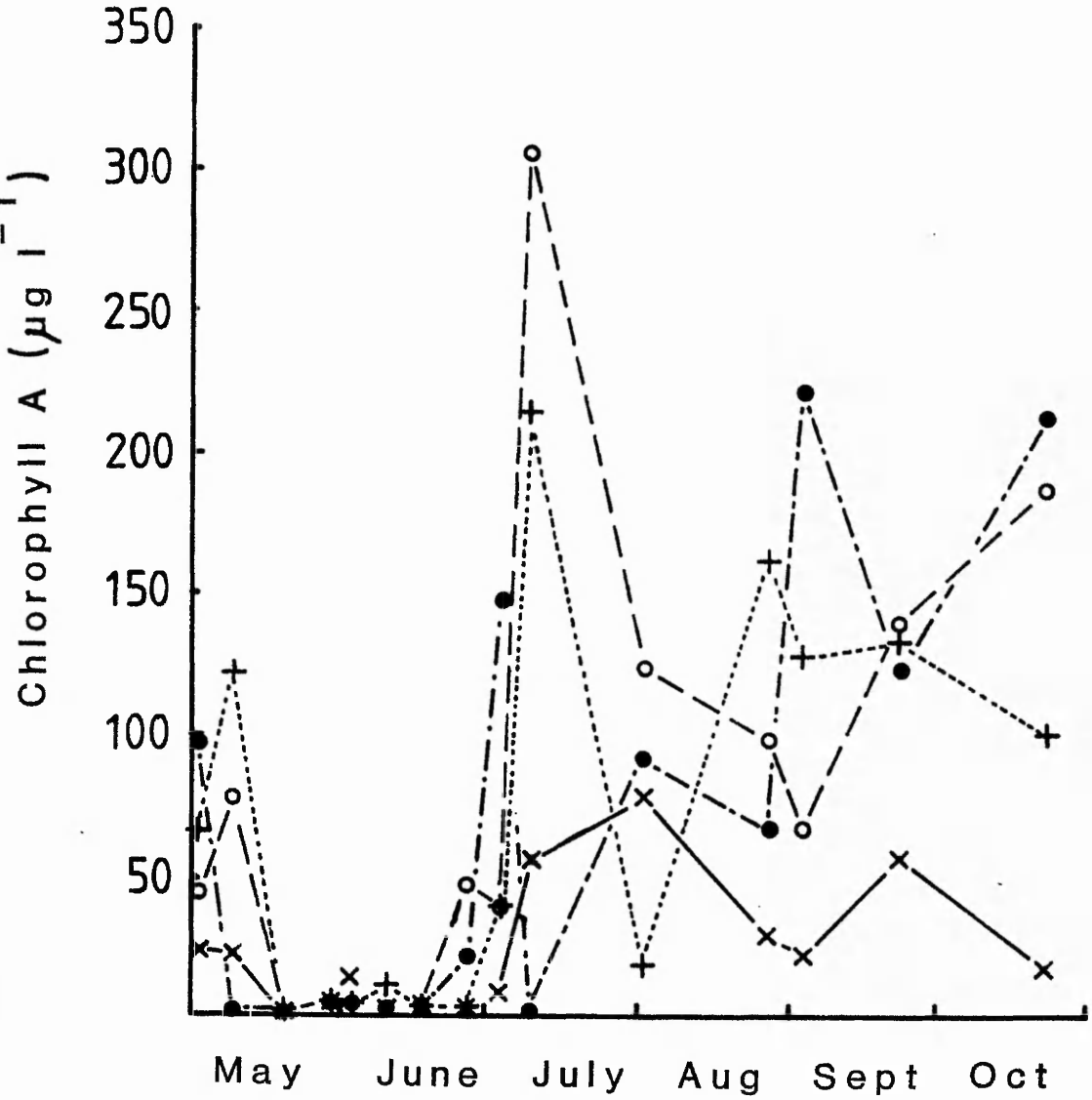


Figure 22. Chlorophyll A concentrations of water samples taken from outside ponds B7 (●—·●), B8 (×—×), C5 (o--o) and C6 (+····+) during the fry rearing experiment (results plotted in Figures 19 and 20).

