DOCTOR OF PHILOSOPHY

Digestion and the systemic control of appitite in the dab, Limanda limanda (L.)

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Award date:
1982

Awarding institution:
Bangor University

DIGESTION AND THE SYSTEMIC CONTROL OF APPETITE

IN THE DAB, Limanda limanda (L.).

A thesis submitted to the University of Wales

by

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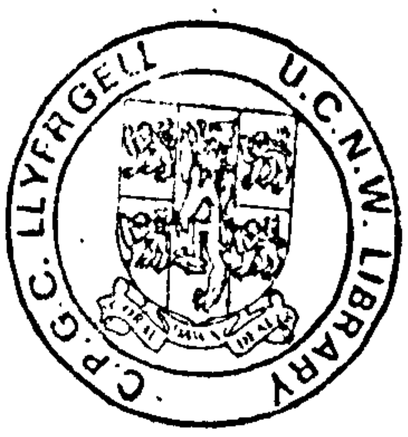
August, 1982.



DEDICATION

Cyflwynaf y thesis hwn i mam, am ei chefnogaeth a'i hannogaeth i mi ar hyd y blynyddoed, ac i Elisabeth am lu o atgofion hyfryd.

Contents

ACKNOWLEDGEMENTS

My sincere thanks are due to my supervisor, Dr. D. J. Grove, for his guidance and encouragement throughout my research programme. I am also grateful to the Chief Technician, Mr. G. Jones, for maintaining supplies of expensive reagents irrespective of the financial cutbacks. Bob Roberts, skipper of the R.V. Lewis Morris, ensured a good supply of healthy animals in frequently unpleasant weather conditions. Dave Vickers and John Mcrobie gave excellent support in maintaining sea water and power supplies in the face of heavy opposition from the maintenance department. Drs. J. Davenport and D. Holland gave helpful advice on respiratory and biochemical analysis techniques. I am also indebted to Police Constable Stephen Berry (North Wales Constabulary) for faithful protection of fish stocks.

Finally, I wish to thank my typists, Mrs. Eileen Williams and Mrs. Pauline Jones for their efficient and skilled typing of this thesis.

ABSTRACT

The effects of season, stress and nutritional state upon plasma glucose and fatty acids were monitored in the sand dab, Limanda limanda (L.), with a view to examining the possible role of these nutrients in controlling appetite return.

A chronic cannulation technique was used to obtain blood samples from animals with minimum disturbance. The annual, mean, 'normal', plasma glucose level (after 72 h food deprivation) was 22.44 ±3.96 mg/100ml (±S.D., n=88). Normal plasma fatty acid levels showed two seasonal peaks. During May to early August, levels ranged from 691.00 to 935.73 µeq/l while a second main peak (859.00 µeq/l) was observed in December. These periods correspond to the post-spawning and vitellogenic seasons respectively.

Mild stress (1 minute emersion to air) caused a significant hyperglycaemic condition (maximum plasma glucose level = 40.25mg/100ml) in 1.0 - 2.0 h, followed by a hypoglycaemic state some 9 h after treatment. Considerable individual variation in the stress response was observed. Trawling stress was reflected in a 230% increase in plasma glucose above normal levels.

Ingestion of 2% body weight meals of Mytilus caused plasma glucose levels to rise to between 34-50 mg/100ml. Feeding of glycogen supplemented (2.4%) fish paste meals gave maximum plasma glucose levels of 78.22 mg/100ml in 12 h. The magnitude of the hyperclycaemia was related to the level of dietary glycogen. A reciprocal relationship was observed between plasma glucose and free fatty acid levels of fish fed 6% body weight meals of Mytilus. The metabolities remained deviated from normal levels for a longer period during the summer, probably due to the greater glycogen content of the food during this season. The glucose/fatty acid reciprocal pattern was replicated by the intra-arterial infusion of a glucose load (0.5g/kg body weight)., which gave a maximum plasma glucose level of 310.4 mg/100ml.

Short-term food deprivation had no effect on plasma glucose levels while plasma fatty acid levels increased from 340.78 µeq/l (25 h post-operative) to 477.50 µeq/l (86 h post-operative). There was no further increase for the next 59 h.

It is likely that the post-prandial metabolite patterns of fish fed several meals within a short period would be very different, since the evacuation rate of a meal is increased in Limanda when followed by subsequent food. Gastric evacuation rates increased with an increase in body size. However, larger fish (120g) still had a longer gastric evacuation time (GET) (15.6h) than smaller fish (30g, 10.1h). In the 120g fish, GET for a single meal was reduced to 13.6 h when it was followed, 3 h later, by a similar sized meal.

The basal respiratory rate for 120g fish was 7.11 ±0.92ml O_2/h. This rate increased to 15.8 ±1.2ml O_2/h and 18.0 ±1.7ml O_2/h after a 3% body weight meal of fish paste and a 6% body weight meal of Mytilus respectively.

Limanda responded positively to a reduction in the level of dietary lipid or carbohydrate by increasing its food intake. Calory intake was maintained at a specific level irrespective of which nutrient was manipulated.

Maximally elevated plasma glucose or depressed fatty acid levels did not totally suppress appetite. This was so whether the plasma nutrient levels were altered naturally (by feeding) or artificially (by glucose infusion). The post-prandial increase in oxygen consumption was equally ineffective for totally inhibiting food intake. In addition, appetite return did not correlate closely with clearance of food from the stomach. It was tentatively concluded that appetite in this species may be principally influenced by available energy reserves.

General Introduction

The literature dealing with the control of appetite in higher vertebrates is both extensive and detailed. Such reviews on the regulation of feeding behaviour (Booth, 1976, 1977, 1979; Blundell et al., 1976; Bray, 1976; Blundell & Latham, 1979) indicate the incomplete understanding in this field and are dealt with in some detail in the following review.

In contrast, examination of the literature on fish appetite is very incomplete. For example, very few comprehensive studies into the physiological control of appetite in fish have been attempted. Essentially much of the published work in this particular area of research has been restricted to investigations of single physiological factors. For instance, the feeding behaviour of several teleosts in relation to the energy value of their food has been examined (Grove et al., 1978; Lovell, 1979). However, little attempt was made to understand how the energy value of the food actually modified appetite or which other appetite signals might be operating simultaneously. Sometimes, a close correlation between food intake and a suspected appetite signal, such as plasma gluclose levels, was not observed. This was interpreted as a strong indication that blood glucose was probably of little relevance in the overall control of appetite.

The present study attempts to examine some of the immediate physiological consequences of feeding in the sand dab, Limanda limanda. This species is readily available in local waters and settles down rapidly in captivity. In addition, Gwyther & Grove (1981) report a strong correlation between stomach fullness and appetite return in this species. The plasma levels of two metabolites, glucose and fatty acids, are examined in relation to the passage of single meals along the intestine. It is hoped that some information concerning the manner in which these metabolites might influence appetite will be obtained. Voluntary food intake of Limanda will therefore be monitored in relation to known plasma nutrient levels. This line of research will also be supported by an examination of the ad lib feeding

behaviour of groups of Limanda offered diets varying in nutrient composition.

Physiological studies of this nature are beset with several problems. Plasma metabolite levels are sensitive to numerous stimuli. These can cause large fluctuations within minutes or over a period of days and even weeks. In particular, the effects of season and stress have to be considered when monitoring the plasma levels of glucose or fatty acids in relation to the nutritional state of the fish. Consequently, in the present study a blood sampling technique had to be perfected which reduced stress to a minimum. In addition, it was hoped that serial sampling of individual fish, for measurement of plasma nutrients, would reduce some of the intraspecific variation often observed in such studies.

Finally, it has to be remembered that the conditions under which the plasma nutrients are investigated are highly artificial. For instance, they are measured following the ingestion of single meals only, whereas wild Limanda may ingest several discrete meals within a feeding period. Plasma metabolite patterns measured in the laboratory may therefore bear little resemblance to the situation in the wild. Consequently, it is difficult to extrapolate any observed relationships between specific metabolites and food intake in laboratory-held animals to wild populations of fish. Such studies may prove more useful for aquacultural practices where feeding is controlled. Irrespective of this fact, an attempt is made to examine one of the likely implications of feeding single meals alone on plasma metabolite patterns. Several factors effect the evacuation rate of food from the stomach in fish. Apart from meal size and temperature, the evacuation of a single meal may be modified by subsequent food intake. Consequently, gastric evacuation rate of meals under single and multiple feeding regimes are investigated. Ultimately, the rate at which nutrients are supplied to the intestine will largely influence subsequent plasma metabolite patterns and other post-prandial changes such as respiratory rates and repletion of energy reserves.

A REVIEW OF APPETITE CONTROL IN VERTEBRATES

1. Maintenance of body weight

It is generally known that warm blooded animals exhibit species-specific rates of growth while approaching adulthood and that, once this stage has been achieved, their body weights may remain relatively stable for many years. Individual adult humans show little variation in body weight, even over considerable periods of time (Davis, 1980). Any variations in the body weight of adult animals is normally due to changes in the level of fat deposits. Rats made obese (by force feeding or lateral hypothalamic stimulation) gradually reduced their voluntary food intake and finally stopped feeding (Cohen and Joseph, 1962; Steinbaum and Miller, 1965). Voluntary feeding was only resumed once normal body weight had been attained after cessation of the respective treatments. Similarly, starved rats gain weight rapidly when food becomes available until pre-starvation weights are reached (Keesey et al., 1976). This evidence has led many workers to believe that there exists in the central nervous system (CNS) a representation or a 'set-point' value of a preferred body weight (Mook et al., 1972; Panksepp, 1974; Scalfani, 1976). What form this 'set-point value' would take is obscure. Toates (1980) suggested that the concentration of a blood-borne factor, acting as a function of the available fat reserves, could be compared with a set-point value and so increase or decrease ingestion rates.

The assumption of a single set-point value as a basis for control of body weight fails in certain situations. Variations in the nature of the diet fed to rats causes alterations in the level at which their weight is regulated (Scalafani, 1976). Furthermore, the normal body weights of a variety of animals, including teleosts, show considerable seasonal variations as they increase or decrease their food intake. This would

require the existence of an adjustable 'set-point value' to explain the above observations. At this juncture, the concept of a single 'set-point value' becomes less convincing. Booth (1980) concluded that "the postulate is arbitrary unless and until independent evidence for a physical comparator mechanism is obtained by anatomical observation, and physiological or biochemical measurement, at the cellular level in the relevant location(s)".

2. The control of appetite

The term "appetite" is generally used to describe an instinctive desire by an animal to fulfil certain essential bodily requirements - particularly that for the ingestion of food. Neither the nature of the inner drive nor the mechanisms by which the intake is controlled are fully understood. Much of the earlier arguments in this area of research have centred around the source of the sensations or stimuli that motivate an animal to feed. The centralist theories advocate the idea that specific neural areas of the CNS alone are responsible for the initiation and termination of a meal as opposed to structures outside the CNS. The latter situation was supported by the peripheralist theories which suggested that, as in any complex form of behaviour, the brain was clearly involved in hunger motivation. However, the nutritional status of an animal, whether it is feeding or fasting, will modify several physiological parameters at any one time. Such changes will be likely to occur outside the immediate sphere of the CNS and would be monitored by the visceral sensory nervous system. The problem of the relative importance of peripheral and central events is not really a question of "either/or". Rather, it is a question of where in the process of regulation do the peripheral and central events modulate feeding behaviour (Novin and Van der Weele, 1976). Many of the peripheral theories, proposed as appetite-control mechanisms, involve fluctuation of certain metabolite levels, such as the glucostatic (Mayer,

1953), aminostatic (Mellinkoff et al., 1956) and lipostatic theories (Kennedy, 1953). Other hypotheses were of a more physical nature involving thermostatic and osmotic phenomena. In most instances these were single factor hypotheses, i.e. a single factor or stimulus, acting independently of all others, was considered to have the overriding influence on food intake. However, on an individual basis many of these theories are now considered to be wholly inadequate in explaining the phenomenon of hunger. For instance, Mayer's (1955) glucostatic theory is based on the principle that the animal is able to detect arteriovenous (A-V) glucose differences. A small difference indicates a low glucose supply and initiates feeding. However, there are several lines of evidence that do not support Mayer's theory (see review by Russek, 1971). These include the observation that exogenous adrenaline decreases the A-V difference, but also causes anorexia (Somogyi, 1951; cited by Russek, 1971). The failure of any individual appetite theory to explain food intake was interpreted by Russek (1971) to reflect a basic lack of physiological knowledge concerning some of the underlying principles of food intake. Basing his ideas on various pieces of experimental evidence (e.g. intra-portal glucose infusions suppress consumption) Russek (1981a,b) presented a strong case for the single factor argument. His theory postulated that food intake was controlled by some metabolic factor reflecting glucose availability. However, it is still the opinion of some workers (Bolles, 1981) that such unifactor arguments have caused much of the confusion surrounding this area of research. This latter viewpoint is largely accepted (Stevenson, 1964; Barnwell, 1974; Novin, 1976; Novin and Van der Weele, 1976; Van der Weele and Sanderson, 1976; Rolls, 1976). Appetite control is now generally considered to be multi-factorial, involving complex physiological interplay at several sites. It may be more fruitful to consider a continuum of nervous, humoral, and metabolic events occurring during and between meals (Van der Weele and Sanderson, 1976). Myers (1975)

presented a dual chemical profile theory which dispensed entirely with the traditional uni-factor concepts of hunger and satiety. He postulated a peripheral profile which consists of the ratio of all blood-borne substances, one to another, that are involved in energy balance, lipid deposition and other aspects of long- and intermediate-term regulation. Changes in the ratios would be monitored by CNS structures. The second profile consists of the chemical activity of transmitter and other neuro-humoral factors within these same structures of the CNS. Myers envisaged that variations in the ratio of the blood-borne constituents could modify the neurochemical profile in terms of the rates of transmitter synthesis, release and degradation. The variations in the ratios in which these neurochemical factors are released could provide appropriate neural signals which serve to activate or inhibit the various, observable activities and events of the feeding mechanism.

Research into appetite-control has; to a large extent, been restricted to studies in the higher vertebrates. In more recent years there has been a greater demand for improved aquacultural techniques in accordance with the developing importance of fish husbandry. The role of many factors, especially plasma metabolites, in food intake regulation of teleosts remains unexplored. This topic requires study since diet composition may influence food intake and subsequent growth-rates of commercially-important species. Prior to considering the available data of direct relevance to appetite-control in fish, it is necessary to review some of the research in higher vertebrates. This is mainly concerned with the identification of:- a) those neural areas of the brain concerned with appetitive behaviour and b) the physiological factors which may influence such regions of the nervous system.

3. Neural control of feeding

Hetherington and Ranson (1943) observed that damage to hypothalamic areas affected food intake in rats. Bilateral lesions to the ventromedial

hypothalamic (VMH) regions, particularly the ventromedial nuclei (VMN), resulted in the animals becoming obese. This was shown by Brobeck et al., (1943) to be due to the development of hyperphagia. A similar condition developed in cats (Anand and Brobeck, 1952) and monkeys (Anand et al., (1955) following the same form of treatment. In addition, it was shown that lesions to the lateral hypothalamus (LH) led to an aphagic condition in rats and cats (Anand and Brobeck, 1951) with the animals starving to death in the presence of food. The latter authors termed the LH region the "feeding centre". Although this area received fibres from other neural regions outside the hypothalamus and was in close proximity to several other important structures (e.g. optic tract, internal capsule), Anand and Brobeck (1951) considered that these other structures were not involved in the actual intake of food. This suggestion was later refuted (Grossman and Grossman, 1963; Gloor et al., 1969) (see later). Anand and Brobeck (1951) also claimed some inhibitory VMH influence over the LH regions since animals developed the hyperphagic condition when one or both LH areas were left intact but both VMH regions were destroyed. Bilateral hypothalamic damage resulted in the aphagic condition. As a result the VMH areas were considered to act as satiety centres. Later, Oomura et al., (1964) recorded a reciprocal electrical relationship between these two sites in the cat, supporting the concept of the VMH/LH interaction.

Delgado and Anand (1953) were among the first workers to improve on the earlier lesion techniques to study neural involvement in food intake. Using permanently implanted neural electrodes, they subjected rats to bipolar electrical stimulation. Treatment of the LH area led to a highly significant, but delayed, increase in both fluid and meat intake. Larsson (1954) similarly observed an increase in food intake following electrical stimulation of the LH areas of sheep and goats. In contrast to earlier lesion studies, Delgado and Anand (1953) observed that only unilateral stimulation of one of the LH areas was necessary to promote a reaction

whereas electrical stimulation of the VMH did not cause any decrease in food intake. Stellar (1954) based his "dual mechanism" theory for the control of appetite on much of this early evidence. This theory encompassed the VMH and LH areas as "satiety" and "feeding" centres respectively, and in fact supported the wholly centralist view of food intake regulation.

In the early 1960s the previously-favoured roles of the respective hypothalamic regions were first seriously questioned. Reynolds (1963, 1965) failed to produce the hyperphagic condition in the rat following VMH lesions induced by radio-frequency currents. This latter technique had two main advantages over earlier surgical and stimulation procedures. Firstly, the lesions are caused by thermocoagulation and so reduce haemorrhages but, more importantly, the method reduces the danger of metallic contamination of the neural tissue. Reynolds (1963, 1965) believed that hypothalamic hyperphagia was more likely to be caused by the irritant side effects of electrolytic lesion methods, i.e. heavy metal deposition or haemorrhage, leading to a chronic stimulation of the neural feeding centres. However, Hoebel (1965) and Stevenson (1969) repeated the electrocauterization experiments on the VMH and did observe development of a general hyperphagic condition. Reynolds (1965) refuted Hoebel's (1965) data as being non-conclusive and Stevenson (1969) conceded that large areas of the VMH could be damaged without effect. He agreed with Reynolds that it was misleading to refer to the VMH as a "satiety centre".

Essentially, the major problem with much of this early work was the lack of specificity of the techniques employed. Many of the neural networks responsible for different forms of behaviour are very tightly interwoven. This makes it practically impossible to remove or stimulate specific neural sites without modifying adjacent structures (Ruiter, 1963). As a result, alterations in feeding behaviour, following neural lesions, were frequently accompanied by modifications in other forms of behaviour (MacLean and Delgado, 1953; Miller, 1960; Grossman and Grossman, 1963; McBurney et al., 1965) which may well lead to a general lowering of arousal

and so alter feeding behaviour indirectly (Blundell and Latham, 1979). Improved histochemical methods have made it possible to map the detailed positions of cell bodies, axons and terminals containing various neural transmitters. Grossman (1960, 1962) adapted a technique whereby he chronically implanted cannulae systems into or slightly above the regions of the brain under investigation so that chemicals could be applied to specific neural structures. This technique had several advantages over earlier methods for research into food intake (Epstein, 1960; Grossman, 1960; Booth, 1967). Eating in satiated animals was promoted following bilateral infusion of the neural depressant procaine into the VMH (Epstein, 1960). However, the effect was only temporary even under conditions of prolonged infusion (Reynolds and Simpson, 1969). These latter authors concluded that the VMH may be responsible for monitoring short-term control factors such as the degree of gastric fullness or meal size. Alternative regulatory mechanisms were considered to exist in other neural areas which were thought to be dependent on the later, post-absorptive effect of the ingested food. While these alternative control mechanisms remained intact, the loss of normal VMH functioning could merely result in changes of meal size.

Evidence for the involvement of extra-hypothalamic regions in food intake control stems from the fact that lesions to several fibre systems serving different regions of the brain, e.g. the medial forebrain, resulted in the development of feeding behaviour similar to that following LH lesions (Morgane, 1961; Gold, 1967; Ungerstedt, 1970, 1971; Grossman and Grossman, 1973; Zigmond and Stricker, 1973; Marshall, 1974; Grossman, 1976). Many of these fibre tracts traverse the LH areas so that damage to these latter areas may ultimately affect tracts which co-ordinate extrahypothalamic activities. Much the same arguments apply to specific tracts traversing the VMH, e.g. the ascending noradrenergic pathway to the forebrain (Grossman and Grossman, 1971; Gold, 1973; Kapatos and Gold, 1973; Ahlskog, 1974; Ahlskog et al., 1975). The above evidence does not necessarily suggest that the

VMH and LH areas are not involved in feeding behaviour, merely that they are not unique in this respect. Ahlskog et al., (1975) demonstrated that damage of the noradrenergic pathway through the VMH, and VMH lesions that leave this pathway intact, each cause hyperphagia but that the combined operations generally cause a greater effect. Finally, electrophysiological studies show that there are both excitatory and inhibitory connections between the VMH and LH (Sutin et al., 1975). In conclusion, present evidence suggests that a simple inhibitory effect of the VMH on the LH, according to Stellar's (1954) theory, is no longer tenable (Peter, 1979) and reference to the VMH and LH as the "satiety" and "feeding" centres respectively is not strictly accurate (Gold, 1966, 1967; Lyon et al., 1968; Blatt and Lyon, 1968; Magnen, 1971; Panksepp, 1971; Rabin, 1972; Ahlskog and Hoeble, 1973; Kapatos and Gold, 1973; Blundell and Latham, 1979). However, there is considerable evidence that hypothalamic areas are involved in the ultimate regulation of plasma glucose and free fatty acid levels (Goodner et al., 1967; Himsworth, 1968; J. Magnen, 1971; Frohman and Bernardis, 1971; Frohman et al., 1971; Steffans et al., 1972; Hawkes and George, 1975; Blundell et al., 1976). It is not exactly clear whether the hypothalamic influence on these metabolites is strictly neural or endocrine, or both. In birds, there is evidence which supports the participation of both mechanisms in the maintenance of plasma fatty acids (Hawkes and George, 1975).

Plasma glucose and plasma fatty acids have been implicated in some of the major 'peripheral' theories of appetite control. In view of the above evidence it is probably incorrect to distinguish between peripheral and central controlling mechanisms of appetite.

4. Physiological factors that influence food intake

Before considering in detail some of the principal appetite control theories, some words of general introduction are required. Many

physiological factors (Table 1a,b) have been incorporated into appetite control theories for higher vertebrates. Some of these are quite likely to be of relevance in teleost studies. Many of the theories are based upon the existence of specialised nerve cells or receptors in peripheral or central neural tissues, which monitor such factors as the levels of blood metabolites, hormones or the degree of gastric distension (Anand et al., 1962, 1964; Oomura et al., 1964; Niijima, 1969; Novin et al., 1973; Müller et al., 1974; Woods et al., 1980). Some of the major hypotheses relating to appetite control concern the post-prandial fluctuations in metabolites such as glucose and amino acids. These nutrients, especially glucose, were considered to be of principal importance in the short-term control of food intake while other signals of oro-gastric origin were implicated in the immediate pre-absorptive phase of satiety. Other authors believe that it is the intact stomach and/or first half of the duodenum that plays a critical role in initiating short-term satiety. In this case, the absorbed nutrients may simply maintain satiety by their action at specific visceral receptor sites (Smith et al., 1974; Koopmans , 1975; Van Itallie et al., 1977). Ultra short-term, short-term and long-term control of food intake are the three main categories to which the various peripheral mechanisms that influence ingestion are often allocated as shown in table 1a (Novin and Van der Weele, 1977; Van Itallie et al., 1977). However, this form of categorisation can only be used as an approximate guide to the apparent influence of a particular stimulus on food intake. There is a considerable amount of data relating to the interaction of oro-gastric and post-absorptive signals (Le Magnen, 1971, 1977; Booth, 1972, 1977) (see later). Furthermore, simple contact of food with the mouth is sufficient oral stimulation to cause a reflex change in plasma glucose and insulin levels before intestinal absorption has actually commenced (Louis-Sylvestre, 1976). Furthermore, Penick et al., (1966) reported a decline in PFA in some human subjects when they were simply allowed to view food. Such subtle

Table 1a - THEORETICAL DIVISION, ON A TEMPORAL BASIS, OF FACTORS WHICH MODIFY APPETITE IN HIGHER VERTEBRATES

	Reference
A) Ultra-short-term factors (involved in early satiation)	
1. palatability	Soulairc, 1944; Mickelson et al., 1955; Campell and Davis, 1974.
2. glucose (chemospecific)	Booth, 1972; Novin and Van der Weele, 1972.
3. gastric distension	Davis and Campell, 1973.
4. gustatory adaptation	Mook et al., 1972.
B) Short-term factors (involved in regulation of meal-to-meal intervals)	
1. osmotic pressure of dietary carbohydrate	Harper and Spivey, 1958.
2. calorific value of food	Carlisle and Stellar, 1969; Booth, 1972 Van der Weele and Sanderson, 1976.
3. nutrient deficiency	Chester and Will, 1973.
4. dietary amino acid imbalance	Harper, 1976.
5. cholecystokinin	Smith and Gibbs, 1976.
6. sex hormones	Fishman, 1976.
7. metabolic flux	Sullivan and Triscari, 1976.
C) Long-term factors (involved in long-term maintenance of body weight	
1. lipid deposits	Kennedy, 1953.
2. prostaglandins	Baile et al., 1973.
3. insulin	Woods et al., 1980.
4. taste	Levitzky, 1980.

Table 1b - EFFECT OF HORMONES ON APPETITE IN HIGHER VERTEBRATES

Hormone and response	Reference
1. adrenaline and noradrenaline applied to central neural regions (+)	Grossman, 1962a,b, 1969.
2. prostaglandins (+)	Baile et al., 1973.
3. cholecystokinin (-)	Gibbs et al., 1973a,b, 1976. Smith et al., 1974; Smith and Gibbs, 1976; Kraly, 1981.
4. growth hormone (+)	Bray, 1976.
5. estrogens (-)	Fishman, 1976.
6. gastric and pancreatic hormones (-)	Steffens, 1980.
7. insulin (-)	Woods et al., 1980.

(+) stimulates appetite

(-) depresses appetite

interactions complicate the investigation of any single potential appetite stimulus.

Clearly, there is a considerable number of factors which modify appetite in the higher vertebrates. A detailed review of each food intake control mechanism is not appropriate in the present context. However, a brief consideration of the principal metabolite hypotheses and the manner in which they may interact is included here. While much evidence exists to implicate carbohydrates, proteins and lipids in the overall regulation of food intake, it is clear that they do so by very different mechanisms (Novin, 1976).

(a) Glucostatic hypothesis

Originally, plasma glucose levels were considered to be of principal importance in the control of ingestion. This belief derived from the known dependence of the CNS on glucose for its energy supply and on the increased hunger sensations following an insulin-induced hypoglycaemia (Mayer and Bates, 1952). However, it was realised that this latter relationship did not always persist.. Mayer (1953, 1968) suggested that it was the glucose availability, measured as the arteriovenous glucose difference (Δ-glucose), that was the stimulus controlling food intake. He proposed that a small Δ-glucose results in feeding while a high Δ-glucose, indicating a readily available glucose supply and high utilization rate of the metabolite, would inhibit ingestion. Mayer (1956) postulated the existence of glucoreceptors in the VMH which would monitor the Δ-glucose. As already mentioned, while there is some evidence in support of this theory, there are a number of areas where this particular mechanism fails to explain much of the detailed observations (Quaade and Juhl, 1962; Russek, 1971 for review; Novin and Van der Weele, 1977).

More recently, glucose has been implicated in another theory proposed by Russek (1963, 1971, 1976). He proposed the existence of hepatic gluco-receptors which monitored the supply rate of glucose from the gut. Russek

(1981a,b) later modified his earlier theory by suggesting that it was, in fact, the hepatic carbohydrate reserves that were monitored. In the formulation of this hypothesis Russek (1981a) proposed that;-

1) the liver possesses receptor cells, possibly the hepatocytes, whose membrane potential would determine the firing rate of nerve fibres. Such fibres have been shown to be in close contact with the hepatocytes (Nicolescue, 1958). "Hunger discharges" would be caused by a depolarisation of the fibres;

2) the liver glycogen content and glucose supply to the liver receptors would be represented by the concentration of a particular metabolite of the glycolytic chain;

3) an increase in the level of this metabolite, during intestinal absorption, would cause a hyperpolarization of the receptor membrane so reducing hunger discharges and inducing satiety;

4) a decrease in its concentration, when intestinal absorption and liver glycogen fall below a certain level, would increase the discharges from the hepatic receptors inducing hunger.

Russek (1981b) believed that an increase in the hepatocyte discharge would also produce a reflex secretion of glycogenolytic and lipostatic hormones. These hormonal changes would spare liver glycogen and so maintain blood glucose levels in the intermeal period. A number of workers have opposed this theory (Stephens and Baldwin, 1974; Bellinger et al., 1976; Strubbe et al., 1977; Bellinger, 1981; Louis-Sylvestre, 1981; Friedman, 1981; Niijima, 1981) but their arguments are often based upon a failure to emulate Russek's data due to variations in the actual techniques employed (Russek, 1981b; Van der Weele and Novin, 1981). For instance, some workers have failed to induce satiety by hepatic-portal glucose infusion. However, the response to such infusions is dependent upon the nutritional state of the experimental animal at the time of infusion (Baile et al., 1971; Novin et al., 1974). The induction of satiety following hepatic-portal

glucose infusions and the finding that intrajugular or systemic administration is ineffective (Russek, 1970) is one of the main areas of support for the involvement of the liver in satiety (Van der Weele and Novin, 1981). However, differential effects according to infusion site have to be demonstrated by biochemical assay to be specific to the hypothesized site of action (Booth, 1979). This author considered Russek's key infusion site result to be ambiguous in that a jugular infusion is liable to produce a larger insulin/glucose ratio in the blood passing through the liver than that produced by a portal infusion. Essentially, glucose from the former infusion would pass via the pancreas, stimulating insulin secretion, before entering the liver while glucose derived from a hepatic-portal infusion would enter the liver directly. Booth (1979) pointed out that it would be hard to predict the consequences of this anomaly on glucose distribution and metabolism around the body. Despite this apparent problem with the hepatostatic theory, other experiments have shown that food-derived glucose does contribute to satiety by its postintestinal action (Booth, 1979). Liver and heart infusions (via jugular vein) of glucose for prolonged periods are likely to generate after-effects in the liver and everywhere else in the body which do not differ according to the site of infusion. Oral, intragastric, duodenal, portal and jugular administration of carbohydrate all induce satiety, although such experiments do not indicate the specific sites involved (Booth and Jarman, 1976; Booth, 1976; Booth, 1979).

Until the experiments of Booth and Jarman (1976) there was no actual proof of a post-absorptive satiety signal. However, the latter authors did finally demonstrate the existence of a satiety signal following glucose infusions in the rat. The satiety effect was dependent on an adequate insulin secretion which indicated that the satiety signal was mediated metabolically. Gastric and duodenal glucose infusions substantially reduced subsequent food intake in the rat once the nutrient had been absorbed. In addition, hepatic-portal glucose infusion showed that most of the post-

absorptive satiety was due to the glucose itself rather than to hormones secreted during the absorption of the intestinal loads (Booth and Jarman, 1976; Booth, 1979). The satiating power of glucose was compared with that of other monosaccharide sugars which conclusively demonstrated that the satiating effect of glucose depended on its metabolism. 3-O-Methyl-glucose is an analogue of glucose which enters cells in the same manner as glucose but is not metabolised. Booth and Jarman (1976) proposed that since it is sufficiently like glucose to enter the cells by the same transport system, 3-O-methylglucose would be likely to have similar effects on the membrane surface of any glucoreceptors. Irrespectively, 3-O-methylglucose infusion had no effect on subsequent food intake. Furthermore, infusion of galactose (which is poorly metabolized) has no satiating effect while fructose (which is metabolised more rapidly than glucose) had an even greater satiety effect than glucose itself. This evidence supports Russek's (1981) theory that a metabolite of the glycolytic pathway might serve as the stimulus for hepatic glucoreceptors. The importance of glucose in the control of intake is further supported by studies with another of its analogues, 2-deoxy-D-glucose, which undergoes incomplete glucose metabolism and also competes with glucose for entry into cells. Doses of this analogue have been shown to block a glucose-induced state of satiety and elicit feeding (Jones and Booth, 1975).

The failure of some authors to induce satiety by glucose infusions (Stephens and Baldwin, 1974; Bellinger et al., 1976; Bellinger et al., 1977; Strubbe et al., 1977) is probably related to variations in the nutritional state of the animals (Novin, 1976) or the size of the glucose dose (Van der Weele and Novin, 1981). Baile et al., (1971) reported that food ingestion by monkeys was unaffected by glucose loads administered prior to feeding but that a 60% reduction in intake was observed if gastric loads were given during ingestion. The reduced intake observed in the monkey and the rat, following glucose infusions, was thought to reflect the

synergistic reaction of the absorbed glucose with the satiety signals arising from the ingestion of food. This would generate an absorption-like signal which is larger than that usually created by the amount of food already eaten (Booth, 1979). This situation ultimately led to an earlier cessation of feeding. These studies, therefore, suggest that the effectiveness of a glucose load is dependent upon the nutritional state of the animal. Furthermore, Van der Weele and Novin (1981) observed that high dose concentrations of glucose (10%) had no effect on the intake of rabbits, while a lower concentration (5%) had a satiating effect.

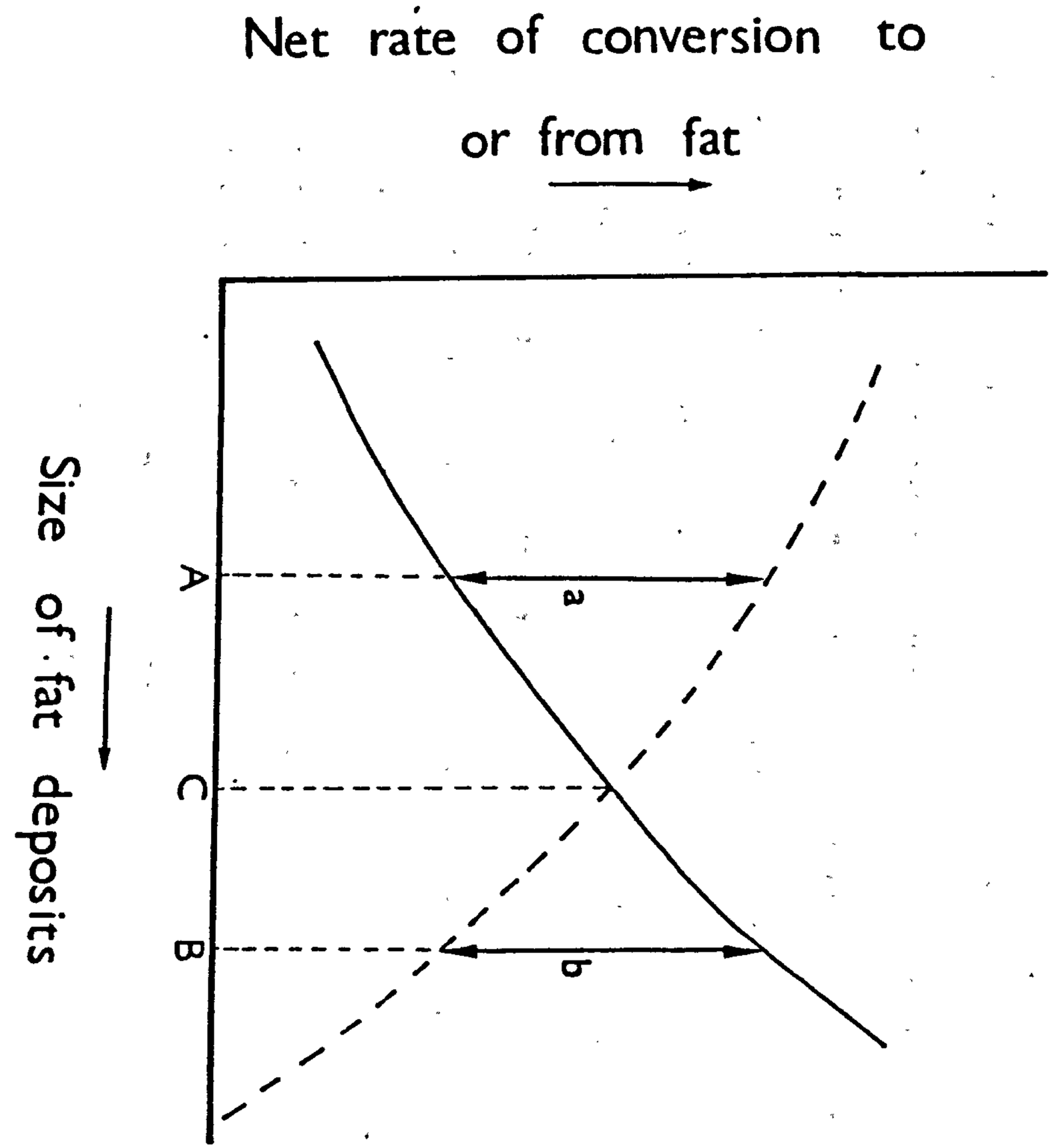
(b) Lipostatic hypothesis

Kennedy (1953) first proposed that the blood level of a factor related to fat reserves may modify food intake. Such a mechanism may operate by interaction with more immediate feeding control mechanisms to maintain stable body weights over long periods. The daily turnover of adipose fat deposits is a constant fraction of the total amount of lipid reserves (Bates et al., 1955) with the processes of lipogenesis and lipolysis occurring simultaneously (Newsholme and Start, 1978). Toates (1975) and Booth (1980) proposed an alternative explanation of long-term stability in body weight which does not require the inclusion of a hypothetical set-point value. The rates of lipolysis and lipogenesis would either increase or decrease respectively as the size of the fat deposits increase (Fig. 1) (Toates, 1975). A balance is automatically reached where there is neither a net gain nor loss of fats and so body weight remains constant. Booth (1980) observed that such opposing processes may vary randomly but tend to have a long-term average rate that can generate some stability stochastically without feedback. He emphasized that the components of such equilibrium systems could adapt. A hormone, whose secretion is stimulated by exogenous factors, may increase the slope of the curve of the transformation it controls. In comparison to the "sliding set-point" concept this system offers a more plausible explanation of the manner in

Fig. 1 - Proposed mechanism for regulation of lipid reserves in higher vertebrates (from Toates, 1975).

lipogenesis(---) decreases and lipolysis(——) increases as a function of fat deposit size.

When fat deposits are at 'A' there is a net lipogenesis of matnitude (a). At 'B' there is a net lipolysis of magnitude (b). The system is at equilibrium at 'C'.


Net rate of conversion to or from fat
Size of fat deposits
A
C
B
a
b

which processes such as lipid metabolism may vary between seasons (Booth, 1980).

Fatty acids and glycerol

During mobilisation of stored lipid reserves, free fatty acids (FA) and glycerol are released. Either of these metabolites may act as a representative signal of the lipid reserves during the long-term control of body weight. Alternatively, either metabolite may effect short-term food intake. Plasma fatty acid (PFA) levels do tend to vary considerably according to the emotional and nutritional state of the animal. On this basis, Russek (1971) and Bray (1976) concluded that this particular nutrient was unlikely to be involved in the control of food intake. In contrast, Booth (1979) considered that such variability in PFA levels did not preclude this nutrient from a role in long-term stabilization of fat stores. A small but persistent background signal underneath all the variability may be detected by the appropriate receptors, and so contribute to a nulti-factorial control system of ingestion. There is little direct evidence for a role of FA in food intake. However, Adair et al., (1968) reported an increase in food intake following intravenous injections of FA into the rat. Oomura (1976) pointed out that hunger-type behaviour was associated with high PFA levels. For instance, obese subjects have high PFAs as do hyperphagic diabetics. Oomura (1976) demonstrated a close relation between blood concentration of FA, effects of PFA on central neuronal activity, and effects of neuronal activity on observable hunger alleviating behaviour.

In contrast to the situation with FA, all the glycerol produced during triglyceride hydrolysis is released into the blood circulation and eventually transported to the liver. It is possible that the availability of glycerol for liver metabolism could be a quantity whose value is transmittable by the liver to the brain. In turn the availability of glycerol would reflect the state of triglyceride reserves (Bray, 1976;

Booth, 1979). Booth (1979) demonstrated a satiating effect of glycerol when administered intraperitoneally and a dose-dependent decrease in food intake following intragastric glycerol loads. Support for the theory that the availability of glycerol for liver metabolism may modify food intake comes from the observation that the action of exogenously-administered glycerol is dependent upon an intact hepatic-portal circulation (Grinker et al., 1980).

(c) Role of insulin in food intake

The rates of carbohydrate and fat metabolism are strongly interrelated with plasma insulin levels. This fact might well indicate a direct role for insulin in the control of food intake in the higher vertebrates. Some workers have considered the possibility that an insulin-sensitive centre may participate in appetitive behaviour (Kennedy, 1953). There is now ample evidence that activity of VMH neurons increases when insulin levels rise in their neighbourhood and both insulin and insulin receptors have been detected in the brain. A good correlation exists between meal pattern and insulin level in the rat which is independent of eating either carbohydrate-rich or carbohydrate-free food (Steffens, 1977). There is, however, some evidence against the argument for a role of plasma insulin in appetite control. Strubbe et al., (1977) failed to reduce spontaneous food intake when plasma insulin was chronically elevated by repeated small glucose infusions. However, the plasma insulin level that is effective in modifying intake is quite critical. Van der Weele and Novin (1981) believe that too little or too much insulin results in hyperphagia while just the correct amount aids the development of satiety. This idea was clearly supported by experiments with rats which were given constant steady insulin doses at various concentrations (Van der Weele et al., 1980, cited in Van der Weele and Novin, 1981).

It has been established that the amount of insulin in the blood in the basal or unstimulated state is positively correlated with body weight

or adiposity (Bagdade, 1968). The leaner an individual, the lower his basal insulin and vice versa. The autonomic nervous system has the potential to regulate the amount of insulin secreted in response to glucose, and hence food intake and body weight, in a very subtle manner (Campfield and Smith, 1980). The latter authors observed that the net effect of altered autonomic neural activity on in vivo insulin secretion is not easily predictable. Consequently, the role of this hormone in the control of food intake and body weight is difficult to determine. Woods and Porte (1980) suggested that, since plasma insulin varies with most behaviours, its immediate value is actually a poor predictor of adiposity. They pointed out that the cerebrospinal fluid (CSF) insulin responds slowly in time to changes of plasma insulin, rather than reflecting the immediate changes of level in response to food ingestion. Even small but sustained increases in plasma insulin cause a maintained increase in CSF insulin whereas large increases of short duration have little effect. These authors argued that CSF insulin may also reflect body weight in a similar manner to basal insulin levels. They, therefore, proposed that CSF insulin might act as a more reliable indicator of body weight. Woods and Porte (1980) demonstrated that infusion of synthetic CSF with insulin into the ventricular system of free-feeding baboons caused a dose-related suppression of food intake and body weight with no concomitant changes in plasma insulin or glucose. Additional experiments further supported their hypothesis that insulin, acting directly at the brain, has a major influence over food intake, although this was likely to be on a long-term rather than a short-term basis.

(d) Aminostatic hypothesis

It is apparent that the plasma amino acid patterns are dependent upon the composition of the diet (Clark et al., 1973). From the observation that a reciprocal relationship existed between the serum amino acid concentration and appetite it was thought that amino acid levels may be

important in regulating ingestion (Mellinkoff et al., 1956). In addition, animals may modify food intake in response to fluctuations of individual plasma amino acids (Harper, 1976). Specific areas of the brain, such as the prepyriform cortex and medial amygdala, are considered to be intimately involved in the food-intake response to diets low in or devoid of a single indispensable amino acid (Rogers and Leung, 1972). Both Myers (1974,1975) and Panksepp (1974,1975) believed these nutrients were important in the control of feeding. However, there is evidence that the response of animals to imbalanced protein diets is not directly related to maintenance of body weight. Although rats respond, by appropriate changes in food intake, to a protein- or amino-acid-imbalanced diet (Harper et al., 1970), the depressed food intake observed on high protein diets is transitory and consumption eventually returns to normal. Harper (1975) considered the reduced intake of low protein diets to be a protective mechanism. Such a response protects the animals, already faced with an inadequate protein consumption, from the adverse effects of a high energy intake in relation to the intake of utilizable protein. The latter situation can lead to obesity. In conclusion, if appetite was regulated by an amino-acid-responsive mechanism, a complex feedback system involving interactions among individual amino acids, nitrogen and energy would be necessary (Harper, 1975). He concluded that such a mechanism would have to be incredibly complex to control food intake.

(e) Energostatic hypothesis

The first three major appetite-control theories all involve a specific metabolite as being of principal importance in the regulation of appetite. However, the failure of any one of these metabolite hypotheses to fully explain the observed patterns of ingestion has led to the development of alternative theories which may involve several nutrients simultaneously. A recent proposal, which incorporates different nutrients simply by their common ability to provide energy, suggests that appetite is controlled by

the supply rate of readily metabolizable energy (Booth, 1972, 1977). This theory is really a modification of the ideas of Ugolev and Kassil (1961) who suggested that it was a deficit in the available energy which stimulated feeding. Their theory was discounted on the grounds that an intermeal-period would cause only a very small depreciation in the available energy supplies of an animal. Such a control mechanism would have to be extremely sensitive to detect such small daily changes (Davis, 1980; Russek, 1981). In contrast Booth's energostatic theory was based on the observation that the intubation of any nutrient substrate into rats reduced their food intake by an equicalorific amount. The decision of whether or not to feed is based upon the examination of the energy supply state. Based on such a system Booth et al., (1976) devised a feeding model for the rat which gave a fairly realistic interpretation of the animal's feeding behaviour (Toates, 1981). Novin and Van der Weele (1977) do not entirely agree with the energostatic theory. In the case of the very short-term control of food intake they did not find that the energy content was the variable to which the animal was responding. They suggested that several mechanisms were involved in satiety. Duodenal infusion of glucose into a vagotomized rat fails to suppress food intake whereas lipid, glycerol and casein are equally effective in both the vagotomized and intact animals (Rezek and Novin, 1976). It appears that the vagus mediates information relating to carbohydrate metabolism (perhaps more specifically glucose) while other macronutrients induce satiety through alternative systems such as the cholecystokinin (CCK) satiety mechanism. Novin and Van der Weele (1977) concluded that short-term satiation may include a mechanism in which glucose modifies a vagal afferent system, together with mechanisms where fats and proteins exert their immediate influence through an endocrine system.

(f) Interaction of stimuli to modify food intake

The general inadequacy of uni-factor arguments to explain food intake may be explained by the considerable interaction between certain sets of stimuli which modify ingestion. For instance, the exterosensory inputs and stimuli reflecting the internal state (e.g. metabolite and hormonal levels) are always necessarily involved in food intake and are inseparable (Toates, 1981). There is clear evidence in support of such interactions. Human subjects reported a "pleasant" sensation when given a sucrose solution to taste. However, following gastric intubations of sucrose solutions the test solution which had earlier elicited "pleasant" sensations were reported to give an "unpleasant" sensation capable of suppressing further intake. The sweet gustative sensation was considered to be composed of two elements; one discriminative, derived from stimulation of gustative receptors, the other affective, derived from some internal stimulation. These two components of sweet sensation appeared relatively independent. The affective component depended upon ingestion and the consequent internal modifications, as indicated by the results of the gastric glucose loads. It was considered that intubation of the glucose activated some mechanism which modified the affective component of the gustative sensation. This ultimately led to a shift in the sensation of sugar from pleasant to unpleasant (Cabanac et al., 1968). The same incoming sensory information has different effects on the organism according to its physiological state (Wyrwicka, 1969). The latter author suggested that the oro-gastric sensations have a range of stimulus intensities. Up to a certain optimum stimulus intensity an animal will show an increase in the strength of its reaction to food, i.e. ingestion rate. Wyrwicka (1969) considered that it was the point of optimum stimulation that various physiological factors of the internal environment could modify. In this manner the two sets of stimuli interact to determine the threshold for ingestion or avoidance of a food item.

Le Magnen (1971), Stunkard (1975) and Booth (1977) obtained evidence to show that an animal may learn to associate specific oro-gastric sensory signals, characteristic of a particular diet, with the post-prandial biochemical changes. In this situation the neutral oro-sensory and gastric signals are repeatedly associated with an unconditioned biochemical satiety stimulus. In this manner they may become conditioned stimuli capable of inducing satiety. This conditioning of oro-sensory signals by post-absorptive stimuli has been demonstrated quite clearly in the rat (Booth 1972, 1977; Booth and Davis, 1973). Furthermore, Booth (1977) observed that rats offered a choice of diets varying in calorific density showed a preference for a particular diet, but this differed according to their internal energy state.

Finally, some authors consider that too great an emphasis has been given to the metabolic determinants of feeding patterns (Collier et al., 1972; Hirsch and Collier, 1974; Collier, 1980). Such homeostatic feeding models ignore both ecological variables and the evolutionary history of a species. These authors demonstrated that when constraints are applied either to meal frequency or ingestion rate, i.e. by varying the amount of work necessary to receive food, there were pronounced changes in the pattern, level and rate of consummatory behaviour in the guinea-pig and rat. Gradually increasing the amount of work necessary to gain access to food resulted in changes of the feeding patterns and meal sizes so that the animals conserved as much energy as possible. Hirsch and Collier (1974) and Collier (1980) concluded that the determinants of the initiation and termination of feeding bouts are not changes in internal states but are ecological variables, such as cost, availability, abundance, and value of food items. Perhaps one of the better examples of ecological determinants of food intake was reported by Galbraith (1967). He reported that Daphnia were not ingested by Salmo gairdneri unless the individual prey exceeded a very distinct threshold in length. This observation suggests that Daphnia below a certain size represent an insufficient caloric reward to the

predator to meet the cost of capture.

5. Control of appetite in fish

Evidence relating to the neural and physiological control of appetite in fish is now reviewed.

6. Neural involvement in fish appetite control

Information concerning the role of central neural regions in the regulation of food intake is sparse. Demski and Knigge (1971) and Demski (1973) evoked feeding behaviour in the bluegill sunfish, Lepomis macrochirus, and the blackchin mouthbreeder, Tilapia heudelotti macrocephala, following electrical stimulation of specific hypothalamic areas. Similar results were obtained following stimulation of the telencephalon in Carassius and L. macrochirus (Grimm, 1960; Fielder, 1968), although this structure is not thought to be essential for normal food intake (Aronson, 1970). Electrical stimulation of the LH resulted in food intake in satiated individuals of Carassius (Savage and Roberts, 1975). Peter (1979) reported reduced growth rates in the same species following lesions applied to the LH, although it is not clear if this was due to reduced consumption. VMH lesions gave no indication of a hyperphagic response. From these few studies it is impossible to draw any firm conclusions concerning the neural role in food intake. In view of the many problems encountered with lesioning and stimulation techniques in higher vertebrates, their usefulness in teleost studies is perhaps even more limited.

7. Physiological control of appetite

There are numerous factors that modify the feeding behaviour of fish, although some are species-specific. In general, these factors are broadly classified as being of ecological, intraspecific, interspecific or of physiological origin. The first three categories account for the majority of factors affecting food intake in fish, but will only be briefly discussed here.

Food intake is modified by tidal cycles in Blenius pholis (Crawford, 1977) and by daylight in the Dover sole, Solea solea (Kruuk, 1963) and L. limanda (Jobling, 1974). Seasonal variations in quantity and composition of prey species affect intake in some species, e.g. Perca fluviatilis and Mugil cephalus (Thorpe, 1977; de Silva and Wijeyarante, 1977). Other ecological determinants of food intake include temperature, e.g. Micropterus salmoides (Niimi and Beamish, 1974), Clupea harengus (de Silva and Balbontin, 1974), salinity, e.g. Mugil cephalus (de Silva and Perera, 1976) and the oxygen supply. The oxygen supply may be limited indirectly by dissolved solids thereby reducing consumption (Webb, 1978).

When food supplies are limited, social hierarchies may develop resulting in an adequate food supply for the dominant members. This behaviour is true for the Atlantic salmon (Fenderson, et al., 1968). In contrast social facilitation perpetuates food intake in Mugil cephalus (Olla and Samet, 1974). There is little information on interspecific effects on feeding behaviour, although food selection was modified due to interaction between cutthroat trout, Salmo clarki clarki, and the dolly varden, Salvelinus malma (Schultz and Northcote, 1972).

In the present context only the physiological factors affecting intake will be considered in any detail, although they are probably less well understood than those in the above categories. Where relevant, the physiological control of fish appetite is discussed in relation to evidence for the higher vertebrates.

(a) Control of body weight and energy intake

In a manner similar to higher vertebrates, fish will respond to food deprivation by a subsequent increased rate and/or volume of intake (Tugenhadt, 1960; de Ruiter and Beukema, 1963; Beukema, 1968; Kariya, 1969; Kariya and Takahashi, 1969; Brett, 1971; Shul'man, 1974; Tyler and Dunn, 1976). This is thought to indicate development of a systemic debt during food deprivation, which is corrected when food becomes available. However,

the compensation is not necessarily precise in that sufficient food may not be immediately ingested to replace that which would normally have been eaten. In addition, the compensation only occurs after a limited deprivation time beyond which intake may decrease or even fail altogether (Bilton and Robins, 1973). An identical response has been observed in the food-deprived rat (Miller, 1955; Toates, 1981). In this animal, the starvation-related decrease in food intake was thought to reflect an adaptive reduction in the animal's metabolism during and after deprivation (Westerterp, 1977), and the fact that the mobilization of lipid reserves during starvation will have led to a depletion of the fat deposits. Their recovery may take a number of days following a return to ad lib feeding (Toates, 1981). Russek (1971) explained this post-starvation anorexia on the basis that appetite is controlled by the available carbohydrate reserves in the liver. During starvation, hepatic glycogen reserves are depleted. However, once this reserve has reached a certain minimum level, the liver glycogen starts to increase by gluconeogenesis. Russek (1971) suggested that this rise in hepatic carbohydrate levels could account for the anorexic behaviour observed after prolonged starvation. The reasons for the decline in consumption by fish during severe starvation is unclear although starvation related increases in liver glycogen have also been observed in teleosts (Robinson et al., 1963).

It is clear, therefore, that fish attempt to maintain species-specific growth rates, or body weights according to sex, age and season. They achieve this by compensating for prior food deprivation, and monitoring their calorific intake (see later). In view of such evidence, Peter (1979) stated that fish regulated body weight and food intake to a set-point level as had been suggested for higher vertebrates. The set-point level would remain static or rise and fall according to the particular phase of the fish's life cycle. The merits of such postulates have already been discussed in relation to mammals. It is equally improbably that the idea of stationary and fluctuating set-point values, involved in the control of body

weight, will serve any useful purpose in appetitive studies of fish.

(b) Role of olfactory and gustatory senses in food intake

Vision is especially important in food-finding and selection, but chemoreception by itself or in conjunction with vision seems dominant in some marine species (Sutterlin, 1975). The flounder, Pseudopleuronectes americanus, finds food by both chemical and visual means. Glycine was a particularly strong olfactory attractant in this species (Sutterlin, 1975) while ingestion in the Dover sole, Solea solea, was stimulated predominantly by glycine betaine, together with glycine or alanine (Mackie et al., 1980). The latter authors observed that the sole exhibited a form of external sensing of the food, by sensory papillae, followed by rejection or uptake into the mouth. In addition, these compounds significantly increased overall food intake in this species. The behaviour of S. solea contrasts markedly with that reported for S. gairdneri (Adron and Mackie, 1978) and Scophthalamus maximus (Mackie and Adron, 1978). These species are essentially 'sight-feeders' which snap at a potential prey item which is then savoured in the mouth. The food is then rejected should it prove unacceptable in terms of taste or texture (Mackie et al., 1980). S. maximus shows a gustatory sensitivity to specific nucleotides like inosine which stimulates acceptance of a food item (Mackie and Adron, 1978). Other species of fish are stimulated to feed by mixtures of chemicals. Glycine betaine, with mixtures of amino-acids, are the feeding stimulants for the pinfish, Lagodon rhomboides (Carr and Chaney, 1976), for the pigfish, Orthopristis chrysoterus (Carr et al., 1977), and the puffer fish, Fugu pardalis (Hikaka et al., 1978). Ina and Higashi (1978) discovered that the strongest feeding stimulants for the sea bream, Chrysophrys major, were to be found in the acetone-insoluble fractions of various marine animals. In contrast, the aceton and water soluble fractions of algae were the most powerful plant stimulants identified.

