ASPECTS OF THE FEEDING BEHAVIOUR OF INTENSIVELY REARED TROUT

Abdul Majid, M.Sc.

A thesis submitted for the award of
Doctor of Philosophy to the
University of Aston in Birmingham

JULY 1980

SUMMARY

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Feeding behaviour of trained rainbow trout was investigated by the use of demand feeders, under different light conditions. The effects of the energy content of diet, and the size, colour and texture of feed pellets, on the feeding behaviour, were studied. An attempt was made to locate the assumed centres for feeding and satiety in the hypothalamus of brain by the intraperitoneal injections of goldthioglucose.

Feeding under nine different constant photoperiods at 160 lux, at a temperature of 13.5°C, showed that trout exhibit a rhythmic pattern of feeding behaviour in all photoperiods except in continuous darkness. Feeding rhythms of trout attributable to the degree of gut distension were formed every eight to ten hours. Further studies by varying levels of light intensity revealed the interaction of light intensity and photoperiod. At shorter photoperiods lower levels of light intensity decreased the feeding activity in terms of food intake but by increasing the photoperiod the same feeding activity was accomplished as by the fish subject to a short photoperiod but under higher light intensity. Simulated effect of increasing and decreasing daylengths did not affect the overall food intake and growth performance.

Trout are quite efficient in adjusting their food intake in terms of energy content. Colour, size and texture of feed pellets affect the feeding responses and elicit preferential food selection behaviour in trout.

Goldthioglucose induced some reversable toxic effects upon general physiology of trout and did not produce any lesions in the assumed areas of feeding and satiety centres in the brain.

It was concluded that the feeding behaviour of trout exhibited selective preferences according to the physical nature of food items and those preferences could be further influenced by the biotic and abiotic factors, light being one of the most important abiotic factors.

KEY WORDS: Rainbow trout - Feeding behaviour - Demand feeding -
Photoperiods - Goldthioglucose

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My wife's endurance and patience deserves all the praise. She also lost her loving and admirable mother a few months ago but she faced all the upheavals and mishaps with renewed determination and always proved herself to be a source of encouragement for me as well.

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CONTENTS

SUMMARY

ACKNOWLEDGEMENTS

CHAPTER 1

1 Introduction 1
1.1. Background 1
1.2. The Aims of the Research 4

CHAPTER 2

2 Review and History 9
2.1. Learning in Fish 9
2.2. Effect of Light on Some Aspects of Fish Life 14
2.2.1. Locomotor and Feeding Activity
2.2.2. Reproduction and Maturation Process
2.2.3. Effect of Light on Early Life of Fish
2.2.4. Growth
2.3. Effect of Some Physical Variants in Food 31
2.3.1. Food Particle Size
2.3.2. Mositure Content
2.3.3. Colour
2.4. Influence of Energy Level in Feed, on Feeding Behaviour 45
2.5. Neural Regulation of Food Intake 48

CHAPTER 3

3 General Materials and Methods 57
3.1. The Experimental Facilities 57
3.2. The Recirculation System 57
3.3. Description of the Recirculation System 62
3.3.1. Header Tank
3.3.2. Experimental Tanks
3.3.3. Faecal Trap
3.3.4. Filter Tank
3.3.5. Light Chambers
3.4. Demand Feeding System 70
3.4.1. Description of the Demand Feeding System
3.4.2. Control Box
3.4.3. Feed Dispenser
3.4.4. Trigger
3.4.5. Event Recorders
3.4.6. Operation of Demand Feeding System
3.4.7. Determination of Feed Dispensed/Trigger Actuation

3.5. Quarantine Procedure 84
3.6. Fish Training, Maintenance & Handling 84
3.7. Tagging and Weighing Procedure 86
3.8. Diet Formulation 87
3.9. Diet Preparation 91
3.10. Methods of Proximate Analysis 92
3.11. Analysis of Experimental Data 93
3.11.1. Specific Growth Rate
3.11.2. Food Conversion Ratio
3.11.3. Statistical Methods

CHAPTER 4

4 Effects of Light Variation on Feeding Behaviour of Rainbow Trout (*Salmo gairdneri*) 95
4.1. General Introduction 95
4.2. Experiment 1 Feeding Behaviour under Constant Photoperiods 98
4.2.1. Introduction 98
4.2.2. Materials and Methods 99
4.2.2.1. The Experimental System and Animals
4.2.2.2. Different Photoperiods Studied
4.2.2.3. Monitoring the Feeding Activity
4.2.2.4. Experimental Diet
4.2.2.5. Weighing Procedure
4.2.2.6. Statistical Methods and Analysis of Growth Data
4.2.3. Results 105
4.2.3.1. Feeding Behaviour under Different Photoperiod Regimes
4.2.3.2. Growth Performance
4.2.3.3. Food Conversion
4.2.4. Discussion and Conclusions 120
4.3. Experiment 2 Feeding Behaviour under Constant Photoperiods and Varying Intensity of Light 126
4.3.1. Introduction 126
4.3.2. Materials and Methods 127
4.3.2.1. The Experimental System and Animals
4.3.2.2. Different Photoperiods and Levels of Light Intensity Studied
4.3.2.3. Monitoring the Feeding Activity
4.3.2.4. Experimental Diet
4.3.2.5. Weighing Procedure
4.3.2.6. Statistical Methods and Analysis of Growth Data

4.3.3. Results 129
4.3.3.1. Feeding Behaviour under Different Photoperiods and Light Intensity
4.3.3.2. Growth Performance
4.3.3.3. Food Conversion
4.3.4. Discussion and Conclusions 142
4.4. Experiment 3 Feeding Behaviour under Continuously Varying Photoperiods 149
4.4.1. Introduction 149
4.4.2. Materials and Methods 149
4.4.2.1. The Experimental System and Animals
4.4.2.2. Varying the Photoperiods
4.4.2.3. Experimental Diet
4.4.2.4. Monitoring the Feeding Activity
4.4.2.5. Weighing Procedure
4.4.2.6. Statistical Analysis
4.4.3. Results 152
4.4.3.1. Feeding Behaviour
4.4.3.2. Growth Performance
4.4.3.3. Food Conversion
4.4.4. Discussion and Conclusions 156

CHAPTER 5

5 Effects of some Physical Variants of Food on Feeding Behaviour of Rainbow Trout *(Salmo gairdneri)* 162
5.1. Experiment 4 Effects of Size of Food Pellets on Feeding Behaviour 162
5.1.1. Introduction 162
5.1.2. Materials and Methods 163
5.1.2.1. The Experimental System and Animals
5.1.2.2. Weighing Procedure
5.1.2.3. The Experimental Feed Particle Sizes
5.1.2.4. Monitoring Feeding Behaviour
5.1.2.5. Statistical Methods
5.1.3. Results 168
5.1.3.1. Feeding Behaviour
5.1.3.1.1. Group 1
5.1.3.1.2. Group 2
5.1.3.1.3. Group 3
5.1.4. Discussion and Conclusions 177

5.2. Experiment 5 Effect of Colour of Feed Pellets on Feeding Behaviour 182
5.2.1. Introduction 182
5.2.2. Materials and Methods 184
5.2.2.1. The Experimental System and Animals
5.2.2.2. The Experimental Plan
5.2.2.3. The Coloured Diets
5.2.2.4. Monitoring Pellet Colour Preference and Feeding Rates
5.2.2.5. Weighing Procedure
5.2.2.6. Statistical Methods
5.2.3. Results 189
5.2.4. Discussion and Conclusions 196
5.3. Experiment 6 Effect of Moisture Content of Feed Pellets on Feeding Behaviour 200
5.3.1. Introduction 200
5.3.2. Materials and Methods 201
5.3.2.1. The Experimental System and Animals
5.3.2.2. Weighing Procedure
5.3.2.3. The Experimental Diets and Moisture Contents
5.3.2.4. Monitoring the Feeding Activity
5.3.2.5. Statistical Analysis
5.3.3. Results 203
5.3.3.1. Feeding Behaviour
5.3.3.2. Growth Performance
5.3.4. Discussion and Conclusions 205

CHAPTER 6

6 Experiment 7 Influence of Energy Level in Feed on Feeding Behaviour of Rainbow Trout (*Salmo gairdneri*) 209
6.1. Introduction 209
6.2. Materials and Methods 210
6.2.1. The Experimental System and Animals
6.2.2. The Experimental Diets
6.2.3. Weighing Procedure
6.2.4. Monitoring the Feeding Activity
6.2.5. Statistical Methods and Analysis of Growth
6.3. Results 214
6.3.1. Feeding Behaviour
6.3.2. Growth Performance
6.3.3. Food Conversion
6.4. Discussion and Conclusions 219

CHAPTER 7

7 Experiment 8 Effect of Goldthioglucose on Feeding Behaviour of Rainbow Trout *(Salmo gairdneri)* 224

7.1. Introduction 224

7.1.1. Preliminary Experiment for Dose Determination

7.2. Materials and Methods 227

7.2.1. The Experimental System and Animals

7.2.2. Tagging and Weighing

7.2.3. Dose Administration

7.2.4. Histology of Brain

7.2.5. Mortality During Experimentation

7.2.6. Examination of Visceral Organs

7.2.7. Monitoring the Feeding Activity

7.2.8. Statistical Methods and Analysis of Growth

7.3. Results 232

7.3.1. Feeding Behaviour

7.3.2. Growth Performance

7.3.3. Food Conversion

7.3.4. Histology of Brain

7.3.5. Effect of GTG on Visceral Organs and General Outlook of Fish

7.4. Discussion and Conclusions 239

CHAPTER 8

8 General Discussion and Conclusions 245

APPENDIX Supplies AND Statistical Analysis DATA. 255

BIBLIOGRAPHY 256

CHAPTER I

SECTION I INTRODUCTION

1.1. Background

The feeding behaviour of any animal can be studied from an ecological or from a physiological viewpoint. Physiological studies seek to explain feeding in terms of internal negative feedback systems that respond to a variety of hypothesized variables. Ecological studies emphasise the external variables that influence feeding. Whatever feeding activity is exhibited by an organism may depend not only on the internal state of hunger but also on the availability, nature, and distribution of food, and on the degree of predation risk associated with the feeding. Therefore, the physiological and ecological approaches ultimately must be integrated to study feeding behaviour.

Ecologists argue that one function for optimal feeding could be to maximise the rate of net energy gain and/or the rate of net gain of important nutrients. The emphasis on an energetic basis for feeding lies in the requirements for energy, for daily activity, maintenance and reproduction. The energy expenditures must be provided from food. A common basis for a variety of approaches to feeding can be illustrated by emphasising the distinction between regulation and control. Several controls could govern feeding but to be effective they must regulate energy. Different organisms are subject to different limitations on their energy balance as the result of evolution in different environments.

Controls may operate at several levels of organisation. Recent interpretations of physiological controls of feeding stress the role of the liver in response to supplies of metabolic fuels. At another level, sensory information from the environment provides input for control of proximate food choice. At a further level, an organism's ability to adjust behaviour to spatial and temporal distribution of food can contribute to control of energy regulation.

Fish as a group have evolved under highly variable conditions in which efficiency of detection, capture and ingestion of natural foods often limits the level of performance of the physiological processes of digestion, energy metabolism and growth. In the natural environment of the fishes a large number of factors influence the feeding habits of fishes, which when studied under the extremely simplified conditions of laboratory culture, give evidence that these factors substantially influence the conversion of food to fish flesh. Evidence from a number of laboratory studies on fish feeding and growth support the view that the growth efficiencies and food conversion rates vary dramatically due to the variety of food types fed to the fish. These differences are attributed to the differences in foraging efficiency on different food types, rather than to major differences in the physiological conversion of foods (see review by Paloheimo and Dickie, 1966). Studies on the dietary habits of fishes show that fishes differ greatly in the character of the food they consume. The size and systematic position of the food organisms show great variability and the range of foodstuffs consumed by fishes is greater than that for other groups of vertebrates (Marshall, 1966; Nikolsky, 1963; Suyehiro, 1942; Scott and Crossman, 1973). Just as there are great varieties of food consumed by fish, so are there great varieties in the responses exhibited by fish when confronted with a particular type of food.

The environmental characteristics of fish, in which the process of feeding is performed plays a direct role on the feeding behaviour. Most of the predators must first locate their food and, therefore, they must possess specialised receptors and respond in a specific way to different stimuli shown by their prey. Sensory capacities of even similar fishes vary so drastically that the characteristics of food items to which one forager responds may be quite different from those by which another animal recognises food. Fishes locate their food through their sensory systems that process visual, electrical, chemical or mechanical stimuli.

Since a complex set of optical, acoustic, chemical electrical and tactile stimuli may be associated with each potential food item, well-defined responses of fishes are usually a consequence of the summation of a complex of signals received through a combination of sensory pathways. However, for most species particular sensory pathways are more important than others in food search.

Feeding periodicity studies based on diet changes in stomach contents provide ample proof that in many species, food is present only during the daylight hours, thereby implying that many fishes rely predominently on vision in searching for food (Woodhead, 1966). Manipulation of food supply and light conditions, under controlled laboratory conditions can provide interesting information on the feeding behaviour of fish.

Location and identification of a stimulus in space are two basic processes required to produce orientation of predators to prey. Specific stimulus features of food objects such as size, shape, colour, texture and motion can determine the probability of detection and the process of identification by the predators. Upon the detection and identification of a food item, depending upon its specific features the motor responses of the predator determine whether to approach, ignore or to actively avoid a particular object. Every sensory system is adapted to respond to certain kinds of stimuli, thus differential exploitation of foods by fishes will often be an outcome of the process of sensory discrimination, that is, fishes will selectively exploit some food items and will show food preferences if they react differently to emitted stimuli. To understand such differences and preferences and to gain further insight into the problems fish face during food acquisition, it would be interesting to plan laboratory experimentation under controlled conditions.

1.2. The Aims of the Research

In the preceeding section of this chapter some of the physiological and ecological factors, which can effect the feeding behaviour have been discussed. The physiological systems of the predator which help in locating and identifying prey by their morphological characters have been outlined. For the present research work, it was decided to experiment upon an intensively cultured salmonid, rainbow trout, *Salmo gairdneri* Richardson. Rainbow trout belongs to a most "sensitive" group of fishes and, therefore, have some of the most demanding environmental requirements for normal growth. Nonetheless, the recycling system (See Chapter 3) used for the experimental work, met nearly all the environmental requirements, except of course, for those which were controlled for experimental purpose.

It was planned to study the controlled feeding behaviour of rainbow trout as there is very little work done on fishes in this field. It was decided to study the feeding response of trout under different photoperiods at constant temperature, since apart from hunger, visual stimuli are apparently of prime importance in the feeding response of this species of fish, although other stimuli such as olfaction and taste may also be concerned (Bardach and Todd, 1970). For behavioural study, great discretion has to be exercised by the experimenter to avoid confounding the variables and to avoid introducing serious uncontrolled or undocumented interfering responses involving activity levels. Thus to avoid any discrepency in the feeding activity of fish, it was decided to use demand feeders, thereby giving every opportunity to the trained fish to perform the feeding activity whenever they desire to do so, and to obtain, whatever quantity of food they wished to consume. Also in the planning of design and construction of the experimental facility, every care was taken to avoid introduction of any serious uncontrolled or undocumented interfering element.

Some experiments were planned to study feeding behaviour when the specific stimulus features of food objects such as size, texture and colour were varied. Also, an attempt was made to locate the feeding centre in the brain of fish and also to see whether rainbow trout can regulate its food intake, when the caloric contents of the diets are varied. Consequently, the following experimental work was planned and undertaken.

In Experiment 1 (Chapter 4), work was undertaken to study the feeding response under nine different photoperiod regimes, with constant light intensity. In fish, it has been demonstrated that the photoperiod has pronounced effects on seasonal and diurnal processes, but there is little data available at present on its effects on the daily feeding pattern. Studies on the diurnal activity pattern of fish, though not necessarily an indication of feeding activity, have shown that under natural conditions many species exhibit a crepuscular rhythm (Alabaster and Robertson, 1961; Young, Tytler, Holliday and MacFarlane, 1972). Hence, it was considered that investigation of the feeding behaviour under different photoperiod regimes would add to the knowledge of understanding the feeding rhythms of rainbow trout.

In Experiment 2 (Chapter 4) the study of feeding behaviour was continued under three different photoperiod regimes but with different light intensities. The selection of these three photoperiod regimes for further study was based upon the results of Experiment 1.

In Experiment 3 (Chapter 4) light was further manipulated by varying day length in order to study the feeding responses of trout. One group of fish was subjected to effects of the increasing daylength and a second group of fish was subjected to effects of decreasing daylength. This study was undertaken to determine the effect of simulated decreasing daylength and increasing daylength, on the feeding activity of trout and the consequent effect on growth and food utilisation was studied.

For Experiment 4 (Chapter 5) the physical property of the food was varied. Food particle size was varied from small to large although the chemical nature of taste property of the food was kept the same. Food particle size preference was investigated. Such food particle size preference experiments could yield interesting results on feeding behaviour of rainbow trout, since the innate capacity of trout, to capture and ingest prey of particular size could be reflected in the behaviour of fish in responding to make its selection of the food particle size. With demand feeders such selection could be easily quantified and an idea of the fish's preference obtained.

Experiment 5 (Chapter 5) was planned keeping in mind the results of experiment 4 on food particle size selection preference. For predatory fish visual cues can be very important in locating and identification of its prey. As previously mentioned rainbow trout is accepted to be primarily a sight feeder and eats a very wide variety of food (Adron, Grant and Cowey, 1973). Colour and contrast differences between otherwise identical food items can frequently alter their probability of consumption by fishes. In order to investigate such probabilities a series of experiments on coloured food pellets were designed and their modulating effect on feeding behaviour and food intake was studied. Colour preference, if any, depicted by rainbow trout can help in standardising a particular colour for preparing the commercial diets for trout hatcheries. Although at present, such commercial diets are usually manufactured in a light or dark tan. Again, demand feeders were considered to be best suited to study the discriminating abilities of rainbow trout in making their choice for a particular coloured diet.

In Experiment 6 (Chapter 5), the effect of texture of feed pellets, on feeding behaviour was studied. The texture of food can have a great bearing upon the amount of food that can be consumed by fish. With a view to investigate if, moisture content of the diet can modulate the feeding behaviour, an experiment was planned in which the level of moisture in the diets were varied significantly.

Fish were given an element of choice between two suitable alternatives of 10% moisture and 25% moisture in the feed pellets. The choice was quantified once more by the use of demand feeders. Such an experimental study can be of great value in determining the optimum moisture level in the diet, keeping in perspective, the difficulties posed in storing such a diet over a long period of time, for use in large commercial trout hatcheries.

Experiment 7 (Chapter 6) was planned to investigate the feeding behaviour of trout, when caloric density of food was varied. Calorie hunger, or what is usually called just plain hunger, has a rather anomalous position as a specific hunger. There is an elaborate machinery, only partly understood, to detect energy imbalance in mammals, and there is evidence that similar control systems are at work in other groups, such as fish (Rozin and Mayer, 1961a, 1964). By varying the energy contents of the experimental diets, it was planned to see if rainbow trout would regulate its food intake in terms of the bulk of food being consumed or whether the food intake is regulated by the calorific value of the diet. Since, the evidence concerning the regulation of food intake in fishes is sparse, the present experiment could be expected to yeild some valuable data.

Experiment 8 was designed to locate the feeding centre in the brain. Extensive work in mammals has demonstrated that the feeding centre is located in the hypothalamus of the brain (Rabin, 1972; Debons, Krimsky, From and Cloutier, 1970; Mayer, 1965). Since very little work has been done on fishes, it was planned to make an attempt, to probe the brain of rainbow trout by intraperitoneal injections of Goldthioglucose (GTG). Just as in rats, lesions bilaterally in the ventromedial nucleus of the hypothalamus, cause hyperphagia leading to obesity, an attempt was made in rainbow trout, to produce similar effects by intraperitoneal GTG injection. Demand feeders were used for feeding, so that any hyperphagic feeding could be monitored and quantified, and the fish could feed *ad libitum*.

CHAPTER 2

SECTION 2 REVIEW & HISTORY

2.1. Learning in Fish

Many animals can be conditioned to perform simple tasks and in return get a reward, such as a supply of certain amount of food, or to avoid an unpleasant experience. Mostly birds and mammals have been involved in such studies. It has been demonstrated, by one of the earliest experiments on fish learning, by Triplett (1901), that when perch and some minnows were kept in the same aquarium, separated by a glass partition, after numerous collisions with the glass, the perch refrained from attacking the smaller fish even when the partition was removed, so that the perch learned not to chase the minnows. Many examples of learning in fish have been cited from incidental observations in studies not explicitly devoted to learning. Noble and Curtis (1939) gave some indications of the many roles which learning may play, and the importance of learning in the normal behaviour of the jewel fish, *Hemichromis bimaculatus*. They demonstrated that learning played an important role in recognition of the male by the female, of eggs, of home territory, of young and of parents by the young. They found suggestions of an involvement of learning in virtually every major function they studied.

One example of most impressive behavioural accomplishment of any fish, well studied and extensively cited in the literature, is the migration of salmon. Harden-Jones (1968) reported that several species of salmon migrate from their home stream to the open ocean when one to a few years of age, and then return to their home stream after a period of years. The return of the fish to their home stream after several years has been suggested to be linked with the process of learning in fish.

Most of the earlier experiments were directed towards the study of fish orientation and locomotor responses. More recently the area of the study has been enlarged and manipulative responses such as lever pressing have commanded more attention. A number of instrumental devices have been developed to study the fish behaviour. An aquatic analog of the "Skinner box" (widely used in the study of rats and pigeons), in which the fish darts at a visual target attached to a lever and thus gains a food reward, has been used by Longo and Bitterman (1959) in the study of learning by goldfish. Rozin and Mayer (1961b) used operant-conditioning techniques that permitted goldfish to control water temperature by pressing against a lever. When placed in a hot tank, goldfish responded for squirts of cold water which transiently lowered the temperature in the tank by about 0.5°C. In other studies by Rozin and Mayer (1961a, 1964) the daily food intake patterns and amount of food intake of goldfish, *Carassius auratus*, were determined by the number of lever presses made by the fish. Van Sommers (1962) also working on goldfish, was able to condition the fish allowing it to control oxygen content of the water by interrupting a photocell light beam. Goldfish learned to break the light beam in presence of a red light in order to get oxygenated water and to avoid breaking the same beam in presence of a green light to get the same reward. Frank and Meyer (1974) used a partitioned shuttlebox monitored by a single photocell to study activity and thermoregulatory behaviour of rainbow trout, *Salmo gairdneri*.

Neill, Magnuson and Chipman (1972) described a two-chamber electronic shuttlebox device with a pair of photocells monitoring the movements of a fish and permitting it to control the temperatures in the two chambers, and thereby to control its own body temperature by shuttling back and forth. A small difference between the temperatures of the two chambers (about 1-2°C) presents an instantaneous spatial gradient to guide the movements of the fish and thereby minimises the necessity of learning, in addition to the potential temporal gradient generated by the movements of the fish through the photocells. However, even with no temperature difference between chambers, according to Neill et al. (1972), fish will learn to control their body temperature in a purely temporal gradient.

It has been shown by Abbot (1972), that rainbow trout, *Salmo gairdneri* could be conditioned to respond to a sound signal operated just prior to feeding to set up an association response, and that fish so conditioned would be recalled from a relatively free swimming environment by a sound signal in anticipation of food. Fish were conditioned to respond to a sound signal of 150-Hz, transmitted underwater in a 0.25 acre pond with 13,000 rainbow trout. Abbot observed that about 90% of the rainbow trout roaming freely in the pond were conditioned to come to feed at the source of an underwater acoustic stimulus. The sound signal was broadcast continuously for one minute before and throughout feeding.

Adron (1972) developed a design for automatic and demand feeders for laboratory experimentation on fish. The feeders could be used to dispense food at regular intervals or could be used to function, as and when, fish desired food. The fish had to be trained to actuate the submerged tip of a trigger to obtain the food. Such demand feeders can be used for studying different behavioural responses of the fish concerned with feeding habits.

Automated and Demand Feeding Compared with Hand Feeding

In the poultry and livestock farms much progress has been made in increased production by using the techniques of *ad libitum* feeding or by increasing the feeding frequencies. In aquaculture, work on improving the feeding techniques has been going on for many years, and it has been shown that the feeding regime has profound effects on growth. A promising method of reducing feed expense is to increase feed use efficiency by feeding fish more than once a day. Deyoe, Tiemier and Suppes (1968) reported that pond reared channel catfish *Ictalurus punctatus*, fed half their ration in the morning and half in the afternoon, used feed more efficiently than did fish, receiving one feeding daily. Similarly, channel catfish reared in cages (Collins, 1971) and those reared in tanks (Andrews and Page, 1975) fed by hand twice daily grew more rapidly than those, fed only once. Andrews and Page (1975) made a study to compare automated feeding at 110 to 120% of satiation 4, 8 and 24 times daily, with hand feeding to satiation once, twice and four times daily. The concluded increased feedings from 2 to 4, 8 or 24 times per day did not result in improved feed use. Hastings (1970) tested demand feeders to assess growth and food consumption by blue and channel catfish in one pond (0.25 acre) and compared it with hand feeding by same number of fish in another pond of same area. He found that blue catfish growth was not significantly changed but channel catfish grew better when fed by the demand feeder.

Sumari and Westman (1972) tested an automatic self-feeder on yearling rainbow trout, *Salmo gairdneri* and made a comparison with hand feeding. In a 146-day experiment using dry feed pellets, the gain in weight was 469.4% for the fish fed with the self-feeder and 435.1% for the fish fed by hand three times daily. They found no marked difference between the two fish groups in the weight gains, weight frequency distribution, mortality, coefficient of condition, or the haematocrit value.

Greenland and Gill (1979) used automated feeding equipment to assess growth and feed conversion rates of channel catfish, *Ictalurus punctatus*. Fish were fed at different feeding frequencies (once, twice or four times in 24 hours), the feeding level being 2% of body weight. Their result showed that increasing the number of automated daily feedings from one to t to four resulted in progressively improved food conversion rates and higher net yields for tank-reared channel catfish.

Recently, Mortensen and Vahl (1979), have described a control system with circuit diagram, for improved automatic feeders. The system can control the amount of rations fed and the length of the feeding periods independently in each of six simultaneous experiments.

The demand feeding system developed by Adron (1972) was used in a later study by Adron _et al._ (1973) to make a quantitative study of the learning capacity and food preferences of rainbow trout, _Salmo gairdneri_. They showed that conditioned populations of trout, when presented with a choice, were able to discriminate between a trigger that supplied food and one that did not. Taste or odour preferences in the diet of trout were studied by comparing the rate of consumption of a standard diet with that of various test diets. They found that fish could discriminate on grounds of taste alone and demonstrated that the demand feeding system could be used to examine taste preferences of the fish. They used the same system to demonstrate that behavioural study pertaining to colours, could also be performed by using differently coloured tips of the triggers, which upon being actuated by fish, resulted in the dispensing of food.

Landless (1976) used the same design, developed by Adron (1972) with the aim of elucidating the behavioural mechanisms involved in demand feeding by rainbow trout in sea-water. Demand feeding by a single fish took seven days of training period, whereas in groups it took two days. Larger automatic feeder and triggers have been used in floating sea cages by Landless. Here trigger actuations in these larger groups of fish (up to 1000 fish) have been suggested to be performed by more than one fish, although direct observations are impossible to note in floating sea cages. Adron and Mackie (1978), by using demand feeders were able to show a marked preference by rainbow trout, for a diet flavoured with a synthetic mixture of chemicals over the unflavoured diet.

2.2. Effect of Light on Some Aspects of Fish Life

2.2.1. Locomotor and Feeding Activity

Most workers have directed their study of light upon the locomotor acivity of fish. Various such activity patterns, subject to phase setting primarily by natural or artificial light cycles have been documented in several fishes.

The synchronising effect of light cycles on the locomotor activity patterns of salmonids in particular has been cited in several instances (Ali, 1964; Swift, 1964; Byrne, 1968; Varanelli and McCleave, 1974).

Cyclic pattern in daily activity in response to changes in environmental factors are shown by fish in fresh water as well as in the ocean. Most species are more active at certain times of the day than at others. Thus fish can be classified into diurnally active species, moving around and performing their daily activities, and relying on their visual acuity, and nocturnal species which rely mostly on their tactile, electrical or chemical senses. In a study by Hasler and Villemonte (1953) on freshwater perch, light intensity has been related to the periodic movements of these fish. Barlow (1958) related the periodic movements of desert pupfish, *Cyprinodon macularis* to temperature. Blaxter (1965) in a review work has related the time of day and light intensity to the feeding activity of herring larvae. Some earlier studies (Sushkino, 1939; Oliphan, 1951) also relate the daily activity pattern of some freshwater species to the daylight conditions. Schooling behaviour of *Hepsitia stipes* and *Bathystoma rimator* showed a daily pattern in the study by Steven (1959). He noted that schools of these fish species dispersed at night and that these showed an activity increase with decreasing illumination. Hunter (1966) kept groups of six fish in constant illumination and observed that these schools became more compact at a time which corresponded to the first hours of darkness of the preceding light regimen. Hoar (1942) studied the effect of light on salmonids under controlled conditions and showed variations in the pattern of feeding and locomotor activity being brought about by the diurnal changes. A pronounced daily rhythm in feeding activity of goldfish under controlled conditions and showed variations in the pattern of feeding and locomotor activity being brought about by the diurnal changes. A pronounced daily rhythm in feeding activity of goldfish was reported by Hirata and Kobayashi (1956) and Hirata (1957). Spencer (1939) listed several species of freshwater fish as diurnally active others as nocturnal. Carp and many other species were showed to be probably arhythmic. Swift (1962, 1964) obtained good records on the diurnal and annual activity pattern of brown trout, *Salmo trutta* L., when the fish were confined in cages in their natural stream. Jones (1956) in a laboratory study on locomotor activity of *Phoxinus sp.* reported that these fish were active during daylight hours but that this pattern reversed if their tank contained a hollow brick, where they could hide from bright light. When cover was provided, the fish were very active around sunrise and sunset. Jones found no evidence for an inherent daily rhythm of locomotor activity under his experimental conditions. Ryder (1977) studied the relationship between ambient surface light intensities and feeding rates of walleye, *Stizostedion vitreum vitreum*. He observed that the feeding rates of walleyes appeared to be related to surface illuminance levels, the greatest feeding activity occurring at the lower illuminations. His results suggested that the diel and annual light regimes govern much of walleye metabolic activity, particularly that of diurnal feeding and annual reproductive behaviour of the adult. Extensive research into feeding periodicities based on diel changes in stomach contents (Woodhead, 1966) confirms that in many species food is present only during the daylight hours, thus implying that many fishes rely predominantly on light, in searching for food, through their visual sense. Laboratory experiments, involving controlled manipulation of food supply and light conditions (Ali, 1959; Blaxter, 1970) have established the essential role of visual cues in successful food location. With some notable exceptions, according to Blaxter (1970), a light intensity of 10^{-1}mc (meter-candle), corresponding to late dusk, is the lower threshold for effective visual location of food by fishes.

Ali (1964) studied the diurnal rhythms of locomotor and feeding activities and oxygen consumption in Atlantic Salmon, *Salmo salar* in relation to light as an exogenous factor. He experimented under different photoperiods. In conclusion he suggested that under controlled conditions a circadian rhythm in oxygen consumption, activity and feeding is brought about as a result of an interplay between the endogenous factors and the natural conditions to which the fish is adjusted. When these natural conditions were experimentally altered the endogenous factors become dominant and when the experimentally produced light durations became aligned with these factors a twelve-hour rhythm was brought about. He summarises that light plays only a minor role in the production of this rhythm in the juvenile Atlantic salmon.

Jenkins (1969b) conducted an elegant series of feeding experiments with brown trout and rainbow trout to determine their degree of predation on terrestrial invertebrates during daylight and at night. Groups of five trout were released into screened 10-m sections of Convict Creek, California. Marked adult ants were then released into the sections through tubes. After fifteen minutes had elapsed the fish were collected and their stomach contents examined. During daytime trials brown trout ate an average of fifty-two percent of the ants drifting by. Rainbow trout intercepted an average of seventy-five percent of the introduced ants. During night trials, feeding by both species was less efficient: nine percent average predation by brown trout and five percent average predation by rainbow trout. Pinhorn and Andrews (1963) studied the reaction of Atlantic salmon under yearlings to various light intensities. They found that salmon were photonegative in light intensities from 0.186 to 18.6 lux and photopositive only in very low intensities from 0.0093 to 0.0186 lux. They observed that the control fish showed very little difference in the intensity of their reactions to intermittent light stimuli in the range 0.1-20.0 ft-c. At 200.0 ft-c there was a marked difference in their response. The light-exposed fish showed no difference in their reactions among any of the light intensities, but, however they observed an apparent trend towards a more intense negative reaction at successively higher light intensities for both control and light-exposed fish over the whole range of intensities (0.1-200.0 ft-c) studied. They concluded that the exposure to photoperiods caused the fish to become more active under the conditions of the experiments and more sensitive to stimuli of all kinds. This caused the reaction of the light-exposed fish to a flashing light stimulus of short duration to be significantly altered so that their negative reaction was less intense and less affected by the absolute light intensity than that of the control fish. Similarly, the behaviour of the two types of fish under continuous light was very different, with the controls again showing the greater negative reaction.

In a similar study by Gibson and Keenleyside (1966) it was observed that salmon showed a positive phototaxis in intensities up to 14.86 lux, and photonegativity only in illuminations greater than 14.86 lux. According to Manteifel, Girsa and Pavlov (1978), the seasonal influences of many abiotic environmental factors occur over prolonged periods and affect metabolic processes resulting in profound physiological changes in the organism. The seasonal changes of the physiological state in fishes are based on the complex influence of rhythmically repeated light and dark periods (of different time) and also of defined temperature. Fish life depends on the manifestation of characteristic activity at the appropriate season of the year. Manteifel et al. studied the response to light in photogradient conditions in an experiment on *Leucaspius delineatus* (Heck). They held two groups of fish at differing photoperiods (17L : 7D, 7L : 17D) each twenty-four hours for one month at the same temperature (17-18^{o}C). Fish experiencing short-days avoided the illuminated zone and were actively concentrated in the darkest part of the photogradient. The long-day fishes showed positive responses to the illuminated zone. Manteifel et al. also held two other groups of fish under continuous light (700 lux), but at lower temperature (temperature not given) and observed that the fish behaved like those held under the short-day conditions. The behavioural reponse of the fish to the short-day or to the low temperature conditions were considered as adaptation to the winter season. They concluded that, light could be treated as a signal of the definitive complex of ecological conditions, favourable for the summer season and unfavourable for fish in the winter period.

Bisson (1978) studied the feeding behaviour of two sizes (2.82g and 45.40g) of rainbow trout, *Salmo gairdneri* in an experimental stream over a period of twenty-eight hours. He compared the availability of different prey taxa with the composition and size distribution of food items in fish stomachs of both the sizes. His observations suggested that both sizes of trout were almost exclusively drift feeders during midday hours (0800 - 1600). Bottom foraging predominated at dawn, although a few individuals fed on both, benthos and drift. At dusk he observed no consistency among preferred feeding modes. During the darkness hours (2000-0400) the fish appeared to feed exclusively off the bottom. A few large sized trout were not observed to feed upon anything during the midnight hours (2400-0400). In general, he concluded, that the drift feeding was associated with periods of highest illumination and food abundance, whereas, bottom foraging occurred at low levels of light and drift density.

Jenkins (1969a) in the study of feeding activity of rainbow trout, postulated that surface feeding was partially dependent on hierarchial behaviour mechanisms. In his study the feeding positions of trout in the stream were consistently located along the principal threads of surface currents, and position choice of trout along these current threads was primarily determined by fish size. Larger fish in local aggregations, within a pool for example, consistently occupied the most-upstream, most-likely points of entry of drifting food. Similar hierarchial effects were observed in trout hatcheries by Brown (1957). She observed different rates of growth of fry in a population, with the larger fish growing more rapidly than the smaller ones, all the fish having started at about the same size. Brown hypothesized that the size hierarchy effect was related to the dominant-subordinate relationships observed to occur among salmon fry, the dominant fish being those that grow fastest, the small subordinate fish were stated to be under stress. Later, Fenderson, Evenhart and Muth (1968) found that, when food supply was limited in quantity, a social hierarchy quickly developed within a small group of Atlantic salmon, with the dominant fish having the greatest food intake. When the situation was changed by removal of the dominant, the food consumption by the previously subordinate animals would increase. Symons (1968) also reported that restricted food supply caused strengthening of the social hierarchy within a small group of Atlantic salmon fry. Slaney and Northcote (1974) found a similar relationship between food availability and level of aggression in the rainbow trout. Feeding behaviour has been showed to be effected by the social facilitation found in striped mullet, *Mugil cephalus* in a study by Olla and Samet (1974). They found that isolated animals, able to periodically view a non-feeding group of their own species took a relatively long time to initiate feeding, whereas isolates that viewed a feeding group initiated feeding very quickly, and isolates that did not view another group took a time intermediate between the other two.

In a study by Swift (1964) the diurnal and annual locomotory activity cycles of four brown trout *Salmo trutta L.*, confined in separate cages on the bed of Windermere Lake and fed artificially from the lake surface, have been described. The diurnal activity patterns for all four fish were found to be very similar throughout the year. The most obvious feature of the diurnal rhythm was the marked rise in activity which occurred at dawn. The increased activity was maintained throughout the hours of daylight and fell at dusk each day. The changes which occurred in the level of activity at different times of the year were caused by a change in the level of activity maintained by the fish during the hours of daylight and also by the length of time for which that activity was maintained. Swift observed seasonal changes occurring in the level of activity at night, but only to a much smaller extent. Swift could find no obvious correlation between the seasonal variations in locomotary activity of the fish, the water temperature or the amount of solar radiation incident on the water surface. Previous workers (Hirata, 1957; Hirata and Kobayashi, 1956; Oliphan, 1951; Sushkino, 1939) associated similar activity cycles in other species of fish, with a feeding cycle. But Swift (1964) showed that, brown trout, which were fed once a day were as active at dawn, as the fish which had been receiving food every two hours and therefore did not associate the diurnal activity of brown trout with the feeding.

In case of mammals and birds rhythmic activities have been known to free-run in constant conditions for months at a time (Aschoff, 1960, 1966), the rhythms termed "endogenous" in various fish studies have usually not been apparent for more than a few days. Activity rhythms have persisted for two or three days in constant conditions in juvenile Atlantic herring, *Clupea harengus* (Stickney, 1972), juvenile sockeye salmon, *Oncorhynchus nerka* (Byrne, 1968), and juvenile Atlantic salmon, *Salmo salar* (Ali, 1964). An exceptional case is the circadian rhythm of the swell shark, *Cephaloscyllium ventriosum*, which continued for about fifteen days in both constant light and constant darkness (Nelson and Johnson, 1970). The same authors did not observe free-running rhythmicity in the horn shark, *Heterodontus francisci*. Circadian rhythms of swimming speed in the bluefish, *Pomatomus saltatrix*, have been reported to dissipate after two or three days in constant dim light, but later to become re-established (Olla and Studholme, 1972). A similar re-establishment of rhythmicity has been suggested for Atlantic salmon (Ali, 1964).

Gibson (1971) found that exposure to light-dark cycles was necessary for as long as two to four months before circadian rhythms which would persist in constant darkness could be entrained in blennies, (*Blennius pholis*). Activity rhythms with a twelve hour period were easily entrained in the same fish by changes in hydrostatic pressure resulting from local tidal cycles. A circadian rhythm of electric organ discharge has been observed to free-run in constant dim light in the electric gymnotid, *Gymnorhamphicthys hypostomous*, (Lissman and Schwassmann, 1965). The appearance of the corresponding locomotor activity rhythm in these fish seems dependent upon the presence of a light-dark cycle and the natural environmental substrate. It was concluded, that the activity rhythm is not a good indicator of the endogenous oscillation, by Schwassmann (1971).

2.2.2. Reproduction and Maturation Process

It has been demonstrated experimentally that the intensity and duration of light has an effect on the maturation processes of fish. By exposing various species of fish to increasing amounts of daily light, decreasing amounts of daily light and continuous light, induced spawning several months before their normal season has been brought about, and this indicates an accelerated rate of maturation (Hoover, 1937; Hoover and Hubbard, 1937; Harrington, 1950, 1956; Medlen, 1951; Corson, 1955). However, upon exposing other species of fish to similar varying conditions of light, it was found that the spawning date was delayed, indicating a decelerated rate of maturation (Allison, 1951; Hazard and Eddy, 1951). From other experiments it was concluded that darkness or small amounts of light greatly depressed maturity and spawning in fish (Bullough, 1940; Rasquin and Rosenbloom, 1954). Rasquin and Rosenbloom (1954) reared a river-dwelling characin, *Astyanax mexicanus* (a near relative of the blind cave fish), in total darkness and observed that spermatogenesis was halted in some, while in others, it was never initiated. This was an indication of the effect of total darkness on spawning activity. These authors found that the dark reared fish were shorter and deeper bodied than those reared in light. They believed that these changes in body form were caused by a loss of calcium since there was a distortion of the vertebral column of the fish. In a few cases light has been found to have no effect on the maturation of fish (Burger, 1939; Mat-hews, 1939; Merriman and Schedl, 1941), while in some species temperature seemed to be more important than light in this respect (Bullough, 1939; Burger, 1939). Burger (1939) found that alteration of light cycles did not change the spawning date of *Fundulus*. He did find, however, that fish receiving no more than 1.5 hours of light per day, and kept at temperature of 6 to 10°C between February and March, remained sexually inactive. Fish receiving similar light treatment but held at a temperature of 14 to 20°C formed large amounts of sperm in the same length of time.

Mathews (1939), also working with *Fundulus*, found that the presence of light was not essential for the complete activation of the testes. No difference was observed between fish that were held in complete darkness, normal daylight conditions, or in additional illumination at night. It is obvious that not all fish species respond to changes in photoperiods in a similar manner.

Many workers have discussed the role of light on the maturation of gonads of fish. In many fish the actual process of spawning, involving release and fertilization of ova, is limited to a relatively brief time span, however, gonadal development is a complicated, long physiological process. A wide variety of breeding behaviour is exhibited by animals at the time of gonadal maturation. In many instances, extensive migrations to special breeding grounds take place preparatory to spawning. Many studies correlate such reproductive behaviour with the annual cycle of seasonal changes. One of the earliest studies was of Rowan (1926), who demonstrated the role played by day-length and temperature in the timing of reproductive rhythms in animals such as birds and fishes. Since then, a large amount of experimental work has been performed, to demonstrate the photoperiodic control of annual cycles. Hoover (1937) used the method of manipulation of day length and was able to advance the natural spawning season, by about four months in case of brook trout, *Salvelinus fontinalis*. Under normal conditions like other salmonids, brook trout spawn from October to December, when the days are shortening. By first increasing and later decreasing the light duration much in advance of the natural photofraction change, the spawning season could be advanced by about four months to August. Hazard and Eddy (1951) were also able to demonstrate similar effects of decreasing the day length by one hour each week for nine weeks in August - September, which resulted in advancement of about one month in spawning time. Combs, Burrows and Bigej (1959) were able to delay the spawning time of *Oncorhynchus nerka* by lengthening the light duration and to advance it by shortening the light duration. Hoar (1953) assumed that the changing photoperiod, by acting on the pituitary, would initiate the transformation to the smolt stage in salmon and that a similar mechanism would bring about prespawning and spawning behaviour in adult salmon.

Henderson (1963) conducted a thorough study on the effects of exogenous factors on the reproductive cycle of brook trout, *Salvelinus fontinalis*, and concluded that daylength plays the predominant role in regulating gametogenesis, whereas temperature plays only a minor or supplementary role. The effect of photoperiod, depended on the phase of gametogenesis in progress at the beginning of the experiment and varies with season. Initiation of gonadal development was apparently independent of environmental factors but short photoperiods during late winter and spring or an accelerated light regime could hasten the initiation of gonadal activity. Once the secondary growth phase was initiated, the rate of ovarian development was retarded by either continuous long or continuous short photoperiods. When Henderson subjected the brook trout to long photoperiods during late winter, spring and early summer, and subsequently exposed to short photoperiods, the growth rate of the ova was accelerated. The stimulating effect of short photoperiods on the terminal part of the growth phase only **occurred** if fish were exposed to long photoperiods during the early stages of the secondary growth phase. Her results suggest that the normal growth of ova in *Salvelinus fontinalis* is dependent upon exposure to long photoperiods followed by short ones. Henderson indicated that only the terminal portion of spermatogenesis can be accelerated or retarded following exposure to photoperiods. She further stated that the maturation cycle of the male germ cells appears to be relatively stable.

Whitehead *et al.* (1978) studied the effects of alterations in photoperiod on ovarian development and spawning in rainbow trout, *Salmo gairdneri*. They indicate that photoperiod acts as a major environmental factor that control the sequence of endocrine and other physiological changes, which ultimately leads to spawning in salmonids. They suggest that rainbow trout may have an endogenous rhythm of reproductive function, which is modified under the influence of photoperiod.

In a study by Hubbs and Strawn (1957) the effect of the duration of the light period on the reproductive rate in a darter fish, *Etheostoma lepidum*, was shown to be unimportant. Whereas, temperature and the condition of the fish were stated to be effecting the reproductive rate. Similarly, Ahsan (1966) on a study of the spermatogenetic cycle of the lake chub, *Conesius plumbeus*, found that temperature was a main factor controlling spermatogenesis, whereas, daylength appeared to be effective only at low temperatures during the later part of the cycle, and an endogenous rhythm seemed partially responsible for the timing of testicular changes. Turner (1957) transported a poecilid fish, *Jenynsia lineata*, to northern hemisphere from their natural habitat in Southern South America, where they spawn during spring and summer, that is October to January - February. After being transported to the northern hemisphere in September, already gravid females initially produced broods at a time corresponding to the summer of their native habitat. Under the influence of a light-dark cycle, shifted by 180° from that in the southern hemisphere, they went through another breeding period corresponding to the northern summer, presumably stimulated by the increasing day length.

In some fishes the time of egg laying is also affected by the light, as shown in a study by Egami (1954), on medaka fish, *Oryzias latipes*, which shows a daily rhythm of oviposition, in which female lays eggs daily just before dawn. Egami correlated the timing of oviposition to the light-dark cycle. He artificially changed the light time to dark and vice versa. It was demonstrated that fish started egg laying according to the new time of artificial dawn, completely out of phase with the egg-laying time prior to the change. In continuous illumination, the spawning rhythm became irregular. In a similar study by Marshall (1967), it was reported that in two fish species *Trichopsis vittatus* and *T. pumilis*, egg laying occurred during last three to four hours of light under a cycle of natural or artificial illumination. When the light cycle was shifted by twelve hours, the fish almost immediately shifted their spawning to the end of the new light phase. When subjected to continuous light spawning occurred less frequently and was not confined to specific times.

2.2.3. Effect of Light on Early Life of Fish

Light plays its role, in several ways, to influence the life of fish, not only at the adult stage, but right from the time of egg laying and early development. Most of the published papers have related the development of fish eggs to water temperature and to dissolved oxygen levels (Garside, 1959), and the influence of light has received only limited attention.

MacCrimmon and Kwain (1969) found that initial mortality of newly fertilised rainbow trout eggs incubated in artificial light increased with intensity whereas, mortality among eyed eggs exposed to the same set of conditions was found to be comparatively low, regardless of intensity. Mortality, time to hatch, metabolic rate, and the number of vertebrae formed correlated positively with visible light intensity but only during the pre-eyed stage of incubation. During the post-eyed incubation period, the numbers of dorsal and anal fin rays were affected by light intensity, the greatest number of rays on these fins occurred in fish incubated at light intensity of 10 lux. The numbers of pectoral and pelvic fin rays were constant at all test light intensities.

Kwain and McCauley (1978), studied the effects of age and overhead illumination on temperatures preferred by underyearling rainbow trout, *Salmo gairdneri*, in a vertical temperature gradient. In their experiment, illumination from above provided by 500w or 15w incandescent bulbs produced light intensities of 2200-1650 lux and 220-22 lux, respectively, from the surface to the bottom of the tank. They observed a definite affinity displayed by fish from the upper part of the tank during the first four months of life under 220 and 2200 lux. Fish under illumination during the remaining eight months observed to be favouring the tank bottom in contrast to fish in total darkness, which were observed to be divided evenly in their orientation to both upper and lower surfaces. They concluded that fish in light gradients during the remaining eight months were photonegative in relation to overhead light. In an earlier study MacCrimmon and Kwain (1966) also found that yearling of rainbow trout, exhibited a negative phototaxis, when given the choice of light or dark areas in a test tank under various light intensities. This contrasted with the positive response displayed by fingerling trout under a wide range of light intensities. They subjected the fingerling and yearling rainbow trout to illumination provided by incandescent daylight bulbs at a series of test intensities of total darkness, 10^{-3} ft-c, 1.0ft-c & 10x2ft-c.A quadrant of each experimental tank was provided with a pie-shaped cover. They found that fingerling trout showed no preference for either the covered or open areas of the tank, being randomly distributed at all of the test light intensities. The yearling trout, demonstrated a significant preference for the covered portion of the tank under all test light intensities except when randomly distributed in total darkness. The reaction of the yearling trout varied with the light intensity when first put in the tank, the movement to beneath the cover being more immediate at the higher, rather than the lower intensity. Also, their subsequent movement between the covered and open areas of the tank was observed to be more pronounced at the higher light intensities. A similar light intensity - age response has been recorded by Andrews (1946) for the common sucker, *Catostomus commersoni*.

2.2.4. Growth

Kwain (1975) studied combined effect of light intensity and temperature on early growth and meristic variation in rainbow trout, *Salmo gairdneri*. He studied the effect of two temperatures (3 and 10°C) and three light intensities (0.2, 2, 20 lux) on growth to 145 days. According to his results growth rate of rainbow trout after feeding varied significantly at test temperatures and light intensities. Percent increase per day of initial measurements (15 days after hatch) between total length and weight was different, the higher temperature always coincided with the higher growth rate. For either temperature, the effect of the three light intensities (20, 2 and 0.2 lux) on growth rate was significant. At low light intensity fish grew slower than those at higher light intensities. Similarly, rainbow trout reared at the lowest light intensity (0.2 lux) gained less length and weight than those at the higher light intensities (20 and 2 lux), regardless of the water temperature. However, he detected no differences in fish reared at 2 and 20 lux. He associated the slow growth at 0.2 lux with the fishs' inability to see clearly in such low light intensity, observing the fish to be apprarently sluggish and not actively looking for food, while those fish at higher light intensities actively sought out food. He concluded that temperature was the main factor controlling the rate of development but light exerted some affect but to a lesser degree.

Stickney and Andrews (1971) studied the influence of photoperiod on growth and food conversion of channel catfish, *Ictalurus punctatus*. They subjected two sizes of channel catfish fingerlings to five different photoperiods and observed a slight tendency for improved growth under a twelve hour photoperiod. In other photoperiods (0, 6, 18 and 24 hour light), they did not notice any substantial influence on growth rate or food efficiency. Gross, Roelofs and Fromm (1965) made a study to determine, if fish growth was influenced by daylength at constant temperature and they also made a comparison of the effect of constant and varying daylengths on growth rate and food conversion efficiency in green sunfish, *Lepomis cyanellus*. They found that fish grew better in sixteen hours than in eight hour constant daylength, although statistically it was marginally significant ($P = 0.10$). However, greater differences in growth were noted between increasing and decreasing daylength, since fish exposed to increasing daylength gained 2.5 times as much weight as those exposed to decreasing daylength, suggesting a stimulative effect of increasing and an inhibitive effect of decreasing daylength. They observed that fish in longer daylengths consumed more food and showed a higher efficiency of food conversion than the fish in shorter daylengths. Higher growth rate in fish exposed to increasing daylength has been attributed to an indirect effect of the photoperiod upon the regulation of metabolic activity by the endocrine system.

Eisler (1957) reared four groups of chinook salmon fry for twelve weeks and exposed them to a continuous photoperiod of four different light intensities of 0.02, 88, 116 and 157 ft-c, using fluorescent light. By the end of the twelfth week, the light-reared groups weighed 56.2%, 58.9% and 56.9% more, and were 40.5%, 41.8% and 41.3% longer than the dark-reared group. He concluded that high intensities of light emitted from white fluorescent tubes would act as a growth stimulator on actively feeding chinook salmon fingerlings. Kilambi, Noble and Hoffman (1970) studied the effect of ten hour and fourteen hour daylength on the growth of channel catfish, _Ictalurus lacustris_ fry (0.07g) over a period of 120 days at temperatures of 26°C, 28°C and 32°C. The mean growth rate for all temperatures at fourteen hour daylength was 16% higher than that at ten hour daylength, but this difference diminished with time and size of the catfish. Knutsson and Grav (1976) studied the effects of increasing photoperiods (6-19, 8-19 and 12-19L) on growth of yearling Atlantic salmon, _Salmo salar_, held in freshwater at three temperatures (7°, 11° and 15°C). The increasing daylengths were applied in the fall-winter season, with the inflection to 12-19L commencing three months ahead of the more normal 6-19L (at Bergen, Norway, 60°N Lat.). As expected, growth was primarily dependent on temperature, but photoperiod was also found to influence growth. Temperature seemed to influence growth independently of photoperiod, while the effect of photoperiod seemed to be dependent on temperature. Optimal growth occurred at 15°C on the 12-19L regime, which was also the regime with the most advanced seasonal inflection. They observed in this species that the effect of temperature was considerably greater than the photoperiod effect.

Adron, _et al._ (1973) used demand feeders to study the feeding behaviour of trained groups of rainbow trout, _Salmo gairdneri_, under three photoperiod regimes of 7L17D, 12L12D and continuous light (light intensity at water water temperature was $15\pm2^{\circ}$C). They observed a regular pattern of feeding activity and noticed peak activity at intervals of eight hours in photoperiods longer than seven hours duration. The eight hour feeding rhythm was also present in fish under continuous light. Such feeding rhythm has been attributed to the overall rate of digestion. Fish in continuous illumination showed higher feeding rates, which in turn gave greater growth rates. Rozin and Mayer (1961a) found a continuous pattern of feeding in the goldfish, *Carassius auratus*, using a similar technique. Landless (1976) observed similar peaks of feeding activity as reported by Adron *et al.* (1973), in rainbow trout kept in tanks with a supply of sea-water temperatures maintained at 11°C in summer and at 5 to 11°C in winter. A well-defined peak of feeding activity was observed at dawn, not specifically related to sunrise, but a well-defined peak was observed at dusk, in relation to sunset, which continued for a variable time thereafter. Landless also observed a high level of feeding overnight, on many occasions, up to 40% of the food being delivered between sunrise and sunset, with a great deal of feeding activity between 00.00 and 06.00 hours. The time interval between two feeding activities was reported as eight hours. Due to variations in light intensity (lux not mentioned) nocturnal feeding took place and another peak of feeding activity was sometimes noticed at or soon after midnight, about eight hours after the dusk period. Landless reports, the satiation time for rainbow trout to be three hours, on demand feeding.

2.3. Effect of Some Physical Variants in Food

2.3.1. Food Particle Size

The increased use of pelleted artificial diets in the aquaculture industry has resulted in the formulation of empirical rules relating the size of pellet to be fed to fish of a particular size class (Hastings and Dickie, 1972). Based initially on subjective evaluation (Phillips, 1956) but later on studies of acceptance-rejection behaviour (Fowler and

Burrows, 1971) the availability of commercial feeds of particular sizes has been governed largely by the economics of manufacturing and size particle separation processes (Robinson, 1971; Stivers, 1971).

Salmonid fish show a high degree of prey selection based on body size (Ricker, 1932; Allen, 1941; Martin , 1952; Lindstrom, 1956; Nilsson, 1957, 1958; Zorbidi, 1970; Moore and Moore, 1974; Bisson, 1978). Depending on fish species and geographical location, selection has been found to be biased towards:

a) the largest available prey (Zorbidi, 1970);

b) the smallest prey individuals found in the prey population (Feller and Kaczynski, 1975); and

c) related to the body size of the salmonid predator (Allen and Clausen, 1960; Egglishaw, 1967; Okada and Taniguchi, 1971).

The importance of the relationship between prey body size and predator body size in fish, from the point of view of the efficiency of energy gathering and utilisation, has been demonstrated in several exercises in mathematical modelling (Paloheimo and Dickie, 1965, 1966; Kerr, 1971a, b) and speculations on the importance of increasing prey size with increase in fish body size have been made by Brett (1970) and Elliot (1975).

Paloheimo and Dickie (1966) in an extensive review of fish feeding and growth experiments, observed that growth efficiency varies dramatically with different food types such as hatchery mash, crustaceans, minnows, etc. They concluded that different growth efficiencies were due to differences in foraging efficiency on different food types rather than to major differences in physiological conversion of foods. According to Paloheimo and Dickie the relative food size was the major factor determining efficient utilisation of rations.

Nakamura and Kasahara (1956) found improved growth in populations of carp (species not cited) fed on mosquito larvae and cladocerans retained by four hundred micron mesh when compared with those fed only on cladocerans smaller than four hundred microns body size. Grassl (1956) found that the smallest of three sizes of pellet, gave the fastest weight recovery on transference of rainbow trout, *Salmo gairdneri* and brook trout, *Salvelinus fontinalis* from natural to artificial diets. Prather (1958) reported that fathead minnows (species not cited) showed highest survival rate, weight gain and conversion efficiency on the largest of three, particle sizes of artificial feed offered. On the other hand LeBrasseur (1969) found that the growth of chum salmon juveniles, *Oncorhynchus keta,* was not significantly affected by the body size of available prey. He fed six different concentrations of size-selected zooplankton for eight weeks to fish of average weight 0.45g (200 fish per tank). The largest size range of zooplankton was 6-20mm total length, mainly composed of euphausids. The second size range was 2.5-4.5mm, mainly composed of copepods and the smallest size was less than 1.5mm composed of small copepods. Chum salmon were offered a mixture of prey sizes ranging from 0.5 to 20mm in length. After one hour of feeding 10-15 fish were killed and their stomach contents were counted. Results indicated that the fish selected copepods 1.6-4.5mm long in proportion to their abundance and rejected copepods which were less than 1.5mm in length. Inspection of the electivity values for large prey from 12 to 20mm suggested that as the fish increased in length, the large prey formed an increasing proportion of the stomach contents. The selection of prey was apparently a matter of choice by the fish.

The results of Bisson (1978) clearly indicated that food selection by two sizes of rainbow trout, *Salmo gairdneri* was due to the size differences among prey organisms. Selection, that is, non consumption of small prey, was apparent at all hours and included periods when both drift feeding and foraging predominated. Bisson observed that fish were size selective, despite having been deprived of food for two days. Ringler (1979) in a study of predation by brown trout, *Salmo trutta*, in a laboratory stream, offered three species of drifting prey over a 7-d trial. These drifting prey simulated aquatic invertebrates dislodged or emerging from the substrate. The trout diets changed daily and during five-minute intervals; responses to the prey species tended to stabilise after 4-6 days and 800-1200 prey captures. Selection was observed to be greatest at high total prey densities, yet no prey species was completely excluded from the diet. Size selective predation was a dominant characteristic of the response. This seemed to involve development of a preference for large prey. Brown trout ultimately achieved 54-91 percent of a hypothetical optimum diet in which prey were ranked in order of size. Metz (1974) showed a close correlation between body length of drifting insects and their selection by rainbow trout in a natural stream.

Ware (1972) reported a strong co-relation between prey size and reaction distance in rainbow trout, *Salmo gairdneri*. He concluded that this property of foraging behaviour contributed to size selective predation. He found that for prey of a given size, predation rate increased linearly with density, but that substrate-oriented search waned when the trout captured fewer than 3.5 prey items per minute. Other fish species which are capable of bottom foraging also exhibit size-selective predation, for example, black bullhead, *Ictalurus melas* (Repsys, Applegate and Hales, 1976), sticklebacks, *Spinachia spinachia* (Kislalioglu and Gibson, 1976b), bluegill sunfish, *Lepomis macrochirus* (Werner and Hall, 1974). O'Brian, Slade and Vinyard, (1976) reported that in bluegill sunfish selective predation is based on a judgement of "apparent size" (prey length / distance to predator) rather than absolute size.

Repsys et al. (1976) studied feeding habits and food selectivity by black bullhead, *Ictalurus melas*, in Lake Poinsett, South Dakota. They observed that the fish preyed upon a wide range of food sources in limnetic, littoral and benthic zones and seasonal differences in diet occurred in response to availability of prey species. Stomach contents of fish comprised different percentages of small organisms and it was observed that fish were most selective for the largest prey (cladocerans) and were least selective for small prey (larvae). Many workers have confirmed that reactive distance of fish to prey, increases in a predictable fashion with prey size (Protasov, 1970; Ware, 1973) which would have significant implications for patterns of prey exploitation. Protasov (1970) by using models of prey not only demonstrated an optimal stimulus size to elicit approach by fish, but also revealed that significant differences exist between species. While the lower size limit of discovery and attraction to prey may often be determined by the visual acuity of predators the upper size limit is more likely to be related to the positive identification of an object as potential food. In addition to size of the prey, the movement of the prey also affected the feeding behaviour. Protasov indicated that piscivorous fish reacted best to baits of high velocity (greater than 5 cm/sec) and that invertebrate eaters reacted best to baits of intermediate velocity (5 cm/sec). Ware (1973) observed that rainbow trout, *Salmo gairdneri* approached moving targets, regardless of size, from further away than stationary objects with identical properties. A model incorporating the visual mechanics of teleosts has been developed by Ware (1973). The model showed that prey activity accounted for the greatest fraction of the variation in diets of rainbow trout, (19%), the remainder being accounted for by degree of exposure (16%), density (11%) and size (1%). Woodhead (1966) stated that because of the poor image transmitting properties of water, high visual acuity can probably only be achieved by fish in a relatively close field; thus, movement perception will be one of the main functions of the visual systems in a large number of fishes.

