DOCTOR OF PHILOSOPHY

Aspects of the regulation of food intake in the dab, Limanda limanda (L.).

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ASPECTS OF THE REGULATION OF FOOD INTAKE

IN THE DAB, *LIMANDA LIMANDA* (L.)

A thesis submitted to the University of Wales

by

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CONTENTS

ABSTRACT

The regulation of food-intake in *Limanda limanda* was investigated, including (a) the role of the stomach as a limiting factor in fish food intake, (b) the qualities of a diet dab respond to and (c) the dynamics of food intake.

Fish fed on squid (4.0 $kJ.g^{-1}$, 76.2% moisture) ate similar daily rations whether fed three times daily or once per day; when fed every three days they were unable to maintain this intake. Fish fed on pellets (18.8 $kJ.g^{-1}$, 8% moisture) could maintain their average daily food intake for all meal intervals tested.

After a satiation meal, food intake broadly increased with deprivation time for at least 96 hours with no clear indication that stomach volume was limiting. However, when the data was re-examined using a return map (where meal *m* is plotted against meal *m*-1) there was evidence that feeding was restricted by stomach fullness as the interval between meals exceeded 25 hours. Surprisingly such limitation did not occur at higher feeding frequencies.

Three models of food intake were used to simulate food-intake data, in which the role of the stomach as a constraining factor was varied:

1) Food intake was assumed always to be completely limited by stomach volume.

2) Food-intake was assumed always to be driven by a systemic need

3) Food intake was assumed to be chiefly limited by a systemic need, but when this was high, stomach volume would constrain intake.

Comparisons of experimental results with these models suggest that when fish are fed frequently, or on a high-energy diet, the stomach volume is probably not limiting, whereas for a low-energy diet, fed infrequently, stomach volume was limiting when systemic need was high.

Dab adapted their food intake to diets of different water content (and therefore energy density). They also adapted the distensibility of their stomachs in response to the increased volume eaten; fish fed on pellets having less distensible stomachs than those fed on squid. Thus it is unlikely that stomach volume can limit food intake in the long term, unless food quality and/or meal timing is variable and the fish cannot adapt their stomachs to the diet/feeding frequency. Methodological trials proved that that observed stomach

volume is a function of the measurement technique, as well as the diet history of the fish, and experiments examining stomach volume should take this into consideration.

The question of what aspect of a diet *L. limanda* adapt to was examined by testing different models using path analysis, a method of inferential modelling of causal relationships, in an attempt to explain how food-intake is regulated. Dab were found to be adapting to both the energy content of the diet and to the individual nutrients.

The dynamics driving food intake in groups of dab were investigated using non-linear time series analyses. These proved to be low-dimensional, significantly non-linear, deterministic systems. The data also suggests that such systems are either capable of occasional chaotic behaviour, or are on the edge of chaos *i.e.* complex dynamical systems. Thus food intake is under the direct control of few (two or three) variables, through which the many known factors that influence food intake must act. Comparisons were made with individuals and groups of *Oncorhynchus mykiss*, with a brief look at *Merlangius merlangus* and *Dicentrarchus labrax*. These results were similar to the dab, and so this dynamical behaviour may be a feature of teleost fish in general. Importantly the fact that individual trout had similar feeding behaviour to groups indicate that the findings were not a function of hierarchical dynamics. The significance of this finding should be that appetite control with these properties allows rapid adjustment of fish according to changes in diet quality.

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CHAPTER ONE: INTRODUCTION

(i) Why try and predict how much food is required by a cage of fish on a day-to-day basis?

Farmed fish, like terrestrial farm animals (Forbes 1995), are usually only offered one diet at a time, either as discrete meals, or *ad libitum* through demand feeders. Individual animals that are offered a diet that is not perfectly balanced will eat an amount that offers the best compromise for its nutritional requirements (Raubenheimer and Simpson 1999), thus it is possible that they will either under- or overeat. If the former is the case a large proportion of the metabolisable energy in the diet is required for maintenance, therefore growth is sub-optimal and the farmer loses money If the latter is true then excessive fat deposition may occur (Forbes 1995). R. Sinnott (Trouw Aquaculture) communicated that the level of lipid in farmed salmon carcasses is considerably greater than in wild fish, so that farmers are required to fast their fish for two weeks in order for it to be suitable to sell for smoking.

Inappropriate feeding methods, or a failure to feed farmed fish according to their natural pattern of feeding behaviour can also lead to sub-optimal production. Talbot *et al.* (1999) point out:

"...the feeding of farmed fish is affected by many husbandry factors, such as cage size, pellet size, feed delivery rate, spatial delivery of food, stocking density, water conditions and recent feeding history (Juell 1995, Ang and Petrell 1998). It is only when there are no restrictions in time, space or quantity of feed that fish can express their preferred feeding behaviour".

Even when fish are allowed to feed as they wish, the satiation ration varies from meal to meal (observed by Pandian 1970, Jobling *et al.* 1989, Juell *et al.* 1994, Ang and Petrell 1997, although all experiments in which fish are allowed to feed voluntarily demonstrate this). The difficulty in judging satiation ration can not only lead to either under- or overfeeding but, given that feeds are by far the greatest production cost in intensive fish farming, a small improvement in the growth per gram of food given (food conversion ratio, or FCR, Jobling

1993) can lead to significant improvements in economic performance (Ruohonen 1994, Talbot *et al.* 1999). A model that could predict daily food requirements of a cage of fish would be a useful tool for this reason. Clearly the study of food intake is of great value to the fish farmer through direct economic benefits and better fish flesh production.

(ii) Predicting day-to-day food consumption in wild fish

Fisheries scientists have an interest in the amount of food consumed daily by fish stocks, as Pitcher and Hart (1982) point out: "The ecologically important feature of digestion is the rate at which food can be processed, as this determines the upper limit to the intake of energy and hence the growth rate." Fisheries scientists and ecologists have attempted to estimate food consumption by using direct methods based on stomach content analysis and rates of gastric emptying (Jobling 1986a); techniques have been reviewed by Elliott and Persson (1978), Elliott (1979) and Fänge and Grove (1979). The two most common methods used were proposed by Bajkov (1935) and Elliot and Persson (1978) (see for example Basimi and Grove 1985b).

(iii) Wild fish eat a great variety of foodstuffs

Only a small proportion of fish species are domesticated and, unlike those that are, fish in the wild are not limited to a single food type. Generally in animals, in order to satisfy the systemic need for the many different nutrient molecules required for survival and reproduction, any particular individual will need to select either a single appropriate diet, or a range of diets which individually offer a sub-optimal nutritional balance, but together offer the best combination possible. Food selection also must take into consideration the necessary compromises with the cost of finding, eating, and processing each food type (Raubenheimer and Simpson 1999).

Pitcher and Hart (1982) estimated that there are something like 20,000 described species of bony fish alone. These species form fish assemblages in different habitats (*e.g* Pitcher and Hart 1982, Figure 1.8 and several examples

in Lowe-McConnell, 1987), though the former point out that most species will live in different places and eat different items during their various life stages. Fish assemblages are part of the ecological community as a whole (Ricklefs 1979) and therefore interact with other species, particularly *via* food webs. The sum of the prey species can be described as a 'feeding-niche', part of the overall niche of a species. It is hypothesised that no two species can occupy the same niche and co-exist in a stable environment (Hutchinson 1958), thus two species in the same geographical location must have different habits. Studies of fish food intake in the wild have demonstrated that fish that exist in the same habitat feed on different prey (trophic partitioning) thus avoiding competition, such as in *Limanda limanda* and *Pleuronectes platessa* (Carter *et al.* 1991). The observed trophic partitioning in this case was modified by the relative numbers of prey, and the two species fed on similar diets at times when the prey species were present in abundance.

Within a single habitat, avoidance of competition is also achieved through spatial and temporal separation of fish species (see for example Ribbink *et al.* 1983).

Trophic partitioning is not always observed; in a study of twelve *Cichlidae* species that are found in the rocky littoral zone of Lake Nyasa, four species were found to have identical niches (Fryer 1959). This was thought to be because their numbers were kept down by predators and therefore food was always present in excess, so that no competition occurred.

The requirement that all species in nature must occupy a different niche when food is not present in excess means that, for species to co-exist, they must feed on a variety of food stuffs. Kapoor *et al.* (1975) review the categories in which fish can be placed depending on their diet. Firstly, categories depend on whether the fish eat plants (herbivores), animals (carnivores) both animals and plants (omnivores) or detritus (detritivores). They can further be classified according to the range of food items taken; generalists (euryphags), specialists with a limited range of food (stenophags) or with only a single food type

(monophags). Many further subdivisions can be used (*e.g.* planktotrophs and piscivores).

Optimal foraging theory suggests that prey items can be selected according to their profitability, which is a function of the energy value of the item, as well as cost of capture, handling and swallowing it. Krebs (1978) suggests that prey should be added to the diet if the net gain contributed by it is greater than the average net gain received from all the prey previously selected. The decision is modified by the relative abundance of prey species (Pyke *et al.* 1977); as the abundance of a profitable food item decreases, the choice of foodstuffs for a species should widen (Pitcher and Hart 1982).

The sand dab, *L. limanda* (L.) are euryphagous carnivores, although at any one time individual fish can be found to have eaten prey of few species. As with other species, food taken varies with fish size (Basimi 1976, Ortega-Salas 1980, Bakhsh 1982, Nedergaard 1995). There is also a variation in food intake with season (Knust 1987, Basimi and Grove 1985b), location (Lozán 1989, Nedergaard 1995) and sex (Lozán 1982, Temming and Hammer 1994).

Relative availability of prey species also has an effect on prey selection in the dab (Whyche and Shackley 1986, Carter *et al.* 1991), although the wide range of prey types taken make it unlikely that an absence of particular prey will affect growth (Weatherly 1972).

(iv) Diet affects the morphology of the gastrointestinal tract

The structure and function of the teleost alimentary canal are reviewed by Barrington (1957), Kapoor *et al.* (1975), Fänge and Grove (1979) and Smith (1979).

Very briefly, most teleosts have a pharynx, oesophagus, stomach, intestine and rectum, although some are stomachless (*e.g.* the Cyprinidae, the blenny, *Lipophrys* (= *Blennius*) *pholis*). Associated with the system is the liver, pancreas and spleen. Some pre-digestion occurs in the stomach which also serves as a storage site (Pitcher and Hart 1982). Most fishes secrete HCl in the gastric fluid, with the main gastric enzyme being the endo-protease pepsin; this

is secreted as pepsinogen which converts to pepsin in an acid environment. A variable number of pyloric caeca are found in many fish species, but their occurrence is not correlated with any particular diet (Kapoor *et al.* 1975). Digestion is completed in the intestine, where digestive enzymes are secreted into the intestine from either the intestine wall or (mainly) the pancreas. The range of enzymes present depends on the species in question and particularly on the diet (Fänge and Grove, 1979), but generally pancreatic juice contains several enzymes serving in the digestion of protein, fat and carbohydrate (Ruohonen 1994). Fish bile is usually weakly alkaline and has high sodium and low chloride concentrations (Fänge and Grove, 1979). In some species the intestine can be divided into the coiled absorptive area and a short straight length (analogous to a large intestine in mammals) leading to the anus (Pitcher and Hart 1982).

The form and function of the alimentary canal determines (or reflects) the type of food that can be eaten and the rate at which it can be processed (Pitcher and Hart 1982). Kapoor *et al.* (1975) review studies on the extent of adaptation of the alimentary tract to a particular kind of natural diet. Whilst the specific adaptation may vary from species to species, the function of the adaptations is often similar. The length of the gut, or in some cases the degree of folding of the gut wall, varies with diet and is usually longest in herbivores and detritivores, with carnivorous fish having a shorter gut.

The range of morphology of Pleuronectiform gastrointestinal tracts was described in a comparative study by de Groot (1971). In this group the relative size of the stomachs and oesophagi is gradually reduced from the Psettodidae (piscivores), with fully developed stomachs and oesophagi, to the Soleidae, with largely reduced ones and the stomachless Australian flounder (polychaete/mollusc feeders) (Grove and Campell 1979a, b). Dab fall mid-way in this scale with still well-developed oesophagus and stomach. Jobling *et al.* (1977) and Basimi and Grove (1985a) found that dab have a stomach volume of around 8 ml per 100 g fish. There are sex-specific differences in stomach

size; Lozàn (1992) found that the weight of the stomach and intestine was greater for the faster-growing female dab than for the males.

The morphology of the gut has been observed to change within species in the short term, as the diet changes (discussed in Ruohonen 1994). Magnan and Stevens (1993) found that the mass of pyloric caeca increased when brook trout, *Salvelinus fontinalis*, was shifted from an adequate diet to a poorer one, whereas in *Oncorhynchus mykiss* high carbohydrate diets induced changes in the anterior intestine and pyloric caeca (Osta Garrido *et al.*1993). Hilton *et al.* (1983), Bromley and Adkins (1984) and Ruohonen and Grove (1996) found that stomach size increased in relation to fish weight when the energy density of the diet was decreased.

The morphology of the gut can change with development; Klust (1938, Cited in Barrington 1957) found that in the Cyprinid *Rhodeus* the ratio of intestinal to body length was 1.55 for a 1.1 cm fish, whereas it was 2.16 for a 6.7 cm fish.

(v) The short term regulation of appetite

Schwartz *et al.* (2000) reviewed the mechanisms within the central nervous system which regulate homeostasis and satiety, and how they interact in humans. Very simply the current model for regulation of food intake in humans is as follows: For the homeostatic regulation mechanism, the hormones insulin and leptin are adiposity signals, and are secreted in proportion to body fat content. High levels of insulin and leptin act in the hypothalamus to stimulate the catabolic effector pathways (which suppress energy intake and promote energy consumption), whilst inhibiting the anabolic effector pathways (which stimulate energy intake and suppress energy consumption). These hormones act *via* several neuropeptide effectors of adiposity signals (Schwartz *et al.* 2000, Friedman 2000).

The major determinant of meal size is the onset of satiety, defined as "a biological state induced by neurohumoral stimuli generated during food ingestion that leads to meal termination" (Schwartz *et al.* 2000). Satiety information generated during a meal is mainly conveyed to the brain by means

of afferent fibres of the vagus nerve and by others passing into the spinal cord from the upper gastrointestinal tract (Ritter *et al.*1994). This information, together with information from the abdominal viscera and the taste buds (Travers and Norgren 1987), converges into the nucleus tractus solitarus (NTS), in the caudal brainstem (Schwartz *et al.* 2000).

Satiety-inducing signals to the NTS are initiated by mechanical or chemical stimulation of the stomach and small intestine by the meal, neuronal input related to energy metabolism in the liver (Friedman *et al.* 1999) and humoral signals such as cholecystokinin (CCK), released upon nutrient stimulation of the neuroendocrine secretory cells lining the intestinal lumen (Moran and Schwartz 1994). This gives a clear role for the upper gastrointestinal tract in the satiety mechanism of humans, involving distension, as well as the nutrient and energy components of a diet.

There is evidence that the signals involved in energy homeostasis (leptin, Matson and Ritter 1999; insulin Figlewitz *et al.* 1986) modulate the response of NTS neurones to meal-related satiety; Leptin also potentiates the effect of CCK on NTS neurones (Emond *et al.* 1999).

Knowledge of the regulation of homeostasis and satiety in fish has lagged behind that of humans or other mammals (Fletcher 1984).

There is now evidence that CCK is involved in the satiety mechanisms of fish (Le Bail and Bouef 1997). However there is a debate about the area on which CCK acts; does it act directly on the brain or does it regulate a peripheral satiety centre or both? Jobling (1986a) argued for the latter:

A fasted fish will cease feeding due to a mechanical distension signal from the stomach (see also Vahl 1979). As food enters the duodenum, CCK is released, triggered by nutrients in the diet. This leads to gall bladder emptying, secretion of pancreatic enzymes and an inhibition of gastric muscular contractions (which results in a cessation of, or a reduction in the flow of, nutrients into the duodenum). The pancreas enzymes include trypsin which provides a negative feedback signal for CCK production. As CCK is inhibited, gastric contractions are no longer inhibited and more food passes into the duodenum. This sequence

is repeated and leads to a pulsed emptying of the stomach, with the frequency of the pulses depending on the nutrient concentration of the food (Jobling 1986a and references therein). This model assumes that the gastric emptying rate is closely related to food intake (see below). If this is the case it also suggests a mechanism for the regulation of food intake for diets of different nutrient composition (see below).

Other hormones which may have a role in satiety and homeostasis in fish were recently reviewed by Le Bail and Bouef (1997). As well as CCK, the hormones peptide YY (PYY), glucagon, and adrenalin may act as short-term (satiation) factors and are generally inhibitory. Growth hormone (GH), thyroid hormone (TH) and leptin require longer to modify food intake behaviour (*i.e.* they regulate homeostasis). Whether insulin or glucocorticoids regulate homeostasis or satiety is not clear and may depend on the hormonal and metabolite environment (Le Bail and Boeuf 1997). In salmonids, GH stimulates appetite for example in the coho salmon, *Oncorhynchus kisutch* (Markett *et al.* 1977, Devlin *et al.* 1999) and rainbow trout, *Oncorhynchus mykiss* (Farbridge and Leatherland 1993, Johnsson and Björnsson 1994). GH has also been found to have this effect in the channel catfish, *Ictalurus punctatus* (Wilson *et al.* 1988). Thyroxine (T_4) was inferred in mechanisms of satiety in *O. mykiss* (Farbridge and Leatherland 1993) whereas 3,5,3' - Triiodotyrosine (T_3) increased appetite (Fagerlund *et al.* 1984). T_3 also had this effect in the Red Sea bream, *Chysophrys major* (Woo *et al.* 1991). Peter (1997) observed that CCK and bombesin had powerful satiety effects in the goldfish, *Carassius auratus*, and that both hormones are prominent in the neuroendocrine secretion of growth hormone. Specific dynamic action (SDA) and plasma metabolites may also have a role in the regulation of appetite of fish (Fletcher 1984) and mammals (Forbes 1995).

When food is present in excess, daily food intake depends on the satiation level and the turnover of food in the gastrointestinal tract (Ruohonen 1994). The latter is dependant on gastric or intestinal emptying rate, and therefore it has been suggested that there is a correlation between emptying rate and food

consumption (Brett 1971, Elliot 1975, Hunt and Stubbs 1975, Hunt, 1980). Furthermore, the volume of the stomach or foregut can be a limiting factor in the meal size of a single feeding bout (Rozin and Meyer 1961,1964, Grove *et al.* 1978, Grove and Crawford 1980, Jobling 1983). Catherill (1979) argued that the rate of stomach (or foregut) emptying is expected to affect both the frequency of meals and the amount taken in each meal. If this is the case then food intake may be the growth-limiting step (Grove *et al.* 1978, Gwyther and Grove 1981).