Finally, the larvae of several fresh water fish (e.g. C. carpio, S. gairdneri, E. lucius) have been shown to react differently to the taste qualities: sweet, sour, bitter and salty. The latter stimulus was stimulatory to all larvae (Appelbaum, 1980).

The above evidence demonstrates that fish respond to specific taste stimuli in a manner similar to that observed for higher vertebrates. In the latter case, oropharyngeal factors can play a powerful role in determining ingestion if they are permitted to operate (Mook, 1969).

(c) Role of food quality on food intake

An imposed decrease in meal frequency resulted in a greater intake per meal by the winter flounder, Pseudopleuronectes americanus (Tyler and Dunn, 1976). Furthermore, some species, e.g. C. carassius, S. gairdneri and the Channel catfish, Ictalurus punctatus, can detect a calory deficit in their food, and compensate by increasing their consumption rate (Rozin and Mayer, 1961; Lee and Putnam, 1973; Grove et al., 1978; Lovell, 1979). In the wild, L. limanda consumes a wide variety of prey species varying considerably in energy value (Gwyther, 1978). This observation was thought possibly to explain the failure of Limanda to detect sudden changes in its calorific intake in the laboratory (Gwyther and Grove, 1981). However, many other species of fish ingest a far greater variety of prey species than Limanda (see review by Hyatt, 1979). This fact does not necessarily mean that they are incapable of detecting short-term changes in their calorific intake. Indeed, a varied diet may quite likely indicate an animal's attempt to maximise its energy intake. Since Limanda is the species under investigation in the present study, the conclusions of Gwyther and Grove (1981) require some comment.

Firstly, on behavioural grounds, optimal foraging theory predicts optimal behaviour when the energy maximisation principle is applicable, i.e. given that the rate of energy gain from food is the only limiting factor. When faced with a choice of different prey species, this argument predicts

that the diets which confer the greatest fitness (optimal diets) are those which maximise the net rate of energy intake. Based on this assumption, it is possible to build models which predict the optimal diets of various animals (see review by Townsend and Hughes, 1981). There is considerable evidence in support of this theory. For instance, the brown trout, S. trutta, was offered a variety of prey species simultaneously, and which varied greatly in energetic value. Initially, the food intake of this species differed little from that predicted by a random feeding model, i.e. there was little selectivity between items of different energy value. After about six days, however, due to selective feeding on the higher energy items, the fish tripled their average energy intake (Ringler, 1979). Alternatively, the food intake of the trout was approaching their optimal diet. Based on such evidence, Townsend and Hughes (1981) concluded that "the rate of energy gain from food has been a significant limiting factor and has constituted an important selection pressure in the evolution of foraging behaviour". However, the optimal diet model does not take into account any time restrictions for foraging. If foraging is restricted for some reason, such as when short feeding periods are advantageous by reducing exposure time to predators, then the optimal diet may be broader than that appropriate to unlimited foraging time (Townsend and Hughes, 1981). Suppose the probability of encountering a high energy item becomes small near the end of the feeding period. Under such circumstances, the energy intake for the remaining feeding period may be increased by eating all the prey encountered, and which can be handled in the time. This would be so even if they were not so valuable in an unlimited foraging period.

The exact feeding behaviour of L. limanda in the wild is unclear, partly due to the lack of feeding synchronisation in the population as a whole (Gwyther, 1978). However, the latter author did obtain some evidence which suggests that the feeding period of Limanda may be restricted to certain periods of the day. The varied prey intake of this species may, therefore,

simply reflect an attempt to maximise energy gain within a limited period.

Finally, in higher vertebrates, much of the evidence from nutrient infusion studies shows that the absolute amount of energy administered is not so important in relation to the short-term control of intake. Rather, it is the rate of energy supply to specific tissues that modifies the short-term control (Booth, 1979). This suggests that, irrespective of the quality of the food ingested, the subsequent changes in the energy reserves/supply are the important elements which are monitored. In both higher vertebrates and fish, low quality meals pass along the intestine at faster rates (see Chapter 1). Presumably, such meals will also result in smaller changes in the overall energy reserves of the animal, leading to an earlier return of appetite. Such a response has been observed in several teleosts.

In conclusion, it would appear that the reported failure of Limanda to respond to dietary dilution in the laboratory is unlikely to be a reflection of its varied diet in the wild. In fish appetite studies, very close attention must be given to the nutrient composition of the diet. Marine and freshwater fish have specific requirements for particular nutrients in their diet. A deficiency of any such nutrient can lead to altered feeding behaviour. Consequently, the feeding response that is being investigated is effectively obscured.

(d) Role of dietary composition on food intake

It is evident that, apart from the energy value of the diet, the actual nutrient composition may also modify consumption. The levels of any one of the three major nutrients may significantly effect the feeding behaviour of fish. However, protein intake is normally related directly to the dietary protein level. When the dietary energy (DE) level of diets was kept constant, there was no significant change in the food consumption of Ictalurus punctatus when offered diets with a protein range of 15 to 45% (Lovell, 1979). Above 45% dietary protein level, food

intake declined significantly. Furthermore, the efficiency with which fish can utilize dietary protein is limited. The maximum protein efficiency ratio (PER = wet fish-weight gain (g)/protein consumed (g)) occurred at dietary protein levels of 40% for P. platessa (Cowey et al., 1977) and 45% for I. punctatus (Lovell, 1979). The reduced food intake and lower PER observed at high protein levels suggests that this nutrient probably has deleterious metabolic effects at high levels.

Not only may high levels of protein reduce food intake, but the digestible energy to percentage protein ratio (DE/P) is an important factor in controlling consumption. At a particular protein level, the addition of lipid or carbohydrate to the diet, so that the DE/P was increased, resulted in decreased consumption by I. punctatus (Page and Andrews, 1973; Lovell, 1979). This ensured that the daily energy intake remained constant. For instance, energy needs were met at a lower feed intake on a 25% protein, 25% corn and 12% lipid diet than on a similar diet with only 6% lipid. Unless the correct nutrient balance is achieved, the reduced food intake following addition of lipid to the diet may result in an overall reduction in protein intake. In extreme cases, this may result in a reduced growth rate because the overall protein intake is too low.

Clearly, the DE/P ratio is another factor to be considered in fish appetite studies. It is probable that the calory-protein ratio requirements will vary for different species. The situation is further complicated by the finding that the calory-protein ratio requirement of a particular species may alter as the animal approaches maturity (Page and Andrews, 1973), or in relation to the water temperature (Ringrose, 1971).

A number of fish species show specific requirements for essential dietary metabolites. The absence of even a trace amount of such factors may considerably impair their appetite and growth. Thiamine is an essential dietary nutrient for S. gairdneri (Steffen, 1970) and

S. maximus (Cowey et al., 1975). A deficiency of this nutrient causes anorexia and poor growth (Murai and Andrews, 1978). More recently, it has become increasingly clear that marine and freshwater species differ considerably in their dietary fatty acid requirements. This fact is particularly relevant to the present study.

Marine fish differ from freshwater species, like S. gairdneri, in their ability to metabolize dietary fatty acids (Cowey et al., 1976). The latter authors observed poor growth in Scophthalamus maximus when fed a diet containing a supply of pre-formed polyunsaturated fatty acids of the ω6 series. In contrast, this species showed good growth when given a supply of polyunsaturated ω3 fatty acids in the form of cod-liver oil. Additions of this lipid to the diet of S. gairdneri similarly improved growth (Castledine and Buckley, 1979). In a comparison of the growth promoting ability of different fatty acids, linolenic acid (18:3ω3) was effective in S. gairdneri but ineffective in Chrysophrys major (Fujii and Yone, 1976; Kanazawa et al., 1977). In contrast, highly unsaturated fatty acids such as eicosapentaenoic (20:5ω3) and docosahexaenoic (20:6ω3) acids considerably improved weight gain in both these species (Kanazawa et al., 1978; Teshima, 1978), with maximal growth being attained in C. major. Kanazawa et al., (1979) considered that 20:5ω3 and 22:6ω3 deriving from dietary 18:3ω3 exerted a higher growth efficiency, as essential fatty acids, than 18:3ω3 in aquatic animals. In addition, they suggested that the effect of 18:3ω3 on weight gain probably varies with the ability of different species to perform the bioconversion of 18:3ω3 to 20:5ω3 and 22:6ω3. It was found that, in contrast to S. gairdneri, some marine species like the rockfish, Sebastiscus marmoratus, globefish, Fugu rubripes rubripes and C. major, appeared to be incapable of synthesizing 20:5ω3 and 22:6ω3 from dietary 18:3ω3 in sufficient amounts to sustain optimum growth. Furthermore, these latter species appeared to be inferior to freshwater fish in their capacity for elongation and desaturation of dietary 18:3ω3 to ω3 highly unsaturated fatty acids.

In view of the above evidence, it is obvious that close attention should be given to dietary composition, if deficiency symptoms are not to influence appetitive behaviour.

(e) Effect of hormones on food intake

Many temperate species of fish show a seasonal mobilization or repletion of body energy reserves normally associated with seasonal variations in the plasma levels of certain hormones. Such periods may obviously be associated with change in food intake. Pickford (1957) reported that injections of beef growth hormone rapidly improved the appetite of hypophysectomized F. heteroclitus. Immediately prior to the onset of rapid growth in Perca fluviatilis L., there was a marked increase in the growth hormone content of the pituitary gland indicating a possible relationship between the hormone and growth.

Thyroxine and prolactin are also strongly implicated in growth control and lipid deposition in particular (see Chapter 2). Therefore, they may modify food intake indirectly. Finally, Baker and Wigham (1979) concluded that low growth rates observed during the reproductive season are probably not directly attributable to any inhibitory effect of sex steroids. They suggested that they arise from a reallocation of metabolites from growth to gamete production or even to a change in feeding activity. A number of species cease to feed during the reproductive season (Purdom, 1979) although it is not clear if this is hormonally-induced. Estrogens certainly have potent inhibitory effects on appetite in higher vertebrates (Fishman, 1976).

(f) Specific dynamic action and food intake in fish

The increase in metabolic rate after feeding is considered by some authors to be of importance in the control of food intake in fish (Beukema, 1968; Muir and Niimi, 1972) and was incorporated into the feeding model of Colgan (1973). Vahl (1979) also stressed the possible

significance of SDA in his hypothesis of food intake in fish. The SDA effect is thought to be caused by the deamination of amino acids during their transformation into new proteins, glycogen or fat. Vahl (1979) and Jobling (1980) suggest that the blood has a maximum carrying capacity for absorbed amino acids during the assimilation of a meal. If this capacity is exceeded, then a toxic situation develops. The rate at which the amino acids can be processed will be partly determined by the available oxygen not required for activity and basic metabolism. The rate of absorption of nutrients from the gut must, therefore, not exceed this maximum processing rate, if the proposed toxic situation is to be avoided. Jobling (1980) thought that this balance might be achieved by a restriction of the blood supply to the gut, thereby reducing absorption. This would presumably reduce the overall rate of gastric clearance which, in conjunction with other satiety stimuli, would reduce food intake.

The reduced consumption rates of fish at high temperatures may result from a decreased dissolved oxygen content, such that insufficient oxygen is available for SDA in competition with other activities. In other words, a decreased dissolved oxygen content may reduce the metabolic scope (Warren, 1971; Muir and Niimi, 1972). However, appetite may also be reduced under super-saturated oxygen conditions, which should increase the metabolic scope (Doudoroff and Shumway, 1970). It is apparent that the true influence of SDA on fish appetite remains unclear.

The idea that the calorigenic effect of food might modify food intake was originally applied to homeotherms, where a depletion of metabolic reserves was presumed to lower body temperature and so induce food intake. Russek (1971) conjectured that, while it was generally the case that homeothermic animals eat more when the temperature is low, and vice versa, this only demonstrates that temperature regulation brings food intake under its control. Equally, he argued that oxygen deficiency increases blood pressure and inhibits feeding, which does not mean that under normal conditions oxygen tension is the main factor controlling blood pressure

or food intake. It is probable that the ability of fish to increase their metabolic rate after feeding will be unaffected by any thermic effects of catabolism; however, the increased supply of oxygen necessary for the deamination of amino acids could be limiting. The SDA observed in fish, measured as a post-prandial increase in oxygen consumption, could therefore indirectly modify further intake on a short-term basis.

(g) Role of the stomach in food intake

In a number of studies it has been observed that the rate of return of appetite in fish is closely related to the time required for the stomach contents to decrease (Bokova, 1938; Brett, 1971; Elliott and Person, 1978; Grove et al., 1978; Grove and Crawford, 1980). Alternatively, hunger was considered to be largely determined by the amount of food in the stomach (Ware, 1972). In contrast, Magnuson (1969) and Catherall (1979) reported that stomach fullness did not closely determine the quantity of food eaten by Katsuwonus pelamis and Blenius pholis respectively. It is clear that, in some species, close correlations are found between the time required to empty the stomach and the return of appetite. This may be so under a variety of experimental conditions of fish size and temperature (Grove et al., 1978; Grove and Crawford, 1980). Furthermore, the reported failure of Limanda to modify its food intake, when its normal diet was diluted with kaolin, was interpreted as an indication that this species may feed for bulk rather than calories (Gwyther and Grove, 1981). The above evidence surely implies that signals generated by an empty stomach play an important role in the initiation of feeding. Some of the earliest theories on appetite control in the higher vertebrates proposed that afferent signals, from an empty stomach, stimulated feeding via the vagus nerve (Cannon, 1929). This was supported to some extent by the finding that exogenous insulin induced hunger and increased gastric contractions (Quigley et al., 1929). Contrary to this evidence, vagal transection eliminated the gastric contractions (Stein and Mayer, 1948)

but not the hunger produced by insulin injection (Grossman and Stein, 1948). In man, and other animals where the stomach has been denervated, or after gastrectomy, normal food intake and regulation of body weight is still maintained (Tsang, 1938; Morgan and Morgan, 1940). In view of such evidence, it is now generally believed that feeding in higher vertebrates is not stimulated by afferent signals from an empty stomach but it is probably reasonable to assume that it can be terminated by a full one (Toates, 1981). Such an inhibitory effect of a full stomach was demonstrated in Ictalurus punctatus. The weight of food taken in this species, at a single feeding, was limited by the density of the diet (Lovell, 1979).

In higher vertebrates, a full stomach may aid satiety. However, if the sensory tracts from the mouth are interrupted or by-passed (e.g. by gastric intubation of food) satiety is delayed. Furthermore, there is increasing evidence which points to a greater importance of tension receptors in the duodenum, rather than the stomach, in the induction of satiation (Davis et al., 1976; Smith and Gibbs, 1976). Therefore, the role of the stomach, either in the initiation of ingestion or satiety, would appear to be minor.

(h) Role of plasma metabolites in food intake

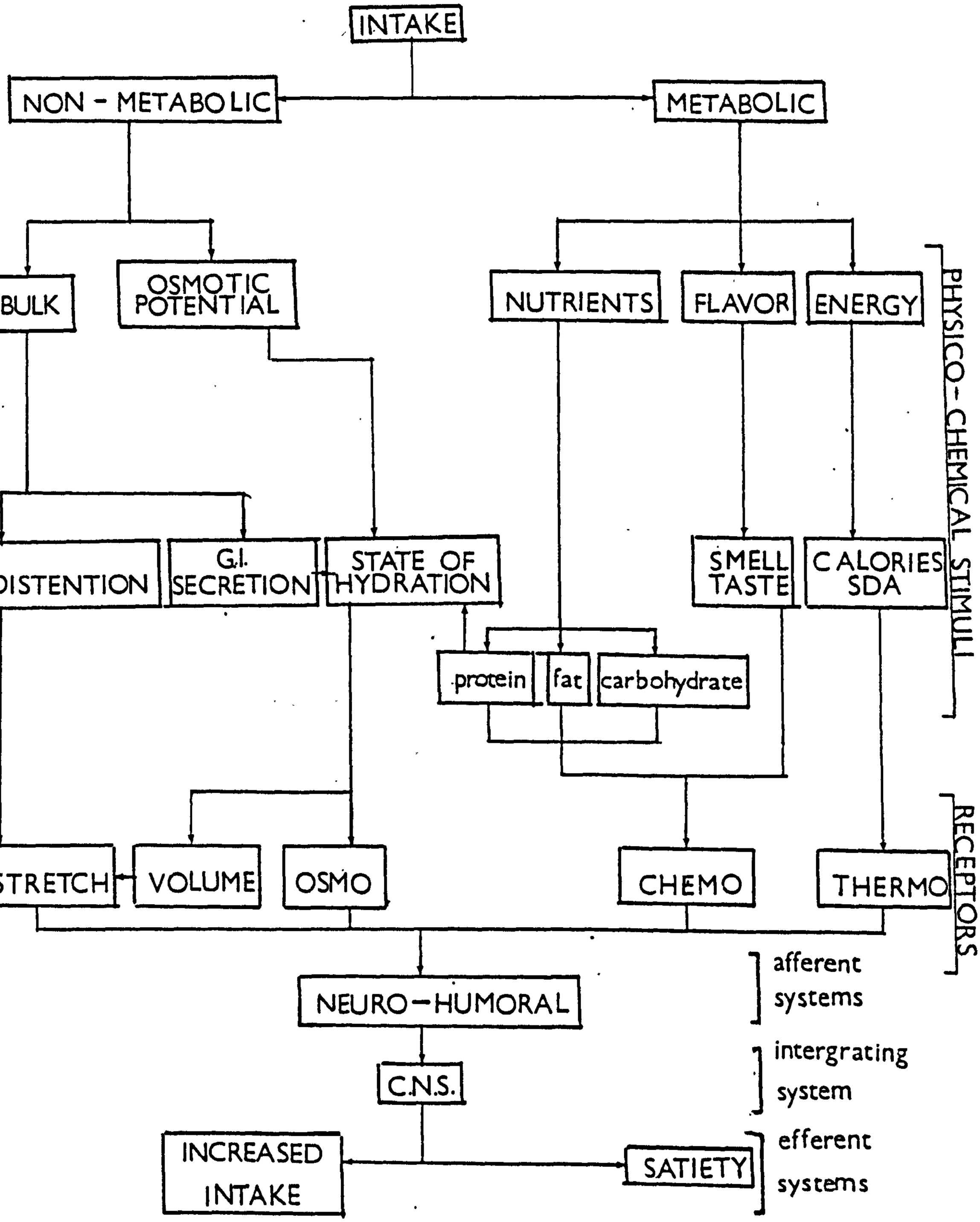
The relevance of plasma metabolites in the control of food intake in higher vertebrates has already been discussed. Research into their possible role in fish appetitive behaviour has been poorly investigated, and the little data that is available is confusing. Kuzmina (1966) observed that infusion of glucose or essential amino acids reduced appetite in Cyprinus carpio, while control injections of saline or non-essential amino acids were ineffective. Infusion of serum, from well-nourished fish, similarly reduced appetite. This response is, therefore, similar to that of higher vertebrates following the same treatment, indicating an influence of plasma metabolites (and possibly humoral factors)

over the control of fish appetite. The greater satiating power of glucose over amino acid infusions observed by Kuzmina (1966) was considered to reflect an adaptation of this species' intake mechanisms to a predominantly vegetable diet. However, glucose loads are also more effective in this respect when administered to higher vertebrates. Booth (1979) suggests that this is because the energy of glucose is more readily available to the relevant tissues, due to the ease with which it is metabolized.

Attempts to correlate endogenous plasma metabolite levels of fish with feeding behaviour have not contributed much to our understanding of their role in this field. Bellamy (1968) reported that in the red piranha, Rooseveltiella nattereri, the lowest plasma glucose levels preceded the greatest food intake. In contrast the skipjack tuna, Katsuwonus pelamis, ingested larger meals when blood glucose levels were increasing than they did after 24 hour deprivation, when glucose levels were actually lower. Failure to observe any post-prandial fluctuations in plasma glucose and PFA was believed to indicate that these metabolites were not relevant in the short-term control of food intake of Limanda (Gwyther, 1978). Furthermore, Peter et al., (1976) could not conclusively demonstrate the existence of hypothalamic glucoreceptors in Carassius and presumed that plasma glucose would not, therefore, be relevant in feeding behaviour. This fact does not rule out the possibility that such receptors might exist in other tissues. Finally, Lovell (1979) found that neither stomach fill nor serum levels of glucose, amino acids and lipids were significantly correlated with the amount of food voluntarily consumed by Ictalurus punctatus. It should be emphasised, however, that in this study, the above parameters were not measured under free-feeding conditions. Consequently, their relation to normal appetite return must be interpreted with caution.

Fig. 2 shows a diagrammatic summary of the various physiological factors known to modify food intake in higher vertebrates.

Fig. 2 - Diagrammatic representation of the various factors known to modify food intake.



after Jacobs (1963)

Chapter 1

Section I

Gastric evacuation rates under single and multiple feeding regimes

Section I

Introduction

The rate at which food passes from the stomach or the gastric evacuation rate (GER) has been studied in a variety of vertebrates and invertebrates. In addition to the estimation of GER (usually measured as g or mg dry weight of material per hour), the time (h) for total clearance of the stomach, the gastric evacuation time (GET), has also been investigated.

In relation to fish, gastric evacuation studies are of considerable interest to fisheries biologists. GER, together with factors such as food quality, feeding frequency, conversion efficiency, digestibility and appetite are important steps in the production of fish tissue (Windell, 1978). GER data may be used to determine rates of energy passage within certain pathways of marine food webs (Tyler, 1970). Food consumption estimates of commercially-important fish species in the wild have been calculated from gastric evacuation measurements obtained in the Laboratory (Thorpe, 1977; Doble & Eggers, 1978; Elliott & Persson, 1978; Diana 1979). Such estimates are based upon the assumption that food intake is directly related to available gastric capacity. However, there is little direct evidence to support this idea and the physiological control of food intake in fish has received only scant attention (see review on appetite control in fish). GER values can still be used to evaluate the relationship between feeding motivation, appetite, feeding activity, and quantity of food in the stomach (Magnuson, 1969). In aquacultural practices, it is important to avoid overfeeding of artificial diets, while still maintaining the ideal feeding schedule for optimum growth (Elliott, 1975; Grove et al., 1978; Vahl, 1979).

Prior to considering the control of GER in fish, the relationship between the nutritive quality of the meal and gastric mobility in higher

vertebrates is briefly discussed. No attempt is made to consider the details of neural control of gastric motility.

I Gastric evacuation in higher vertebrates

In the higher vertebrates, the amount of food forced into the duodenum from the stomach depends upon the strength with which the antral muscles contract. This contraction is influenced by the amount of food in the stomach, so that GER is proportional to the volume of food in the stomach at any given time (Vander et al., 1975). However, in terms of control of gastric evacuation in man, there is increasing evidence that the volume of food is not the most important factor.

GER is strongly modified by the biochemical composition or calorific density of the diet, and the volume of duodenal chyme. Changes in the osmolarity or nutrient composition of the duodenal contents is detected by specialised receptors which can modify gastric motility (Vander et al., 1975). In particular, the anion of fatty acids appears to be the most important lipid fraction controlling GER (Hunt, 1975). The potent inhibitory action of lipids is partly initiated through their stimulatory effect on cholecystokinin secretion. This hormone, along with other duodenal hormones like secretin, modify gastric motility. In addition to the chemospecific control of GER, motility is also modified by a neural reflex, the enterogastric reflex. This reflex reduces the rate at which osmotically active molecules are formed in the duodenum by lowering the amount of material leaving the stomach which becomes available for digestion. The duodenal osmolarity must not rise too high, otherwise fluid will enter the duodenum, from the plasma, and the blood volume will be subsequently reduced. Finally, it is clear that the greater the nutritive density of a meal, the slower is the rate of transfer to the duodenum (Hunt & Stubbs, 1975; Hunt, 1975). After the intake of a meal, the stomach starts to deliver its contents at a high rate. As mentioned earlier, the presence of

digested food in the duodenum causes the evacuation rate to be progressively inhibited, such that the stomach contents are eventually emptied at a low constant rate (maximum inhibition). In man, the time lapse from meal intake until any signs of inhibition can be recorded is influenced by meal composition. The promptness with which the inhibition is initiated influences the time taken before the constant emptying state is achieved. Johansson (1975) demonstrated that a slow inhibition of the early emptying rate resulted in a rapid appearance of the constant evacuation rate. Therefore, the early delivery of a large caloric load should lead to an earlier achievement of maximum inhibition. Proteins in food contribute to the slowing of gastric emptying in such a way that isocaloric amounts of carbohydrate and mixed protein have the same effect (Burn-Murdoch et al., 1978). This evidence obviously supports the existence of mechanisms which control GER in direct relation to meal quality, and not simply meal volume.

II Mathematical descriptions of gastric evacuation

A number of models have been proposed to describe the rate at which food is eliminated from the stomach in a variety of animals. At least five different mathematical models have been postulated to describe gastric evacuation in fish. The simplest of these is represented by a linear decrease in stomach contents with time. This model suggests that food flows into the intestine at a constant rate, and appears adequate for a number of teleosts, eg. Megalops (Pandian, 1967), Gobius (Healey, 1971), various gadoids (Daan, 1973; Jones, 1974) and Rutilus rutilus (Hofer et al., 1982).

The next model assumes that the rate of emptying is proportional to stomach fullness. The larger the original volume of the meal then the greater is the initial emptying rate of the stomach (Hunt & MacDonald, 1954). Therefore, the instantaneous evacuation rate is dependent upon the amount of food in the stomach so that:

$$dV/dt \equiv -cV^{b} \qquad .....(1)$$

here the differential dV/dt describes the rate of emptying, V is the volume of food in the stomach and c and b are constants. This type of mathematical description has been used to describe gastric evacuation in a variety of vertebrates and invertebrates. An exponential equation (where b=1) gave a good fit to the data for the lizard, Lacerta vivipara (Avery, 1973), Octopus cyanea (Boucher-Rodoni, 1973), the blowfly, Phormia regina (Thomas & Holling, 1974) and several teleosts, eg. Oncorhychus nerka (Brett & Higgs, 1970), Salmo trutta (Elliott, 1972); Platichthys flesus (Kiorboe, 1978), Blennius pholis (Grove & Crawford, 1980) and Perca fluviatilis (Persson, 1981).

Tyler (1970) observed that the surface area of the food would have a profound influence on the way it was evacuated from the stomach. This idea was further developed by Fänge & Grove (1979). Since the digestive enzyme attack occurs at the outer surface of the food bolus, the evacuation rate might be expected to be proportional to the surface area of the food remaining, such that:

$$dV/dt = -KV^{0.67} \qquad .....(2)$$

where the differential dV/dt describes the evacuation rate, V is the volume of food in the stomach, and K is a constant which depends on such factors as the species and size of the individual fish, temperature, and food type. Fänge & Grove concluded that a larger meal would be evacuated at a faster rate and that a double-log plot of evacuation rate against meal size would have a slope of 0.67. The time required to empty the stomachs (t_λ) of fish fed to the same relative level may be estimated by integration of equation (2) which gives:

$$t_\lambda = K'V_o^{1/3} \qquad .....(3)$$

where V_o is the meal size given to the fish.

A straight line of slope 0.33 is obtained following a plot of log t_λ against log V_s when the same experimental conditions apply ie. same size fish fed identical food type, at the same temperature. Finally, Fänge & Grove (1979) argued that this relationship should persist where different sized fish are fed to the same percentage of their body weight. The larger food bolus in bigger fish would present a relatively smaller surface area to gastrointestinal secretions. Consequently, although larger fish would digest a stated percentage bodyweight meal at a faster absolute rate than smaller fish, the time required to complete digestion would be greater.

There is a certain amount of evidence to support the above argument. For instance Jobling et al. (1977) reported for Limanda that increasing the meal size from 1% to 5% body weight led to only a fourfold increase in the time to clear the stomach. In this case GER and GET altered with meal size as predicted by the model. In an analysis of published data, Fänge & Grove (1979) found that the exponent describing the change in digestion rate (g/hr) with wet weight of meal lies between 0.5 (as estimated for Limanda, Jobling et al., 1978) and 1.0 (e.g. Lepomis, Windell, 1966).

Jobling (1981) reviewed the various models describing gastric evacuation in fish. He believes that the surface area model has little practical application. Jobling points out that when large meals consist of a number of small prey items (which is often the case), the surface area of the meal will increase in direct proportion to the amount of food eaten. Therefore, the surface area does not increase in proportion to meal size to the power 0.67. Jobling (1981) suggests that the instantaneous rate of evacuation is more dependent on the volume rather than the surface area of the food. This leads to the final model which has been used to describe gastric evacuation in a variety of animals. The square root model was first proposed by Hopkins (1966). In this instance, the model proposes that

gastric motility is associated with the radial distension of the stomach and the circumferential tension so developed is proportional to the radius. Since the radius of a cylinder varies with the square root of the volume, the tension developed in the stomach wall will also be proportional to the square root of the volume of food in the stomach. The 'square root' model may be expressed mathematically as:

$$dV/dt = -cV^{0.5} \qquad .....(4)$$

(dV/dt, c and V, as in equation (2)).

This model describes GER significantly better in man (Hopkins, 1966), rat (Booth, 1978), Raja (de Souza, 1978), P. platessa (Jobling & Davies, 1979) and Scyliorhinus canicula (Lyle, 1981). In addition, the recent review of the literature by Jobling (1981), shows that the gastric evacuation data for several other teleosts is most appropriately described by the square root model. However, it should also be pointed out that in the Jobling & Davies' study of P. platessa, the argument for the square root transformation is based on data from just a few (n = 9), force fed animals.

Sibly (1981) considered the square root model to be somewhat naive in its conception of physiological possibilities, since it proposes that the rate of peristaltic contraction is necessarily linearly proportional to the distension of the stomach wall. The model is based on the assumption that the stomach approximates to an elastic cylinder of fixed length. However, following the ingestion of food the stomachs of some fish species, such as Limanda, increase in length as well as radially (pers. obs.).

In conclusion, it is likely that both surface and volume effects modify the emptying rate of fish (Tyler, 1970) and the two models cannot be held as being mutually exclusive (Jobling, 1981). It is possible that the relative importance of the surface area effect increases with the time that the meal remains in the stomach.

The usefulness of gastric evacuation data in related physiological studies, and for estimation of digestion rates in the wild, is limited according to how representative it is of the natural rates. A comparison of gastric evacuation rates of some fish species in the laboratory and in the wild suggests that the former are indeed slower (Gwyther, 1978; Basimi, 1978). Hofer et al. (1982) also reported substantial deviations between rates of evacuation measured in wild and laboratory populations of roach, R. rutilus. The failure of some workers to take into account the known effects of stress, food deprivation and meal presentation methods upon gastric evacuation may account for such observations.

III Factors modifying gastric evacuation rates in fish

The various factors which modify GER have been reviewed by Kapoor et al. (1975), Windell (1978) and Fänge & Grove (1979). It is apparent that temperature, meal size and fish size have received the greatest attention. Some of the published data relating to these variables is given in Table 1. Clearly such studies have contributed greatly to our understanding of how these latter parameters effect GER. However, it is also clear that if GER values are to be of any use in either physiological or ecological investigations, then closer attention must be given to meal presentation methods and food quality.

a) Temperature

GER in poikilotherms may show considerable modification following a change in the ambient temperature (Kinne, 1964) until a new basal, steady rate is achieved. The acclimation temperature is likely to effect peristaltic and enzymic activity, and rates of absorption along the intestine. In general, GER tends to increase in a curvilinear fashion with increasing temperatures until a point below the upper lethal limit is reached (Fábián et al., 1963; Jobling & Davies, 1979; Jobling, 1980;

TABLE 1 EMPTYING TIME OF THE STOMACH IN FISHES (Data taken from Fänge & Grove, 1979).

Species	Temperature (°C)	Time to 100% evacuation (h)	Fish size (g) or (cm)	Reference
Ictalurus punctatus	10	24	380g	Shrable et al (1969)
Clupea harengus	20	10	-	Blaxter & Holliday (1963)
Esox lucius	18.23	50	40cm	Seaburg & Moyle (1964)
Oncorhynchus nerka	14.9	23	30-40g	Brett & Higgs (1970)
Pleuronectes platessa	14	12	280-320g	Edwards (1971)
Platichthys flesus	17-18	16	-	de Groot (1971)
Scophthalamus maximus	10	96-100	-	de Groot (1971)
Perca Flavescens	15	6-12	6cm	Nobel (1973)
Barbus liberiensis	22-25	3-5	3-10	Payne (1975)
Salmo trutta	12-15	3	7-15	Otto (1976)
Salmo gairdneri	15	22	30	Grove et al (1976)

Elliott, 1972; Brett & Higgs, 1970; Molnár et al., 1967; Molnár & Tolg, 1962). Both temperature and GER follow an exponential curve, such that a positive linear relationship exists between temperature and log GER (Persson, 1979, 1981; Jones, 1974). A complementary, negative relationship exists between temperature and log GET (Windell et al., 1976). Molnár & Tölg (1962) and Grove et al., (1978) found that a log-log transformation between GET and temperature produced a linear relationship. Backiel (1971) presents data on temperature and GET interactions in the form of Krogh's curve (Krogh, 1914) with a respiratory quotient (Q10) value of about 2.6. Digestion in flatfish appears to vary with temperature with Q10 values close to 2 (Data in Jobling et al., 1977; Gwyther, 1978; Basimi, 1978). Another form for presenting the GET data was used by Fábián et al. (1963), who plotted the log-reciprocal of evacuation time with the reciprocal of temperature. They demonstrated that their data on predatory fish followed the Arrhenius equation as with enzyme kinetics.

b) Meal size

A large amount of evidence exists which shows that the GER of fish is normally faster with increasing meal size (Elliott, 1972; Beamish, 1972; Tyler, 1970; Windell, 1966, 1969; Jones, 1974, Flowerdew & Grove, 1979; Jobling & Davies, 1979; Grove & Crawford, 1980; Gwyther & Grove, 1981). In a few extreme cases, the increased rate of evacuation is such that the total clearance time at a particular temperature is the same whatever the meal size (Windell, 1966; 1969; Rosenthal & Paffenhöfer, 1972; Grove & Crawford, 1980; Kitchell & Windell, 1968; Brett & Higgs, 1970). In contrast, Steigenberg & Larkin (1974) reported a reduced GER with increasing meal size for Ptychocheilus. However, in the majority of species an increase in meal size does not lead to a complete compensation in GER so that the larger the meal, the greater the total GET. For instance, a five-fold increase in meal size only trebled the emptying-time in Limanda (Jobling et al., 1977).

Windell (1978) observed that increased evacuation rates can only be achieved by changes in the volume of gastric contents pumped per peristatic stroke or an increase in the number of strokes per unit time.

c) Fish size

Several authors have shown that for species like Scophthalmus (Flowerdew & Grove, 1979), Limanda (Jobling et al., 1977), Stizostedion (Swenson & Smith, 1973) and a variety of gadoid species (Jones, 1974), larger individuals evacuated a standard meal (in grams) more rapidly than smaller fish of the same species. This is in contrast to the situation for Lepomis (Windell, 1966), Perca fluviatilis (Schneider, 1973, Persson, 1979, 1980) and P. platessa (Jobling, 1980) where for a given amount of food GER and total GET are unaffected by different fish sizes. In Limanda, a linear relationship was found between body weight and stomach volume (Jobling et al., 1977). Consequently, the latter authors concluded that where such relationships were established it was more valid to feed percentage body weight meals to evaluate the influence of animal size on GER. In this manner different size groups should receive an equal volume stimulus from their respective meals. Smaller fish tend to eliminate a percentage body weight meal quicker than larger fish though there may be partial compensation in larger fish through increased elimination rates (Pandian, 1967; Jobling et al., 1977; Flowerdew & Grove, 1979).

d) Meal composition

Separate food fractions such as digestible organic matter and indigestible chitin or plant material may show differential movement through the stomach (Windell, 1966; Windell et al., 1969; Jones, 1974; Windell, 1978). The chitinous exoskeletons of invertebrate prey species often remain in the stomach long after the digestible component has been evacuated. This is particularly true for large pieces of chitin which require softening prior to passing through the pyloric sphincter (Kionka & Windell, 1972).

The inhibitory effect of dietary lipid on gastric evacuation is probably of greater significance in gastric evacuation studies, since this nutrient can modify the overall rate at which the organic component passes from the stomach. Windell et al. (1972) observed no difference in GER of S. gairdneri fed diets ranging in lipid content from 6.5 to 14.5%. However, mealworms, which contain 35% lipid, were evacuated more slowly by both Lepomis macrochirus and the roach, Rutilus rutilus L:, in comparison to other natural food items (Windell, 1967; Hofer et al., 1982).

The slowing of GER by dietary lipid may be due to the higher calorific value of this nutrient. In agreement with the findings for higher vertebrates an increase in the dietary calorific level reduces GER in fish. In several teleosts (eg. S. gairdneri, Scophthalmus maximus, P. platessa and R. rutilus) dilution of the basal diet with inert material or the offering of low energy food items has resulted in enhanced GER (Grove et al., 1978; Flowerdew & Grove, 1979; Jobling, 1980; Hofer et al., 1982). This evidence suggests that the calorific level, rather than the nutrient composition, of the diet may be of greater significance in regulating GER. Such a system may act to control the delivery rate of a specific level of energy to the intestine.

e) Meal presentation

One of the major difficulties encountered during the investigation of gastric evacuation rates/times has been the problem of coaxing fish to accept a given weight of food. This is especially true when feeding animals with standard body weight meals of unnatural nutrient composition. In general, the majority of workers have solved this problem by resorting to force-feeding (Molnár & Töly, 1962; Windell, 1966; Swenson & Smith, 1973; Steigenberger & Larkin, 1974; Jobling et al., 1977; Flowerdew & Grove, 1979). However, a few authors have clearly shown that force feeding significantly decreases GER (Windell, 1966; Lyle, 1981). Swenson & Smith (1973) reported

an approximate two-fold difference in GERs when comparing voluntary with force-fed fish. Fish are very sensitive to even slight disturbance (see Chapter 2) and so it is unlikely that even prior adaptation to force-feeding (Gwyther, 1978) will completely eradicate this problem.

f) Feeding history and meal succession

In many gastric evacuation studies it has been the normal procedure to deprive the fish of food prior to feeding the test meal. Deprivation periods are known to cause distinct morphological changes of the intestinal tract of some species. Windell (1966) reported a shrinking of the pyloric caeca of bluegill sunfish, Lepomis macrochirus, which advanced progressively with time. A similar condition was observed in the brown trout, Salmo trutta, (Elliott, 1972). Since the caeca have both an enzyme secretory and absorptive function in fish, any morphological change might be expected to effect digestion and clearance rates.

The above observation has been confirmed in several studies. Until a certain level of deprivation is reached, an increase in deprivation causes a decrease in GER and an increased total clearance time (Windell, 1966). Even short-term starvation (3 days) can lead to a lower GER (Sarokon, 1975 - cited in Windell, 1978). Sarokon (1975) found that a four-day period of daily feeding was necessary for GERs to normalise in S. gairdneri following a three-day fast. Clearly, the immediate feeding history can have significant effects on GER in fish. This fact also questions the validity of monitoring the passage of individual meals through the intestine, separated either side by a deprivation period. This type of feeding schedule is different from the natural conditions of many species, especially for omnivores and herbivores (Hofer et al., 1982).

Laurence (1971) reported a GER twice as fast in larval largemouth bass, Micropterus salmoides, when fed continuously than that for single meals. Similarly, Nobel (1973) observed that an initial meal was evacuated

faster in the yellow perch, Perca flavescens, when it was then offered an excess of food. In contrast, neither S. trutta nor S. gairdneri showed an enhanced GER under a daily, multiple-meal feeding regime (Elliott, 1972; Sarokon, 1975). The latter author did however record a significant increase in GER for S. gairdneri when they were fed two daily meals, 8 hours apart, on alternate days. This was confirmed by Possompes et al., (1975), who observed a decreased transit and retention time for this species on a multiple meal schedule. Using a slightly different experimental approach, Tyler (1970) compared the dry stomach contents of the cod, Gadus morhua, following three single daily meals, with the expected value from the GER measured for a single meal only. He reported an enhanced rate in the former instance while Persson (1981), using an identical technique, was unable to demonstrate such an effect for the perch, Perca fluviatilis. Finally, in a closely controlled series of experiments with wild and laboratory populations of R. rutilus, Hofer et al. (1982) demonstrated similar evacuation rates in fish fed voluntarily. Furthermore, gut passage time was shorter when the fish were allowed to feed ad libitum.

Methods and Materials

I Capture and care of animals

Limanda limanda were captured by trawl in the Beaumaris Bay area of the Irish Sea. The fish were transferred to a 4000 ℓ outdoor pool with aerated, partially re-circulated sea water, at ambient temperature. It was found that the optimum trawl-time, to catch the fish, was 10-15 minutes. Trawls in excess of this period resulted in excessive scale damage and high post-capture mortality. Given well aerated, filtered sea water and uncrowded conditions, the fish could normally be induced to feed on freshly chopped whiting and Mytilus edulis tissue within three to four days of arrival at the laboratory. The animals were kept in the outdoor pool for at least three weeks. Following this initial acclimatization period, groups

of fish (8-12) were transferred to 200 l tanks supplied with re-circulating sea-water (30 l/minute). The water temperature was raised (2-3^{o}C day^{-1}) until the desired experimental temperature (15.0^{o}C) was attained. 1 kw semi-submersible heaters controlled by mercury contact thermometers (Gallenkamp) were used for this purpose. Occasionally, during July and August, ambient sea-water temperatures rose to excessively high levels (18-19^{o}C). During such periods, Churchill coolers were incorporated into the system which, in conjunction with the heater/thermostat units, maintained constant water temperatures. The fish were acclimatised to the experimental temperature for at least four weeks prior to being used in experiments. During this period the fish were given daily satiation meals of fresh whiting or Mytilus edulis.

II. Preparation of diets for gastric evacuation experiments

Fillets of muscle tissue were taken from whiting, Merlangus merlangius, captured during May/June, and freeze-dried. The tissue was then ground-up into a fine powder, which was passed through a 600 μ sieve. The freeze-dried whiting (FDW) powder was then stored at -20^{o}C, and used as required. Fish tissue does not normally form a significant part of the diet of Limanda. However, whiting was considered suitable for the purpose of the gastric evacuation experiments since:

a) the FDW was voluntarily accepted by Limanda in the form of a paste,

b) whiting were available in large quantities, such that a single, basic stock diet could be prepared. This ensured a constant nutrient composition of the test meals.

c) where the addition to the FDW of 'inert markers' (chromic oxide) was necessary, a homogeneous distribution of these substances was more readily achieved. This was confirmed in the present study by measuring the level of chromic oxide in several subsamples of the diet.

Finally, the overall aim of this experiment was to study the effect of meal presentation on gastric evacuation, and not the effect of nutrient composition.

III Gastric evacuation of single and multiple meals

a) Experimental technique

Gastric evacuation was examined in three size groups of fish, small (11-50g), medium (51-100g) and large (101-200g). All experiments were performed at 15.0°C. Following acclimation in the laboratory, each fish was individually weighed and placed in sparate compartments (33x57x25 cms.) of 140 l. tanks. The fish were fed daily for a further five days on fresh whiting (2% body weight rations). They were then starved for 48 hrs. prior to being offered the test meals. This ensured complete evacuation of the intestine. A 48 hr. deprivation period should not have proved too severe in terms of morphological changes of the gut (see Introduction).

In an earlier gastric evacuation study, Jobling (1974) demonstrated that the stomach volume of Limanda increased in proportion to body weight. The same relationship holds true for several teleosts. Therefore, to achieve an equal gastric-feeding stimulus it is preferable to feed individual animals to the same percentage body weight. Despite this fact, it has been common practice in earlier studies to feed the individuals of a particular size group of fish to the average body weight of the group. This means that individuals at the extreme ends of the size range will receive meals either in excess or below the desired ration level. Considering the large number of factors affecting GER in fish this source of variation is undesirable. Consequently, in the present study each fish was voluntarily fed a meal according to its own individual weight.

To monitor the evacuation of a single meal, fish in each size group were offered a 1% body weight meal composed of 30% freeze-dried whiting (FDW), 4% chromic oxide, 2% binder (methyl cellulose) and 64% water. The

chromic oxide could be determined chemically at a later stage. The moisture level of the meals may seem rather high. However, in a number of trial studies with freeze-dried whiting diets of lower moisture content, it was found that even a 1% body-weight meal of FDW paste underwent considerable post-ingestive swelling in the stomach. This may have contributed to the frequent reluctance of some fish to ingest consecutive meals of these diets within a short period (3 h). After feeding the test meals, groups of fish (4-8) were gently anaesthetised in a weak quinaldine solution (0.04 ppm) and frozen at -20°C. This procedure was repeated at specific time intervals after feeding.

The stomach and intestines were later dissected from the fish in a semi-thawed condition. In this manner, the entire contents of the stomach could be separated cleanly from the stomach lining. The stomach residium was placed in pre-weighed vials, dried at 60°C for 30 h., and re-weighed.

When the pattern of gastric evacuation had been established for a single meal, the entire experiment was repeated in an identical manner for the two larger size groups of fish. However, in this instance, a second 1.0% body weight meal (M_2) was voluntarily fed, 3h. after the first meal (M_1). The second meal was similar in composition to the earlier meal, except that the chromic oxide was substituted with white kaolin powder. The chromic oxide permitted the amount of the two meals remaining in the stomach to be estimated. The procedure enables the impact of the arrival of a second meal on the evacuation of an earlier meal to be monitored. In addition, the influence of the presence of food already in the stomach upon the evacuation of a subsequent meal can be observed.

The addition of binder to the diet considerably reduces the degree of mixing between the two separate meals. Consequently, the two meals tend to remain largely discrete and this can easily be seen upon dissection of the fish.

The effect of allowing free mixing of both meals was monitored in the two larger size groups. This was simply achieved by repeating the above

single and multiple feeding experiments, in an identical manner, except that the binder was omitted from the diet.

IV Chemical analysis

Chromic oxide (Cr_2O_3) was determined by the oxidative method of McGinnis & Kasting (1964). The chromic oxide was oxidised to dichromate ions, the concentration of which was measured spectrophotometrically following development with diphenylcarbazide reagent.

Reagents:

a) Wet oxidation mixture

2g sodium molybdate ($Na_2MoO_4 2H_2O$) was dissolved in 30 ml distilled water followed by 30 ml of concentrated sulphuric acid. When the solution was cool, 40 ml 70% perchloric acid was added.

b) Diphenylcarbazide reagent

0.25% 1,5-diphenylcarbazide (w/v) was prepared in 50% aqueous acetone immediately prior to use.

Duplicate 5 or 10 mg subsamples (accurate to 3 decimal places) of the stomach contents, in the case of the single or multiple meal feeding regimes respectively, were weighed out into digestion tubes. 2 ml wet oxidation mixture were added to the samples which were then heated for 30 minutes at 220°C. After cooling, the mixture was diluted to one litre. 0.5 ml diphenylycarbazide reagent was then forcibly injected, to ensure thorough mixing, into a 10 ml subsample of the digested sample. After allowing a 3 minute colour development period, the absorbance was read at 540 nm, against a reagent blank. A Cecil CE 303 spectrophotometer was used for the absorbance measurements.

Results

I General observations

The majority of fish readily accepted entire, proferred meals although considerable care and patience was necessary during the actual presentation of the food. One problem associated with the isolation of Limanda in individual compartments was that some fish were easily stressed by the least disturbance. This problem has also been reported for the bass, Dicentrachus labrax, where isolation lowered readiness to feed and increased sensitivity to external stimuli (Stirling, 1977). In the present study, most of the isolated animals settled down quite readily and ate normally. However, some individuals were clearly nervous and would not accept food in isolation. One method of solving this problem was to accompany each sand dab with P.plattesa individuals. The latter species does not accept food voluntarily in captivity for considerable periods after it is first brought into the laboratory. Consequently, the plaice do not compete with Limanda for the pre-weighed meals. However, their presence reduced the nervousness of any stressed Limanda which subsequently commenced feeding.

II Analysis of data

The aim of this gastric evacuation study was not to identify the model which most adequately described gastric evacuation in Limanda. Rather, it was the influence of multiple feeding on the evacuation rate of discrete meals that was of interest. The merits of the various mathematical models describing gastric evacuation rates have already been discussed. For the purposes of the present study, a previously unreported model will be used for analysis of the data. In this model, two factors are assumed to control emptying rate of a chosen food type at a given temperature for the dabs. The stomach will be stretched isometrically in three dimensions by the arrival of a meal (S) but the perceived stimulus for this mass of

food depends on the size of the stomach. It is known for several species, including the dab, that stomach volume is proportional to body weight (W). The stretch stimulus in the gut wall, which initiates peristalsis and gastric juice secretion, can be assumed proportional to $(SW/)^{0.33}$. The response of the organ, primarily in terms of gastric juice secretion, depends on the area of the gastric epithelium. Response will then be proportional to $W^{0.66}$. Accordingly, emptying rate can be formulated as:-

$$dS/dt = -K(W)^{0.33}(S)^{0.33} \qquad .....(5)$$

where the differential dS/dt describes the emptying rate, S is the absolute meal size, W is the fish weight and the constant K is a measure of the efficiency with which the food is digested. The value of K depends upon factors such as fish species, temperature and food type. The volume of food in the stomach at any particular time (St), and for a fish of known weight (W), may be determined by integration of the above equation which gives:

$$S_t^{2/3} = So^{2/3} - \frac{2K}{3} W^{1/3} t \qquad .....(6)$$

where So is the weight of food fed to the fish.

Since the meal size fed to individual fish in the present work varied directly with body weight, it was first necessary to normalise the data for each size group before a comparison of evacuation rates could be made. Consequently, equation (6) is solved at each observation for K such that:

$$\frac{3(So^{2/3} - St^{2/3})}{2W^{1/3}t} = K \qquad .....(7)$$

Prior to reporting the results of the present gastric evacuation study, it may help to outline some of the predictions, from equations (5) and (6), of the consequences of multiple feeding regimes. In Fig. 1 line 'A' represents the emptying from the stomach of a single meal (M) fed at

Fig. 1. Predicted evacuation patterns of various meals according to equations (5) and (6)

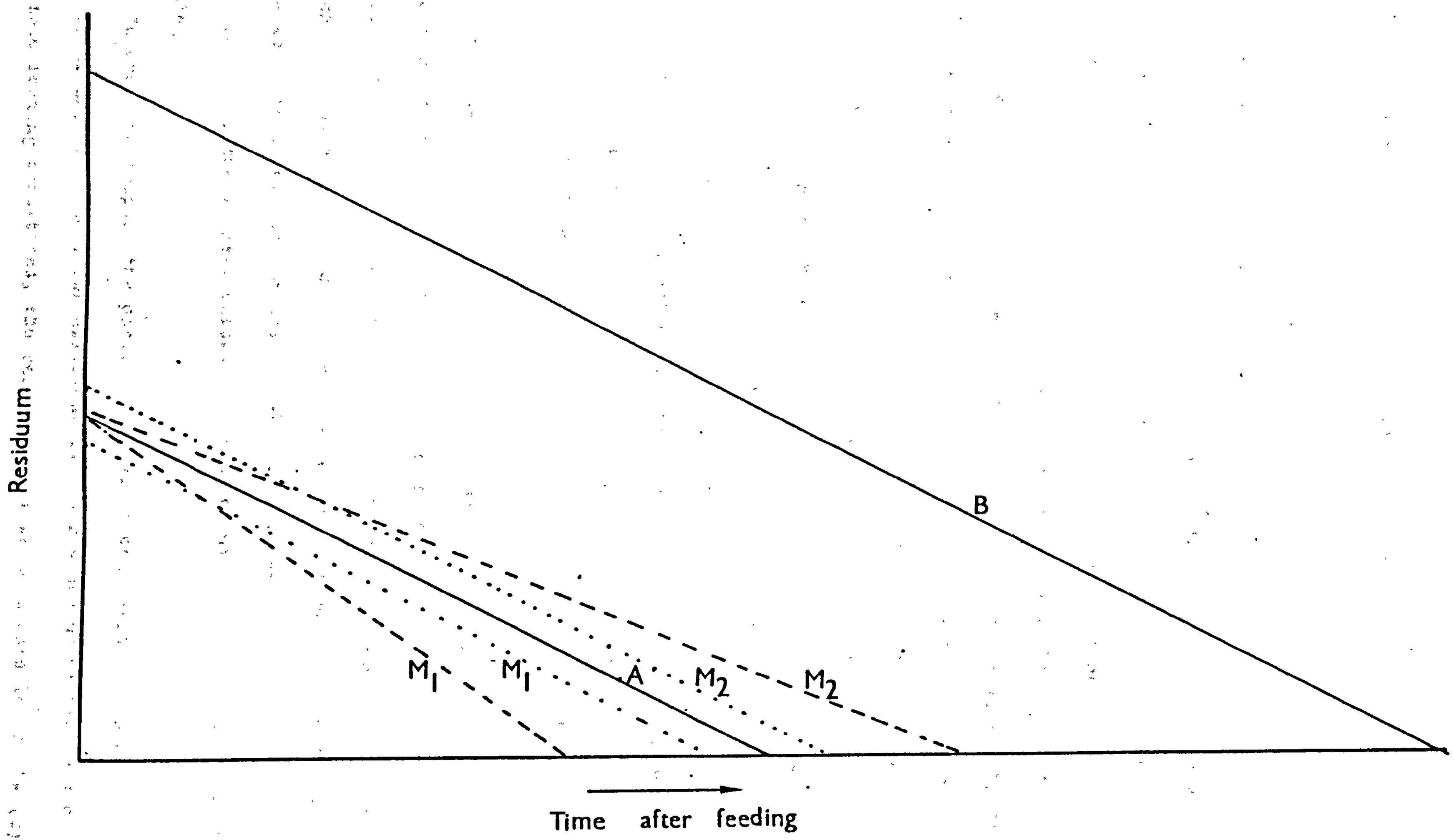
A = single meal (M)

B = meal 2xsize (M)

--- meals (M_1) and (M_2) with binder

... meals (M_1) and (M_2) with no binder

) Evacuation patterns of respective meal types


Residuum
B
M_1
M_1
A
M_2
M_2
Time after feeding

time zero after transforming the residual stomach contents to the $^{2}/_{3}$ power function as required by equation (6). If a meal, in all respects identical, but of twice the size were presented (M_3) the stomach is expected to empty following the parallel line 'B' (slope = $\frac{2}{3}KW^{1/3}$ is independent of meal size). If, instead, two meals of size M (M_1 and M_2) are given simultaneously and were to mix rapidly in the stomach, each would empty at a rate parallel to lines A and B, and identical with that of the single meal M. These are shown as dotted lines in Fig.| . A further modification is now considered whereby the two meals M_1 and M_2 are presented together but are physically prevented from mixing. This would be equivalent to ingestion of, for example, two small crustaceans and was achieved in this study by using a binding agent in preparing M_1 and M_2 separately. Furthermore, M_1 could be given a little earlier than M_2 ensuring that 1) it is treated with gastric juices in advance of M_2, and 2) it lies in the more posterior region of the stomach. If by virtue of pre-treatment with gastric juices and physical position in the stomach, M_1 is preferentially evacuated (dashed line in figure) then M_2 must be decreased in emptying rate to achieve the overall curve which is described by the line 'B'.