2.3.2. Moisture Content

In the early stages of fish farming, attempts were made to duplicate the composition of natural foods. This was laboriously accomplished by growing insects, harvesting small fish or other aquatic animals or by using weak or dying domestic animals (Hastings and Dickie, 1972). Fresh or frozen beef liver was the main diet used in hatcheries to grow trout to stocking size. McCay and Dilley (1927) attempted to grow trout fingerlings on various levels of purified protein, fat, carbohydrate and salts supplemented with known vitamins.

The use of pelleted feeds containing intermediate and low levels of moisture (30 and <10%, respectively) has become widespread on a production basis commonly known as the "Oregon Moist Pellet" (Crawford, Law, McKee and Westgate, 1973). Laboratory diets however, usually have a high moisture content of 50% or more (Wolf, 1951; Halver, 1969) and due to this high level the diets require refrigeration or have to be prepared at short intervals of time. Commercial, intermediate or high-moisture diets cannot be stored for any extended period of time and are subject to fungal growth (Crawford et al., 1973).

Poston (1974) fed brown trout *Salmo trutta*, pelleted diets varying in moisture levels, to study their effect upon growth and feed utilisation. The dry and moist-pelleted diets contained 9.6 and 55.0% water respectively. Fish (initial mean weight 6.6g) were fed four times a day. Fish fed the moist diet grew slightly faster than those fed the dry diet and consumed almost twice as much feed/gram of gain as those fed the dry diet, on a wet feed basis. However, on a dry matter basis, consumption of feed/gram of gain was not different for the two diets. The two diets were equally accepted by the fish. Poston found no significantly different growth rates or feed conversion rates. Windell and Norris (1969) fed natural food organisms (oligochaetes and mealworms) and trout feed pellets to rainbow trout *Salmo gairdneri*, to study the rate of gastric digestion and evacuation. They observed that delay in evacuation was much greater for feed pellets than for oligochaetes. They suggested that the dry composition of pellets may be responsible for the delayed digestion and evacuation of pellets as compared to natural organic foods. Increased moisture content may facilitate a more rapid dissolution of pellets, enhancing enzyme action, digestion and absorption.

The use of low-moisture experimental diets can help in overcoming many problems associated with high moisture diets, however, there is a paucity of published work on the study of the effect of varying the water level of a pelleted experimental diet on the feeding behaviour of salmonids.

2.3.3. Colour

The earliest work on the problem of colour vision in fishes, was reviewed by Warner (1931). This review presented the work of the earliest experimenter, Graber who used the preference method in the study of two freshwater fishes, *Cobitus barbatula* & *Alburnus spectabilis*. He found that the two fish, showed a preference for the darker rather than the light, for blue without ultraviolet rather than blue with ultraviolet, for red rather than green and for green rather than blue with ultraviolet. Graber experimented on two marine species *Gasterosteus spinchia L.* and *Syngnathus acus L.* and showed that it was possible to reverse the preference for red over blue by using an intensity of the former twenty times as great as that of the latter. Graber produced these colours by pigmented glass and "equated" the intensity by using pairs of colours, which appeared to be equally bright to the human eye. The source of illumination was not mentioned. Graber's work appeared to indicate wavelength discrimination, but his work was criticised by later workers, due to technical flaws.

Bauer (c.f. Warner, 1931) using the preference method, concluded that teleost fish and man differed but little with respect to colour vision. For his experiments on colour vision he devised a "phototaxis basin", painted black on all sides but one. The basin, containing the experimental fish was placed within a black box, one side of which (that corresponding to the clear glass side of the basin) contained openings into which two filters could be fastened, each extending over one half of that side. The filters were of glass, paper or gelatine and the wave length for each was noted. On the side opposite the filters was a hole for the use of the observer. The light intensity was regulated by moving the source of the light back and forth. Bauer also used spectral lights in a part of this work. He experimented on *Charaz puntazzo Gm.*, *Atherina hepsetus L.* and *Mugil sp*. If unfiltered light was thrown into one half of the basin, the other half being dark, neither positive nor negative phototaxis was exhibited. The fish swam back and forth into and out of the lighted part of the basin. A positive response was given, however, to change of almost any kind. If a piece of white paper was placed at the open side the fish assembled at that point. They again assembled when the paper was removed or replaced by another. However, if the intensity of the lights was made very strong, the fish rushed to the darker part of the basin. The above behaviour was found in both photopic and scotopic animals. When photopic fish were tested with coloured lights, using an intensity too low to arouse negative responses, strongly positive movements were observed in the case of blue, green and light yellow. Red light called forth immediate and violent negative reactions. When spectral lights were used, starting from the violet end of the spectrum and gradually increasing the wave-length, the negative reaction was first definitely observed at 610 mμ. If the two halves of the basin were illuminated by different colours, one of which was red, the fish in the red half gave pronounced negative reactions to the source of light until they happened to get into the other half when they at once reacted positively. Once in this light they never returned to the red. Bauer did not control the intensity factor directly but maintained that this differential behaviour was not due to intensity differences since, when the two halves of the basin were illuminated by white light of different intensities, the animals swam back and forth from one half to the other, indifferently. Bauer's general conclusion was that photopic fish could distinguish differences in wave-length, whereas scotopic fish might or might not be able to make such a discrimination.

The work of earlier investigators covered in the review by Warner (1931) were concerned mostly with phototactic responses of fish to the directional stimuli of different wavelengths of light. Different investigators presented conflicting results and none could satisfactorily control the intensity factor of the colours used. However, data presented by them did show evidence that some fish, at least, do respond to colour as such, and that the capacity to discriminate colours was far less developed in some species than in others.

Experiment by Washburn and Bentley (cf. Warner, 1931) on colour discrimination in fish, shows an attempt to eliminate brightness cues. They trained creek chub, *Semotilus astromaculatus* to associate red colour with food by feeding them from red coloured forceps, which were presented to fish, simultaneously with empty green coloured forceps. The fish, subsequently, became trained and started choosing the red forceps even though they were presented without food and the shade of red was changed considerably from trial to trial.

In a later study by Brown (1937) it was observed that the smallmouth bass, *Micropterus sp.* freshly captured from the field were attracted in order of decreasing effect to red, yellow, white, green, blue and black targets.

Some earlier simple colour vision experiments were done by Frisch (c.f. Warner, 1931). A number of species such as the minnow *Phoxinus laevis*, the ide *Idus*, the bitterling *Rhodeus* and tench *Tinca* were trained to take food from a coloured container placed amongst a large number of other containers of different shades of grey. Without colour vision one or more of the grey containers would have been confused with the coloured one in terms of brightness. These experiments give good proof of colour vision in these species, and showed, in the minnow, an ability to discriminate between twenty different colours within the visible spectrum.

Hurst (1953) performed an experiment to investigate, whether fish can see colour or not. He kept the bluegill sunfish, *Lepomis macrochirus*, in a fifty-gallon glass aquarium, with an open wire box at each end. At feeding time the lights in the room were extinguished and a beam of coloured light was shot into each food box, one of red light and the other of green light. If a fish went into the box with red illumination, it was fed a worm and the lights were turned out. If fish went into the box with green light, it was not fed and the lights were turned out. The lights were alternated randomly. The intensities of both the red and green lights varied over a range from 1 to 45 foot candles. Hurst was able to demonstrate that bluegills could discriminate between red and green lights. He observed that the fish chose red over green in a ratio of 94:7 in a series of 101 test trials.

Wolf (1953) in an attempt to make fish-food pellets more appealing to fish, coloured them green, blue, yellow and red. These coloured pellets together with some standard commercial pellets (tan coloured) were fed to rainbow trout, *Salmo gairdneri*. The fish reacted to the green, blue and yellow pellets as they did to the standard pellets. The fish ate some but allowed many to fall to the pond bottom, where they were then ignored. When red pellets were fed, fish reacted in the same vigorous manner as when they were fed meat. Further observations revealed that rainbow trout showed a vigorous reaction to the red pellets and red pellets which fell on the pond bottom were also picked off and were consumed by fish. Yaeger (1967) used sophisticated learning techniques to obtain spectral sensitivity and spectral saturation functions from goldfish while studying their colour vision. In the spectral saturation study, the fish was presented with two levers. One lever was trans-illuminated by white light, the second by white light, to which a small amount of monochromatic light was added (both were balanced for brightness). Yaeger obtained thresholds for discrimination by rewarding the fish with food for pressing the lever containing the light mixture and systematically varying the wavelength of the added monochromatic light. When a trial was over, the fish had to press a third lever located at the opposite end of the tank, whereby the stimuli would light up again to start a new trial.

Protasov (1970) reported that significant differences in selection of baits, distinguished only by colour, existed among a variety of fishes. He claimed that colour selection by various species was a reflection of the colours of natural foods, but close examination of data from a range of studies indicated that association was not very precise. According to Protasov, those studies did not indicate that species-specific preferences for colour existed and that colour stimuli might often play a significant role in biasing the food exploitation patterns.

Ware (1971) in order to examine whether the reactive distance of experienced animals was associated with the property of food contrast, experimented upon rainbow trout, *Salmo gairdneri* and conditioned the fish to white prey. Fish were then switched at random to either light grey, dark grey or black food. He found that the maximum distance of reaction observed in each group appeared to be inversely related to food colour. He observed that the fish exposed to light and dark grey food reacted immediately and the initial distance of reaction did not improve significantly with additional experience. Trout switched to black prey responded like an inexperienced fish and required several days of experience to approach an unfamiliar colour of the food. Ginetz and Larkin (1973) used coloured eggs of rainbow trout to study colour preference by rainbow trout, *Salmo gairdneri*. Eggs were fed in lots of 70, comprising 35 each of all combinations of two colours from the series: red, orange, yellow, blue, green, brown and black. They presented all combinations once to each of four groups of fish over a 28-day experimental period. They observed, that rainbow trout showed preference for darker colours and this preference tended to decline as contrast between the food and background colour declined. There was an indication that red and blue colours elicited a greater feeding response than other colours regardless of the environmental surroundings. Upon further experimentation to examine effects of background colouration and light intensity, on colour preference of rainbow trout, they observed that consumption of colour that matched the background was low, except for the blue background. For the red background, the consumption of yellow was highest and vice versa. For the black background, consumption of red was highest, closely followed by yellow. Their results suggested the importance of contrast with the background colour as a factor influencing choice. For a blue background, however, they observed the highest consumption of blue eggs, closely followed by red and yellow eggs. At high light intensities blue and red coloured eggs were the most preferred. At low light intensities, consumption was highest for yellow, even though the background was pale greenish-blue. They concluded that rainbow trout indicated a capacity for colour discrimination that could have significance for the rate of predation on prey of various colours and the relative attractiveness of various kinds of artificial lures in sport fishing.

By using differently coloured tips of triggers, Adron et al.(1973) demonstrated that conditioned populations of rainbow trout, *Salmo gairdneri*, could differentiate between certain colours. The fish were pre-trained to actuate a trigger, whose tip was submerged 3mm under the surface of tank water in order to obtain the food which was delivered by a food dispenser. The tips of the triggers were coloured red, blue green or yellow. By using two triggers (one empty, one with food) with differently coloured tips (red and green, red and blue, red and yellow) at a time in one tank Adron et al.showed that fish could differentiate by colour of the tip of the trigger as to which trigger would reward them with food and which would not. But they postulated that the fish were distinguishing differences in brightness and not in colour. This idea is in line with the choice experiments by different workers which perpetuate to show that colour vision exists in fish. Many experiments provide only circumstantial evidence. Because according to Blaxter (1970) experimentation in which fish are offered, and make, a choice between coloured lights are not valid, as proof of colour vision unless they are designed to remove the possibility of choice by a brightness difference. It is not sufficient to adjust the brightness of two coloured lights photometrically; they must be adjusted "subjectively" according to the spectral sensitivity of the light-adapted fish. Alternatively, a correct choice must be made between one colour against a wide range of intensities of another colour. Where either of these criteria has been satisfied and a successful choice of colour made, a colour sense would appear to exist. Choice experiments of this sort have shown that many species of fish prefer the blue or blue-green.

The physiological or anatomical requirements for colour vision are the presence of cones reacting to three primary colours, different combinations of which will give any colour in the visible range and electrophysiological studies (Jacobsen, 1964; Cronly-Dillon and Muntz, 1965) strongly suggest that the retina of the goldfish eye possesses at least three types of photoreceptor with different spectral sensitivities. Such suggestions can be tested by observing the behaviour of fish, and in particular by determining its ability to discriminate between pairs of different colours. Muntz and Cronly-Dillon (1966) trained goldfish (*Carassius auratus*) in order to test their ability to see colour. Goldfish were trained to discriminate between blue, green and red painted panels which varied greatly in brightness. The stimuli were chosen in such a way that the discriminations could not be solved in terms of brightness. All the discriminations were easily learned by the fish, in spite of the large brightness differences. Their results showed that the goldfish had a preference for blue colour over red and green colours.

2.4. Influence of Energy Level in Feed, on Feeding Behaviour

Most of the work by nutritionists is directed towards feed formulation with a varying of the levels of macro and micro-nutrients. The influence of such varied diets on growth have been extensively studied. Very little attention, however, has been devoted towards the study of the feeding behaviour by altering the level of the energy content in the diet. Most of the work on the regulation of food intake has been done on mammals but a little has been carried out on fish. The earliest work on the regulation of food intake in fish appears to be by Rozin and Mayer (1961a) on the goldfish, *Carassius auratus*. They showed that lower energy-containing diets were consumed more readily and the fish compensated for the low level of diet-energy by consuming more food. They trained goldfish to press a bar for food and thus provided individual fish to regulate its own food intake. There was marked variation between individuals, and each fish established its own cycle of feeding activity and level of food intake. During experimentation when food pellets of two different sizes were provided for alternate three-day periods, the ratio of the larger to smaller pellets eaten by the fish was such that three out of five fish, very accurately compensated in order to maintain the same level of nutrient intake. In further experiments fish were provided with food pellets, made up with kaolin as a non-nutritive filler, added to them so as to decrease the overall energy content of each pellet. Goldfish were fed the regular pellets or the kaolin-containing pellets for alternative four-day periods for a total of thirty-two days. The ratio of kaolin-containing pellets to regular pellets eaten was such that there was quite an accurate compensation for the difference in nutritive value of the two kinds of food.

It has been observed in different experimental studies that fish were capable of making up nutritional deficiencies in their body reserves very quickly, when provided with the opportunity. Such investigations were carried out on goldfish, in a further study by Rozin and Mayer (1964). They showed that in starved fish, which were deprived of food from 0 to 4 hours, there was a clear tendency towards increased food intake. Their data indicated that goldfish would eat about the same amount of food in one hour after deprivations ranging from four to forty-seven hours. When food deprivation was carried out beyond a certain point compensation by increased food intake was no longer possible. In similar experiments performed on sockeye salmon fry, *Oncorhynchus nerka* by Bilton and Robins (1973), fry were starved for up to seven weeks and then fed *ad libitum* for eight weeks. It was noted that the body weight and length of the fry was the same, or nearly the same as those of the control. Fry starved for four weeks or longer were not able to overcome the deficits and could not grow rapidly, when they were refed. Mortality rate was also observed to be higher in fry starved for four weeks or longer, which indicated that the more prolonged starvation had caused some irreversible effects.

Tyler and Dunn (1976) demonstrated the effects of deprivation of feeding in winter flounder, *Pseudopleuronectes americanus*. They found that the flounder was able to compensate to some extent for decreased meal frequency by increasing meal size. However, once the meal size was less than a certain level, wieght loss was evident which showed that the fish were starved. Specifically, either 32, 16, 8, 4 or 2 meals were given per 32 day period, intake per meal was increased such that although growth did not occur the fish were able to maintain their body weight. With only two meals per thirty-two days, weight loss was evident. When the flounders were given daily meals, that is, 32 meals per 32 days, growth took place. Johnson (1966) provided evidence in the pike, *Esox lucius* for the regulation of both food intake and growth on a seasonal basis, as well as seasonal changes in food intake to provide the body maintenance requirement. Fish were held individually in tanks and were fed live minnows of known weights. The water temperature in tanks changed according to the changes in the ambient seasonal temperatures. The amount of food required for body maintenance, varied on a seasonal basis. Of significance was, that the maintenance food requirement varied independent of temperature to some extent. The peak requirement was in June, followed by a fall over the next month or so to a base level that was constant until the next spring. The peak requirement occurred at the same time of the year as the maximum growth and maximum food intake of fish fed _ad libitum_. For the fish fed _ad libitum_, food intake decreased to zero and some weight was lost during the winter months of January and February. The peak in food intake and growth was in June, before the maximum in temperature occurred. When temperature was the highest in July of the next year, food intake and growth rate were observed to be decreasing very markedly. Thus in pike, food intake and growth were apparently regulated on an annual basis, somewhat independent of temperature changes.

Page and Andrews (1973) pointed out that high dietary levels of energy and protein resulted in decreased feed consumption in channel catfish _Ictalurus punctatus_. Lee and Putnam (1973) studied the response of rainbow trout, _Salmo gairdneri_ to varying protein/energy ratios in the diet. They observed that the food intake was regulated by the caloric content of the food. Fish fed the low energy diets were able to gain weight at a rate comparable to those fed high energy diets by increasing their feed intake. It was indicated that rainbow trout eat to satisfy their energy requirement. They concluded that "caloric intake regulated food consumption".

Recently Marais and Kissil (1979) studied the influence of energy level on feed intake and growth of gilthead bream, *Sparus aurata*. They fed low energy diets and high energy diets to the fish and observed a larger intake of lower energy-containing diets with a good response to growth.

2.5. Neural Regulation of Food Intake

Most experimental work on the neural regulation of food intake has been concentrated on the role of the hypothalamus. Hetherington and Ranson (1940) showed that lesions of the hypothalamus of rats including the ventromedial nucleus (HVM), resulted in increased weight gain. Brobeck, Tepperman and Long (1943) found that this weight increase resulted from an increase in food intake following lesions in the region of the ventromedial nucleus of the hypothalamus. Lesions of the ventrolateral region of the hypothalamus (HVL), have the opposite effect, causing aphagia and adipsia, which can result in the death of the animal (Anand and Brobeck, 1951; Morrison and Mayer, 1957). Whereas, when the ventrolateral region of the hypothalamus is stimulated through permanently implanted electrodes, the animal increases its daily food intake (Delgado and Anand, 1953). Similarly, if the ventromedial nucleus of the hypothalamus is stimulated, it causes a cessation of eating in a food deprived animal (Smith, 1956).

Similar kinds of lesions can be induced in the brain of the various strains of mice with injections of goldthioglucose (GTG). GTG is considered to effect the feeding centres in the brain, when injected intraperitoneally in the case of mice. The GTG induces lesions in the region of the ventromedial nucleus of the hypothalamus (Brecher, Laquer, Cronkite, Edelman and Schwartz, 1965; Debons, Silver, Cronkite, Johnson, Brecher, Tenzer and Schwartz, 1962; Debons, Krimsky and From, 1970; Drachman and Tepperman, 1957; Liebelt and Perry, 1957; Marshall, Barrnett and Mayer, 1955). Such treated mice tend to become hyperphagic and thus get obese. Similar obesity occurred in rats following intrahypothalamic implants of GTG (Sandrew and Mayer, 1973). Mayer (1965) suggested that GTG selectively destroys specialised cells in the HVM which have a particular affinity for glucose, thus disrupting the modulating system which mediates food intake. Such cells he called glucoreceptors. However, when studying the physiological and behavioural syndrome following systemic doses of GTG, a confounding factor is the general toxicity of the compound to peripheral organs in rats and often is lethal (Browning and Kwan, 1965; Deter and Liebelt, 1964; Larsson, 1959; Liebelt, Sekiba, Liebelt and Perry, 1960; Liebelt, Sekiba, Ichinoe and Liebelt, 1966; Petersson and Hellman, 1962), although HVM lesions and hyperphagia usually occur in those that survive a high dose (Baile, et al. 1970; Deter and Liebelt, 1962; Talbert and Hamilton, 1954; Wagner and DeGroot, 1963). Debons et al, (1970) have shown that GTG enters the HVM through oligodendrocytes which become destroyed and this in turn results in the dissolution of the adjacent neuropile. The disintegration of oligodendrocytes also causes damage to adjacent capillaries and thus decreases blood supply to the area. Arees, Veltman and Mayer, (1969) have also observed decreased blood supply to HVM following GTG treatment.

Dogs, sheep, goats (Baile, Mayer, Baumgardt and Peterson, 1970) and guinea pigs (Luparello, 1969) do not develop HVM lesions following a GTG treatment, though sheep and guinea pigs have some damage to peripheral organs. GTG administered systemically in Japanese quail (Carpenter, Stein, Silverstein and Tienhoven, 1969) or chickens (Svacha and Reid, 1973) has no significant effects on food intake or body weight, and caused no observable lesion in the HVM of Japanese quail (Carpenter et al. 1969).

Stimulation of the HVM results in the cessation of eating and its destruction results in overeating, therefore, it is considered an inhibitory or "satiety centre". The stimulation of HVL causes an animal to eat and an intact HVL seems to be a necessary condition for an animal to eat, therefore, this region has been commonly considered to be the "feeding centre". This evidence can be considered to provide the basic support for a dual-centre conception of hypothalamic function in the regulation of food intake.

Several assumptions have been made concerning ventromedial hypothalamic function and its relationships with other hypothalamic areas, based upon these behavioural lesion and stimulation studies. The conception of hypothalamic function developed in the dual-centre theory presupposes a series of inhibitory interconnections between the HVM and HVL so that as the electrical activity of one "centre" increases, it inhibits the activity of the other (Stellar, 1954). The validity of this theory depends upon the anatomical and electrophysiological demonstration of fibres running from the HVL to the HVM and from the HVM to the HVL. Experimental attempts to verify these interconnections have been generally unsuccessful but still, these presumed interconnections have achieved the status of fact. Some workers (Dahl and Ursin, 1969; Han and Young, 1964; Lewinska and Romaniuk, 1966; Valenstein, Cox and Kakolewski, 1969), have questioned the adequacy of the conception of the HVM as the "satiety centre" for the regulation of food intake as proposed in the dual-centre theory. Reynolds (1959, 1963), Rabin (1968) and Rabin and Smith (1968) have suggested that the hyperphagia observed, following HVM lesions is the result of an "irritative side effect" of the lesion technique and not the result of a specific loss of tissue.

Since the dual-centre theory attempts to account for a specified behaviour in terms of its neural mechanisms it must be consistent with the known neuroanatomy and neurophysiology of the hypothalamus as well as with the behavioural deficits observed following its destruction. Neuroanatomical evidence with regard to the presence of fibres running from the HVM to the HVL is somewhat contradictory. Most workers (Bleier, Bard and Woods, 1966; Guillery, 1957; Kaelber and Leeson, 1967; Wolf and Sutin, 1966), have been unable to find any evidence of such fibres. Bleier et al. (1966) found no significant degeneration in the HVM following extensive ablation of the forebrain of cats which included the HVL. They argue that this lack of degeneration in the HVM indicates that most of the axons of HVM cell groups cannot project to the HVL, but more probably are involved in the regulation of the anterior pituitary as part of the hypothalmo-hypophyseal system.

Arees and Mayer (1967) and Mayer and Arees (1968) provided some evidence for fibres running from the HVM to the HVL. They injected mice with goldthioglucose, which produced lesions in the HVL. They observed degenerating axon terminals, primarily within the perifornical region of the HVL but they could not trace the degenerating fibres running from HVM to HVL. In contrast to the anatomical studies, electrophysiological work has provided some evidence of conduction from medial to lateral hypothalamus. In a study by Oomura, Ooyama, Yamamoto and Naka, (1967) recording microelectrodes were placed in the HVM and stimulating electrodes were placed in the HVL. The effect of stimulating the HVL was to cause a reduction in the frequency of unit activity in the HVM. When the electrode positions were reversed, it was observed that when the HVM was stimulated there was a decrease in the frequency of firing of ventrolateral neurons. These results have been interpreted as providing electrophysiological support for the dual-centre theory by showing that as the activity of neurons in the HVL is increased, unit activity in the HVM is decreased; while as the activity of neurons in the HVM is increased, the firing rate of units in the HVL is decreased. Murphy and Renaud (1969) failed to replicate the finding that stimulation of the HVL caused a decrease in HVM unit activity. Data presented by these authors do not permit adequate interpretation and cannot, therefore, be considered as providing reasonable support for transmission from medial to lateral hypothalamic centres.

There is much controversy regarding the neural pathways from the HVM to the HVL for inhibition of feeding. Hyperphagia and obesity were observed by various workers when cuts were made lateral to the HVM of the rat, (Albert and Storlien, 1969; Palka, Liebelt and Critchlow, 1971; Pauxinos and Bindra, 1973; Storlien and Albert, 1972). The results of these workers were also interpreted to show a pathway running directly from the HVM to HVL, just as was demonstrated by Arees and Mayer (1967) and Mayer and Arees (1968). However, the latter results have been questioned in a thorough review of the literature by Rabin (1972). He concluded that most of the evidence failed to support the idea of a direct inhibitory connection of HVM with the HVL and that the available evidence did not clearly support the idea of a HVM satiety centre, in that this region serves to organise and control the phenomenon of satiation. Studies by Gold (1973); Sclafani and Maul (1974) and Sclafani, Berner and Maul, (1975) also failed to support a direct inhibitory connection between the HVM and HVL. They made cuts between the HVM and HVL and these lesioning experiments make it clear that lesions anterolateral to HVM and posterior to both the HVM and HVL can cause hyperphagia. It was proposed that a ventrally located noradrenergic pathway ascending to the free brain through the HVM region was the source of the inhibitory effect of HVM lesions on feeding (Kapatos and Gold, 1973;

Ahlskog and Hoebel, 1973; Gold, 1973). The earlier study by Panksepp (1971a) also showed that lesions specifically in the HVM failed to block the occurrence of post-prandial inhibition of feeding.

In spite of all these doubts there is firm evidence for a role of the HVM in satiation. Ahlskog, Randall and Hoebel, (1975) have shown that destruction or damage of the noradrenergic pathway through the H.V.M. and electrolytic lesions of the HVM that leave the noradrenergic pathways intact, each cause hyperphagia and obesity, with the combined operations generally causing an even greater effect. Sutin, McBride, Thalman and Van Atta (1975) made electrophysiological studies and showed that there were both excitatory and inhibitory connections between the HVM and HVL.

As compared to work on mammals there is very little work done which is concerned with the neural control of feeding in the fish. Grimm (1960) found that feeding behaviour could be evoked in goldfish, _Carassius auratus_ by stimulation of locations in the telencephalon. He also found that plugging the olfactory pits of goldfish would result in the blockage of the occurrence of arousal and bottom searching for food, after the introduction of a food odour. He suggested that olfactory input and then telencephalon output could serve to activate or arouse feeding behaviour.

Studies on the ablated telencephalon in goldfish by Peeke, Peeke and Williston, (1972) showed that the forebrain exerted modulating influences on memory consolidation and that short-term memory remained intact in the ablated fish, but the long-term memory required to retain the habituation from one day to the next was severely impaired. Peeke _et al._ studied habituation in the normal and ablated goldfish using live bait, isolated from the fish but in clear view. The number of bites attempted were counted in daily fifteen minute trials. They found that for the exposure period each day the ablated fish successfully habituated, but the response rates in the subsequent days were significantly higher for the ablated fish compared to the normal. Kaplan and Aronson, (1967) used an avoidance conditioning model to train *Tilapia h. macrocephala* to swim through a hole in a partition to avoid electric shock, using a light as the continued stimulus. They observed that after ablation of the telencephalon, the number of avoidances were markedly reduced and the latencies of responses became erratic. They found that the ablated fish no longer maintained efficient intertrial behaviours such as waiting near the hole for the onset of the light.

Demski and Knigge (1971) electrically stimulated a number of forebrain regions, via chronically implanted electrodes and made observations on the evoked behaviours of the bluegill sunfish, *Lepomis macrochirus*. They observed feeding-like behaviour, such as surface-searching, bottom-searching, snapping up and spitting out of gravel or debris, and snapping up, chewing and swallowing prey. Such behaviours were caused by stimulation of particularly the region around the lateral recess of the third ventricle in the inferior lobe of the hypothalamus. Feeding and aggressive, after-responses were evoked by stimulation more dorsally in the nucleus rotundus. Demski (1973) while working on the cichlid *Tilapia heudelotti macrocephala*, besides being able to confirm the above observations, was also able to evoke feeding behaviour by stimulation anteriorly along the midline at about the thalamic-hypothalamic junction. He interpreted his results as being due to activation of fibres in the medial forebrain bundle, a pathway interconnecting olfactory regions in the telencephalon with the preoptic region, and posteriorly with the subglomerulosal region of the inferior lobe of the hypothalamus. Thus his work also implicates the telencephalon in feeding behaviour and raises the possibility that lateral hypothalamic stimulation evokes feeding by activation of fibres in the medial forebrain bundle. However, earlier work done by Aronson (1963, 1970); Hale (1956); Segaar (1965) and Savage (1969) shows that the telencephalon is not an essential part of the neural apparatus for feeding in the teleosts because they observed feeding in fish with an ablated telencephalon. In later work by Demski (1977), it was demonstrated that feeding responses, represented by the biting and mouthing of food, were consistently evoked by electrical stimulation of the inferior lobe of the hypothalamus of the nurse shark *Ginglymostoma cirratum*. Biting and/or mouthing of food were inconsistently evoked by stimulation of the dorsal hypothalamus and basal regions of the telencephalon. His results suggest that homologous hypothalamic regions may be involved in feeding behaviour in sharks and teleosts.

In a recent study by Savage and Roberts (1975) on goldfish, *Carassius auratus* involvement of the lateral hypothalamus (HVL) has been convincingly demonstrated. They observed the evoked behaviours of goldfish that were electrically stimulated, via chronically implanted electrodes, in various hypothalamic, thalamic and midbrain regions. Although, they recorded a wide number of behavioural responses, a full feeding response according to their notation involved orientation towards food, assumption of a feeding posture, taking food into the mouth and ingesting the food. In this study the different areas of the hypothalamus involved in evoking feeding behaviour have been pinpointed. Stimulation of the region lateral or dorsal to the lateral recess of the third ventricle, posterior to the connection of the lateral recess with the central and main part of the third ventricle, was quite effective in evoking feeding responses. Stimulation more dorsally in the nucleus subpraglomerulosus region and the corpus interpeduncularis, both were quite effective in arousing feeding behaviour. These results are similar to those of Demski and Knigge (1971) and Demski (1973).

In mammals, extensive work on study of feeding behaviour has been made by the technique of lesioning different areas in the telencephalon, but in teleosts there are no comparative published studies dealing with the effects of lesioning on food intake. The only work on fish is by Peter, Monckton and McKeown (1976), who administered goldthioglucose to goldfish, *Carassius auratus*, by intraperitoneal injection or by intraventricular brain injection. This caused a dose-dependent hypophagia and slowing of growth. These workers did not observe any lesions in the ventromedial hypothalamic region (HVM). After brain injection of GTG there was a dose-dependent hypertrophy of the forebrain ependyma. Amongst some animals, showing low growth rates, which received a high dose of brain injection there was disruption of the ependymal lining of the lateral-dorsal aspect of the lateral recess of the third ventricle, accompanied by some hypertrophy in the region around the lateral recess of the third ventricle in the inferior lobe of the hypothalamus. They also observed that the effects of GTG on food intake and body weight were time dependent to some extent, since some fish recovered normal growth and showed tendency towards normal feeding. However, when these workers injected the fish an equimolar amount of gold in the form of $AuCl_3$, all fish died within forty-eight hours. This shows that the free ionic form of gold is quite toxic and soon fatal to goldfish. The effects of GTG might be due to the toxicity of gold as suggested by Peter et al. and was not necessarily related to the specific uptake of GTG due to the glucose moiety of the molecule.

CHAPTER 3

SECTION 3 GENERAL MATERIALS AND METHODS

3.1. The Experimental Facilities

In order to study the feeding behaviour of rainbow trout under controlled conditions in the laboratory planning and equipment is required. All the different experiments described in this thesis were conducted in two different recirculation systems, both built on the same plan. Throughout the different experiments *ad libitum* feeding was maintained by the use of demand feeders. To monitor the feeding frequency event recorders were used.

3.2. The Recirculation System

Mains tap water (for chemical analysis of tap water see Table 1) was the sole supply to the laboratory, therefore, to minimise the quantity of water needed it was decided that water recirculation systems would be required. Holding fish in a restricted volume of water causes deleterious changes in water quality that must be rectified. These changes include increases in the concentrations of dissolved ammonia, dissolved organic material, solid faecal matter and dissolved carbon dioxide as well as a decrease in the dissolved oxygen content of the water.

The first limiting change is the depletion of oxygen due to the metabolic requirements of the fish. This situation is easily corrected by mechanical agitation and aeration of the water.

TABLE 1

Analysis of Tap Water Used in the Present Study

pH	7.0
O_2	8 ppm
Na^+	6 ppm
K^+	1 ppm
Ca^{++}	8 ppm
Mg^{++}	1 ppm
Mn^{++}	<0.1 ppm
Fe^{+++}	<0.1 ppm
Cl^-	10 ppm
NO_3^{2-}	2 ppm
SO_4^{2-}	4 ppm
PO_4^{2-}	0.5 ppm
$(NH_4)^+$	0.05 ppm

TABLE 2

Water Quality in Recirculation System

Temperature	15.0°C ± 2.5°C
Dissolved Oxygen	>7 mg/l
Total Ammonia	<0.1 mg/l
Total Nitrate/ Nitrite	<50.0 mg/l
pH	6.7 - 7.2

The second limiting change is the production of ammonia, by the fish, which is their primary method of nitrogen elimination. Unless steps are taken, potentially harmful levels are quickly reached. Smith (1972) reports a value of 1 mg/l as "safe" for rainbow trout *Salmo gairdneri*, with oxygen levels in excess of 7mg/l. As the dissolved oxygen level of the water falls, so the threshold of ammonia toxicity falls (Larmoyeaux and Piper, 1973). It must, however, be borne in mind that the toxicity of the ammonium ion (NH_4^+) is low, but the toxic action of ammonia (NH_3), with which it is in equilibrium, is much greater. This dissociation is pH dependent; the higher the pH the greater the proportion of ammonia (Lloyd, 1961). There is also a slight dependence of the equilibrium on temperature; increasing the temperature increases the proportion of ammonia (Huisman, 1969).

On the basis of the above considerations it was considered that if the total ammonia remained below 1 mg/l it would have no toxic or growth inhibitory effects on rainbow trout at an approximate pH of 7 and a level of dissolved oxygen in excess of 7 mg/l.

Several water quality parameters were measured twice weekly throughout all of the experimental periods and the values recorded (See Table 2). A great deal of information on water purification, by various methods of recirculation, for fish culture has been published (Spotte, 1970; Hirayama, 1966; Hirayama, 1974; Parker and Simco, 1973; Meske, 1971, 1976; Parker and Broussard, 1977). These methods vary widely in principle, degree of complexity and capital cost. Previous researchers in the University of Aston Fish Culture Unit had successfully used, for rainbow trout, recirculation systems based on a downflow submerged gravel filter (Roberts, 1976; Atack and Matty, 1978). This system had the proven advantages of relatively low capital cost, reliability and simplicity of construction and operation. Such a method of biological filtration involves the passage of ammonia laden water down through a gravel substrate which provides a large surface area on which nitrifying bacteria may grow.

Bacteria growing on a filter bed are chemosynthetic autotrophs which oxidise simple organic compounds to more complex carbohydrates, lipids and proteins, in this case by using ammonia as their energy source. The oxidation of ammonia produces nitrate and nitrite ions, thus a base is required to combine with these ions. Birmingham tap water is extremely soft and, for this reason, crushed cockle shells were mixed with the gravel filter medium to provide the required base. Absence of sufficient base would cause a fall in the pH due to the accumulation of nitrous and nitric acids. The calcium carbonate from the crushed cockle shells reacts with the nitrate ions thus:

$$NH_4^+ + 1.5O_2 \xrightarrow[\text{NITROSOMONAS}]{} 2H^+ + H_2O + NO_2^-$$

$$4NH_3 + 7O_2 \xrightarrow{} 4NO_2^- + 6H_2O$$

$$2NO_2^- + O_2 \xrightarrow[\text{NITROBACTER}]{} 2NO_3^-$$

$$2CaCO_3 + 4NO_3^- \xrightarrow{} 2Ca(NO_3)_2 + 2CO_2 + O_2$$

The toxicity of the nitrate, produced by the biological oxidation of ammonia, to fish is fairly low. In view of this, it was decided to regulate nitrate levels in the recycling systems by the simple expedient of adjusting the quantity of make-up water flowing through the system rather than attempting to incorporate a denitrifying stage.

3.3. Description of the Recirculation System

Two recirculation systems were built. Each system (Figure 1) constituted three experimental tanks, a header tank, a faecal trap and a filter tank. The experimental tanks were placed in independent wooden environmental chambers, each fitted with two fluorescent tubes of two feet twenty watts each, positioned four feet from surface of water and fitted with diffusers. The length of the photoperiods was directly controlled by "Sangamo" 24-hour dial synchronous time switches, which were individually fitted, one to each of the environmental chambers. The transition between darkness and light and vice versa, was sudden and complete. A steady flow of water was maintained in the recirculation systems. Flow-rates employed in each system are shown in Table 3.

3.3.1. Header Tank

A 300 l header tank (Figure 2) was supported on a platform 3.0m above the floor, which supplied water to three experimental tanks, through a 1.27cm bore PVC pipe. Header tank received water from filter tank through an electric pump. Make-up water was continuously added to the header tank both to compensate for losses through splashing and evaporation and to keep nitrate levels within acceptable limits. Excessive water flowed back to filter tank through a 1.27cm bore PVC pipe (for flow-rates see Table 3).

3.3.2. Experimental Tanks

Each tank was a circular 91.44 cm diameter and 38.10 cm deep with a water holding capacity of 240 l (Figure 3). It was made of 0.32 cm thick, white polypropylene sheet and was fitted to a tripod stand. The bottom of each tank had a slope which slightly tapered from the base towards the centre of the tank. Through the centre, a hole was drilled and a 1.91 cm bore PVC pipe with 33.02 cm length was inserted and welded at

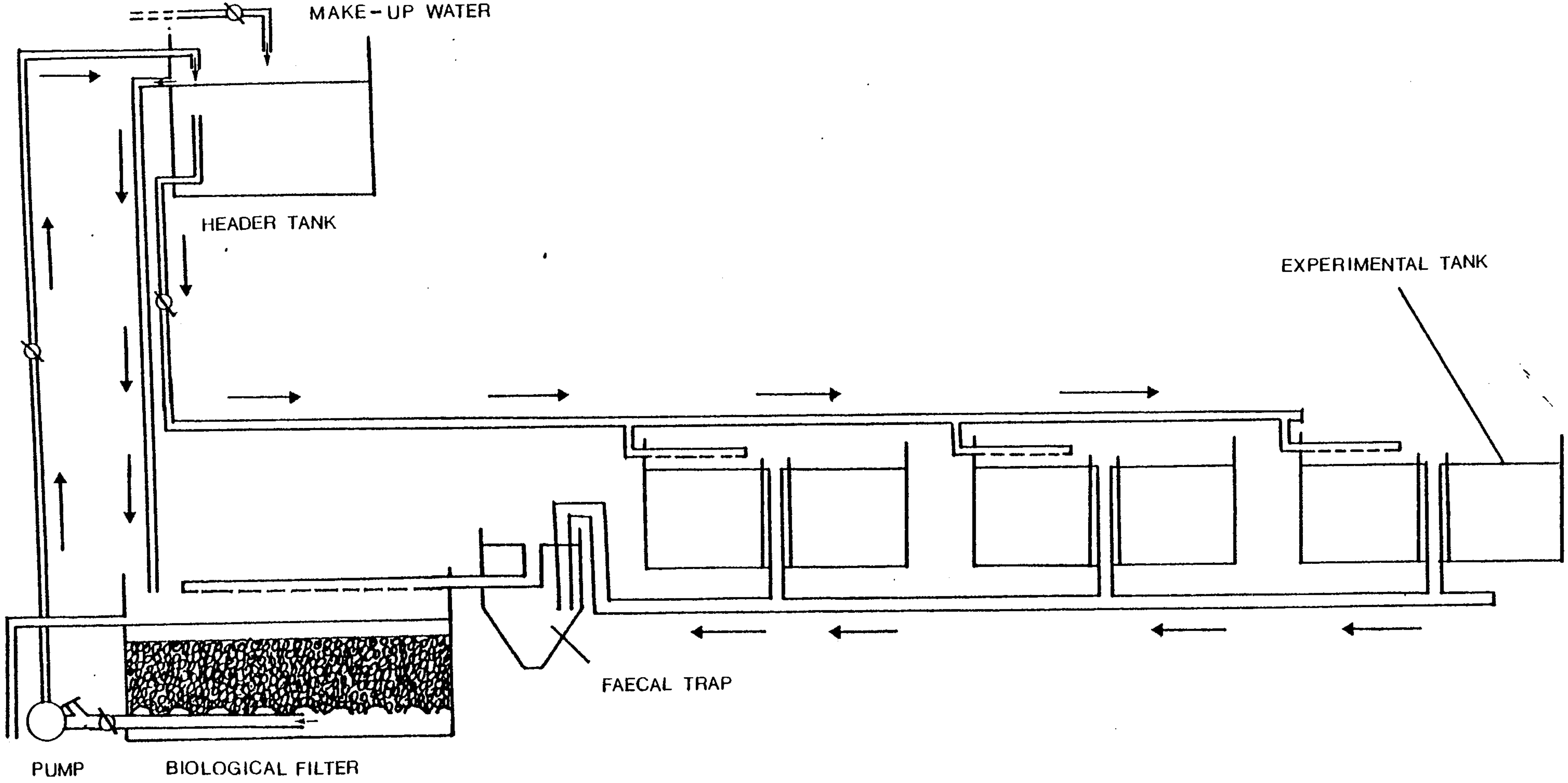


FIGURE 1 A diagrammatic representation of a Recycling System

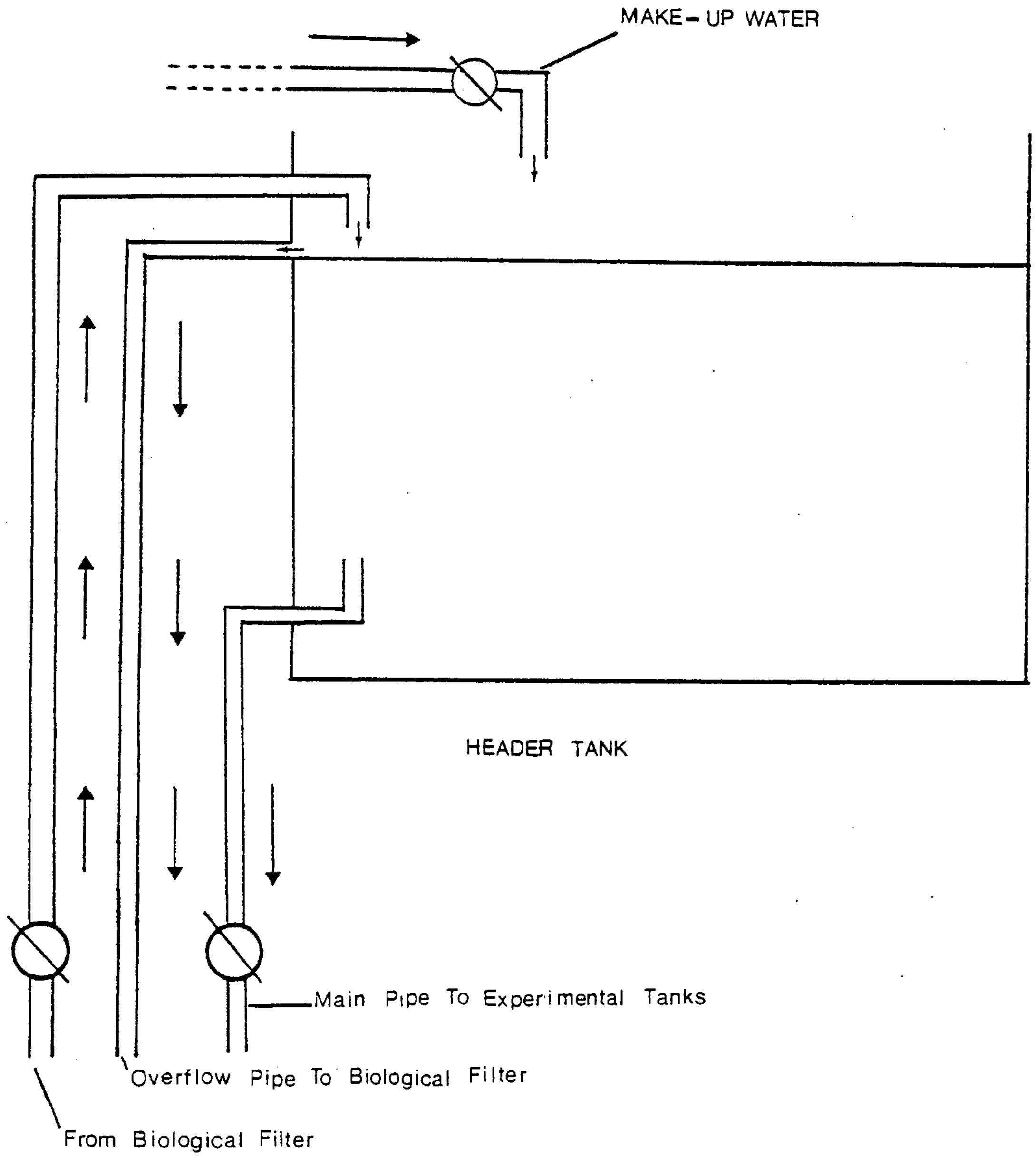


FIGURE 2 A Header Tank of the Recycling System

TABLE 3

Flow-Rates Employed in Recirculation Systems

Make-up water (from mains supply) into Header Tank	0.6 l/min
Header to Experimental Tanks	15.0 l/min
Header to Filter	15.0 l/min
Experimental Tanks to Faecal Trap	15.0 l/min
Faecal Trap to Filter Tank	15.0 l/min
Filter to Header Tank (via Pump)	30.0 l/min
Filter Tank to Drainage (excessive water)	0.6 l/min

the base. Over this central pipe a 7.62 cm diameter PVC pipe of 35.56 cm length was placed and at bottom two wedge shaped holes were cut, so that water along with faeces of fish could flow through. Any dead fish in the tank could have no chance of getting into the pipe and blocking it. The inlet pipe in each tank ran up to the centre of the tank and was drilled with six small holes, through which water flowed into the tanks with a jet stream and a "circular flow pattern" was developed in the tank, thus helping the gradually shelving base to create a self-cleaning action. Such an inflow also aided in oxygenation of the water and prevented the formation of "dead spaces" where waste products could accumulate. Flow and water pressure could be controlled and angle of jets were adjustable. Each tank was covered by 0.64 cm diamond nylon mesh fastened to a flange around the circumference of the tank. From the experimental tanks the water flowed into a conical plastic tank which served as a faecal trap.

3.3.3. Faecal Trap

Overflow water from the three tanks drained into a common pipe which carried the water to a 68 l conical faecal trap (Figure 4). Water was introduced into the faecal trap at an angle to induce a circular flow which spun solids to the outside edge where they then sank to the bottom. Solid material collecting in this trap was siphoned out three times in a week. Relatively solid-free water flowed into the biological filter tank through a 2.54 cm bore PVC pipe.

3.3.4. Filter Tank

Water passed from an abstraction point in the centre of the faecal trap onto the surface of the filter tank (Figure 5). The filter was made up of a 600 l oblong tank containing 400 l of 1 to 1.5 cm broken gravel supported on a corrugated, perforated plate which was, in turn, supported

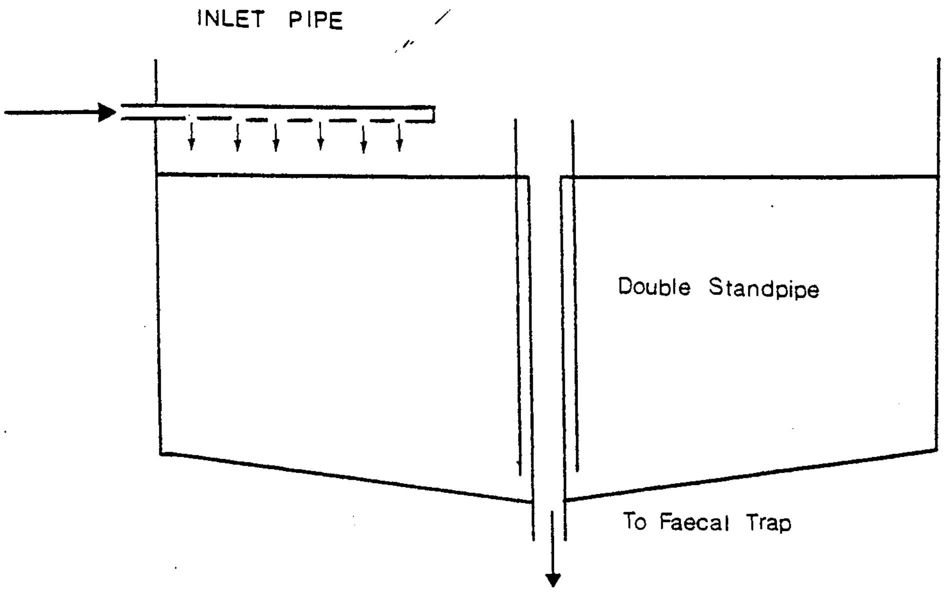


FIGURE 3 A transverse section of an Experimental Tank

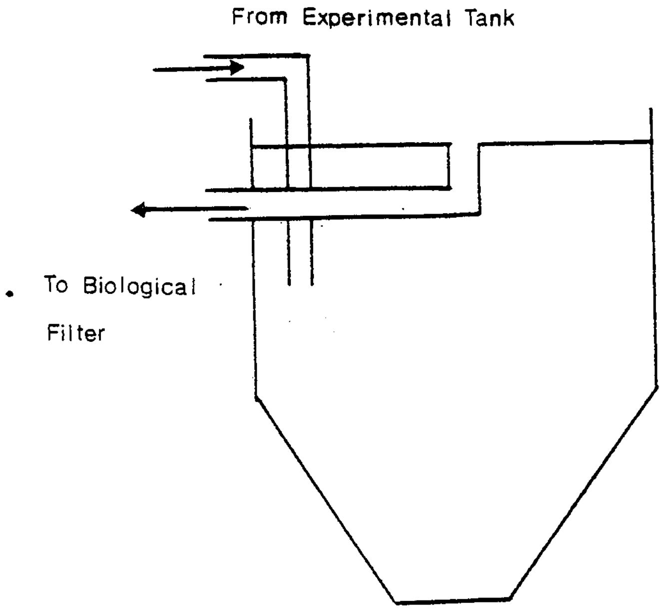


FIGURE 4 A transverse section of the Faecal Trap.

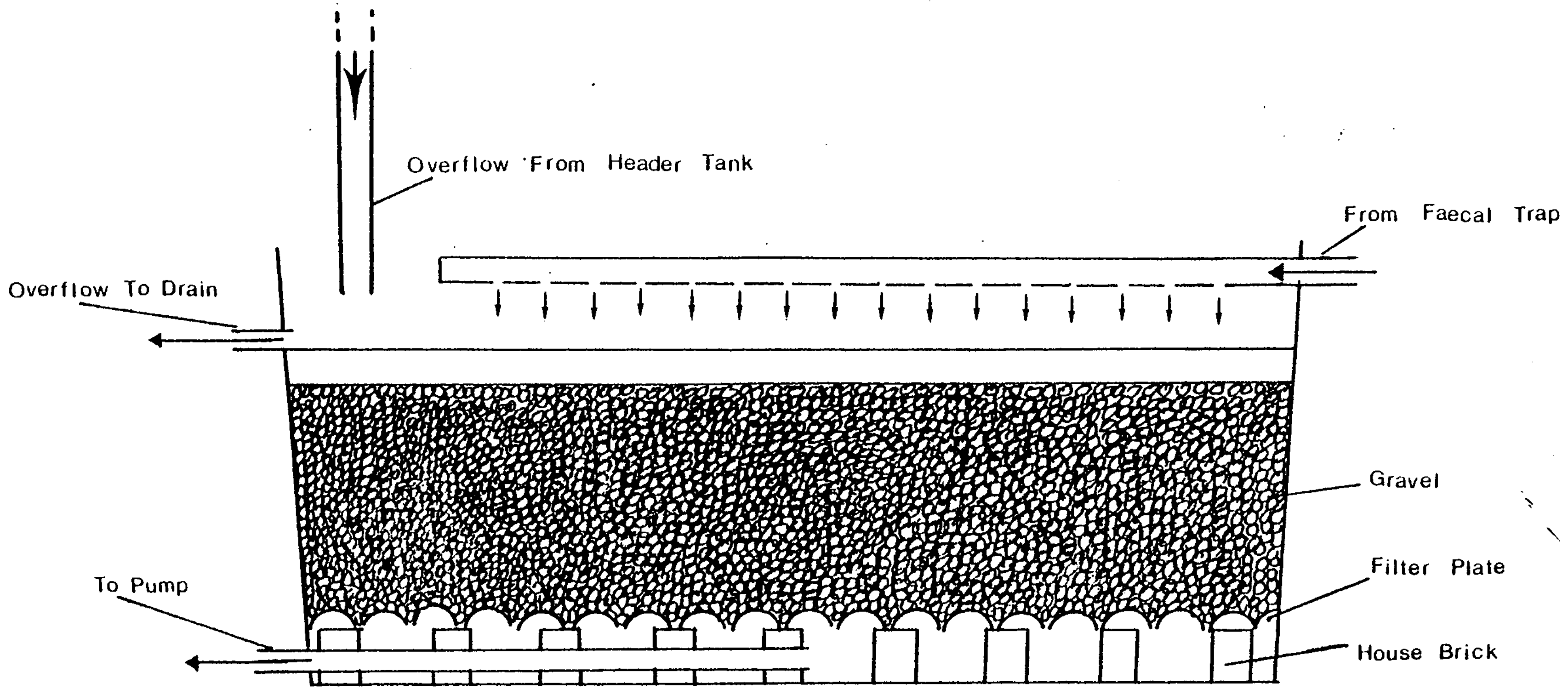


FIGURE 5 A transverse section of the Biological Filter

on house bricks. Water was drawn down through the filter gravel into the cavity below the filter plate by a pump. This pump drew water through an in-line filter (to prevent gravel and detritus obstructing the pump) and returned it to the header tank where it was jetted in to increase aeration. The pumping rate was double that of the flow to the experimental tanks, the remainder being returned to the surface of the filter via the header tank overflow.

Once a month, two thirds of the total depth of gravel in the filter was dug over and the displaced detritus siphoned off. This was performed as the suction of the pump tended to pack the gravel down very tightly and, in addition, prolific growth of micro-organisms on the filter began to restrict the flow. The water from the faecal trap flowed into the filter tank through small holes in the PVC pipe, in the form of jet stream, so that it helped in aeration of water. Excess water in the system over-flowed from the filter tank into the drain, through a PVC pipe.

The recirculation system worked perfectly throughout the experimental period without causing any concern for the flow rates (as shown in Table 3) or for the water quality (as shown in Table 2). The water temperature in the system during severe winter period went down to 10.5^{o}C. Since all the experimental tanks were enclosed in wooden light-proof chambers (to be described later) the ambient temperature did not fluctuate much. Besides, the experimental facility was located inside the main fish culture unit of Aston University, which is maintained at a fairly constant temperature during severe winter by the heating system of the building. During summer time the water temperature in recirculation system was not much affected and did not go above 16.0^{o}C at any time throughout the summer.

During experimental period maintenance of the recirculation system was performed by cleaning the filter tank once during each month. PVC pipes from experimental tank to faecal trap and from faecal trap to filter tank were cleaned, with a long wired brush once a week. Stand pipes in the experimental tanks were removed and cleaned once a week.

3.3.5. Light Chambers

Six wooden chambers, three each for the two recirculation systems were constructed. Each wooden chamber measured 1.22 x 1.52 x 2.29m (Figure 6). Each chamber was light proof and was double walled in order to help in keeping the temperature constant. The light proof chambers were made of ply-wood supported by wooden frames. Each chamber had a door, which was cut through, by a window measuring 50.96 x 35.00 cm. Through these windows, food dispensers were filled with food, with the least disturbance to the fish. On the ceiling of each chamber a rectangular space was cut through with two fluorescent tubes of 60.96 cm 20 watts each, positioned 122 cm from surface of water and fitted with diffusers, were fixed. On the roof, over the cut part of the ceiling, wooden frames were fitted and were covered over with heavy duty black polythene, to prevent any outside light entering the light-proof chambers during the dark period.

The lights were connected to the mains through "Sangamo" 24-hour dial synchronous time switches, which were individually fitted to each of the light chambers, so that photoperiod could be controlled automatically at present regime.

3.4. Demand Feeding System

It has been shown by previous workers (Adron et al, 1973; Landless, 1976) that rainbow trout can be trained to press a trigger to obtain food. Such training or conditioning of the fish can be exploited by fish

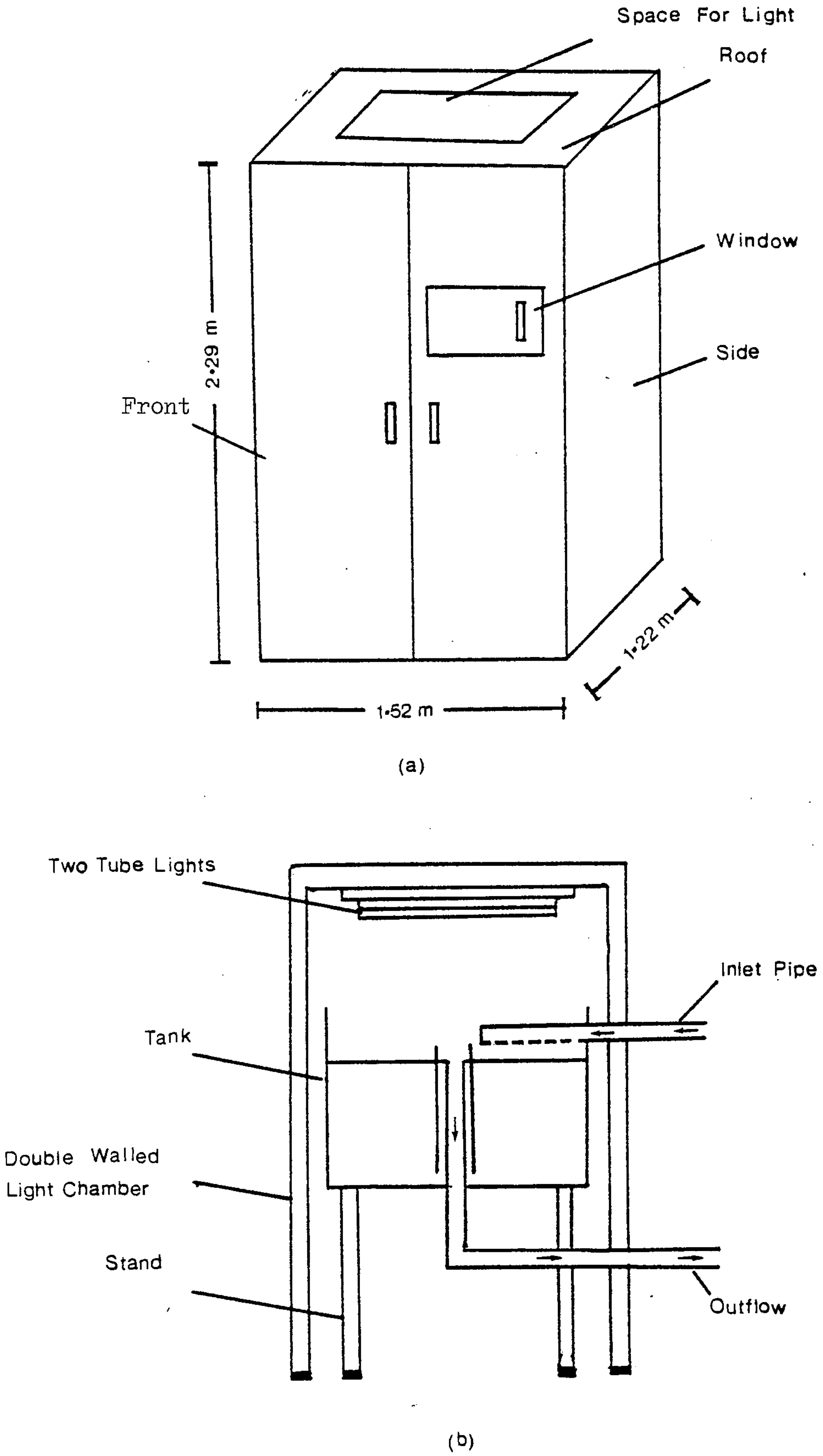


FIGURE 6 (a) An outside view of the Light Chamber

(b) An inside view of the Light Chamber

culturists for better stock management methods. The cost of feed is one of the factors that most influence production costs of intensively cultured rainbow trout. Demand feeding can be an attractive alternate to fish culturists interested in replacing labour-intensive hand feeding. Demand feeding can provide the fish with food, whenever desired by the fish, so that the feeding frequency would be much higher than hand feeding. Due to the increased feeding frequency it would be expected that an improved feed conversion would be achieved, as has been found by previous workers (Deyoe _et al.,_ 1968; Collins, 1971; Andrews and Page, 1975).