Correlations have been observed between gastric emptying rate (GER) and appetite return, and between meal frequency and gastric emptying time (GET) in certain species. For example, in *O. mykiss* Grove *et al.* (1978) observed a close inverse relationship between contents of stomach and appetite, whereas Adron *et al.* (1973) and Landless (1976) observed a correlation between GET and meal frequency in demand-fed fish. This relationship was also observed by Elliot and Persson (1978), Jobling *et al.* (1977), Gwyther and Grove (1981), Grove *et al.* (1985), Ruohonen (1994), Seyhan *et al.* (1998) and Sims *et al.* (1996). Contrary to previous studies in the dab (Gwyther and Grove 1981), Fletcher (1982) found that whilst a good correlation was found in the dab up to ten hours after a meal, after 24 hours the amount consumed was not enough to compensate for the food evacuated. It should be noted however that GER data and appetite return data were derived from different trials.

(vi) Models of gastric evacuation rate and modifying factors

Not only is GER possibly important in the short term regulation of food intake, it is also an essential variable used in methods estimating food consumption in the wild (*e.g.* Bajkov 1935, Elliott and Persson 1978). The original method of Bajkov (1935) assumed a linear GER, whilst subsequent modifications of this method (Doble and Eggers 1975) and the method of Elliott and Persson (1978) assume an exponential GER. It is important for these estimations of food consumption that the correct GER is used, and therefore a number of studies have examined the shape of the gastric evacuation curve.

Olsen, (1989) and dos Santos (1990) discuss the various models used to describe digestion and gastric evacuation. Much of the review below is based on these works.

Jobling (1986a, 1987) proposed that there is a pulse-rate modulation of stomach evacuation in fish in the same way as in mammals. This would mean that gastric emptying is not a smooth process and therefore models can only be approximations. Fish will ideally control the rate of evacuation to achieve long enough storage time in the stomach and optimal continued digestion and absorption along the intestine. If the rate is too high, digestion will not be complete and absorption along the intestine will suffer. On the other hand, the evacuation should be fast enough to yield space so that more food can be ingested (Jobling 1986b, 1987).

The gastric digestion of newly- ingested food can be divided into three phases (Karpevitch and Bokova, 1937). An initial delay time (t_d), in which the gastric juices start penetrating and acting on the food. This is followed by the emptying phase, of duration t_{end}. Finally the third stage is the emptying of the last remnants of the meal, in normal diets this may represent materials of low digestibility. Time delay was modelled as being temperature dependant by Grove *et al.* (1985), being therefore a constant at constant temperature for a given diet. Basimi and Grove (1985a) stated that t_d was dependant on temperature and the friability of the diet.

Grove (1986) suggested that the emptying function for a particular food type consists of two major phases:

(a) a temperature sensitive delay time (t_d) during which newly ingested food would be digested in the stomach; and

(b) an emptying phase of duration t_{end} which depends on:

(1) temperature,

(2) the distention of the sac-like stomach,

(3) the secretory surface of the stomach, and

(4) the surface area of the meal.

Energy density is also important (Al Aradi 1986). It is further assumed that the temperature effect is exponential, the stomach volume increases linearly with fish weight (w), the gastric secretions are proportional to the stomach surface, and the secretion stimulus is proportional to the increased stomach radius. This leads to a first order differential equation:

$$dV/dT=-Ke^{bT}w^{0.33}V \quad \text{.....(equation 1.1)}$$

and the solution

$$\ln V_t=\ln V_0-(Ke^{bT}w^{0.33})t \quad \text{.....(equation 1.2)}$$

or

$$V_t = V_0\, e^{-ct} \qquad \text{where } c = k\, e^{bT}w^{0.33} \quad \text{.....(equation 1.3)}$$

where V_t is volume of food in the stomach at time t, V_0 is the volume of food fed to the fish, T is temperature and w is fish weight. This curve is monotonic (as are the curves described below), however if the underlying exponential emptying curve is interrupted by feed-back from the intestine, the curve will change shape and appear 'stepped' (see below).

If fish size and temperature are kept constant, the principal emptying curve can be studied, and the general differential equation describing the evacuation rate is (see Jones 1974)

$$dV/dT=-cV^b \quad \text{.....(equation 1.4)}$$

Jobling (1981a, 1987), From and Rasmussen (1984), Holmgren *et al.* (1983) and others discuss the value of b that best fits the experimental data. Four models emerge:

Exponential model. The assumption behind this model is that the emptying rate is proportional to the content of the stomach:

$$dV/dt = -aV^{1.0} \quad \text{.....(equation 1.5)}$$

or, after integration,

$$V_t = V_0 e^{-at} \quad \text{.....(equation 1.6)}$$

Square root model. This model assumes that the stomach can be viewed as a cylinder, where the wall tension will be proportional to the radius. The radius varies with the square root of the volume, and the tension of the stomach wall will also be proportional to the square root of the volume:

$$dV/dt = -aV^{½} \quad \text{.....(equation 1.7)}$$

or , after integration,

$$V_t^{½} = V_0^{½} - ½at \quad \text{.....(equation 1.8)}$$

Rectilinear model. While the exponential and square root models are volume dependant, the rectilinear model is surface dependant. This stems from the assumption that digestion operates on the surface of the food bolus. The rate of digestion and presumably the rate of evacuation, will therefore depend on the surface area of the bolus. The surface of a sphere varies with volume$^{2/3}$:

$$dV/dt = -aV^{2/3} \quad \text{.....(equation 1.9)}$$

or , after integration,

$$V_t^{1/3} = V_0^{1/3} - 1/3at \quad \text{.....(equation 1.10)}$$

Linear model. This model assumes that the evacuation rate is constant, possibly because the emptying rate is independant of digestion rate and / or stomach forces, (Olsen, 1989).

Seyhan *et al.* (1998) demonstrated that in whiting fed small meals of natural prey items the emptying rate is linear but varies according to the number, n, of similar weight items given, so that the gut emptying time is similar whatever the value of n. A model given by Grove *et al.* (1985), in which n food particles are digested as a single particle presents a similar argument.

The GER of different species of fish have been found to differ (Fänge and Grove 1979, Grove *et al.* 1985). To give two examples, flatfish have been found to fit a curvilinear model (*e.g.* Edwards 1971, Goddard 1974, Jobling *et al.* 1977, Grove *et al.* 1978, Flowerdew and Grove, 1979, Moctezuma 1982, Fletcher 1982 and Hailstone 1984) whereas the gadidae are claimed to demonstrate a linear evacuation (Jones 1974, Bromley 1988, Robb 1990, Singh-Renton 1990 and Seyhan *et al.* 1998). Recently however Andersen (1998, 1999) found the emptying to follow a power curve in whiting, especially for large meals.

Jobling (1986a) proposed a model in which the power term, b, was not constant but varied according to the energy density and the size of the diet. Thus for low energy or small diets gastric emptying would be curvilinear, whereas for high energy or large diets a linear model would offer the most efficient delivery to the intestine. Olsen (1989) points out that the model that best fits the experimental data is dependant on several factors; for example, fish species, emptying phase, meal size and feed properties like particle size and energy content. The least refractory fraction may be digested first, thus yielding a varying nutritional time profile of the flow. This selectivity may be species dependant. The diets fed to cultured fish are very homogenous, and the selectivity will therefore be expected to be less pronounced than in fish fed whole natural prey organisms, especially if refractory material is present in the latter. Beamish (1972) observed that lipids were held back with respect to protein in the stomach of the largemouth bass, *Micropterus salmoides*.

Gastric emptying can be modified by a number of factors (Ruohonen 1994). Increasing temperature increases the GER up to a point below the upper lethal limit, when the trend either ceases or reverses (Mölnár and Tölg 1962a, b, Brett and Higgs 1970, Elliott 1972, Jobling *et al.* 1977, Persson 1979, Jobling and Davies 1979, Jobling 1980, Seyhan 1994)

The relation is curvilinear and Q_{10} values of close to 2 have been found for several species (Jones 1974, Brett and Higgs 1970, Fauconneau *et al.* 1983), including the dab (Jobling *et al.*1977 and Basimi and Grove 1985a). Tyler (1970) however found that cod switched from a lower to higher rate abruptly within its physiological range.

The effect of fish size on GER has been found to be variable, depending on the study (Ruohonen 1994 and references therein). In the case of flatfish, larger fish evacuate a meal faster in absolute terms (g h^{-1}), but the relative rate of evacuation (g.g^{-1}fish h^{-1}) is slower (Jobling *et al.* 1977, Flowerdew and Grove 1979).

Gastric emptying is normally faster with increasing meal size (*e.g.* Windell 1966, Elliot 1972, Jones 1974, Flowerdew and Grove 1979, Jobling *et al.* 1977, Jobling and Davies 1979, Gwyther and Grove 1981). In most cases the increase in GER does not compensate fully for the increase in meal size, and this is the case in the dab (Jobling *et al.* 1977). Sometimes however the GET is the same irrespective of meal size (*e.g.* Windell 1966).

The effect of meal quality on the power function of a gastric evacuation curve has been discussed by Jobling (1986a), generally higher calorific densities lead to slower GER (*e.g.* Grove *et al.* 1978, Flowerdew and Grove 1979, Jobling 1980). Al Aradi (1986) noted that this effect only occurred in diets of up to 1.4 kcal g^{-1} in the turbot (*Scophthalmus maximus* L.), above which the instantaneous rate of evacuation remained constant. This suggests that turbot are unable to compensate their GER for changes in dietary calorific value much above that normally found in the wild.

The surface area of a meal, or equivalently the size of the food particles, has been shown to have an effect on the GET, with greater surface areas per unit

weight resulting in faster GET (Swenson and Smith 1973, Grove *et al.* 1985, Jobling 1986a, Seyhan 1994). Recently the surface area of ration was found not to have an effect in the northern pike, *Esox lucius* (Nilsson and Brönmark 2000).

Previous feeding history has an effect on GER; it is modified by starvation (Windell 1966, 1967, Bellamy 1968, Ishiwata 1969, Tyler 1970, Brett 1971, Elliott 1972, Larson and Lewander 1973, Goddard 1974, Fänge and Grove 1979, Talbot *et al.*1984), whilst multiple meals increase the GER in many cases (Tyler 1970, Laurence 1971, Noble 1973, and Fletcher 1982). The outcome of multiple meals depends to some extent on how the diet is processed in the stomach (Grove *et al.* 1985). If particles from two meals mix to form a single bolus, the GER is similar to a single meal of similar bulk, whereas if the particles remain separate, and therefore have a bigger surface area, the emptying rate either increases or decreases, depending on the gastric secretion capacity of the species.

(vii) Fish can adapt to novel diets

Evidence presented above demonstrates that fish are able to feed to satiety in the short term whilst maintaining homeostasis in the longer term. As in humans (Schwartz *et al.* 2000), if energy homeostasis is to be achieved, either the amount of food consumed during individual meals or the frequency of the meals must be regulated. The observation that GER changes with the nutrient quality of the diet, together with evidence of a correlation between GET and meal interval (above) means that physiologically, the hypothesis that fish have the ability to adapt to novel diets is reasonable. In fact there is a good deal of evidence in the literature that this is the case.

A number of species have been observed to respond to dilution of dry diets with kaolin by increasing food intake to maintain calorific intake (for example Rozin and Meyer 1961, Grove *et al.* 1978, Marais and Kissil 1979, Jobling, 1980, Hilton *et al.* 1983). Bromley and Adkins (1984) found that in rainbow trout, such compensation occurred only up to 30 % addition of indigestible

cellulose into their diet by increasing their food intake, failing to compensate for 40-50 % dilution. Turbot, *S. maximus,* did not respond to dietary dilution when 33 % kaolin was present, however it did compensate adequately to a 50 % dilution (Al-Aradi 1986). Experiments in several species have shown the ability to adapt to changes in the dietary lipid content (*e.g.* Bromley 1980b, Koskela *et al.* 1998, Ogata and Shearer 2000), with an increase in lipid leading to a decrease in food intake. Fish have also been observed to adapt to dietary protein (*S. salar*, Sveier *et al.* 1999: hybrid *Clarius* catfish, Jantrarotai *et al.* 1998).

Increasing the dietary energy:protein ratio reduces food intake *e.g.* in the channel catfish, *Ictalurus punctatus* (Lovell 1979) and rainbow trout, *O. mykiss* (Lee and Putnam 1973). Jobling (1981 b) and Jobling and Wadsvick (1983) suggest that lipid is more important than proteins in the regulation of food intake, presumably because of its higher energy content.

Dilution of diet by water also has an effect on food intake (Bromley 1980a, Bromley and Smart 1981, Ruohonen and Grove 1996, Ruohonen *et al.* 1997). The ability to compensate for dilution with water failed when dilution reached 67% water, a level comparable with the water content of many natural prey items (Bromley and Smart 1981). In the study by Grove *et al.* (1978), increased feeding was achieved through more frequent meals, whereas in Bromley and Adkins (1984) the fish ate more at each meal.

Gwyther and Grove (1981) found that dab did not respond to dietary dilution. Fletcher (1982) observed a rapid feeding adaptation to decreases in dietary lipid and, to a much lesser extent, carbohydrate in the dab; they responded by feeding more frequently. In that study the diet was not simply diluted, but the relative amounts of the nutrients differed from diet to diet.

Factors other than the quality of the diet can modify food intake. A period of starvation or restricted diet results in an increased daily food intake (hyperphagia) and compensatory growth (Bilton and Robins 1973, Nicieza and Metcalfe 1997, Christensen and McLean 1998, Jobling and Johansen 1999, Saether and Jobling 1999, Qian *et al.* 2000).

When considering the effects of diet quality on food intake, one must also take into account other factors affecting meal size. Fish size has an effect on food intake, with larger fish eating more in absolute terms, but less in relative terms. This is because the relationship between fish size and absolute food intake is not linear; for most species, food intake has been found to increase with body weight raised to the power 0.6 - 0.8 (Jobling 1993).

The principle abiotic factor affecting food intake is temperature. Food intake increases with temperature, up to an optimum, after which it drops as temperature approaches the upper thermal limit of the species. (*e.g.* Elliott 1979, 1982, reviewed in Brett 1979 and Jobling 1993). Fish are able to adapt to water temperature at several physiological levels (Cossins and Bowler 1987) and fish kept at low temperatures, where food intake is low, eventually increase their food intake (Brett and Higgs 1970).

Other abiotic factors influence food intake, for example a reduction in oxygen saturation below a critical value results in lower feeding levels (Adelman and Smith 1970, Andrews *et al.* 1973). Pollutants, high dissolved nutrient levels in the environment and high or low pH can inhibit food intake (Svobodova 1993). Temperature and fish size may interact; in turbot (*S. maximus*) larger fish have optimum growth rates at lower temperatures than small individuals (Martinez-Tapia and Fernandez-Pato 1991).

(viii) Energy budgets in individual fish.

Once the food has been selected and ingested, the various components of the energy budget of an individual fish can be divided up as follows:

$$C = F + U + R + P \quad \text{.....(equation 1.11)}$$

where C is the total energy consumed, F is the energy value of the faeces, U is the energy value of the excretory products, R is the total energy of metabolism, which can be divided into standard metabolism (the metabolic rate when no activity is observed), energy required for activity and energy required for

digestion, movement and deposition of food, and P is the total change in the energy content of the body and can be divided into somatic growth and gonadal growth (Elliott 1979 cited in Ruohonen 1994, see also similar equations in Krebs, 1972 and Brett and Groves, 1979).

The portion of C allocated to F, U, R and P is dependant on several factors including diet composition, (a large research area; recent examples include Aksnes *et al.* 1996, Helland and Grisdale-Helland 1998a, b, c, Jobling *et al.* 1998a, Koskela *et al.* 1998, Hemre and Sandnes 1999, Ruohonen *et al.* 1999, Vergera *et al.* 1999), environmental factors such as temperature (Koskela *et al.* 1997a, b, Lyytikäinen and Jobling 1998), seasonal effects (*e.g.* Jobling *et al.* 1998b), feeding regime (Boujard *et al.* 1996, Koskela *et al.* 1997c, Johansen and Jobling 1998) and meal timing (see below).

Equation 1.11 can also be considered in reverse. The amount of food eaten (C) can be influenced by the 'systemic need' for nutrient energy to allow metabolism, maintenance and growth.

(ix) Circadian feeding rhythms and feeding behaviour in teleosts

There have been several reviews on this area of research (Boujard and Leatherland 1992, and references therein). This discussion draws largely on Boujard and Leatherland (1992).

When allowed to feed using on-demand self-feeders (demand feeders, Rozin and Meyer 1961, Adron 1972, and Adron *et al.* 1973), all fish except the goldfish (*C. auratus*, Rozin and Meyer 1961) have been observed voluntarily to eat discrete meals (Boujard and Leatherland 1992). The timing and behaviour of feeding rhythms are important considerations in aquaculture and fisheries models. Most authors distinguish between exogenous and endogenous rhythms; the former are under the influence of an external periodic factor, analogous to forced oscillations, whilst the latter originate from within the organism. Exogenous rhythms rapidly disappear under constant conditions, whilst endogenous rhythms are sustained with a period close to 24 h.

Boujard and Leatherland (1992) considered the value of using activity rhythms as a reference point for feeding activity and concluded that it was unwise to infer too close a relationship between these rhythms. This was because some fish species have locomotor activity peaks at a different time to feeding peaks. For example activity in *Ictalurus nebulosus* was found to be both diurnal and nocturnal in the same population, however feeding was observed to be crepuscular (at dawn and dusk) for both the nocturnal and diurnal fish (Eriksson 1978). Boujard *et al.* (1990) observed a 180^0 phase-angle difference between the activity rhythms of fed and unfed *Hoplosternum littorale*.

The timing of meals differs between and within species in captivity (Spieler 1999). Examples of species feeding during the photophase are *Anabas testudineus* (Patra 1993), *Oreochromis niloticus* (although mainly crepuscular; Toguyeni *et al.* 1997) and *C. auratus* (Hirati 1973). Feeding during the scotophase was observed in *Anguilla japonica* (Hirati 1973), *I. nebulosis*, (Eriksson and van Veen 1980) and *Siluris glanis* (Boujard 1995).

In commercially-valuable species a greater number of studies have been carried out, and these have revealed that meal times for a particular species are by no means fixed. The sea bass, *Dicentrarchus labrax*, has been found to feed during the photophase (Begout-Anras 1995, Sánchez-Vásquez and Tabata 1998). This species has also been found to shift its feeding rhythm spontaneously between scotophase and photophase as light : dark regime, season and temperature are varied (Sánchez-Vásquez 1994, 1995a, 1995b, 1997a, Madrid *et al.* 1997, Azzayadi *et al.* 1998).

Salmo trutta (Neveu 1980) were found to be crepuscular feeders, although when these species were offered food only for 2 h in the photophase and 2 h in the scotophase, both were observed to feed in the photophase (Jobling *et al.* 1998c).

S. salar has been found to feed in the photophase at approximately $10\,^0$C in a natural (summer) photoperiod by Higgins and Talbot (1985), whilst they were found to be crepuscular by Paspatis and Boujard (1996) under a 12 : 12 (L : D) photoperiod at $16\,^0$C.