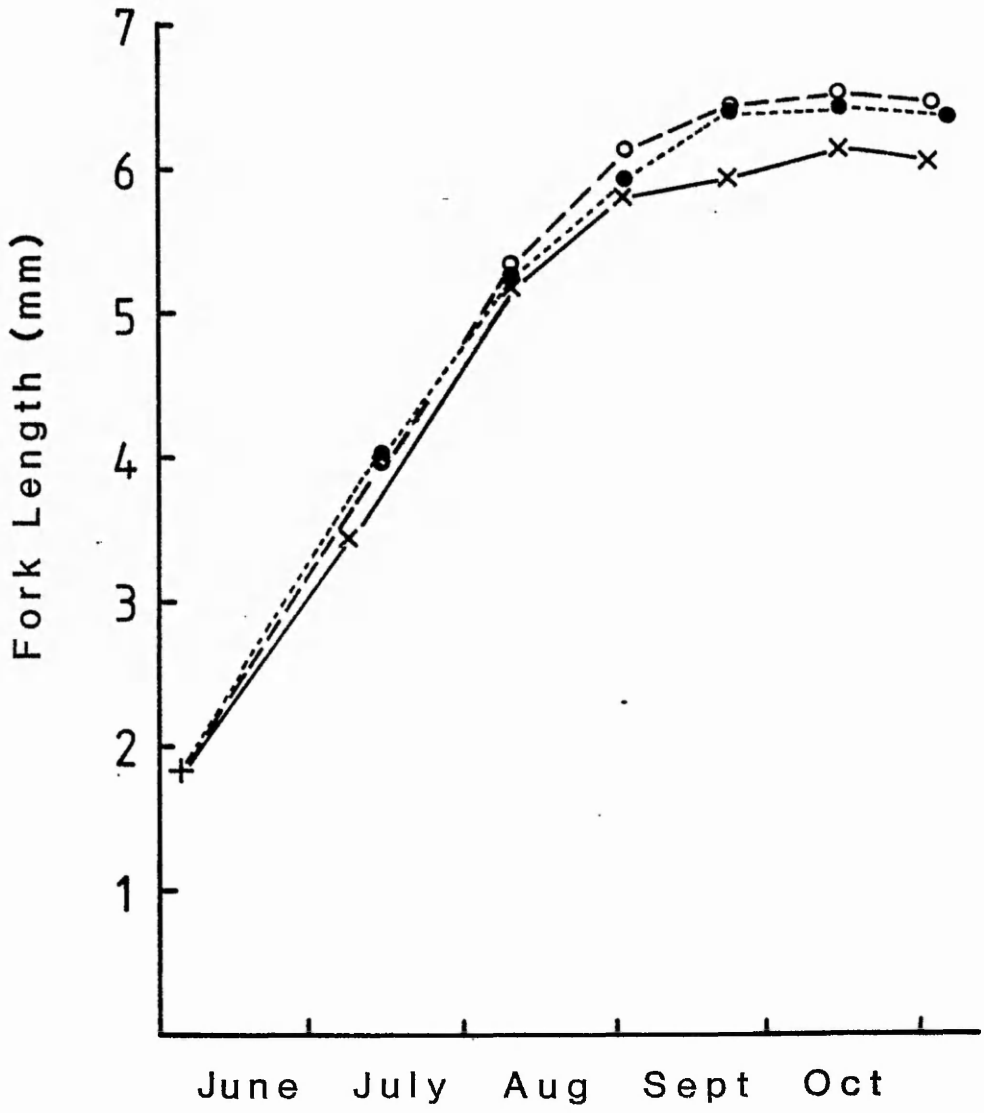


Figure 23. Growth in terms of mean fork length for fry stocked as larvae into outside ponds (338 m^2) at three stocking densities: B7 (●·····●) 15 m^{-2}, B8 (×—×) 10 m^{-2}, B9 (o--o) 20 m^{-2}. Although not plotted, all SEM's < 1mm (Appendix 9). These fry were not overwintered.

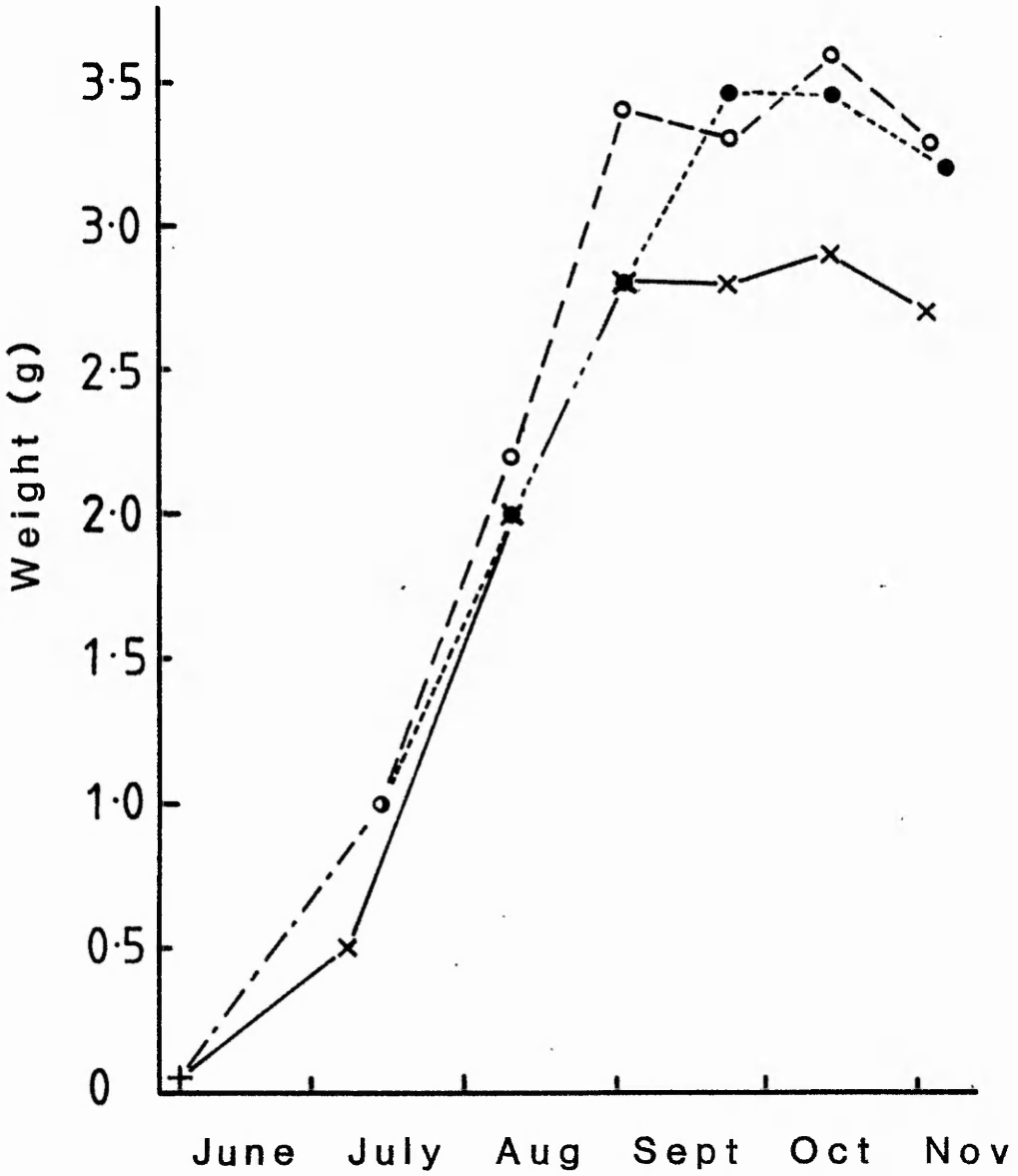


Figure 24. Growth in terms of mean weight (from batch weights) of fry stocked as larvae in outside ponds (338 m^2) at three stocking densities: B7 (●·····●) 15 m^{-2}; B8 (×—×) 10 m^{-2}; B9 (o––o) 20 m^{-2}. These fry were not overwintered.

Plate 13

Plate 13 illustrates pond B7 after draining. The pond bottom is very muddy and is largely covered with macrophytes. Prior to complete draining, excessive macrophytes were piled on the banks.

Plate 14 shows pond B8. This pond was particularly badly affected with considerable quantities of macrophytes and filamentous algae, as seen in the picture.

Plate 14

Plate 15

The pond shown in this plate was almost clear of macrophytes during the course of the experiment due to the grazing of carp the year prior to the experiment. Large, bare patches of the pond bottom can be seen between the discrete patches of macrophytes.