Demand feeding can give a fairly accurate assessment of the trouts' preferred rate of food intake. Since the amount of food dispersed at every trigger actuation can be adjusted, therefore, the level of food intake can be accordingly adjusted without resulting in any food wastage.

3.4.1. Description of the Demand Feeding System

Different parts of the demand feeding system were constructed in the laboratory from components and with tools which are cheap and are readily available. Only the electrical components, which did not cost much, had to be bought. Demand feeding unit was built on the same principle as described by Adron _et al._ (1973), with some minor changes.

3.4.2. Control Box

Demand feeding unit consisted of a control box (Plate 1) which contained all the electrical components connected up as shown in the circuit diagram (Figure 7). Connected to the control box were:

1. Triggers
2. Solenoids that controlled the action of the plunger of the feed dispenser; and

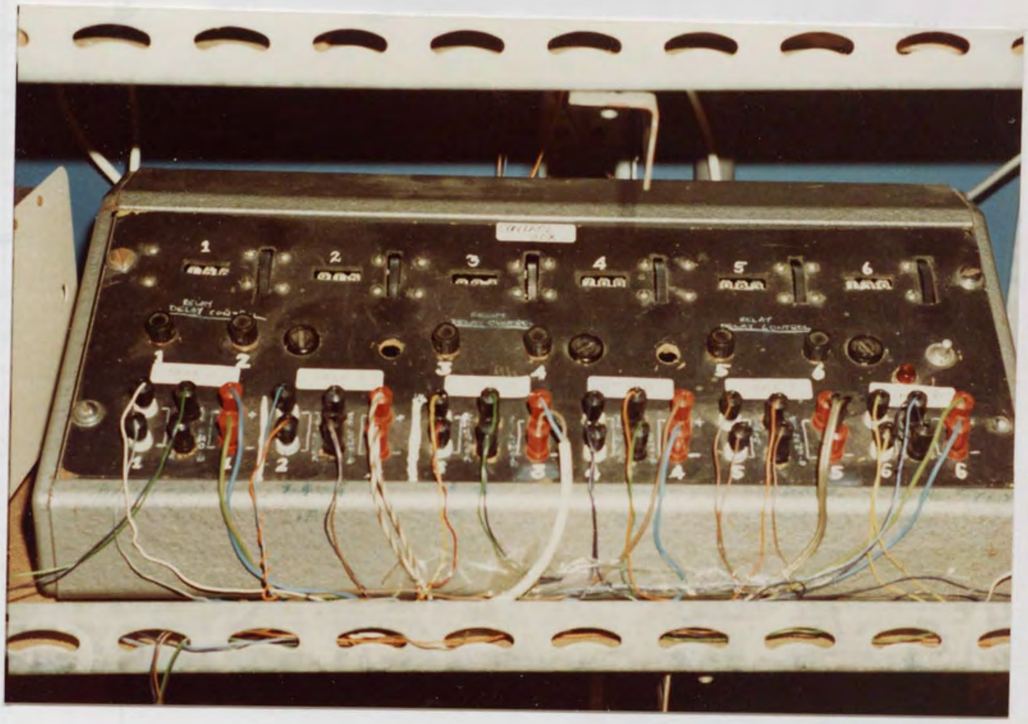


PLATE 1 CONTROL BOX

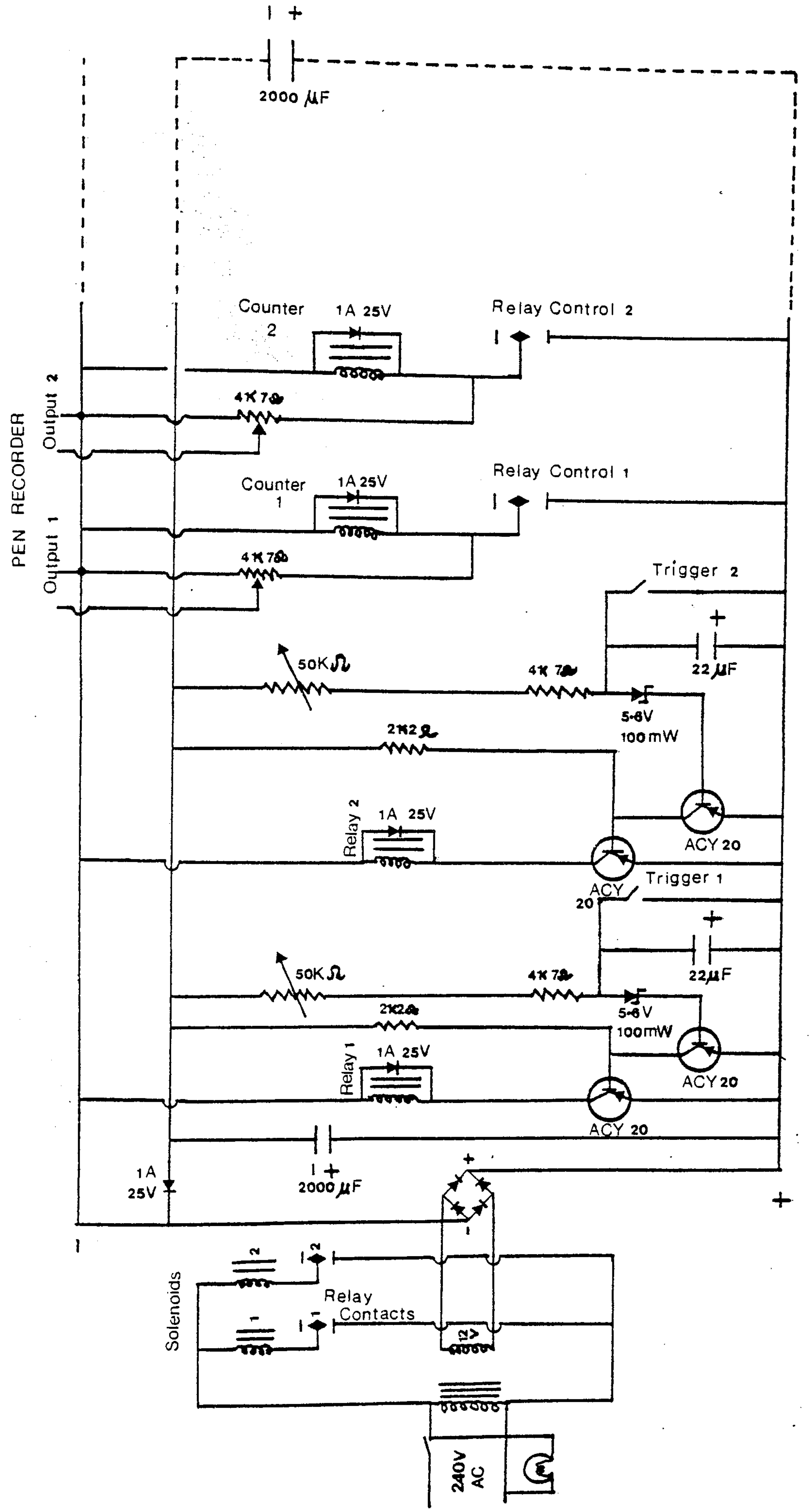


FIGURE 7 The Circuit Diagram of the Demand Feeding System

3. Recorders, which recorded the trigger actuations.

A red indicator light was included in the circuitary of the control box, which was lit up all the time, when the demand feeding system was working in perfect condition.

The demand feeding system worked perfectly well, during the whole time of different experiments. The Control box was fitted with an electrical circuitary for six independent experimental tanks, with different outlets for triggers, solenoids and event recorders for each of the six experimental tanks. The Trigger actuations of each of the six experimental tanks were shown on six independent counters attached on the control box. Counter readings were noted every day for each tank during the experimental period.

3.4.3. Feed Dispenser

The feed dispensers were constructed, using 20 ml disposable plastic syringes, funnel shaped plastic cups, 6 BA threaded brass rods, brass nuts, 0.32 cm thick perspex sheet, 5.08 cm long 2 lb extension springs, solenoids and plastic glue (Araldite).

The feed dispenser was constructed on a simple principle of food pellets being "pushed and dropped" by an advancing plunger. Upon retraction of the plunger, pellets dropped from the hopper to occupy space vacated by retracted plunger and eventally pushed out slowly, into the experimental tank, upon the advancement of the plunger.

Each feed dispenser (Figure 8) had a hopper made up of a funnel shaped disposable plastic cup. The bottom of the cup was cut to a shape, such that, it could sit directly on the tubular portion of the unit, at an appropriate angle. The tube was a 20 ml disposable plastic syringe, from

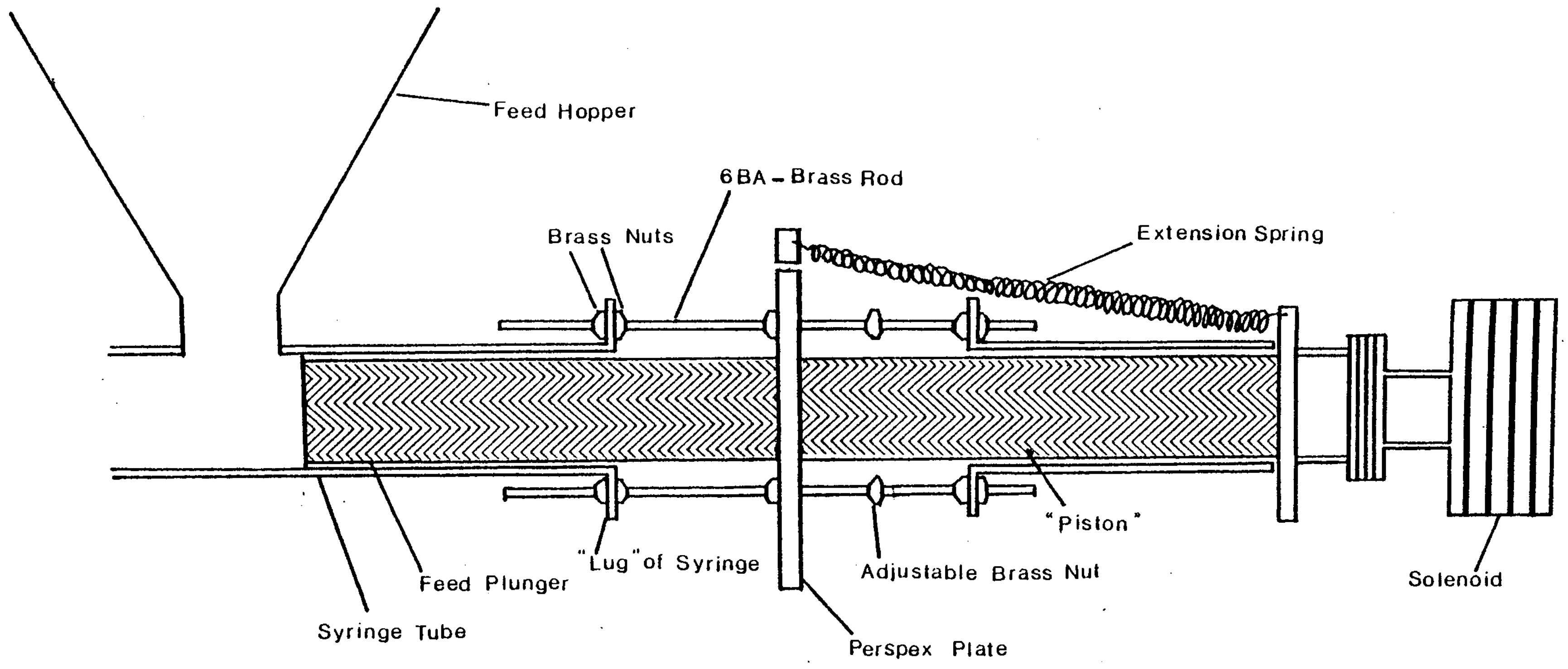


FIGURE 8 A Diagrammatic representation of a Feed Dispenser Unit

which the "closed" end had been cut. A hole was made in the syringe near the cut end using a 1.27 cm heated cork borer. It was trimmed to a suitable size using a knife and file. The cup was then sellotaped in position over the hole. The cup can be permanently fixed in position by brushing over the sellotape with a suitable hardner, such as fibre glass resin. This keeps the feeder intact in the dampest conditions.

The plunger was the normal syringe plunger cut to an appropriate length 6.35 cm in this instance. A plastic disc, slightly smaller in diameter than the tube, was fixed onto the end of the plunger to prevent pellets escaping. Movement of the plunger was powered by a second syringe fixed rigidly to the first by two 6 BA threaded brass rods inserted into holes drilled into the "lugs" of each syringe tube and held by brass nuts fixed on each side. The second syringe was cut off from the closed end and its plunger was inserted through the cut end. This second syringe and the plunger (henceforth called the piston) together worked as a "power-unit". The dispenser plunger and piston were glued to a shaped piece of 0.32 cm thick perspex, which accommodated one end of a 5.08 cm, 2 lb extension spring.

Brass nuts were threaded onto the brass rods connecting the two syringe tubes and passing through the shaped piece of perspex joining the plunger to the piston. Nuts inserted on the piston side allowed for slight alteration of tension in the extension spring whilst nuts inserted on the plunger side acted as stops and could be adjusted to give a variable traverse of the plunger/piston unit.

Feed dispenser was attached upon a suitably sized wooden board, which was fixed with an angular hold, so that the feed dispenser could be adjusted to any suitable angle. The wooden board was fixed firmly to the rim of the experimental tank. The working of the food dispenser was controlled by a solenoid. The feed dispenser piston was connected to the armature of an A.C. solenoid with a 3 lb pull and a maximum 2.54 cm travel. The Extension spring was connected to the end of the piston and the piece of perspex in between glued ends of piston and food plunger. The position of the solenoid was adjusted so that the solenoid armature travelled 1.27 cm - 1.91 cm, and in doing so, pulled the piston from its "resting position" to its "action position". It was required that the extension spring be powerful enough to return the piston to its "resting position" so that the feed pellets from the hopper which occupied the space vacated by the plunger, be pushed out and dropped into the experimental tank. As the piston returned to its "resting position" the armature of the solenoid travelled back to its original position, under the force of the pull of the extension spring.

The solenoid was activated only when the fish would activate the tip of a trigger, by a gentle push with its mouth.

3.4.4. Trigger

A trigger worked simply like an electric switch. Essentially, it was a contact made by two wires to complete an electric circuit. In this case, it was the trained fish which made the contact, by touching the tip of the trigger.

Each trigger was made from a 20 ml syringe (Figure 9). The plunger of the syringe was removed and discarded and the bottom of the syringe was cut off the outer case. The solid rubber seal from the end of the plunger was then removed. A small hole was burned in the seal and a 15.24 cm length of insulated rigid copper wire was pushed halfway through the hole. The wire was held tightly in the rubber seal. When the wire

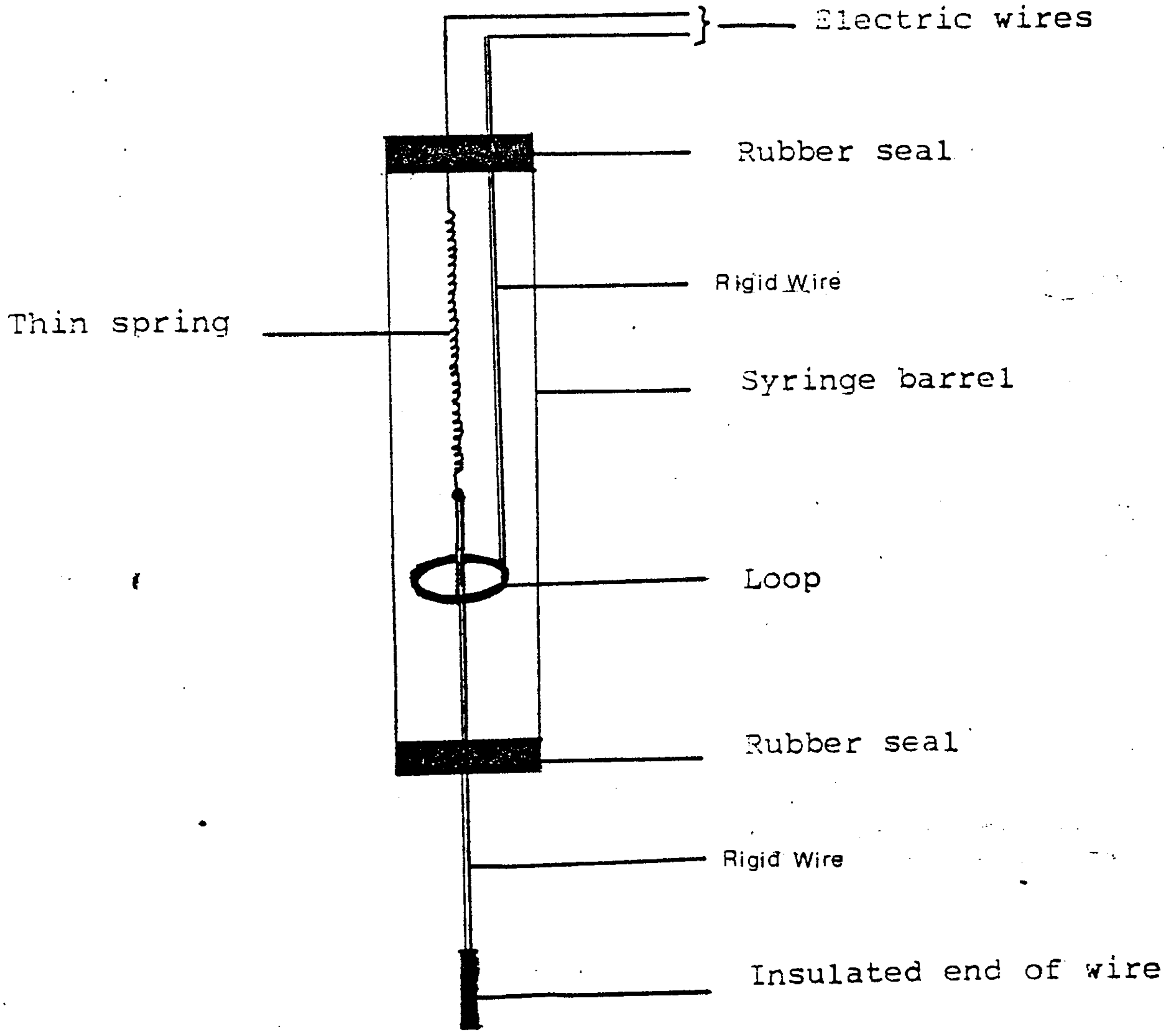


FIGURE 9 A Diagrammatic view of the Trigger

was pushed laterally at one end, there was an equal and opposite movement at the other end. When released, the wire returned to a central position by virtue of the elasticity of the rubber seal. Also a 5 cm long thin spring was attached at the inner end of the copper wire by making a small loop at its end which helped in preventing the trigger becoming stuck and making continuous contact. The rubber seal was then pushed into the bottom of the syringe case, where it fitted tightly, sealing the bottom end of the syringe case. A rubber seal was removed from a second 20 ml syringe. A small hole was burned in the seal and another 15.24 cm length of insulated rigid copper wire was pushed through the hole. The insulation of the copper wire was removed from the tip and a loop of 6 mm diameter was formed. Through the rubber seal another small hole was burned adjacent to the first one, through which 2.0 cm straightened length of the thin spring was protruded out and this was first passed through the 6 mm diameter loop. Care was taken that a portion of 5 mm of the top end of the lower 15.24 cm long copper wire passed through the loop without making any contact. The rubber seal was then forced into the top of the 20 ml syringe case. The position of the lower 15.24 cm long rigid copper wire was adjusted so that its upper end was just inside the loop of the upper copper wire but did not touch it, and a small movement in any direction was sufficient to make an electrical contact. The lower tip of the wire up to 15 mm length, was covered with a water-proof red plastic tape. The free ends of the straightened portion of the spring and the looped copper wire, which protruded out of the upper rubber seal of the syringe, were connected independently to the control box.

3.4.5. Event Recorders

In addition to recording the number of trigger actuations per unit time, it was considered desirable to record the distribution of trigger actuations during a 24 hour period. In view of this desirability event recorders (Plate 2) were purchased (See Appendix). Whenever the trigger was actuated, feed dispenser, counter and recorder operated simultaneously. In this way a series of short black lines were recorded on the chart of the recorder, each time food was dispensed by trigger actuations. The speed by which the chart moved on the recorder could be adjusted and exact time of trigger actuations could be determined.

3.4.6, Operation of Demand Feeding System

The demand feeding system which was constructed from different electrical components and readily available inexpensive materials, operated quite satisfactorily over the different experimental periods, without needing much servicing and replacement of components. It was capable of dispensing a range of food sizes up to 8 mm pellets without jamming. Amounts of pellets dispensed could be varied and no dusting of the pellets occurred during dispensing. The hopper of the feed dispenser could hold enough quantity of feed for a minimum of two days at the maximum feeding rate envisaged.

The control box was fitted with electrical circuitary for six independent demand feeding units. Each unit was comprised of one trigger and one feed dispenser with connections to the trigger actuation counter and the event recorder. Any number from one to six, of these demand feeding units could be used in a single experimental tank, depending upon the nature of the experiment (up to four demand feeding units were used in a single experimental tank for coloured food pellets experiments). The trigger was fixed in the experimental tank, with its tip submerged just up to 5 mm on the surface of water. The Feed dispenser along with the solenoid was attached upon the experimental tank about 30 cm in front of the

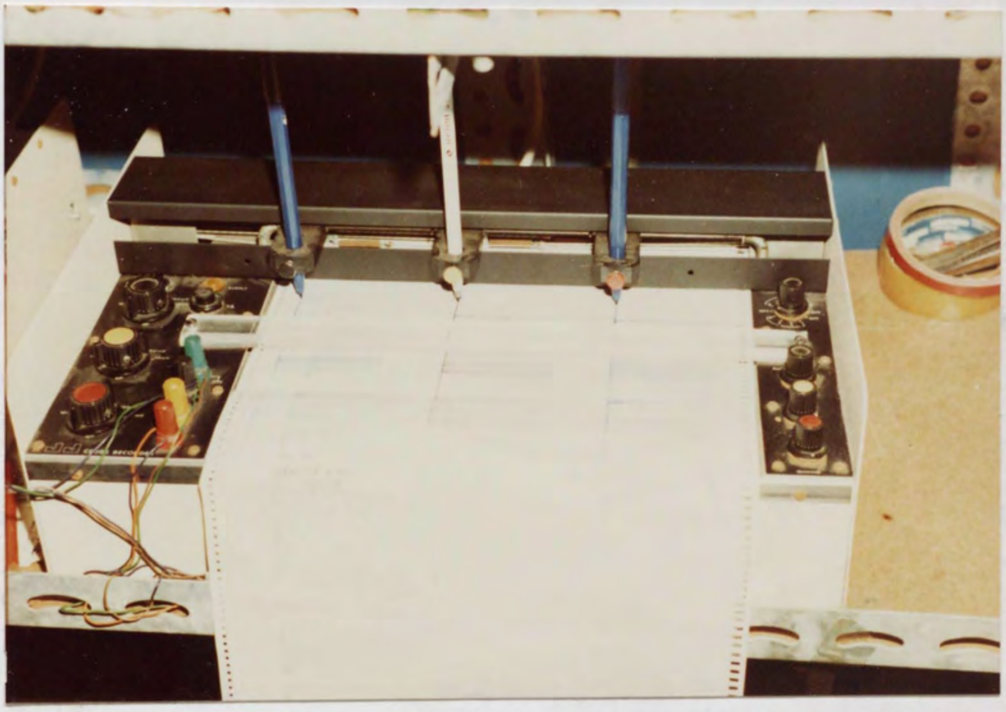


PLATE 2 EVENT RECORDER

trigger.The Rest of the electrical components were incorporated inside the control box. When the trigger was actuated by fish, by gentle push with its mouth, electrical contact was made in the syringe of the trigger and a relay was actuated. This operated a counter in the control box and also the solenoid which was attached to the feed dispenser unit. The solenoid retracted the piston along with the plunger into "action position" from the "resting position", so that the space vacated by the retracted plunger was occupied by the feed pellets which drop down from the hopper. The extension spring would start pulling the piston back to its original position, a small quantity of feed pellets which had occupied the space in the barrel of feed dispenser, was consequently pushed into the experimental tank through the open end. The quantity of feed dispensed during each operation could be adjusted, by altering the traverse of the plunger. The traverse of the plunger could be shortened or lengthened by moving backwards or forwards the adjustable brass nuts (See Figure 8) on the threaded brass rods of the feed dispenser.

3.4.7. Determination of Feed Dispensed / Trigger Actuation

The food dispensers in the experimental tanks were tested before starting any experiment and at biweekly intervals during the experimentation period for proper functioning and for checking the amount of feed being dispensed at every trigger actuation. For testing, the trigger in the experimental tank was manually actuated and feed collected and weighed to determine the amount dispensed. Twenty repeat trials were performed and meaned and standard deviations calculated and thus feed dispensed / trigger actuation was determined.

3.5. Quarantine Procedures

Quarantine facilities existed in a building separate from the main fish culture facilities. Access to this area was via a sterilised footpath and all equipment used was sterilised and restricted for use in the quarantine area only.

Upon arrival each batch of fish was placed in a prepared sterilised tank. For the first eight days the fish were fed a specially prepared antibiotic diet, to eliminate bacterial pathogens, and for the remainder of the time in quarantine, were fed a commercial fish feed.

To eliminate any external parasites, the fish were bathed in 150 ppm formalin for one hour, after seven days of their arrival in the quarantine facility. After a further five days the fish were bathed on three successive days in 2 ppm malachite green for one hour, under vigorous aeration.

Fish were kept in quarantine for three weeks before being transferred to the experimental tanks and during this period, were carefully observed for any abnormalities and samples were examined microscopically for pathogens.

3.6. Fish Training, Maintenance and Handling

Rainbow trout *Salmo Gairdneri* (Richardson) were used as experimental animals for all the different experiments. Upon transference from the quarantine into the experimental tanks the fish were left to be conditioned to actuate the trigger in order to obtain the food. This conditioning period lasted for not more than ten days, since the fish acclimatised and adapted itself in experimental tanks within a week. The number of trigger actuations were noted from the readings on the counters, on the control box and by the tenth day trigger actuations were noted to be fairly stable. Before starting every experiment, a period of ten days was counted as the training time. The trout were very quick in learning to actuate the trigger and obtain food. This confirms the findings of previous workers such as Adron _et al_. (1973). For training of fish to obtain its food, by actuating the trigger no effort upon the part of the experimenter were required. The fish when introduced into the experimental tank started to bite aggressively at the small submerged tip of the trigger needle. That biting was done with a certain show of restraint, at the beginning of the training period. Perhaps the fish considered the tip of the needle to be a food pellet but during that attempt for food, when the fish was rewarded with food pellets, it learned the beneficial effects of touching the trigger needle. After a week's time, aggressive biting behaviour changed and thereafter, the fish were seen to touch the trigger tip, with a gentle push with the mouth. The number of trigger actuations did not deviate much after a week's training time.

During all the experiments, except where mentioned, the fish were provided with commercially manufactured, pelleted hatchery diet (Edward Bakers, Sudbury, Suffolk). In all cases size of food pellets was determined according to the recommended size, in the feeding tables of the manufacturers, except for experiments on size of food pellets. Food hoppers of the feed-dispensers were filled up with food pellets, as and when required, often after two days. Care was taken not to exert any undue exertion or fatigue upon the fish during the experimental periods. Any routine maintenance and repairs inside the light-proof chambers were done with least disturbance to the fish and in as little time as possible.

3.7. Tagging and Weighing Procedure

For experiment in Chapter 7, the fish were individually tagged. 5mm wide embossing "Dymo" tape was used as tags and each tag was embossed with numerical number starting with one. The tape was trimmed to an oval shape for individual tag and 1mm diameter hole was punched on one side. The tags were attached by a loop of 0.5mm diameter silver wire inserted just in front of, and below, the dorsal fin.

Fish were anaesthetised with Benzocaine (Ethyl-4-aminobenzoate, BDH) at a concentration of 50 mg/l. They were then removed from the anaesthetic and a 1mm diameter hypodermic needle inserted through the fish at the desired tag attachment position. One end of a 5cm length of silver wire was inserted into the needle and drawn through the fish by withdrawal of the needle. A tag was then threaded onto the silver wire and the wire bent over the back of the fish, allowing a generous loop for growth of fish, the free ends were then securely twisted together and the excess trimmed off.

Tagging of fish enabled identification of each fish and the keeping track of its growth rate during the course of the experiment. Also, tagging enabled instant identification of any mortality in the population.

For anaesthetising the fish for other experiments MS-222 (tricaine methane - sulphonate, Sandoz, Basle) at a concentration of 50 mg/l was also used. In Experiments which necessitated individual weighing, it was done by first subjecting the fish to starvation for twelve hours and then anaesthetising the fish in groups of two to four at a time. After being anaesthetised, the fish was removed from the anaesthetic, lightly blotted on a paper towel, and placed on a tared small rectangular pad of absorbent material situated on the pan of a Sartorius 3719 MP dual range balance. The fish and pad were weighed to an accuracy of ± 0.01g and the fish returned to its tank where complete recovery occurred within three to four minutes. The absorbent pad was then weighed and the difference between the weight and the weight of the pad and fish was taken as the live weight of the fish.

For some experiments the fish were first starved for twelve hours and then batch weighed, one tank at a time. Again the fish were anaesthetised, a whole tank at a time, in a 10 l plastic container, they were then allowed to drain for ten to fifteen seconds in a net, with gentle shaking, before being transferred to a tared bucket of water on a Sauter 10 kg side pan balance weighing to 1g. The fish when returned to their tanks, recovered completely from the anaesthetic within three to four minutes.

3.8. Diet Formulation

For experiments in Chapter 5 on colour of food pellets and Chapter 6 on calorific value of food semipurified rations were prepared in the laboratory.

Diet formulated for Experiment 7 contained a simple protein source (herring meal) to which were added purified ingredients to form a nutritionally complete ration.

The method of formulation adopted would, it was hoped, minimise the effects of dietary ingredient variation and thus allow preparation of isonitrogenous diets. The protein source (herring meal) to be used was first analysed for moisture, crude lipid, crude protein, ash and nitrogen free extractives (N.F.E.) as detailed in Section 3.10.

The amount of protein source required (xg), per 100 g of diet, in order to give the desired level of protein was then calculated.

The quantity of crude lipid in x g of the protein source was then calculated and the amount of herring oil to be added, to 100 g of diet, to achieve the desired final lipid level, found. Similarly, the quantity of ash in x g of the protein source was balanced to the desired level with a mineral mixture (Table 4). NFE in the protein source was assumed to be a mixture of carbohydrates with an average digestibility equal to that of starch with which the NFE was balanced to the desired level.

The amount of moisture in each dietary ingredient was determined analytically and the quantity in the diet subtracted from the running total so that formulation was on a dry weight basis for all ingredients. To both diets, 1% binder (carboxymethylcellulose, sodium salt, high viscosity), 1.5% vitamin and trace element supplement (Table 5) were added. The remainder of the diet was filled with a mixture of glucose, starch and α-cellulose to achieve the desired energy level.

Basic ingredients used in the formulation of the diet for Experiment 5 in Chapter 5 for studying the effect of coloured food on the feeding behaviour, instead of using herring meal as protein source, casein was used. Similarly, instead of herring oil, cod-liver oil was used as the main source of lipid. Main reason for this being that casein besides being very rich in protein is also white in colour and therefore was best suited an ingredient which could be dyed to any desired colour. The other ingredients as well, were all nearly white and therefore, the desired coloured food was easily prepared.

TABLE 4

Mineral Supplement Composition

From: test diet H440 Western Fish Nutrition Laboratory (NFE, 1973)

A combination of:

Premix No. 5 (Mineral) in grams

Aluminium chloride	0.015
Potassium iodide	0.015
Cuprous chloride	0.010
Manganese sulphate	0.080
Cobalt chloride	0.100
Zinc sulphate	0.300

and

Salt Mixture No. 2 in grams

Calcium biphosphate	13.58
Calcium lactate	32.70
Ferric citrate	2.97
Magnesium sulphate	13.20
Potassium phosphate	23.98
Sodium biphosphate	8.70
Sodium chloride	4.35
TOTAL	100.00

TABLE 5

Composition of Vitamin Premix

This premix was supplied as a commercial fish diet additive by B.P. Nutrition.

	mg/kg of premix
Vitamin A	0.012 m.i.u.
Vitamin D_3	0.0015 m.i.u.
Vitamin E	60
Vitamin K	15
Thiamine	10
Riboflavin	25
Pyridoxine	15
Biotin	60
Vitamin B_{12}	2
Nicotinic acid	150
Pantothenic acid	50
Folic acid	4
Choline chloride	1130
Vitamin C	60
BHT (antioxidant)	1000
Iron	20
Cobalt	200
Manganese	30
Copper	200
Zinc	50
Iodine	4500

3.9. Diet Preparation

For Experiments number 5 and 7 diets had to be formulated and prepared. The amount of diet required for each experiment was estimated from the starting weight of the fish and by the duration of the experiment and from the expected maximum growth rate assuming a food conversion ratio of 1(g dry feed fed per g live weight gain). Five percent was added to this figure to allow for losses during pelleting, drying and sieving of the dry diet to the required size, as well as the taking of samples for analysis.

All dietary ingredients were sieved to a particle size of less than 1mm prior to weighing and pelletising to ensure that a homogeneous mixture was obtained. The dry ingredients were then weighed out, according to the formulation, placed in the bowl of a Hobart A200 (Hobart Limited, London) food mixer and thoroughly blended for three minutes. To this mixture was added the weighed quantity of oil (Herring Oil for Experiment 7 and Cod Liver Oil for Experiment 5) and blending continued for a further three minutes. Warm water (28°C) was then added to the diet, with mixing, until a stiff dough was obtained. To the diet used for the experiment on coloured food pellets concentrated edible liquid colourants bought from a local supermarket (Asda) were added along with the warm water.

After addition of the water, the stiff dough was extruded through the mincer attachment of the food mixer, using a 3 mm die, into long sphagetti-like strands. These were dried on porous trays by having warm air (40°C) blown over them from an electric fan convector heater.

When the diet was almost dry to the touch, these strands of diet were broken up into smaller lengths by rubbing between the hands and then further dried. The dry diets were seived to remove very small bits and any powdered diet, and only approximately 4mm pellets of diet were collected for storage at -20°C, inside sealed polythene bags, until required for feeding.

3.10. Methods of Proximate Analysis

Proximate analysis of diets and dietary ingredients were carried out by the following procedures:

Moisture

Moisture content was determined by air drying the samples in an oven at 105°C for forty-eight hours.

Crude Lipid

Crude lipid content was determined by extracting dried samples for four hours, using a Soxhlet apparatus (A Gallenkamp and Company Limited), with forty to sixty degrees centigrade boiling range petroleum ether and measuring, by weight difference, the amount of ether soluble material extracted.

Crude Protein

Crude protein content was determined by the microkjeldahl method for determining nitrogen (A.O.A.C. Methods, 1970) and applying the empirical factor of 6.25 to the results to convert total nitrogen to total crude protein.

Ash

Ash content was determined by heating samples in a muffle furnace (FR 610A, A Gallenkamp and Company Limited) for twelve hours at a temperature of 500-550°C.

Nitrogen Free Extractives (NFE)

NFE : was determined by calculation.

NFE = 100 - (% moisture + % crude lipid + % crude protein + % ash)

Energy

Energy contents were determined using a ballistic bomb calorimeter (A Gallenkamp and Company Limited).

3.11. Analysis of Experimental Data

3.11.1. Specific Growth Rate

In the early stages of life the growth of a fish under controlled conditions follows an exponential curve the equation of which is:

$$W_2 = W_1 \cdot e^{g} \quad (T-t)$$

Where W_2 is the final weight (at time T) and W_1 the initial size (at time t). T and t are expressed in units of time (usually days); W_2 and W_1 in units of weight (usually grams); e is the base of natural logarithms; g is a constant for a particular curve known as the specific, instantaneous, geometrical or multiplicative growth rate.

Rearrangement of the above equation, to obtain g, and multiplying by 100 gives the rate of change in weight of the fish, expressed as percent per day; this is commonly termed the specific growth rate (SGR).

$$\text{SGR} \quad (\% / \text{Day}) = \frac{\log_e W_2 - \log_e W_1}{T - t} \times 100$$

(Brown 1957)

3.11.2. Food Conversion Ratio

The food conversion ratio (FCR) is defined as the amount of food fed per unit live weight gain of fish:

$$\text{FCR} = \frac{\text{Food Fed. (g)}}{\text{Live Weight Gain (g)}}$$

3.11.3. Statistical Methods

Statistical comparisons between means were made by analysis of variance and by using Duncan's Multiple Range F-Test (Duncan, 1955). Standard Errors (± SE) were calculated from the residual mean square in the analysis of variance and are presented, where relevant, to indicate the range of means tested.

CHAPTER 4

Effect of Light Variations on Feeding Behaviour of Rainbow Trout

4.1. General Introduction

In many physical and chemical aspects open water bodies present a relatively uniform environment for the fish. Fishes, through the course of their evolution, have adapted themselves to a wide range of external stimuli, that exert direct effects upon their behaviour and physiology. Light can be considered to be one of the most important factors that play a significant part in determining the behaviour of the majority of fish. Light provides the necessary conditions for the existence of fishes and permits orientation for many animal species. The way in which light may affect fish are poorly understood and complex. The light intensity, angular distribution, polarisation, spectral composition and the duration of daylight, may all exert direct effects upon behaviour and physiology of fishes. Due to its speed, light gives the earliest possible information concerning the distant environment, and its directional properties are also of great value in the perception of form and movement, as well as in orientation. But it must be realised that in water, light becomes scattere and consequently its value in providing early information is reduced. Like any other stimulus, especially constant ones, light plays a signal role, preceding the biotic and abiotic changes in the environment of organisms. Under experimental conditions the switching on of a light can set in train daily activity rhythms during the night hours. Fishes gather in schools, begin to hunt for food and feed on plankton. The specific form of daily activity is the behavioural adaptation of animals to their environment. The daily rhythms of feeding in fishes depends upon the more or less favourable periods for discovery and capture of food. The gradual increase in the light intensity in the early morning hours and the gradual decrease as the night sets in, serve as signals of change in biotic environmental conditions. This is reflected in the behaviour of fishes from different ecological groups belonging to different links of the food chain of the water body.

Many studies have been made to investigate the combined effect of light and temperature on growth. Light periodicity has been manipulated in relation to the question of smolt transformation or of inducing early maturation with growth responses being additionally recorded. There are very few studies in which light has been manipulated to study the feeding behaviour of fish. There are multiple ways in which light can be manipulated, its quality, quantity and periodicity can be varied to study the feeding behaviour under controlled conditions in the laboratory. It's interaction with other environmental factors, particularly temperature, and the possible harmony or disharmony with endogenous rhythms of fish can also be studied.

In the present work, effect of light on the feeding behaviour of rainbow trout *Salmo gairdneri*, was investigated under different constant photoperiod regimes. The feeding behaviour was studied under constant light periodicity (Experiment 1) and also under the effect of varying daylength (increasing and decreasing daylengths, Experiment 3). Effect of different levels of light under different constant photoperiods, on feeding behaviour were studied (Experiment 2) to provide additional information on the relation between light intensity and behaviour of rainbow trout. Since for all the experiments recycling systems (See Section 3.2) were used and the experimental tanks were enclosed within the light proof wooden cabinets (Section 3.3.5), there was little variation in temperature. Demand feeders (Section 3.4) provided equal opportunity for fish in each experimental lot to feed ad libitum because food was constantly available throughout the experimental period. The whole work on the study of the effect of light on feeding behaviour was divided up into three separate experiments.

In Experiment 1, nine groups of fish were subjected to nine different photoperiod regimes of the same level of light intensity.

In Experiment 2, based upon the results of the first experiment, feeding behaviour of rainbow trout was further investigated on nine groups of fish under three different photoperiods, with three different levels of light intensity for each photoperiod.

In Experiment 3, effect of increasing and decreasing photoperiods on feeding behaviour of two groups of rainbow trout were studied. Fish were subjected to simulating effects of increasing and decreasing daylength and their feeding responses were monitored throughout the experimental period.

4.2. Experiment 1 : Feeding Behaviour Under Constant Photoperiods

4.2.1. Introduction

Rhythmicity is a characteristic of many physiological processes in both plants and animals. Some of these rhythms appear to be under endogenous control, although many are influenced by external factors such as lunar tidal, rainfall and temperature cycles. In latitudes where seasonal changes in daylength are apparent, the photoperiod has been shown to be an important factor in the control of rhythmicity. In fish, it has been demonstrated that the photoperiod has pronounced effects on seasonal and diurnal processes (See also Section 4.1. General Introduction), but there is little data available at present on its effects on the daily feeding pattern.

Studies on the diurnal activity pattern of fish, though not necessarily an indication of feeding activity, have shown that under natural conditions many species exhibit a crepuscular rhythm (Alabaster and Robertson, 1961; Young, Tyler, Holliday and MacFarlane, 1972). Such studies suffer from the lack of control of other factors that can affect rhythmicity, such as the availability of food and temperature. This can be overcome in the laboratory by the use of demand feeders, which in effect make food constantly available, and temperature control being especially easy in recycling systems with experimental tanks being placed in double walled insulated environmental chambers. Adron et al. (1973) and Landless (1976) have shown that fish soon learn to operate demand feeders, and also that demand feeders are of great value in the study of various aspects of feeding behaviour.

The purpose of the following experiment was to observe the influence of nine different lighting regimes on the feeding behaviour of rainbow trout maintained in recycling systems.

4.2.2. Materials and Methods

4.2.2.1. The Experimental System and Animals

The experimental facility used in this study was the recirculation system as described in detail in Section 3.2.

The experiment was performed on nine groups of twenty-five fish each. Fish (10-15cm) were obtained from Burwarton Fish Farm, Cleobury, Shropshire. Fish were quarantined, as detailed in Section 3.5 and removed to the experimental facility.

Fish were left for acclimation and training to learn to actuate the trigger for food supply (Section 3.6), which lasted for at least ten days for each group of fish.

Water quality for all trials was maintained as shown in Table 2 and regular water quality measurements were taken, as described in Section 3.2. Temperature of water in the experimental tanks did not fluctuate much during different trials. Average temperature of water was $13.0^{0} \pm 2^{0}$C.

4.2.2.2. Different Photoperiods Studied

It was planned to study nine photoperiods on nine different groups of twenty-five fish each (average weight 38.16g±0.77) over a period of four weeks for each group. Each successive photoperiod regime differed from one another, by three hours in duration of light period.

Nine photoperiods studied were three hour light (3L)/21 hour dark (21D), 6L18D, 9L15D, 12L12D, 15L9D, 18L6D, 21L,3D, 24L0D (continuous light) and 0L24D (continuous dark). For all photoperiods, light intensity at the water surface was 160 lux during light regime.

For every photoperiod, light in the wooden light chambers was controlled by automatic time switches as described in detail in Section 3.3.5. Before starting a study of each photoperiod, the individual time switch had to be pre-set once to the desired photoperiod regime, so that during each day of the trial, transition between darkness and light and vice versa was sudden and complete.

4.2.2.3. Monitoring the Feeding Activity

A demand feeding system described in detail in Section 3.4 was used for studying the feeding behaviour under the different photoperiod regimes.

Each experimental tank was provided with one feed dispenser connected to one trigger, according to the relative positions as shown in Figure 10. The trigger was left in the tank at all times and positioned in such a way that, it's tip just broke the water surface to minimise accidental contact by the fish. Each trigger was given a routine testing, as described in Section 3.4.7. Food delivered at each trigger actuation was determined for each group and is shown in Table 6. Total amount of food consumed in a day was worked out by total number of trigger actuations for that day and average amount of food consumption/fish/day was calculated for each group and is shown in Table 7. The number of trigger actuations performed by groups of fish under different photoperiod regimes were noted daily by the readings shown by counters on the control box of the demand feeding system (See Section 3.4.2.). For each group mean number of trigger actuations/day were calculated from total number of daily

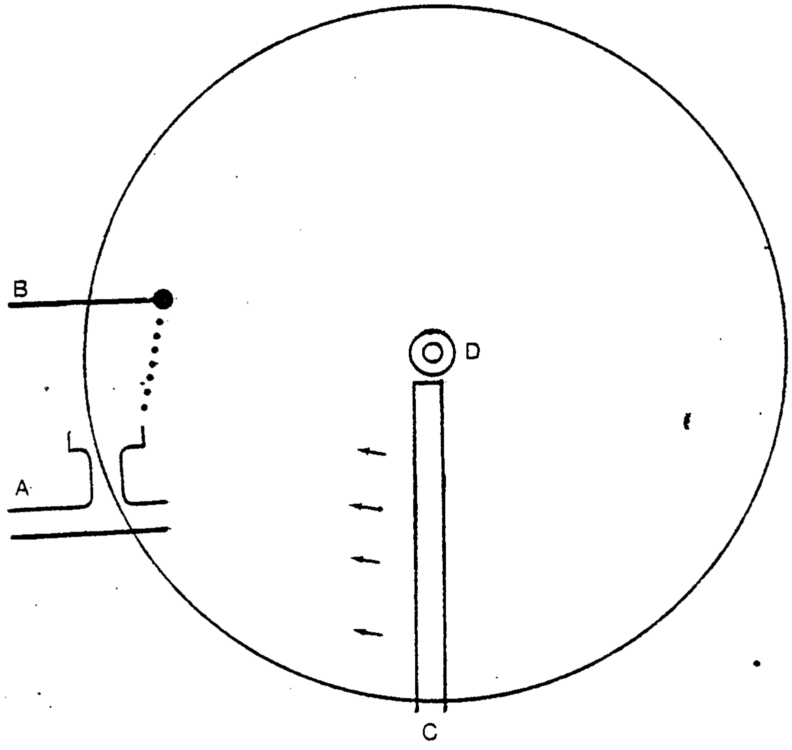


FIGURE 10 The relative positions of the Feed Dispenser and the Trigger in the Experimental Tank for Experiment 1.

A = FEED DISPENSER

B = TRIGGER

C = INLET PIPE

D = OUTLET PIPE

TABLE 6

Daily mean trigger actuations and amount of food delivered per trigger actuation in Experiment 1 (Means and standard deviations shown for 28 days)

Photoperiods	Mean Trigger actuation/day	Food Dispensed per trigger actuation g.
3L21D	70 S.D. ± 14	0.35
6L18D	84 S.D. ± 21	0.34
9L15D	92 S.D. ± 20	0.34
12L12D	99 S.D. ± 18	0.37
15L9D	124 S.D. ± 15	0.35
18L6D	136 S.D. ± 16	0.33
21L3D	115 S.D. ± 32	0.37
24L0D	105 S.D. ± 18	0.37
0L24D	4 S.D. ± 2	0.33

TABLE 7

Growth and Food Utilisation Data from Experiment 1

25 Fish/Group Photoperiod	Average Food Consumed/fish/ day	Average Weight Gain after 28 days	FCR[1]	SGR[2]
3L21D	0.99	18.09	1.53	1.38
6L18D	1.14	20.18	1.58	1.51
9L15D	1.25	25.62	1.37	1.81
12L12D	1.46	20.79	1.97	1.54
15L9D	1.74	24.14	2.02	1.72
18L6D	1.80	21.87	2.30	1.62
21L3D	1.70	24.58	1.94	1.80
24LOD	1.55	14.10	3.08	1.17
OL24D	0.04	-4.56	x	x

X = Not calculated due to weight loss

1 Food Conversion Ratio

2 Specific Growth Rate

actuations performed in twenty-eight days of the experiment, as shown in Table 6.

Trigger actuations were also recorded on a chart recorder over a period of fifteen days for every photoperiod regime. The chart recorder was run at a rate of 12cm/hr, which allowed trigger presses more than 10-15 seconds apart to be easily distinguished. From these recordings on a chart, feeding frequency of any hour of the day, of a particular photoperiod regime could be worked out and the number of trigger actuations for the whole day could be double checked by total number of actuations noted for that particular regime from the counters on the control box.

Separate days under each lighting regime were considered to be replicate treatments. Thus, when calculated as a percentage of the total daily actuations, the number of presses occurring during similar hours on separate days could be meaned and standard deviations calculated. It was necessary to express hourly actuations as a percentage to obviate small differences in the total number of presses occurring within individual days.

4.2.2.4. Experimental Diet

Feed dispensers were stocked with commercially available floating feed pellets, manufactured by Edward Baker Limited, Sudbury, Suffolk under the trade name of "Omega Trout Food". Feed dispensers were re-stocked, as often as required, usually three times a week.

4.2.2.5. Weighing Procedure

Details of the weighing procedure are presented in Section 3.7. Fish were individually weighed (± 0.01g), under anaesthesia, after twelve hours starvation for the initial average weights. Thereafter, fish were individually weighed again, after every fourteen days following the same procedures as for initial weighing.

4.2.2.6. Statistical Methods and Analysis of Growth Data

These were performed as detailed in Section 3.11. Data on the increase in body weight under different photoperiods was used for calculating regression equations and projected values of growth data were compared with the observed values of the growth data.

4.2.3. Results

4.2.3.1. Feeding Behaviour Under Different Photoperiod Regimes

Fish exposed to different photoperiod regimes fed actively only during the light phase. Occasional trigger presses did occur during the dark phase on all regimes, though they appeared to be random and not aggregated into groups as found during the light phase. It is unlikely that feeding could have occurred during the dark phase, since the level of light was below that required for visual feeding to take place. For these reasons such actuations were assumed to be accidental and ignored in the analysis of results. For every group under each photoperiod the percentage of the total daily actuations which occurred during similar hours on fifteen separate days were meaned and plotted against the hours of light period and are shown in Figures 11 to 18. Results obtained for the feeding behaviour of different groups under different photoperiod regimes is given below.

Three Hours Light / Twenty-One Hours Dark (3L21D) (Figure 11)

The fish started feeding immediately the lights came on. Feeding which was at its highest rate during the first hour of the three hours light kept on taking place till the lights went off. During the second and third hour the rate of feeding declined progressively. During the twenty-one hours darkness, occasional trigger actuations were noticed.

Six Hours Light / Eighteen Hours Dark (6L18D) (Figure 12)

The fish started the feeding activity within minutes of exposure to light, the feeding being at its maximum in the first hour. During the second and third hours, feeding was at a fairly high rate, but showed a declining tendency. Fish maintained the feeding activity up to the sixth hour of the light regime but at a much slower rate than during the first three hours of the light regime. Occasional and random trigger actuations took place during the eighteen hour dark period.

Nine Hours Light / Fifteen Hours Dark (9L15D) (Figure 13)

Again the feeding activity started as soon as lights came on, being the highest in the initial hour and then declining every hour till the fourth hour when it was at its lowest level, remaining so, for at least three hours before starting to rise again. During the last hour of this photoperiod, the feeding activity was fairly high. It stopped completely as the lights went off. During the fifteen hour dark period only random trigger actuations (two-three) took place on certain days.

Twelve Hours Light / Twelve Hour Dark (12L12D) (Figure 14)

The initial pattern of feeding was the same as in nine hour light / fifteen hour dark photoperiod. Two distinct peaks of feeding activity were present being separated from each other by a nine hour interval.

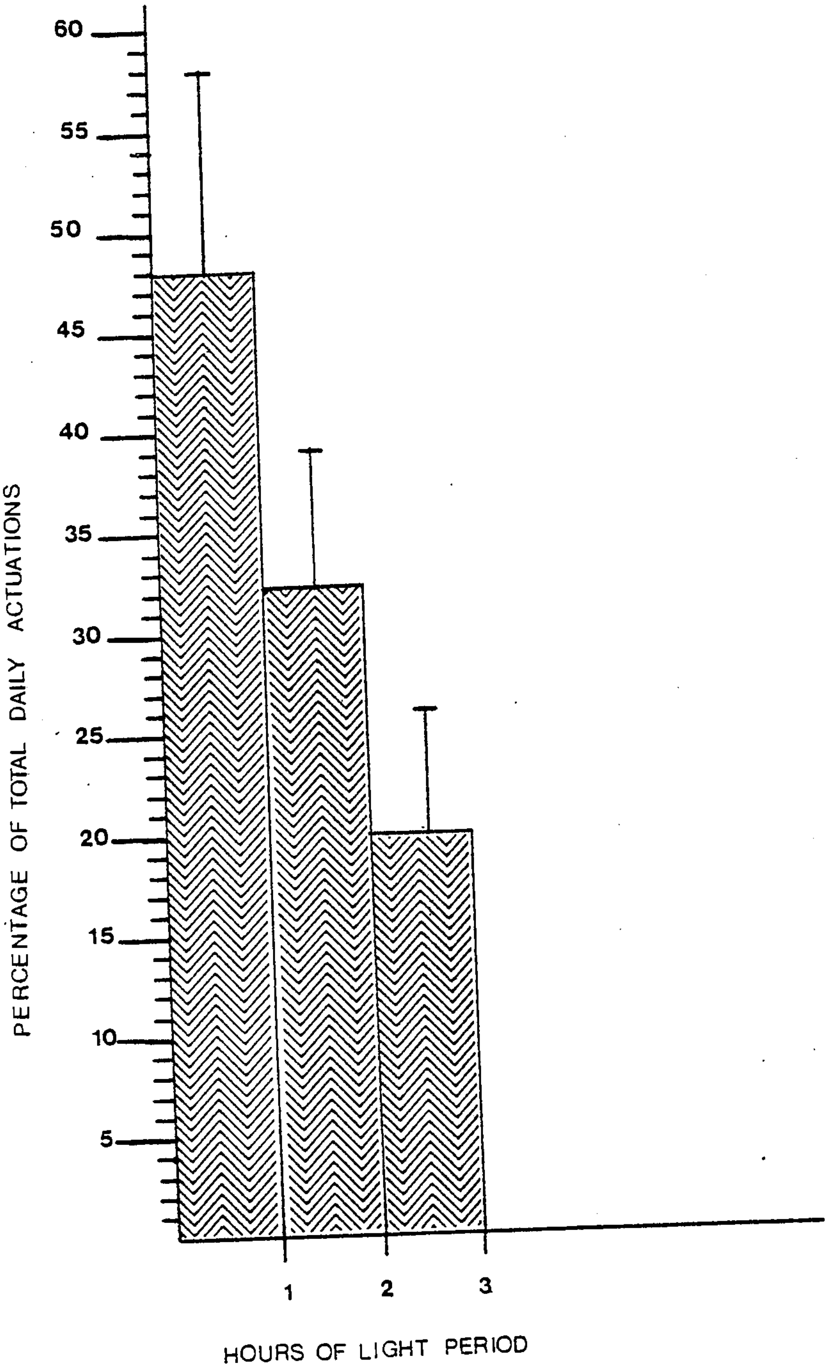


FIGURE 11 Feeding Activity of rainbow trout under 3 hours light/ 21 hours dark photoperiod.
(Means and standard deviations shown for 15 repeat Trials)

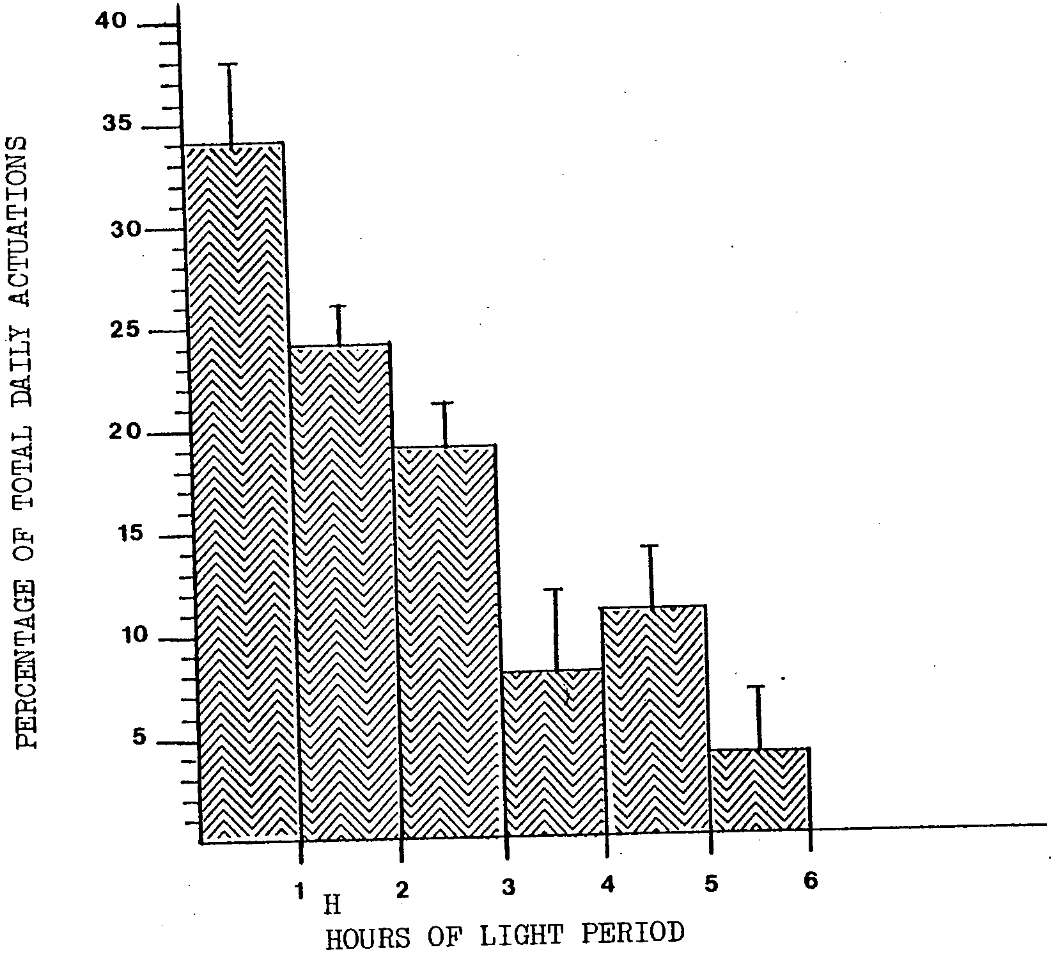


FIGURE 12 Feeding activity of rainbow trout under 6 hours light/18 hours dark photoperiod (Means and standard deviations shown for 15 repeat trials).

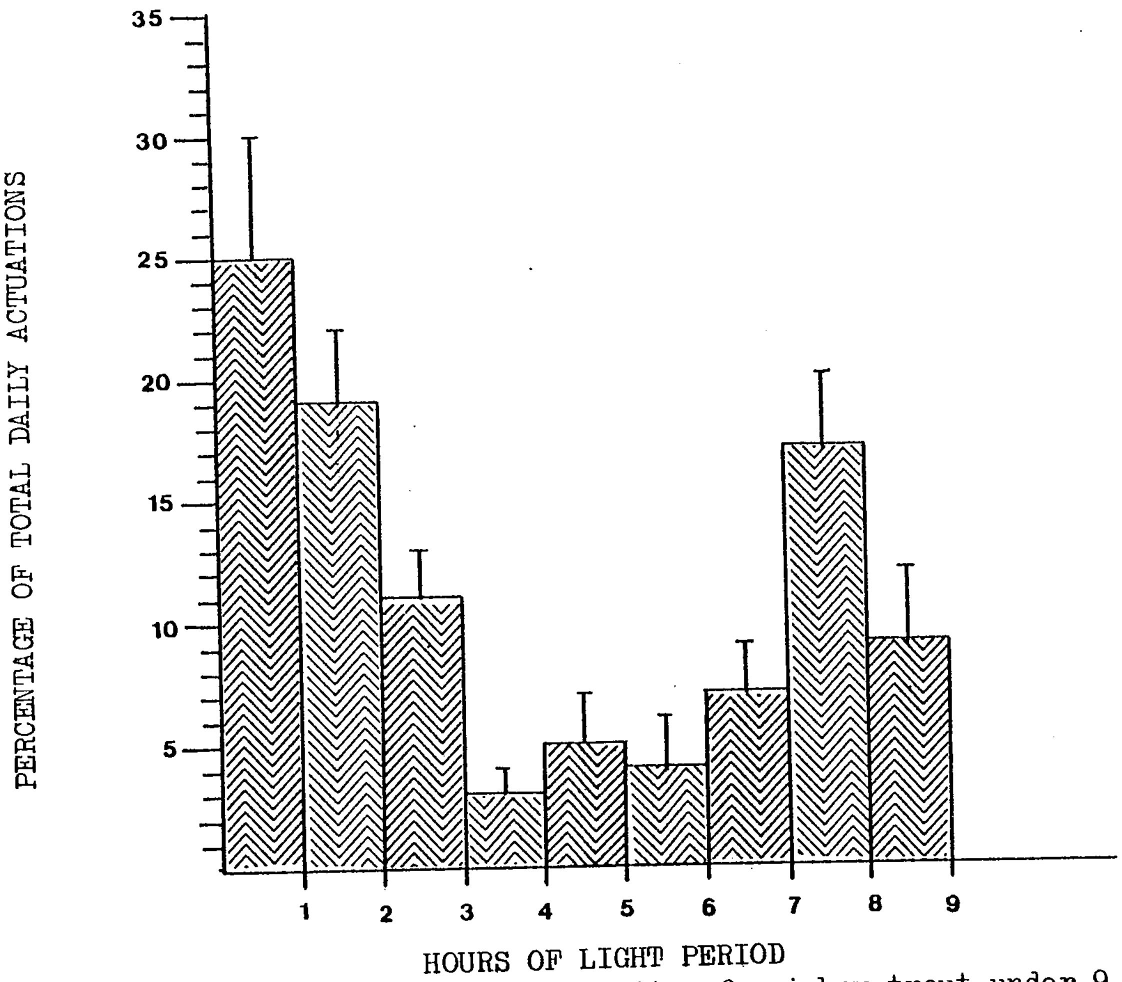


FIGURE 13 Feeding activity of rainbow trout under 9 hours light/15 hours dark photoperiod (Means and standard deviations shown for 15 repeat trials).

