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STUDIES ON THE NUTRITION AND METABOLISM OF TURBOT (*Scophthalmus maximus* L) WITH REFERENCE TO FISH FARMING

T H E S I S

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by

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GLOSSARY

Efficiency of Energy Utilisation (EEU) The relation between the total energy consumed (ΣE_{cons} kj) and the total body energy accrued over the experimental period (ΣE_{accr} kj)

$$EEU\ \% = \frac{\Sigma E_{accr}}{\Sigma E_{cons}} \times 100$$

Efficiency of Protein Conversion (EPC) The relation between the total amount of protein consumed (ΣP_{cons} g) and the total body protein accrued over the experimental period (ΣP_{accr} g)

$$EPC\ \% = \frac{\Sigma P_{accr}}{\Sigma P_{cons}} \times 100$$

Food Conversion Ratio (FCR) The relation between the amount of food consumed (ΣF_{cons} g) and the change in body weight (ΔWg) over the experimental period. Food and body weight may be given in wet weight or dry weight or a combination. The commonly used form is wet weight of food : wet weight of fish gain.

$$FCR = \frac{\Delta W}{\Sigma F_{cons}}$$

Net Protein Utilisation (NPU) This relationship accounts for losses of nitrogen from the body as faecal nitrogen (F), metabolic nitrogen (F_k), urinary nitrogen (U) and endogenous urinary nitrogen (U_k) in relation to the nitrogen intake (I). Thus the nitrogen available for metabolism is determined as

$$NPU = \frac{I - (F - F_k) - (U - U_k)}{I}$$

Protein Efficiency Ratio (PER) This relationship is a quicker method for assessing protein utilisation and is determined as the ratio between body weight gain over the experimental period (ΔW wet weight g) and the amount of protein consumed over the experimental period (ΣP_{cons} g).

$$PER = \frac{\Delta W \text{ wet weight}}{\Sigma P_{cons}}$$

Specific Dynamic Action (SDA) The effect resulting from ingestion of a meal and caused by biochemical reactions on food substrates; manifested in fish as an increase in oxygen consumption above the basal rate.

Specific Growth Rate (SGR) This relationship applies when fish are growing exponentially. Therefore two growth points ($\bar{W}_{t1}$ and $\bar{W}_{t2}$) separated by a time period t (days) may be expressed in logarithmic form and divided by the time period to give the SGR

$$SGR = \frac{\log_n \bar{W}_{t2} - \log_n \bar{W}_{t1}}{t}$$

Studies on the nutrition and metabolism of turbot (*Scophthalmus maximus* L) with reference to fish farming

James Arnold Graham Brown PhD 1980

SUMMARY

This thesis provides the first detailed study of maximal oxygen consumption of turbot on a fish farm over a range of fish sizes and temperatures. Also provided is a study of the diets used in turbot farming and the development of a diet that contains no fresh fish.

A detailed study of previous research on flatfish nutrition, identified fresh fish, sprat in particular, as the optimum diet for turbot farming. A series of experiments was undertaken that confirmed this and also identified one possible explanation for the optimum performance of sprat, as a function of high non-protein energy ratios in sprat. This factor was exploited in the production of a diet containing no fresh fish and which produced superior results to diets containing fresh fish; the optimum level of lipid in the diet was determined as 18%.

The study of oxygen consumption was on fully-fed fish so that maximum demand could be quantified. Continuous monitoring of tank water oxygen levels enabled the calculation of the Specific Dynamic Action (SDA) effect in turbot and the relation of it to dietary energy. Variation of SDA with the dietary energy profile was identified as a contributing factor to differential fish growth on various diets.

Finally, the implications of this work to fish farming were considered. Economic appraisal and comparison of the diets routinely used in turbot farming identified that the diet developed as a result of this work, ie the diet containing no fresh fish protein, was more cost effective on the basis of the production of one tonne of turbot. The study of oxygen consumption enables water supply to be calculated for any fish size between 1g and 1000g between the temperatures of 7°C and 16°C. The quantification of SDA enables correct adjustment of oxygen flows according to the feeding status of the fish.

Key Words : Turbot, nutrition, metabolism, fish farming.

PREFACE

PREFACE

The impetus for this project stemmed from the research programme initiated by Spillers Farm Feeds Limited into fish feed manufacture. Dr. Philip Smith recognised marine fish farming as an area of development and thus with the co-operation of Shearwater Fish Farming Limited, the project was commenced. Shearwater had initiated a marine flatfish programme in 1974 and this was run initially utilising recycling systems on a trout farm at Low Plains near Carlisle. In 1977, an opportunity arose to lease a fish farm from the CEGB at Wylfa Power Station. This enabled a scaling-up of the marine fish programme for Shearwater and the author accepted the position of site manager. Thus the research described in this thesis was conducted at two locations using different tank systems. The pressures of running a pilot-scale turbot farm and working for a PhD were such that the research was organised to fit in with the farm operation, in such a way that neither programme suffered as a result, but the project required five years for completion.

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ACKNOWLEDGEMENTS

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1. GENERAL INTRODUCTION

1. General Introduction

Aquaculture has received considerable coverage by the media in recent years and has become a very topical subject. The farming of fish by man is by no means an innovation, in fact the only thing new about aquaculture is the word. The Chinese have reared fish in ponds since around 2000 BC and the actual process differed very little from that which is in operation there still and indeed in parts of Europe. In developing countries, fish farming exists today in the same capacity as it has for thousands of years, to provide a source of protein. Cowey and Sargent (1972) regarded as very significant that those fish which have been farmed for many generations, eg the carp, are vegetarians, as are many of the animals farmed by man. Fish farming in these countries largely takes place in ponds with very little extra food being provided by man, the usual procedure being to manure the pond to enhance natural productivity. In more developed countries, consumer demand for high-quality fish products led to the development of farming such species as rainbow trout, (*Salmo gairdneri*), catfish (*Ictalurus punctatus*), Pacific salmon (*Oncorhynchus spp*) and Atlantic salmon (*Salmo salar*). The farming of these carnivorous fish presented different problems from that of carp, since they require a diet that is high in protein and typical trout rations contain 45% protein compared with 35% for carp.

The need for supplemental feeding of farmed fish and in some cases the requirement for a complete diet, where natural productivity

was not enhanced, led to an increase in research into fish nutrition, in order to optimise diets for fish growth. The importance of optimising diets is illustrated by the fact that food represents about 60% of the production cost of fish farming where supplemental feeding is employed. In the review published by Cowey and Sargent (1972), the success of USA catfish farming was attributed to, amongst other things, the availability of pelleted rations formulated in accordance with the basic nutritional requirements of the fish. In 1963, 17,000 acres were under intensive warm water cultivation of which 2,370 acres were catfish. In 1969, 68,000 acres in the USA were under intensive warm water cultivation of which 39,000 acres were catfish (Anon 1970).

It is some 20-25 years ago that fish nutrition research really gathered momentum, with authors such as Philips in the USA. As much of the early research was conducted in the USA, it follows that a large proportion of our knowledge is about the requirements of species that are farmed there, eg channel catfish, rainbow trout and Pacific salmon. The Japanese have also an intense nutrition research programme, working on such species as rainbow trout, eels and yellowtail (*Seriola quinqueradiata*). Whilst fish nutrition knowledge has made considerable advances over the past few years, the understanding is still less advanced than that of other animals. One has to remember though, that the effort expended on fish nutrition research is lower than that of other farmed animals because the actual production of fish is much less than that of, for example, broiler chickens.

In the face of the fish nutrition research, how does production from fish farming compare with that from the fishing industry? According to figures published by Pillay (1975), the world aquaculture production for 1975 was six million tonnes; however, one has to remember that the word aquaculture embraces all forms of aquatic organisms and therefore includes seaweeds, crustaceans and molluscs as well as fish. This production figure only represents about 9% of the harvest from natural fisheries (FAO 1977) and about 40% of the aquaculture production took place in China. However, the world aquacultural production doubled between 1970 and 1975 and is projected to double again by 1985 (FAO1977).

In the UK, fish farming as an industry started in the early 1960's (McAnuff 1979) although fish farming as an activity has been recorded since Roman times, the main species being carp. The decline in fish farming during the 19th century is attributed to the increase in the fishing industry, as the productive home waters such as the North Sea produced a large supply of fish. The production of farmed fish for human consumption in the UK is currently estimated at 4,000 tonnes for 1979 (McAnuff), although about 80% of this is rainbow trout (*Salmo gairdneri*). The production of 2,400 tonnes of farmed fish in 1976 compared to UK fish landings of 933,000 tonnes (MAFF 1976), thus representing a contribution of about 0.3% of fish supplies. If one assumes that approximately 70% of fish landed are for human consumption (McAnuff) then fish farming produced about 0.4% of fish consumed. Thus compared with a world figure of 10% there is much scope for increase in the UK.

Over the past 10-15 years, marine fish farming has been gathering momentum in the UK. At present, the only significant production of truly marine fish in the world, discounting anadromous salmonids, is that of yellowtail (*Seriola quinqueradiata*) in Japan, the 1972 production being in excess of 74,000 tonnes Pullin (1976). The species of marine fish being farmed world wide is very extensive from Pompano (*Trachinotus carolinus*) in the USA to Milkfish (*Chanos chanos*) in the Philipines. In Europe, a number of marine species have been investigated, particularly Bass (*Dicentrarchus labrax*) and Bream (*Sparus aurata*). In the UK, the main candidates to date for marine farming are flatfish. This work on marine flatfish dates back to the end of the 19th century, when fish hatcheries were set up to release young fish to augment natural fisheries. Due to the inability to produce the millions of juveniles needed to make a significant effect on the fishery, the programme was shelved by 1920. Some work was continued on hatchery improvement and a major advance was made by Rollefsen (1939), who demonstrated that *Artemia* nauplii were an acceptable food for larvae of the plaice (*Pleuronectes platessa*). This work was progressed by Shellbourne (1964, 1967), who demonstrated rearing methods for the plaice and sole (*Solea solea*) in larger numbers than previously. The White Fish Authority (WFA) adopted two methods of plaice culture:

(i) in a dammed off 2ha sea loch at Ardtoe Ardnamurchan, Argyll,

(ii) in $50m^3$ tanks at Hunterston Power Station, Ayrshire.

Of these two, the latter proved the most suitable. For more detailed

descriptions see Kingwell (1974) and Howard and Kingwell (1975). Having demonstrated a technique for on-growing plaice, the programme was halted by a major factor: the costs of production calculated in 1972 could not be recovered by the sales value of the fish.

A study by Jones (1972) gave the following as the most important criteria for evaluating candidate species for farming:

(i) a high market price,

(ii) a fast gain in weight on a cheap and readily available food,

(iii) ease of breeding and rearing of young in captivity.

The species that most seemed to fit these criteria for the UK were turbot (*Scophthalmus maximus*) and sole (*Solea solea*). Accordingly, most further farming research was evaluated with these species. Interestingly, Anthony (1910) had identified turbot as being the most suitable species for UK farming. It is with the second of Jones' criteria, that fish nutrition can be involved. As mentioned previously, food accounts for around 60% of production costs in fish farming and so it is critical to formulate a diet that is a compromise between cost and growth.

Little research on marine flatfish nutrition had taken place before that initiated by Cowey et al (1970), most of the work having been concerned with ecological perspectives, ie the feeding of natural diets and relation of results to natural environments. The main researchers were Dawes (1930, 1931), Buckmann (1952) and Colman (1970), who fed plaice on a diet of mussel (*Mytilus edulis*). Birkett (1969) performed experiments to determine the nitrogen

balance of plaice and sole. Cowey and his co-workers in the '70s (1970, 1970a, 1971, 1972, 1974, 1976, 1976a) ran a programme on the nutrition of marine flatfish, examining such aspects as essential amino acid requirements and protein utilisation. Whilst these experiments gave information on specific nutritional aspects, there was only slow progress towards developing an optimum diet for use in fish farming. The White Fish Authority (WFA) initiated a programme of diet development, that culminated in the current WFA7 diet which is used for on-growing turbot in their sea cages at Ardtoe. This diet consists of a mixture of sprats, queen scallop offal and fish meal and vitamin binder that is mixed and extruded as a moist pellet. Bromley (1974, 1974a) performed experiments with turbot, to determine such factors as optimum dietary moisture level and dietary fat and protein levels. Purdom et al (1972) described trials with turbot fed on industrial fish in minced and bound form for small fish and chopped for large fish. This, and previous published and unpublished evidence, indicated that certain moist and wet diets containing industrial fish, notably sprat (*Sprattus sprattus*), resulted in better growth and conversion when fed to turbot, than other diets such as dry pellets and moist diets, not containing industrial fish.

Much of the previous research had used very small numbers of fish and often no replicates of diet treatments. One of the main objects of the first section of this thesis was to try and investigate and explain the differences suggested above. This would then enable a diet to be formulated, suitable for use on a fish farm, with a performance equivalent to, but containing no raw, industrial fish. If the good performance of turbot on industrial fish could not be

matched, one of the objectives was to formulate a diet that produced the best balance between growth and diet cost.

The second section of the thesis is concerned with metabolism and oxygen consumption. This section is directly related to the previous section as metabolism and oxygen consumption mirrors diet composition and consumption. Knowledge of oxygen consumption rates and carbon dioxide production rates are key issues in fish farming, particularly where an intensive system is employed and it is critical to have control over water quality, as reduced waterflow rates are used.

There is still a lot of basic information to be determined before marine fish farming can approach the broiler chicken industry in terms of scale and profitability. It is important that the farmer is able to predict food consumption and therefore oxygen consumption and water usage from temperature information. The main aim of this section was to investigate oxygen consumption over the operational temperature range and size range of the fish. Two other aspects examined were the effect of feeding levels and diet composition on oxygen consumption and diurnal fluctuations of oxygen consumption.

Oxygen consumption rates have been extensively examined for salmonids in the laboratory by Beamish (1964a, 1975) and Brett (1964, 1965). Also some work has been conducted on marine fish, plaice and cod (Edwards 1969, 1972). The experiments in this section were performed on large groups of fish in tanks, in order to ensure relevance of results to a fish farm situation ie they were not transferred to a special respirometer. The relative merits of several methods of

oxygen consumption determination are discussed.

The final section of the thesis concerns the significance of the findings to fish farming. By virtue of the fact that all the research in this thesis was carried out on fish farms, the underlying direction has been the application of its findings to turbot farming. Presented in this section are production, growth and conversion data for some experimental diets from the first section. As mentioned previously, food is a major cost item and so is water supply, where a pumped system is used. The ability to specify and predict these (water requirements from oxygen metabolism work) will be a major step in quantifying the constraints of turbot farming. If the fish farming business is going to approach that of the broiler industry in terms of scale and profitability, it is vital to predict performance as accurately as possible and thereby enable the utilisation of computer-linked production planning.

2. GROWTH, FEEDING RATE AND NUTRIENT UTILISATION OF TURBOT FED DIETS OF VARYING FISH PROTEIN SOURCES

2.1 Introduction

A number of recent reviews have been written on general fish nutrition eg Cowey and Sargent (1972) and Braekkan (1977). The principle species covered in these reviews have been those on which most research has been performed, eg Pacific Salmon (*Oncorhynchus spp.*), Rainbow trout (*Salmo gairdneri*) and Catfish (*Ictalurus punctatus*). In order to illustrate the differences between UK marine flatfish and these species, the following review is presented as a background to past research and as an indication of the future requirements. As the terminology of fish nutrition is very confusing and contradictory, the terms used in this thesis are as defined in Utne (1978). The nutrition of post-metamorphosed fish only is discussed, as work on larval fish is considered to be out of the scope of this thesis. The review is divided into three sub-sections:

(i) feeding and growth experiments utilising natural diets and the ecological implications of the results,

(ii) feeding and growth experiments utilising natural and compounded diets and the commercial implications of the results,

(iii) experiments to define nutritional requirements and limits.

The first sub-section covers most of the early work utilising such diets as mussel (*Mytilus edulis*). The earliest major contribution was that of Dawes (1930, 1931) on plaice (*Pleuronectes platessa*). One of the main problems encountered was that the efficiency of food

Author	Fish Species	Fish Size (g)	Temp °C	Food Conversion Ratio (wet weight food)		Date
Dawes (1930)	*P. platessa*	25-149	12-20	*M.edulis*	7.4	1928
		30-203			11.7	1928
		13-185			9.5	1929
		17-82			10.9	1930
		17-205			14.4	1930
Buckmann (1952)	*P. platessa*	10-80	15-19	*M.edulis*	5.8	1950
					8.5	1951
Hatanaka et al (1956a)	*Limanda yokohomae*	42-120	17-21	*Tyrlorryhncus heterochaetus* (annelids)	5.0	1955-56
		139-215			7.3	"
		68-124	11-15		5.5	"
		89-207			7.5	"
		193-279			9.6	"
		74-107	7.7-9.5		6.2	"
		129-227			8.8	"
		110-222	11-15	*Vererupis japonica* (clams)	7.4	"
Hatanaka et al (1956b)	*Kareius bicoloratus*	102-175	7-13	*V.japonica*	6.6	"
		120-212	14-18		5.2	"
		72-275	18-19.5		5.6	"
		87-234	11-18	*T.heterochaetus*	5.2	"
Bowers and Landless (1969)	*Scophthalmus maximus*	5.8-27.7	17	*Pecten maximus*	5.2	1969
Colman (1970)	*P.platessa*	0.3-2.6	15	*M.edulis*	4.0	1965
		0.4-5.7	16		5.0	1966
		0.7-5.1	13		6.2	1966
Edwards et al (1969)	*P. platessa*	0.8-8.0	17	*M. edulis*	4.0	1968

TABLE 2.1

Summary of early work on flatfish food conversion rates

utilisation varied between individual fish and within the same individual at different periods. This is obviously one of the hazards of experimenting with live animals and is still a problem today. Dawes' work on body maintenance in plaice has been used and extrapolated by various workers such as Colman (1970), to apply to different sized fish. The data from these early workers is summarised in Table 2.1. The main purpose of these experiments was to examine feeding and growth rates of fish in the laboratory and relate the results to natural productivity. As will be seen later, conversions of around 6:1 are regarded as being intolerable to the fish farmer, but are no doubt accurate reflections of dynamics in natural habitats.

Some interesting general points do emerge from these experiments. Dawes (1930,1931) observed that plaice required 1-2% body weight per day of mussel at 15°C to maintain their initial weight. Both Colman (1970) and Edwards et al (1969) obtained conversion rates of around 4·0:1 at 15°C when plaice were fed on mussel flesh. Bowers and Landless (1969) recorded a conversion rate of 5.2:1 for turbot fed on scallops (*Pecten maximus*).

Dawes (1930,1931) found that plaice fed on restricted rations utilised their food more efficiently than those fed to satiation. Hatanaka et al (1956a,b) showed that as fish increased in size, conversion efficiency decreased. These data are presented in Table 2.1. Maintenance requirements and digestion rates are also presented in these papers. These factors are considered in the section on metabolism later in the thesis.

Most of this work was performed using very small numbers of fish and such factors as temperature were not controlled very closely. In

several cases, only single fish were used for experiments. The extrapolation of this data to large groups of fish, as in fish farms, would be very suspect. The data are useful as a baseline, to see what improvements can be made by manipulating diet ingredients.

The work reported in the second sub-section concerns the search for a diet that could be fed commercially to flatfish. A foodstuff that proved an excellent food for '0' group flatfish, at the MAFF hatchery in the Isle of Man, was the enchytraeid worm *Lumbricillus rivalis*. Kirk (1972,1973a,b) performed a series of experiments with sole (*Solea solea*) and plaice (*Pleuronectes platessa*) using a variety of natural diets including enchytraeid worms. It was proposed that these worms could be commercially produced. However large scale culture techniques have so far been unsuccessful (Kirk 1971), although Thain (pers communication) manages to rear sufficient numbers for large scale sole weaning. Kirk (1972) reported that plaice only grew well on diets of fresh *Lumbricillus*. In a later paper (Kirk 1973b), the food value to sole of other enchytraeids was investigated. The four species tried were *Enchytraeus albidus*, *Lumbricillus rivalis*, a combination of the two, and *L. reynoldsoni*. *L. rivalis* and *E. albidus* produced similar growth rates - 4.63% and 4.46% daily weight gain respectively for 0.4g sole at 18°C, but *L. reynoldsoni* produced no growth. It was proposed that these diets could be used to wean newly metamorphosed fish on to an artificial diet. Subsequent failure to mass culture enchytraeids led to investigation of other natural diets (Kirk 1973a). The two diets tried were mussel (*Mytilus edulis*) and slipper limpet (*Crepidula formicata*). Once again, no food conversion data were presented, only growth data. Slipper limpet produced the best growth for sole of about 4g at 18°C. The mean daily

% change in weight on slipper limpets was 1.24% and 0.97% on fresh mussels.

Purdom et al (1972) conducted trials to test the suitability of turbot (*Scophthalmus maximus*) for fish farming. During the first six months, the fish were fed on a diet of minced fish bound with 2.5% methofas (carboxy-methyl cellulose binder, ICI). In later months, when the fish were larger, a diet of chopped fish of various species was used, including blue whiting (*Micromesistius poutassu*), sandeels (*Ammodytes spp.*) and sprats (*Sprattus sprattus*). The predominant diet was sprat and sandeel. The overall wet weight food conversion rate over 20 months was 3.08 to 1. The fish grew from 3.2g to 1027g in this period at a mean temperature of 16.3°C ± 3.1°C. A range of food conversion rates was experienced over this period due to, among other things, different diets. The lowest food conversion was 2.31. Purdom (1977) later attributed one of the higher wet weight food conversions, at 3.81, to the feeding of gadoids such as blue whiting. He states that these fish are calorifically much poorer than sprat. Food conversion information presented also supports the evidence of Hatanaka et al (1956a,b) that food conversion decreases with increasing fish size.

The first published work on attempts to feed plaice on an artificial diet was Kirk and Howell (1972). Three artifical diets were made up by adding 30% water, 10% guar gum and 60% of one of the following: Aberdeen meal, commericial white fish meal and Aberdeen meal plus soya bean meal. Growth and conversion on these diets was compared to that on *Lumbricillus rivalis*. At 12°C, the fish growth on *L. rivalis* was far superior to that on the other three diets. The fish had grown from 6g to 19g in eight weeks on *Lumbricillus* and only reached about 8g in

the case of the three artificial diets. Wet weight food conversion rates were very poor with the artificial diets. In the fourth two-week period, they were: Aberdeen meal 6.8:1; commercial whitefish meal 6.7:1; Aberdeen meal and soya bean meal 9.0:1. These conversions were marked improvements on the preceding periods, where conversions had been as high as 34.9:1. In a further experiment at 15°C in the paper, the wet weight food conversion rate of plaice on *Lumbricillus* was found to be 2.02. The poor performance of the artificial diets, in terms of food conversion and growth, was suggested as being due to the fish taking a long time to adapt to the new diets. The fact that food conversions were improving when the experiment was terminated was used as evidence to support this. However, an initial weaning period of three weeks was allowed and the change over to the artificial diets was reported as being successful.

Deniel (1973) formulated moist diets similar to those above, but more balanced in terms of formulation. The three diets he made were FP_{40} (main ingredients: herring meal, starch and soya oil); LEP (main ingredients: herring meal, milled wheat and maize oil) and CPFP (main ingredients: soluble fish protein concentrate, herring meal, glucose and maize oil).

The diets were fed to turbot (*Scophthalmus maximus*) that were maintained at a temperature of 14°C. The following food conversions were recorded:

Diet	FCR
FP_{40}	1.6
LEP	1.2
CPFP	1.4

These results are averages for various sizes of fish. This research showed that turbot would grow and convert fairly well on a diet containing no fresh fish. Further research on dry diets was performed by Jones (pers. commn.). A dry diet was formulated by Spillers Farm Feeds Limited in an attempt to produce a commercial ration for turbot farming. The diet had the following composition:

Ingredient	% Present
Herring meal	35.0
Whitefish meal	23.0
Soyolk	11.0
Wheatfeed	11.0
Distillers soluble	5.0
Whey powder	5.0
Yeast	5.0
Cod Liver Oil	4.0
Vitamins and minerals	1.0

The moisture content of this diet was 10%. The diet was fed to turbot maintained at 18^{o}C. The fish grew from a mean weight of 19.8g to 63g in two months. The mean conversion ratio was 1.29:1. The diet was tested again Experiment 1.1 of this thesis and these results could not be replicated. The use of a dry pellet on a large scale farm has a number of advantages over a moist pellet. Among these are such things as easier storage, ability to be automatically fed and that there is no need to have specialist equipment to manufacture the dry diets on site, as they can be bought from a feed manufacturer. As yet, fresh moist pellets are not produced commercially in the UK, unlike in the USA where

several companies produce for example the oregon moist pellet. The nearest we have in the UK is a stabilised moist pellet (22% water) produced by Bakers Ltd, which is still in the testing stage.

Bromley (1974a) examined the effects of dietary water content on the growth of turbot and sole. The diets were all based on whiting (*Merlangius merlangus*). Four dietary water levels and three feeding rates were tested. The moist and dry diets were made by making fish meal from the whiting; water and binder were then added to make up dietary water levels of 20 and 40%. The dry meal provided the 0% water diet and fresh whiting provided the 75% water diet. The diets were fed at 2, 4 and 6% of the fish body weight daily. At the different feeding rates, the actual amount of food fed was adjusted so that fish on different dietary treatments received the same amount of dry matter. The problem when making this type of adjustment is, that at any % feeding level, the fish on the high moisture content have to eat more in terms of wet weight of food (in order to consume an equivalent dry weight of food to fish on a dry diet, eg in this experiment, if the fish on the dry diet are eating 50g a day, the fish on the 75% (water diet would have to eat 200g of food. This may be the reason why the dry diet produced the best growth at the 2% and 4% feeding rates. At 6% feeding level, the 20% moisture diet produced the greatest growth rate. Reasons are not given for this anomalous performance. Bromley states that the moist diets may have resulted in poorer growth due to fragmentation of the diet during feeding.

The body responsible for much of the commercial flatfish diet development work over the past few years is the White Fish Authority (WFA). Their diet development work has culminated in their most recent diet WFA7. This is of the following composition:

Ingredient	% Present
Fish Meal	40
Queen Offal	20
Sprats	20
Vitamin binder	20

The WFA expect a wet weight conversion ratio of 1.8:1 on this diet with turbot. Feeding trials on three different diets were reported by WFA (1976), in which the three diets used were sprat, reclaimed cod flesh sausage and WFA6. The feeding levels were adjusted, so that the turbot received the same amount of food on a dry weight basis. The following food conversions were recorded:

Diet	FCR (wet weight basis)	FCR (dry weight basis)
Sprat	2.44:1	0.76:1
RCF sausage	5.94:1	1.42:1
WFA6	2.37:1	1.33:1

As can be seen, it is often misleading to compare FCRs of different diets on an as fed basis, particularly where large differences in dietary moisture content are present. This information also highlights earlier evidence, that turbot convert sprat extremely efficiently to flesh.

The third sub-section, as defined earlier, is concerned with nutritional requirements and limits in flatfish. The necessary dietary components can be broadly divided into the following categories:

protein
lipid
carbohydrate
miscellaneous - including vitamins and minerals.

Studies on the protein requirements of fish have shown that they are relatively high when compared with those of man and domestic animals.

Animal	Protein requirement	Reference
Chinook salmon	40% at 5°C 55% at 14°C	DeLong et al (1958)
Channel catfish	40%	Dupree & Sneed (1966)
Carp	38%	Ogino & Saito (1970)
Pigs	17%	BP Nutrition (UK) Ltd (1976)
Poultry	22%	
Cows	12%	

It is for this reason that studies were conducted on protein requirement and utilisation in turbot. Cowey et al (1970) regarded an investigation of the essential amino acid requirements of flatfish, a prerequisite to studies of protein utilisation and requirements. Plaice were given intraperitoneal injections of $|U - {}^{14}C|$ glucose and then kept under controlled conditions. After six days, the animals were sacrificed and the protein isolated. The protein was then hydrolysed and the constituent amino acids separated. One problem of acid hydrolysis is that trypotophan is destroyed so the experiment was unable to determine any incorporation of $|U - {}^{14}C|$ glucose into this amino acid. There was found to be little or no incorporation of ^{14}C into arginine, methionine, valine, isoleucine, leucine, lysine, histidine and phenylalanine. These requirements are in line with those of Halver et al (1957) for Pacific salmon.

There are various ways of examining the utilisation of and quantitative requirements for dietary protein. One of these approaches is to examine the assimilation rates of dietary protein and hence calculate the efficiency with which food is converted into flesh. What is involved

therefore is an examination of the nitrogen balance of the fish. Birkett (1969) examined the nitrogen balance in plaice and sole at 17°C. By evaluating the nitrogen content in the food, faeces and the fish, various functions were derived. The results can be summarised as follows:

Species	Gross efficiency of nitrogen absorption	Maintenance requirement	Basal excretion rate
		(mgNg body wt^{-1} day^{-1})	
small plaice (approx 20g)	0.275	0.203	0.056
yearling plaice (approx 2g)	0.417	0.499	0.208
small sole (approx 30g)	0.391	0.381	0.149
yearling sole (approx 1g)	0.490	0.244	0.120

Several anomalies are evident in the above data. It appears that small plaice absorb a larger proportion of nitrogen than small sole; however, they have a lower gross efficiency. Both species were fed on the same diet ie *Arenicola*. However, the yearling plaice and sole were fed on *Artemia*. Hence one cannot make a direct comparison between the two sizes of fish. Birkett reworked Dawes' (1930,1931) data to calculate the nitrogen efficiency. The value was 0.259 which is very similar to that for his own small plaice.

As mentioned previously, fish have a relatively high dietary protein requirement when compared with other animals. One of the first requisites when specifying a flatfish diet, is to know the optimum protein level for fish growth. A first attempt was made by Cowey et al (1970a) using a semi-purified diet, the protein content of which was supplied by Caesin. Six levels of protein were used 20, 30, 40, 50, 60 and 70% and the plaice

maintained at 15°C. The diets were fed isocalorically on a total energy basis. The growth rate appeared to increase linearly as the dietary protein content increased, up to the highest level at 70%. Conversion was lowest at 60% and 70% protein levels (1.6:1). The protein efficiency ratio (PER) was highest at 50% protein level (1.16).

In a further experiment, Cowey et al (1972) examined the dose-response curve of plaice to freeze-dried cod muscle and the net protein utilisation (NPU) at each dietary protein level, by the procedure of Bender and Miller (1953). Six levels of protein were tested, as detailed in the previous experiment, and the fish maintained at 15°C. The curve which was fitted to the results was an ordinary polynomial in the nutrient levels. The maximum of the response curve occurred at a protein level of 52% ie this was the requirement for optimum growth. The NPU values determined were plotted against the % dietary calories and this showed a decline from an NPU of about 57 at 40% protein calories, to an NPU of about 31 at 90% protein calories. This relationship is similar to that in carp (Ogino and Saito 1970). However the relationship between PER and % protein in the diet is very different to that of carp. With the freeze-dried cod muscle, the PER for plaice peaked at about 40% protein in diet (PER 1.7). When caesin was fed to plaice, peak PER (1.16) was at about 50% protein in diet (Cowey 1970). For carp, PER is highest at low dietary protein levels (around 10%) and falls approximately linearly as % dietary protein increases (Ogino and Saito 1970).

Having determined the optimum protein level for plaice, the next logical step was to examine various sources of protein to see if an alternative to fish meal could be found that cost less. Cowey et al (1971) evaluated seven protein sources, viz low temperature dried cod meal, leaf protein, soya bean meal, BP protein concentrate, fish protein

concentrate, freeze dried cod muscle and leaf protein. The proteins were made up in various combinations. The leaf protein concentrate was tried at six levels ranging from 0-63% of total dietary protein. The BP protein concentrate and soya bean meal were incorporated at about 45% of total dietary protein. The diets were balanced with L.T. cod meal and α cellulose. At 15^{o}C, the growth rates demonstrated that as the proportion of leaf protein in the diet increased, so the growth rate diminished. This effect was most pronounced when leaf protein was incorporated at 40%, or over, of total protein in diet. Growth on the diet containing BP protein, was superior to that on soya bean meal. PER values ranged from < 1 on diets containing soya bean meal and a high proportion of leaf protein, to 1.5 on diets containing BP protein and < 40% leaf protein. The highest PER was on the diet containing freeze-dried cod muscle (1.8). The amino acid profile of LT cod meal was similar to that of freeze-dried cod, yet their PER's differed (1.5 : 1.8). This shows up the difference between processing methods. LT cod meal is very carefully prepared, so one can understand how commercial fish meal sometimes gives a rather poor performance. The fish protein concentrate gave a very poor performance, both in growth and PER (0.7). It was speculated that processing had rendered some essential amino acids unavailable.

In a further experiment, Cowey et al (1974) examined NPU's by plaice on another series of proteins. As the relationship between NPU and protein intake in similar is plaice to that in mammals (Cowey et al 1972), it was thought that at high protein intakes, NPU values for various dietary proteins would be similar in plaice. Seven dietary proteins were evaluated, viz, freeze-dried cod muscle, white fish meal, BP protein

concentrate, Promine D, fish protein concentrate, extracted herring muscle and extracted sprats. The diets were formulated to contain 50% protein on a dry weight basis. Once again, large differences were evident. The fish protein concentrate produced the worst PER and NPU, 0.6 and 0.23 respectively. This contrasted to the experimental fish protein concentrates, eg extracted sprats that produced a PER of 0.98 and an NPU of 0.33. Once again freeze-dried cod muscle had the greatest PER at 1.78 and NPU at 0.42. Digestibilities were also determined by the inert indicator method (Knapka et al 1967). No major differences in digestibility were apparent, the mean value was about 0.9. The main difference was for soya-bean meal, which had a digestibility ratio of 0.68. There also appeared to be no major differences in amino acid content between the different proteins. The main problem is determining differences in biological availability between various proteins.

In mammals, the level of dietary protein has effect at cellular level, eg on hepatic concentrations of ribonucleic acid (RNA) (Munro and Clarke 1960). Cowey et al (1974a) examined the effect of dietary protein level on certain cell components and enzymes in the liver of plaice. The analyses were also compared to wild plaice. The major difference was in total hepatic RNA, protein and phospholipid levels. These were highest in plaice fed a 50% protein diet and lowest in wild fish. Wild fish livers contained a smaller amount of alanine aminotransferase, aspartate aminotransferase and glucose-6-phosphatase activity, than those of cultured fish.

Little is known of the fatty acid requirements of marine fish. A fair amount of work has been undertaken with rainbow trout (*Salmo gairdneri*), (Castell et al 1972 and Watanabe et al 1974). These workers

showed that linolenic acid was superior to linoleic acid, producing lower feed conversions and greater growth rates in rainbow trout. The lipids of marine fish contain high levels of polyunsaturated fatty acids of the ω3 series, which are of dietary origin (Cowey et al 1976). In an initial experiment to examine turbot fatty acid requirements, Cowey et al (1976) fed diets containing three different lipids. The lipids were chosen for their different characters. Hydrogenated coconut oil was chosen because it aggravates essential fatty acid deficiency symptoms in mammals (Holman 1968), cod liver oil was chosen because it contains polyunsaturated fatty acids of the ω3 series and corn oil was chosen because it contains mainly linoleic acid. These lipids were incorporated into moist diets, made up principally of white fish meal, at a rate of 40g kg diet^{-1}. The experiment lasted for 16 weeks and the fish were maintained at 15^{o}C. Weight gain was largest with the fish fed on the cod liver oil diet and lowest on the hydrogenated coconut oil diet. PER was also highest (1.65) on the cod liver oil diet and lowest on the hydrogenated coconut oil diet (0.97). There was little evidence of metabolic transformation of fatty acids, eg there was little conversion of linoleate to arachidonate in the fish given corn oil and little accumulation of eicosatrienoic acid in the fish given hydrogenated coconut oil. The relatively large amounts of some ω3 polyunsaturated fatty acids, found to be present in the fish fed on corn oil diet and coconut oil diet, were attributed to their dietary consumption prior to the experiment. The fish fed on the hydrogenated coconut oil diet underwent fairly extensive pathological changes. Marked lipid infiltration and ceroid deposition occurred in the livers of the turbot. Other changes were thickening and distortion of fat cell membranes,

increased vascularisation and some loss of cellular integrity in the adipose tissue surrounding the lateral lymphatic sinus. This also happened to some small extent in the turbot fed on the corn oil diet. Experiments with plaice by Owen et al (1972) also showed that when the fish were fed diets containing linoleic acid as the main dietary fatty acid, little 20:4ω6 or 22:5ω6 was found in the lipids of either hepatic or extra-hepatic tissues.

In a further experiment, Owen et al (1975) examined elongation and desaturation of dietary fatty acids in turbot and rainbow trout. The turbot were fed a lipid free diet for 16 weeks and the trout fed the same diet for six weeks. The turbot were then fed on [1 - ^{14}C] 18:1ω9, 18:2ω6 or 18:3ω3 fatty acid. The trout were fed on [1 - ^{14}C] 18:3ω3 fatty acid. The fish were then sampled after six days, as this was considered long enough for the isotopes to enter metabolic pathways. In the trout, 70% of the radioactivity was present in 22:6ω3 fatty acid. In the turbot, a large proportion (70-90%) of radioactivity remained in the unchanged acid fed. The main product formed, of longer chain length than the acid fed, was by addition of two carbon atoms to the chain. Very small amounts of radioactivity were found in acids of shorter chain length. It is apparent then, that turbot can only chain-elongate to a very limited degree and cannot desaturate fatty acids. It is necessary, therefore, to supply long chain polyunsaturated fatty acids of the ω3 series in the diet.

A further criterion to examine in fish diets is the ratio of ω3/ω6 acids. Examination of fatty acid ratios in various fish oils by Ackman (1967) showed the following ω3/ω6 ratios, fresh water species (maria, alewife and culliber) mean ratio 3.3; marine species (Atlantic

herring and cod) mean ratio 8.3. Hence one would expect a requirement for a high ω3/ω6 ratio in the diets of marine fish. Cowey and Sargent (1972) reported an experiment to examine ω3/ω6 ratios in plaice. Fish were fed on a diet containing corn oil and lipids present in the dietary cod muscle, the dietary ratio of ω3/ω6 being 0.35. After 12 months, some of the fish were given a fat free diet for six months whilst the remainder of the fish were fed on the original diet. Fish on the fat-free diets were found to lose their polyenoic acids readily, particularly from the triglycerides, the phospholipids retaining slightly more. The fish continued on the corn oil/cod muscle diet had similar levels of 22:6ω3 acids to wild fish, particularly in the triglycerides. It was noted that these fish also had elevated levels of 16:0, 16:1, 18:0, 18:1 and 18:2ω6 acids, compared with wild fish. The total triglyceride levels were also elevated in the fish on the corn oil diet. It was speculated that this was to maintain normal levels of 22:6ω3 acid. This excess deposition of lipid, caused no pathological conditions or depression of growth rate.

The last major nutrient to be discussed is carbohydrate. In the wild, marine flatfish are carnivorous and hence normally consume a high protein diet. The utilisation of carbohydrate to spare protein is discussed later. Cowey et al (1975) examined the metabolism of glucose by plaice. This was investigated by injecting the fish intraperitoneally with [U - ^{14}C] glucose. Eighteen hours later, the distribution of radioactivity in different biochemical fractions from the fish was examined. Two groups of fish were used, those that had been on a high dietary energy regime (14.28MJ) and those that had been on a low dietary energy regime (7.95MJ). After 18 hours, the fish on the high energy

diet had respired 23% of ^{14}C as $^{14}CO_2$ and the fish on the low energy diet had respired 12.2% as $^{14}CO_2$. This compares to 82.5% after only eight hours in the mouse (Vrba 1966). This amounts to about a 20 fold difference. Although there is a tissue temperature difference between the species mice function at 40°C whilst the plaice were maintained at 15°C this would only account for about a 5-fold difference in reaction rate. Another difference between plaice and mice is that only 3% of radioactivity present in the acid-soluble fraction in the mouse remains after eight hours compared with over 50% in plaice after 18 hours. Other differences highlighted are that more glucose C is converted into lipids in the mouse and more glucose C is converted into protein in the plaice. It was also apparent that more glucose C was converted into glycogen in the high energy diet (8.2%) as opposed to the low energy diet (2.4%). Assimilation rates of carbohydrates at different levels are reported by Cowey and Sargent (1972). It was found that as the level of dextrin in the diet increased, the amount assimilated, as a percentage of that consumed, declined. At 10% inclusion, the % assimilation was 83.5% whilst at 60% inclusion, the % assimilation was only 35%.

The final section of nutrients in the diet concerns vitamins, minerals and appetite stimulants. Studies on vitamin requirements of salmonids, Chinook salmon in particular, were undertaken by Halver in the late 50s and 60s. The methods developed by Halver for studying vitamin requirements have been applied to other fish species. A summary of existing knowledge of daily vitamin requirements and the pathology of vitamin deficiency symptoms is contained in a paper by Hashimoto and Okaichi (1969). The vitamin levels in diets formulated by Cowey

et al (1970) for plaice were based on the published data by Halver (1957a) with two modifications. Choline chloride was replaced by choline bitartrate and vitamin A was supplied in cod liver oil.

The first study on specific vitamin requirements in turbot was undertaken by Cowey et al (1976a). Two methods of assessing the effect of dietary thiamine levels on turbot were tested. The first method was to examine the growth of the fish on diets containing seven levels of thiamin and one diet containing pyrithiamin (thiamin antagonist). Good growth was obtained on all diets containing thiamin, except in the group containing the lowest thiamin level (0.19 mg kg^{-1}). These fish grew at the same rate as the others until the twelfth week, but thereafter their weight did not increase. The group of fish on the diet containing pyrithiamin (40 mg kg $diet^{-1}$) grew as the other groups up until the sixth week. After this, mortalities began to occur and all the fish died by the tenth week. No deficiency signs were observed in the turbot given pyrithiamin or the lowest level of thiamin. The characteristic signs in the free swimming fish are loss of equilibrium and instability. Signs like this are obviously less noticeable in bottom living fish like turbot. The other method of determining the effects of dietary levels of thiamine on the fish was to examine erythrocyte transketolase activity and the percentage stimulation of erythrocyte transketolase by thiamin pyrophosphatase (TPP), as described by Brin et al (1960) for rats and humans. The results demonstrated that at a dietary level of 2.6 mg thiamin kg $diet^{-1}$, all the erythrocyte transketolase was saturated with TPP. These data and the growth data suggest that the dietary thiamin level for turbot lies between 0.62 and 2.60 mg kg dry $diet^{-1}$. This is in line with the level reported for carp

by Aoe et al (1969) which is 0.5mg kg dry diet^{-1}. However Halver (1972) reported that Chinook salmon require 10-15mg thiamin kg dry diet^{-1}. This discrepancy may be due to the presence of thiaminases in the gut and tissues of fish. Thiaminase is present in the tissues of carps but is absent from salmonids (Harris 1951; Hashimoto et al 1970).

A final piece of work is concerned with growth stimulation. The oral application of stilboestrol, as a growth stimulant, to trout was examined by Ghittino (1970). The fish were fed a range of levels that provided from 50µg to 5mg diethyl stilboestrol 100g live weight^{-1} day^{-1}. The growth of the fish was less than that in control groups. Cowey et al (1972) reported growth trials with plaice fed on 1.2 to 5.0µg 100g live weight^{-1} day^{-1}. At the lowest treatment level, there was a significant increase in growth rate with no undesirable side-effects over a ten week period, (growth rate was almost doubled).

So far, the nutrient groups have been considered in isolation. As all three groups ie protein, oil and carbohydrate are energy sources, it is obvious that they are interlinked. As mentioned previously, marine flatfish are principally carnivores; their natural source of energy is protein . As fish protein is a high priced commodity, one can either replace it with alternative proteins,or replace it in part with oil or carbohydrate. Studies of the former have so far proved unsuccessful (see Cowey et al 1971). This leaves the latter approach open to exploration.

Bromley (1974) investigated the effect of various levels of oil on the growth and protein conversion of turbot at a temperature of 17^{o}C. The diets were based on pouting (*Trisopterus esmarkii*), enriched with various levels of cod liver oil and corn oil (50:50 mixture). The dietary

oil levels were 1%, 4%, 7% and 10% on a wet weight basis. Bromley gives no value for the moisture content of the diet. Assuming this to be around 70%, the dietary oil levels become 3, 13, 23 and 33% on a dry weight basis. The corresponding dry weight protein levels are 57, 55, 53 and 50%. These four diets were fed at four different levels, 25, 50, 100 and 150 cal g fish^{-1} day^{-1}. The growth rates of the 13 and 23% diets levelled off at a feeding rate of 100 cal g fish^{-1} day^{-1}. The growth rate of the fish on the 13% oil diet levelled at 3.4% day^{-1} and 23% oil diet levelled at 2.7% day^{-1}. The growth rate of the fish on the 3% oil diet was still increasing at a feeding level of 150 cal g fish^{-1} day^{-1} (the value was 4% day^{-1}). The energy and protein conversion efficiencies also showed a peak value at a feeding rate of 100 cal g fish^{-1} day^{-1}, for fish on all oil levels. The peak value for energy conversion efficiency, was 40% on the 13% oil diet. The lowest peak value was 24%, on the 33% oil diet. The peak values for protein conversion efficiency were around 44% for the 13, 23 and 33% oil diets, but lower, at 34%, for the 3% oil diet. The summary of this is that at 17^{o}C, turbot have an optimum feeding rate of 100 cal g fish^{-1} day^{-1}. The diet which resulted in optimum performance was the one containing 13% oil and 55% protein. This protein level is similar to the 52% optimum level for plaice reported by Cowey et al (1972). Important data missing are the effect of the diets on the body composition. High levels of fat can cause an increase in the carcass fat levels.