The value of K for individual fish, was calculated using the estimated dry weight of food remaining in the stomach at each time interval ie. based on the chromic oxide content of the residuum and the assumption that both food and chromic oxide are emptied at the same rates. There was a tendency for the mean K value to decrease as digestion proceeded, with the largest values occurring within the first hours after feeding.

In order to compare the pattern of gastric evacuation of specific amounts of dry food (So), for different fish sizes and under single and multiple feeding conditions, the residium (St) at 30 minute intervals (t)

was calculated for standard fish sizes (W) of 120, 60 and 30g body weights. This was achieved by incorporating the normalized K values, for the respective time intervals after feeding, into equation 6. The residium of M_2 in the multiple feeding experiment was derived by subtraction of the estimated amount of M_1 from the total dry weight of gastric contents. This method would probably have given a slight underestimation of the GER of M_2 since no allowance was made for any material secreted on to the meal after ingestion.

III Comparison of evacuation rates of 1% body weight meals for 120, 60 and 30g fish

The data for the fish fed 1% body weight meals is considered first. The relationship between the residium and the time interval after feeding was curvilinear on an arithmetic scale for each fish size (Fig. 2). The curves were transformed into near-linear form by taking the cube root of the square of residual meal size (Fig. 3) as required by equation (6). A comparison of the slopes for the regression functions of each fish size, fed a 1% body weight meal, shows a significant increase in the gradient with increased body weight, as predicted by equation (6). Given the normalised data, it can be seen that the digestive capacity per unit area of gastric epithelium, does remain fairly constant in different sized dabs as predicted by the model. (Table 2). Jobling et al. (1977) showed that stomach volume (ml) is 8% of total body weight (gm). With the size

Table 2. Comparison of digestive capacity (K) for 30, 60 and 120g fish.

W	$^{2}/_{3}\ W^{1}/_{3}$	Slope	$K = \text{slope}/_{^{2}/_{3}W^{1}/_{3}}$
30	2.07	1.965	0.949
60	2.61	2.79	1.071
120	3.29	2.922	0.888

Fig. 2. Relationship between dry weight of food remaining in the stomach (residuum, mg) and time after feeding for 120, 60 and 30g Limanda voluntarily fed 1% body weight meals.

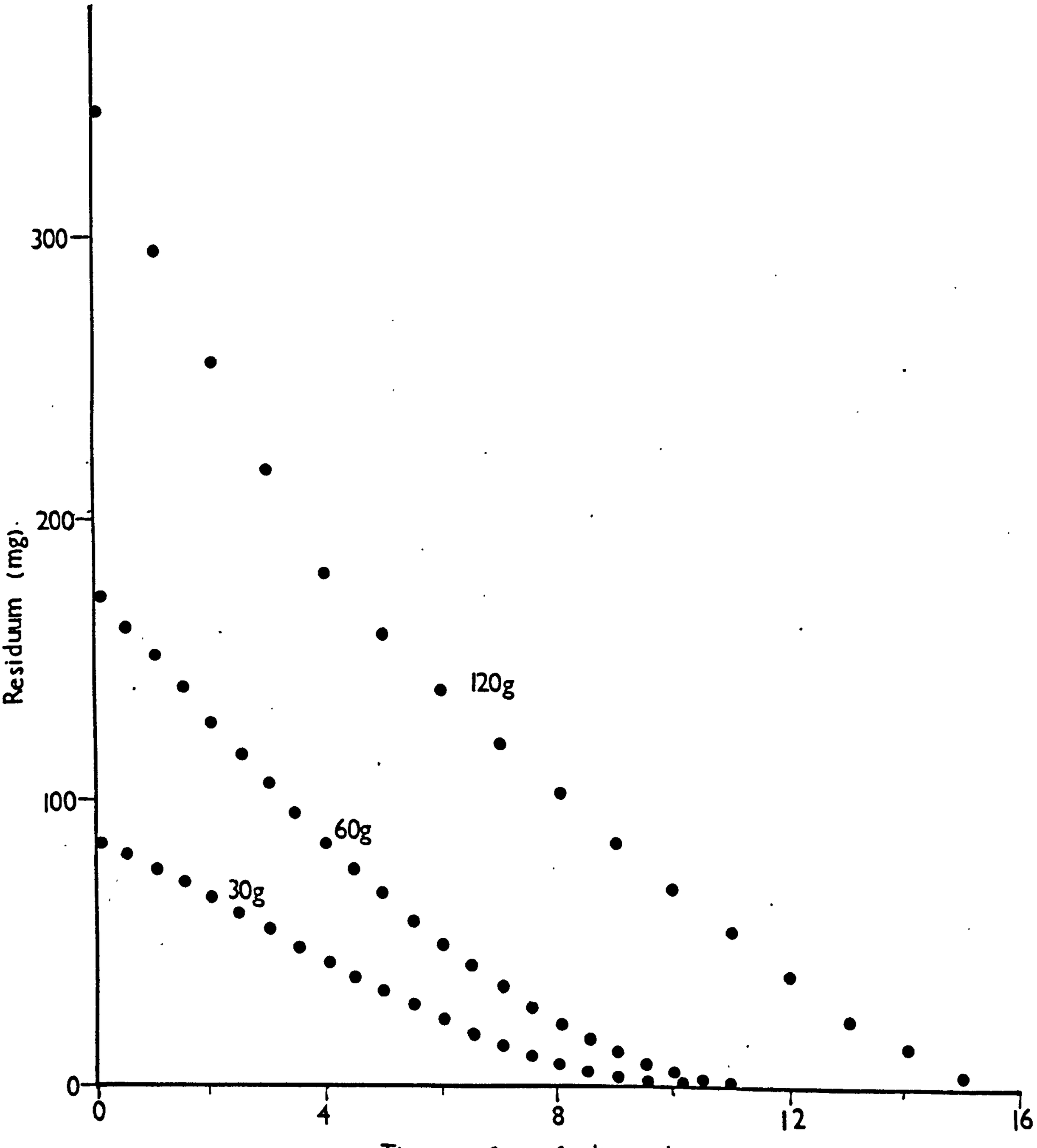

300
200
100
0
Residuum (mg)
120g
60g
30g
0
4
8
12
16
Time after feeding (h)

Fig. 3. Comparison of regression lines following cube squared root transformation of residuum data for 120, 60 and 30g fish fed 1% body weight meals.

($Y = S_t^{2/3}$ where S_t is the residuum after x hours)

a) 120g

$Y = 45.728 - 2.922\ x \quad (r = -0.9953)$

b) 60g

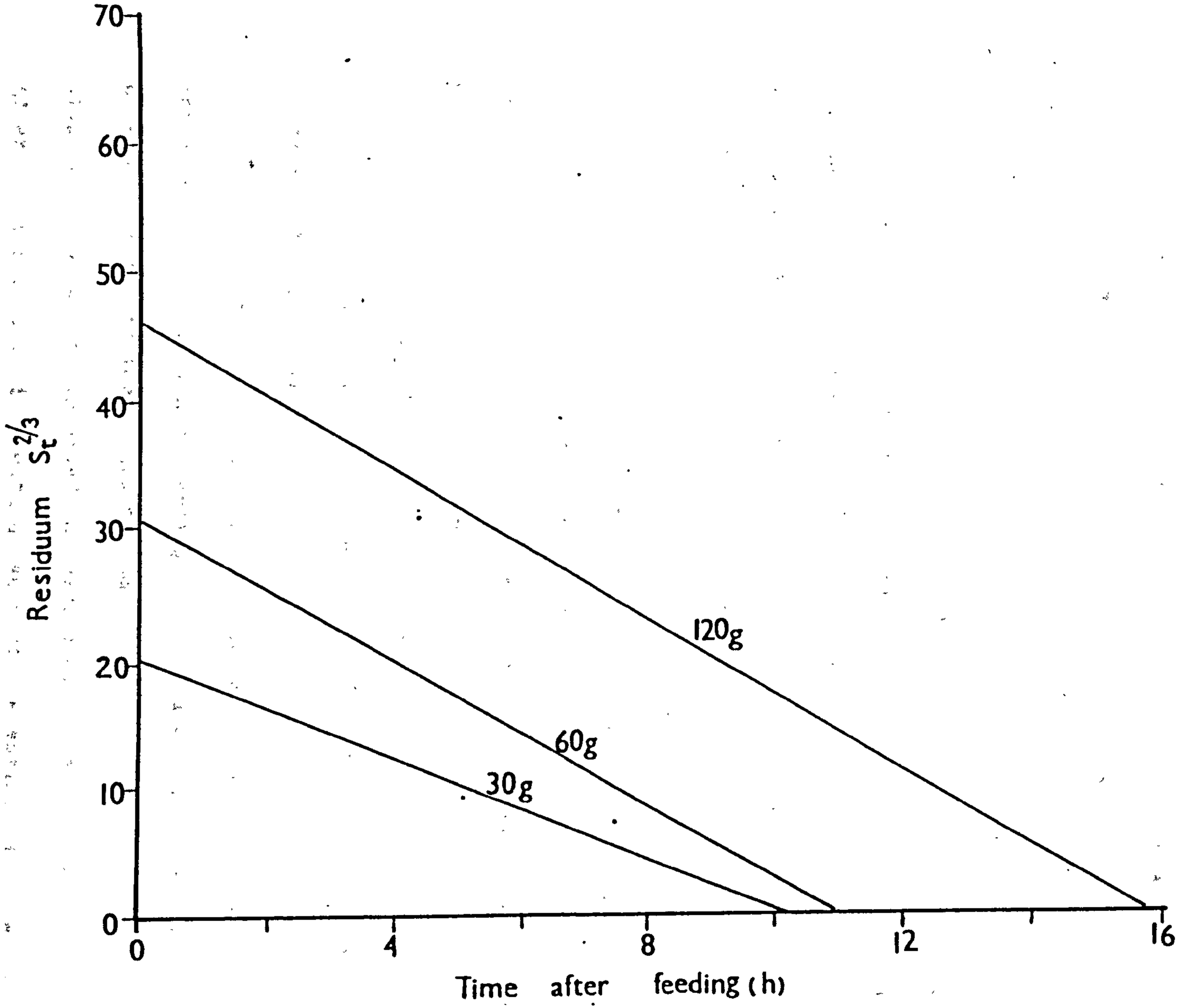
$Y = 30.682 - 2.796\ x \quad (r = -0.9993)$

c) 30g

$Y = 19.980 - 1.965\ x \quad (r = 0.9991)$

Comparison of slopes for all regressions: F-value = 32.230,

$\therefore$ slopes significantly different ($p<0.01$).


70
60
50
40
30
20
10
0
Residuum $S_t^{2/3}$
120g
60g
30g
0
4
8
12
16
Time after feeding (h)

range used here, the available gastric epithelium will increase from $f(0.08x30)^{2/3}$ to $f(0.08x120)^{2/3}$, a range of f(1.79) to f(4.52) or 2.5 fold.

It is calculated from the above results that fish of sizes 30, 60 and 120g will, on average, evacuate 8.7, 15.9 and 23.7 mg food per hour from the stomach in the 5h period beginning 3h after food was ingested. Despite the slower absolute evacuation rates of the smaller fish, they achieved complete evacuation of the 1% body weight meals earlier than the 120g fish. The calculated GETs (the intercept on the x-axis) compare very closely with the observed values (Appendix I). The 120, 60 and 30g fish evacuated the entire meal from the stomach in approximately 15.5, 11.0 and 10.0h respectively. Gwyther (1978) earlier reported gastric evacuation times (GET) of 17.3h (120g), 12.5h (60g) and 9.0h (30g), at 15.5°c for Limanda. The data of Jobling et al. (1977) also compares closely with the present findings for this species. This is probably surprising in view of the fact that the latter authors force-fed their animals and used diets which were approximately 28% higher in calorific value. Both force-feeding and increased calorific density generally extends GET. However, close comparison with the present study is possibly not valid since Jobling (1977) and Gwyther (1978) measured GET by x-radiography. This technique may underestimate GET since it is difficult to detect the final remnants of a meal.

Prior to considering the data for the multiple feeding experiments, there is one observation concerning the estimated and observed dry weights of stomach contents that requires some comment. A comparison of the original data for the observed and estimated stomach contents revealed that after the first hour of digestion, some of the estimated values were considerably lower than the observed dry weights. This was true for the diets both with and without binder added but only for the two larger fish sizes. This clearly indicates that some dry material has been added to the meal during the first hour of digestion. The extra material probably

originates from a combination of gastric juices and some of the epithelial lining of the stomach. This phenomenon has been reported in earlier studies. Lyle (1980) was able to induce a significant rise in the gastric dry weight contents of Scyliorhinus canicula by inserting a piece of sponge into the stomach. Similarly, therefore, if the dry weight of the stomach contents is used to measure the meal residium, the secretion of material to the stomach contents may result in a lower apparent rate of gastric evacuation. This may be especially so when working with small animals and at low ration levels.

A similar comparison of the two sets of values after the first hour of digestion revealed a reverse situation where the estimated weights tended to be higher than the observed values. This pattern was again apparent only in the two larger size groups of fish fed both diet types. This discrepancy probably arises due to the easier passage of soluble components of the diet into the intestine than the less soluble chromic oxide marker. This preferential retention of chromic oxide label has been reported in nutritional studies with ruminants (Knapka et al., 1967). However, this problem should not invalidate a comparison of the evacuation rates of individual meals, under single and multiple feeding regimes, since the respective rates are both based on the chromic oxide technique. Furthermore, a comparison of the 'slopes for the respective regression functions, based on observed and estimated dry weights, revealed no significant differences in the overall evacuation rates.

IV Comparison of evacuation rates of 1% body weight meals under single and multiple feeding regimes.

The same transformation of the data for the residium of meals M_1 and M_2, fed to the 120 and 60g fish, gives the curves shown in Figs. 4-7. These are compared with the curves representing the evacuation of the single meal and the combined meals ($M_1 + M_2$) from the time at which M_2

Fig. 4. a - d. Cube root of square of the residium (120g fish, + binder).

a) single meal (O—O)

$Y = 45.73 - 2.922\,x$ ($r = -0.9953$)

b) M_1 (Δ—Δ)

$Y = 48.47 - 3.549\,x$ ($r = -0.9991$)

c) M_2 (▼—▼)

$Y = 48.34 - 2.675\,x$ ($r = -0.9989$)

d) $M_1 + M_2$ (●—●)

$Y = 66.48 - 4.117\,x$ ($r = -0.9987$)

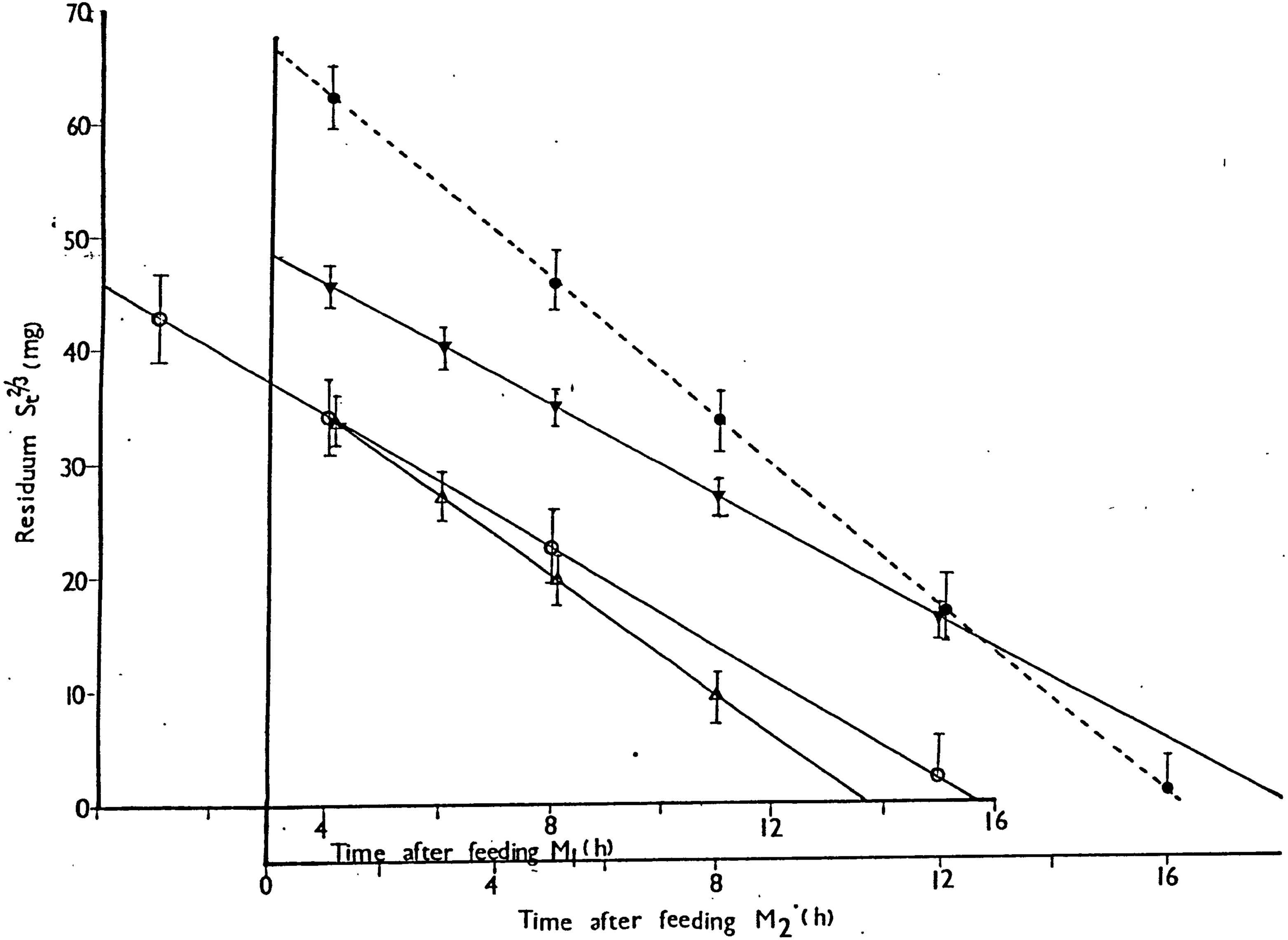

70
60
50
40
30
20
10
0
Residuum $S_t^{2/3}$ (mg)
4
8
12
16
Time after feeding M_1 (h)
0
4
8
12
16
Time after feeding M_2 (h)

Fig. 5. a-d. Cube root of square of the residium (120g fish, - binder).

a) single meal (0—0)

$Y = 49.40 - 3.326\ x \quad (r = -0.9993)$

b) M_1 (Δ—Δ)

$Y = 46.34 - 2.614\ x \quad (r = -0.9897)$

c) M_2 (▼—▼)

$Y = 38.32 - 2.058\ x \quad (r = -0.9594)$

d) $M_1 + M_2$ (●—●)

$Y = 61.07 - 3.499\ x \quad (r = -0.9808)$

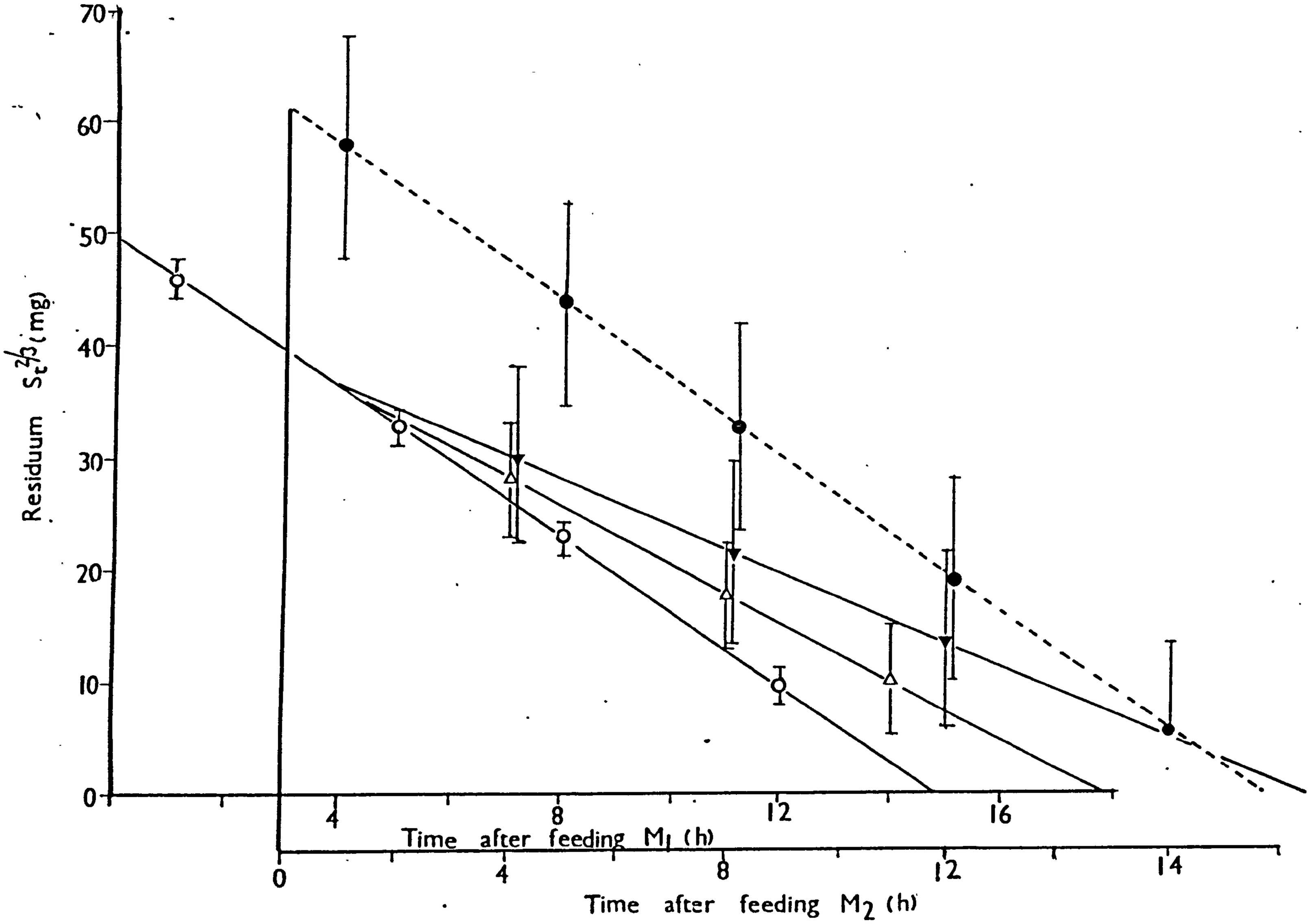

70
60
50
40
30
20
10
0
Residuum $S_t^{2/3}$ (mg)
4
8
12
16
Time after feeding M_1 (h)
0
4
8
12
14
Time after feeding M_2 (h)

Fig. 6 a-d. Cube root of square of the residium (60g fish, + binder)

a) single meal (O—O)

$Y = 30.68 - 2.796\ x$ $(r = -0.9993)$

b) M_1 (Δ—Δ)

$Y = 30.69 - 2.909\ x$ $(r = -0.9997)$

c) M_2 (▼—▼)

$Y = 29.63 - 1.725\ x$ $(r = -0.9981)$

d) $M_1 + M_2$ (●—●)

$Y = 40.25 - 2.864\ x$ $(r = -0.9973)$

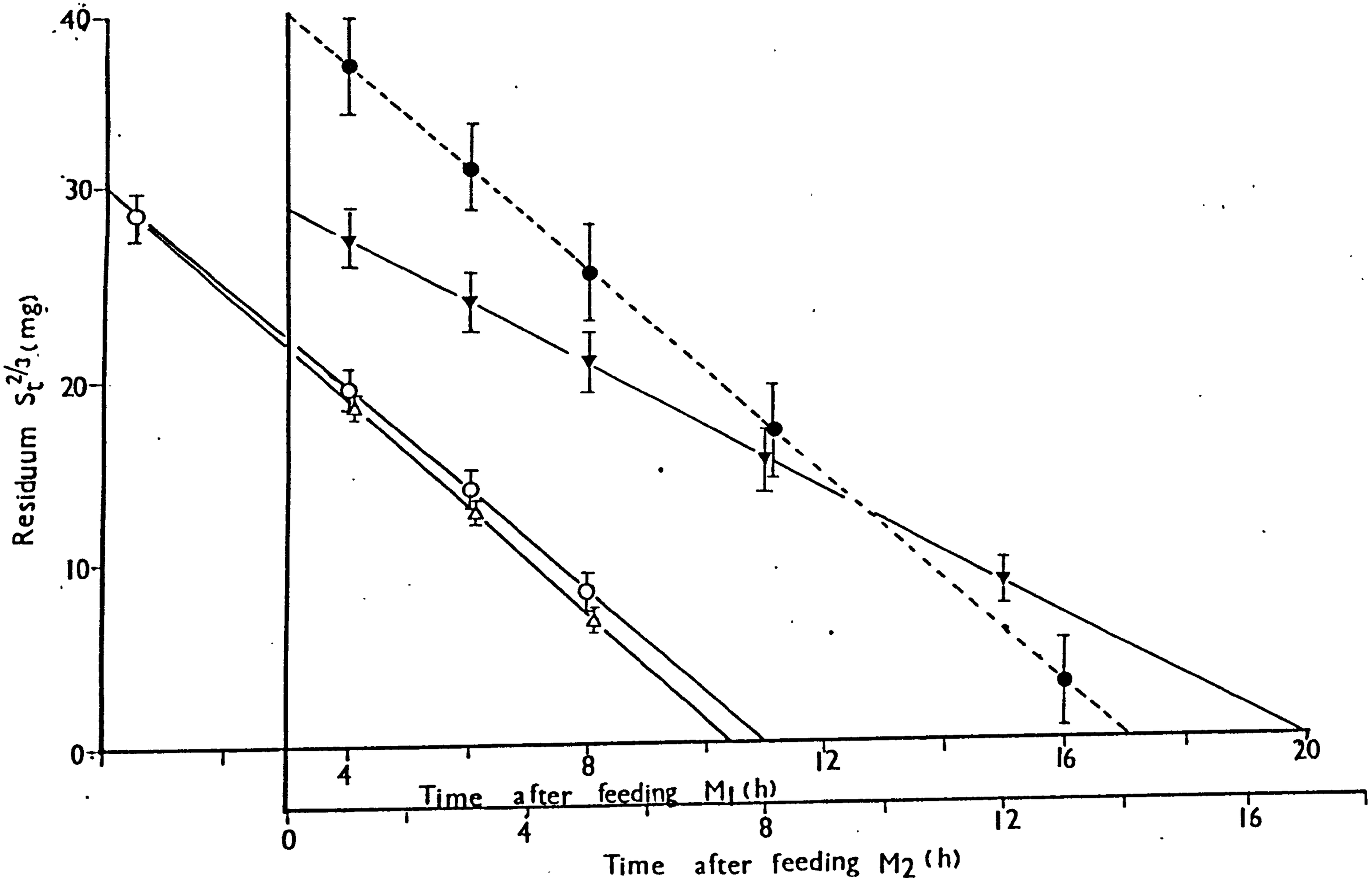

40
30
20
10
0
Residuum $S_t^{2/3}$ (mg)
4
8
12
16
20
Time after feeding M_1 (h)
0
4
8
12
16
Time after feeding M_2 (h)

Fig.7 a-d Cube root of square of the residium (60g fish, - binder)

a) single meal (O—O)

$Y = 33{:}48 - 3.010\,x$ (r = -019926)

b) M_1 (Δ—Δ)

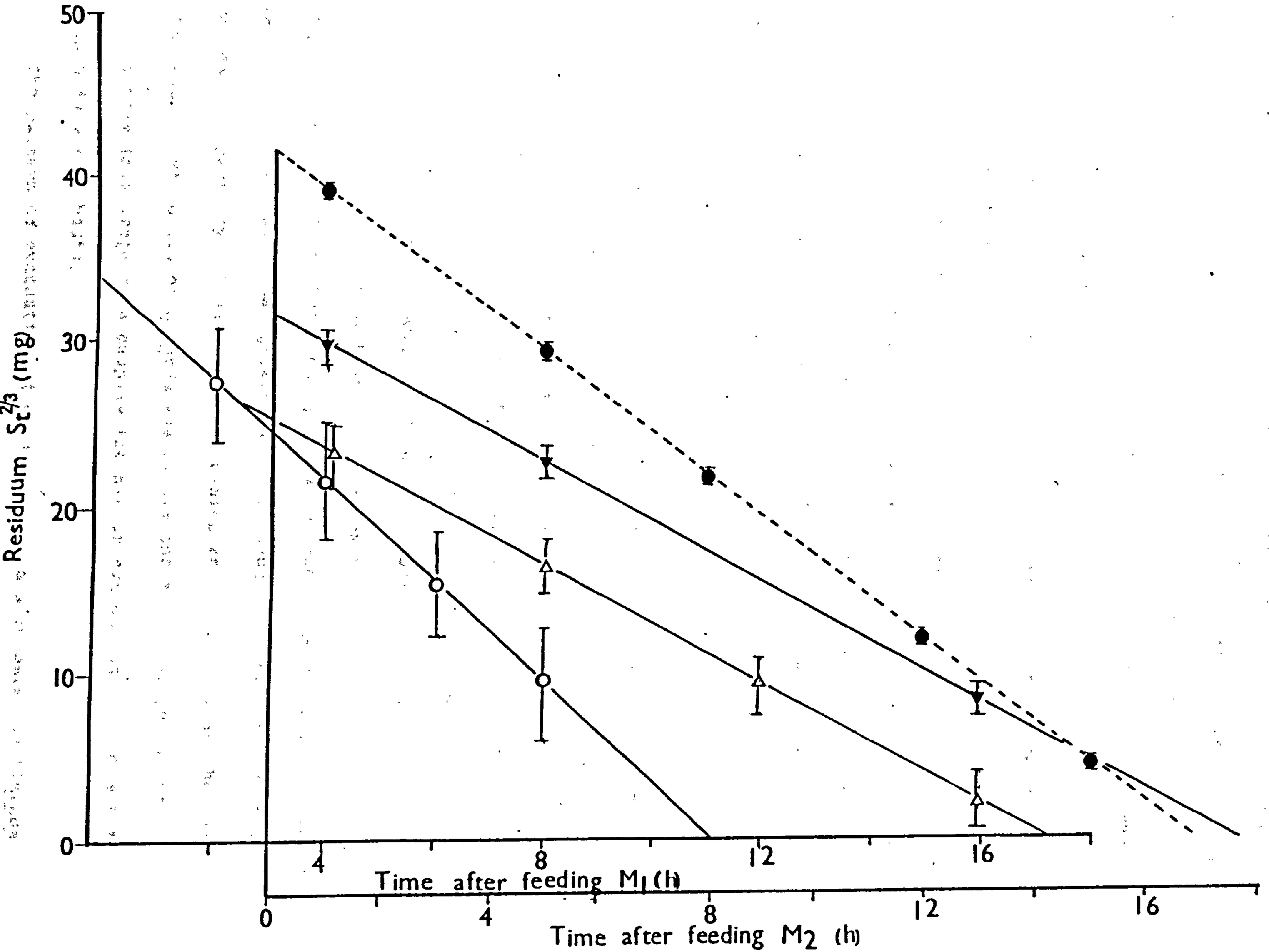
$Y = 30.61 - 1.781\,x$ (r = -0.9974)

c) M_2 (▼—▼)

$Y = 31.50 - 1.527\,x$ (r = -0.9990)

d) $M_1 + M_2$ (●—●)

$Y = 41.38 - 2.862\,x$ (r = -0.9955)


50
40
30
20
10
0
Residuum $S_t^{2/3}$ (mg)
4
8
12
16
Time after feeding M_1 (h)
0
4
8
12
16
Time after feeding M_2 (h)

was fed. A comparison of the regression functions of the single-meal and M_1 indicated that the latter was evacuated at a significantly faster rate but only when the meals remained discrete after ingestion. In the absence of binder, M_1 was evacuated more slowly than a single meal alone. In both instances, the evacuation rate of M_2 was lower in comparison to a single meal (Tables 3 &4). The GET of M_2, for 60 and 120g fish, increased from 11.0 to 17.2h and 15.5 to 18.0h respectively.

Finally, notwithstanding the preferential emptying or otherwise of portions of a meal (depending on whether the portions mix or are prevented from doing so), equation (6) predicts that the overall emptying rate of the stomach should have the same slope as a smaller, single meal. In two of the four comparisons which are possible in this study this is true (Table 4). The slopes were significantly different in the large group fed meals containing binder and in the smaller group fed meals without binder.

Discussion

Large Limanda evacuate a given percentage body weight meal more slowly than the smaller size groups as predicted by equations (5) and (6) of the model. This finding agrees with the earlier reports on gastric evacuation studies for this species (Jobling, 1977; Gwyther, 1978) and for several other teleost species (Grove et al., 1978; Flowerdew & Grove, 1979; Grove & Crawford, 1980). Pandian (1967) believed that the faster evacuation rate, in proportionate terms, among the smaller individuals of Megalops resulted in higher feeding rates for such size classes. It would be expected that the higher growth rates of young fish would be reflected, at least in part, by a higher food processing rate. It is also the case that small individuals of Limanda assimilate their food slightly more efficiently than large fish despite the higher evacuation rate on a per gram body weight basis.

The pattern of evacuation of consecutive meals ingested by Limanda appears to depend largely upon the physical nature of the meals themselves.

Table 3 Gastric evacuation data for Limanda fed single and multiple meals (M_1 + M_2) at 15.0°c.

a) Standard 120g fish (with binder)

	Single meal	First multiple (M_1)	Second multiple (M_2)	Combined (M_1 + M_2)
meal weight (mg) at t=0	343	343	343	584
Y intercept	309	337	336	542
GET (h)	15.65	13.66	18.07	16.15
slope	2.9220	3.5492	2.6749	4.1166

b) Standard 120g fish (no binder)

	Single meal	First multiple (M_1)	Second multiple (M_2)	Combined (M_1 + M_2)
meal weight (mg) at t=0	343	343	343	596
Y intercept	347	315	237	477
GET (h)	14.85	17.73	18.62	17.45
slope	3.3261	2.6138	2.0578	3.4989

c) Standard 60g fish (with binder)

	Single meal	First multiple (M_1)	Second multiple (M_2)	Combined ($M_1 + M_2$)
meal weight (mg) at t=0'	171	171	171	276
Y intercept	170	170	161	255
GET (h)	10.97	10.55	17.17	14.05
slope	2.7962	2.9087	1.7254	2.8643

d) Standard 60g fish (no binder)

	Single meal	First multiple (M_1)	Second multiple (M_2)	Combined ($M_1 + M_2$)
meal weight (mg) at t=0	171	171	171	301
Y intercept	194	169	177	266
GET (h)	11.12	17.19	20.63	14.66
slope	3.0098	1.7805	1.5270	2.8617

When the meals are allowed to mix with each other (in the absence of binder), the GER of M_1 is, as predicted, considerably reduced in comparison to a single meal. This is in contrast to the situation when binder is added to the diet to prevent meals M_1 and M_2 from mixing freely. This suggests that, in the presence of binder, M_1 maintained its position near to the pyloric sphincter. (This was observed to be the situation during extraction of the stomach contents. Even when M_1 was almost completely evacuated there was only a very slight mixing of the two meals at their interface). Therefore, any increase in peristaltic activity due to the arrival of M_2 would have had a greater impact on M_1 in terms of its emptying rate. As might be expected, this pattern was most clearly shown in the larger fish (Table 2). This size group (101-200g) evacuate standard percentage body weight meals at absolutely faster rates than small animals (mg/h). Consequently, any enhancement effect of multiple feeding may be expected to be proportionately greater in the large fish. The increase in evacuation rate of M_1 only represented a further 3.4 mg (dry weight) or 12.2 cals. per hour being delivered to the intestine. This may appear to be a rather insignificant increase in the supply rate of nutrients for absorption. However, it should be remembered that in the wild Limanda may ingest several discrete meals within a feeding period. Under these conditions, any enhancement effect of subsequent food intake on GER of earlier meals may be even greater.

The natural diet of the dab is very diverse but there appears to be a shift in the relative importance of prey type with size (Jobling, 1977). This author observed that large Limanda prey upon the larger polychaetes such as Arenicola and Lanice, larger crustacea and echinoderms while smaller fish depend more heavily on bivalve siphons and small nereid polychaetes. With such feeding habits it is quite feasible that the enhancement effect of subsequent food intake on the GER of earlier meals would readily operate. This may be particularly so for the larger animals

feeding on larger prey items which do not readily become mixed with later meals. In the smaller size groups, the operation of such a system may not be so apparent although this may be compensated for by their already faster processing rates (on a proportional body weight basis).

The one instance where the combined meal (M_1 + M_2) was evacuated more slowly when compared to a single meal (in the 51-100g fish fed meals without binder) is unusual. It may be explained by the fact that when M_2 arrived in the stomach, a certain proportion of the gastric secretions had already been utilised by the first meal. The enzymic degradation rate of the combined meals (which mixed readily) may therefore not have been as efficient had it been exposed to the entire gastric secretions. Norris et al. (1973), working with Lepomis macrochirus, observed that a meal in excess of 1% body weight exhausted the secretory capabilities of the stomach. Furthermore, El-Shamy (1976), in a multiple feeding study of this species, reported a nearly complete release of enzymes following the ingestion of the first meal. These observations may lend some support to the above conclusion.

Finally, the most important implication of these findings in terms of the present study are considered briefly. The speed with which changes in the rate of respiration, levels of blood metabolites and energy reserves occur after feeding will ultimately depend upon the rate at which nutrients are delivered to the intestine. Clearly, the supply rate of the nutrients from the stomach will depend on the numerous factors already discussed in the introduction. In addition to these factors such as meal size, fish size and energy value of the meal, the interaction of individual meals within the stomach are important. An increased evacuation rate of one meal, due to the arrival of subsequent food in the stomach, is likely to influence the pattern of several post-prandial parameters. This fact must be remembered in the following physiological studies which are all restricted to the 101-200g size range of fish.

Section II

Post-prandial metabolic rate of *Limanda limanda* in relation to gastric and intestinal clearance

Introduction

When feeding, fish must process their food and this requires energy for digestion, assimilation, transportation, biochemical treatment and incorporation. Therefore, only a proportion of the food ingested by fish is available for such bodily functions as maintenance, growth and activity (Tandler and Beamish, 1981). Fish may dissipate part of the ingested energy as faeces, metabolic excretion and as heat or specific dynamic action (SDA). SDA was first defined in homeotherms by Rubner (1902) as the heat increment resulting from the ingestion of a meal. This increment was thought to reflect the processing of the meal. SDA in fish is usually measured by indirect calorimetry (Beamish, 1974) as the post-prandial oxygen consumption. The oxygen consumption rate may then be converted into caloric units of energy by applying oxycalorific equivalents (Brett & Groves, 1979). Beamish (1974) termed this post-prandial rise in oxygen consumption, reflecting an increased energy expenditure, as the apparent SDA. Apparent SDA incorporates both the energy requirements for the biochemical processing of the meal and the mechanical aspects of feeding. The latter portion will include grasping, chewing, swallowing and peristalsis (Tandler & Beamish, 1979 a, b; 1981). The contribution of mechanical processes to apparent SDA may be quite significant although the relative energy expenditure for peristalsis may decline with increased meal size (Tandler & Beamish, 1979a). However, Jobling & Davies (1980) consider peristalsis to be relatively unimportant in this respect. Apparent SDA is influenced by fish size (Beamish, 1974; Tandler & Beamish, 1981), meal size (Muir & Niimi, 1972; Pierce & Wissing, 1974) and environmental temperature (Brett, 1976). More recently, the influence of nutrient composition and dietary energy level on apparent SDA has been investigated in Micropterus salmoides (Tandler & Beamish, 1979, 1981) and also in P. platessa (Jobling & Davies, 1980; Jobling, 1982).

SDA has been measured for several teleast species (see review by Brett & Groves, 1979). Unfortunately, interspecific comparison is difficult due to

the variety of techniques used to measure respiratory rates (Jobling, 1982). The post-prandial increase in oxygen consumption is represented by a gradual rise to a maximum rate followed by a slow decline. Consequently, there are three parameters of the post-prandial rise and fall in oxygen consumption that are of interest; the maximum or peak level of oxygen consumption, its duration and the magnitude of the SDA effect (Jobling & Davies, 1980). The latter component is measured as the total post-prandial increase (over basal levels) in consumption for the complete assimilation of the meal.

(a) Peak level of SDA

Muir & Niimi (1972) and Beamish (1974) recorded an elevation in peak metabolic rate in aholehole, Kuhlia sandvicensis, and M. salmoides respectively with increasing ration. Oxygen consumption continued to increase in these species up to a maximum level of food or energy intake. This situation contrasts with the findings of Jobling & Davies (1980) for P. platessa where the peak of oxygen consumption increased up to a maximum level. This level was induced by ration sizes below the satiation amount and resembles the findings for sockeye salmon, Oncorhynchus nerka (Brett & Zala (1975)) and menhaden, Brevoortia tyrannus (Hettler, 1976). Jobling & Davies (1980) concluded that the rate of the assimilatory processes represented by SDA has a saturation level which is limited by cellular metabolism rather than the respiratory system.

It is thought that the maximum level of oxygen consumption represents a metabolic response to the total nutrient concentration of the diet rather than to its composition (Tandler & Beamish, 1980). These authors showed that the magnitude of SDA in M. salmoides was generally independent of an increase in dietary protein. This does not entirely agree with the findings for P. platessa (Jobling & Davies, 1980). These authors suggested that the overall SDA magnitude in plaice is determined both by the protein content and, to a lesser extent, the level of carbohydrate and lipid.

(b) Duration of SDA

The duration for which the post-prandial metabolic rate remains elevated above the resting (pre-feeding) rate is modified by several factors. The metabolic rate remained elevated for longer periods with increasing ration size in M. salmoides (Beamish, 1974; Tandler & Beamish, 1981), Kuhlia sandvicensis (Muir & Niimi, 1972) and P. platessa (Jobling & Davies, 1980). For a given gross energy intake the duration of elevated oxygen uptake was negatively related both to increased body weight for the bass (Tandler & Beamish, 1981) and also to increased temperature for Gadus and P. platessa (Sanders, 1963; Jobling & Davies, 1980). In relation to the present study, perhaps the most significant factor to modify SDA duration is dietary composition. While Schalles & Wissing (1976) reported that dietary composition had no effect on SDA duration, more recent evidence opposes this view. Tandler & Beamish (1979, 1981) observed that the metabolic rate remained elevated for a longer period of time following a 100% protein meal than after an isocaloric 100% carbohydrate meal. A similar response was observed for P. platessa where SDA duration increased with dietary protein level.

As discussed in the preceding section, the rates of gastric and total intestinal clearance of a meal are temperature-dependent. It is therefore to be expected that the SDA duration would itself be related to the delay before gastric emptying starts, to the rate of gastric emptying and to the rate of passage of food along the intestine. This relationship seems to hold true for the few species studied to date, although oxygen consumption may be elevated for a time slightly longer than the period when the digestion products are being absorbed (Jobling & Davies, 1980; Jobling, 1982).

(c) The overall magnitude of SDA

The magnitude of the SDA effect in P. platess was not affected by temperature (Jobling & Davies, 1980) but has been shown to increase

exponentially with temperature in the coho salmon, Oncorhynchus kitsutch (Averett, 1969) and M. salmoides (Tandler & Beamish, 1979). Several species show a linear increase in apparent SDA with meal size. This is true for the cod, Gadus morhua (Edwards et al., 1972), Cyprinus carpio (Hamada & Ida, 1973) and Oncorhynchus nerka (Brett, 1976). Tandler & Beamish (1981) also reported that the magnitude of the apparent SDA (mechanical & biochemical) was influenced by the proportion of dietary protein and carbohydrate. Apparent SDA was positively correlated with dietary protein levels in M. salmoides. The magnitude of apparent SDA was significantly higher when this species was fed isocaloric meals of 100% protein than when 75% carbohydrate and 25% protein diets were offered. Similarly, the latter diet induced a greater overall increase in oxygen consumption than did a diet consisting of carbohydrate alone. Biochemical SDA for largemouth bass fed a 100% protein meal was 7 to 10-fold higher than that measured following an isocaloric meal of 100% carbohydrate. Tandler & Beamish (1979) observed a lower apparent SDA than expected when fish were fed a diet where carbohydrate formed 25% of the caloric content of the meal. However, this 'protein-sparing' effect was not observed at carbohydrate levels above 25%. They concluded that the sparing effect may only operate at specific threshold levels of protein and carbohydrate.

The biochemical component of apparent SDA is principally due to the protein element of the diet. Non-protein nutrients also contribute to the SDA effect, but to a lesser extent (Tandler & Beamish, 1980; Jobling & Davies, 1980). The higher SDA of protein for isocaloric meals, compared to carbohydrate meals is largely due to the energy required for the oxidative deamination of amino acids. It may also be the case that a considerable proportion of apparent SDA may be due to de novo protein synthesis leading to growth (Ashworth, 1969; Tandler & Beamish, 1979).

Materials and Methods

I The respirometer

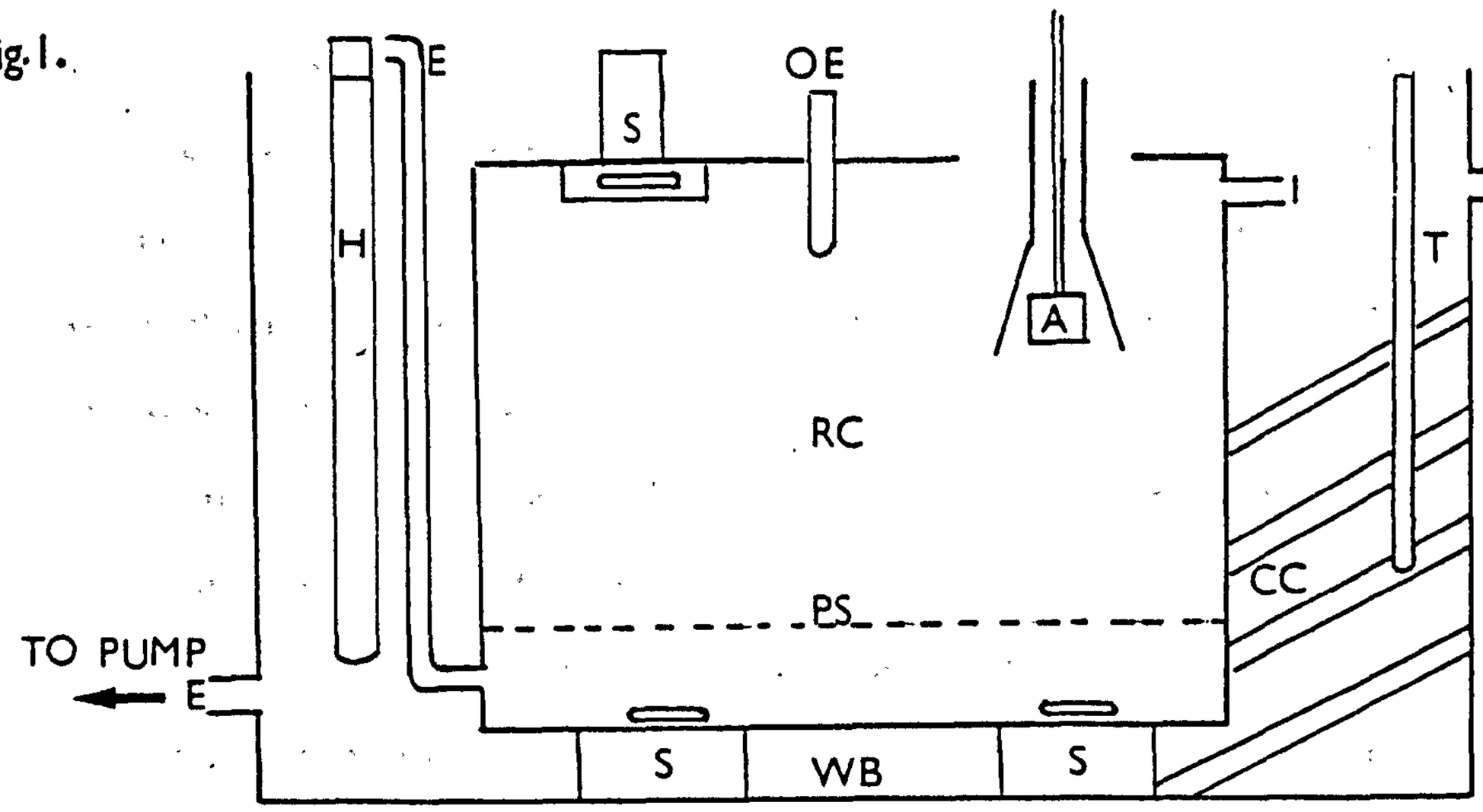
A closed chamber respirometer was used to measure the resting and post-prandial rates of oxygen consumption by Limanda (Fig. 1). The test chamber 35ℓ) was built of Perspex (30x30x39 cms) with a single lid (12.5 cm diam.) sealed by means of an 'O' ring. A water inlet valve was situated at the top of the chamber with an exit valve at the base, on the opposite side. These valves allowed a periodic flushing of the chamber without disturbing the experimental animal. The test chamber was almost completely submerged in a freshwater-bath maintained at 15.0°C. The water-bath temperature was accurately controlled using a combination of a Churchill cooler unit and a 1 kw Vitreosil heater/mercury-contact-thermostat unit (Gallenkamp). The water in the bath was continually circulated by a pump unit (Totton Electrical Products Ltd.) with a flow rate of 30ℓ per minute. The water within the test chamber was adequately mixed using magnetic followers. Two magnetic followers (air-operated) were located at the base of the unit, and a third (electrically-operated) was situated at the top of the chamber, about 8 cms. from a Radiometer E5046 oxygen electrode. Signals from the electrode were relayed through a Radiometer PHM 71 Mk II pH meter to a Smith's Servoscribe chart recorder set on the 0-10 mV range.

Immersing the electrode in a saturated sodium dithionite solution gave a baseline corresponding to 0% oxygen saturation. The 100% saturation level was obtained by immersing the electrode in the fully aerated test chamber with no fish present. The available oxygen in the chamber under these conditions was calculated from the nomogram of Green & Carritt (1967) (salinity 34%, temperature 15.0°C).

II Experimental tecnnique

Prior to use in an experiment, the fish were acclimatised in the

Fig. 1.



RC - RESPIRATORY CHAMBER

WB - WATER BATH

H - HEATER

T - THERMOSTAT

S - STIRRER

A - AERATOR

CC - COOLING COILS

E - EXIT VALVE

I - INLET VALVE

OE OXYGEN ELECTRODE

PS PLASTIC SCREEN

laboratory for four weeks, in groups of five to six individuals, at the experimental temperature (15.0°C). During this period the fish (101-200g) were fed twice-daily on both fresh whiting and Mytilus edulis tissue. When the fish were feeding normally they were weighed and placed individually in an open Perspex acclimation chamber of similar dimensions to the respirometer chamber. The individual fish remained in this chamber for seven days and were fed as before. This latter period ensured that the animals were accustomed to accepting food in isolation from other fish. On the seventh day, the fish were fed a satiation meal of M. edulis and then left unfed for 24h before transferring to the respirometer chamber and deprived of food for a further 48h. During this latter period the respiratory rate of the fish was observed to follow a gradual decline in oxygen uptake. Oxygen consumption during this period would have been elevated both by the satiation meal and probably also as a result of handling-stress. The initial 48h in the test-chamber allowed the animal to settle down and become accustomed to any minor disturbance during the re-aeration of the chamber (see below). Preliminary trial tests had shown that, after a satiation meal of M. edulis, oxygen uptake remained at the level measured 72h after a meal for at least a further five days.

When oxygen consumption was steady (≃ 72h after feeding) the fish was voluntarily fed a satiation meal of whole Mytilus edulis (collected during February). The subsequent respiratory rate was than monitored until it returned to the pre-feeding rate. At this juncture, the animal was fed a 1% body weight meal of freeze-dried whiting paste (Section I) and oxygen uptake was again recorded. This procedure was repeated at 2 and 3% body weight rations. Each of these latter ration levels was interspersed with a satiation meal of Mytilus and a rest period of 72h during which the oxygen electrode was recalibrated. This ensured that the immediate pre-feeding history of the fish was similar before each test meal was consumed. Approximately 60% of the water was also changed in between the test meals.

Occasionally, an individual fish would abruptly cease feeding and therefore had to be substituted.

Since the respirometer was of the closed type, it was necessary to reaerate the chamber at regular intervals. It was generally necessary to reaerate the seawater at 2.5-3.0h intervals. More frequent (2.0h) reaeration was required during the period when post-prandial oxygen uptake was maximal. During the periods when oxygen consumption was being monitored, the fish were not allowed to extract more than 18% of the available oxygen. This precaution should have prevented the oxygen tension itself from affecting oxygen uptake by Limanda (see Jobling, 1982). Reaeration was normally achieved within 45 minutes and was signified by a steady equilibration-line of oxygen tension.

III Observation of gastric and intestinal clearance rates

In conjunction with the above respiratory studies, individual fish were voluntarily fed either satiation meals of M. edulis or 1, 2 or 3% body weight meals of freeze-dried whiting. Three to six fish were then killed (see Section I) at specific time intervals after feeding. The gastric and intestinal contents were then extracted and treated in an identical manner to that described in Section I. However, in this instance the observed dry weights of the stomach contents were used for comparison with the SDA effect.

Results

Individual Limanda settled down quite quickly in the respirometer chamber. They remained quiescent on the plastic grid partition for long periods. This contrasts with the behaviour of '0' group Lamanda (10g) (Edwards et al. 1969). Therefore, in the present study, there was no problem of distinguishing between active and resting or post-prandial respiratory rates. The animals did not appear to be disturbed by the proximity of the magnetic

stirrers. In trial experiments, the fish normally accepted food within 24h. after which they remained largely immobile. This was particularly so under the low prevailing light intensity used during these experiments.

In the present study, the post-prandial increase in oxygen consumption represented the apparent SDA (biochemical & mechanical components, see Introduction). The peak rate of oxygen uptake, the magnitude and the duration of the SDA effect all increased as food or gross energy intake increased (Table 2). The mean (±S.D.) rate after about 72h food deprivation was 7.11 ± 0.92 ml $O_2 h^{-1}$ (n120) for fish of 120-130g body weight at 15.0°C. Oxygen consumption rose rapidly to a mean (±S.E.) maximum level of 12.2 ± 1.0, 12.7 ± 1.1 and 15.8 ± 1.2 ml $O_2 h^{-1}$ following 1, 2 and 3% body weight meals of freeze-dried whiting respectively. There then followed a more gradual decline to prefeeding levels. Maximum oxygen uptake (18.0 ± 1.7ml$O_2 h^{-1}$), after a satiation meal of Mytilus, was achieved at 14.30 ± 7.28h (mean ± S.D.) after ingestion. In this instance, four of the fish showed a second smaller peak level of oxygen uptake about 30h post ingestion (Fig. 3). The peak levels of oxygen consumption, for the fish fed the freeze-dried whiting rations, represented an increase of from 1.7 to 2.2 times the 'resting' rate. In addition, this peak rate persisted for only a short period of time (< 10h). These findings agree with those for the other teleost species (see Jobling, 1982). Compared with a 3% ration level of whiting, a meal (6% body weight) of Mytilus induced a 2.2 to 2.4 (mean ± S.D. = 2.29 ± 0.11) times increase in respiration within 4h which was maintained in excess of 24h.