Frazer *et al.* (1993, 1995) found that Atlantic salmon were diurnal feeders when the temperature was above 10 ^{0}C and nocturnal when it was below 10 ^{0}C. *Pagrus major* also switches its feeding activity between the photophase and scotophase, depending on the length of the photoperiod and temperature (Tabata *et al.* 1997).

In *O. mykiss,* Landless (1976) found that, during winter, individual fish fed mainly during the photophase with a second peak eight hours later. In the autumn however, there was little food intake during the day. Diurnal feeding with a marked feeding period at dawn was also observed by Boujard and Leatherland (1992b). In contrast Grove *et al.* (1978) and King and Grove (unpublished) found that trout feeding occurred nocturnally, whilst Alanärä and Brännäs (1997) and Brännäs and Alanärä (1997), who monitored individual fish demand-feeding within a group, found that within a tank, fish fed either in the photophase or in the scotophase depending on the individual. Tabata (1998) observed that there was a great deal of plasticity in feeding time in this species, but that once a feeding time was established, it tended to be quite persistent.

Thus feeding time in fish varies according to season, length of photoperiod and temperature. Within species it can also vary inter- and intra- individually, and it is also modified by diet quality.

This variation in meal timing presumably has evolved to allow maximum fitness of each fish species. For example, in the wild, fish-foraging periodicity has been found to correlate with daily changes of diet quality. The red spotted blenny, *Parablennius sanguinolentus,* was found to feed in the early afternoon when its main food, algal turf, was richest in starch and energy. Where pollution had led to the algal turf being replaced as the main foodstuff by *Ulva lactuca* feeding time was found to correlate with highest starch levels and energy levels for that species, which was at a different time of day than the turf. Meal time did not correlate well with the tidal cycle. The fact that pollution had only killed the turf in the last 20 years is a further sign of plasticity or adaptability in fish feeding (Zoufal and Taborsky 1991).

In laboratory experiments, feeding schedule has been proved to affect growth and tissue composition in several species (discussed by Spieler 1977, 1990, 1992, 1999, Boujard and Leatherland 1992a). Spieler (1977), Noeske *et al.* (1981) and Noeske and Spieler (1984) found that the goldfish, *C. auratus*, had differences in growth depending on the feeding time relative to the light:dark cycle. However each study was conducted at a different time of year (November, March and January), and the actual differences observed were different depending on season, with feeding being most conducive to growth at dawn, 4-8 h after light onset or 18 h after light onset (*ie* 6h after dark onset). Noeske-Hallin *et al.* (1985) observed differential fattening of channel catfish (*Ictalurus punctatus*) when they offered fixed ration meals at different times (L : D = 12 : 12). In this example the feeding schedule which resulted in the greatest abdominal fat weight was not that which resulted in the greatest body weight. However no differential growth was noted when the same species were allowed to feed to satiation under a natural photoperiod over six months (Robinson *et al.* 1995). *D. labrax* also had differing results between studies; the optimum time of feeding has been found to be the scotophase in the winter, with improved specific growth rate (SGR) and food conversion ratio (FCR) (Azzayadi *et al.* 1998), whereas Boujard *et al.* (1996) found that there was no optimal feeding time in the summer, when fish were fed at restricted times using demand-feeders. *O. mykiss* has been found to have slightly more consistent results, with improved SGR, FCR, total lipid, and protein conversion efficiency all being found around dawn (Boujard *et al.* 1995, Gélineau *et al.* 1996,1998), although Gélineau *et al.* (1998) found no difference in weight gain between meal times. The results of Reddy *et al.* (1994) differed from the above; they found that rainbow trout grew more when fed at either midday or dusk.

There is obviously a good deal of variation of results within species, and because the trials were not designed for comparison with each other it is not possible to determine why this should be the case. Any explanation for this variation requires a mechanism by which meal timing effects SGR and other

physiological parameters and consistently explains reported findings. Spieler (1999) hypothesised that the best growth would be observed when the internal activity, feeding and reproductive rhythms (which he referred to as the "internal milieu") were at "the best alignment to process and incorporate a food stuff into growth". He recognised that feeding *per se* alters an array of circadian variables, the levels of which have an impact on the digestive process, and concluded that the internal milieu "must be one that is receptive to specific feeding evoked alterations at specific circadian times".

There is little information on the role of feeding as a synchroniser of other rhythmic cues in fish (Spieler 1977). Locomotor activities have been found to be modified in response to time of feeding. Goldfish, which when fed at random times have a daily diurnal acrophase, when fed once a day 'phase shift' their locomotor activity to the meal time regardless of when during the photoperiod the meal is presented (Spieler and Noeske 1984, Spieler and Clougherty 1989, Sánchez-Vasquez *et al.* 1997b). Davis and Bardach (1965) observed a pre-feeding peak of activity which persisted when feeding time was shifted relative to the photophase. When feeding time was shifted by 6h, a residual peak of activity persisted just prior to the previous feeding time. Davis and Bardach (1965) concluded that feeding could act as an endogenous cue for pre-feeding activity. Thus feeding rhythms may be under endogenous control, but it may be disrupted by scheduled feeding.

Boujard and Leatherland (1992) observe that when food is continuously available meal timing appears to be light entrained. However when food is offered at discrete intervals (such as when food is available cyclically in the wild, or in aquaculture), pre-feeding activity might be food-entrained. They argue that because when meal times are shifted fish show differential growth, only part of the temporal organisation of the fish is shifted by the feeding time and conclude therefore that feeding time is under the control of multiple oscillators. Underwood (1989) showed that light and temperature are the main Zeitgebers, acting first at the level of the pineal, as well as at other locations. There is strong evidence that the hypothalamus might also be involved in the

control of circadian rhythms, but that these cannot be the only organs involved in the control of circadian rhythms, and that others might function as food-entrainable organs (Boujard and Leatherland 1992).

(x) Inter- and intra-individual ration size in feeding populations

Variation in food intake is known to occur in individual fish in a population; this variation is both intra-individual and inter-individual (*e.g.* Davis and Olla 1987, Jobling *et al.* 1989, Carter *et al.* 1992, McCarthy *et al.* 1992, 1993, Jobling 1993, Jobling and Koskela 1996, Carter and Shelverton 1997). Both types of variability have been ascribed to the effects of social hierarchies. The strength of the social hierarchy tends to decrease with increasing ration (McCarthy *et al.* 1993, Brännäs and Alanärä 1994, Carter and Shelverton 1997, Moutou *et al.*1998). Stocking density has also been observed to reduce the strengths of hierarchies (Alanärä and Brännäs 1996, Carter and Shelverton 1997) and increasing the scatter of food on a pond reduced the strength of a hierarchy in tilapia (Ryer and Oller 1995, McCarthy et al 1999).

Kristiansen (1999) observed that in juvenile salmonids no fish had a well-defined rank position when share of meal was used as the ranking index. This probably indicates that not only do groups of fish have variable daily intakes (see above) but individuals in a group do too, and that these variations are not synchronised.

In experiments where fish were self-feeding using demand-feeders, the triggers were used only by a few fish (Brännäs and Alanärä 1993, Alanärä *et al.*1998) In such situations, the dominant fish in these situations showed the highest growth rates and the least stress (Alanärä *et al.* 1998). Alanärä and Brännäs (1996) found that increased stocking density led to the trigger being activated by a small group of individuals, rather than only one or two.

Wirtz (1974) observed that, in *B. pholis*, when individuals were placed in a tank with conspecifics they demonstrated greater competition with fish of their own size than with fish of dissimilar size, because hierarchical dominance could not be achieved. This in turn effected food intake.

(xi) Dynamics of time series of food intake

In exploratory studies (King unpublished), a tank of six *L. pholis* were fed at random time intervals for two months, in an attempt to gain information for hypothesis formulation. Figure 1.1 plots the data from the randomly-fed fish as an 'appetite return curve' (Grove and Crawford 1980). The results show a large scatter of data and it is obvious that if an attempt were made to predict an individual meal size, whilst it would be possible to state a mean and a confidence limit for any particular time after a meal, it would be impossible to predict the exact amount taken.

In a follow up study (King unpublished) individual *L. pholis* were fed at regular time intervals (Figure 1.2). The resulting **time series** demonstrated that, even though the fish were fed a single type of diet at predictable times, food intake still did not settle to a predictable amount. (The example shown is a 21 g fish, fed at 24 h intervals, at 11:00 h, on squid mantle pieces at 14 ^{0}C). If the data from Figure 1.2 is taken and the food eaten at time *t* is plotted against food eaten at time *t-1* (a return map), it can be seen that such time series have an element of orderliness which lasts for a number of days, before the feeding behaviour takes an apparently unpredictable and random direction. In this case feeding levels 'wander' before settling to a periodic behaviour for meals 11-17 (periodic data sets appear as a circle in return maps), after which food intake suddenly drops, to oscillate around a lower level, until meal 36 at which time the feeding level starts to increase, failing to settle again before the end of the study. This behaviour was also observed in preliminary trials using *P. platessa* (King unpublished)

The large variation in day-to-day feed intake and the great plasticity observed in feeding times, within species and even individuals (see above), mean that it is possible that there is some undetermined mechanism yet to be postulated and examined on the causality of satiation amount and meal times (Spieler 1999).

To date little work has been carried out describing the dynamics of time series of food intake in teleosts (Ruelle, 1980, defines the dynamics of a system as the time evolution of that system; a study of appetite dynamics would be a study of

those forces that produce the meal-to-meal change in food intake). A qualitative exploration of these driving mechanisms may allow a hypothesis to be generated which explains not only the variation in food intake but other, as yet unspecified, aspects of the regulation of appetite.

The reasons for the scatter around the trend in Figure 1.1, and the daily variation in appetite in Figure 1.2 are unknown; is it random or deterministic and if it is deterministic, does its apparent randomness infer chaos (Cvitanovic 1989)?

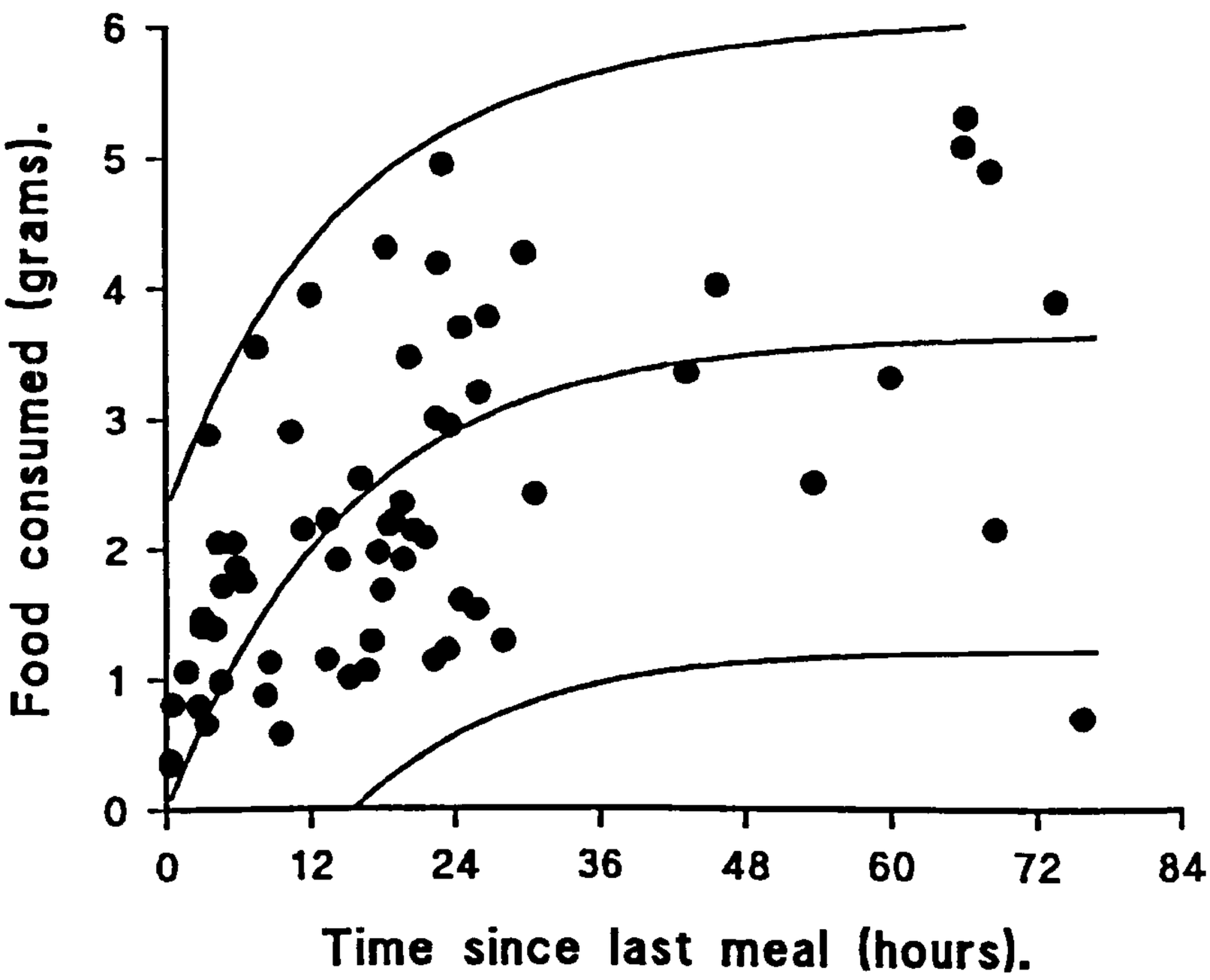


Figure 1.1 Appetite return curve for a group of *L. pholis* fed at random time intervals, showing the variable nature of food intake in fish. The exponential curve was fitted, using P-fit version 6.0c (Biosoft), with 95% confidence limits shown (n = 7, mean weight = 37.51g ± 7.11 SD. Temperature range = 13.5 - 16°C)

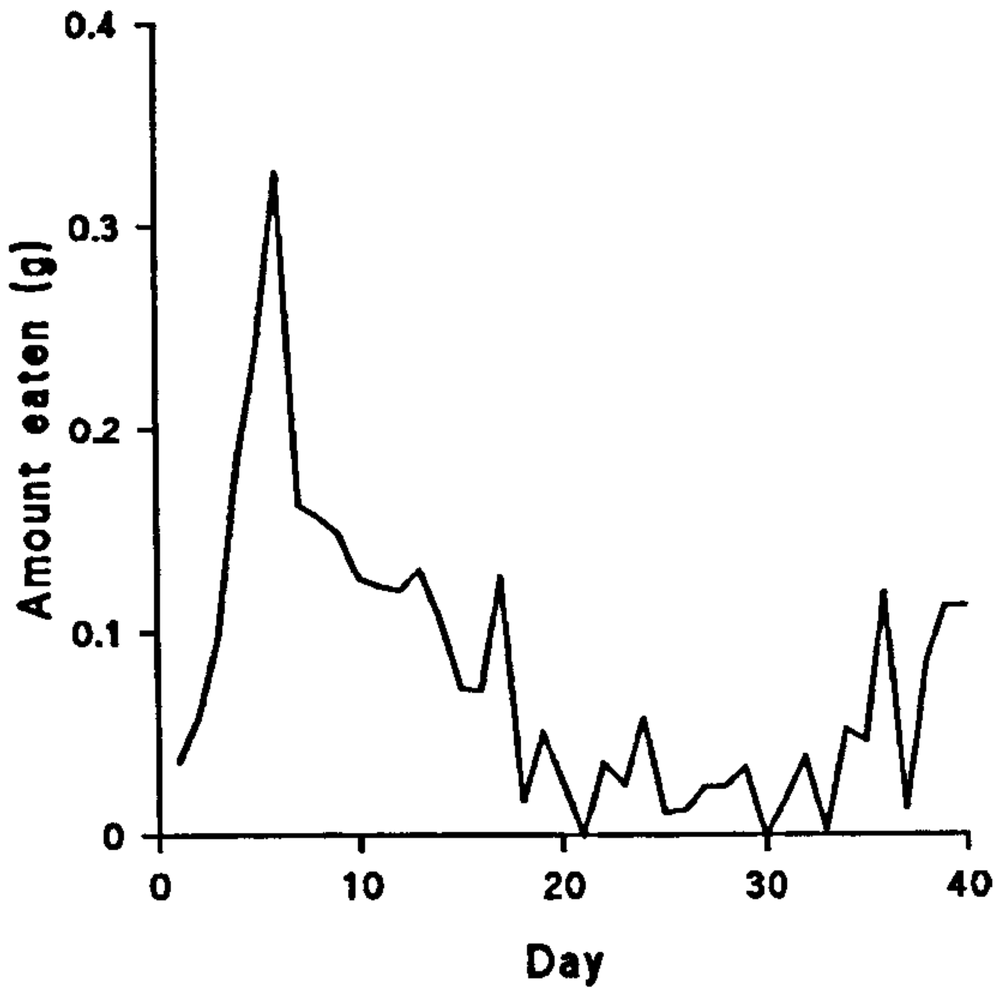


Figure 1.2. Real time plot of time series of food intake of an individual *L. pholis* fed a single discrete meal once every 24hrs. (Weight = 21 g, temperature range = 15.5 - 17 ^{0}C)

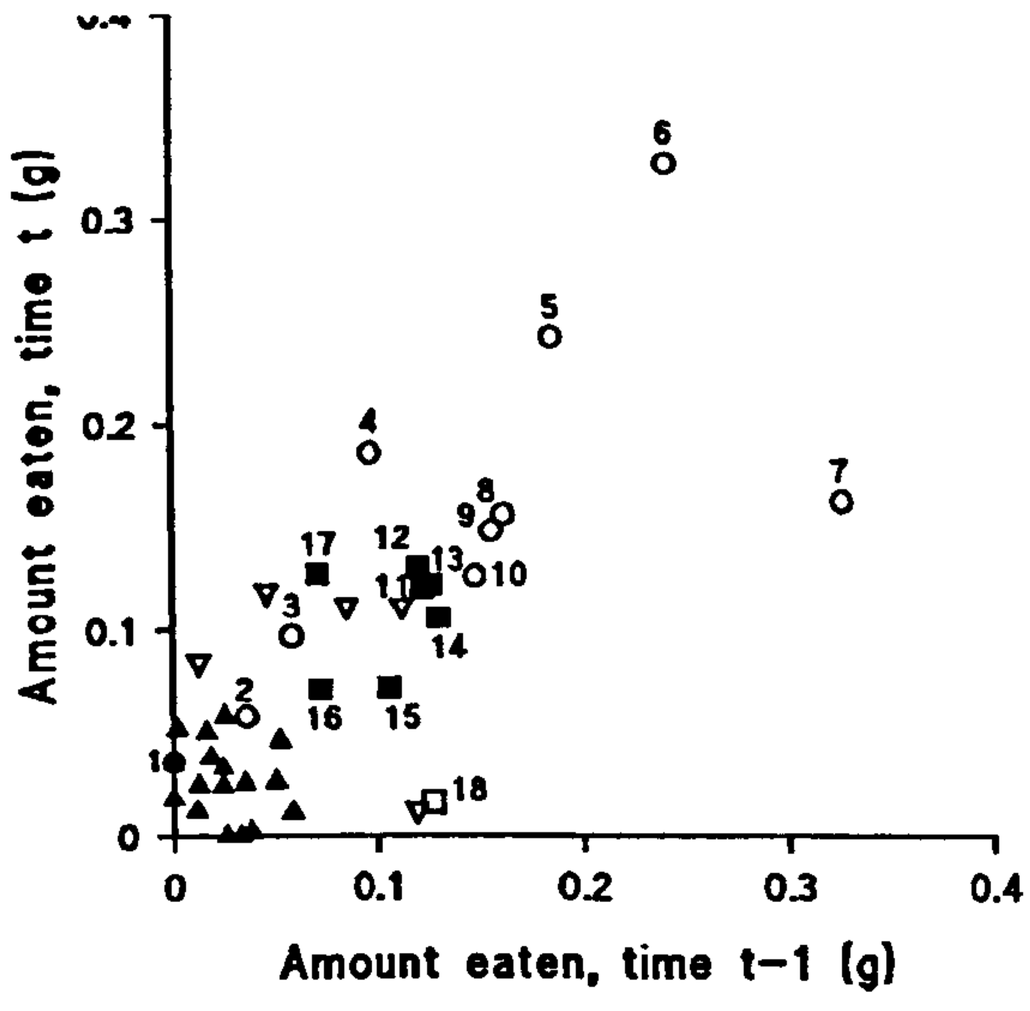


Figure 1.3 Return map of the food-intake time series (figure 1.2), with first 18 meals numbered.

o = meals 1-10, ■= meals 11-17, □ = meal 18,

▲= meals 19 -35, ▽= meals 36-40

Deterministic data is driven by relatively simple equations, whereas systems that are driven by many forces are considered random (or stochastic). Chaotic dynamics appear to be random but are in fact highly deterministic. The latter type of systems can be defined as a superposition of a very large number of unstable periodic orbits. This leads to time series that appear to fall into periods of almost periodic behaviour before returning to more random motion (Ditto and Spano 1995); this is exactly what is observed in feeding behaviour in Figure 1.3.