Further demonstrations of the protein sparing effects of carbohydrate and lipid, are reported by Walne (1973). The protein component, freeze-dried cod muscle, was kept constant at 35% and variations of capelin oil and carbohydrate (1:1 dextrin:glucose) were tested. No growth rates or food conversions were reported. However the following PER's were

determined.

		% lipid		
		3	6	9
% carbohydrate	0	1.94	-	-
	9	2.05	2.44	2.54
	18	2.22	2.35	2.64

Once again, no body carcass analyses were made on completion. The diet with 18% carbohydrate and 9% lipid obviously shows a maximum PER, as there is a high level of non-protein energy. However, this usually leads to a higher level of fat in the carcass.

Cowey et al (1975) also examined the effect of dietary energy source on protein utilisation in plaice. All the diets contained 40% protein. One series of diets contained a range of lipid levels from 5.6 to 17.6%. The other series contained two levels of carbohydrate (10 and 20%) at each of two levels of lipid (5.6 and 8.6%). The nine diets were then fed to satiation over a growth period of eight weeks, at 15^{o}C. For the diets containing protein and lipid as energy sources, there were no significant differences in average growth between treatments. Weight gains of those given diets containing carbohydrate, as well as protein and lipid, were all greater than those given just protein and lipid, although only the fish given 8.6% lipid and 20% carbohydrate gained significantly more weight ($P<0.01$). Both NPU and PER were negatively correlated with the protein energy : total energy ratio in the diet. This was first shown in trout by Lee and Putnam (1973). At any given total energy or protein energy : total energy ratio, higher values of both PER and NPU were obtained with diets containing carbohydrate

than for those without it. Itwas recognised by Cowey that there was a difference in energy intake between the fish receiving the different diets. The intakes were higher in the fish eating the diets containing carbohydrate. This phenomenon in itself may have contributed to the lower efficiency of protein utilisation in the fish given diets lacking carbohydrate. The body composition data demonstrated a positive correlation between the dietary lipid, in the fish given lipid and protein energy diets, and increase inbody lipid. The body lipid contentwas highest (6.1%) in the fish that had consumed the diets with the highest energy contents, ie 17.6% lipid diet and diet containing 8.6% lipid, 20% carbohydrate. Cowey gives no food conversions but these can be worked out from the feeding data. The conversions decrease with increasing lipid content, from a value of 2.40 at 5.6% oil to 1.87 at 17.6% oil. The food conversions were lower on the diets containing carbohydrate. The conversions were fairly similar (average 1.56) except for the diet containing 5.6% oil, 10% carbohydrate which was 1.68.

In a further experiment, Adron et al (1976) examined the effects of dietary energy level and source on growth, feed conversion and body composition of turbot maintained at $15^{o}C$. Seven diets were formulated, containing 35% protein and energy levels varying from 1860 to 3150 k cal g^{-1}. An additional diet contained 50% protein and 3150 k cal g^{-1}. At a constant dietary protein level, weight gain and protein utilisation increased with increasing dietary energy level. The mean weight gain on Diet 2 (1860 k cal g^{-1}) was 28.13g, the PER was 1.82 and the food conversion ratio was 1.57:1. Diet 8 (3150 k cal g^{-1}) resulted in a mean weight gain of 41.59g, a PER of 2.73 and a conversion of 1.05:1. At comparable dietary energy levels (approximately 3000k cal g^{-1}),

protein utilisation was superior with the 35% protein diet compared to the 50% protein diet (2.73 compared to 1.86). Weight gains of the two groups were similar (mean weight gain approximately 41g) as were food conversions (1.05 and 1.08 respectively). The fish were fed 25g dry diet kg wt fish^{-1} day^{-1}. With this dietary regime, it is obvious that the fish on the lower energy diets are receiving less energy per kg body weight, compared to the fish on the high energy diets. The protein sparing action of dietary carbohydrate, was less marked than that of dietary lipid. This is demonstrated, for example, by comparing diet 2 (3% lipid, 0% carbohydrate) with diet 3 (3% lipid, 9% carbohydrate). The PER's were 1.82 and 2.05 respectively. However, a higher PER value was obtained from the fish given diet 5 (9% carbohydrate) than on those fish given diet 6 (18% carbohdyrate). The PER values were 2.44 and 2.35 respectively. It would appear therefore, that carbohydrate exhibits less protein sparing effect in turbot, than in plaice. The body composition of the turbot also varied with dietary energy level. The lipid content of the fish increased as the non-protein calorie content of the food rose. However, even the turbot given the highest level of non-protein calories (50%), contained less lipid than that of the wild turbot.

Having reviewed the literature, the published nutritional requirements of flatfish can be summarised as follows.

Protein: Optimum level for NPU 52%. This level can be reduced by addition of lipid and carbohydrate. Amino acid requirements appear to be in line with those of salmonids.

Lipid: Long chain polyunsaturated fatty acids of the ω3 series must be present in the diet. Protein utilisation can be increased by increasing dietary lipid levels to 18% without any significant effect on body composition.

Carbohydrate: Only simple carbohydrates have been tested, eg glucose and dextrin. It appears that there is a difference in utilisation efficiencies between turbot and plaice. As with lipid, dietary carbohydrate demonstrates protein sparing action. Diets containing up to 20% carbohydrate have resulted in similar growth in plaice, to diets of equal energy level without carbohydrate. There has been no demonstrable ill effect of the addition of carbohydrate. In turbot, a higher PER was recorded when fish were fed diets containing 9% carbohydrate, as opposed to diets containing 18% carbohydrate.

Vitamins: As yet, only one vitamin has been studied - thiamin. First indications are that the dietary requirement of turbot for thiamin is about 0.62-2.60 mg kg dry diet^{-1}. This is about ten times less than the requirement of Chinook salmon.

The problem facing the fish farmer is to achieve a balance of all the factors that define diet performance. Essentially, the requirement is for a diet that results in the best growth, conversion and PER at the least cost. The most practical solution is often a compromise because, as mentioned previously, cost is a major factor. A comparative review of

all the diets tested is difficult as many differences occur, such as temperature, nomenclature and data accuracy.

The diet that resulted in the most effective balance was that of Adron et al (1976). This diet resulted in an FCR of 1.05, a PER of 2.73 and a growth rate of 3.93% day^{-1} (at 15°C). The diet composition was:

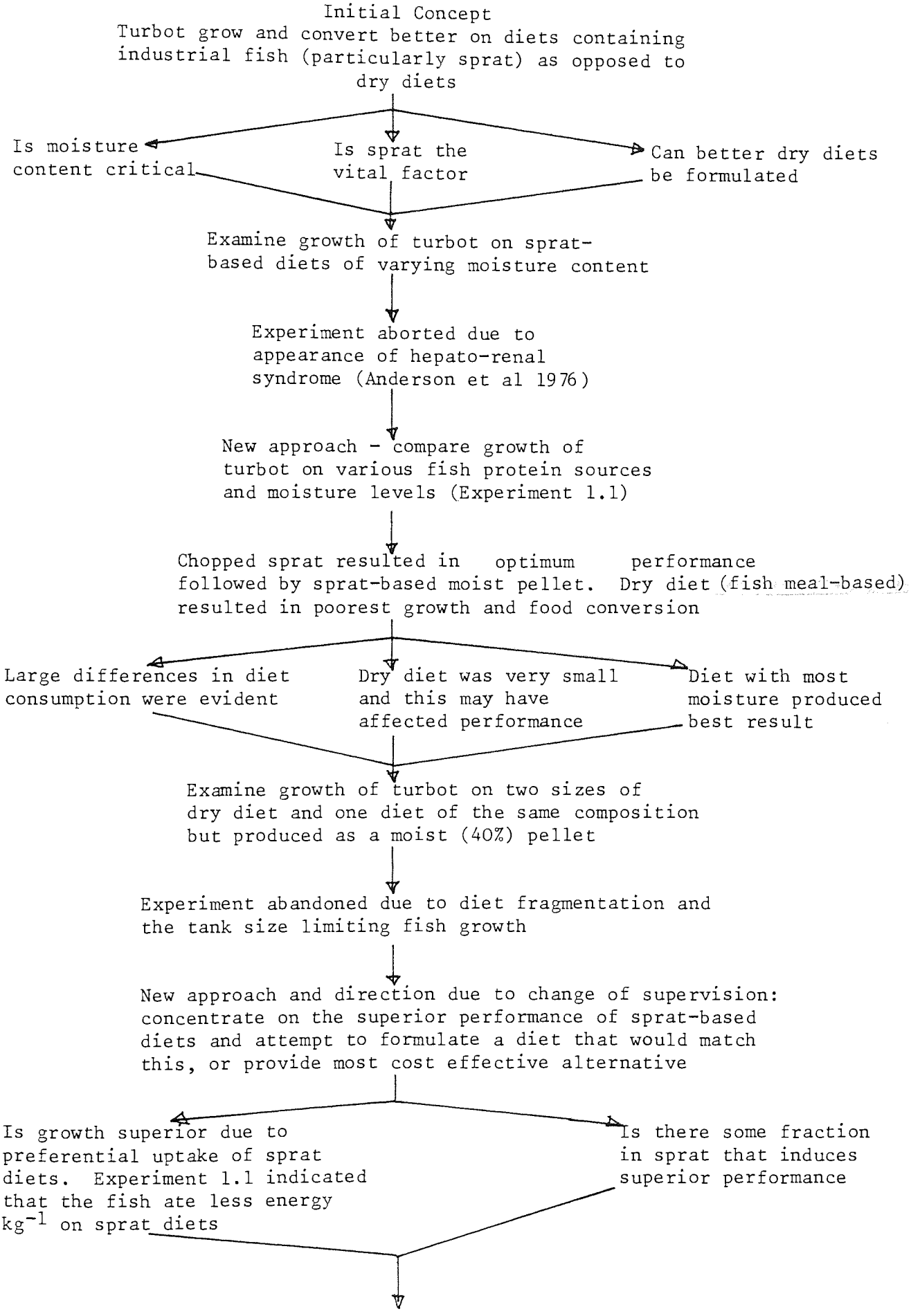
Ingredient	% Present
Freeze-dried cod muscle	41.2
Capelin oil	8.1
Dextrin	9.0
Glucose	9.0
Vitamin premix	2.8
α cellulose	24.4
Cellofas (binder)	5.0
Mineral premix	0.5
Water	44g per 56g dry diet

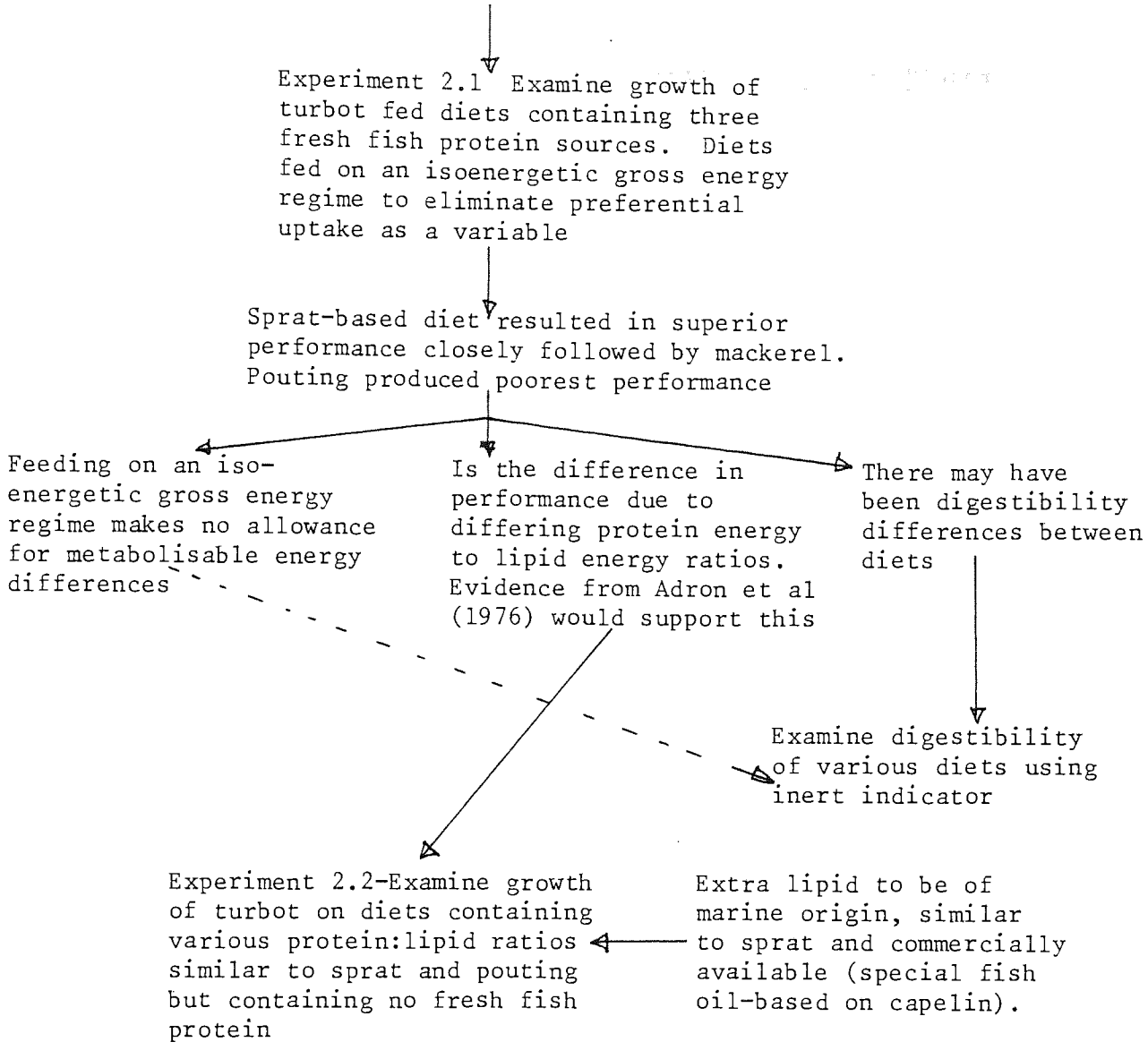
This compares with the data of Purdom et al (1972) for turbot fed on industrial fish, whose maximum conversion efficiency was about 43%. This is equivalent to an FCR of 2.32:1. Assuming industrial fish to have a moisture content of 70%, the FCR on a dry diet weight basis becomes 0.70:1. The comparison of the costs of these two diets is discussed in Section 4 of this thesis.

One of the main purposes of this thesis, was to develop a diet that could be used in commercial fish farming. At present, the turbot farming business utilises moist pellets made from industrial fish and compound

feed, or just industrial fish. This is in contrast to the UK trout farming business which uses mainly dry diets.

This thesis was originally sponsored by Spillers Farm Feeds Limited and so there was an interest in commercial diet production for turbot farming. As reported previously, a dry diet had been fed to turbot with reasonable growth and FCR. The first experiment was designed to try this dry diet again and compare the fish growth to diets of sprat and pouting (*Trisopterus esmarkii*). The complete sequence of experiments can be represented by the flow chart shown overleaf. The experimental design was such as to meet the requirements of the ICES working group on mariculture ICES (1977).


Initial Concept
Turbot grow and convert better on diets containing industrial fish (particularly sprat) as opposed to dry diets
Is moisture content critical
Is sprat the vital factor
Can better dry diets be formulated
Examine growth of turbot on sprat-based diets of varying moisture content
Experiment aborted due to appearance of hepato-renal syndrome (Anderson et al 1976)
New approach - compare growth of turbot on various fish protein sources and moisture levels (Experiment 1.1)
Chopped sprat resulted in optimum performance followed by sprat-based moist pellet. Dry diet (fish meal-based) resulted in poorest growth and food conversion
Large differences in diet consumption were evident
Dry diet was very small and this may have affected performance
Diet with most moisture produced best result
Examine growth of turbot on two sizes of dry diet and one diet of the same composition but produced as a moist (40%) pellet
Experiment abandoned due to diet fragmentation and the tank size limiting fish growth
New approach and direction due to change of supervision: concentrate on the superior performance of sprat-based diets and attempt to formulate a diet that would match this, or provide most cost effective alternative
Is growth superior due to preferential uptake of sprat diets. Experiment 1.1 indicated that the fish ate less energy kg-1 on sprat diets
Is there some fraction in sprat that induces superior performance


Experiment 2.1 Examine growth of turbot fed diets containing three fresh fish protein sources. Diets fed on an isoenergetic gross energy regime to eliminate preferential uptake as a variable
Sprat-based diet resulted in superior performance closely followed by mackerel. Pouting produced poorest performance
Feeding on an iso-energetic gross energy regime makes no allowance for metabolisable energy differences
Is the difference in performance due to differing protein energy to lipid energy ratios. Evidence from Adron et al (1976) would support this
There may have been digestibility differences between diets
Examine digestibility of various diets using inert indicator
Experiment 2.2-Examine growth of turbot on diets containing various protein:lipid ratios similar to sprat and pouting but containing no fresh fish protein
Extra lipid to be of marine origin, similar to sprat and commercially available (special fish oil-based on capelin).

2.2 Materials and Methods for Series 1 Experiments at Low Plains

2.2.1 Tank Design

The trout farm at Low Plains is situated some 20 miles from the nearest sea water source and so in order to conduct experiments with marine fish, it was necessary to construct a recirculation system. Clearly, only a limited supply of sea water could be stored and so a 6000 gallon tank (Purley Pool) was built at the rear of the farm to provide a month's supply. This storage tank was replenished once a month by sea water brought to the farm by a road tanker from Morecambe Bay.

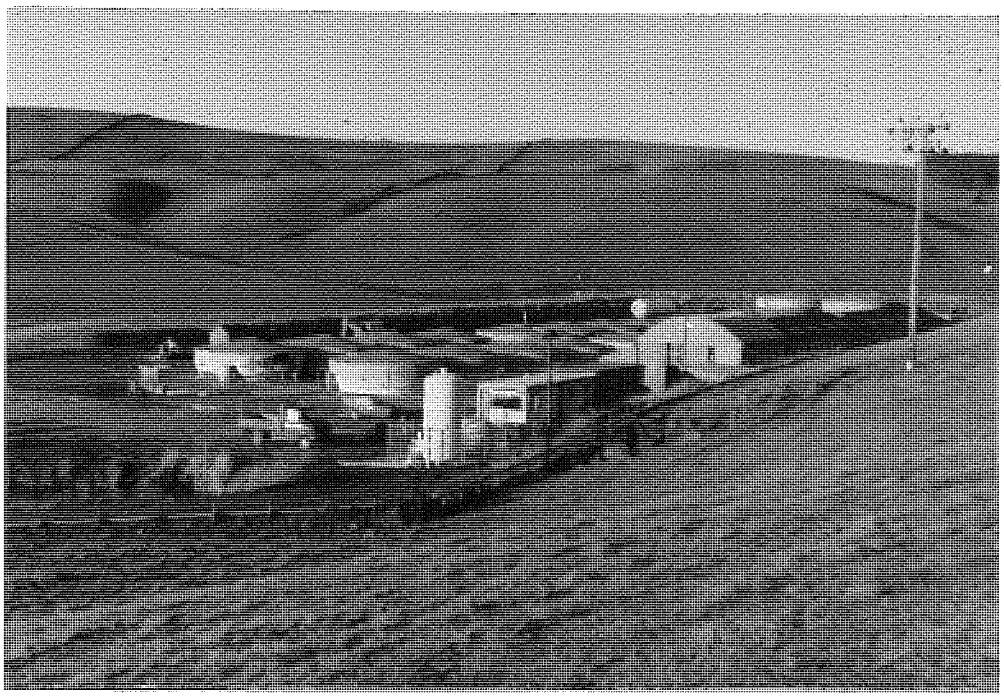
The tanks chosen were circular plastic domestic water tanks, of 250ℓ capacity, as experience with round tanks had shown a better self cleaning action than with square tanks, ie faecal matter was removed more easily. The tanks were covered with plastic mesh, to prevent the fish from escaping and a central mesh screen was also present to prevent escape, where the water left the tank. Twelve tanks were used in the experiment, comprising of three groups of four, each group with its own pumpwell, heater and biofilter. This tank layout enabled an experimental design, of testing four diets in triplicate. The temperature chosen for the experiments was 16^{o}C, as this is optimum for turbot growth (Jones et al 1980). The water was heated by 1 kw vitreosil immersion heaters (Thermal Syndicate Ltd) controlled ±1^{o}C with 'aquarium' thermostats (Uno Products Ltd). To minimise heat losses, and therefore power requirement, the whole system was lagged with polystyrene. From the central pumpwell, the water was raised to the top of the biofilter and allowed to run back to the tanks. The pumping was performed by PV21 pumps (Beresford Pumps Ltd), which gave a flow rate of $2m^3hr^{-1}$ thus

permitting a flow of $0.5m^3hr^{-1}$ to each tank. Where the outflow from each tank spilled into the pumpwell, there was a tray containing filter material (Crystal Clear Products Ltd), the purpose of which was to remove solids; this material was changed daily. When recycling, one must also employ some form of treatment to remove soluble waste products formed by the fish. The principal nitrogenous excretory product of fish is ammonia, acceptable levels of which in trout farming are <0.1 ppm NH_3-N, (Muir 1975). The biofilter incorporated in the experimental system was a 50 gallon water butt (Harcoster Products Ltd), containing <1" river gravel. The gravel was innoculated with nitrifying bacteria from established biofilters. To check the filter was working efficiently, weekly checks were made on ammonia and nitrite levels. Ammonia nitrogen was measured by the phenol-hypochlorite method and nitrite nitrogen by diazotisation - for further details see Appendix 1. The levels of ammonia nitrogen were typically around 0.005 ppm and the levels of nitrite nitrogen were typically around 0.03 ppm. To remove nitrate, the by-product of ammonia degradation, the tanks were flushed daily and the volume made up with sea water from the external reservoir. The mean daily water change was approximately 5%. Pure oxygen was bubbled into each tank through a 1" cube airstone to maintain a dissolved oxygen level of around 100%. The fish were kept on a photo-period of approximately 12 hours light per day. For further details of tank design and layout see Photograph 1 and Photograph 2.

2.2.2 Fish Stocks

The first experiment had to be abandoned because of a similar problem to that reported by Anderson et al (1976). The fish used in experiment 1.1 were kindly donated by Dr. B. Howell (MAFF Port Erin) and

Photograph 1 Low Plains trout farm



Photograph 2 Experimental system in fry shed at Low Plains

were 1 group hatchery fish, the approximate mean weight being 90g. The fish were apportioned equally between the twelve tanks, so as to give approximately equal biomasses in all twelve tanks (2 kg). Prior to their transport to Low Plains, they had been used in growth experiments and had been maintained on a moist diet of minced trash fish and fish meal.

2.2.3 Diet Preparation and Analysis

The dry diet, the size of which was 3mm, was produced by Spillers at their experimental unit at Kennet, near Cambridge, and then fat coated at Low Plains in a Hobart A40 mixer. Sufficient dry diet was made in one batch to last the whole experiment. The sprat for experiment 1.1 was obtained from Fosketts of N. Shields - the same sample was used throughout the whole experiment. The pouting used in experiment 1.1 was obtained from the WFA at Hunterston. The moist diets, the size of which was 3mm, were made up fortnightly using a Hobart A40 mixer and stored in air-tight containers in a deep-freeze, along with the sprat diets. The compound feed used in these diets had been blended at Kennet. The moisture content of the diets was checked weekly by drying at 100°C in a forced-draught oven to constant weight. The procedure for preparing the sprat for feeding was to thaw about 1 kg, chop into pieces about 5mm thick and then refreeze it.

The analytical work was also conducted at Kennet, where the following components were determined.

(i) Protein - by kjeldahl digestion

(ii) Fat - by soxhlet extraction

(iii) Ash - by combustion in a muffle furnace at 500°C

(iv) Moisture - by drying in a forced draught oven

(v) Carbohydrate - by subtraction

(vi) Gross energy - by bomb calorimetry

For more detailed descriptions, refer to Appendix 1.

At the beginning and end of experiment 1.1, five fish from each tank were taken for body carcass analysis as detailed previously. These samples were minced, homogenised, dried and then ground before analysis.

2.2.4 Fish Weighing

After an initial settling-in period of four weeks in the new tanks and on new diets, the fish were weighed. This was conducted by first removing all the fish from a tank and placing them in a 150ℓ bin full of sea water. This bin had a continuous supply of oxygen passed through a diffusing stone. A fish was removed from the holding bin and then placed on a measuring board and the length recorded. The fish was then 'dried' with a moist cloth and placed on a pad of synthetic foam and weighed on a top-pan balance to ±0.1g. The fish was then returned to the tank. Turbot are very docile fish and usually lie very still on the balance whilst being weighed. No adverse effects resulted from this weighing procedure and the fish were weighed every two weeks by this method.

2.2.5 Feeding Procedure

In an attempt to examine preferential diet consumption rates and levels of energy intake, a modified form of *ad libitum* feeding was employed. At 09.00 hrs each day, a certain amount of food was weighed

out into a container for each tank; the amount weighed out was usually in excess of the amount consumed. At 10.00 hrs, ie when the food had thawed out, the fish were fed. A few particles of food were dropped into each tank in turn and this procedure was continued until the fish ate no more. Remaining food was returned to the deep-freeze. At 15.00 hrs, the food was again removed from the deep-freeze and left for an hour to defrost. At 16.00 hrs, the same feeding procedure as detailed before was performed. At the end of the feed, the food remaining in the containers was weighed and the amount of food consumed during the day was recorded. When a third feed was tried at 13.00 hrs, no significant difference in the amount of food consumed was apparent, so the twice daily feeding regime was adopted.

2.3 Materials and methods for Series 2 Experiments at Wylfa

2.3.1 Tank Design

The fish farm design at Wylfa is fully described in Jones et al (1980). The experiments were conducted in the fry shed in shallow circular tanks of 300ℓ capacity. For tank design and layout see Photograph 3 & Photograph 4 . Considerable problems were experienced during experiment 2.1, due to the incidence of gas bubble disease (Jones et al 1980). Consequently the temperature was maintained at 11°C so as to reduce the amount of power station cooling water supplying the fry shed. Also, on a number of occasions, the power station reduced load on the turbines, therefore reducing the temperature to ambient sea temperature.

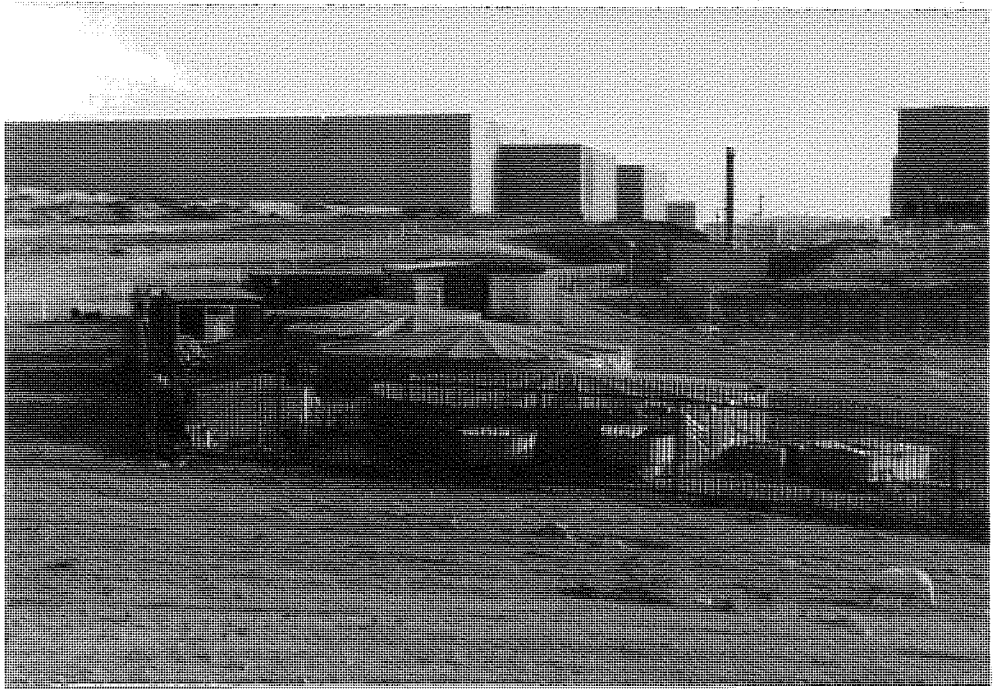
TABLE 2.3.1

Temperature data for Experiment 2.1

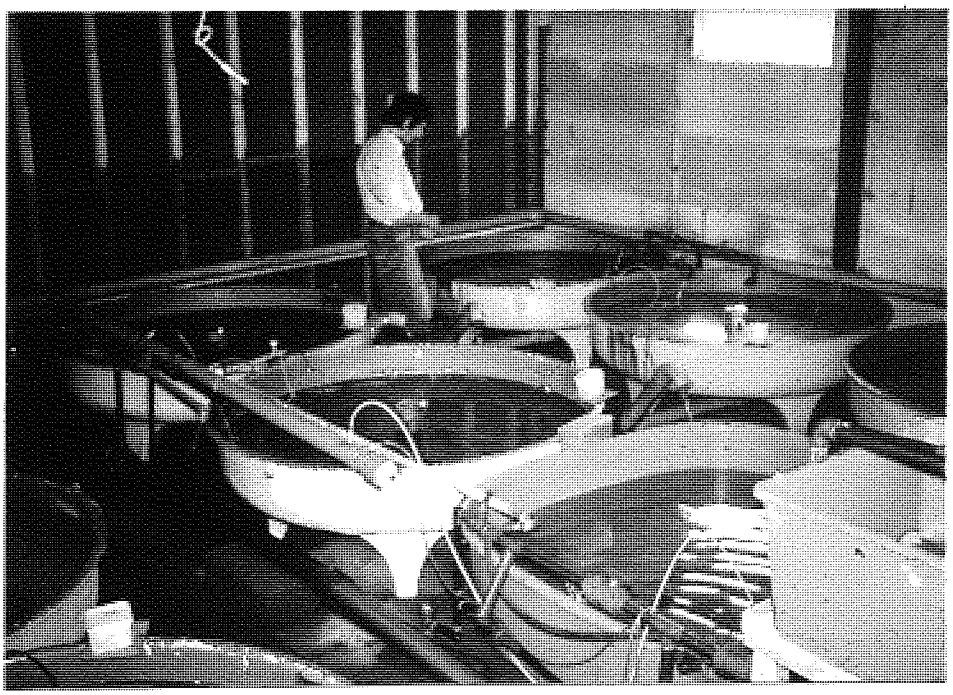
Period (see Appendix 2)	Average Temperature °C
1	9.4
2	11.0
3	11.0
4	9.3
5	11.2
6	12.2
Mean	10.7

During experiment 2.2 a much more stable temperature could be maintained at 12°C, as the nitrogen super-saturation problem had been circumvented and the power station had remained 'on load' throughout the course of the experiment. The system at Wylfa involved no recirculation, as a

Photograph 3 Wylfa turbot farm



Photograph 4 Experimental tanks in fry shed at Wylfa



continuous supply of sea water was available. The flow rate through the tanks was approximately 30ℓ min^{-1}, thereby maintaining an oxygen level of around 85%. The fish were subjected to a photo-period of about 10 hrs light per day. Six tanks were available for experiment 2.1 (three diets in duplicate) and nine tanks for experiment 2.2 (three diets in triplicate).

2.3.2 Fish Stocks

The fish used in experiments 2.1 and 2.2 were '0' group turbot of wild origin. The fish had been seine-netted from Borth beach, near Aberystwyth, Dyfed, during the month of September. The fish for experiment 2.1 were the 1977 year class and 1978 year class for experiment 2.2, in each case the starting mean weight was approximately 12g. After capture, the fish were returned to Wylfa and weaned onto a moist diet containing sprat and compound feed. Prior to the start of the experiments, 400 (for experiment 2.1) or 500 (for experiment 2.2) fish were weighed into each tank. The fish were graded to give approximately equal biomasses in each tank (4.5 kg for experiment 2.1 and 6.9 kg for experiment 2.2). Where differences in initial biomass occurred (as in experiment 2.2), the diet allocation was such that no differences could occur due to different starting weights.

2.3.3 Diet Preparation and Analysis

The compound feed for experiment 2.1 was blended on site with ingredients supplied by BP Nutrition (UK) Limited. The compound feed for experiment 2.2 was blended and supplied by BP Nutrition. The sprats and mackerel used in experiment 2.1 were supplied by Brekkes (North Shields). The pouting was supplied by Celtic Fisheries (Bangor, Gwynedd),

the same source of fish was used throughout the whole experiment. The special fish oil used in experiment 2.2 was supplied by Marfleet Refining Company (Hull). For both experiments, sufficient diet for the whole period was prepared prior to the experiment commencing. The fish was allowed to thaw out and then minced in a Hobart E4532 mincer and cut through a 4mm die plate. The fish or oil and water was then blended with the compound feed to a homogenous mixture, in a Hobart SE401 mixer. The diet was then extruded through a 2mm die in the E4532 and the diets were sealed and stored at $0^{o}F$ in a cold store. Moisture contents of the diets were checked weekly, by drying to constant weight at $100^{o}C$ in a forced draught oven.

The analytical work was conducted by the technical staff in the Biological Sciences Department at Aston University and the same analyses were determined as detailed in 2.2.3. Samples were dried and ground at Wylfa prior to despatch to Aston. At the beginning and end of experiments 2.1 and 2.2, ten fish were taken from each tank for body carcass analysis. These samples were treated as in 2.2.3.

2.3.4 Fish Weighing

After an initial settling-in period of four weeks, the fish were weighed. A bucket of water was filled and tared to 5kg on a counter-balance. Fish were then netted from a tank, allowed to drain ten seconds, placed into the bucket and the weight was then noted. The fish were returned to a cage within the fry tank, as not all the fish could be weighed in one session. The accuracy of weighing was $\pm 1g$ and this procedure was adopted for both experiments, as it had no advserse effects on the fish after the initial shock was overcome. A sub-sample of fish

(10%) was taken from each tank at the beginning and end of each experiment to measure individual weights and lengths, as detailed in 2.2.4. The fish were weighed every two weeks, except at the beginning of experiment 2.1 where the water temperature was very unstable.

2.3.5 Feeding Procedure

One of the problems of *ad libitum* feeding was the prevention of food wastage and also different people fed the fish to differing levels of satiation. Consequently, in experiments 2.1 and 2.2, a fixed ration level was used so that fish were fed isoenergetically. This regime was also used to try to eradicate different fish growth that might be due to differential gross energy consumption rates between diets. The ration level was set according to previous experiments and experience and varied with temperature, (see tables 2.3.2 and 2.3.3). The food was weighed out the previous day to enable it to defrost overnight. Rations were adjusted fortnightly when the fish were fed. To ensure that all the food was consumed, the fish were fed 5-6 times daily - a little of the ration being used on each occasion.

2.3.6 Digestibility Trial

Experiment 2.2 diets were formulated as detailed previously except that chromic oxide was blended with the compound feed before the addition of water and oil. The amount of chromic oxide added was to give approximately 1% level in the finished diet. The fish were then fed on the chromic oxide diet for a period of four weeks. Trials showed that the best time for collecting faeces was at 20.00 hrs, when a number of fish were selected from each tank for faeces collection. The method

TABLE 2.3.2

Feeding rate for experiment 2.1

Period (see Appendix 2)	Feeding Rate kj kg fish^{-1} day^{-1}
1	170
2	175
3	175
4	125
5	160
6	175

TABLE 2.3.3

Feeding rate for experiment 2.2

Period (see Appendix 2)	Feeding Rate kj kg fish^{-1} day^{-1}
1	280
2	250
3	275
4	250
5	250
6	250

These rations had to be modified on occasional days and hence the total food fed for the period does not always equal the amount in above tables multiplied by time.

used was to apply gentle pressure to the body cavity with thumb and index finger and the faeces were expelled onto tissue paper, so that any urine expelled would be soaked up in the tissue paper. It was extremely difficult to expel faeces from fish of ~ 30g and so faeces was pooled from fish on the same diet. Thus three samples of faeces were collected from 20 fish, from each of the nine tanks. The faeces was then scraped carefully off the tissue paper and dried to a constant weight at 100°C, in a forced draught oven. Three sub-samples of each sample were weighed to assess the moisture content of the faeces. The dried material was then sent to Aston for analysis, where the chromic oxide was determined by optical density (see Appendix 1 for further details).

2.4 Results

2.4.1 Diet Formulations and Analytical Results

Due to the change in location of the experimental facilities during the programme, the experiments were divided into two series.

The first series were conducted at Low Plains as detailed previously. The main aim of these experiments was to determine such factors as growth rate of turbot on sprat in an experimental design that permitted statistical analysis of results. Also diets were formulated with the aim of reproducing the values obtained for sprat. The diet formulations are detailed in Table 2.4.1. The mineral and vitamin premixes used in all experiments were supplied by BP Nutrition (UK) Limited. The premixes were based on the requirements of trout and as Cowey et al (1976a) pointed out that turbot have lower requirements than trout, the premixes were considered to provide adequate supplies for the turbot.

The second series of experiments were conducted at Wylfa and most of the results are considered separately from series 1, as different size fish were used and the fish were maintained at different temperatures. The second series of experiments was designed to examine why sprat produces better growth rates when fed to turbot and also an attempt was made to formulate a diet that matched the performance of sprat-based diets but contained no fresh fish. For experiment 2.2, a pregelatinised potato starch binder (Lyogel, Alwitt Ltd) was incorporated, as fragmentation problems had been experienced in earlier experiments when no fresh fish was present in the diet. The special fish oil used in experiment 2.2 was supplied by Marfleet Refining Company (Hull). In specifying a lipid ingredient in a diet formulated to compare with sprat diets, it was desirable

TABLE 2.4.1

Diet Composition and Formulation for Experiment 1.1

% Dietary Component	Diet Code			
	1(i)	1(ii)	1(iii)	1(iv)
Sprat	57.0			100.0
Pouting			57.0	
Herring meal		35.0		
White fish meal		23.0		
Soyolk	12.0	11.0	12.0	
Wheatfeed	12.0	10.5	12.0	
Distiller's solubles	5.0	5.0	5.0	
Whey powder	5.0	5.0	5.0	
Brewer's yeast	5.0	5.0	5.0	
Water				
Cod liveroil	2.5	4.0	2.5	
Vitamin mix	1.0	1.0	1.0	
Mineral mix	0.5	0.5	0.5	
Moisture content %	50.2	10.4	48.7	79.7
Protein content % (dry)	54.9	47.9	51.1	73.5
Ash content % (dry)	10.3	10.7	11.0	16.1
Lipid content % (dry)	14.3	13.5	8.3	11.5
Gross energy kjg^{-1} (dry)	22.325	22.640	22.616	19.768

The difference between these diets was that the fish protein originated from three different sources, ie fish meal, pouting flesh and sprat flesh. Diets 1(i), 1(ii) and 1(iii) were so formulated as to have equal levels of fish-derived protein and diet 1(iv) was used as a target for maximum growth.

The composition and formulation of series 2 diets are detailed in Table 2.4.2 below.

TABLE 2.4.2

Diet Composition and Formulation for Experiments 2.1 & 2.2

% Dietary Component	Diet Code					
	2(i)	2(ii)	2(iii)	3(i)	3(ii)	3(iii)
Herring meal	17.5	17.5	17.5	21.2	23.2	25.2
White fish meal	11.5	11.5	11.5	13.9	15.3	16.5
Defatted soya flour	8.0	8.0	8.0	9.1	9.9	10.8
Dried Brewers Yeast	2.5	2.5	2.5	3.0	3.3	3.6
Wheatfeed	5.0	5.0	5.0	4.2	4.6	5.0
Delactosed Whey	5.5	5.5	5.5	6.1	6.7	7.2
Lyogel				3.0	3.3	3.6
Special fish oil				13.5	7.7	2.1
Water				24.3	24.3	24.3
Sprat	50.0					
Mackerel		50.0				
Pouting			50.0			
Vitamin mix	0.75	0.75	0.75	1.1	1.1	1.1
Mineral mix	0.25	0.25	0.25	0.6	0.6	0.6
Moisture content %	39.3	40.8	41.0	32.5	33.3	34.1
Protein content % (dry)	64.4	64.4	67.5	46.1	51.1	56.1
Ash content % (dry)	13.2	12.8	12.9	8.8	9.6	10.4
Lipid content % (dry)	25.6	19.5	13.6	26.1	18.3	10.6
Gross energy kjg^{-1} (dry)	20.20	20.5	20.10	22.51	21.29	19.94

to obtain an oil with a similar Free Fatty Acid (FFA) profile to that of sprat. The special fish oil is prepared principally from Capelin (*Mallotus villosus*) and the FFA profile is shown in Table 2.4.3. This FFA profile compares to one for sprat shown in Table 2.4.4. Most of the fish oils available are liver oils such as cod liver oil. For the purpose of the experiment, a whole body oil was required (similar to sprat) so this is why the special fish oil was chosen.

2.4.2 Comparison of growth rate of turbot fed diets of varying fish protein sources

Fish growth rate is generally expressed in an exponential form and represented by the equation -

$$\text{specific growth rate} = \frac{(\log_e \bar{W}_{t2} - \log_e \bar{W}_{t1})}{t}$$

where $\bar{W}_{t1}$ = mean weight at start

$\bar{W}_{t2}$ = mean weight at time t

t = time period .

The weight (kg) data from which the results of Table 2.4.5 were extracted are presented in Appendix 2. Mean weights for each diet were then calculated as in Table 2.4.6, $\log_e$ values calculated and the data presented in Figure 2.4.1. This and subsequent growth data figures are presented only as illustrations as the statistical analysis was applied to Table 2.4.7.

It is apparent from figure 2.4.1, that large differences occurred between diets 1(i), 1(iv), 1(iii) and 1(ii). The other method of analysis applied to these data was to calculate specific growth rates for each individual growth period and then to apply Analysis of Variance to the results to qualify statistically differences between the results. These data are presented in Table 2.4.7.