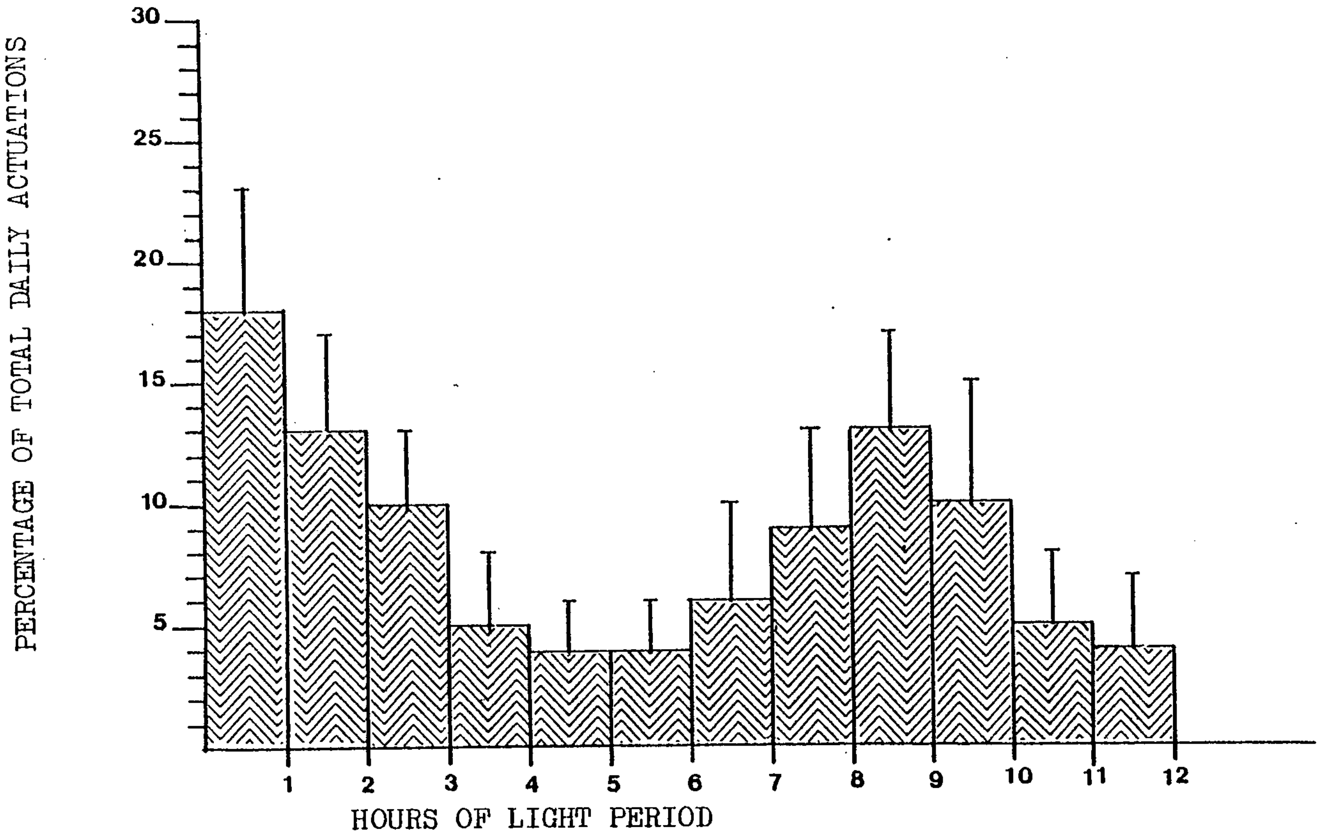


FIGURE 14 Feeding activity of rainbow trout under 12 hours light/12 hours dark photoperiod (Means and standard deviations shown for 15 repeat trials).

The maximum feeding activity was noticed in the first hour of the light period, declining hour by hour till the fourth hour, after which the feeding level remained fairly constant at four to five percent of the total daily actuations, for around three hours, after which the feeding activity started rising, reaching a maximum level of thirteen percent of total daily actuations in the ninth hour. During the last three hours, the feeding activity dropped steadily. During the twelve hour dark period, only random trigger actuations took place.

Fifteen Hour Light / Nine Hour Dark (15L9D) (Figure 15)

During the fifteen hour light period, only two peaks of feeding activity took place, again separated by nine hours intervals. As during the twelve hour light / twelve hour dark photoperiod, the feeding was at its maximum intensity in the first hour of the light period, reaching twenty-two percent of the total daily actuations. In the second feeding peak, the actuations reached the maximum of twelve percent of the total daily actuations during the ninth hour. Around three to four hours before lights switched off, very few trigger actuations were noticed. Occasional actuations took place during nine hour dark period on certain days.

Eighteen Hour Light / Six Hour Dark (18L6D) (Figure 16)

Feeding activity started as soon as the lights switched on and steadily slowed down within the first three hours. A second peak of feeding activity occurred after ten hours, with a third peak starting in the

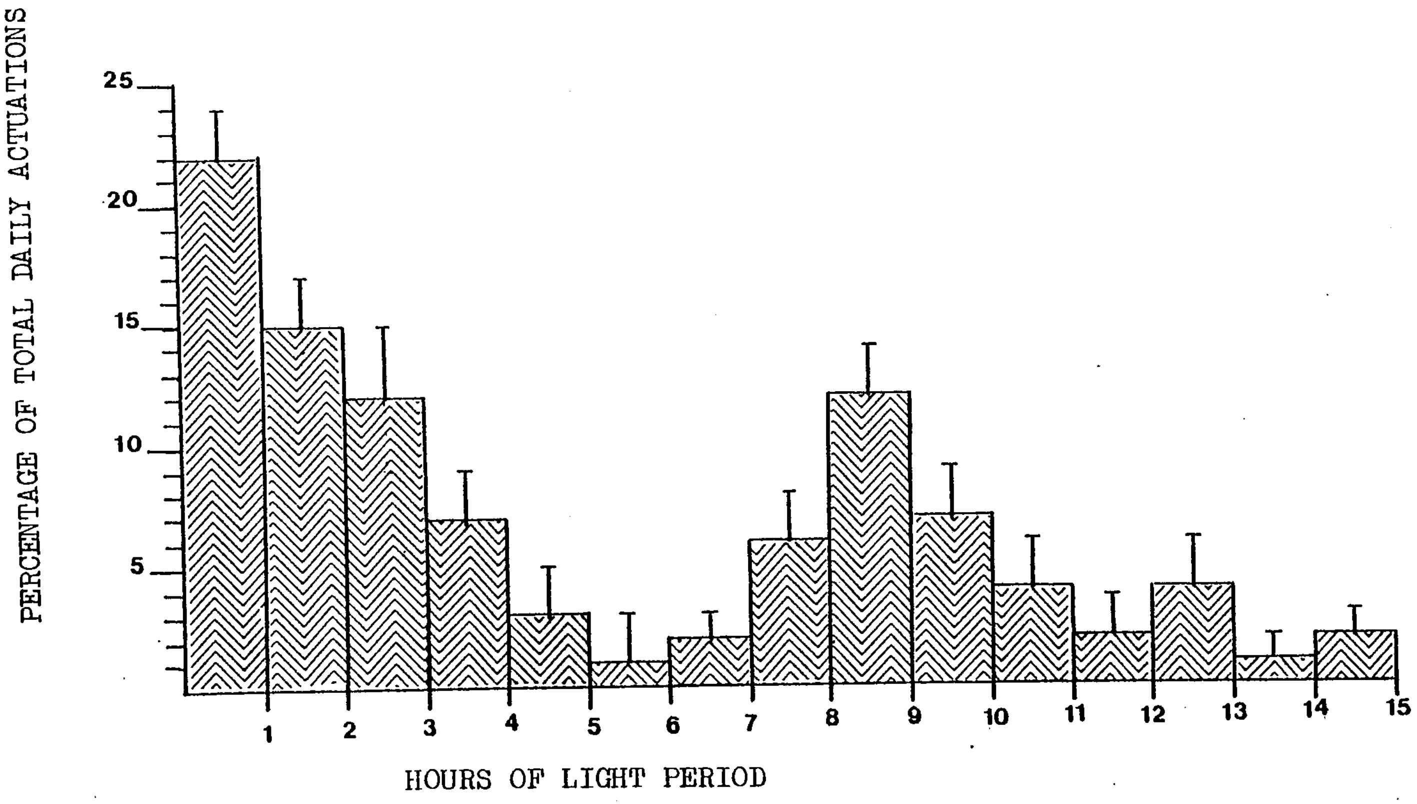


FIGURE 15 Feeding activity of rainbow trout under 15 hours light/9 hours dark photoperiod (Means and standard deviations shown for 15 repeat trials).

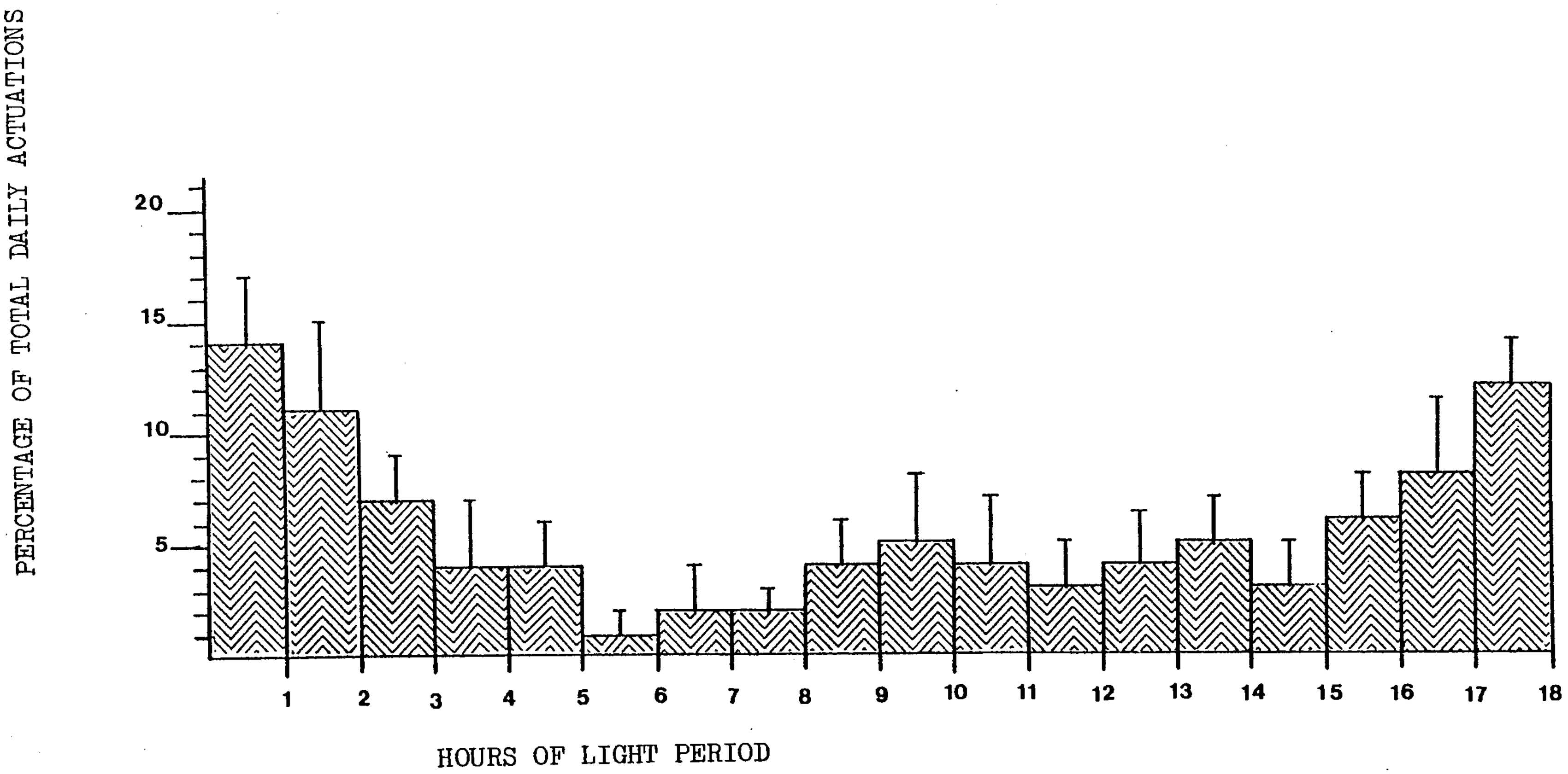


FIGURE 16 Feeding activity of rainbow trout under 18 hours light/6 hours dark photoperiod (Means and standard deviations shown for 15 repeat trials).

seventeenth hour and reaching its maximum level in the eighteenth hour. Feeding stopped suddenly at the end of the eighteenth hour upon the lights being switched "off". Very occasional actuations took place during the six hour dark period.

Twenty-one Hour Light / Three Hour Dark (21L3D) (Figure 17)

Feeding activity started within minutes of the coming "on" of the lights. Three distinct peaks of feeding activity were present. The first peak was formed in the very first hour of the light period (12% of the total daily actuations). From the fourth to eighth hour feeding was at its lowest level and started rising again in the ninth hour, reaching a maximum of nine percent of the total daily actuations in the tenth hour, thus forming a second peak. The third peak of feeding activity was formed in the eighteenth hour (7% of the total daily actuations). In the last three hours of the lighting period feeding was significantly at a low level. During the three hour dark period very random trigger actuations were noticed on certain days.

Continuous Lighting (24LOD)

For this trial, the results were plotted as trigger actuations/hour against time, since there was little to be gained from converting them to percentages. Trigger actuations performed over a period of eighty-five hours towards the end of the trial were recorded by a chart recorder and results were plotted, as shown in Figure 18. Once plotted in this way, a peak of feeding was considered to have occurred within any hour or group of hours where the number of actuations were greater than the hourly mean for the whole period. Peaks thus defined varied in duration, intensity and periodicity, but the mean time between the median points of consecutive peaks was found to be 10.7 hours (S.E. ± 0.56 hours).

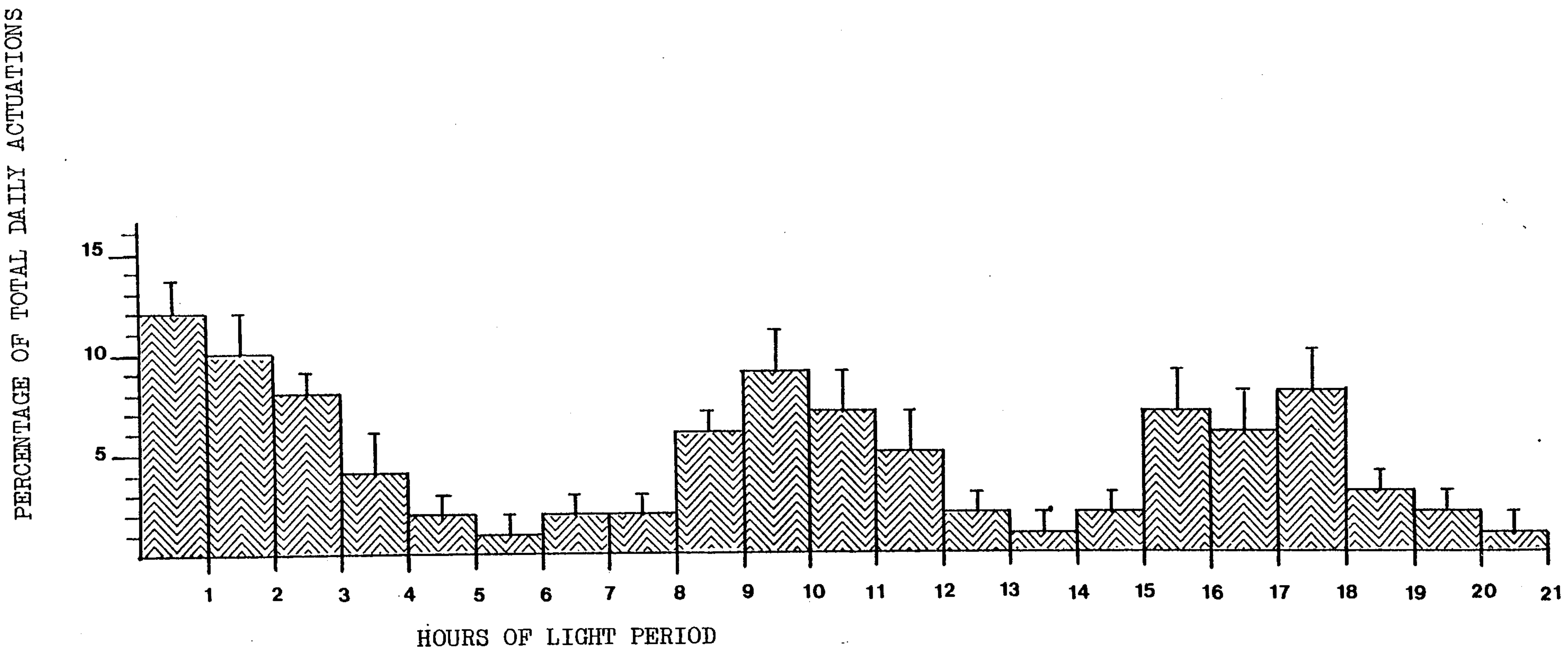


FIGURE 17 Feeding activity of rainbow trout under 21 hours light/3 hours dark photoperiod (Means and standard deviations shown for 15 repeat trials).

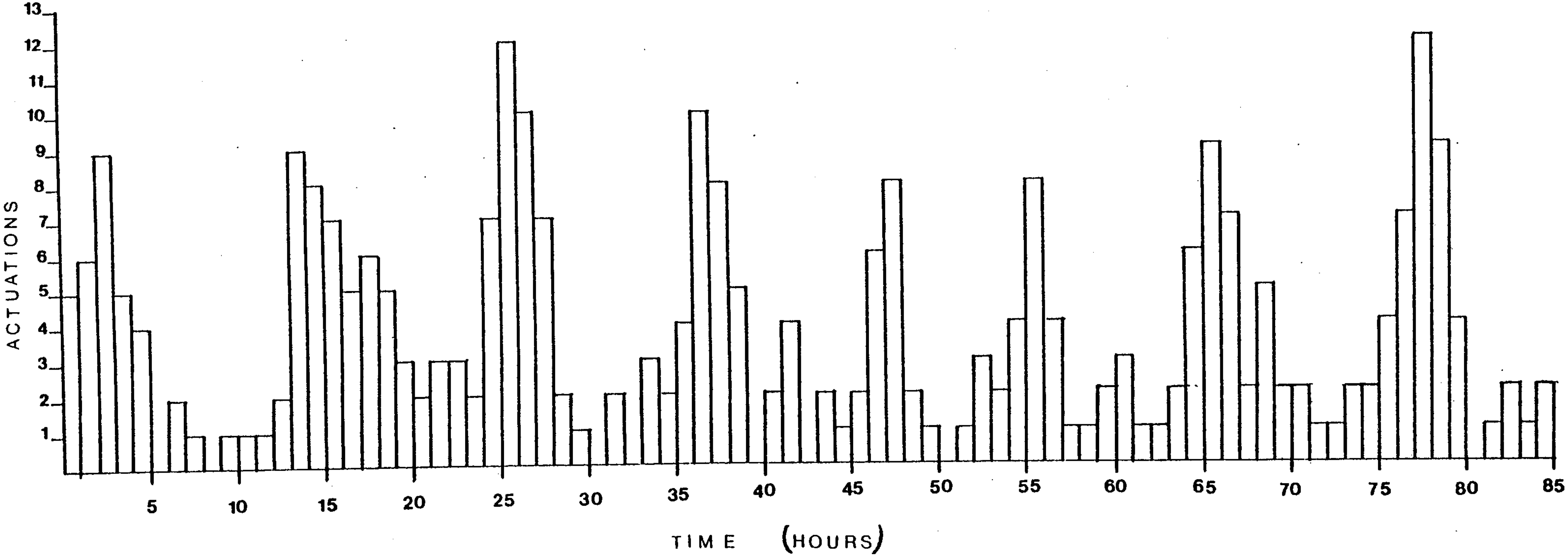


FIGURE 18 Feeding activity of rainbow trout under conditions of continuous light for a period of 85 hours towards the end of the trial.

Continuous Dark (OL24D)

Feeding activity did not take place as vigourously as in the case of groups under the light and dark phases of any photoperiod. There was complete absence of a feeding rhythm, except for occasional and random trigger actuations. During the continuous dark photoperiod the level of light intensity was so low that it is unlikely that feeding could have occurred.

Due to very inactive feeding, no recording of the feeding activity was calculated. An average of only four trigger actuations per day were noted over the whole period of twenty-eight days of the experiment.

4.2.3.2. Growth Performance

Growth responses of groups of fish subjected to different photoperiod regimes are represented graphically in Figure 19, where the increase in average fish weight is plotted for every photoperiod regime studied, except for the group of fish which were subjected to continuous dark regime which lost weight. Figure 20 shows the regressions of the observed against the predicted gain in fish weights of the groups under different photoperiod regimes. It shows that there is a general increase in growth with the increase in photoperiod. After initial increase in growth, the growth rate decreases with increasing photoperiod.

The group of fish under 9L15D showed a better growth performance than the rest of groups under different light regimes. The group of fish under 21L3D photoperiod showed the same growth performance as the group under 9L15D but the group on a 21L regime consumed more quantity of food than the group on a 9L regime.

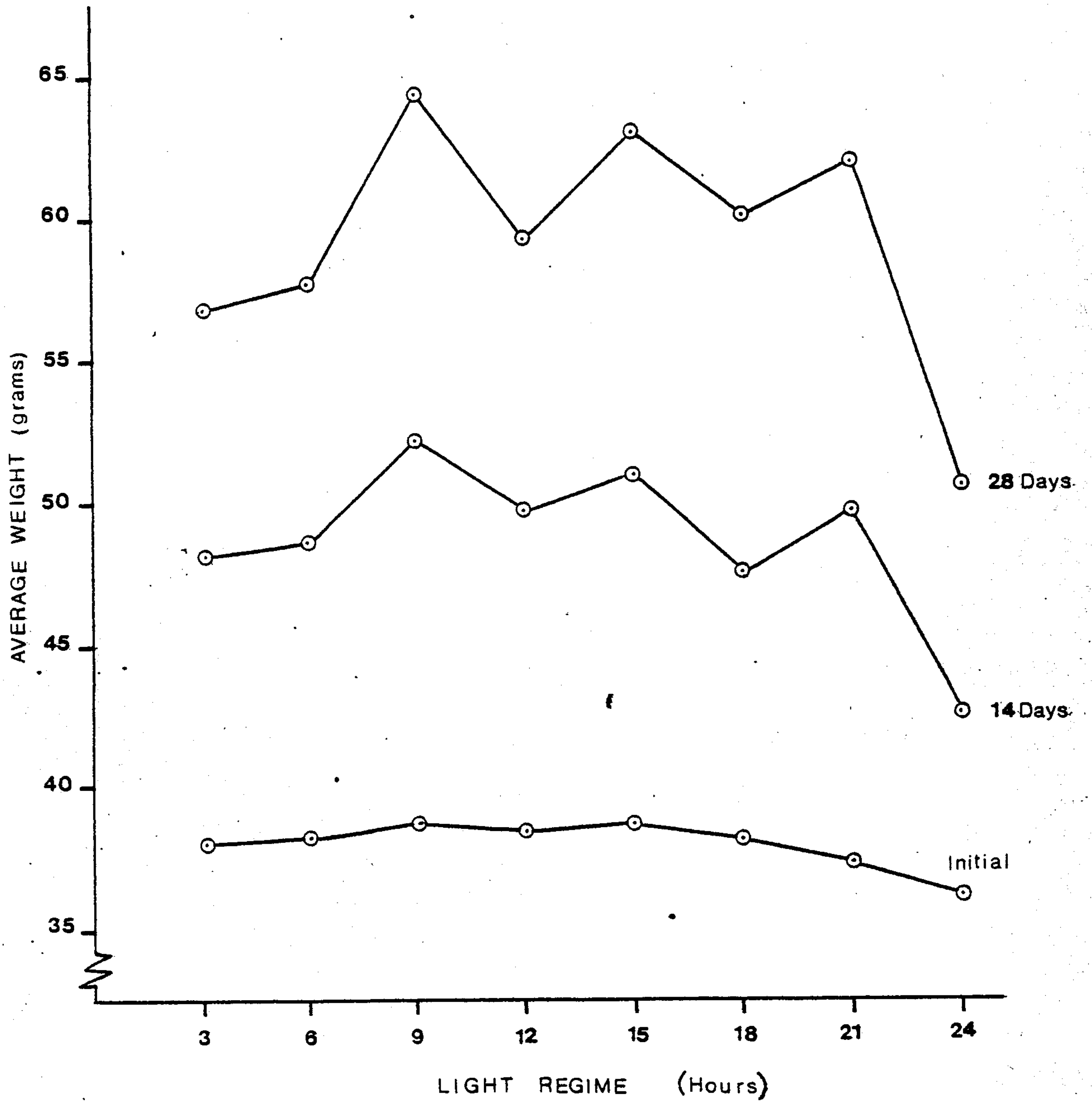


FIGURE 19 Growth responses of rainbow trout exposed to different photoperiods in Experiment 1

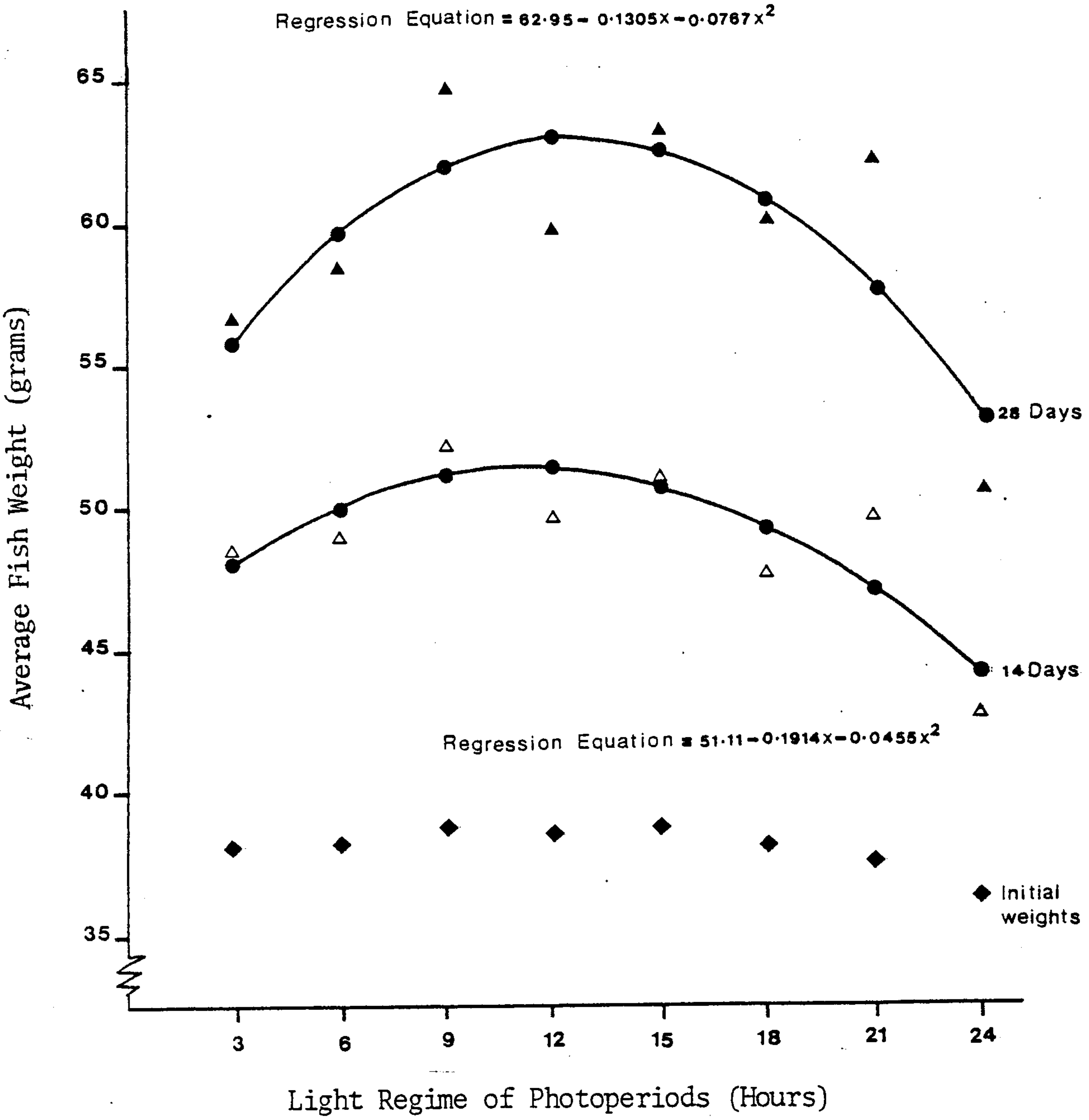


FIGURE 20 Relationship of weight-gains and different photoperiod regimes in rainbow trout. Curvilinear curve of solid line represents the response-curve.

(Appendix table 1 for statistical analysis).

The Specific Growth Rates (SGRs, Section 3.11.1) of all groups under different photoperiods are shown in Table 7. The highest growth rate is shown by group of fish on 9L regime, though for other groups of fish under different photoperiods, there is a general trend of increasing SGRs with increasing photoperiods. Under continuous lighting (24LOD) growth rate was the lowest of all the groups. The group of fish in continuous dark regime did not show any gain in weight, as mentioned previously on the contrary an average of 4.56g was lost, over the whole period of experiment (28 days).

4.2.3.3. Food Conversion

Food Conversion Ratios (FCRs, Section 3.11.2) were calculated for all the groups under different photoperiods and are shown in Table 7. The lowest food conversion ratio was shown by the group under 9L15D photoperiod. A general increase in food conversion ratios occurred with the increasing photoperiod. Group under continuous lighting (24LOD) showed the highest conversion ratio than the groups under other different photoperiods. For the group under continuous dark regimes, food conversion ratio was not calculated, due to very poor feeding rate and weight loss, over the whole period of the experiment.

4.2.4. Discussion and Conclusions

The results show that trout exhibit a rhythmic pattern of feeding behaviour in all photoperiods. The feeding peaks were formed every eight to ten hours depending upon the photoperiod regime. Such peaks have been observed by previous workers (Adron et al, 1973) who found an eight hour cycle of feeding in continuous lighting, which they attributed to the rate of digestion and stomach emptying. In rainbow trout fed on dry pellets digestion is a little slower, 40% of the stomach content being evacuated after eight hours at 12°C in 30g trout (Windell et al., 1972). In Pacific Salmon *Onchorynchus nerka* of about 30g the stomach is about 50% empty after eight hours at 10°C (Brett and Higgs, 1970) and 75% empty after eight hours at 15°C.

In the present study, a peak of feeding activity was observed in the very first hour of every photoperiod regime except of course in the continuous lighting regime. This first peak was of the higher intensity than subsequent peaks formed in the same photoperiod. Although trigger actuations occurred during the dark phase, these were so random and fragmented that no pattern of feeding could be formed, hence such trigger actuations were considered accidental. It cannot be said that the fish had no feeding urge during the dark phase, but the light is the limiting factor, depriving fish of the opportunity to feed, since rainbow trout are primarily sight feeders and will eat a wide variety of food (Ware, 1973). The longer the period of this deprivation, the more intense was the feeding when subjected to light. In the continuous light since the dark regime was eliminated, fish did not experience any period of food deprivation. Therefore under continuous light peaks of feeding activity were not as conspicuous as in the photoperiods with light and dark regimes. Perhaps the most serious criticism of these experiments is that largely due to lack of time replicate experiments were not made. Because of the variability seen in Figure 19 conclusions are difficult to make from one experiment.

In the shortest 3L21D photoperiod, light after twenty-one hours of darkness, caused the fish to start trigger actuations at once with very active feeding. In the first hour 48% (S.D. ± 10%) trigger actuations of the total three hour actuations were recorded, in contrast to only 12% (S.D. ± 2%) in the corresponding first hour of the longest photoperiod.

of 21L3D. Similarly as the lighting phase of the different photoperiods increased, there was a corresponding decrease in the percentage of total daily actuations in the first hour of the onset of the lighting phase. Nevertheless, in every photoperiod regime the first peak of feeding activity, formed immediately after the lights were on, was of higher intensity than any subsequent peak in the same photoperiod. This appears to be related to the length of the dark regime of the photoperiod because during this period owing to absence of light fish experience deprivation subsequently resulting in a high intensity of the feeding activity at very first opportunity of the onset of the light regime. Studies by other workers on different species of fish also report similar observations. Brett (1971) observed in sockeye salmon (*Oncorhynchus nerka*) that within limits feeding increases with prior deprivation and decreases with satiation. Beukema (1968) found that total daily (eight hour) food intake by three-spined stickleback (*Gasterosteus aculeatus* L.) following deprivation over the range of sixteen to eighty-eight hours was not affected but that more was eaten in the first hour with greater deprivation. Tugendhat (1960), also in the three-spined stickleback (*Gasterosteus aculeatus* L.) observed that with increasing deprivation from one to three days the total number of completed feeding responses increased, the mean duration of a response became shorter, and the total time spent did not change in a one hour bout. In skipjack tuna (*Katsuwonus pelamis*) the proportions of prey items attended to and attacked decrease as satiation progresses (Magnuson, 1969).

In all photoperiods, irrespective of their duration and the number of feeding peaks it was observed that the initial peak declined at a gradual rate for three hours, after which the trigger actuations remained around the 5% level of total daily actuations. Thus in demand feeding around three hours of feeding activity was required by rainbow trout of 40g at 13.5°C to reach a point of satiation. Ishiwata (1968) gives a figure of 60 min for rainbow trout of 135g at 10°C using hand feeding. When demand feeding, the satiation time is longer because the rate of food delivery is determined by the fish rather than by the experimenter.

As in all photoperiods studied an initial peak of feeding always occurred within the first three hours of light, irrespective of the duration of the scotoperiod, it can be correlated with the increased activity noted at dawn amongst many species of fish in natural daylight (Alabaster and Robertson, 1961; Swift, 1962; Young et al., 1972). In contrast, there was no definite increase in feeding rate associated with the last hour of light under any of the photoperiods studied, whereas high activity at dusk is exhibited by fish in the wild (Alabaster and Robertson, 1961). The 12L12D photoperiod is comparable to a natural day length period and in this study the second peak of feeding activity formed prior to darkness can be comparable to the dusk feeding activity in the natural environments. Such dusk feeding activity was noted in 15L, 18L and 21L photoperiods as well.

The presence of this "dusk feeding" activity in all photoperiods from 9L to 12L photoperiods does not mean that trout possess a biological clock for dusk feeding but rather that the rhythm is related to the degree of satiation since in all cases a feeding peak had occurred within the previous five to six hours.

Overall, the results agree with the hypothesis that the feeding rhythm of trout is mainly governed by the degree of gut distension. This is a particularly attractive explanation since goldfish, which have no storage stomach, exhibit a diurnal monophasic rhythm of feeding activity (Rozin and Mayer, 1961a), as opposed to a diurnal polyphasic rhythm of feeding activity in trout, which possess a stomach and retain the food in the gut for a considerable duration of time, depending on the water temperature.

Adron et al., (1973) have shown that, compared to shorter photoperiods, food consumption and growth are increased under continuous lighting, without a loss of food conversion efficiency. In the present study, on the contrary, fish under continuous lighting showed the poorest growth performance and the highest food conversion ratio than the groups under other different photoperiods, except group under continuous dark. It is not known whether the eight to ten hours rhythm is maintained over extended periods of continuous light but the poor growth performance and a higher food consumption give indications that due to the lack of a scotoperiod, fish eventually start experiencing stress and strain.

The presence of a rhythmic pattern of feeding behaviour in all photoperiods can be favourably manipulated in the promotion of growth. The presence of a peak of feeding activity every eight to ten hours in different photoperiods is suggestive of a point at which appetite is approaching a peak. Thus the size of ration and time of presentation can be favourably manipulated to maximize the daily intake by taking advantage of this earliest point when appetite is at its peak. Under such a condition it can be expected that fish would utilise the feed more efficiently and would show the best growth performance at a photoperiod with the light regime of ten to twelve hours. Results shown for the 9L15D photoperiod in the present study support this view. In this photoperiod fish get the opportunity of satiating themselves at least twice in twenty-four hours. At the same time, during the dark period of the photoperiod fish can conserve their energy by being comparatively inactive as compared to the higher activity and a higher energy consumption expected under longer light regimes or under continuous light.

It can be concluded that the feeding behaviour of rainbow trout under all photoperiods, except in continuous dark, exhibited a positive response to the coming on of the light by initiation of a peak of feeding activity lasting at least three hours. Subsequently depending upon the length of the light regime, peaks of feeding activity were formed approximately every eight to ten hours. Since the best growth performance and feed utilisation was shown under 9L15D photoperiod, thus longer photoperiods than 9L15D or 10L14D can be of no advantage from the growth and feed utilisation point of view. In absence of the light regime under continuous dark conditions feeding activity could not be performed by rainbow trout and fish lost considerable body weight, thus total absence of light is harmful for fish from the feeding point of view. In this experiment since light intensity was at the same level (160 lux) for all photoperiods, it was, therefore, planned to study the feeding behaviour under different levels of light intensity (Experiment 2).

Demand feeding experiments must be viewed with caution because of fish being subject to environmental changes prior to the experiments. Also hierarchical effects that may develop during the experiment are difficult to eliminate and finally in this particular protocol the normal dawn-dusk light changes were not simulated.

4.3. Experiment 2': Feeding Behaviour Under Constant Photoperiods & Varying Intensity of Light

4.3.1. Introduction

In the previous Experiment 1, from the results obtained, it was observed that rainbow trout exhibited rhythmic feeding activity and a peak of feeding activity occurred after every eight to ten hours, depending upon the length of the photoperiod. On all regimes with a dark and light phase, under photoperiods of more than eighteen hours light, three peaks of feeding activity were observed, whereas under 9L15D, 12L12D and 15L9D photoperiods only two peaks were observed. For Experiment 2 only those photoperiods under which groups of fish exhibited two peaks of feeding activity, were selected for further investigations because these groups showed better growth rates and food conversion ratios than the groups of fish under photoperiods, which exhibited three peaks of feeding activity.

The level of light intensity can be a limiting factor for feeding activity, especially for fish which are visual feeders (see also Section 4.1. General Introduction). The present experiment was planned to investigate effects of three levels of light intensity on the feeding behaviour of trout. Groups of fish were exposed to light intensities of 10 lux, 80 lux and 320 lux, under three photoperiods of 9L15D, 12L12D and 15L9D for each light intensity. Results obtained in Experiment 1 for these three photoperiods under the light intensity of 160 lux were incorporated with the results of the present experiment. In the present experiment the effects of changing the light intensity and the photoperiod on feeding activity of rainbow trout were studied. Subsequent effects of feeding activity on growth were also studied.

4.3.2. Materials and Methods

4.3.2.1. The Experimental System and Animals

All the conditions were the same as described for Experiment 1 under Section 4.2.2.1. except that average temperature of water was $14.0^{\circ} \pm 2^{\circ}C$.

4.3.2.2. Different Photoperiods and Levels of Light Intensity Studied

The feeding behaviour of trout was studied under three photoperiods of 9L15D, 12L12D and 15L9D, each at three light intensities of 10 lux, 80 lux and 320 lux. Under each photoperiod, each level of light intensity was studied for a period of four weeks for each group of fish.

For every photoperiod light in the light chambers was controlled by automatic time switches as described in detail in Section 3.3.5. Before starting study of feeding behaviour under each photoperiod and light intensity, the individual time switch was pre-set as described in Section 4.2.2.2.

For setting the intensity of light to the desired level, the diffusers of fluorescent tubes in the light chambers (See Section 3.3.5.) were partly covered by opaque black PVC sheet. Intensity of light at the water surface was measured by a photometer (See Appendix).

4.3.2.3. Monitoring the Feeding Activity

Demand feeding system described in detail in Section 3.4. was used for studying the feeding behaviour under different photoperiods and light intensities.

Each experimental tank was provided with one feed dispenser and one trigger according to the same plan as for Experiment 1 and as shown in

Figure 10. The rest of the methods for monitoring the feeding activity were also the same as described in Section 4.3.2.3. Food delivered at each trigger actuation and the mean number of trigger actuations/day for each group, (calculated from total number of daily actuations performed in twenty-eight days of the experiment) are shown in Table 8. Average amount of food consumption/fish/day by fish in each group is shown in Table 9.

Recording of the feeding activity by different groups under different photoperiods and light intensity was made on the chart recorder for fifteen days for each group, following the same procedures as described in Section 4.3.2.3. for Experiment 1. Data collected by recording of the feeding activity over the fifteen days for every photoperiod and level of light intensity was used for calculating the percentage of daily trigger actuations for different hours of the light period of the day and is presented in Figures 21, 22 and 23.

4.3.2.4. Experimental Diet

The same diet was used as in Experiment 1. Feed dispensers were filled and re-stocked as described in Section 4.2.2.4.

4.3.2.5. Weighing Procedure

Details of the weighing procedure are presented in Section 3.7. Same plan was used as described in Section 4.2.2.5. for Experiment 1.

4.3.2.6. Statistical Methods and Analysis of Growth Data

These were performed as detailed in Section 3.11.

4.3.3. Results

4.3.3.1. Feeding Behaviour under Different Photoperiods and Light Intensity

Fish exposed to varying light intensities under different photoperiods were observed to feed actively only during the light regime, though occasional and rare actuations did occur on certain days during the dark regime as well. During the dark regime, because trigger actuations were not aggregated into conspicuous groups as observed in the light regime, therefore as in the previous experiment these random actuations were considered to be accidental and ignored in the analysis of results.

For each group, the mean trigger actuations/day for different photoperiods and levels of light intensity were calculated from the daily number of total trigger actuations performed over twenty-eight days and are shown in Table 8. Mean number of trigger actuations performed in each photoperiod at different levels of light intensity show an increasing trend with the increase in length of light period and the level of light intensity, with a corresponding increase in the rate of feeding activity, except for the group of fish under 9L15D photoperiod at light intensity of 160 lux, in which case mean number of trigger actuations/day was lower than the mean number of trigger actuations/day for the rest of the groups ($P<0.001$).

The average amount of food consumed/fish/day for different groups under three photoperiods at different levels of light intensity is shown in Table 9. From the amount of food shown for each group in the Table, it is evident that there was an increase in the amount of food consumed, with increase of level of light intensity, except in the case of the group of fish under 9L15D photoperiod at light intensity of 160 lux, in which case average food consumed by each fish in a day was lower than

TABLE 8

Daily mean trigger actuations and amount of food delivered per trigger actuation in Experiment 2

Photoperiod	Light Intensity							
	10 lux		80 lux		160 lux		320 lux	
	Average Trigger Act/day	Food Deliv./ Trigger Act. g.	Average Trigger Act/day	Food Deliv./ Trigger Act. g.	Average Trigger Act/day	Food Deliv./ Trigger Act. g.	Average Trigger Act/day	Food Deliv./ Trigger Act. g.
9L15D	119 S.D.±21	0.36 S.D. ± 0.01	122 S.D.±22	0.37 S.D. ± 0.03	92 S.D.±20	0.34 S.D. ± 0.01	139 S.D.±21	0.36 S.D. ± 0.02
12L12D	125 S.D.±19	0.36 S.D. ± 0.02	122 S.D.±19	0.38 S.D. ± 0.05	99 S.D.±18	0.37 S.D. ± 0.07	142 S.D.±28	0.38 S.D. ± 0.08
15L9D	133 S.D.±23	0.35 S.D. ± 0.01	139 S.D.±26	0.35 S.D. ± 0.02	124 S.D.±15	0.35 S.D. ± 0.04	146 S.D.±29	0.38 S.D. ± 0.05

TABLE 9

Growth and food utilisation data from Experiment 2

Photoperiod	Light Intensity															
	10 Lux				80 Lux				160 Lux				320 Lux			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
9L15D	15.40	1.71	1.18	3.11	14.84	1.81	1.09	3.41	25.62	1.25	1.81	1.37	18.56	2.00	1.35	3.00
12L12D	14.10	1.80	1.12	3.57	22.86	1.85	1.71	2.27	20.79	1.46	1.54	1.97	26.32	2.16	1.86	2.30
15L9D	23.29	1.86	1.59	2.24	24.36	1.95	1.70	2.24	24.14	1.74	1.72	2.02	26.60	2.22	1.85	2.34

1 = Average Weight Gain in Twenty-eight Days (grams)

2 = Average Food Consumed by Fish each day (grams)

3 = Specific Growth Rate

4 = Food Conversion Ratio

the average amount of food consumed by fish in the rest of the groups under different photoperiods and levels of light intensity.

Feeding behaviour of trout under the three photoperiods, irrespective of the level of light intensity was observed to be aggregated into peaks of feeding activities. Two peaks of feeding activity took place, again separated by an average of nine hours interval as found in Experiment 1, (Section 4.2.) for the three photoperiods. Fish started the feeding activity within minutes of the switching "on" of the lights in all photoperiods and light intensities. This feeding activity continued at a peak rate for the initial hour and then declined every hour until the fourth hour, after which the feeding level remained fairly low, with only a few trigger actuations during each hour. Feeding activity towards the end of the seventh hour started to rise again reaching a fairly high level in the eighth and ninth hours. It stopped completely as the lights switched "off" at the end of the ninth hour in 9L15D photoperiod whereas, in 12L12D and 15L9D photoperiods, feeding activity continued into the tenth hour. Thereafter, it started declining and stopped completely for the group of fish in 12L12D at the end of the twelfth hour, when the lights switched "off". Group of fish in 15L9D showed fairly low feeding activity for four hours after the second peak of feeding activity and stopped feeding completely after the lights switched "off".

Under all three photoperiods and levels of light intensity studied in this experiment, the first peak of feeding activity was of a higher intensity than the second peak of feeding activity, which started after approximately eight to nine hours interval.

Effect of Light Intensity on Feeding Rates

Percentages of daily trigger actuations at different hours of the light regime of each photoperiod at different levels of light intensity are represented in Figures 21, 22 and 23.

The feeding rates exhibited during the first peak of the feeding activity at switching "on" of the light under every photoperiod at different light intensities, seem to be related to the level of light intensity. Under the higher light intensity of 320 lux feeding rate was much higher than the lower light intensities. Analysis of the data obtained by recording the feeding frequency of all the groups the feeding rates, irrespective of the length of dark and light phases of the photoperiods, in relation to the level of light intensity can be ranked in the order of 320 lux >160 lux >80 lux >10 lux for the first two hours of the first peak of the feeding activity. During the third hour of the first peak of feeding activity, all the groups showed somewhat similar rates of feeding activity. During the fourth, fifth and sixth hours of the light regime feeding activity remained fairly low for all groups but groups of fish under lower levels of light intensity were feeding at a higher level than the groups of fish under light intensity of 320 lux and according to data presented in Figures 21, 22 and 23 feeding activity during the fourth, fifth and sixth hours of the light regime, during all photoperiods at different levels of light intensity can be ranked in relation to light intensity in the order of 80 lux >10 lux > 160 lux >320 lux. During the seventh hour feeding rates of all groups, under different photoperiods and light intensities, started showing an upward trend. During the eighth, ninth and tenth hours, a second peak of feeding activity was exhibited by all groups, except the groups under 9L15D photoperiod, in which case feeding activity ended suddenly upon switching "off" of the lights at the end of the ninth hour, so that, these groups showed the second

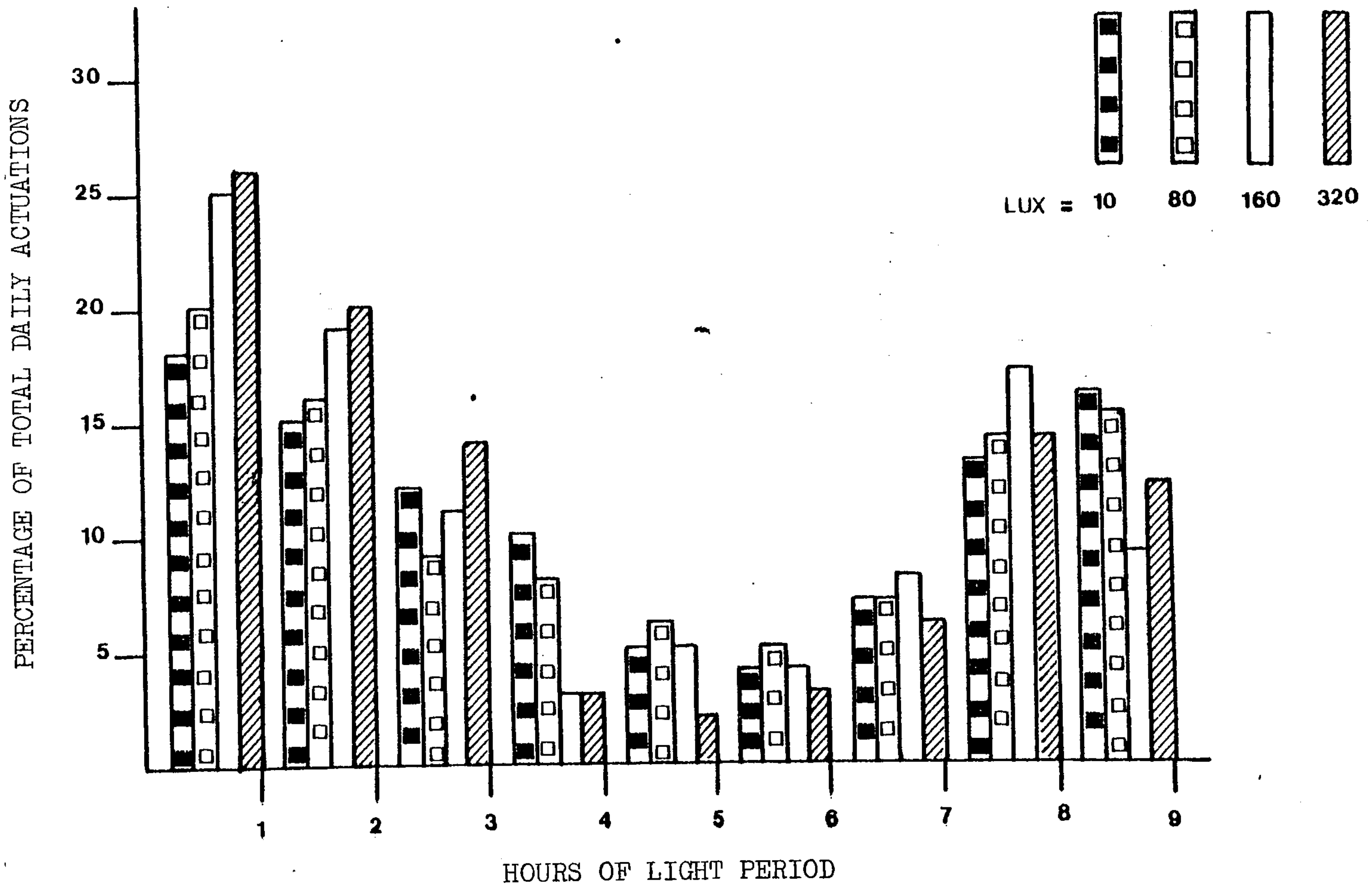


FIGURE 21 Feeding activity of rainbow trout under 9 hours light/ 15 hours dark photoperiod and 4 different light intensities in Experiment 2

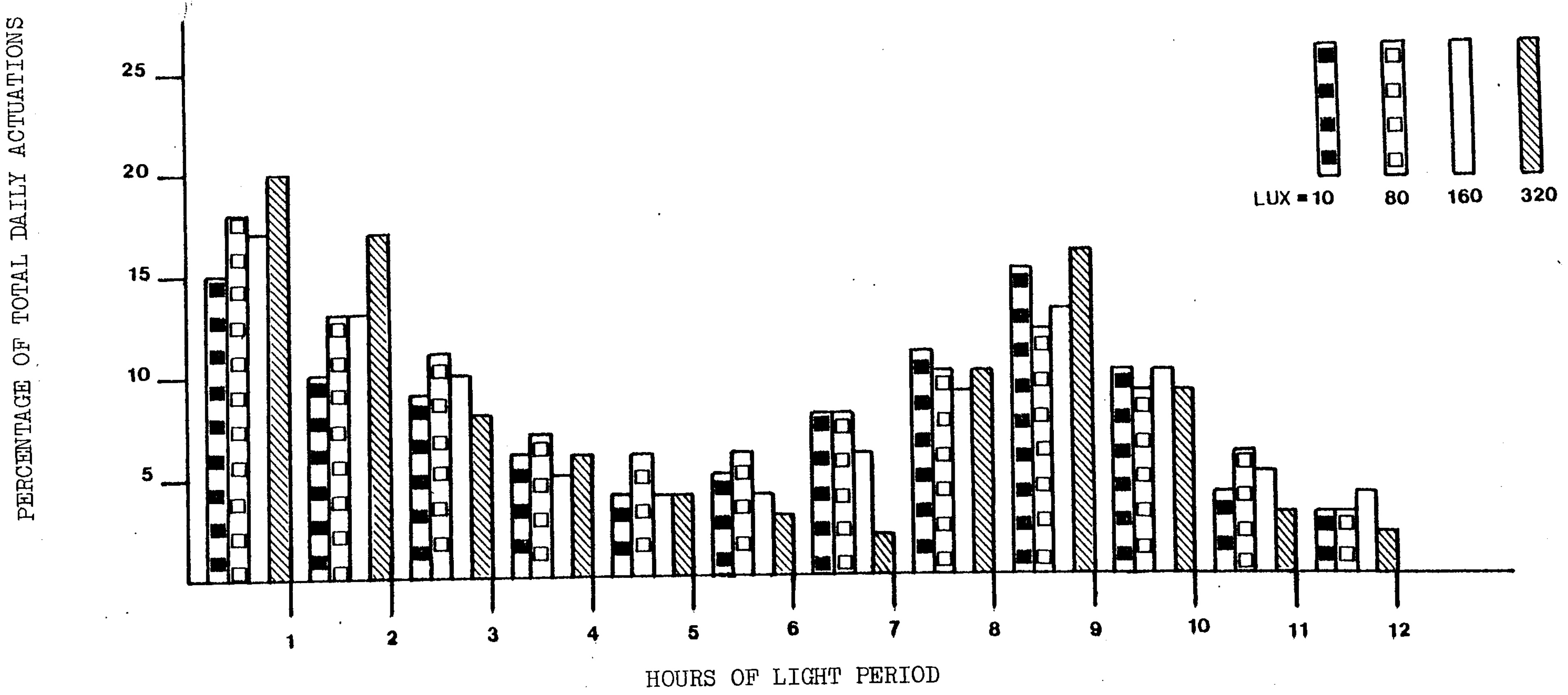


FIGURE 22 Feeding activity of rainbow trout under 12 hours light/ 12 hours dark photoperiod and 4 different light intensities in Experiment 2.

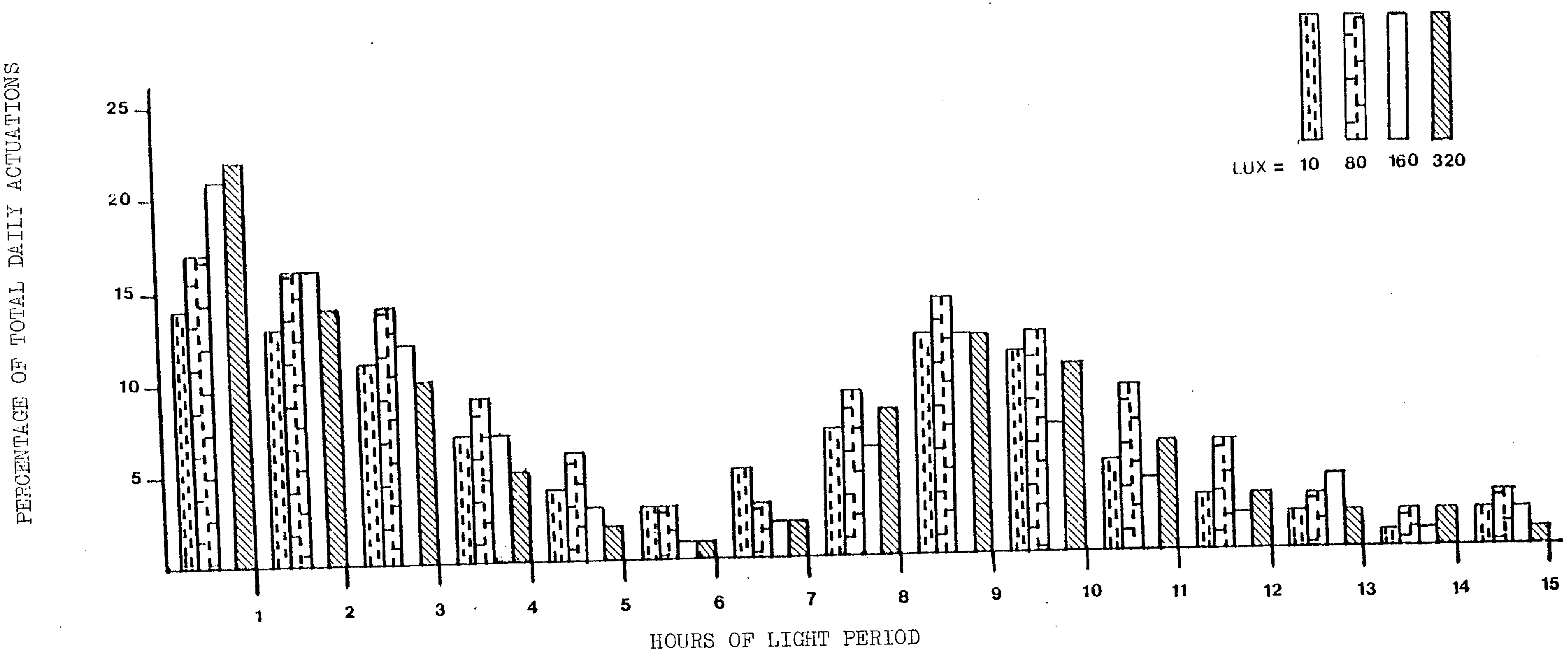


FIGURE 23 Feeding activity of rainbow trout under 15 hours light/ 9 hours dark photoperiod and 4 different light intensities in Experiment 2

peak of feeding activity of only two hours duration (Figure 21).

In groups under 12L12D photoperiod, feeding rates declined after the tenth hour and stopped altogether after the lights were switched "off" at the end of the twelfth hour. During the second peak of feeding activity, under different levels of light intensity, feeding rates remained fairly similar for all groups (Figure 22).

In groups under 15L9D photoperiod, during the eleventh to the end of the fifteenth hour, feeding activity at all different levels of light intensity remained fairly low and stopped altogether suddenly at switching "off" of the lights (Figure 23).

The different levels of light intensity did not effect the rhythmic pattern of feeding activity of the different groups. Two peaks of feeding activity as observed under the corresponding three photoperiods in Experiment 1 were separated by an interval of nine hours.

4.3.3.2. Growth Performance

Growth responses of groups of fish subjected to different photoperiods and different levels of light intensity are represented graphically in Figures 24, 25 and 26, where in each figure the increase in average fish weight is plotted against time.

Fish on 9L15D photoperiod (Figure 24) at light intensity of 10 lux and 80 lux showed no significant difference ($P>0.05$) in their growth rates but showed a significantly poorer growth ($P<0.01$) than the group at light intensity of 160 lux and 320 lux. Fish at 160 lux light intensity showed a significantly higher growth response ($P<0.05$) than the group at 320 lux light intensity.