The respective groups of fish fed the three ration levels of freeze-dried whiting were closely matched in size. Consequently, at each ration level, the individual fish received very similar sized meals. Despite this fact, the maximum respiratory rates and SDA magnitude varied considerably between individual fish as indicated in Table 2. At ration levels of 1 and 2% body weight of freeze-dried whiting, the SDA magnitude represented around

TABLE 2 RESPIRATION DATA* FOR LIMANDA FOLLOWING MEALS OF WHITING PASTE OR MYTILUS

Meal size (% b.w.)	Energy intake (k.cal)	Fish size (g)	Time (h) to maximum rate of O_2 uptake	Peak rate O_2 uptake as % resting level	Magnitude of apparent SDA: Post-prandial O_2 cons. above resting level (ml)	Magnitude of apparent SDA: SDA magnitude (k.cal)	Duration of SDA effect (h)	% ingested energy expended as SDA	G.E.T. (h)	Total Clearance Time (h)
(a) Freeze-dried whiting diet										
1.0	1.01 ±0.12	126.0 ±7.2 (n = 4)	5.0 ± 1.1	62.42 ± 6.60	50.0 ± 4.1	0.23 ± 0.02	25.0 ±3.2	22.77 ±1.98	15.5	20-23
2.0	1.97 ±0.07	120.0 ±6.5 (n = 5)	7.2 ± 2.2	81.38 ± 30.44	88.4 ± 46.6	0.41 ± 0.22	36.2 ±8.5	20.81 ±11.17	25.0	29-31
3.0	3.15 ±0.30	129.0 ±10.5 (n = 6)	10.3 ± 4.4	119.22 ± 25.24	186.2 ± 82.6 (range 110.6 - 301.7)	0.86 ± 0.38	58.0 ±11.5	27.30 ±12.06 (range 16.26 - 44.33)	36.0	39-41
(b) Mytilus edulis diet										
6.0	5.21 ±0.36	124.2 ±12.1 (n = 5)	(Peak 1) 14.3 ± 7.28 (Peak 2) 30.0 ± 3.5	141.40 ± 30.23	344.8 ± 42.1	1.60 ± 0.19	71.6 ±2.61	30.71 ±3.64	≅48-55	≅60-64

* Mean ± S.D. G.E.T. - gastric evacuation time.

20% of the ingested energy. Two of the six fish fed at 3% rations exhibited far higher SDA magnitudes than other members of the group. They repeatedly showed higher SDA magnitudes in excess of 280 ml O_2, when fed 3% rations. Consequently, at this ration level, 29% of the ingested energy was expended as SDA. Limanda fed to satiation on Mytilus expended 30.60 ± 3.46% of the ingested energy as the cost of apparent SDA.

Conversion of the individual SDA magnitudes, at each ration level, into energy units (assuming an oxycalorific coefficient of 4.63 k cal/ ℓO_2 or 19.377 k J/ℓO_2, (Brett & Groves, 1979) and plotting against the energy content of the food (measured by bomb calorimetry) produces a line, the slope of which corresponds to the energy cost of SDA for the whiting diet (Fig.2). The slope of the line, the SDA coefficient, may be used for comparison with other published data. In the present instance, the two high SDA values observed at the 3% ration level are excluded from the calculation of the coefficient which had a value of 0.198.

Relationship between passage of food along the intestine and specific dynamic action

Figs. 3 & 4 depict the relationship between the percentage increase of respiratory rates above resting oxygen uptake and the clearance of food from the alimentary canal. As might be expected, the greatest post-prandial oxygen uptake occurs during or just after the period when maximum amounts of food are being processed in the intestine. Respiratory rates remained well elevated after complete gastric evacuation. According to the energy intake they were still significantly higher for a variable period after total clearance of the alimentary canal (Table2). Duration of the SDA effect is therefore not exactly correlated with the period that food remains in the intestine.

Fig. 2: Effect of ingested energy upon magnitude of specific dynamic action in Limanda (120-130g).

SDA = 0.198 ingested kJ. + 0.079.

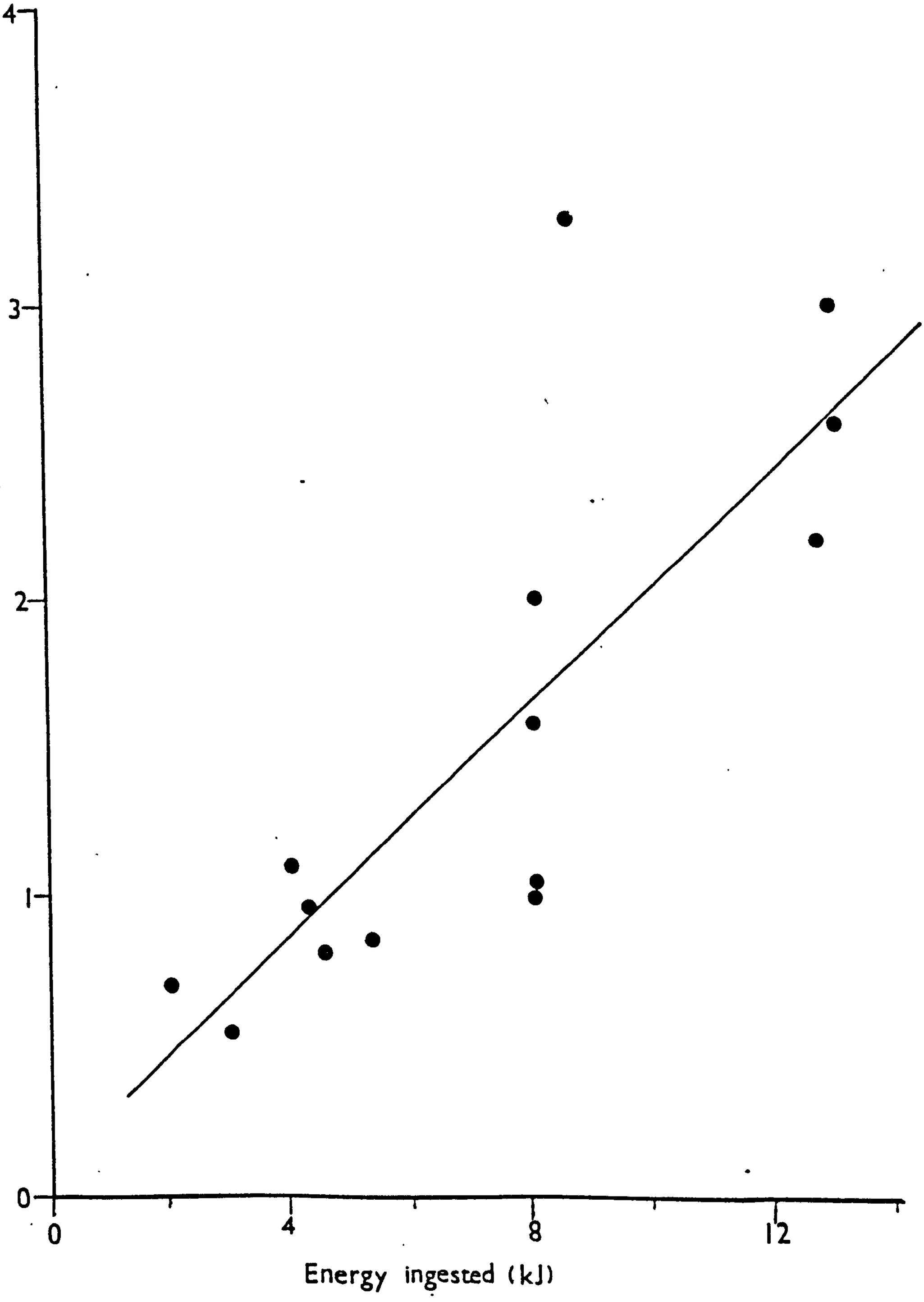

SDA magnitude (kJ)
0
1
2
3
4
0
4
8
12
Energy ingested (kJ)

Fig. 3: Relationship between post-prandial elevation of respiration rate and passage of a meal along the alimentary canal (mean±s.e.).

A-% meal evacuated from stomach

B-% meal in intestine

1% Meal

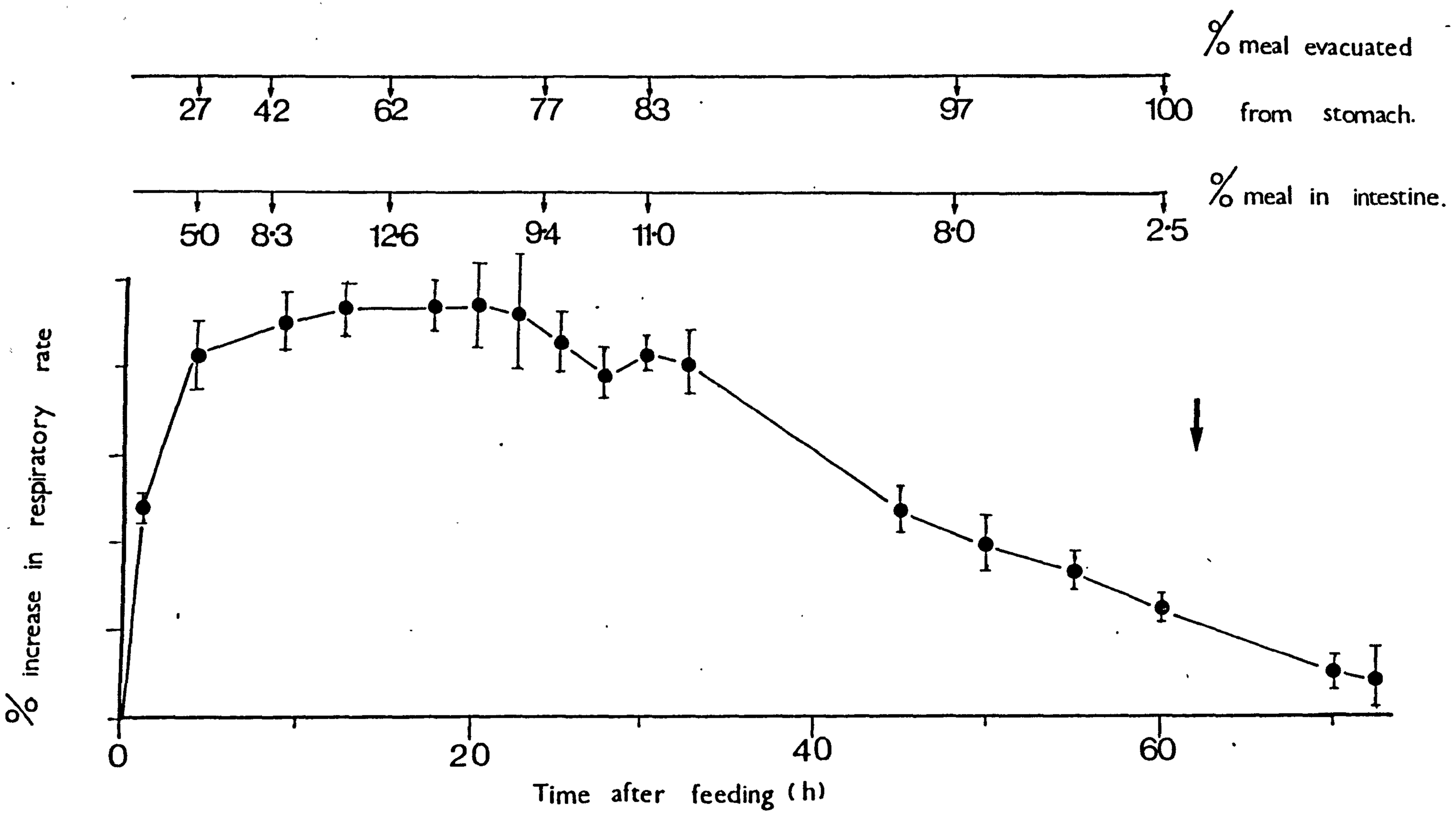
2% Meal

3% Meal

% increase in respiratory rate above resting level

Time after feeding (h)

Fig. 4: Relationship between post-prandial elevation of respiration rate and passage of a satiation meal of Mytilus along the alimentary canal, (mean±s.e.).


% meal evacuated from stomach.
27
42
62
77
83
97
100
% meal in intestine.
5·0
8·3
12·6
9·4
11·0
8·0
2·5
% increase in respiratory rate
0
20
40
60
Time after feeding (h)

Discussion

The principal purpose of the present respiratory study was to examine the pattern of oxygen uptake by Limanda in relation to food intake and passage of a meal along the intestine. The post-prandial rise in oxygen uptake can also be examined in relation to plasma metabolite levels and food intake (Chapter 4).

Beamish (1964) defined the 'resting' rate of oxygen consumption as that in the absence of spontaneous activity in the unfed state. The resting rate for Limanda (7.11 ml O_2 h^{-1}) is only slightly higher than that recorded for P. platessa individuals of a similar size (Priede & Holliday, 1980). Edwards et al. (1969) earlier recorded a resting rate of 2.08 ml O_2 h^{-1} for Limanda (10g) at 10°C. The 'resting' metabolic rates of flatfish species are generally lower than round-bodied teleosts (Wood et al., 1979; Jobling, 1982). The latter author suggested that this may be due to the smaller gill surface area of flatfish. However, it is equally likely to reflect the truely sessile nature of such species when they are not actually involved in feeding or swimming activity.

The three main elements of the post-prandial increase in oxygen uptake, i.e. the magnitude, duration and peak level of SDA, all increase with meal size as observed for other teleosts. The SDA coefficient of 0.198 compares closely with the value of 0.161 for P. platessa which was also fed on white fish-meal paste (Jobling & Davies, 1980). Values for other species range from 0.127 for Lepomis macrochirus, fed on mayfly larvae (Pierce & Wissing, 1974), to 0.22 for Cichlosoma bimaculatum fed on tubifex (Kraeger et al., 1968). Cho et al. (1975) and Jobling & Davies (1980) reported that the SDA coefficient increased in proportion to the digestible protein content of the diet. In the present study approximately 20% of the ingested energy is expended as SDA at the three ration levels of freeze-dried whiting. This value is comparable to the SDA magnitudes of

other teleosts fed high protein meals (Flowerdew & Grove, 1980). When Limanda was fed to satiation on Mytilus, it expended just over 30% of the ingested energy as SDA.

The peak post-prandial level of oxygen uptake was achieved after feeding a 3% body weight meal of fish paste. This level was approximately double the resting metabolism, and did not increase after ingestion of the Mytilus meals despite their greater energy value. This ratio of feeding to resting metabolic rate is similar to that for P. platessa (Jobling & Davies, 1980), while other teleosts exhibit a range from less than 1.0 for submaintenance rations up to 5.8 for small fish on high rations (Brett & Groves, 1979).

In agreement with the observations for P. platessa (Jobling & Davies, 1979), Limanda would appear to have an upper saturation level, in relation to peak oxygen uptake, beyond which it is unable to increase its metabolic rate in the face of an increased energy intake. In P. platessa, the level of cellular metabolism was thought to be the factor limiting the maximum level of oxygen uptake. This was supported by the report that this species can increase its respiratory rate during swimming to at least five times its resting rate (Priede & Holliday, 1980). An alternative explanation to that of Jobling & Davies (1980) may be that the supply rate of nutrients from the stomach is controlled by the energetic density of the food. The maximum level of oxygen uptake may therefore reflect the fact that the supply and absorption rate of nutrients for assimilation is limited.

While an increased energy intake above a certain level may not cause a proportionately greater peak rate of oxygen consumption, it may result in the peak rate being maintained for longer periods.

In the present study, a second peak rate of oxygen consumption was observed in some of the fish after feeding. This was particularly so after the satiation meals of Mytilus. A similar pattern was reported for C. auratus, Acheilognatus lanceolate, and Tridentiger obscurus (Hamada

& Ida, 1973) and also for Crenimugil labrosus (Flowerdew & Grove, 1980). There is no clear explanation for this 'double peak' feature of the SDA effect. The former authors reported a greater nitrogen excretion associated with the later peak which may reflect a period of maximum amino acid deamination. Certainly, the two peak levels of oxygen uptake in Limanda fed on Mytilus occur when maximum amounts of food are being processed in the intestine.

The duration of the overall SDA effect is proportional to the meal size or energy ingested. This is the standard pattern that is now emerging from several teleost studies and is clearly related to the period during which dietary nutrients are being absorbed and assimilated. It is also clear, however, that oxygen uptake remains elevated beyond the point when the entire alimentary canal has been evacuated. This period must reflect the purely biochemical, assimilatory cost of the last nutrients to be absorbed. Factors such as temperature, food energy and nutrient composition, which all modify gastric evacuation rates in fish, will obviously have considerable bearing on the duration of SDA.

In the preceding section of this chapter, it was shown that the evacuation rate of a meal was increased if it was followed shortly by anotner meal. Indeed, under multiple feeding conditions, if the two separate meals (M_1 + M_2) are considered together then the evacuation rate was faster than a single meal alone in 120g fish. This fact will obviously have repercussions for the pattern of the post-prandial SDA effect. An increased evacuation rate will clearly result in a faster delivery of energy from the stomach. While this fact is unlikely to affect the overall magnitude of the SDA effect it is feasible that both its duration and the peak rate of oxygen consumption will be modified. In addition, the SDA effect of a second meal would probably be additive and maintain oxygen uptake at a level above the resting rate. Such a situation was demonstrated in S. gairdneri where the respiratory rate remained 60% above routine rates in regularly-fed

animals (Staples & Nomura, 1976). The possible influence of the SDA effect on appetite is more fully discussed in Chapter 4.

CHAPTER 2

PLASMA METABOLITE LEVELS OF *LIMANDA limanda* IN RELATION TO SEASON AND STRESS.

Introduction

Research into the physiology and biochemistry of fish plasma has greatly increased in recent years. The various studies have included examinations of plasma enzyme activities and the concentrations of several plasma metabolites, hormones and electrolytes. The literature concerning the blood biochemistry of some teleosts is now quite extensive (see review by Hille (1982) for S. gairdneri).

1) Collection of blood samples from fish

Several methods of blood withdrawal have been used during biochemical investigations of fish plasma. The actual technique employed is of considerable importance since the results of biochemical analysis may vary accordingly (Hille, 1982). For instance, the activity of certain plasma enzymes is greater in blood samples extracted by caudal puncture than by direct cardial sampling (Gaudet et al., 1975). Other methods for the collection of fish blood samples ranged from crude collections of blood from the caudal vessels after severing the tail to a rapid puncturing of a blood vessel immediately after capture (Falkmer and Matty, 1966; Minick and Chavin, 1970). Several authors who have used this latter technique considered that a blood sample so obtained would contain normal physiological levels of the various components under study. However, such techniques may also lead to a contamination of the blood samples. In addition, the finding that plasma corticoids became elevated by stress within minutes or even seconds in some species (Pickford, 1973; Strange, 1977) does cast some doubt on the validity of this method for physiological studies. Even if a blood sample can be taken very quickly after capture, this method does have one serious drawback. Following the first sample, various blood parameters undergo significant modification due to handling stress. This means that considerable intervening periods are required between blood samples from any individual fish before the normal physiological levels of a particular

component can be measured again (Zohar, 1980). This fact has not always been taken into account. Using this technique to study the influence of factors such as season, photoperiod or feeding on plasma constituents would require consecutive samples to be taken from several different groups of fish. This approach introduces the problems of the often quite considerable individual variation encountered in some species. Ideally, for physiological studies, one should work only with fish which are in a well-defined condition. Unfortunately, this is not always feasible. For instance, the temporal patterns of various reproductive hormones in the circulation of the rainbow trout, Salmo gairdneri, are not synchronised in different fish of the same population (Billard et al., 1978; Fostier et al., 1978). In Limanda, the onset of vitellogenesis does not necessarily commence at the same time amongst all the individuals of a population (Baksh, pers. comm.). Significant changes in the level of plasma nutrients are usually associated with this reproductive process in teleosts (see later). Apart from vitellogenesis, other physiological parameters, such as the rates of food passage along the gut and even the response to stressors, do vary between individuals as described in the present study. These factors may well modify the rate at which metabolites and hormones appear in the circulation of individuals or even their final concentration.

Measurement of the normal level of a physiological parameter demands a technique which provides samples obtained from animals which are physiologically representative of that species under natural conditions. In addition, Thorpe and Ince (1976) considered that for hormonal and metabolite studies a very careful consideration of both sampling techniques, to minimise stress, and the nutritional state of the animal was necessary. Chronic cannulation techniques have, therefore, been used by several workers in order to obtain serial blood samples for hormonal or metabolite measurements in fish (Smith and Bell, 1964; Mackay and Beatty, 1968; Thorpe and Ince, 1974; Ince and Thorpe, 1974, 1975, 1977; Zohar, 1980). However,

even this method is not claimed to be wholly without fault. Aortic cannulation of rats resulted in modified patterns of prolactin and luteinising hormone during the oestrus cycle, in contrast to the situation when these components were measured following swift decapitation (Neill, 1972). This may have been due to the use of heparin in the cannulae (Zohar, 1980) which has been observed to interfere with the binding of luteinising hormone to gonadal receptors (Fox and Wisner, 1979 - cited in Zohar, 1980). In the present study, heparin was not used to reduce the occasional formation of clots due to its reported influence on plasma fatty acid (PFA) levels. Injection of heparin into humans (Newholme and Start, 1972) and rats (Dole, 1956) releases a lipoprotein lipase from the capillary walls of the adipose tissue causing subsequent hydrolysis of circulating triglycerides (TG).

2) Blood metabolites

Plasma metabolite levels are influenced by a variety of stimuli in both the higher (Table 1) and the lower (Tables 2 to 5) vertebrates. In fish, two metabolites, glucose and fatty acids, have received considerable attention, possibly because they are one of the principal forms in which carbohydrates and lipids are translocated around the body in higher vertebrates (Fridickson and Gordon, 1958). Elevations in plasma glucose have been associated with spawning, storage of energy reserves, feeding and stress. Similarly, high PFAs are generally encountered during spawning and times of energy storage, and in response to starvation in some species. Low PFA levels are normally associated with feeding or during stress.

Essentially, before the effect of any particular treatment on a haematological parameter can be investigated, it is necessary to attain measurements of its normal serum levels. The normal serum levels are generally viewed as the concentration of a particular blood component measured under conditions resembling as near as possible those of the test organism in nature. Such natural conditions are difficult to assess when working with fish. This probably explains why so few attempts have been

TABLE 1 - FACTORS MODIFYING PLASMA METABOLITIES IN HIGHER VERTEBRATES

Metabolite measured	Treatment	Change in concn.	Reference
1) Man			
FA (μeq/l)	insulin dose (0.1μ/kg)	933±272-438±135	Dole, 1956.
"	A (5mg/patient)	854±80-±742±346	"
"	oral glucose (50g/person)	850±226→240	"
"	Fasting (10-12 hr)	943±240→1236±196	"
	i.v.i.glucose (25g/person)	540-210	Crofford et al.,1964
essential amino acids (μM/100ml)	"	valine 24.2→19.2 leucine 12.6→9.4	"
glucose (mg/100ml)	i.a.i.glucose<100 mg/100g body wt.)	80→160	Trémolières, 1977
2) Rattus norvegicus			
FA (μeq/l)	% body wt. loss→ 20% loss	215→515	Walker & Remley,1970.
glucose (mM)	Fasting - 2 days	7.1±0.3→5.8±0.4	Freminet & Leclerc, 19 .
FA μm/l	Fasting - 7 days	336±25→513±19	Yaffe et al., 1980.

i.v.i. - intravenous injection. i.a.i. - intra-arterial injection.

F.A. - fatty acids. A - adrenaline.

made to study plasma metabolite changes in relation to different nutritional states in teleosts. It is evident that teleosts exhibit much interspecific variation in their normal plasma glucose and PFA levels. However, it is likely that some of this variation stems from the failure of many workers to consider the influence of a variety of other factors, apart from the nutritional state, on normal metabolite levels. Larsson and Fänge (1977) in a survey of the plasma cholesterol and PFA of seventeen teleost species observed a remarkable species range in the levels of both these metabolites. They concluded that the level of PFA in different species was a reflection of both the activity and the storage site (muscle or liver) of lipid reserves. However, these conclusions were based upon metabolite measurements of fish sampled immediately after capture. Obviously, such a technique makes no allowances for the influences of stress or of nutritional state. In addition, they did not pay sufficient regard to the sex of the fish sampled. In view of the considerable influence of the reproductive season alone on plasma lipids (Shatunovskiy, 1971; Petersen and Emmerssen, 1977) it is necessary to describe fully the condition of the individual when reporting plasma lipid parameters. Much the same arguments apply for many of the plasma glucose studies. It is, therefore, apparent that there are a number of important factors which must be considered before the influence of the nutritional state on plasma metabolites can be evaluated (see review by Hille, 1982).

I Seasonal variation

Several teleosts have been found to undergo seasonal changes in plasma metabolite levels (Tables 2 & 3), plasma hormonal levels (Wingfield and Grimm, 1977) and even in various cellular components of the blood (Bridges et al., 1976). The factors instrumental in controlling these fluctuations are not entirely understood, nor are they necessarily the same in all species (Mackay and Beatty, 1968).

TABLE 2 - EFFECT OF SEASON ON PLASMA GLUCOSE IN FISH

Species	Season	Change in concn. (mg/100ml)	Reference
Catostomus commersonii	♀ No change	-	Mackay & Beatty, 1968
	♂ May/June	153±50	
	October	91±10	
Opsanus tau	June-August	42±6-20±5	Tashima & Cahill, 1968.
Esox lucius	December/January	46.9±1.8	Thorpe & Ince, 1974.
	February/March	109.0±5.5	
	April	68.9±1.8	
	May/June	53.3±2.2	
Clarias batrachus	January	72.8±8.9	Tandon & Joshi, 1974.
	May	60.9±11.0	
	July	68.0±13.5	
Platichtys flesus	December	* 25	Petersen & Emerssen, 1977.
	January	30	
	February	60	
	March	45	
Carassius auratus	July-August	*38-30	Delahunty et al., 1978.
Carassius auratus	October-March	41±3-82±10	Prack et al., 1980.
Spicara chryselis	February	* 50	Fernandez & Planas, 1980.
	March	100	
	April	140	
	August/September	50	

*Data taken from figs. in respective reference.

TABLE 3 - EFFECT OF SEASON ON PLASMA LIPID FRACTIONS IN FISH

Species	Season	Lipid fraction and change in concentration		Reference
		*(cholesterol)		
Salmo trutta ♀	October	359	(e)	McCartney, 1967.
	January	860		
		*(phospholipid)		
"	September	68.2	(e)	
	May	30.4		
Gadus morhua callarius(L)	maturation of gonad from early stage IV to late stage IV	6% total lipid as FFA rising to 12%, 21% total lipid as TG rising to 32%		Shatunovskiy, 1971.
Zoarces viviparus	May	(FA) 613±84	(a)	Pekkarinen & Kristoffersson, 1975.
	June	952±111		
	September	316±48		
	December	890±222		
Platichthys flesus	August	*(lipid) 1380	(e)	Petersen & Emerssen, 1977.
	November	890		
	February	1480		
	March	950		
Spicara chryselis	March	*(FA) 120	(a)	Fernandez & Planas, 1980.
	April	260		
	September	60		
	October	175		
Oncorhynchus kisutch	May	(FA) 422±55.2	(a)	Leatherland & Sonstegard, 1980.
	August	1342.2±40.2		
	September	436±32.1		

(e) mg/100ml. (a) µeq/l. FA - fatty acids.

*Data taken from figs. in respective reference.

a) Plasma metabolites

Seasonal changes in plasma glucose, total lipid and PFA have all been associated with energy storage periods and reproductive processes in teleosts (Fernandez and Planas, 1977; Petersen and Emmerssen, 1977). Some species like the mummichog, Fundulus heteroclitus, are thought to become hyperglycaemic in response to low seasonal temperatures (Umminger, 1971). Other authors have attributed this physiological response to a combination of both reproductive demands and the direct or indirect effects of temperature (Nace et al., 1964; Tandon and Joshi, 1974). Mackay and Beatty (1968) observed no correlation between seasonal temperature and variations in blood glucose of the white sucker, Catostomus commersonii, and interpreted the changes in blood glucose as being related to reproduction. Hyperclycaemic states during the reproductive season have been observed only in the males of C. commersonii (Mackay and Beatty, 1968) and Spicara chryselis (Fernandez and Planas, 1977) and these levels declined gradually after breeding. In other species both sexes show elevated blood sugar levels in association with gonadal growth (Table 1). There is little information concerning the significance of spawning-related hyperglycaemia. Leach and Taylor (1977) considered that the maximum rates of standard metabolism observed in some species during spawning may require a mobilization of energy reserves to cope with the increased demand for energy substrates. Alternatively, the glucose may be used for incorporation as glycogen in the maturing egg follicles as suggested for Clarias lazera (Yanni, 1961) or fat deposition in the testes of C. commersonii (Mackay and Beatty, 1968). In the latter species, the post-spawning glucose peak gradually declines during the summer months when there is a very rapid build-up of fat deposits in the testes. This may explain the summer decline in plasma glucose by an increased turnover rate of glucose. The increases in plasma glucose observed in some species have been associated with a simultaneous decrease in liver glycogen (Tandon and Joshi, 1974; Fernandez

and Planas, 1977; Srikar et al., 1979). The liver glycogen was consequently considered to be the source of the elevated plasma glucose. However, Petersen and Emmerssen (1977) pointed out that there is no such simple correlation between the glycogen concentration in the liver and glucose levels in the blood. The concentration of liver glycogen is dependent upon the activity of the phosphorylase and glycogen synthetase enzyme systems, while blood glucose levels are influenced by the rate of demand from the tissues, supply from diet, glycogenolysis or gluconeogenesis (Petersen and Emmerssen, 1977).

Seasonal plasma lipid patterns have not been investigated quite so thoroughly. Where such a variation was observed it was interpreted in terms of mobilisation of energy reserves in connection with gonadal growth or replenishment of nutrient reserves following spawning (Plack and Woodhead, 1966; Takashima et al., 1972; Pekkarinen and Kristoffersson, 1975; Petersen and Emmerssen, 1977; Fernandez and Planas, 1977). The exact role of PFA in teleost gonadal recrudescence is unclear (Wiegand and Peter, 1980a). They have been shown to act as precursors of other plasma lipids such as triglycerol (TG) and phospholipids (Robinson and Mead, 1973) both of which are actively involved in gonadal growth (Shatunskiy, 1971; Wiegand and Peter, 1980 b,c).

It was generally believed that the main source of these increased supplies of metabolites for reproduction depended on the category of 'lean' or 'fat' fish to which a particular species belonged (Pollard, 1972). The 'lean' fish are characterised by having far less muscle fat in contrast to the 'fat' fish (Shchepkin, 1971). Consequently, in the so-called 'lean' species, lipid metabolism is principally confined to the liver which undergoes seasonal variations in weight (Pollard, 1972). This possibly implies that during the reproductive season, one of the most likely sources for the increased energy demand would be the liver. Certainly, this would appear to be the situation in the eel-pout, Zoarces vivparus (Pekkarinen and Kristoffersson, 1975) and Gadus (Plack and Woodhead, 1966; Shatunovskiy,

1971) where towards the end of oogenesis 25% of the lipid expended by the liver is concentrated in the gonads. During this period plasma lipids of the female remain at significantly higher levels than those found in male fish. This is thought to be achieved through hormone action since the fish are not feeding at this time (Plack and Woodhead, 1966). Shatunovskiy (1971) observed that during the resting period in Gadus, the principal fractions of reserve lipids which accumulated in the liver were TG followed by FA. In the initial stages of vitellogenesis he found that the TG were expended at far higher rates than any of the other lipid fractions. A similar situation was observed in the scorpion fish, Scorpaena porcus (L.) (Shchepkin, 1977). These seasonal plasma metabolite variations are undoubtedly hormone-mediated. A complete review of seasonal changes in hormonal levels will not be attempted apart from instances where specific hormone/metabolite interactions have been reported. A thorough review of teleost endocrine rhythms is given by Simpson (1978).

b) Plasma hormones

A seasonal change in the activity of interrenal tissue and in the levels of plasma corticosteroids have been reported for many species of fish (Chavin, 1965; Johnson, 1972; Fuller et al., 1974; Woodhead, 1975; Hunt et al., 1982). During the 'resting' summer period, the pleuronectids have low levels of cortisol, testerosterone and estradiol. The levels of these hormones rise with gonadal recrudescence (Wingfield and Grimm, 1977). In P. platessa plasma estradiol levels begin to decrease just before the peak spawning season in contrast to cortisol and testerosterone which reaches a maximum concentration at the onset of the peak spawning period. An increased interrenal activity has been associated with a spawning-related hyperglycaemia and marked changes in cortisol levels in some salmonids. This condition was thought to reflect a mobilisation of energy reserves in the fasting fish as they migrated to the spawning grounds

(Robertson et al., 1961). However, the appearance of seasonal cortisol peaks in immature specimens of P. platessa indicates that cortisol levels may be independent of the gonadal cycle (Wingfield and Grimm, 1977; Billard et al., 1978). Some authors have suggested that the above situation may suggest a purely 'permissive' or 'supporting' role of cortisol in gonadogenesis (Leach and Taylor, 1977; Simpson, 1978).

The artificial administration of sex steroids has led to a clearer understanding of the manner in which plasma metabolites are regulated during the reproductive season. Injections of cortisol have been shown to enhance the breakdown of TG and stimulate the metabolism of FA in Anguilla species (Butler, 1973; Dave et al., 1978). Estradiol-17-β caused increases of vitellogenin, total plasma lipid, phospholipid, TG and PFA in a dose-related manner when injected into P. flesus. Plasma glucose levels were also affected although the direction of change was dependent upon the hormone dose (Petersen and Korsgaard, 1978). Oestrone and estradiol-17-β are shown to be active in mobilising lipid reserves and testerosterone injections similarly raised PFA levels in Carassius (Wiegand and Peter, 1980 a,c). In addition, gonadotrophins, presumably via sex steroids, were shown to cause a net mobilisation of lipid in fish with small ovaries and accelerated ovarian uptake of lipid in fish with large ovaries (Wiegand and Peter, 1980 b).

Finally, the mechanism by which sex steroids may raise PFA both in teleosts and in the higher vertebrates remains unclear, although studies have shown that the hypothalamus does influence fat mobilisation in mammals (Bray and Nishizawa, 1978). A number of endocrine glands under pituitary control have been observed to enhance lipolysis (Kastin et al., 1975). In the higher vertebrates it was argued that sex steroids could act directly on the hypothalamus to modify the secretion of pituitary hormones (Holm, 1967). In the case of teleosts it is true that oestrogen target cells have been located in the forebrain and have been shown to take up sex steroids (Kim et al., 1978; Morell and Pfaff, 1978). However, a great

deal more research will be required before a clear understanding of the hormonal control of plasma metabolites during reproduction is attained.

II Photoperiod

The adenohypophysis of the pituitary gland produces a number of hormones, including the gonadotrophins, thyrotrophic hormone, adreno-corticotrophic hormone, growth hormone and prolactin. The production site of most of these hormones in the teleosts has been identified (Matty, 1978) and it has been shown that there is a diurnal variation in the secretory activity of many of the pituitary cells (Ball and Baker, 1969). Since some of these hormones have been demonstrated to modify plasma metabolite levels, a knowledge of their activity is desirable in any metabolite study. While it may not always be feasible to monitor hormonal rhythms for any particular species, circadian fluctuations of a plasma metabolite may signify such a rhythm. Leatherland et al., (1974) discovered a circadian rhythm in the plasma growth hormone and prolactin of juvenile salmon, Oncorhynchus nerka, which they correlated with diurnal variations in PFA. The peak levels of the hormones and PFA occurred during the dark and light phase respectively. Serum prolactin varied in a circadian manner in Carassius and the rhythm was modified depending on the photoperiod length (McKeown and Peter, 1976). Injections of prolactin into some cyprinodont species caused fat deposition or mobilisation according to the time of injection relative to the onset of light(deVlaming and Sage, 1972). In the golden top minnow, Fundulus chrysotus, the response to prolactin could be entrained by daily injections of cortisol, with either fat deposition or mobilisation occurring according to the time interval between the two injections (Meier, 1972). The behaviour of plasma metabolites in direct relation to cortisol levels was mentioned earlier, but it would now appear that the picture is further complicated by a possible interaction between this hormone and prolactin. In addition Simpson (1978) and Baker and Rance (1980) demonstrated significant diurnal variations of plasma cortisol in S. gairdneri.

Thyroxine has been shown to phase the fattening response to prolactin in F. chrysotus (Meier, 1970). Furthermore, some studies have demonstrated diurnal changes in thyroid activity. Osborn et al., (1977) measured diurnal variations in thyroxine levels in rainbow trout blood and found that the time of maximum concentration altered according to the season. A seasonal variation in the fattening of fish, under the control of prolactin, would require that both hormones show a diurnal rhythm and that at least one should show a seasonal change in its timing (Simpson, 1978). These complex interactions of prolactin with other hormones may, therefore, suggest that diurnal variation of a plasma metabolite encountered during one season may not necessarily persist throughout the year. This may explain some of the contrasting results that have been obtained by different authors when working with the same species. Apart from the higher vertebrates (Pauly and Schering, 1967) circadian rhythms in levels of plasma lipid fractions and glucose have been observed in several teleosts (Leatherland et al., 1974; Sharpiro and Hoffman, 1975; Delahunty et al., 1978 a,b; Carillo et al., 1980). Carassius showed a diurnal variation in both plasma glucose and total lipid when held on a 16L:8D regime. In both instances, the actual point of the photoperiod at which the animals were fed was found to be implicit in deciding both the timing and magnitude of respective metabolite levels (Delahunty et al., 1978a). The latter authors also demonstrated, by surgical ablation of the pineal organ, that during specific seasons this organ participated in regulating levels and daily fluctuations of some metabolites (Delahunty et al., 1978 b). In contrast to their findings, other workers could detect no diurnal variations in the plasma metabolites of this species (Chavin & Young, 1970; Minick and Chavin, 1972), whilst Nace et al. (1964) only observed a daily glucose rhythm during reproductive periods. Carassius also exhibited circadian rhythms in the majority of its plasma amino acids, some of which were clearly associated with the photoperiod (Carilla et al., 1980).

There is conflicting evidence concerning the mobilizing effect of exogenous growth hormone (GH) on lipid reserves in fish. GH had a lipolytic action in C. auratus and Salmo gairdneri (Minick and Chavin, 1970; Leatherland and Nuti, 1981) but was without effect on PFA in Oncorhynchus nerka (McKeown et al., 1975). Leatherland et al., (1974) reported an inverse relationship between PFA and endogenous GH in O. nerka. In S. gairdneri the rise in PFA was correlated with a decrease in hepatic lipid reserves only (Leatherland and Nuti, 1981). There is some evidence supporting a GH/insulin interaction (Donaldson et al, 1979) which may further complicate the situation.

In conclusion, it appears that the seasonal mobilization or deposition of energy reserves depends not only on the action of individual hormones, but also on their synergistic cooperation. Environmental influences would ultimately control the synergism by bringing the diurnal peaks of various hormones into or out of phase with one another according to the season. Alternatively, such factors may also control the seasonal increases in the plasma levels of such hormones (Baker and Wigham, 1979).

III Feeding History

Long-term maintenance on diets with either an excess or deficiency of a particular nutrient can apparently lead to changes in the blood chemistry and even modify certain biochemical pathways involved in general metabolite homeostasis. John et al., (1979) maintained S. gairdneri for 53 weeks on diets supplemented with varying levels of ascorbate (160-1280mg/kg feed). Up to a certain level (640mg/kg feed) of ascorbate they found that the mean level of PFA rose significantly higher than that in fish fed on ascorbate-free diets. An even more subtle phenomenon was revealed by Yone (1976) and Shimeno et al., (1978) in the red sea bream, Chyrsophrys major, and the yellow tail, Seriola dumerillii, respectively. They discovered that fish fed during a 30-day pre-experimental period with diets containing low carbohydrate levels (10-20%) demonstrated an enhanced tolerance to high glucose loads than fish fed beforehand on either

carbohydrate-free or carbohydrate rich (40%) diets. It is also apparent that even short periods of starvation may have a pronounced effect on plasma metabolites in several teleosts. For instance, a 5-day starvation period resulted in a significant elevation of PFA levels in S. gairdneri (Bilinski and Gardener, 1968).

In view of the above evidence, it is important that experimental fish for metabolite studies are acclimatised on diets which resemble both in quality and quantity the food which they would normally ingest in the wild.

IV Temperature

Both high and low glycaemic and lipaemic states have been associated with low or high acclimation temperatures respectively (Table 4). A low temperature-induced hyperglycaemic condition has been observed in the toadfish, Opsanus tau (Nace and Schuh, 1961), Fundulus heteroclitus (Leach and Taylor, 1977), Clarias batrachus (Tandon and Joshi, 1974) and other teleost species (Dean and Goodnight, 1964; Umminger, 1970, 1971). Umminger (1969, 1970, 1971) undertook a detailed study of low-temperature adaptation in F. heteroclitus where, at sub-zero temperatures, blood glucose was elevated to 350mg/100ml. Fish acclimated to fresh water at 0.1^{o}C portrayed an even greater hyperglycaemia up to 1066mg/100ml. The glycaemia developed as a result of an increased rate of liver glycogenolysis. Umminger (1971) observed that freshwater-adapted fish suffered a partial osmoregulatory failure at low temperatures. This resulted in a net loss of serum electrolytes to the medium which was partly compensated for by the elevated serum glucose. At sub-zero temperatures Umminger (1969, 1970) concluded that saltwater-adapted fish had elevated blood sugar to reduce the possibility of ice-crystal formation. However, Leach and Taylor (1977) working with the same species, but under natural field conditions, proposed that the hyperglycaemic state provided a means of regulating metabolic processes at low temperatures, presumably by first ensuring a readily available energy

TABLE 4 - EFFECT OF ACCLIMATION TEMPERATURE ON PLASMA GLUCOSE AND FATTY ACIDS

Species	Acclimation Temp. °C	Metabolite Concentration	Reference
(a) Plasma glucose (mg/100ml)			
Lepomis macrochirus	20 - 5	88.32 - 50.19	Dean & Goodnight, 1964.
Ictalurus melas	- exercise 20	47.87	"
	5	61.68	
	+ exercise 20	67.86	
	5	82.17	
Micropterus salmoides	- exercise 20	43.95	"
	5	44.92	
	+ exercise 20	81.84	
	5	75.25	
Pomoxis annularis	- exercise 20	91.40	"
	5	179.62	
	+ exercise 20	121.09	
	5	192.00	
Cyprinus carpio	8 - 22	100	Motelica, 1965.
	3.9	244	
Anguilla rostrata (C)	5	107.7	Mayerle & Butler, 1978.
	15	50.3	
Salmo gairdneri	8	75.5±4.6	Connors et al., 1978.
	12	66.7±1.6	
	16	59.7±1.9	
Cyprinus carpio	15	103.63±7.30	Smit et al., 1981.
	20	61.81±4.45	
	25	58.18±3.64	
Salmo gairdneri	15	53.33±8.16	"
	20	33.84±5.22	
	25	43.33±3.12	
Sarotherodon mossambicus	15	48.33±1.67	"
	20	64.99±6.12	
	25	46.66±5.65	
(b) Plasma FA (µeq/l)			
Carassius auratus	12	290	Mazeaud, 1973.
	27	160	

- without exercise. + with exercise.

supply. Connors et al., (1978) believed that elevated glucose levels at low temperatures were a consequence of retarded growth and a possible adjustment to anaerobic metabolism. However, this contention was not upheld by the blood lactate levels of low temperature-acclimated fish (Smit et al., 1981).

Low glucose levels are usually associated with high temperatures and were thought to reflect the greater metabolic rate under such circumstances (Mayerle and Butler, 1971). It is clear that whatever the adaptive significance of elevated or depressed serum glucose, different species respond in a variety of ways to the same acclimation temperature. At low temperatures, Micropterus salmoides maintains normoglycaemia whereas Salmo gairdneri, Ictalurus melas, Pomoxis annularis and Cyprinus carpio become hyperglycaemic, and Lepomis macrochirus becomes hypoglycaemic. S. gairdneri becomes increasingly hypoglycaemic with rising temperature but only up to a certain point. At around 20°C this species shows a rise in blood sugar (Connors et al, 1978; Smit et al, 1981) which may be associated with a developing asphyxia due to a lowering of the oxygen content.

V Stress

The final and possibly greatest problem to consider when investigating blood metabolites is their high susceptibility to stress-induced changes (Table 5). Stress may be defined as:- constraining influences which change the existing physiological equilibrium. The initial response of fish to stress is basically similar to that found in higher vertebrates in that circulating concentrations of catecholamines and glucocorticoids increase in level (Leloup-Hatey, 1958; Nakano and Tomlinson, 1967). The possible implications of these hormonal increases for plasma metabolites may be inferred from direct research into the effects of artificially-induced stress. Alternatively, a more controlled procedure involves the infusion of fish with those hormones whose endogenous levels are normally found to fluctuate

TABLE 5 - EFFECT OF STRESS ON PLASMA GLUCOSE IN FISH

Species	Type of stress	*% elevation	Reference
Cyprinus carpio	2 hr hypoxia	216	Mazeaud, 1969.
Carassius auratus	Aquarium transfer	88	Minick & Chavin, 1970.
Salmo gairdneri	Struggling & hypoxia	100	Mazeaud, 1973.
Heteropneustes fossilis	Capture and transport	190	Tandon & Joshi, 1973.
Pseudopleuronectes americanus	" "	174	Fletcher, 1975.
Pseudopleuronectes americanus	Inversion in shallow water	37	"
Lapeo capensis	Capture and transport	168	Hattingh, 1977.
Perca fluvens	" "	217	Haux et al., 1981.
Salmo trutta	Acute handling (2 mins)	27	Pickering et al., 1982.

* Calculated from published data in respective references.

following an applied stress. In this manner the behaviour of individual blood parameters may be evaluated in relation to a particular catecholamine or corticosteroid.

The rise in concentration of certain hormones following stress has been termed by Mazeaud et al., (1977) as the 'primary' response to stress. Stress also has a significant impact on several other blood parameters including various metabolites, e.g. glucose, and cellular components (Minick and Chavin, 1972; Fletcher, 1975; Pickering et al., 1982). It may also cause severe osmoregulatory disturbances (Maetz, 1974). This author suggested that alterations in circulating adrenaline are probably responsible for the disruption of water balance in fish resulting in weight variations. These latter disturbances were termed the secondary response to stress (Mazeaud et al., 1977). Wedemeyer and McLeay (1981) extended the system of Mazeaud et al., (1977) by a tertiary category which includes many behavioural modifications such as greater susceptibility to disease and decreased growth rates.

The development of a hyperglycaemic condition is one of the classic symptoms following stress. Serum glucose levels are so sensitive to stress that Sibergeld (1974) suggested their use as indicators of environmental pollution. Artificially-applied stresses including capture and transportation, sham injections, handling, hypoxia and increases in ammonium ion concentrations have all resulted in hyperglycaemia (Table 5).

PFAs are also modified by stress but, unlike the immediate glucose response, there is a considerable interspecific variation in the stress response pattern. Hypoxic stress in Salmo gairdneri caused a concomitant increase in plasma glucose and PFA concentrations (Mazeaud, 1973). In contrast, hypoxia in Cyprinus carpio was followed by hyperglycaemia, as in S. gairdneri, but a pronounced decline in PFA (Mazeaud, 1969). The reasons for this interspecies variation to stress is not fully understood. The evidence to date suggests that most species show the same qualitative

response to stress in that they exhibit increases in catecholamines and corticosteroids (Mazeaud et al., 1977). However, the similarity ends here and, interestingly, there also appears to be a considerable variation in the response of different species to exogenous doses of these hormones.

The plasma metabolite patterns normally associated with stress may be duplicated in some species by injections of adrenaline (A) or noradrenaline (NA) (Mazeaud and Mazeaud, 1965). Consequently, the underlying mechanisms governing the stress response of metabolites can be investigated. The catecholamines are particularly efficient in inducing hyperglycaemia (Mazeaud, 1965; Young and Chavin, 1965) and have been shown to cause a rapid depletion of liver and muscle glycogen (Nakano and Tomlinson, 1967). In the majority of species, administration of exogenous A or NA results in a pronounced rise in blood glucose (Table 6). In contrast, these hormones have divergent effectsupon PFA (Table 7). Catecholamines may either increase (Leibson et al., 1968; Mazeaud, 1969; Larsson, 1973) or depress PFA levels (Farkas, 1969; Ince and Thorpe, 1975). The depression of PFA observed in some species is normally accompanied by an elevation in plasma glucose. In such instances it has been suggested that an enhanced insulin secretion due to the elevated glucose levels may explain the PFA depression (Minick and Chavin, 1973). However, catecholamine administration to higher vertebrates causes a concurrent hyperglycaemia and hyperlipaemia. Farkas (1969) attributed the antilipolytic effect of catecholamines in some species (e.g. Cyprinus carpio) to an essential difference between the biochemical mechanisms governing lipid metabolism in the tissues of lower and higher vertebrates. It is likely that the interspecific variation in the location of lipid reserves may account for some of the observed variations. In addition the physiological condition of a fish may determine its response to catecholamine infusion (Plisetskaya, 1980). An elevation or depression of PFA following catecholamine infusion may be determined by the initial level of the metabolite in the circulation (Plisetskaya, 1980). In birds, PFAs were either elevated or lowered, in response to catecholamine infusions,

TABLE 6 - EFFECT OF CATECHOLAMINE INJECTIONS ON PLASMA GLUCOSE IN FISH

Species	Treatment	*Change in glucose concn. (e)	Reference
Cyprinus carpio	i.p.i. A (100µg/kg)	40 - 115	Mazeaud, 1964.
	i.p.i. NA (1mg/kg)	75 - 200	Mazeaud, 1965.
	i.m.i. A (2mg/0.45kg)	75.3 - 126.2	Farkas, 1967.
	i.p.i. NA (2mg/0.45kg)	75.3 - 132.9	"
Squalus acanthius	i.m.i. A (0.5mg/kg)	58 - 73	Patent, 1970.
	i.m.i. NA (0.5mg/kg)	No effect	"
Hydrolagus colliei	i.m.i. A (0.5mg/kg)	53.6±6.7 - 72.6±8.1	"
	i.m.i. NA (0.5mg/kg)	74.0±10.3→ 47.5±4.8	"
Anguilla anguilla	i.p.i. A (0.5mg/kg)	50 - 140	Larsson, 1973.
	i.p.i. A (5.0mg/kg)	30 - 200	"
	i.p.i. NA (5.0mg/kg)	47.1 - 82.1	"
Esox lucius	i.a.i. A (0.05mg/kg)	60 - 140	Thorpe & Ince, 1974
	i.a.i. NA (1.0mg/kg)	50 - 160	"
Anguilla anguilla	i.a.i. A (25µg/kg)	50 - 160	Ince & Thorpe, 1977
	i.a.i. NA (50µg/kg)	50 - 140	"

i.p.i. - intraperitoneal injection; i.m.i. - intramuscular injection;
i.a.i. - intra-arterial injection; (e) mg/100ml).
*Data taken from figs. in respective references.

TABLE 7 - EFFECT OF CATECHOLAMINE INJECTIONS ON PLASMA FATTY ACIDS IN FISH

Species	Treatment	Change in concn.		Reference
Cyprinus carpio	i.p.i. A (100µg/kg)	0.82 - 0.51	(f)	Mazeaud, 1965.
	i.m.i. A (2mg/kg)	No change		Farkas, 1967.
	i.p.i. NA (2mg/kg)	2.196 - 0.976	(f)	"
Scorpaena porcus (L)	i.p.i. A	Increase		Leibson et al., 1968.
Carassius auratus	i.m.i. NA (100µg/kg)	576±41-305±39	(a)	Minick & Chavin,
	i.m.i. A (10µg/kg)	605±41-329±67	(a)	1973. "
Anguilla anguilla	i.p.i. A (5.0mg/kg)	400 - >700	(a)	Larsson, 1973.
	i.p.i. NA (5.0mg/kg)	No change		"
Esox lucius	i.a.i. A (0.05mg/kg)	266±17-177±11	(a)	Ince & Thorpe, 1975.
	i.a.i. NA (1mg/kg)	261±16-328±11 -155	(a)	"

(f) µm/ml; (a) µeq/l; i.p.i. - intraperitoneal injection; i.m.i. - intramuscular injection; i.a.i. - intra-arterial injection; A - adrenaline; NA - noradrenaline.

depending on whether their initial levels were low or high respectively (Mazina et al., 1970 - cited in Plisetskaya, 1980).

Less is known about the direct effects of stress on corticosteroid levels, although appreciable rises in cortisol (e.g. from 2.0 to 150.0ng/ml plasma in S. gairdneri) have been observed (Mazeaud et al., 1977; Strange et al., 1977; Strange and Schreck, 1978; Barton et al., 1980; Barton and Peter, 1982). This response has been associated with high mortalities in salmonids (Barton et al., 1980). Reduction in plasma thyroxine in S. gairdneri and P. platessa may have been due to an inhibition of the release of thyroid stimulating hormone due to stress-induced rises in corticosteroid levels (Osborn and Simpson, 1972, 1974). Again, it is likely that changes in these hormones will modify plasma metabolite levels. Exogenous thyroxine elevated PFA from 250 to 400 µeq/l in Cyprinus carpio (Murat and Serfaty, 1970). Similarly, cortisol injections cause both alterations in the composition of PFA and in the total level of plasma TG in A. anguilla (Dave et al., 1979) while administration to S. acanthias resulted in hyperglycaemia (Patent, 1970).

The timing and magnitude of the stress responses in teleosts is dependent upon the severity, duration and type of stress (Mazeaud, 1977; Barton et al., 1981). In S. gairdneri, barely detectable levels of cortisol (< 2ng/ml plasma) were detected in non-stressed fish whereas levels rose to 40-50 ng/ml and 150-200 ng/ml following mild and severe disturbances respectively (Barton et al., 1980). In addition, the relative increases in plasma cortisol and glucose following identical stress may be modified by the acclimation temperature (Strange, 1980). The magnitude of the hyperglycaemic response in Carassius was determined by the time of exposure to air (Chavin and Young, 1970). A single saline injection into this species caused a decline in PFA (Minick and Chavin, 1972) whereas single injections for several days had the opposite effect (Wiegand and Peter, 1980). However, such divergent responses may be due to variations in the actual timing of

of the treatment. Fundulus chrysotus was found to either lose, gain or maintain weight according to the time of day that it suffered repeated handling. This suggested that stress has different physiological consequences for the animal depending on the time of day that the event occurs (Meier et al., 1973). This has been recently supported by Sokolowska and Bieniarz (1981) who found that the impact of an electrical stress on the circadian rhythm of hypothalamic catecholamine content in A. anguilla was dependent upon the timing of the applied stress. It appeared that there was a diverse intensity of the response to the same stress agent depending on whether it was applied during the high or low locomotor activity period of the animals.

A further complication arises from the apparent intraspecific variation in the physiological response of individuals to the same stressor. Mazeaud et al., (1977) observed a range in blood adrenaline from 4.20 to 21.60 µg/ml plasma in equally stressed individuals of the coho salmon, Oncorhychus kisutch. Similarly, Nakano and Tomlinson (1967) discovered that S. gairdneri with low pre-test liver glycogen concentrations showed only slight rises in plasma glucose following stress. These fish recovered gradually and glucose levels were normal within 30-50 hr. Fish with high pre-test liver glycogen content developed appreciably greater glycaemic levels which remained significantly above resting levels for the first 48 hr of the recovery period.