7.4 Discussion

7.4.1 Stocking densities 10 and 30 m^{-2}; 1980

During the first two months of the experiment, similar growth between groups suggests that intraspecific competition for resources did not begin to act before August. Thereafter, it is clear that the higher stocking densities (B8, C6) were considerably disadvantaged. The most plausible explanation for this is that competition between the fish reduced the available food to a level which restricted growth, that is, food supply became the growth limiting factor in place of another. This other factor was probably temperature, since the rates of metabolic processes, including growth, are determined by environmental temperature in fish and other poikilotherms, which are unable to regulate body temperature. In the 10 m^{-2} groups on the other hand, it is likely that temperature and not food supply limited growth. This is supported by the fact that it took four to five weeks longer for fish grown in these outside ponds (B7, C5) to reach 5 cm than fish kept in greenhouse ponds (A23) at 20 m^{-2} (67 m^{-3}), although the latter fish were fed supplementarily (see 6.2). Unfortunately, it was not possible to conduct surveys of invertebrate food items which could have confirmed these ideas.

Comparison of growth of the groups stocked at the same density (10 or 30 m^{-2}), suggests that the fish in the ponds of greater area have an advantage. However, the larger ponds are also deeper, and the ascending order of number of fish per cubic metre (Table 12) corresponds to the descending order of growth during the growing season: C5, B7, C6, B8. Despite the fact that pond area is more important than volume, since incident sunlight and consequently primary production are related to area, increased volume does lead to a marginal increase in production of fish. This is probably because the greater volume contains more phytoplankton nutrients for primary

production, and also serves to dilute metabolic waste products. Tang (1979) states that the most productive Chinese fish ponds are between 3 and 4 metres deep.

The survival figure for B8 was considerably underestimated because many fish were lost during pond drainage. Consequently, the yield figure was depressed. Although growth achieved by the more densely stocked fish was appreciably reduced, the yield in the C6 group (30 m^{-2}) despite losses through disease, was 27°/o higher than the next group (C5). This is a well-known phenomenon in fish farming, and was demonstrated as long ago as 1933 by Schaperclaus. He showed that as the stocking density of carp was increased, the individual growth decreased, but total exploitation of the natural food supply increased. Furthermore, Wolny (1962) demonstrated that there was an optimum density for yield of carp fry. As density is increased from low levels yield increases rapidly, levels-out to an optimum than falls slowly but at an accelerating rate to zero. In conclusion, by increasing the stocking density, high yield and efficient utilisation of food resources may be had at the expense of individual growth and hence size. The reverse is obtained by reducing the density.

Relative to fish at 10 m^{-2}, those at 30 m^{-2} grew poorly. Nevertheless, the smallest fish, B8 (mean length 45 mm, mean weight 1.1 g) were 60°/o longer and more than 300°/o heavier than fry from a wild population in the River Hull, 1978 (Worthington, unpublished). The latter fish were sampled during the same month. On the other hand, Huet (1970) states that, according to conditions, roach of one summer should range from 6 to 10 cm long and weigh 2 to 8 g, although he refers to the warmer, continental summers.

The small amount of growth over winter is probably insufficient to compensate for the increased risk of disease (as in C6). In addition, the ponds could otherwise be drained, disinfected and refilled for the following season.

It would be better to overwinter the fish in smaller ponds (which could be aerated), where they could be managed more easily.

The precise extent and effects of the unwanted plants (Lemna, Elodea, etc,) was not ascertained, although they would obviously compete with the phytoplankton which is useful indirectly to the fish. Clear differences were observed in the luxuriance of the macrophytes between ponds. For example, C6, B8, and to a lesser extent, B7, were affected by dense Lemna growths. These would reflect and absorb light (reducing water temperature) and use nutrients at the expense of phytoplankton. This would clearly reduce fish growth according to the severity of the problem.

As described in 6.3, the fertilisation policy failed to perpetuate a phytoplankton bloom, as evidenced by fluctuating chlorophyll A levels. In addition, the straw content of the calf manure clogged the nets during sampling, and consequently fish were damaged. For these reasons, the fertilisation policy was modified according to 7.2.2.4 - adding inorganic fertilisers once only prior to introduction of fish, and excluding the use of organic manure.

7.4.2 Stocking densities 10, 15, and 20 m^{-2} in 1981

It is apparently paradoxical that fish stocked at the highest density (B9) should also grow to be the largest. Consideration of the characteristics of each pond suggests a possible explanation. Unfortunately, exaggerated differences were observed to develop between the flora of each pond. B8 (stocked at 10 m^{-2}) had particularly profuse growths of Lemna and macrophytes, as indeed it had the previous year. B7 (stocked at 15 m^{-2}) was similarly affected, but to a much lesser extent. In contrast, B9 (stocked at 20 m^{-2}) was almost devoid of macrophytes. The consequences of excessive weed growth have already been discussed in 7.4.1. The major effect is to deprive

phytoplankton of nutrients and light, thus reducing primary production in the form ultimately useful to roach fry.

Growth of fish stocked at 15 or 20 m^{-2} in 1981 was equal or slightly greater than that of fish stocked at 10 m^{-2} in 1980, suggesting that factors other than food supply (probably mainly temperature) limit growth at these densities. Indeed, further experiments might show that fry may be stocked more densely than 20 m^{-2}, to give a higher yield, without detracting significantly from the growth of individuals.

The "survival" figures were somewhat distorted in each group. More fish apparently were removed from B8 and B9 than were put in, but this can be attributed to over-estimation of larvae during stocking. 4 to 6 cm fry are much easier to count than larvae, therefore the number of fry removed is regarded as being more accurate. Hence, with the exception of B7, where a quantity of fish was not recovered, the intended stocking densities were slightly exceeded. The yield of the B7 group was consequently an underestimate. The yield of fish stocked at 20 m^{-2} (691 kg ha^{-1}) was excellent, and exceeded the maximum from 1980 (C6, 10 m^{-2}; 371 kg ha^{-1}) by 86°/₀. It is suggested that the 20 m^{-2} group performed so well because B9 was the only pond occupied the previous year by carp, and was virtually unaffected by Lemna and macrophytes. Indeed, this observation warrants further study because "crop rotation" between carp and other species could greatly improve yields.

This study is by no means definitive, but it does indicate for example, that stocking at 10 m^{-2} under-exploits a pond's resources. Furthermore, with correct pond preparation and management (fertilisation and macrophyte control), growth and survival (and hence yield) of fish stocked at 20 m^{-2} makes this a very satisfactory stocking density.