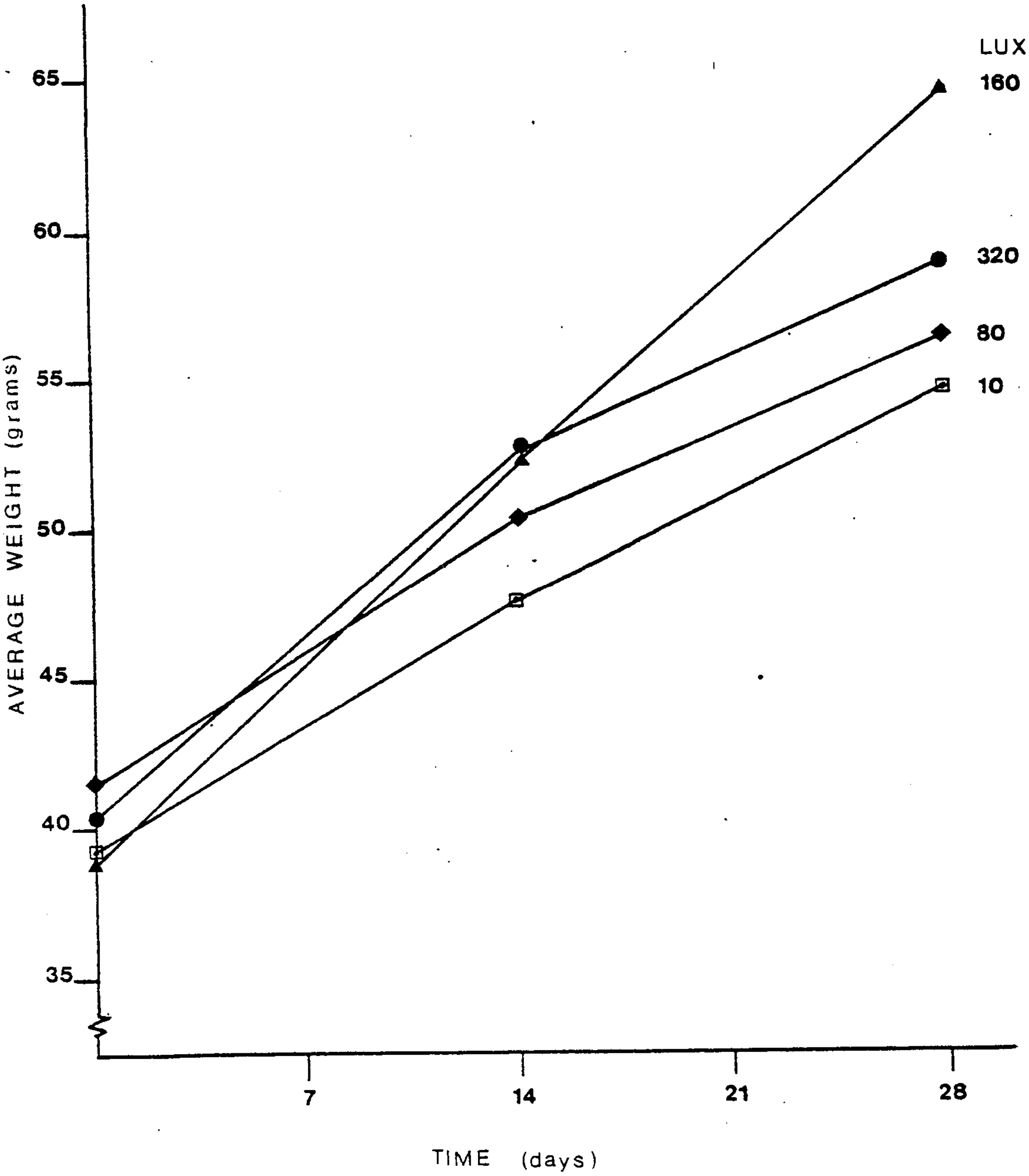


FIGURE 24 The growth responses of rainbow trout exposed to 9L15D photoperiod under 4 different light intensities in Experiment 2

Fish on 12L12D photoperiod (Figure 25) at light intensity of 80 lux showed a significantly better growth ($P<0.01$) than the group at light intensity of 10 lux. There was no significant difference in the average weight gained by groups of fish at light intensities of 80 lux and 160 lux ($P>0.05$). But fish at light intensity of 320 lux showed a better growth response than the fish at light intensities of 10, 80 and 160 lux.

Fish on 15L9D photoperiod (Figure 26) at different light intensities showed no significant difference ($P>0.05$) in average gain in weight. All groups showed better growth responses than groups at shorter photoperiods of 9L15D and 12L12D.

The differences in final average fish weights were reflected by differences in Specific Growth Rates (SGRs Section 3.11.1) which are presented in Table 9. SGRs of fish, under photoperiod of 9L15D at light intensity of 160 lux and under photoperiods of 12L12D and 15L9D at light intensity of 320 lux each, were much better than the groups of fish under other light intensities of different photoperiods.

4.3.3.3. Food Conversion

Food Conversion Ratios (FCRs Section 3.11.2) were calculated for all the groups under different photoperiods and light intensities and are shown in Table 9. The lowest food conversion was shown by the group under 9L15D photoperiod at light intensity of 160 lux. Amongst other groups of fish there was a general trend of higher FCRs for groups at shorter photoperiods irrespective of the level of light intensity.

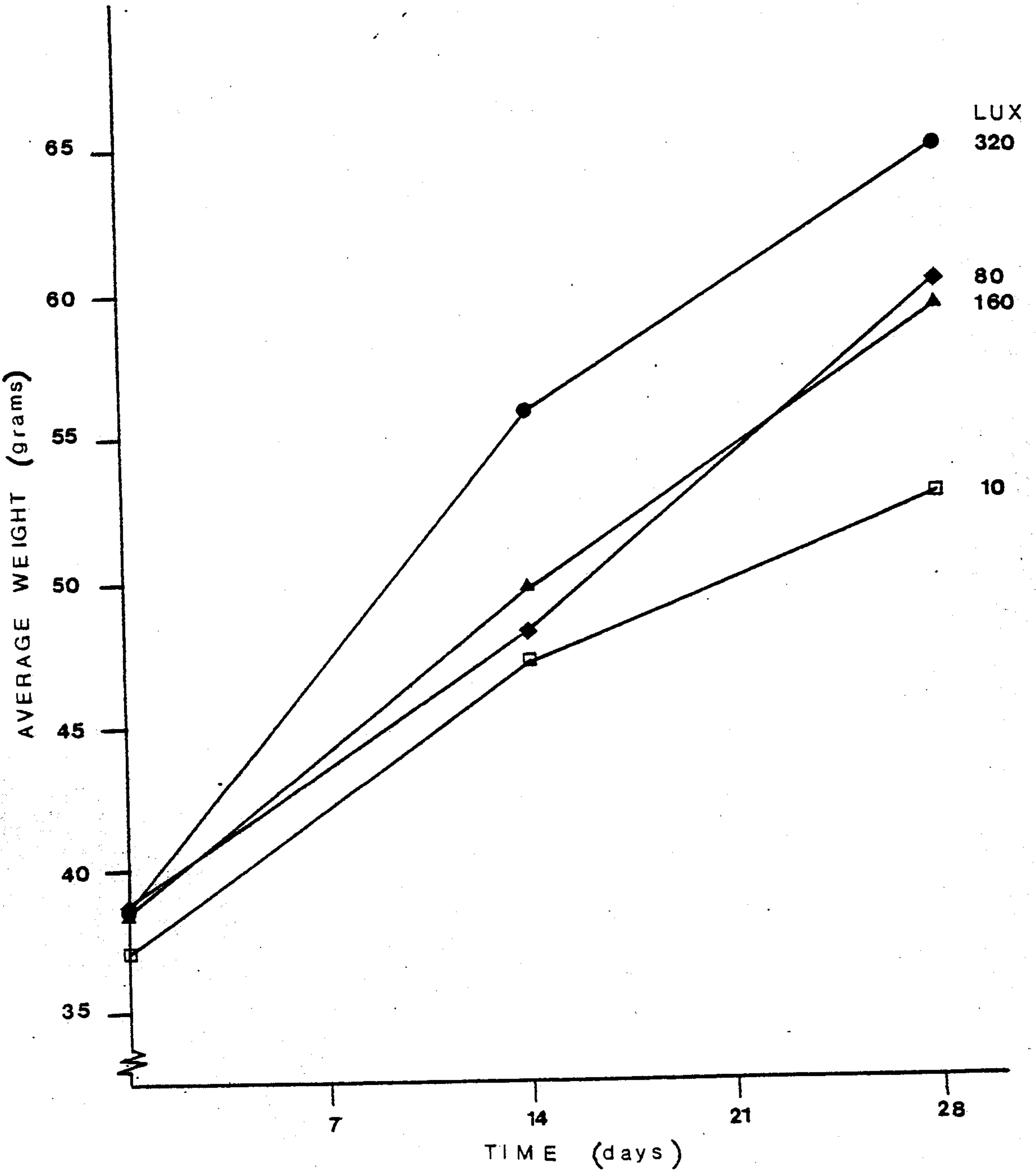


FIGURE 25 The growth responses of rainbow trout exposed to 12L12D photoperiod under 4 different light intensities in Experiment 2

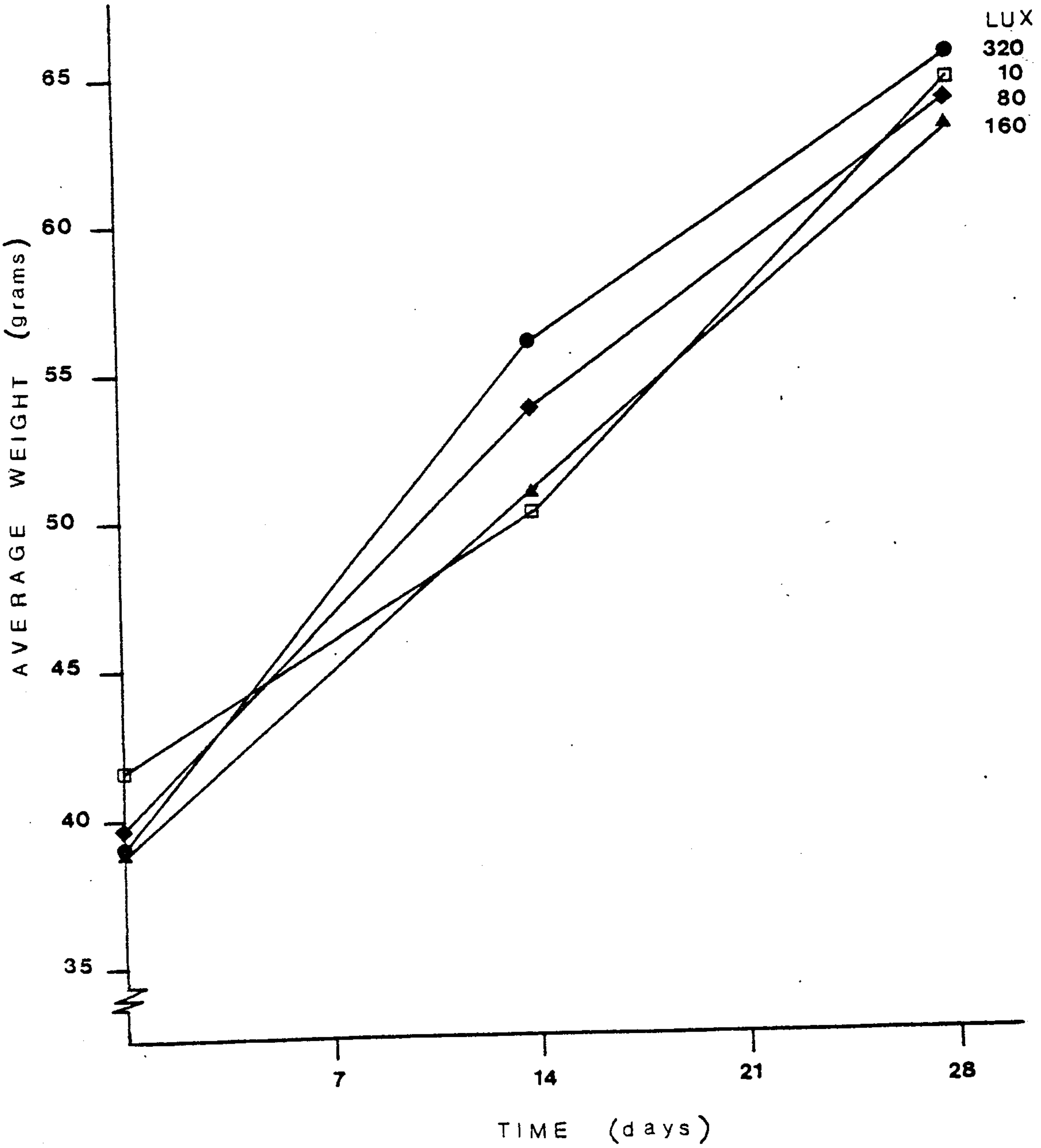


FIGURE 26 The growth responses of rainbow trout exposed to 15L9D photoperiod under 4 different light intensities in Experiment 2

4.3.4. Discussion and Conclusions

There was no effect of the different levels of light intensity on the rhythmic pattern of feeding activity. Under all photoperiods and levels of light intensity two peaks of feeding activity were observed separated by an interval of eight to nine hours. But again as in the previous experiment, replication of the experiment would have substantiated this conclusion.

The effect of increasing the level of light intensity on feeding behaviour is quite evident by the rate of feeding activity performed under each level of light intensity shown in Table 8. The mean number of daily trigger actuations under the low levels of light intensity (10, 80 and 160 lux) were significantly less than those under the highest intensity of light (320 lux) at photoperiods of 9L15D and 12L12D. Feeding frequency is effected by the lower level of light intensity which is evident by the fact that upon the onset of lights during the first two hours under all the photoperiods, fish under the light-intensities of 160 and 320 lux feed at a higher rate than fish under light intensity of 10 and 80 lux (see Figures 21, 22 and 23). During the third hour, the groups under 10 and 80 lux maintained the same feeding frequency as during the first and second hours but groups under the light intensity of 160 and 320 lux slowed down the feeding rates and maintained the same rates as groups under 10 and 80 lux light intensities. This shows that groups under the higher light intensities owing to their higher feeding level become satiated in less time than groups under the lower light intensities. Also, during the next three hours of the light regime, it was observed that groups under lower light intensities continue their feeding activity at a slightly higher level than the groups under the higher light intensity of 160 and 320 lux.

The feeding by groups under the lower light intensities reaches an apparently higher level during fourth, fifth and sixth hours because groups under higher light intensity owing to their more rapid satiation (due to faster rate of feeding during the initial three hours) slow down their feeding activity to such an extent that it appears as if the groups under the low light intensity are feeding at a higher level. This effect is attributable to a slow and steady feeding activity of the low light intensity groups. Such a feeding behaviour further supports the view that under the higher light intensity fish become satiated in a lesser time than the fish under low light intensity. The only plausible reason for such a feeding behaviour can be that light intensity seems to play a dominant role for the feeding activity by rainbow trout. Blaxter (1970) has also pointed out that light above a certain threshold is essential for certain patterns of behaviour; around this threshold there is a gradual reduction in behavioural performance and usually a general reduction in activity as well.

For rainbow trout which are visual feeders (Ware, 1973; Protasov, 1970), light intensity can play a crucial part in modulating the feeding behaviour. As observed in Experiment 1, under continuous dark feeding activity was very much restricted and the absence of light resulted in some loss of body weight owing to restriction imposed on feeding by the light. In the present experiment under the low light intensities of 10 and 80 lux feeding activity is much slower but steady as compared to the feeding activity of groups under the higher light intensities of 160 and 320 lux. The group under light intensity of 160 lux, under all photoperiods, was feeding significantly at a lower rate ($P<0.05$) than the other groups at 10, 80 and 320 lux, as is evident by the daily mean number of trigger actuations (Table 8). This gives an indication that at the light intensity of 160 lux, the feeding responses of trout compromise with the environmental factor of light. At a light intensity of 320 lux under all the three photoperiods the daily mean number of trigger actuations were at a significantly higher rate ($P<0.05$) than those of groups under the lower light intensities of 10 and 80 lux. Level of light intensity lower than 160 lux appears to have an inhibitory effect upon the feeding activity, whereas conversely a light intensity above 160 lux appears to have a stimulative effect upon the feeding activity.

The present work shows that not only the light intensity seems to modulate the feeding behaviour but also the length of the photoperiod effects the feeding behaviour. As the length of the light regime of the photoperiod was increased the daily mean number of trigger actuations also increased correspondingly. As mentioned in Section 4.3.1. results of Experiment 1 for photoperiod of 9L15D were incorporated in this experiment. Data in Table 8 shows an anomaly as compared to data for other light intensities and this coupled with lack of replication can cast some doubt on the results of the experiments. Groups under light intensity of 10, 80 and 160 lux at 15L9D photoperiod started to feed at a significantly higher rate ($P<0.05$) than the groups under the same light intensities at 9L15D photoperiod. There was no significant difference ($P>0.05$) between the feeding rates at 9L15D and 12L12D at light intensities of 10, 80 and 160 lux. Groups under 320 lux at all the three photoperiods did not show any significant differences in the number of mean daily trigger actuations ($P>0.05$). Therefore, there is good evidence to show that the inhibitory effects of low light intensity on the feeding activity of trout can be compensated in respect of the total amount of food that is consumed in the whole day, by an increase in the daylength. But by increasing the daylength, there is no effect on the pattern of feeding frequency. The feeding pattern of the groups under the low light intensities remains slow and steady, taking a longer time for the fish to be satiated as discussed before.

The changes in the feeding behaviour of the trout in the present controlled conditions of the laboratory are hard to compare with those which may be observed in their natural habitat owing to a large number of inter-dependent factors that influence the feeding behaviour. A comparable study by Bisson (1978) suggests that the feeding pattern of rainbow trout changes according to changing intensity and spectral composition of light in its natural habitat. Bisson in comparisons of the availability of different prey taxa with the composition and size distribution of food items in fish stomachs, has reported that trout were almost exclusively drift feeders during the midday hours (0800-1600). At dusk there was no consistency among the preferred feeding modes. At dawn bottom foraging predominated. During darkness hours (2000-0400) the fish fed exclusively off the bottom. Large sized trout did not eat anything during the 2400-0400 period. Since at different times of the day light intensity is different therefore such feeding behaviour of trout may be attributed to light intensity.

The present work shows that light intensity and photoperiod studies on the effect of feeding behaviour cannot be performed separately. If the light intensities are at low levels of 10 or 80 lux, increasing feeding activity (in terms of food intake) was accomplished at longer photoperiods of 15L9D, and if the light intensity is at a higher level of 320 lux then, an increased feeding activity was accomplished even at a shorter photoperiod of 9L15D. At an intermediate light intensity of 160 lux under all the three photoperiods, the mean number of daily trigger actuations were significantly lower ($P<0.05$) than the groups under the higher as well as the lower light intensities. There was no effect of 160 lux light intensity upon the feeding frequency, during the initial or the later hours of the coming "on" of the lights. Thus it appears as if the fish seem to stabilise their feeding activity at a light intensity of 160 lux.

The growth performance of different groups also reflects how the light intensity and photoperiod can effect the fish life. At low light intensities of 10 and 80 lux at shorter photoperiods growth was the poorest. But when the length of the photoperiod was increased then even at the low light intensities of 10 and 80 lux a significantly better growth was achieved. Similarly at a higher light intensity of 320 lux when photoperiod was changed from 9L15D to 12L12D or 15L9D, a significantly better growth performance was achieved. In the present study the food consumption and food conversion efficiency were also determined to find a possible mechanism through which photoperiod and the light intensity can effect growth. Results presented in Table 9 show that as the light regime was increased food consumption was also increased. Consequently maintenance requirements of fish increase as daylength increases. Increased maintenance requirements may be satisfied by increasing food consumption or improving conversion efficiency. In the present study food consumption was related to the length of the light regime of the photoperiod therefore, under longer light regime it appears that fish compensated for increased energy requirements by consuming more food. The extent that this increased activity affected food consumption is unknown, but the inter-relationships among food consumption, photoperiod, light intensity and growth suggest that the influence of photoperiod and light intensity on growth may be partially effected through stimulating food consumption in fish.

At longer photoperiod of 15L9D irrespective of the light intensities, similar growth performance was observed in different groups with a corresponding increase in the food conversion ratio for groups under 160 and 320 lux and a decrease in food conversion ratio for groups under lower light intensities of 10 and 80 lux. Thus due to these differences in food conversion efficiency it is evident that light does affect the physiological abilities of fish body in converting the food to fish flesh. Other workers have also pointed that the light intensities and wavelengths perceived by the eyes of fish vary according to species but the nature of the reaction may exert a strong influence on the physiology and behaviour of the fish (Hoar, 1958; Nikolsky, 1963). In present work the higher food conversion ratio results seen in the shorter photoperiods with low light intensities show that there is a certain physiological stress being experienced by the fish body. Under stronger light conditions, fish seem to become overactive and again appear to be over stressed. At photoperiod of 9L15D under a light intensity of 160 lux the food conversion ratio was the lowest, but as the light regime of the photoperiod was lengthened to 12L and 15L there was a corresponding increase in the food conversion ratio. This points to a certain threshold level of photoperiod and light intensity at which the lowest food conversion ratio can be expected with the best growth performance.

In a study of the influence of photoperiod on growth of the fingerlings of channel catfish (*Ictalurus punctatus*) by Stickney and Andrews (1971), a slightly better growth performance and food conversion under 12L12D photoperiod was observed. They however, suggested that growth rate or food efficiency would not be significantly enhanced by controlling this variable. Past studies involving light and fish growth have yielded conflicting results. Brown (1946) demonstrated that in brown trout, (*Salmo trutta*) there is an inverse relationship between growth and the daylength. Gross *et al*,(1965) reported in green sunfish

(Lepomis cyanellus), that increasing the photoperiod stimulates growth and decreasing photoperiod inhibits growth. Some studies suggest that there is no relationship between daylength and growth (Anderson, 1959; Bjorklund, 1958). Kwain (1975) studied the effect of light intensity and temperature on early growth of rainbow trout and reported that the fastest growth occurred at 10°C and 2 lux and the lowest growth at 3°C and 0.2 lux.

In conclusion it can be said that the singular influence of light cannot be isolated from that of other factors operating simultaneously and undoubtedly, the physiological and behavioural response of the organism is a resultant of the interaction of several variables. The present work indicates that light intensity and photoperiod studies cannot be performed separately. If the light intensity is low then under a longer photoperiod fish can adjust their food intake to show the same growth performance as can be achieved under a short photoperiod but at a high light intensity. Although the present study points towards a photoperiod of 9L15D at light intensity of 160 lux being an ideal situation for feeding activity better growth and low food conversion ratio by rainbow trout further studies should be carried out in order to determine the optimal pattern of light intensity and photoperiod in relation to changes in temperature. Presently however it can be positively suggested that in trout hatcheries adverse illumination levels and unfavourable light regimes could contribute to feeding inefficiency and poor growth performance by fish with the associated high mortality rate that can be expected.

4.4. Experiment 3 : Feeding Behaviour under Continuously Varying Photoperiods

4.4.1. Introduction

In the present experiment an attempt was made to simulate the natural effects of the increasing daylength and decreasing daylength in the light chambers (Section 3.3.5.). In the previous two experiments, photoperiods were of constant duration, but in this experiment from the initial day of the experiment to the final day, photoperiod was shortened or lengthened by ten minutes every day for decreasing and increasing photoperiods respectively, so that over the whole duration of the experiment, the photoperiod varied by eight hours in either direction (decreasing and increasing photoperiods). One group of fish experienced shortening days of "autumn to winter" and the other group experienced lengthening days of "winter to spring", over a period of forty-eight days.

Demand feeders facilitated the monitoring of feeding rates of the two groups and the feeding activity could be quantified by the number of trigger actuations performed by each group of fish.

4.4.2. Materials and Methods

4.4.2.1. The Experimental System and Animals

The experimental facility used in the present study was the recirculation system as described in detail in Section 3.2.

Fish (13-16cm) were obtained from the same fish farm as mentioned in Section 4.2.2.1. After quarantine procedures, as detailed in Section 3.5., two batches of twenty-two fish each were allocated to two experimental tanks. Fish were acclimatised and trained as described in Section 3.6. Temperature of the water in the tanks was $13.5^{o} \pm 2^{o}C$.

4.4.2.2. Varying the Photoperiods

Two groups of fish were subjected to conditions of varying photoperiods under the light intensity of 160 lux.

One group of twenty-two fish (average initial weight = 39.71g ± 1.40) was initially subjected to seven hour light (7L) and seventeen hour dark (17D) on the first day of experimentation. Thereafter, every day till day forty-eight of experiment the length of photoperiod was increased by ten minutes at the end of the light period, so that during forty-eight days, this group of fish experienced the effect of varying daylength (increasing daylength) from 7L on the first day to 15L on day forty-eight. After forty-eight days the photoperiod was kept at constant 15L9D for a further seventeen days.

A second group of twenty-two fish (average initial weight = 40.95g ± 1.15) was initially subjected to fifteen hour light (15L) and nine hours dark (9D) on the first day of experimentation. Thereafter, every day till day forty-eight of experiment, the length of photoperiod was decreased by ten minutes at the end of the light period, so that during forty-eight days, this group of fish experienced the effect of varying daylength (decreasing daylength) from 15L on the first day to 7L on day forty-eight. After forty-eight days, the photoperiod was kept at a constant 7L17D for seventeen days.

4.4.2.3. Experimental Diet

It was the same diet as used for Experiment 1. Feed dispensers were filled and re-stocked as described in Section 4.2.2.4.

4.4.2.4. Monitoring the Feeding Activity

The demand feeding system, described in detail in Section 3.4. was used for studying the feeding activity. Each experimental tank was fitted with one feed dispenser and one trigger according to the same plan as in Experiment 1 and shown in Figure 10.

Feed dispensers were tested, as described in Section 3.4.7. Feed dispensed/trigger actuation for the two groups is shown in Table 10.

Trigger actuations performed by fish in both groups were separately noted daily. Trigger actuations performed over each period of twelve days were added up for up to day forty-eight of experiment and their daily means and standard deviations were worked out as shown in Table 10. Average amount of food consumed per day by each fish in the group was calculated by the number of trigger actuations performed over the period of twelve days, in order to work out the food utilisation data, as shown in Table 10. After day forty-eight of the experiment up to day sixty-five when constant photoperiod was experienced by the two groups of fish, trigger actuations were again noted daily and daily mean number of trigger actuations for the period of seventeen days was worked out for both the groups and are shown in Table 10.

4.4.2.5. Weighing Procedure

Details of the weighing procedure are presented in Section 3.7. After acclimation and completion of the initial training period, fish in the two tanks were individually weighed (± 0.01g) for initial weights, and after every twelve days up to day forty-eight. Final weighing was done on day sixty-five at the conclusion of the experiment.

4.4.2.6. Statistical Analysis

Statistical analysis were performed involving a student's t-test comparison of the means of pooled samples of the weights of individual fish from each photoperiod. Similarly means of the other data were statistically compared using student's t-test. In the analysis of the results, P = 0.05 was used as the level of significance.

4.4.3. Results

4.4.3.1. Feeding Behaviour

Fish in both the groups fed actively throughout the experimental period of sixty-five days. Feeding records of both the groups is shown in Table 10 by the number of trigger actuations and the amount of food consumed by fish, under different regimes of the varying photoperiods.

During initial twelve days of experiment there was a conspicuous variation in the amount of daily food consumption of both groups, but thereafter, fairly consistent rates of feeding activity were established by fish, as can be observed by the daily mean number of trigger actuations shown in Table 10.

The group of fish subjected to a gradual increase in daylength over forty-eight days were observed to show a consistent increase in food consumption. The other group of fish subjected to a gradual decrease in daylength over the same period of days as the previous group of fish, were observed to show a consistent decrease in food consumption. These observations can be noted by the daily mean number of trigger actuations, as shown in Table 10, performed by the two groups for the forty-eight days of the varying photoperiods. At the end of forty-eight days the total amount of food consumed by both the groups did not show

TABLE 10

Growth rate and food utilisation data of two groups of rainbow trout subjected to increasing and decreasing photoperiods in Experiment 3

		1st 12 days	2nd 12 days	3rd 12 days	4th 12 days	5th17 days
INCREASING DAYLENGTH	Increasing Photoperiod from 7L to 15L	9L 15D	11L 13D	13L 11D	15L 9D	Constant 15L9D
	Total Trigger Actuations	611	750	911	941	1966
	Daily Mean Actuations S.D. ±	51 21	63 15	76 13	78 17	123 9
	Total Food Consumed (g) (at rate of 0.76g/trig.act.)	464.36	570	692.36	715.16	1494.16
	Av.Wt. Food Consumed/Fish/ 12 days (g)	21.11	25.91	31.47	32.51	67.92
	Av. Wt. Food Consumed/Fish/ day (g)	1.76	2.16	2.62	2.71	4.00
	Food Conversion Ratio	2.92	2.04	1.92	3.50	2.14
	Specific Growth Rate (%/Day)	1.39	1.99	2.03	0.96	1.85
DECREASING DAYLENGTH	Decreasing Photoperiod from 15L to 7L	13L 11D	11L 13D	9L 15D	7L 17D	Constant 7L17D
	Total Trigger Actuations	919	871	648	343	828
	Daily Mean Actuations S.D. ±	77 22	73 12	54 12	29 7	52 10
	Total Food Consumed (g) (at rate of 0.80g/trig.act.)	735.20	696.80	518.40	274.40	622.40
	Av. Wt. Food Consumed/Fish/ 12 days (g)	33.42	31.67	23.56	12.47	30.11
	Av. Wt. Food Consumed/Fish/ day (g)	2.78	2.64	1.96	1.04	1.77
	Food Conversion Ratio	2.79	3.68	1.56	1.48	0.86
	Specific Growth Rate (%/Day)	2.14	1.26	1.83	0.87	2.04

a significant difference ($P>0.05$). Although under different light regimes both the groups were consuming significantly different quantities of food ($P<0.05$), except when both groups were at the same photoperiods after twenty-four days ($P>0.05$). For the first twelve days the daily mean number of trigger actuations by both the groups under increasing and decreasing daylength showed a significant difference ($P<0.01$). Group under decreasing daylength were consuming significantly higher quantities of food than the group under increasing daylength. For the next twelve days, daily mean number of trigger actuations by both the groups did not show a significant difference ($P>0.05$). For each twelve days again at third and fourth weighings of the fish, the daily mean number of trigger actuations by group under decreasing daylength was significantly at a lower rate than the group under increasing daylength ($P<0.001$).

After forty-eight days, for the group of fish under constant photoperiod of 15L9D, the daily mean number of trigger actuations for seventeen days was significantly higher ($P<0.01$) than the group of fish under constant photoperiod of 7L17D. Average daily trigger actuations for group of fish in 15L9D photoperiod was 123 S.D.± 9 and fish consumed the food at an average of 4.00g/day/fish. In contrast, other group of fish under constant 7L17D photoperiod average daily trigger actuations were 52 S.D.± 10 and average food consumption was 1.77g/day/fish.

4.4.3.2. Growth Performance

Growth responses of fish in both the groups is represented graphically in Figure 27, where average fish weights are plotted against time.

Statistical analysis of initial fish weights showed no significant differences ($P>0.05$) between the two groups. Fish in both groups showed a marked gain in final average weights over the initial average weights,

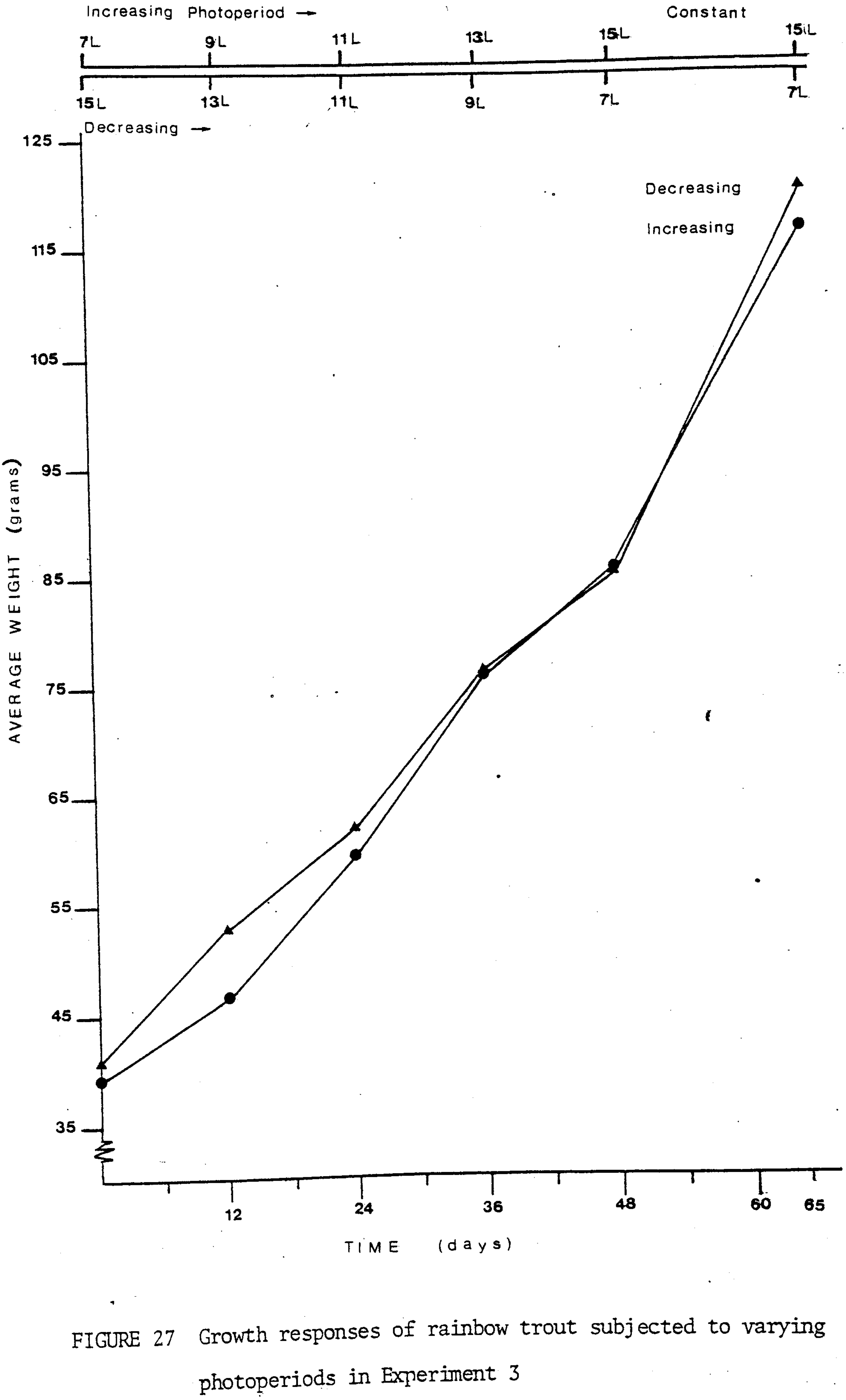


FIGURE 27 Growth responses of rainbow trout subjected to varying photoperiods in Experiment 3

but the final average weights of fish in both the groups did not show a significant difference (P>0.05).

The Specific Growth Rates (SGRs Section 3.11.1.) of the two groups over the sixty-five days of the experiment are shown in Table 10. Mean Specific Growth Rates of the two groups, after forty-eight days did not show a significant difference (P>0.05). For the period of days thirty-seven to forty-eight, fish in both groups showed a poor growth response, which was caused due to circumstances beyond control (electricity was cut off on several occasions due to strike by employees of Electric Board, which caused several problems, though on every occasion electricity was restored by the use of portable generators in the fish culture unit).

4.4.3.3. Food Conversion

Food Conversion Ratios (FCRs Section 3.11.2.) for every twelve day period for both the groups is presented in Table 10. Mean values of FCRs of the two groups after forty-eight days did not show a significant difference (P>0.05). FCRs showed consistent decrease with decreasing daylength after twenty-four days of experimentation in case of fish subjected to decreasing photoperiod. After day forty-eight group of fish under constant 7L17D photoperiod for seventeen days, showed the lowest conversion ratio of 0.86, as compared to 2.14 in case of fish under constant 15L9D photoperiod for same period of time.

4.4.4. Discussion and Conclusions

The effect of changing the daylength on feeding behaviour is perhaps best demonstrated by comparing the feeding activity of fish in the forty-eight days period of the "increasing group" with that of the "decreasing group". Both the groups experienced the same amount of light periods at the end of the varying photoperiod (7-15 hour and 15-7 hour respectively), but fish exposed to increasing daylength consumed progressively higher amounts of feed corresponding to the gradual increase of daylength (ten minutes each day). On the other hand, fish exposed to decreasing daylength consistently consumed lower amounts of feed corresponding to gradual decrease in the daylength. Apparently at different photoperiods fish in the two groups were feeding at different rates. However, when the mean values of the food consumed over the total period of forty-eight days were compared there was no significant difference ($P>0.05$) in the amounts of feed consumed by the two groups. Thus a clear effect of the varying daylength on the feeding behaviour of rainbow trout is apparent in the present experiment. For both the groups at different times when the photoperiod was also different, the feeding activity was mainly restricted by the daylength. Increasing daylength seems to have a stimulative effect upon feeding activity and decreasing daylength appeared to show an inhibitive effect upon the feeding activity.

Since rainbow trout are visual feeders their feeding behaviour can be expected to be modulated by the daylength. In longer light regimes of the photoperiods fish remain comparatively more active than during the dark regime, so that the metabolic rate of the body demands higher energy intake which is met by increased food intake. Fish under the shorter light regime of the photoperiod remain comparatively less active and therefore the longer rest periods induced by darkness reduce the energy required for maintenance and offer an explanation for the observed lower food consumption following periods of reduced light exposure. A similar phenomenon was also observed by Brown (1946) in brown trout *(Salmo trutta)*, when she exposed the fish to different photoperiods.

In the present experiment since the feeding was ad libitum, the feeding responses of the fish in both the groups were better understood in relation to the effects of their environment. The only environmental variable was the daylength, hence leaving little doubt about the effects of the daylength on the feeding behaviour. Swift (1964) found that brown trout *(Salmo trutta)* exhibit a diurnal rhythm in their locomotory activity which is stimulated by the light. Diurnal activity pattern influenced by light is also reported by Alabaster and Robertson (1961), who associated this activity with a feeding cycle in roach *(Rutilus rutilus)*, bream *(Abramis bramis)* and perch *(Perca fluviatilus)*.

The effect of the varying daylength was further studied after forty-eight days by keeping the daylengths constant for a further seventeen days for both groups. Thus the group under 15L regime remained at this constant photoperiod of 15L9D and the other group remained under constant photoperiod of 7L17D for seventeen days. During the seventeen day period it was observed that under constant photoperiods the group under longer light regime of 15L9D fed at a significantly higher rate ($P<0.01$) than the group under shorter light regime of 7L17D. Fish under longer photoperiod increased their daily mean number of trigger actuations whereas the other group under shorter photoperiod was able to actuate the trigger at the same rate as they were actuating under 9L15D photoperiod.

The only explanation of a greater number of trigger actuations by fish under longer photoperiod can be that the environmental conditions of the fish provided them a greater opportunity to actuate by allowing them a longer time of visual encounter. Fish adapt to the longer daylength and start feeding greater quantities of food than the minimum level required for growth. Thus a poor food conversion ratio is shown by the group as is evident from Table 10. The group under shorter daylength adapts itself by feeding to satiation in as little time as possible, as observed in Experiment 1, under shorter photoperiods of 3L21D and 6L18D. Feeding intensity during the initial two hours of the light regime is very high in order to maximise the food intake in the shortest time possible. Thus light imposes great restrictions on the level of feeding activity that can be performed by rainbow trout. As discussed in Section 5.1.4., due to a longer period of deprivation, the hunger level is increased which in turn effects the feeding behaviour of rainbow trout. Group under shorter photoperiod consumed significantly less quantity of food during the seventeen days of constant photoperiod and showed a better food conversion efficiency as compared to the group under longer photoperiod of 15L9D. Thus, it shows that shorter daylength does not effect the food conversion efficiency of the fish as long as the other variables are not limiting. The prior exposure of the two groups to the varying daylengths did not appear to effect the feeding level. Under varying conditions of daylength a corresponding variation in mean number of daily trigger actuations was observed during the experiment, within and between the two groups at different times of the photoperiods, as shown in Table 10. This variation is explicable on the basis of the changes in the light regimes. When the varying conditions of the daylength were changed to constant photoperiods for both groups, the daily trigger actuations stabilised according to the constant duration of the light regimes. Group under longer constant light regime showed greater number of daily mean trigger actuations and the other group under shorter constant light regime showed a less number of daily trigger actuations, with little variation each day during the seventeen days of the constant photoperiod. Thus it can be said that it is not any prior photoperiod history that modulated the feeding behaviour under constant photoperiods, but it was the effect of light which elicitated different feeding responses under varying conditions of the photoperiods.

The increasing and decreasing daylengths did not show any effect on the growth performance of fish in the two groups. Fish in the increasing daylength were progressively feeding at higher rates than the other group under the decreasing daylength, and were therefore expected to gain more weight than the other group at the end of the experiment. But contrary to expectations the final average weights of the fish in the two groups did not vary significantly ($P>0.05$). Gross et al. (1965) observed in green sunfish *(Lepomis cyanellus)* that the growth was influenced by the type of daylength. They observed a better growth in the sixteen hour daylength than in the eight hour constant daylength and under increasing and decreasing daylength a significant difference in growth performance of the two groups was observed. Thus results of the present study disagree with those of Gross, et al., but complement the findings of Bjorklund (1958), who found that in goldfish *(Carassius auratus)* length of the day did not have any effect upon the growth performance.

The group of fish under decreasing daylength was actuating the trigger at a consistently decreasing rate along with the decreasing daylength and was apparently consuming lower quantities of food but was able to achieve the same growth performance as the group feeding at an apparently higher rate. This indicates that the varying effect of the photoperiod did not affect the growth performance of the two groups. Fish under the shorter constant photoperiod were deprived of the opportunity to feed for a longer period owing to the restriction imposed by the shorter period of the light regime. Nevertheless, fish under shorter photoperiod made up the deficiency by utilising the feed with a better efficiency than the other group. It is quite possible that fish in the longer constant photoperiod due to their increased activity did not show a better feed conversion than the fish in shorter photoperiod, or conversely the fish in the shorter photoperiod due to their decreased activity were able to show a better feed conversion than the fish in longer photoperiod. Whatever be the reasons, it is evident that both the groups in the increasing and also the decreasing photoperiod showed the same growth performance over the forty-eight days of varying conditions and also under constant photoperiods for seventeen days.

In conclusion the results show that varying daylength does effect the feeding behaviour of fish but does not effect the growth performance. Improved efficiency in food conversion ratio results in the group subjected to a shorter constant photoperiod and poor feed conversion results in the group subjected to a longer constant photoperiod, thus showing that light can effect the food conversion efficiency of rainbow trout. Since the one common variable in both the groups was the variation in the day length, it would seem that it is the light which stimulates these fish to elicit different feeding responses and thus modulate their feeding behaviour accordingly.

CHAPTER 5

Effect of Some Physical Variants of Food on Feeding Behaviour of Rainbow Trout *(Salmo Gairdneri)*

5.1. Experiment 4 : Effect of Size of Food Pellets on Feeding Behaviour

5.1.1. Introduction

There is much evidence that salmonid fish, in common with many other teleosts, select prey on the basis of body size. Both physical and physiological factors can be postulated as playing possible limitations on the size range of food material that can be efficiently handled.

A wide variety of organisms are available as food, during the year to trout inhabiting the freshwater. The substrate of streams or lakes are grazed to obtain these foods, while free-swimming forms in midwater are pursued and captured. Salmonids also feed on organisms passively transported by water currents or wave action along the bottom, in midwater or at the water surface. This charactertistic diversity of diet and breadth of feeding behaviour has led many scientists to classify salmonids as "opportunistic feeders", fish that tend to eat whatever they can capture, whenever it is available (Pentelow, 1932; Needham, 1969; Hunt, 1965; Mann and Orr, 1969; Miller, 1946).

Food particle size has been recognised as of practical importance in fish culture for many years. Empirical rules have been worked out for determining an appropriate schedule of pellet sizes of prepared food with fish growth (Hastings & Dickie, 1972). There has been very little work done to demonstrate the role which food particle size plays in either fish culture or nature. It is quite possible that food particle size plays a major role in controlling growth efficiency and information on its effects can be taken into consideration for interpretation of other experiments dealing with the chemical nutrient quality of the food with which the particle size effects are likely to interact. The characteristics of physical distribution and availability for grazing may have an influence on food value for growth, which are as important as substantial changes in the chemical nutrient or bulk characteristics.

The kinds of natural food eaten by a given species of fish are commonly determined by examining stomach contents. Such data, however, cannot be used as a measure of food preference unless quantitative methods are applied. The measurement of the relative preference given to different kinds of food organisms by a given species of fish must be based on two important things:

1. The relative numbers of these organisms eaten by the fish; and
2. The relative numbers of these organisms available.

The accuracy of this measure of preference, then, depends upon the accuracy of the methods that are used for determining the number of organisms available and the number eaten by the fish. In the present study demand feeders were used (described in detail in Section 3.4) so that free options were provided to fish to select their own preferred food particle size. Thus a fairly accurate account of preferred food particle size opted for, by fish could be obtained by knowing the number of trigger actuations for different food particle sizes. Throughout different trials two or more than two pellet-size options were made available to fish at a time for the study of their food particle size preference.

5.1.2. Materials and Methods

5.1.2.1. The Experimental System and Animals

The experimental facility used in the present study was the recirculation system as described in detail in Section 3.2.

Fish (13-18 cm) were obtained from the same source as mentioned in Section 4.2.2.1. After quarantine procedures (Section 3.5.), two batches of twenty-one fish each were allocated to two of the 240 l experimental tanks and one batch of forty-two fish were allocated to another experimental tank at the prevailing ambient temperature of $14.0^{\circ} \pm 2^{\circ}C$. Fish were trained for trigger actuations as outlined in Section 3.6.

Photoperiod was controlled at twelve hours light and twelve hours dark (12L12D) throughout the experimental period.

5.1.2.2. Weighing Procedure

Details of the weighing procedure are presented in Section 3.7. Fish were individually weighed and their total body lengths were taken at the start and conclusion of the experiment.

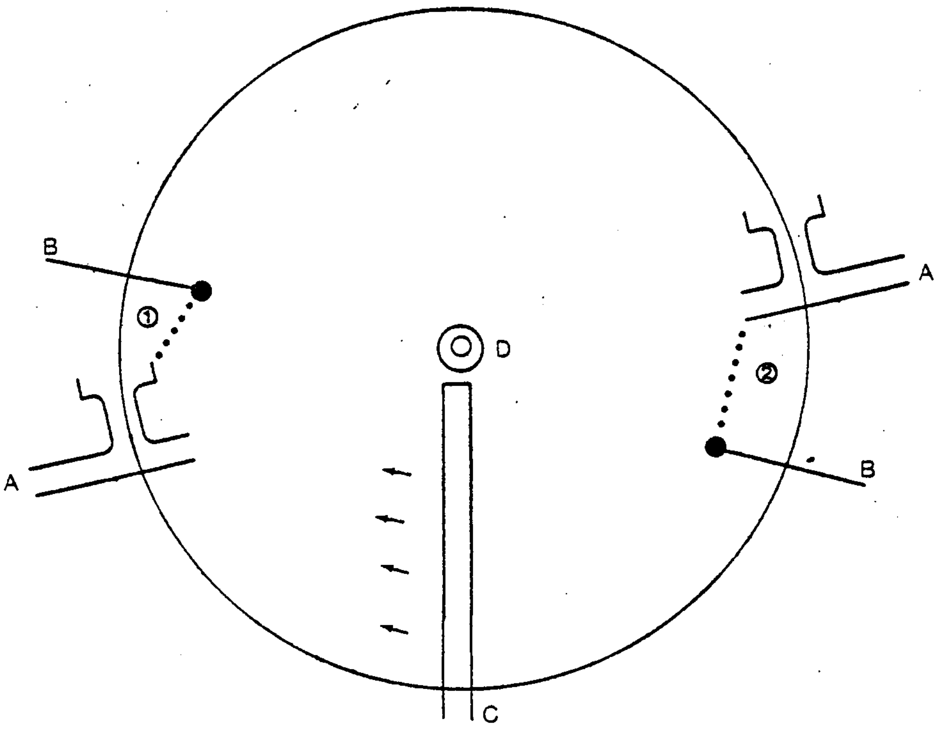
5.1.2.3. The Experimental Feed Particle Size

Food consisted of the same commercial diet as described in Section 4.2.2.4. The calculated dietary components, as given by the manufacturers are shown in Table **13**. The recommended feed pellet size for different fish size group by the manufacturers has been shown in Table 11. For this study three different feed pellet sizes in different combinations were studied in three different trials as shown in Table 12. During each of the two trials for fish in Groups 1 and 2, one feed pellet size was according to the manufacturer's recommendation, and the other pellet size was one to two numbers higher than the recommended size. At the start of the trial for fish in Group 3, according to the manufacturer's recommendation, pellet size five was suitable, but the group was provided with one size lower and one size higher than the recommended size, alongwith the recommended size of feed pellet. It was intended to prolong the trial in order to enable the fish to grow in weight and size to an extent, that they became entitled to the next size of feed pellet as recommended by the manufacturers. Thus a transition in the feeding behaviour of fish in response to pellet size could be expected towards the last stages of the experiment, by fish opting to select the higher size of pellet due to their increase in weight and size.

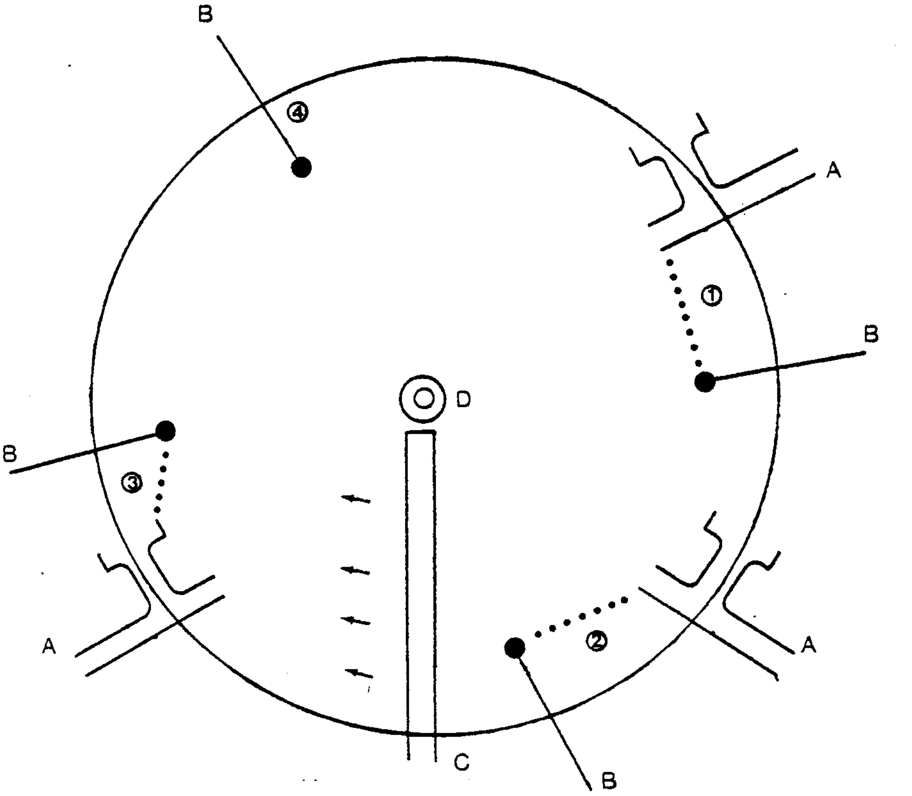
Manufacturer's recommendation chart mentioned the feed pellet's sizes by numbers only and did not mention the measurements in inches or centimetres. Thus in order to convert these numbers into millimetres, measurements of different food pellet sizes were taken in mm by picking 40 pellets at random from a handful of pellets of each size number. Diameters and length sizes measured in mm of forty pellets of each size number was meaned and standard errors calculated and are shown in Table 11.

5.1.2.4. Monitoring Feeding Behaviour

Demand feeding system described in detail in Section 3.4. was used for studying feeding behaviour. Two feed dispensers and two triggers were provided for fish in each experimental tank of Group 1 and Group 2, according to the relative positions of triggers and feed dispensers as shown in Figure 28a. Whereas, fish in Group 3 were provided with three feed dispensers and four triggers in the experimental tank according to the relative positions of triggers and feed dispensers as shown in Figure 28b. A fourth trigger was not connected to a feed dispenser and was considered to be a blank. Hence, fish in Groups 1 and 2, were each provided with two options of pellet sizes to choose from, and fish in Group 3 had four options of pellet sizes to select including the blank trigger, as shown in Table 12.



ⓐ GROUPS 1 & 2



ⓑ GROUP 3

FIGURE 28 Relative positions and numbers of feed dispensers (A) and triggers (B) for studying feed pellet size preference of rainbow trout during 3 different trials in Experiment 4. Arrows show direction of flow of water in the tank.

C = Inlet Pipe   D = Outlet Pipe

Feed dispensers of three groups were stocked with feed pellets of different sizes, as shown in Table 12, and refilled when required, usually after two days. At every trigger actuation approximately 0.25g of feed pellets were dispensed by the respective feed dispenser except the trigger which was not connected to a feed dispenser. This blank trigger was introduced to the tank in order to study whether the fish can discriminate between a trigger that supplies food and one that does not. Feed dispensers of all tanks were occasionally tested, at least once every week, as discussed in Section 3.4.7.

The daily number of trigger actuations by fish in Groups 1 and 2 were noted for three weeks and are shown in Figures 28 for Group 1 and Figure 29 for Group 2. For fish in Group 3, daily trigger actuations were noted for eighty-four days of experimentation. Daily percentages of trigger actuations by fish in Group 3 for the initial fifteen days and for the final fifteen days of experimentation were calculated from actual number of total daily trigger actuations for these periods and are presented in Figure 30. The study of the feeding behaviour of fish in this group was prolonged for eighty-four days, so that the effect, if any, of the increase in body weight and body length may be conspicuously reflected in a change of feeding behaviour in response to feed particle size.

Fish in all three groups had every opportunity of obtaining food at any time and as many times as desired. There was no check on the quantity of food that could be consumed by fish by continuously actuating the triggers, so that it can be said that fish were feeding *ad libitum*.

5.1.2.5. Statistical Methods

These were performed as detailed in Section 3.11.

5.1.3. Results

5.1.3.1. Feeding Behaviour

5.1.3.1.1. Group 1

Feeding record of this group is represented graphically in Figure 29, where the number of trigger actuations are plotted against time (days). Initial average weight of fish was 48.00g S.D. ± 9.54 and initial average total body length of fish was 15.73 cm S.D. ± 0.78 as shown in Table 12, and according to manufacturer's recommended feed pellet size as shown in Table 11, it was expected that fish would prefer to consume feed pellet size five. But as shown in Figure 29, fish opted to actuate the trigger connected to feed dispenser number one, containing feed pellet size number four. For the first two days of the trial, number of trigger actuations for pellet sizes four and five were nearly the same, but from day three to day fifteen a significant difference ($P<0.01$) in number of trigger actuations for size four and size five became apparent. From day sixteen and onwards until the end of the trial, the apparent difference in number of trigger actuations for size four and size five started narrowing down and there was a tendency to show equal preference for both sizes of feed pellets. Statistical analysis of feeding behaviour from day sixteen to day twenty-one of the trial period showed no significant difference ($P>0.05$) for selection preference of sizes four and five of the feed pellets. Statistical analysis of feeding for both sizes of feed pellets, over the whole period of twenty-one days, show a significant preference for size four ($P<0.05$).

At the end of the trial final average weight of fish was 65.09 g S.D. ± 13.61 and average body length was 16.99 cm S.D. ± 1.23.

TABLE 11

Trout feed pellet size as recommended by manufacturers for rainbow trout and used in Experiment 4

Pellet Size Number	Measurement* of Pellet Size in mm		Recommended Weight of Fish g	Recommended Length of Fish cm
	Diameter	Length		
4	3.00 S.E. ± 0.01	3.33 S.E. ± 0.01	25 to 40	12.70 to 15.25
5	4.00 S.E. ± 0.01	4.26 S.E. ± 0.02	40 to 90	15.25 to 20.32
6	5.00 S.E. ± 0.01	5.28 S.E. ± 0.04	90+	20.33+

*Actual measurements of pellet size were not mentioned by the manufacturers. These were measured by selecting 40 pellets at random and their standard errors were worked out.

TABLE 12

Trout feed pellet sizes and average fish weight and length experimented on, for study of feeding behaviour of rainbow trout in Experiment 4

Group Number	Feed Dispenser Number	Pellet Size Number	Fish No. in Group	Initial Average Weight of Fish g	Initial Average Length of Fish cm	Final Av. Weight of Fish g	Final Av. Length of Fish cm
1	1	4	21	48.00 S.D. ± 9.54	15.37 S.D. ± 0.78	65.09 S.D. ± 13.61	16.99 S.D. ± 1.23
	2	5					
2	3	4	21	48.54 S.D. ± 5.31	15.80 S.D. ± 0.52	63.35 S.D. ± 7.49	16.74 S.D. ± 0.69
	4	6					
3	5	4	42	61.69 S.D. ± 8.68	16.47 S.D. ± 0.81	163.96 S.D. ± 36.05	22.16 S.D. ± 1.68
	6	5					
	7	6					
	8	BLANK					

TABLE 13

Calculated dietary components of feed pellets used in Experiment 4 as given by manufacturers (Edward Baker Limited)

Component %	Diet (Feed Pellet Size) Number 4	Diet (Feed Pellet Size) Numbers 5 & 6
Oil	8.0	6.0
Protein	47.0	41.0
Fibre	4.5	4.5
Ash	10.0	10.5
Moisture	8.0	9.0
Carbohydrate (NFE)*	22.5	29.0

* NFE = Nitrogen Free Extractives

5.1.3.1.2. Group 2

Feeding record of this group is represented graphically in Figure 30, where number of trigger actuations are plotted against time (days). Initial average weight of fish was 48.54g S.D. ± 5.31 and total body length was 15.80 cm S.D. ± 0.52 as shown in Table 12. According to manufacturer's recommended food pellet size as shown in Table 11, fish should have been provided with feed pellet size five, but this group was provided with feed pellet sizes four and six.

Fish in this group opted to actuate the trigger connected to feed dispenser number three containing pellet size four. For the first two days of the trial, number of trigger actuations for pellet size four and six were nearly the same but an apparent difference became clear from day three and onwards until the end of the trial. Very few trigger actuations were performed for feed pellet size six, but whatever quantity of pellets were dispensed, they appeared not to be consumed by fish, since some of them were always found floating on surface of water, whenever inspection of the experimental tank was done.

At the conclusion of the trial, final average weight of fish was 63.35g S.D. ± 7.49 and final average total body length was 16.74 cm S.D. ± 0.69.

Statistical analysis of feeding behaviour for both sizes of feed pellets over the whole period of twenty-one days, shows a significant preference for size four ($P<0.01$).