Finally, there is one other situation relating to the stress response of fish which is particularly relevant in metabolite studies. This concerns the interaction of catecholamines with other hormones. Catecholamine infusion into higher vertebrates suppresses insulin secretion (Edgar et al., 1969). Consequently, plasma insulin levels decrease. Some time after the infusion, insulin levels normally exhibit a rise in concentration well above the normal resting level (Porte and Williams, 1966). This pattern has also been demonstrated in A. anguilla where 30 minutes after injections of A or NA plasma insulin levels fell to about half the resting value. However, at

180 minutes post-injection, plasma insulin was approximately three times the pre-injection level (Ince and Thorpe, 1977). Following catecholamine injections, E. lucius (Thorpe and Ince, 1974) and H. colliei (Patent, 1970) developed significant hypoglycaemic conditions after the normal rise in glucose levels. Ince and Thorpe (1977) considered that the biphasic response of insulin to increased plasma catecholamines might possibly explain the delayed hypoglycaemia found in these species. A similar biphasic insulin response was also observed in the lamprey, Lampetra fluviatilis, and scorpion fish, Scorpaena porcus (Plisetskaya et al., 1976). In this instance, however, the impact of the A dose depended upon the original plasma insulin levels of the fish. When insulin levels were high (during the autumn and spawning periods in the lamprey and scorpion fish respectively) A caused an initial decrease in insulin levels followed by an increased rate of insulin secretion. When insulin levels were naturally low, A had only a minor inhibitory effect and a stimulatory effect on insulin secretion predominated.

It is quite clear that there are a number of factors which may individually modify plasma metabolite levels in a complex and often unexplained manner. Although several of these factors are likely to be acting simultaneously in the natural state, their mode of interaction remains largely uninvestigated. Irrespective of this fact it is important to be aware of the possible significance of exogenous stimuli such as season, temperature and stress when investigating the relationship of the nutritional state to levels of plasma metabolites.

Materials and Methods

1) Cannulation procedure

Blood samples for metabolite analyses were obtained via chronically-implanted cannulae from 101-200g female fish. The animals were acclimatized to laboratory conditions and the experimental temperature for four weeks.

During this period they were fed on both freshly chopped whiting and whole Mytilus edulis tissue. Approximately six hours prior to the insertion of the cannulae the fish were fed a satiation meal of fresh whiting. Immediate exposure to the anaesthetic after feeding was avoided since this normally caused regurgitation. The fish were fed satiation meals before operating, since preliminary trials had demonstrated that, while most individuals recovered from the operation within 24 hr and commenced voluntary food intake, a more reliable recovery period was 48-72 hr. However, a starvation period of such duration may have resulted in the mobilization of stored energy reserves and so ingestion of a satiation meal prior to the cannulation operation reduced the likelihood of such an event occurring. Consequently, 72 hr were allowed to elapse before the commencement of any tests. Houston et al., (1969) also considered a three-day post-operative recovery period to be necessary before blood samples could be withdrawn via chronically implanted cannulae.

In the present study, the fish were anaesthetised in 10% quinaldine dissolved in acetone(v/v) which was then diluted in sea-water to give a final quinaldine concentration of 5 ppm. It normally required three minutes' immersion in the solution to induce anaesthesia. The animal was then placed on an adjustable operating table and covered with a wet cloth, leaving the area immediately anterior to the caudal fin exposed. Using a 23 gauge hyperdermic needle, a single scale along the second row of scales ventral to the lateral line and 2.5 cms anterior to the caudal peduncle of the fish was removed. The exposed skin was then perforated by a needle pointing forward along the vertebral axis but entering the skin at an angle of 100° to the surface. Once a vertebral rib had been detected, the needle was withdrawn without rupturing the caudal vessels. 32 cm lengths of polythene tubing (800/100/160/100 Arnolds Vet. Products Ltd.) were used for the cannulation. The end for insertion into the artery was drawn out to approximately half the original external diameter and the tip was cut obliquely. A 23 gauge needle was inserted at the opposite end and the cannula was filled

with non-heparinised saline. The saline was prepared according to the formula of Cobb et al., (1973) for P. platessa except that the glucose component was omitted. The saline pH was adjusted to about 8.0 just prior to use. The pointed end of the cannula was inserted along the track left by the needle. Normally, the pliable cannula tip would slip readily between the vertebral ribs and into the caudal artery, within the haemal arch, if the correct site for puncturing the body musculature had been selected. After successfully penetrating the artery the cannula was easily slipped along the vessel for a distance of about 1.5 cms. It was secured to the body surface, ventral to the lateral line and running posteriorly, by two nylon sutures. Blood was drawn up the cannula and into a saline-filled 1 ml syringe to remove any trapped air bubbles. The saline and 5-10µl of blood were discarded and the blood in the cannula was returned to the artery with fresh saline using a syringe. The cannula, with a 'dead' volume of approximately 60 µl, was kept full of saline. The needle was plugged with a cork stopper which ensured that it floated on the water surface for ease of sampling. An attempt was made to avoid complete obstruction of the blood supply to the area posterior to the cannulation point. This was usually achieved by varying the diameter of the cannula portion to be inserted into the artery according to the size of fish. The fish were returned to a 33 x 57 x 25 cm compartment of a 140 l tank supplied with recirculating sea-water. Each compartment was vigorously aerated. This not only ensured a good supply of oxygen to the fish but had the added advantage of agitating the water surface. This avoided disturbance of the animals by the closeness of the experimenter during the sampling procedure.

The gills were not flushed with sea-water during the cannulation procedure since, with practice, the actual operation took no longer than 1.5-2.0 minutes. On returning the animals to fresh sea-water the gill opercula were depressed manually several times to flush the gill filaments with water and so expel any traces of the anaesthetic. The characteristic

bright red colour of the arterial blood usually confirmed the location of the cannula in the caudal artery rather than the vein. However, at the completion of the experiment, the caudal region was dissected to confirm its exact position.

Originally, a cannulation technique developed by Cech Jr. and Rowell (1976) was employed. This method involved the actual exposure of the caudal artery by making a 1.0 cm incision above the artery and then cutting through the muscle layers towards the backbone. While this technique had the obvious advantage of enabling the operator to penetrate the artery visually, it had a number of serious drawbacks:

(a) the actual operation took longer and the time for post-operative recovery was extended;

(b) the fish were less inclined to feed, even after 3 or 4 days post-operative recovery;

(c) the technique was probably more traumatic involving greater tissue damage, the incision requiring several stitches;

(d) post-operative infection was a greater problem and the cannulae were not held so firmly by the muscle tissue.

2) Sampling procedure

A standard sampling procedure was adhered to for all experiments. The saline in the cannula and about 5-10 µl of blood were drawn up using a 10 ml syringe and discarded. A heparinised 1 ml syringe was then attached to the cannula and the approximate volume of blood required was withdrawn. The sample was transferred to a 2 ml plastic "Eppendorf" tube and centrifuged at 2000 revs./minute for two minutes. The clear plasma was then separated from the red blood cells and assayed immediately for PFA determinations or frozen at -20°C for subsequent glucose studies. Blood samples taken at sea were obtained by exposing the caudal artery of stunned fish. The artery was pierced by a 23 gauge needle and the blood

was drawn into a 5 ml heparinised syringe and treated in the normal manner.

The blood sampling frequency was such that maximum blood volume removed within a 24 hr period did not exceed 12-14% of the total blood volume. This situation was only approached in the PFA studies, the glucose experiments necessitated the removal of only about half the above volume.

In view of the earlier evidence concerning the interactions of feeding times, photoperiod and stress in some fish species, all experiments were commenced at approximately the same time of day. Each experiment started after the third daily sample (72 hr) had been taken and sampling frequency then varied according to the particular experiment. All experiments were performed at 15.0°C under prevailing photoperiodic conditions unless otherwise stated. At the termination of all sampling regimes, each fish was offered food to assess any gross detrimental effects of the experimental procedure on the animals' well being.

3) In the present study, it was not feasible to monitor the influence of all the factors discussed earlier on plasma metabolite levels of Limanda. Consequently, only those factors most likely to be of significance in later experiments were examined.

(a) Effect of capture stress on plasma glucose

During the months of August and September, when coastal water temperatures for the capture area (Beaumaris Bay) would have been approximately 10-12°C, female fish (101-200g) were captured in 15 minute trawls. The fish were dispatched by a blow to the head immediately upon arrival on the deck of the research vessel. Blood samples were taken as already described and assayed for glucose only. The trawls took place between 14.00 and 18.00 hr. GMT.

(b) Effect of cannulation and 6-day fasting on plasma metabolites

To monitor the immediate effects of cannulation and short term food

deprivation on plasma glucose and PFA levels, two separate groups of nine fish were sampled as outlined in Table 8a and b below.

TABLE 8(a) - SAMPLING REGIME FOR PLASMA GLUCOSE

Fish No.	Hours post-operative											
	5	25	48	72	76	80	84	88	94	105	132	145
1	+	+	+	+	+	-	-	-	-	+	+	+
2	+	+	+	+	-	-	-	-	-	+	+	+
3	+	+	+	+	-	-	-	-	-	+	+	+
4	+	+	+	+	-	-	-	-	-	+	+	+
5	+	+	+	+	-	-	-	-	-	+	+	+
6	+	+	+	+	-	-	-	-	-	+	+	+
7	-	-	-	-	+	+	+	+	+	-	-	-
8	-	-	-	-	+	+	+	+	+	-	-	-
9	-	-	-	-	+	+	+	+	+	-	-	-
10	-	-	-	-	+	+	+	+	+	-	-	-
11	-	-	-	-	+	+	+	+	+	-	-	-
12	-	-	-	-	+	+	+	+	+	-	-	-

(b) - SAMPLING REGIME FOR PLASMA FATTY ACIDS (PFA)

Fish No.	Hours post-operative								
	25	50	72	76	80	86	100	120	145
1	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+
5	+	+	+	-	-	-	-	-	-
6	+	+	+	-	-	-	-	-	-
7	+	+	+	-	-	-	-	-	-
8	+	+	+	-	-	-	-	-	-
9	+	+	+	-	-	-	-	-	-

\+ Sample taken.

\- No sample taken.

As indicated in Table 8(a), 6 fish were sampled at 4 hr intervals to

ascertain the existence of any daily glucose rhythm. The 72 hr samples were taken as being physiologically representative of the "normal" levels of both metabolites. This experiment was performed during the months of late August to September, 1979.

(c) Effect of season on plasma glucose and plasma fatty acids (PFA)

3-9 fish maintained at ambient water temperature were cannulated in the usual manner, normally around the middle of each month. Blood samples were taken 72 hr after cannulation for estimation of monthly PFA and glucose levels. During the winter months, when water temperatures were low (minimum 4-5^{o}C), the fish were only fed approximately 1.0 - 1.5% body weight meals prior to cannulation. This ensured that even at these low temperatures the pre-operative meal would have been digested and assimilated completely before the monthly 72 hr sample was taken.

(d) Effect of artificial stress on plasma glucose

The effects of an artificially applied stress on plasma glucose were examined by capturing the individually cannulated animals in a hand-net and exposing them to the atmosphere for 1 minute. Since a more frequent sampling regime was found to be necessary to monitor metabolite changes in individual fish, a total of 13 animals was used, divided into 3 groups. Each group was treated in an identical manner, although the sampling regime did vary as follows. The first group of five fish was sampled at 0.5, 1.0, 2.0, 4.0 and 7.0 hr after the applied stress, while the other two groups of four fish were sampled at 0.5, 1.0, 4.0, 7.0, 9.0, 16.0 and 24.0 hr, and 2.0, 9.0 and 30 hr respectively. The fish were tested during late July to August, 1981.

4. Biochemical assays

(a) Measurement of blood glucose

Blood glucose was measured by the glucose-oxidase/Peroxidase technique

of Raabo and Terkildsen (1960) using a Sigma biochemical test combination kit (Cat. No. 510-DA). The actual procedure is based upon the following coupled enzymatic reaction:

1. $\text{Glucose} + 2H_2O + O_2 \xrightarrow[\text{oxidase}]{\text{glucose}} \text{Gluconic acid} + 2H_2O_2$

2. $H_2O_2 + \underset{\text{(colourless)}}{\text{O - Dianisidine}} \xrightarrow{\text{peroxidase}} \underset{\text{(brown)}}{\text{Oxidised O - Dianisidine}}$

The intensity of the brown colour measured at 450 nm is proportional to the original glucose concentration (Sigma Technical Bulletin No. 510, 1976).

25µl clear serum was diluted in 0.5 ml H_2O and mixed thoroughly with 5,0 mls of the combined enzyme-colour reagent solution (5 international units of glucose oxidase + 1 purpurogallin unit peroxidase enzymes and 2.5 mg O - Dianisidine dihydrochloride colour reagent per ml). The solutions were incubated at 37.0°C for 30 minutes in a thermostatically controlled Grant water bath. After incubation the absorbance of the test solutions were read in a 1 cm path-length cuvette at 450 nm against a blank (0.5 ml water and 5.0 ml combined enzyme-colour reagent solution) using a Cecil CE 303 grating spectrophotometer. The absorbance of the test solutions were compared with that of a known glucose standard. Each sample was analysed in duplicate.

(b) Measurement of plasma fatty acids

Serum PFAs were measured using a Boehringer-Mannheim test combination kit (Cat. No. 126055). The principal of the method, after Duncombe (1964), is that the non-esterified fatty acids are converted into colourless complex copper salts which are soluble in chloroform. Addition of diethyldithiocarbamate causes the formation of a coloured complex salt the absorbance of which is proportional to the concentration of PFA. Using a Griffin and George mechanical shaker, duplicate 50 µl samples of serum were mixed thoroughly with 2.5 ml chloroform and 0.5 ml buffer/cupric nitrate solution (triethanolamine buffer 0.45 mol/l; pH 7.8, Cupric nitrate

0.27 mol/l). The mixture was then transferred to pyrex centrifuge tubes and spun for 5 minutes at 2000 revs./minute. Using a fine-tipped glass pipette connected to a water-jet aspirator, the blue-green aqueous layer together with the white protein layer were completely removed. 2.0 ml of the chloroform layer were then mixed with 0.2 ml diethyldithiocarbamate (9mmol/l) solution. The absorbance was read 10 minutes later in a 1 cm path-length cuvette, at 435 nm, against a blank prepared from 2.5 ml chloroform, 50 µl water and 0.5 ml buffer/cupric nitrate solution. The absorbance of the test solutions was compared with that of a known palmitic acid standard (Sigma). A Cecil CE 303 grating spectrophotometer was used to read the absorbance and all glassware was scrupulously cleaned in detergent and Decon prior to use in the PFA analyses.

Results

The cannulation technique was reasonably successful and only rarely did a cannula become irreversibly blocked by blood clots. Occasionally, fish did manage to dislodge the cannula by snagging the tubing on the tank partitions, although they normally remained in position for at least 3-4 weeks. Some animals continued to feed and retained their cannulae for periods in excess of 2 months, even though the sutures generally parted from the tissue within 12-15 days. However, it appeared that the technique caused the very minimum of discomfort to the fish as suggested by their normal post-operative feeding behaviour. Indeed, some fish would accept food within 24 hr of cannulation and during initial trials some animals continued moving freely around the tank, ingesting food, while blood was actually being withdrawn from the artery. A few fish were even witnessed to approach the floating hypodermic needle and attempt to ingest it as a potential food item.

(a) Effect of capture stress on plasma glucose

Plasma glucose levels of fish sampled immediately after capture averaged

76.6±8.9 mg/100ml (mean±S.E). The range was quite considerable (22-148 mg/100ml) with some individuals exhibiting glucose levels in excess of 120mg/100ml. In comparison to the glycaemic state measured in animals 72 hr after cannulation, the glucose level observed in freshly caught fish represented a 230% increase.

(b) Effect of cannulation and 6-day fasting on plasma metabolites

Table 9 shows the respective daily concentrations of glucose and PFA in groups of fish for 6 days following cannulation in Aug./Sept. 1979. It can be seen that 5 hr after the operation the fish exhibited glucose levels which were quite normal when compared to those measured 72 hr post-operation. At 72 hr the influence of both the pre-operational meal and handling stress were assumed to be no longer active. Glucose levels did tend to fluctuate for a period after cannulation before finally settling some 48 hr after the operation. There were no further fluctuations during the succeeding 4 days. PFA levels at 50 and 72 hr after the operation were significantly higher than the levels measured at 25 hr, thus indicating a general upward post-operative trend in this metabolite. Regular sampling of animals 72 hr after the operation revealed a continued increase in PFA such that the levels observed at 86 hr were significantly higher than those observed at 72 hr. PFA levels appeared to remain steady thereafter and, within the limits of the sampling regime used, did not alter significantly for the next 59 hr.

Fish sampled every 4 hr, in the period 72-96 hr after cannulation, showed no significant variation in their plasma glucose levels.

(c) Effect of season on plasma glucose and plasma fatty acids (PFA)

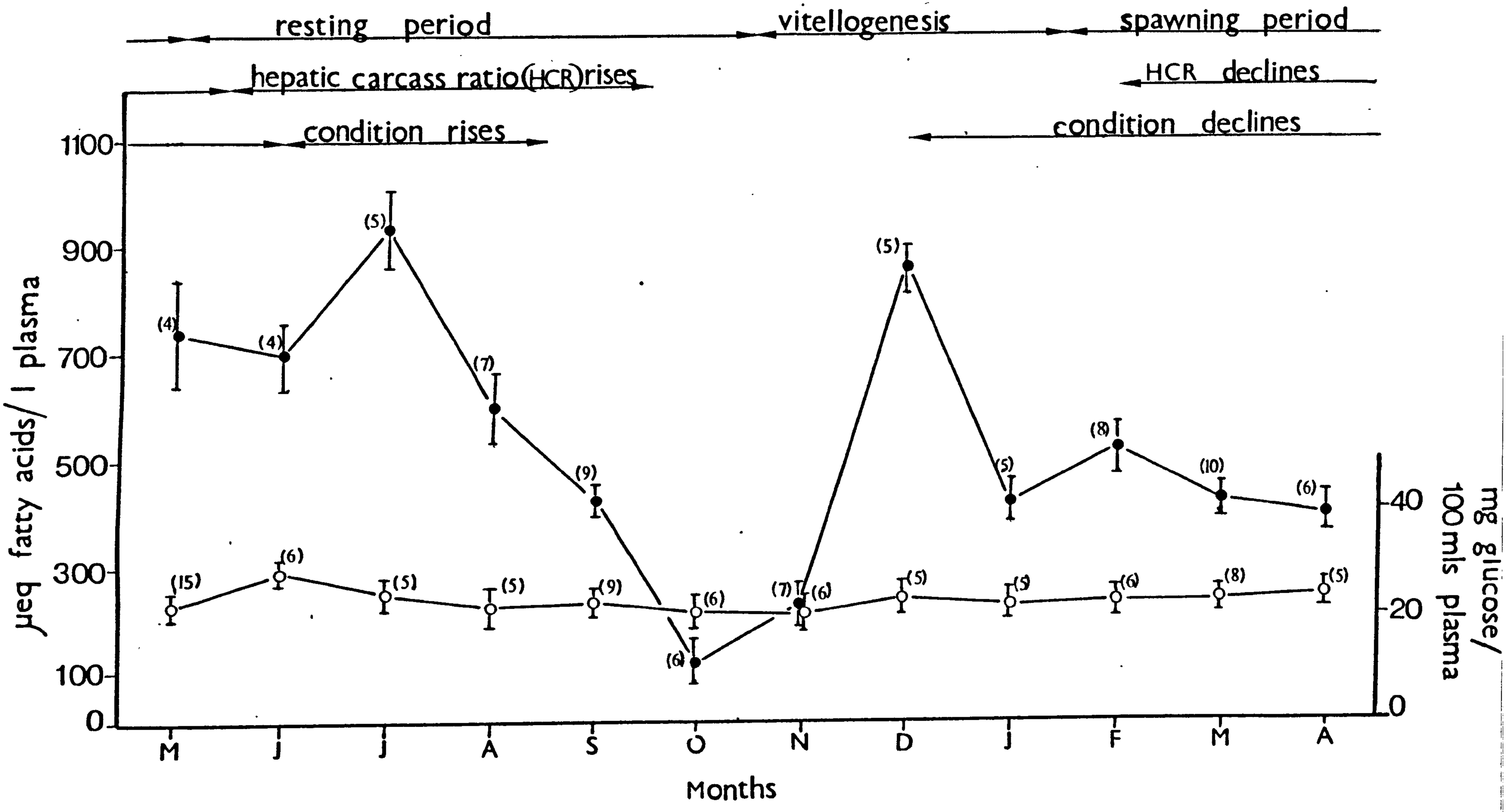
Glucose concentrations did not exhibit any obvious seasonal changes through the year, remaining between 20-25 mg/100ml for the whole period (Fig. 1) with an annual mean and standard deviation of 22.44±3.96 mg/100 ml (n=88). This pattern was in contrast to that exhibited by PFA (Fig. 1).

TABLE 9 - POST-OPERATIVE LEVELS OF PLASMA GLUCOSE AND FATTY ACIDS IN LIMANDA DURING THE MONTHS OF AUGUST/SEPTEMBER

Hours post-operative	Plasma glucose mg/100ml (n = 6)	Plasma FFA µeq/l	
5	22.0 ± 1.58	-	
25	33.0 ± 5.40	340.78 ± 10.57	(9)
48	24.4 ± 2.29	-	
50	-	401.30 ± 9.04	(9)
72	21.5 ± 2.35	405.75 ± 14.76	(9)
76	24.33 ± 2.35	433.80 ± 19.50	(4)
80	22.75 ± 1.70	450.00 ± 20.70	(4)
84	23.42 ± 1.93	-	
86	-	477.50 ± 27.90	(4)
88	23.50 ± 2.03	-	
94	23.67 ± 1.89	-	
100	-	490.00 ± 13.24	(4)
105	19.66 ± 2.41	-	
120	-	453.30 ± 52.40	(4)
132	22.00 ± 3.22	-	
145	19.30 ± 2.61	476.25 ± 32.10	(4)

Figures in brackets represent number of fish.

Fig. 1 - Seasonal variation in plasma glucose (o—o) and plasma fatty acid (●—●) levels of Limanda in relation to the reproductive cycle. (Numbers in brackets indicate number of fish samples; points represent mean ± S.E.).


resting period
vitellogenesis
spawning period
hepatic carcass ratio(HCR)rises
HCR declines
condition rises
condition declines
1100
900
700
500
300
100
0
µeq fatty acids/ l plasma
40
20
0
mg glucose/ 100mls plasma
(4)
(4)
(5)
(7)
(9)
(6)
(7)
(6)
(5)
(5)
(8)
(10)
(6)
(15)
(6)
(5)
(5)
(9)
(6)
(6)
(5)
(5)
(6)
(8)
(5)
M
J
J
A
S
O
N
D
J
F
M
A
Months

The period from May to early August was characterised by high concentrations ranging from 691.00±65.90 to 935.20±73.4 µeq/l. A second peak (859.00± 45.13 µeq/l) of shorter duration appeared in December which was preceded during the autumn by the lowest monthly levels encountered. The remaining months, January to April, showed a moderately stable concentration ranging from 390.16±35.74 to 515.10±37.80 µeq/l.

(d) Effect of artificial stress on plasma glucose

There was a significant rise in glucose within 0.5 hr of the 1 minute emersion (Fig. 2, Table 10). The maximum hyperglycaemia (40.25 mg/100 ml) was attained in 1.0 - 2.0 hr, whereupon there was a rapid decline towards normal pre-emersion levels at 4-5 hr. However, the plasma glucose did not return to normal levels immediately. Instead levels continued to decline below the pre-emersion plasma glucose concentration, of about 21.00 mg/100ml, to develop a significant hypoglycaemic condition 9 hr after treatment. After this point had been attained, levels returned slowly to normal, there being no significant difference from pre-emersion levels at 24 hr. It is clear from the large standard deviations of the mean glucose levels observed at 1.0, 2.0 and 3.0 hr after emersion that there was considerable variation in the individual response of fish to the same stressor. Some fish exhibited little or no rise in glucose levels although the development of a hypoglycaemic condition appeared to be common in most fish monitored at 7 hr and later.

Discussion

1) The 'normal' plasma metabolite levels of Limanda

Blood glucose levels in Limanda showed little fluctuation throughout the year. The annual mean compares reasonably well with the values quoted by Gwyther (1978) for this species but are much lower than the concentrations (40.8 mg/100 ml plasma) observed by Thorpe and Ince (1977). However, the

Fig. 2 - Effect of one minute emersion upon the plasma glucose levels of Limanda. (Temperature = 15.0°C, numbers in brackets indicate number of fish sampled, points represent mean ± S.E.)

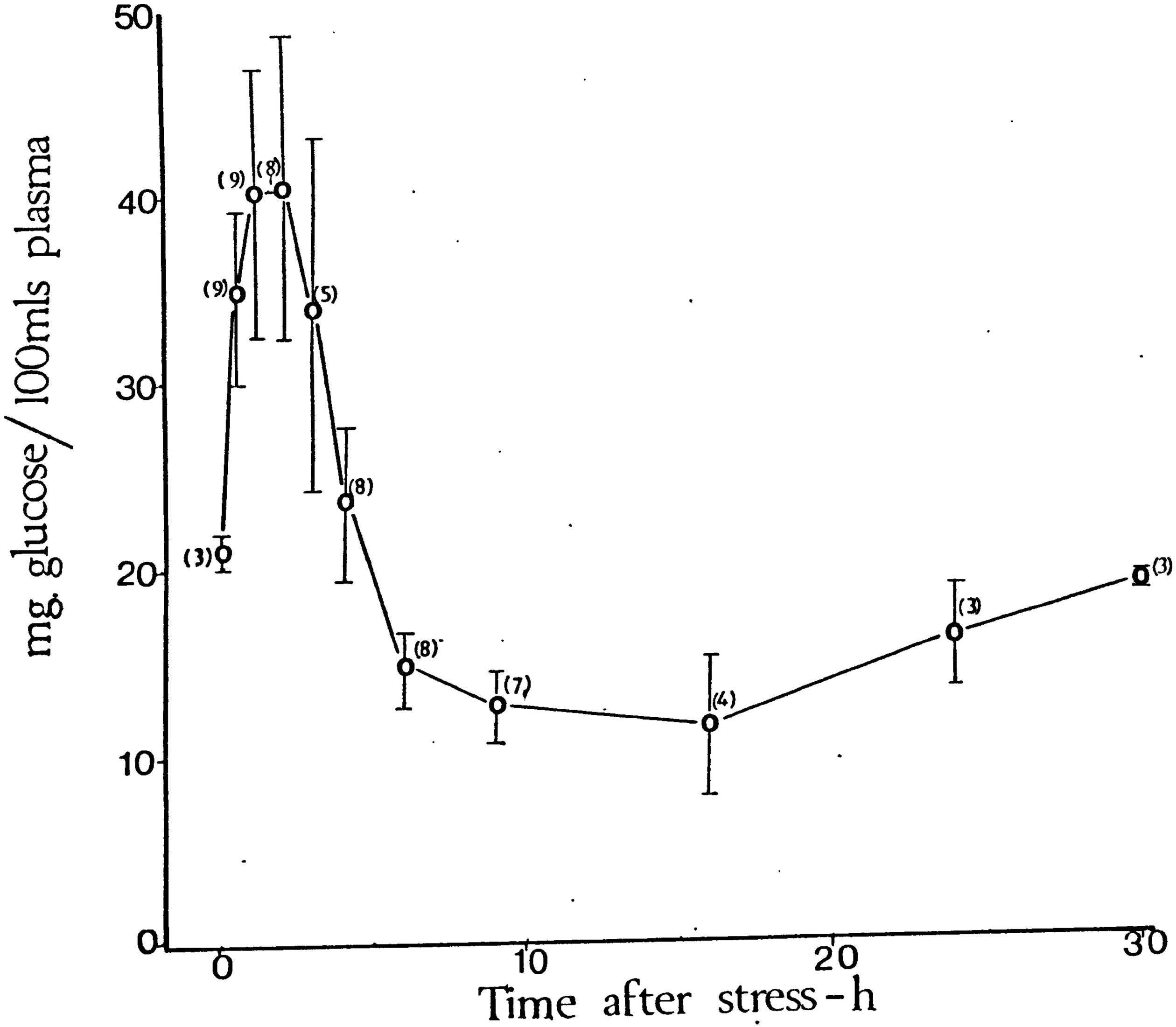

mg. glucose/100mls plasma
50
40
30
20
10
0
0
10
20
30
Time after stress-h
(3)
(9)
(9)
(8)
(8)
(5)
(8)
(3)
(7)
(4)
(3)

TABLE 10 - EFFECT OF 1-MINUTE EXPOSURE TO AIR ON PLASMA GLUCOSE (mg/100ml) LEVELS OF INDIVIDUAL FISH

Fish Weight (g)	Hours post-stress										
	0.0	0.5	1.0	2.0	3.0	4.0	7.0	9.0	16.0	24.0	30.0
180	21.5	21.5	21.5	19.0	16.0	13.5	15.5	-	-	-	-
220	21.5	54.0	63.0	51.0	42.0	29.0	21.0	-	-	-	-
135	24.0	30.0	27.0	23.0	18.5	14.0	12.0	-	-	-	-
140	25.0	39.0	35.0	30.0	24.5	18.0.	17.0	-	-	-	-
200	21.6	30.0	33.0	-	-	23.0	9.0	4.0	2.0	15.0	-
160	21.0	26.0	25.0	-	-	-	-	-	18.0	-	-
230	14.0	19.0	25.0	-	-	14.0	7.0	10.0	8.0	12.0	-
180	23.0	39.0	39.0	-	-	31.0	16.0	13.0	18.0	21.0	-
160	20.5	-	-	46.0	-	-	-	17.0	-	-	19.0
140	25.0	-	-	27.0	-	-	-	15.0	-	-	19.0
170	20.0	-	-	35.0	-	-	-	16.0	-	-	18.5
170	20.0	-	-	-	-	-	-	14.0	-	-	-
160	16.0	55.0	92.0	91.0	68.5	46.0	20.0	-	-	-	-
Mean 172.7	21.0	34.83	40.06	40.25	33.90	23.56	14.69	12.71	11.50	16.0	18.83
±S.E. 8.17	0.88	4.35	7.70	8.22	9.74	4.02	1.77	1.69	3.95	2.65	0.17

exact nutritional state of the fish at the time of sampling in the latter study is unclear. The normal plasma glucose levels observed in the present study are at the lower end of the scale of values quoted for other species (see Chavin and Young, 1970, for review) which range from 9.3 mg/100 ml in Cottus scorpius (Falkmer, 1961) to 179.6 mg/100 ml in Pomoxis annularis (Dean and Goodnight, 1964). The normal blood glucose levels have been interpreted by some workers as reflecting the mode of life of a particular species (Leibson et al., 1967). The relatively sessile inactive species, like the bottom-dwelling flatfish, generally have lower blood glucose concentrations in contrast to more active species (Table 11). In a similar manner the locomotory activity of different species is believed to influence the normal PFA level. It is generally the case that highly active species like the sea bass, Spicara smaris, have high PFA levels to provide a high energy fuel source for sustained swimming (Table 12). Furthermore, Larsson and Fänge (1977) suggested that the site of the lipid depots may also dictate whether a species has a high or low PFA level under normal conditions. They postulated that those species with lipid stores located mainly in the skeletal muscle might be able to utilise these reserves without releasing FA into the general circulation. The PFA levels of such species would, therefore, be low in comparison to those species which have to transport FA, from liver and adipose lipid stores, for oxidation by the muscle tissue. In highly active species, like the mackerel, Scomber scombrus, with large muscle lipid deposits, direct utilisation of these reserves might well occur. While such a mechanism has only been demonstrated in higher vertebrates to date (Issekutz et al., 1964) it is apparent that some species do possess the necessary enzyme complexes for the full utilisation of local lipid reserves (Bilinski and Gardner, 1968; Bilinski and Lau, 1969).

Limanda exhibited a considerable seasonal range in PFA, the full significance of which is discussed later. The presence of a chronic cannula did not appear to cause any variation in the 'normal' physiological

TABLE 11 - REPORTED 'NORMAL' PLASMA GLUCOSE LEVELS IN TELEOSTS

Species	Glucose concn. (mg/100ml)	Reference
(x) *Pleuronichthys coenosus*	18	White, 1928.
(x) *Scophthalamus aquosus*	31	Gray & Hall, 1930.
Salmo gairdneri	71	Kiermeir, 1939.
(y) *Oncorhynchus tshawytscha*	210	Robertson *et al.*, 1961.
(x) *Cottus scorpius*	42.0	Falkmer, 1961.
(y) *Salmo gairdneri*	75	Nakano & Tomlinson, 1967.
Esox lucius	210	Mackay & Beatty, 1968.
(x) *Limanda limanda*	2.5	Gwyther, 1978.
"	22.44 ± 3.96	Present study.

(x) - sedentary species. (y) - active species.

levels of the two metabolites studied. As in the case of plasma glucose, PFA values compare favourably with those recorded by Gwyther (1973) for Limanda where the blood samples were obtained by the rapid puncturing of the caudal vessels (Tables 11 and 12). The glucose concentration measured in the 72 hr post-operative sample appears representative of the normal physiological metabolite levels, since the levels did tend to fluctuate in the preceding recovery period but not after 48-72 hr. The PFAs, however, show a steady post-operative increase which may have reflected a gradual return to normal levels following the influence of both post-ingestive and operative factors. Similarly, Ince and Thorpe (1975) detected a post-operative depression of PFA in Esox lucius which they attributed to surgical stress and contrasted this effect with the PFA pattern observed in mammals after surgery. After 72 hr the levels continued to show an increase (Table 9). Therefore, it may have been more valid to use the 86 hr samples as being truly representative of the normal PFA levels since they did not fluctuate significantly after this point. However, in such studies it is necessary to strike a balance between the points when post-operative and ingestive factors no longer operate and actual starvation begins to influence the physiological levels of a metabolite. Considering the susceptibility of PFA levels to food deprivation in the higher vertebrates, it was considered that the 72 hr sample reflected a point when the pre-operative meal and operational stress factors no longer influenced the circulating PFA levels. This conclusion may be further supported by the steady plasma glucose levels at this point. Strong reciprocal relationships, probably hormone-mediated, between plasma glucose and PFA have been demonstrated in teleosts (Palmer and Ryman, 1972). In addition the pre-operative meal would have been cleared completely from the intestine within the 72 hr period. The likely significance of the rise in PFA is discussed more fully in Chapter 3.

The failure to detect any variation in plasma glucose levels when fish were sampled on a four-hourly basis within a 24 hr period argues

TABLE 12 - REPORTED 'NORMAL' PLASMA FATTY ACID LEVELS IN TELEOSTS

Species		Fatty acid concentration		Reference
Opsanus tau		430 ± 20	(a)	Tashima & Cahill, 1968.
Salmo gairdneri		286 ± 33	(a)	Bilinski & Gardener, 1968.
Spicara smaris	(y)	1150 ± 90	(a)	Leibson et al., 1968.
Trachurus mediterraneus	(y)	610 ± 30	(a)	"
Carassius auratus		910 ± 9.4	(a)	Minick & Chavin, 1972.
Anguilla anguilla		384 ± 14	(a)	Larsson & Lewander, 1973.
Esox lucius		266	(a)	Ince & Thorpe, 1975.
Oncorhynchus nerka	(y)	1297.7 ± 101.7	(a)	McKeown et al., 1975.
Lophius piscatorius	(x)	212 ± 70	(g)	Larsson & Fänge, 1977.
Trigla gurnadus		299 ± 40	(g)	"
Scomber scombrus		430 ± 43	(g)	"
Gadus poutassou		1061 ± 145	(g)	"
Gadus virens		1078 ± 213	(g)	"
Clupea harengus		840 ± 77	(g)	"
Pleuronectes platessa	(x)	567 ± 41	(g)	"
Limanda limanda	(x)	220 380	(a)	Gwyther, 1978.
Salmo gairdneri		81 ± 5	(a)	Leatherland & Nuti, 1981.
Spicara chryselis		60 → 260	(a)	Fernandez & Planas, 1980.
Limanda limanda	(x)	113.33 ± 38.45 → 932.2 ± 73.4	(a)	Present study.

(a) - μeq/l; (g) - μ mole/l; (x) - sedentary species; (y) - active species.

against the existence of any circadian rhythm for this metabolite. However, this most certainly does not imply that such a rhythm might not persist at other times of the year. The steady glucose levels observed during this period also suggest that such frequent withdrawal of small samples of blood did not cause any obvious physiological stress to the fish. The gradual rise in PFA observed after the 72 hr sample was probably a quite normal physiological phenomenon. The levels plateaued at a certain point (86 hr) and did not alter significantly during the succeeding 48 hr. The maximum volume of blood removed from individual fish falls within the range extracted from other fish species (Thorpe and Ince, 1974; Ince and Thorpe, 1977; Zohar, 1980). No effect of serial sampling on plasma metabolite or hormonal levels was detected in these latter studies, although other plasma elements may be affected (see Cairns and Christian, 1978).

In conclusion, it is emphasised that so-called 'normal' metabolite levels quoted without reference to the physiological state and acclimation conditions of the fish are of little use. Bern and Nandi (1964) emphasised this point, in relation to plasma glucose studies, when they observed that: "in general, the normal blood glucose concentration reported for a few specimens of a particular species was probably a meaningless biological datum".

2) Effect of capture and handling on blood glucose of Limanda

The elevated glucose levels observed in freshly captured Limanda agree with the post-capture findings for other species (Table 5). While such an increase cannot be attributed entirely to stress, since all the fish had a certain amount of food in their intestines, the considerable individual variation is indicative of stress-induced hyperglycaemia. This situation emphasises two points: (i) it is clearly pointless to take samples from freshly caught animals for measurement of 'normal' physiological parameters and (ii) the importance of reducing trawl times to the very minimum to reduce the degree of stress.

3) Seasonal changes in plasma metabolites of Limanda

In many of the seasonal studies of plasma metabolites there was a failure to avoid stressing the animals prior to sampling. Ideally, blood samples for measurements of seasonally-induced changes in certain blood parameters should be taken from animals of known physiological state and under conditions resembling as near as possible their natural environment. In the present study it was pointless to extract blood from fish in the field for seasonal studies due to stress-related hyperglycaemia following trawling and their unknown nutritional state.

Female Limanda show relatively stable plasma glucose levels throughout the year with no apparent seasonal variations. This agrees with the findings of Mackay and Beatty (1968) for the female white sucker, Catostomus commersonii. The latter authors did recognise that the considerable variability (annual mean ± S.D. 129±40, n = 66) in the glucose levels between individual fish may have masked any seasonal variation. There was little such individual variation in plasma glucose levels of Limanda.

Other teleosts which have been studied in this respect have shown significant rises in plasma glucose in association with spawning periods (Yanni, 1961; Tandon and Joshi, 1974; Thorpe and Ince, 1974; Petersen and Emmerssen, 1977). Unfortunately, in some of these latter studies the fish had either been deprived of food for several days or fed within 48 hr of sampling. The exact influence of the nutritional state of the fish on plasma metabolite levels was, therefore, unknown. The apparent absence of any seasonal fluctuations in blood glucose of Limanda would suggest that, at least in the female sex of this species, there is no increased demand for carbohydrate during the reproductive season. Alternatively, an enhanced glucose supply may be masked by an increased turnover rate.

Seasonal elevations in plasma lipids and PFA have been recorded in other teleosts. In Platichthys flesus, total plasma lipid ranged from about 20 mg/100 ml to around 150-180 mg/100 ml according to the time of year (Petersen and Emmerssen, 1977). Fernandez and Planas (1977) observed two

main PFA peaks in Spicara chryselis and a similar seasonal pattern was observed in Limanda. The high plasma levels of lipid fractions are thought to be associated with the storage of energy reserves, vitellogenesis and spawning. In the present study, the size group of fish examined (101-200g) undergoes a resting period, commencing about May, following the cessation of spawning (Baksh, 1982; Fig. 1). The resting period lasts until October/November when gonadal growth or vitellogenesis commences once more prior to the next reproductive season. In several teleost species the resting period is characterised by an improvement in the condition of the animals as the tissue and liver reserves are replenished. This takes the form of increases in the level of stored lipid and glycogen utilised from the body tissues during reproduction (Srikar et al., 1979; Dawson and Grimm, 1980). In Limanda, the condition factor (bodyweight-gonadweight/Length^3 x 100) and the liver somatic index (liverweight/bodyweight) start an upward trend in May and reach peak values in the August and September/October periods respectively before the onset of the next vitellogenic period (Baksh, 1982). Therefore, the elevated PFA levels, from May until late August, occur during the period when the liver shows a 125% increase in lipid reserves.

Petersen and Emmerssen (1977) measured total lipid in P. flesus so it is not feasible to make direct comparisons with the PFA data for Limanda. However, it is interesting that, like the PFA pattern in Limanda, P. flesus showed high plasma lipid levels, during the pre-vitellogenic period, which were thought to be derived mainly from food. In this latter study it is unclear whether or not the fish were fed in the intervening 7-day period between capture and blood sampling. If the animals were deprived of food, then it is unlikely that their blood would contain any nutrients absorbed from the gut just prior to sampling. In the present study this problem does not arise since the nutritional state of the fish was known (see Materials and Methods). It is also evident that PFA levels in Limanda do not rise following the ingestion of food (see Chapter 3) so that the summer PFA levels observed in this species are unlikely to be directly related to its

nutritional state. Rather, it might be more likely that, at the completion of spawning, there is a partial reabsorption and translocation of any remaining gonadal material. Htun (1977) described degenerating intra-ovarian oocytes (corpora atrecia) in Limanda whose number reached a maximum when spawning was complete. During the post-spawning period they gradually degenerated until complete resorption had occurred before the onset of the next vitellogenic phase. It is, therefore, possible that the post-spawning PFA peak observed in Limanda reflects the mobilisation and translocation of the oogonial TG fraction. The mobilisation of TG in higher vertebrates results in the release of PFA into the circulation (Newsholme and Start, 1973).

The early vitellogenic PFA peak observed during December in Limanda was also recorded in Spicara chryselis (Fernandez and Planas, 1977) and in Zoarces viviparus (Pekkarinen and Kristoffersson, 1975). Vitellogenesis in the Baltic cod, Gadus morhua callarias (L), was preceded by a considerable transport of phospholipids in the blood. Their level declined at the onset of vitellogenesis but at the same time there was a relative increase in transport of PFA and TG. It is apparent that, during early vitellogenesis in this species, TG reserves are expended at a far higher rate than any other lipid fractions (Shatunoskiy, 1971). The brief December elevation in PFA may indicate a similar pattern of events in Limanda with alternative lipid fractions participating to a greater or lesser extent in gonad growth as vitellogenesis proceeds. However, the brief duration of the December PFA peak rather limits physiological interpretation unless its repeated occurrence can be demonstrated. PFAs decline in January and remain stable throughout the spawning season until mid-May when the 101-200g size group enter the resting period.

Pleuronectids like Platessa and Limanda belong to the 'lean' category of fish and both species undergo considerable changes in their liver weight during vitellogenesis and spawning (Dawson and Grimm, 1980; Baksh, 1982). This suggests an important energy contribution by the liver to the growth

of the gonada as might be expected in 'lean'fish. However, Dawson and Grimm (1980) reported that the liver's total lipid contribution to gonad growth was negligible in comparison with the carcass. Baksh (pers. comm.) supplies data which would allow the conclusion that during the periods when their respective energy reserves are being depleted, the liver supplies slightly more energy than the general carcass on a per gram basis. It is apparent though, that the total energy gained from catabolism of hepatic tissue (for 20 cm fish) during the October to February reproductive period was approximately 6.1 k.cals., whereas about 55.6 k.cals. were lost from the carcass. As in P. platessa it would appear that the main energy reserves for gonadal growth in Limanda originate from the general body carcass and not from the liver. Baksh (1982) observed an early decline in the carcass condition at the onset of vitellogenesis while the liver lipid was not significantly depleted until late vitellogenesis. The early vitellogenic PFA peak may, therefore, reflect mobilisation of carcass TG reserves.

4) Effect of stress on plasma glucose of Limanda

Limanda showed a characteristic rise in glucose following application of a mild stressor. This is in agreement with findings for other teleosts, although the hyperglycaemic condition was maintained for only a short period. This is probably due to the very mild nature of the stressor. It has been clearly shown that the intensity of the response to stress is dependent upon the nature of the treatment (see Introduction). In the present study, although all animals were treated identically and within the same two-week period, some fish showed little or no rise in plasma glucose whilst other specimens developed highly significant hyperglycaemic states. This situation has also been observed in relation to catecholamine levels following stress in teleosts (Mazeaud et al., 1977). It appears that the variable glucose response observed in Limanda may be due to differences in the primary response of the fish to the stressor. Alternatively, variations

in the amount of stored glycogen reserves may account for the above observations in this species, as suggested for S. gairdneri (Nakano and Tomlinson, 1967). While liver glycogen studies have not been performed in Limanda, both seasonal (Ottolenghi et al., 1981) and daily fluctuations in liver glycogen (Delahunty et al., 1978) have been encountered in other species.

In Limanda the hyperglycaemic response to stress was succeeded by a significant hypoglycaemia 9 hr after treatment. Plasma glucose did not return to normal levels for 24 hr. This hypoglycaemic response was recently observed in Salmo trutta following acute handling stress (Pickering et al., 1982). However, in this latter study the fish were offered food daily throughout the experiment. The hypoglycaemic response was, therefore, thought to be due to the observed suppression of appetite for 72 hr after the applied stress. A hypoglycaemic response, subsequent to the hyperglycaemia condition, was witnessed in Esox lucius following catecholamine injections (Thorpe and Ince, 1974). As discussed in the Introduction, the biphasic insulin response to catecholamine treatment observed in some teleosts may explain the delayed hypoglycaemic condition. It is apparent that some of the secondary responses to stress, like plasma metabolite fluctuations, can be mimicked by catecholamine injections (Mazeaud et al., 1977). In the present study, the hypoglycaemic condition cannot be attributed to food deprivation since plasma glucose remains stable for at least six days in Limanda when food is unavailable (see Chapter 3). Therefore, the biphasic nature of the plasma glucose responses in Limanda may be tentatively interpreted in terms of the conclusions drawn from the catecholamine infusion studies. In Table 10 the results show that the hyperglycaemic response varies greatly between individual fish, yet the later hypoglycaemia is much more constant. This pattern may be explained on the assumption that (a) catecholamines mobilise glucose from liver glycogen reserves; (b) catecholamines also suppress insulin release; (c) there is a release of insulin following the fall in blood catecholamine levels and in response to the

elevated plasma glucose level; (d) the magnitude of the hyperglycaemia is limited by the liver reserves. From the present study, it is quite clear that even mild stress will cause significant changes in plasma glucose. It is quite likely that other metabolites, particularly PFA, will also be affected. In studies of intermediary metabolism the possibility of chronic non-specific stressors cannot be overlooked. Plasma metabolites may fluctuate for considerable periods after the catecholamines have returned to normal levels (Mazeaud and Mazeaud, 1965). In addition, variations in the response to stress may vary according to the season (Plisetskaya et al., 1976) or even the time of day (Meier et al., 1973).

CHAPTER 3

RESPONSE OF PLASMA GLUCOSE AND PLASMA FATTY ACIDS TO INGESTION OF FOOD AND METABOLITE LOADS.

Introduction

It is sometimes difficult to evaluate much of the research relating plasma metabolite levels to the nutritional state of fish. Such studies have often failed to account for some of the problems associated with the measurement of plasma metabolites (Chapter 2). In particular, insufficient attention has been given to blood sampling techniques, type of diet or the manner in which the fish were fed. These problems have been appreciated by some workers, who have perfected sampling methods which avoid stress-associated changes in plasma nutrients (Thorpe and Ince, 1974; Bergot, 1979).

1) Effect of feeding on plasma glucose and plasma fatty acids (PFA)

Much of the research concerning the response of plasma metabolities to food intake in fish has been restricted to studies on the influence of long-term fasting and subsequent refeeding on metabolite levels (Tables 1, 2 and 3). This particularly applies to those species which undergo natural fasting periods in the wild. A few studies have observed the immediate post-prandial changes of plasma metabolites such as glucose (Table 4) and amino acids (Nose, 1972; Schlisio and Nicolai, 1978). However, the ability of fish to assimilate dietary carbohydrate does vary quite considerably according to their natural food preference. This fact may partially account for the varied response of plasma glucose between different fish species.

The enzyme equipment of fish is adapted to their respective food and feeding habits both qualitatively and quantitatively (Goel, 1975). A higher amylase activity was detected in the intestine of the coral-feeding Scaridae than in the molluscivorous labrid fishes (Gohar and Latif, 1960). Similarly, a higher carbohydrase activity was found in herbivores when compared to carnivorous species (Goel, 1975). Highly carnivorous species, like S. gairdneri, are unable to utilize high concentrations of dietary

TABLE 1 - EFFECT OF FASTING ON PLASMA GLUCOSE IN FISH

Species	Duration of fast	Change in glucose concn.(e)	Reference
Gadus morhua	16 days	108±8→72±4	Kamra, 1966.
	30 "	→75±4	
Opsanus tau	1-3 months	No change	Tashima & Cahill, 1968
Anguilla anguilla	8 days	42.5±2.1	Larsson & Lewander, 1973.
	95 "	→19.1±2.6	
	145 "	→32.6±2.8	
Esox lucius	0 "	46.6±2.6	Ince & Thorpe, 1976.
	≅92 "	→28.3±1.8	
Chrysophrys major	0-90 days	No change	Sakamoto & Yone, 1978.
Oncorhynchus maculatus	3 "	77.8±8.7→94.2±17.8	Woo & Cheung, 1980
	33 "	→40.4±4.4	

(e) mg/100 ml.

TABLE 2 - EFFECT OF FASTING ON PFA IN FISH

Species	Duration of fast	Change in PFA concn.	Reference
Salmo gairdneri	1 day	286±33 (a)	Bilinski & Gardner, 1968.
	5 "	511±41	
Opsanus tau	1 month	0.43±0.02 (b)	Tashima & Cahill, 1968.
	3 "	0.12±0.01	
Anguilla anguilla	8 days	384±14 (a)	Larsson & Lewander, 1973
	95 "	328±49	
	545 "	545±34	
Oncorhynchus nerka	0 "	1297.7±101.7(a)	McKeown et al., 1975.
	30 "	1063.0±31.0	
Esox lucius	0-3 months	No change	Ince & Thorpe, 1976.
Carassius auratus	1 day	0.058±0.016 (c)	Wiegand & Peter, 1980.
	17 "	0.245±0.027	
Oncorhynchus maculatus	0 "	0.35±0.07 (c)	Woo & Cheung, 1980.
	33 "	0.14±0.04	
	145 "	0.73±0.14	
Salmo gairdneri	0 "	81±5 (a)	Leatherland & Nuti, 1981.
	30 "	113±12	
	0 "	47.1±4.2	
	60 "	107.6±17.6	

(a) µeq.l (b) meq/l (c) mM.

TABLE 3 - EFFECT OF FASTING ON PLASMA PROTEIN IN FISH

Species	Period of fast	Change in metabolite concentration	Reference
Gadus morhua	0 - 16 days	5.42±0.33→4.73±0.40 [d]	Kamra, 1966.
	- 30 "	→ 4.76±0.54	
Esox lucius	1 - 3 months	No change	Ince & Thorpe, 1976
Oncorhynchus maculatus	0 - 66 days	3.37±0.71→5.78±0.39 [d]	Woo & Cheung, 1980

d) g/100ml.

TABLE 4 - EFFECT OF FEEDING ON PLASMA GLUCOSE IN FISH

Species	Feeding details	Change in glucose concn.	Reference
Myxine glutinosa	Force fed M.edulis brei	No change	Falkmer & Matty, 1966
Roseveltiella nattereri	Voluntary fed fish	*58 → 90 [e]	Bellamy, 1967
Katsuwonus pelamis	" " "	*132 (7.0hr. post-prandial) to 81 (10-24 hr. post-prandial) [e]	Magnuson, 1969
Squalus acanthias	Force fed minced fish	*40 → 105 [e]	Patent, 1970
Chrysophrys major	-	*50 → 200 [e]	Furuichi & Yone, 1981
Esox lucius	Force fed minced fish after 3 months' starvation	28.3 ± 1.8 → 57.0 ± 3.8 [e]	Ince & Thorpe, 1976
Limanda limanda	Voluntary fed trout feed pellets	No change	Gwyther, 1978
Salmo gairdneri	30% glucose pellets	*95 → 200 [e]	Bergot, 1979.

e) mg/100ml. *Data taken from figs. and tables in respective reference.

carbohydrate without developing pathological symptoms (Philips et al., 1948), although it appears that it is the polysaccharides that present the main problem to such species (Singh and Nose, 1967). In contrast, P. platessa showed an enhanced growth rate when its diet was supplemented with up to 20% carbohydrate (Cowey et al., 1975). While there may be considerable interspecific variation in the ability of fish with different feeding habits to utilise dietary carbohydrates, the overall picture suggests that they assimilate this nutrient less efficiently than mammals. This is supported by the fact that during starvation substrates other than glucose are preferentially oxidised (Nagai and Ikeda, 1972). There may, therefore, be a limited capacity of fish to oxidise glucose aerobically (Cowey and Sargent, 1979) as indicated by the more rapid glucose oxidation in mammals in comparison to the equivalent rates observed in some carnivorous fish (Cowey et al., 1975). This situation may explain the higher dietary protein requirement of some fish which is indicated by the efficiency with which they assimilate various nutrients.

Low PFA levels are often associated with a normal feeding state in fish, although there are some exceptions (Tashima and Cahill, 1968; McKeown et al., 1975). Reduced PFA levels observed in fed fish are comparable to the situation encountered in higher vertebrates. In the latter case, the activity of the lipogenic enzymes are modulated by the lipid and carbohydrate components of the diet. The changes in the activity of the lipogenic enzymes, acetyl-COA carboxylase and fatty acid synthetase, have been shown to be related to their rates of biosynthesis. Lipid decreases this latter process while insulin and carbohydrate enhance it (Volpe, 1966; Newsholme and Start, 1972). The decline in PFA following feeding is considered to reflect an enhanced rate of esterification of tissue FA. (This process is discussed later in greater detail.)