8 FINAL DISCUSSION

8.1 Husbandry of Brood Fish

Although this work has discovered a good deal about the roach and its culture, further research is clearly needed. This study has emphasised the importance to successful fish culture of maintaining the good health of brood fish and reducing stress imposed upon them. For example, adequate nutrition is essential to keep the fish in good condition. During starvation, blood corticosteroids will increase to mobilise carbohydrate stores, but will also suppress the immune system concomitantly (review: Ellis, 1981). Furthermore, low temperatures also reduce defence capability (O'Neill, 1980; Ellis, 1981). Thus, the warming of recently-captured wild fish not yet feeding under captive conditions (Chapter 2), in combination with the stress induced by capture and transport, renders them more susceptible to disease. Highfields Lake has, until recently, been overstocked with fish (K.Easton, pers. comm.). Consequently, the roach were thin and in poor condition when compared to those fish raised on the fish farm. Yet both stocks of fish seemed to adapt well to captivity and began feeding on artificial food within days of introduction to the laboratory, in contrast to those from the River Sence. It is possible that Highfields fish, having experienced considerable competition for food, were keener to feed than those from the River Sence. Those fish raised in captivity (Calverton fish farm), on the other hand, had experience of capture, handling and artificial food and appeared to acclimate to laboratory conditions quite quickly. Thus, the feeding response may not only indicate the existing degree of stress, but it may also prevent exacerbation of that stress. This former phenomenon is familiar to many fish stockmen, who suspect fish are in some way stressed when they refuse food. The present study again underlines the importance of having good quality, "domesticated" brood fish, that is, raised entirely in captivity.

8.2 Reproduction

The validity of the study of the reproductive cycle may be questioned since the derived data were from a different population to those with which it was compared (fish farm-raised fish and those from the River Sence). However, taking the plasma calcium data for example, although the absolute values were lower in Highfields fish than those from the fish farm, the trends are likely to be the same over a reproductive cycle. It is possible that the differences seen between populations are again attributable to nutritional effects. Fish in the laboratory were fed *ad libitum*, whereas Highfields fish (as already stated in Chapter 3) were somewhat poorly nourished. Since it has often been demonstrated that fecundity can be reduced when food is scarce (Scott, 1962; review: Bagenal, 1978), Highfields female roach may have produced fewer or smaller eggs, requiring less yolk. This is supported by a four-year study conducted on female roach (Kuznetsov and Khalitov, 1978). They found that in the years of plentiful food, fecundity and the lipid content of eggs was high. When food was in short supply, the reverse was true, and under both circumstances egg weight was unaffected. This may result in lower blood levels of vitellogenin and hence calcium, the latter being observed in Highfields fish. It would be interesting to determine the fecundity of fish from this population, and attempt the reversal of reduced fecundity by artificial feeding.

Plasma calcium is easy to measure when compared with many other blood parameters, and the technique could play a useful role in both fish culture and fishery management. It is only possible to sex roach morphologically for approximately one month of the year (spawning time). However, the seasonal plasma calcium profile suggests that sexually mature females could be distinguished in the Highfields population from November to June with a reasonable degree of certainty. This possibility would need to be evaluated more rigorously, particularly in samples

containing more males. In addition, oestradiol (which increases in the blood during vitellogenesis) causes plasma calcium to rise at the expense of calcium stores in the scales of goldfish and killifish, Fundulus heteroclitus (Mugiya and Watabe, 1977). Since this effect would only naturally occur in females, it may be possible to sex fish (during the vitellogenic phase of ovogenesis) on the basis of reduced scale thickness/density relative to body size, however this is speculative.

The cortisol data (Chapters 2 and 3) are open to the criticism that plasma levels of this hormone are the net effect of a number of processes, including secretion, degradation and clearance (Donaldson, 1981). However, it would not be possible to determine rates of these processes in the study of the reproductive cycle of fish in the wild.

In most cases the use of any exogenous hormone preparations to induce spawning artificially is considered to be an admission of failure to either understand or simulate those cues which naturally trigger spawning (including maturation, ovulation or spermiation). This is particularly true where potential brood fish have been kept under captive conditions for some time and have had time to acclimate. However, there are situations where hormones are necessary: for example, where brood fish are unavoidably stressed, perhaps after collection from the wild, or where a large number of brood fish need to be spawned simultaneously for artificial fertilisation. The problems encountered with gonadotrophin preparations (eg, species-specificity, storage and potency problems) justify continued investigation of artificial compounds for induced spawning. Tamoxifen, for example, may possible be administered orally by incorporating it into the food since, unlike gonadotropins, it is not digested in the gut. Also, because the drug is soluble in alcohol but virtually insoluble in water, it could be soaked into powdered trout food in alcohol solution. After drying, this food could be reconstituted by mixing the food with water, pelletised and dried. The tamoxifen is unlikely to

leach-out of the food uneaten by the fish. One drawback to the use of antioestrogens like tamoxifen is that in addition to inducing the release of pituitary GtH, they may block GtH synthesis and storage by opposing the positive feedback effects of sex steroids. Successive treatments might exhaust the GtH store, thus interfering with subsequent reproductive function.

With regard to the advancement of spawning, the simplicity and effectiveness of the environmental manipulation technique suggest that this method is preferable to hormone treatment. Thus, there is an obvious requirement for comprehensive study on the environmental factors controlling reproduction in roach, like the extensive work done on the stickleback by B. Baggerman, for example. However, the regime described in 5.2.2 is clearly very effective and relatively easy to implement, and is consequently of practical use to fish farmers. When the factors which control reproductive cycles are investigated, it is important to refer to the ecology of the animal and interpret results in this context. Failure to do this may lead to mistaken conclusions. For example, Scott (1979) found that by subjecting minnows to simulated natural photoperiod and temperature regimes, full gametogenesis (compared to wild fish) did not take place. However, gametogenesis was completed after subjecting fish to photoperiods changing rapidly from 8L:16D to 16L:8D within a week. This was explained by reference to the behaviour of this fish in the wild. It spends the winter months in subdued light under stones, and emerges in spring (when the temperature reached 8°C) to swim in shoals in the daylight. Many laboratory experiments make no provision for such behavioural influences.