Chaotic systems are non-linear, dissipative systems which show self-similarity at different scales (this would mean that, if food-intake were chaotic, the pattern of food intake over a long period would look very similar to the pattern over a shorter period). This self-similarity is explained by universal properties exhibited by chaotic systems.

A description of an experimental system that is capable of exhibiting chaos (Libchaber and Maurer 1980) is given below, explaining how a large class of simple deterministic systems can exhibit highly erratic or statistical properties and to demonstrate some of their universal properties. The explanation of the theory is largely drawn from May (1976), Feigenbaum (1980) and Cvitanovic (1989). (A mathematical example of a chaotic system is also briefly discussed in Chapter five).

Libchaber and Maurer (1980) demonstrated that chaos is possible in a Raleigh-Bénard experiment, in which a small box (1 mm high) containing liquid helium is heated from below. At low temperature gradients, although there was a heat flow across the cell, the liquid was static. At a critical temperature gradient, a convective flow commenced, with the hot liquid rising in the middle and the cool liquid flowing down the sides; this led to two convective rolls which were cylindrical in shape. A thermometer was placed on the uppermost ridge of one of these convective rolls, which at this stage recorded a constant temperature. As the temperature gradient was increased further, a wave started to run along the convective roll, so that the uppermost point of the ridge moved from side to

side, resulting in a fluctuation in the recorded temperature and at this stage a sinusoid was observed (Figure 1.4 a).

As the temperature gradient was once again increased, a further instability appeared, that is a period doubling occurred (Figure 1.4 b) Increasing the temperature gradient further led to a further series of period doublings until the trajectory is aperiodic and the system is exhibiting chaos (figures 1.4 c and 1.4 d).

The next step in considering the universal properties of chaotic systems is to construct a Poincaré map. This is done by looking not at the whole trajectory, but at its point of intersection with a given surface (Figure 1.5). The sequence of the distance of the orbits from the centre of the attractor is universal (Figure 1.5).

If a Poincaré map is plotted against a continuum of the temperature gradient, the result is a bifurcation tree (Figure 1.6) which reveals two universal constants in chaotic systems, $\alpha = 2.5029$ and $\delta = 4.6692$ (see Figure 1.6 for an explanation of these constants).

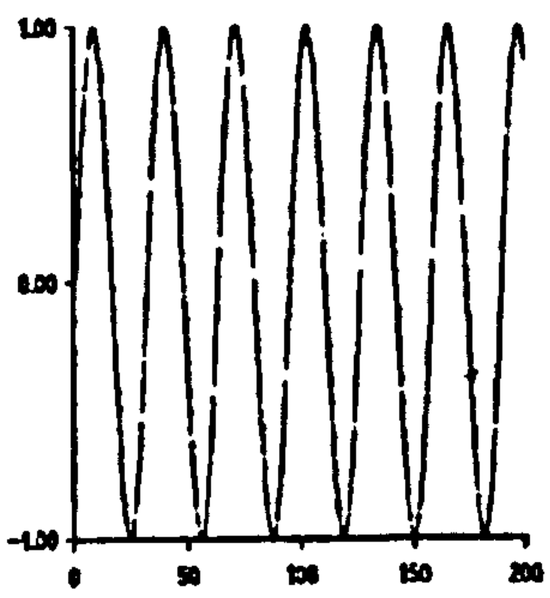
It is unlikely that the type of dynamics observed above , or in any other biological system, could be described in quite such a clear cut way as 'deterministic', 'random' or 'chaotic'; other options include nonchaotic but still nonlinear determinism, linear correlations, and the noise could either be in the dynamics or the measurement of food intake (Theiler *et al.* 1992a).

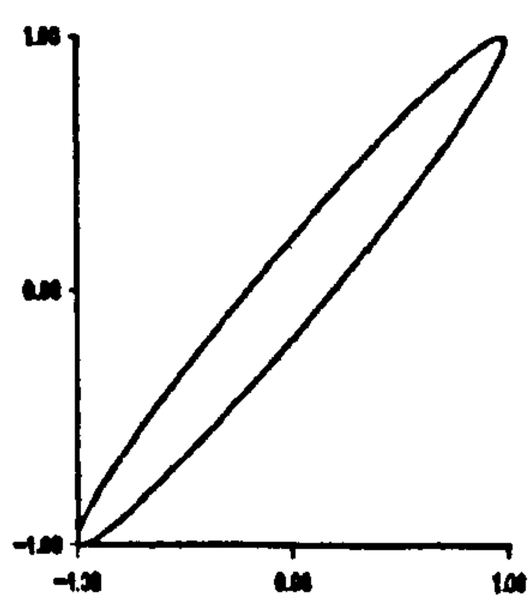
Furthermore it is probable that a time series of food intake could result from a combination of some analogues of the above possibilities. Thus any attempt to analyse food-intake dynamics are likely to prove difficult.

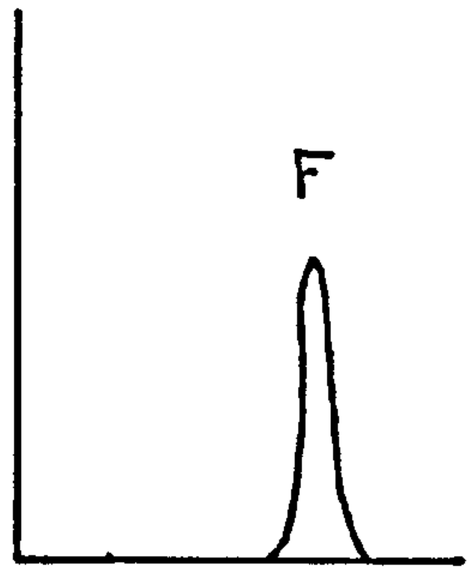
Figure 1.4a Sinusoidal behaviour demonstrated at the first instability of the convective rolls, with the trajectory repeating itself every t seconds. This periodic rhythm can be displayed in three ways; as a real time plot (left) as a phase space plot (centre), or as a frequency spectrum (right), where X = the frequency and Y = the amplitude

Figure 1.4b After the first period doubling the time series develops a new wave on the original sinusoidal instability. The trajectory now repeats itself after 2t seconds and a new wave band appears with half the frequency of the original.

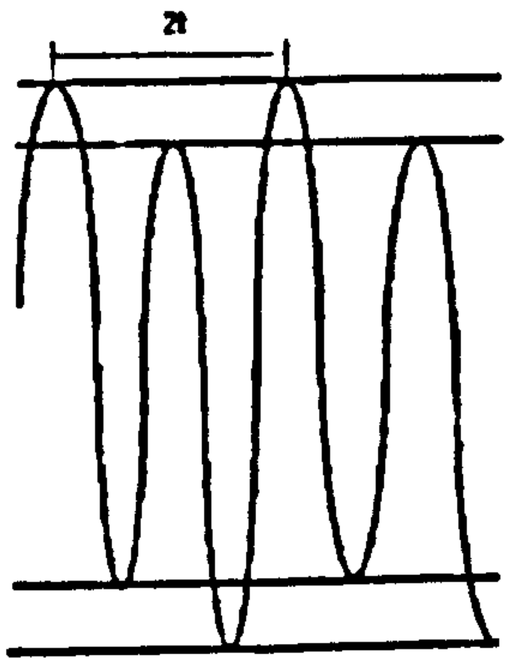
t

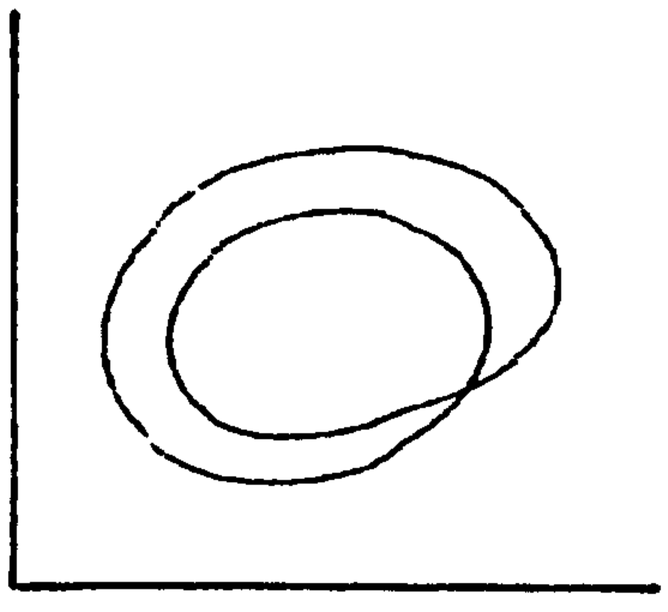






2t





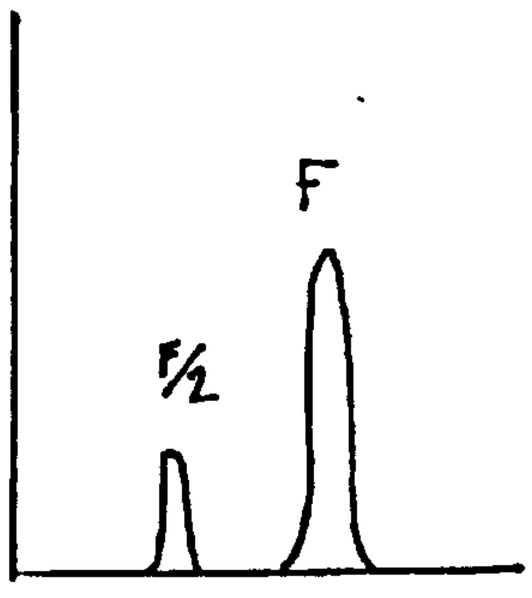
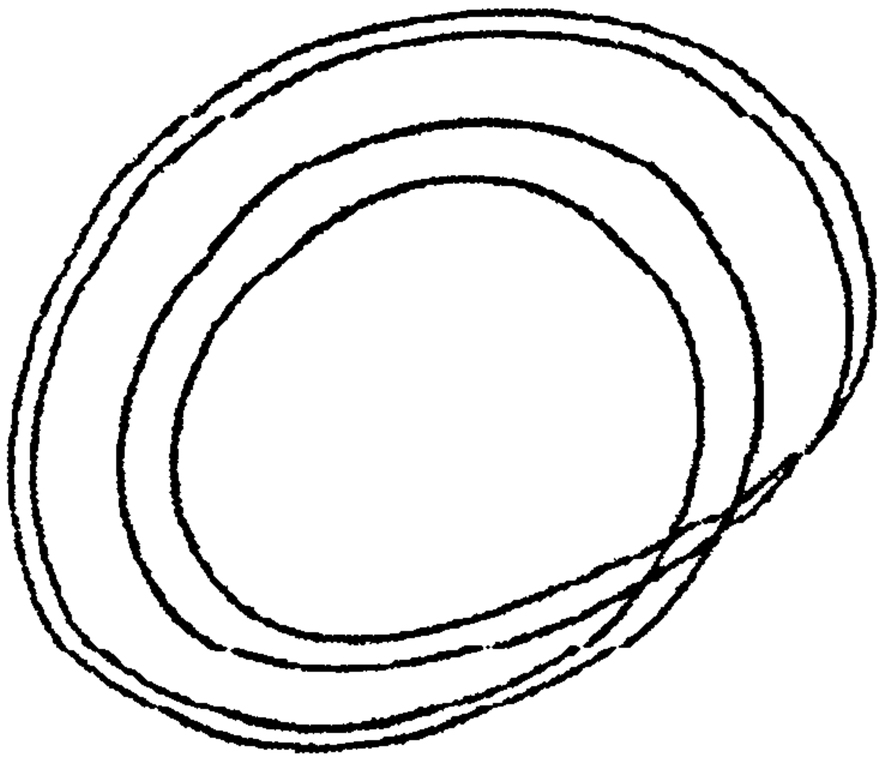
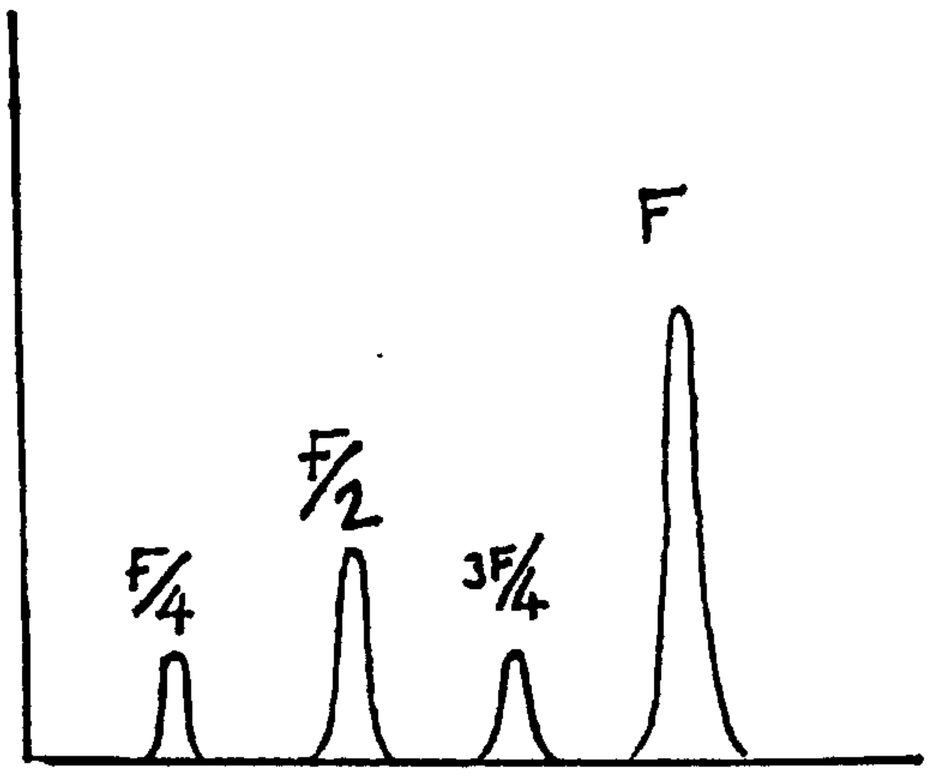


Figure 1.4c A further increase in the temperature gradient leads to another period doubling. The trajectory now repeats itself after 4t seconds and two new wave bands occur.

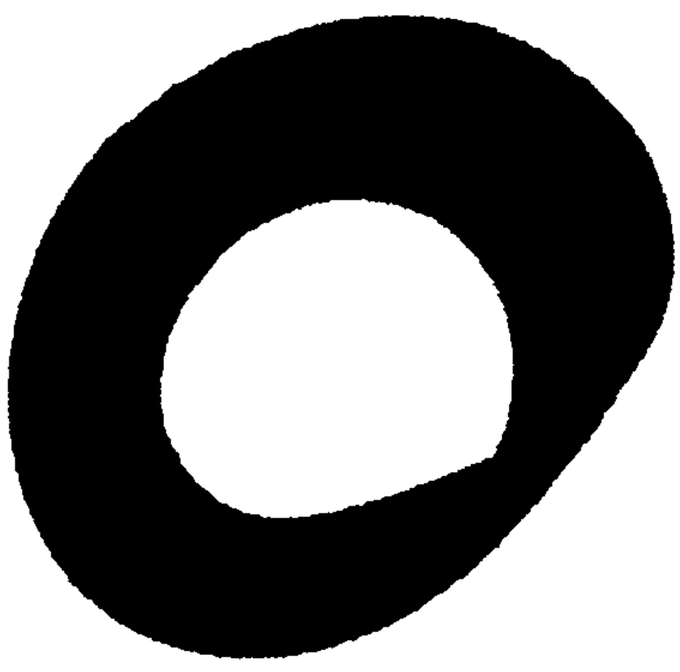
Figure 1.4d Successive period doublings occur until the system is aperiodic, in other words it is exhibiting chaotic behaviour.