There is little information about the form in which lipids pass from the gut lumen into the blood circulation in teleosts. Fish possess an intestinal lipase which, unlike the lipases of omnivorous mammals, is

non-specific so that FA and glycerol are the main end-products of triacylglycerol (TG) breakdown (Patton et al., 1975). Robinson and Mead (1973) and Kayama and Iijima (1976) fed labelled palmitic acid to trout and carp and observed that the radioactivity first appeared in the PFA fraction. In S. gairdneri, this pattern persisted for the first 2 hrs after feeding, with the TG fraction becoming labelled at 4 hrs. At this point, activity in the PFA fraction had subsided. Robinson and Mead (1973) proposed that, rather than direct absorption into the blood circulation, the FA are converted to TG in the intestinal mucosal cells and then exported to the lamina propria where they undergo hydrolysis before absorption into the circulation as FA. In whatever form the lipids are translocated, the above evidence suggests that, unlike the situation in the higher vertebrates, dietary lipids are absorbed from the intestine as FA in fish. Robinson and Mead (1973) offer no explanation for the delayed appearance of the radioactivity in the TG fraction. Fish do not have a very well-developed lymphatic system, or thoracic duct, and may not be able to deliver assimilated TG to the blood circulation as rapidly as is found in mammals. The active TG fraction observed by Robinson and Mead (1973) must, therefore, originate from the mobilization of labelled hepatic TG synthesised from absorbed PFA (Cowey and Sargent, 1979). However, Robinson and Mead (1973) did not record any change in the total plasma TG fraction. If Cowey and Sargent (1979) are correct, and the labelled TG originated from the liver, then a preferential release of the labelled hepatic TG might be implied. In mammals, FA are converted into TG before absorption into the blood circulation (Newsholme and Start, 1972). It should be remembered that in the study of Robinson and Mead (1973) the palmitic acid was fed to the fish as a pure lipid meal. The initial appearance of the radioactivity in the PFA fraction may reflect a shortage of the necessary precursors for their immediate esterification to TG prior to absorption. Further research is obviously needed in this area using labelled lipid fractions, preferably incorporated into mixed nutrient meals.

2) Effects of food deprivation on plasma glucose and plasma fatty acids (PFA)

The deprivation of food for periods varying from a few days to several weeks has been shown to have a significant impact on various plasma metabolites in fish. However, there is considerable interspecific variation in the metabolite response to starvation (Tables 1, 2 and 3). No change was detected in plasma glucose of Opsanus tau (Tashima and Cahill, 1968) and Ophiocephalus maculatus (Woo and Cheung, 1980) after 1-3 months and 33 days starvation respectively. Such constancy of plasma glucose during starvation indicates an efficient regulatory system (Falkmer and Matty, 1966). Sakamoto and Yone (1978a,b) detected elevated levels of plasma gluconeogenic enzymes in Chrysophrys major which they related to the increased rate of gluconeogenesis in the liver during starvation. This is similar to the pattern observed in mammals when food is withheld (Lardy et al., 1965). Other species of fish show starvation-related depressions in plasma glucose (Table 1).

Starvation in teleosts is often associated with a decline in liver and muscle lipids indicating a general mobilization of energy reserves (Table 5). In some species, e.g. E. lucius, lipid mobilization is not necessarily reflected by any variation in the level of PFA (Mazeaud, 1973; Ince and Thorpe, 1976), while other species may show either a decline (Tashima and Cahill, 1968; Mayerle and Butler, 1971; McKeown et al., 1975), an elevation or even a biphasic response (Larsson and Lewander, 1973; Table 5). However, the magnitude of starvation-related rises in PFA may vary according to the duration of food deprivation (Bilinski and Gardner, 1968; Mazeaud, 1973). Bilinski and Gardner (1968) recorded the most significant rise in PFA during the first two weeks of starvation rather than after prolonged periods. It is also apparent that the PFA response to starvation may vary according to the season in which the animals are tested. Mazeaud (1973) recorded either a rise or fall in PFA according to whether Cyprinus carpio were

TABLE 5 - EFFECT OF FASTING ON ENERGY RESERVES IN FISH

Species	Storage organ and energy reserve	Duration of fast	% decrease in energy reserve *	% increase (I) or decrease (D) in plasma nutrients and starvation period *	Reference
Anguilla japonica	liver glycogen	15 days	15		Hatey, 1951.
	liver lipid	30-40 "	50		
Carassius auratus	liver glycogen	8 "	50		Stimpson, 1965.
(1.5 yr old)	muscle glucogen	16 "	No change		
	muscle & liver lipid	16 "	"		
(5.0 yr old)	liver lipid	25 "	40		
	liver glycogen	25 "	No change		
	muscle glycogen & lipid	25 "	"		
Gadus morhua	liver glycogen	37 "	37	Glucose - 36(D)	Kamra, 1966.
Clupea harengus	muscle lipid	4 months	98		Wilkins, 1967.
Salmo gairdneri	lateral line muscle FA	5-56 days	77-33 (Increase)	PFA, 79-86(I)5 days	Bilinski & Gardner, 1968.
Tilapia mossambica	liver glycogen	1-6 days	75		Swallow & Fleming, 1969.
	muscle glycogen	6-9 "	31		
Anguilla anguilla	liver glycogen	11-164 days	82	Glucose-40(D)11-47days	Dave et al., 1975.
	liver triglycerides	11-96 "	54	Protein-34(D)11-164 "	
	muscle protein	11-164 "	No change	Cholesterol-31(D) 11-96 "	
	muscle triglycerides	11-164 "	35	PFA-(No change) 11-164 "	

(Table 5 cont →

TABLE 5 (cont.)

Species	Storage organ and energy reserve	Duration of fast	% decrease in energy reserve *	% increase (I) or decrease (D) in plasma nutrients and starvation period *	Reference
Esox lucius	liver glycogen	1 month	34	glucose-39(D) 3 months	Ince & Thorpe, 1976.
	" "	3 "	77	protein-(No change) "	
	muscle glycogen	3 "	54	PFA-(No change) "	
	muscle lipid	1-3 "	No change		
	" "	1-3	" "		
Chrysophrys major	liver lipid	0-90 days	39	glucose-(No change)	Sakamoto & Yone, 1978.
	liver protein	0-90 "	11	protein-25(I) 0-9 days	
	liver glycogen	0-30 "	76	protein-57(D) 90 "	
	dorsal muscle lipid	0-90 "	50	cholesterol-63(D) 70 days	
	dorsal muscle protein	0-90 "	33		
	vertebrae lipid	0-90 "	91		
Oncorhynchus maculatus	liver lipid	142 "	No change	glucose-(No change) 33 days	Woo & Cheung, 1980.
	liver protein	33 "	58	glucose-48(D) 142 "	
	liver glycogen	142 "	45	protein-71(I) 66 "	
	muscle protein	142 "	No change	PFA-59(D) 33 "	
	muscle glycogen	0-142 "	" "	PFA-112(I) >33 "	

* Values calculated from published data in respective references. PFA - plasma fatty acids.

deprived of food in the autumn or summer respectively.

Low insulin levels are usually associated with starvation in mammals which leads to a lipid mobilization and subsequent rise in PFA (Sharpiro, 1965). As a result there is an increase in FA oxidation (Newsholme and Start, 1972) and a reduction in glucose metabolism and FA re-esterification (Krahl, 1974). The above system conserves glucose for those tissues dependent upon this metabolite for energy and encourages the oxidation of FA by the heart and muscle. Similarly, low insulin levels have been observed in fasted fish (Thorpe and Ince, 1976). This may explain the increase in PFA reported for some fish species when deprived of food.

Many species of fish undergo natural periods of starvation during their normal life cycles. The metabolic strategies that have developed to cope with this period show considerable interspecific variation as indicated by the depletion of the different energy reserves (Table 5). This fact probably accounts for much of the variation encountered in the plasma chemistry of fish during food deprivation. Both the mode of feeding and mechanism of storing energy reserves are thought to dictate the ability of fish to withstand starvation and to account for the associated changes in blood biochemistry (Woo and Cheung, 1980). The failure to observe any variation in PFA during starvation in some species may indicate a local utilization of lipids which is not reflected by circulating PFA concentrations (see later).

3a) Effect of glucose infusions on plasma glucose

Metabolite infusion studies have been used to examine the interaction of plasma nutrients in various species of fish (Tables 6 and 7). A severe hyperglycaemia is the normal pattern observed in fish following a glucose load. In Platichthys, maximum plasma glucose levels were not achieved until 6 hr after an intraperitoneal glucose infusion (Korsgaard et al., 1981). This contrasts with the situation reported in other species where the normal delay was of 0.5 - 1.0 hr duration following identical

TABLE 6 - EFFECT OF METABOLITE LOADS ON PLASMA GLUCOSE IN FISH

Species and Treatment	*Change in glucose concn.[a]	*Time (hr.) until maximum effect	*Time (hr.) until normoglycaemia	Reference
Myxine glutinosa				
i.m.i. glucose (0.5g/kg)	18 - 115	1.0	28	Falkmer & Matty, 1966.
Opsanus tau				
i.m.i. glucose (0.5g/kg)	42 - 156	0.5	>6	Tashima & Cahill, 1968.
Salmo gairdneri				
i.l. glucose (1g/100g)	81.6 - >500	6.0	-	Palmer & Ryman, 1972.
Esox lucius				
i.a.i. glucose (0.5g/kg)	50 - 305	0.5	48	Thorpe & Ince, 1974.
i.a.i. glucose (0.5g/kg)	35.7 ± 1.5	0.5	6	
+ insulin (10Iu/kg)	-344 ± 17.5			
Anguilla anguilla				
i.a.i. glucose (0.5g/kg)	45 - 415	0.5	24	Ince & Thorpe, 1974.
i.l. glucose (0.5g/kg)	45 - 75	3.5	24	
i.l. glucose (0.5g/kg)	54 - 68	-	Hyperglycaemic >48	
i.a.i. amino acid (0.25g/kg)	52 - 113	1		
	- 34	9	24	
Seriola quinqueradiata				
oral glucose (5mg/100g)	54 - 150	1 - 2	7	Shimeno et al., 1979.
Cyprinus carpio				
oral glucose (5mg/100g)	48 - 110	2	5	
Cyprinus carpio				
oral glucose (167mg/100g)	48 - 170	1	5	Furuichi & Yone, 1981.

Table 6 cont. →

TABLE 6 (cont.)

Species and Treatment	*Change in glucose concn. [a]	*Time (hr.) until maximum effect	*Time (hr.) until normoglycaemia	Reference
Chrysophrys major				
oral glucose (167mg/100g)	48 - 175	2	>5	Furuichi & Yone, 1981.
Seriola quinqueradiata				
oral glucose (167mg/100g)	100 - 190	3	>5	

i.m.i. = intramuscular injection; i.a.i. = intra-arterial injection; i.l. = intraluminal.

a) mg/100 ml. *Data taken from figs. and tables in respective references.

TABLE 7 - EFFECT OF METABOLITE LOADS ON PLASMA LIPIDS IN FISH

Plasma lipid fraction	Species and treatment	Change in concentration	Time (hr) until maximum effect	Time to attain normal concn.	Reference
PFA (μeq/l)	Opsanus tau				
	i.m.i. glucose	No change	-	-	Tashima & Cahill, 1968.
	Salmo gairdneri	1000 →	5.0	-	
	1 ml oral dose (1g/ml)	200			Palmer & Ryman, 1972.
	Carassius auratus	910 ± 9.4			Minich & Chavin, 1972.
	i.m.i. glucose (0.75g/kg)	→ 170 ± 30			
	Esox lucius				
	i.a.i. amino acid mixture (0.25g/kg)	266 ± 17 → 119 ± 10	3.0	9 - 24	Ince & Thorpe, 1975.
cholesterol (mg/100ml)	Anguilla anguilla				
	i.a.i. glucose (0.5g/kg)	458 → 365	1.5	24	Ince & Thorpe, 1974.
	i.a.i. amino acid (0.25g/kg)	470 → 370	9.0	24	

i.m.i. = intramuscular injection; i.a.i. = intra-arterial injection.

loads at similar temperatures. This anomaly probably arises as a result of the varied infusion sites used in the different studies (Table 8). Normally, there is a rapid and immediate decline in plasma glucose once the maximum hyperglycaemic condition has been achieved. This decline in glucose levels, known as the 'phase of equilibration', is followed by the 'phase of utilization' where the glucose is gradually metabolized by the tissues (Thorpe and Ince, 1974).

It is not clear what proportion of a glucose load may actually be excreted via the urine. This metabolite is normally absent from the urine of marine teleosts (Malvin et al., 1965). However, an increased renal excretion of glucose was detected in the goosefish, Lophius americanus, and in the hagfish, Myxine glutinosa, following glucose loads (Malvern et al., 1965; Falkmer and Matty, 1966). Only a very small proportion of a glucose load was lost via this route in the dogfish, Squalus acanthias, with 94-98% of filtered glucose being reabsorbed (Boylan and Antkowiak, 1966). Sato et al., (1967) reported that the yellow tail excreted uric sugar when fed a glucose-containing diet.

3b) Hormonal control of plasma glucose and fatty acids

The excretion of uric sugar, and the generally slow assimilation of plasma glucose following glucose loads probably reflect an inability of fish to assimilate this nutrient efficiently. The recovery to normoglycaemia is much slower than that observed in mammals (Thorpe and Ince, 1974; Ince and Thorpe, 1974), although the glucose doses administered to fish were generally higher than in the case of mammals (Bergot, 1979). However, the response of fish to glucose loads was considered to indicate a glucose intolerance due either to an insulin deficiency or normal adaptation to a diet low in carbohydrate relative to protein or fat content (Falkmer, 1961; Tashima and Cahill, 1968; Thorpe and Ince, 1974). This was supported by the finding that a glucose load was assimilated more rapidly if it had been preceded by an earlier glucose infusion (Wardle, 1972; Ince and Thorpe, 1974). It was likely that the first glucose load

TABLE 8 - PUBLISHED DATA ON GLUCOSE INFUSION STUDIES IN FISH

Species	Infusion site and glucose load	T° (°C)	*Hyperglycaemic peak (mg/100 ml) & time to attain (hr.)	% volume glucose diffusion space	*Delay to commence equilibration (hr.)	$t_{\frac{1}{2}}$ (hr.)	K (%/hr.)	Reference
Myxine glutinosa	i.m.i. (0.5g/kg)	14-18	115 (1.0 - 1.5)	-	1.0 - 2.0	-	-	Falkmer & Matty, 1966.
Salmo gairdneri	stomach (1 mg)	12-15	500 (0.5-1.5)	-	6.0 - 8.0	-	-	Palmer & Ryman, 1972.
Pleuronectes platessa	renal portal vein (0.13g/kg)	9	167 (0.5)	29.4	0.5 - 1.0	-	-	Wardle, 1972.
Esox lucius	heart (0.5g/kg)	10-15	305 (0.5)	45	0.5 - 1.0	12	-	Thorpe & Ince, 1974.
Anguilla anguilla	heart (0.5g/kg)	10-15	410 (0.5)	33	0.5 - 1.0	4.0	14.96	Ince & Thorpe, 1974.
	heart (0.25g/kg)	10-15	1st injection 2nd injection				16.19 58.58	
Platichthys flesus	i.p.i. (150mg/fish)	-	- (6.0)	-	6.0	-	-	Korsgaard et al., 1981.
Limanda limanda	i.a.i. (0.5g/kg)	15	310 (1.0)	22.6	6.0	4.0	16.0	Present study

i.m.i. = intramuscular injection; i.p.i. = intraperitoneal injection;

i.a.i. = intra-arterial injection. * Data taken from figs. and tables in respective references.

activated the glucose-metabolising enzymes so that the subsequent infusion was dealt with more efficiently. Furthermore, simultaneous- or pre-injection of fish with insulin also improved the removal rate of plasma glucose. This was thought to support the idea that fish may be insulin-deficient (Palmer and Ryman, 1972; Thorpe and Ince, 1974). Further evidence supporting this idea was presented by Yone (1978) and his co-workers. In a comparative study of glucose tolerance in the omnivorous carp, Cyprinus carpio, the highly carnivorous yellowtail, Seriola quinqueradiata, and the semi-carnivorous red sea bream, Chrysophrys major, the response of blood sugar to glucose load was highest in the yellowtail. Furthermore, the maximum plasma glucose response was greater when these species had been previously maintained on high (45%) rather than low (10%) carbohydrate diets (Yone, 1978; Shimeno et al., 1979, Furuichi and Yone, 1981). The latter authors also observed that the time to attain maximum hyperglycaemia and return to normoglycaemia was delayed in the order yellowtail, red sea bream and carp. Therefore, the yellowtail was the least tolerant to a glucose load. The above evidence clearly indicates that the response of fish to a glucose load is largely dictated by their normal dietary preference. This was further reflected in a comparison of the respective insulinogenic indeces of the above species. This index represents the ratio of the post-glucose increment of serum insulin level to the increment of blood sugar level, and is used as a measure of the diabetic condition. Yone (1978) reported the lowest ratio for the yellowtail followed by the red sea bream and the carp. This indicated that the yellowtail was the most 'diabetic' of the three species studied. In addition, the above species showed a lower insulin response to a glucose load when previously maintained on high carbohydrate diets. This fact probably reflects their lower resistance to such loads when maintained on high carbohydrate diets.

In view of the above evidence, Yone (1978) concluded that the species studied were potentially diabetic in nature and a low insulin secretion

resulted in a reduced carbohydrate utilization. It is apparent that higher circulating insulin levels are associated with the fed rather than the unfed state in fish. Furthermore, it is the amino acid or protein component of the diet that is most effective in inducing the rise in this hormone (Thorpe and Ince, 1976; Ahmad and Matty, 1975; Murat et al., 1981). This may reflect the importance of protein intake for maintenance of optimum growth in many species (Murat et al., 1981). Cowey et al., (1977) observed that a high protein diet had a greater insulinogenic power than a high carbohydrate diet in S. gairdneri. These authors concluded that glucose probably did not act as a signal for insulin release in this species. However, it is also the case that fish maintained on abnormally high carbohydrate diets do have a weaker insulinogenic capability (see Yone, 1978). Furthermore, Cowey et al., (1977) reported that the failure of S. gairdneri to maintain normal plasma glucose levels, on high carbohydrate diets, was partly due to an absence of an enhanced glucose uptake by the peripheral tissue. In addition, they did not observe any increase in glucose phosphorylating capacity of the liver. Both these latter points do reflect a weak insulinogenic power of glucose. It should be pointed out, however, that in an earlier study (Palmer and Ryman, 1972) both an increased liver glycogen biosynthesis and a reduced PFA level was recorded following glucose loads in S. gairdneri. Both these observations may imply an enhanced insulin activity induced by glucose.

It may be concluded that, while glucose is not a potent stimulator of insulin secretion, it still causes rises in the plasma level of this hormone. Even in highly carnivorous species like the yellowtail, considerable increases in plasma insulin occur in response to glucose (Furuichi and Yone, 1981). Ince and Thorpe (1977) measured a dose-dependent increase in plasma insulin levels following infusion of glucose loads directly into the blood circulation of A. anguilla.

Exogenous insulin normally depresses both plasma glucose and PFA, although there is some interspecific variation (Tables 9 and 10), some of

TABLE 9 - EFFECT OF HORMONAL TREATMENT ON PLASMA GLUCOSE IN FISH

Species	Treatment	Change in concentration	Reference
Carassius auratus	i.m.i. bovine insulin (1u/kg)	44.4±3.8→13.3±3.1 [a]	Minick & Chavin, 1972.
	i.m.i. Lung fish (Protopterus aethiopicus) insulin (1u/kg)	43.0±2.9→15.5±4.6 [a]	
Anguilla anguilla	glucagon (1.0mg/kg)	52.0±4.2→91.1±10.0 [a]	Larsson & Lewander, 1972.
Esox lucius	i.a.i. codfish insulin (2Iu/kg)	* 90 → 50 [a]	Thorpe & Ince, 1974.
	i.a.i. bovine insulin (2Iu/kg)	* 48 → 35 [a]	
	i.a.i. bovine glucagon (1mg/kg)	* 70 → 125 [a]	
Anguilla anguilla	i.a.i. cod insulin (2Iu/kg)	* 55 → 3 [a]	Ince & Thorpe, 1974.
Salmo gairdneri	High protein diet/ i.p.i. insulin (4u/kg)	1.56±0.33→1.56±0.17 [c]	Cowey et al., 1977.
	High carbohydrate diet/ i.p.i. insulin (4u/kg)	6.11±1.22→1.89±0.39 [c]	
Anguilla anguilla	i.a.i. glucagon (50µg/kg)	* 50 → 105 [a]	Ince & Thorpe, 1977.

i.m.i. = intramuscular injection; i.a.i. = intra-arterial injection;

i.p.i. = intraperitoneal injection. a) mg/100 ml. c) mmol/l.

* Data taken from figs. and tables in respective reference.

TABLE 10 - EFFECT OF HORMONAL TREATMENT ON PLASMA LIPIDS AND PROTEINS

Species	Treatment	*Change in concentration		Reference
1) FATTY ACIDS				
Carassius auratus	i.m.i. bovine insulin (1u/kg)	910±9.4→147±23.0	a	Minick & Chavin, 1972.
	i.m.i. Lungfish insulin (1u/kg)	910±9.4→229±124	a	
	i.m.i. Bonito insulin (1u/kg)	910±9.4→367±37	a	
	i.m.i. Ratfish insulin (1u/kg)	910±9.4→277±48	a	
Esox lucius	i.a.i. codfish insulin (2Iu/kg)	266 → 109	a	Ince & Thorpe, 1975.
	i.a.i. glucagon (2mg/kg)	266±17→329±18	a	
Anguilla anguilla	i.p.i. insulin (100Iu/kg)	Increase % palmitoleic acid and decrease % oleic acid		Dave et al., 1979.
2) CHOLESTEROL				
Anguilla anguilla	i.a.i. codfish insulin (2Iu/kg)	440 → 340	e	Ince & Thorpe, 1974.
3) PHOSPHOLIPID				
Carassius auratus	i.m.i. bovine insulin (1u/kg)	271±7.1→71±20	e	Minick & Chavin, 1972.
4) TOTAL PROTEIN				
Anguilla anguilla	glucagon (1.0mg/kg)	No change		Larsson & Lewander, 1972.
5) AMINO ACIDS				
Anguilla anguilla	i.a.i. bovine insulin (2Iu/kg)	3.8 → 1.6	e	Ince & Thorpe, 1974.
	i.a.i. codfish insulin (2Iu/kg)	3.5 → 1.8	e	
Esox lucius	i.a.i. bovine insulin (2Iu/kg)	6.3 → 2.2	e	Ince & Thorpe, 1975.
	i.a.i. codfish insulin (2Iu/kg)	7.6 → 2.6	e	
Salmo gairdneri	High protein diet/ i.p.i. insulin (4u/kg)	10.20 → 3.09	c	Cowey et al., 1977.
	High carbohydrate diet/i.p.i. insulin (4u/kg)	3.05 → 0.94	c	

i.m.i. = intramuscular injection; i.a.i. = intra-arterial injection;
i.p.i. = intraperitoneal injection. a) µeq/l. e) mg/100 ml. c) mmol/l.
*Data taken from figs. and tables in respective references.

which may derive from the dose rate or insulin specificity (Thorpe and Ince, 1974). Insulin stimulated the *in vitro* uptake of ^{14}c-glucose into liver glycogen of *Opsanus tau* (Tashima and Cahill, 1968). However, a decrease in this reserve was observed in *S. gairdneri* following insulin treatment and this hormone also promoted the oxidative clearance of glucose in this species (Ablett *et al.*, 1981 a,b). Lipogenesis was stimulated in liver slices of *Notemigonus crysoleucas* (Vlaming and Pardo, 1975).

The role of glucagon in metabolite homeostasis of fish is also not fully understood, particularly in the case of lipid metabolism. In higher vertebrates glucagon stimulates lipolysis, glycogenolysis and gluconeogenesis. However, its activity in fish is species-dependent (Epple, 1969). Glucagon injection in *A. anguilla* caused a marked hyperglycaemia but failed to incur any demonstrable lipolytic role in this species (Larsson and Lewander, 1972). There was, however, a significant rise in PFA following a high exogenous dose of this hormone into *Esox lucius* (Ince and Thorpe, 1975). Finally, both insulin and glucagon may interact with other hormones to modify plasma metabolite levels (Baker and Wigham, 1979).

The effect of other hormones, such as catecholamines and corticosteroids, and the manner in which they might interact with insulin to modify metabolite levels, have been discussed in Chapter 2. Less is known about the effect of growth hormone on plasma metabolites of fish. Mammalian growth hormone raised plasma glucose when injected into *Cottus scorpio* (Matty, 1962). Simultaneous ovine growth hormone and prolactin injections had no effect on PFA in *Oncorhynchus nerka* while this metabolite was increased following such treatment in *Carassius* (Minick and Chavin, 1970; McKeown *et al.*, 1975).

3c) Reciprocal plasma glucose/PFA relationship

Glucose loads have been observed to cause a reciprocal decline in PFA in higher (Dole, 1956) and lower (Farkas, 1967) vertebrates, including a

variety of fish species (Table 7). A variety of mechanisms have been established in the higher vertebrates through which glucose-loading may lower PFA (Steinberg and Vaughn, 1963). In general, these mechanisms reduce the rate of lipolysis and release of fatty acids from the adipose tissue. The effect was thought to be achieved by glucose through two basic mechanisms:

I. Triglyceride (TG) synthesis (lipogenesis) can be enhanced through stimulation of insulin secretion and a ready supply of glucose to the adipose tissue (Newsholme and Start, 1972). In this tissue, lipogenesis and lipolysis normally occur simultaneously. In addition to an enhanced TG synthesis, insulin can inhibit the lipolysis of preformed TG (Jungas and Ball, 1963).

II. The production of hormonal lipolytic substances such as growth hormone and glucagon may be inhibited by the action of glucose at distant sites (Unger et al., 1963).

Goodner et al., (1967) considered that most of the above mechanisms would be active following the large glucose loads normally used in such infusion studies. These authors provided evidence for a third basic mechanism, involving a glucose-sensitive centre in the brain, which regulates lipolysis in the fasting individual independently of insulin secretion. This mechanism is probably mediated by the sympathetic nervous system.

The relationship between glucose and lipid metabolism in fish tissues is perhaps not so well understood. The response of PFA and other lipid fractions to glucose infusions does vary between species (Table 7) and so the mechanisms involved in a glucose-associated PFA decline are unclear. This is particularly so when glucose is considered to be only weakly insulinogenic, in some species, in contrast to other substrates. However, clear glucose/PFA reciprocal relationships have been demonstrated in fish. Farkas (1969) observed the expected PFA depression after in vivo glucose treatment in C. carpio. In this fish under in vitro conditions, the adipose tissue from fish treated earlier with glucose produced less FA than

the tissue from starved animals, even although the rate of TG lipolysis was the same in both groups of animals. In the glucose-treated animals, a proportion of the FA was immediately re-esterified to TG instead of being released from the cells. At first hand this evidence would appear to support an insulin-mediated PFA depression, as observed in the higher vertebrates. This might be confirmed by the glucose-induced insulin increase that has been demonstrated in C. carpio (Furuichi and Yone, 1981). Highly carnivorous species such as S. gairdneri also exhibited a decline in PFA following glucose loads (Palmer and Ryman, 1972). However, in two species of carnivorous South American catfishes, Pimelodus maculatus and Arius felis, it was observed that the activities of the liver lipogenic enzymes were always low, regardless of the lipid or carbohydrate content of the diet, and could not be raised by insulin administration (Warman and Bottino, 1978). While the activity of lipogenic enzymes in other tissues was not monitored, it is likely that many species of fish would lie between the two extremes suggested for carp and these South American catfish.

Methods and Materials

Animals were cannulated as described in Chapter 2. Individual fish were monitored to follow the patterns of plasma glucose and PFA following ingestion of food or administration of metabolite loads.

1) Effect of feeding standard meals (as % bodyweight) of natural and artificial diets on plasma glucose

Dabs (101 - 200g) accepted 2% body weight meals of Mytilus edulis mantle (paper dried) voluntarily at three acclimation temperatures (5.5, 9.5 and 12.5°C). The blood-sampling frequencies varied according to the temperature and are indicated in fig. 1. Blood samples were analysed for glucose levels only. These experiments were performed during the period February to early March (1979) using shellfish collected at that time.

Similarly, the effect of feeding 2% body weight meals of freeze-dried

whiting tissue supplemented with M. edulis glycogen extract on plasma glucose level was also monitored at 15°C. Whiting fillets were freeze-dried and then ground up into a fine powder which would pass through a 600µ sieve. A moist paste containing 30% freeze dried whiting, 67.6% water and 2.4% glycogen extract was prepared and offered to the fish in the form of pellets which could be swallowed whole. The post-prandial glucose pattern was compared with that obtained for fish fed the whiting paste without the glycogen extract.

2) Effect of feeding satiation meals of whole Mytilus edulis tissue on plasma glucose and plasma fatty acids

Individual fish were voluntarily fed satiation meals of M. edulis tissue during the winter and summer seasons (viz. February-March and August-September respectively). The mussels, collected during the corresponding seasons, were blotted dry and fed whole to the fish which were allowed to eat freely for 10 minutes. During each season separate groups of animals were used for the respective glucose and PFA assays. Experiments were performed at 15.0°C during both seasons.

3) Effect of intra-arterial glucose injections on plasma glucose and plasma fatty acids

Individual fish, at 15°C, were injected with 125 µl saline containing glucose (0.5g/kg body weight) via the cannula. The glucose was administered by drawing the blood up the cannula and into a 1 ml syringe containing the dose solution. The blood was then immediately returned into the artery followed by the required volume of glucose solution. The cannula was then gently flushed with normal saline to ensure complete delivery of the glucose dose. Control injections of pure saline were administered to another group of fish. Separate experiments were performed to assay the immediate post-injection levels of glucose and PFA. The infusions were made over a period of 2-3 minutes and the biochemical assays were performed as in Chapter 2.

Results

1) Effect of feeding standard body weight meals on plasma glucose

(a) Ingestion of 2% body weight meals of M. edulis caused a gradual increase in blood glucose at all three temperatures (Fig. 1). The maximum glycaemia at 9.5 and 5.5°C was between 34-36 mg/100 ml at 14 and 23 hr respectively. Fish at 12.5°C developed a significantly higher glycaemia, 50 mg/100 ml after only 6 hr, than either of the other two groups. It was apparent that the rate of development of the hyperglycaemic condition and the subsequent rate of removal of the glucose from the blood circulation was modified by the acclimation temperature. The respective rates were enhanced with increasing temperature.

(b) The feeding of glycogen-supplemented meals at 15°C resulted in a similar rate of development of hyperglycaemia to that observed at 12.5°C (in 1(a) above) but continued to rise for a longer period, reaching maximum glucose levels of 78.22 ± 2.57 mg/100 ml in 12 hr (Fig. 2). Normoglycaemia was achieved 30 hr after ingestion. Fish fed the non-supplemented diets developed a slight but significant hyperglycaemia in 2-4 hr, returning to normal pre-feeding levels in 12-18 hr.

2) Effect of satiation meals of Mytilus edulis on plasma glucose and plasma fatty acids (PFA)

Figs. 3 and 4 show the post-feeding changes in plasma metabolities in fish collected in summer and in winter but maintained at 15°C. Individual animals ingested Mytilus tissue equivalent to about 6.0% of their body weight. Both groups developed a slow hyperglycaemia with maximum levels of 100.7 ± 13.0 mg/100 ml and 59.0 ± 12.0 mg/100 ml in the summer and winter groups respectively. Normoglycaemia was attained at about 60 hr post-feeding in the former group while the winter fish showed an earlier return to pre-feeding levels in 40-48 hr. PFA underwent a decline following feeding. However, there was a seasonal variation both in the duration of

Fig. 1 - Plasma glucose levels of Limanda after ingestion of 2% body-weight meals of Mytilus edulis mantle at (A) 12.5°C (n=6), (B) 9.5°C (n=6), and (C) 5.5°C (n=4). Fish weight 101-200g.

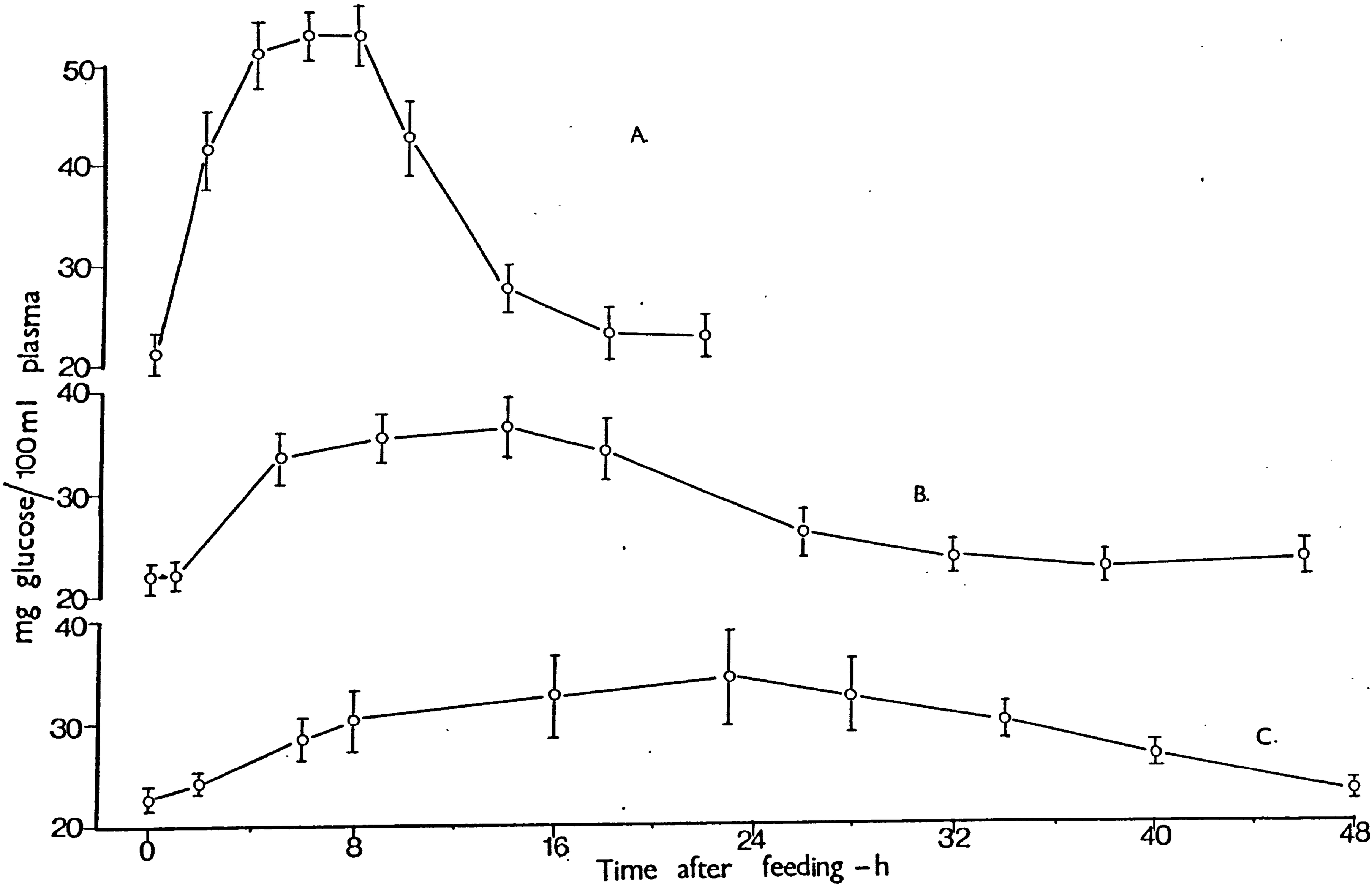

mg glucose/100ml plasma
50
40
30
20
A.
40
30
20
B.
40
30
20
C.
0
8
16
24
32
40
48
Time after feeding – h

Fig. 2 - Plasma glucose levels of Limanda after ingestion of 2% body-weight meals of freeze-dried whiting tissue containing 2.4% Mytilus glycogen extract o—o (n=10) and without glycogen supplement ●—● (n=5).

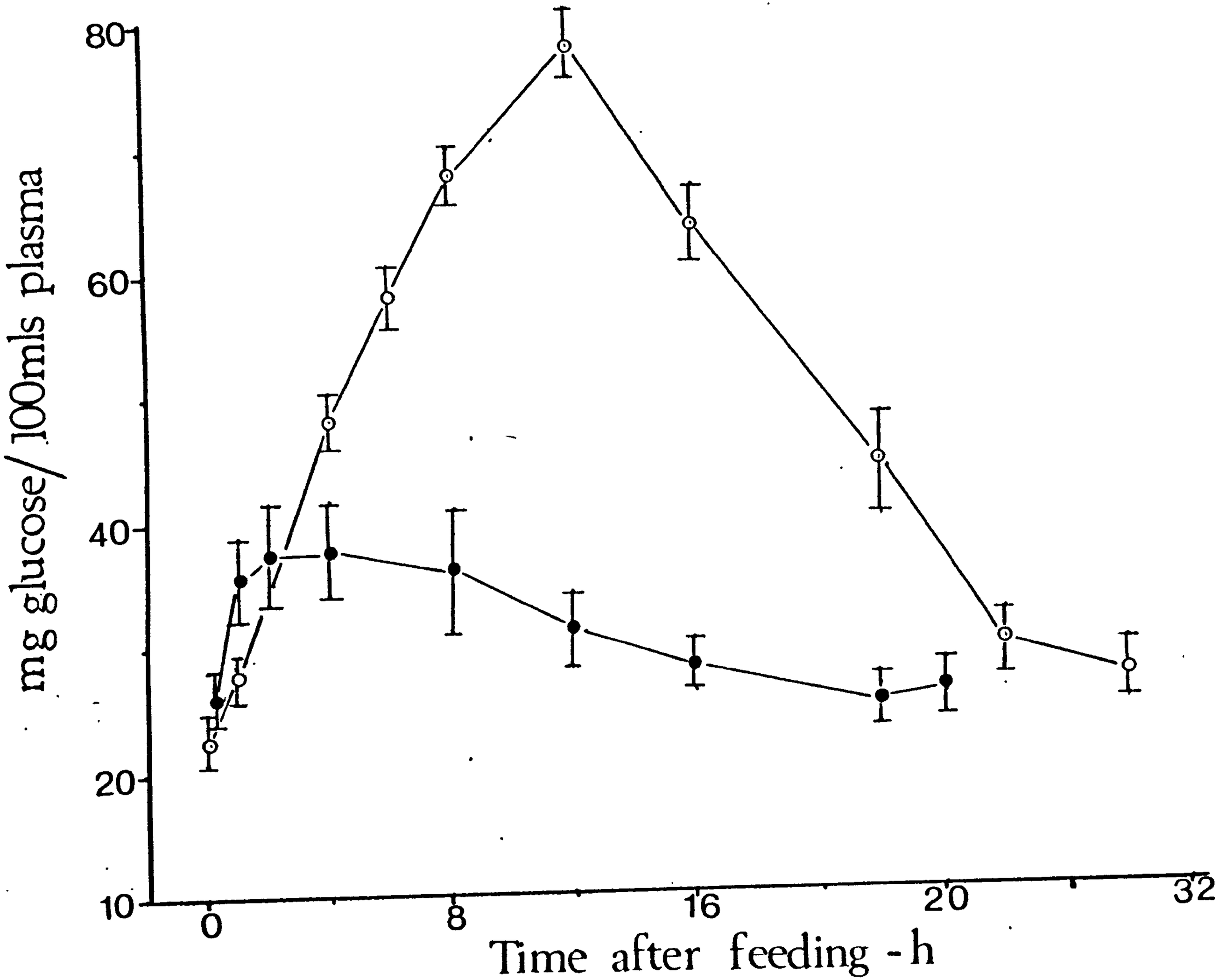

mg glucose/100mls plasma
80
60
40
20
10
0
8
16
20
32
Time after feeding - h

Fig. 3 - Plasma glucose o—o (n=6) and plasma fatty acid ●—● (n=5) levels of Limanda after ingestion of a satiation meal of Mytilus edulis tissue during the August/September period. Arrow indicates time of meal (= 6.3% body-weight) after cannulation. Fish weight = 171.2 ± 12.1g (mean ± S.E.). All points represent mean ± S.E.

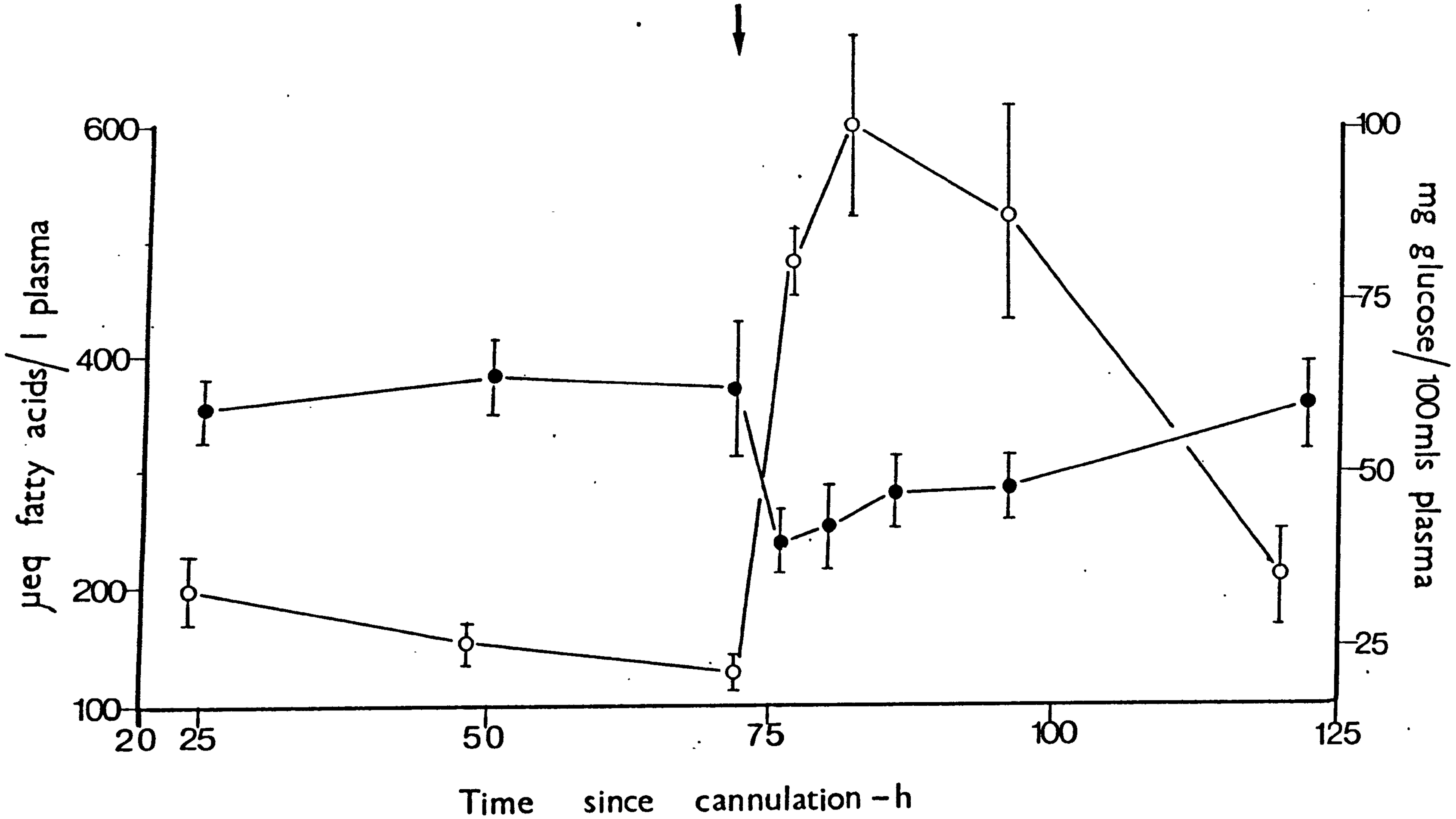
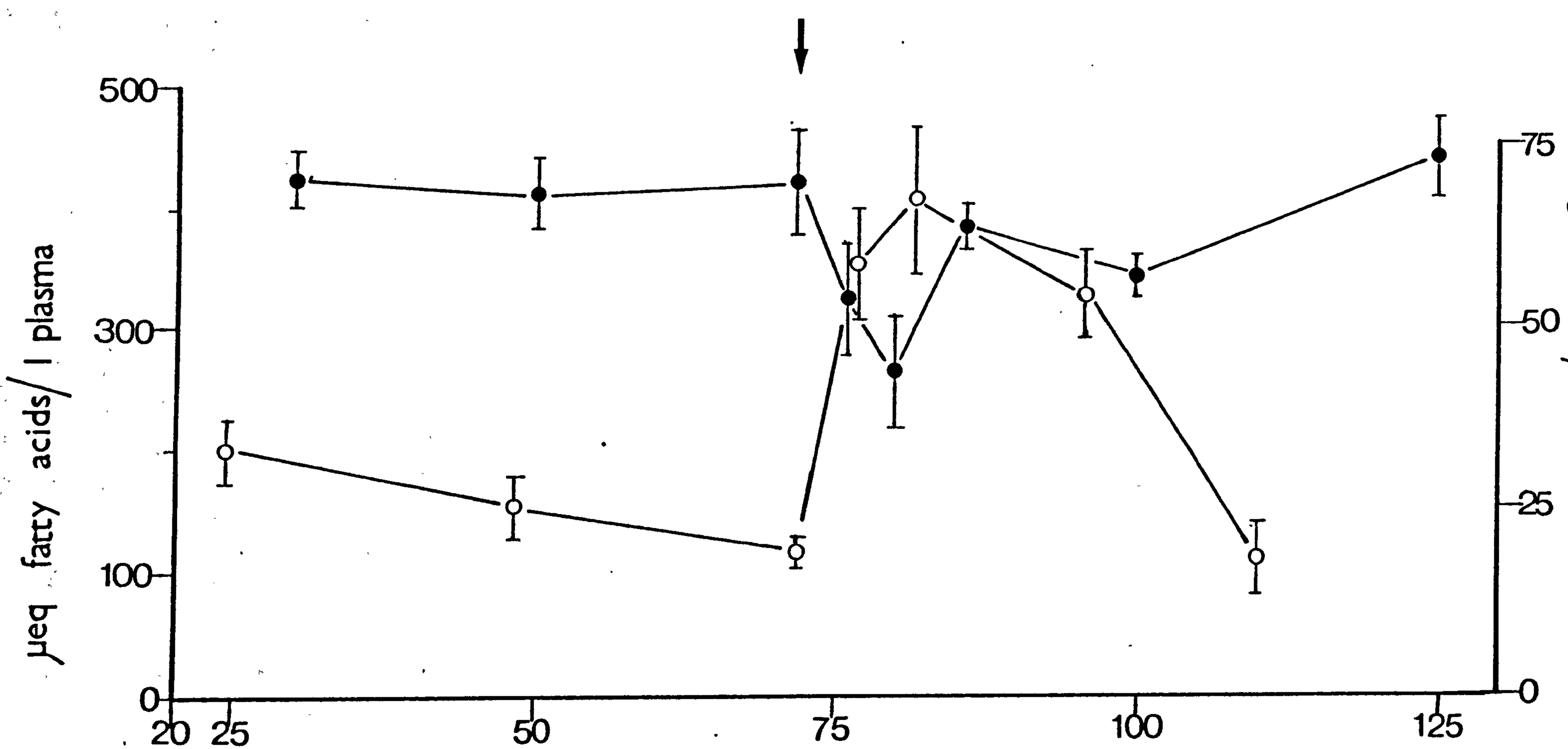
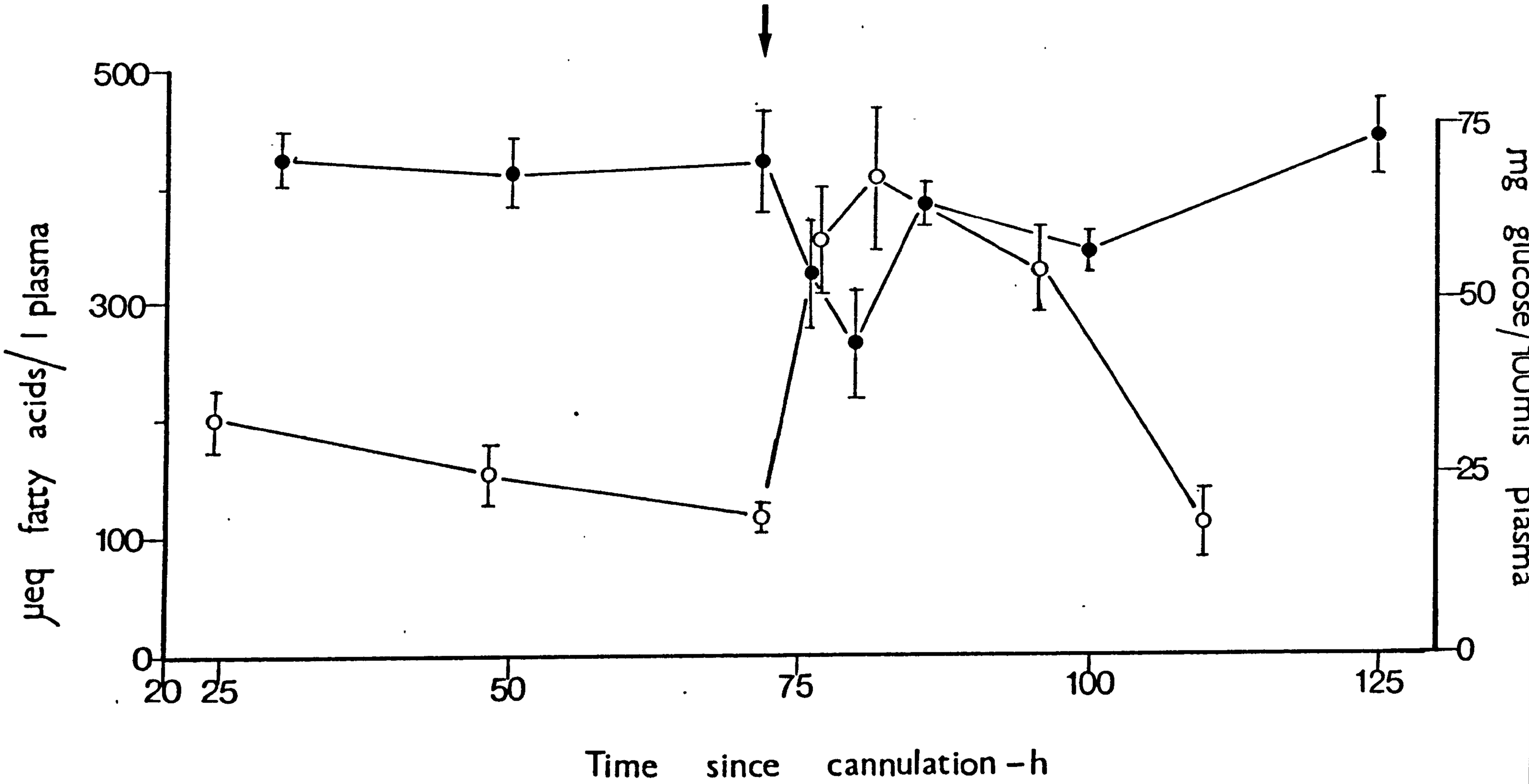

600
400
200
100
µeq fatty acids/ l plasma
100
75
50
25
mg glucose/ 100mls plasma
20
25
50
75
100
125
Time since cannulation – h

Fig. 4 - Plasma glucose o—o (n=6) and plasma fatty acid ●—● (n=6) levels of Limanda after ingestion of a satiation meal of Mytilus edulis tissue during the January/February period. Arrow indicates time of meal (= 6.3% body-weight) after cannulation. Fish weight = 198.0 ± 5.6g (mean ± S.E.). All points represent mean ± S.E.


500
300
100
0
20
25
50
75
100
125
75
50
25
0
µeq fatty acids/l plasma
Time since cannulation – h


500
300
100
0
μeq fatty acids/ l plasma
75
50
25
0
mg glucose/100ml plasma
20
25
50
75
100
125
Time since cannulation – h

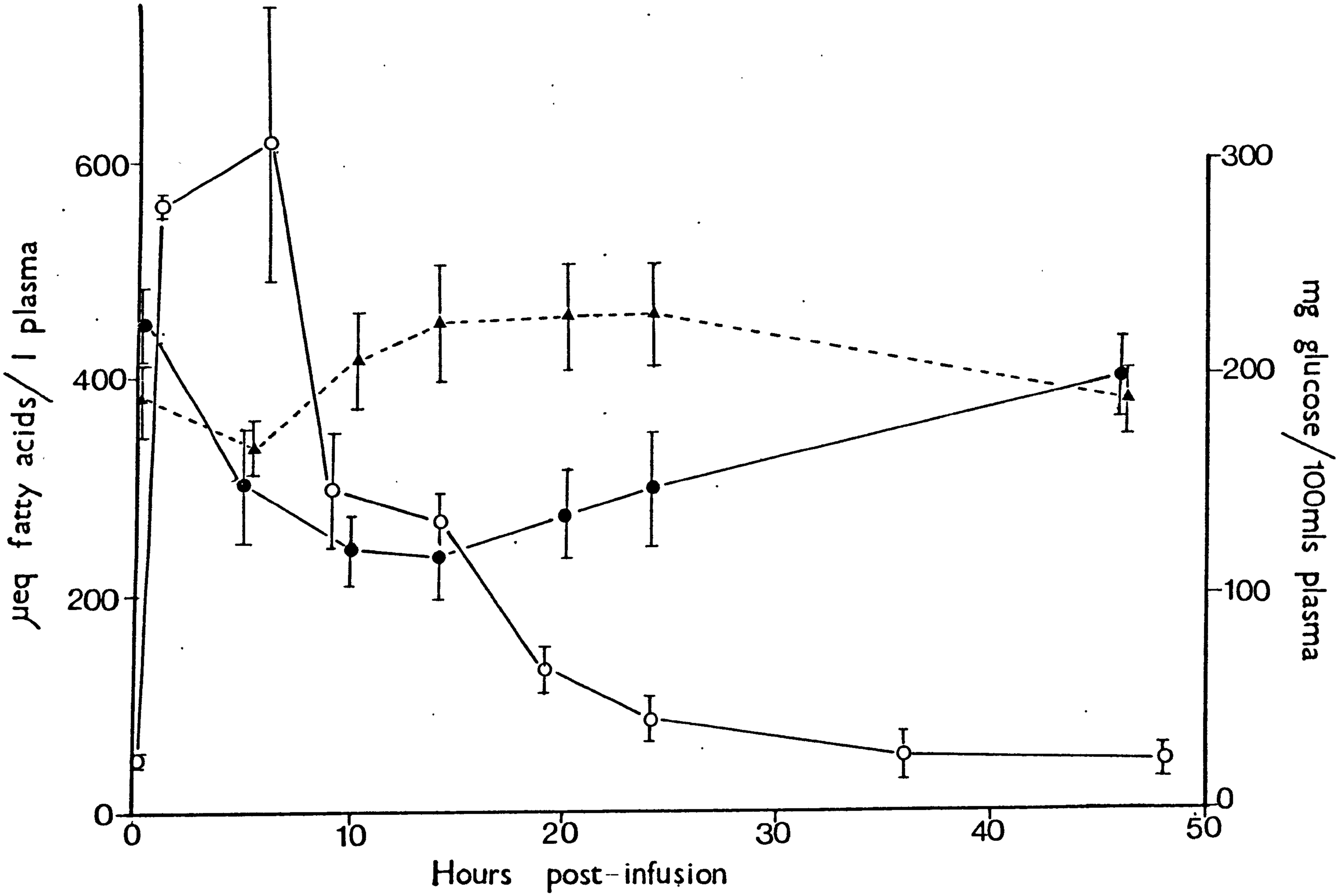
the depressed PFA levels and the initial rate of decline. In the winter fish PFAs were significantly lower 8 hr after feeding ($P < 0.05$) but had returned to normal some 6 hr later (14 hr post-feeding). In contrast, the summer fish showed a significant decline in PFA 4 hr after ingestion of the meal. Levels did not alter significantly in the following 20 hr but had returned to normal some 50-55 hr after feeding. A seasonal comparison of the PFA peaks 14 hr after feeding shows that at this time the winter fish exhibited quite significantly higher concentrations than the summer fish.