8.3 Rearing of Larvae and Fry

The pond rearing of larvae is evidently very effective. However, if larval rearing is to be integrated with the advanced spawning technique, then larvae must be reared in a

hatchery since the earliest that greenhouse ponds can be stocked with larvae is usually early May, when the temperatures are favourable to survival. If a successful hatchery technique were to be found which would bridge the gap between advanced spawning and the beginning of the growing season in greenhouse ponds, then it is likely that 12 cm roach could be produced in one season. Under normal circumstances, the nauplii of Artemia cannot be consumed by roach larvae because it was assumed that they were too large (Dolben, 1979). However, roach larvae have been reared on Artemia by using the simple expedient of chilling the nauplii in a refrigerator before offering them to the fish (E.A.Huisman, pers. comm.). This has the effect of retarding the motility of the nauplii and the larvae are apparently then able to catch them. If this method fails, then it would probably be necessary to culture an invertebrate, possibly a freshwater rotifer, although this of course requires further research. In the event of success in this regard, roach culture would become much more flexible and larval rearing would not be restricted to the warmer summer months. Despite this prospect, larval rearing in greenhouse ponds is likely to remain a viable technique, although it could be improved. For example, various chemicals are used in Hungarian and Polish fish culture in order to manage the pond biocoenoses. Trichlorophone marketed variously as Dipterex, Dylox or Masoten selectively kills arthropods, leaving the protozoans and rotifers to thrive unharmed (Tamas, 1979; Grygierek and Wasilewska, 1979). This means that most of the production of bacteria and phytoplankton would be channelled into food organisms available to the larvae. Furthermore, some parasites (eg, Argulus) would be eliminated simultaneously.

The design of all of the pond experiments is open to criticism because they ostensibly assessed growth and survival. Since the sampling methods employed are bound to affect survival, strictly speaking both of these parameters cannot be studied in the same population. Despite this, the survival figure is evidently not very precise, and

furthermore, the limited availability of ponds and fish larvae to stock them precluded investigation of this aspect.

The experiments involving the production of fry in ponds have contributed to our knowledge of how fry grow in ponds under different conditions. The fish farmer must decide which is the most appropriate stocking density which will maximise the production of a certain size of roach. Firstly, this target size needs to be identified. For example, if high yield rather than growth rate is preferred, then larvae should be stocked more densely. On the other hand, the objective may be to produce 10 or 12 cm fish in one year, in which case early spawning (February) might be combined with rearing in a hatchery, greenhouse or outside ponds, at densities unrestrictive to growth (in outside ponds, possibly 20 m^{-2}). Further experiments would be worthwhile to investigate density effects between 20 and 30 m^{-2}. This would help determine the point where increasing stock density restrains growth unacceptably. From all viewpoints, it is highly desirable to replicate each treatment group, since the flora and fauna of apparently identical ponds may vary considerably. For example, Swingle (1947) observed that the flora of 27 adjacent, apparently identical ponds with a common water supply, when fertilised, developed different phytoplankton populations. Thus, in experiments where fish are raised in ponds, and therefore dependant on the flora and fauna which develop therein, replicate groups would help to reduce aberrant results.

Pond experiments in the first year employed organic manure as a source of enrichment but this was discarded in the second year in favour of inorganic fertilisers. However, the fish farmer may prefer to employ liquid cow manure because it is cheap, easily-procurable and has other desirable properties. For, example, Schroeder (1978) fertilised ponds with either cow or chicken manure and found that the production of all organisms large enough to be used directly by fish accounted for less than half of the

measured fish growth. On the other hand, the production of microbes could account for this discrepancy, and it appeared that the fish harvested the microorganisms (bacteria and protozoa) by ingesting the small straw-like particles of the manure which served as a substrate for microbial growth. Clearly, inorganic fertilisers do not have the same properties. Furthermore, organic fertilisers give much greater yields of fish if applied in small quantities on a regular, frequent (weekly) basis rather than in large quantities once or twice per year (review: Wohlfarth and Schroeder, 1979).

It is likely that the farming of roach will take place in parallel with other species. If this is the case, crop rotation or polyculture with other species may yield considerable benefits. For example, experience at the STWA experimental fish farm has demonstrated the ability of carp to eliminate macrophytes, by consuming them and also muddying the water, thus inhibiting light penetration. As already discussed, the management of ponds containing young roach is rendered difficult when macrophytes grow luxuriantly. Thus, rearing carp in ponds the year prior to rearing roach could increase the yields of roach. Moreover, the carp would probably benefit from the macrophytes as supplemental feed. Polyculture offers more-efficient exploitation of resources, particularly when considering the culture of species such as roach and carp, which frequently depend heavily on natural food production. Under these circumstances, it is clear that the one species of a monoculture, having evolved to exploit a particular environmental niche and food supply, cannot exploit the whole potential food supply efficiently. Chinese polyculture is high developed. Feedstuffs are not readily available in China and the Far East (Wohlfarth and Schroeder, 1979), consequently pond manuring and natural food production are of great importance. In order to exploit this natural food efficiently, one pond may be used to culture as many as seven species of carp including common, silver, grass and bighead carp (Lin, 1982). One

observation from the present study is that 0+ roach cannot consume the larger insects, including corixids which are often present in large numbers. Common carp on the other hand do consume these and may therefore thrive in polyculture with roach. Furthermore, grass carp, although not entirely herbivorous, thrive on some types of plant, particularly Lemna spp (Porath, Hepher and Koton, 1979). This plant, as already discussed, can be a considerable nuisance in roach rearing ponds, not least because it reproduces so rapidly. Thus, the culture of roach with grass carp and possibly common carp could be mutually beneficial to each species and also considerably simplify pond management. A great deal of further research is required on this and other topics, but the benefits are likely to be very worthwhile.

8.4 Recommendations

The results of this study, though by no means definitive, indicate a series of procedures which could be implemented with success by fish farmers to culture roach:

8.4.1 Brood fish husbandry

Ideally, brood fish should be raised from eggs, larvae or fry under artificial conditions in order to ensure adequate nutrition, early sexual maturity and the exclusion of pathogens and parasites. Since it takes three or four years for roach to become sexually mature, it may be necessary to collect brood fish from the wild.

Great care should be then taken during capture, transport and introduction to quarantine aquaria, in order to minimise stress and physical damage, thus reducing the risk of disease. Quarantine should entail immersion in a 25 mg l^{-1} solution of a mixture of malachite green and formalin (3 g l^{-1}) for more than two weeks to eradicate ecto-parasites. Capture and acclimation should ideally take place in the summer post-spawning when the fish are fit and

temperatures high enough to encourage them to consume artificial food. Aquaria should contain some form of refuge, which tends to reduce the self-inflicted damage caused by fright reflexes.