Tables 2.4.3 and 2.4.4

Free fatty acid profiles of special fish oil (2.4.3 Marfleet Refining Company) and sprat (2.4.4 Leslie Barton)

C No	Sprat % Present	Special Fish Oil % Present
12 : 0		0.05
14 : 0	1.45	7.13
15 (total)		0.23
16 : 0	13.36	9.54
16 : 1	6.55	10.35
17 (total)		0.45
17 : 1		0.54
18 : 0	1.22	0.68
18 : 1	6.01	12.44
18 : 2		1.10
18 : 3	5.31	0.47
18 : 4	3.13	
20 : 1 ω 5	1.39	
20 : 1 ω 9	3.39	20.20
20 : 3	2.83	
20 : 4	0.85	
20 : 5	2.22	7.35
22 : 1	6.80	24.08
22 : 5	0.92	0.36
22 : 6	3.06	3.68
24 : 1	1.15	1.00

Figure 2.4.1 Specific growth rates for Experiment 1.1

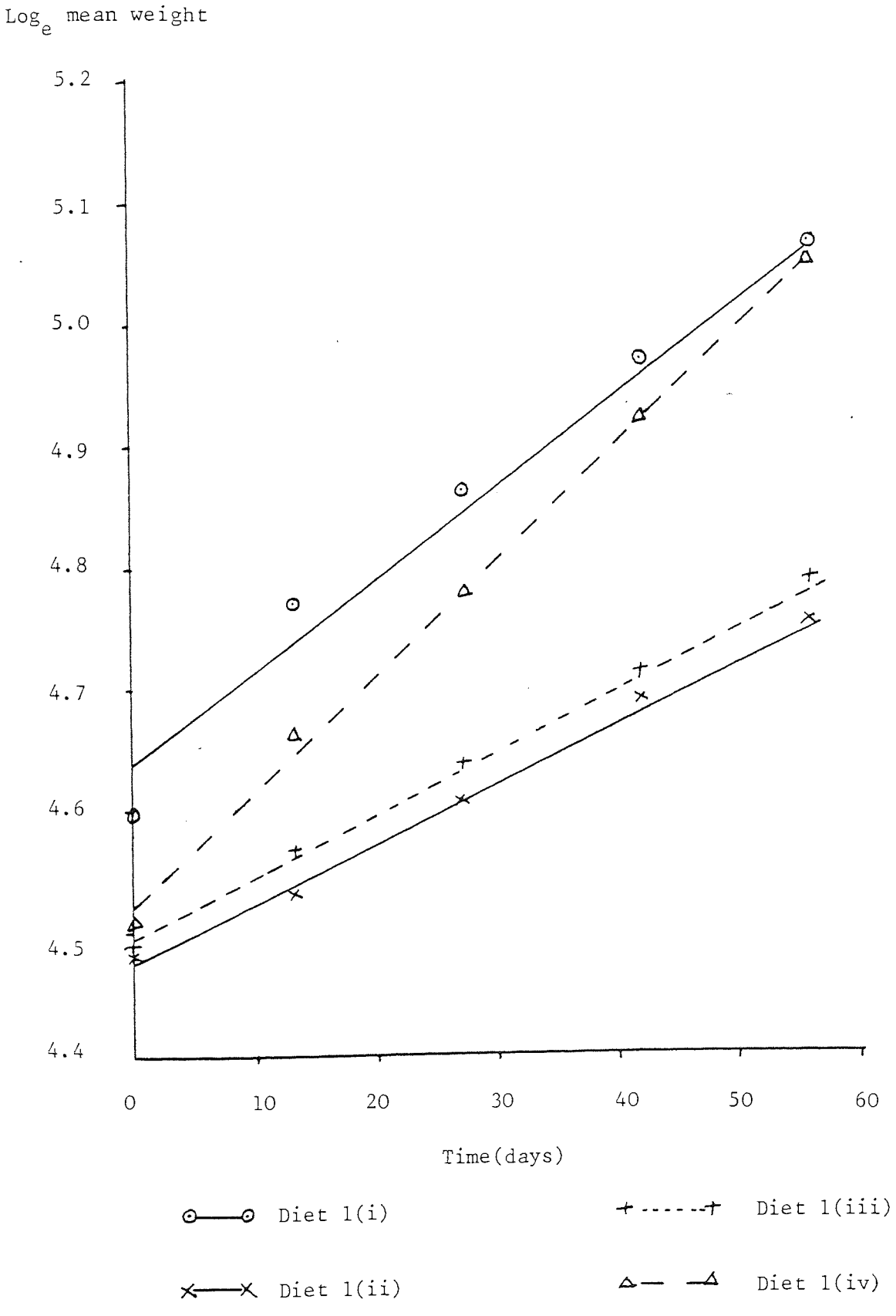


TABLE 2.4.5

Mean Weight (g) Data for Experiment 1.1

Diet	Time(Days)				
	0	14	28	43	57
1(i)	95.8	118.1	126.2	141.9	158.2
1(i)	92.4	103.3	112.9	122.0	135.1
1(i)	109.8	132.7	149.6	170.2	185.8
1(ii)	84.6	88.4	94.9	104.4	112.2
1(ii)	75.4	78.0	83.2	87.4	91.9
1(ii)	105.5	112.0	123.2	136.1	145.3
1(iii)	87.0	91.7	97.5	104.6	113.4
1(iii)	96.8	104.5	111.3	119.8	130.2
1(iii)	83.3	93.0	101.3	110.4	118.1
1(iv)	104.5	117.3	136.8	159.0	183.0
1(iv)	90.7	113.7	121.0	138.4	157.0
1(iv)	77.9	87.7	100.6	115.8	131.5

TABLE 2.4.6

Average Diet Mean Weights (g) and Log_e Mean Weight Values for Experiment 1.1

Time(Days)		Diet			
		1(i)	1(ii)	1(iii)	1(iv)
0	Mean weight(g)	99.4	88.5	89.0	91.1
	Log_e mean weight	4.599	4.483	4.489	4.512
13	Mean weight(g)	118.0	92.8	96.4	106.2
	Log_e mean weight	4.771	4.530	4.569	4.665
27	Mean weight(g)	129.6	100.4	103.3	119.5
	Log_e mean weight	4.864	4.609	4.638	4.783
42	Mean weight(g)	144.7	109.3	111.6	137.7
	Log_e mean weight	4.975	4.694	4.715	4.925
56	Mean weight(g)	159.7	116.5	120.6	157.2
	Log_e mean weight	5.073	4.758	4.792	5.058

TABLE 2.4.7

Specific Growth Rates for Experiment 1.1 ($x10^{-3}$)

Diet	Experimental Period			
	1	2	3	4
1(i)	14.9	4.7	7.8	7.7
1(i)	7.8	6.3	5.1	7.2
1(i)	13.4	8.5	8.5	6.2
1(ii)	3.1	5.0	6.3	5.1
1(ii)	2.4	4.6	3.2	3.5
1(ii)	4.4	6.8	6.6	4.6
1(iii)	3.8	4.4	4.6	5.7
1(iii)	5.4	4.5	4.9	5.9
1(iii)	7.9	6.1	5.7	4.8
1(iv)	8.2	11.0	10.0	10.0
1(iv)	16.1	4.4	8.9	9.0
1(iv)	8.4	9.8	9.7	9.0

Analysis of variance of these results yielded the following results.

Diet	1(ii)	1(iii)	1(i)	1(iv)
Mean specific growth rate ($x10^{-3}$)	4.63	5.31	8.18	9.54
At 5%	———		———	

N.B. Data joined by a line are not significantly different.

So diets 1(ii) and 1(iii) were not significantly different but were significantly different from diets 1(i) and 1(iv) (which were not significantly different). Thus the two sprat-based diets produced the highest growth rates.

The results from experiment 2.1 are presented in Table 2.4.8.

TABLE 2.4.8

Mean Weight (g) Data for Experiment 2.1

Diet	Time Period (Days)						
	0	35	51	63	82	98	112
2(i)	11.0	13.8	15.7	17.3	18.3	20.6	23.1
2(i)	11.3	13.8	15.3	16.4	17.2	20.0	21.4
2(ii)	11.9	15.0	16.9	18.5	19.0	21.2	23.1
2(ii)	10.9	13.4	14.9	16.1	16.9	19.0	20.8
2(iii)	11.3	14.0	15.5	16.4	17.2	18.9	20.8
2(iii)	13.2	15.8	17.7	18.7	19.5	21.3	23.2

The weighing data from which these results were extracted are presented in Appendix 2. Diet means were calculated, $\log_e$ values calculated and the data presented in Table 2.4.9 and Figure 2.4.2.

It is apparent from Figure 2.4.2, that there was little difference in growth rates between diets. Analysis of variance of specific growth rates in Table 2.4.10 confirmed that there was no significant difference between diet means, although the specific growth rate for the sprat-based diet (2(i)) was numerically greatest.

As detailed previously in the materials and methods section, there was a temperature drop in period 4 due to a fault at the power station, which affected all tanks. This had a marked depressant affect on growth but because it affected all tanks, the results were left in the analysis.

The growth results from experiment 2.2 are presented in Table 2.4.11.

Figure 2.4.2 Specific growth rates for Experiment 2.1

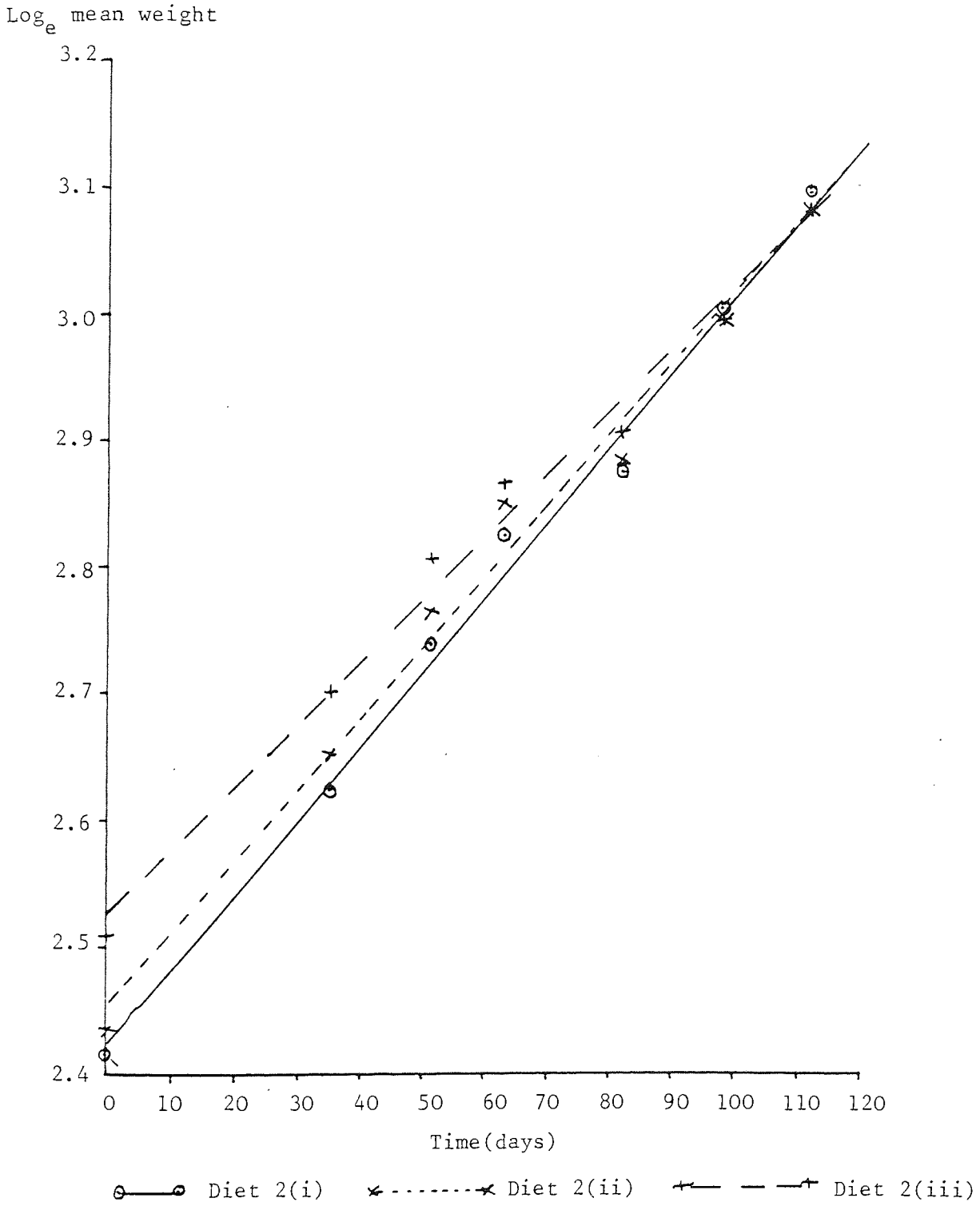


TABLE 2.4.9

Average Diet Mean Weights (g) and Log_e Mean Weight Values for Experiment 2.1

Time (Days)		Diet 2(i)	2(ii)	2(iii)
0	Mean weight(g)	11.2	11.4	12.3
	log_e mean weight	2.416	2.434	2.510
35	Mean weight(g)	13.8	14.2	14.9
	log_e mean weight	2.625	2.653	2.701
51	Mean weight(g)	15.5	15.9	16.6
	log_e mean weight	2.741	2.766	2.809
63	Mean weight(g)	16.9	17.3	17.6
	log_e mean weight	2.827	2.851	2.868
82	Mean weight(g)	17.8	18.0	18.4
	log_e mean weight	2.879	2.890	2.912
98	Mean weight(g)	20.3	20.1	20.1
	log_e mean weight	3.011	3.001	3.001
112	Mean weight(g)	22.3	22.0	22.0
	log_e mean weight	3.105	3.091	3.091

TABLE 2.4.10

Specific Growth Rates for Experiment 2.1 ($x10^{-3}$)

Diet	Experimental Period 1	2	3	4	5	6	Mean Value
2(i)	6.5	8.1	8.1	3.0	7.4	8.2	6.33
2(i)	5.7	6.5	5.8	2.5	9.4	4.8	
2(ii)	6.6	7.5	7.5	1.4	6.3	6.1	5.90
2(ii)	5.9	6.6	6.5	2.6	7.3	6.5	
2(iii)	6.1	6.4	4.7	2.5	5.9	6.8	5.25
2(iii)	5.1	7.1	4.6	2.2	5.5	6.1	

TABLE 2.4.11

Mean Weight (g) Data for Experiment 2.2

Diet	Time (Days)						
	0	15	28	42	57	70	86
3(i)	15.3	17.8	19.7	22.3	25.6	26.7	29.8
3(i)	14.7	17.3	19.0	20.5	23.8	25.4	28.0
3(i)	14.1	16.4	18.1	19.9	23.4	24.6	27.1
3(ii)	16.6	19.4	22.0	24.3	27.7	30.0	34.0
3(ii)	14.7	16.7	18.8	20.8	24.3	26.3	29.8
3(ii)	11.4	13.2	15.3	16.7	20.1	21.6	24.5
3(iii)	17.5	19.4	22.1	24.0	26.8	29.5	32.9
3(iii)	13.4	15.5	17.6	19.8	22.9	25.2	28.9
3(iii)	14.2	16.4	17.0	20.6	24.0	26.2	29.7

The weighing data from which these results were extracted, are presented in Appendix 2. Mean weights were calculated for each diet and $\log_e$ values determined. These data are presented in Table 2.4.12 and Figure 2.4.3.

It is apparent from Figure 2.4.3, that there was little difference in growth rate between the diets. Analysis of variance of the specific growth rates in Table 2.4.13, demonstrated that there was no significant difference between the diets but diet 3(iii) had the highest numerical value.

In summary of the preceding data, the sprat diet produced the fastest growth rate in experiment 1.1, followed by the sprat-based pellet. In experiment 2.1, the sprat based pellet once again produced the fastest growth rate, followed closely by the mackerel diet. In experiment 2.2, all

the growth rates were faster than in experiment 2.1, which was the aim of the experiment as all three diets (3(i), (ii) and (iii)) contained no fresh fish.

TABLE 2.4.12

Average Diet Mean Weights (g) and Log_e Mean Weight Values for Experiment 2.2

Time (Days)		Diet 3(i)	3(ii)	3(iii)
0	Mean weight(g)	14.7	14.2	15.0
	log_e mean weight	2.688	2.653	2.708
15	Mean weight(g)	17.2	16.4	17.1
	log_e mean weight	2.845	2.797	2.839
28	Mean weight(g)	18.9	18.7	19.6
	log_e mean weight	2.939	2.929	2.976
42	Mean weight(g)	20.9	20.6	21.5
	log_e mean weight	3.040	3.025	3.068
57	Mean weight(g)	24.3	24.0	24.6
	log_e mean weight	3.190	3.178	3.203
70	Mean weight(g)	25.6	26.0	27.0
	log_e mean weight	3.243	3.258	3.296
86	Mean weight(g)	28.3	29.4	30.5
	log_e mean weight	3.343	3.381	3.418

Figure 2.4.3 Specific growth rates for Experiment 2.2

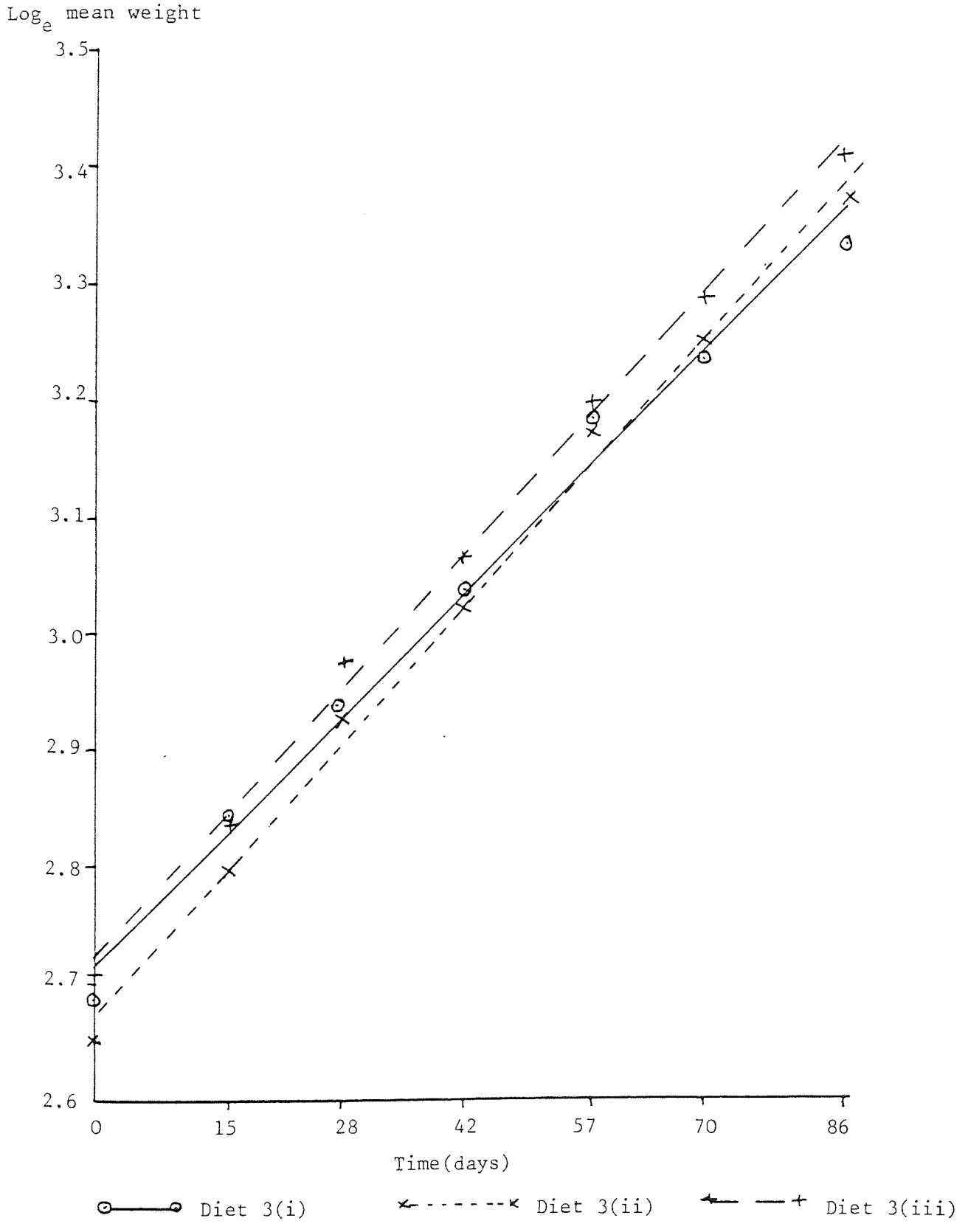


TABLE 2.4.13

Specific Growth Rates for Experiment 2.2 ($x10^{-3}$)

Diet	Experimental Period 1	2	3	4	5	6	Mean Value
3(i)	10.1	7.8	8.9	9.2	3.2	6.9	
3(i)	10.9	7.2	5.4	10.0	5.0	6.1	7.54
3(i)	10.1	7.6	6.8	10.8	3.8	6.0	
3(ii)	10.4	9.7	7.1	8.7	6.1	7.8	
3(ii)	8.5	9.1	7.2	7.0	6.1	7.8	8.27
3(ii)	9.8	11.4	6.3	12.4	5.5	7.9	
3(iii)	6.9	10.0	5.9	7.4	7.4	6.8	
3(iii)	9.7	9.8	8.4	9.7	7.4	8.6	8.30
3(iii)	9.6	11.3	5.8	10.2	6.7	7.8	

2.4.3 Comparison of feeding rate of turbot fed on diets of varying fish protein sources

Experiment 1.1 was designed to examine the amount of food turbot would consume when fed on a modified *ad libitum* regime. One problem with adopting this approach is that the results are very much dependent on the skill of the person who is feeding the fish. With diets such as chopped fish, it was easy to remove uneaten food; however this was not easy with other diets, as they tended to fragment. Several approaches were used to examine the rate of uptake of food as a function of body weight. The first approach was to examine the relationship between wet weight food intake and body weight. These data are presented in Tables 2.4.14, 15, 16 and 17. The values in the tables represent mean values of food intake and body weight between experimental periods. Food consumption is usually plotted as $\log_e$:$\log_e$ function and consequently the food (wet weight)

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consumption data from the preceding tables are plotted in Figures 2.4.4 (diets 1(iv) and 1(iii)) and Figure 2.4.5 (diets 1(i) and 1(ii)) to provide an illustration of differences between diets, as statistical analysis was applied to Table 2.4.18. The weighing and feeding data are presented in Appendix 2.

TABLE 2.4.14

Food intake, body weight and $\log_e$ data for Diet 1(i)

Mean Total Weight (g) $t_1 \rightarrow t_2$	$\log_e$ Total Weight	Mean daily ration(wet wt g $t_1 \rightarrow t_2$)	$\log_e$(ww) mean daily ration	Mean daily ration(dry wt g $t_1 \rightarrow t_2$)	$\log_e$(dw) mean daily ration
2590	7.859	60.03	4.095	29.92	3.399
2350	7.762	52.83	3.967	26.33	3.271
2980	8.000	68.26	4.223	34.02	3.527
2970	7.996	48.41	3.880	24.13	3.183
2600	7.863	41.25	3.720	20.56	3.023
3470	8.152	58.78	4.074	29.30	3.378
3330	8.111	55.75	4.021	27.79	3.325
2810	7.941	50.36	3.919	25.10	3.223
3990	8.292	75.46	4.324	38.74	3.657
3710	8.219	62.11	4.129	30.96	3.433
3070	8.029	55.61	4.018	27.72	3.322
4440	8.398	73.28	4.294	36.52	3.598

Figure 2.4.4 Food consumption (ww) as a function of body weight for Diets 1(iii) and 1(iv)

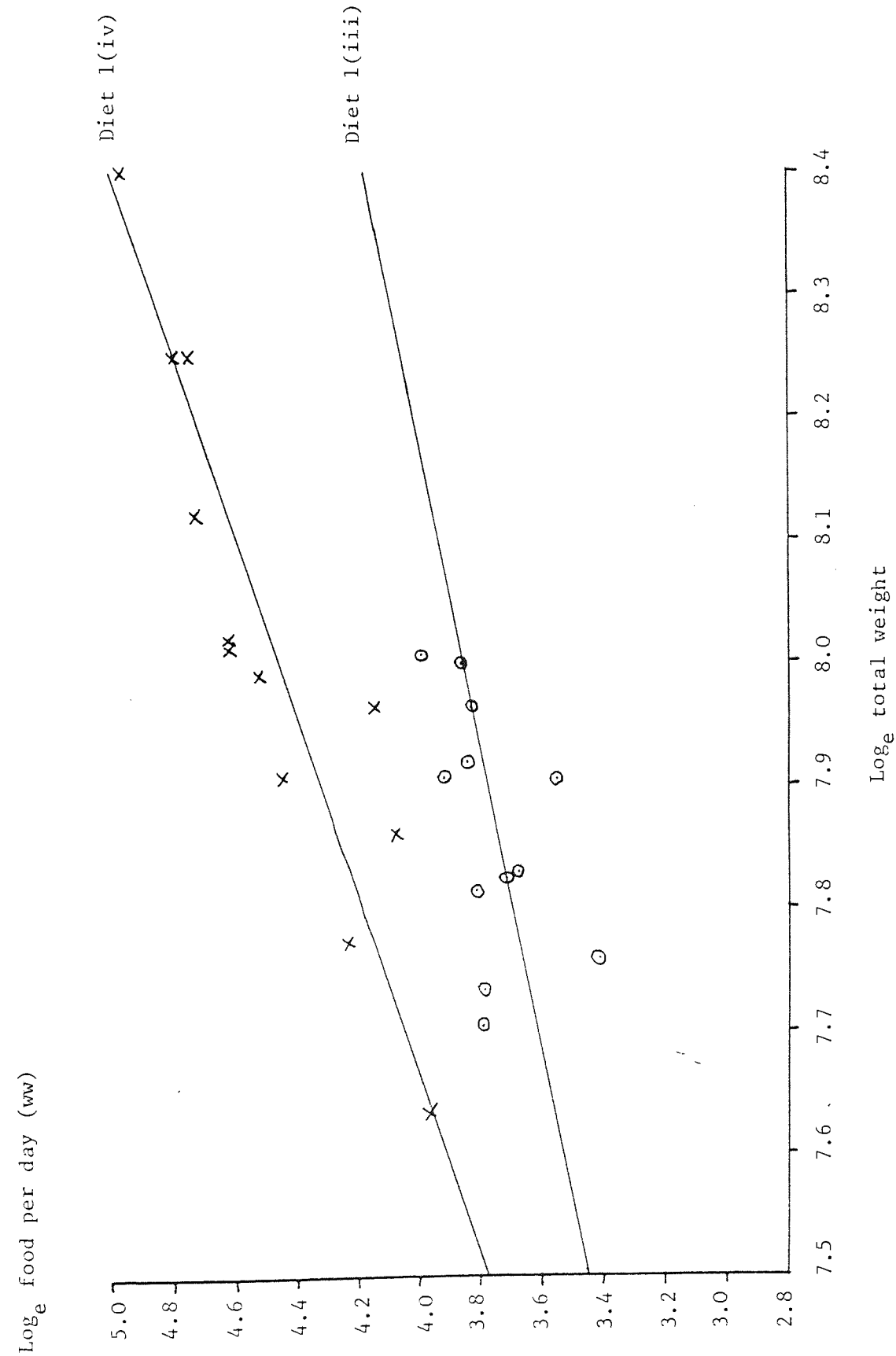


TABLE 2.4.15

Food intake, body weight and $\log_e$ data for Diet 1(ii)

Mean Total weight (g) $t_1 \rightarrow t_2$	$\log_e$ Total Weight	Mean daily ration(wet wt g $t_1 \rightarrow t_2$)	$\log_e$(ww) mean daily ration	Mean daily ration(dry wt g $t_1 \rightarrow t_2$)	$\log_e$(dw) mean daily ration
2240	7.714	30.77	3.427	27.58	3.317
1990	7.596	24.36	3.193	21.83	3.083
2610	7.867	31.88	3.462	28.57	3.352
2370	7.771	20.22	3.007	18.12	2.897
2090	7.645	16.86	2.825	15.11	2.715
2830	7.948	23.98	3.177	21.49	3.068
2590	7.859	29.72	3.392	26.64	3.282
2210	7.701	24.98	3.218	22.39	3.109
3110	8.042	34.59	3.544	31.00	3.434
2820	7.944	31.72	3.457	28.43	3.347
2330	7.754	23.69	3.165	21.23	3.055
3410	8.314	31.22	3.441	27.98	3.311

TABLE 2.4.16

Food intake, body weight and $\log_e$ data for Diet 1(iii)

Mean Total weight (g) $t_1 \rightarrow t_2$	$\log_e$ Total Weight	Mean daily ration(wet wt g $t_1 \rightarrow t_2$)	$\log_e$(ww) mean daily ration	Mean daily ration(dry wt g $t_1 \rightarrow t_2$)	$\log_e$(dw) mean daily ration
2220	7.705	44.50	3.795	22.85	3.129
2470	7.812	45.24	3.812	23.23	3.145
2280	7.732	44.34	3.792	22.76	3.125
2340	7.758	30.40	3.414	15.61	2.748
2700	7.901	34.72	3.547	17.83	2.881
2500	7.824	36.78	3.605	18.88	2.938
2510	7.828	35.96	3.582	18.46	2.916
2870	7.962	45.79	3.824	23.51	3.157
2740	7.916	46.66	3.843	24.00	3.178
2710	7.905	50.01	3.912	25.68	3.246
3110	8.042	54.56	3.999	28.01	3.333
2970	7.996	47.57	3.862	24.42	3.195

TABLE 2.4.17

Food intake, body weight and $\log_e$ data for Diet 1(iv)

Mean Total weight (g) $t_1 \rightarrow t_2$	$\log_e$ Total Weight	Mean daily ration(wet wt g $t_1 \rightarrow t_2$)	$\log_e$(ww) mean daily ration	Mean daily ration(dry wt g $t_1 \rightarrow t_2$)	$\log_e$(dw) mean daily ration
2870	7.962	63.63	4.153	12.93	2.560
2590	7.859	59.22	4.081	12.03	2.487
2070	7.635	52.70	3.965	10.71	2.371
3270	8.093	102.01	4.625	20.72	3.031
2940	7.986	92.46	4.527	18.79	2.933
2370	7.771	69.24	4.238	14.07	2.644
3810	8.245	123.10	4.813	25.01	3.219
3350	8.117	114.85	4.744	23.34	3.150
2710	7.905	86.13	4.456	17.50	2.862
4410	8.392	145.34	4.979	29.53	3.385
3810	8.245	116.87	4.761	23.75	3.168
3030	8.016	102.40	4.629	20.81	3.035

Large differences were evident in the amount of food eaten amongst the four diets. This variation was quantified by calculating the feeding rate as a percentage of body weight for each feeding period and then applying analysis of variance to the results. The rates for wet weight of food consumed, were calculated from the data in Tables 2.4.14 - 2.4.17 and are presented in Table 2.4.18.

Analysis of variances yielded the following:

	1(ii)	1(iii)	1(i)	1(iv)
Mean % consumption	1.06	1.64	1.85	2.98
At 5% significance			———	

Figure 2.4.5 Food consumption (ww) as a function of body weight for Diets 1(i) and 1(ii)

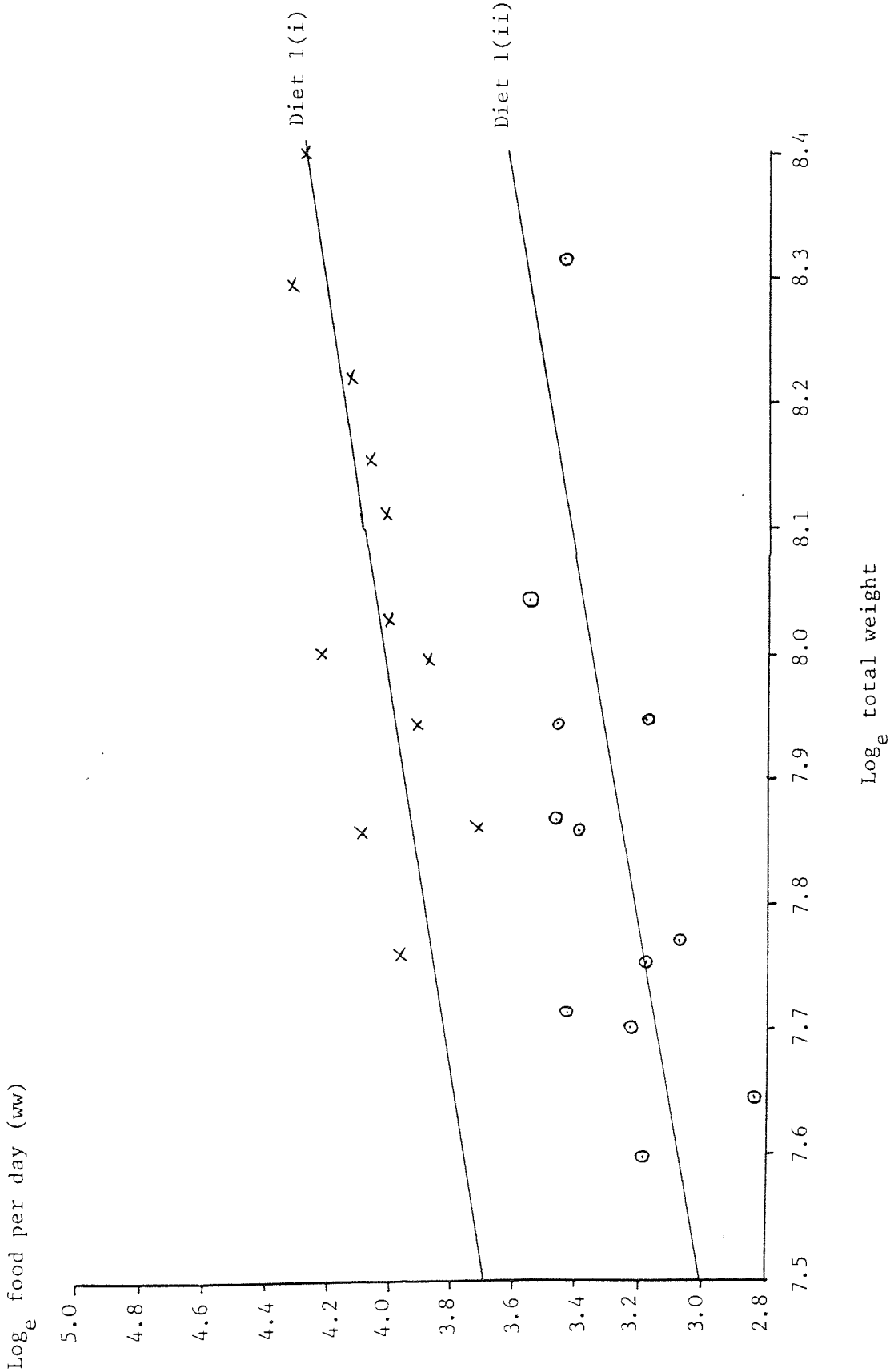


TABLE 2.4.18

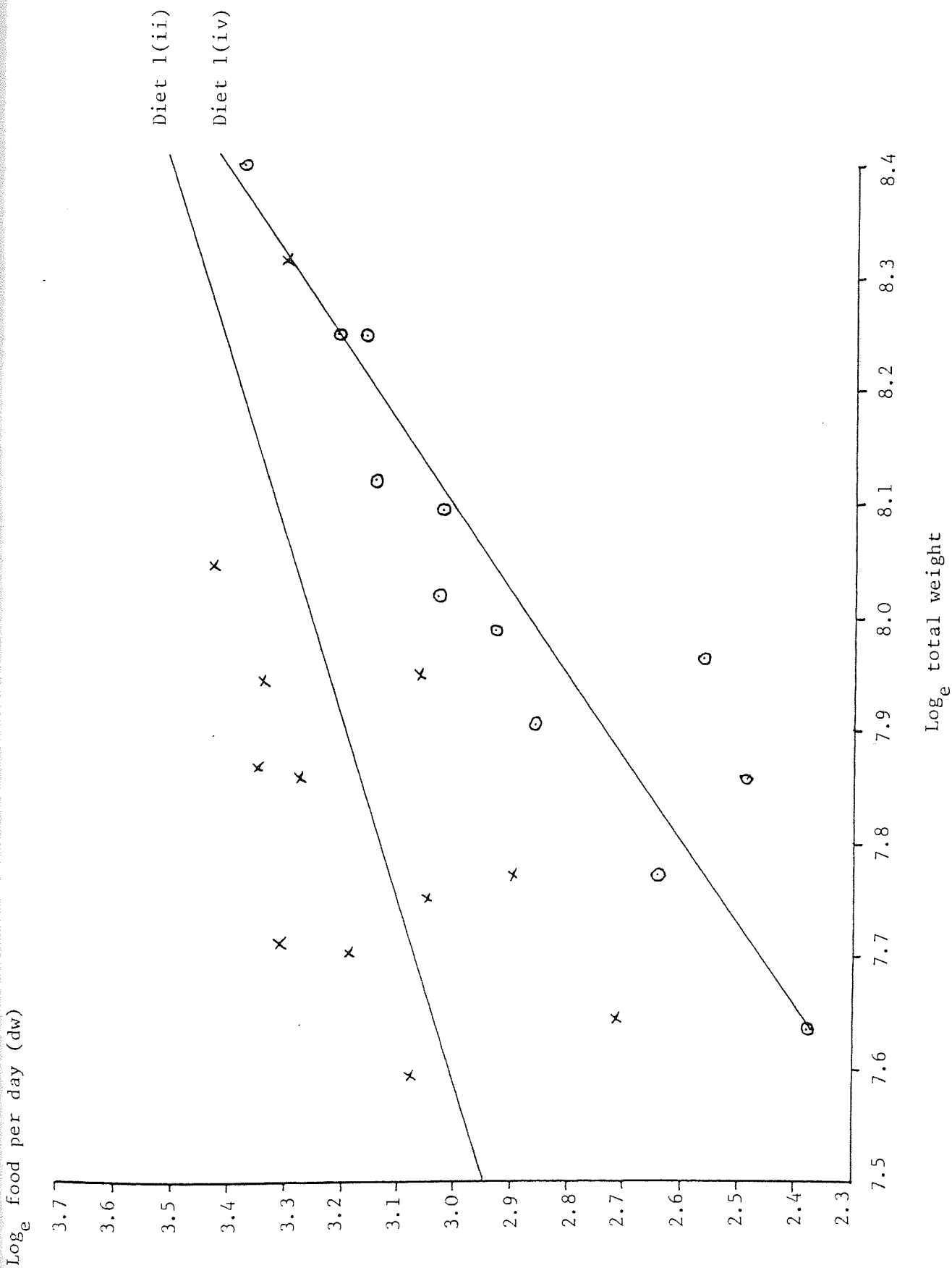
Wet weight food consumption as % body weight

for Experiment 1.1

Diet	Experimental Period			
	1	2	3	4
1(i)	2.31	1.62	1.67	1.67
1(i)	2.24	1.58	1.79	1.81
1(i)	2.29	1.69	1.89	1.65
1(ii)	1.37	0.85	1.14	1.12
1(ii)	1.22	0.80	1.13	1.01
1(ii)	1.22	0.84	1.11	0.91
1(iii)	2.00	1.29	1.43	1.84
1(iii)	1.83	1.28	1.59	1.75
1(iii)	1.94	1.47	1.70	1.60
1(iv)	2.21	3.11	3.23	3.29
1(iv)	2.28	3.14	3.42	3.06
1(iv)	2.54	2.92	3.17	3.37

Thus diets 1(iii) and 1(i) were not significantly different, but were significantly different from diets 1(ii) and 1(iv) which were significantly different. Here possibly is one reason in accounting for differences in specific growth rate between diets, ie the fish on diet 1(iv) were eating nearly three times as much as those on diet 1(ii). However, there were very large differences between moisture content of the diets (Table 2.4.1). Further analysis of consumption rates was performed, by converting the ration to dry weight of food consumed and then relating this to body weight. These data are presented in Tables 2.4.14 - 2.4.17 and in Figures 2.4.6 (Diets 1(iv) and 1(ii)) and 2.4.7

Figure 2.4.6 Food consumption (dw) as a function of body weight for Diets 1(ii) and 1(iv)



(Diets 1(i) and 1(iii)). Once again, the data were transformed to percentage body weight consumption to qualify statistically the results (Table 2.4.19), the Figures only being presented as illustrations.

TABLE 2.4.19

Dry weight food consumption as % body weight for Experiment 1.1

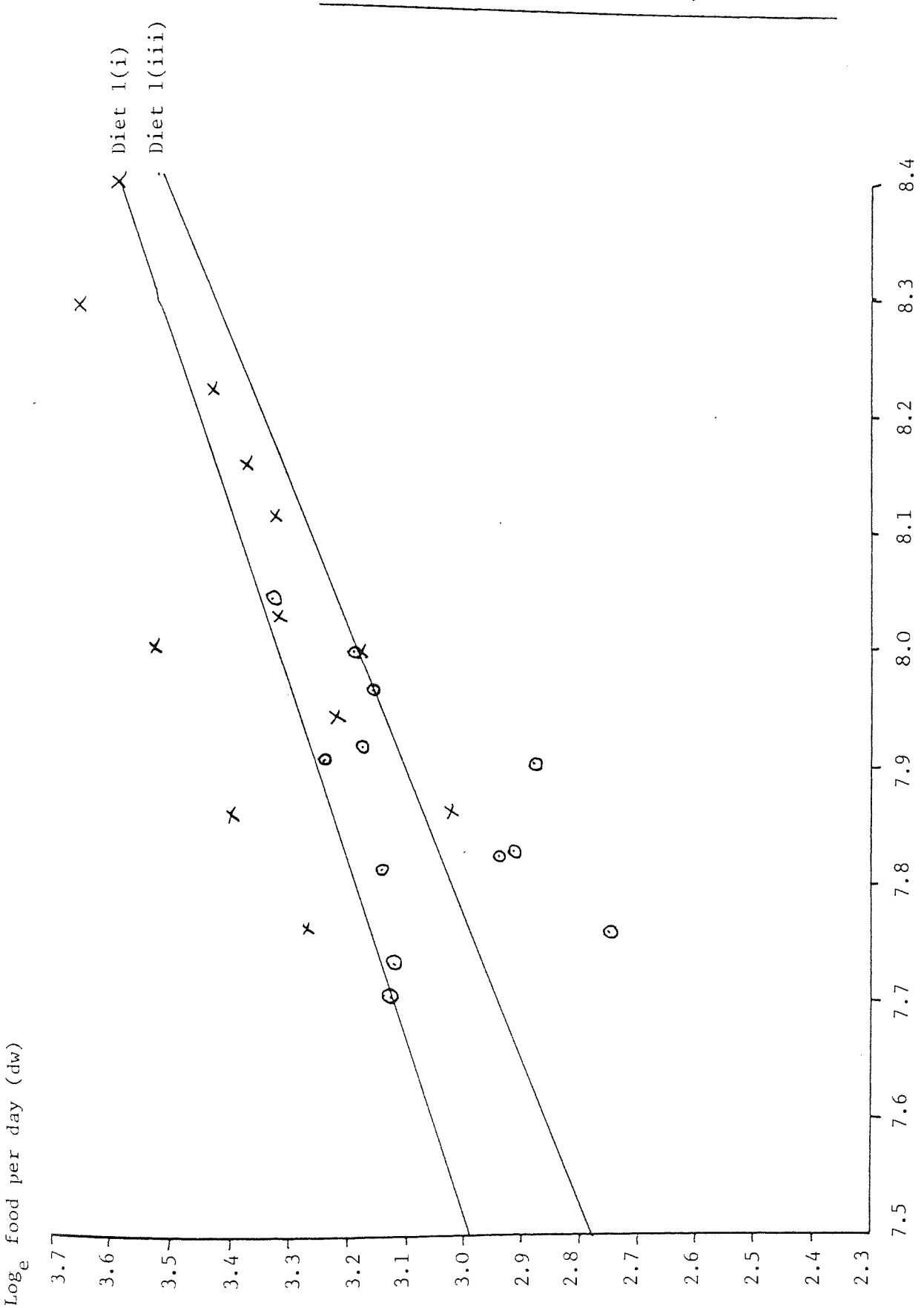
Diet	Experimental Period			
	1	2	3	4
1(i)	1.15	0.81	0.83	0.83
1(i)	1.12	0.79	0.89	0.90
1(i)	1.14	0.84	0.94	0.82
1(ii)	1.22	0.76	1.02	1.00
1(ii)	1.09	0.72	1.01	0.91
1(ii)	1.09	0.75	0.99	0.82
1(iii)	1.03	0.66	0.73	0.94
1(iii)	0.94	0.66	0.82	0.90
1(iii)	1.00	0.75	0.87	0.82
1(iv)	0.45	0.63	0.66	0.67
1(iv)	0.46	0.64	0.69	0.62
1(iv)	0.52	0.59	0.64	0.68

Analysis of variance of the results yielded the following:

	1(iv)	1(iii)	1(i)	1(ii)
Mean % consumption	0.604	0.796	0.922	0.948
At 5%				

Hence the fish were not eating equivalent amounts of food on a dry weight basis either. This tends to dismiss the theory put forward by Bromley (1974a), that the rate of consumption is controlled by the amount

Figure 2.4.7 Food consumption (dw) as a function of body weight for Diets 1(i) and 1(iii)



of dry matter consumed. A final approach was to examine the data in respect of the gross energy consumed, in relation to body weight, to identify whether this is the determinant factor in controlling food consumption rate. These data are presented in Tables 2.4.20 and 2.4.21.