3. Effects of intra-arterial glucose injection (0.5g/kg) on plasma glucose and plasma fatty acids (PFA)

The reciprocal relationship between post-prandial plasma glucose and PFA concentrations suggested that the elevation in glucose levels may be responsible for the observed decline in PFA. Infusion of a glucose load of 0.5g/kg body weight resulted in an immediate and gross hyperglycaemia in 1 hr (Fig. 5). This glycaemia (280.6 ± 4.8 to 310.4 ± 64.7 mg/100 ml) compares with the maximum glucose level of 78.22 ± 2.57 mg/100 ml observed in fish fed 2% freeze-dried whiting meals supplemented with glycogen extract (glycogen at 2.4% level ≡ oral glucose dose of ≅0.5g/kg body weight). The hyperglycaemia following intra-arterial glucose loading was maintained without a significant change for 5 hr whereupon there was a rapid decline in concentration to 148.4 mg/100 ml by 9 hr post-injection. This latter period termed by Thorpe and Ince (1974) as the "phase of dilution of the load in its final volume of distribution" was followed by a more gradual decline, the "assimilation phase" of the load, to normoglycaemia. The latter condition was just reached by 24 hr post injection.

By extrapolation of the linear portion of the exponential curve to zero time (log of the mean excess blood glucose value vs. time), the rate of glucose assimilation (K), the half-life ($t_{\frac{1}{2}}$) period of the load and

Fig. 5 - Effect of intra-arterial glucose (0.5g/kg body-weight)/saline (125 µl) infusion on plasma glucose (o—o) and plasma fatty acids (●—●), and intra-arterial saline (125 µl) infusion alone on plasma fatty acids (▲—▲). Points represent mean ± S.E. of 3 groups of 6 fish each.


600
400
200
0
μeq fatty acids/ l plasma
300
200
100
0
mg glucose/100mls plasma
0
10
20
30
40
50
Hours post-infusion

the glucose diffusion space were estimated. In calculating the diffusion space it was necessary to correct for the considerable post-infusion delay (6 hr, see Fig. 8) before the glucose load had fully equilibrated.

For Limanda K, $t_{\frac{1}{2}}$ and the diffusion space were calculated as 16.0% h^{-1}, 4.0 hr and 22.6% respectively. These values do not differ strikingly from the data of Ince and Thorpe (1974) who recorded K, $t_{\frac{1}{2}}$ and diffusion space values of 14.96% hr^{-1}, 4.0 hr and 33% respectively for Anguilla (10 - 15°C).

Monitoring of PFA patterns following a glucose load revealed a significant decline, from a pre-injection level of 450.8 µeq/l to 305.5 µeq/l in 5 hr. Levels continued to decrease and showed a 47% reduction 14 hr post-injection. Levels were still significantly lower after a further 10 hr but returned to normal after 46hr. While control infusions of saline caused some fluctuation of PFA levels, these changes were not significant.

Discussion

The original aim of this section was to observe whether the ingestion of food caused significant changes in circulating metabolites which might, directly or indirectly, generate signals to modify subsequent feeding behaviour. The timing of such metabolite changes could themselves indicate whether the return of appetite is closely linked with such systemic factors. As will become apparent, while the simplistic nature of the experimental design does provide relevant information on this subject, it does in itself raise a number of complex biochemical questions far beyond the original aim of the study. Before considering the possible relationship between plasma nutrients and feeding behaviour, an attempt will be made to discuss the plasma metabolite patterns in relation to other vertebrate studies on carbohydrate and lipid metabolism.

1) Effects of feeding on plasma glucose and plasma fatty acids (PFA)

It is clear that the ingestion of both synthetic meals and Mytilus

significantly modifies the immediate post-prandial levels of both plasma glucose and PFA. Meals of Mytilus mantle caused moderate rises in plasma glucose. The rate of removal of excess glucose from the plasma appears to be temperature-dependent; the rate increasing with higher acclimation temperature. Apart from the fact that digestion and absorption must increase relative to sequestration and metabolism, the reason for the greater level of glycaemia at 12.5°C is not clear. The magnitude of the hyperglycaemia is known to be modified by the carbohydrate level of the diet (Bergot, 1979; present study). However, the experiments at the three different temperatures were performed when the carbohydrate reserves of Mytilus are normally stable (Gabbott and Bayne, 1973). The slow rise to maximum hyperglycaemia in all feeding studies agrees with the findings of Bergot (1979).

Limanda is essentially a carnivorous species. A large proportion of its diet is comprised of bivalves, gastropods and polychaete worms (Gwyther, 1978) all of which have appreciable glycogen reserves. This suggests that Limanda would possess the necessary enzyme complement for the digestion and metabolism of dietary glycogen. This is supported by the highly significant hyperglycaemic condition that develops following glycogen-supplemented meals.

1(a) Estimation of proportion of dietary glycogen that appears in the peripheral circulation

An approximate estimation of the proportion of assimilated dietary glycogen (experiment 1, Methods and Materials) that appears in the peripheral circulation can be calculated. Two manipulations of the observed blood glucose levels were used. Points on the decreasing limb of the curve in Fig. 2 were used in a semi-logarithmic plot to estimate the instantaneous rate of decrease (D). Over the whole experiment, the amount of glucose transferred to the blood can be estimated from the blood glucose concentration. Using the equation of Elliot and Persson (1978),

originally developed to estimate gastric evacuation rates in fish, the amount of glucose transferred in each time interval is given by:-

$$T_g = \frac{(S_t - S_o\, e^{-Dt})\, Dt}{1 - e^{-Dt}}$$

where S_o and S_t = glucose concentrations at start and end of a time interval (t) respectively. This method assumes that

I) the instantaneous rate of glucose absorption from the intestine is constant within each time interval and

II) the rate may reset to a different level as time passes after feeding.

The total amount of glucose transferred from the gut to the blood in 33 hr is estimated as 1.36 mg/ml. On the assumption that ≅ 70% of the fish is water (Baksh - pers. comm.) of which ≅ 3% is represented by blood, a 167g fish would contain ≅ 3.51 ml blood. Therefore, the total glucose transferred in 33 hr. = 4.79 mg. Allowing for the glucose derived from the whiting tissue = 0.8 mg (calculated in an identical manner from the glucose data following 2% meals of freeze dried whiting), the total glucose derived from the glycogen supplemented meal, as represented by the peripheral levels, was 3.99 mg. At a dietary glycogen level of 2.4% (wet wt.), a 167g fish would have received about 40.8 mg glycogen. Therefore, the total glucose absorbed from the meal, as represented by the peripheral measurements, represents only 9.8% of the Mytilus glycogen ingested.

The negligible proportion of the glycogen represented by the peripheral blood glucose levels would suggest either a rapid removal of glucose at specific sites, such as the hepatic tissue, or a very weak amylolytic activity in the intestine of this species. It is feasible that a considerable proportion of the blood glucose may undergo glycogenesis in the liver before reaching the peripheral circulation. Equally, a weak amylolytic activity in the gut would be in accordance with the findings for other carnivorous species (see Introduction). The cod, Gadus morhua, has only low amylolytic activity (Overnell, 1973) and Cowey et al., (1974) report a similar finding for marine flatfish.

1(b) Effect of satiation meals of Mytilus on plasma glucose and plasma fatty acids (PFA) during different seasons

Satiation meals of Mytilus caused a significant rise and fall in both plasma glucose and PFA respectively in the summer and winter groups of fish. In each case it is clear that the respective metabolites return to their pre-feeding levels at approximately the same time. Interestingly, the post-prandial fluctuations in the plasma metabolites were of far shorter duration in the winter animals. Since the two groups of animals were fed with Mytilus collected during the respective seasons, it is likely that the biochemical composition of their respective meals differed. The early autumn glycogen reserves of Mytilus have been shown to be more than 500% higher than the winter levels (Gabbott and Bayne, 1973). Although the winter group of fish ingested exactly the same amount of Mytilus as the summer animals, their maximum recorded glycaemia was about 40% lower. In addition, the plasma glucose remained elevated above pre-feeding levels for nearly twice as long in the summer fish. These observations do indicate that the Mytilus fed to the winter fish probably contained lower carbohydrate reserves. It has been shown in the previous section that the addition of glycogen to test meals significantly elevates the post-prandial glucose levels of the blood.

The reciprocal plasma glucose/FA relationship will be discussed later in more detail.

2) Effect of short-term food deprivation on plasma glucose and plasma fatty acids (PFA)

In Chapter 2 the post-operative plasma metabolite levels were monitored daily for six days to assess the immediate effects of short-term cannulation and brief starvation (Table 9, Chapter 2). Short-term deprivation had no effect on plasma glucose levels in Limanda, which agrees with the response described in several other teleosts (Ince and Thorpe, 1976; Woo and Cheung, 1980). It appears that Limanda can regulate blood glucose quite efficiently

when deprived of food for short periods, possibly by an increased rate of hepatic gluconeogenesis. PFA levels rose significantly above the 'normal' 72 hr post-operative level after about 92 hr without food. They were maintained at this elevated level during the next 59 hr. This suggests that, in Limanda, lipid reserves are mobilized during short deprivation periods as a source of energy. This is similar to the response of S. gairdneri after only 120 hr starvation (Bilinski and Gardner, 1968).

Under the experimental conditions described in Chapter 2, Limanda was observed to have two seasonal peaks in PFA. It was suggested that the post-spawning peak may be related to a re-absorption of gonadal material. The second peak, in December, is likely to be associated with the mobilization of lipid reserves in relation to vitellogenesis. However, it is interesting that Gwyther (1977) recorded a period of natural 'fast' in Limanda collected during the vitellogenic period and from the same locality (January, 1976). He related this 'fast' to a seasonal scarcity of normal prey species. In view of this evidence it is possible that the winter mobilization of lipid reserves is influenced jointly by the shortage of food and reproductive processes.

3) Effect of glucose infusion on plasma glucose and the glucose/fatty acid reciprocal relationship

The infusion of glucose into individual fish was performed in an attempt to understand the reciprocal glucose/FA relationship observed in the feeding studies. It is appreciated that the glucose load infused (0.5g/kg body weight) resulted in physiologically abnormal glycaemic states. However, there were a number of unknown factors in this study, such as the influence of the infusion site on the general distribution of the load.

3(a) Effect of glucose infusion on plasma glucose

The gross hyperglycaemic condition which developed in Limanda after a

glucose infusion and the slow return to normoglycaemia is the normal response of teleosts to such treatment. As in other studies the maximum hyperglycaemic response was achieved within 1.0 hr. Surprisingly, this condition was maintained for at least another 5.0 hr, a response which is not normally observed following glucose infusions into the blood circulation of fish (Table 7). Usually, once the maximum glycaemic condition has been achieved there follows a rapid decline reflecting the 'equilibration phase'. An examination of the various infusion sites used by different workers reveals that glucose loading *via* the renal portal vein (Wardle, 1972) or heart (Thorpe and Ince, 1974) results in the onset of the equilibration phase soon after the maximum hyperglycaemia. Glucose infusion into the peritoneal cavity (Korsgaard *et al.*, 1981) or stomach (Palmer and Ryman, 1972) appears to incur some delay before the complete load enters the circulation. This ultimately prolongs the onset of the 'equilibration phase'. In the present study, although the glucose was injected directly into the caudal artery, the entire load would not immediately pass into the general circulation. Initially, the glucose would have to pass through the capillary bed of the body musculature, posterior to the site of infusion, prior to entering the caudal and renal portal vein system. The delay to the onset of the 'equilibration phase' in *Limanda* may, therefore, be explained by a limited transfer rate of the glucose to the general visceral circulation.

The assimilation rate and half-life period of the glucose load in *Limanda* are in close agreement with the findings for *Anguilla* (Ince and Thorpe, 1974) although in comparison with the data for *Esox* (Thorpe and Ince, 1974), *Limanda* would appear to deal more efficiently with an equal glucose load. This may reflect some physiological adaptation of *Limanda* to an invertebrate diet which can contain significant amounts of carbohydrate. This conclusion is supported by the more rapid utilization of a glucose load by the omnivorous carp in contrast to the highly carnivorous yellowtail

(Furuichi and Yone, 1981).

3(b) The glucose/FA reciprocal relationship

A concurrent decline in PFA occurred following both a glucose infusion and ingestion of a Mytilus meal. In both instances the PFA level showed a reciprocal relationship with glucose levels (Figs. 3, 4 and 5) although other metabolites, such as amino acids, were not monitored in the latter study. A number of mechanisms have been suggested to explain this relationship in higher vertebrates (see Introduction) and have also been discussed in some teleost studies (Farkas, 1969). Probably, an insulin-mediated antilipolytic response is implied, since glucose infusions have been demonstrated to stimulate pancreatic activity of fish. This may be particularly so in these feeding studies since glucose and amino acids may have a synergistic effect on insulin secretion (Patent and Foa, 1971; Ince and Thorpe, 1977).

In view of the present knowledge relating to the control of plasma glucose and PFA in fish, it is not clear whether the glucose/FA relationship can indeed be explained along similar lines as those for higher vertebrates. However, a glucose/FA reciprocal relationship has been clearly demonstrated. In addition, increases in plasma insulin are generally associated with the fed state in fish. Therefore, the following explanation of the post-prandial PFA pattern in Limanda is offered on the tentative assumption that lipid metabolism in fish is modified by glucose availability and plasma insulin levels.

In the higher vertebrates, the conversion of FA to TG in the tissues depends upon a supply of glycerol phosphate. This substrate is largely derived from glucose, so it might be argued that TG synthesis would be stimulated when glucose availability is increased by the elevated circulating insulin (Newsholmeand Start , 1972). In Limanda, the degree of hypolipaemia was similar during both seasons, although the duration of this condition was far longer in the summer fish. This would indicate that,

although the plasma glucose was not elevated to the same extent in the respective groups, there was an equivalent antilipolytic effect at some stage after feeding. This may suggest that the maximum plasma insulin levels attained by the respective groups of fish were similar in each instance. Ince and Thorpe (1977) observed that while insulin secretion in Anguilla was dose-dependent, an increase in the maximum level of this hormone was not achieved above a certain plasma glucose level. Interestingly, they also noted that the maximum plasma insulin level was achieved faster at the higher glucose doses. This may explain the more rapid decline of PFA in the summer fish when higher plasma glucose levels were also witnessed.

The earlier return of PFA to pre-feeding levels in the winter fish may simply reflect the shorter period during which an elevated glucose supply is available to the tissues to modify the esterification rate. The duration of elevated plasma glucose does not necessarily reflect the period of increased plasma insulin levels in fish (Ince and Thorpe, 1977). Irrespective of this fact, the earlier return to normoglycaemia in the winter fish may also have meant a shorter period of enhanced insulin activity. This might help to explain the more brief hypolipaemic response observed in these fish. However, examination of the SDA pattern, following satiation meals of Mytilus, shows that the respiratory rate is elevated far beyond the time when PFA levels have returned to normal. This indicates that plasma amino acids may remain elevated long after the plasma glucose and PFA have returned to pre-feeding levels. Amino acids are generally more powerful insulin releasors than glucose. In addition, Ince and Thorpe (1977) pointed out that certain amino acids also stimulate secretion of growth hormone and glucagon. Both these latter hormones promote insulin secretion. Consequently, it is doubtful whether the post-prandial PFA pattern of the winter fish is due to an earlier decline in plasma insulin levels. At the same time, it is appreciated that any enhanced insulin secretion, due to a synergistic action of glucose and amino acids (Ince and

Thorpe, 1977), would almost certainly operate for a shorter period of time in the winter fish.

Finally, the summer and winter feeding experiments were performed during the resting and peak vitellogenic periods respectively. Even though the two groups of fish were maintained at the same temperature, it is possible that their physiological states could have accounted for the different post-prandial metabolite patterns observed. Pre-spawning increases in plasma insulin levels have been recorded in some species (Plisetskaya et al., 1976). Such hormonal changes during the vitellogenic period may allow for a more rapid utilization of dietary nutrients.

In conclusion, the reciprocal plasma glucose/FA relationship observed in Limanda compares with the findings for the higher vertebrates following food intake. This only implies that similar mechanisms may be responsible. In the higher vertebrates, the reciprocal glucose/FA relationship demands that the dietary FA are esterified to TG in the gut wall prior to absorption into the blood circulation from the lymphatic system. This process effectively 'conceals' the FA from various tissues (adipose) which will then be stimulated to take up blood glucose in the presence of elevated insulin levels. This ultimately enhances the re-esterification of tissue FA, so that there is a fall in plasma levels of this metabolite. The form in which dietary lipids are absorbed into the blood circulation in fish is not fully understood. Robinson and Mead (1973) suggest that dietary lipid may be absorbed predominantly as free fatty acids (FA). If this is the situation for teleosts in general, then the reciprocal glucose/FA pattern observed in Limanda would be more difficult to explain along the same lines as those for higher vertebrates. However, some caution is necessary when interpreting the data of Robinson and Mead (1973) (see Introduction). Furthermore, Kayama and Iijima (1976) present evidence which demonstrates that the post-prandial composition of plasma lipid fractions is modified by the nutritional history of the fish. Initially, after

feeding a labelled lipid meal to Cyprinus carpio, there was a greater incorporation of the lipid into the PFA fraction in those fish which had previously been deprived of food. Fish which had not been starved, prior to being fed the experimental meal, exhibited a lower incorporation of lipid into the PFA fraction. In this instance, there was a more rapid appearance of dietary lipid in the plasma TG fraction. Kayama and Iijima (1976) concluded that the passage of dietary lipid into the circulation involves two distinct pathways: a) the direct release of FA without esterification and b) the discharge of esterified forms such as TG and phospholipids after esterification and incorporation into lipoproteins. The predominance of either system would depend upon the nutritional state of the fish. Low density lipoproteins have been observed in trout intestinal cells, intercellular spaces of the lamina propria and the blood capillaries that drain the intestine (Bergot and Fléchon, 1970; Leger et al., 1979). These authors considered that these particles left the intestine via the portal route and lymphatic system.

In the present study, the fish were only deprived of food for a sufficiently long period to empty the intestine. In addition, they were fed a mixed nutrient meal so that the necessary precursors for re-esterification of FA in the intestinal wall should have been available. In view of the evidence of Kayama and Iijima (1976) and Leger et al., (1979) it is feasible that, during the initial stages of digestion of the meal, dietary lipid is absorbed as FA into the blood circulation. However, as digestion proceeds dietary FA are re-esterified to TG and incorporated into lipoproteins, in the intestinal wall, prior to release into the circulation. Such a system would allow an increased uptake of blood glucose in peripheral tissues thereby enhancing the re-esterification of tissue FA. This would explain the observed decline in PFA after the ingestion of food by Limanda.

Chapter 4

Section I

Control of appetite in

Limanda limanda

Introduction

One of the principal techniques employed to study feeding behaviour in teleosts has involved examination of their voluntary food intake using demand feeders. Adron (1972) designed an automatic demand feeder which he used to condition groups of S. gairdneri to operate a simple trigger so as to receive a food reward. This system has proved useful in various studies of fish behaviour. These include food preferences, learning capacity and circadian feeding rhythms, in addition to hierarchial and territorial behavioural studies (Adron et al., 1973; Loizides, 1975; Landless, 1976). Jobling (1974) and Gwyther (1978) trained groups of Limanda to operate demand feeders and observed feeding rhythms under natural photoperiodic or continuous lighting conditions. The latter author also demonstrated the effects of fish size, temperature, "diluted" diets and autonomic drugs on the feeding behaviour of this species. Other species of marine flatfish, including P. platessa and the turbot, Scothphalamus maximus, have also been successfully trained to operate demand feeders (Grove, unpubl; Moctezuma, 1982).

Earlier research in higher vertebrates indicates that considerable caution is required when using demand-feeding systems in behavioural studies. This applies both to the experimental design and subsequent interpretation of the feeding patterns. This has become especially evident during demand feeding studies with the rat. Le Magnen and Tallon (1966) found that the size of meals was reliably correlated to post-prandial intervals (intermeal-period) but not to the pre-prandial interval. This suggested that the amount of food eaten at each feeding bout was not controlled by a time dependent depletion signal (e.g. some factor indicating state of energy reserves or energy supply rate) but that the amount eaten did determine how long hunger was quelled. However, Hirsch and Collier (1974) and Panksepp (1976) question the validity of this post-prandial relationship on statistical grounds. Panksepp (1973, 1976) believes that the method of data analysis used by Le Magnen and

Tallon (1966) tends to reduce the normal variability of intermeal intervals. He also points out that a number of workers have subsequently failed to detect the correlations claimed by Le Magnen and Tallon (1966). Panksepp (1976) did note that some animals do show reliable post-prandial correlations but that this only occurred under certain experimental conditions, e.g., where animals are required to expend some moderate effort to obtain food, or when fed on diluted diets. Panksepp (1973) suggested that the post-prandial correlation may be characteristic of a depletion type pattern of feeding under such conditions. Diluted diets, or moderate effort to obtain food, may enhance the likelihood of animals regulating at the "depletion (or lower) end of their normal body-weight regulatory range". Furthermore, Panksepp (1976) believes that the above experimental conditions, which increase correlations between meals and post-meal intervals, also reduce the diurnal variability of feeding normally practised by the rat. Therefore, the correlation may be due to "a normalizing effect of this kind of feeding pattern on the typically bimodal distribution of intermeal intervals".

Panksepp (1976) concluded that "while satiety can be correlated with the amount ingested (in only a small percentage of animals tested and under restricted experimental conditions) the phenomenon is too weak to ascribe any important overall regulatory meaning to it. Certainly, it cannot be used as a precise and reliable dependent measure in studies of energy balance regulation". It is true that systematic regulating patterns have been abstracted from meal-taking data for teleosts, as well as higher vertebrates. However, because of the variety of factors that may modify these feeding patterns, Panksepp doubted whether such studies would reveal the manner in which meals are terminated or initiated. Some of the most likely factors to influence the ad. lib. feeding patterns of fish in the laboratory may include their social setting, the strength of competing motivations, activity level, arousal state and the degree of food availability. These factors have to be considered when interpreting feeding patterns,

before the possible influence of nutrient composition, or dietary energy on feeding behaviour can be deduced.

Materials and Methods

1. Effect of dietary composition on voluntary food intake

Groups of 6 fish (60-85g) were trained to bite or nudge a hand-held, p.v.c. (red colour)-coated platinum wire which was to be later incorporated as the trigger mechanism in the demand feeder. Each time a fish touched the wire intentionally a few pellets of a synthetic diet (see below) were dropped into the water. The training sessions were repeated 3 to 4 times each day until the desired association had developed between the trigger and food for all members of the group. This situation was usually reached within sixteen days, whereupon the demand feeders were put into operation. The demand feeders were constructed according to the design of Adron (1972). The trigger mechanism was so arranged that food was delivered no matter from which direction the trigger was deflected. The exposed regions (contact points) of the platinum wire were polished with fine sand-paper and the whole trigger-mechanism was sprayed with WD-40 (Cadulac Chemicals Ltd.) to reduce corrosion and maintain a good contact between the electrical points. This reduced the amount of necessary maintenance and hence the disturbance to the animals. Activation of the trigger completed an electrical circuit and released a known quantity of food into the water. A plastic loop (20 cm in diameter), suspended from the feeder and floating on the water surface around the trigger, prevented any floating food-pellets escaping *via* the over-flow pipe.

An event recorder was connected to the trigger circuit such that the number and frequency of actuations could be monitored. The fish were maintained under a 24 hr lighting regime so that feeding behaviour could be monitored in the absence of photoperiodic cues.

Preparation of diets

The synthetic diet was based on the formula of Cowey et al., (1975). Prior to adding the lipid fraction of the diet, the remaining components were mixed dry in the proportions shown in Table 1. Sufficient water (50ml/100g dry diet) was added to obtain a stiff paste to which the cod-liver oil was then added. The mixture was then subjected to a 10 minute homogenization to ensure an even distribution of the dietary nutrients and vitamins. Finally, the paste was compressed, with a pallet knife, into 0.25x0.25x30 cms. grooves cut into Perspex plates. The diet was then dried at 30°C for 24 hr. The dried strands of diet were then simply cut into approximately 0.5 cm long pellets and frozen at -20°C.

Initially, casein was used as the protein source but this proved to be totally unacceptable to the fish and had to be substituted by freeze-dried whiting muscle (see Chapter 1 for preparation).

1a) Analysis of data

The periodogram analysis of Enright (1965), which was further developed by Williams and Naylor (1968), was used to analyse the actuations recorded by the event recorder. The number of actuations in each succeeding hour were scanned for possible rhythms ranging from 4-hourly to 28-hourly feeding bouts. For instance, to test for feeding every f hours, the data was arrayed as:

$$\begin{array}{cccc} x_1 & x_2 & \cdots & x_f \\ x_f + 1 & x_f + 2 & \cdots & x_{2f} \\ \hline \Sigma X_1 & \Sigma X_2 & \cdots & \Sigma X_f \end{array}$$

From this array ('form estimate') the mean

$$= \left(\frac{\Sigma X_1 + \Sigma X_2 + \cdots \Sigma X_f}{f}\right)$$

and its standard deviation was calculated. Lack of rhythmicity at frequency

TABLE 1 - COMPOSITION (g/100g DRY DIET) OF SYNTHETIC DIETS USED IN DEMAND-FEEDING EXPERIMENTS

	Control diet	Test diets		
		1	2	3
Cellulose	22	22	22	22
Freeze-dried whiting	30	30	30	30
Cod-liver oil	20	20	12	5
Dextrin	25	5	25	25
Kaolin	0	20	8	15
Vitamin mixture†	2.7	2.7	2.7	2.7
Mineral mixture#	0.3	0.3	0.3	0.3

†To provide per 100g dry diet: thiamine hydrochloride 6 mg, riboflavin 20 mg, pyridoxine hydrochloride 4 mg, nicotinic acid 80 mg, calcium pantothenate 28 mg, myo-inositol 400 mg, biotin 600 µg, folic acid 1.5 mg, p-aminobenzoic acid 40 mg, choline chloride 800 mg, ascorbic acid 200 mg, α-tocopherol 40 mg, menaphthone 4 mg, cyanocobalamin 9 µg.

#To provide per 100g dry diet: $Ca(H_2PO_4)_2.H_2$= 40 mg, calcium lactate 100 mg, ferric citrate (hydrated) 10 mg, $MgSO_4.7H_2O$ 40 mg, K_2HPO_4 70 mg, $NaH_2PO_4.H_2$= 25 mg, $AlCl_3.6H_2O$ 2 mg, $ZnCl_2$ 6 mg, $CuSO_4.5H_2O$ 3 mg, $MnSO_4.4H_2O$ 2 mg, KI 2mg.

f is indicated when the total values in each column approximate the overall mean. However, when the column totals distribute as a sine curve about the mean, and the standard deviation is large, a rhythm is indicated.

Dr. J. A. Williams (Port Erin) kindly made available his computer programme for the periodogram analysis. Further to calculating the standard deviations for each of the frequency values, the programme incorporated a method for testing each result for signifance. This was achieved by randomizing the original data to produce a periodogram with no rhythmic component. The regression line and 95% fiducial limits were then calculated for this latter periodogram and transposed on to the periodogram of the original data to give a final computer line plot (see results). The regression and 95% fiducial limits gave a quantitative assessment of the periodogram which, in its original form, was purely qualitative. Any values above the upper fiducial limit were considered significant and indicative of the presence of a feeding rhythm within this period. The computerised programme for periodogram analysis presents results as a graph of the coefficient of variability (c.v.) for each frequency tested:

$$\text{c.v.} = \frac{\text{(Standard deviation of form estimate columns)}}{\text{Mean of form estimate columns}} \times 100$$

It was the original intention of this particular line of study to examine the effect of diets of various nutrient composition on voluntary food intake. It will be appreciated that the successful completion of such long-term experiments demands not only the continual cooperation of the fish but constant undisturbed experimental conditions. Indeed, during this study a number of factors were found to behave as strong appetite suppressants. These included frequent use of pneumatic drills three metres below the experimental tanks (courtesy Maintenance Department, UCNW), chronic infusion of copper salts into sea-water supply (courtesy Maintenance Department, UCNW), sabotage of fresh sea-water supplies (student extremists) and, finally, death of experimental animals (deranged 'antivivisectionist').

1b) Effect of reducing carbohydrate component on food intake

A group of Limanda (n=6, 60-80g, Group I), trained to operate the demand feeders as described earlier, were allowed to feed on the control diets for about three weeks until their daily food intake was fairly constant. Their hourly intake was then recorded for 5-6 days before the carbohydrate component of the diet was reduced from 25% to 5% (test diet 1). This was achieved by substituting the dextrin with white kaolin (china clay) powder. The subsequent food intake was then monitored for a further 4-6 days. The fish were then offered the control diet and their feeding behaviour was similarly monitored.

1c) Effect of reducing lipid component on food intake

Another three groups of fish (n=6, 60-80g, Groups II, III and IV) were similarly trained and conditioned to the control diet. The feeding behaviour of each group was then monitored for 4-6 days on this diet. Subsequently, the dietary lipid component was reduced from 20% to 12% (test diet 2) for Group II, and from 20% to 5% (test diet 3) for Groups III and IV. This was achieved by substituting the appropriate amounts of cod-liver oil with white kaolin. The subsequent feeding behaviour was again recorded for 4-5 days before returning the fish to the control diet.

Disturbance of the animals was kept to a minimum and restricted to periods when the food hopper required replenishing. All experiments were performed at 15.0^{o}C.

2. Effect of glucose loads (0.5g/kg) on 'hunger'

It had earlier been demonstrated that the ingestion of Mytilus tissue could significantly elevate the levels of plasma glucose and that the observed plasma glucose/PFA relationship could be'mimicked' by a glucose load. Either or both of the above nutrients may act as post-absorptive signals to modify appetite in fish. A preliminary study into the influence

of elevated plasma glucose levels on appetite was very simply tested in ten cannulated fish (101-200g) held individually at 15.0°C (Nov. - Dec., 1980). The cannulated animals were fed daily 2-3% body-weight meals of fresh whiting and mussel tissue for one week. This ensured that the animals were feeding normally. They were then deprived of food for 72 hr. A crude measure of their 'hunger' was then obtained by timing the 'speed of the food reaction'. This was recorded as the time-delay between the arrival of food in the water until the point just prior to its ingestion. A piece of Mytilus tissue was suspended in mid-water by a length of nylon line. As soon as the fish was about to ingest the tissue, the latter was immediately withdrawn from the tank. The fish was subsequently given an intra-arterial glucose load (0.5g/kg body-weight) (see Chapter 3). Following this treatment, the 'hunger' of each fish was tested (as above) at approximately 5.0 hourly intervals for the duration that the plasma glucose levels were elevated (≈36 hr - from Chapter 3).

3. Return of appetite following a satiation meal

Individual fish (101-200g) were deprived of food for 70 hr to ensure complete emptying of the alimentary tract. They were then offered whole Mytilus tissue from a pre-weighed portion until food was refused. This stage was usually reached within six minutes. The return of appetite was then measured by offering the fish more food, after pre-determined deprivation intervals, following the satiation meal. Every fish was tested at each deprivation interval (3.5, 8.0, 15.0, 24.0, 36.0, 48 and 70 hr) on two to three occasions. The deprivation intervals after each satiation meal were arranged in a different order for each fish. This avoided any sequential bias. The experiment was performed during late January to March at 15.0°C.

Results

1. Effect of dietary composition on voluntary food intake

Table 2 shows the 24-hourly voluntary food intake data for the different groups of Limanda, at 15.0°C, trained to operate demand feeders. On average, each fish (60-85g) ingested about 1.5% body-weight of the control diet on a daily basis. This was equivalent to a daily calorific intake of approximately 4.0 k cal. per fish. This compares with a daily average intake of 2.4% body-weight (≡ 8.5 k.cal./74g fish) recorded for Limanda by Gwyther (1978) at 15.5°C.

A slight increase in daily food intake of groups I and II was indicated when fish were offered test diets 1 (5% dextrin) and 2 (12% cod-liver oil). Both these diets represented an equicalorific dilution (20%) of the control diet. However, the average daily intake on test diets 1 and 2 were not significantly different from the respective control values (Table 2).

A 36% reduction in the calorific value of the diet, obtained by reducing the cod-liver oil component to 5% (test diet 3), resulted in a considerably increased daily consumption of food. Food intake rose by 83 and 40% in groups III and IV respectively. The response was rapid, with an increased consumption during the first 24 hr on test diet 3 (Table 3). However, both groups III and IV reduced their intake on day 5 after presentation of the 5% lipid diet. The latter group continued to reduce their intake up to day 8 when the experiment was terminated. Group III fish briefly resumed an elevated intake on day 6 but consumption on the following two days was again reduced (Table 3). Consequently, food intake per feeding bout was no longer significantly different from the control values.

2. Periodogram analysis of feeding data

A plot of the coefficient of variability (c.v.) for each hourly frequency tested was obtained from the periodogram analysis. Occasionally,

TABLE 2 - VOLUNTARY FOOD INTAKE DATA, FOR LIMANDA (60-81g), ON DIFFERENT SYNTHETIC DIETS

Group No.	Group I		Group II		Group III				Group IV		
Diet type	Control diet	Test diet 1	Control diet	Test diet 2	Control diet	Control diet	Test diet 3		Control diet	Test diet 3	
Period (days) on control diet†	5	-	4	-	6	7	-	-	6	-	-
Period (days)on test diet†	-	8	-	4	-	-	4	6	-	4	5
Average food intake (g)/ group/24 hr	4.57 ±0.45	5.92 ±2.10	6.98 ±0.73	8.12 ±1.18	7.41 ±0.69	5.59 ±0.62	10.22* ±0.47	9.14* ±0.89	8.38* ±1.07	6.91 ±0.41	9.65 ±0.67	8.76 ±1.03
Average % body-weight ingested/fish/24 hr	1.2± 0.21	1.6± 0.16	1.7± 0.18	2.0± 0.30	1.8± 0.69	1.5± 0.16	2.6±* 0.12	2.3±* 0.25	2.1±* 0.27	1.6± 0.10	2.2± 0.15	2.0± 0.22
Energy (k.cal.)/(g) dry weight diet	3.99	3.18	3.99	3.23	3.99	3.99	2.56	2.56	2.56	3.99	2.56	2.56
Average energy (k.cal) intake/group/24 hr	18.0 ±1.8	18.7 ±1.8	27.9 ±2.9	26.2 ±3.8	29.6 ±2.8	22.31 ±2.46	26.15 ±1.22	23.4 ±2.28	21.46 ±2.73	27.5 ±1.64	24.7 ±1.72	22.4 ±2.65
Feeding frequency (hr)	16	17	-	9-10	15	14	-	-	-	24	6	6

* S.D. from respective controls.

† Where several test or control diet periods are listed for a group, these refer to consecutive periods of testing.

TABLE 3 - WEIGHT (g) FOOD, % BODY-WEIGHT AND CALORIE INTAKE PER GROUP PER DAY

	Fish weight (g) (n)	Group I 61 (6)			Group II 81 (5)			Group III 64 (6)			Group IV 74 (6)		
	Day	(g) food eaten	%food eaten	k.cals. ingested	(g) food eaten	% body weight	k.cals. ingested	(g) food eaten	% body weight	k.cals. ingested	(g) food eaten	% body weight	k.cals. ingested
CONTROL DIET PERIOD	1	5.90	1.6	23.6	6.48	1.6	25.9	3.76	1.0	15.0	7.28	1.6	29.0
	2	4.18	1.2	16.7	7.56	1.9	30.2	5.84	1.5	23.3	7.54	1.7	30.1
	3	5.17	1.4	20.6	8.64	2.1	34.5	7.33	1.9	29.3	7.02	1.6	28.0
	4	3.77	1.0	15.1	5.22	1.3	20.8	6.93	1.8	27.7	4.81	1.1	19.2
	5	3.53	1.0	14.1	-	-	-	6.53	1.7	26.1	7.93	1.8	31.6
	6	-	-	-	-	-	-	2.97	0.8	11.9	6.89	1.5	27.5
	7	-	-	-	-	-	-	5.74	1.5	22.9	-	-	-
TEST DIET PERIOD	1	6.04	1.7	19.2	10.92	2.7	35.3	11.04	2.8	28.3	10.14	2.3	26.0
	2	3.57	1.0	11.4	5.46	1.3	17.6	9.08	2.3	23.2	10.53	2.3	27.0
	3	6.04	1.7	19.2	8.96	2.2	28.9	10.95	2.8	28.0	7.67	1.7	19.6
	4	5.10	1.4	16.2	7.14	1.8	23.1	9.79	2.5	25.1	10.27	2.3	26.3
	5	8.59	2.3	27.3	-	-	-	5.07	1.3	13.0	5.20	1.2	13.3
	6	4.42	1.2	14.1	-	-	-	8.90	2.3	22.8	-	-	-
	7	7.65	2.2	24.3	-	-	-	3.83	1.0	9.8	-	-	-
	8	5.50	1.5	17.6	-	-	-	3.56	0.9	9.1	-	-	-
CONTROL DIET PERIOD	1	5.10	1.4	20.3	9.44	2.3	37.7	-	-	-	-	-	-
	2	3.74	1.0	14.9	5.92	1.5	23.6	-	-	-	-	-	-
	3	2.47	0.7	9.8	5.28	1.3	21.1	-	-	-	-	-	-
	4	-	-	-	9.28	2.3	37.0	-	-	-	-	-	-
	5	-	-	-	7.20	1.8	28.7	-	-	-	-	-	-
	6	-	-	-	7.36	1.8	29.4	-	-	-	-	-	-

Comparison of mean daily food and energy intake on control and test diets.

Group I a) food N.S. (t=1.65) b) energy N.S. (t=0.23) } control (day 1-5)/ test (day 1-8).

Group II a) food N.S. (t=0.83) b) energy N.S. (t=0.33) } control (day 1-4)/ test (day 1-4).

Group III a) food S.D. (P<0.001) b) energy N.S. (t=1.1) } control (day 1-7)/ test (day 1-4).

Group IV a) food S.D. (P<0.01) b) energy NS (t=1.1) } control (day 1-6)/ test (day 1-4).

multiple significant feed frequencies (harmonics) were obtained with a significant feeding frequency appearing at value (f) with a second frequency at 2f. In such situations, the correct frequency was identified by comparison with the original data and selecting the most significant of the frequency values.

In general, the intermeal interval on the control diet lay between 14 and 16 hr with the exception of the group IV animals which fed with a 24 hr frequency. There was no change in the feeding frequency of group I fish when they were offered the 12% dextrin diet (Fig. 1A & B). Initially, a significant feeding frequency was not detected when group II were offered the control diet (Fig. 2A). However, the intermeal period increased from between 9 and 10 to 15 hr when these fish were transferred back from the 12% lipid diet to the control diet (Fig. 2B & C).

In the case of the group III fish, a significant feeding frequency was not detected by periodogram analysis (Fig. 3A & B) on the 5% lipid diet. However, fig. 4 shows the hourly actuations for the whole experimental period. When the 5% lipid diet was offered an increased meal frequency is strongly implied. In the periodogram analysis, the number of actuations in each succeeding hour were only scanned for possible rhythms ranging from 4-hourly to 28-hourly feeding bouts. It is, therefore, possible that an intermeal period of shorter duration than 4 hr would remain undetected. This conclusion is supported by the observation that a considerable shortening of the intermeal period, from 24 to 4 hr (Fig. 5 A & B), occurred when the group IV fish were offered the same 5% lipid diet (test diet 3). It is also possible that the group III fish were in fact feeding asynchronously.

Finally, there was no significant difference between the daily calorific intakes of groups I and II on the control diets and test diets 1 and 2 for the periods studied. Initially, no difference was detected in the daily energy intake of groups III and IV when they were transferred onto the 5% lipid diet. However, concurrent with the reduced consumption which

Figs. 1-3, 5. Periodogram analysis of continuous recordings from demand feeders. Limanda limanda (60-81g) were offered a variety of diets at 15.0°C. Points lying above the upper (95%) fiducial limits of the randomized data are considered to be true rhythms and these are indicated by arrows.

Fig. 1 - Periodogram analysis of recordings for Group I on control diet (A) and test diet 1 (5% dextrin) (B).

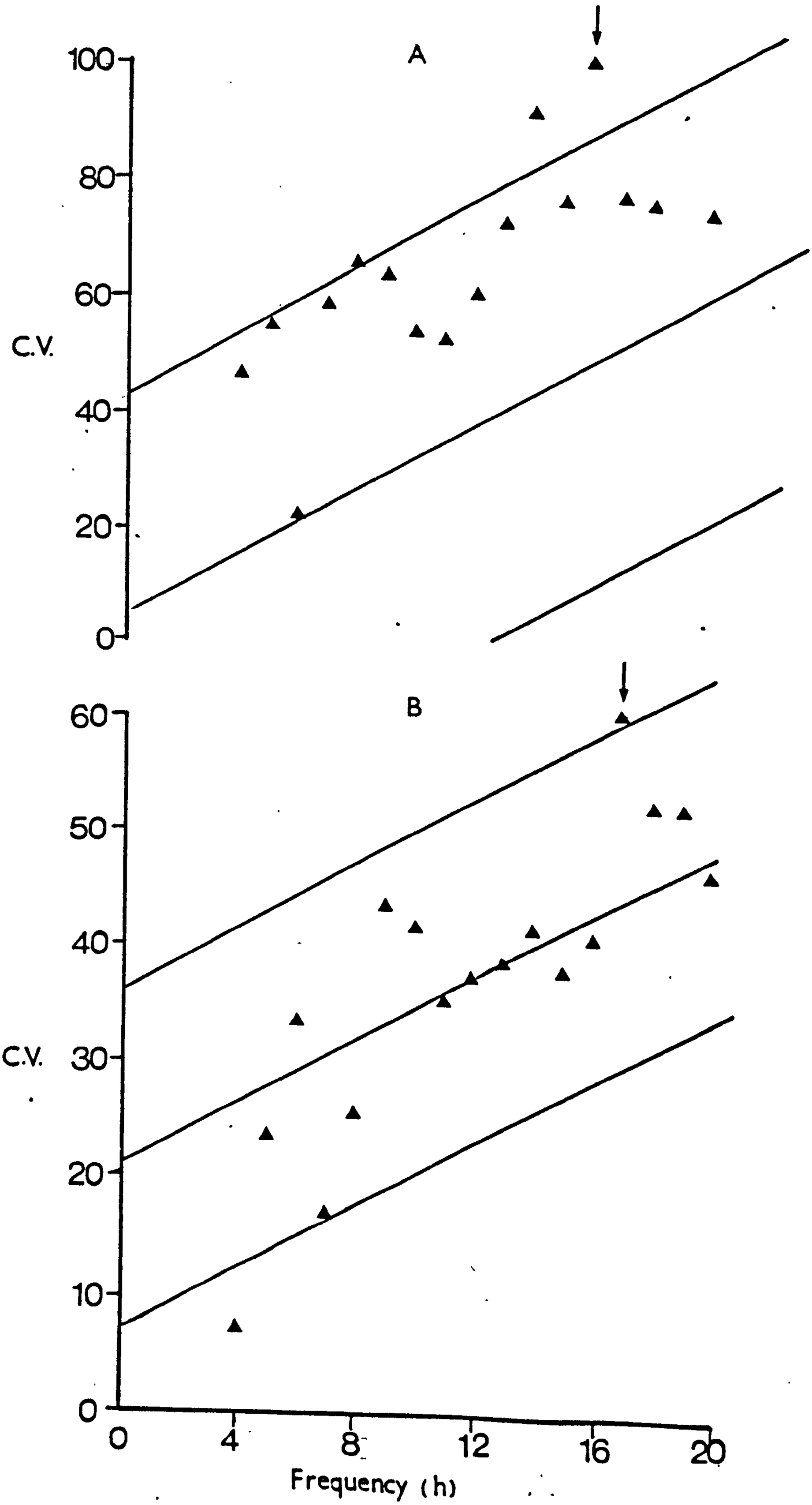
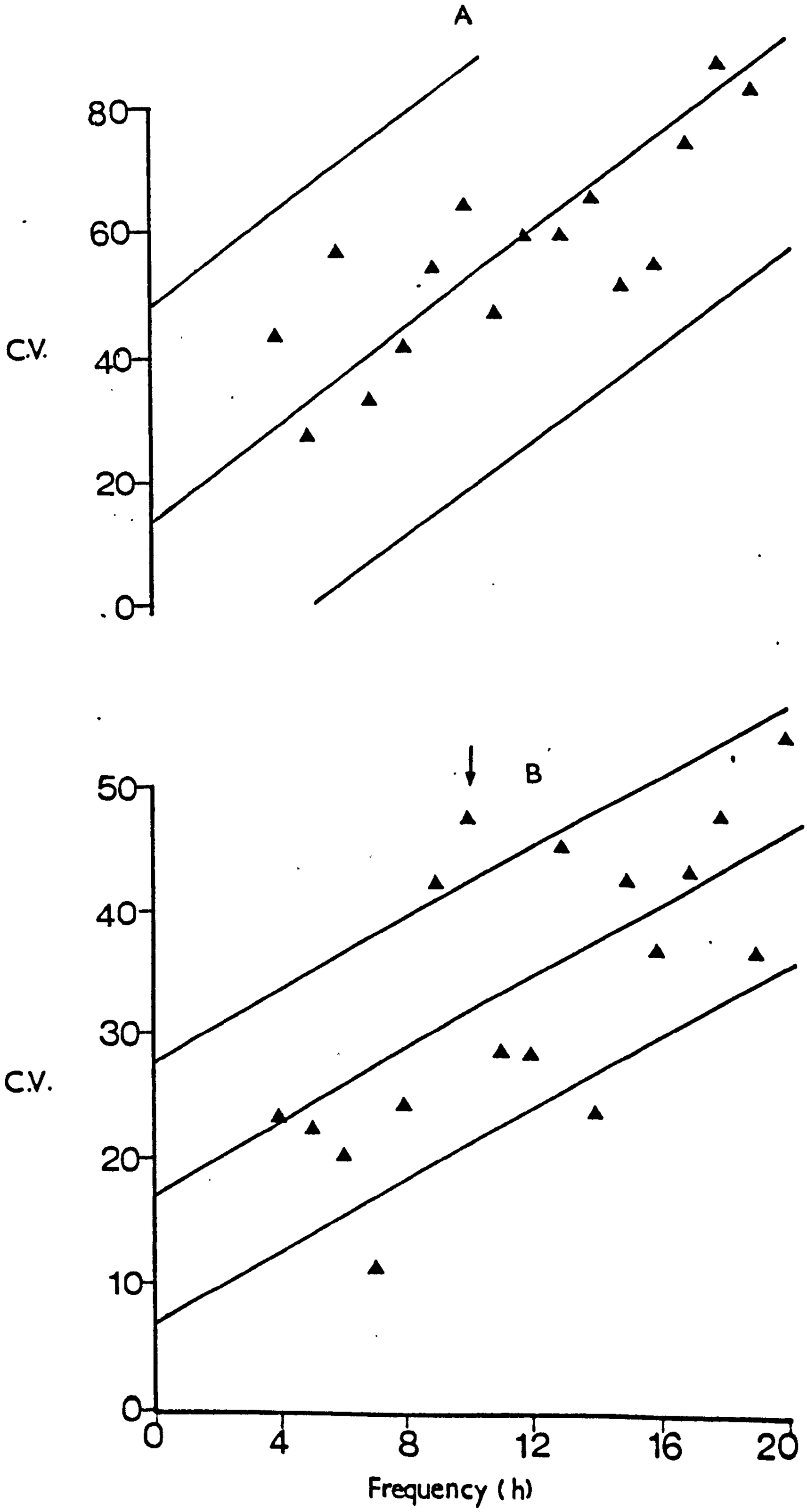

A
100
80
60
40
20
0
C.V.
B
60
50
40
30
20
10
0
C.V.
0
4
8
12
16
20
Frequency (h)

Fig. 2 - Periodogram analysis of recordings for Group II on control diet (A), test diet 2 (12% lipid) (B) and after returning to control diet (C).


A
80
60
40
20
0
C.V.
B
50
40
30
20
10
0
C.V.
0
4
8
12
16
20
Frequency (h)

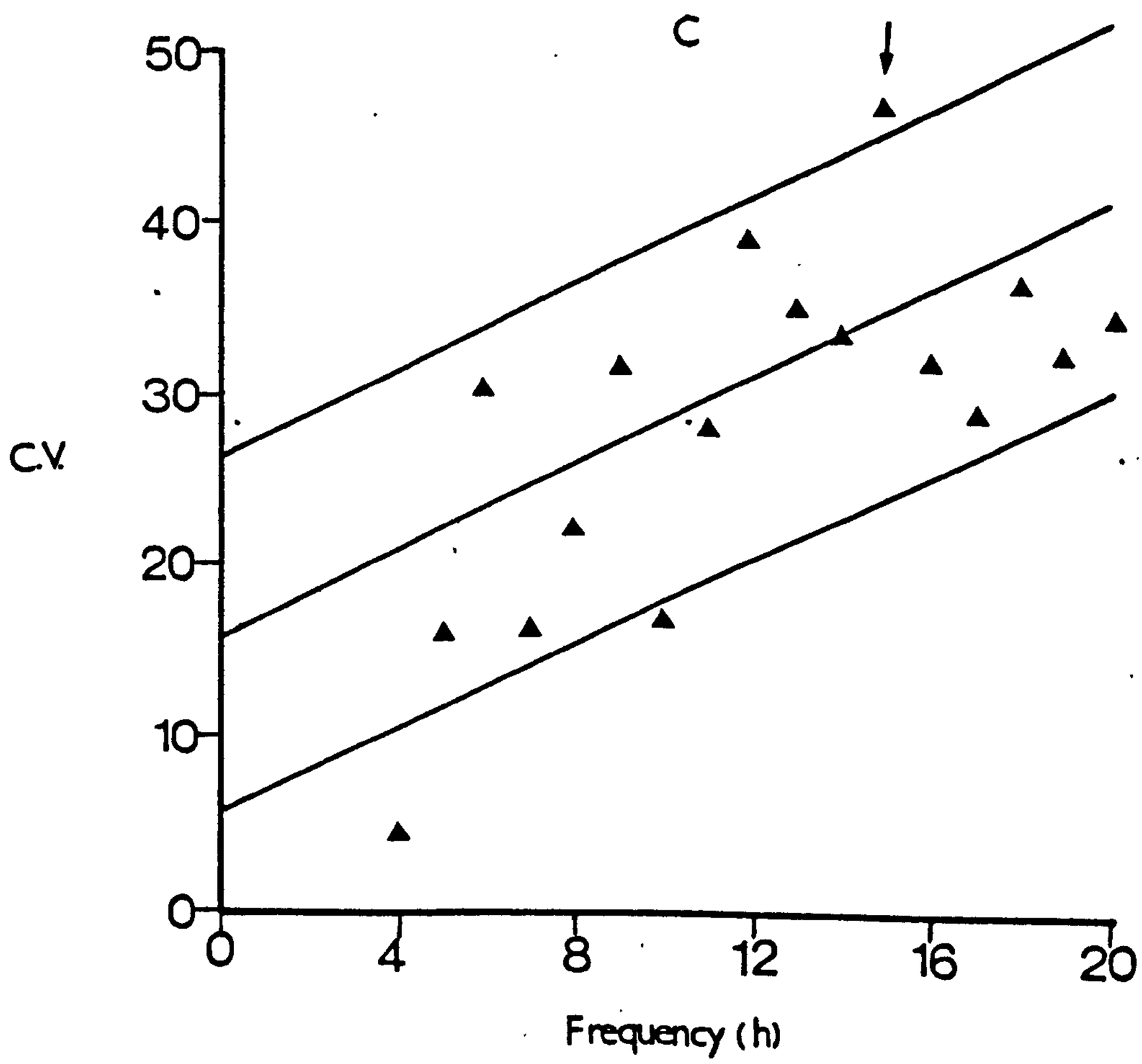

C
50
40
30
C.V.
20
10
0
0
4
8
12
16
20
Frequency (h)

Fig. 3 - Periodogram analysis of recordings for Group III on control diet (A) and test diet 3 (5% lipid) (B).

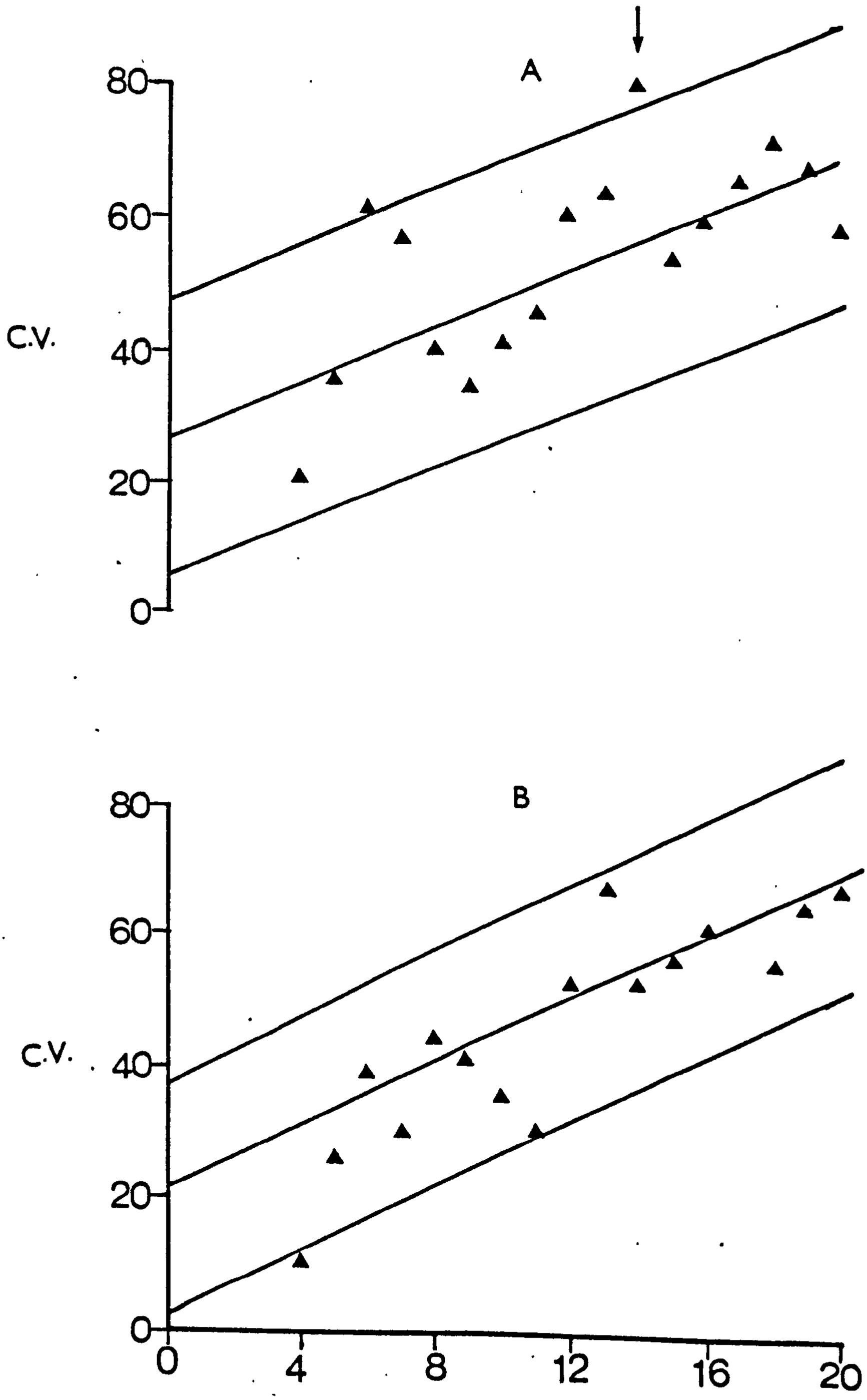

A
80
60
40
20
0
C.V.
B
80
60
40
20
0
C.V.
0
4
8
12
16
20
Frequency h

Fig. 4 - Feeding activity per 24 hours by Group III on control diet and test diet 3 (5% lipid). Arrow denotes change-over to test diet 3 from control diet.