Increases of temperature should be gradual to minimise stress and enable the immune system to keep pace with proliferation of pathogens. In addition to these practices, normal husbandry methods should be employed, that is, maintaining a clean, uncrowded environment with plenty of food and adequate oxygen replenishment.

8.4.2 Controlled reproduction

Ideally, fish should be spawned spontaneously by providing appropriate conditions throughout the reproductive cycle. Post-spawning, the brood fish should be placed in a pond, where natural food would be supplemented with artificial food. It is desirable to identify the sexes by dye-marking or tagging in order to segregate them if necessary and also control the sex ratios of spawning groups (naturally, 1 male to 2 females in the roach reared by STWA). The approximate timing of reproduction may be dictated by providing an increasing photoperiod, with 14.5L:9.5D scheduled for spawning time. Actual spawning may then be finely controlled by temperature manipulation, the spawning threshold temperature of approximately 14°C to be surpassed at the desired time. Spawning could be advanced or retarded in the long-term by providing either compressed or expanded natural light cycles. Given that successful hatchery rearing could be developed, then the earliest spawning for pond rearing of fry would be approximately early February. This would allow nearly three months for the fish to reach 2 cm (when they would be large enough to consume most items of zooplankton), and for the outside ponds to reach a suitable temperature to ensure survival (12° to 15°C). If spawning did not take place spontaneously, then it could be induced with either carp pituitary extract or tamoxifen treatment. Once ovulation and spermiation had been induced,

it may be desirable to fertilise the eggs artificially, for the purposes of genetic improvement, for example. Although several species, including carp, have been reproduced by this method, it has not been very successful with roach, and further research is therefore needed. The eggs should be incubated at approximately 20°C in order to minimise the period when the eggs are vulnerable to infection or predation. In the absence of hatchery rearing techniques, from May onwards, eggs may be hatched and larvae reared to 2 cm in greenhouse ponds which ensures good growth and survival.

8.4.3 Rearing of fry

Because of the expense of polythene greenhouses, large-scale production of fry should be carried-out in carefully prepared outside ponds. These should have been disinfected and cleared of macrophytes either manually or by stocking the previous year with carp. Ponds should be manured on a weekly basis with liquid cow manure. The ponds should have been refilled and manured one month before stocking to allow plankton to develop, and should be "seeded" with appropriate organisms if necessary. In most cases, outside ponds should be stocked at 20 m^{-2}. Supplementary feeding may be advantageous but the cost-effectiveness of this would need to be assessed. To produce larger first year roach (10 to 12 cm), the techniques of early spawning, hatchery and greenhouse pond rearing should be combined.

8.4.4 Other species

These investigations and the recommendations made, specifically concern the roach. However, the findings could be applied usefully to many other species of coarse fish, particularly those which are closely related, such as rudd (Scardinius erythrophthalmus), bream (Abramis brama), chub (Leuciscus cephalus), and dace (Leuciscus leuciscus). Clearly, since each of these occupy distinct ecological niches, each will have slightly different physiological

requirements and life cycles. Although these must be investigated if these species are to be cultured successfully, this study might form a useful basis for such investigations.

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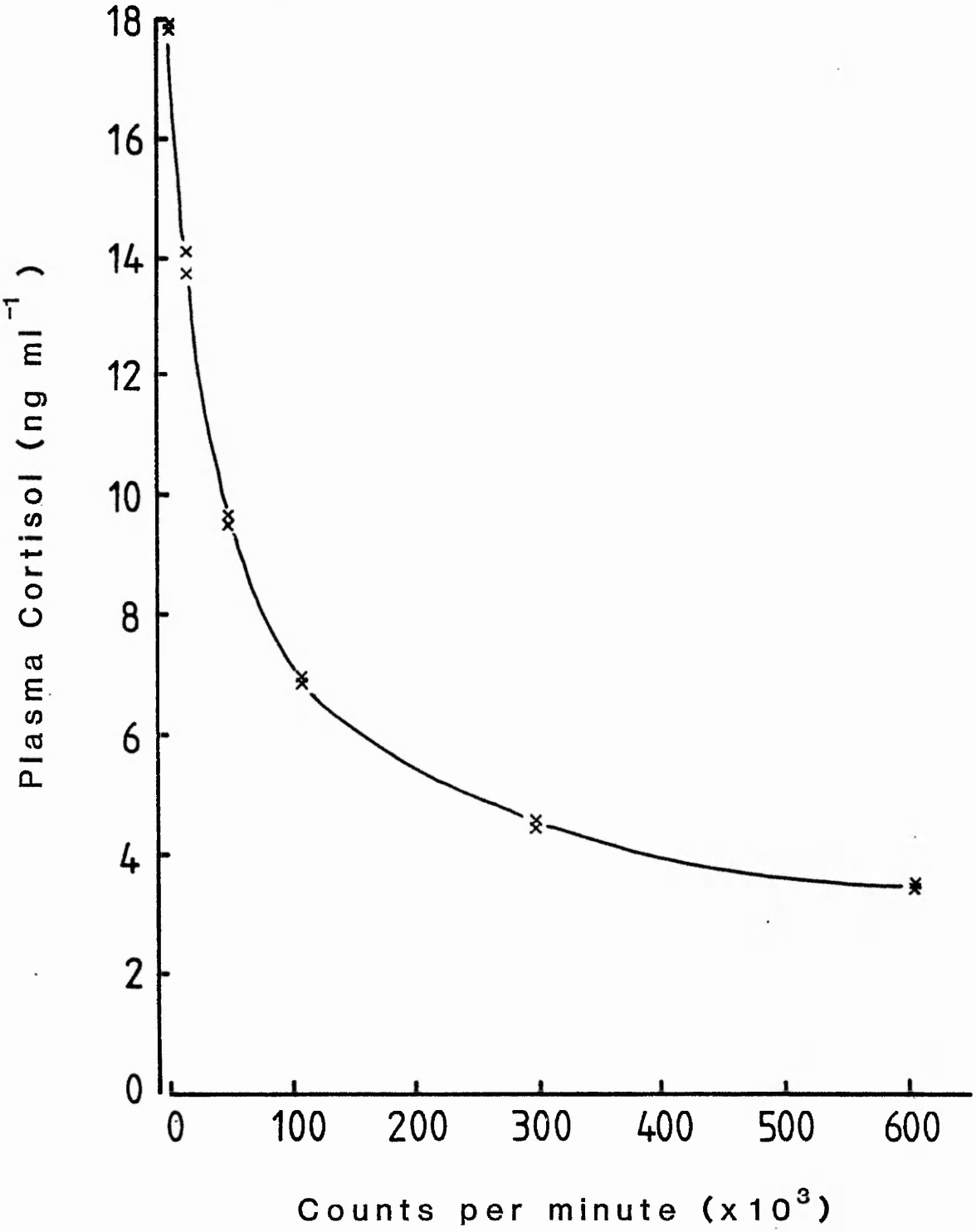

Appendix 1. An example of a standard curve obtained during the cortisol RIA procedure. Each standard was analysed in duplicate in order to establish reproducibility.