TABLE 2.4.20

Energy consumption and body weight data for Diets 1(i) & 1(ii)

Diet 1(i)				Diet 1(ii)			
Energy Consumed kj day^{-1}	Log_e energy consumed	Total body wt (g)	Energy Consumed kjg body $wt^{-1} day^{-1}$	Energy Consumed kj day^{-}	Log_e energy consumed	Total body wt (g)	Energy Consumed kjg body $wt^{-1} day^{-1}$
667.96	6.504	2590	0.2579	624.41	6.436	2240	0.2788
587.82	6.376	2350	0.2501	587.82	6.376	2350	0.2484
759.80	6.633	2980	0.2549	646.82	6.472	2610	0.2478
538.70	6.289	2970	0.1814	410.24	6.017	2370	0.1731
459.00	6.129	2600	0.1765	342.09	5.835	2090	0.1637
654.12	6.483	3470	0.1885	486.53	6.187	2830	0.1719
620.41	6.430	3330	0.1863	603.13	6.402	2590	0.2329
560.36	6.329	2810	0.1994	506.91	6.228	2210	0.2294
864.87	6.763	3990	0.2168	701.84	6.554	3110	0.2257
691.18	6.538	3710	0.1863	643.66	6.467	2820	0.2282
618.85	6.428	3070	0.2016	480.65	6.175	2330	0.2063
815.31	6.704	4440	0.1836	633.47	6.451	3410	0.1856

Figure 2.4.8 Energy consumption as a function of body weight for Diets 1(i) and 1(iv)

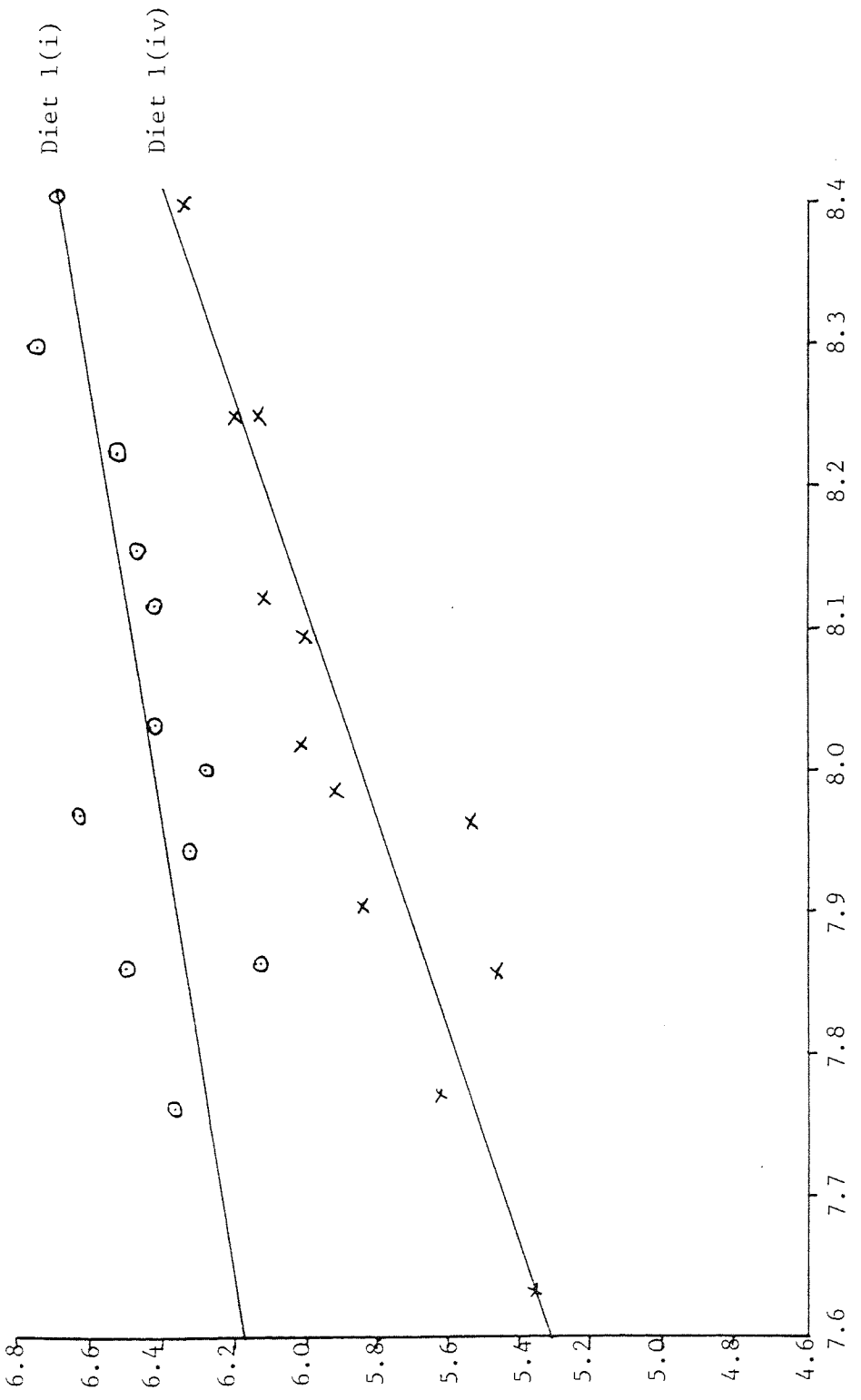


TABLE 2.4.21

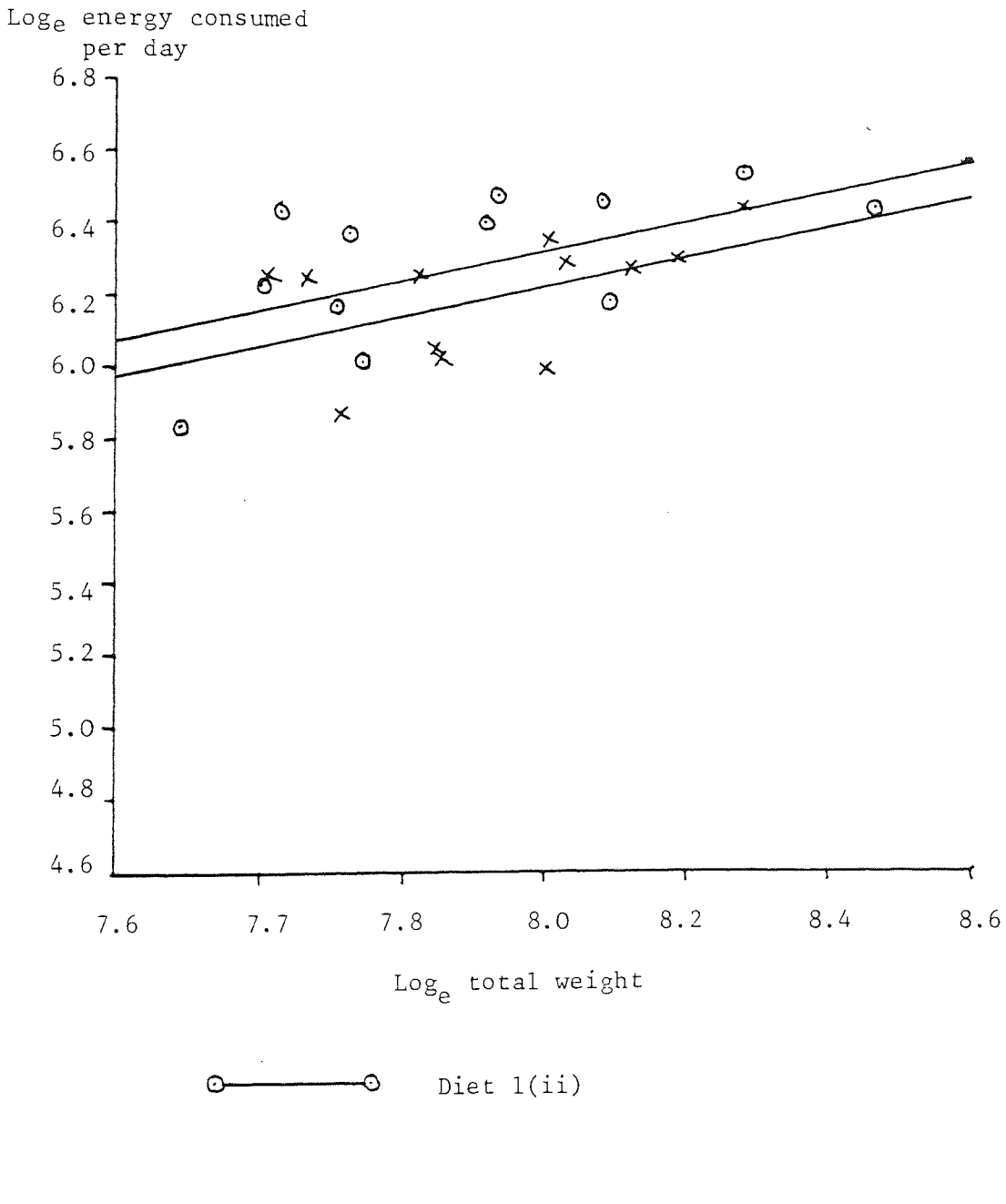
Energy consumption and body weight data for Diets 1(iii) & 1(iv)

Diet 1(iii)				Diet 1(iv)			
Energy Consumed kj day^{-1}	Log_e energy consumed	Total body wt (g)	Energy Consumed kjg body $wt^{-1}day^{-1}$	Energy Consumed kj day^{-1}	Log_e energy consumed	Total body wt (g)	Energy Consumed kjg body $wt^{-1}day^{-1}$
516.78	6.248	2220	0.2328	255.60	5.544	2870	0.0891
525.37	6.264	2470	0.2127	237.81	5.471	2590	0.0918
514.74	6.244	2280	0.2258	211.72	5.355	2070	0.1023
353.04	5.867	2340	0.1509	409.59	6.015	3270	0.1253
403.24	6.000	2700	0.1493	371.44	5.917	2940	0.1263
426.99	6.057	2500	0.1708	278.14	5.628	2370	0.1174
417.49	6.034	2510	0.1663	494.40	6.203	3810	0.1298
531.70	6.276	2870	0.1853	461.39	6.134	3550	0.1377
542.78	6.297	2740	0.1981	345.94	5.846	2710	0.1277
580.78	6.364	2710	0.2143	583.75	6.369	4410	0.1324
633.47	6.451	3110	0.2037	469.49	6.152	3810	0.1232
552.28	6.314	2970	0.1860	411.37	6.019	3030	0.1358

For further details see Appendix 2.

The energy consumption data are plotted against body weight in Figures 2.4.8(Diets 1(i) and 1(iv)) and 2.4.9(Diets 1(ii) and 1(iii)), to serve as an illustration of differences between diets. Transformation of the data in this manner produced very similar levels of consumption for diets 1(i), 1(ii), and 1(iii), but the rate for diet 1(iv) was markedly different. Analysis of variance of the energy consumption rate

Figure 2.4.9 Energy consumption as a function of body weight for Diets 1(ii) and 1(iii)



data in Tables 2.4.20 and 2.4.21 yielded the following:

	1(iv)	1(iii)	1(i)	1(ii)
Energy consumption rate	0.1199	0.1913	0.2069	0.2160
At 5%		———		———

Thus the energy consumption on diet 1(iv) was significantly different from that on the other three diets. Diets 1(iii) and 1(i) and 1(i) and 1(ii) were not significantly different but diet 1(iii) was significantly different from diet 1(ii). Clearly then, the fish were not eating equivalent rates of gross energy, particularly of diet 1(iv). The indications were that fish required less of a sprat diet, on a gross energy basis, than of a compounded diet containing sprat. The possible reason for this, may be due to differences in metabolisable energy between the diets, ie if one could plot energy consumption rate as metabolisable energy against body weight, a different relationship might be evident.

This experiment demonstrated that there were differences in food consumption rate amongst the four diets, which could not be explained on a dry weight of food fed basis or a gross energy consumed basis. However, the three diets containing a portion of compounded feed (1(i), (ii) and (iii)) did indeed demonstrate similar consumption rates, on a gross energy basis. Clearly, there is some difference in nutrient availability between diets containing compound feed and those which may be considered to be more natural (in a wild habitat definition) such as sprat. It was for this reason, that subsequent experiments were fed on an isoenergetic gross energy basis, as all had some element of compounded feed. However, there was a marked difference in gross energy consumption rates between experiment 2.1 and 2.2 which was unprecedented.

2.4.4 Comparison of food conversion ratio of turbot fed diets of varying fish protein sources

One of the most frequently used indices of efficiency with which food is converted into fish flesh, is the Food Conversion Ratio (FCR) where -

$$FCR = \frac{\Sigma F_{t1 \rightarrow t2}}{\Sigma wt_2 - \Sigma wt_1}$$

where $\Sigma F_{t1 \rightarrow t2}$ = Amount of food consumed over time period $t_1 \rightarrow t_2$

Σwt_1 = Total fish weight at start of experiment t_1,

Σwt_2 = Total fish weight at end of experiment t_2.

In view of the fact that the diets used all had varying moisture contents, FCR's were compared on a dry weight of food fed basis. The data for FCR calculations for experiment 1.1, are presented in Tables 2.4.22 (Diets 1(i) and 1(ii)) and 2.4.23 (Diets 1(iii) and 1(iv)).

TABLE 2.4.22

Food Conversion Data for Diets 1(i) and 1(ii)

Diet		Experimental Period			
		1	2	3	4
1(i)	Δwt	555.60	202.90	392.23	407.71
	ΣFood	388.92	294.21	392.23	291.4
	FCR	0.70	1.45	1.00	0.96
1(i)	Δwt	258.88	231.95	218.29	314.44
	ΣFood	344.31	250.51	353.63	352.17
	FCR	1.33	1.08	1.62	1.12
1(i)	Δwt	572.90	422.17	513.44	391.73
	ΣFood	446.86	358.84	533.98	462.24
	FCR	0.78	0.85	1.04	1.18
1(ii)	Δwt	99.26	169.45	292.53	203.24
	ΣFood	359.32	216.90	371.51	365.83
	FCR	3.62	1.28	1.27	1.80
1(ii)	Δwt	68.18	135.71	108.63	115.91
	ΣFood	282.27	181.85	315.03	273.55
	FCR	4.14	1.34	2.90	2.36
1(ii)	Δwt	156.05	268.98	309.74	220.95
	ΣFood	372.96	258.22	433.64	360.15
	FCR	2.39	0.96	1.40	1.63

where Δwt = w.weight gain over experimental period (g)

$\Sigma food$ = dry weight of food eaten during experimental period

FCR = food conversion ratio.

For further details of weights etc., see Appendix 2.

TABLE 2.4.23

Food Conversion Data for Diets 1(iii) and 1(iv)

Diet		Experimental Period			
		1	2	3	4
	Δwt	119.09	144.76	176.47	221.29
1(iii)	ΣFood	300.11	188.19	261.18	325.30
	FCR	2.52	1.30	1.48	1.47
	Δwt	191.35	170.30	212.62	259.95
1(iii)	ΣFood	304.25	214.58	331.69	356.13
	FCR	1.59	1.26	1.56	1.37
	Δwt	251.87	215.04	236.62	200.38
1(iii)	ΣFood	299.73	225.79	338.37	310.59
	FCR	1.19	1.05	1.43	1.55
	Δwt	331.27	508.76	575.32	624.48
1(iv)	ΣFood	172.26	234.03	350.95	387.18
	FCR	0.52	0.46	0.61	0.62
	Δwt	597.15	190.47	450.39	484.72
1(iv)	ΣFood	161.23	211.42	324.28	310.22
	FCR	0.27	1.11	0.72	0.64
	Δwt	244.45	321.18	381.13	391.93
1(iv)	ΣFood	144.23	157.38	243.92	274.35
	FCR	0.59	0.49	0.64	0.70

where Δwt = ω.weight gain over experimental period (g)

$\Sigma Food$ = dry weight (g) of food eaten during experimental period

FCR = food conversion ratio.

Analysis of variance of the FCR's yielded the following:

Diet	1(iv)	1(i)	1(iii)	1(ii)
FCR	0.59	1.09	1.48	2.09
At 5%		———		

The FCR on the chopped sprat diet (1(iv)) was significantly different to all the other diets. Diets 1(i) and 1(iii) were insignificantly different, but the others were all significantly different, diet 1(ii) (dry diet) resulting in the highest FCR.

The FCR data for experiment 2.1 diets, are shown in Table 2.4.24.

TABLE 2.4.24

Food Conversion Data for Experiment 2.1

Diet		Experimental Period					
		1	2	3	4	5	6
2(i)	Δwt	1084	771	633	382	928	947
	ΣFood	1279	648	589	703	761	852
	FCR	1.18	0.84	0.93	1.84	0.82	0.90
2(i)	Δwt	1017	594	468	318	1096	597
	ΣFood	1281	665	590	684	734	842
	FCR	1.26	1.12	1.26	2.15	0.67	1.41
2(ii)	Δwt	1286	731	643	196	854	772
	ΣFood	1299	716	637	751	811	865
	FCR	1.01	0.98	0.99	3.83	0.95	1.12
2(ii)	Δwt	926	590	465	267	766	670
	ΣFood	1306	631	558	643	705	750
	FCR	1.41	1.07	1.20	2.41	0.92	1.12
2(iii)	Δwt	1100	593	369	331	645	771
	ΣFood	1287	658	594	685	761	794
	FCR	1.17	1.11	1.61	2.07	1.18	1.03
2(iii)	Δwt	1028	725	379	271	625	734
	ΣFood	1306	725	663	740	788	851
	FCR	1.27	1.00	1.75	2.73	1.26	1.16

where Δwt = weight gain over experimental period (g)

$\Sigma Food$ = dry weight (g) of food eaten during experimental period

FCR = food conversion ratio.

For further details see Appendix 2.

Analysis of variance of the FCR's, failed to prove any significant differences bewteen the diets. The diet means were as follows:

2(i) 1.20

2(ii) 1.23

2(iii) 1.45

So once again the sprat-based diet produced the lowest FCR although it was extremely closely matched by the mackerel diet.

The FCR data for experiment 2.2, are presented in Table 2.4.25. The data from which these values were extracted are presented in Appendix 2. In the table;

Δwt = weight gain (kg) over experimental period

$\Sigma food$ = dry weight (kg) of food eaten during experimental period

FCR = food conversion ratio.

Analysis of variance of the FCR's in Table 2.4.25, failed to prove any significant differences between the diets. The diet means were as follows:

3(i) 1.76

3(ii) 1.40

3(iii) 1.48

Thus no diet resulted in such a low FCR as chopped sprat (1(iv)), but diet 3(ii) resulted in a lower FCR than all the pouting based diets

(1(iii) and 2(iii)). This was the result aimed for originally, as diet 3(ii) contained no fresh fish ingredient.

TABLE 2.4.25

Food Conversion Data for Experiment 2.2

Diet		Experimental Period					
		1	2	3	4	5	6
3(i)	Δwt	1.247	0.977	1.287	1.649	0.483	1.483
	ΣFood	2.606	1.192	1.673	1.863	1.913	1.913
	FCR	2.09	1.22	1.30	1.13	3.96	1.29
3(i)	Δwt	1.279	0.947	0.729	1.574	0.760	1.235
	ΣFood	2.622	1.146	1.611	1.716	1.763	1.815
	FCR	2.05	1.21	2.21	1.09	2.32	1.47
3(i)	Δwt	1.153	0.864	0.870	1.749	0.617	1.245
	ΣFood	2.444	1.097	1.540	1.662	1.721	1.780
	FCR	2.12	1.27	1.77	0.95	2.79	1.43
3(ii)	Δwt	1.424	1.262	1.174	1.631	1.136	1.981
	ΣFood	2.164	1.249	1.961	2.137	2.147	2.278
	FCR	1.52	0.99	1.67	1.31	1.89	1.15
3(ii)	Δwt	0.991	1.040	1.019	1.712	1.023	1.687
	ΣFood	1.516	1.175	1.681	1.832	1.893	2.008
	FCR	1.53	1.13	1.65	1.07	1.85	1.19
3(ii)	Δwt	0.863	1.061	0.685	1.675	0.773	1.453
	ΣFood	1.320	0.923	1.349	1.457	1.461	1.642
	FCR	1.53	0.87	1.97	0.87	1.89	1.13
3(iii)	Δwt	0.936	1.320	0.938	1.354	1.337	1.693
	ΣFood	1.207	1.452	2.082	2.234	2.059	2.387
	FCR	1.29	1.10	2.22	1.65	1.54	1.41
3(iii)	Δwt	1.022	1.050	1.121	1.504	1.172	1.790
	ΣFood	1.635	1.155	1.659	1.850	1.852	2.041
	FCR	1.60	1.10	1.48	1.23	1.58	1.14
3(iii)	Δwt	1.100	1.303	0.847	1.685	1.012	1.683
	ΣFood	1.980	1.238	1.813	1.955	1.984	2.137
	FCR	1.80	0.95	2.14	1.16	1.96	1.27

2.4.5 Comparison of protein utilisation of turbot fed diets of varying fish protein sources

Two indices of protein utilisation are presented here, viz Protein Efficiency Ratio (PER) and Efficiency of Protein Conversion (EPC)

where $PER = \frac{\Delta W_{t1 \rightarrow t2}}{\Sigma \text{Protein eaten}}$

$\Delta W_{t_1 \rightarrow t_2}$ = weight increase of fish over experiment

ΣProtein eaten = total amount of protein consumed during experiment

and $EPC = \frac{\Sigma protein_{t2} - \Sigma protein_{t1}}{\Sigma protein\ eaten} \times 100 \%$

$\Sigma protein_{t2}$ = total fish protein at end of experiment

$\Sigma protein_{t1}$ = " " " " start " "

Σprotein eaten = total amount of protein consumed during experiment.

PER is the most usually quoted index of protein utilisation, however EPC is a more accurate reflection of how efficiently dietary protein is being used to produce body protein, as it takes into account weight change as a function of protein composition. The PER and EPC data for experiment 1.1, are presented in Table 2.4.26. For further details see Tables 2.4.1, 2.4.22, 2.4.23 and Appendix 2.

Analysis of variance of the EPC failed to demonstrate any significant differences between the diet means which were as follows -

1(i)	23.8
1(ii)	16.0
1(iii)	18.0
1(iv)	30.0

TABLE 2.4.26

PER and EPC Data for Experiment 1.1

Diet	Total fish Protein start (g)	Total fish Protein end (g)	Protein accrued (g)	Total Protein consumed(g)	PER	EPC
1(i)	390.6	624.8	234.2	807.0	1.93	29.0
1(i)	361.6	492.0	130.4	714.0	1.43	18.3
1(i)	447.5	686.2	238.7	985.5	1.93	24.2
1(ii)	358.4	474.8	116.4	631.8	1.14	18.4
1(ii)	319.5	370.9	51.4	505.3	0.85	10.2
1(ii)	352.6	485.8	133.2	683.8	1.40	19.5
1(iii)	354.3	413.9	59.6	549.8	1.20	10.8
1(iii)	394.5	538.9	144.4	617.5	1.35	23.4
1(iii)	353.1	472.2	119.1	599.8	1.51	19.9
1(iv)	443.0	776.9	333.9	906.8	2.25	36.8
1(iv)	384.5	586.9	202.4	801.5	2.15	25.3
1(iv)	317.6	498.6	181.0	648.9	2.06	27.9

However the PER values showed the following:

Diet	1(ii)	1(iii)	1(i)	1(iv)
Mean PER	1.13	1.35	1.76	2.15
At 5%	———			

So diets 1(ii) and 1(iii) were not significantly different, but the other two were with the sprat-based diet resulting in the highest PER.

The PER and EPC data for experiment 2.1, are presented in Table 2.4.27.

TABLE 2.4.27

PER and EPC Data for Experiment 2.1

Diet	Total fish Protein start (g)	Total fish Protein end (g)	Protein accrued (g)	Total Protein consumed(g)	PER	EPC
2(i)	529	1175	646	2003	2.37	32.3
2(i)	373	1128	755	1995	2.05	37.8
2(ii)	386	1170	784	2103	2.13	37.3
2(ii)	346	1019	673	1904	1.98	35.3
2(iii)	368	1032	664	2174	1.75	30.5
2(iii)	415	1099	684	2307	1.63	29.7

For further details of these data, see Tables 2.4.2, 2.4.24 and Appendix 2.

Analysis of variance of the PER and EPC data, failed to demonstrate any significant differences but the sprat and mackerel diets (2(i) and 2(ii)) appeared toproduce superior PER's and EPC's.

The diet means were as follows:

	PER	EPC
2(i)	2.21	35.1
2(ii)	2.06	36.3
2(iii)	1.69	30.1

The PER and EPC data for experiment 2.2, are presented in Table 2.4.28. For further details of these data see Tables 2.4.2, 2.4.25 and Appendix 2.

TABLE 2.4.28

PER and EPC Data for Experiment 2.2

Diet	Total fish Protein start (g)	Total fish Protein end (g)	Protein accrued (g)	Total Protein consumed(g)	PER	EPC
3(i)	1073	2251	1178	5840	1.47	18.2
3(i)	989	1879	890	4893	1.42	17.7
3(i)	1122	2164	1042	6534	1.46	18.1
3(ii)	944	1758	814	4610	1.48	20.2
3(ii)	914	1726	812	4491	1.46	19.9
3(ii)	953	1976	1023	5140	1.60	21.8
3(iii)	859	1639	780	5584	1.16	15.9
3(iii)	731	1619	888	4066	1.38	14.0
3(iii)	917	1960	1043	6040	1.28	17.3

Analysis of variance of this data yielded the following.

Diet	Avg PER	At 5%	Avg EPC	At 5%
3(i)	1.51	|	18.0	
3(ii)	1.45	|	20.6	
3(iii)	1.27		15.7	

Consequently all the EPC values were significantly different, with the highest being attributed to Diet 3(ii). The PER values for diets 3(i) and 3(ii) were not significantly different, but they were from diet 3(iii).

PER is a function of dietary energy and protein levels and a fuller discussion of differences between diets is presented in 2.5. Once again, a sprat diet (2(i)) produced the greatest PER.

2.4.6 Comparison of energy utilisation of turbot fed diets of varying fish protein sources

It must be stressed at the start of this section that energy utilisation is described here only in terms of gross energy as determined by bomb calorimetry. The Efficiency of Energy Utilisation (EEU) data for Experiment 1.1 are presented in Table 2.4.29.

TABLE 2.4.29

EEU Data for Experiment 1.1

Diet	Total fish energy start J	Total fish energy end J	Energy accrued J	Total energy consumed J	EEU %
1(i)	10918.9	18421.6	7502.7	32815.3	22.9
1(i)	10109.6	13413.2	3303.6	29039.4	11.4
1(i)	12510.8	21397.9	8887.1	40078	22.2
1(ii)	10020.8	16156.8	6136.0	29850.3	20.6
1(ii)	8931.2	11428.1	2496.9	23875.2	10.5
1(ii)	11152.7	15364.6	4211.9	32308.7	13.0
1(iii)	9905.3	11114.2	1208.9	24347.1	5.0
1(iii)	11027.4	14685.7	3658.4	27346.5	13.4
1(iii)	9872.4	13091.9	3219.5	26560.7	12.1
1(iv)	12384.8	22570.0	10185.2	22752.6	44.8
1(iv)	10749.8	17024.7	6274.9	20110.8	31.2
1(iv)	8878	14363.7	5485.8	16280.9	33.7

For further details of these data, see Tables 2.4.1, 2.4.14-17, 2.4.20-21, 2.4.32 and Appendix 2. Analysis of variance of these EEU data yielded the following.

Diet	1(iii)	1(ii)	1(i)	1(iv)
Avg EEU %	10.2	14.7	18.8	36.6
At 5%				

So diet 1(iv) was significantly higher than the other three, which were not significantly different.

The EEU data for Experiment 2.1 are presented in Table 2.4.30.

TABLE 2.4.30

EEU Data for Experiment 2.1

Diet	Total fish energy start J	Total fish energy end J	Energy accrued J	Total energy consumed J	EEU %
2(i)	14561	33143	18626	97552	19.1
2(i)	15484	31621	16326	97159	16.8
2(ii)	16007	34303	18663	104030	17.9
2(ii)	14355	29939	15819	94175	16.8
2(iii)	15261	30392	15211	95963	15.9
2(iii)	17226	32237	15222	101833	14.9

For further details, see Tables 2.4.2, 2.4.24, 2.4.34 and Appendix 2. Analysis of variance of the EEU results failed to demonstrate any significant differences (at 5%). The average values were:

Diet 2(i) 18.0

2(ii) 17.4

2(iii) 15.4.

The EEU data for experiment 2.2, are presented in Table 2.4.31.

Analysis of variance of the EEU data (Table 2.4.31) yielded the following.

Diet	3(iii)	3(i)	3(ii)
Avg EEU %	16.9	23.1	23.2
At 5%			

TABLE 2.4.31

EEU Data for Experiment 2.2

Diet	Total fish energy start J	Total fish energy end J	Energy accrued J	Total energy consumed J	EEU %
3(i)	34254	71923	37669	161363	23.3
3(i)	32696	67301	34605	152040	22.8
3(i)	31659	66046	34387	148111	23.2
3(ii)	37130	73881	36751	162244	22.7
3(ii)	32995	64879	31884	142787	22.3
3(ii)	25304	53160	27856	112956	24.7
3(iii)	38836	64754	25918	152892	17.0
3(iii)	29752	49025	19273	130675	14.7
3(iii)	31753	58650	26897	141333	19.0

For further details, see 2.4.2, 2.4.25, 2.4.36 and Appendix 2. Consequently diets 3(i) and 3(ii) were not significantly different, but were from diet 3(iii). EEU is also a function of dietary energy levels and also the source of energy, ie fat, protein or carbohydrate. On a gross energy utilisation basis, the sprat diet (1(iv)) resulted in the highest value. However, two of the diets containing no fresh fish (3(i) and 3(ii)) resulted in higher values than any of the other fresh fish-based diets (1(i), 1(iii), 2(i), 2(ii) and 2(iii)).

2.4.7 Comparison of body composition and condition factor of turbot fed diets of varying fish protein sources

Two other factors that can be used to monitor the effect of different feeds, are the condition factors and carcass analyses. It is desirable for a feed to induce a maximum weight gain; however it is important that

this weight gain does not significantly alter the body composition. This is particularly important in turbot, which are not naturally fatty (usually between 7-12%). In turbot, fat is stored along the fin margins and increased dietary fat can cause excessive deposition and mortality has been associated with this in plaice (Roberts 1970). The feed also has an affect on the physical dimensions of the fish. The relationship between fish weight and length is termed the 'condition factor' K and is usually represented by the equation

$$K = \frac{100W}{L^3}$$

To ensure that the relationship was similar in turbot, a series of length-weight measurements were made on the fish farm at Wylfa Power Station. The averaged results are presented in Table 2.4.32 and Figure 2.4.10. The relationship between length and weight can be written thus:

$$\text{Log}_e \text{ weight} = 3.0259 \times \text{Log}_e \text{ length} - 3.9025$$

$$\text{or } W = L^{3.0259}$$

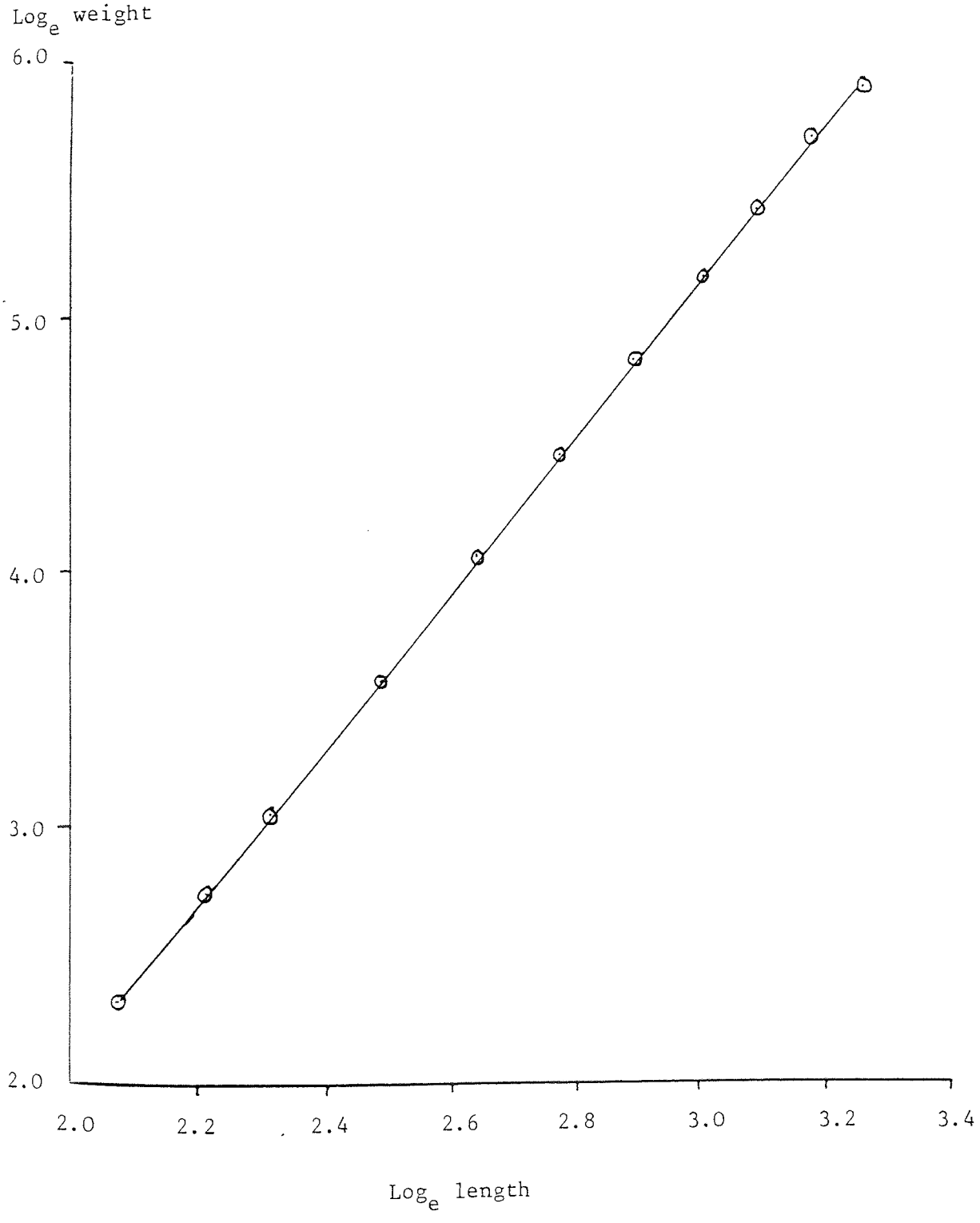
Thus the power to which L is raised is similar to that given previously and consequently the K values were calculated accordingly.

TABLE 2.4.32

Length-weight Data for Farmed Turbot

Weight(g)	Log_e Weight	Length(cm)	Log_e Length
10	2.3026	8	2.0790
15	2.7081	9	2.2083
21	3.0445	10	2.3026
35	3.5553	12	2.4849
58	4.0604	14	2.6391
87	4.4659	16	2.7726
127	4.8442	18	2.8904
180	5.1930	20	2.9957
236	5.4638	22	3.0910
305	5.7203	24	3.1781
375	5.9269	26	3.2581
445	6.0981	28	3.3322

Figure 2.4.10 Length-weight relationship for farmed turbot



The body composition data for Experiment 1.1 are presented in Table 2.4.33.

TABLE 2.4.33

Body Composition Data for Experiment 1.1

Diet	% Moisture		% Lipid (dry weight)		% Protein (dry weight)		% Ash (dry weight)		Gross (dry weight) Energy Jg^{-1}	
1(i)	78.1		11.2		72.2		16.6		21281	
1(i)	78.5	78.2	10.2	12.6	70.6	70.0	19.2	17.4	19236	20500
1(i)	78.0		16.4		67.3		16.3		20975	
1(ii)	75.8		16.3		67.1		16.6		22830	
1(ii)	77.0	74.0	14.1	15.0	67.6	67.2	18.3	17.8	20821	21700
1(ii)	69.2		14.5		67.0		18.5		21301	
1(iii)	80.2		8.0		73.8		18.3		19798	
1(iii)	77.0	78.3	9.5	10.6	71.9	71.6	18.6	17.8	19606	18500
1(iii)	77.8		14.3		69.1		16.6		19163	
1(iv)	77.8		10.7		73.5		15.9		21340	
1(iv)	79.8	78.9	12.4	11.6	71.3	72.4	16.3	16.0	20671	21000
1(iv)	79.1		11.6		72.5		15.9		20878	
Pre expt.	77.3		8.5		71.8		19.7		20083	

Analysis of variance failed to demonstrate any significant differences between the fish, except for Gross Energies:

Diet	1(iii)	1(i)	1(iv)	1(ii)
Average Gross Energy Jg^{-1}	18500	20500	21000	21700
At 5%				

So the only significant difference was between 1(iii) and 1(ii). This difference was probably a reflection of the dietary lipid levels.

The condition factors for experiment 1.1 are presented in Table 2.4.33.

TABLE 2.4.34

Condition Factors for Experiment 1.1

Diet	K Start	K End
1(i)	1.76	2.07
1(i)	1.80	2.18
1(i)	1.94	2.11
1(ii)	1.85	1.97
1(ii)	1.86	2.00
1(ii)	1.92	1.96
1(iii)	1.76	1.93
1(iii)	1.85	1.92
1(iii)	1.90	2.00
1(iv)	1.93	2.34
1(iv)	1.81	2.20
1(iv)	1.85	2.13

For further details see Appendix 2.

Analysis of variance showed no significant difference in condition factor for the fish at the start of the experiment. At the end of the experiment, the following analysis was evident.

Diet	1(iii)	1(ii)	1(i)	1(iv)
Average K	1.95	1.98	2.12	2.22
At 5%	———		———	

So 1(iii) and 1(ii) were not significantly different, but both were from 1(i) and 1(iv) which were not significantly different; so the sprat diets resulted in the greatest K values.

Body composition data for Experiment 2.1 are presented in Table 2.4.35.

TABLE 2.4.35

Body Composition Data for Experiment 2.1

Diet	% Moisture	% Lipid (dry weight)	% Protein (dry weight)	% Ash (dry weight)	Gross Energy Jg^{-1} (dry weight)
Pre-Expt	81.7	9.0	66.3	15.1	18200
2(i)	80.9	8.7	67.5	17.2	19058
2(ii)	80.7	8.3	65.4	16.0	19342
2(iii)	81.0	9.3	64.7	16.4	19113

Because of problems with analytical facilities, no replicate analyses were made - the samples represent pooled specimens from the duplicate tanks on the same diet. Consequently no statements about significant differences may be made. As can be seen there were no large numerical differences between the diets and pre-experimental fish.

The condition factors for Experiment 2.1 are presented in Table 2.4.36.

TABLE 2.4.36

Condition Factors for Experiment 2.1

Diet	K Start	K End	
2(i)	1.96	2.06	2.11
2(i)	1.97	2.15	
2(ii)	1.97	2.09	2.05
2(ii)	1.96	2.00	
2(iii)	1.97	2.00	2.00
2(iii)	1.98	1.99	

For further details see Appendix 2.

Analysis of variance showed no significant differences between the condition factors of fish on different diets and from the fish at the start of the experiment.

The body composition data for Experiment 2.2 are presented in Table 2.4.37.

TABLE 2.4.37

Body Composition Data for Experiment 2.2

Diet	% Moisture	% Lipid (dry weight)	% Protein (dry weight)	% Ash (dry weight)	Gross Energy Jg^{-1} (dry weight)
Pre-expt	78.0	10.8	58.5	14.4	20300
3(i)	78.3	15.7	58.5	17.2	22400
3(ii)	78.6	10.0	62.0	16.6	20400
3(iii)	79.7	10.5	65.5	17.6	19600

Once again, no replicate samples were analysed because of lack of analytical facilities. There is an interesting dietary effect here, viz Diet 3(i), the high lipid diet, caused an increase in carcass lipid levels which was quite marked. Also protein variations were evident in reflection of dietary protein variations.

The condition factors for Experiment 2.2 are presented in Table 2.4.38.

TABLE 2.4.38

Condition Factors for Experiment 2.2

Diet	K Start	K End	
3(i)	1.98	1.92	
3(i)	1.97	1.97	1.92
3(i)	1.99	1.87	
3(ii)	1.99	2.02	
3(ii)	1.98	2.07	2.02
3(ii)	1.78	1.96	
3(iii)	1.97	2.06	
3(iii)	1.98	1.98	2.01
3(iii)	1.97	1.99	

For further details see Appendix 2.

Analysis of variance of the condition factors showed no significant differences between condition factors of fish on different diets. It would appear from this data, and that of Experiment 2.1, that fish of around 20g did not vary in condition on the six diets tested. Variation in condition appeared to be more a factor in larger (100g) fish.

2.4.8 Digestibility of three diets containing no fresh fish protein

As some indications were evident in earlier experiments (1.1 and 2.1) that there were digestibility differences between diets, a digestibility test was conducted at the end of experiment 2.2, The analytical results are presented in Table 2.4.39.

TABLE 2.4.39

Chromic Oxide Analyses for Experiment 2.2

	% Chromic Oxide		% Protein		% Lipid	
Diet	Diet	Faeces	Diet	Faeces	Diet	Faeces
3(i)	3.02	7.82	46.1	15.26	26.1	4.24
3(ii)	3.06	6.25	51.1	16.94	78.3	3.68
3(iii)	3.10	5.11	65.1	18.57	10.6	1.03

From these data, the digestibilities of protein and lipid were calculated as follows.

$$\text{Digestibility (\%)} = 100 - 100 \frac{\text{\% indicator in feed}}{\text{\% indicator in faeces}} \times \frac{\text{\% nutrient in faeces}}{\text{\% nutrient in feed}}$$

These data are presented in Table 2.4.40.

TABLE 2.4.40

Digestibility Coefficients for Experiment 2.2

Diet	Protein % Digestibility	Lipid % Digestibility
3(i)	87.3	93.7
3(ii)	83.8	90.2
3(iii)	79.9	94.1

Due to the small amount of faeces obtained, only limited analyses were possible. The protein digestibility decreased with increasing dietary protein levels. This is a further indication of some defect, or indigestibility, in the compound feed and has implications when considering results from former experiments. The digestibility of the lipid differed very little with increasing dietary fat, even up to 26%.

2.5 Discussion

This series of experiments was beset by a number of complications, such as a change of location and the initial utilisation of a recycling system. It is apparent when comparing results that more replication should have been used; however the constraints of carrying out research on a fish farm were such that it was not always possible to have more than duplicates. It was also evident from the results that wide variation occurred within replicates of one dietary treatment. It was for this reason that analysis of variance was applied to the results, to make statistical statements about the resulting data.

It was stated previously that the aims of this research were to quantify the claimed superior nutritional qualities of sprat and to formulate a diet that could come as close as possible to replicating this without containing any fresh fish component. Obviously, one attempts to specify a diet that results in maximum performance in all facets, such as growth rate, food conversion, protein utilisation, etc., whilst not affecting the body composition.

Comparison of growth rates is only valid at the same temperature for similar sized fish, as growth rate is a function of mean weight and temperature. In Experiment 1.1, the sprat-based diets produced significantly higher growth rates than the other two diets (Table 2.4.7). The maximum growth rate was for diet 1(iv) at 9.54 which compares to 11.1 in Purdom et al (1972), for turbot fed on trash fish at 18°C. In Experiment 1.1, fish were fed on a modified satiation regime, so this is a possible reason for differences in growth rates, ie fish eat more of a certain diet and therefore grow faster. Examination of the data in Table 2.4.18, shows that there were very marked differences between wet weight % diet consumption

rates. However, as discussed previously, there were also very marked differences in moisture content between the diets and so it is more correct to compare food consumption rates on a dry weight of food-fed basis, as in Table 2.4.19. This transformation tended to rationalise the data for three of the diets, so that they were insignificantly different but diet 1(iv), of which more was consumed on a wet weight % basis, was transformed to being the lowest % consumption. Clearly then, the amount of dry food consumed was not the controlling factor when considering a fish diet, but may have been influential when considering diets with a compound feed fraction. Bromley (1974a) suggested that the factor controlling feed intake was the amount of dry matter in the food. However it is usually considered that fish eat to meet their energy requirements. Accordingly, if one converts the amount of food fed into energy units, one would expect to see similar relationships for all the diets. These data are presented in Tables, 2.4.20 and 2.4.21 and Figures 2.4.8 and 2.4.9. Whilst gross energy intake rates were fairly similar for diets 1(iii) and 1(i) and 1(i) and 1(ii), they were significantly different from diet 1(iv). It must be remembered that the energy units quoted here are gross energy units. Not only were the rates of energy consumption different but the levels also. It would appear from Figures that the fish fed diet 1(iv) consumed much less, on a gross energy basis, than those on the other three diets. There are two possible explanations of this anomaly. Firstly, it was difficult to quantify waste food when feeding diets 1(i), (ii) and (iii), as they tended to break up in the water; however, diet 1(iv) waste could be removed and weighed. The second explanation may lie in differences in digestibility and hence metabolisable energy between the diets. Diet 1(iv) was a fairly natural diet and thus one might

intuitively expect the gross energy to be a more accurate reflection of the metabolisable energy, when compared to the other three diets which have a compounded fraction in them. A more accurate way to compare diet performance would be to feed on an isoenergetic metabolisable energy regime. In Experiment 1.1, one of the aims was to examine and quantify preferential diet uptake rates. It was evident that the fish ate less of the whole fish diet on a dry weight of food fed basis and gross energy basis, than of the other diets. Conversely, this diet produced the greatest growth rate.