4t





∞n



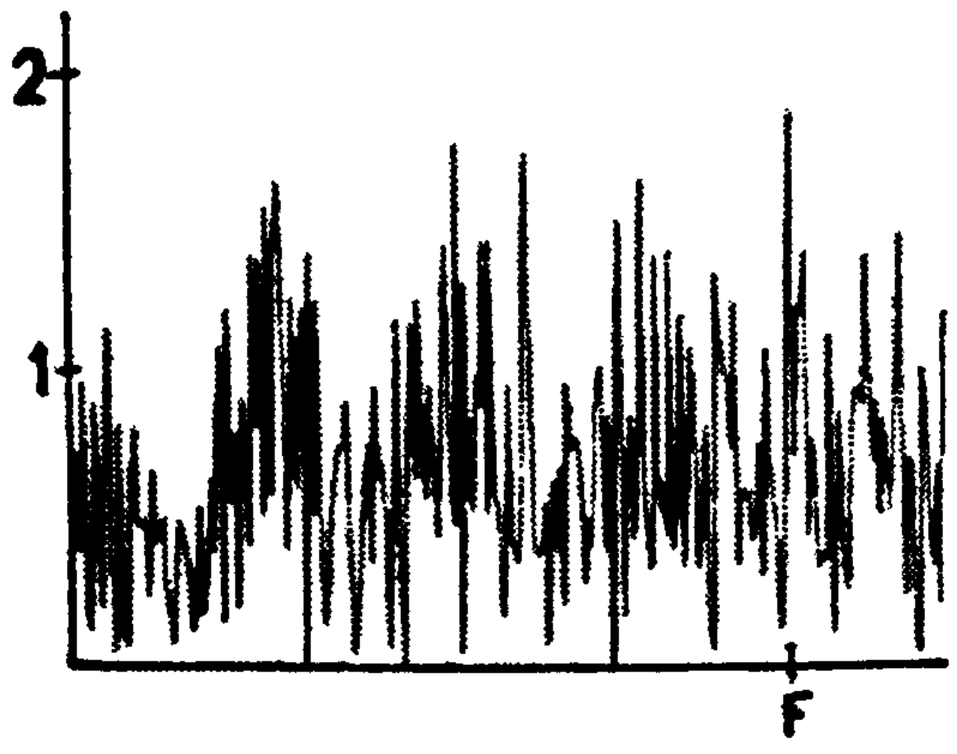
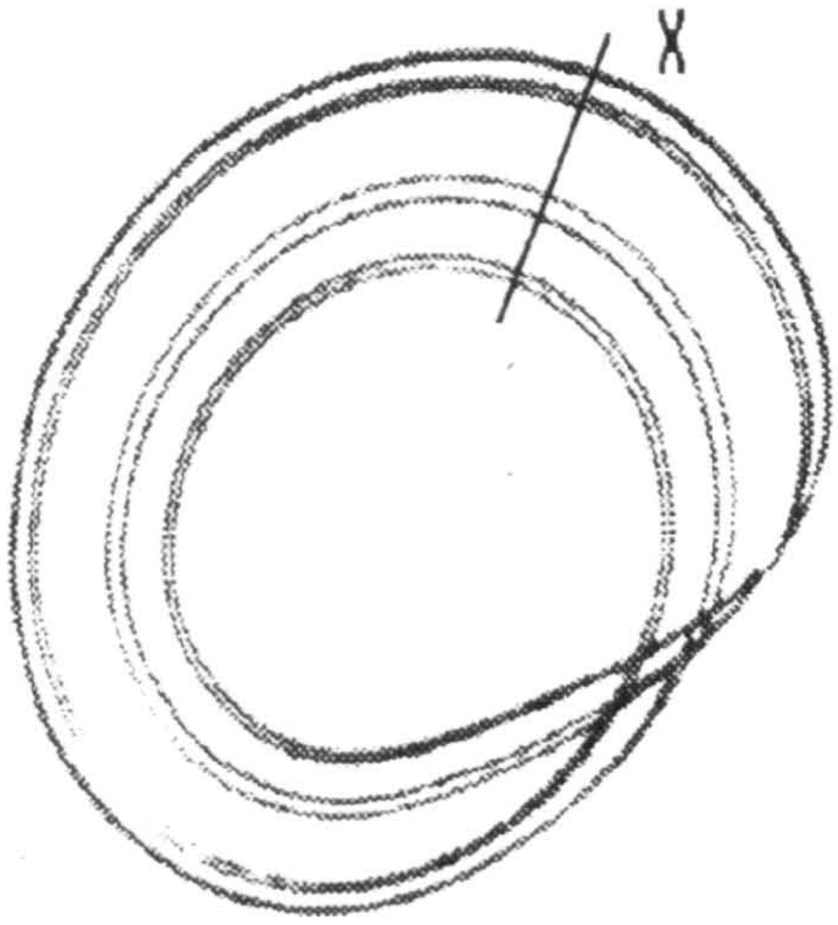


Figure 1.5 A Poincaré map. This is a map of the trajectory at its point of intersection with a given surface, the sequence of the orbits on the surface is universal.


X

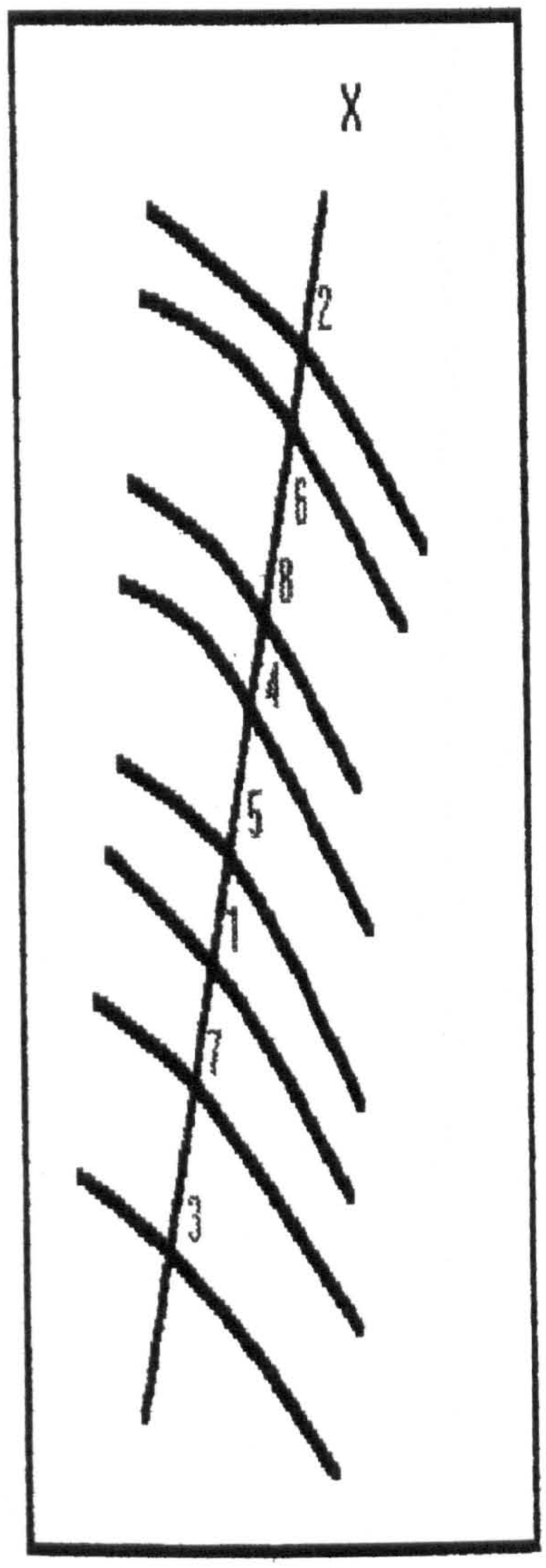
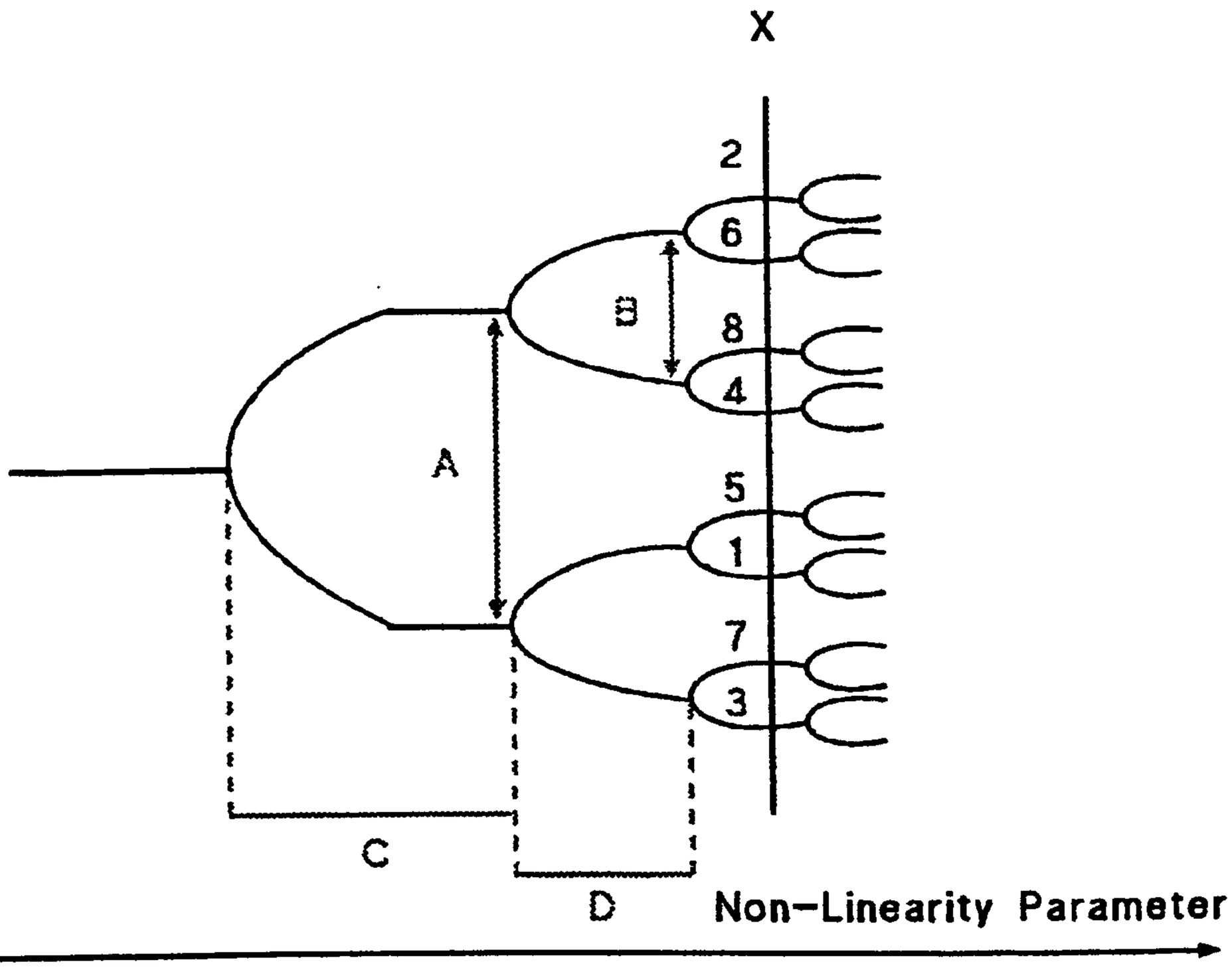

X
2
6
8
4
5
1
7
3

Figure 1.6 A bifurcation tree. These are plots of a continuum of Poincaré maps against an increasing non-linearity parameter (in this case temperature gradient). Two universal constants are revealed. The convergence parameter, δ and the scaling parameter a. The line 'x' is the Poincaré map from Figure 1.5.



Universal constants (quantitative)

$A / B = \alpha = 2.5029....$

$C / D = \delta = 4.6692...$

(Feigenbaum 1980)

(xii) Aims of the present study

It is clear that a greater understanding of the general factors regulating appetite and its endogenous and exogenous modifiers in teleosts is desirable in aquaculture, as well as in the study of fish in the wild. This thesis looks at three areas of the study of food intake in the dab.

1. The role of the stomach in the regulation of food intake.

Chapter two considers various hypotheses presented in the literature (see above) in which stomach content or stomach fullness have a role in the regulation of food intake. Specifically, does stomach fullness lead to meal termination, and does the frequently observed correlation between GER and appetite return prove that stomach content has a role in determining meal size? Chapter three asks whether dab adapt their stomach volume to diets of different energy densities. If this is the case then in the long-term stomach volume would be unlikely to be limiting.

2. Modelling responses to differences in dietary nutrients

Several papers have been published in recent years examining the effects of changing the levels of dietary nutrients on level of food intake (see above). One problem in this kind of study is the fact that by changing the amount of one ingredient it is also necessary to change another, and unless the experimenter is careful the energy content of the diet will also change.

The second study (Chapter 4) illustrates this problem by using path analysis (Sokal and Rohlf 1981) to test three structural models explaining how dab adapt their food intake to novel diets with different ratios of ingredients. The first assumes the nutrients alone are important, the second that both nutrients and energy content are important, and the third that energy has been observed to be correlated to food intake simply because it is a function of the nutrient content of the diet.

3. The nature of the dynamics driving food intake in the dab

Finally, in Chapter 5, in order to try and understand the mechanisms behind appetite control, time series of food intake of dab are studied using qualitative non-linear time series analyses. Data was taken from fish fed once daily and

from fish fed using demand feeders. In the latter the interval between each feeding event was used, as well as food intake per unit time. To assess whether the findings for dab may be generally applicable in teleosts, comparisons were made with rainbow trout, sea bass and whiting (Chapter 6).

One element of the studies that follow is an attempt to examine data using approaches which allow critical examination of assumptions which are frequently made in the literature on fish feeding, but which may not be justified.

CHAPTER TWO: ON THE ROLE OF THE STOMACH IN THE REGULATION OF FOOD INTAKE

(i) INTRODUCTION

It has previously been suggested that the upper limit of the ability of fish to adapt to their diets depends largely on stomach volume and gastric emptying rate (Vahl 1979). Jobling (1986a) suggested that food type determines the type of regulation of gastric emptying, with low-energy diets having a volume-based regulation, whereas high-energy diets have an energy-based regulation. The former strategy requires that fish maintain a high degree of stomach fullness and it is here that stomach volume may become a factor that limits short-term food intake. An inability of fish adapted to pelleted diets to compensate to low-energy diets has been observed in a number of fish species, this has been attributed to the stomach volume being limiting (*e.g.* Yamamoto *et al.* 1995, Ruohonen and Grove 1996, Ogata and Shearer 2000).

Several authors have reported a correlation between GER and appetite return, as well as between GET and meal interval in demand-fed fish (*e.g.* Brett 1971, Adron *et al.* 1973, Elliot 1975, Landless 1976, Hunt and Stubbs 1975, Grove *et al.* 1978, Hunt, 1980). Fletcher (1982) determined GER and appetite return in two different studies on dab fed on *Mytilus edulis*, and compared curves fitted from the data. He found that whilst there was a good correlation between food intake and GER up to 12 h after a meal, as well as after 48 h, during the intervening period food intake was lower than the amount of food that had been evacuated from the gut. Fletcher therefore concluded that there was not a good correlation between the two curves. This contrasted with previous work in which demand-fed dab were observed to have a meal interval of similar length to their GET (Gwyther and Grove 1981). This correlation could be an indication that volume based regulation is occurring, however it could also be interpreted as the return of appetite and stomach emptying being correlated with a third unknown factor. In other words correlation does not necessarily mean causation (Sokal and Rohlf 1982).

Fletcher (1982) found that the emptying curve (and appetite return) of dab fed a low-energy diet followed a curvilinear pattern. This was in keeping with the hypothesis of Jobling (1986a), who proposed that the gastric evacuation curve would vary in shape depending on the diet consumed. For low energy diets gastric emptying would be curvilinear, whereas for high energy or large diets a linear model would offer the most efficient delivery to the intestine.

This Chapter aims to examine further the role of the stomach in short-term food-intake regulation; does the stomach volume limit food-intake *per se*, how variable is meal size and is food intake a function of stomach content?

Firstly, in **experiment 2.1**, the question was asked whether food intake is limited by stomach volume. This was carried out by examining food intake of fish fed either squid mantle or Trouw Aquaculture pellets (Table 2.1) at regular intervals with different inter-meal duration. If they were able to adapt their feeding at each meal, so that they maintained their overall food intake, this would indicate that stomach volume was not limiting. Models are presented which attempt to explain the results

Secondly, **in experiment 2.2.1**, the shape of appetite return curves for dab was re-examined using squid (4 $kJ.g^{-1}$) and commercial pellets (18.8 $kJ.g^{-1}$). Appetite return curves were generated to see if they are curvilinear or linear, and whether food intake peaks at a maximum intake.

It was hoped to test whether GER and appetite return in individual fish were correlated, and whether gastric emptying was linear for a high energy diet, by carrying out an *in situ* X-radiography study. Here fish would be fed a satiation meal and then after time *t* fed again to satiation. They would be X-rayed just after they had taken the second meal, to see how much food remained in their stomach from previous meals. Different meals would be marked with different markers. The use of *in situ* X-radiography, where the diet contains radio-opaque markers that pass out of the stomach at the same rate as the food, would require that the radio-opaque markers are a true reflection of the stomach content. Whilst this is not a problem for the prandium (Koskela and Jobling in press), measuring how much food remains in the stomach from the pre-prandium requires that the marker

is evacuated from the stomach at the same rate as the food. This requirement was tested in **experiment 2.2.2**.

Several studies have successfully used radio-opaque markers in studies of GER (Talbot and Higgins 1983, Talbot 1985, Flowerdew and Grove 1979, Grove *et al.* 1978). Radio-opaque materials used have included iron filings and glass beads of different sizes (Koskela and Jobling, in press). In some cases however, retention of the markers occurs, leading to an underestimate of GER (Jørgensen and Jobling 1988, arctic charr, Al Aradi 1986, turbot). dos Santos and Jobling (1991) found that, in *G. morhua*, PVC and glass beads were retained, with larger beads being retained more than smaller ones. The time of retention was longer in fish fed multiple meals than in fish fed a single meal. Jørgensen and Jobling (1988) recommended that careful preliminary trials should be carried out to make sure that retention of radio-opaque markers did not occur. To determine whether the markers and the food passed out of the stomach at the same rate, a serial slaughter was carried out. As well as this, *in situ* X-ray studies were used to look for signs of retention of radio-opaque markers by the stomach. Different amounts of the binder methyl-cellulose were used to see if harder pellets were less prone to marker-retention than moist pellets.

Experiment 2.3 looked for a correlation between gastric emptying and appetite return in individual fish, using a serial slaughter technique. Fish were given a pre-prandial meal, followed *t* hours later by the prandium, and the relationship between the two meals examined. A negative correlation between the two would indicate that either the stomach is limiting the amount eaten, or a systemic regulation that is closely correlated to stomach content is in play. No relationship, or a different relationship, between the amount eaten in the first and second meals, would indicate that the stomach volume is not a limiting factor.

(ii) METHODS

(ii)a General methods

Dab were captured in one of two ways, firstly in otter trawls carried out on the RV 'Prince Madog'and secondly using a hand-pulled beam trawl on a small hard-boat, the 'Sand Pebbler'. The latter was used for samples taken in the shallower waters close to shore. Fish were captured in Red Wharf Bay, on the East coast of Anglesey, (53^0 18 50 N, $4^0$11 00 W) unless otherwise stated. These fish were housed in 250 l tanks, water was recirculated *via* a biofilter and a trickle-feed of new water slowly replaced the existing tank water, to avoid the build up of nitrites. Two diets were used; Trouw Aquaculture commercial pellets and squid mantle (table 2.1). For the squid mantle, the energy per gram wet weight (4.0 $kJ.g^{-1}$) is a little higher than the value estimated by Clarke *et al.* (1994) from carbon content, (3.7-3.9 $kJ.g^{-1}$) but is a little low compared to their estimate using proximate analyses (4.6 - 4.8 $kJ.g^{-1}$). Water content compares well with previous studies (75 - 82 %; Grove unpublished).