Appendix 2 Reproductive cycle data - females

Date	Number of Fish	Gonosomatic index % $\bar{x} \pm$ SEM	Hepatosomatic index % $\bar{x} \pm$ SEM	Condition factor $\bar{x} \pm$ SEM	Plasma calcium mmol l^{-1} $\bar{x} \pm$ SEM
12.8.81	19	2.5 ± 0.1	-	1.5 ± 0.0(2)	2.28 ± 0.02
7.9	19	4.2 ± 0.3	1.7 ± 0.1	1.6 ± 0.0(4)	2.40 ± 0.05
7.10	16	5.8 ± 0.5	1.7 ± 0.2	1.4 ± 0.0(3)	2.50 ± 0.06
4.11	17	6.2 ± 0.4	1.8 ± 0.0(4)	1.5 ± 0.1	2.75 ± 0.06
2.12	1	8.3	1.5	1.6	2.67
6.1.82	10	8.7 ± 0.4	2.3 ± 0.1	1.5 ± 0.0(3)	2.91 ± 0.06
11.2	20	7.6 ± 0.4	2.5 ± 0.1	1.5 ± 0.0(2)	2.74 ± 0.07
11.3	17	8.8 ± 0.4	2.3 ± 0.2	1.5 ± 0.0(2)	3.06 ± 0.10
7.4	12	10.2 ± 0.5	2.0 ± 0.2	1.6 ± 0.1	3.16 ± 0.08
14.4	4				
19.5	13	14.3 ± 0.6	1.5 ± 0.1	1.7 ± 0.0(2)	3.15 ± 0.13
9.6	30	2.2 ± 0.1	1.3 ± 0.1	1.6 ± 0.0(2)	2.47 ± 0.02
19.7	19	2.4 ± 0.2	1.7 ± 0.1	1.4 ± 0.0(3)	2.54 ± 0.06

Statistics: Plasma calcium

Aug - July: $t = 4.11$, $p < 0.001$

Aug - Sept: $t = 2.23$, $p < 0.05$

May - June: $t = 5.17$, $p < 0.001$

Appendix 3 Reproductive cycle data - males

Date	Number of Fish	Gonosomatic index % $\bar{x} \pm$ SEM	Hepatosomatic index % $\bar{x} \pm$ SEM	Condition factor $\bar{x} \pm$ SEM	Plasma calcium mmol l^{-1} $\bar{x} \pm$ SEM
12.8.81	1	1.0	-	1.4	2.25
7.9	1	1.8	2.2	1.6	2.51
7.10	4	4.4 ± 1.4	1.7 ± 0.4	1.4 ± 0.0(4)	2.21 ± 0.10
4.11	2	3.6 ± 0.1	1.6 ± 0.0(4)	1.6 ± 0.0	2.24 ± 0.11
2.12	1	2.6	2.4	1.5	2.03
6.1.82	4	4.2 ± 0.4	2.2 ± 0.3	1.5 ± 0.1	2.50 ± 0.08
11.2	3	4.9 ± 1.1	2.4 ± 0.4	1.4 ± 0.1	2.45 ± 0.24
11.3	3	7.5 ± 0.6	1.4 ± 0.5	1.4 ± 0.1	2.38 ± 0.17
7.4	1	8.9	3.0	1.7	2.68
19.5	13	4.4 ± 0.9	1.4 ± 0.1	1.5 ± 0.0(2)	2.64 ± 0.06
19.7	5	0.9 ± 0.1	1.6 ± 0.2	1.2 ± 0.0(4)	2.55 ± 0.05

Appendix 4 Seasonal changes in plasma cortisol of roach captured from Highfields lake

Plasma cortisol (ng ml^{-1})

Date	$\bar{x} \pm$ SEM	Sample (n)	$\bar{x} \pm$ SEM	Sample (n)
12.8.81	5 ± 2	16	0	1
7.9	1 ± 1	19	0	1
7.10	7 ± 4	16	7 ± 5	4
4.11	2 ± 1	17	7 ± 7	2
6.1.82	2 ± 1	13	0	4
11.2	4 ± 1	27	5 ± 3	3
11.3	4 ± 1	18	5 ± 2	3
7.4 14.4	6 ± 2	15	187	1
19.5	17 ± 8	11	306 ± 71	13
9.6	9 ± 4	30	-	0
19.7	11 ± 6	19	3 ± 2	4

Statistics (females) Student's t-test

April - May t = 1.33 df = 25 Not significant (at p = 0.05 level)

April - June t = 1.79 df = 44 " "

April - July t = 0.67 df = 33 " "

July - August t = 0.95 df = 34 " "

Appendix 5 Plasma calcium ($\bar{x}$ + SEM) recorded from roach kept under different photoperiod regimes

Date	Group 3 Advanced photoperiod Plasma calcium mM		Group 2 Natural photoperiod Plasma calcium mM	
	(n = 16)	(n = 8)	(n = 15)	(n = 9)
1.10.81	3.95 ± 0.10	2.92 ± 0.10	3.69 ± 0.11	3.18 ± 0.07
24.11	3.70 ± 0.07	2.93 ± 0.02	3.39 ± 0.07	2.84 ± 0.06
15.12	3.11 ± 0.07	2.61 ± 0.03	3.33 ± 0.08	2.75 ± 0.06
8.1.82	3.22 ± 0.05	2.71 ± 0.05	3.37 ± 0.06	2.69 ± 0.05
29.1	4.09 ± 0.17	2.75 ± 0.04	3.54 ± 0.07	2.73 ± 0.05
18.2	2.99 ± 0.08	2.67 ± 0.04	3.41 ± 0.13	2.70 ± 0.04
23.2	2.49 ± 0.06	2.40 ± 0.03	2.94 ± 0.17	2.45 ± 0.02