To try and eliminate preferential uptake as a factor in altering growth rate, the feeding in Experiment 2.1 was on an isoenergetic gross energy basis. No digestibilities or metabolisable energy data were available for the diets so, as they contained equivalent levels of fresh fish and compound feed, an isoenergetic gross energy feeding regime was used. The specific growth rate data from this experiment are presented in Figures 2.4.2 and 2.4.10. No great differences were apparent, although the sprat-based diet (Diet 2(i)) resulted in the fastest growth rate. Direct comparisons between this experiment and Experiment 1.1 are not valid, as different fish sizes and temperature were used. However in Experiment 2.2, similar fish size and temperature were used and also a similar feeding regime. There was no significant difference in growth rate between the diets in Experiment 2.2 (Figure 2.4.3 and Table 2.4.13); however all the rates recorded were higher than those for Experiment 2.1. The reason for this difference, is doubtless due to the different feeding rates employed in the two experiments, see Tables 2.3.2 and 2.3.3. The temperatures and mean weights were similar yet the fish in Experiment 2.2 ate far more than in Experiment 2.1. The actual reason for this

remains unexplained. These growth rates may be compared to those recorded by various other workers. The specific growth rate recorded by Purdom et al (1972) for 8-16g fish fed a minced fish diet at 13°C was 12.6. Deniel (1973) recorded specific growth rates of 9.8 for the FP_{40} diet, 7.8 for the FH_{40}-FP_{40}, 9.1 for the FP_{40}-LEP-CPFP diet and 10.0 for a fish diet (ranging from whiting (*Merlangius merlangus*) to mackerel (*Scomber scombrus*)) for similar sized turbot to Experiments 2.1 and 2.2 at 14°C. Comparisons are difficult, as a number of feeding regimes were used. In Deniel's and Purdom's work, the fish were fed to satiation and this may well be the cause of higher specific growth rates. However, by using satiation feeding, it was possible for wastage to occur and so in striving for maximum growth rate, conversion rate is sometimes poorer. The economic and technical implications of these results are discussed in section 4 of this thesis.

As mentioned previously, the most accurate way of comparing food conversion ratios is to convert the amount of food fed to a dry weight. The food conversion ratio data for Experiment 1.1 are presented in Tables 2.4.22 and 2.4.23. The whole sprat diet (1(iv)) returned a far lower food conversion than all the other diets, with the sprat-based moist pellet second. The performance of the dry diet (1(ii)), was very poor. This food conversion for diet 1(iv), was coupled with the greatest specific growth rate and so here was a diet which resulted in maximum growth rate and minimum food conversion. This excellent food conversion on sprat has also been reported by Purdom et al (1972) and Deniel (1973). It is interesting that diets 1(iii) and 1(i) produced different responses, as they both contained the same level of fish but from different sources. The sprat-based diet 1(i) demonstrated superior growth and food conversion

rate, compared to the pouting-based diet (1(iii)). This difference between different fish sources was further investigated in Experiment 2.1. As the protein requirements for marine flatfish is high (Cowey et al 1972), it was decided to increase dietary protein by the addition of fish meal to the compound feed (see Table 2.4.2). Also, in order to eliminate differences in dietary uptake, the fish were fed a restricted ration. The food conversion data are presented in Table 2.4.24. Although no significant differences in food conversion were recorded, the sprat-based diet (2(i)) gave the superior result. This conversion was, however, matched extremely closely by that of diet 2(ii), which was a mackerel-based diet. The conversion rate on the diet 2(i), was marginally greater than diet 1(i) which was also sprat-based. Diet 2(iii) gave a very similar result to Diet 1(iii), which was also a pouting-based diet. So the addition of fish meal had not radically altered food conversions. Experiment 2.2 was an attempt to match the performance of a sprat-based diet, without any fresh fish ingredient. Experiments with rainbow trout (eg Higuera et al (1976) and turbot (Adron et al 1976) have demonstrated the beneficial effect of increasing dietary non protein energy, on conversion and protein utilisation. The protein : fat energy ratios chosen in Experiment 2.2 were such as to reflect the composition of sprat. Once again the feed intake was controlled. Diet 3(ii) produced the lowest food conversions (see Table 2.4.25), which was a close reflection of the superior growth rate. However, the conversion at 1.40 was still poorer than that for a sprat-based diet (1.20) and particularly whole sprat (0.59). However, the conversion for Diet 3(ii) does compare favourably with those reported by Deniel (1973) which were 1.6 (FP_{40}), 1.2 (LEP) and 1.4 (CPFP) and diet 1(iii) and 2(iii), which contained pouting. Clearly all

these conversions were still a long way behind that for sprat at 0.59. The economic and technical implications of these results are discussed further in Section 4 of this thesis.

As protein is one of the most expensive components of the diet, it is obviously of importance to ensure that a maximum rate of conversion of dietary protein to fish protein occurs. Two indices of protein utilisation were evaluated and the results from the whole experimental series are presented in Table 2.5.1.

TABLE 2.5.1

PER and EPC Data for all Experiments

Diet	PER	EPC %	% Diet Protein	% Non Protein Energy	% Fresh Fish Protein (of total protein)
1(i)	1.76	23.8	54.9	53.7	25.1
1(ii)	1.13	16.0	47.9	60.2	0
1(iii)	1.35	18.0	51.1	57.5	21.3
1(iv)	2.15	30.0	73.5	30.0	100
2(i)	2.21	35.1	64.4	40.0	34.6
2(ii)	2.06	36.3	64.4	40.9	34.6
2(iii)	1.69	30.1	67.5	36.8	37.7
3(i)	1.51	18.0	46.1	42.9	0
3(ii)	1.45	20.6	51.1	32.2	0
3(iii)	1.27	15.7	56.1	19.6	0

As can be seen, the EPC generally reflected the PER ranking. For Experiment 1.1, the EPC data showed no significant differences whilst the PER values did. EPC is a better reflection of what is happening to dietary protein, as the body composition of the fish is accounted for. Protein is the principal energy source in turbot but it can, to some extent,

be replaced by lipid and carbohdrate (Adron et al 1976). The increase of non protein energy leads to more protein being available for building fish protein, as opposed to being an energy source. Cowey et al (1970a), determined the optimum PER for plaice at 50% dietary protein. The relation between PER and dietary protein level for plaice and carp is presented in Cowey and Sargent (1972). The relationship for the experiments reported in this thesis are presented in Figure 2.5.1. The data reported in Cowey et al (1972) demonstrated a PER asymptote around 40% for plaice fed a diet based on freeze-dried cod muscle. This is a possible explanation of expression(2) on Figure 2.5.1, as demonstrating decreasing PER with increasing dietary protein above 40%. However expression(1) demonstrates increasing PER with increasing dietary protein. The data for experiments 1.1 and 2.1 were further investigated, as the relationship between PER and fresh fish protein % (as distinct from fish meal protein) in the diet. These data are presented in Figure 2.5.2. In order to further clarify this relationship, the data from other workers were recalculated and plotted on Figure 2.5.2. Reference numbers on the Figure are as follows:

(1.) WFA (1979)

(2.) Bromley (1974a)

(3.) WFA (1976)

(4.) Purdom et al (1972)

(5.) Walne (1973)

(6.) Jones et al (1980).

It is possible to conclude, that differences in the quality of the protein source induced this effect on the PER.

Cowey et al (1972) used freeze-dried cod muscle as a fish protein

Figure 2.5.1 PER and % dietary protein relationships for all Experiments

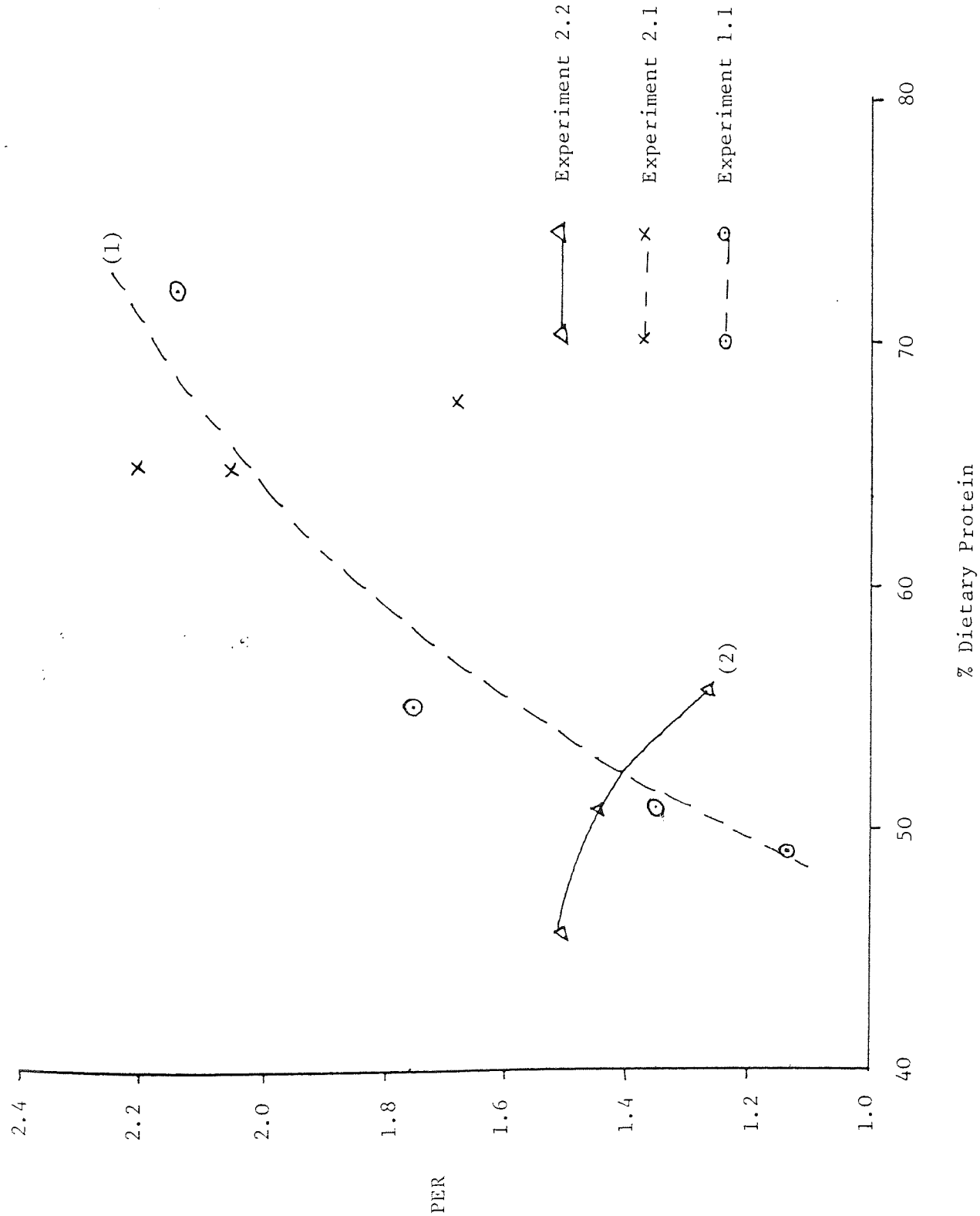
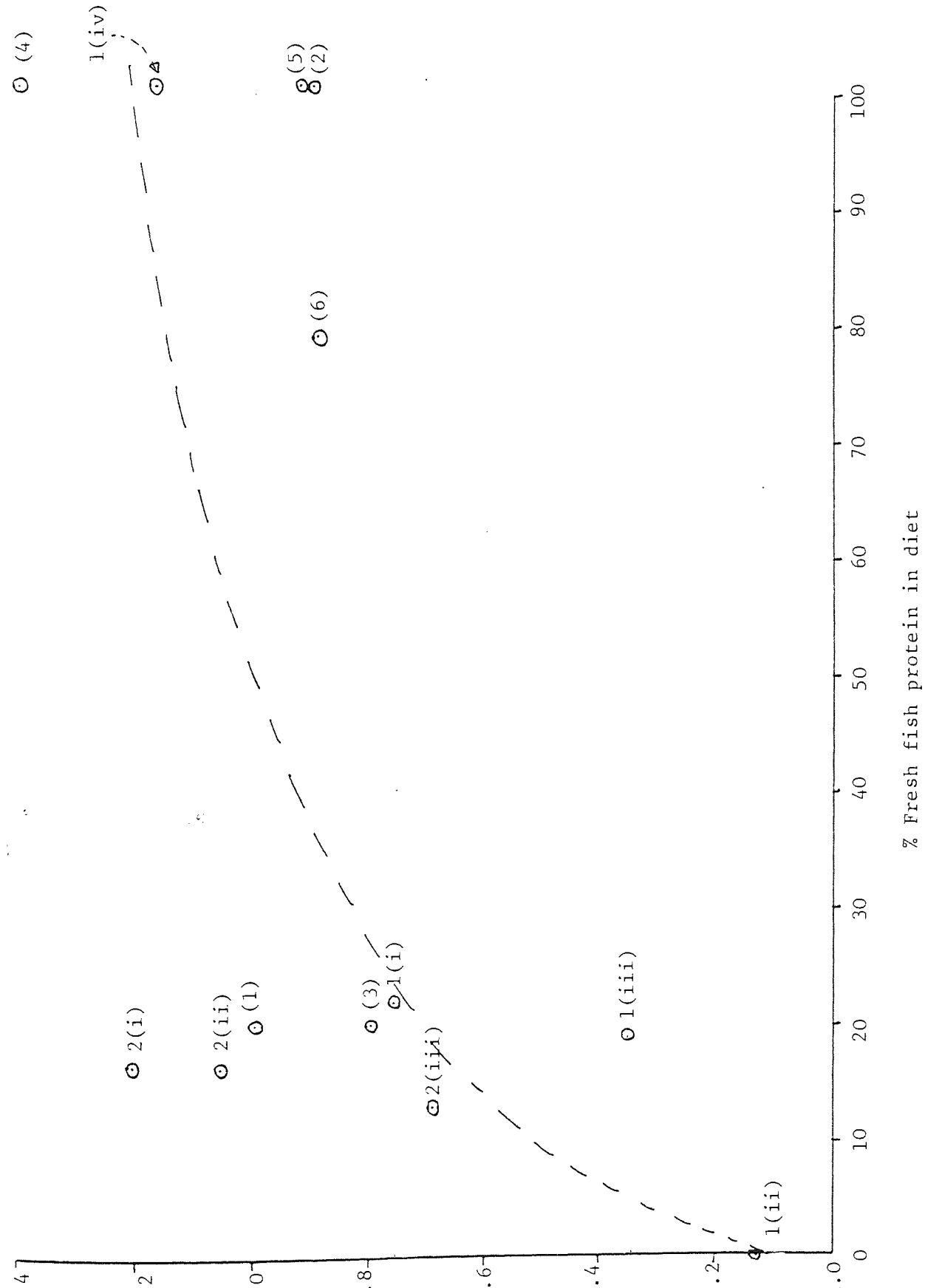


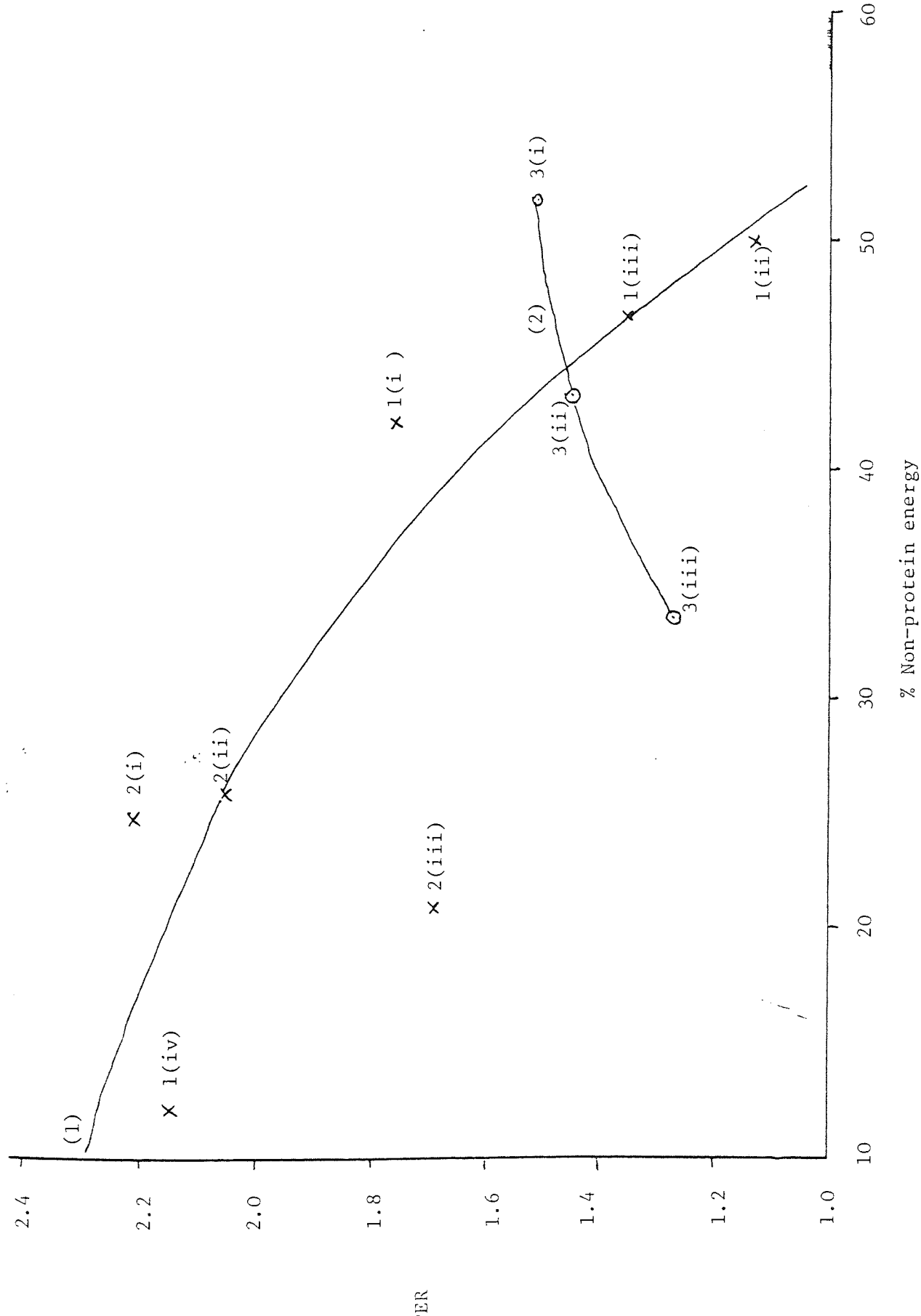
Figure 2.5.2 PER as a function of % fresh fish dietary protein



source and Bromley (1974a) used specially prepared trash fish meal, as he claimed that commercially prepared fish meals were nutritionally inferior. It would seem necessary therefore to supplement the fish meal with certain amino acids that are deficient. Finally, PER values can be examined as a function of the energy content of the diet. As mentioned before, lipid exhibits a protein-sparing capacity up to certain levels. These data are presented in Figure 2.5.3, where once again two relationships appeared to be evident.

Relationship (1) shows a steady decline in PER with increasing % non protein energy. This is similar to the relationship in Figure 2.5.1 and may be due to differing dietary protein sources. Relationship (2) is for Experiment 2.2 where the same protein and fat source were used, but in differing ratios. Here the protein sparing effect of lipid is demonstrated up to the highest level used (26%) although it is likely that the relationship was reaching an asymptote here. This result is in line with that reported by Adron et al (1976), who recorded a maximum PER (2.73) on a diet containing 50% non protein energy. Bromley (1974) reported a maximum PER (2.6) at a 16% dietary lipid level, with turbot fed a lipid-supplemented pouting diet. Increasing the lipid level to 28% and 40% failed to increase this. The actual composition of the lipid used to supplement the diets is critical, as turbot have very limited ability to chain elongate and desaturate dietary fatty acids (Owen et al 1975, Cowey et al 1976). Hence preformed long chain polyunsaturated ω3 fatty acids must be supplied in the diet. The effect of different dietary lipids, at 4% of diet, on PER was reported in Walne (1973). Hydrogenated coconut oil gave a PER of 0.89, corn oil gave 1.23 and cod liver oil 1.65. The decision to use special fish oil for Experiment 2.2 was taken as it is

Figure 2.5.3 PER as a function of non-protein energy



one of the few commercial whole fish oils available and because it is similar to sprat oil (See 2.4.1).

In Experiment 1.1, diet 1(iv) demonstrated a large difference in energy conversion efficiency with the sprat-based pellet diet (1(i)) second, although itwas not significantly different from the other two diets. The results from Experiments 1.1 and 2.1 reflect the findings of the protein utilisation data and may therefore be reflections of protein quality. Bromley (1974a), reported a maximal energy conversion (40%) at a dietary energy level of 22.9 kjg dry diet^{-1}. In Experiment 2.2, the energy utilisation efficiency reached 23.2%, at a dietary energy level of 21.3 kjg dry weight^{-1} and did not significantly alter at the higher dietary energy level of 22.5 kjg dry weight^{-1}.

It is important that diet manipulation to ensure maximal growth rate etc., does not significantly alter the body composition of the fish. Turbot are not naturally a fatty fish (typical levels are around 12% - Adron et al 1976) and so it is important that increasing dietary energy levels do not render this higher. Adron et al (1976) found that dietary energy levels up to 13.167 kjg dry diet^{-1} (containing 50% non protein energy) did not increase the carcass lipid level above that of wild fish. Taking the value of 12% as a standard, in Experiment 1.1 only one diet produced fish with a value in excess of it, ie the fish on diet 1(ii) (dry pellet) at 15.0%. The lipid levels for Experiment 2.1 were all similar to the pre-experimental levels. In Experiment 2.2, the high lipid diet (26% diet 3(i)) produced fish with fatty carcases - 15.7%, which was higher than that for the other diets (at 10%) and for the pre-experimental fish (10.8%). To use diet 3(i) on a fish farm would be unacceptable, because of the effect on the carcass.

Turbot of the range 1.0 - 2.0 kg are marketed generally as fillets. One of the main bonuses of farmed turbot are that they are generally heavier for their length, ie have a higher condition factor than their wild counterparts. This leads to better fillets and a more favourable market reaction. Condition factor is a function of fish size, as mentioned previously, so one can not compare condition factors between Experiments 1.1 and 2.1 and 2.2. In Experiment 1.1, diet 1(iv) (sprat) produced the highest K value, with diet 1(i) (sprat pellet) second. These two were significantly different from diets 1(ii) and 1(iii). In Experiment 2.1, the sprat diet (2(i)) again produced the highest K value, although it was not significantly different from the other two diets. In Experiment 2.2, diet 3(ii) produced the highest K value, although there was no significant differences between the diets. The value of diet 3(ii), was less than that for 2(i) and so the sprat-based diet still produced the greatest condition factor.

The data for all the experiments are presented in Table 2.5.2, in order to draw comparisons between all the factors tested.

In comparing all the factors tested, diet 1(iv) (sprat) was the optimum diet, except in one factor (PER) where it was exceeded by diet 2(i), which was a sprat-based pellet. The aim of this experimental series was to produce a diet that would induce similar performance to fresh fish-based diets, without containing any fresh fish. Diet 3(ii) induced faster growth than any of the fresh fish based diets in Experiment 2.1 Food conversions were lower for 3(ii) than for any of the pouting based diets (1(iii) and 2(iii)). The PER was also greater for 3(ii) than for 1(iii). Finally the EEU values were greater for 3(ii) than any of the other fresh fish based diets (1(i), 1(iii), 2(i), 2(ii) and 2(iii)).

TABLE 2.5.2

Summary of all factors tested

Diet	Specific growth rate x 10^{-3}	FCR (dry weight)	PER	EPC	EEU	K
1(i)	8.18	1.09	1.76	23.8	18.8	2.12
1(ii)	4.63	2.09	1.13	16.0	14.7	1.98
1(iii)	5.31	1.48	1.35	18.0	10.2	1.95
1(iv)	9.54	0.59	2.15	30.0	36.6	2.22
2(i)	6.33	1.20	2.21	35.1	18.0	2.11
2(ii)	5.90	1.23	2.06	36.3	17.4	2.05
2(iii)	5.25	1.45	1.69	30.1	15.4	2.00
3(i)	7.54	1.76	1.51	18.0	16.9	1.92
3(ii)	8.27	1.40	1.45	20.6	23.1	2.02
3(iii)	8.30	1.48	1.27	15.7	23.2	2.01

Such factors as FCR have important implications in considering the commercial application of diet formulations. For further discussion of these matters see Section 4.

3. OXYGEN CONSUMPTION AND METABOLISM OF TURBOT

3.1 Introduction

Oxygen consumption of salmonids is well documented over a range of temperatures and fish sizes. To date, little information has been published on the oxygen consumption of marine flatfish (see Edwards et al 1969). Furthermore, recent research has demonstrated (Harmon 1978, Muller-Feuga et al 1978) that there are large variations between the data published for oxygen consumption determined in the laboratory and those determined in the field, ie on fish farms. A knowledge of the oxygen consumption rate of a species of fish is imperative when that species is farmed, as it is instrumental in determining the water flow rate required to a tank to maintain life. It is for these reasons therefore, that a comprehensive study of the oxygen consumption of turbot was conducted, at temperatures from 7°C to 16°C and over a range of fish sizes from 1g to 1000g. Also important in intensive fish farming is the affect of feeding and the influence of diet on oxygen consumption. Having determined and supplied the oxygen requirements of the fish, the actual water requirement is then a function of the tolerance of turbot to carbon dioxide and ammonia. Consequently, the flow rates are adjusted so as to ensure that these metabolites do not reach toxic or sublethal concentrations.

The respiratory metabolism of salmonids has been intensively studied by Brett (1964, 1965, 1970) and Liao (1971). The metabolic rate of fish is influenced by many factors and Brett (1970) drew up a list, with some indication of the influence of each factor. This is illustrated in Table 3.1.1.

TABLE 3.1.1

Various factors which may affect metabolic rate, with estimates of the extent of possible influence

	ABIOTIC			BIOTIC	
1	Temperature (+10°C)	+ 5∿3x	10	Activity (max)	+5∿15x
2	Salinity (0→30‰)	-10∿15%	11	Weight($M=aW^b$)	b = 0.7∿1.0
3	Oxygen (10-18ppm)	→ -100%	12	Sex (gonad = 12%W)	?
4	Carbon dioxide (2ppm)	10x-20%	13	Age (vs size)	?
5	Ammonia (0.3ppm NH_3)	⇌ toxic	14	Group (Schooling)	-20% ?
6	pH (0.5-0.9)	?	15	O_2 Debt (Normalload)	±20% ?
7	Photoperiod	30%	16	Condition(exercised)	A + 30% ?
8	Season	30%	17	Starving (10 days)	S = 20% ?
9	Pressure	?	18	Diet (fat ratio)	± 6%

Symbols denote increase (+), decrease (-), times (x), approximate range (∿), potential influence (→), dissociation involved (⇌), active rate (A), and standard rate (S).

It is obvious that some of these factors are interrelated and Brett considered the most important influencing factors to be temperature, mean weight, activity and diet. Oxygen consumption rates are generally defined in three categories: resting, routine and active (Beamish 1964). The 'resting' rate describes when there is no spontaneous activity, the 'routine' rate is when spontaneous activity occurs and 'active' rate is induced by forcing the fish to swim. Considerable attention has been focussed on active respiration in salmonids and relating oxygen consumption

to swimming speed.

The actual methods employed by Brett (1964) and others, have generally involved placing single fish in specially constructed respirometers. In these chambers fish have no free swimming space; ie they can only swim in one direction. It seems unlikely therefore that these results would apply to a fish farm where freedom of movement is allowed. Also considering Table 3.1.1, many of the factors are relatively constant on a fish farm and so Muller-Feuga et al (1978) point to feeding level, fish size and temperature, as being the most important variables in determining oxygen consumption. Two approaches have been adopted for determining oxygen consumption on fish farms, by Harmon (1978) and Muller Feuga et al (1978).

Harmon's work was part of a study to investigate excessive oxygen utilisation on an intensive trout farm at Low Plains, Cumbria. As mentioned previously, the fish farming method employed by Shearwater Fish Farming Limited is very intensive, whereby tanks are stocked very highly and pure oxygen is dissolved in the tank water, to supplement that in the inlet water (see Harmon 1978 for further details). At these high stocking densities, interruption of the oxygen supply results in a rapid fall in dissolved oxygen saturation (100% - 60% in 25 minutes). Harmon (1978) used this procedure and recorded the subsequent reduction in oxygen concentration on a chart recorder, which showed a linear decrease down to 50% saturation. The water supply to the tank was maintained and measured. From the chart trace, a rate of oxygen depletion was calculated and given the water flow rate, tank volume and fish biomass, oxygen consumption in terms of $gO_2 kg\ fish^{-1}\ hr^{-1}$ could be calculated. Allowance was also made for the water displacement made by the fish. This procedure was repeated with several fish sizes from 15g to 155g at

8.5°C. According to Harmon (1978) the two main errors of this method were diffusion of oxygen from the atmosphere into the tank and also Biochemical Oxygen Demand (BOD). The maximum rate of mass transfer was calculated as being only 4% of the oxygen demand and BOD accounted for about 2%. Harmon calculated the overall error (including tank volume estimation error and tank biomass estimation errors)as being ± 10%.

The other approach has been that of Muller-Feuga et al (1978), who applied multiple regression analysis to their results, to determine the effect of temperature and size on oxygen consumption under standard feeding conditions. Various groups of rainbow trout, with an average weight of 12-925g, were acclimated for 2-3 days in ponds at densities of 5-10 kgm^{-3}. Prior to measurements of oxygen consumption, the water flow was adjusted so as to have 6 $mg\ell^{-1}$ dissolved oxygen at the outlet to the pond. Every three minutes for at least one hour, the difference in oxygen content between inlet and outlet water was recorded and corrected for surface exchange, by the method of Gorin et al (1977). The average relative error when measuring oxygen demand was calculated as 4%. The fish were fed according to standard trout feeding tables and oxygen demand was measured when the fish had settled down after feeding activity.

The data from these determinations of oxygen consumption shows considerable variation, as each experimental series seems to differ. Harmon (1978) illustrated the difference between his data and that of Liao (1971) and Willoughby et al (1972) at one temperature. Whilst the data of Liao and Willoughby were similar at low fish mean weights (0.24 $gO_2\ kg^{-1}\ hr^{-1}$ for 10g fish), the subsequent relationship between oxygen consumption and mean weight differed such that for 200g fish Liao recorded

an oxygen consumption rate of 0.17 $gO_2\ kg^{-1}\ hr^{-1}$, whilst Willoughby et al recorded a rate of 0.11 $gO_2\ kg^{-1}\ hr^{-1}$. Harmon (1978) demonstrated a similar relationship between oxygen consumption rate and fish size to Willoughby et al (1972), but the values recorded were considerably higher, eg for 200g fish, Harmon recorded a value of 0.18 $gO_2\ kg^{-1}\ hr^{-1}$, whilst Willoughby et al recorded 0.11 $gO_2\ kg^{-1}\ hr^{-1}$. However, these values differ to those given by Muller Feuga et al (1978) who recorded 0.08 $gO_2\ kg^{-1}\ hr^{-1}$ and Bass (1978) who recorded 0.10 $gO_2\ kg^{-1}\ hr^{-1}$ for 200g fish at 9°C. The main reasons for the discrepancies between all these data is the lack of standardisation. Harmon (1978) listed some of these differences.

1. It must be stated whether the data are derived from an average 24 hour value, or for when the fish have been fed their daily ration (the latter was the case with Harmon and Muller-Feuga).
2. The diet must be specified in terms of both its energy content and protein content, as increasing protein content increases oxygen consumption.
3. The conditions under which the experiments were conducted must be specified with particular reference to water current. Harmon gives the high peripheral water velocity in the fish tanks as a possible reason for his data being higher than that of other workers, as the fish tend to be fairly active, compared to a pond where circulation rates are much less.
4. The stocking density under which the fish are kept (kgm^{-3}) should be given, as the subsequent effect on water quality (particularly CO_2) may have an effect on metabolic rate.

The conclusions that one reaches is that whatever farming system one is employing, the relevant data must be consulted.

Studies of oxygen consumption rates of UK marine fish, have been made by Edwards et al (1969) on plaice and dabs, Edwards et al (1972) on cod and Jobling and Spencer Davies (1980) on plaice.

Edwards and his co-workers used a closed circuit respirometer for both the cod, plaice and dab experiments with an incorporated oxygen probe. Also estimates of active metabolism were made, by inducing the fish to swim in a current of about 7.5 cm sec^{-1}. The 'resting' experiments lasted for about two hours (% drop 5%) and the 'active' experiments lasted about 30 minutes (% drop 14%). Oxygen consumption was determined for resting and active plaice at 10°C, for a range in body size from 0.1 to 10g. Also consumption was measured for three fish sizes at 5, 10, 15 and 20°C for 'resting' plaice and for one fish size at 5, 10 15 and 20°C with active plaice. Similar determinations were made with 'resting' dabs at 10°C and for 0.4g dabs at 5, 10 and 15°C. The same apparatus was also used by Edwards and his colleagues, for determining the oxygen consumption of 'resting' cod at 12°C (the resting state was induced with MS222 as detailed by McFarland (1959)).

The experimental data for resting plaice at 10°C yielded the following relationship:

$$Q_{O_2} = 0.214\ W^{0.721}$$

where Q_{O_2} = oxygen consumption in $m\ell\ O_2\ fish^{-1}\ hr^{-1}$, W = live wt g. This is equivalent to 0.0445 $gO_2\ kg^{-1}\ hr^{-1}$.

For active plaice at 10°C the following relationship was apparent.

$$Q_{O_2} = 0.737\ W^{0.590}$$

This is equivalent to 0.062 $gO_2\ kg^{-1}\ hr^{-1}$.

The corresponding equation for resting dabs was

$$Q_{O_2} = 0.449\ W^{0.666}$$

This is equivalent to 0.064 $gO_2\ kg^{-1}\ hr^{-1}$ and for 'resting' cod

$$Q_{O_2} = 0.245\ W^{0.82}$$

This is equivalent to 0.101 $gO_2\ kg^{-1}\ hr^{-1}$. The relationship between oxygen uptake and temperature is known as Q_{10} and for plaice was determined as

5-10°C	10-15°C	15-20°C
2.6	3.6	5.6

The Q_{10} value for dabs at 10-15°C was 2.1. Saunders (1963) gave a Q_{10} value for cod at 10-15°C of 2.48. These correspond to values for rainbow trout of 3.5 below 10°C and 1.7 above 10°C. (Muller-Feuga et al (1978)).

The equation $Q_{O_2} = aW^b$, is the general equation relating resting oxygen consumption to body weight and the value of b is usually around 0.8 (Winberg 1956). The value of a is dependent on the environment and condition of the fish. One of the main problems when relating values to the above equation is that it represents 'resting' metabolism and any slight activity will affect the values of a and b. Thus it can be seen that several values of b have been determined for plaice that were supposed to be resting. The data for cod are also variable as Saunders gave 0.791 for starved cod and 0.886 for fed cod. Also Sundnes (1957) gave a value of 0.71 for cod and pollack.

It is obvious, then, that one must try and specify exactly the conditions under which the determination was performed. Flatfish are normally fairly sedentary, particularly turbot on a fish farm, and much

of the time is spent lying on the tank bottom, so one could intuitively expect the oxygen consumption rates to be lower than that of fish that continuously swim, such as cod and trout.

Consider now the effect of one particular aspect on metabolism viz feeding. For calculating energy budgets, Winberg (1956) assumed that the metabolic rate of feeding fish was about twice the rate associated with non-feeding fish subject to undisturbed activity (routine metabolism). This was a fairly subjective measure, which depended on the actual method of determination of metabolic rate. Paloheimo and Dickie (1966) demonstrated, using an indirect method of assessing total metabolism, that *ad libitum* feeding required an energy expenditure of 6-8 times that for maintenance feeding. They therefore considered that routine metabolism characterised fish on a maintenance ration and active metabolism was characterised by those fish on a full ration. However, two areas of controversy were identified by Brett (1970) in this analysis. Firstly, the evidence was based on the assumption that the utilisable or net energy derived from food is independent of the size of the ration. Secondly, that a decrease in the gross conversion efficiency of food is shown to be dependent on ration size. Preliminary experiments by Brett (1970) with sockeye salmon at 20°C, indicated that oxygen metabolism rose from about 100 mg kg^{-1} hr^{-1} when starving, to 300 mg kg^{-1} hr^{-1} when feeding on a maintenance ration, to 450 mg kg^{-1} hr^{-1} when feeding on a maximum ration. Brett concluded that continuous feeding on a high ration, induced about one half of the active metabolic rate and about four times the standard metabolic rate in sockeye salmon. Relationships between metabolism and food intake have also been shown for plaice (Edwards et al 1969) and cod (Edwards et al 1972). In order to remove differences in

fish weight as a variable in the relationship, Edwards and his co-workers assumed the value of b, in the expression $Q_{O_2} = aW^b$, to be constant at 0.721 for plaice and therefore the value of a varied with experimental conditions. In practice, the value of Q_{O_2} could be represented by:

$$Q_{O_2} \ (m\ell\ O_2\ hr^{-1}) = 0.214\ W^{0.721}$$

Metabolic rate M was related to food F and growth G, thus :

$$\frac{F}{Q_{O_2}} = \frac{G}{Q_{O_2}} + \frac{M}{Q_{O_2}}$$

The experimental data was used to derive the following regression equation:

$$\frac{M}{Q_{O_2}} = 0.42\ \frac{F}{Q_{O_2}} + 0.34$$

Thus, when the fish were starved (F=0), the metabolic rate was 0.34 Q_{O_2}; this agreed with the actual result from the first period of experimental starvation. Also maintenance (G=0), would be obtained with a food intake and oxygen uptake of 0.62 Q_{O_2}. However, there was evidence that as the fish grew larger, there was a decline in the food taken and an increase in metabolic rate relative to a given level of food intake. The combined effect of these, was a marked decrease in time with the efficiency of growth, as a proportion of food assimilated ($\frac{G}{F}$), as had been indicated previously by Dawes (1930, 1931). A similar relationship between metabolism ($\frac{M}{Q}$) and food intake ($\frac{F}{Q}$), was determined for cod by Edwards et al (1972) and the data fitted the following regression:

$$\frac{M}{Q_{O_2}} = 0.784\ \frac{F}{Q_{O_2}} + 0.134$$

A more recent examination of the effect of feeding on metabolic rate

in plaice, is that of Jobling and Spencer-Davies (1980). The rate of oxygen consumption in plaice was found to rise after feeding and did not decline to a resting level until 24-72 hours later. This post-prandial increase in the rate of oxygen consumption corresponds to the Specific Dynamic Action (SDA). Quantitative approaches to SDA have been made by Muir and Niimi (1972) with aholehole (*Kuhlia sandvicensis*), Beamish (1974) with largemouth bass (*Micropterus salmoides*) and Miura et al (1976) with biwamasu (*Oncorhynchus rhodurus*). Jobling and Spencer-Davies (1980) used plaice of 16-50g and determined SDA at 10°, 15° and 20°C in a continuous flow respirometer. Neither the maximal stimulation of oxygen consumption (expressed as a percentage of the resting rate), nor the magnitude of the SDA were influenced by the experimental temperature. However, the duration of the SDA was found to be temperature dependent (51.4 hours at 10°C and 26.0 hours at 20°C). The shorter duration of SDA at higher temperature was correlated with the correspondingly higher rates of digestion and gastric evacuation (Jobling and Spencer-Davies 1979). In comparing the magnitude of the SDA with that of other published work, Jobling and Spencer-Davies proposed that the most useful SDA coefficient was the relationship between the SDA magnitude and the digestible energy, not ingested energy. The reason for this is that it is generally agreed that the SDA is a post-absorptive phenomenon and so one must account for differences in digestibility between test diets. The coefficient of magnitude of SDA against digestible energy, obtained by Jobling and Spencer-Davies, was 0.192 on white fish paste. This compares to the value of 0.17 for *Micropterus salmoides* (Beamish 1974) and 0.23 for *Oncorhynchus rhodurus* (Miura et al 1976), fed fish-based diets. Jobling and Spencer-Davies also demonstrated that, the SDA coefficient

increased in proportion to the digestible protein content of the diet, as shown by Cho et al (1975, 1976) for *Salmo gairdneri*.

With all the preceding data for the apparent SDA in salmonids, cod, etc., an experimental programme was initiated, to examine the SDA in turbot. As has been discussed, SDA varies with the nutrient composition and so oxygen consumption determinations were run in parallel to Experiment 2.2, as different levels of protein energy were used. Furthermore, SDA data were calculated from the chart traces from continuously monitoring Production Tank 3's oxygen level.

3.2 Materials and Methods

3.2.1 Fry Tank Determinations

The fry shed at Wylfa, which has already been described (2.3.1), contains a number of 300ℓ circular fibre glass tanks. Two supplies of water were available, so a range of temperatures could be obtained by adjusting the proportion of ambient and power station cooling water. The tanks were stocked with young '0' group turbot varying in size from 1g to 66g. Some of the oxygen consumption studies were conducted in parallel with Experiment 2.2 and these data are discussed in 3.3.2. The fish were usually fed on a diet similar to 2(i), as this is the usual production diet at Wylfa. Two methods were used to measure oxygen consumption in the fry tanks: a static system and a dynamic system. The static system consisted of turning off the incoming water to the tank and monitoring the decrease in dissolved oxygen level over a certain period of time, using a portable oxygen meter (Phox Systems Ltd). A time period of 30-40 minutes was usually sufficient to induce an appreciable reduction (20-30%), from a 100% dissolved oxygen level starting point. The oxygen demand was obviously a function of the temperature and stocking density in the tank.

If the decrease in oxygen level was rapid, oxygen readings were taken every minute, as opposed to every five minutes if the decrease was slower. To ensure that the water in the tank remained homogenously mixed, a small submersible pump (Beresfords Ltd) was placed in the tank to maintain the original water velocity, created by the incoming water. The dynamic system was operated by simultaneously measuring the dissolved O_2 level in the inlet and

outlet water with the portable oxygen meter The water flow rate to the tank was also measured by making a timed volume collection. Accurate weights of fish in the tank were known, as was the amount of food consumed. Using the dynamic system, an experiment was performed to investigate oxygen consumption on starved fish, fish after a days feed, three hours after last feed and 16 hours after last feed. In the case of each oxygen consumption determination, the temperature was recorded and the salinity determined with a refractometer.

3.2.2 Production Tank Determinations

The water supplies, oxygenation system and physical tank dimensions are fully described in Jones et al (1980). The production tanks contained a rangeof fish sizes from 25g in Tank 4,to 2000g in Tank 1 and supplies of cooling and ambient sea water were available to each tank.

In order to carry out an oxygen consumption determination, the water supply was turned off to a tank as was the oxygen supply. The oxygenation pump was left on so as to maintain water circulation around the tank; the pump itself had no reoxygenation effect merely circulation. Each tank had an oxygen monitor (Benridge Developments Ltd) and the oxygen readings were recorded at regular time intervals, until an appreciable decrease in dissolved oxygen level had occurred (20-30%) from the starting point of 100% dissolved oxygen. As in the fry shed, the actual rate of oxygen level decline, was a function of temperature and stocking density. The stocks in the production tanks were weighed monthly and accurate records kept of food consumed. Generally, oxygen consumption determinations were performed as near as possible

to these weighing exercises, in order to have an accurate figure for the fish biomass in the tanks. The salinity and temperature were also recorded for each determination, as detailed previously. Accurate records were also made of the internal dimensions of the tanks, as all tanks varied slightly in proportions. Errors involved in this method, such as surface exchange, were small and calculated by Harmon (1978) as being approximately ±10%.

In order to monitor diurnal fluctuations in oxygen demand, the portable oxygen meter was connected to a variable speed chart recorder (Heathkit Ltd) This equipment was left running for several days, during which the water flow rate was maintained constant, as was the oxygen supply to the oxygenation pump. In order to quantify oxygen demand from this chart read-out, the efficiency of the oxygenation pump was determined. The first requirement was to determine the oxygen consumption of the fish, (as detailed previously but using a continuous chart read-out as opposed to manual recording) and then re-instate a known flow rate of oxygen to the oxygenation pump. The chart read-out then showed a gradual incline (see 3.3.2) and the experiment was ceased when the oxygen level returned to 100% in the tank. By recording the decline in dissolved oxygen level, due to oxygen consumption by the fish on a chart recorder, it was possible to demonstrate that it was linear over the range used, ie the oxygen consumption rate did not alter over the operating range of dissolved oxygen levels. Subsequent tests demonstrated that there was no significant difference in the efficiency of the oxygenater up to 200% dissolved oxygen levels.