Composition (%)	Pellets[1]	Squid
Moisture	8	76.17
Protein	48	15[2]
Lipid	19	1[2]
Carbohydrate / fibre	16	-
Ash	9	2.95[3]
Energy ($kJ.g^{-1}$)	18.83	4.0

Table 2.1: Proximate analyses of diets used in this chapter

([1]Trouw Aquaculture, [2]Oliva unpublished [3]Abitia- Cardenas and Galvan-Magaña unpublished)

Fish were fed by hand, the exact method varying according to the diet offered: Squid mantle was pre-weighed and offered a few pieces at a time and this was replenished as soon as the fish had taken them. This was to keep the feeding stimulus constant, because Colgan (1972) noted that the amount of food taken by the pumpkinseed fish was in part determined by the amount offered until all the fish in the tank were sated. The remaining pieces were carefully removed, patted with a paper towel and weighed. The hydration rate of squid mantle in sea water was estimated prior to this trial, by taking known weights of mantle and placing it in a petri-dish of sea water for fixed periods before removing it, patting it dry with a towel and re-weighing. The meal was ended once food had been left uneaten for five minutes, as in this time the squid mantle increased in weight due to hydration by less than 5 %.

In the case of the pelleted diet, the average weight of each pellet was measured (0.132 g $\pm$ 0.007 SD, n = 10) and the number of pellets eaten counted.

(ii) b Methods specific to experiments

Experiment 2.1 The effect of inter-meal interval on food intake

This experiment examined the ability of dab to compensate their long term food intake to changes in feeding frequency, by adjusting individual meal size.

Data was collected in two trials, in the first, four groups of fish were fed once per day for eight weeks and then once every three days for a further two weeks (n = 16, mean weight = 88.2 g $\pm$ 22.8 SD). In the second, two groups of fish were fed once per day and another two groups were fed three times per day (n = 24, 121.94 g $\pm$ 29.5 SD) Temperatures were 16 ^{0}C and 15 ^{0}C respectively. In all cases feeding was carried out at regular time intervals (exactly 8 h, 24 h or 72 h), with the amount taken measured as described in section 2 (ii) a. Whether dab were able to adjust their food intake to lower meal frequencies was tested with a linear regression of meal size against meal interval. A t-test was also carried out on the food intake per meal in the groups of fish fed every 72 h. To further examine the nature of the response, for fish fed once per day and those fed three times per day, the amount eaten in a given meal (m) was plotted against the amount eaten in the

previous meal (m-1), and the resulting return maps examined for evidence of stomach volume influencing the feeding behaviour of dab.

Experiment 2.2.1 Appetite return curves for high- and low-energy diets

This experiment examined appetite return curves to determine whether there is an upper limit of feeding for these diets, and whether diet quality affects the shape of the curve.

Fish were captured from Red Wharf Bay and randomly allocated to four 250 l tanks, where they were acclimated for six months on Trouw Aquaculture pellets (table 2.1). One month prior to the start of the trial fish were weighed to the nearest gram, groups of fish (n = 3) were placed into four tanks and half of the groups (chosen randomly) were changed to a diet of squid mantle (table 2.2).

Tank	Number of individuals	Mean weight (g)	±SD	Diet
1	3	309	40	pellets
2	3	211	48	squid
3	3	167	20	squid
4	3	116	18	pellets

Table 2.2 Number of individuals, mean group weight (g) ± standard deviation and diet allocation for each replicate in experiment 2.2.1

Fish were fed using the methods described in section 2 (ii) a. The intervals for **every other meal** were chosen randomly, with alternate meals offered after an interval of 24 h (Table 2.3). This is for two reasons; firstly, in previous appetite return studies, the pre-interval meal has been at a fixed time, leading to the post-interval meals being at a different time of day as well as a fixed time after the prandium. For example Fletcher (1982) fed dab an initial meal at 09:00 h (t_0); if the post-interval meal was offered at $t = 4$, it will have always been at 13:00 h. It is possible therefore, that the effect of different inter-meal periods on appetite return is confounded or modified by the effect of the time of day that the post-interval

meal was offered and this may influence the shape of the appetite curve. The second reason was to enable a return map to be constructed in which the influence of longer term factors was minimised (see Table 2.3)

Meal	Interval
1	
	X_1 hours
2	*m*-2
	24 hours
3	*m*-1
	X_2 hours
4	*m*
	24 hours
j	
	X_i hours

Table 2.3 Experimental design for appetite return study. X_1 .... X_i are randomly selected time intervals. Data points for the return map, *m* and *m*-1, are those meals which have a random time interval in between. The preceding interval (between *m*-1 and *m*-2) is always 24 h, reducing the influence of long-term factors

During the course of the experiment temperature ranged between 15 °C and 16 °C and fish were kept on a winter photoperiod (light : dark = 8 h : 16 h).

Results were plotted as amount eaten against time since previous meal. Both exponential and linear curves were fitted to the data with P-FIT (BIOSOFT 1991). The coefficient of variation, V (Sokal and Rohlf 1981), was calculated for those meals preceded by an inter-meal interval of 24 h.

Return maps were examined for topographical evidence of a relationship between meal size and the amount of food remaining in the stomach from the previous meal.

The relationship between meal size and time of day, size of the previous meal, was examined using multiple regression:

$$Y = a + b_{Y1.}X_1 + b_{Y2.}X_2 + b_{Y3.}X_3 \quad \text{.....(equation 2.1)}$$

where Y is meal size, a is a constant, X_X are the independent variables (time of day, size of previous meal and meal interval) and $b_{YX.}$ are the partial correlation coefficients.

Experiment 2.2.2 On the use of particulate radio-opaque markers to monitor GER in the dab: Preliminary trials for X-radiography studies.

Preliminary experiments were carried out on dab to determine whether radio-opaque markers passed out of the stomach at the same rate as the food. Two markers were studied for their suitability for monitoring GER; glass beads ('ballotini', 0.4 mm-0.52 mm diameter, Jencons) and Barium sulphate spheroids ($BaSO_4$ powder encapsulated in plastic, 1 mm, ICI).

The radio-opaque markers were presented to the fish in several forms, resulting in six diets in total. Details of the ingredients of each diet are given in table 2.4, together with details of the trial in which they were used.

Diets were made by grinding the Trouw Aquaculture diet (table 2.1) to a powder and mixing with the other ingredients listed in table 2.4. For the pellets, (diets 1 and 3 to 5) just enough distilled water was added to make a stiff paste, before the mix was passed through a mincer. The resulting pellets were oven dried at 40 °C to the required moisture content. Diet 2 was a 1 % (dry weight) meal made up of 53.5 % ground Trouw aquaculture feed (Table 2.1), 15 % $BaSO_4$ powder and 32.5% distilled water, so that 1 g (dry weight) of the resultant paste had a volume of 2 ml. In diet 6 the powder was simply placed in a gelatine capsule (#4, Gallenkamp).

The diets that were labelled with $BaSO_4$ spheroids contained approximately 30 per meal; the large size of the spheroids and the relatively poor contrast observed with *in situ* X-radiography meant that more than this proved difficult to count. Those labelled with ballotini contained approximately 60 per meal, except for diet one, which contained around 150 per meal.

For each experiment, fish were starved for 48 h before they were offered the test diet, a 1% BW meal, and allowed five minutes to eat it.

	Use	Particulate Marker	Methyl cellulose	Ba SO_4	Water	Present-ation
Diet 1	Serial slaughter	Spheroids	1%	5%	26%	pellet
Diet 2	X-ray study (emmersed subjects)	Ballotini (0.4-0.52mm diameter)	-	15%	32.5%	paste
Diet 3	X-ray study (*in situ*)	Ballotini (0.4-0.52mm diameter)	1%	10%	10%	pellet
Diet 4	X-ray study (*in situ*)	Spheroids	1%	10%	10%	pellet
Diet 5	X-ray study (*in situ*)	Spheroids	2%	10%	10%	pellet
Diet 6	X-ray study (*in situ*)	Spheroids	-	10%	10%	powder in gelatin capsule

Table 2.4 Details of diets used in preliminary trials of radio-opaque markers in the comparative study of appetite return and GER in individual fish.

Experiment 2.2.2(a) Serial slaughter

Fish were captured from Red Wharf Bay and acclimated to the 250 l tanks for four weeks. During this time fish were fed daily on a Trouw aquaculture commercial pellet (Table 2.1). Prior to the experiment fish were starved for 48 h, they were then offered a 1 % body weight meal of diet 1. Those that accepted the full ration were used in the experiment (n = 19, mean weight = 126.6 g $\pm$ 21.6 SD).

Temperatures for the experiment ranged from 8.5 ^{0}C and 10.5 ^{0}C and fish were kept under a winter photoperiod (8L : 16D).

Once the prandium had been accepted, at various times after the prandium, individual fish were anaesthetised using 2-phenoxyethanol (2.5 ml.20 l seawater^{-1}), placed onto an Agfa-Curix 18 cm x 24 cm photographic plate and X-rayed with a PLH Medical X-protector (model ZAR/PLH; 70kV, 10mA) X-ray unit. An

exposure time of 1 s was used, with the X-ray camera at a distance of 60 cm from the subject. The fish were then slaughtered whilst still anaesthetised by destroying the brain. Their gastrointestinal tracts were dissected out and frozen, with a ligature having first been tied around the pyloric sphincter. Next the frozen samples were allowed to partially thaw (this made it possible to remove the entire stomach contents) and the contents of the stomach and of the intestine were placed on to separate tared weighing boats and weighed. The $BaSO_4$ spheroids from the stomach and the intestine were counted to see how accurate X-ray counts were. The samples were next oven dried to constant weight at 40 ^{0}C. The wet : dry ratio of the weights of the stomach and intestine contents and the wet : dry ratio of the diet were used to determine what portion of the food remaining in the whole of the gut was to be found in the stomach. Finally this was compared with the estimate derived from spheroid counts.

Films were developed using Agfa G150 developer, Kodak 'Max' stop bath and Agfa G354 fixer. The temperature of these was kept at approximately 25 ^{0}C by placing the developing trays in water baths. Plates were put in the chemicals for the following times; developer 2.5 minutes, stop bath 0.5 minutes, fixer 5.0 minutes (clearer results were found if the plate could be left longer in the fixer bath, if convenient). Each plate was then rinsed in running fresh water for approximately 5 - 10 minutes and hung up to dry. Plates were examined to see if retention of the markers was occurring.

Experiment 2.2.2(b) X-radiography

For the remainder of the diets fish were X-rayed *in situ,* to visually monitor the progress of the markers. This was with the exception of the fish fed on diet 2, which were emersed and placed (without being returned to the water first) onto an Agfa-curix 18 cm x 24 cm photographic plate and X-rayed, using an exposure time of 1 second, with the X-ray camera at a distance of 60 cm from the subject. The fish were then replaced in water to recover from the anaesthetic.

For the *in situ* study, fish within a suitable size range for the tanks (35-160 g) were acclimated for several weeks; any fish which proved unable to adapt to the tanks were removed at an early stage and replaced, until eight fish were in place and

feeding readily within the system. Those fish that had eaten the whole ration were X-rayed immediately, and at regular time intervals (which varied from trial to trial) until the stomach was empty.

The *in situ* X-ray apparatus is illustrated in Figure 2.1. Two of these tanks were made, each containing four 24 cm x 18 cm compartments in which a single fish was placed. Partitions were made of transparent perspex to allow the fish to see each other, so that they would feed since otherwise individual dab do not feed well in the laboratory (Jobling 1974). The compartment walls were perforated to allow sea water to pass through the tanks from one side to the other and each compartment was individually aerated.

X-rays were taken with the X-ray unit set to an exposure time of 0.3 seconds and placed 40 cm above the surface of the water. A space under the tank-bottom allowed an X-ray plate to be slid underneath. The front of the tank was blacked out to minimise stress to the fish when the camera was being operated. Agfa-Curix RP1 18 cm x 24 cm plates were used, which were placed in Agfa-Curix cartridges

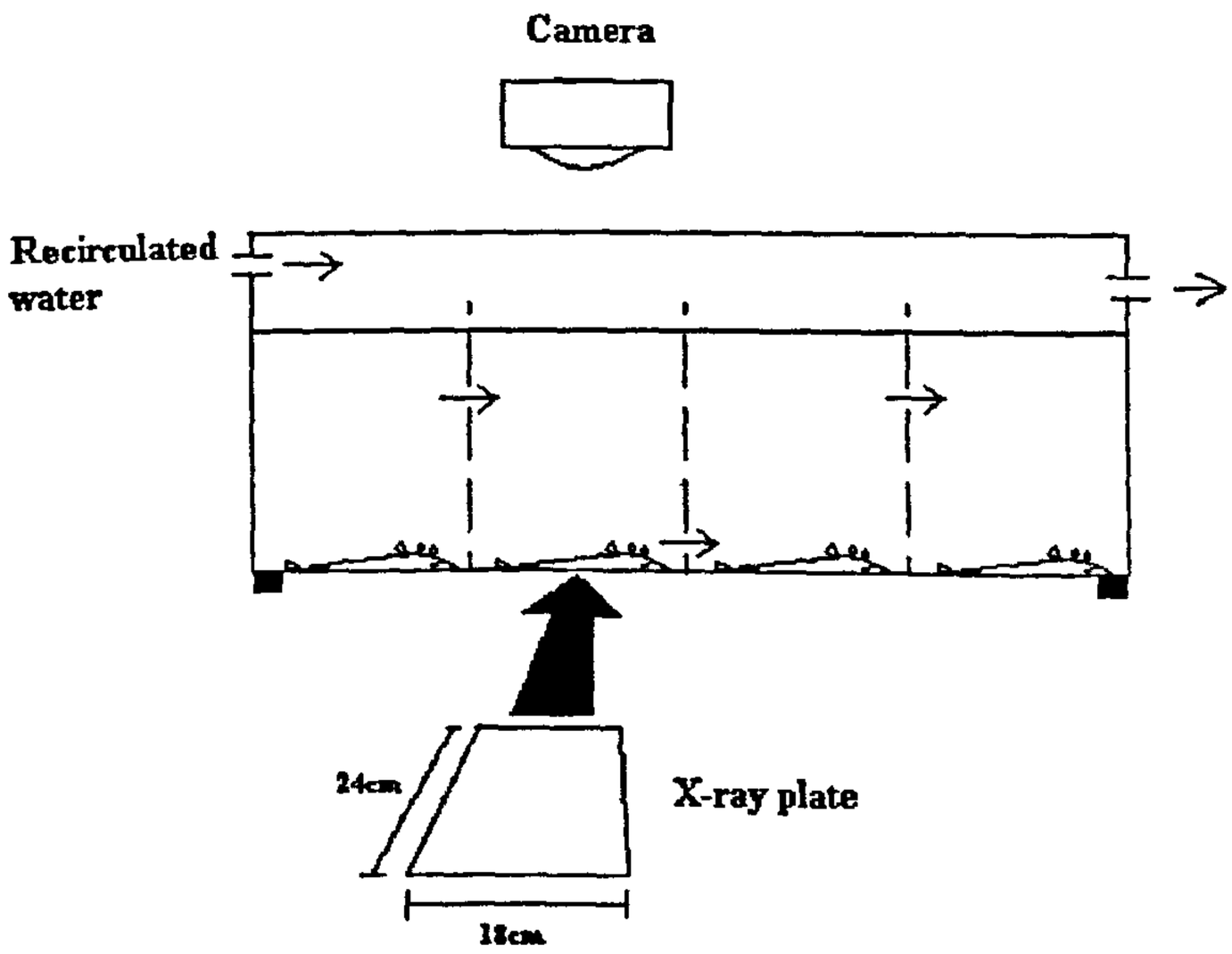


Figure 2.1 Compartmentalised *in situ* X-ray tanks for monitoring food content of fish stomachs. A space under each compartment allows the X-ray plate to be placed under the fish without disturbance. Water is aerated and recirculated *via* a biofilter, with a trickle feed to flush out nitrates.

Experiment 2.3 A study of the relationship between stomach content and food intake in *Limanda limanda* using serial slaughter.

This study was to determine whether meal size is correlated with stomach content in individual fish.

Twenty-five fish were captured from Red Wharf Bay during April 1997 and acclimated for seven weeks at 17 °C, in 24 h daylight, having been sorted into groups in six tanks so that each fish in each tank could be easily recognised (n = 25, average weight = 83.88 g $\pm$ 42.3 SD). The experiment was carried out in June 1997.

Frozen squid was thawed and the mantles removed and chopped into squares of approximately the same size. Each piece was weighed individually and placed on trays in weight ranges of 0.05 g; all pieces below 0.3 g were rejected. This process led to the squid mantle pieces on each tray being of very similar weight. Fish in each tank were offered fed squid mantle from only one size range and the number of pieces taken by each fish was observed. An initial meal was offered at 10:00 h, and the second at 20:00 h; in each case the amount of food taken by each fish was recorded by counting the number of pieces eaten.

At 20:30 h fish were very gently anaesthetised in 2-phenoxyethanol (1.5 ml per 20 l seawater; it was a minimum dose to reduce the chance of vomiting) and killed with a sharp blow to the head and the brain destroyed. Their gastrointestinal tract was then dissected out as described in Chapter 3. The squid pieces were removed and sorted into their original meals on tared weigh-boats. This was quite straightforward as the squid pieces from meal one were milky / opaque in appearance, with slightly rounded edges, whereas the squid from meal two was still slightly translucent and still as square shaped as when it was eaten. The wet weight of squid from each meal was recorded before each sample was oven-dried at 40 °C to constant weight. The resulting dry weight : wet weight ratio, together with the known moisture content of freshly thawed squid (Table 2.1) were used to plot the amount of food eaten in the second meal against the stomach contents remaining from the first meal. Linear regression was used to test for a negative correlation.

To ensure that there was no error caused by fish being of different sizes, this test was repeated after the data had been transformed using:

$$\text{Food intake} = a\ \text{Fish weight}^{b} \quad \text{.....(equation 2.2)}$$

where b = 0.58 (Jobling 1974).

(iii) RESULTS

Experiment 2.1 The effect of inter-meal interval on food intake

The pellet-fed fish proved able to increase their ration so as to maintain their long-term average food intake (Figure 2.2, Appendix 2.1). This is apparent because regression analysis of food intake plotted against meal interval showed that there was a significant linear relationship between meal interval and size for the pellet-fed fish ($r^2 = 0.92$, $P = 0.038$).