5.1.3.1.3. Group 3

Feeding record of this group is represented graphically in Figure 31, where percentages of the total daily trigger actuations for four triggers

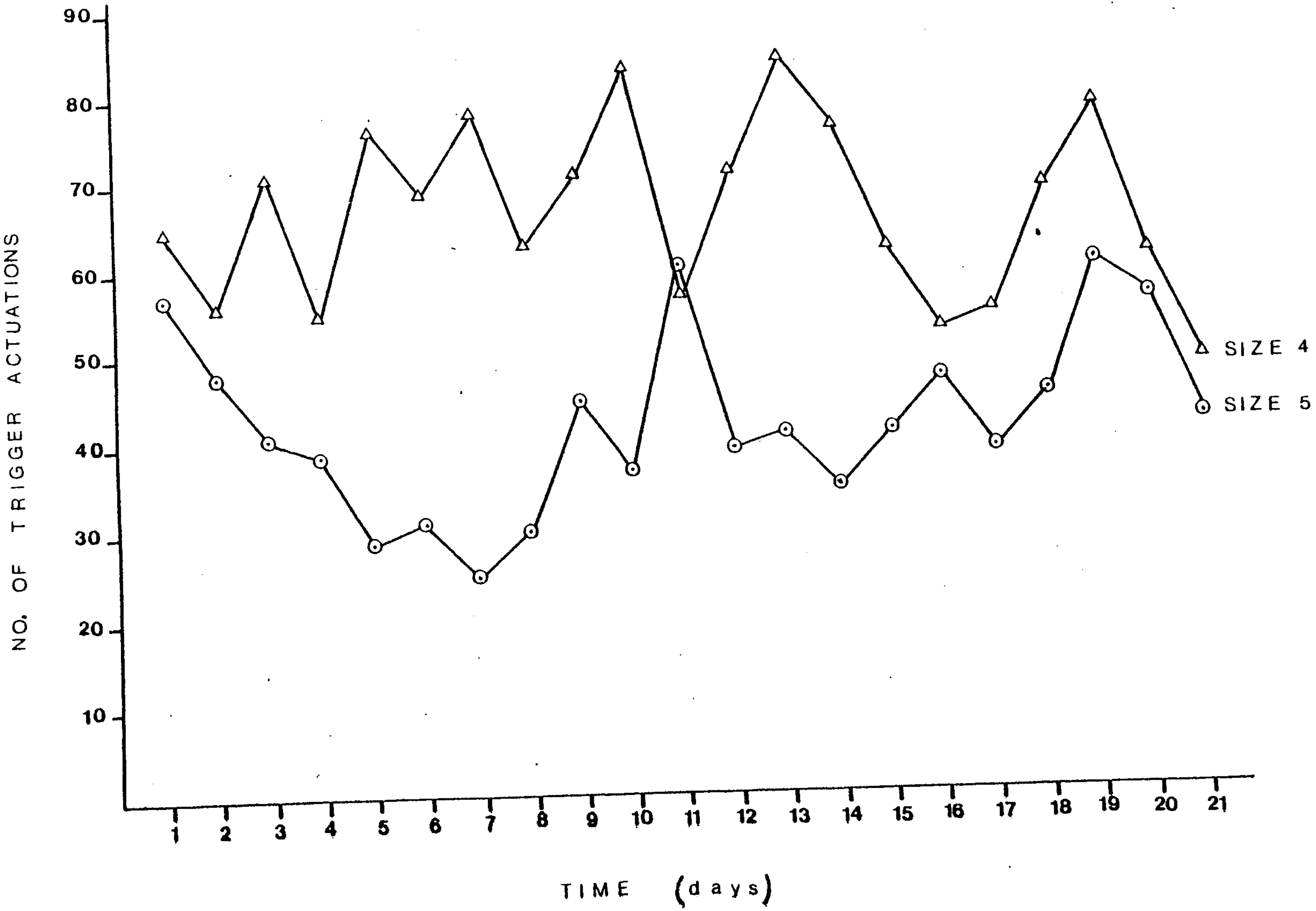


FIGURE 29 Trigger actuations performed for 2 sizes of the feed pellet by rainbow trout in Group 1 of Experiment 4

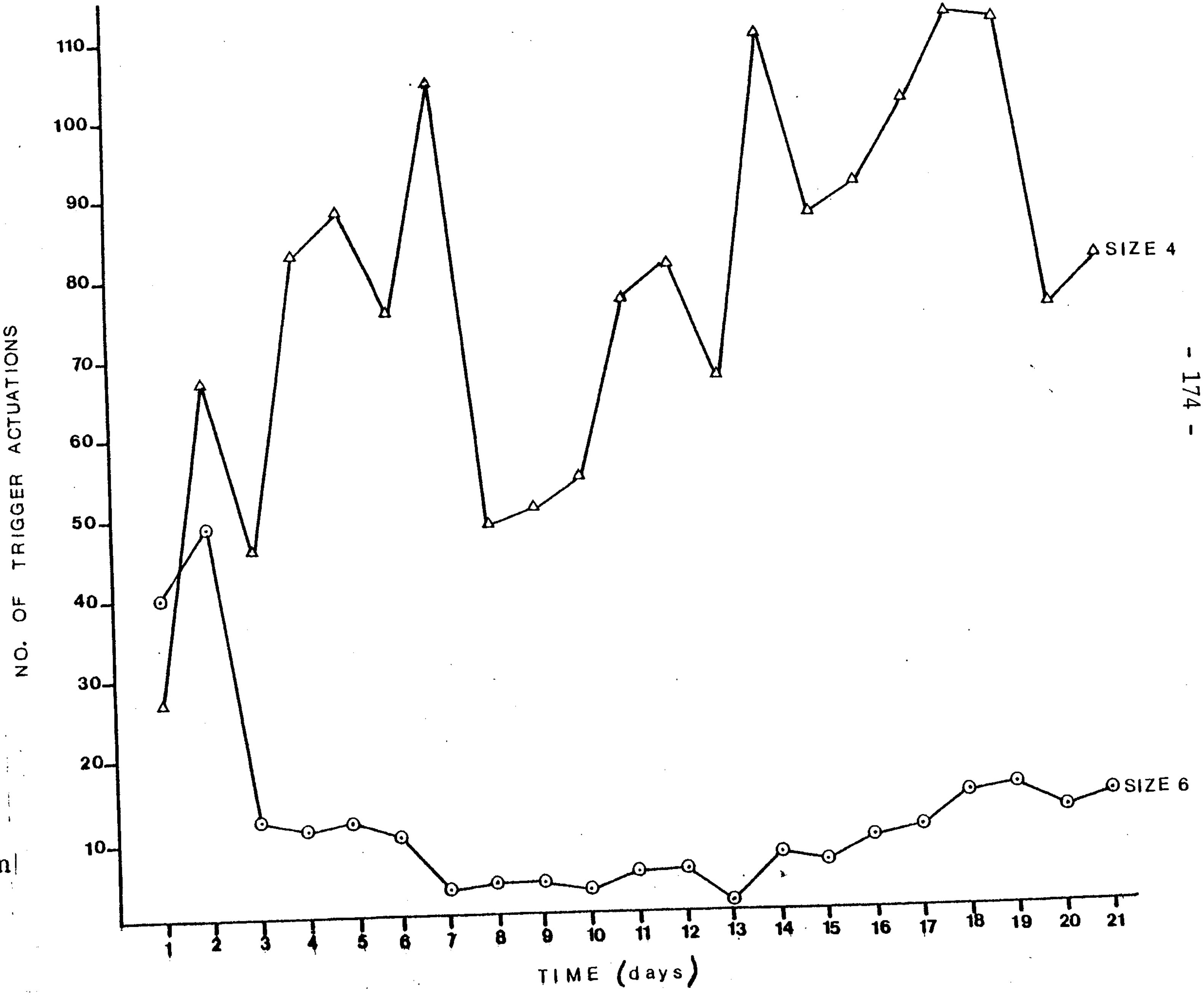


FIGURE 30 Trigger actuations performed for 2 sizes of the feed pellet by rainbow trout in Group 2 of Experiment 4

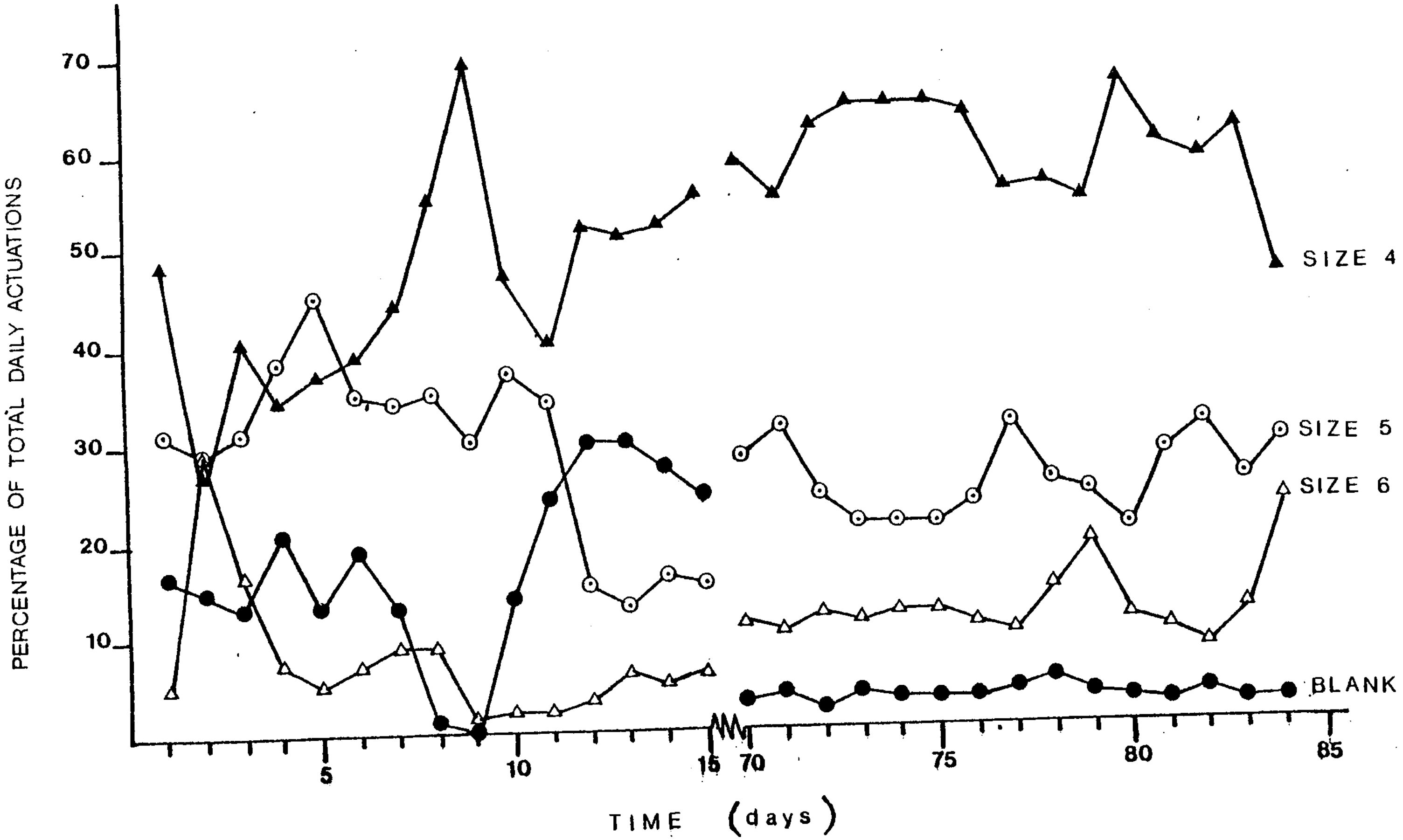


FIGURE 31 Trigger actuations performed by rainbow trout in Group 3 for 3 different sizes of feed pellets and 1 blank feed dispenser in Experiment 4 (Trigger actuations shown for the first 15 days and the last 15 days of the trial)

in the tank (three triggers connected to feed dispensers and one blank trigger) are plotted against time (fifteen initial days and fifteen final days of the experiment).

Initial average weight of fish was 61.69 g S.D. ± 8.68 and initial average total body length was 16.47 cm S.D. ± 0.81 as shown in Table 12. According to manufacturer's recommended feed pellet size as shown in Table 11, it could be expected that fish of this weight and size would prefer to consume feed pellets of size five. Trigger actuations for initial fifteen days of the trial shown in Figure 31, depicts a confused picture at start, but after two days it is apparent from Figure 31 that fish were actuating the triggers connected to feed dispenser number five, holding pellet size four (See Table 12) and feed dispenser number six holding pellet size number five more frequently than the trigger connected to feed dispenser number seven holding pellet size six and the blank trigger.

Trigger actuations for the last fifteen days of the trial shows a preference of an average of 59% S.D. ± 5.11% for feed pellet size four. Preference for feed pellet size five was at an average of 26% S.D. ± 3.81% as compared to an average of 12% S.D. ± 3.68% for pellet size six. The blank trigger was actuated at an average of 3% S.D. ± 0.85%.

Statistical analysis of feeding for the last fifteen days of the trial shows significant differences ($P<0.05$) in size selection preferences by rainbow trout and ranks the feed pellet size preference in the following order $4>5>6>$Blank. (Appendix table 2).

At the end of the trial final average weight of fish was 163.96 g S.D. ±

36.05 and final average body length was 22.16 cm S.D. ± 1.68.

5.1.4. Discussion and Conclusions

The feeding behaviour of rainbow trout seems to be modulated by the food particle size. In this study when the trained fish were given the opportunity of ad libitum feeding, a food particle size - dependent preference was elicitated in the feeding behaviour. During all the trials a particular feed pellet size preference over other feed pellet sizes was observed. Feed pellet size preference seems to bear some relationship to the size of the fish body. But as feed pellet size 4 had 6.0% more protein than the other pellet sizes this possibly could have influenced the results.

In Group 3 larger sized fish were used and were subjected to a longer period of experimentation (eighty-four days). When fish in Group 3 were subjected to four triggers in the tank, the number of trigger actuations for at least ten days appeared to be indiscriminate. On the first three days, even the blank trigger was actuated for nearly the same number of times as the triggers connected to feed dispensers containing different sizes of feed pellets. As time passed a selection preference started to emerge, since the number of trigger actuations for every feed pellet size showed a certain degree of consistency. This implies that feeding responses of rainbow trout had stabilised with experience. Fish had become experienced in terms of familiarity with particular sizes of the feed pellets and also with the ability to identify the food triggers solely by their spatial relationship to each other and to the other space parameters, such as the inlet and outlet pipes in the tank. The time period to get familiarised with particular size of feed pellets and also for the ability to identify the food triggers is dependent upon the number of choices offered to the fish. In Groups 1 and 2, only two triggers were presented in the tanks of the respective groups. It took only two days for each group to be able to discriminate the triggers according to the feed pellet size delivered by the respective triggers. In Group 3, there were four triggers and the time period for the fish to be able to show discrimination was at least ten to twelve days. Therefore, irrespective of the actual basis of learning, trout can improve their response to prey if they obtain sufficient experience. This conclusion may help to explain why salmonids tend to feed predominantly on one or two main food organisms and to a lesser extent on alternative prey (Allen, 1941).

The feeding behaviour of the fish during the indiscriminate trigger actuations, during the initial few days of the experiment, is comparable to the similar observations by other workers. Rainbow trout (*Salmo gairdneri*) averaged twenty-four exposures to artificial prey (chicken liver) over a four day period before approaches were observed, and some fish required up to eleven days (Ware, 1971). Brown trout (*Salmo trutta*) required fifty to one hundred and fifty exposures to crickets and mealworms during fifteen minutes before significant numbers of attacks occurred. These fish required 1200-1800 exposures during four to six days before responses stabilised (Ringler, 1975). Failure to attack prey upon first encounter appears to be a general phenomena (Ivlev, 1961). However there is almost no information on learning to forage in particular regions of prey abundance, or on the mastery of specific kinds of foraging behaviours. Surprisingly little is known of the rates at which various fishes learn to respond to prey, nevertheless it seems clear that learning is not instantaneous.

For fish in Group 3, it was found that by the last two weeks of the experiment fish had considerably increased in body weights and lengths, and along with this increase in body length fish started to show a tendency for an increasing preference for the next higher size (size five) of the feed pellet than the size (size four) they were consuming previously. This tendency gives indications that if the experiment had been prolonged for Group 3, the shift towards size five feed pellets would have become clear and conclusive. This increase for the next higher size feed pellet (size five) was corresponded by a fall in the number of trigger actuations, towards the end of the experiment, for the lower size of the feed pellet (size four). The largest feed pellet (size six) for Group 3 was also observed to be consumed with an increasing demand towards the end of the experiment, by some fish in the group. Presumably, the fish which had grown at a faster rate than the other fish in the group were consuming this largest size feed pellets (size six, 5mm). Towards the end of the experiment there was much variation in the body size and weight of the fish in the whole group, as is evident from Table 12. Therefore, an increase in the demand for size six feed pellets is not surprising since larger sized fish can be expected to demand a relatively larger feed pellet size. The switch over to a larger feed pellet size is also suggestive that the underlying mechanism involves the efficiency of the grazing, that is, the caloric reward for a particular behaviour pattern, rather than a simple physiological or digestive efficiency change. The increase in size of the fish was importantly related to the fact that the fish were obtaining food in larger feed pellet size and therefore were obtaining a higher calorie intake per expenditure of energy for grazing. While such explanations for the importance of the feed particle size are still mere speculation, there is a need for studying them in relation to production in natural communities. This need is further strengthened by the findings that the pattern of change may show the influence of seasonal and year-to-year differences in the food arrays (Ivlev, 1961) or in the amounts available per grazer (Nikolsky, 1962). However, there is still little doubt that they are importantly linked with growth phases or stanzas (LeCren, 1958; Parker and Larkin, 1959).

In Group 2, when fish had two options of feed pellet size, it was observed that fish preferred to consume size four (3mm) at a significant rate than the size six (5mm) feed pellets. Though some trigger actuations took place for size six feed pellets but whatever quantity of pellets dropped in the tank, they were not accepted by fish for consumption. At times visual observations were done and it was noticed that some of the fish would capture the size six feed pellet but soon it was ejected out of the buccal cavity. This ejection was not due to difficulty in ingestion since no ejective behaviour imposed by the feeding apparatus, as outlined by Wankowski (1979) was noticed. Though to a certain extent physical factors do place possible limitations on the size range of material that can be satisfactorily handled. The rejection of size six feed pellets can be attributed to the fact that fish had a second option provided in the tank and rainbow trout develop a preference for the better of the two or more choices. Size four feed pellets were preferred more than size six feed pellets perhaps due to a comparatively greater ease of capture, manipulation and ingestion. Experiment replication once more could have confirmed these results. The internal nutritional state of fish, such as hunger can also be speculated to be responsible for eliciting a size selective feeding behaviour. In the present study fish experienced no deprivation of food for a longer period than twelve hour dark regime of the photoperiod. Thus, the degree of hunger is not of an intense nature. If fish experience a considerably long period of food deprivation, then the level of hunger is increased and fish can be expected to be less selective in respect of food. It would then be expected, that fish would consume even the less preferred foods. Studies by other workers also, stress that the prey selection is highly dependent on hunger level.

The effects of hunger on predation rates are well documented in some species of fish. In rainbow trout (*Salmo gairdneri*) predation rates declined at low hunger levels, apparently because the fish took progressively longer time to manipulate food (Ware, 1972). Handling time in sticklebacks (*Spinachia spinachia*) also, increased with declining hunger (Kislalioglu and Gibson, 1976a). Beukema (1968) found that hungry sticklebacks (*Gasterosteus aculeatus*) captured the most prey because they grasped and ate a greater proportion of discovered prey and they swam more actively than satiated fish and thus had more prey encounters. All such studies support to speculate that if rainbow trout in the present study, would have been deprived of food for a prolonged period and then subjected to feed pellet size six, then the fish would have consumed this size as well. But when two or more options are provided to the fish then in due course of time rainbow trout can learn to choose the most preferred option with some experience.

At every trigger actuation, for all the groups approximately 0.25g of feed pellets were dispensed by the respective feed dispensers. Since the sizes of feed pellets were different, therefore, the number of feed pellets of different sizes in each unit of weight measure would also be different. Thus at each trigger actuation, for size six feed pellets, approximately five feed pellets were dispensed as compared to eleven for size five, and eighteen for size four. One aspect of this dispension of feed pellets in unequal numbers at each trigger actuation was that fish experienced a variation in relative prey abundance (feed pellets in this case). Due to this variation in relative prey abundance, it can be hypothesized that there is a selection preference for the most abundant prey item. Thus, at different trials for the three groups, when the three sizes of feed pellets were introduced to the fish, size four feed pellets were observed to be most preferred than the other higher sizes, owing to their relatively greater abundance. On the contrary, if the fish had experienced a scarcity of prey items, such a prey-abundance-selection preference would not have been exhibited in the feeding behaviour. Within the morphometric limitations imposed by their feeding apparatus, fish could be expected to consume the prey, as encountered, without showing any selection-preference.

In conclusion it can be said, that the feeding behaviour of rainbow trout, in respect of preferences for different sizes of food particles is modulated by a number of prey characteristics. Prey size and abundance play a central role in diet selection by rainbow trout. The diet of predators will be determined by the availability and distribution of food but the potentially powerful effect of learning to be size-selective in respect of food particle cannot be overlooked, because variations in the experiences and motivational levels of predators, will influence the rate at which they show such size-selective preferences. Predicting variations in feeding patterns requires an understanding of the processes of prey selection, but relatively few detailed studies have been made. Available knowledge of morphological and behavioural adaptations reveal varied modes of prey detection, acquisition and handling among predatory fishes.

5.2. Experiment 5 : Effect of Colour of Food Pellets on Feeding Behaviour of Rainbow Trout *(Salmo gairdneri)*

5.2.1. Introduction

Among the many factors influencing selection of a suitable food item by a predatory fish, the physical characteristics of that food item may form one of the basis for selection or rejection. Some of these factors probably include shape, size, odour, movement and colour of prey. The relative importance of these factors can vary from one environment to the other, for example, odour within a stream would probably be unimportant as an immediate stimulus, movement would probably be insignificant in some habitats where organisms remain attached to the substrate or their passive drift with current is by far the greatest component of their movement. In streams where the water is clear, the colour, size and shape of the food item can be considered important characteristics, that may enable the predator to locate and detect the prey. Several species of fish have been found to have colour vision and experiments have been done on some of the usual functions associated with colour vision.

Throughout the world fish farming is emerging as a significant animal husbandry industry. Intensive fish culturing is being practised in most of the developed countries of the world. Feed manufacturing is also being practised on a commercial basis and trout-hatchery food is also manufactured in the form of pellets. The method of introduction of the pellets to the trout has posed a problem, since when pellets are introduced a training period is usually required. Even after the fish have become conditioned to feeding on pellets, still quite a considerable amount of feed-pellets are noticed to be wasted, since the fish appear to be reluctant to feed upon the pellets which sink to the bottom of the pond. Although the food wastage can be avoided to a certain extent by slow and careful dispensing of pellets but in large production hatcheries such slow and time consuming process would be very expensive and laborious in terms of cost and manpower.

Perhaps the use of demand feeders at different points in the pond can solve the problem of labour and cost. Also a further look is required to find out the colour preference of the food of trout. Light reflected from the surface of food can exhibit a wide range of colours and contrast. Colour and contrast differences between otherwise identical food items may frequently alter their probability of consumption by fishes. Usually the commercial food pellets of trout are light tan in colour. It is quite possible that trout may respond more favourably to some other colour. To evaluate the colour preference or preferences of trout, demand feeders were used and fish were given a choice of blue, red, orange, yellow, green and brown colours in different combinations.

5.2.2. Materials and Methods

5.2.2.1. The Experimental System and Animals

The experimental facility used in the present study was the recirculation system as described in detail in Section 3.2.

The experiment was performed in four separate trials. Each trial period was run for twenty-one days. Fish (12-15 cm) were obtained from the same source as mentioned in Section 4.2.2.1. After quarantine procedures (Section 3.5.) fish were removed to the experimental tanks and were trained for trigger actuations as outlined in Section 3.6.

Water quality for all trials was maintained as shown in Table 2 and regular water quality measurements were taken, as described in Section 3.2. Photoperiod was controlled at twelve hours light and twelve hours darkness (12L12D) throughout the four trials of the experiment.

5.2.2.2. The Experimental Plan

It was planned to study six different coloured diets over a range of four separate trials. In two initial trials run simultaneously in two experimental tanks, six coloured diets in combination of orange, yellow and green for Trial 1 and blue, red and brown for Trial 2 were tested on two batches of twenty-two fish each, at a prevailing ambient temperature of $14.0^{o} \pm 2^{o}C$.

In Trials 1 and 2 each experimental tank (240 l) was fitted with three feed dispensers connected to three individual triggers located in the experimental tanks according to the plan as shown in Figure 32A for Trials 1 and 2.

In Trial 1, three different coloured feed pellets were introduced to a group of twenty-two fish (average weight 45.18 g). Three different colours of feed pellets were orange, yellow and green.

In Trial 2, in the other experimental tank (240 l) another group of twenty-two fish (average weight 47.20 g) were introduced to blue, red and brown coloured feed pellets.

From the results obtained in Trials 1 and 2, it was planned to investigate the feed pellet colour preference of the two most preferred colours out of three studied in each of the Trials 1 and 2. Thus in Trial 3, the four combinations of pellet colours studied were blue, red, orange and yellow. These four different coloured pellets were introduced to a batch of thirty fish (average weight 44.63 g), at the prevailing ambient temperature of $14.5^{o} \pm 2^{o}C$, in four different feed dispensers each connected to an individual trigger and located in the experimental tank according to the plan as shown in Figure 32B.

In Trial 4, based upon the results of Trial 3, the two most preferred coloured diets namely red and blue were tested on a batch of twenty-two fish (average weight 46.22 g) in one of the experimental tanks (240 l),

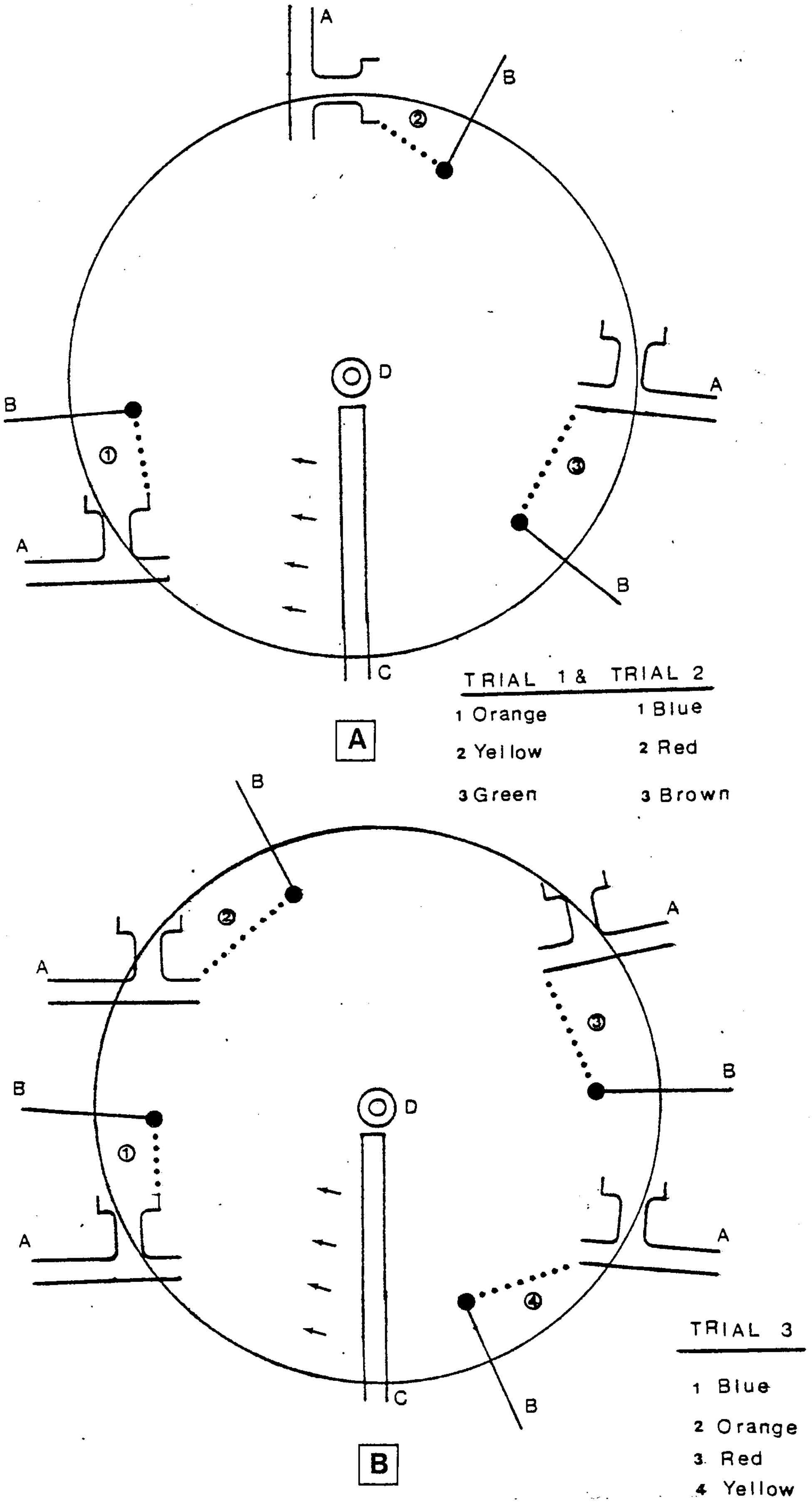


FIGURE 32 Relative positions and numbers of feed dispensers and triggers used for studying the food colour preference of rainbow trout during different trials in Experiment 5. Arrows show direction of flow of water in the tank.

A = Feed Dispenser, B = Trigger, C = Inlet Pipe,
D = Outlet Pipe

at the prevailing ambient temperature of 15.0° ± 2°C. Two feed dispensers connected to two individual triggers were located in the experimental tank, according to the same plan as in Experiment 4 and shown in Figure 28a.

5.2.2.3. The Coloured Diets

Six different coloured diets were formulated by the general method described in Section 3.8. Basic ingredients used, as shown in Table 14 were identicle to each other except in colouration. Colours used for dyeing were harmless food colours in concentrated liquid form, manufactured by Rayner and Company Limited, London.

The basic ingredients were mixed and prepared by the general method described in Section 3.9.

5.2.2.4. Monitoring Pellet Colour Preference and Feeding Rate

The demand feeding system described in detail in Section 3.4. was used for studying the colour preference and the feeding rates.

The daily number of trigger actuations, performed by the groups of fish for the different coloured pellets, during the four trials were noted for the whole period of the trials (twenty-one days for each trial). At each trigger actuation approximately 0.25g of feed was dispensed.

For Trials 1, 2 and 3 daily percentages of trigger actuations for consumption of different coloured feed pellets were calculated from the actual number of total daily trigger actuations and are presented in Figure 33 for Trial 1, Figure 34 for Trial 2 and Figure 35 for Trial 3.

TABLE 14

Ingredient composition of the diets used in Experiment 5

Ingredient (dry weight)	%
Casein	44.0
Cod Liver Oil	8.0
Mineral Mix[1]	1.5
Starch	4.5
Binder[2]	1.0
Glucose	23.0
α-cellulose	17.0
Vitamins[3]	1.0
TOTAL	100.0

1 = Composition given in Table 4

2 = Carboxymethylcellulose, sodium salt, high viscosity.

3 = Composition given in Table 5

N.B. Edible food colourants were used to dye the pellets, which were first mixed in water and then added to the ingredients during diet preparation.

For Trial 4, since there were only two coloured diets which were introduced to fish, the percentages of daily trigger actuations were not worked out. The actual number of daily trigger actuations are presented in Figure 36.

5.2.2.5. Weighing Procedure

Fish were batch weighed according to the procedure detailed in Section 3.7. Before starting every trial average initial fish weights for the groups of fish in four trials were worked out from the total weight of the batch. No weighing was done during or at the conclusion of the trials because the growth parameters were not studied. The initial weights were taken in order to make adjustment of tank weights, by redistribution of fish, to ensure a uniform starting weight for each batch of fish in different trials.

5.2.2.6. Statistical Methods

These were performed as detailed in Section 3.11.3.

5.2.3. Results

During all the four trials, the different feed pellets were fed actively by fish in all the groups. Fish showed preference for different colours upon introduction to different combinations of coloured pellets.

For Trials 1, 2 and 3 the calculated percentages of the daily trigger actuations for different feed pellets are shown in Figures 33, 34 and 35 respectively. The mean and the standard deviations of all observations in the period of twenty-one days for the trigger actuations of the four trials are presented in Table 15.

TABLE 15

Daily mean trigger actuations for twenty-one days, during four trials by rainbow trout for different coloured diets used in Experiment 5

Trial	Colour of Food Pellets	Daily Mean Trigger Actuations	S.D. ±
1	Orange	99	16
	Yellow	87	16
	Green	57	12
2	Blue	76	16
	Red	79	21
	Brown	43	16
3	Blue	98	18
	Red	92	18
	Orange	60	10
	Yellow	36	7
4	Blue	73	8
	Red	71	14

Trial 1

Statistical analysis of the trigger actuations during Trial 1 for orange and yellow coloured feed pellets shows no significant difference ($P>0.05$) in preference for either of the colours, both orange and yellow coloured pellets were consumed at nearly the same rates. Whereas orange and yellow coloured feed pellets were consumed at much higher rate ($P<0.01$) than the green feed pellets. In Trial 1, the comparison of the daily mean trigger actuations for twenty-one days, for three different coloured diets ranks the colour preference in the following order orange$\geqslant$yellow>green. (Appendix table 3).

Trial 2

The statistical analysis of the trigger actuations during Trial 2 for blue and red coloured feed pellets shows no significant difference ($P>0.05$) in preference for either of the colours, both blue and red coloured pellets were consumed at nearly the same rates. Whereas blue and red coloured feed pellets were consumed at a significantly higher rate ($P<0.01$) than the brown pellets. In Trial 2 the comparison of the daily mean number of trigger actuations for twenty-one days for three different coloured diets ranks the colour preference in the following order red$\geqslant$blue>brown.(Appendix table 4).

Trial 3

The statistical analysis of the trigger actuations during Trial 3 for blue and red coloured feed pellets shows no significant difference ($P>0.05$) for either of the colours, both blue and red coloured pellets were consumed at nearly the same rates. Whereas blue and red coloured feed pellets were consumed significantly at a higher rate ($P<0.01$) than the orange or yellow feed pellets. The orange coloured pellets were consumed at a significantly higher rate ($P<0.01$) than the yellow feed

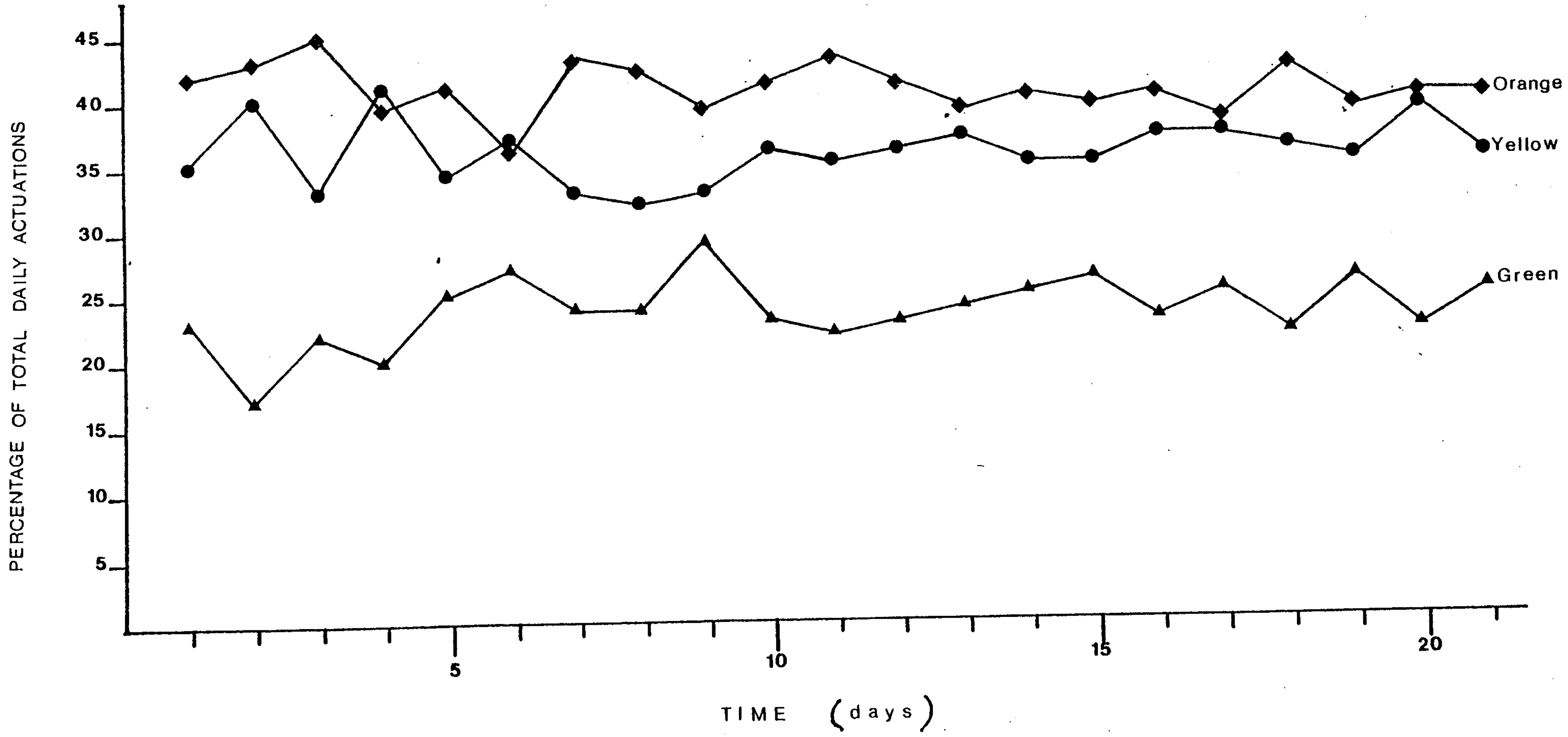


FIGURE 33 The daily trigger actuations by rainbow trout introduced to three different coloured feed pellets during Trial 1 in Experiment 5.

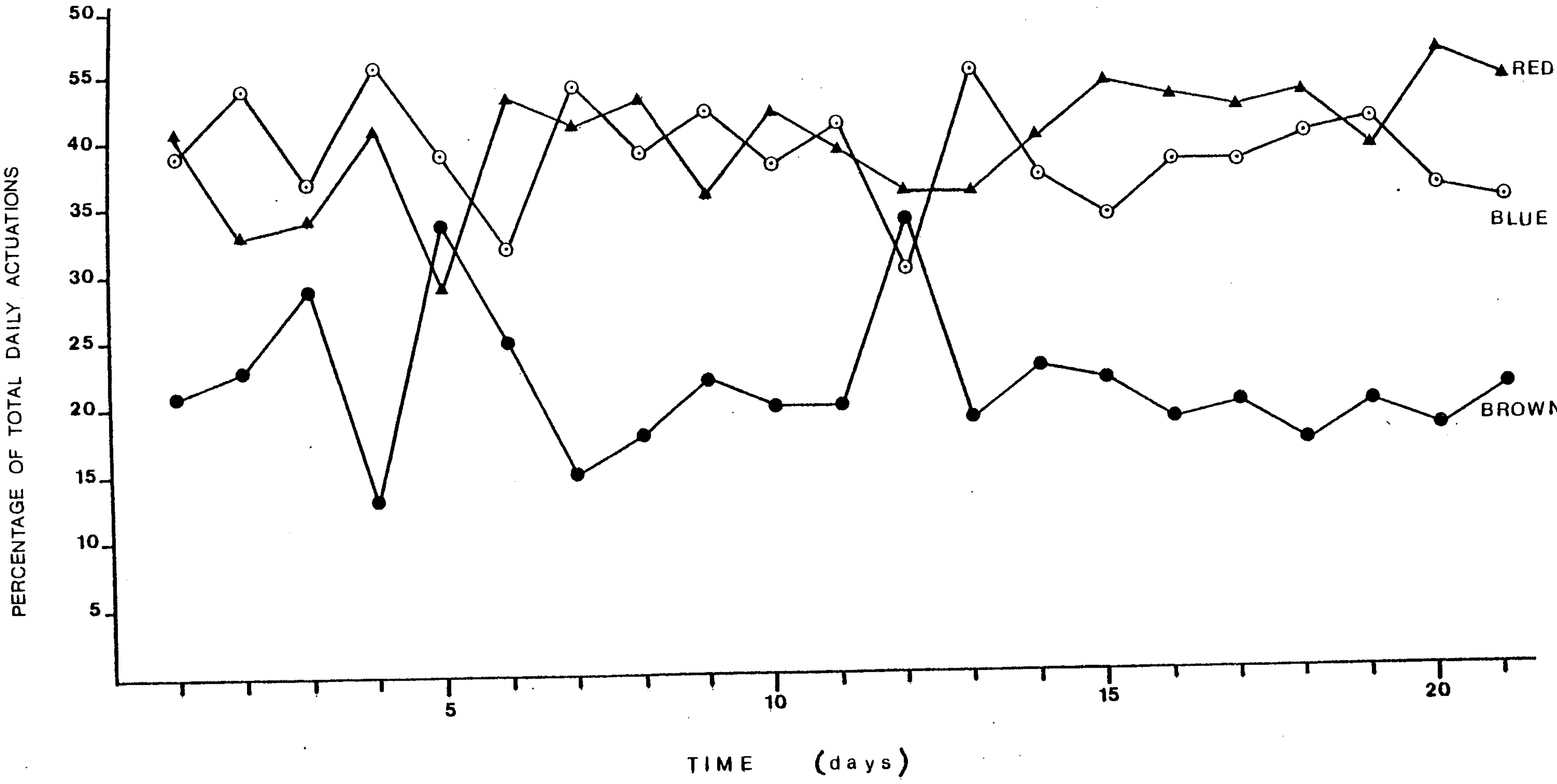


FIGURE 34 The daily trigger actuations by rainbow trout introduced to 3 different food pellets during Trial 2 in Experiment 5

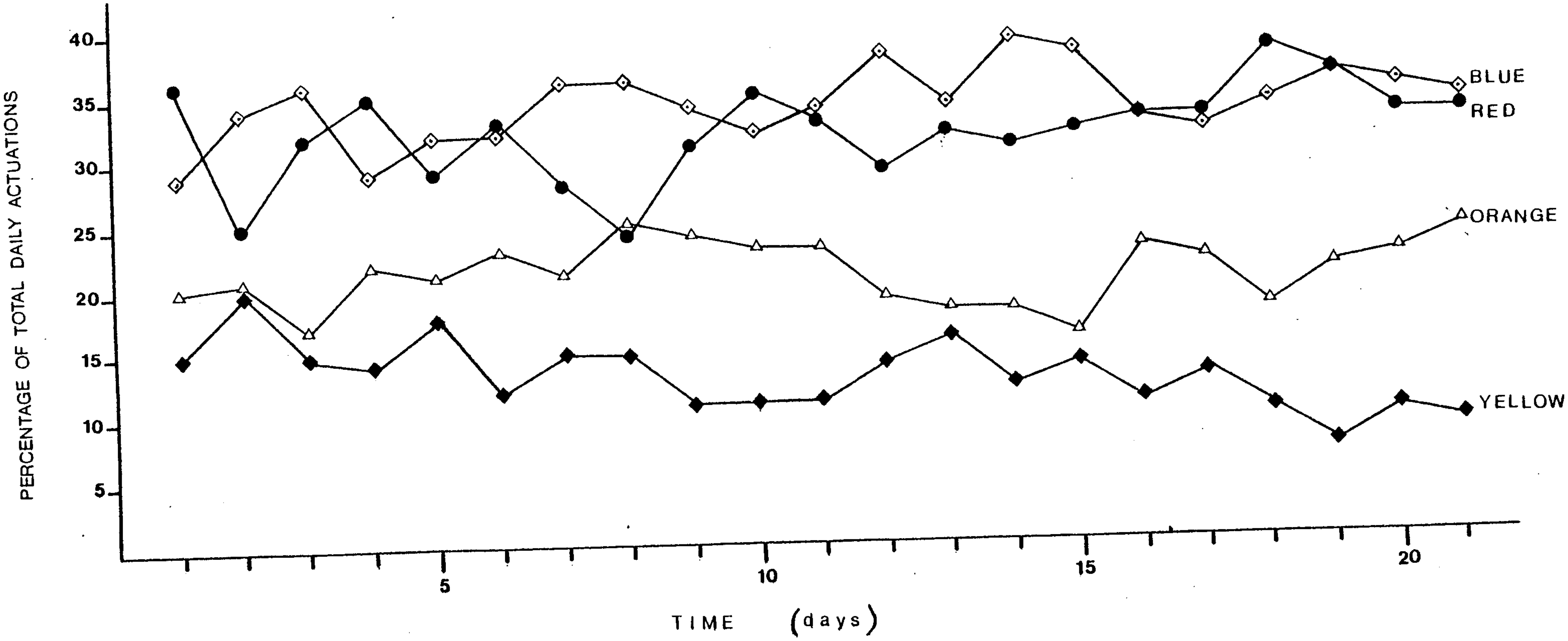


FIGURE 35 The daily trigger actuations by rainbow trout introduced to 4 different coloured food pellets during Trial 3 in Experiment 5

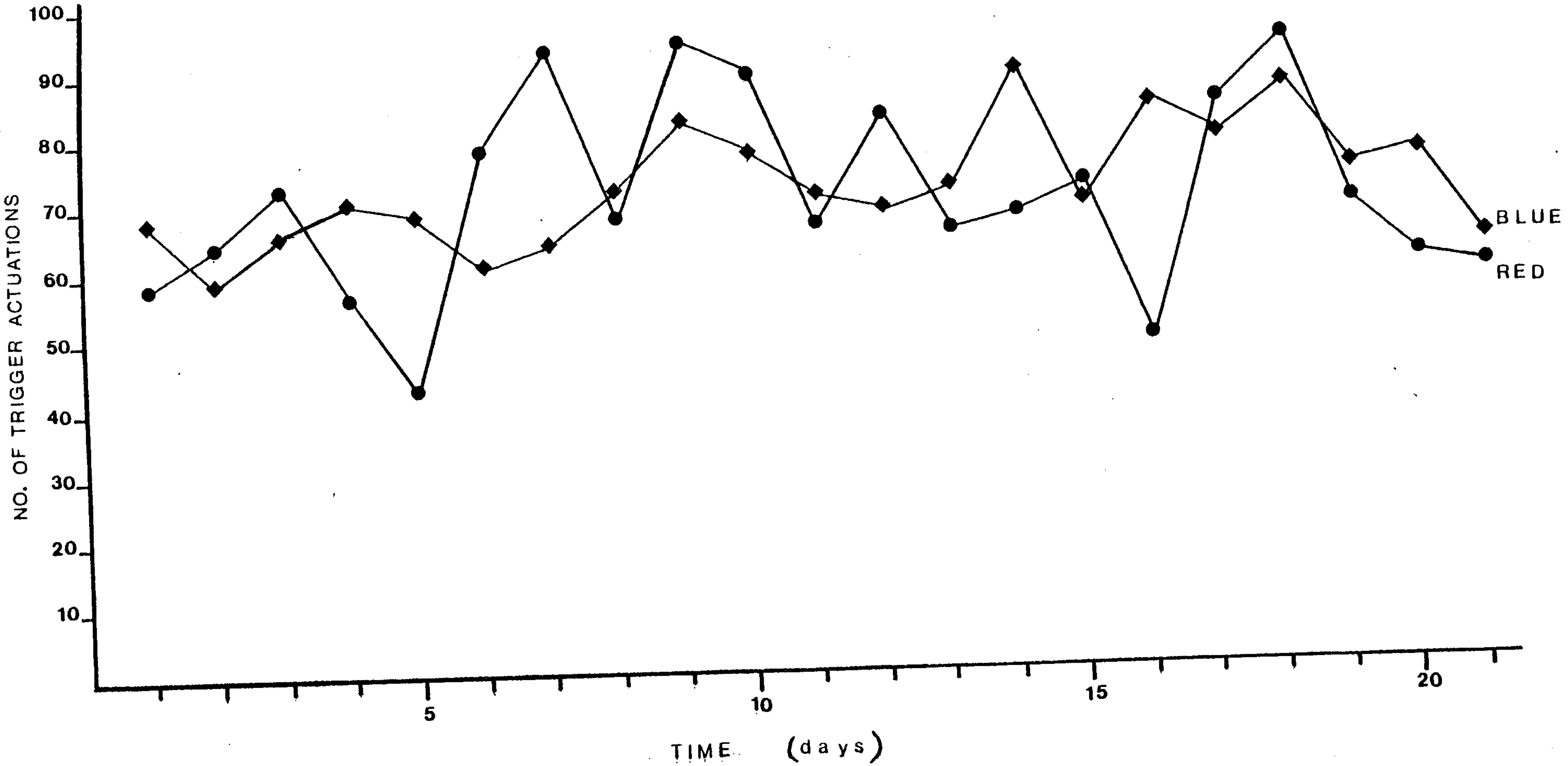


FIGURE 36 The daily trigger actuations by rainbow trout introduced to 2 different coloured feed pellets during Trial 4 in Experiment 5

pellets.

The comparison of the daily mean number of trigger actuations for four different coloured diets ranks the colour preference in the following order blue≥red>orange>yellow (Appendix table 5).

Trial 4

The statistical analysis of the trigger actuations during Trial 4 for blue and red coloured feed pellets shows no significant difference (P>0.05) in the feeding rates of the two coloured diets. Both the coloured diets were consumed at nearly equal rates, though the daily average means of the trigger actuations for the red and blue feed pellets, show a slight preference for blue coloured pellets.

During the four separate trials, the comparisons of the most preferred coloured diets, show that out of all the different combinations, the most preferred coloured diets are blue and red. When blue and red coloured diets are absent then orange and yellow are preferred over the green coloured diet. From the results of the four trials for six different coloured diets, when introduced in different combinations, the colour preference of rainbow trout can be ranked in the following order blue≥red>orange>yellow>green>brown.

5.2.4. Discussion and Conclusions

The results of this experiment indicate that, when given two or more options of colours of food to select from, rainbow trout trained to operate the trigger to obtain food, exhibit preferences for particular colours in relation to others. In Trial 1 amongst the three colours offered for selection by trigger actuations, orange and yellow coloured feed pellets were opted for at a significantly greater rate than the green coloured feed pellets. Similarly in Trial 2, amongst the three colours offered, red and blue coloured feed pellets were consumed at a significantly greater rate than the brown or tan coloured feed pellets. In Trial 3, when the most preferred coloured pellets in Trials 1 and 2, that is blue, red, orange and yellow were offered in four feed dispensers, it was found that the most preferred coloured feed pellets were blue and red. Thus in different combinations of colours, the colour preference of rainbow trout is different, but when red and blue coloured pellets were given together in two separate feed dispensers, fish preferred to select both the blue and red coloured pellets at the same rate.

The colour preferences were elicitated by the trained trout within one to two days of the start of each trial. Since in salmonids feeding is accomplished by visual location of prey (Ware, 1973; Ali, 1959; Protasov, 1970), followed by subsequent capture and ingestion, it can be said that rainbow trout have a predisposition to respond to the visual stimulus on the basis of its colour, since in the present study, feed pellets were identicle in all respects, except for their colour.

Light intensity during all trials was kept at a constant level of 160 lux. In a study by Ginetz and Larkin (1973) under four light intensities of apparently insignificant differences, there was no major pattern of change observed in colour preference of rainbow trout. Although under different experimental backgrounds of the tank, Ginetz and Larkin observed certain food colour preferences were related to the background colour. In the present study the colour of the background (internal colour of tank bottom and sides) remained white for all the tanks in different trials. The sum result of all the trials showed that rainbow trout display innate preference for blue and red coloured feed pellets during feeding. This complements the findings by Ginetz and Larkin (1973), who regardless of surrounding environmental conditions, also observed a greater preference by trout for blue and red coloured eggs. Wolf (1953) also observed that rainbow trout preferred red coloured feed pellets more than even blue pellets. Colour discrimination has also been observed by Muntz and Cronly-Dillon (1966) in trained goldfish *(Carassius auratus)*. They found that the goldfish has a preference for blue, since the blue-green discrimination was learnt significantly faster when blue was the positive stimulus than when green was the positive stimulus.

Colour of the prey has been shown to effect the rate of predation by some workers. Moodie (1972) observed greater rates of trout predation on sticklebacks with red markings. Kislalioglu and Gibson (1976b) observed that sticklebacks *(Spinachia spinachia)* appeared to respond to stimuli associated with mysids in the order movement ⩾length> colour >shape. Attacks on the head region were attributed to its greater thickness, presence of appendages, and darker colour. Limited data suggested that colour and shape stimuli were additive in their effect on fish predation. Ware (1971) found that reaction distance of rainbow trout *(Salmo gairdneri)* was related to contrast of artificial prey, coloured chicken liver, with a dark background. Furthermore, trout that were trained on white food and then provided with black food responded as if they had never experienced this kind of prey, whereas the fish did exhibit "transfer of learning" to light grey and dark food. Ginetz and Larkin (1973) suggested that colour preference by rainbow trout could be manipulated in reducing predation by fish on salmon fry. Adron *et al.* (1973) also used four differently coloured tips of the triggers in their experiment on demand feeding by rainbow trout but their results cannot be considered for determining the colour discrimination ability of trout. The purpose of their experiment was to determine the discriminatory ability of trout in respect of whether trout can differentiate between a blank and a food trigger rather than to determine the colour discriminatory ability. Nevertheless they concluded that trout can differentiate between certain colours.

The effect of varying the background colouration on food colours can play a profound role on the colour selection ability of trout. The brightness factor of different coloured feed pellets might also account for colour discrimination by trout. Also, it is quite possible that the dyeing agents differed in taste, although the manufacturer was the same. All these factors combined together can create some doubt regarding the results of the present study. Nevertheless a colour discriminatory ability was exhibited by the trout in the present study. Kwain and McCrimmon (1967) demonstrated that yearling trout can distinguish between black and white bottom colourations. When exposed to a series of white light intensities over either white or black backgrounds, preference was for black.

In the present experiments, predators were of hatchery origin and of the same age class. No information was obtained on variability of colour preference with age or origin. Nevertheless, in the present study it can be concluded that rainbow trout exhibited the capacity for colour discrimination and showed an equal preference for blue and red colours. This points to possible methods for reducing costs and wastage of food in hatcheries based on the self-demand feeding system with environmental settings of similar type as in the present experimental trials.

5.3. Experiment 6 : Effect of Moisture Content of Feed Pellets on Feeding Behaviour of Rainbow Trout *(Salmo gairdneri)*

5.3.1. Introduction

In the previous two experiments on the effect of physical variants of food on feeding behaviour, effect of the feed particle size and effect of different combinations of colour of feed pellets were studied. In order to study another physical variant, an experiment was planned to study the effect of the texture of feed pellets on feeding behaviour of rainbow trout.

For a long time in past years fresh and frozen animal products constituted the main source of hatchery production rations. Such products bore a heavy burden of expenditure on preservation, preparation and storage. A large quantity of food was wasted during feeding. All such factors stimulated extensive search for substitutions, which have resulted these days in the common use of pelleted dry or semimoist feeds.

The importance of the physical characteristics of the pelleted diets cannot be denied a thorough investigation, since these characteristics can form the basis for selection or rejection of the diets, as already discussed in Sections 5.1.1. and 5.2.1.

To study the effect of texture of the feed pellets on feeding behaviour of rainbow trout, it was planned to introduce to fish, two similar diets of same composition but varying in moisture content. To study the preference for either one of the two diets, differing in moisture level, demand feeding system was used, so that fish in the same group were provided two options to choose from.

5.3.2. Materials and Methods

5.3.2.1. The Experimental System and Animals

The experimental facility used in the present study was the recirculation system as described in detail in Section 3.2.

Fish (14-16 cm) were obtained from a stock of fish which were reared in the Fish Culture Unit of the University where the present study was conducted. These fish, therefore, were not subjected to normal quarantine procedures and were removed directly to the experimental facility from their rearing tanks.

Fish were left for acclimation and training to learn to actuate the trigger for food supply, which lasted for ten days.

Water quality was maintained as shown in Table 2 and regular water quality measurements were taken, as described in Section 3.2. Temperature of water remained at $14.5^{o} \pm 2^{o}C$, throughout the experimental period of twenty-one days.

Photoperiod was controlled at twelve hours light and twelve hours darkness, through twenty-four hour dial synchronous time switches as described in Section 3.3.5.

5.3.2.2. Weighing Procedure

Details of the weighing procedure are presented in Section 3.7. Upon completion of acclimation and initial training period, group of twenty-one fish were batch weighed (± 0.1g), under anaesthesia after twelve hours starvation. At the end of the experiment after twenty-one days, fish were starved for twelve hours and were batch weighed (± 0.1g), under anaesthesia to determine the final average weight of the fish in the group.

5.3.2.3. The Experimental Diets and Moisture Contents

Two identical diets manufactured by Edward Baker Limited, Sudbury, Suffolk were used for the experiment. Diet 1 contained approximately 10% moisture and in texture, pellets were relatively harder to touch than the pellets of Diet 2. In Diet 2 approximately 25% moisture was present and feed pellets floated relatively for less time than the pellets of Diet 1. In texture, although feed pellets of Diet 2 were relatively soft to touch than the pellets of Diet 1, nevertheless, no problems were confronted when they were dispensed through the feed dispensers of the demand feeding system.

5.3.2.4. Monitoring the Feeding Activity

Demand feeding system described in detail in Section 3.4. was used for studying the feeding behaviour. Two feed dispensers and two triggers were provided to fish in the experimental tank, according to the same relative positions of triggers and feed dispensers, as for Experiment 4, and shown in Figure 28a. One feed dispenser was stocked with Diet 1 containing 10% moisture level and the other feed dispenser was stocked with Diet 2 containing 25% moisture level. Fish in the experimental tank, therefore, had two options of moisture levels to select from, with every provision to actuate either of the two or both the triggers, any number of times and at any time during the experimental period, so that feeding could be termed _ad_ _libitum_.

Trigger actuations performed by fish for both the diets were noted daily. At every trigger actuation approximately 0.24g of feed pellets were dispensed by the feed dispenser. Feed dispensers were occasionally checked and tested at least once every week, as discussed in Section

3.4.7. Feed dispensers were refilled as often as required, so that they were never empty at any time during experimentation.

5.3.2.5. Statistical Analysis

These were performed involving a student's t-test comparison of the means of the daily trigger actuations performed by the two groups, using P = 0.05 as the level of significance.

5.3.3. Results

5.3.3.1. Feeding Behaviour

Fish were observed to be feeding actively throughout the experimental period of twenty-one days. Feeding record for the group of fish is shown in Table 16.

For initial four days of the experiment number of trigger actuations for Diet 1 (10% moisture) and Diet 2 (25% moisture) did not differ significantly ($P>0.05$). From day five and onwards, fish were actuating the trigger connected to feed dispenser containing Diet 2 at a much higher rate than the trigger connected to feed dispenser containing Diet 1. Thus showing a greater preference for feed pellets containing 25% moisture level. For the whole period of twenty-one days the statistical analysis of the number of trigger actuations for the two diets show a significant difference ($P<0.001$) in consumption of two diets by the group of fish. Mean daily number of trigger actuations for Diet 1 for the whole period of twenty-one days was 36±20, thus average daily consumption of Diet 1 was 8.64g by the group of twenty-one fish. The mean daily number of trigger actuations for Diet 2 was 118±30 thus average daily consumption of Diet 2 was 28.32g. Therefore Diet 2 with 25% moisture level was consumed 3,28 times more than Diet 1 with 10% moisture level over the whole period of twenty-one days of

TABLE 16

Trigger actuations by rainbow trout for two experimental diets containing different levels of moisture used in Experiment 6

Days	Trigger Actuations		Total Actuations
	Diet 1*	Diet 2**	
1	58	49	107
2	63	75	138
3	77	61	138
4	69	71	140
5	55	98	153
6	49	121	170
7	55	113	168
8	42	127	169
9	29	131	160
10	35	117	152
11	21	142	163
12	19	149	168
13	27	121	148
14	31	140	171
15	18	116	134
16	11	132	143
17	23	150	173
18	30	141	171
19	17	127	144
20	11	152	163
21	21	143	164
TOTAL	761	2476	3237
Mean Daily	36	118	154
S.D.	±20	±30	±17

* 10% Moisture

** 25% Moisture

experimentation.

5.3.3.2. Growth Performance

Fish feeding on two diets showed a marked gain in final average weight. Growth and food utilisation data was as shown below:

Initial Average Weight =	50.88g
Final Average Weight =	69.90g
Specific Growth Rate (%/day) =	1.51
Food Conversion Ratio (dry basis) =	1.53

Fish showed a growth rate of 1.51%/day by feeding on the two diets, though, over the whole period of experimentation Diet 2 was consumed at a significantly higher rate than Diet 1, hence, this growth rate and the feed conversion ratio of 1.53 could be attributed to higher rate of consumption of Diet 2. Therefore, the moisture level of 25% does not appear to show a marked inhibitory effect upon the growth parameters, nor were there any inhibitory or deleterious effects of a high moisture level upon the feeding rates of the fish.

5.3.4. Discussion and Conclusions

The feeding activity of rainbow trout, as observed during the initial few days gave all indications as if the fish would prefer to consume the two diets irrespective of the moisture content of the diets. As the experiment progressed, the fish started showing a significant preference for the diet that contained 25% level of moisture content (Diet 2) over that of the diet containing 10% moisture. This consumption of Diet 2 was maintained until the conclusion of the experiment after twenty-one days. In the experiment where a diet containing 10% moisture was solely fed to the fish it was found that they consumed this diet at the same rate as the moisture rich diet (25% moisture). Thus, it can be said that when a trout has two, or more than two, options for selection of food then the fish selects only that food which has a greater appeal for acceptability, thereby showing a preference for a particular food over the others - in this case the moisture rich diet.

The texture of the food pellets with 25% moisture were much softer and this probably formed the basis for their better selection than the comparatively hard textured food pellets of the 10% moisture content diet (Diet 1). Such a feeding behaviour can also be expected in the natural environment of rainbow trout, where food is in abundance and is of diversified nature.

Under such conditions rainbow trout can be expected to show preferences for soft bodied and easily consumable prey items, although a number of other choice elements, such as taste, size, shape, colour and nature of the prey can also of course be accountable for feeding behaviour.

It was after four to five days that the trout started showing a preference for Diet 2, so that it can be said that trout can learn by experience which food to select or to reject.

Similar effects were observed by Bryan (1972) amongst trained rainbow trout. A number of animals select preferred foods to different degrees depending upon their physiological state such as hunger level. The usual generalisation is that animals are less selective when very hungry (Ivlev, 1961; Beukema, 1968). Hunger levels can be defined relative to the duration of time an animal was deprived of food by an experimeter (Holling, 1966; Beukema, 1968). In the present study such hunger did not have a measurable influence on the degree of selection by trout with the experience of knowing which trigger to actuate for the preferred diet. However, hunger is difficult to define in a way which is general, yet meaningful because different measures of hunger have different characteristics (Marler and Hamilton, 1966; de Ruiter, 1967). Similar problems arise in defining food preferences, particularly as the word "preference" has many shades of meaning. Consequently, it is important to use the term with reference to specific circumstances and to recognise that they may apply only to other situations which are very similar.

In the present experiment though the two diets were basically made up of the same ingredients (except for moisture contents) the feeding behaviour of trout seems to be responding to some set of stimuli from the food. The only variable of the diet which differed was the moisture content of the two diets and thus the degree of hardness or softness of the diet.