3.3 Results

3.3.1 Oxygen consumption of turbot over the operational temperature and size range

Little work has been published on turbot oxygen consumption, see Jones et al (1980). The aim of this study was to provide operational information on maximum oxygen consumption on the fish farm, to assist in calculating water requirements and stocking densities. Thus most of the determinations were made on fully fed fish. The results are divided into a series of temperature ranges, viz 7-8°C, 9-10°C, 11-12°C, 13-14°C, 15-16°C. As mentioned previously, where a static system was employed, checks were made to ensure that the decrease in dissolved oxygen level was linear. Such a chart trace is presented in Figure 3.3.1. The method used was to take oxygen readings at timed intervals, then subject the results to linear regression, in order to determine the gradient. The actual method of calculation of oxygen consumption is best illustrated with a worked example.

Tank 3 - 3.11.79

Biomass = 1668 kg Mean weight = 142 g

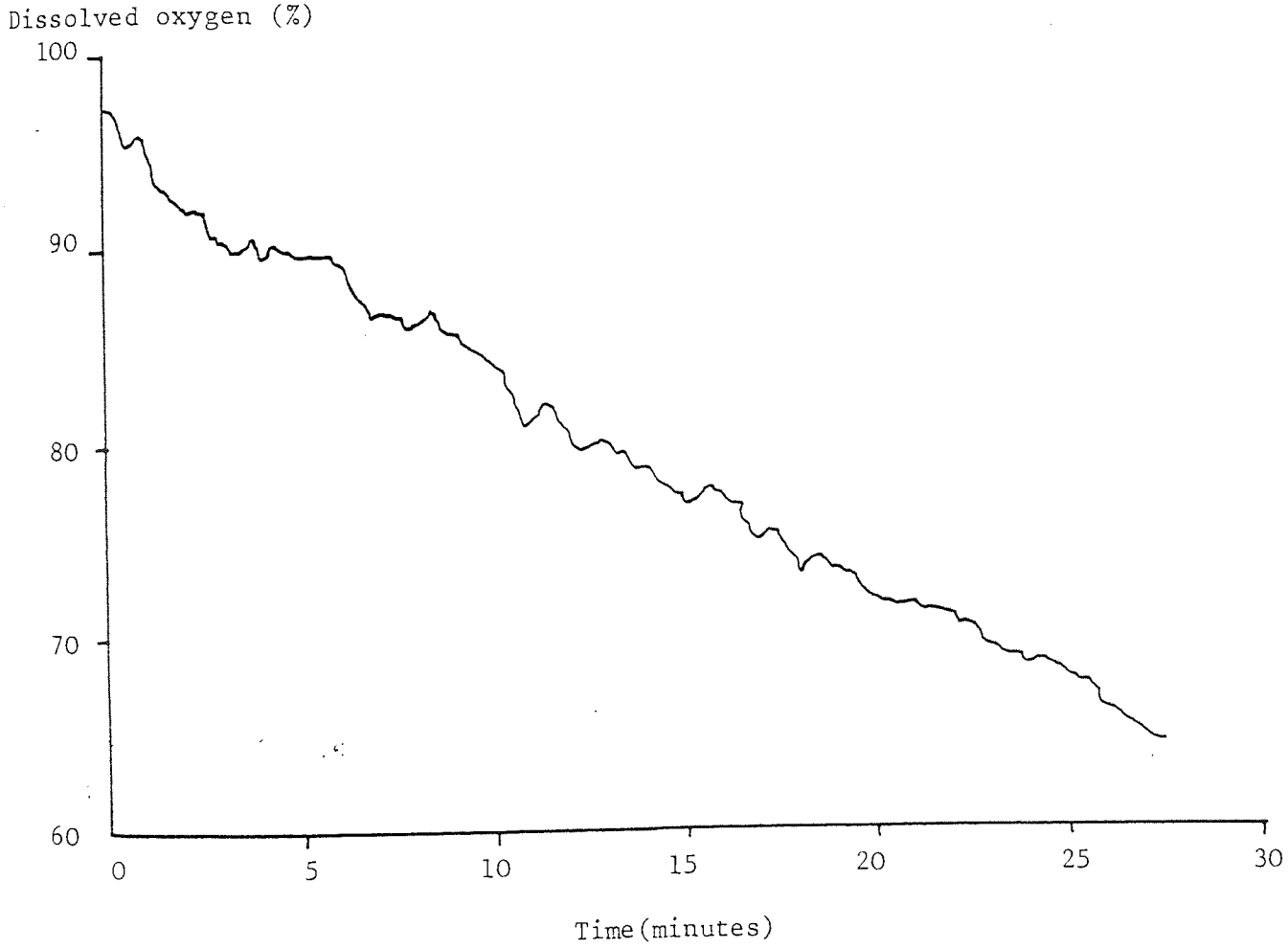
Temperature = 12.5°C Food consumed = 16 kg

Salinity = 35 ‰ Tank volume = 42.43 m^3

100% oxygen saturated water at above conditions contains 8.605 mg O_2 ℓ water

Time (minutes)	% Dissolved Oxygen
0	98
3	96
7	92
11	89
15	82
19	80
26	73

Figure 3.3.1 A typical dissolved oxygen decline in production tank 3



Gradient from above data = 0.9918 (by linear regression)

Therefore % O_2 drop in 1 hour = 59.51%

= 5.121 mg ℓ^{-1}

Tank volume = 42.43 m^3

Therefore total oxygen consumed by fish = 217.27g

Tank biomass = 1668 kg

Therefore oxygen consumption = 0.1303 g kg $fish^{-1}$ hr^{-1}

The calculation may be summarised :

$$O_{2\ cons} = \frac{G \times 60 \times \frac{|O_2|}{100} \times V}{M}$$

where G = regression gradient of dissolved oxygen fall against time

$|O_2|$ = dissolved oxygen concentration (mg ℓ^{-1}) at experimental conditions

V = tank volume m^3

M = tank biomass kg

$O_{2\ cons}$ = oxygen consumption rate: g O_2 kg $fish^{-1}$ hr^{-1}

The dependence of dissolved oxygen concentration on water temperature and salinity has already been mentioned. At Wylfa, the salinity is fairly constant and so the oxygen concentration is a function of water temperature, as presented in Table 3.3.1.

The oxygen consumption data are presented in Table 3.3.2 and 3.3.3, according to temperature ranges. For further details of these data, see Appendix 3. The data reference numbers that appear in these tables relate to Appendix 3.

Table 3.3.1

Oxygen concentration at 100% saturation for 35‰ salinity and varying temperature

Temperature °C	Oxygen concentration (mg ℓ^{-1})
7	9.648
8	9.435
9	9.243
10	9.051
11	8.868
12	8.686
13	8.524
14	8.362
15	8.200
16	8.049
17	7.897

Source : Montgomery Thom & Cockburn (1964)

Table 3.3.2

Oxygen consumption at temperatures 7-8°C, 9-10°C, 11-12°C

Temperature range (°C)	Mean weight (g)	Oxygen consumption g O_2 kg $fish^{-1}$ hr^{-1}	Data Reference number
7-8	4	0.1082	1
"	20	0.0812	2
"	177	0.0514	3
"	186	0.0547	4
"	660	0.0393	5
"	686	0.0459	6
9-10	5	0.1646	7
"	30	0.1230	8
"	45	0.0916	9
"	165	0.0858	10
"	195	0.0528	11
"	690	0.0558	12
"	1000	0.0419	13
11-12	4	0.2280	14
"	55	0.1558	15
"	143	0.0917	16
"	312	0.0728	17
"	577	0.0835	18
"	1000	0.0521	19

These data are presented in Figure 3.3.2 along with the data from Table 3.3.3. The data were fitted by $\log_e/\log_e$ regression analysis ($P < 0.01$).

Table 3.3.3

Oxygen consumption at temperatures 13-14°C, 15-16°C

Temperature range (°C)	Mean weight (g)	Oxygen consumption g O_2 kg fish^{-1}hr^{-1}	Data Reference number
13-14	4	0.2882	20
"	28	0.1890	21
"	123	0.1429	22
"	148	0.1451	23
"	482	0.0896	24
"	1000	0.0567	25
15-16	4	0.4881	26
"	29	0.2607	27
"	44	0.2412	28
"	229	0.1498	29
"	1000	0.0977	30

The regression lines in Figure 3.3.2, may be represented by the following equations:

$$7\text{-}8^{\circ}C \quad \log Q_{O_2} = -0.1837 \log \bar{w} - 1.9699$$

$$9\text{-}10^{\circ}C \quad \log Q_{O_2} = -0.2509 \log \bar{w} - 1.3650$$

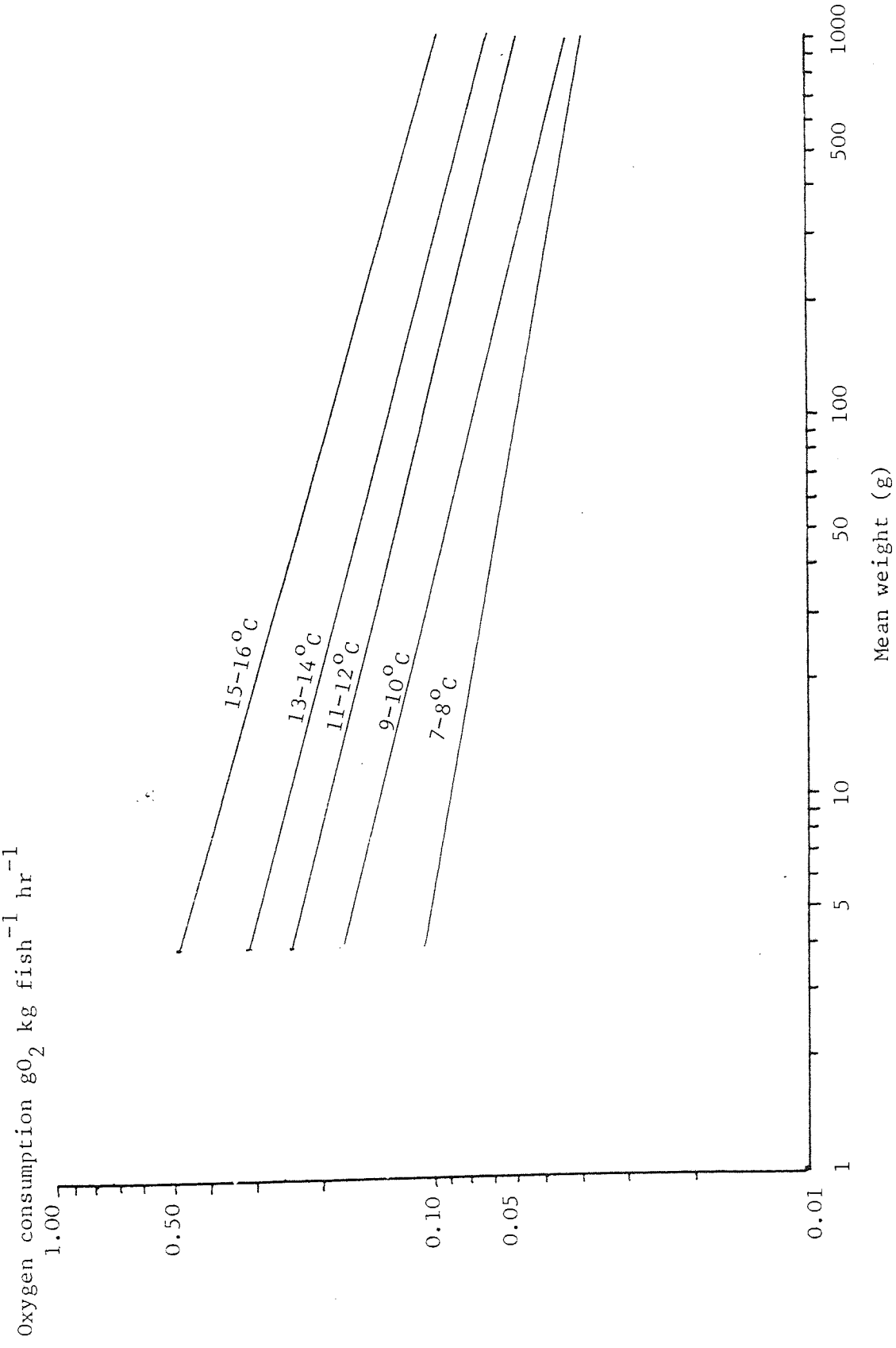
$$11\text{-}12^{\circ}C \quad \log Q_{O_2} = -0.2567 \log \bar{w} - 1.0413$$

$$13\text{-}14^{\circ}C \quad \log Q_{O_2} = -0.2742 \log \bar{w} - 0.7493$$

$$15\text{-}16^{\circ}C \quad \log Q_{O_2} = -0.2891 \log \bar{w} - 0.3345$$

All the determinations detailed previously, were made on populations

Figure 3.3.2 Oxygen consumption over operational size and temperature range



of fish, approximately one hour after the final feed of the day. As will be seen in the next section, this usually corresponds to the maximum oxygen demand and was used as a standard time in all the determinations.

3.3.2 Diurnal fluctuations in oxygen consumption and the correlation of these to feeding

The first examination of diurnal fluctuations in oxygen consumption, was made in the fry shed, as described in 3.2.1, at a temperature of 12.8°C. The results are presented in Table 3.3.4. For further details see Appendix 3.

Table 3.3.4

Oxygen consumption at various feeding states in the fry shed

Mean Weight (g)	Oxygen consumption g O_2 kg $fish^{-1}$ hr^{-1}			
	Starved	After 8 hrs feeding	3 hrs after last feed	16 hrs after last feed
32	0.1820	0.2851	0.2292	0.2141
29	0.1678	0.3094	0.2669	0.1496
30	0.1683	0.2716	0.2573	0.2076
27	0.2003	0.2465	0.2090	0.1847
28	0.1463	0.2486	0.2719	0.1634
31	0.1991	0.3139	0.2927	0.1626
27	0.2087	0.2909	0.2603	0.2061
66	0.1415	0.1900	0.1901	0.2010
12	0.2076	0.2736	0.3161	0.4135

The 'starved' fish had been deprived of food for 24 hours prior to the experiments and the determinations were made at 09.30 hrs. Consequently, the determinations "16 hours after last feed" were made at 09.30 hours the following morning. Seven of the above series of determinations were made

on fish of similar mean weight and so the results may be summarised as follows:

Mean weight 29g

Starved fish	0.1818	g O_2 kg fish^{-1} hr^{-1}
After 8 hrs feeding	0.2809	" " " "
3 hrs after last feed	0.2553	" " " "
16 " " " "	0.1840	" " " "

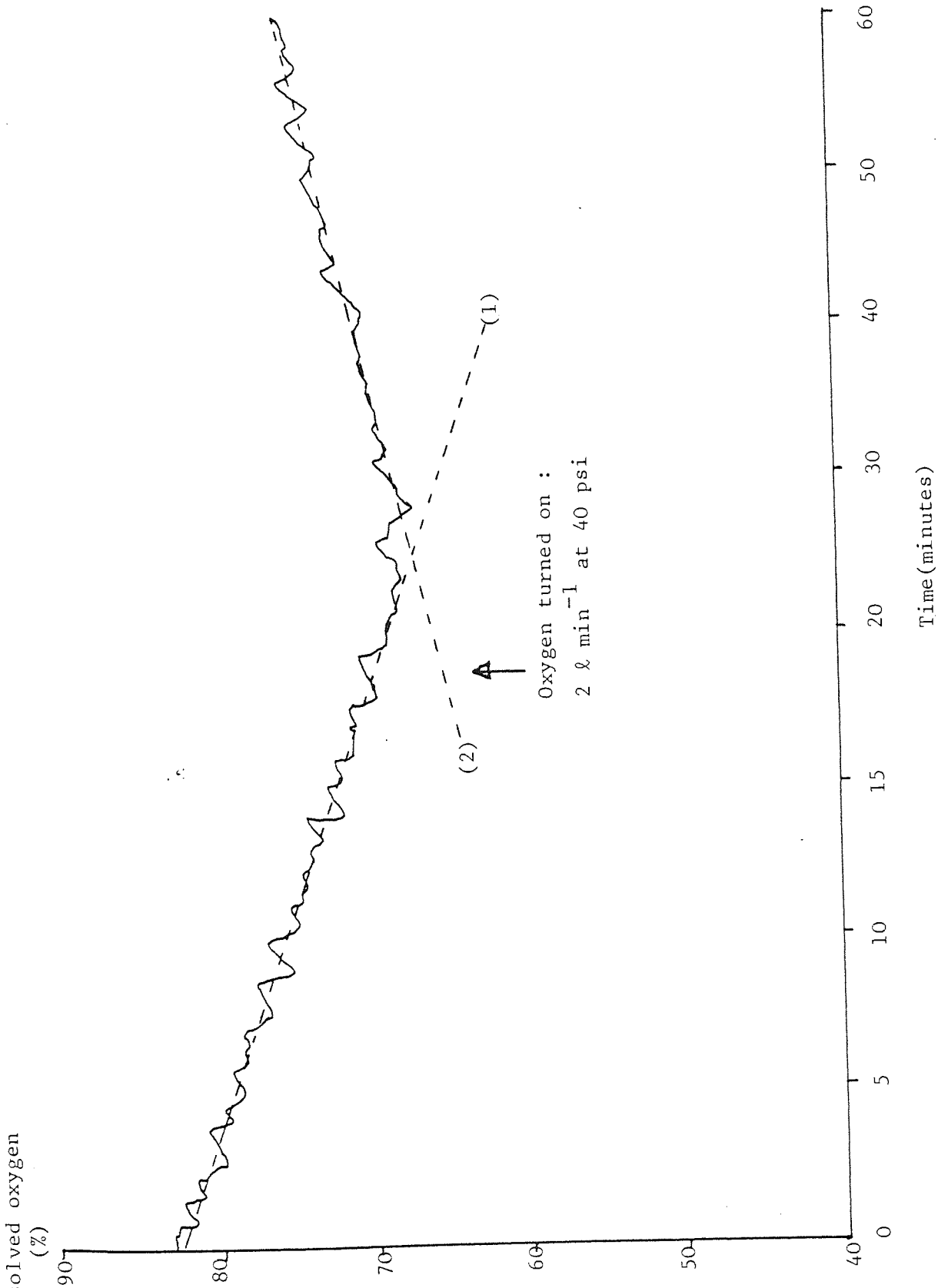
This data suggests that maximum oxygen demand occurs, when all the daily food ration has been consumed. The data for the 12 g fish was somewhat different, as these fish were more excitable than the 29g fish and the presence of a person at the side of the tank induced a marked behavioural change ie the fish swam up from the bottom of the tank and awaited feeding. As will be seen in the next series of determinations, this feeding response resulted in a marked increase in oxygen consumption.

Due to the somewhat discontinuous nature of these determinations, a series of experiments were set up to monitor dissolved oxygen levels in one of the production tanks. The tank chosen for this was tank 3, which contained a large biomass (1893 kg) and consequently significant fluctuations in dissolved oxygen levels could be recorded. As mentioned in 3.2.2, the efficiency of the oxygenation system was established by the method described. A typical chart trace from such an exercise is presented in Figure 3.3.3. From this figure, the efficiency was worked out as follows:

Tank 3 21.11.79

Biomass	=	1893 kg			
Temperature	=	10.4°C	Food consumed	=	19 kg
Salinity	=	35 ‰	Tank volume	=	42.43 m^3

Figure 3.3.3 Oxygen consumption and re-oxygenation in production tank 3



100% oxygen saturated water at above conditions contains 8.96 mg O_2 ℓ water^{-1}.

Gradient (1) = 0.6900

Therefore % O_2 drop in one hour = 41.4% = 3.709 g O_2 ℓ$^{-1}$

Therefore O_2 consumed = 157.4 g hr^{-1}

Rate of increase in oxygen level due to oxygenater = (2)

Gradient = 0.5000

Therefore dissolved oxygen increased by 114.1 g hr^{-1}

Therefore total oxygen supplied by oxygenater = 271.5 g hr^{-1}

Oxygen flow supplied to oxygenater = 2 ℓ min^{-1} at 40 psi

Converting this to atmospheric pressure

$$\frac{P_1V_1}{T_1} = \frac{P_2V_2}{T_2}$$

$$\frac{14.8 \times V_1}{273} = \frac{40 \times 2}{283}$$

Therefore V_1 = 5.21 ℓ min^{-1} = 312.6 ℓ hr^{-1}

converting this to g.

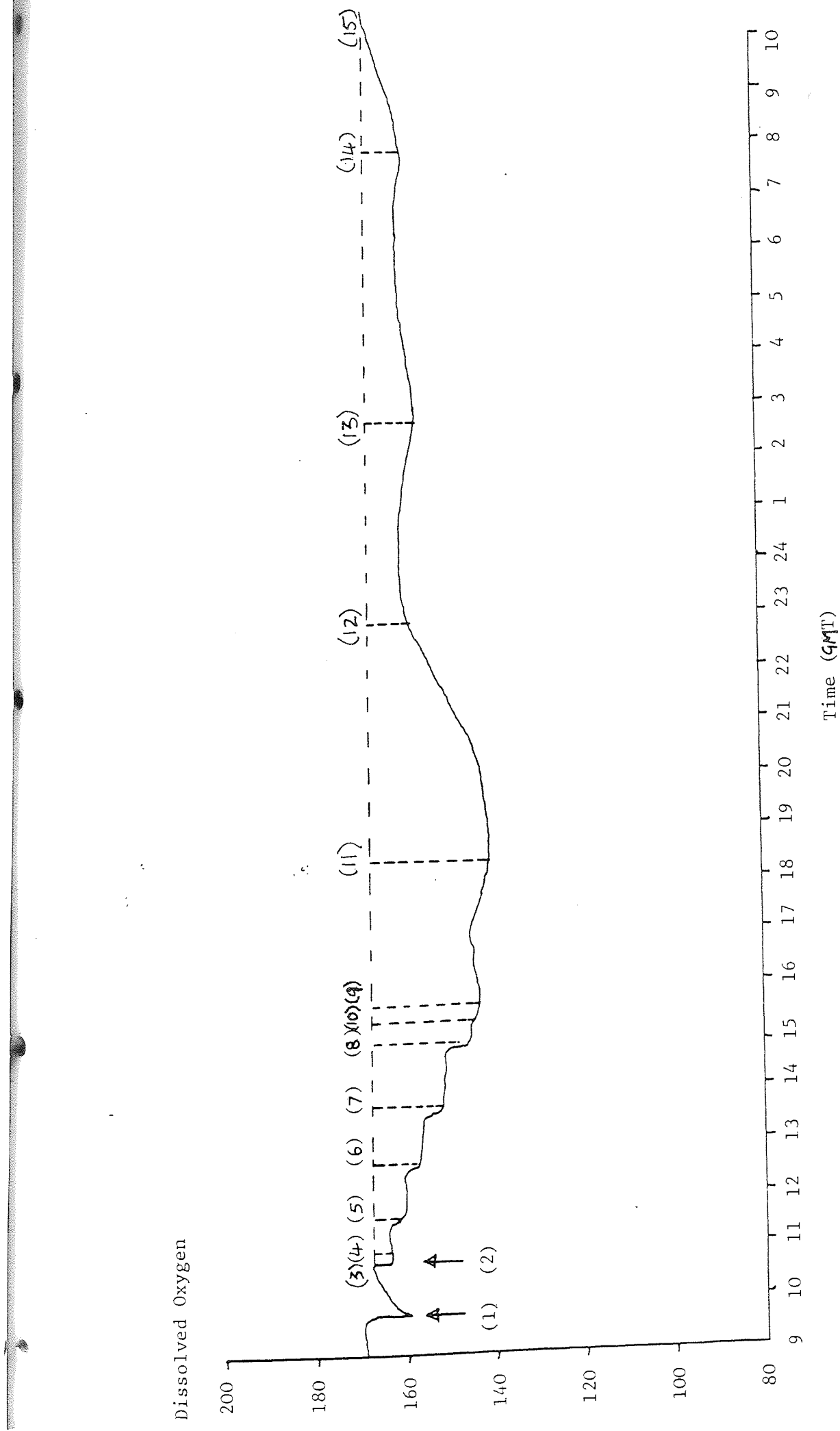
1 ℓ of O_2 = 1.429g

Therefore O_2 supplied by oxygenater = 446.7 g hr^{-1}

Therefore oxygenater efficiency = $\frac{271.5}{446.7} \times 100 = 60.8$ %

This figure was applied throughout the series, as a short time period was used and the conditions for oxygen solubility did not alter. A typical 24 hour chart trace is presented in Figure 3.3.4. During the duration of this trace, the water flow rate to the tank was maintained at 8.45 m^3 hr^{-1} and the oxygen flow to the oxygenater maintained at 1.2 ℓ min^{-1} at 40 psi.

Figure 3.3.4 Dissolved oxygen chart trace 18.11.79 to 19.11.79 from tank 3



The figure was drawn as an average line through fluctuations on the chart trace, which amounted to ± 2% from the drawn line. The figure shows very clearly the effect of feeding on oxygen consumption, both at each feed and the cumulative effect (Specific Dynamic Action SDA, see 3.1). Point (1) on the figure represented routine husbandary in the tank, including cleaning of the oxygen electrode. Point (2) represented feeding activity and it can be seen that the dissolved oxygen level dropped very sharply during feeding, as the fish were very active. This activity contrasted sharply to the normal tank status, in which most fish were lying on the tank floor. This period of activity corresponds to active metabolism (as defined in other determinations). The duration of this activity was fairly short, approximately 10 minutes, and the intensity was a function of the amount of food fed, eg the reduction in % saturation at 14.35 hrs was greater (8%) than that at 15.10 hrs (4%), the corresponding rations being 5 kg and 2 kg of food fed respectively. It is possible to calculate from one of these feeding activity increases in oxygen consumption, a value for active metabolism in turbot.

Tank 3 18.11.79

Biomass = 1867 kg Tank volume = 42.43 m^3

Temperature = 10.5°C Salinity = 35 ‰

100% saturated water at above conditions contains

8.96 mg O_2 ℓ $water^{-1}$

Oxygen supply to oxygenater = 1.2 ℓ min^{-1} at 40 psi

Oxygenater efficiency = 60.8% (previous determination)

Water flow to tank = 8.449 $m^3 hr^{-1}$

% oxygen drop = 8% in 10 minutes, therefore gradient = 0.8

Therefore extra oxygen consumption = 0.8 x 60 x 0.089 x 42.43 g hr^{-1}.

$$= \underline{182.48} \text{ g hr}^{-1} \qquad (1)$$

This was in excess of the oxygen supplied by the water and the oxygenater.

Oxygen supplied by water = 8.449 x 8.96 g hr^{-1}

= 75.70 g hr^{-1} (2)

Oxygen supplied by oxygenater :

Volume of oxygen = $\frac{40 \times 1.2 \times 273 \times 60}{14.8 \times 283}$ ℓ hr^{-1}

= 187.72 ℓ hr^{-1}

Mass of oxygen = 187.72 x 1.429 = 268.25 g hr^{-1}

However oxygenater was only 60.8% efficient,

therefore O_2 supply = 163.1 g hr^{-1} (3)

Therefore oxygen consumed = (1) + (2) + (3) = 421.3 g hr^{-1}

Therefore consumption rate = $\frac{421.3}{1867}$ g O_2 kg $fish^{-1}$ hr^{-1}

= 0.2256 g O_2 kg $fish^{-1}$ hr^{-1}

This was for 158 g fish.

The corresponding value from Figure 3.3.2, for the same conditions, is 0.09 g O_2 kg $fish^{-1}$ hr^{-1}. Thus a trebling of metabolic rate was induced during active metabolism, as opposed to routine metabolism. It is further evident that after each feed, the dissolved oxygen level in the tank remained at a reduced level, until the next feed reduced it further. Thus over a day's feeding regime, there was a gradual reduction in dissolved oxygen level. As the water flow and oxygen flow were maintained constant, this reduced oxygen level was due to increased oxygen demand by the fish. This post-prandial increase in oxygen consumption, corresponds to the SDA. The SDA commenced immediately after the first feed at 10.30 hrs and reached a peak at around 18.30 hrs (ie minimum dissolved oxygen concentration). A similar sequence of events is presented

The calculation becomes :

Dissolved oxygen level at 09.00 hrs = 170 %

= 15.23 g m^3

Therefore oxygen in water = 290.93 g

Time between inflow and probe measurements = $\frac{19.1}{8.449}$

= 2.26 hrs

Therefore oxygen supplied by water + oxygenater = 2.26 x 238.8

= 539.69 g

Therefore oxygen used by fish = 248.76 g in 2.26 hrs

= 110.1 g hr^{-1}

Oxygen consumption rate = $\frac{110.1}{840.4}$ = 0.1310g O_2 kg $fish^{-1}$ hr^{-1}

Dissolved oxygen level at 18.30 hrs = 139%

Therefore dissolved oxygen in water = 237.88 g

Oxygen supplied (see previous calculation) = 539.69 g

Therefore oxygen used by fish = 301.81 g

= 133.54 g hr^{-1}

Therefore oxygen consumption rate = 0.1589 g O_2 kg $fish^{-1}$ hr^{-1}

Similar calculations for Figure 3.3.5 yield the following:

Minimum rate at 09.00 hrs = 0.1184 g O_2 kg $fish^{-1}$ hr^{-1}

Maximum rate at 16.00 hrs = 0.1544 g O_2 kg $fish^{-1}$ hr^{-1}

It is obvious, therefore, that feeding the fish increased the oxygen consumption rate due to SDA and the effect was 1½ times the oxygen consumption rate before feeding. This increased oxygen consumption, appeared to last for approximately 24 hours after first feeding. This indication was also evident from Table 3.3.4, although different diets and

in Figure 3.3.5. Knowing the efficiency of the oxygenation system and the water and oxygen flows, it is possible to calculate the oxygen consumption rate at minimum (09.00 hrs) and maximum (18.30 hrs) levels as follows:

Tank 3 18.11.79

Biomass = 1867 kg

Temperature = $10.5^{o}C$ Salinity = 35 ‰

100% oxygen saturated water at above conditions contains

$8.96 \text{ mg } O_2 \text{ } \ell \text{ water}^{-1}$

Oxygen supply to oxygenater = $1.2 \ell \text{ min}^{-1}$

Water flow to tank = $8.449 \text{ m}^3 \text{ hr}^{-1}$

Oxygen supply from water = 75.7 g hr^{-1} (see previous working)

Oxygen supplied by oxygenater = 163.1 g hr^{-1}

Therefore total oxygen supply = 238.8 g hr^{-1}

A number of assumptions were made concerning the time lapse between water entering the tank and coming into contact with the oxygen electrode and also the distribution of fish within the tank.

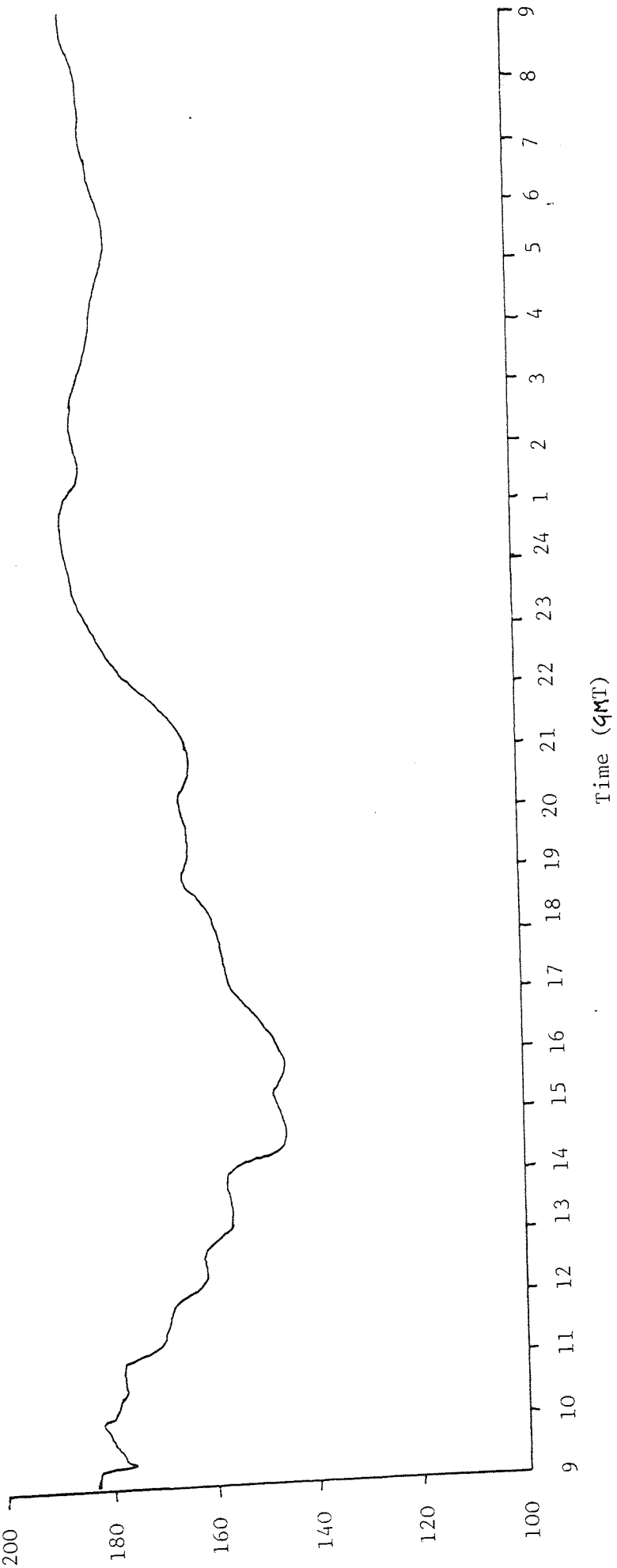
(i) The electrode was placed 1/3 way across the tank on the bottom. This means that the volume from here to the side of the tank was 19.1 m^3.

(ii) The fish were evenly distributed throughout the tank, therefore biomass in this section of the tank was

$$\frac{19.1}{42.43} \times 1867 = 840.4 \text{ kg.}$$

(iii) The oxygenater efficiency was constant and the incoming water 100% saturated with oxygen (checked periodically and substantiated).

Figure 3.3.5 Dissolved oxygen chart trace 20.11.79 to 21.11.79 from tank 3



temperatures were being used.

Not only is the peak and duration of the SDA effect important, but also the magnitude of it. It is possible to calculate the extra oxygen consumed as a result of SDA by calculating oxygen consumption at various intervals on Figure 3.3.4 and then integrating this data.

The oxygen consumption data are presented in Table 3.3.5.

Table 3.3.5

Oxygen consumption data from Figure 3.3.4

Data Reference Number	Time (hours)	Dissolved oxygen %	Oxygen consumption ghr^{-1}	Deviation from resting cons $g\ hr^{-1}$
3	0	168	110.1	0.0
10	4.60	145	129.0	18.9
11	7.57	141	132.0	21.1
12	12.00	158	119.2	9.1
13	15.77	156	120.7	10.6
14	21.00	159	118.4	8.3
15	23.33	168	110.1	0.0

These data are plotted in Figure 3.3.6. The oxygen consumption figures were calculated by the method detailed previously. Integration of the area below the line in Figure 3.3.6, yielded a total oxygen consumption of 268.95 g. However, this was for only a proportion of the fish biomass (840.4 kg). If the whole biomass is considered, the oxygen consumed was 597.49 g. Assuming an oxycalorific coefficient of 20.13 kj ℓ O_2^{-1} (Brody 1945), the oxygen consumed was equivalent to 8417 kj. Similar calculations were applied to Figure 3.3.5 and the SDA was 6894 kj. On each

Figure 3.3.6 Oxygen consumption from Figure 3.3.4

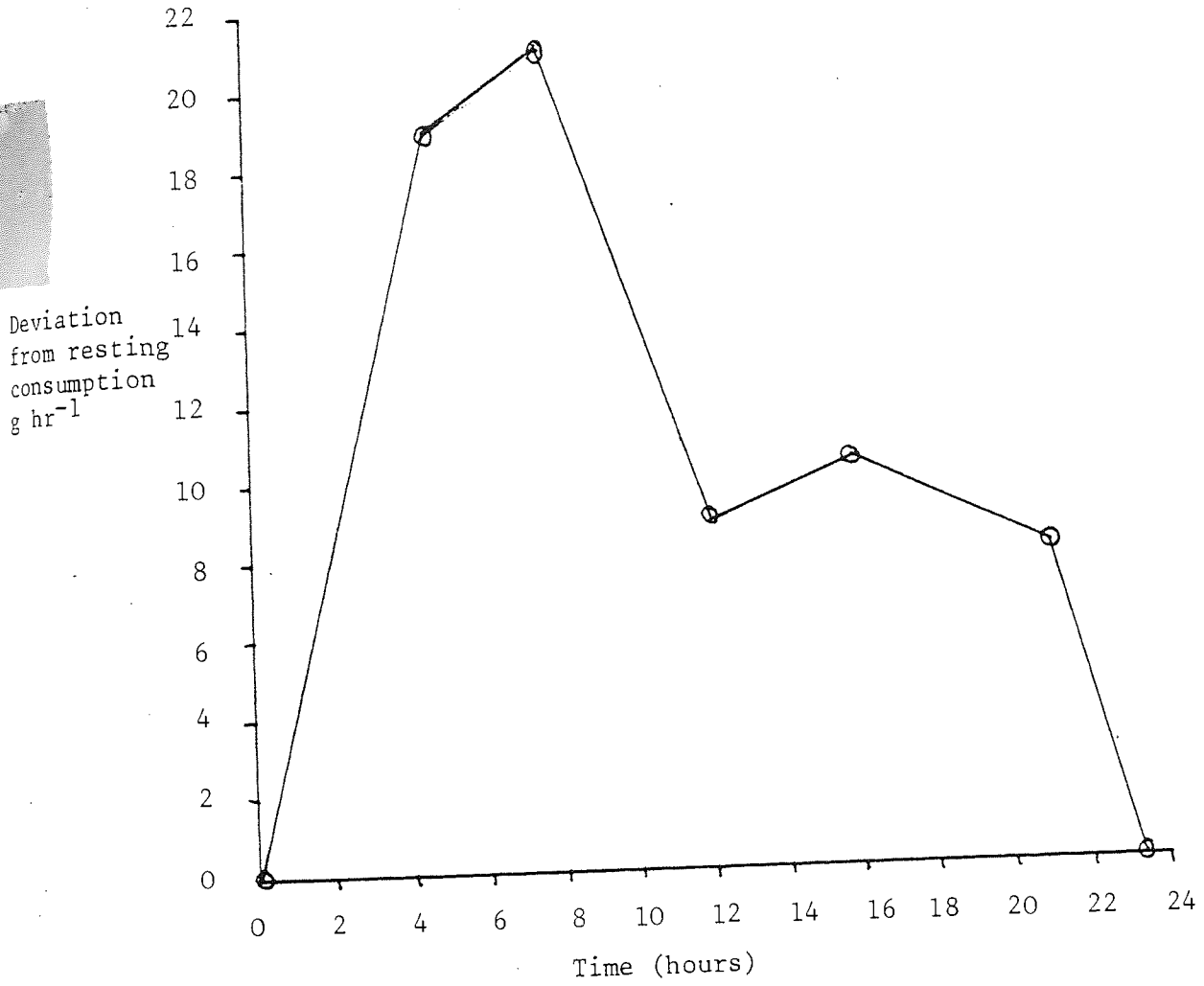
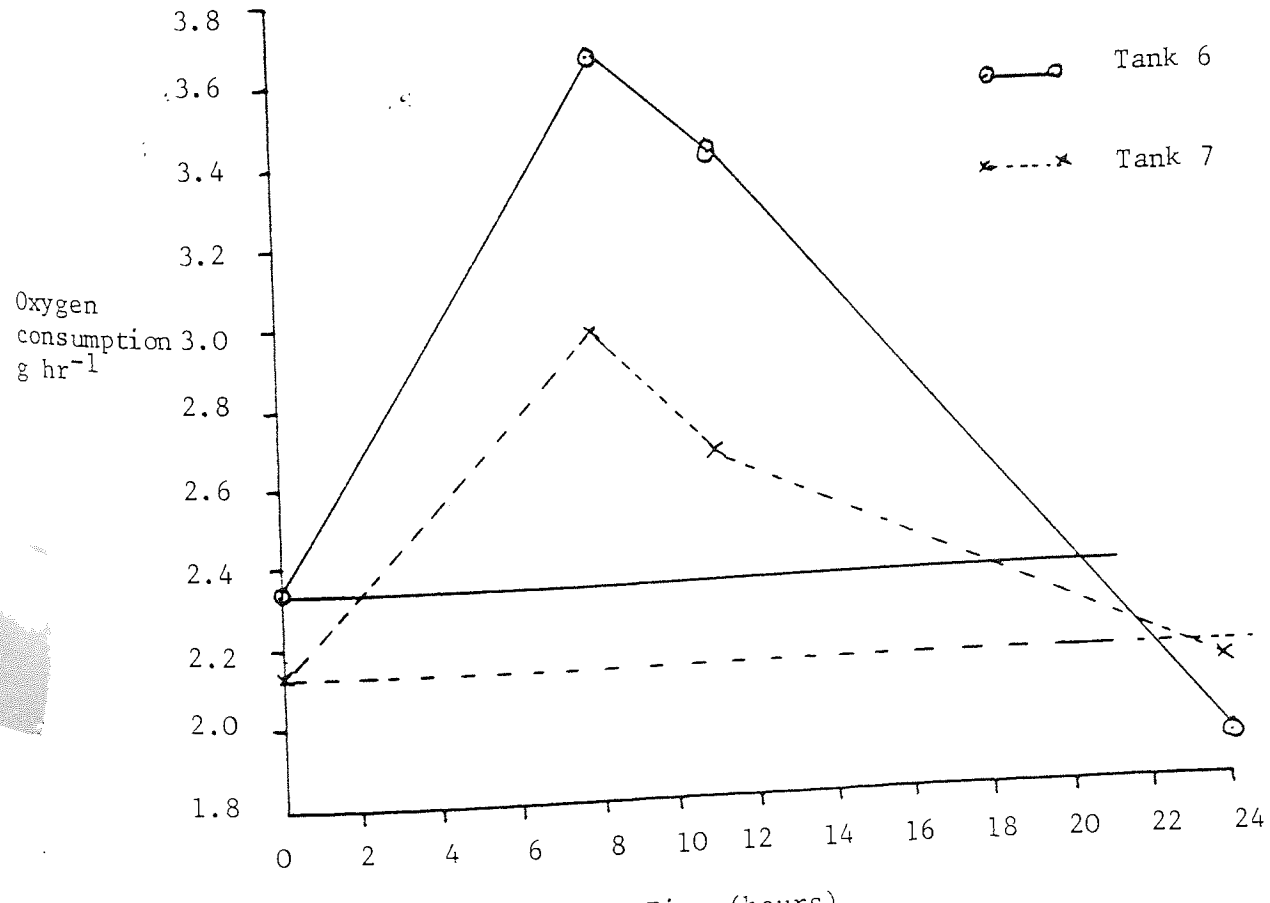


Figure 3.3.7 Reanalysis of fry shed oxygen consumption



day, the ration consumed was 19 kg of food with a gross energy value of 14,202 kj kg food^{-1} (Diet 3(ii)). Thus the amount of energy consumed was 269,838 kj. The extra oxygen consumed above the resting level was not only due to SDA, but also feeding activity caused a considerable increase in oxygen consumption. During the day represented by Figure 3.3.4, there were six feeds, the duration of activity being around ten minutes on each occasion. From the calculation detailed previously, the extra oxygen consumed as a result of 6 x 10 minute feeding periods was 253g. Converting this to energy units yields a figure of 3566 kj. Thus approximately 50% of the extra oxygen consumed as a result of feeding, the fish may be attributed to feeding activity.

Thus it has been possible to define three levels of oxygen consumption:

(i) Resting : when fish have not been fed and remain quiescent, under the conditions described here = 0.1184 g O_2 kg fish^{-1} hr^{-1}.

(ii) Routine : when fish have been fed to satiation and some activity is occuring, under the conditions described here = 0.1589 g O_2 kg fish^{-1} hr^{-1}.

(iii) Active : when fish are constantly swimming, eg at feeding time, under these conditions = 0.2256 g O_2 kg fish^{-1} hr^{-1}.

The data presented in Table 3.3.4 were similarly analysed although fewer points were taken during a 24 hour cycle. It has been demonstrated already that maximum oxygen demand occurred shortly after the last feed of the day and so the lines drawn on Figure 3.3.7 are based on the 24 hour trace in Figure 3.3.4. The data for Figure 3.3.7 are presented in Table 3.3.6.

of SDA to dietary energy, is presented in Table 3.3.8.

Table 3.3.8

Relation of SDA to Ingested Energy for Tanks 6 & 7

Tank	Extra oxygen consumed (g)	Oxygen energy (SDA kj)	Food consumed(g)	Energy consumed (kj)
6	14.313	201.62	206.3	4167.3
7	8.209	115.64	191.0	3858.2

Similar analysis for the rest of the data yielded the following.