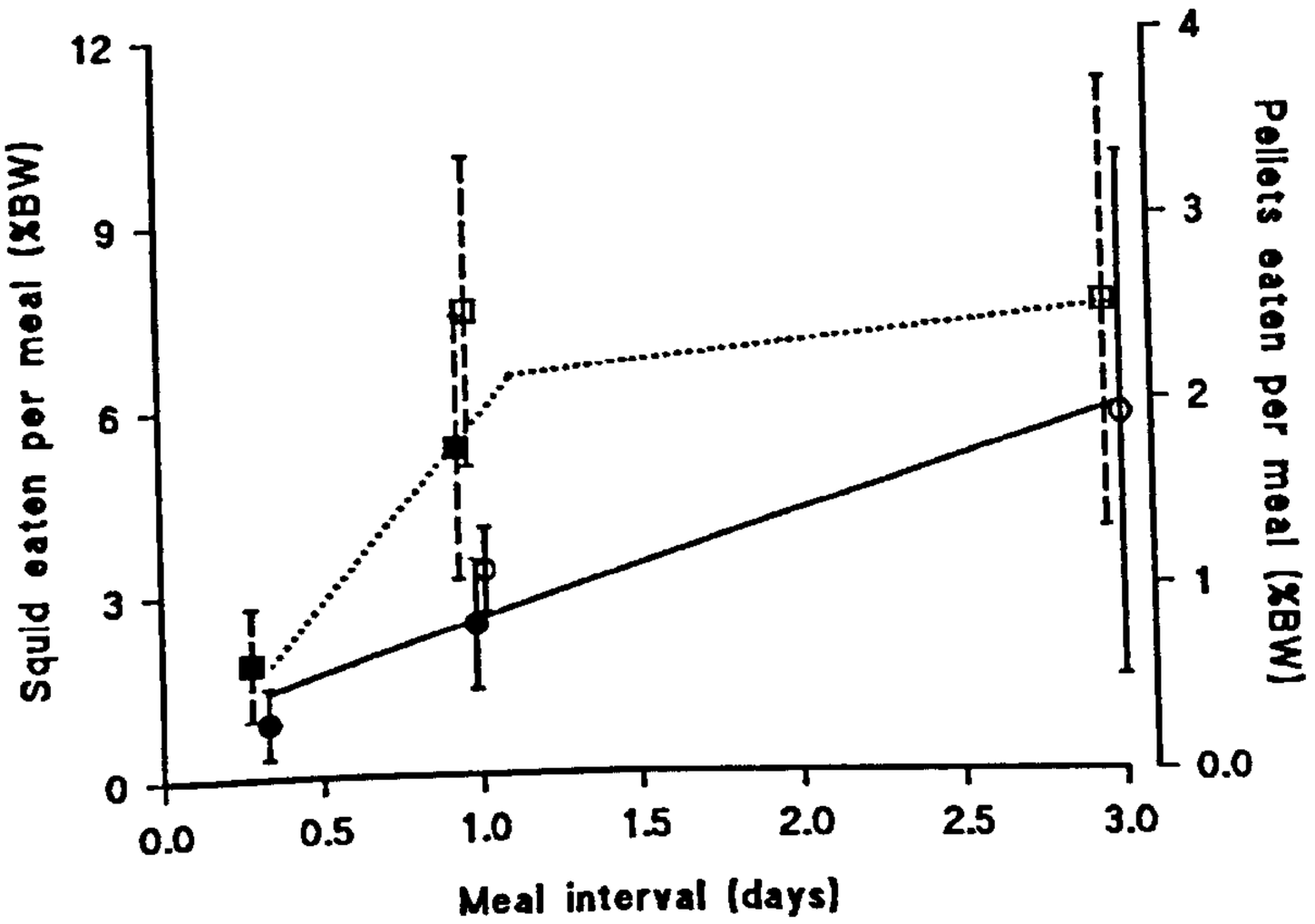


Figure 2.2 Amount of diet eaten (%BW) for different meal frequencies. Error bars are one standard deviation. Fish fed on pellets (o,●, solid line) compensate for less frequent meals even when fed only once every three days, unlike fish fed on squid (□, ■ broken line) which cannot compensate when fed once every three days. Mean values for trial one are indicated by open symbols, those for trial two by solid symbols.

This was not the case for the squid-fed fish. Whilst this group compensated their food intake to maintain total daily ration when offered food daily or three times per day, when fed every three days they failed to increase their ration (Figure 2.2, $r^2 = 0.53$, $P = 0.27$); a t-test showed that there was no significant difference between single meal size in fish fed squid once daily and those fed once every three days ($T = 1.48$, $P = 0.17$).

Return maps of the fish fed once per day and three times per day on pellets and on squid (Figure 2.3 a - d, where meal m is plotted against meal m-1) revealed a good deal of variability, with fish rarely eating the maximum amount observed in a single meal (M_{max}). As in Figure 1.3, figure 2.3e illustrates that there are periods of apparently periodic behaviour, with periods of apparently random behaviour and long excursions in between (see also Chapter 5).

Figure 2.4 presents the outcome of a theoretical model (model 2.1) in which meal size is considered to be limited by the stomach volume, *i.e.* fish are assumed to eat until their stomachs are full at every meal, so that meal size (M_x) is a function of stomach volume and food remaining in the stomach from previous meals:

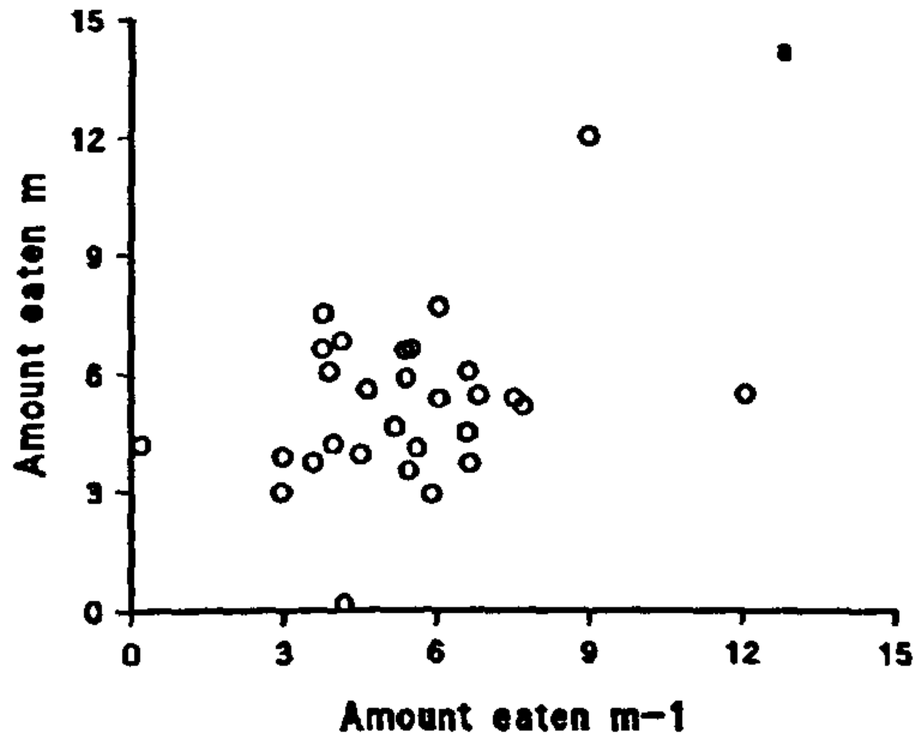
$$M_x = M_{max} - F_x \quad \text{.....(equation 2.3)}$$

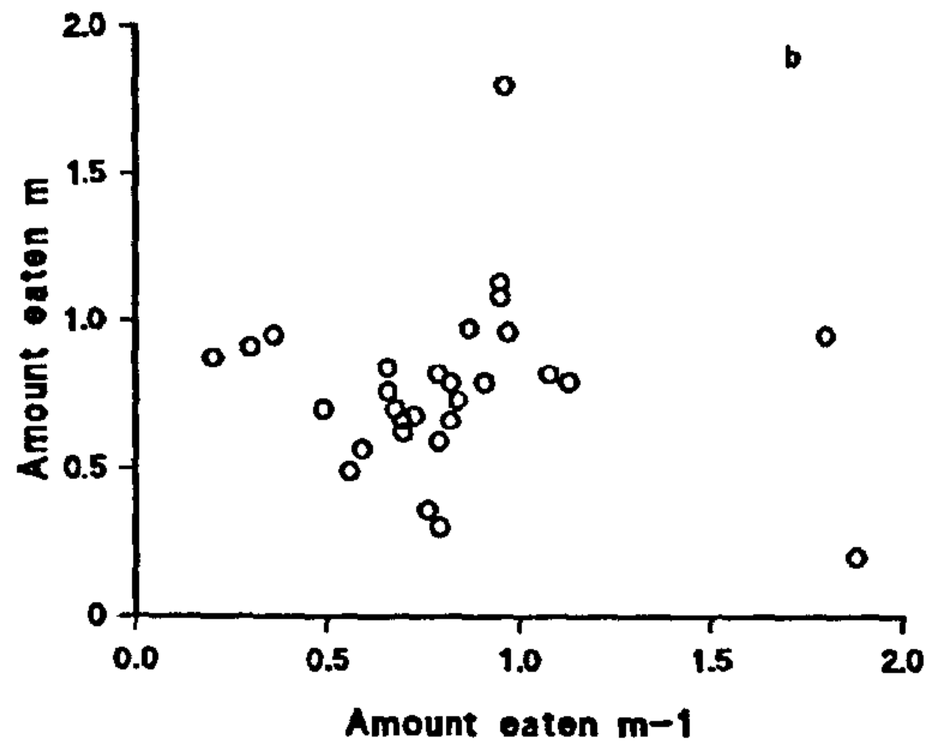
where M_{max} is the maximum meal size that the stomach can accommodate (assumed to be 10 g) , and F_x is the amount of food (g) remaining in the stomach from the previous meal, determined by an exponential model described by Fletcher *et al.* (1984):

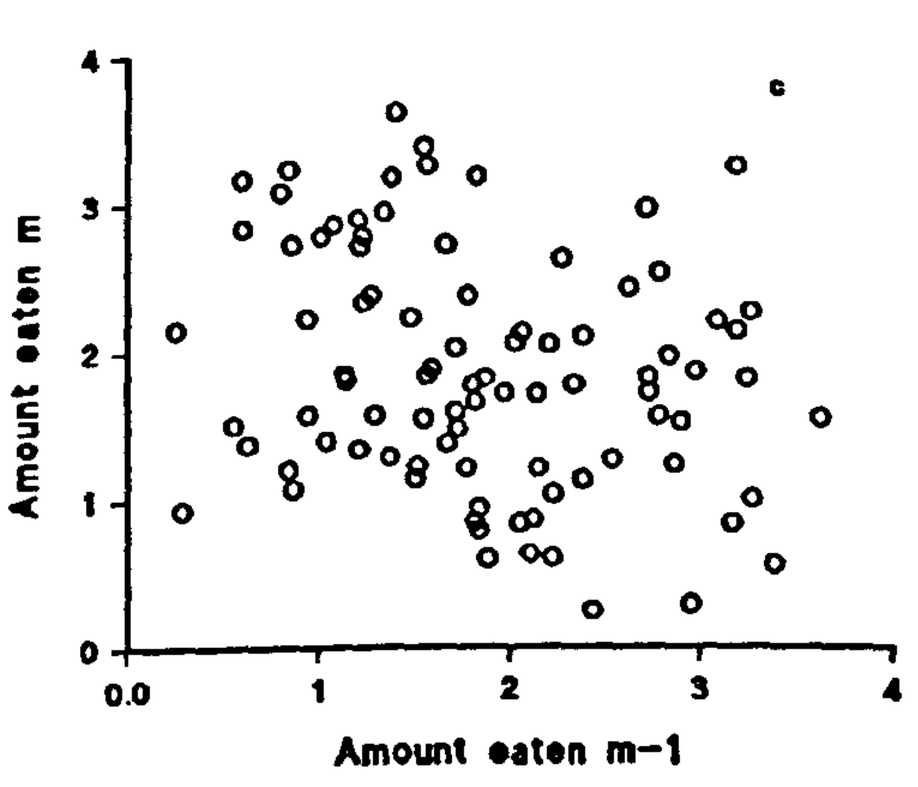
$$F_x^{0.75} = F_{x-1}^{0.75} - 0.0068W^{0.43}\, e^{0.041T}t \quad \text{.....(equation 2.4)}$$

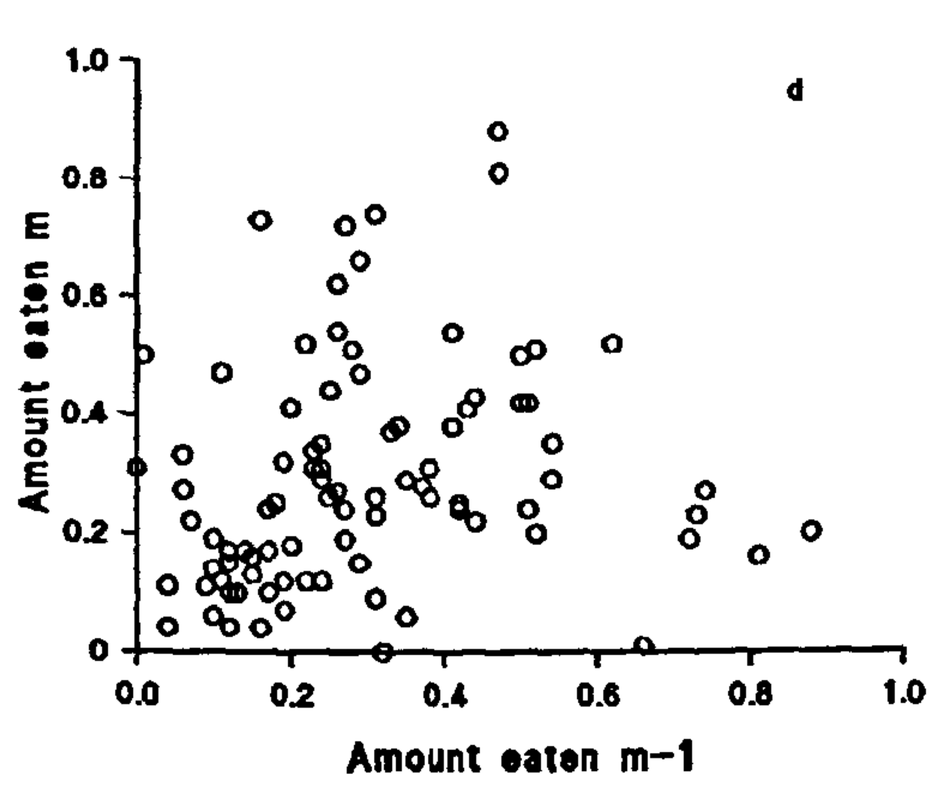
Fish weight, W, was assumed to be 100 g, temperature, T, was 16 °C, meal interval, t, was 24 h, M_{max} was 10 g and the first meal was assumed to be M_{max} (*i.e.* for the first meal $F_{x-1} = M_{max}$). Constants were taken from Fletcher *et al.* (1984). It is clear from Figure 2.4 that if the fish feed to stomach fullness the amount of food eaten per meal initially falls on a diagonal line, before rapidly

stabilising to a single food intake value (the closed circle). This clearly is not what happening in figures 2.3 a - d, and therefore fish cannot be feeding to stomach replenish fullness alone.









Figures 2.3a-d Return maps of food intake (%BW of fish) for fish fed on squid or pellets, once or three times a day. (a = squid, once per day; b = pellets, once per day; c = squid, three times per day; d = pellets, three times per day)

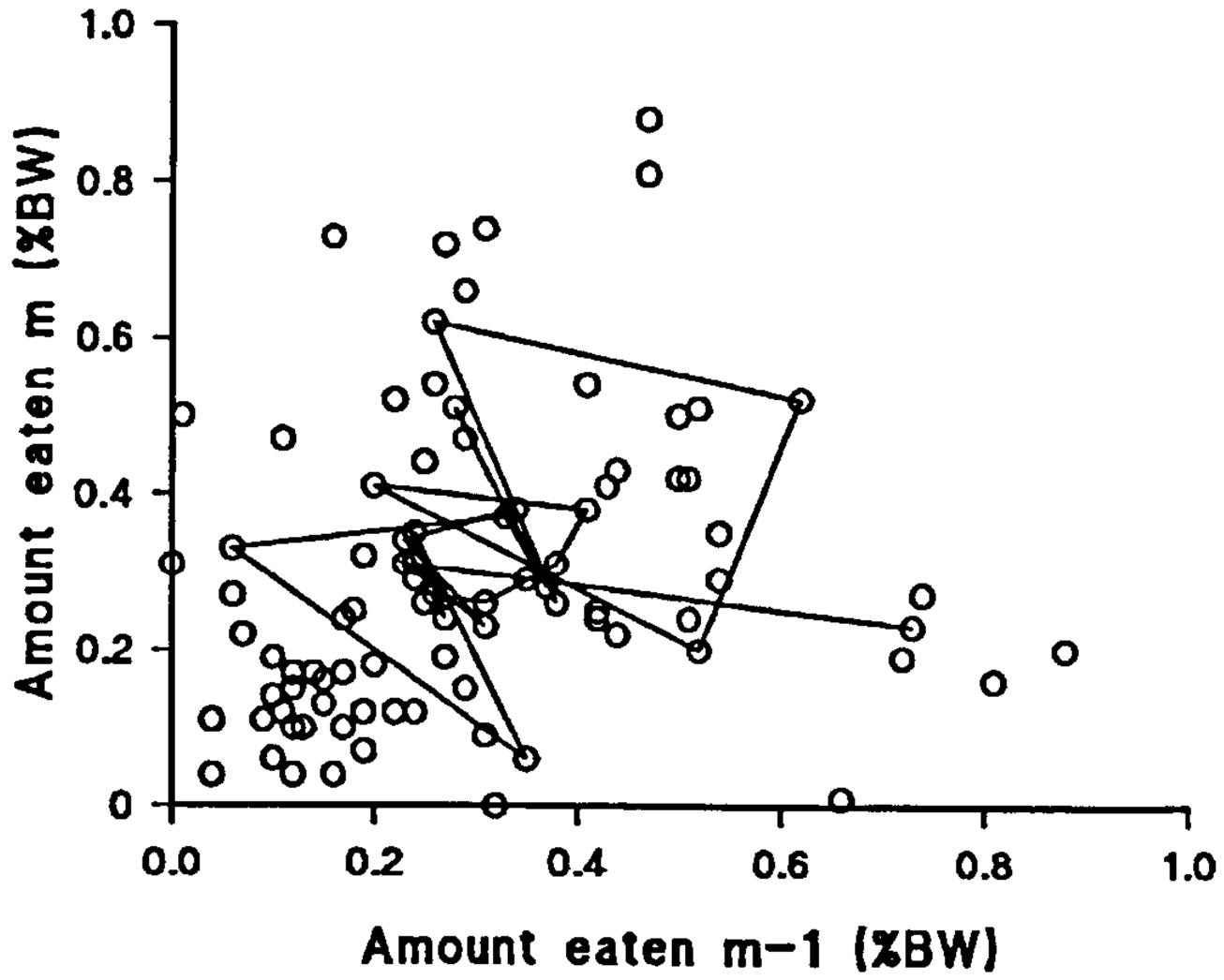


Figure 2.3e is as for Figure 2.3.d, but in this case a sequence of 20 points (meals 20-40) are connected to show the apparent intermittent periodicity with excursions in between.