In natural situations, from amongst the abundant prey types available, rainbow trout might be expected to show selection preference/s for soft textured preys over the hard textured preys. In this laboratory study trout were provided with two simultaneous choice situations and food distributions were simplified, and many components of searching behaviour were ignored. However, in natural situations potential prey species are distributed heterogeneously in space (Allen, 1941; Ivlev, 1961) and time (Mundie, 1959). Consequently, different trout which search for, and capture prey in exactly the same manner might eat different prey because they happened to hunt in different areas or at different times of day. Food heterogeneity and food specialisation seem to be a related phenomena in trout. Bryan and Larkin (1972) found that brook trout *(Salvelinus fontinalis)*, cutthroat trout *(Salmo clarki)* and rainbow trout (*Salmo gairdneri*) show food specialisation and by the analysis of the food eaten by individuals in a stream and ponds observed that prey types were eaten by the three species in proportions which were characteristic for an individual.

The effect of moisture content in the diet of brown trout (*Salmo trutta*) was studied by Poston (1974), who found that brown trout fed the moist diet (55% moisture) consumed almost twice as much feed as those fed the dry diet (9.6% moisture). In the present study although the moisture content was 25% in one diet and 10% in the other diet the total diet containing the higher level of moisture was more than three times greater than the lower moisture diet. This contrasts with a similar experiment by Poston (1974) where he found that the quantity of high moisture diet consumed was only twice that of a low moisture diet. This difference may be due to the different methods of feeding and moisture levels used. Poston also used hand feeding whereas demand feeders were used in the present study.

In conclusion the present study suggests that a high level of moisture in the diet makes it more acceptable to rainbow trout. Fish preferred to consume the moist diet at a greater rate than the comparatively dry diet. A moisture rich diet does not have any inhibitory effects upon the growth performance of the fish or upon the food utilisation by the fish.

CHAPTER 6

Experiment 7 : Influence of Energy Level in Feed on Feeding Behaviour of Rainbow Trout *(Salmo gairdneri)*

6.1. Introduction

Most animals are capable of maintaining stable health and nutritional balance in the face of great dietary diversity. This accomplishment is understandably due to the simple fact that many natural foods contain a balance of essential nutrients and also because the animal body is capable of meeting its needs by the degradative and synthetic processes of intracellular metabolism. Also animals can exhibit substantial adaptive behavioural change to sustain the health of the body and many animals can make appropriate dietary choices, both qualitatively and quantitatively; when required to consume essential nutrients from separate sources. Some animals can learn whether new foods sustain health or produce illness.

A great deal of existing work in this area is restricted to mammals, especially rats. There are very few studies on fishes, although a great deal of work has been done in the field of fish nutrition to modify dietary constituents and to evolve better balanced diets. Such studies, no doubt, are very important for formulating a balanced feed for fish in culture, but these studies do not reflect the behavioural attitude of the fish, because no attention is paid generally to the behavioural response of the fish. Since the food rations and the feeding frequencies are being determined by the experimenter, the innate requirements (or needs and desires) of the fish are overlooked. It is well established that carnivorous fish such as rainbow trout, normally require a higher energy level than domestic monogastric animals. Most of this energy is obtained from their natural proteinaceous diet. Gerking (1955) also suggested that a large part of the calorific requirements of fish are derived from proteins, whereas homothermous animals can use larger proportions of carbohydrates and fats for this purpose. Fish are able to store fat quickly and in large quantities which may indicate that protein is normally preferred to fat for oxidative metabolism.

To study the feeding behaviour of rainbow trout that is, to see if the fish could regulate food intake in response to a varying amount of energy level in the diet, an experiment was planned. The fish were given a choice of diets with high energy and low energy contents, under ad libitum conditions using demand feeders.

6.2. Materials and Methods

6.2.1. The Experimental System and Animals

The experimental facility used in the present study was the recirculation system as described in detail in Section 3.2.

Fish (12-15 cm) were obtained from the same Fish Farm as mentioned in Section 4.2.2.1. After quarantine procedures, as detailed in Section 3.5., two batches of twenty fish each, were then allocated to two of the 240 l experimental tanks at the prevailing ambient temperature of $14.5^{o} \pm 2^{o}C$. Fish were acclimatised and trained as described in Section 3.6.

Water quality was maintained as shown in Table 2, and regular water quality measurements were taken as described in Section 3.2. Photoperiod was controlled at twelve hours light and twelve hours darkness (12L12D) throughout the experimental period.

6.2.2. The Experimental Diets

Formulation of the diets was carried out by the general method described in Section 3.8. Two diets were prepared, with particular consideration to the fact that one diet should be lower in energy content than the other, but without causing any deleterious effect upon the condition of the fish. Basic ingredients used for the two diets, as shown in Table 17, were identicle to each other. Diet 1 and Diet 2, both contained a calculated value of 45% protein. The two diets differed in the lipid contents, the calculated value in Diet 1 being 6.0% and in Diet 2 being 18.0%. Two diets differed in their energy contents, which was determined by using a ballistic bomb calorimeter. Diet 1 contained 4.55kcal/g and Diet 2 contained 5.99kcal/g. Before starting the experiment, proximate analysis of the diets was done as described in Section 3.10. and the values for the components shown in Table 18. During acclimation and training period, fish in the two tanks, were fed the experimental diets, which were observed to be readily accepted. The diets were in pelleted form and were of a floating type. No mortality occurredduring the experimental period of five weeks.

6.2.3. Weighing Procedure

Details of the weighing procedure are presented in Section 3.7. Fish were individually weighed for the initial weights and every seventh day for five weeks.

6.2.4. Monitoring the Feeding Activity

Demand feeders were used to monitor the feeding responses of two groups of fish, throughout the experimental period. The tanks were fitted with the demand feeding system as detailed in Section 3.4. Each tank was provided with one feed dispenser and one trigger in relative

TABLE 17

Ingredient composition of the diets used in Experiment 7

Ingredient (Dry Weight)	Diet 1 %	Diet 2 %
Herring Meal	63.35	63.35
Herring Oil	1.34	13.34
Mineral Mix[1]	1.50	1.50
Starch	4.81	4.81
Binder[2]	1.00	1.00
Glucose	14.00	14.00
α-cellulose	13.00	1.00
Vitamins[3]	1.00	1.00
TOTAL	100.00	100.00
Calculated:		
Crude Protein (%)	45.0	45.0
Crude Lipid (%)	6.0	18.0

1 = Composition given in Table 4

2 = Carboxymethylcellulose, sodium salt, high viscosity.

3 = Composition given in Table 5

TABLE 18

Proximate analyses of the diets used in Experiment 7

Component	Diet 1 (%)	Diet 2 (%)
Moisture	8.13	7.17
On a moisture free basis:		
Crude Lipid	6.41	19.01
Crude Protein	45.45	45.37
Ash	16.59	16.57
NFE*	31.55	19.05
Energy	4.55 kcal/g	5.99kcal/g

* NFE = Nitrogen Free Extractives.

position to each other as shown in Figure 10. The feed dispensers were stocked with feed as and when required often after two days. The feed dispensers were adjusted to deliver approximately 0.50g feed/trigger actuation and tested (See Section 3.4.7.). At each actuation the amount of feed-pellets dispensed (during testing trials) by the feed-dispenser containing low calorie diet was 0.49g ± 0.039. For the feed dispenser containing high calorie diet, the amount of feed dispensed was also 0.49g ± 0.036. For both groups, for the calculations of total feed consumed daily by number of trigger actuations, 0.50g feed was considered to be dispensed/trigger actuation.

The daily number of trigger actuations were noted for five weeks for both the groups. Triggers were left in the two experimental tanks for the whole period of the experimentation, so that, fish had the opportunity of obtaining food at any time and as many times as desired. In a sense, the feeding can be termed ad libitum. At no time, food was observed to be left unconsumed in the tanks.

6.2.5. Statistical Methods and Analysis of Growth

These were performed involving a student's t-test comparison of the means of the data, using P = 0.05 as the level of significance.

6.3. Results

6.3.1. Feeding Behaviour

Fish in both groups fed actively throughout the experimental period of five weeks. Fish on low calorie Diet 1, consumed more food than the fish on high calorie Diet 2. The feeding record for the two groups is shown by mean daily number of trigger actuations per week in Table 19. Average food consumption/day for both groups was calculated and is shown in Table 20. The food consumption rates of

TABLE 19

Growth, food utilisation and trigger actuations data from Experiment 7

Diet Number		WEEKS 1	2	3	4	5
1 (4.55 kcal/g)	Initial Mean Weight = 47.65 g S.D. ± 3.40	54.19g S.D.±8.81	59.32g S.D.±6.95	65.15g S.D.±7.61	74.36g S.D.±10.05	82.13g S.D.±8.98
	Mean Trigger Actuations	91	84	126	132	147
	S.D. ±	5	14	8	11	12
	S.G.R.[1] (% Day)	1.84	1.29	1.34	1.89	1.42
	F.C.R.[2] (dry basis)	2.25	2.65	3.50	2.32	3.06
2 (5.99 kcal/g)	Initial Mean Weight = 47.23 g S.D. ± 3.26	55.99g S.D.±9.73	61.40g S.D.±8.71	67.09g S.D.±11.96	76.84g S.D.±11.65	85.36g S.D.±9.21
	Mean Trigger Actuations	61	65	101	101	105
	S.D. ±	4	13	5	10	18
	S.G.R.[1] (% Day)	2.43	1.32	1.27	1.94	1.50
	F.C.R.[2] (dry basis)	1.14	1.96	2.90	1.69	2.01

1 = Specific Growth Rate

2 = Food Conversion Ratio

TABLE 20

Consumption of low and high calorie diets by rainbow trout and their comparisons in terms of energy value of diet

Average Daily Food Consumption	WEEK 1	WEEK 2	WEEK 3	WEEK 4	WEEK 5	MEANS
Low Calorie Diet 1 (g/Fish)	2.28	2.10	3.15	3.30	3.68	2.902S.D.±0.609
High Calorie Diet 2 (g/Fish)	1.53	1.61	2.53	2.53	2.63	2.166S.D.±0.489
In Terms of Calorific Value kcal/g						
Diet 1 (4.55 kcal/g)	10.37	9.56	14.33	15.02	16.74	13.204S.D.±2.77
Diet 2 (5.99 kcal/g)	9.16	9.64	15.15	15.15	15.75	12.970S.D.±2.93
Energy Intake Ratio "R" $= \frac{\text{Diet 1 (g/fish/day)}}{\text{Diet 2 (g/fish/day)}}$	1.49	1.30	1.25	1.30	1.40	1.348S.D.±0.09

two groups were then compared with one another to work out the energy ratio of the two groups as shown in Table 20. In order to get an estimate of the quantitative effect of the compensation for low calorie diet, the ratio of the average amount of food consumed/day by the low calorie diet group of fish, to the average amount of food consumed/day by the high calorie diet group of fish, was worked out. This ratio being termed in this case, as the Energy Intake Ratio (R). For perfect calorie balancing it would be 1.32, since the energy content, determined by bomb calorimeter (See Section 3.10), for the low calorie diet was 4.55 kcal/g, and for high calorie diet was 5.99 kcal/g, (See Table 19).

Thus in theory, for two groups of fish, for a perfect counterbalancing of energy intake, fish on low calorie diet should be consuming 1.32g of food for every 1g of food consumed by the fish on the high calorie diet. The energy intake ratio R of the two groups as shown in Table 20 provide good evidence that the fish were responding primarily to obtain the same amount of calories for their metabolic activities. The two groups of fish were consuming statistically significant ($P<0.05$) different quantities of food as worked out from mean daily trigger actuations for five weeks, but in terms of calorie-intake, both the groups were consuming nearly equal value of food as shown in Table 20.

6.3.2. Growth Performance

Growth responses of the two groups fed the two diets are represented graphically in Figure 37, where the average fish weights are plotted against time. Statistical analysis of the initial fish weights showed no significant differences ($P>0.05$) between the two groups. Fish in both groups showed a marked gain in final weights over the initial weights, but the final fish weights showed no significant difference

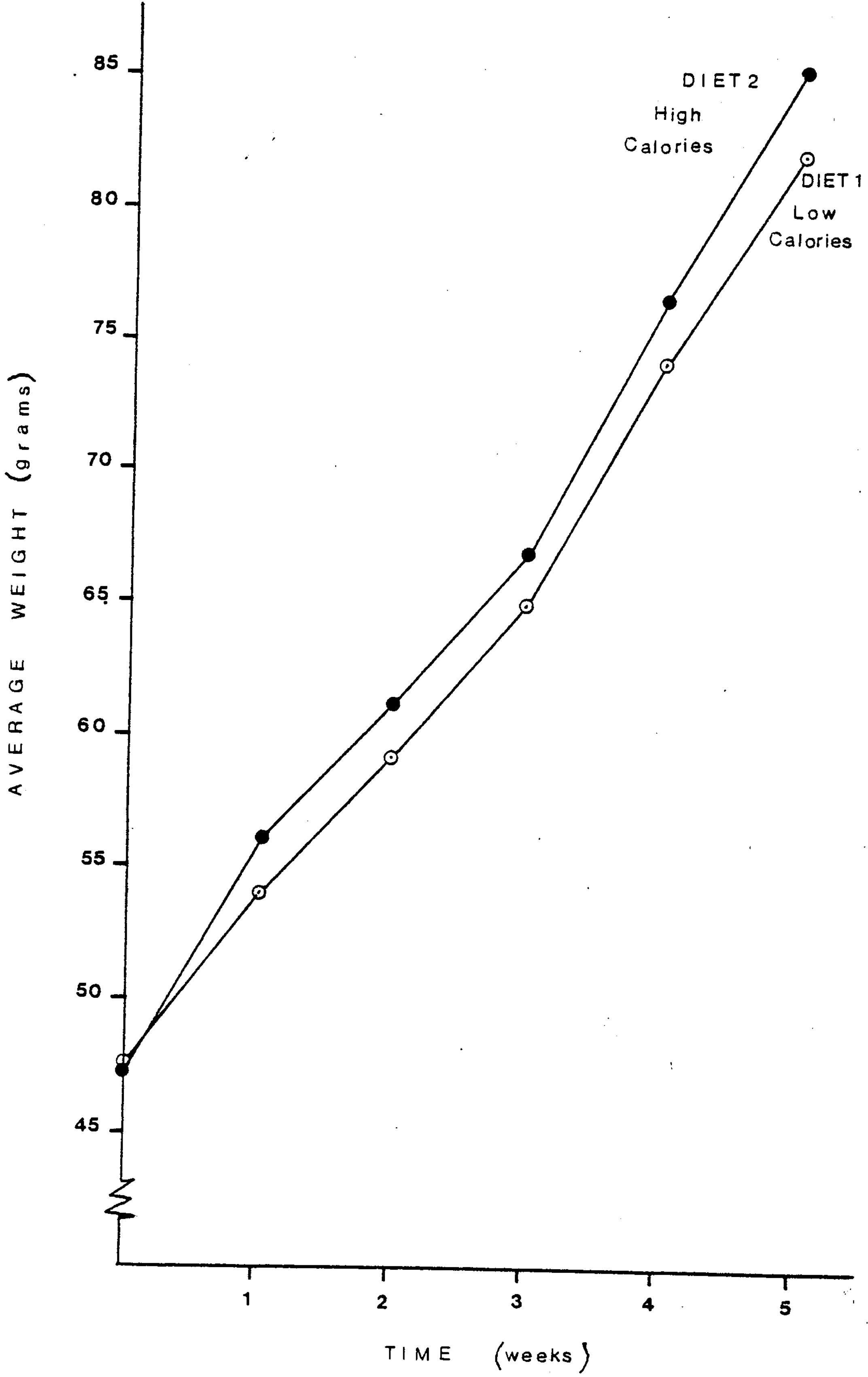


FIGURE 37 The growth responses of rainbow trout fed 2 diets with different levels of calorific value

$(P>0.05)$ between the two groups. The Specific Growth Rates (SGRs, Section 3.11.1.) of the two groups over the five week period of experimentation are shown in Table 19.

6.3.3. Food Conversion

Food Conversion Ratios (FCRs, Section 3.11.2.) were obtained for the two groups and are presented in Table 19. Fish on low calorie diet consumed significantly more food $(P<0.05)$ than the fish fed the high calorie diet, although the gain in weights at the end of experimentation period between the two groups did not show a significant difference $(P>0.05)$. FCR of low calorie diet group is higher than the FCR of fish on high calorie diet. High energy content of the diet resulted in decreased FCR and low energy content of the diet resulted in increased FCR.

6.4. Discussion and Conclusions

In the present study the difference in the feeding rates of the two groups of rainbow trout seems to be related to the difference in the calorific values of the two diets. Fish which were fed a lower level of calorific value diet consumed significantly larger quantity of food than the fish which were fed a diet of higher calorific value, although the supply of both the diets were without any restriction and both the diets were identical in respect of their basic dietary ingredients. Results of the present study are in general agreement to the work performed by Rozin and Mayer (1961a) on goldfish *(Carassius auratus)*. They observed that goldfish responded to dietary dilutions and adjusted their feed intake according to the calorific value of the diets.

The results of the present work suggest that rainbow trout are quite capable of self-regulating the amount of nutrient ingested according to the metabolic needs of the body. Such regulation of food in the pike *(Esox lucius)* has been observed on an annual basis, somewhat independent of temperature changes by Johnson (1966). Pike have been observed to consume maximum quantity of food at the same time of the year as the maximum growth in the month of June. In the months of January and February food intake decreased substantially alongwith the decrease in growth. These findings may seem contrary to those of Rozin and Mayer (1961a), who found that food intake of goldfish was adjusted closely with changes in temperature. However, in the work of Rozin and Mayer, the growth rates of the goldfish were apparently not measured, thus making it difficult to compare this work with that of Johnson on pike. A feeding behaviour similar to one found in pike may also be exhibited by rainbow trout if a prolonged study is done. The present study was of such a short duration that it is not possible to authentically state that rainbow trout can also regulate its food intake on seasonal basis as well.

The growth performance of fish, which were fed the two diets did not show a significant difference, thus implying that fish on low calorie diet were able to regulate the amount of nutrients ingestion and achieved the same growth level as the fish on high calorie diet. This observation is further supported by the different amounts of food which were consumed by the two groups of fish. Lee and Putnam (1973) in their study of varying protein/energy ratios in the diets of rainbow trout, also found that fish fed low energy diets were able to gain weight at a rate comparable to those fed high energy diets by increasing their food intake. Similar observations were reported by Page and Andrews (1973), when they fed channel catfish *(Ictalurus punctatus)*, a high dietary level of energy and protein.

Besides growth the feed conversion, which is also a standard measure of fish performance, was also correlated with the energy content of the diets. Fish which were fed the low calorie diet showed a higher conversion rate, which is also attributable to the mechanism of regulation of the food intake. Since the energy content of the diet was lower than the energy content of the other diet, fish made up the loss by ingesting a higher quantity of food. But when the food intake of the two groups was calculated on the basis of calorie-intake, it was found that both the groups were consuming nearly the same amount of calories. Although in the present study the two experimental diets were not below the substantial nutritive values required for rainbow trout but still, since the two diets did not contain isocalorific values, the fish responded to the difference and exhibited different levels of food ration consumption.

It can be hypothesised that when the food supply is not limited then the internal physiological state of the rainbow trout can modulate its feeding behaviour according to the calorific value of the diet by adjusting its ration level on a self-regulatory basis. It appears that there is an elaborate machinery, only partly understood to detect energy imbalance in fishes. Evidence that such control systems are at work in goldfish is also provided by Rozin and Mayer (1961a and 1964). Similar temperature-adjusted food intake has been found in many other species (Beukema, 1968). However, Beukema found that sticklebacks *(Gastreosteus aculeatus)* feed until the stomach is full and hence the total amount ingested daily may be independent of the caloric value of the food. Recent interpretations of physiological controls of feeding stress the role of liver in response to supplies of metabolic fuels (Friedman and Stricker, 1976). At another level, sensory information from the environment provides input for control of proximate food choice. At a further level, an organism's ability to adjust behaviour to spatial and temporal distribution of food can contribute to control of energy regulation.

Food intake by various species of fish is increased following periods of starvation or deprivation (Rozin and Mayer, 1964; Ware, 1972). Thus, deficits in body reserves are made up as quickly as possible when provided with the opportunity to do so. But when deprivation proceeds beyond a certain point, compensation by increased food intake is no longer possible (Bilton and Robins, 1973; Tyler and Dunn, 1976). Although the concept of homeostasis underlies much feeding research, it appears that food intake by a fish is regulated by a homeostatic system, which works within certain limits of a minimum and maximum level of food rations. This homeostatic system can regulate the energy intake by adjusting the quantity of food intake. The goldfish has been shown in free operant varying temperature and food concentration to eat for calories by regulating the quantity of food intake (Rozin and Mayer, 1961a).

The energy requirement of the fish would depend upon the physiological state of the fish body, which in turn is affected by so many environmental factors - both biotic and abiotic, so that, the whole process of the regulation of food intake is a complicated system. Physiological studies on the feeding behaviour have tried to explain the food regulation in the animals through a number of hypothesised variables such as "glucostatic", "thermostatic", "lipostatic" and "hepatostatic" theories (Novin, Wyrwicka and Bray, 1976). Ecological studies emphasise the external variables that influence feeding. Both physiological and ecological approaches to comparative studies of feeding behaviour have a common basis in energy regulation.

Satia (1974) reported the possibility that the protein requirements of a fish may in part depend on the energy content of its diet. Thus in a diet with a high energy content in relation to the percent of dietary protein, less protein should be wasted as an energy source, which in turn means that less protein would need to be consumed to meet the protein requirements of the fish. The present study shows that rainbow trout seems to feed for satisfying its energy requirement rather than to fill up its stomach with as much quantity of feed that can possibly be ingested. Some workers have labelled rainbow trout as "opportunistic feeders", fish that tend to eat whatever they can capture whenever it is available (Pentelow, 1932; Hunt, 1965; Mann and Orr, 1969; Miller, 1946). Such a claim may be true under conditions where food supply is restricted, but in the present study since the food supply was not restricted feeding behaviour of trout did not show any signs of "opportunistic feeding" by consuming the two diets at equal rates.

In conclusion it can be said that feeding behaviour of trout changes when the fish are fed the diets which contain different levels of energy contents. Fish when fed a diet with low energy level consumes more food than the fish fed a diet containing high energy level. There is a vast scope of work that can be undertaken in the area of dietary self-selection, most of them having to do with the generality of certain demonstrated phenomena. The manner in which physiological states and environmental conditions modify self-selection **still** leaves a large number of conditions unstudied. Demand feeders can be used for all such types of study of the rainbow trout and also other species of fish which can be conditioned to actuate the trigger for food as the reward.

CHAPTER 7

Experiment 8 : Effect of Goldthioglucose on Feeding Behaviour of Rainbow Trout *(Salmo gairdneri)*

7.1. Introduction

Much work has been done on mammals to study the neural control of food intake. The hypothalamus is usually regarded to be the centre, from where the food intake is regulated, and most of the evidence is provided by the experimental work done on the laboratory rat (Anand, 1961). The existence of a medial "satiety centre" and a lateral "feeding centre" in the hypothalamus has been postulated by several workers (See review by Rabin, 1972). The ventro-medial nuclei of the hypothalamus have been described as a "major centre" for affective reactions and feeding behaviour. Lesions in the ventro-medial area of the hypotholamus result in overeating and obesity whereas in the lateral hypothalamus, cause aphagia or hypophagia in rats and other mammals. In several studies goldthioglucose has been utilised to produce localised damage in the hypothalamus of rats.

There are relatively very few studies dealing with the neural control of feeding in teleosts. No published studies dealing with the effects of lesioning the ventro-medial area and the lateral hypothalamus area on food intake in teleosts, could be found. However, some work has been done on feeding behaviour of fishes after electrical stimulation of a number of forebrain regions. Peter et al. (1976) have performed a series of experiments to determine the effects of goldthioglucose on feeding and growth of goldfish.

In view of the fact that virtually no other work has been done on fishes, it was decided to study the effect of goldthioglucose on feeding behaviour, forebrain histology and growth of rainbow trout, *Salmo* *gairdneri*. To determine the level of dose to be systematically administered a pilot experiment had to be performed to determine the LD 50 dose, which is described in the next section.

7.1.1. Preliminary Experiment for Dose Determination

A pilot experiment was carried out to determine the dosage of goldthioglucose (GTG) to be administered to fish. Fish (12-16 cm) were obtained from the same Fish Farm as mentioned in Section 4.2.2.1.

After quarantine procedures as detailed in Section 3.5. were assigned to four experimental tanks in recycling system fitted with demand feeders (as described in Section 3.2.) in four groups of fifteen fish each. Fish were acclimated and trained for trigger actuations for ten days, at ambient temperature of $14.5^{o} \pm 2^{o}C$ and photoperiod of 12L12D. At the end of the tenth day, triggers were removed from the four tanks, to deprive fish from the food for overnight and subsequent weighing the next day.

After twelve hours of starvation, on the eleventh day, fish in each group were individually weighed under anaesthesia, as described in Section 3.7. and intraperitoneal injection of GTG was administered to each fish according to dosage shown in Table 21. Injections of GTG were prepared by dissolving GTG in a physiological solution (PS) and administered to fish (Burnstock, 1958). Each group of fish was assigned to its respective experimental tank after GTG injections. Feeding activity was monitored by number of trigger actuations per day for two weeks and mortality was noted daily as shown in Table 22. At every trigger actuation approximately 0.15g feed pellets (Baker's Commercial trout hatchery diet, floating pellets) were dispensed.

TABLE 21

Goldthioglucose (GTG) dosage injected to rainbow trout for dose determination experiment

Group	Dose
1	0.1mg GTG/g body weight, with 0.1 mg GTG/0.01 ml PS
2	0.5mg GTG/g body weight, with 0.5 mg GTG/0.01 ml PS
3	0.7 mg GTG/g body weight, with 0.7 mg GTG/0.01 ml PS
4	0.9 mg GTG/g body weight, with 0.9 mg GTG/0.01 ml PS

Results of the experiment in Table 22, showed that a dose of 0.9mg GTG/g body weight proved fatal for 100% of fish in Group 4. Nearly 50% of fish in this group died by day two post-injection and all the fish were dead by the fifth day. For fish in Group 3, a dose of 0.7mg GTG/g body weight proved fatal for 80% of fish. For fish in Group 2 a dose of 0.5mg GTG/g body weight, mortality was observed to be 40% by the end of the fourteenth day. Feeding activity of this group was low. Amongst fish in Group 1, with the lowest dose of 0.1mg GTG/g body weight, no mortality occurred. Feeding activity of this group was low during the initial three days after GTG injection but showed signs of recovery from the fourth day, as evident by higher number of trigger actuations than the previous three days (See Table 22 for the number of trigger actuations).

It was concluded from the results of this experiment that dose of 0.5mg GTG/g body weight should be used for the main experiment.

7.2. Materials and Methods

7.2.1. The Experimental System and Animals

The recirculation system described in detail in Section 3.2. was used for this experiment. Fish (12-17 cm) were obtained from the same Fish Farm as mentioned in Section 4.2.2.1. After quarantine procedures, as detailed in Section 3.5., three groups of fish were allocated to three of the 240 1 experimental tanks at the prevailing ambient temperature of 14.5° ± 2°C.

One group of twenty-four fish were selected as the experimental animals. Two other groups of fourteen fish each were assigned, one each as normal control and sham control. The fish were left for acclimation and trigger actuation training as described in Section 3.6.

TABLE 22

Mortality rate and feeding activity of rainbow trout after injection of goldthioglucose (GTG).

DAYS Post Injection	Group 1 = 15 Fish Average Weight = 29.85 g		Group 2 = 15 Fish Average Weight = 31.56 g		Group 3 = 15 Fish Average Weight = 30.67g		Group 4 = 15 Fish Average Weight = 31.15 g	
	A	B	A	B	A	B	A	B
1	-	65	2	4	4	2	6	-
2	-	71	1	8	2	2	3	3
3	-	69	1	6	1	5	1	2
4	-	96	-	13	2	3	2	1
5	-	110	1	9	-	4	2	1
6	-	89	-	8	2	7	-	-
7	-	115	1	7	-	6	-	-
8	-	128	-	11	-	10	1	-
9	-	119	-	8	1	5	-	-
10	-	130	-	10	-	4	-	-
11	-	111	-	15	-	6	-	-
12	-	142	-	12	-	10	-	-
13	-	119	-	18	-	8	-	-
14	-	127	-	17	-	7	-	-
Mortality Rate (%)	0		40		80		100	

A = Number of Fish Dead
B = Number of Trigger Actuations

During acclimation and training period trigger actuations were noted daily for the three groups of fish. By the eighth day all the three groups showed normal feeding activity and seemed well adjusted in the experimental tanks.

Regular water quality measurements were taken as described in Section 3.2. and water quality was observed to be as shown in Table 2. Photoperiod was controlled at twelve hours light and twelve hours darkness (12L12D) throughout the experimental period.

7.2.2. Tagging and Weighing

The three groups of fish before being removed into experimental tanks were individually tagged as described in Section 3.7. No losses were experienced during tagging or acclimation period of ten days. On the tenth day fish were weighed individually (± 0.01g), as detailed in Section 3.7. Subsequently, after twelve hours of starvation, every second week, upto week twelve, fish in three groups were individually weighed under anaesthesia.

7.2.3. Dose Administration

Goldthioglucose was purchased from Sigma Chemical Company, Poole, Dorset. Dosage used was based on the literature and results of the pilot experiment, described in Section 7.1.1. Goldthioglucose (GTG) was injected intraperitonealy at the initial weighing. Injection of GTG was prepared by dissolving GTG in the physiological solution (Burnstock, 1958) in the proportion of 0.5mg GTG per 0.01ml of physiological solution (PS). A dose of 0.5mg GTG/g body weight was administered intraperitonealy to twenty-four fish under anaesthesia.

Sham control group of fourteen fish were injected at initial weighing, intraperitonealy with physiological solution (PS) in proportion of 0.01ml PS/g body weight.

7.2.4. Histology of Brain

On the second day post injection, two fish from each group were sacrificed by spinal section, and the brain of each fish was rapidly dissected out and fixed in Bouin's solution for forty-eight hours. Subsequently, on the third and four days (post injection) two fish each day were sacrificed from GTG group and their brains fixed in Bouin's for section cutting. Similarly, at the end of the experiment in week twelve, after final weighing, four fish from the GTG injected group were killed and their brains were fixed in Bouin's solution for section cutting. Following standard histological procedures, the brain of each fish was embedded in paraffin wax. The forebrain of each fish was then serially cross-sectioned at seven micron thickness on a rotary microtome. The serial sections were stained with haematoxyline and counterstained with eosin.

7.2.5. Mortality During Experimentation

Mortality occurred amongst the GTG injected fish. On day two after GTG injection, four fish died and on days eleven and twelve, one fish died each day thus showing a 25% mortality rate.

In the sham control group, two fish died during week two, thus showing 14.29% mortality rate. No mortality occurred in normal control group, but to maintain an equal number of fish in each group, two fish were removed from normal control group at weighing time in week two.

Thus at the start of week three (post injection) only ten fish were left in each group. No mortality occurred after week three in any of the three groups.

7.2.6. Examination of Visceral Organs

Fish sacrificed for histology of brain and those which died post injection were examined for any apparent abnormality of kidney, liver and alimentary canal.

7.2.7. Monitoring Feeding Activity

The demand feeding system described in detail in Section 3.4. was used for monitoring the feeding activity. Each experimental tank was fitted with one feed dispenser and one trigger according to the same plan as in Experiment 1 and shown in Figure 10. Food consisted of the same commercial diet as used for Experiment 1 and described in Section 4.2.2.4. Feed dispensers were adjusted to deliver approximately 0.15g feed per trigger actuation and were tested as described in Section 3.4.7.

Daily number of trigger actuations were noted for twelve weeks for all groups. Daily food consumption by each group was obtained by multiplying total number of daily actuations with amount of food dispensed per trigger actuation (0.15g).

Triggers were left in experimental tanks for the whole duration of experimentation, except for twelve hours prior to weighing time, in order to deprive the fish from food for starvation, prior to weighing. Thus, one day prior to weighing (after every two weeks), triggers from three experimental tanks were removed, usually at the end of the day, so that next morning, after at least twelve hours of starvation, fish could be weighed.

Since fish in all three groups could obtain food by trigger actuation at any time and as many times as desired, it can be said that fish had every opportunity to perform unlimited feeding activity. In a sense, feeding could be termed ad libitum. At no time, food was observed to be left unconsumed by fish in any of the three experimental tanks.

7.2.8. Statistical Methods and Analysis of Growth

These were performed as detailed in Section 3.11.

7.3. Results

7.3.1. Feeding Behaviour

Fish in normal control and sham control groups fed actively throughout the experimental period. GTG injected fish virtually stopped feeding for the first two weeks after injection of GTG as can be observed by daily mean trigger actuations shown in Table 23. GTG injected fish started actuating the trigger from the second week, but at a significantly lower rate than the normal and sham control groups.

Fish in the GTG injected group were observed to be inactive and hypophagic, whereas fish in normal control group were feeding normally (Table 25). Fish in the sham control group also showed active feeding, though during week one, they apparently were not as active as fish in normal control group, which could be due to stress following injection of physiological solution.

7.3.2. Growth Performance

Growth responses of the groups are represented in Figure 38 where average fish weights are plotted against time.

Fish in GTG injected group showed poor growth and had an obvious decrease in growth rate, showing a decrease in body weight after the injection of GTG. During the sixth week, post injection, as shown in Table 24 and Figure 38, the fish in this group began once again to have an increase in body weight. Some fish in the group continued to show decrease in body weight even after the sixth week, whereas others apparently recovered from the effects of GTG and began increasing body weight rapidly once again, as it can be seen in Table 24, that the variation in the group increased with time.

Fish in normal and sham control group showed much better growth. Final average weights of two control groups did not vary significantly ($P>0.05$), but were significantly different ($P<0.05$) when compared to final average weight of GTG injected fish as shown in Table 24 (Appendix table 6).

Statistical analysis of the Specific Growth Rates (SGRs Section 3.11.1.) presented in Table 24 showed no significant difference ($P>0.05$) between growth rates of two control groups up to the termination of experiment but showed a significant difference ($P<0.05$) up to the end of week eight when compared with poor growth rates of GTG injected fish. Thereafter, growth rates of the three groups did not show a significant difference ($P>0.05$) till the end of the experiment in week twelve.

It can be seen from the growth data in Table 24, that a dose of 0.5 mg GTG/g body weight slowed down the growth rates and even reversed weight gain in the rainbow trout. This effect was temporary to some extent since some of the fish started to show some growth from week ten to the end of the experiment.

TABLE 23

Daily mean trigger actuations per week by rainbow trout after injection of Goldthioglucose (GTG)

Group		WEEK NO.											
		1	2	3	4	5	6	7	8	9	10	11	12
Normal Control		74	80	69	75	112	83	84	100	133	149	161	173
	S.D. =	±7	±14	±19	±19	±16	±8	±8	±10	±10	±7	±7	±7
	Fish No. in Group	12	12	10	10	10	10	10	10	10	10	10	10
Sham Control		77	77	78	85	101	105	106	97	114	144	148	155
	S.D. =	±7	±10	±10	±6	±11	±8	±8	±8	±11	±8	±14	±9
	Fish No. in Group	12	12	10	10	10	10	10	10	10	10	10	10
GTG Injected		4	6	22	27	55	60	68	52	75	107	124	116
	S.D. =	±2	±1	±4	±3	±2	±5	±3	±11	±21	±16	±31	±26
	Fish No. in Group	12	12	10	10	10	10	10	10	10	10	10	10

TABLE 24

Mean changes in body weight, specific growth rate and food conversion ratio of rainbow trout in Experiment 8

Group		Initial Weight (g)	WEEK 2	4	6	8	10	12
Normal Control	Weight (g)	32.05	41.16	52.59	67.58	85.79	108.85	137.14
	S.D. ±	1.37	2.05	4.08	4.34	3.77	7.24	18.84
	No. of fish	12	12	10	10	10	10	10
	S.G.R.[1]		1.79	1.75	1.75	1.70	1.70	1.65
	F.C.R.[2]		1.64	1.34	1.36	1.07	1.29	1.24
Sham Control	Weight (g)	31.86	39.04	49.94	63.25	80.94	103.90	126.61
	S.D. ±	1.68	1.97	3.62	5.61	7.13	9.68	9.06
	No. of Fish	12	12	10	10	10	10	10
	S.G.R.[1]		1.45	1.76	1.69	1.76	1.78	1.41
	F.C.R.[2]		2.26	1.58	1.63	1.21	1.18	1.41
GTG Injected	Weight (g)	32.34	29.86	31.22	34.89	41.68	52.72	67.05
	S.D. ±	1.65	2.54	5.02	7.20	5.13	10.03	11.16
	No. of fish	12	12	10	10	10	10	10
	S.G.R.[1]		x	0.32	0.79	1.27	1.68	1.72
	F.C.R.[2]		x	3.81	3.32	1.88	1.74	1.76

1 = Specific Growth Rate

2 = Food Conversion Ratio

TABLE 25

Average daily food intake by rainbow trout after injection of Goldthioglucose (GTG). Calculated from daily trigger actuation (0.15g food / trigger) means and standard deviations calculated for each week.

Week Number	G R O U P		
	Normal Control Food g/Fish/Day	Sham Control Food g/Fish/Day	GTG Injected Food g/Fish/Day
1	0.95 ± 0.10	0.97 ± 0.10	0.05 ± 0.03
2	1.10 ± 0.24	0.95 ± 0.15	0.07 ± 0.01
3	1.04 ± 0.28	1.18 ± 0.15	0.33 ± 0.06
4	1.13 ± 0.28	1.28 ± 0.09	0.40 ± 0.04
5	1.68 ± 0.23	1.52 ± 0.16	0.83 ± 0.03
6	1.24 ± 0.12	1.58 ± 0.12	0.90 ± 0.07
7	1.26 ± 0.12	1.59 ± 0.13	1.03 ± 0.04
8	1.51 ± 0.14	1.46 ± 0.12	0.79 ± 0.17
9	2.00 ± 0.10	1.71 ± 0.16	1.13 ± 0.32
10	2.24 ± 0.10	2.16 ± 0.12	1.60 ± 0.24
11	2.42 ± 0.11	2.22 ± 0.22	1.86 ± 0.47
12	2.59 ± 0.11	2.33 ± 0.14	1.73 ± 0.39
Means	1.596	1.580	0.893
S.D. ±	0.554	0.443	0.590

7.3.3. Food Conversion

Food Conversion Ratios (FCRs Section 3.11.2.) were obtained for each group at intervals of two weeks and are presented in Table 24. GTG injected group showed poor food conversion rates till the end of week six, thereafter conversion rates improved. Sham control group showed poor food conversion rate upto week two but improved rates till the end of the experiment. Normal control group showed the best food conversion rates.

Mean food conversion ratios were obtained for each group at the end of week twelve, and upon statistical analysis, each group showed a significant difference ($P<0.05$) in conversion rates.

7.3.4. Histology of Brain

The histological effects on forebrain of 0.5 mg GTG/g body weight, on two fish at second day, two fish at third day, two fish at fourth day and four fish at the end of week twelve post injection were observed. Histological study on the forebrain of normal and sham control fish was also carried. The Hypothalamus region of the brain of GTG injected group was scanned and compared with cross-sections of the similar regions of the brain of normal and sham control groups for any observable effects of GTG in the regions of ventro-medial or latero-hypothalamus. There were no observable effects on the histology of the forebrain in fish that received the dose of GTG.

7.3.5. Effect of GTG on Visceral Organs and General Outlook of Fish

The visceral organs such as kidney, liver and alimentary canal of sacrificed animals of the three groups upon examination did not show any apparent abnormality in structure. The stomachs of these GTG injected fish were empty which showed that virtually no feeding activity

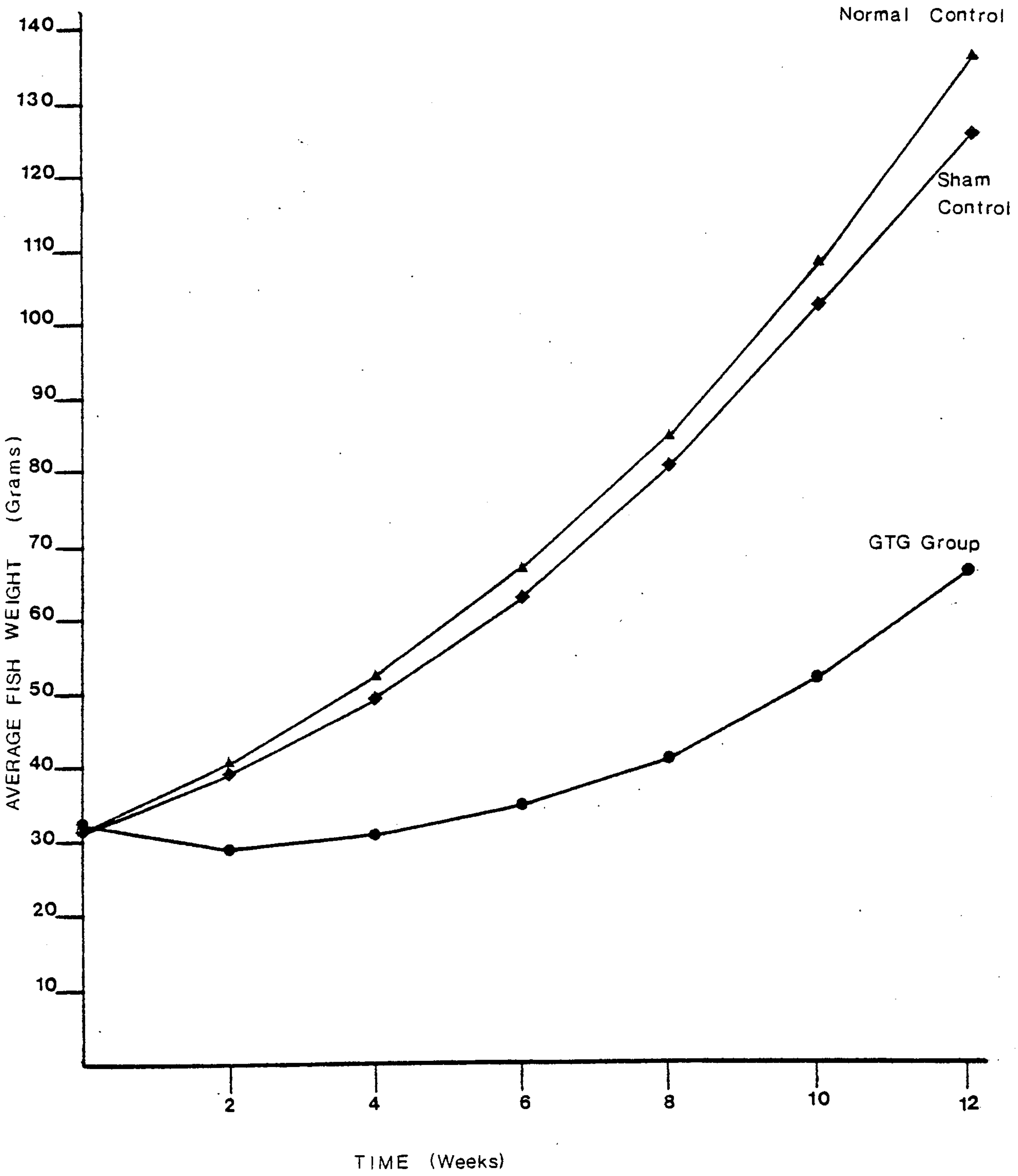


FIGURE 38 The growth responses of rainbow trout in the 3 groups of Experiment 8

had been performed, after the injection. In contrast some quantities of food were present in stomachs of the fish belonging to normal and sham control groups.

Fish which died during the course of the experiment were dissected to ascertain the cause of death. Liver, kidney and alimentary canal of GTG injected fish and sham control group fish showed no apparent abnormality. Stomachs of GTG injected fish were empty and shrivelled due to aphagia. Death of these animals appeared to occur due to aphagia and some toxic effects of GTG. Death of two fish in sham control group during the second week of the experiment appeared to be due to stress and strain of the injection of physiological solution.

Body colour of all the rest of the fish in GTG injected group was pale yellow and all the fish showed general weakness and inactivity till about the seventh week post injection. Thereafter, fish seemed to have recovered from the effects of GTG and started showing gradual improvement in general activity.

7.4. Discussion and Conclusions

The control fish showed a normal feeding activity throughout the whole period of the experiment, whereas GTG treated fish were observed to be hypophagic and inactive soon after the administration of GTG injection. Development of the hypophagic condition in the rainbow trout is contrary to the conditions of hyperphagia and obesity that occur in rats and mice (Brecher *et al.*, 1965; Debons *et al.*, 1962; Debons *et al.*, 1970; Drachman and Tepperman, 1957; Liebelt and Perry, 1957; Marshall *et al.*, 1955). Hypophagic conditions observed in the present study on rainbow trout persisted for eight weeks, which is a long period as compared to the hypophagia in mice which has been noted to occur within the first one or two days post injection but thereafter, the feeding activity is observed to be higher than the pre-treatment levels (Gray and Liebelt, 1961).

The results of the present study indicate that intraperitoneally injected GTG causes a decrease in growth in rainbow trout. This decreased growth induced by GTG is due to hypophagia. The effects of GTG on food intake and body weight are time dependent to some extent since some fish in the group started to show normal feeding activity and gain in body weight after the sixth week and the tendency seemed to be for recovery of normophagia. Similar effects were observed by Peter et al., (1976) on goldfish, when they administered intraperitoneal injections of GTG to the fish.

The control fish which were killed for brain sectioning, upon examination of the visceral organs were found to have large amounts of adipose tissue lining the peritoneal cavity as well as surrounding the intestines. GTG injected fish which died on the eleventh and twelfth day post injection had lost an appreciable quantity of body weight and were generally inactive and pale in body colour showing all apparent signs of weakness. The examination of their visceral organs showed very little observable fat tissue in the peritoneal cavity, and their stomachs were completely empty and reduced in size. The loss in body weight of the GTG injected fish presumably reflected loss of body reserves, such as fat. No lesions were observed in liver, intestine or kidney at dissection of any of the GTG injected fish, indicating that GTG had no apparent damaging effect upon these organs.

In rats it has been observed by Deter and Liebelt (1964) that the GTG-induced hypothalamic lesions have been associated with ulcerogenesis.

Similarly in mice, cessation of normal vaginal cyclicity was observed (Liebelt, Sekiba and Taylor, 1961), and in certain strains of mice, an enhancement of tumorigenesis has been reported (Waxler, Tobar and Melcher, 1953). In the case of fish the direct or indirect effects of GTG on various aspects of metabolism need to be further investigated in order to understand more fully the observed changes in body weight.

In the present study, no histological effects of GTG on the forebrain of rainbow trout have been observed. There were no localised lesions observed in the hypothalamus of the brain. This is quite contrary to the condition found in mice but similar to the conditions found in the case of Japanese quail (Carpenter et al., 1969), or chickens (Svacha and Reid, 1973) or dogs, sheep, goats (Baile et al., 1970) and guinea pigs (Luperello, 1969). In the case of mice a single injection of GTG has been repeatedly shown to produce localised lesions in the hypothalamus and other areas of the brain with the subsequent development of hyperphagia and obesity (Deter and Liebelt, 1964). In goldfish, Peter et al., (1976) also did not find any observable lesion in the ventro-medial hypothalamus (HVM) when fish were intraperitoneally injected with a low dose (0.1mg GTG/g body weight) or a high dose (0.5mg GTG/g body weight) of GTG. They, however, observed some hypertrophy of the ependyma of the forebrain in a few fish which were treated with the high dose of GTG. This ependymal hypertrophy was not a consistent feature amongst the fish injected intraperitoneally, but a dose dependent ependymal hypertrophy was present amongst those fish, which were given brain injections of GTG. From the results of the present study and work by Peter et al., (1976), it can be deduced that GTG does not produce any lesions in the forebrain of the fish but it can be agreed that GTG does play a significant role in causing hypophagia in fish, whether injected directly into the brain (intraventricularly) as by Peter et al., (1976) or systemically (intraperitoneally) injected, as in the present study (and also by Peter et al.). The mechanism by which GTG acts on rainbow trout cannot be fully explained, since the areas of the assumed centre of feeding activity in the brain remained unaffected by GTG injections. Significantly, GTG does not induce the formation of a lesion in the HVM of rainbow trout and thus does not give a visual clue to authentically state that an interference in the neural mechanism, directly related to some aspect of feeding behaviour, has taken place and thus make it easy to localise the specific area or areas in the hypothalamus which can be held responsible for the neural control of food intake.

Amongst the mammals it is widely accepted that a centre for regulation of feeding activity is present in the region of the lateral hypothalamus, although the existence of a satiety centre in the HVM of mammals has been looked upon with doubt by a number of workers in this field (Ahlskog and Hoebel, 1973; Gold, 1973; Panksepp, 1971a and 1971b; Rabin, 1972). In the bluegill, *Lepomis macrochirus* (Demski and Knigge, 1971), and the cichlid fish *Tilapia heudellotti* (Demski, 1973) feeding-like responses were observed during electrical stimulation of the area around the lateral recess of the third ventricle, which suggests that the lateral hypothalamus may be involved in feeding behaviour in teleost fishes. A better understanding of the neural control of food in fish may be achieved by producing direct lesions in the ventrolateral and ventromedial regions of the hypothalamus. However, there is no published work on the teleosts in this field.

In the case of mice the formation of a HVM lesion by GTG is insulin dependent (Debons et al., 1970), suggesting that glucoreceptors are present in this area of the brain. The absence of such lesions in rainbow trout suggests that HVM glucoreceptors are not present in this species. In mice systematically administered gold thiomalate and other gold thio compounds have no effect on weight gain and do not induce a HVM lesion (Marshall, Barrnett and Mayer, 1955; Mayer and Marshall, 1956) whereas, when GTG is administered, it causes lesions in the HVM, which shows that the uptake of the drug particularly by the HVM region is presumably related to the glucose moiety, followed by the toxic effects of the high concentration of gold intracellularly. In rats systematically administered GTG, gold thiomalate and gold chloride, all induced lesions in the kidneys but only GTG caused HVM lesions and obesity (Deter and Liebelt, 1962). The effects on the kidneys in rats were presumably related to the general toxicity of the gold compounds, whereas the effects of GTG on the HVM were likely due to the same mechanism as outlined above with regard to mice.

Peter _et al._, (1976) concluded from the results of the study of the effects of three gold compounds on food intake by goldfish, that the free ionic form of gold is quite toxic and proved fatal to the fish. Also in the present study on rainbow trout, GTG appears to have a strong toxic effect but this effect is temporary and non-fatal, perhaps due to a slow release of gold by degradation of the GTG compound, injected at a dose of 0.5mg GTG/g body weight and not necessarily related to the glucose moiety of the molecule and the resulting high intracellular concentration of gold. But when GTG is given in a higher dose than 0.5mg GTG/g body weight as in the pilot experiment (Section 7.1.2.), then the toxic effect of gold becomes irreversible and proves fatal to the majority of the fish. The toxic effects of the dose of 0.5mg GTG/g body weight seemed to dissipate after about eight weeks in the present experiment and the fish started showing normal feeding activity and started gaining body weight.

In conclusion it can be said that the effects of GTG observed in the present study resulted in hypophagia and loss of body weight. Due to the absence of any observable lesions in the hypothalamus of the brain, it can be concluded that the assumed feeding centre of rainbow trout remained unaffected and that hypophagia resulted due to some reversible toxic effect of the gold compound.

CHAPTER 8

8.1. General Discussion and Conclusions

The demand feeding system used in the present work posed no problem during the whole period of the different experiments. The fish also adjusted their feeding habits to the use of the demand feeders. The present use of the demand feeders was on a laboratory scale but the whole mechanism of the system has the potential to be developed on a trout hatchery scale as well. Demand feeders can only be useful for fish that can be conditioned to operate the system, therefore other cultured species of fish have to be first studied for their suitability to the demand feeding system. Not many species of fish are known which can be conditioned to operate the system. Goldfish (Rozin and Mayer, 1961a) and channel catfish (Randolph and Clemens, 1976; Hastings et al., 1972) have been shown to obtain food by pressing a lever. Rainbow trout have been shown by other workers also to obtain food by the use of demand feeders (Adron et al., 1973; Sumari and Westman, 1972; Landless, 1976) without any adverse effects upon their growth and normal feeding activity.

It was observed in the present study that the trout did not overeat because when the data for the trigger actuations during different experiments were analysed and food consumption calculated, it was within the normal limits. The calculated maximum amount of food eaten in different experiments was up to 5% of body weight daily and the minimum was around 2.5% except for fish under continuous dark photoperiod and the GTG injected fish in Experiment 8. It can be said that the trout fed on the demand feeders following their own feeding rhythm thus fulfilling their body needs. If, however, the fish are fed by hand at some determined ration there is a possibility that they would be underfed or overfed. For determining a good ration by hand feeding, one has to take into consideration a number of factors related to feeding frequency such as satiation time, stomach capacity and feeding interval, and other abiotic and biotic factors, among which light, temperature and fish size are of greatest importance. Fish on demand feeders can adjust their food intake according to their behavioural responses to all such factors and conditions. Also the experimenter can adjust the reward level dispensed at each trigger actuation by making the necessary adjustments on the feed dispensers (described in Section 3.4.3.). By the use of demand feeders food is distributed whenever it is required and in pre-determined quantities, which can improve the utilisation of diets by the fish and thus would result in better growth, besides being economical to be used in a fish hatchery.

In the different experiments, the training of the fish to learn to actuate the trigger did not require any skill or special efforts upon the part of the experimenter. The time period of this training is also of very short duration which further makes it simple for adopting this practice of demand feeding in fish farming. Some rainbow trout in the group started to use the trigger within hours of the introduction of the trigger in the tank.
After four to six days an increase in the number of trigger actuations per day was noticeable in all the groups. The increased number of trigger actuations were noted to stabilise at a certain level after seven to eight days and thus the fish were observed to feed normally thereafter, forming peaks of feeding activity.

It was claimed by Landless (1976) that the trigger actuations were performed by only one or two dominant fish in a group of twenty, whereas food that was dispensed was consumed by all the fish in the group, thus food intake remained unaffected. In the present work, although a record of trigger actuations by each fish in the group was not maintained through visual observations, it can be said that there were more than 50% of the fish in each group which were actuating the trigger. Although it is hard to single out the fish in a group of twenty to thirty freely swimming fish, but particularly in Experiment 8, where the individual fish were tagged and could be identified in a freely swimming group of ten fish in the tank, it was observed that at least seven individual fish were taking part in the trigger actuations. The social effect does seem to be operative in a group where the presence of one or more fish actuating the trigger facilitates the other fish in the group to do the same. Such a phenomenan of social facilitation amongst striped mullet has been shown by Olla and Samet (1974) where the presence of a group of fish feeding initiated other non-feeding fish to feed. However, in every group of fish in different experiments at least a few individuals did not actuate the trigger. This may be due to a hierarchical effect because it was noted that in some groups although the fish were not faced with any inadequacy of food some comparatively larger fish tried to dominate over the smaller fish by showing an aggressive behaviour of biting at the tail of the smaller fish. Also, in some tanks quite often during the off time of the feeding peak, it was observed that the fish were grouped together in a particular area of the tank whereas two or three fish would be in isolation in the other areas of the tank. When any isolated fish moved in to join the group then the fish in the whole group would become scattered with a rapid movement.

Newman (1956) observed similar aggressive behaviour in trout which was more marked in smaller tanks. Thus a certain hierarchical relationship seems to be present in the groups of trout, but any such hierarchical effect did not have a conspicuous influence on the feeding pattern of the trout in the present study. Demand feeding technique can be utilised for obtaining information on hierarchical effects of stocking mixed sizes of fish and also separately amongst fish of similar size range in a group. The influence of stocking mixed sizes of fish and the inter-relations of adverse environmental factors on the feeding routine can be assessed by using demand feeders.

In this study the feeding pattern of the trout showed a rhythmic activity as already discussed in Section 4.2.4. In contrast, in similar studies by Rozin and Mayer (1961a and 1964) using individual goldfish, it was found that such a pattern of rhythmic activity of feeding was not present in goldfish. Although goldfish also pressed a lever to obtain food they showed a regular pattern of feeding at intervals throughout the day or night. This difference is related to their physiology and different feeding strategy. The rainbow trout is predatory and has a storage stomach. A carnivorous fish can be expected to be a verocious feeder and is expected to feed as much as it can get hold of subject to the limitation imposed by its storage system - the stomach. When due to digestion the stomach was emptied to a certain degree after eight to ten hours the fish again engaged in the feeding process. This active feeding lasted for about three hours and virtually ceased upon reaching the satiation level. Thus on demand feeding satiation time is about three hours at $13.5^{o} \pm 2^{o}C$ as compared to hand feeding which is reported to be one hour for rainbow trout of 135g at $10^{o}C$ (Ishiwata, 1968). An eight hour cycle of feeding on demand feeders was also reported by Adron _et al._ (1973) in rainbow trout maintained at a temperature of $15^{o}C$ and light of 10 lux. In the present study during Experiments 1 and 2 water temperature was at least $2^{o}C$ lower than Adron _et al's._ experiment and since the rate of digestion is also attributable to temperature therefore, a longer interval of time can be expected between two consecutive peaks of feeding activity. During Experiment 1 the constant photoperiod of 9L15D at a light intensity of 160 lux appears to be in harmony with the conditions required for an efficient food consumption and optimal conversion by rainbow trout at a temperature of $13.5^{o} \pm 2^{o}C$. The presence of an eight to ten hours time interval between the peaks of feeding activity gives an indication that in rainbow trout the time sequence for return to full appetite in relation to the rate of stomach evacuation can be strategically manipulated to increase daily food intake which can yeild the best growth rate and feed utilisation by trout in hatcheries and fish farms. By using automatic feeders that dispersed food from one to twenty-four times per day the growth and conversion efficiency of catfish were discovered to be highest on satiation feeding twice per day at $28^{o}C$ (Andrews and Page, 1975). This is in contrast to the behaviour of young salmon where continuous feeding for fifteen hour/day at $20^{o}C$ produced significantly greater growth rate than feeding to satiation three times daily (Shelbourn, Brett and Shirahata, 1973). An explanation for these differences was given by Kono and Nose (1971) who examined the effect of various feeding frequencies on six different species of fish. They concluded that the suitability of different time sequences was influenced by stomach size, with the smallest stomachs requiring most frequent feedings. This also explains the monophasic rhythm of feeding activity by goldfish (Rozin and Mayer, 1961a) which does not possess a storage stomach and therefore does not feed in bouts but at regular intervals throughout day and night. Thus the time sequence of feeding can be strategically manipulated to increase daily intake, taking into consideration also the size of the fish.

The experiment on light intensity and photoperiod (Experiment 2) was not planned originally but the results of Experiment 1 on constant photoperiods created the idea of studying feeding behaviour under different levels of light intensity. Since in Experiment 1 the light intensity of 160 lux was produced by the fluorescent tubes (day light type) fitted in the light chambers, it was decided to study feeding behaviour at double and half of this light intensity together with at a lower light intensity of 10 lux. Further studies at lower levels than 10 lux and at higher than 320 lux light intensity would yield more information but owing to time factor these could not be undertaken. For measuring light intensity different authors have used different units of light intensity. Most commonly experimentalists have used foot candles (ft.c.) or metre candles (mc) also called lux and occasionally millilamberts (mL), which is a measurement of brightness or quanta. Blaxter (1970) in comparing different units of measure standardised the different units in metre candles according to; 1mc = 0.1ft c = 0.1mL and justified small discrepancies in standardisation by suggesting that since logarithmic changes of light have to be considered in the study of light reactions of fish, therefore, small discrepancies can be ignored.

In the natural environment there are continuous changes in quality and intensity of light both during its diurnal cycle and seasonal cycle. These changes are noticeable during night as well. At different time of the month, light intensity at night varies with the natural phases of the moon. The minimum light intensity for initial detection of food and visual recognition and selection are of great importance. Although under the controlled laboratory conditions the trout did not exhibit any active feeding during the dark regime of the photoperiods in the natural environment under certain conditions it is quite possible that trout might be able to feed even during the night. Hoar (1942) pointed out that in a confined space a learning factor may be involved and feeding by night under tank conditions may take place. In the present study no such effect of the learning was observed and no night feeding took place.

Rainbow trout dwell in shallow clear waters and are predatory by nature. In shallow clear waters during clear full moon nights especially in the surface layers light intensity may be sufficient for visual orientation. On moonlit nights the light intensity at the water surface reaches about 0.1 lux (Manteifel _et_ _al._, 1978). In the natural habitat during the dark hours at a light intensity of 0.1 lux very restricted feeding by trout has been reported by Bisson (1978). It can, therefore be speculated that because of their adaptation to a comparatively stable natural environment during the course of their ontogeny, rainbow trout might be quite capable of performing some nocturnal feeding in their natural habitat. Rainbow trout are considered to be diurnally active fish and most of the workers consider them as a visual feeder. The dusk and dawn light conditions were not artificially simulated in these laboratory studies - the light coming on to full intensity upon the onset of the light regime and switching off to complete darkness upon the end of the light regime of the photoperiod. Nevertheless the coming on of the light induced a peak of feeding activity under every photoperiod irrespective of the length of the light and dark regimes. Total absence of light restricted the feeding activity of the trout whereas continuous light disorientated the fish in time and space. In this later state fish experienced a monotonous period of unchanging environment and were cut off from stimuli which induce periods of activity and rest.