Table 3.3.9

Relation of SDA to Ingested Energy for Fry Shed

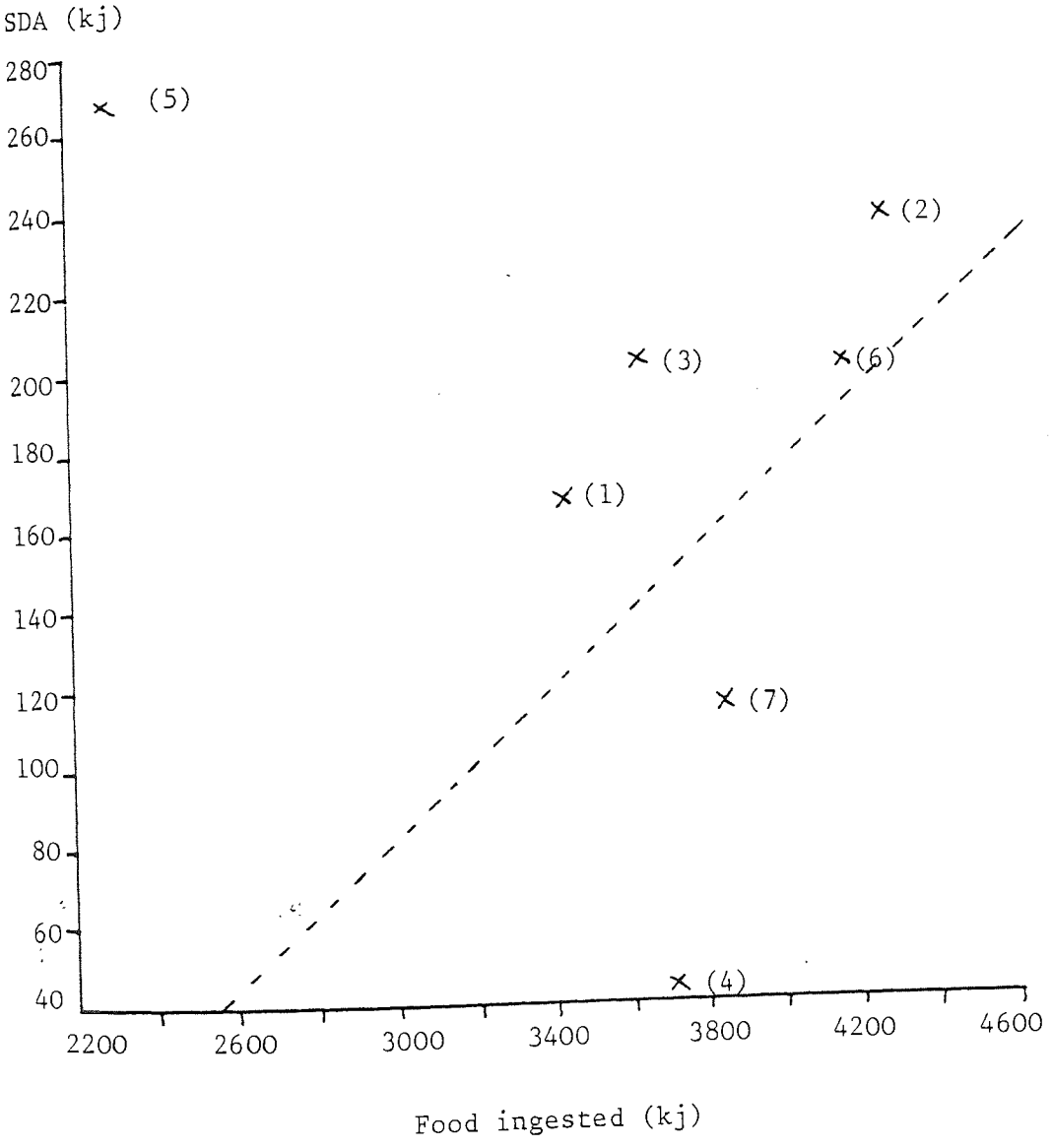
Tank	SDA kj	Energy consumed kj
1	167.5	3436.0
2	240.3	4282.4
3	215.8	3642.1
4	44.5	3718.8
5	265.5	2397.7

The data in Table 3.3.9 are presented in Figure 3.3.8. The relationship can be represented by the following equation, determined by regression analysis. ($P < 0.01$).

$$\text{SDA (kj)} = 0.0924 \times \text{energy ingested (kj)} - 191.48$$

The data from Tank 5 wereomitted from the analysis, since they appeared to be based on faulty determinations.

Figure 3.3.8 Relationship between SDA and ingested energy for fry shed



Further analysis of Figures 3.3.4 and 3.3.5, also demonstrated a relationship between ingested energy and SDA. On Figure 3.3.4, points a-f represent six feeds, the quantity of each being recorded. As SDA is proportional to gastric evacuation time (Jobling and Spencer-Davies 1980) and therefore to ration size (Flowerdew and Grove 1979), the duration of SDA for different feeding states was calculated by proportioning the total ration SDA duration. Oxygen consumption was calculated from the dissolved oxygen levels in the tank by the method detailed previously. The data extracted from Figure 3.3.4 are presented in Table 3.3.10.

Table 3.3.10

SDA duration and oxygen consumption from Figure 3.3.4

Data reference	Time (GMT)	Food consumed(kg)	Oxygen consumed(ghr^{-1})	SDA duration(hrs)	Total oxygen consumed(g)
3	10.44	-	110.10	-	-
4	10.54	3	114.61	3.71	18.59
5	11.36	6	116.88	7.41	55.81
6	12.40	9	119.16	11.12	111.90
7	13.40	12	123.70	14.82	223.89
8	14.52	17	129.00	21.00	440.87
9	15.32	19	130.52	23.47	532.35

The total oxygen consumed for each feeding state was calculated by integrating the excess oxygen consumption figure, eg 114.61 - 110.10 = 4.51 g hr^{-1} against the SDA duration (3.71 hrs) with a peak oxygen consumption at the feeding time (10.54 hrs); this method was detailed previously. The same values as detailed previously for food and oxygen

energy, were applied and the results presented in Table 3.3.11.

Table 3.3.11

SDA magnitude (kj) at increasing feeding levels (kj) from Figure 3.3.4

SDA magnitude g O_2	SDA magnitude kj	Food consumed kg	Food consumed kj
18.59	261.9	3	42606
55.81	786.2	6	85212
111.90	1576.3	9	127818
223.89	3153.9	12	170424
440.87	6210.4	17	241434
532.35	7499.1	19	269838

Similar analysis of Figure 3.3.5, yielded the results in Table 3.3.12.

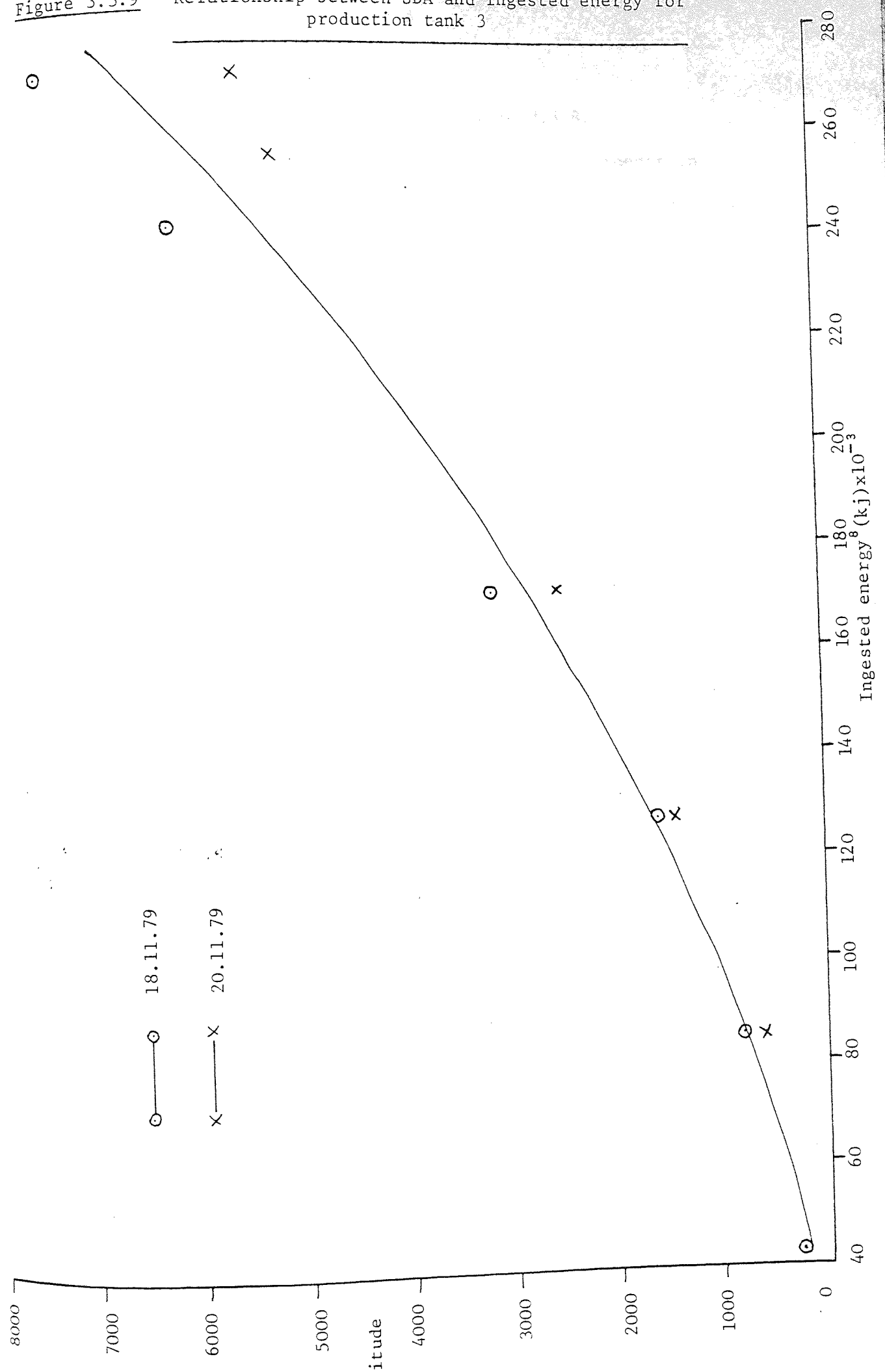
Table 3.3.12

SDA magnitude (kj) at increasing feeding levels (kj) from Figure 3.3.5

SDA magnitude g O_2	SDA magnitude kj	Food consumed kg	Food consumed kj
3.69	51.98	2	28404
41.45	583.90	6	85212
99.48	1401.35	9	127818
176.72	2489.41	12	170424
373.05	5255.07	18	255636
393.64	5545.12	19	269838

The data from Tables 3.3.11 and 3.3.12 are presented in Figure 3.3.9. There appears to be a curvilinear relationship in Figure 3.3.9 as opposed

Figure 3.3.9 Relationship between SDA and ingested energy for production tank 3



to the linear relationship presented in Figure 3.3.8.

Some indications had been evident from the experiments in Section 2 of this thesis that there were differences in metabolisable energy between diets and that this was in part responsible for differential growth rates, food conversions, etc. During the course of Experiment 2.2, a number of oxygen consumption determinations were made on fish in tanks in the fry shed being fed on the three different diets. In all cases, the determinations were made on populations that had been fully fed. The oxygen consumption determinations are presented in Table 3.3.13.

Table 3.3.13

Oxygen consumption determinations in Experiment 2.2 ($gO_2 kg\ fish^{-1}\ hr^{-1}$)

Diet 3(i)	Diet 3(ii)	Diet 3(iii)
0.2340	0.2390	0.2190
0.2857	0.2477	0.2679
0.2840	0.1890	0.2910
0.2820	0.2450	0.2650

For further details see Appendix 3. Analysis of variance of these data, failed to demonstrate any significant difference between the diets. The average values were as follows:

Diet 3(i)	0.2714	$g\ O_2\ kg\ fish^{-1}\ hr^{-1}$
Diet 3(ii)	0.2302	$g\ O_2\ kg\ fish^{-1}\ hr^{-1}$
Diet 3(iii)	0.2607	$g\ O_2\ kg\ fish^{-1}\ hr^{-1}$

These were peak rates of oxygen consumption measured ater eight hours feeding, as described previously in Table 3.3.4. If one were to assume a similar pattern of oxygen consumption to Figure 3.3.6, a resting metabolic

rate of 0.1818 g O_2 kg fish^{-1} hr^{-1} (mean value from Table 3.3.4), a feeding rate of 250 kj kg fish^{-1} day^{-1} and a biomass of 10 kg, the oxygen consumed becomes -

Diet 3(i)	10.752 g
Diet 3(ii)	5.808 g
Diet 3(iii)	9.468 g

This may be related to energy consumed as detailed in Table 3.3.11.

Table 3.3.14

SDA Data for Diets used in Experiment 2.2

	SDA kj	Energy consumed kj
Diet 3(i)	151.5	2500
3(ii)	81.8	2500
3(iii)	133.4	2500

Thus large differences in SDA were evident for the different diets.

3.4 Discussion

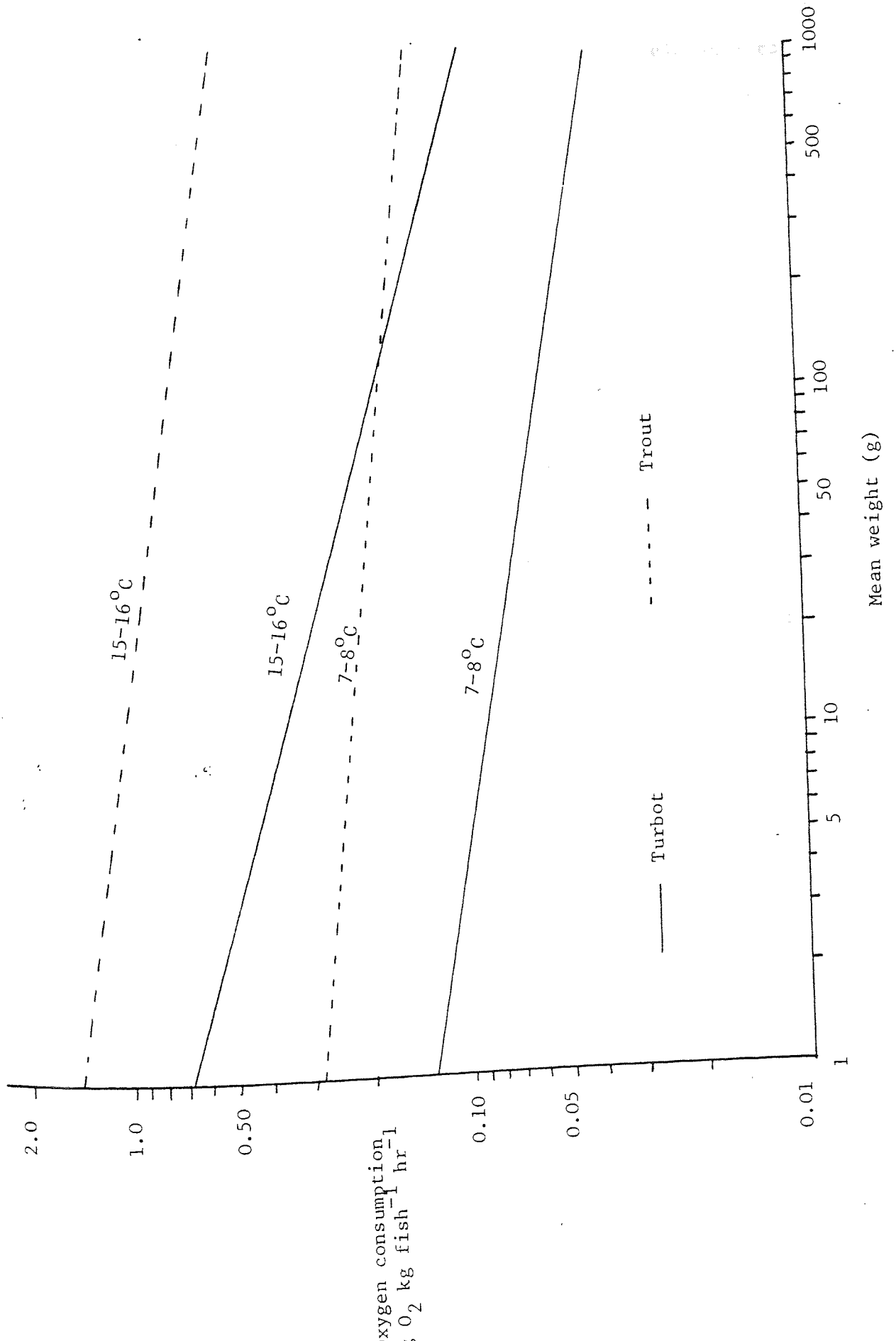
The data presented in Figure 3.3.2 represent one of the very few comprehensive studies of the relationship between oxygen consumption and mean weight at differing temperatures for any fish species. As mentioned in 3.1, most of the previous work was performed with rainbow trout. These data are extremely confusing as different relationships are evident in each paper. It is evident from the regression equations of the data on Figure 3.3.2, that the relationship between oxygen consumption and mean weight varies at differing temperature. The oxygen consumption rate increased with decreasing weight at a higher rate at high temperatures (9-16°C) than at low temperatures (7-8°C). Muller Feuga et al (1978). determined a somewhat different relationship for rainbow trout, whereby the rate of oxygen consumption varied in a similar fashion for temperatures from 4°C to 10°C. However, from 12-21°C the rate of change was lower, but similar for all data points within this temperature group. No explanation was given for this anomaly between 10 and 12°C and it seems that some facet of the experimental procedure must have induced this affect. To demonstrate the magnitude of this anomaly, if one compares the oxygen consumption rate of 100g fish at 10°C and 12°C, the corresponding values are 110 mg kg^{-1} hr^{-1} and 260 mg kg^{-1} hr^{-1} respectively. Thus for a rise of 2°C, the metabolic rate increased by a factor of 2.4. Data from other workers on rainbow trout failed to corroborate this anomaly and further demonstrated the differences between the data of workers using different methods. Liao's (1971) data for 100g fish at 10° and 12°C yielded consumption rates of 204 mg kg^{-1} hr^{-1} and 267 mg kg^{-1} hr^{-1} respectively and data given by Bass (1978) yielded consumption rates of 125 and 145 mg kg^{-1} hr^{-1} respectively. The data

presented in Figure 3.3.2 yield consumption rates for 100g fish at 9-10°C and 11-12°C as 80 and 105 mg kg hr^{-1} respectively. It is apparent, therefore, that the oxygen consumption rate in turbot is much lower than that of rainbow trout. This is a reasonable assumption as turbot generally lie still on the bottom of the tank, whereas trout are generally in continuous swimming motion. An illustration of the different rates of oxygen consumption is presented in Figure 3.4.1, the trout data used being from Liao (1971).

The literature on flatfish oxygen consumption is very limited - the major work being that of Edwards et al (1969) on plaice. As described in 3.1, the oxygen consumption rate for 100g fish can be calculated from the data presented, as being 85 mg kg hr^{-1} for resting plaice and 159 mg kg^{-1} hr^{-1} for active plaice at 10°C. The value for resting plaice is very similar to the value determined from Figure 3.3.2, although this was for fed turbot and the data from Edwards et al (1969) were for starved plaice. As feeding has a marked effect on oxygen consumption (3.3.2), it would appear that oxygen consumption rates determined in this series of experiments (Figure 3.3.2) were much lower than those reported by Edwards and his colleagues. As discussed previously, this is probably due to different experimental conditions and techniques.

Following the ingestion of a meal, the rate of metabolism in the fish increases and over the course of a days feeding, the rate is considerably increased (Figure 3.3.4). The biochemistry of this process, which is referred to as SDA (Specific Dynamic Action), is not completely understood but is generally assumed to be largely the result of the deamination of proteins (Beamish et al 1975). SDA is difficult to measure experimentally and all studies to date have dealt with 'apparent specific

Figure 3.4.1 Comparison of oxygen consumption of turbot and trout at two temperatures



dynamic action'. For a recent review of research on apparent specific dynamic action in fish, see Braaten (1978).

The most recent research, which is also of direct relevance to this thesis, is the work of Jobling and Spencer-Davies (1980) on plaice, reported in 3.1. The Figure 2 of Jobling and Spencer-Davies may be compared to Figure 3.3.7 in this thesis. The slope of these figures represents the SDA coefficient (Miura et al 1976). Jobling and Spencer-Davies determined a value of 0.161 for plaice, whereas the gradient from Figure 3.3.7 is 0.0924. As has already been mentioned, the magnitude of the SDA increases with increasing dietary protein content. However, Jobling and Spencer-Davies determined that diets with a high proportion of their digestible energy derived from non-protein components gave a higher SDA than would be predicted from their protein content. The diets used during the course of the determination of oxygen consumption and SDA in 3.3.3, had quite a large proportion of their energy derived from a non-protein source (up to 40% in the case of diet 3(i)). This is a possible reason for discrepancies between the values for SDA coefficient determined in this thesis and the data for Jobling and Spencer-Davies (1980). Furthermore, the methods of determination of SDA were very different, particularly the feeding method. No published information is available on SDA determinations in large groups of fish, as in a production tank. Comparison of the SDA coefficient determined in Table 3.3.5 and Table 3.3.9, also shows considerable variation within the two types of determination, ie production tank and fry tank. Using the relationship determined from Figure 3.3.8, ie

$$\text{SDA (kj)} = 0.0924 \times \text{energy ingested (kj)} - 191.48$$

the SDA induced by feeding 269,838 kj (Figure 3.3.4 and 3.3.5) should

have been 24,742 kj. The calculated value was only 8417 kj, almost 50% of which was attributed to feeding activity. The data in Figure 3.3.7, failed to take into account any extra oxygen consumed due to feeding activity. However, the feeding regime employed in the fry shed was such that all the tanks had the same number of feeds and thus feeding activity as a variable was standardised in Figure 3.3.7. The duration of the SDA effect in both determinations was considerably shorter than that reported by Jobling and Spencer-Davies (1980) (24 hours as opposed to 35/75 hours). Furthermore, most research to date has been performed on fish that had been given a single feed. The multiple feed regime employed on a fish farm appears to induce a lower SDA than one single large feed. Also the duration of the SDA effect reported here was considerably shorter than that reported by other workers. This is discussed further, later in this section.

Further evidence of the influence of dietary energy composition on SDA is presented in Table 3.3.10 and the subsequent analysis of the data. Diets 3(i), 3(ii) and 3(iii) had very different energy composition profiles ranging from 20-40% non-protein energy (Figure 2.5.3). Table 3.3.11 demonstrates the effect of increasing non protein energy level on SDA. If one were to assume the hypothesis presented earlier, that SDA increased with increasing dietary protein, one would expect the SDA levels to increase in the order 3(i), 3(ii), 3(iii). It is evident from Table 3.3.11 that this was not the case. In order to investigate further these differences, it was necessary to recalculate the energy content of the diets. All the gross energy values presented in Table 2.4.1 were determined by bomb calorimetry. Clearly the oxidation of foodstuffs in a bomb calorimeter is not a true representation of the situation

occurring within a fish. For further discussion of this see Braaten (1978). Consequently the values determined by Elliot and Davison (1975) were 3.53 cal mg^{-1} for carbohydrate (4.10), 3.28 cal mg^{-1} for lipid (9.45) and 3.20 cal mg^{-1} for protein (5.65). The values in brackets are those determined by Brody (1945) in a bomb calorimeter. Thus the data in Table 3.3.11 were reanalysed with these values. These data are presented in Table 3.4.1.

Table 3.4.1

Reanalysis of data in Table 3.3.11
Incorporating Data from Elliot and Davison (1975)

Diet	Food consumed (dry weight g)	Energy consumed kj	SDA kj
3(i)	111.1	1394.3	151.5
3(ii)	117.4	1461.2	81.8
3(iii)	125.4	1547.3	133.4

Thus it is apparent that the fish were not fed on an isoenergetic regime. The relationship between food consumption and SDA was still unclear and so the feeding data were further analysed by utilising the digestibility figures from Table 2.4.40. These data are presented in Table 3.4.2.

So there was no evidence of correlation between SDA and digestible protein energy as presented in Jobling and Spencer-Davies (1980). Indeed SDA appeared to be most closely related to the level of digestible energy in the diet. It is possible that the reason for this discrepancy, is that

Table 3.4.2

Re-analysis of data in Table 3.3.11 incorporating digestibility data

Diet	Food consumed (dry weight g)	Protein digested(g)	Protein energy kj	Lipid digested(g)	Lipid energy(kj)	Digestible energy(kj) (Includes carbohydrate)
3(i)	111.1	44.7	598.1	24.5	335.9	1065.4
3(ii)	117.4	50.3	673.0	16.5	226.2	1037.9
3(iii)	125.4	56.2	752.0	10.0	137.1	1036.7

previous research has involved the use of diets whose principle energy component was protein, eg Jobling and Spencer-Davies (1980) used diets based on sprat with a high protein content and also Beamish (1974) fed largemouth bass (*Micropterus salmoides*) on emerald shiners (*Notropis atherinoides*), which are also high in protein content.

The maximum increase in post-prandial oxygen consumption appeared to be approximately $1\frac{1}{2}$ times the resting rate for production stocks (Table 3.3.5) and fry shed stocks (Table 3.3.6). This compares with an approximate doubling recorded by Jobling and Spencer-Davies (1980), for plaice (*Pleuronectes platessa*) and by Brett and Zala (1975) for sockeye salmon (*Oncorhynchus nerka*). The fish stocks used in the SDA determinations reported in this thesis were fed to satiation. Muir and Niimi (1972) determined that the peak rate of oxygen consumption of aholehole (*Kuhlia sandvicensis*) increased with increasing ration size up to the maximum that the fish would consume. Some evidence of this in turbot can be ascertained by further examination of Figure 3.3.4. After the initial increase in oxygen consumption due to feeding activity, the tank oxygen

level remained at a lower level than before feeding. This sequence of events repeated itself until the day's ration had been fed. Thus a step-wise increase in SDA was induced until the complete ration had been consumed. This feeding regime was different from that usually employed in SDA studies, where the fish is usually force fed.

The duration of the SDA was shown to be correlated with the rate of passage of food through the gut by Jobling and Spencer-Davies (1980). What this means is that the duration is proportional to the quantity of food eaten and inversely proportional to the temperature. The duration of the SDA's observed in Figures 3.3.4, 3.3.5 and 3.3.6 were all around 23-24 hours. According to the data of Jobling and Spencer-Davies (1980), this would correspond to a Gastric Evacuation Time of 11 hours. This compares with a value from Flowerdew and Grove (1979), for 159g turbot at 10.5°C, of about 17 hours. However, the data given in Flowerdew and Grove were for turbot fed a 2% body weight ration (dietary water content 67%). This compares with a feeding rate of 1.02% (dietary water content 33.3%) in Figure 3.3.4. If these feeding rates are converted to a dry weight basis, they become 0.68% for Figure 3.3.4 and 0.60% for Flowerdew and Grove (1979). Thus there seems to be fairly good correlation between the SDA/gastric emptying time relationship in turbot and plaice. The larger SDA coefficients determined by other workers, compared to the ones in Section 2.3, may possibly be attributed to larger rations and consequently increased duration of the SDA effect.

As SDA is generally considered to be an energy loss in an energy budget, it also becomes a very important factor to consider when evaluating diets. In specifying an optimum diet, it is important to consider all aspects of performance. With the data presented in Table

3.4.1 it is apparent that there were large differences between the SDA's of the three different diets. The fact that diet 3(ii) induced the lowest SDA may account, at least in part, for the better performance of the turbot on this diet.

Section 3 therefore has presented the most comprehensive study of oxygen consumption in turbot and indeed, as far as the author is aware, of any marine fish. The data on SDA is also the first published account of this effect in turbot.

4. GENERAL DISCUSSION AND CONCLUSIONS

4. General Discussion and Conclusions

Having considered technical aspects in Sections 2 and 3 of this thesis, discussion is now presented of the commercial implications of these results.

It has already been stated that food is a major cost to the fish farmer. Experimental results from feeding trials with small numbers of fish, eg in fry tanks, are not always replicated when applied to production stocks. Consequently three of the diets tested in Experiments 1.1, 2.1 and 2.2 were also tested in production tank trials. The diets used were whole sprat, sprat-based moist pellet (2(i)) and the 18% oil diet (3(ii)). The first two diets were routinely used on the fish farm, until Experiment 2.2 identified an alternative diet (2(ii)) which returned growth rates and FCR's that were comparable to fresh fish based diets.

Tables 4.1, 4.2 and 4.3 represent growth and food conversion rates in the production tanks at Wylfa, using three different diets, viz diet 3(ii), diet 2(i) and sprat.

TABLE 4.1

Production Tank Growth and Food Conversion on 18% Oil Diet (3(ii))

Date	Total weight (kg)	Mean weight (g)	Biomass increase (kg)	Food consumed (kg)	FCR	% mean wt increase
25.09.79	1595	110				
			297	469	1.58	0.84
25.10.79	1621	138				
			390	550	1.41	0.75
27.11.79	2011	171				
Fish graded and moved						
18.02.80	1445	159				
			425	794	1.85	0.82
26.03.80	1870	207				
			336	656	1.95	0.66
23.04.80	2206	245				

Thus an average FCR of 1.70 ± 0.25 was returned with an average % mean weight increase of 0.79 ± 0.08. The mean temperature over this period was 13.8°C ± 1.4.

TABLE 4.2

Production Tank Growth and Food Conversion on Sprat-Based Diet (2(i))

Date	Total weight (kg)	Mean weight (g)	Biomass increase (kg)	Food consumed (kg)	FCR	% mean wt increase
18.05.79	678	44				
18.06.79	815	55	141	392	2.78	0.74
25.07.79	1060	68	245	511	2.08	0.64
23.08.79	1250	81	190	427	2.20	0.66
25.09.79	1595	110	386	411	1.07	1.08

Thus an average FCR of 2.03 ± 0.71 was returned with an average % mean weight increase of 0.78 ± 0.20. The mean temperature over this period was 12.7°C ± 2.2.

TABLE 4.3

Production Tank Growth and Food Conversion on Sprat

Date	Total weight (kg)	Mean weight (g)	Biomass increase (kg)	Food consumed (kg)	FCR	% mean wt increase
12.08.77	742	680				
15.10.77	1172	970	351	720	2.05	0.79
07.12.77	1294	1070	122	715	5.86	0.15
25.01.78	1746	1460	452	1012	2.24	0.74
Fish graded						
07.03.78	1594	1530				
25.04.78	1811	1790	249	827	3.32	0.35
15.06.78	1860	1970	194	415	2.14	0.20

Thus an average FCR of 3.12 ± 1.6 was returned with an average % mean weight increase of 0.45 ± 0.30. The mean temperature over the period was 12.7°C ± 2.0.

Thus, on an as-fed basis, the 18% oil diet gave the best growth and the lowest FCR. Assuming that the FCR's detailed in the preceding tables could be consistently maintained, it is possible to calculate the amount of food required to produce one tonne of turbot as detailed in Table 4.4.

TABLE 4.4

Food Required to Produce One Tonne of Turbot

Diet	Average FCR	Food required to produce 1 tonne of turbot (tonnes)	Time of production
18% oil diet	1.70	1.70	7 months
sprat based pellet	2.03	2.03	4 months
sprat	3.12	3.12	10 months

N.B. The FCR's are based on production trials

Thus less food is required, on an as-fed basis, to produce one tonne of turbot from the 18% oil diet, than the other two diets.

It is important to evaluate the cost of the diet as, although it may take more of one diet to produce a tonne of turbot, the cost of that diet can be considerably lower than the cost of the diet that results in the lowest FCR. It is in this analysis that many of the diets formulated in earlier experiments eg Adron et al (1976) become unviable, due to the excessive cost of the ingredients, such as freeze-dried cod muscle, or their lack or availability. The following comparative economic analysis of the three diets discussed previously is based on the following assumptions:

(i) A 100 tonne turbot production unit with a high electricity demand, therefore reduced tariff.

(ii) The same food preparation room and handling equipment is used for all three diets and is therefore not included in the capital costs.

(iii) The two moist diets are prepared five days a week.

(iv) Sprats can only be fed to fish above 100g - below this a pellet diet must be used.

(v) Cold storage at the site must be able to hold a minimum of one months sprat.

(vi) Sprats can only be bought for a limited time of year (4 months) and thus the requirements for the whole year must be bought in this period and stored in contracted cold-storage. Also to obtain a reasonable price, sprats must be purchased in 18 tonne consignments, ie one lorry load.

1. Costings for 18% Oil Diet

Food requirement = 100 x 1.7 = 170 tonnes

Weekly food requirement = 3.30 tonnes

This is made in five days therefore food preparation machinery must be capable of producing 660 kg day^{-1}. No cold storage required as diet contains no frozen fish.

The composition of the diet is detailed in Table 2.4.2 earlier in this thesis.

Ingredient	% Present	Amount required for 170 tonnes diet (Tonnes)
Compound feed	68.0	116
Special fish oil	7.7	13
Water	24.3	42

Therefore storage capacity for compound feed must be 10 tonnes and storage capacity for special fish oil must be 1.1 tonnes.

(i) Capital:	£'s K
(a) Mixing/Pelleting machine	5.0
(b) Food storage bin (compound feed)	8.0
(c) Chill room (1 tonne)	1.5
TOTAL	14.5

(ii) Recurrent :	£'s K
(a) 116 tonnes compound feed at £315 tonne^{-1}	36.5
(b) 8 tonnes special fish oil at £370 tonne^{-1}	3.0
(c) Power mixer/mincer and chill room 68.8 kwH per day at 1.875p kwH^{-1} Period requirement 14649 kwH	0.3
(d) Labour 1 man	1.8
(e) Depreciation on capital	0.9
(f) Maintenance	0.6
TOTAL	43.1

Therefore cost per tonne of turbot produced = £431.

2. Costings for Sprat-Based Diet

Food requirement = 100 x 2.03 = 203 tonnes

Weekly food requirement = 3.9 tonnes

Ingredient	% Present	Amount required for 203 tonnes of diet (tonnes)
Sprats	45%	92
Compound feed	55%	111

Storage capacity for compound feed must be 10 tonnes and cold storage must be a minimum of 8 tonnes.

	£'s K
(i) Capital:	
(a) Mixing/Pelleting machine	5.0
(b) Food storage bin (compound feed)	8.0
(c) Cold Store (10 tonnes)	7.1
(d) Chill room (1 tonne)	1.5
TOTAL	21.6

	£'s K
(ii) Recurrent :	
(a) 90 tonnes of sprat at £160 tonne^{-1}	14.4
(b) 111 tonnes compound feed at £315 tonne^{-1}	35.0
(c) Power mixer/mincer 44.8 kwH per day at 1.875p kwH^{-1} Period requirement 5451 kwH	0.1
(d) Power cold store and chill room 72 kwH per day at 1.875p kwH^{-1} Period requirement 8760 kwH	0.2
(e) Labour 2 men	2.0
(f) Depreciation on capital	0.7
(g) Maintenance	0.5
(h) Contracted-out cold storage at 1.75p per tonne per week	0.6
(i) Haulage of sprats from cold store to site at £20 per tonne	1.6
TOTAL	55.1

Therefore cost per tonne of turbot produced = £551.

3. Costings for Sprat Diet

The fish must first be grown to 100g on a pellet diet. If the market size is 2kg, then the amount of food required to achieve this will be 8.5 tonnes. Using the value determined in 1 above, this will cost £2·3K.

Food requirement (sprats) = 95 x 3.12 = 297 tonnes

Therefore cold storage capacity must be a minimum of 25 tonnes

(i) Capital:

	£'s K
(a) Cold store (25 tonnes)	8.4
TOTAL	8.4

(ii) Recurrent:

	£'s K
(a) 306 tonnes of sprats at £120 per tonne	36.7
(b) Power cold store 120 kwH per day at 1.875p kwH^{-1} Period requirement 36500 KwH	0.8
(c) Contracting out cold storage	5.8
(d) Haulage of sprats from cold store to site at £20 per tonne	4.0
(e) Labour 1 man	2.5
(f) Maintenance	0.4
(g) Depreciation on capital	0.8
(h) 8.5 tonnes 18% Oil diet at £254 per tonne	2.2
TOTAL	53.2

Cost per tonne of turbot produced £532.

Thus the following cost per tonne of turbot produced are calculated:

Diet	Food cost of producing 1 tonne of turbot
18% oil diet	£431
Sprat-based diet	£551
Sprat diet	£532

Thus the 18% oil diet represents a major cost saving in the production of turbot.

One of the most cost-sensitive items in the preceding analysis is the price and availability of sprats. The ability to buy large quantities (around 50 tonnes per day as by fish meal plants) results in a considerable reduction in the cost of sprat, reducing it to about £80 per tonne. However, the sprat used for fish meal is generally of very poor quality, as it tends to be bulk handled and therefore is often crushed. This quality of sprat would be unacceptable for feeding to fish. Also, in recent years, the sprat fishery in the UK has been declining and this has been the main impetus in formulating a diet that contained no fresh sprat. Furthermore, the quality of sprat varies very much according to the time of year at which it is caught (Wallace & Hulme 1977) and the length of time between capture and freezing. Inconsistent results on other fish farms have been correlated with poor quality sprats. The ability to formulate a diet which will produce consistent performance is of great assistance in production planning on a fish farm. Furthermore, the lower labour requirement and the ability to scale up the preparation of the 18% oil diet with bulk deliveries of oil and compound feed are major advantages.

From a knowledge of the oxygen requirement of turbot, it is possible to calculate the flow rate of water required to maintain a tank stocked with fish of a given total weight and mean weight at any temperature. This calculation is best demonstrated with a worked example.

Total biomass of fish = 2,000 kg

Mean weight of fish = 200 g

Temperature = 7-8°C

From Figure 3.3.2.

Oxygen consumption = 0.053 g O_2 kg fish^{-1} hr^{-1}

Therefore oxygen requirement = 106 g O_2 hr^{-1}

From Table 3.3.1.

Oxygen concentration in water at 7-8°C = 9.042 gm^{-3}.

However a minimum oxygen level of 4 ppm is required in the tank.

Therefore water supply =

$$\frac{106}{9.042} \times 2.5 = 29.31 \text{ m}^3 \text{ hr}^{-1}$$

$$= 6447 \text{ gallons hr}^{-1}.$$

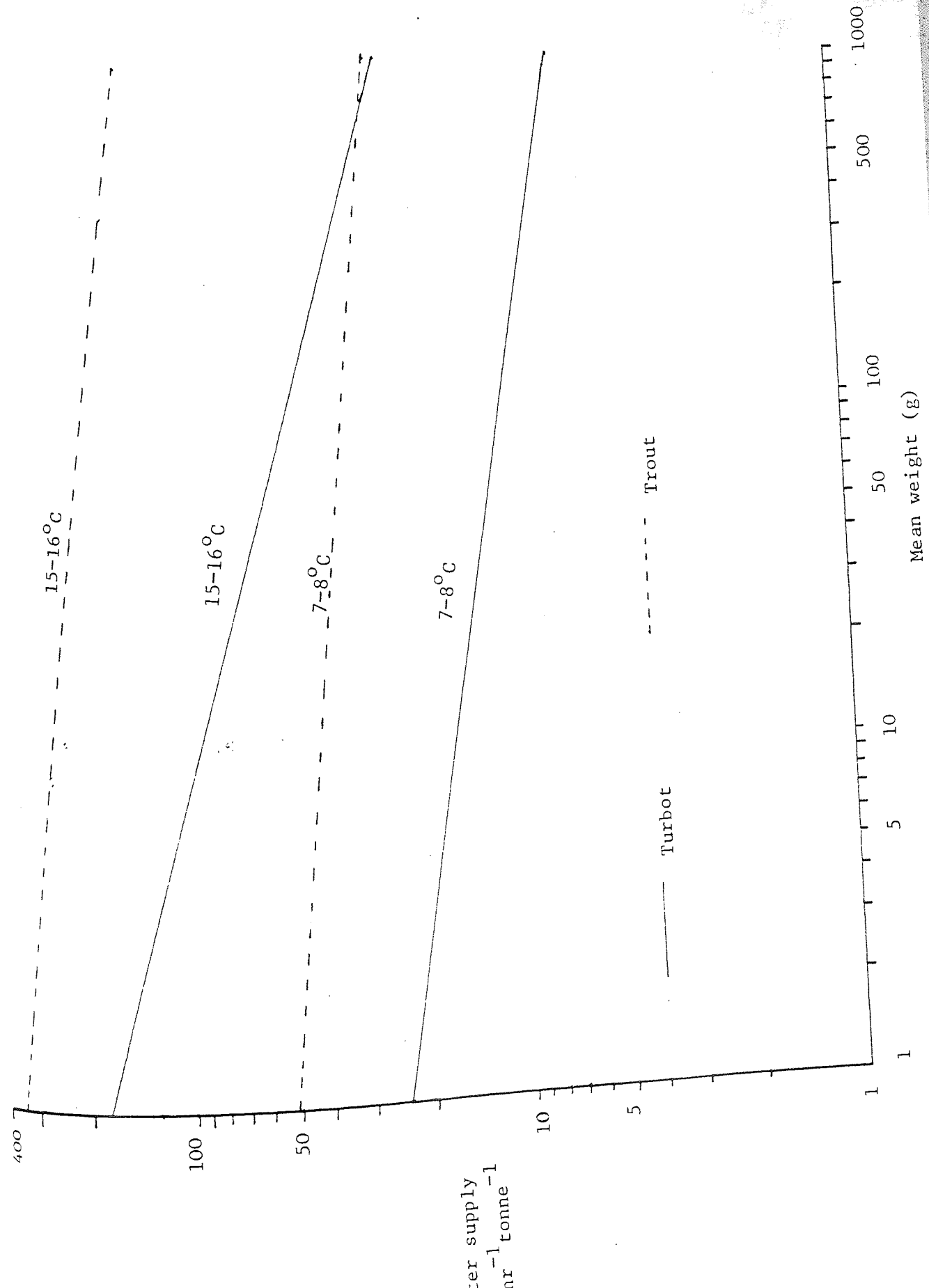
At a higher temperature, the oxygen demand is greater and the amount of oxygen dissolved in the water less than at 7-8°C.

At 15-16°C, the water supply requirement is 95.4 m^3hr or 20,985 gallons hr^{-1}. A further illustration is presented in Figure 4.1, using data from Figure 3.4.1.

It is obvious, therefore, that the amount of water available determines the stocking densities that can be maintained within the tanks. The cost of pumping water varies according to location, in particular tidal range and height of tanks above sea level, eg at Wylfa there is a total head

Figure 4.1 Water supply required to satisfy the oxygen requirements of turbot and trout at 7-8°C and 15-16°C

(Note calculation on previous page based on two tonne production unit



of 30m. On sites where pumping costs are high it may be advantageous to use aeration or oxygenators, eg as at Wylfa. Thus in order to maintain higher stocking densities than the limited water flow would support (approximately 50 kgm^{-3}), a re-oxygenation system is employed. The system, which was described in Section 3 and Harmon (1978), enables a 7-fold reduction to be made in the water supply to the tank. Having thus developed and utilised a system by which the oxygen is supplied to the tank independently of the water supply, the purpose of the water supply is to remove waste products of metabolism such as carbon dioxide and ammonia.

Problems have already been experienced in intensive rainbow trout farming due to high levels of carbon dioxide in the tank water causing a condition termed nephrocalcinosis (Smart et al 1979). At Wylfa, the outgoing water from each tank is checked daily after the days ration has been consumed. In view of the growth-retardant effect of carbon dioxide on turbot (Jones et al 1980), it is important to maintain the tank pH above a critical level of carbon dioxide, calculated as the effect upon pH (ΔpH). Clearly, the amount of carbon dioxide produced is a function of food, temperature and fish biomass in the tank. The relationship between these three parameters has been expressed by plotting ΔpH against flow rate per unit of food energy fed (Figure 6, Jones et al 1980). Thus in economising in water supply by increasing oxygenation, it is important to avoid high levels of carbon dioxide and reduced growth rates.

The levels of ammonia excretion were also reported in Jones et al (1980), for a production tank of turbot. The critical value when considering ammonia toxicity is the level of unionised ammonia present

(Smart 1975). As this is dependent upon pH and salinity (Smart 1975) and the production tanks are generally at a low pH (around 7.2), unionised ammonia is generally at a very low level (0.0039 mg NH_3-Nℓ^{-1}). Thus there is a benefit in maintaining a fairly low pH, in that it inactivates ammonia as a toxin.