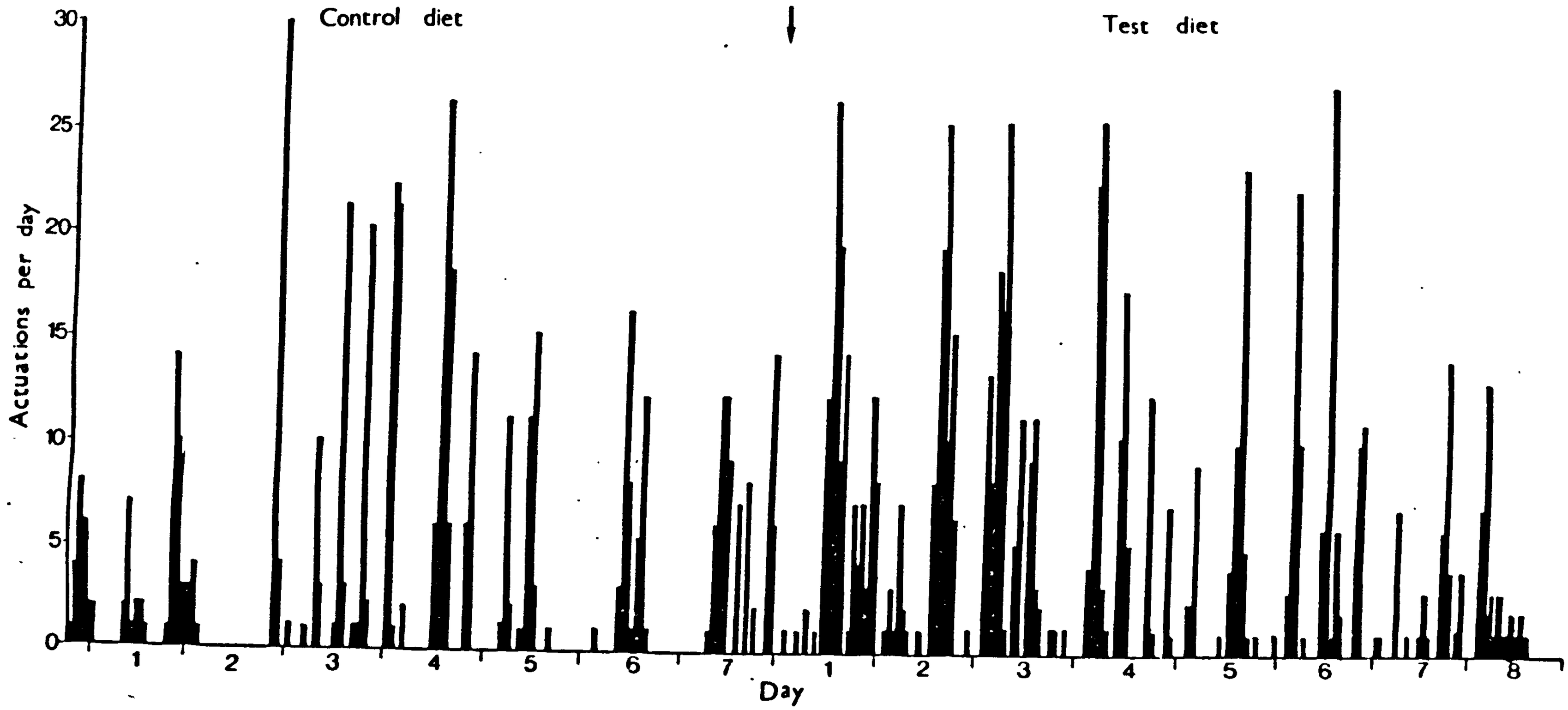
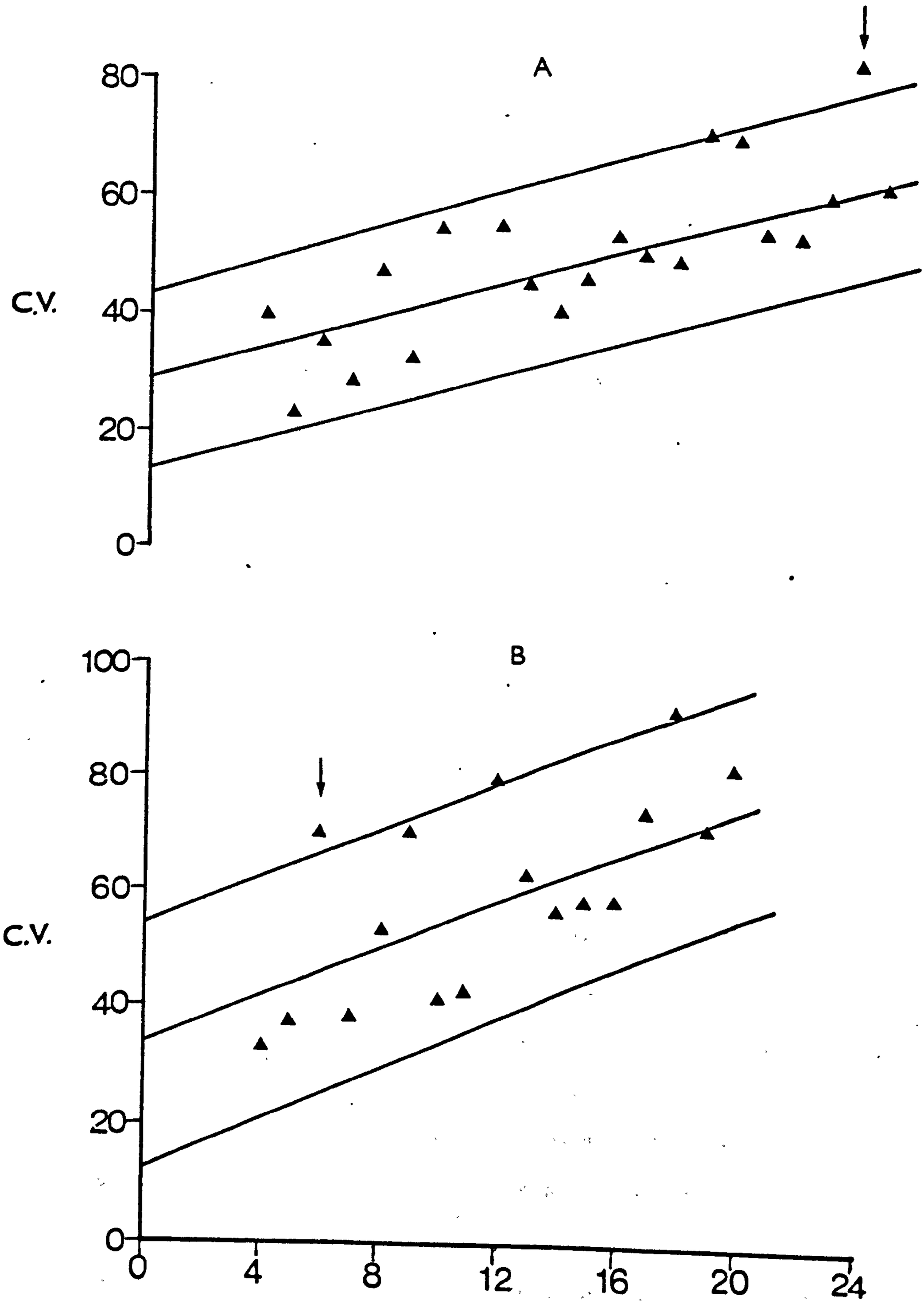

Control diet
Test diet
Actuations per day
30
25
20
15
10
5
0
1
2
3
4
5
6
7
1
2
3
4
5
6
7
8
Day

Fig. 5 - Periodogram analysis of recordings for Group IV on control diet (A) and test diet 3 (5% lipid) (B).


A
80
60
40
20
0
C.V.
B
100
80
60
40
20
0
C.V.
0
4
8
12
16
20
24
Frequency (h)

began on day 5 of the latter diet, there was a concomitant decline in calorie intake (Tables 2 & 3).

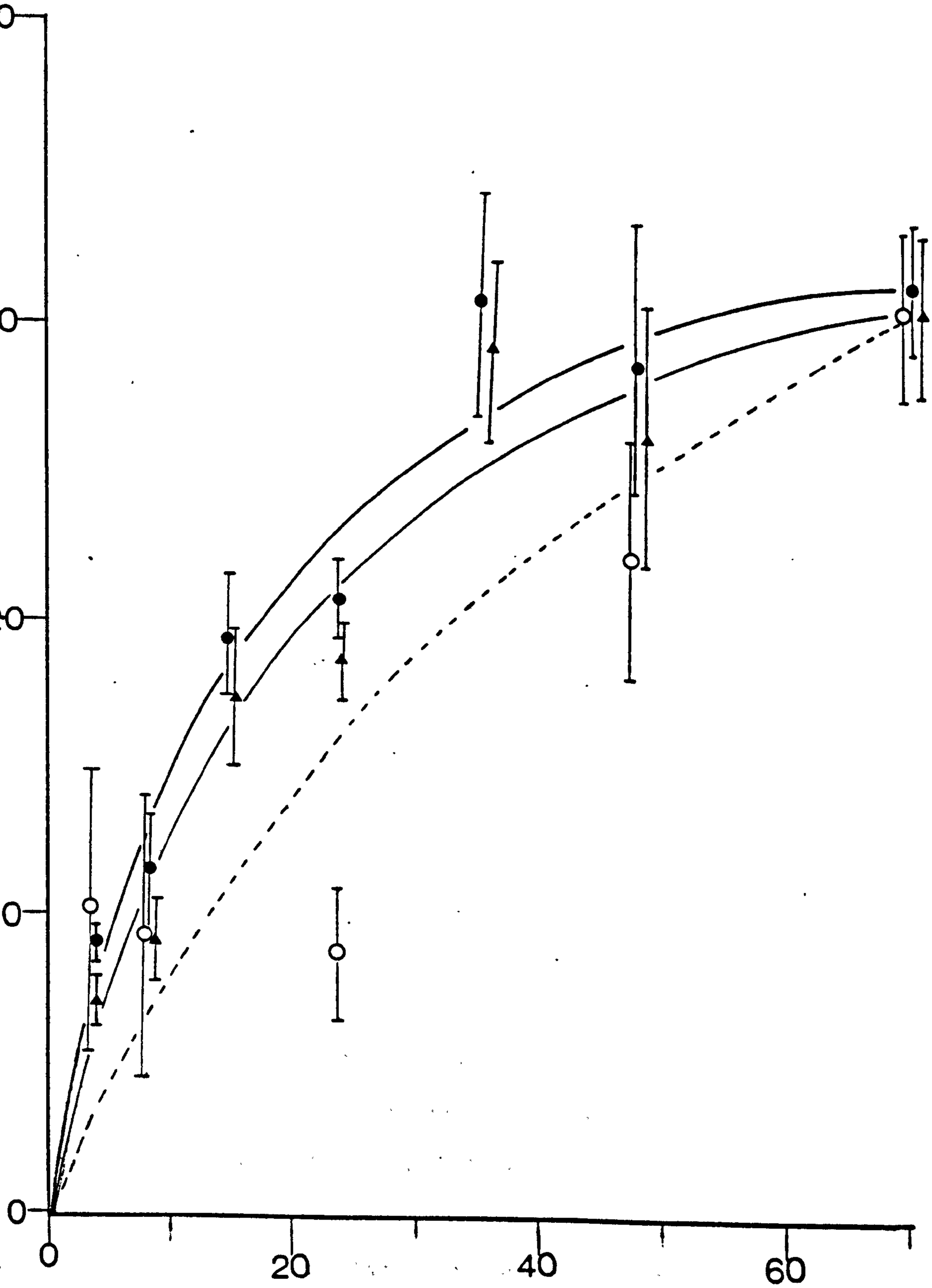
3. Effect of glucose loads (0.5g/kg) on 'hunger'

The initial 'speed of the food reaction', prior to glucose loading, was very rapid, taking about 3 to 4 seconds, following 70 hr food deprivation. This compares with 2.73 and 0.57 seconds for fed and starved individuals of C. carpio (Kuz'mina, 1966). Subsequent 'hunger' measurements, following the glucose infusions, showed no modification of the 'speed of the food reaction' by individual fish. This was true for the entire period that the plasma glucose would have been elevated. On every occasion, the fish responded immediately to the arrival of food in the tank. The repeated removal of the mussel did not appear to de-sensitise the response to a potential food item.

4. Return of appetite following a satiation meal

Fig. 6 depicts the dry weight of Mytilus ingested by fish (101-200g) at various deprivation intervals following a satiation meal. It can be seen that approximately 50% of a satiation meal may be ingested after about 30 hr deprivation. This data may be used as a rough measure of the return of appetite for Limanda. It is possible to compare the observed return of appetite with the various other physiological parameters measured in the present study (see later). However, in the immediate instance, the return of appetite is compared only with gastric emptying time (GET) and total clearance data for satiation meals of Mytilus (Fig. 6). It is apparent that, during the immediate post-prandial period (3-10 hr), the fish compensate quite closely for the volume of food that has been evacuated from the stomach. However, approximately 18-30 hr after ingestion of the meal, the animals clearly do not ingest sufficient food to fill the available stomach capacity. They neither compensate for the volume of

Fig. 6 - Voluntary ingestion (o—o) of Mytilus edulis tissue in relation to evacuation of a satiation meal of Mytilus from the stomach (●—●) and entire alimentary canal (▲—▲). (mean±se)


4·0
3·0
2·0
1·0
0
0
20
40
60
Mytilus (g. dry weight) in ingested and evacuated
Time since feeding – h

food evacuated from the stomach or indeed from the entire alimentary canal.

Discussion

1. Effect of dietary composition on voluntary food intake

The demand feeding studies clearly show that Limanda can detect a change in the nutrient content or calorific level of its food. In view of the contrary finding of Gwyther (1978) for this species, the present study raises a number of complex questions which will be discussed later.

In the present study, the compensatory response to a diluted diet was reflected by an increased daily food intake. This was particularly evident on test diet 3 (5% lipid) and ensured that the energy intake remained constant. However, no such obvious increases in daily consumption were observed on test diets 1 and 2 (5% dextrin and 12% lipid respectively). This would probably be expected, since a very large increase in consumption was unnecessary (when the dietary calory level is reduced by 20%) if maintenance of a particular energy intake was the prime factor regulating consumption (see later).

An increase in food intake may be achieved either by reducing the inter-meal period or the size of the individual meals. In the present study, an increase in the daily number of meals is indicated when the animals are offered a 12% or 5% lipid diet. This pattern agrees with that observed for S. gairdneri (Loizides, 1978).

To examine if the increased daily intake was achieved by increasing meal size, in addition to reducing the inter-meal period, the amount of food eaten per meal was estimated (Table 4). Where a significant feeding frequency was detected by periodogram analysis, the total number of meals within that period could be calculated. Consequently, the amount of food eaten per meal is obtained. It can be seen from Table 4 that on test diets 2 and 3 (12 and 5% lipid) there is in fact a reduced food intake per meal.

TABLE 4 - COMPARISON OF FOOD INTAKE PER MEAL ON DIFFERENT DIETS

Diet type	Group I		Group II			Group III		Group IV	
	Control	Test Diet 1	Control	Test Diet 2	Control	Control	Test Diet 3	Control	Test Diet 3
Period on diet (hr)	120	192	96	96	144	168	96	144	96
Intermeal period (hr)	16.0	17.0	-	10.0	15.0	14.0	-	24.0	6
Total number meals/period	7.5	11.4	-	9.6	9.6	12.1	-	6.0	16.6
Total food intake (g)	22.85	47.36	27.92	32.48	44.46	39.13	40.88	41.46	38.6
Food intake (g)/meal	3.05	4.15	-	3.38	4.63	3.23	-	6.91	2.31

However, the more frequent meals ensure an overall greater daily food intake. The fish offered test diet 1, with a reduced carbohydrate content (25 to 5% dextrin), show no change in their feeding frequency. In contrast to the behaviour of fish on test diet 2 (12% lipid), they maintain a constant calorific intake by increasing their intake per feeding bout. This is despite the fact that both the latter diets were equally reduced in their calorific content.

Naturally, on the basis of a few experiments it is not feasible to draw any firm conclusions concerning these different feeding responses to test diets 1 and 2. However, considering the wealth of relevant information concerning this topic in higher vertebrates, some further detailed discussion is justified.

Possible explanation of the feeding response of groups I and II to equicalorific dilution

As discussed above, groups I and II responded in a different way to an equicalorific reduction in the dietary carbohydrate and lipid levels respectively. This suggests that these latter nutrients may each modify appetite in a different manner. Two to four hours after the ingestion of a 2% body-weight meal, Limanda showed a significantly elevated plasma glucose level (see Chapter 3). This was so despite the fact that the meals contained only about 2.4% carbohydrate (wet weight). In addition, these fish had been starved for 72 hr which may have delayed assimilation of the food (see Chapter 1). In the demand-feeding studies, it is not clear how soon the ingestion of the food pellets would be reflected by a post-absorptive rise in plasma glucose. The group I fish took approximately 4 hr to complete each meal (Appendix 2), both on the control diet and test diet 1. It is, therefore, conceivable that the plasma glucose levels of these fish would have been elevated before the end of a meal. This would be particularly likely when using 25% carbohydrate diets, and in the absence of any food deprivation effects. It might, therefore, be argued

that the fish were able to detect a reduction in the dietary carbohydrate level during a feeding bout. This might explain the larger meals taken by group I fish in contrast to the smaller, but more frequent meals ingested by group II. If this is the case, then Limanda must be able to detect a reduction in dietary carbohydrate level earlier than when a lower lipid content is used.

The manner in which dietary-derived glucose might act to modify appetite in fish is unclear. In higher vertebrates, it appears that it is the immediate supply rate of readily metabolizable energy that is important in the short-term regulation of appetite (Booth, 1979). A reduced glucose metabolism, at the 5% carbohydrate level (test diet 1), may therefore have been detected during a meal, should the energy supply rate also be important in controlling appetite in Limanda. Unfortunately, glucose is thought to be metabolized very slowly by fish (Cowey et al., 1975, 1977). The latter authors recorded a half-life ($t_{\frac{1}{2}}$) of 6 hr for this nutrient in S. gairdneri, while Mazeaud (1973) measured a $t_{\frac{1}{2}}$ value of 12 hr for Cyprinus carpio. Limanda showed a quicker assimilation of a glucose load ($t_{\frac{1}{2}}$ = 4.0 hr, Chapter 3), although this value still reflects a low rate of glucose metabolism. This suggests that the larger meals taken by Limanda, on the 12% dextrin diet, cannot be explained in terms of this species' ability to detect a reduction in the availability of glucose for metabolism rapidly.

However, it is interesting to note that, in higher vertebrates, glucose is unique in inducing satiety via the vagus nerve. Vagotomy destroys this particular satiety power of glucose (Novin, 1976). Furthermore, the latter author showed that the satiating effect of glucose is greater proportionally than its caloric value. This evidence demonstrates that glucose can modify consumption before it undergoes post-absorptive metabolism. Such a mechanism has not been ruled out in fish and may explain the response of Limanda to a reduction in dietary carbohydrate level as opposed to its behaviour following a lipid reduction.

Clearly, considerably more work is required on this subject to clarify the situation.

3. Feeding behaviour of groups III and IV on the fifth day of test diet 3

The observed feeding behaviour of fish from groups III and IV, on the fifth day of test diet 3, itself requires some comment. The increased feed intake observed for the first four days on the 5% lipid diet (test diet 3) was followed by a sudden decline in consumption on the fifth day by both groups. Any one, or a combination of the following explanations, may be responsible for this observation:

(a) Although all members of a group of fish were trained to operate the demand feeder, it was usually the case that 2-3 individuals became the dominant operators. It is possible that the increased energy expenditure necessary to maintain a constant energy intake on the 'diluted' diets became excessive. This might be particularly so if only 2 or 3 dominant fish were involved in activating the trigger, while supplying food for all members of the group. However, this is unlikely to be the sole reason for this sudden change in feeding rate, although it may have complemented a response to an imbalanced diet.

(b) Reducing the cod-liver oil component from 20 to 5% may well have resulted in an imbalanced diet such that an essential lipid fraction was limiting. Despite an overall increase in daily food intake, for the first four days on test diet 3, the daily lipid intake was still reduced by about 100% in comparison to the control levels. It is feasible that, in such a situation, an essential lipid fraction was limiting which eventually led to a depression of food intake. In higher vertebrates, for instance, a diet lacking in an essential component may result in the animal losing its 'appetite' for that diet even although it is perfectly capable of meeting their energy needs (Harper and Boyle, 1976). A learned 'preference' or 'aversion' to a particular diet may develop according to whether or not it

is properly balanced. It is conceivable that such a learned 'aversion' to an imbalanced diet may take a few days to develop, especially in poikilotherms.

(c) Substitution of the lipid with kaolin had no effect on the relative proportions of the other major nutrients. However, the increased food intake for the first four days on test diet 3 would have meant a 100 and 94% increase in the daily carbohydrate and protein intake respectively. The decline in consumption on the fifth day might have been associated with some toxic side effect from the ingestion of excessive amounts of either of these nutrients. Dextrin was incompletely assimilated by P. platessa at dietary levels in excess of 20% (Cowey et al., 1972). In addition, the latter authors also found that high dietary protein levels reduced food intake. Consequently, the increased protein intake, observed in the present study on test diet 3, may have had a cumulative toxic effect which caused the sudden appetite depression.

In conclusion, the early feeding behaviour of groups III and IV, on the 5% lipid diet, probably reflects a response to an overall deficit in energy intake. This latter period is then followed by a sudden decline in consumption, probably generated by a deficient intake of some essential lipid fraction or an excessive intake of protein.

4. Comparison of present observations with those of Gwyther (1978)

The present findings for the voluntary food intake of Limanda differ from the earlier work on this species (Gwyther, 1978) in two distinct areas:

I Daily intake of control diets by Limanda in the present study were much lower than those recorded by Gwyther (1978).

II The latter author detected no response to dietary dilution.

I) The difference in food intake between the two studies cannot be explained in terms of differences in fish size or temperature. Neither are variations in food delivery per actuation likely to be responsible. Limanda has been

TABLE 5 - NUTRIENT COMPOSITION OF CONTROL DIETS USED IN PRESENT STUDY AND BY GWYTHER (1978)

Components of diet %	Cooper Diet (Gwyther, 1978)	Control diet (present study)
Protein	58.0	30.0
Carbohydrate	10.0	25.0
Lipid	7.5	20.0
Fibre/ash	16.5	0.0
α-Cellulose	0.0	22.0
Vitamin	-	2.8
Mineral mixture	-	0.2
†Dietary energy (k.cal/100g)	478	399
% body weight intake/day	2.4	1.5
Fish weight (g)	74	60-81

†Calorific value of freeze-dried whiting tissue = 3.58 k.cal/g (by direct bomb calorimetry).

lipid = 9.45 k.cal/g)
carbohydrate = 4.1 k.cal/g) (Brody, 1945.
)

shown to be quite capable of adjusting the number of daily actuations to maintain a constant level of food intake (Gwyther, 1978). Furthermore, the 'Cooper' salmonid diet used by Gwyther has a higher calorific value than the diet used in the present study. Therefore, if calorific density does modify consumption in Limanda, a greater volume of the latter, not the former, diet should have been eaten. There are several likely reasons to explain this discrepancy:

(a) Table 5 compares the nutrient composition and energy characteristics of the diet used in the present study with the 'Cooper' salmonid diet. Clearly, there are considerable differences in the relative proportions of the major nutrients.

Fish can utilise protein and simple carbohydrates as an energy source. A large proportion of ingested protein may be used in this manner by some species (Cowey et al., 1974). However, lipid is often used more readily than protein as an energy source. The low dietary lipid levels in the salmonid diet may have required the fish to utilise a greater proportion of their dietary protein for energy purposes. However, the ability of fish to metabolize protein can depend both on the type, and level, of dietary protein (Cowey et al., 1974). If for a particular reason Limanda was unable efficiently to assimilate the protein component of the Cooper diet, then proportionately more food may have had to be ingested in an attempt to satisfy the animal's requirements. P. platessa achieved a maximum protein efficiency ratio (PER) at a 40% dietary protein level, when the lipid and carbohydrate were present at 13.5 and 10.0% respectively (Cowey et al., 1972). A further increase in the protein level, with a concurrent decrease in the lipid content of the diet, resulted in a lower PER for P. platessa. This is supported by the findings of Page and Andrews (1973) where a similar lowering of the digestible energy/protein (DE/P) ratio resulted in a poorer PER for I. punctatus.

(b) In view of the reduced consumption in I. punctatus, following an increase

in the lipid component of the diet (Page and Andrews, 1973), it is conceivable that the lower feed intake observed in the present study may have been due to the higher levels of both dietary carbohydrate and, particularly, dietary lipid. It is possible that the higher levels of these latter nutrients, in the control diet, resulted in the immediate energy requirements of the fish being satisfied at a lower feed intake. Certainly, it is considered that lipid is the preferred energy source of marine fish (Cowey and Sargent, 1979), and it is known to spare the oxidation of dietary protein as an energy source. In this respect, dietary lipid should form between 10 and 20% of the diet of cultured species to obtain optimum growth. This range probably reflects the level at which lipid is ingested by marine flatfish in the wild (Cowey and Sargent, 1979).

(c) Finally, an explanation of this first discrepancy between the two studies may lie in the source of lipid used in the respective diets. Cod-liver oil contains about 10% linolenic fatty acid, but, more importantly, it contains 10.0% and 11.4% of eicosapentaenoic (20:5ω3) and docosahexaenoic (20:6ω3) fatty acids respectively (Castledine and Buckley, 1980). The latter two fatty acids are essential for optimum growth in a number of marine fish (Kanazawa et al., 1979; Cowey and Sargent, 1979). In the present study, at the 20% lipid level, 22:6ω3 and 20:5ω3 would have formed over 4.0% of the control diet, which should have satisfied the requirements of Limanda for these essential fatty acids. This is supported by the findings of Yone and Fujii (1975a,b) who reported both an enhanced growth rate, and food conversion efficiency in Chrysophrys major, when dietary lipid was supplemented with a 2% mixture of 22:6ω3 and 20:5ω3.

The exact composition of the lipid component of the 'Cooper' salmonid diet used by Gwyther (1978) is unknown. However, it is perhaps relevant to note that in an analysis of several commercial salmonid feeds, Sinnhuber (1974) measured a maximum level of 0.74 and 0.58% of 22:6ω3 and 20:5ω3 respectively. These fatty acids were absent from some feeds. While this fact may be irrelevant in relation to fresh-water fish (see review), a

deficiency of these fatty acids in the diet of marine animals could well affect their feeding behaviour.

In conclusion, the different food intake, in these two separate studies, may be explained by a specific response to the level of readily metabolizable energy and/or the level of a specific lipid fraction. Possibly, the former explanation is nearer the truth since Gwyther (1978) reported no deficiency symptoms of the fish such as a gradual decline in food intake. Alternatively, if the dietary deficiency was not too extreme, a lowered assimilation efficiency may have necessitated a greater consumption rate. Just such a response was observed in the bass, Dicentrarchus labrax (Carrillo et al., 1982). This species ingested greater quantities of a commercial feed than it did of a natural diet. This was despite the fact that the commercial feed was far higher in energy value. The bass fed the natural diet had a greater energy utilisation efficiency so that they were able to satisfy their requirements at lower feed intake levels. The fish on the commercial diet had to augment their food intake with a daily mobilisation of their lipid reserves.

II) The final problem relating to the respective studies concerns the ability of Limanda to detect changes in food quality. There are two possible explanations:

(a) In the present study, a reduction of the lipid content to 5%, while maintaining a constant protein level, was followed by an increase in food intake as observed for I. punctatus (Page and Andrews, 1973; Lovell, 1979). This evidence indicates that the level of immediately metabolizable dietary energy (lipid and carbohydrate) is particularly important in relation to the amount of food that is ingested. This would agree with the findings for higher vertebrates. The Cooper diet already had low levels of lipid (7.5%) and carbohydrate (10%). Consequently, even at the maximum dietary dilution (50%) investigated by Gwyther, the level of metabolizable energy, as carbohydrate and lipid, would have been only moderately reduced (10 to 15%). In the present study, a 20% reduction in the energy level (by reducing either the carbohydrate (test diet 1)

or lipid (test diet 2)) appeared to be the lower limit at which a feeding response to dietary 'dilution' could be detected experimentally.

The dietary levels of lipid and carbohydrate determine the overall digestible energy/protein (DE/P) ratio of the diet. Food consumption by fish increases when this ratio is reduced (see review). Since Gwyther (1978) added kaolin to the complete feed to reduce the calorific value of the food, the 'diluted' diets probably had very similar DE/P ratios to the control diet. Irrespective of this fact, it might be expected that a 50% reduction in the energy value of the diet would have been detected. Some other factor may have contributed to the reported failure of Limanda to respond to dietary dilution.

(b) Failure to increase ingestion of a diluted feed (Gwyther, 1978) may be partly due to some dietary characteristic not related to its nutrient content. The absorption of fluid by dry feeds after ingestion causes the food to expand in the stomach. This expansion of the food may inhibit further food intake (Lovell, 1979). If, in the first instance, more food has to be ingested to satisfy the immediate energy requirements, there may be less room for adjustment of meal size should the dietary calorific value be further reduced. This would be particularly so in the case of dry feeds that expand after ingestion. Such a situation may prevent the animal from adjusting its feed intake when presented with a lower quality diet.

In the rat, adjustment to a diluted diet is initially achieved by reducing the intermeal period. This is followed, a few days later, by a return to normal meal frequencies but increased meal sizes. However, if the animal is initially prevented from reducing its intermeal period, it fails completely to compensate for a reduced calory intake (Le Magnen, 1963).

It is, therefore, likely that the reported failure of Limanda to respond to dietary dilution (Gwyther, 1978; Gwyther and Grove, 1981) was related to some nutrient imbalance of the control diet. This fact emphasises the caution necessary when interpreting demand feeding data.

5. Effect of glucose loads on hunger

Kuzmina's (1966) findings for the carp contrast with the behaviour of

Limanda when presented with food during the assimilation phase of a glucose load. The failure of high plasma glucose (and low PFA) levels to modify the response to food may suggest that normal, post-absorptive variations in plasma concentrations of these two metabolites would not affect feeding behaviour. The eagerness with which the glucose-loaded fish approached the food also indicates that they would have ingested a normal satiation meal under these circumstances. However, intra-arterial infusion effectively eliminates the participation of any satiety signals that might be elicited during the intestinal absorption of glucose. In addition, it must be emphasised that the fish in the present study were tested in a single nutritional state, i.e., only after 72 hr food deprivation. In view of the evidence relating to the interaction of the nutritional state and the glucose suppression of food intake in the higher vertebrates (Booth, 1979), no firm deductions can be made from the present observations. However, the technique of using chronically implanted cannulae for the infusion of metabolite loads should prove useful in fish appetite studies.

6. Return of appetite following a satiation meal

The feeding behaviour of fish, following a satiation meal of Mytilus, strongly suggests a post-absorptive satiety mechanism. This is indicated by the failure of Limanda to compensate adequately for the amount of food that had already passed from the stomach. These findings are in contrast to those of Grove et al., (1978) for S. gairdneri, and Grove and Crawford (1980) for Blennius pholis, where strong correlations were observed between the return of appetite and evacuation data. However, in a separate study, Catherall (1979) reported a similar suppression of appetite in the blenny but did not detect a close correlation between appetite and gut fullness.

Much of the above discussion relating to the demand feeding studies is based upon the assumption that food intake is influenced by the energy content of its food. It is quite true that the fish in the present study responded very accurately to a reduction in the energy value of its food.

However, there is no direct evidence, as in the higher vertebrates, to show that it is the caloric value of food that is monitored by fish. In the final section of this Chapter, all the present physiological measurements on Limanda are discussed in relation to their possible influence on appetite.

Section II

Evaluation of data in relation to appetite control in *Limanda limanda*

SECTION II

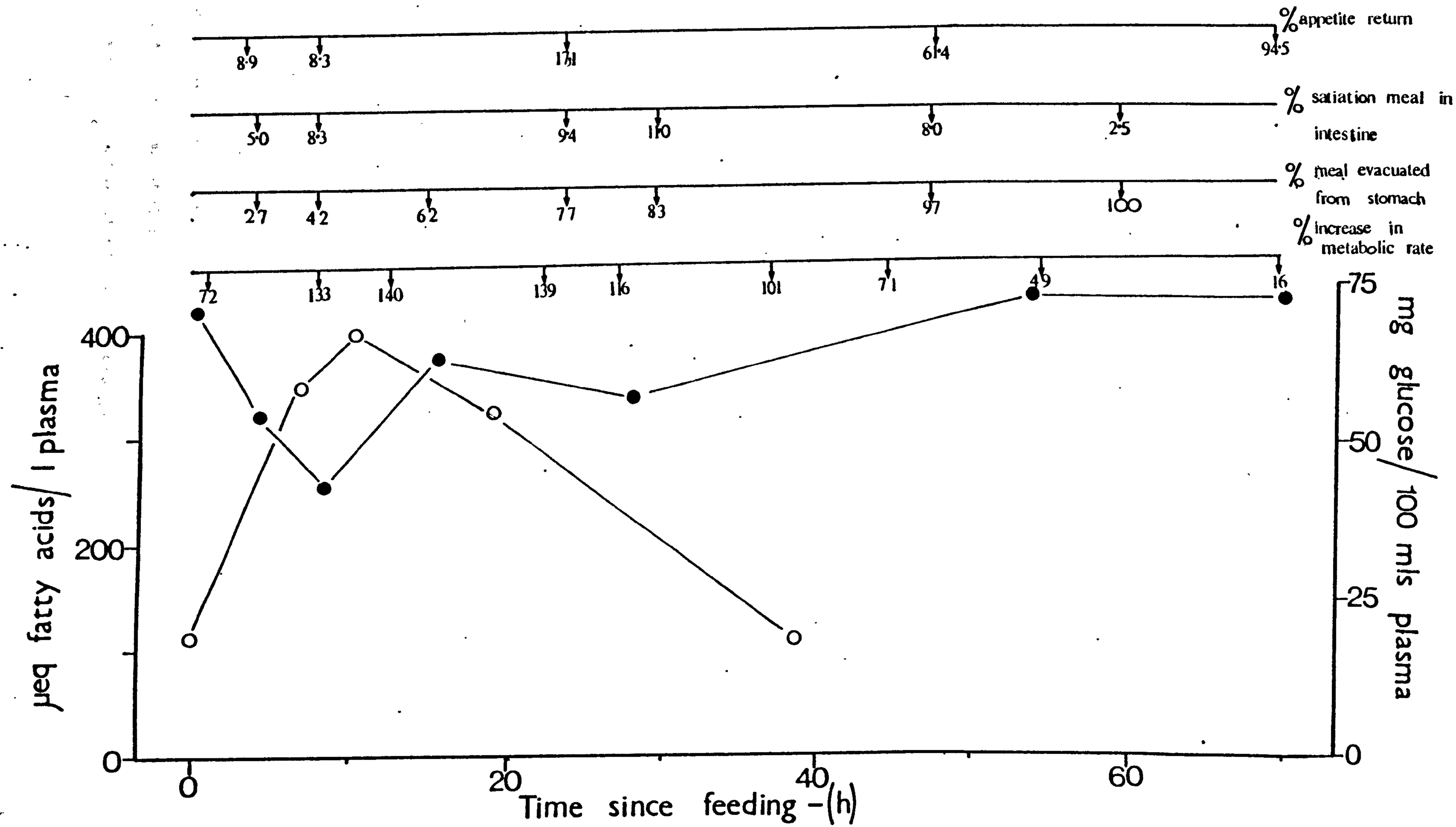
In this final section the data from the previous Chapters is discussed in relation to appetite control in Limanda.

Under ad lib feeding conditions Limanda can detect variations in the quality of its diet and adjust food intake accordingly - usually within 24 hr. The maintenance of a constant calory intake by this species, when offered diets varying in energetic value, strongly suggests that Limanda monitors its gross daily energy intake. This conclusion agrees with the findings for a variety of other fish species (Cowey et al., 1972; Page and Andrews, 1973; Lee and Putnam, 1973), where consumption appears to be adjusted according to total energy intake rather than digestible energy intake (Jobling, 1981). While this may be true, it is also likely that feeding behaviour is modified according to the levels of specific dietary nutrients. These observations imply that post-absorptive signal(s) influence food consumption. This is supported by the post-prandial suppression of food intake witnessed in the return of appetite study. Obviously, stomach capacity was not the sole factor limiting intake in this instance.

Fig. 7 demonstrates the relationship between the various physiological parameters measured in Limanda during the present study. In order for such a comparison to be valid, it is crucial that the various parameters are measured in animals acclimatized under identical laboratory conditions, and during the same season. Fig. 7 depicts the data collated for Limanda (101 - 200g), at 15.0°C, during the months of January to March (1980 and 1981) inclusive. Consequently, the animals used for each experiment should have been in a similar physiological condition.

An examination of the intestinal clearance data, and the levels of plasma glucose and FA, reveals that the latter are returning to normal while a proportion of the meal still remains in the stomach. In addition, the intestine is still processing maximum amounts of food during this period. This indicates that metabolites other than glucose, i.e. lipids, and amino

Fig. 7 - Relationship of various physiological parameters of Limanda limanda following a satiation meal of Mytilus edulis during the January to February period. (Temperature = 15.0°C, fish (♀) weight = 101-200g, meal size ≈ 6% body weight, o—o plasma glucose, ●—● plasma fatty acids).


% appetite return
8·9
8·3
17·1
61·4
94·5
% satiation meal in intestine
5·0
8·3
9·4
11·0
8·0
2·5
% meal evacuated from stomach
2·7
4·2
6·2
7·7
8·3
9·7
10·0
% increase in metabolic rate
7·2
13·3
14·0
13·9
11·6
10·1
7·1
4·9
1·6
400
200
0
μeq fatty acids/l plasma
75
50
25
0
mg glucose/100 mls plasma
0
20
40
60
Time since feeding – (h)

acids in particular, are still being absorbed from the intestine and undergoing assimilation. This is supported by the respiratory rate, which remains elevated well beyond the point when plasma glucose has returned to the pre-feeding level. A similar pattern emerges from a comparison of the intestinal contents, SDA and plasma glucose levels of fish fed a 2% body-weight meal of freeze-dried whiting (Chapters 1 & 3). It is, therefore, probable that dietary amino acids are continually absorbed until total intestinal clearance is approached. This would explain why SDA remains elevated until after the entire meal has been cleared from the intestine since deamination is a major process demanding increased oxygen uptake.

In contrast to the findings for aholehole (Muir and Niimi, 1972), voluntary food intake by Limanda was not inhibited during the long period that oxygen uptake remained maximally elevated. This confirms earlier ideas that the increased oxygen demand associated with the assimilation of metabolites does not necessarily prevent further consumption. However, it is apparent that, similarly to P. platessa (Jobling, 1981), Limanda may have an upper limit beyond which it is unable to further increase its oxygen consumption purely for the energy consuming process of SDA (Chapter 1). In other words, the rate of assimilation of dietary metabolites is limited, so that the duration of peak oxygen consumption rates are determined by meal size and/or composition. In view of this fact, SDA could indirectly affect appetite by its limiting influence on the assimilation rate of a meal.

One final point relating to the possible influence of SDA on general feeding behaviour concerns its effect on other aerobic activities (Tandler and Beamish, 1981). It is likely that during peak periods of post-prandial oxygen uptake in Limanda, the available oxygen for activities such as prey location may be restricted. This is supported by the observation that peak oxygen uptake rates associated with food processing in P. platessa amounted to 30-40% of the metabolic scope for activity (Jobling, 1981). Furthermore, peak rates in a number of other species have actually equalled the active

rate of oxygen uptake (Job, 1954; Averett, 1969; Niimi and Beamish, 1974).

The present study has conclusively shown fluctuations in plasma glucose and fatty acid levels following a meal. These latter nutrients, especially glucose, are of considerable importance in the appetite control of higher vertebrates. Equally, they may prove to be relevant in the control of feeding rates in fish. Examination of the post-prandial nutrient patterns in fig. 7 demonstrates that food is still consumed even when these plasma nutrients are maximally deviated from the pre-feeding level. Up to this point, the fish do appear to be feeding to fill the available stomach capacity, as originally suggested by Gwyther and Grove (1981). Some 24-30 hr after feeding, plasma glucose and PFA are approaching their pre-feeding levels. Coincident with this latter period appetite was strongly suppressed. In this instance, the fish did not fill their stomachs to full capacity.

In view of the above observations, there is no real evidence for a simple relationship between a specific level of either of the two metabolites examined and inhibition of food intake. This complies with the evidence from artificially-induced hyperglycaemic studies. However, appetite is clearly suppressed after a proportion of dietary carbohydrate has been metabolised, and after a period of enhanced lipogenic activity. In higher vertebrates, plasma/glucose had to be first metabolised before it suppressed appetite. The gradual return of plasma glucose and FA to pre-feeding levels may also indicate that the energy reserves have been replenished at this juncture. In addition to the greater lipogenic activity, probably due to the elevated plasma glucose levels, it is likely that dietary lipids will also have contributed to the available energy reserves. The strong influence of dietary lipid on appetite was clearly demonstrated under ad lib feeding conditions. A reduction in the level of this nutrient led to an earlier return of appetite.

The main conclusion to be drawn from the present evidence is that it is the overall post-prandial change in energy availability/reserves, rather

than the specific levels of particular plasma nutrients which modifies the return of appetite in Limanda. This is currently the most favoured theory for appetite control in higher vertebrates (Russek, 1981). An increased feeding frequency, following dietary dilution, may be explained by a more rapid depletion of energy reserves in specific tissues. However, this point is only speculative and concrete evidence is currently non-existent. Furthermore, it is appreciated that the above conclusion is based on a rather crude measure of appetite return, although the more accurate demand-feeding studies do demonstrate the importance of dietary energy in this field.

It is accepted that the caloric value of the food will probably be just one of many physiological elements forming a multifactorial system of appetite control in fish. In addition to the physiological factors modifying the appetite of fish, their feeding behaviour in the natural state will be influenced by the types of food available, experience and a whole range of environmental and social stimuli to which they are exposed. Irrespective of this fact, the calorific value of food has a strong influence on fish appetite. It is, therefore, important to establish the optimum ratio of the major dietary nutrients for different fish species under intensive culture.

SUMMARY

1. Limanda limanda evacuated a standard body weight meal at a faster absolute rate with increasing body size. However, this rate of elevation was not enough to prevent the large fish from having a longer gastric evacuation time. Under multiple feeding regimes, a meal was evacuated faster when followed three hours later by a second meal. This was only true where meals remained discrete after ingestion.

2. The faster evacuation of food from the stomach when followed by more material will affect a variety of physiological parameters that are influenced by food intake.

3. The respiratory rate increases rapidly after food intake. Both duration and magnitude of the SDA effect vary according to the energy ingested, while the peak rate of oxygen uptake appears to have an upper limit. Peak post-prandial oxygen consumption occurs during the period when maximum amounts of food are being processed. Plasma glucose and fatty acid levels had returned to normal while oxygen consumption still remained elevated.

4. Chronic cannulation techniques were very effective for the taking of serial blood samples from Limanda while avoiding stress-induced changes in plasma metabolite levels.

5. A seasonal variation in the level of plasma fatty acids was likely to have been associated with the reproductive cycle of the fish. The winter (859.00 µeq/l) and spring (691.0 - 935.2 µeq/l) peaks probably reflected mobilisation of lipid reserves for gonadal growth, followed by a reabsorption of gonadal material respectively. No seasonal variation in plasma glucose was observed and the annual average was 22.44 mg/100ml plasma.

6. Limanda responded to mild handling stress by developing a significant hyperglycaemia followed later by a hypoglycaemic condition.

7. Ingestion of meals of Mytilus mantle resulted in elevated plasma glucose levels. The duration of the hyperglycaemia was dependent upon the temperature. Glycogen-supplemented meals demonstrated that both the duration of a feeding-related hyperglycaemia and the peak level of plasma glucose attained were related to dietary glycogen levels.

8. Ingestion of satiation meals of Mytilus resulted in a reciprocal rise in plasma glucose and a fall in plasma fatty acids. There was some seasonal variation in the duration of the metabolite response, probably reflecting differences in dietary glycogen level.

9. The glucose/free fatty acid relationship was replicated by infusion of a glucose load (0.5g/kg body weight). The reciprocal decline in plasma fatty acids with a rise in glucose levels was thought to be insulin-mediated as in higher vertebrates.

10. Limanda adapted readily to a self-feeding system and showed distinct feeding rhythms under a 24 h lighting regime.

11. There was a positive response by groups of dabs to reductions in the dietary levels of carbohydrate or lipid. The fish compensated very accurately to maintain a specific calory intake. This was essentially achieved by the ingestion of smaller but more frequent meals. However, in the case of a reduction in the carbohydrate component, larger meals were taken at the same frequency as the control diet.

12. Maximum post-prandial increases in oxygen uptake did not inhibit further food intake.

13. Food intake was not totally inhibited during the period when plasma glucose and fatty acids were maximally deviated from 'normal' prefeeding levels

14. Artificially induced hyperglycaemia did not affect the food reaction time of Limanda.

15. Appetite was maximally suppressed even after plasma metabolite levels had returned to normal. This may indicate that some indicator of energy reserves is more important in relation to short term control of food intake in Limanda than simply the plasma levels of glucose or fatty acids.

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APPENDIX 1a) GASTRIC EVACUATION DATA FOR FISH (1-50g) FED SINGLE MEAL (WITH BINDER)

Fish wt (g)	Time (h)	Dry wt meal fed (mg)	Observed (mg)	Estimated (mg)
50	1	143	118	128
44	1	114	112	114
27	1	86	80	81
50	1	143	113	113
49	2.5	143	96	96
34	2.5	86	113	67
50	2.5	143	114	97
42	4	114	72	59
36	4	114	67	59
50	4	143	72	69
50	4	143	87	89
49	8	143	40	47
49	8	143	6	00
43	8	114	17	19

b) GASTRIC EVACUATION DATA FOR FISH (51-100g) FED SINGLE MEAL (NO BINDER)

Fish wt (g)	Time (h)	Dry wt. meal fed (mg)	Estimated (mg)	Observed (mg)
93	4	257	211	213
71	4	200	82	83
80	4	229	140	110
57	4	171	82	81
78	4	229	180	155
72	4	200	123	144
63	4	171	107	98
90	4	257	194	165
91	4	257	190	180
85	4	257	119	153
74	4	200	96	84
84	4	229	110	129
91	4	257	131	126
84	4	229	171	153
86	4	257	171	157
64	4	171	104	93
86	8	257	4	11
75	8	229	24	21
57	8	171	21	23
80	8	229	35	39
69	8	200	32	35
90	8	257	23	26
70	8	200	0	0
92	8	257	23	45
85	8	257	20	22
97	8	286	10	15
89	8	257	34	46
89	8	257	27	26
99	8	286	0	0
86	8	257	1	4
63	10	171	4	0
97	10	286	0	0
92	10	257	0	0
80	10	229	35	31
99	10	286	52	53

/Over

b) Contd.

Fish wt (g)	Time (h)	Dry wt. meal fed (mg)	Estimated (mg)	Observed (mg)
88	1	257	254	253
99	1	286	269	303
94	1	257	249	293
98	1	286	283	282
66	1	200	199	197

GASTRIC EVACUATION DATA FOR FISH (51-100g) FED 2 MEALS 3h APART (NO BINDER)

Fish wt (g)	Time (h)	Dry wt. meal fed (mg)	Estimated (mg)	Observed (mg)
94	4	257	161	423
100	4	286	172	447
95	4	286	182	503
94	4	257	164	384
76	8	229	118	286
94	8	257	59	279
99	8	286	156	315
82	8	229	72	238
73	8	200	81	199
100	11	286	131	315
75	11	229	137	246
66	11	200	49	195
64	11	171	33	124
80	15	229	58	86
56	15	171	70	94
83	15	229	14	120
75	15	229	0	82

c) GASTRIC EVACUATION DATA FOR FISH (101-200g) FED SINGLE MEAL (NO BINDER)

Fish wt (g)	Time (h)	Dry wt. meal fed (mg)	Estimated (mg)	Observed (mg)
132	1	371	321	327
112	1	314	270	297
110	1	314	279	269
138	1	400	355	364
162	4	457	283	253
138	4	400	255	258
101	4	286	184	171
136	4	400	276	256
122	4	343	263	272
119	4	343	232	234
116	8	343	108	106
111	8	314	0.00	0.00
117	8	343	80	75
176	8	486	170.7	137
101	8	286	45	74
129	8	371	130	111
104	8	286	2	7
113	8	314	140	99
104	8	286	42	28
102	8	286	97	87
144	11	400	93	130
142	11	400	131	124
124	11	343	27	24
114	11	314	95	77
123	15	343	0	0
135	15	400	0	0
118	15	343	0	0
156	15	457	0	0
171	15	486	0	0.

d) GASTRIC EVACUATION DATA FOR FISH (101-200g) FED 2 MEALS 3h APART (NO BINDER)

Fish wt (g)	Time since M1 (h)	Wt. meal fed/meal (mg)	Total dry wt. observed (mg)	Est. dry wt. M1 (mg)	Wt. M2 (mg)
110	4	314	600	258	342
104	4	286	332	169	163
113	4	314	420	189	231
137	4	400	535	233	302
139	4	400	526	231	295
127	8	371	329	116	213
115	8	343	229	84	145
117	8	343	348	148	200
116	8	343	272	85	187
102	8	286	268	103	165
102	8	286	256	89	167
112	8	314	226	62	164
137	8	400	296	118	178
129	8	371	231	142	89
118	11	343	246	54	192
120	11	343	235	40	195
122	11	343	357	117	240
115	11	343	327	106	221
112	15	314	191	72	119
123	15	343	292	70	222
105	15	314	131	6	125
121	15	343	302	126	176

e) GASTRIC EVACUATION DATA FOR FISH (101-200g) FED SINGLE MEAL(WITH BINDER).

Fish wt (g)	Time (h)	Dry wt. fed (mg)	Estimated (mg)	Observed (mg)
111	1	314	258	298
151	1	429	403	417
135	1	400	331	377
135	1	400	373	425
124	1	343	273	342
101	1	286	281	263
110	4	314	152	146
125	4	371	208	244
158	4	457	239	216
135	4	400	266	254
111	8	314	153	122
102	8	286	98	81
118	8	343	174	146
103	8	286	101	79
118	15	343	15	8
132	15	371	67	45
131	15	371	74	60

17

f) GASTRIC EVACUATION DATA FOR FISH FED (101-200g) 2 MEALS 3h APART (WITH BINDER)

Fish wt (g)	Time hr. since M1.	Dry wt. meal fed (mg)	Total dry wt. observed (mg)	Est. dry wt. M1 (mg) ↓
116	4	343	445	198
117	4	343	521	176
140	4	400	559	213
136	4	400	572	194
109	4	314	441	158
122	4	343	493	215
117	4	343	460	149
127	4	367	396	125
164	6	457	612	210
130	6	371	463	146
144	6	400	512	140
147	6	429	495	172
144	6	400	452	144
105	6	314	396	126
115	8	343	420	155
101	8	286	304	132
160	8	457	485	114
111	8	314	251	58
124	8	343	274	84
127	11	371	180	33
118	11	343	267	39
134	11	371	265	70
151	11	429	193	2
106	11	314	195	32
108	11	314	144	15
150	15	429	45	1
125	15	371	89	28
101	15	286	91	7

g) GASTRIC EVACUATION DATA FOR FISH (51-100g) FED SINGLE MEALS (WITH BINDER)

Fish wt (g)	Time (h)	Dry wt. meal fed (mg)	Observed (mg)	Estimated (mg)
94	1	257	243	222
100	1	286	238	219
89	1	257	231	227
63	1	171	154	167
95	1	286	266	286
78	4	228	115	117
86	4	257	153	156
69	4	200	80	90
84	4	240	99	110
72	4	200	98	106
85	8	257	57	63
78	8	240	35	40
78	8	240	58	58
100	8	286	65	69
94	11	257	00	00
54	11	143	14	13
76	11	240	59	54

h) GASTRIC EVACUATION DATA FOR FISH (51-100g) FED 2 MEALS 3h APART (WITH BINDER)

Fish wt. (g)	Time (h)	Dry wt. meal fed (mg)	Observed (mg)	Estimated wt. M1 (mg)
90	4	257	353	123
97	4	286	523	150
91	4	257	329	102
69	4	200	270	98
92	4	257	317	124
94	4	257	349	112
90	6	257	274	109
99	6	286	398	150
100	6	286	343	134
60	6	171	215	65
51	6	143	141	18
71	8	200	138	5
74	8	200	185	27
100	8	286	302	69
80	8	229	201	27
70	8	200	131	6
75	8	229	265	44
95	8	286	271	70
83	11	229	105	8
70	11	200	100	11
83	11	229	22	2
64	15	171	103	11
67	15	200	97	15
72	15	200	20	10
77	15	229	102	12
78	15	229	49	8

APPENDIX 2 DEMAND FEEDING DATA FOR GROUP 3 (TEST DIET 3 = 5% LIPID)

Day	Actuations per hour																							
1	5	8	5	0	4	1	0	1	2	0	1	0	2	28	8	1	6	0	0	0	0	0	0	0
2	0	0	0	1	1	2	1	2	3	1	6	1	0	0	0	0	0	0	0	2	31	0	0	0
3	0	0	0	0	0	0	0	0	0	0	15	6	9	0	0	7	0	3	0	0	2	21	0	0
4	8	0	0	1	9	7	1	2	0	3	0	0	0	0	0	0	0	0	0	0	0	2	5	8
5	8	3	5	5	1	0	0	1	0	0	0	0	12	6	0	0	2	0	0	0	0	0	0	0
6	5	22	12	11	9	3	0	0	0	0	1	1	0	0	0	0	0	0	0	0	5	0	9	0
7	1	0	3	1	0	2	4	0	0	3	3	0	0	0	0	0	0	0	0	0	1	4	5	2

Control diet

1	0	0	0	0	1	0	9	7	9	6	6	8	11	1	1	2	0	0	0	6	1	0	3	0
2	3	0	0	0	0	0	4	2	5	1	0	0	0	0	0	12	2	0	0	0	7	6	0	0
3	0	4	0	0	0	0	0	9	9	3	3	0	0	0	19	11	0	9	0	4	0	0	0	0
4	0	0	16	0	0	0	3	0	2	0	0	0	0	0	0	0	0	0	6	11	15	3	2	2
5	0	0	0	0	5	24	0	0	0	0	0	8	6	17	23	2	2	0	0	0	0	1	11	2
6	1	3	7	11	0	0	2	0	0	4	0	4	0	0	0	0	0	0	0	0	2	12	6	0
7	0	1	5	8	16	11	4	1	0	0	7	0	0	8	0	10	3	5	7	0	4	0	0	0
8	0	0	2	15	17	0	0	1	11	9	3	0	1	1	2	0	0	0	0	2	1	0	0	0

Test diet