The estimations of diurnal feeding rhythms have usually been based upon the examination of stomach and gut contents and must be of limited reliability even under the best conditions. It is often difficult to obtain critically timed samples; the fullness of stomachs may vary widely, and digestion rates possible change in relation to size, season, temperature, and so on. Even in cases where the rhythms may appear clearly established it need not necessarily indicate a direct rhythm of feeding activity so much as diurnal changes in the vulnerability or availability of the prey to the predator. In the present study by the use of demand feeders, food was made continuously available to fish at all times thus eliminating all chances of the scarcity or unavailability of food which could possibly hinder trout in showing up their diurnal feeding rhythms.

The results of the experiments on different levels of light intensity give an indication that the quantity of food consumption is related to the intensity of light as already discussed in Section 4.3.4. It has been suggested that light usually has an indirect effect upon the physiology of fish involving regulation of metabolic activity by the endocrine system. The existence of a specific growth hormone secreted by the fish pituitary gland has been established (Hoar, 1957). It is well demonstrated by many experiments (reviewed by Pickford and Atz, 1957) involving light manipulation and production of gonadotropic hormones that the pituitary gland is affected by photoperiod. Possibly due to increased production of growth hormone there is a corresponding increase in feeding appetite and thus an increased food consumption was noticed as the light intensity was increased from 10 lux to 320 lux. It can be simply stated that the prime demand for food is imposed by the maintainance requirements of the fish. But due to the potential growth capacity (induced by growth hormone) of the fish a further demand in increased food intake is made. Perhaps these interdependent requirements control the voluntary food intake by fish.

An attempt to locate an area in the brain that controls the food intake, by inducing lesions through goldthioglucose in the assumed areas of medial "satiety centre" and the lateral "feeding centre" in the hypothalamus did not produce any positive results except that normal feeding behaviour was interrupted.

That the physical nature of food of fish can influence diet selection has been shown in the different Experiments (numbers 4, 5 and 6) and discussed in Chapter 5. In the present study, although the effect of the size, colour and texture of the feed pellets were studied, a number of other characteristics of prey (food) can influence diet selection. Prey movement, distribution and relative abundance also form some other important criteria of prey detection and capture by visual predators such as rainbow trout. Experiments on colour of feed pellets give an important clue of the importance of prey-colours in the natural environment of trout, also it can be suggested that in the manufacturing of commercial trout feeds, due consideration should be given to the colour preferences of the consumers. In natural environments the colour preferences of trout may vary according to their surroundings in respect of the colours of the prey items and the background they live in, also taking into consideration that to what extent light can penetrate in water to facilitate fish to easily locate its prey items. Size of the prey item is another important criterion of prey selection. Particularly for smaller fish, size of the prey item can impose certain limitations on efficient handling and capturing and subsequent ingestion. The present work shows that trout do exhibit preferential selection when given an opportunity of size selection as already discussed in

Section 5.1.4.

The experiment on colour preference of trout could be further analysed by changing the positions of the feed dispensers containing the different coloured diets at about half way through the experiment. This form of "cross-over" experimental protocol would however have taken a longer time period but could have helped in eliminating any hierarchical effects in the tanks and also could have helped in further understanding of the learning capacity of the trout.

Finally it would not be wrong to conclude that trout are quite capable of regulating their food intake in relation to the caloric content of the diet.

APPENDIX 1

Materials and their Suppliers used in the Present Study

Pumps

Beresford PV52, James Beresford and Sons Limited, Birmingham.

Tanks

Header, experimental and filter tanks by "Supaglass", White Lund, Morecombe.

Event Recorders

Type CR500 and CR503 Recorders. Educational Measurements Limited, Warsash, Southampton.

Photometer

Portable photoelectric photometer. Diffusion Systems Limited, Hanwell, London.

Time Switches

"Sangamo" 24-hour Dial Synchronous Time Switches. Sangamo Weston Limited, Enfield, Middlesex.

Electric Components

A. Gallenkamp and Company Limited, London.

Appendix table 1

Analysis of variance on the effect of different photoperiods on growth of trout in Experiment 1, (a) After 14 days, and (b) After 28 days period.

(a) Source of Variance	d.f.	SS	MS	F	Significance
Between Photoperiods	7	58.85	8.41	12.55	<1%
Linear	1	13.85	13.85	20.67	<1%
Quadratic	1	28.19	28.19	42.07	<0.1%
Remainder	5	16.81	3.36	5.01	ns
Error	7	4.71	0.67		

(b) Source of Variance	d.f.	SS	MS	F	Significance
Between photoperiods	7	136.28	19.47	29.06	<0.1%
Linear	1	6.43	6.43	9.60	<1%
Quadratic	1	79.97	79.97	119.36	<1%
Remainder	5	49.88	9.98	14.90	<1%
Error	7	4.71	0.67		

d.f. = degrees of freedom;

SS = sums of squares;

MS = mean square = SS/f;

F = ratio of MS of effect/error MS

Appendix table 2

(a) Analysis of variance on the effect of different sizes of feed pellets on feeding behaviour of trout in group 3 of experiment 4, and (b) Duncan's new multiple range test of comparisons between treatment means.

(a) Source of variance	d.f.	SS	MS	F
Treatments	3	27008.81	9002.94	612.49 ***
Residual	56	823.19	14.70	
(Total	59	27832.00)		

(b)

Treatments	Blank	Size 6	Size 5	Size 4
Treatments means (Trigger actuations)	3	12	25	59

d.f. = degrees of freedom;

SS = sums of squares;

MS = mean square = SS/f;

F = ratio of MS of effect/residual MS.

*** = Significant at 0.1% level.

_____ = Significantly different (1%) from mean not underlined.

Appendix table 3

(a) Analysis of variance on the effect of the coloured feed pellets on the feeding behaviour of rainbow trout during trial 1 in experiment 5, and (b) Duncan's new multiple range test of comparisons between treatment means.

(a) Source of variance	d.f.	SS	MS	F
Treatments	2	19037.36	9518.68	17.45 ***
Residual	60	32728.05	545.47	
(Total	62	51765.41)		

(b) Treatments	Green	Yellow	Orange
Treatments means (Trigger actuations)	57	87	99

d.f. = degrees of freedom;

SS = sums of squares;

MS = mean square = SS/f;

F = ratio of MS of effect/residual MS.

*** = significant at 0.1% level

_____ = significantly different (1%) from mean not underlined.

Appendix table 4

(a) Analysis of variance on the effect of the coloured feed pellets on the feeding behaviour of rainbow trout during trial 2 in experiment 5, and (b) Duncan's new multiple range test of comparisons between treatment means.

(a) Source of variance	d.f.	SS	MS	F
Treatments	2	16580.55	8290.28	24.84***
Residual	60	20022.88	333.71	
(Total	62	36603.43)		

(b) Treatments	Brown	Blue	Red
Treatment means (Trigger actuations)	43	76	79

d.f. = degrees of freedom;

SS = sums of squares;

MS = mean square = SS/f;

F = ratio of MS of effect/residual MS.

*** = significant at 0.1% level;

_____ = significantly different (1%) from mean not underlined.

Appendix table 5

(a) Analysis of variance on the effect of the coloured feed pellets on the feeding behaviour of trout during trial 3 in experiment 5, and
(b) Duncan's new multiple range test of comparisons between treatment means.

(a) Source of variance	d.f.	SS	MS	F
Treatments	3	52624.32	17541.44	82.59 ***
Residual	80	16991.24	212.39	
(Total	83	69615.56)		

(b) Treatments	Yellow	Orange	Red	Blue
Treatments means	36	60	92	98

(Red and Blue means underlined together.)

d.f. = degrees of freedom;

SS = sums of squares;

MS = mean square = SS/d.f.;

F = ratio of MS of effect/residual MS.

*** = significant at 0.1% level;

______ = significantly different (1%) from mean not underlined.

Appendix table 6

(a) Analysis of variance on the effect of the GTG injection on growth of trout, and (b) Duncan's new multiple range test of comparisons between means.

(a) Source of variance	d.f.	SS	MS	F
Treatments	2	28570.26	14285.13	66.53***
Residual	27	5797.84	214.73	
(Total	29	34368.10)		

(b) Treatments	GTG treated	Sham Control	Normal Control
Treatment means (Body weight) g	67.05	126.61	137.14

d.f. = degrees of freedom;

SS = sums of squares;

MS = mean square = SS/d.f.;

F = ratio of MS of effect / residual MS;

*** = significant at 0.1% level

______ = significantly different (1%) from mean not underlined.

BIBLIOGRAPHY

ABBOTT, R.R. 1972 Induced aggregation of pond-reared rainbow trout (*Salmo gairdneri*) through acoustic conditioning. Trans.Am.Fish Soc., 101 : 35-43

ADRON, J.W. 1972 A design for automatic and demand feeders for experimental fish. J.Cons.Perm.Int.Explor.Mer.34 : 300-305

ADRON, J.W., Grant, P.T. and Cowey, C.B. 1973 A system for the quantitative study of the learning capacity of rainbow trout and its application to the study of food preference and behaviour. J.Fish.Biol. 5 : 625-636

ADRON, J.W. and Mackie, A.M. 1978 Studies on the chemical nature of feeding stimulants for rainbow trout (*Salmo gairdneri*) Richardson. J.Fish.Biol. 12p 303-310

AHLSKOG, J.E. and Hoebel, B.G. 1973 Overeating and obesity from damage to a noradrenergic system in the brain. Science, 182 : 166-169

AHLSKOG, J.E., Randall, P.K. & Hoebel, B.G. 1975 Hypothalamic hyperphagia : Dissociation from hyperphagia following destruction of noradrenergic neurons. Science 190 : 399-401

AHSAN, S.N. 1966 Some effects of temperature and light on the cyclical changes in the spermatogenic activity of the Lake Chub (*Conesius plumbeus* Agassiz). Can.J.Zool. 44 : 161-171

ALABASTER, J.S. & Robertson, K.G. 1961 The effect of diurnal changes in temperature dissolved oxygen and illumination on the behaviour of roach (*Rutilus rutilus* L.) bream (*Abramis brama* L.) and perch (*Perca fluviatilis* L.) Animal Behaviour 9 : 187-192

ALBERT, D.J. & Storlien, L.H. 1969 Hyperphagia in rats with cuts between the ventromedial and lateral hypothalamus. Science 165 : 599-600

ALI, M.A. 1959 The ocular structure retinomotor and photobehavioural responses of juvenile Pacific salmon. Can.J.Zool. 37 : 965-996

ALI, M.A. 1964 Diurnal rhythm in the rates of oxygen consumption, locomotor and feeding activity of yearling Atlantic salmon (*Salmo salar*) under various light conditions. Proc.Indian Acad. Sci. Section B 60 : 249-263

ALLEN, G.H. & Clausen, L.G. 1960 Selectivity of food by brook trout in a Wyoming beaver pond. Trans.Am.Fish.Soc. 89 : 80-81

ALLEN, K.R. 1941 Studies on the biology of the early stages of the salmon (*Salmo salar*) J.Anim.Ecol. 10 : 47-76

ALLISON, L.N. 1951 Delay of spawning in eastern brook trout by means of artificially prolonged light intervals. Prog. Fish-Cult. 13 : 111-116

ANAND, B.K. & Brobeck, J.R. 1951 Hypothalamic control of food intake in rats and cats. Yale J.Biol.Med. 24 : 123-140

ANAND, B.K. 1961 Nervous regulation of food intake. Physiol.Rev. 41 : 677

ANDERSON, R.O. 1959 The influence of season and temperature on growth of the bluegill *(Lupomis machrochirus* Rafinesque). PhD Thesis, University of Michigan . 140p

ANDREWS, C.W. 1946 Effect of heat on the light behaviour of fish. Proceedings and transactions of the Royal Society of Canada. Volume XL : 27-31

ANDREWS, J.W. & Page, J.W. 1975 The effects of frequency of feeding on culture of catfish. Trans.Am.Fish.Soc. 104 : 317-321

AREES, E.A. & Mayer, J. 1967 Anatomical connections between medial and lateral regions of the hypothalamus concerned with food intake Science, 157 : 1574-1575

AREES, E.A., Veltman, B.I. and Mayer, J. 1969 Hypothalamic blood flow following goldthioglucose-induced lesions. Expl.Neurol. 25 : 410-415

ARONSON, L.R. 1963 The central nervous system of sharks and bony fishes with special reference to sensory and integrative mechanisms In "Sharks and Survival" (P.W. Gilbert, ed.) p. 165-241 Heath, Boston, Massachusetts.

ARONSON, L.R. 1970 Functional evolution of the forebrain in lower vertebrates. In "Development and Evolution of Behaviour" (L.R. Aronson, E. Tobach, D.S. Lehrman, and J.S. Rossenblatt, eds.) p. 75-107 Freeman, San Fransisco, California.

ASCHOFF, J. 1960 Exogenous and endogenous components in circadian rhythms. Cold Spring Harbour Symp.Quant.Biol. 25 : 11-25

ASCHOFF, J. 1966 Circadian activity with two peaks. Ecology, 47 : 657-672

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS METHODS 1970 Official methods of analysis of the association of official analytical chemists. Ed. Horwitz, W. 11th Edition Publ. by A.O.A.C., Washington, D.C.

ATACK, T.H. and Matty, A.J. 1978 The evaluation of single-cell proteins in the diet of rainbow trout.II-The determination of net protein utilisation, biological value and true digestibility E.I.F.A.C. Symp. Fin-Fish Nutri. and Feed Technol. Hamburg, June 1978, E.I.F.A.C. / 78 / Symp. E/59

BAILE, C.A., Mayer, J., Baumgardt, B.R. and Peterson, A. 1970 Comparative goldthioglucose effects on rats, sheep, dogs, goats and mice. J.Dairy Sci. 53 : 801-807

BARDACH, J.E. and Todd, J.H. 1970 Chemical Communication in fish. In Advances of Chemoreception (Eds. Johnston, J.W., Moulton, D.G. and Turk, A.) Volume 1 p 205-240 New York : Meredith Corporation

BARLOW, G.W. 1958 Daily movements of the desert pupfish *(Cyprinodon macularis)* in shore pools of the Salton Sea, California Ecology 39 : 580-587

BEUKEMA; J.J. 1968 Predation by the three-spined stickleback *(Gasterosteus aculeatus L.)* The influence of hunger and experience Behaviour 31 : 1-126

BILTON, H.T. and Robins, G.L. 1973 The effects of starvation and subsequent feeding on survival and growth of Fulton Channel sockeye salmon fry *(Oncorhynchus nerka).* J.Fish.Res.Board Can. 30 : 1-5

BISSON, P.A. 1978 Diel food selection by two sizes of rainbow trout *(Salmo gairdneri)* in an experimental stream. J.Fish.Res.Board Can. 35 : 971-975

BJORKLUND, R.G. 1958 The biological function of the thyroid and the effect of length of day on growth and maturation of goldfish *(Carassius auratus)* Linn. PhD Thesis, University of Michigan 171p.

BLAXTER, J.H.S. 1965 Effect of change of light intensity on fish. Spec.Publs.int.Commn. NW. Atlant.Fish., 6 : 648-661

BLAXTER, J.H.S. 1970 Light. In "Marine Ecology" (O.Kinne, ed.), Vol. 1, Part 1, p.213-320 Wiley, New York

BLEIER, R., Bard, P. and Woods, J.W. 1966 Cytoarchitectonic appearance of the isolated hypothalamus of the cat. J.Comp. Neurol., 128 : 255-312

BRECHER, G., Laquer, G.L., Cronkite, E.P., Edelman, P.M., Schwartz, I.L. 1965 The brain lesion of goldthioglucose obesity. J.Exp.Med. 121 : 395-491

BRETT, J.R. 1970 Fish - The energy cost of living. In "Marine Aquaculture" (W.J. McNeil, ed.) Oregon State Univ. Press

BRETT, J.R. 1971 Satiation time, appetite and maximum food intake of sockeye salmon *(Oncorhynchus nerka)*. J.Fish.Res.Board Can. 28 : 409-415

BRETT, J.R. and Higgs, D.A. 1970 Effect of temperature on the rate of gastric digestion in fingerling sockeye salmon *(Oncorhynchus nerka)* J.Fish.Res.Board Can. 27 : 1767-1779

BROBECK, J.R., Tepperman, J. and Long, C.W.H. 1943 Experimental hypothalamic hyperphagia in the albino rat. Yale.J.Biol.Med. 15 : 831-853

BROWN, F.A.Jr. 1937 Responses of the large-mouth black bass to colours. Ill.Nat.Hist.Surv.,Bull.21 : 37-55

BROWN, M.E. 1946 The Growth of Brown Trout *(Salmo trutta)*. The Growth of two year old trout at a constant temperature of 11.5°C. J.Exp.Biol. 22 : 130-144

BROWN, M.E. 1957 Experimental studies on growth. In "The Physiology of Fishes" (M.E. Brown, ed.) Volume 1 p. 361-400 Academic Press, New York

BROWNING, H.C & Kwan, L.P. 1965 Abnormal pituitary-ovarian relationship with mammary gland stimulation, in goldthioglucose treated mice Tex.Rep.Biol.Med. 118 : 253-255

BRYAN, J.E. 1972 Feeding history, parental stock and food selection in rainbow trout. Behaviour 45 (1/2) : 123-153

BRYAN, J.E. and Larkin, P.A. 1972 Food specialisation by individual trout. J.Fish.Res.BoardCan. 29 (ii) : 1615-1624

BULLOUGH, W.S. 1940 The effect of the reduction of light in spring on the breeding season of the minnow *(Phoxinus laevis)* Ibid., 110 (10) : 149-157

BURGER, J.W. 1939 Some experiments on the relation of the external environment to the spermatogenetic cycle of *Fundulus heteroclitus (L.)* Biol.Bull., 77 : 96-103

BURNSTOCK, G. 1958 Reversible inactivation of nervous activity in a fish gut. J.Physiol.Lond. 141: 35-45

BYRNE, J.E. 1968 The effects of photoperiods and temperature on the daily patterns of locomotor activity in juvenile sockeye salmon *(Oncorhynchus nerka)* PhD Thesis, University of British Columbia, Vancouver, 125p

CARPENTER, J.W., Stein, C.M., Silverstein, A. and Van Tienhoven 1969 The effect of goldthioglucose on food consumption and reproduction of the Japanese quail *(Coturnix coturnix japonica)* Poult.Sci. 48: 574-578

CRAWFORD, D.L., Law, D.K., Mckee, T.B. and Westgate, J.W. 1973 Storage and nutritional characteristics of modified Oregon moist rations as an intermediate-moisture product. Prog.Fish-Cult. 35 : 33-38

COLLINS, R.A. 1971 Cage culture of catfish in reservoir lakes. Proc.Annu.Conf.Southeast.Assoc.Game.Fish.Comm. 24 : 489-496

COMBS, B.D., Burrows, R.E. and Bigej, R.G. 1959 The effect of controlled light on the maturation of adult blueback salmon. Prog. Fish-Cult. 21: 63-69

CORSON, B.W. 1955 Four years' progress in the use of artificially controlled light to induce early spawning of brook trout. Prog. Fish-Cult. 17 (3) : 99-102

CRONLY-DILLON, J.R. and Muntz, W.R.A. 1965 The spectral sensitivity of the goldfish and the clawed toad tadpole under photopic conditions. J.Exp.Biol., 42 : 481-494

DAHL, E. and Ursin, H. 1969 Obesity produced by iron and tissue destruction in the ventromedial hypothalamus. Physiol.Behav. 4 : 315-317

DEBONS, A.F., Silver, L., Cronkite, E.P., Johnson, K.A., Brecher, D., Tenzer, D., and Schwartz, I.L. 1962 Localisation of gold in mouse brain in relation to goldthioglucose obesity. Am. .Physiol. 202 : 743-750

DEBONS, A.F., Krimsky, I., and From, A. 1970 A direct action of insulin on the hypothalamic satiety centre. Am.J.Physiol. 219 : 938-943

DEBONS, A.F., Krimsky, I., From, A., and Cloutier, R.J. 1970 Site of action of goldthioglucose in the hypothalamic satiety centre. Am.J.Physiol. 219: 1397-1402

DELGADO, J.M.R. and Anand, B.K. 1953 Increase of food intake induced by electrical stimulation of the lateral hypothalamus. Amer.J.Physiol. 172 : 743-750.

DEMSKI, L.S. and Knigge, K.M. 1971 The telencephalon and hypothalamus of the bluegill (Lepomis macrochirus) : Evoked feeding aggressive and reproductive beahviour with representative frontal sections. J.Comp.Neurol. 143 : 1-16

DEMSKI, L.S. 1973 Feeding and aggressive behaviour evoked by hypothalamic stimulation in a cichlid fish. Comp.Biochem.Physiol. A44 : 685-692

DEMSKI, L.S. 1977 Electrical stimulation of the shark brain. Am.Zool. 17 : 487-500

DETER, R.L. and Liebelt, R.A. 1962 The development of acute gastric ulcers in rats treated with goldthioglucose. Gastroenterology 43 : 575-584

DETER, R.L. and Liebelt, R.A. 1964 Goldthioglucose as an experimental tool. Tex.Rep.Biol.Med. 22 : 229-243

DEYOE, C.W., Tiemeier, O.W. and Suppes, C. 1968 Effects of protein, amino acid levels and feeding methods on growth of fingerling channel catfish. Prog.Fish-Cult. 30 (4) : 187-195

DRACHMAN, R.H. and Tepperman, J. 1957 Auro-thioglucose obesity in the mouse. Yale J. Biol. Med. 29 : 565

DUNCAN, D.B. 1955 Multiple range and multiple F-tests. Biometrics 11 : 1-42

EGAMI, N. 1954 Effect of artificial photoperiodicity on time of oviposition in the fish Oryzias latipes. Annotationes Zool. Japon. 27 : 57-62

EGGLISHAW, H.J. 1967 The food, growth and population structure of salmon and trout in two streams in the Scottish highlands. Freshw.Salmon Res. Scotland 38 : 32p.

EISLER, T. 1957 The influence of light on the early growth of chinook salmon. Growth 21 : 197-203

ELLIOTT, J.M. 1975 Number of meals in a day maximum weight of food consumed in a day and maximum rate of feeding of brown trout, Salmo trutta L. Freshwat. Biol. 5 : 287-303

FELLER, R.J. and Kaczynski, V.W. 1975 Size selective predation by juvenile chum salmon, Oncorhynchus keta, on epibenthic prey in Puget Sound. J.Fish.Res.Board.Can. 32 : 1419-1429

FENDERSON, O.C., Evenhart, W.H. and Muth, K.M. 1968 Comparative agonistic and feeding behaviour of hatchery-reared and wild salmon in aquaria. J.Fish.Res. Board Can. 25 : 1-14

FOWLER, L.G. and Burrows, R.E. 1971 The Abernathy Salmon diet. Prog. Fish.Cult. 33 (2) : 67-75

FRANK, L.H. and Meyer, M.E. 1974 Behavioural thermoregulation in hypophysectomised and sham-operated rainbow trout, (Salmo gairdneri) Behav.Biol. 11 : 101-108

FRIEDMAN, M.I. and Stricker, E.M. 1976 The physiological psychology of hunger : A physiological perspective. Psychol.Rev. 83 : 409-431

GARSIDE, E.T. 1959 Some effects of oxygen in relation to temperature on the development of lake trout embryos. Can.J.Zool. 37 : 689-698

GERKING, S.D. 1955 Influence of rate of feeding on body composition of protein metabolism of blue gill sunfish. Physiol.Zool. 28 : 267-282

GIBSON, R.N. 1971 Factors affecting the rhythmic activity of *Blennius pholis L.* (Teleostei). Anim. Behav. 19 : 336-343

GIBSON, R.J. and Keenleyside, M.H.A. 1966 Responses to light of young Atlantic Salmon *(Salmo salar)* and brook trout *(Salvelinus fontinalis)* J. Fish Res. Board Can. 23 : 1007-1021

GINETZ, R.M. and Larkin, P.A. 1973 Choice of colours of food items by rainbow trout *(Salmo gairdneri)* J. Fish. Res. Board Can. 30 : 229-234

GOLD, R.M. 1973 Hypothalamic obesity : The myth of the ventromedial nucleus. Science 182 : 488-490

GRASSL, E.F. 1956 Pelleted dry rations for trout propagation in Michigan hatcheries. Trans.Am.Fish.Soc. 86 : 307-322

GRAY, G.F. and Liebelt, R.A. 1961 Food intake studies in goldthioglucose obese CBA mice. Tex.Rep.Biol.Med. 19 : 80-88

GREENLAND, D.C. and Gill, R.L. 1979 Multiple daily feedings with automatic feeders improve growth and feed conversion rates of channel catfish. Prog.Fish-Cult. 41 (3) : 151-153

GRIMM, R.J. 1960 Feeding behaviour and electrical stimulation of the brain of *Carassius auratus*. Science 131 : 162-163

GROSS, W.L., Roelofs, E.W. and Fromm, P.O. 1965 Influence of photoperiod on growth of green sunfish *(Lepomis cyanellus)*. Fish Res. Board Can. 22(a) : 1379-1386

GUILLERY, R.W. 1957 Degeneration in the hypothalamic connexions of the albino rat. J. Anat. (Lond.) 91 : 91-115

HALE, E.B. 1956 Social facilitation and forebrain function in maze performance of green sunfish *(Lepomis cyanellus)*. Physiol.Zool. 29 : 93-107

HALVER, J. 1969 Vitamin requirements, p.209-232. In "O.W. Neuhaus and J.E. Halver (ed.) Fish in Research" Academic Press, Inc., New York, N.Y.

HAN, P.W. and Young, T.K. 1964 Obesity in rats without hyperphagia following hypothalamic lesions. Chin.J.Physiol. 19 : 143-154

HARDEN-JONES, F.R. 1968 "Fish Migration" St. Martin's Press, N.Y.

HARRINGTON, R.W. 1950 Preseasonal breeding by the bridled shiner, *Notropis bifrenatus,* induced under light-temperature control. Copeia 4 : 304-311

HARRINGTON, R.W. 1956 An experiment on the effects of contrasting daily photoperiods on gametogenesis and reproduction in the centrarchid fish, *Enneacanthus obesus* (Girard). J.Expl.Zool. 131 : 203-223

HASLER, A.D. and Villemonte, J.R. 1953 Observations on the daily movements of fishes. Science 118 : 321-322

HASTINGS, W.H. 1970 Demand Feeders. Resource, Publ. Bur. Sport Fish Wildl. U.S. 88 : 145-146

HASTINGS, W.H. and Dickie, L.M. 1972 Feed formulation and evaluation in "Fish Nutrition" (ed. Halver, J.E.) Academic Press, New York, and London.

HASTINGS, W.H., Hinson, B., Tackett, D., and Simco, B. 1972 Monitoring channel catfish use of a demand feeder. Prog.Fish-Cult., 34 (4) : 204-206

HAZARD, T.P. and Eddy, R.E. 1951 Modification of the sexual cycle in brook trout *(Salvelinus fontinalis)* by control of light. Trans. Am. Fisheries Soc. 80 : 158-162

HENDERSON, N.E. 1963 Influence of light and temperature on the reproductive cycle of the eastern brook trout, *(Salvelinus fontinalis)*. J. Fish.Res. Board Can. 20 : 859-897

HETHERINGTON, A.W. and Ranson, S.W. 1940 Hypothalamic lesions and adiposity in the rat. Anat.Rec., 78 : 149-154

HOAR, W.S. 1942 Diurnal variations in feeding of young salmon and trout. J.Fish.Res.Board Can. 6 : 90-101

HOAR, W.S. 1953 Control and timing of fish migration. Biol.Rev. 28 : 437-452

HOAR, W.S. 1957 Endocrine organs, p. 245-285 "The Physiology of Fishes" Vol 1 (Brown, M.E. ed.) Academic Press Inc., N.Y.

HOAR, W.S. 1958 Endocrine factors in the ecological adaptation of fishes p.1-23 "Comparative Endocrinology" (A. Gorbman, ed.) Wiley, N.Y.

HOLLING, C.S. 1966 The functional response of invertebrate predators to prey density. Mem.Ent.Soc.Can. 48 : 1-86

HOOVER, E.E. 1937 Experimental modification of the sexual cycle in trout by control of light. Science 86 : 425-426

HOOVER, E.E. and Hubbard, H.E. 1937 Modification of the sexual cycle in trout by control of light. Copeia 4 : 206-210

HIRATA, H. and Kobayashi, S. 1956 Diurnal rhythm of the feeding activity of goldfish in autumn and early winter. Bull.Fac.Fish. Hokkaido Univ. 7 : 72-84

HIRATA, H. 1957 Diurnal rhythm of the feeding activity of goldfish in winter and early spring. Bull.Fac.Fish. Hokkaido Univ., 8 : 96-107

HIRAYAMA, K. 1966 Studies on water control by filtration through sand beds in a marine aquarium with a closed circulating system. II Relation of grain size of filter and purification of breeding water. Bull.Jap.Soc.Scient.Fish 32 : 11-19

HIRAYAMA, K. 1974 Water control by filtration in closed culture systems. Aquaculture 4 (ii) : 369-385

HUBBS, C. and Strawn, K. 1957 The effects of light and temperature on the fecundity of the greenthroat darter, *(Etheostoma lepidum)* Ecology 38 : 596-602

HUISMAN, E.A. 1969 A study of the possibility of breeding carp in flow recirculation tanks (1968-1969). Ann. Rep. of the Organisation for Improvement of Inland Fisheries (O.V.B.) Lelystad, Holland 45p (C.E. Transl. 5503)

HUNT, R.L. 1965 Surface-drift insects as trout food in Brule River. Wis. Acad. Sci. Arts Lett. 54 : 51-61

HUNTER, J.R. 1966 Procedure for analysis of schooling behaviour. J.Fish.Res. Board Can. 23 : 547-562

HURST, P.M. Jr., 1953 Can Fish See Colour? Prog.Fish-Cult.15 (3) : 95

ISHIWATA, N. 1968 Ecological studies on the feeding of fishes - IV. Satiation curve. Bull.Jap.Soc.Sci.Fish., 34 : 691-694

IVLEV, V.S. 1961 Experimental ecology of the feeding of fishes. Yale Univ. Press, New Haven, 302p

JACOBSEN, M. 1965 Spectral sensitivity of single units in the optic tectum of the goldfish. Q.Jl. exp. Physiol. 49 : 384-393

JENKINS, T.M. 1969a Social structure, position choice and microdistribut of two trout species *(Salmo trutta* and *Salmo gairdneri)* resident in mountain streams. Anim.Behav.Monogr. 2 : 57-123

JENKINS, T.M. 1969b Night feeding of brown and rainbow trout in an experimental stream channel. J.Fish.Res.Board Can. 26 : 3275-3278

JOHNSON, L. 1966 Experimental determination of food consumption of pike *Esox lucius*, for growth and maintenance. J.Fish.Res.Board Can. 23 : 1495-1505

JONES, F.R.H. 1956 The behaviour of minnows in relation to light intensity. J.Exptl.Biol. 33 : 271-281

KAELBER, W.W. and Leeson, C.R. 1967 A degeneration and electron microscope study of the nucleus hypothalamicus ventromedialis of the cat. J.Anat.(Lond.) 101 : 209-221

KAPATOS, G. and Gold, R.M. 1973 Evidence for ascending noradrenergic mediation of hypothalamic hyperphagia. Pharmacol.Biochem.Behav. 1 : 81-87

KAPLAN, H. and Aronson, L.R. 1967 Effect of forebrain ablation on the performance of the conditioned avoidance response in the teleost fish, *Tilapia h, macrocephala*. Anim. Behav. 15 : 436-446.

KERR, S.R. 1971a Prediction of fish growth efficiency in nature. J.Fish.Res.Board Can. 28 : 809-814

KERR, S.R. 1971b A simulation model of lake trout growth. J.Fish. Res.Board Can. 28 : 815-819

KILAMBI, R.V., Noble, J. and Hoffman, C.E. 1970 Influence of temperature and photoperiod on growth, food consumption and food conversion efficiency of channel catfish. Proc.Annu.Conf. Southeast Assoc.Game Fish Comm. 24th pp 519-531 (Aquat.Sci.Fish. Abstr. 4, 4Q5139F, p.231)

KISLALIOGLU, M. and Gibson, R.N. 1976a Prey "handling time" and its importance in food selection by the 15-spined stickleback, (*Spinachia spinachia* L.) J. Exp. Mar. Biol. Ecol. 25 (2):151-158

KISLALIOGLU, M. and Gibson, R.N. 1976b Some factors governing prey selection by the 15-spined stickleback, (*Spinachia spinachia* L.) J. Exp. Mar. Biol. Ecol. 25 (2) : 159-169

KNUTSSON, S. and Grav, T. 1976 Seawater adaptation in Atlantic salmon (*Salmo salar* L.) at different experimental temperatures and photoperiods. Aquaculture 8 : 169-187

KONO, H. and Nose, Y. 1971 Relationship between the amount of food taken and growth in fishes. Frequency of feeding for a maximum daily ration. Bull.Jap.Soc.Sci.Fish. 37 (3) : 169-175

KWAIN, W. 1975 Embryonic development, early growth, and meristic variations in rainbow trout (*Salmo gairdneri*) exposed to combinations of light intensity and temperature. J.Fish.Res. Board Can. 32 : 397-402

KWAIN, W.H. and MacCrimmon, H. 1967 The behaviour and bottom colour selection of rainbow trout, (*Salmo gairdneri*) Richardson, exposed to different light intensities. Anim.Behav. 15: 75-78

KWAIN, W. and McCauley, R.W. 1978 Effects of age and overhead illumination on temperature preferred by underyearling rainbow trout, (*Salmo gairdneri*) in a vertical temperature gradient. J.Fish.Res.Board Can. 35 : 1430-1433

LANDLESS, P.J. 1976 Demand feeding behaviour of rainbow trout. Aquaculture 7 (1) 11-25

LARMOYEAUX, J.D. and Piper, R.G. 1973 Effects of water reuse on rainbow trout hatcheries. Prog.Fish.Cult. 35 (1) : 2-8

LARSSON, S. 1959 Inactivation of insulin in livers of mice with gold-thioglucose induced obesity. Acta Physiol.Scand. 46: 159-164

LEBRASSEUR, R.J. 1969 Growth of juvenile chum salmon (*Oncorhynchus keta*) under different feeding regimes. J.Fish.Res.Board Can. 26 : 1631-1645

LeCREN, E.D. 1958 Observations on the growth of perch *(Perca fluviatilis L.)* over twenty-two years with special reference to the effects of temperature and changes in population density. J.Animal Ecol. 27: 287-334

LEE, D.J. and Putnam, G.B. 1973 The responses of rainbow trout to varying protein to energy ratios in a test diet. J.Nutri. 103 (6) : 916-922

LEWINSKA, M.K. and Romaniuk, A. 1966 Is the ventromedial nucleus of the hypothalamus a satiation centre? Acta.Biol.exp. (Warszawa) 26: 285-297

LIEBELT, R., Sekiba, K. and Perry, J.H. 1960 Genetic susceptibility to goldthioglucose induced obesity in mice. Proc.Soc.exp.Biol.Med. 104: 689-694

LIEBELT, R.A., Sekiba, K. and Taylor, H.G. 1961 Persistent vaginal cornificiation in mice following the administration of goldthioglucose. Anat.Rec. 139 : 249

LIEBELT, R.A., Sekiba, K., Ichinoe, S., and Liebelt, A.G. 1966 Persistent vaginal cornification in goldthioglucose treated Bald-c-ki mice. Endocrinology 78 : 845-854

LIEBELT, R.A. and Perry, J.H. 1957 Hypothalamic lesions associated with goldthioglucose induced obesity. Proc.Soc.exp.Biol. Med. 95 : 774-777

LINDSTROM, T. 1956 On the relation of fish size - food size. Rep. Inst.Fw.Res. Drottningholm, 36 : 133-147

LISSMANN, H.W. and Schwassmann, H.O. 1965 Activity rhythm of an electric fish, *(Gymnorhamphicthys hypostomous)*. Z. Vergl.Physiol. 51 : 153-171.

LLOYD, R. 1961 The toxicity of ammonia to rainbow trout *(Salmo gairdneri)*. Waste Water Treatment J. 8 : 278-279

LONGO, N. and Bitterman, M.E. 1959 Improved apparatus for the study of learning in fish. Am.J.Psychol. 72 : 616-620

LUPARELLO, T.J. 1969 Studies on goldthioglucose ulcerogenesis. J. Psychosom.Res. 13: 113-118

MacCRIMMON, H.R. and Kwain, W.H. 1966 Use of overhead cover by rainbow trout exposed to a series of light intensities. J.Fish. Res.Board Can. 23: 983-990

MacCRIMMON, H.R. and Kwain, W.H. 1969 Influence of light on early development and meristic characters in the rainbow trout *(Salmo gairdneri)* Richardson. Can.J.Zool. 47 : 631-637

MAGNUSON, J.J. 1969 Digestion and food consumption by skipjack tuna *(Katsuwonus pelamis)*. Trans.Amer.Fish.Soc. 98: 379-392

MANN, R.H.K., and Orr, D.R.O. 1969 A preliminary study of the feeding relationships of fish in a hard-water and a soft-water stream in southern England. J.Fish.Biol. 1 : 31-44

MANTEIFEL, B.P., Girsa, I.I. and Pavlov, D.S. 1978 On rhythms of fish behaviour. In "Rhythmic Activity of Fishes" (J.E. Thorpe, ed.) p.215-224 Academic Press, London, New York, San Fransisco ISBN 0-12-690650-5

MARAIS, J.F.K. and Kissil, G.W. 1979 The influence of energy level on the feed intake, growth, food conversion and body composition of *Sparus aurata*. Aquaculture, 17 : 203-219

MARLER, P. and Hamilton, W.J. 1966 Mechanisms of animal behaviour - Wiley, New York p.114-158

MARSHALL, J.A. 1967 Effect of artificial photoperiodicity on the time of spawning in *Trichopsis vittatus* and *T. pumilis* (Pisces, Belontiid Anim. Behav. 15 : 510-513

MARSHALL, N.B. 1966 The Life of Fishes, World, New York

MARSHALL, N.B., Barrnett, R.J. and Mayer, J. 1955 Hypothalamic lesions in goldthioglucose injected mice. Proc.Soc.exp.Biol.Med. 90 : 240-244

MARTIN, N.V. 1952 A study of the lake trout, (*Salvelinus mamaycush*) in two Algonquin Park, Ontario, lakes. Trans.Am.Fish.Soc. 81: 111-137

MATHEWS, S.A. 1939 The effects of light and temperature on the male sexual cycle in *Fundulus heteroclitus*.. Biol.Bull., 77 : 92-95

MAYER, J. 1965 The ventromedial glucostatic mechanism as a component of satiety. Postgrad.Med. 38: A101-A109

MAYER, J. and Arees, E.A. 1968 Ventromedial glucoreceptors system. Fed.Proc. 27 : 1345-1348

MAYER, J. and N.B. Marshall 1956 Specificity of goldthioglucose for ventromedial hypothalamic lesions and obesity. Nature 178 : 1399

McCAY, C.M. and Dilley, W.E. 1927 Factor H in the nutrition of trout. Trans.Amer.Fish.Soc. 57 : 250-260

MEDLEN, A.B. 1951 Preliminary observations on the effects of temperature and light upon reproductions in *Gambusia affinis*. Copeia 2 : 148-152

MERRIMAN, D. and Schedl, H.P. 1941 The effects of light and temperature on gameotogenesis in the four-spined stickleback, (*Apeltes quadracus*) (Mitchill). J.Exp.Zool. 88 : 413-449

MESKE, C.H. 1971 Warm-water pisciculture, new processes of water treatment. Naturwissenschaften. 58 (6) : 312-318

MESKE, C.H. 1976 Fish culture in a recirculation system with water treatment by activated sludge. FAO Tech.Conf. on Aquaculture Kyoto, May 26-June 2, 1976. FIR:AQ/CONF./76/E.62.

METZ, J.P. 1974 The invertebrate drift on the surface of a prealpine stream and its selective exploitation by rainbow trout (*Salmo gairdneri*). Oecologia 14 (3) : 247-267

MILLER, R.B. 1946 Notes of the Artic Grayling, (*Thymallus signifier)* Richardson. Copeia

MOODIE, G.E.E. 1972 Predation, natural selection and adaptation in an unusual three spine stickleback. Heredity 28 : 155-167

MOORE, J.W. and Moore, I.A. 1974 Food and growth of anadromous artic char, (*Salvelinus alpinus L.*) in the Cumberland Sound area of Baffin Island. J.Fish.Biol.6, 79-92

MORRISON, S.D. and Mayer, J. 1957 Adipsia and aphagia in rats after lateral subthalamic lesions. Amer.J.Physiol. 191 248-254

MORTENSON, W. and Vahl, O. 1979 A programmed controller for automatic feeders. Aquaculture, 17: 73-76

MUNDIE, J.H. 1959 The diurnal activity of the larger invertebrates at the surface of Lac La Ronge, Saskatchewan. Can.J. Zool. 37: 945-956

MUNTZ, W.R.A. and Cronly-Dillon, J.R. 1966 Colour discrimination in goldfish. Anim.Behav. 14 : 351-355

MURPHY, J.T. and Renaud, L.P. 1969 Mechanisms of inhibition in the ventromedial nucleus of the hypothalamus. J. Neurophysiol. 32 : 85-102

N.A.C. 1973 Nutrient requirements of trout salmon and channel catfish National Academy of Sciences, Subcomm. on Fish Nutrition No. 11 57p

NAKAMURA, N. and Kasahara, S. 1956 A study of the phenomenon of the Tobi-Koi or Shoot Carp II on the effect of particle size and quality of the food. Bull.Jap.Soc.Sci.Fish. 21 : 1022-1024

NEEDHAM, P.R. 1969 Trout Streams. San Fransisco:Holden Day Inc.

NEILL, W.H., Magnuson, J.J. and Chipman, G.D. 1972 Behavioural thermoregulation by fishes : a new experimental approach. Science 176 : 1443-1445

NELSON, D.R. and Johnson, R.H. 1970 Diel activity rhythms in the nocturnal bottom dwelling sharks (*Heterodontus francisci* and *Cephalosyllium ventriosum)* Copeia : 732-739

NEWMAN, M.A. 1956 Social behaviour and interspecific competition in two trout species. Physiol. Zool. 29 : 64-81

NIKOLSKY, G.V. 1962 On some adaptations to the regulation of population density in fish species with different types of stock structure p. 265-282. "The exploitation of natural animal populations" LeCren and Holdgate (ed.) Blackwell Scientific Publ.Oxford, 339p

NIKOL-SKY, G.V. 1963 The Ecology of Fishes Academic Press, NY p. 44-56, 182

NILSSON, N.A. 1957 On the feeding habits of trout in a stream of northern Sweden. Rep.Inst.Fw.Res. Drottningholm 38: 154-166

NILSSON, N.A. 1958 On the food competition between two species of *Coregonus* in a north Swedish lake. Rep.Inst.Fw.Res. Drottningholm 36 : 146-161

NOBLE, G.K. and Curtis, B. 1939 The social behaviour of the jewel fish, *(Hemichromis bimaculatus)* Gill. Bull.Am.Museum Nat.Hist. 76: 1-46

NOVIN, D., Wyrwicka, W. and Bray, G.A. 1976 "Hunger: Basic mechanisms and clinical implications" Raven, New York

O'BRIEN, W.J., Slade, N.A. and Vinyard, G.L. 1976 Apparent size as the determinant of prey selection by bluegill sunfish *(Lepomis macrochirus)* Ecology 57: 1304-1310

OKADA, S. and Taniguchi, A. 1971 Size relationships between salmon juveniles in shore waters and their prey animals. Bull.Fac. Fish. Hokkaido University 22 : 30-36

OLIPHAN, V.A. 1951 Diel feeding rhythms in the fry of the Baikal grayling and others (A.I.B.S. translation) Doklady, Biological Sciences Section, 114 : 591-593

OLLA, B.L. and Studholme, A.L. 1972 Daily and seasonal rhythms of activity in bluefish *(Pomatomus saltatrix)*. Pages 303-325 in H.E. Winn and B.L. Olla, Eds., Behaviour of Marine Animals Volume 2, Vertebrates. Plenum Press, New York.

OLLA, B.L. and Samet, C. 1974 Fish to fish attraction and the facilitation of feeding behaviour as mediated by visual stimuli in striped mullet *Mugil cephalus*. J. Fish.Res.Board Can. 31: 1621-1630

OOMURA, Y., Ooyama, H., Yamamoto, T. and Naka, F. 1967 Reciprocal relationship of the lateral and ventromedial hypothalamus in the regulation of food intake. Physiol.Behav. 2 : 97-115

PAGE, J.W. and Andrew, J.W. 1973 Interactions of dietary levels of protein and energy on channel catfish *(Ictalurus punctatus)*. J.Nutr. 103: 1339-1346

PALKA, Y., Liebelt, R.A. and Critchlow, V. 1971 Obesity and increased growth following partial or complete isolation of ventromedial hypothalamus. Physiol. Behav. 7 : 187-194

PALOHEIMO, J.E. and Dickie, L.M. 1965 Food and growth in fishes. A growth curve derived from experimental data. J.Fish.Res.Board.Can. 22: 521-542.

PALOHEIMO, J.E. and Dickie, L.M. 1966 Food and growth of fishes. III, Relations among food, body size and growth efficiency. J.Fish. Res.Board Can. 23 : 1209-1248

PANKSEPP, J. 1971a Is satiety mediated by the ventromedial hypothalamus? Physiol.Behav. 7: 381-384

PANKSEPP, J. 1971b A re-examination of the role of the ventromedial hypothalamus in feeding behaviour. Physiol.Behav. 7: 385-394

PARKER, N.C. and Broussard, M.C. Jr. 1977 Selected bibliography of water reuse systems for aquaculture. Publ. by Texas Agricultural Experiment Station, Texas USA, June 1977 33p

PARKER, N.C. and Simco, B.A. 1973 Evaluation of recirculating systems for the culture of channel catfish. Proc. 27th Ann.Conf.S.E. Assoc. Game and Fish Commrs. 27 : 474-487

PARKER, R.R. and Larkin, P.A. 1959 A concept of growth in fishes. Ibid. 16: 721-745

PAXINOS, G. and Bindra, D. 1973 Hypothalamic and midbrain neural pathways involved in eating, drinking, irritability, aggression, and copulation in rats. J.Comp.Physiol.Psychol. 82: 1-14

PEEKE, H.V.S., Peeke, S.C. and Williston, J.S. 1972 Long-term memory deficits for habituation of predator behaviour in the forebrain ablated goldfish *(Carassius auratus)*. Exp.Neurol. 36 : 2, 288-294

PENTELOW, F.T.K. 1932 The food of the brown trout *(Salmo trutta L.)* J. Animal Ecol. 1 (2) : 101-107

PETER, R.E., Monckton, E.A. and McKeown, B.A. 1976 The effects of goldthioglucose on food intake, growth and forebrain histology in goldfish, *Carassius auratus*. Physiol.Behav. 17: 303-312

PETERSSON, B. and Hellman, B.O. 1962 The pancreatic islet tissue in mice with obesity induced by goldthioglucose. Acta path.microbiol. scand. 55 : 401-406

PHILLIPS, A.M. 1956 The nutrition of trout. I,General feeding methods. Prog.Fish.Cult. 18: 113

PICKFORD, G.E. and Atz, J.W. 1957 The physiology of the pituitary gland of fishes. New York Zool.Soc., New York 613p

PINHORN, A.T. and Andrews, C.W. 1963 Effect of photoperiods on the reactions of juvenile Atlantic salmon *(Salmo salar L.)* to light stimuli. J.Fish.Res.Board Can. 20 : 1245-1265

POSTON, A.H. 1974 Effect of feeding brown trout *(Salmo trutta)* a diet pelleted in dry and moist forms. J.Fish.Res.Board Can. 31 : 1824-1826

PRATHER, E.E. 1958 Further experiments on feeds for fathead minnows. Proc.12thAnn.Conf.S.E.Assoc. Game and Fish Commissioners 176-178.

PROTASOV, V.R. 1970 "Vision and Near Orientation of Fish" Isr. Programme Sci.Transl., Jerusalem.

RABIN, B.M. 1968 Effect of lesions of the ventromedial hypothalamus on the electrical activity of the ventrolateral hypothalamus. Electroenceph.clin.Neurophysiol. 25: 344-350

RABIN, B.M. 1972 Ventromedial hypothalamic control of food intake and satiety; A reappraisal. Brain Res. 43: 317-342

RABIN, B.M. and Smith, C.J. 1968 Behavioural comparison of the effectiveness of irritative and non-irritative lesions in producing hypothalamic hyperphagia. Physiol.Behav. 3 : 417-420

RANDOLPH, K.N. and Clemens, H.P. 1976 Some factors influencing the feeding behaviour of channel catfish in culture ponds. Trans. Am. Fish.Soc. 6 : 718-724

RASQUIN, P. and Rosenbloom 1954 Endocrine imbalance and tissue hyperplasia in teleosts maintained in darkness. Bull.Amer.Mus. Nat.Hist. 104 : 359-426

REPSYS, A.J., Applegate, R.L. and Hales, D.C. 1976 Food and food selectivity of the black bullhead, *Ictalurus melas,* in Lake Poinsett, South Dakota. J.Fish.Res.Board Can.33 : 768-775

REYNOLDS, R.W. 1959 The effect of amphetamine on food intake in normal and hypothalamic hyperphagic rats. J.Comp.Physiol.Psychol. 52: 682-684

REYNOLDS, R.W. 1963 Radio-frequency lesions in the ventrolateral hypothalamic "feeding centre". J.Comp.Physiol.Psychol. 56: 956-967

RICKER, W.E. 1932 Studies of speckled trout in Ontario. Publ.Ont. Fish.Res. Lab. 44 : 69-110

RINGLER, N.H. 1975 The mechanism of selective predation in drift-feeding brown trout *(Salmo trutta)* PhD Dissertation, University of Michigan, Ann.Arbor. 198p

RINGLER, N.H. 1979 Selective predation by drift-feeding brown trout J.Fish.Res. Board Can. 36: 392-403

ROBERTS, J.K. 1976 The metabolism and growth of rainbow trout *(Salmo gairdneri)* in fresh and saline waters. PhD Thesis, University of Aston in Birmingham 327p

ROBINSON, R.A. 1971 The what, how and why of pelletting. Resour. Publ.U.S. Bur.Sport Fish and Wildl. 102, 43-63

ROWAN, W. 1926 On photoperiodism reproductive periodicity and the annual migrations of birds and certain fishes. Proc.Boston Soc. Nat.Hist. 38 : 147-189

ROZIN, P. and Mayer, J. 1961a Regulation of food intake in the goldfish. Am.J.Physiol. 201 : 968-974

ROZIN, P. and Mayer, J. 1961b Thermal reinforcement and thermo-regulatory behaviour in the goldfish. *Carassius auratus.* Science 134 : 942-943

ROZIN, P. and Mayer, J. 1964 Some factors influencing short-term food intake in the goldfish. Am.J.Physiol. 206 : 1430-1436

RUITER, L. de 1967 Feeding behaviour of vertebrates in the natural environment p. 97-116 "Handbook of Physiology"(Amer.Physiol.Soc.) Volume 1, Section 6

RYDER, R.A. 1977 Effects of ambient light variations on behaviour of yearling, subadult and adult walleyes *(Stizostedion vitreum vitreum)* J.Fish.Res.Board Can. 34: 1481-1491

SANDREW, B.B. and Mayer, J. 1973 Hyperphagia induced by intrahypothalamic implants of mercury thioglucose. Physiol.Behav. 10 : 1061-1066

SATIA, B.P. 1974 Quantitative protein requirements of rainbow trout. Prog.Fish-Cult. 36 (2) : 80-85

SAVAGE, G.E. 1969 Some preliminary observations on the role of the telencephalon in food-reinforced behaviour in the goldfish *(Carassius auratus)* Anim.Behav. 17 : 760-772

SAVAGE, G.E. and Roberts, M.G. 1975 Behavioural effects of electrical sti lation of the hypothalamus of the goldfish, *(Carassius auratus)*. Brain Behav. Evol. 12 : 42-56

SCHWASSMANN, H.O. 1971 Biological rhythms. Pages 371-416 in W.S.Hoar and D.J. Randall, Eds. Fish Physiology, Volume 6, Academic Press, New York

SCLAFANI, A. and Maul, G. 1974 Does the ventromedial hypothalamus inhibit the lateral hypothalamus? Physiol.Behav. 12 : 157-162

SCLAFANI, A., Berner, C.N. and Maul, G. 1975 Multiple knife cuts between the medial and lateral hypothalamus in the rat. A reevaluation of hypothalamic feeding circuitry. J.Comp.Physiol. Psychol. 88 : 210-217

SCOTT, W.B. and Crossman, E.J. 1973 The freshwater fishes of Canada. Fish.Res.Board Can., Bull No. 184

SEGAAR, J. 1965 Behavioural aspects of degeneration and regeneration in fish brain. A comparison with higher vertebrates. Prog. Brain Res. 14 : 143-231

SHELBOURN, J.E., Brett, J.R. and Shirahata, S. 1973 Effect of temperature and feeding regime on the specific growth rate of sockeye salmon fry, *(Oncorhynchus nerka)* with a consideration of size effect. J.Fish.Res. Board Can. 30: 1191-1194

SLANEY, P.A. and Northcote, T.G. 1974 Effects of prey abundance on density and territorial behaviour of young rainbow trout *(Salmo gairdneri)* in laboratory stream channels. J.Fish.Res.Board Can. 31: 1201-1209

SMITH, C.E. 1972 Effects of metabolic products on the quality of rainbow trout. Am.Fishes of U.S. Trout News 17 (3) : 7-21

SMITH, O.A. 1956 Stimulation of lateral and medial hypothalamus and food intake in the rat. Anat.Rec. 124 : 363-364

SPENCER, W.P. 1939 Diurnal activity rhythms in freshwater fishes. Ohio J.Sci. 39 : 119-132

SPOTTE, S.H. 1970 Fish and invertebrate culture; Water management in closed systems. John Wiley and Sons Inc. 145p

STELLAR, E. 1954 The physiology of motivation. Psychol.Rev. 61: 5-22

STEVEN, D.M. 1959 Studies on the shoaling behaviour of fishes. Responses of two species to changes in illumination and to olfactory stimuli. J.Exptl.Biol. 36 : 261-280

STICKNEY, A.P. 1972 The locomotor activity of juvenile herring *(Clupea harengus)* in response to changes in illumination. Ecology, 53: 438-445

STICKNEY, R.R. and Andrews, J.W. 1971 The influence of photoperiod on growth and food conversion of channel catfish. Prog. Fish-Cult. 33 (4) : 204-205

STIVERS, R.E. 1971 Feed manufacturing. Resour.Publ.U.S. Bur.Sport Fish and Wildl. 102; 14-42

STORLIEN, L.H. and Albert, D.J. 1972 The effect of VMH lesions, lateral cuts and anterior cuts on food intake, activity level, food motivation and reactivity to taste. Physiol.Behav. 9 : 191-197

SWENSON, W.A. and Smith, L.L. Jr. 1973 Gastric digestion, food consumption, feeding periodicity and food conversion efficiency in walleye *(Stizostedion vitreum vitreum)*. J.Fish.Res.Board Can. 30 (9) : 1327-1336

SWIFT, D.R. 1962 Activity cycles in the brown trout *(Salmo trutta)* Fish feeding naturally. Hydrobiologia 20: 241-247

SWIFT, D.R. 1964 Activity cycles in the brown trout *(Salmo trutta)* Fish artificially fed. J.Fish.Res.Board Can. 21: 133-138

SUMARI, O. and Westman, K. 1972 Comparative feeding experiment on rainbow trout designed to test an automatic self-feeder. Finnish Fish.Res. 1 : 1-9

SUSHKINO, A.P. 1939 The nutrition of the Caspian migratory herring larvae during the river period of their life. Zool.Zh. 182 : 221-230

SUTIN, J., McBride, R.L., Thalman, R.H. and Van Atta, E.L. 1975 Organisation of some brainstem and limbic connections of the hypothalamus. Pharmacol., Biochem. Behav. 3 Suppl.1 : 49-59

SUYEHIRO, Y. 1942 A study on the digestive system and feeding habits of fish. Jpn.J.Zool. 10: 1-303

SVACHA, A.J. and Reid, B.L. 1973 Effect of goldthioglucose in the domestic fowl. Poult.Sci. 52 : 926-930

SYMONS, P.E.K. 1968 Increase in aggression and in strength of the social hierarchy among juvenile Atlantic salmon deprived of food. J.Fish.Res.Board Can. 25 : 2387-2401

TALBERT, G.B. and Hamilton, J.B. 1954 Failure to produce obesity in the rat following goldthioglucose injection. Proc.Soc.exp.Biol. Med. 86 : 376-378

TRIPLETT, N. 1901 The educability of the perch. Am.J. Psychol. 12 : 354-360

TUGENDHAT, B. 1960 The normal feeding behaviour of the three-spined stickleback *(Gasterosteus aculeatus L.)* Behaviour 15: 284-318

TURNER, C.L. 1957 The breeding cycle of the South American fish, *Jenynsia lineata*, in the northern hemisphere Copeia : 195-203

TYLER, A.V. and Dunn, R.S. 1976 Ration, growth and measures of somatic and organ condition in relation to meal frequency in winter flounder, *(Psuedopleuronectes americanus)*, with hypotheses regarding population homeostasis. J.Fish.Res.Board.Can. 33: 63-75

VALENSTEIN, E.S., Cox, V.C. and Kakolewski, J.W. 1969 Sex differences in hyperphagia and body weight following hypothalamic damage. Ann. N.Y. Acad. Sci., 157 : 1030-1048

VAN SOMMERS, P. 1962 Oxygen-motivated behaviour in the goldfish *(Carassius auratus)*. Science 137 : 678-679

VARANELLI, C.C. and J.D. McCleave 1974 Locomotor activity in Atlantic salmon *(Salmo salar)* in various light conditions and in weak magnetic fields. Animal Behav. 22 : 178-186

WAGNER, J.W. and deGroot, J. 1963 Effect of goldthioglucose injections on survival, organ damage and obesity in the rat. Proc.Soc. exp. Biol. Med. 112 : 33:37

WANKOWSKI, J.W.J. 1979 Morphological limitations, prey size selectivity, and growth response of juvenile Atlantic salmon *(Salmo salar)*. J.Fish.Biol. 14 : 89-100

WARE, D.M. 1971 Predation by rainbow trout : the effect of experience. J.Fish.Res.Board Can. 28 : 1847-1852

WARE, D.M. 1972 Predation by rainbow trout : the influence of hunger, prey density and prey size. J.Fish.Res.Board Can. 29 : 1193-1201

WARE, D.M. 1973 Risk of epibenthic prey to predation by rainbow trout. J.Fish.Res.Board Can. 30(6) : 787-797

WARNER, L.H. 1931 The problem of colour vision in fishes. Quart.Rev. Biol. 6 : 329-346

WAXLER, S., Tobar, P. and Melcher, L.R. 1953 Obesity and time of appearance of spontaneous mammary tumors in C3H mice. Cancer Res., 13 : 276

WERNER, E.E. and Hall, D.J. 1974 Optimal foraging and the size selection of prey by the bluegill sunfish *(Lepomis macrochirus)*. Ecology, 55 : 1042-1052

WHITEHEAD, C., Bromage, N.R., Forster, J.R.M., and Matty, A.J. 1978 The effects of alterations in photoperiod on ovarian development and spawning time in the rainbow trout *(Salmo gairdneri)*. Ann. Biol. anim. Bioch. Biophys. 18 (4) : 1035-1043

WINDELL, J.T. and Norris, D.O. 1969 Gastric digestion and evacuation in rainbow trout. Prog. Fish -Cult. 31 (1) : 20

WINDELL, J.T., Hubbard, J.D. And Horak, D.L. 1972 Rate of gastric evacuation in rainbow trout fed three pelleted diets. Prog. Fish -Cult. 34 : 156-159

WOLF, L.E. 1951 Diet experiments with trout : A synthetic formula for dietary studies. Prog.Fish-Cult. 13 : 17-20

WOLF, H. 1953 Coloured Fish Food Pellets. Prog.Fish.Cult. 15 : 182

WOLF, G. and Sutin, J. 1966 Fiber degeneration after lateral hypothalamic lesions in the rat. J.Comp.Neurol., 127: 137-156

WOODHEAD, P.M.J. 1966 The behaviour of fish in relation to light in the sea. Oceanogr. Mar. Biol. Annu. Rev. 4 : 337-340

YAEGER, D. 1967 Behavioural measures and theoretical analysis of spectral sensitivity and spectral saturation in the goldfish, *Carassius auratus*. Vision Res. 7 : 707-727

YOUNG, A.H., Tytler, P., Holliday, F.G.T. and MacFarlane, A. 1972 A small sonic tag for measurement of locomotor behaviour in fish. J.Fish.Biol. 4 : 57-65

ZORBIDI, Zh. Kh. 1970 Seasonal and ontogenic changes in the feeding of coho salmon juveniles in the Plotnikova and Avacha rivers. Izv. T.I.N.R.O. 78, 129-150. Fish.Res.Board Can.Transl.Ser. 2440, 33pp 1973