When discussing water flow requirements, as in Figure 4.1, it is important to remember that this represents the maximum daily requirement. This maximum occurs at peak oxygen requirement, ie shortly after the final feed of the day. As illustrated in Figure 3.3.4, this increased oxygen requirement is manifested by a doubling in oxygen consumption. Thus the water supply to the farm, where no oxygen support is used, must meet this maximum requirement. On a farm where water supplies are pumped, this obviously leads to a waste of energy as, after the excess oxygen consumption due to feeding has ceased, the water supply is double that necessary to maintain adequate oxygen levels. On a farm where oxygen support is used, the water supply is not the main source of oxygen and so the water supply is not in excess. At Wylfa, the oxygen supply to each tank is increased at approximately 14.00 hrs and then reduced again at approximately 22.00 hrs. The study of diurnal fluctuations of oxygen consumption in 3.3.2 has enabled the extra oxygen required, due to the SDA effect, to be calculated and the oxygen flow settings to the oxygenaters, to be adjusted accordingly. On larger production sites, this adjustment of oxygen flow rates is performed automatically, by oxygen monitors switching solenoid valves to increase oxygen supply (see Harmon 1978 for further details).

The magnitude and duration of the SDA effect are clearly of major importance to the fish farmer, as is the affect of increased activity on oxygen consumption at feeding times. The preliminary analysis of Table

3.3.13, indicates the difference in the SDA effect of varying dietary energy profiles. Before drawing any conclusions from this data, it is important that the trials are conducted on a much larger scale, eg with stocks in a production tank. The implications of the affect of dietary energy profile are important for the fish farmer, as substantial savings in oxygen utilisation could be made, by using the diet that caused the lowest SDA effect (Diet 3(ii)).

Thus the main commercial contributions of this work, have been the development of a diet that is more cost-effective than sprat or sprat-based diets, whilst maintaining the quality of the end-product. Further research is required to determine whether the diet could be stabilised by reducing the water content, so that it could be manufactured commercially and distributed to fish farmers. The studies on oxygen consumption have permitted the calculation of water flow requirements to the fish farm to match the maximum requirement of the fish after they have been fed. Also a saving in oxygen consumption has been made, by quantifying diurnal fluctuations in oxygen demand and thus calculating the reduced oxygen flows required, to maintain a 4 ppm oxygen minimum in the tanks during the night. The analysis of the magnitude of the SDA effect as a function of the food fed, produced different results in the fry shed compared to the production tanks. Furthermore, the resulting SDA coefficients were lower than those recorded by other workers. This area of research requires further investigation to examine and explain these differences. Preliminary analysis of the SDA effect of different diets demonstrated that significant savings in oxygen utilisation could be made by using a diet that resulted in the lowest SDA effect, but maintained satisfactory growth and food conversions. The relationship between SDA and food conversion is a future field of research. The most

effective way to study these relationships would be to construct energy budgets for turbot on different diets, incorporating SDA as an energy cost.

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APPENDIX 1 ANALYTICAL PROCEDURES

Appendix 1 Analytical Procedures

(i) Ash

Silica dishes were heated in a muffle furnace at 500°C for 30 minutes. The dishes were then weighed to the nearest mg (W_1). 5g of the minced sample was then transferred to the dish and the dish reweighed (W_2). The dish was then heated in a water bath to drive off most of the water, then the sample was charred over a bunsen burner. The dish was then placed in a muffle furnace overnight at 500°C ± 15°C. The dish was cooled the following morning in a desiccator and reweighed (W_3).

$$\% \text{ Ash} = \frac{(W_3 - W_1)}{(W_2 - W_1)} \times 100$$

(ii) Moisture

An aluminium dish and lid was weighed (M_1). 10g of the sample was added and the dish reweighed (M_2). The dish was then placed in a forced draught oven with temperature controlled at 101°C ± 1°C. The dish was left overnight in the oven and reweighed in the morning (M_3) after cooling in a desiccator.

$$\% \text{ Moisture} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

(iii) Oil - Soxhlet Extraction

(W_1) 5g of the sample was weighed and transferred to a soxhlet thimble (Whatman cellulose extraction thimbles single thickness). The top of the thimbles were closed with absorbent cotton wool. A clean dry flask was then weighed (W_2). Sufficient solvent (4 : 1 Methanol : Chloroform) was placed in a flask (150 ml) to allow the extraction to proceed without going dry. The flask was heated on a hot plate, so that the sample was extracted by a steady stream of condensing solvent, in the butt type extractor and at least 10 siphoning cycles were completed in an hour in the siphoning soxhlet. The extraction was allowed to proceed for four hours. The flask was then put in an oven at 100°C for 30 minutes and then placed in a desiccator to cool and be weighed. This process continued until a constant weight was obtained (W_3).

$$\% \text{ Oil} = \frac{W_3 - W_2}{W_1} \times 100$$

(iv) Protein - Kjeldahl Determination of Nitrogen

0.5g of the sample was weighed into a clean, dry kjeldahl flask and two kjeltabs 'M' added. 20 ml of concentrated sulphuric acid was then added, so that any residue on the inside of the flask was washed down into the bulb.

The neck of the flask was closed with a loosely fitting bulb stopper and heated on a suitably designed digestion stand, linked to an efficient exhaust system to remove the acid fumes. Strong heat continued until the acid digest became clear (about 20 minutes) and then

for a further period of 45 minutes. The flask was then cooled and a little distilled water added. When the flask had cooled again, the contents were diluted to 600 ml. A sample was then taken for nitrogen determination on a Technicon Auto Analyser. To determine % protein, % nitrogen was multiplied by 6.25.

(v) Gross Energy

Samples were made into pellets weighing between 1.2g and 1.6g. A 2" length of cotton was then combusted in an adiabatic bomb calorimeter (Gallenkamp Ltd) and the temperature rise recorded (T_1). The bomb was then calibrated using a benzoic acid tablet and the temperature rise recorded once more (T_2). The calibration factor was then calculated.

$$\text{Calibration factor (C)} = \frac{6321.6 \times \text{Mass benzoic acid}}{T_2 - T_1}$$

A pellet of the test sample was then combusted and the temperature rise recorded (T_3). The gross energy of the sample was

$$\text{GE sample} = \frac{(T_3 - T_1) \times C}{\text{mass sample}} \quad \text{cals}$$

(vi) Ammonia Analysis

1. Stock solutions

a) Sodium nitroprusside solution

0.4 gms sodium nitroprusside were dissolved in deionised water and made up to 100 ml in a volumetric flask.

b) NaOH stock solution

68 gms NaOH were dissolved in deionised water and made up to 250 ml

after allowing the initial solution to cool.

c) Phenol stock solution

156 gms A.R. Grade Phenol were dissolved in methanol and made up to 250 ml in a volumetric flask.

2. Analytical reagents

a) Citrate Buffer

The following were dissolved in deionised water and made up to 250 ml in a volumetric flask -

Sodium citrate	50 gms
EDTA	25 gms
NaOH	5 gms

Because of the large volume of the reagent solids, they were transferred to the volumetric flask using the minimum of deionised water. Solution of these chemicals took 24 hours at room temperature.

b) Phenate reagent

15 ml of the phenol stock solution and 10 ml sodium nitroprusside solution were made up to 100 ml, with deionised water. This was then transferred to a clear glass bottle and stored in a refrigerator.

c) Alkaline hypochlorite

30 ml NaOH stock solution and 5 ml sodium hypochlorite solution were diluted to 100 ml with deionised water, transferred to an amber glass bottle and stored in a refrigerator.

Procedure for sample analysis

To 5 ml of the test sample in a 50 ml flask, the following were added:

a) 2.5 ml citrate buffer

b) 2.5 ml phenate reagent

c) 2.5 ml alkaline hypochlorite

This was then made up to 50 ml with deionised water, stoppered, and mixed well. It was allowed to stand for at least 20 minutes at room temperature, then the absorption was measured using 1 cm cells at a wavelength of 635 nm.

(vii) Nitrate Analysis

Analytical Reagents

a) Sulphanilamide reagent

1.0g of sulphanilamide was dissolved in 90 ml of distilled water and 10 ml of concentrated hydrochloric acid.

b) Napthylethylenediamine reagent (NAD)

A 0.1% (W/V) solution of N-1- napthylethylenediamine dihyrochloride in distilled water was prepared and stored in an amber glass bottle.

Analytical Procedure

1.0 ml of sulphanilamide solution was added to 5 ml of the test sample in a 50 ml graduated cylinder. After five minutes 1.0 ml of NAD solution was added, mixed and the cylinder made up to 50 ml with distilled water. After 15 minutes, the absorption of the solution was measured at 543 nm in a 10 cm cuvette.

(viii) Chromic oxide determination.

100mg of the faecal sample (w_1) was placed in a kjeldahl flask and 2.5 mℓ of concentrated nitric acid added. After five minutes

the flask was heated until a white precipitate formed and all the black deposit had dissolved. The flask was then cooled to room temperature, 1.5 mℓ of concentrated perchloric acid added and then reheated for ten minutes. After the flask had cooled, it was made up to 50 mℓ and the organic matter was allowed to precipitate. The absorbance of the solution was measured at 350 nm against a distilled water blank.

$$\% \ Cr_2O_3 = \frac{(\text{Absorbance of solution} - 0.0032) \times 100}{417.8 \times w_1}$$

APPENDIX 2 ANCILLARY DATA FOR SECTION 2

Appendix 2

Ancillary Data for Section 2

Experimental Periods for Experiment 1.1

Period	Dates
1	5 July 1976 - 19 July 1976
2	19 July 1976 - 2 Aug 1976
3	2 Aug 1976 - 17 Aug 1976
4	17 Aug 1976 - 31 Aug 1976

Experimental Periods for Experiment 2.1

Period	Dates
1	1 Mar 1977 - 5 Apr 1978
2	5 Apr 1977 - 21 Apr 1978
3	21 Apr 1977 - 3 May 1978
4	3 May 1977 - 22 May 1978
5	22 May 1977 - 7 Jun 1978
6	7 Jun 1977 - 21 Jun 1978

Experimental Periods for Experiment 2.2

Period	Dates
1	12 Feb 1978 - 27 Feb 1979
2	27 Feb 1978 - 12 Mar 1979
3	12 Mar 1978 - 26 Mar 1979
4	26 Mar 1978 - 10 Apr 1979
5	10 Apr 1978 - 23 Apr 1979
6	23 Apr 1978 - 9 May 1979

Fish Numbers for Experiment 1.1

Tank	Period					Diet
	0	1	2	3	4	
1	25	25	25	25	25	1(i)
2	26	26	26	26	26	1(ii)
3	26	26	26	26	26	1(iv)
4	25	25	25	25	25	1(iii)
5	26	26	26	26	26	1(iv)
6	24	24	24	24	24	1(i)
7	25	25	25	25	25	1(iii)
8	25	26	26	26	26	1(ii)
9	26	26	26	26	26	1(iii)
10	25	25	25	25	25	1(iv)
11	24	24	24	24	24	1(ii)
12	25	25	25	25	25	1(i)

Fish Numbers for Experiment 2.1

Tank	Period							Diet
	0	1	2	3	4	5	6	
1	397	396	396	396	396	396	395	2(i)
2	412	407	407	407	406	405	405	2(i)
3	406	403	403	403	400	398	397	2(ii)
4	398	398	398	396	391	388	385	2(ii)
5	406	406	406	406	404	403	403	2(iii)
6	392	392	391	391	386	383	383	2(iii)

Fish Numbers for Experiment 2.2

Tank	Period							Diet
	0	1	2	3	4	5	6	
2	501	501	501	501	498	498	498	3(ii)
3	502	501	501	501	501	499	497	3(i)
4	496	496	496	495	495	495	495	3(iii)
5	502	502	502	502	497	497	494	3(i)
6	503	502	502	502	502	502	501	3(i)
9	501	501	501	501	501	501	499	3(ii)
10	498	497	497	497	497	497	495	3(iii)
11	497	496	496	496	496	496	496	3(ii)
12	502	502	502	502	502	498	496	3(iii)

Biomass (g) Data for Experiment 1.1

Tank	Period				
	0	1	2	3	4
1	2396	2952	3155	3547	3955
2	2199	2298	2468	2715	2918
3	2718	3049	3558	4133	4758
4	2174	2293	2438	2614	2835
5	2359	2956	3147	3597	4082
6	2218	2477	2709	2928	3243
7	2420	2611	2782	2994	3254
8	1960	2028	2164	2272	2388
9	2166	2418	2633	2870	3070
10	1948	2193	2514	2895	3287
11	2531	2688	2957	3266	3487
12	2745	3318	3740	4254	4646

Biomass (g) Data for Experiment 2.1

Tank	Period						
	0	1	2	3	4	5	6
1	4372	5446	6217	6850	7232	8160	9105
2	4649	5626	6220	6688	6999	8090	8687
3	4806	6062	6793	7436	7600	8434	9189
4	4310	5326	5916	6370	6613	7370	8020
5	4582	5682	6275	6644	6965	7598	8369
6	5172	6200	6918	7297	7543	8143	8877

Biomass (g) Data for Experiment 2.2

Tank	Period						
	0	1	2	3	4	5	6
2	8314	9738	11000	12174	13805	14941	16922
3	7670	8917	9894	11181	12830	13313	14796
4	8696	9632	10952	11890	13244	14581	16274
5	7321	8600	9547	10276	11850	12610	13845
6	7089	8242	9106	9976	11725	12342	13587
9	7388	8379	9419	10438	12150	13173	14860
10	6662	7684	8734	9855	11359	12531	14321
11	5666	6529	7590	8275	9950	10723	12176
12	7110	8210	9513	10360	12045	13057	14740

Length Weight Data for Experiment 1.1

System 1 Pre-experiment

Tank 1		Tank 2		Tank 3		Tank 4	
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
17.7	89.83	19.2	120.09	19.4	143.39	18.6	112.48
17.1	84.43	14.6	84.05	16.8	98.58	18.3	103.83
18.6	128.13	18.8	112.57	17.8	108.09	17.6	86.66
16.1	70.14	18.7	112.55	18.3	115.00	18.5	108.00
17.2	92.40	19.5	132.56	17.5	104.88	16.2	72.01
18.4	124.24	19.6	139.13	17.9	99.56	18.3	126.06
20.7	164.59	13.8	43.32	19.5	121.95	18.0	102.89
19.0	116.89	19.3	120.81	17.2	108.74	16.8	81.14
16.3	73.00	13.8	52.36	18.5	123.80	16.0	70.71
18.2	103.89	18.2	104.46	19.7	152.13	16.5	75.74
15.1	61.80	16.9	85.84	17.5	95.22	15.0	57.27
18.0	107.96	14.7	59.00	19.2	124.19	16.1	70.94
17.3	91.00	16.6	77.07	14.7	54.00	21.5	178.62
21.2	171.08	15.5	72.31	18.5	113.47	16.5	72.67
16.8	81.48	17.4	89.57	14.1	58.64	16.6	29.84
18.7	109.85	13.9	43.47	18.9	116.55	18.3	100.38
14.7	59.91	15.2	64.90	17.0	93.76	17.5	97.76
15.6	59.58	16.8	81.60	18.9	142.00	15.0	61.38
17.1	91.05	16.8	89.18	21.8	186.28	17.1	81.45
17.1	85.26	17.9	98.33	17.1	92.76	17.8	97.66
18.8	124.77	17.3	94.41	14.5	51.12	16.5	74.47
19.8	137.70	14.4	54.10	16.3	72.00	15.6	65.78
17.4	89.96	13.1	35.24	17.4	92.64	15.5	58.46
16.0	100.74	16.5	76.61	17.3	88.54	14.8	52.11
16.5	75.78	15.2	71.22	17.7	99.38	16.8	85.00
		17.4	84.92	12.7	60.86		

Length Weight Data for Experiment 1.1

System 2 Pre-experiment

Tank 5		Tank 6		Tank 7		Tank 8	
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
16.4	75.97	21.9	191.42	18.3	109.00	20.3	146.17
19.5	134.47	19.9	135.55	17.1	101.74	17.3	92.52
15.7	65.68	21.3	177.57	21.3	177.80	12.5	33.82
12.9	35.55	21.8	178.94	18.8	117.00	16.3	73.76
18.4	113.96	17.9	107.49	19.1	129.15	13.4	46.07
17.6	90.78	19.8	133.00	19.3	139.11	17.6	90.77
19.4	136.94	17.4	79.62	18.4	109.28	15.6	69.79
17.9	112.94	17.6	100.31	16.3	75.00	14.4	45.90
14.7	51.78	18.1	111.90	18.6	110.74	17.2	94.11
18.4	111.56	18.6	117.20	18.5	107.44	17.8	112.31
19.1	125.12	17.1	81.92	16.6	77.77	16.9	82.40
15.3	69.21	18.2	115.10	16.3	68.95	16.3	94.22
14.3	54.09	14.2	46.41	18.4	117.10	17.3	90.52
16.5	83.62	16.5	75.28	18.7	106.50	14.6	52.60
20.1	100.33	15.1	66.67	18.1	100.00	15.8	61.45
16.3	101.96	12.1	30.52	17.3	80.49	15.2	60.40
17.6	94.42	15.6	67.80	16.7	78.61	15.4	62.99
17.2	89.18	16.6	78.72	17.7	108.52	15.0	54.77
20.0	144.80	13.4	63.06	19.0	132.40	14.8	66.20
17.3	76.01	15.2	54.86	15.0	51.14	19.3	120.48
19.4	124.93	16.6	73.17	12.3	27.89	17.6	94.10
16.0	68.41	15.7	65.18	14.2	47.18	14.3	61.97
16.8	89.86	17.2	90.93	14.1	47.33	17.0	89.44
15.7	69.18	16.0	65.85	17.0	93.77	14.2	59.59
17.4	95.34			17.2	123.73	12.2	30.44
15.3	62.47						

Length Weight Data for Experiment 1.1

System 3 Pre-experiment

Tank 9		Tank 10		Tank 11		Tank 12	
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
20.3	145.96	16.9	87.39	19.7	148.94	20.3	165.75
16.1	65.41	16.6	77.78	16.0	29.60	16.5	83.33
19.7	149.17	17.7	105.26	18.5	114.40	16.4	76.16
18.5	117.71	17.6	106.17	21.3	185.04	18.2	124.50
17.2	93.40	19.2	121.99	17.0	96.44	19.6	134.41
19.0	117.58	16.4	76.74	19.5	137.48	22.1	198.55
19.0	142.16	18.1	82.28	21.9	193.48	22.9	228.37
19.4	143.71	15.8	69.92	19.0	128.87	18.4	111.08
18.0	105.91	16.3	82.11	20.5	173.69	14.4	50.20
15.8	73.53	15.6	64.88	16.0	75.58	17.2	86.49
18.1	104.10	16.3	72.71	18.2	97.97	17.7	120.45
16.4	66.90	19.7	151.67	18.6	81.30	20.1	144.18
15.1	57.81	14.8	59.35	13.8	49.06	16.1	75.62
16.3	73.89	14.7	72.36	20.7	165.37	17.4	86.33
15.6	59.95	16.2	69.89	17.6	93.27	18.1	102.25
15.2	60.70	15.8	79.31	16.7	77.00	17.9	119.99
14.0	55.68	14.9	54.00	15.2	60.72	17.6	99.64
17.0	86.86	14.7	55.46	18.0	108.57	18.4	114.18
14.4	46.52	16.9	84.04	12.5	48.61	18.8	129.06
14.7	49.16	16.5	78.80	17.6	102.18	15.9	74.55
16.1	81.82	15.9	81.69	17.3	101.23	15.5	71.13
13.1	39.00	13.4	45.86	16.1	67.96	14.9	73.91
13.5	45.82	14.5	54.00	17.5	93.00	18.2	106.82
15.1	54.74	14.1	48.08	14.2	52.08	15.8	82.50
12.4	46.52	14.9	65.27			17.0	86.29
15.4	82.30						

Length Weight Data for Experiment 1.1

System 1 Post-Experiment

Tank 2		Tank 1		Tank 3		Tank 4	
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
21.1	170.70	19.0	132.56	24.2	346.75	19.1	116.97
20.0	140.47	22.9	272.98	21.5	204.20	19.4	123.62
17.7	103.97	22.7	227.31	19.1	164.48	17.5	99.02
19.6	127.80	21.5	188.76	21.2	183.42	18.1	105.79
20.3	150.13	18.1	113.76	18.7	129.17	18.1	100.37
16.4	64.60	19.3	146.50	18.0	120.28	16.1	72.04
20.9	169.90	21.7	187.98	20.1	152.28	19.9	146.59
15.6	70.10	16.7	76.48	18.2	119.63	16.7	87.61
19.0	124.15	16.4	94.11	20.1	169.32	16.1	80.50
19.0	126.09	17.4	103.51	18.0	147.18	17.4	98.36
21.1	187.54	17.6	94.99	20.4	205.18	23.7	271.04
13.3	31.84	20.8	165.54	25.7	380.00	19.5	165.50
18.7	115.78	17.7	104.87	24.0	288.65	17.7	95.91
16.3	87.00	18.4	110.10	17.2	91.55	19.1	135.50
17.0	95.79	21.0	177.27	20.8	157.54	18.9	120.32
14.9	59.45	18.8	132.62	22.5	124.45	18.0	97.24
17.8	101.85	18.9	118.67	19.7	166.43	15.9	67.99
13.9	40.11	22.0	215.16	18.2	107.74	16.0	76.74
21.5	176.59	18.2	159.53	21.6	258.40	20.6	148.80
17.9	111.21	19.8	150.09	15.0	57.32	18.4	113.32
16.4	80.13	20.5	159.91	19.5	144.64	16.9	83.88
18.4	125.55	20.4	175.41	15.1	85.38	17.0	86.74
19.0	121.62	19.1	147.80	19.2	136.07	19.7	144.67
20.4	155.90	21.1	189.72	21.8	249.26	16.4	79.25
16.8	84.89	24.5	308.87	21.9	249.94	18.8	117.49
16.3	92.24			23.8	318.33		

Length-Weight Data for Experiment 1.1

System 2 Post-Experiment

Tank 5		Tank 6		Tank 7		Tank 8	
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
20.4	168.96	22.8	225.18	21.0	175.22	18.8	107.32
17.3	89.10	16.1	94.65	17.2	87.74	17.9	101.88
21.0	186.94	18.3	106.82	18.4	110.60	19.2	153.27
18.2	116.36	12.5	37.20	18.2	152.60	15.1	75.39
23.9	276.72	17.1	115.60	20.2	151.37	22.8	213.48
16.9	96.82	17.6	102.27	21.5	192.25	16.9	87.10
14.4	53.06	24.7	301.47	17.6	96.38	17.5	101.94
22.4	225.49	22.0	208.25	22.2	210.27	14.4	53.35
23.2	259.14	19.8	152.78	20.3	180.49	15.4	62.65
18.6	173.66	17.4	90.32	18.0	119.30	13.4	39.00
15.8	68.28	16.1	71.40	15.9	64.99	13.5	42.70
19.7	150.00	18.7	127.40	14.8	55.91	15.5	70.99
20.9	200.95	18.7	127.45	20.9	183.07	17.9	104.70
17.9	120.30	24.4	287.92	13.1	34.38	17.4	100.00
21.0	175.48	20.5	180.24	19.0	112.26	18.7	151.00
21.8	212.77	16.0	74.18	19.7	142.53	17.5	102.47
22.9	261.48	24.3	295.29	20.1	128.08	19.1	116.60
19.3	136.10	15.0	91.80	20.0	150.25	16.7	88.52
23.8	293.00	15.9	83.87	19.9	140.20	15.0	56.66
19.7	164.37	16.9	79.72	19.2	123.36	14.6	48.28
19.1	139.49	16.8	85.98	18.2	129.93	15.9	65.50
18.4	95.46	17.4	98.23	19.7	134.57	15.0	71.67
16.8	89.02	15.8	66.08	19.7	135.10	17.1	88.34
16.9	96.05	19.9	137.93	19.7	164.55	15.0	64.39
17.6	124.69			17.0	78.69	19.4	139.58
17.6	108.00					15.9	81.54

Length-Weight Data for Experiment 1.1

System 3 Post-Experiment

Tank 9		Tank 10		Tank 11		Tank 12	
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
21.9	214.73	17.8	112.73	13.5	61.97	20.0	160.41
13.5	42.05	19.3	151.79	22.0	209.57	22.4	223.91
21.8	217.80	20.6	186.93	24.4	287.64	25.5	323.88
18.9	120.09	18.5	127.40	19.5	131.82	19.9	137.72
17.6	96.27	15.7	78.79	24.5	287.47	16.9	113.26
19.1	133.52	16.6	103.86	20.0	150.15	17.5	114.21
20.1	154.14	19.6	162.60	18.9	140.98	20.3	160.89
16.8	106.42	19.6	153.66	16.7	85.44	17.2	95.94
18.5	114.89	20.4	169.19	17.8	100.07	19.2	133.70
16.5	68.12	21.7	233.31	18.9	126.78	20.5	164.51
21.8	188.69	22.9	239.64	18.8	135.81	18.3	120.80
19.1	135.44	18.1	110.66	23.0	274.00	21.0	211.07
19.7	132.10	18.5	116.81	15.0	67.70	20.4	174.70
14.5	56.73	17.7	109.56	19.9	165.82	20.6	206.35
18.8	104.81	16.9	88.90	15.8	67.24	19.0	140.00
21.6	220.26	15.3	68.70	17.4	94.29	19.2	170.81
18.5	136.97	18.3	113.65	20.7	143.99	19.8	142.49
24.0	274.47	17.5	98.85	20.2	159.77	22.2	226.28
14.9	74.73	20.3	150.45	19.0	137.96	20.9	188.40
15.9	61.44	20.0	200.98	19.8	163.99	22.2	203.42
17.4	92.90	14.3	62.13	21.8	198.66	23.1	258.06
16.0	75.17	19.1	147.42	19.2	130.26	20.5	163.36
15.5	65.04	16.7	112.59	17.4	88.33	21.0	203.19
15.7	62.83	16.0	87.51	16.3	77.50	19.4	154.53
15.6	68.05	17.2	98.78			26.9	453.76
15.0	52.67						

Length-Weight Data for Experiment 2.1 - Pre-Experiment

Length (cm)	Weight (g)	Length (cm)	Weight (g)
7.9	9.9	7.9	10.5
7.9	10.3	8.4	12.1
8.2	11.0	7.7	9.2
8.0	8.5	7.4	7.7
7.8	9.2	7.7	9.6
8.3	11.6	7.3	7.4
7.6	9.8	8.1	12.1
8.4	12.3	8.6	13.8
8.3	11.6	8.9	14.1
7.7	9.2	8.4	12.6

Length-Weight Data for Experiment 2.1 - Post-Experiment

Tank 1				Tank 2			
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
12.7	42.9	11.2	29.1	11.5	39.1	12.2	38.0
9.9	17.0	11.0	25.9	9.6	22.2	8.5	12.3
9.2	16.6	9.7	17.5	10.1	23.2	9.4	18.8
12.7	40.2	11.4	27.3	10.1	27.5	12.0	33.9
10.7	22.1	12.4	37.1	8.4	12.2	11.9	34.9
7.8	9.1	10.2	22.0	9.6	19.7	11.0	28.0
9.8	18.8	10.4	22.0	10.7	29.3	9.7	18.3
13.2	48.7	8.7	14.5	11.0	25.2	9.2	16.3
10.5	20.9	11.0	37.2	10.0	19.9	9.8	19.7
11.1	28.3	11.7	28.4	8.8	14.6	9.0	17.1

Length-Weight Data for Experiemnt 2.1 - Post-Experiment

Tank 3				Tank 4			
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
10.7	35.0	11.2	27.6	7.5	9.1	9.3	17.9
8.6	12.0	12.6	39.5	11.3	32.0	10.0	20.8
10.4	23.2	11.4	31.1	8.5	12.4	10.6	26.5
10.6	26.1	10.1	21.0	12.3	38.3	11.8	37.7
8.5	13.0	10.2	22.0	10.9	28.9	10.3	22.1
10.1	20.5	9.9	20.7	11.0	27.5	9.2	14.8
13.7	57.1	10.2	31.6	10.2	22.6	9.0	14.6
13.2	46.0	11.2	26.4	8.9	15.3	7.9	9.7
10.2	22.9	11.8	30.9	10.5	25.1	10.9	25.9
9.7	17.7	8.9	16.1	11.2	27.8	10.9	25.0

Length-Weight Data for Experiment 2.1 - Post-Experiment

Tank 5				Tank 6			
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
12.2	35.9	10.7	24.2	10.5	22.5	11.5	29.1
10.3	20.8	10.9	25.6	10.8	10.5	10.9	23.7
9.8	19.9	12.0	35.3	13.3	45.8	11.0	26.3
14.3	56.1	9.7	19.5	13.6	23.6	12.9	41.3
12.1	36.0	9.6	18.6	10.0	19.3	10.8	25.5
8.0	9.0	8.9	16.3	12.3	38.5	12.7	39.0
10.0	18.7	9.9	19.4	12.1	36.9	12.0	33.9
11.0	27.5	10.4	33.7	10.7	25.2	8.5	11.1
12.4	40.9	9.8	16.9	12.9	50.2	11.4	33.2
13.4	53.3	9.1	15.9	7.3	7.8	9.1	14.7

Length-Weight Data for Experiment 2.2 - Pre-Experiment

Length (cm)	Weight (g)	Length (cm)	Weight (g)
7.7	11.8	7.4	8.3
8.6	12.6	7.2	8.1
7.2	9.0	7.5	9.1
8.7	11.9	7.7	10.1
7.8	9.6	7.2	9.5
7.6	9.0	7.9	10.5
7.4	8.3	8.2	10.6
7.9	9.4	8.0	11.7
7.8	10.0	8.5	12.5
8.1	10.5	8.6	12.8

Length-Weight Data for Experiment 2.2 - Post-Experiment

Tank 2				Tank 3			
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
9.9	16.1	11.8	32.6	11.5	31.4	13.5	53.2
12.2	33.0	10.6	20.4	10.8	22.9	12.2	35.6
13.3	48.6	12.8	42.7	11.7	19.9	10.8	25.0
11.1	23.6	14.3	65.4	11.5	29.1	14.0	60.4
14.5	61.3	12.3	36.2	11.5	34.0	13.4	47.3
8.9	11.0	11.9	31.7	12.6	37.7	13.8	42.5
13.0	40.1	13.0	42.1	12.2	33.2	13.5	44.0
11.6	29.8	11.6	27.3	12.5	36.0	12.6	36.3
12.7	37.8	10.6	24.1	12.0	31.5	11.8	24.3
14.8	64.7	10.4	20.3	14.5	62.6	11.0	23.5
11.4	29.1	11.6	26.6	12.3	32.1	12.1	32.3
13.4	50.4	9.7	18.6	13.5	46.6	12.2	32.7

Length-Weight Data for Experiment 2.2 - Post-Experiment

Tank 4				Tank 5			
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
14.5	59.3	13.4	48.7	12.9	42.0	13.9	44.3
13.4	50.6	13.1	48.6	13.6	51.6	11.1	23.7
13.6	50.7	11.4	27.8	12.4	41.8	11.6	26.3
11.0	24.0	11.1	26.8	13.9	49.4	11.1	25.0
12.6	45.8	13.8	52.0	12.7	41.5	11.8	29.0
11.2	27.0	12.4	46.1	11.8	25.9	12.3	37.5
12.5	35.8	14.7	59.2	12.1	32.1	11.6	33.0
11.4	28.5	13.2	50.5	13.9	60.3	12.0	33.6
13.4	53.1	14.0	55.0	11.8	32.4	11.5	29.7
13.3	47.0	12.6	40.6	11.5	31.6	11.6	32.7
12.6	35.6	11.3	28.0	10.5	23.6	10.4	22.6
13.2	45.9	12.0	33.4	12.5	38.4	12.5	37.0

Length-Weight Data for Experiment 2.2 - Post-Experiment

Tank 6				Tank 9			
Lcm	Wg	Lcm	Wg	Lcm	Wg	Lcm	Wg
12.7	38.9	10.9	24.8	11.8	32.6	12.9	36.8
11.3	31.9	13.0	40.2	12.9	50.8	11.0	25.0
12.3	32.5	13.0	42.5	13.1	47.9	9.8	24.6
11.2	27.4	11.7	29.6	10.9	25.3	11.6	33.5
12.3	28.5	13.2	44.9	11.4	25.5	10.8	21.7
12.6	28.0	9.5	16.0	11.6	29.1	13.0	45.8
11.5	21.3	12.7	35.4	13.6	51.1	11.7	29.2
12.7	28.2	13.6	47.7	11.5	28.2	12.6	35.7
11.6	32.6	10.0	18.7	11.6	30.0	12.0	30.3
11.8	30.2	12.1	35.1	11.7	34.6	13.3	52.0
11.2	27.4	12.5	41.0	13.1	47.5	12.1	37.0
11.4	27.0	11.8	29.3	11.5	41.0	12.3	37.8

Length-Weight Data for Experiment 2.2 - Post-Experiment

Tank 10		Tank 11		Tank 12	
Lcm	Wg	Lcm	Wg	Lcm	Wg
11.0	25.8	11.9	34.7	14.5	58.8
13.1	41.2	12.4	40.0	14.2	56.8
14.2	65.6	11.1	27.1	13.0	39.1
12.8	41.0	12.0	33.8	14.4	57.3
13.8	33.0	11.2	24.1	10.1	22.0
13.1	42.7	10.9	22.4	13.5	47.3
13.2	45.4	11.8	32.1	13.4	48.8
12.2	34.0	9.9	15.5	12.9	39.4
13.0	42.5	12.8	43.5	11.9	33.2
12.9	40.1	10.6	23.0	11.5	28.4
13.0	41.9	10.6	21.2	12.6	37.6
12.6	38.0	12.2	34.8	12.5	35.5
13.1	44.0	11.6	30.0	11.8	26.1
11.0	21.7	11.3	27.5	11.9	39.7
12.2	38.1	13.5	44.8	11.5	28.5
12.9	41.7	11.9	29.6	12.0	32.3
11.9	31.0	10.5	21.9	12.7	37.1
11.8	31.1	12.6	40.5	12.5	41.1
13.4	48.5	10.9	24.7	13.3	44.0
12.0	36.1	11.2	28.6	11.4	30.9
12.5	41.1	10.6	23.4	12.3	39.9
13.1	45.3	10.8	20.9	10.4	22.9
11.0	31.6	9.7	17.8	13.2	46.7
12.6	46.7	9.8	17.0	12.6	39.7

APPENDIX 3 ANCILLARY DATA FOR SECTION 3

Appendix 3

Ancillary data for Section 3

Identification of tanks, dates and biomasses from Tables 3.3.2 and 3.3.3

Data Reference Number	Date	Tank	Total fish weight kg	Gradient from fall in oxygen saturation
1	28.3.79	FS 7	9.1	0.4036
2	26.4.79	FS 7	9.1	0.2876
3	21.12.79	P 3	2063	0.4729
4	3.1.80	P 3	2159	0.5167
5	21.12.79	P 2	2410	0.3996
6	3.1.80	P 2	2503	0.5033
7(i)	12.1.80	FS 1	7.6	0.7571
7(ii)	12.1.80	FS 2	7.1	0.7667
8	30.5.79	FS 12	6.1	0.4500
9(i)	16.5.79	P 4	700	0.934
9(ii)	31.5.79	P 4	733	0.897
10	10.1.80	P 3	2163	0.8125
11	4.1.79	P 4	668	0.1429
12	10.1.80	P 2	2517	0.6130
13	11.12.77	P 2	1299	0.2180
14	11.1.79	FS 7	7.3	0.7285
15(i)	11.6.79	P 4	766	0.1320
15(ii)	26.6.79	P 4	835	0.6381
16(i)	3.11.79	P 3	1668	0.9918
16(ii)	7.11.79	P 3	1695	0.6476
17	24.6.79	P 3	1135	0.3738
18	7.11.79	P 2	2124	0.7758
19(i)	12.12.77	P 2	1301	0.3211
19(ii)	13.12.77	P 2	1304	0.2422

Data Reference Number	Date	Tank	Total fish weight kg	Gradient from fall in oxygen saturation
20	16.01.79	FS 7	7.9	0.9669
21	12.04.79	FS 2	13.9	1.7500
22(i)	11.10.79	P 4	1422	0.7321
22(ii)	12.10.79	P 4	1432	0.9560
23	11.01.80	FS 2	7.1	0.6594
24	11.09.79	P 3	1751	0.7469
25(i)	16.12.77	P 2	1313	0.2483
25(ii)	11.07.79	P 2	1545	0.4645
26	25.01.79	FS 7	8.9	1.9107
27(i)	25.04.79	FS 3	13.6	2.8848
27(ii)	25.04 79	FS 4	14.6	2.2909
28	23.04.79	P 4	543	2.412
29	23.04.79	P 3	900	1.498
30(i)	17.12.77	P 2	1317	0.4250
30(ii)	12.12.77	P 2	1301	0.3211

P = Production Tank, FS = Fry Tank

Details of decline in oxygen concentration over time

The records were accidentally destroyed for data reference numbers 13, 19(i), 19(ii), 20, 25(i), 30(i) and 30(ii).

Data Reference Number

1		2		3		4	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	84	0	88	0	100	0	97
5	81	5	84	10	95	5	95
10	77	10	82	15	93	10	92
15	76	15	81	20	90	15	88
20	73	20	80	25	87	20	85
25	71	25	79	30	85	25	83
30	70	30	79	35	83	30	80
35	70	40	74	40	81	35	79
40	67	50	72	45	79	40	76
45	64						

Data Reference Number

5		6		7(i)		7(ii)	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	90	0	97	0	89	0	96
10	85	5	95	5	85	5	92
15	83	10	92	10	81	10	88
20	84	15	88	15	77	15	85
25	82	20	85	20	74	20	81
30	79	25	83	25	70	25	78
35	76	30	85	30	66	30	75
40	73	35	79				
45	72	40	76				
50	70						

Data Reference Number

8		9(i)		9(ii)		10	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	103	0	103	0	84	0	96
5	101	5	102	5	82	5	92
10	100	10	99	10	79	10	89
15	98	15	99	15	78	15	85
20	93	20	97	20	76	20	82
25	92	25	96	25	75	25	78
30	90	30	91	30	74	30	72
		35	91	35	73	40	63
		40	90	40	72		
		45	90	45	71		
		50	90	50	70		
		55	89	55	68		
		60	88	60	66		

Data Reference Number

11		12		14		15(i)	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	92	0	97	0	86	0	84
5	90	5	93	3	83	5	76
10	88	10	89	6	81	10	73
15	86	15	88	10	77	15	72
20	85	20	84	14	73	20	71
25	84	25	82	18	71	25	69
30	84	30	79	22	69	30	65
35	84	40	71	26	66	35	63
40	85			30	64	40	62
45	84					45	61
50	82					50	60

Data Reference Number

15(ii)		16(i)		16(ii)		17	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	92	0	98	0	102	0	79
5	89	3	96	3	96	5	73
10	84	7	92	6	94	10	70
15	83	11	89	12	91	15	69
20	78	15	82	15	88	20	68
25	76	19	80	18	92	25	67
30	72	26	73	21	91	30	66
35	70			24	84	35	63
				28	78		

Data Reference Number

18		21		22(i)		22(ii)	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	89	0	81	0	92	0	100
3	87	2	77	2	85	3	98
6	83	4	74	5	83	6	98
9	83	6	71	8	80	11	83
12	80	8	67	11	81	14	89
15	76	10	63	14	79	19	82
18	75	12	60	17	74	22	81
21	72			20	73	25	78
24	71			23	69	28	72
27	68						

Data Reference Number

23		24		25(ii)		26	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	95	0	89	0	85	0	92
5	91	3	86	5	83	2	88
10	88	6	84	10	79	4	84
15	85	9	83	15	76	6	81
21	81	12	81	20	74	8	77
25	78	18	77	25	74	10	73
30	75	25	69	30	70	12	69
				35	69		
				40	69		

Data Reference Number

27(i)		27(ii)		28		29	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	94	0	87	0	95	0	78
1	93	1	85	5	95	5	74
2	89	2	84	10	92	10	70
3	87	3	81	15	91	15	66
4	84	4	79	20	86	20	63
5	80	5	77	25	84	25	63
6	77	6	74	30	81	30	58
7	75	7	72	35	75	35	54
8	72	8	70	40	73		
9	69	9	67	45	70		
		10	64	50	68		
				55	64		

Ancillary Data to Table 3.3.4

Tank Biomass and Mean Weight Data

Tank	Mean weight (g)	Total fish weight (kg)
1	32.28	12.591
2	28.96	11.584
3	30.66	11.957
4	27.02	10.916
5	28.11	11.130
6	30.91	11.685
7	26.56	10.254
8	66.35	13.601
9	12.12	8.494

Starved Fish

Tank	% DO In	% DO Out	% Decrease	Decrease $mg\ell^{-1}$	Flow rate $\ell\ min^{-1}$
1	98.5	73.5	25.0	2.1375	17.86
2	97.5	73.0	24.5	2.0950	15.46
3	98.5	75.5	23.0	1.9665	17.05
4	98.0	59.5	38.5	3.2918	11.07
5	99.0	71.5	27.5	2.3513	11.54
6	98.0	78.5	19.5	1.6673	23.26
7	100.0	74.0	26.0	2.2230	16.04
8	100.0	83.5	16.5	1.4108	22.73
9	99.0	73.0	26.0	2.2230	13.22

After 8 hours feeding

Tank	% DO In	% DO Out	% Decrease	Decrease $mg\ell^{-1}$	Flow rate $\ell\ min^{-1}$
1	108.0	73.0	35.0	2.993	20.00
2	108.0	78.5	30.5	2.608	22.90
3	108.0	76.5	32.5	2.779	19.48
4	104.5	80.5	24.0	2.052	20.41
5	106.0	81.0	25.0	2.138	21.58
6	108.0	78.5	30.5	2.608	23.44
7	106.0	81.0	25.0	2.138	23.26
8	103.5	79.5	24.0	2.052	20.98
9	100.0	79.0	21.0	1.796	21.58

3 Hours After Last Feed

Tank	% DO In	% DO Out	% Decrease	Decrease $mg\ell^{-1}$	Flow rate $\ell\ min^{-1}$
1	102.0	76.5	25.5	2.180	22.06
2	102.0	78.5	23.5	2.009	25.64
3	102.0	76.0	26.0	2.223	20.83
4	100.0	80.0	20.0	1.710	22.22
5	102.0	79.0	23.0	1.967	25.64
6	101.0	75.0	26.0	2.223	25.64
7	103.0	81.5	21.5	1.838	24.19
8	100.0	78.5	21.5	1.838	23.44
9	100.5	78.0	22.5	1.924	23.26

16 Hours After Last Feed

Tank	% DO In	% DO Out	% Decrease	Decrease $mg\ell^{-1}$	Flow rate $\ell\ min^{-1}$
1	100.5	70.5	30.0	2.565	17.14
2	100.5	78.0	22.5	1.924	20.0
3	99.5	72.5	27.0	2.308	16.76
4	100.0	76.5	23.5	2.009	17.96
5	99.5	76.5	23.0	1.967	19.48
6	102.0	77.0	25.0	2.137	20.27
7	99.5	77.5	22.0	1.881	16.04
8	99.5	73.0	26.5	2.266	14.93
9	99.5	68.0	28.5	2.437	12.50

Identification of tanks, biomasses and dates from Table 3.3.13

Tank	Diet	Data Reference Number	Total fish wt kg	Gradient from fall in oxygen saturation	Oxygen cons $gO_2 kg\ fish^{-1}\ hr^{-1}$
3	3(i)	1	11.274	1.7500	0.2340
3	3(i)	2	12.870	2.4429	0.2857
3	3(i)	3	12.976	2.4484	0.2840
3	3(i)	4	10.599	1.9857	0.2820
2	3(ii)	5	12.148	1.9286	0.2390
2	3(ii)	6	13.802	2.2714	0.2477
2	3(ii)	7	13.923	1.7483	0.1890
2	3(ii)	8	11.180	1.8214	0.2450
4	3(iii)	9	11.958	1.7428	0.2190
4	3(iii)	10	13.243	2.3571	0.2679
4	3(iii)	11	13.369	2.5847	0.2910
4	3(iii)	12	11.116	1.9571	0.2650

Details of decline in oxygen concentration over time

Data Reference Number

1		2		3		4	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	83	0	100	0	89	0	91
2	81	2	95	2	84	2	88
4	77	4	90	4	79	4	83
6	73	6	86	6	75	6	80
8	69	8	81	8	69	8	76
10	66	10	75			10	71
12	63						

Data Reference Number

5		6		7		8	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	97	0	100	0	81	0	81
2	92	2	95	2	77	2	80
4	87	4	91	4	74	4	77
6	84	6	86	6	71	6	73
8	81	8	82	8	67	8	70
10	77	10	77	10	73	10	64
12	73			12	60	12	60

Data Reference Number

9		10		11		12	
Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %	Time(mins)	DO %
0	88	0	90	0	92	0	85
2	83	2	86	2	89	2	81
4	79	4	81	4	84	4	77
6	77	6	76	6	79	6	73
8	73	8	71	8	74	8	70
10	70	10	67	10	69	10	65