Appendix 6 Growth data for 0+ roach held in greenhouse pond A17

Date	Age (days)	Weight $\bar{x}$ (g)	Length $\bar{x}$ ± SEM (mm)	Sample n	MDWI %
15.5.80	3	0.002	8.5 ± 0.1	15	-
19.5	7	0.011	12.8 ± 0.1	16	34.6
20.5	8	0.014	13.5 ± 0.1	31	24.0
28.5	16	0.013	22.1 ± 0.5	11	20.1
3.6	22	0.29	28.6 ± 0.2	26	12.7
6.6	25	0.39	31.4 ± 0.2	14	9.8
9.6	28	0.46	32.6 ± 0.3	13	5.5
17.6	36	0.9	38.5 ± 0.2	22	7.6
24.6	43	1.0	41.5 ± 0.4	27	2.7
3.7	52	1.3	44.9 ± 0.6	41	2.2
9.7	58	1.5	47.4 ± 0.4	68	-
8.8	88	3.2	59.9 ± 0.4	63	3.7
27.8	107	5.2	70.2 ± 0.7	41	2.4
9.9	120	6.0	75.6 ± 0.6	35	1.2
18.9	129	6.0	76.1 ± 0.7	43	-0.15
14.10	154	7.4	82.6 ± 0.7	58	0.87
31.10	171	7.3	82.4 ± 0.8	38	-0.08
19.11	190	7.5	82.7 ± 1.0	37	0.14
24.4.81		7.9	83.5 ± 0.9	35	-

Appendix 7 Growth data for 0+ roach held in greenhouse pond A23

Date	Age (days)	Weight $\bar{x}$ (g)	Length $\bar{x}$ + SEM (mm)	Sample n	MDWI %
15.5.80	3	-	8.4 ± 0.06	10	-
19.5	7	-	12.1 ± 0.4	6	-
20.5	8	0.018	13.6 ± 0.3	6	-
17.6	36	0.95	40.1 ± 0.3	42	9.6
24.6	43	1.44	44.7 ± 0.6	18	5.9
3.7	52	1.46	47.3 ± 0.4	52	0.2
10.7	59	1.5	48.5 ± 0.3	40	0.7
29.7	78	2.2	54.6 ± 0.4	44	2.0
8.8	88	2.7	57.3 ± 0.6	17	2.0
27.8	107	3.2	61.8 ± 0.5	40	0.8
9.9	120	3.6	65.5 ± 0.8	29	1.0
18.9	129	4.2	66.6 ± 0.8	41	1.7
14.10	154	4.7	71.2 ± 0.7	38	0.5
31.10	171	5.1	72.4 ± 0.6	36	0.5
19.11	190	5.0	72.0 ± 0.7	44	-0.1

Appendix 8 Growth data for 0+ roach stocked in outside ponds at various densities

Pond	Date	Length $\bar{x}$ + SEM (mm)	Weight $\bar{x}$ (g)	Sample n
Common sample taken prior to stocking	13.6.80	14.0 ± 0.2	0.02	70
B7 ($10\ m^{-2}$)	8.7.80	25.2 ± 0.3	0.2	60
	1.8.80	36.7 ± 0.6	0.8	46
	28.8	51.4 ± 0.4	2.0	67
	18.9	56.6 ± 0.5	2.6	49
	10.10	59.4 ± 0.5	2.6	47
	29.10	59.7 ± 0.7	2.6	40
	21.1	60.6 ± 0.8	2.7	42
	25.3.81	58.3 ± 0.6	2.7	45
	22.4	62.0 ± 0.5	3.2	40
B8 ($30\ m^{-2}$)	21.7.80	27.6 ± 0.3	0.3	40
	1.8	32.4 ± 0.4	0.5	49
	28.8	39.7 ± 0.5	0.9	60
	18.9	45.0 ± 0.3	1.2	47
	10.10	44.4 ± 0.6	1.1	34
	30.10	45.4 ± 0.6	1.1	44
	21.1.81	47.2 ± 0.5	1.2	39
	25.3	43.8 ± 0.5	1.1	48
	24.4	45.8 ± 0.5	1.2	32
C5 ($10\ m^{-2}$)	7.7.80	23.4 ± 0.5	0.2	25
	1.8	38.8 ± 0.4	0.9	41
	28.8	55.5 ± 0.5	2.4	57
	18.9	61.2 ± 0.4	3.2	45
	10.10	64.0 ± 0.5	3.3	44
	30.10	64.4 ± 0.8	3.3	36
	21.1.81	63.0 ± 0.7	3.1	41
	25.3	63.4 ± 0.5	3.3	35
	24.4	62.9 ± 0.6	3.1	34
C6 ($30\ m^{-2}$)	7.7.80	23.6 ± 0.4	0.2	33
	6.8	36.8 ± 0.5	0.7	46
	28.8	42.2 ± 0.5	1.2	49
	18.9	45.2 ± 0.5	1.3	47
	10.10	45.7 ± 0.4	1.2	56
	30.10	47.3 ± 0.7	1.3	43
	21.1.81	48.7 ± 0.7	1.5	40
	25.3	45.9 ± 0.5	1.3	35
	6.5	47.5 ± 0.4	1.4	100

Appendix 9 Growth data for 0+ roach stocked in outside ponds at various densities

Pond	Date	Length $\bar{x}$ + SEM (mm)	Weight $\bar{x}$ (g)	Sample n
Common sample taken prior to stocking	4.6.81	18.2 ± 0.28	0.05	33
B7	15.7	40.7 ± 0.5	1.0	35
(15 m^{-2})	10.8	52.6 ± 0.6	2.0	34
	2.9	59.5 ± 0.6	2.8	38
	23.9	64.8 ± 0.8	3.4	33
	14.10	64.2 ± 0.8	3.4	39
	6.11	63.9 ± 0.5	3.2	100
B8	8.7	34.6 ± 0.4	0.5	35
(10 m^{-2})	10.8	52.4 ± 0.6	2.0	40
	2.9	58.2 ± 0.7	2.8	37
	23.9	59.5 ± 0.6	2.8	38
	14.10	61.7 ± 0.7	2.9	36
	2.11	60.7 ± 0.4	2.7	100
B9	15.7	40.1 ± 0.5	1.0	39
(20 m^{-2})	10.8	53.5 ± 0.4	2.2	39
	2.9	61.8 ± 0.6	3.4	35
	23.9	64.6 ± 0.6	3.3	35
	14.10	65.2 ± 0.6	3.6	35
	3.11	64.6 ± 0.4	3.3	100