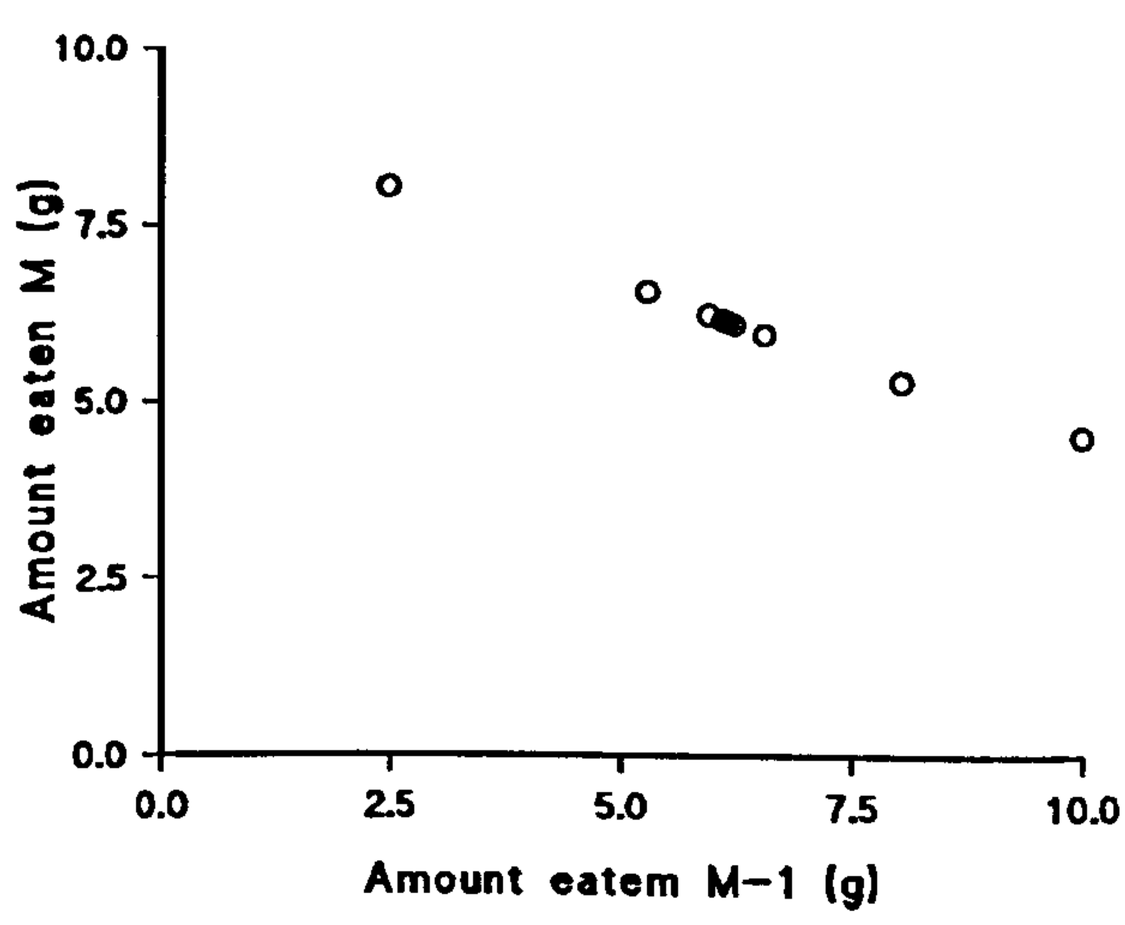


Figure 2.4 Results of Model 2.1 in which fish feed to stomach fullness alone. Initially feeding falls on a negatively-sloped line, before quickly stabilising to a fixed point.

If food intake is ever limited by stomach intake it is clearly not happening for every meal. Model 2.2 assumes that stomach volume is not limiting, but that fish still modify their food intake to allow for existing food in the stomach (F_x). The food

intake in this case is determined by a systemic need, assumed to be driven by a sinusoidal rhythm with noise superimposed upon it. A long term non-stationarity (an extra sinusoid with noise, which repeats itself roughly every 60 days) is also superimposed on the sinusoidal rhythm.

Thus fish in the model will have a meal size of:

$$M_x = [\ (e^{(\sin k\, t)}) + \text{noise} + \text{non-stationarity}] - F_x \quad \text{.....(equation 2.5)}$$

The noise was given a mean of 0.5 g (normal distribution, range = 0.0 g to 1.0 g); 16% of the average value of food intake from the sinus rhythm (3.2 g). Values were superimposed on the long-term non-stationarity, which ranged from 1 g to 7 g. The resulting time series of M_x is given in Figure 2.5, whilst a return map of the same is shown in Figure 2.6. Even if the remains of the previous meal, F_x, are discounted the resultant pattern is little changed, so stomach content may be unimportant in this model.

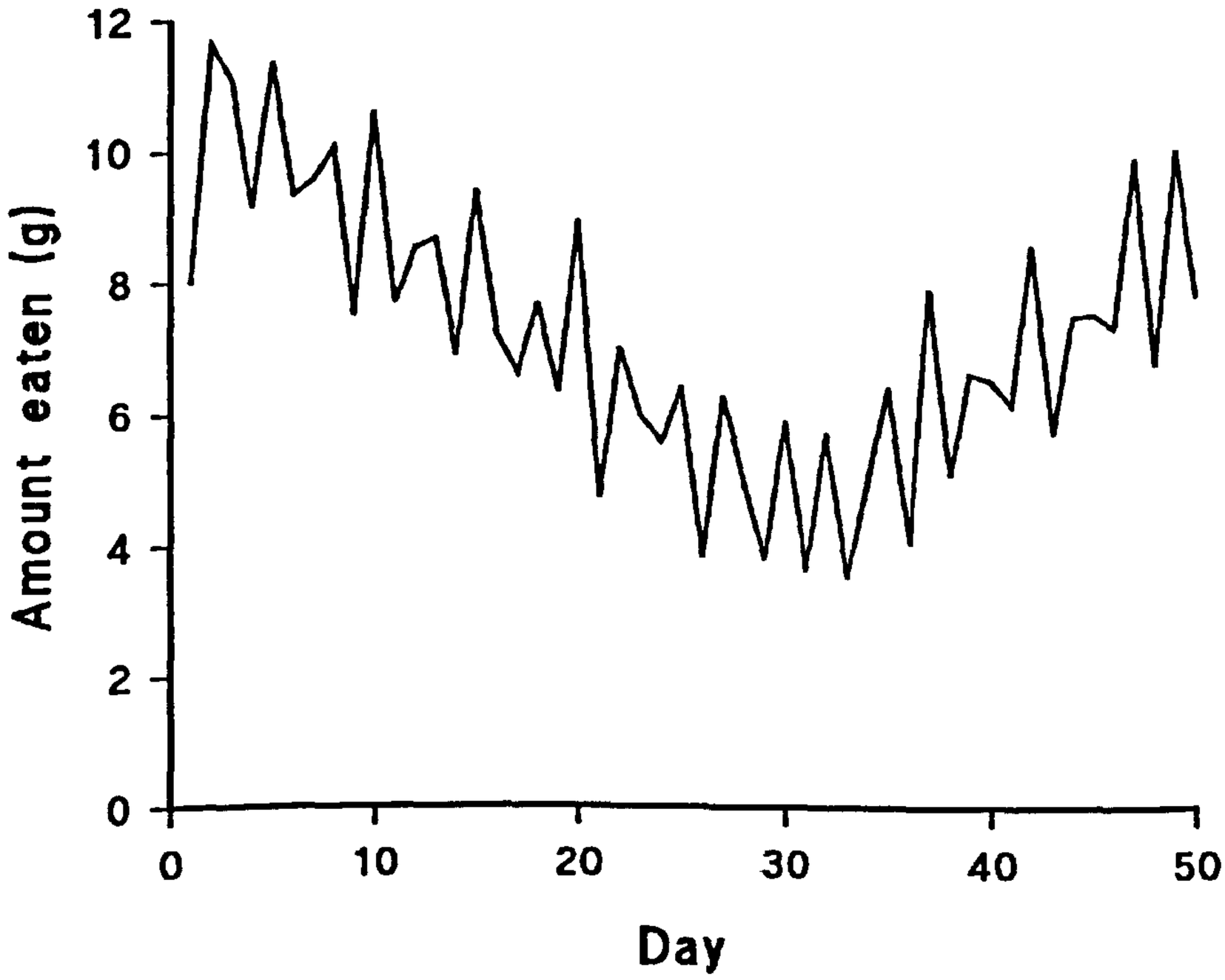


Figure 2.5 Time series of model 2.2, in which food intake is based on a sinus rhythm with noise, superimposed on a longer term non-stationarity (see text). No upper limit to food intake is imposed.

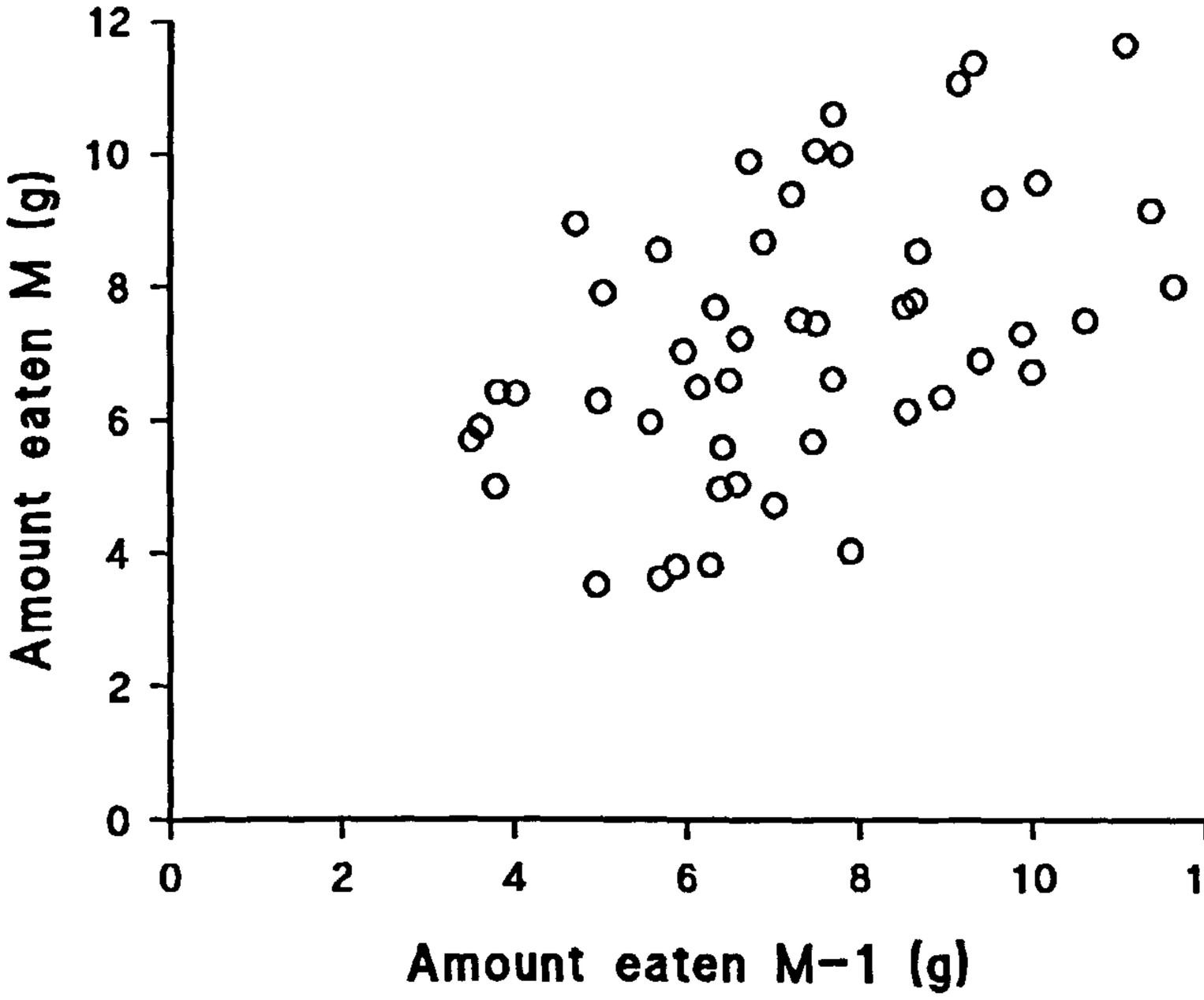


Figure 2.6 Return map of model 2.2, in which food intake is based on a sinus rhythm with noise, superimposed on a longer term non-stationarity (see text). No upper limit to food intake is imposed.

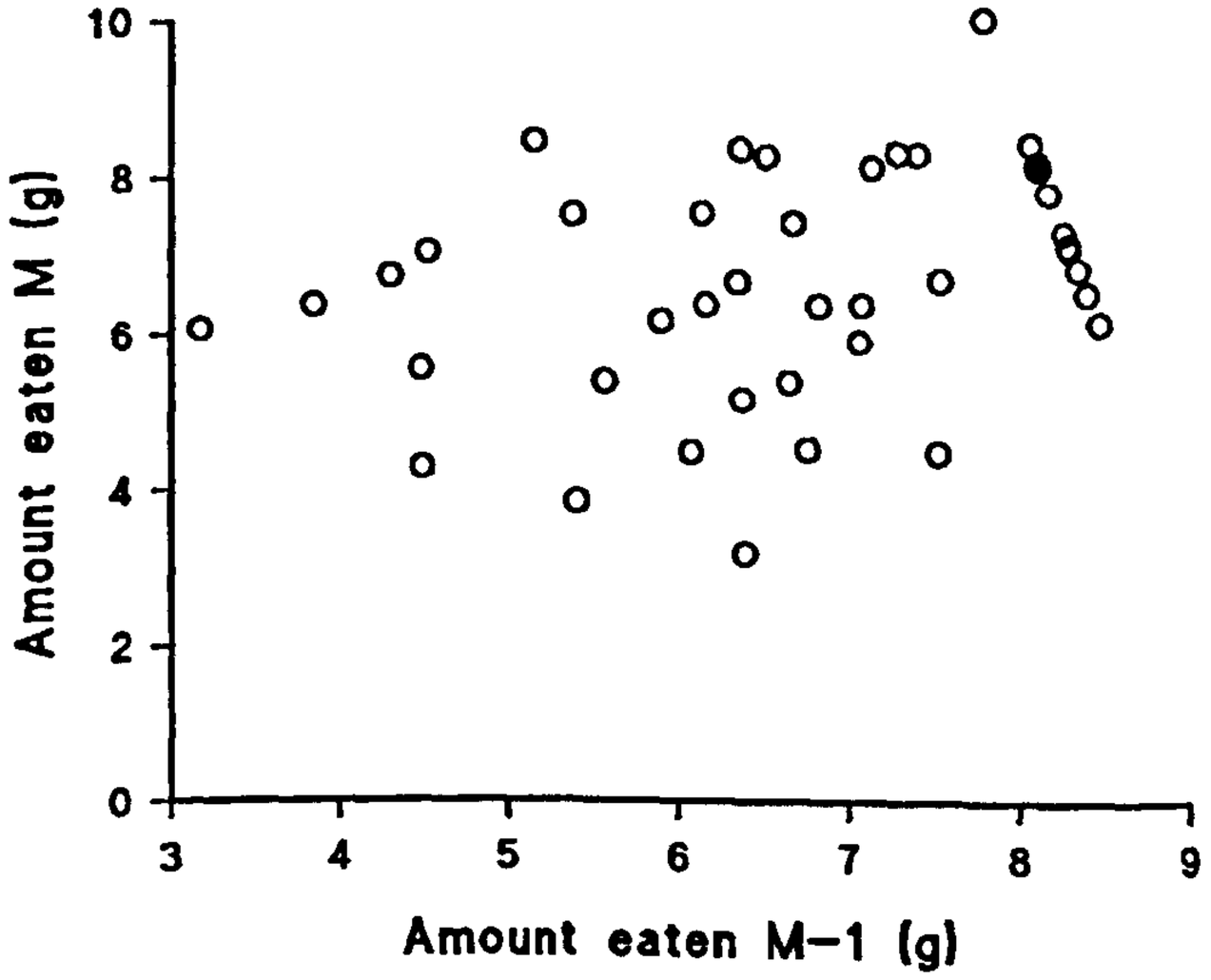


Figure 2.7 Model 2.3, in which food intake is based on sinus rhythms with noise, superimposed on a longer term non-stationarity, as in model 2.2 (see text). However, in this case an upper limit to food intake is imposed.

A final model (model 2.3) is presented in Figure 2.7. This model is the same as model 2.2, except that a maximum stomach content of 10 g is imposed. When the fish have a high feeding drive for a long period the food intake is driven to a single point, as in model 2.1 (Figure 2.5, solid circle). When there is low systemic need, the feeding behaves as in model 2.2 (Figure 2.6, points on lower left). However, when the systemic need is such that fish-feeding is oscillating just below the maximum limit, the larger meals are actually limited by stomach volume, and a negatively-sloped diagonal line is produced by the bigger meals. The smaller meals in this situation are found to the lower left of this line.

Re-examination of figures 2.3 a -d would suggest that stomach volume is never limiting at the feeding frequencies examined, as no limiting slope is apparent.

Experiment 2.2.1 Appetite return curves for high- and low-energy diets

Unfortunately, during the course of the experiment, additional fish were accidentally introduced into one tank by a well-meaning fisherman, halving the data for the pellet-fed group.

Experiment 2.2.1 (a) Shape of the appetite return curves

In the case of the squid-fed fish, both linear regression ($r^2 = 0.34$, $P < 0.01$) and the curvilinear fit ($r^2 = 0.37$, $P < 0.01$, appendix 2.2) explained a similar amount of the data variation and both were significantly correlated with the data (Figure 2.8). The residuals were also similar (linear regression = 197.86, curvilinear fit = 194.22) It is therefore not possible to say whether appetite return was linear or not in this case. Data is strongly influenced by the two data points at 94.33 h and more points here would have been useful, however including several long time intervals may have caused a build up of hunger which would have influenced the result. The maximum meal size was 49.5 g (7.8 % body weight) and the average meal size was 19.29 g $\pm$ 10.57 SD (3.04 % body weight). The average meal size when the previous meal was at given 24 hours previously was 20.92 g $\pm$ 14.15 SD, giving a coefficient of variation of 68%.

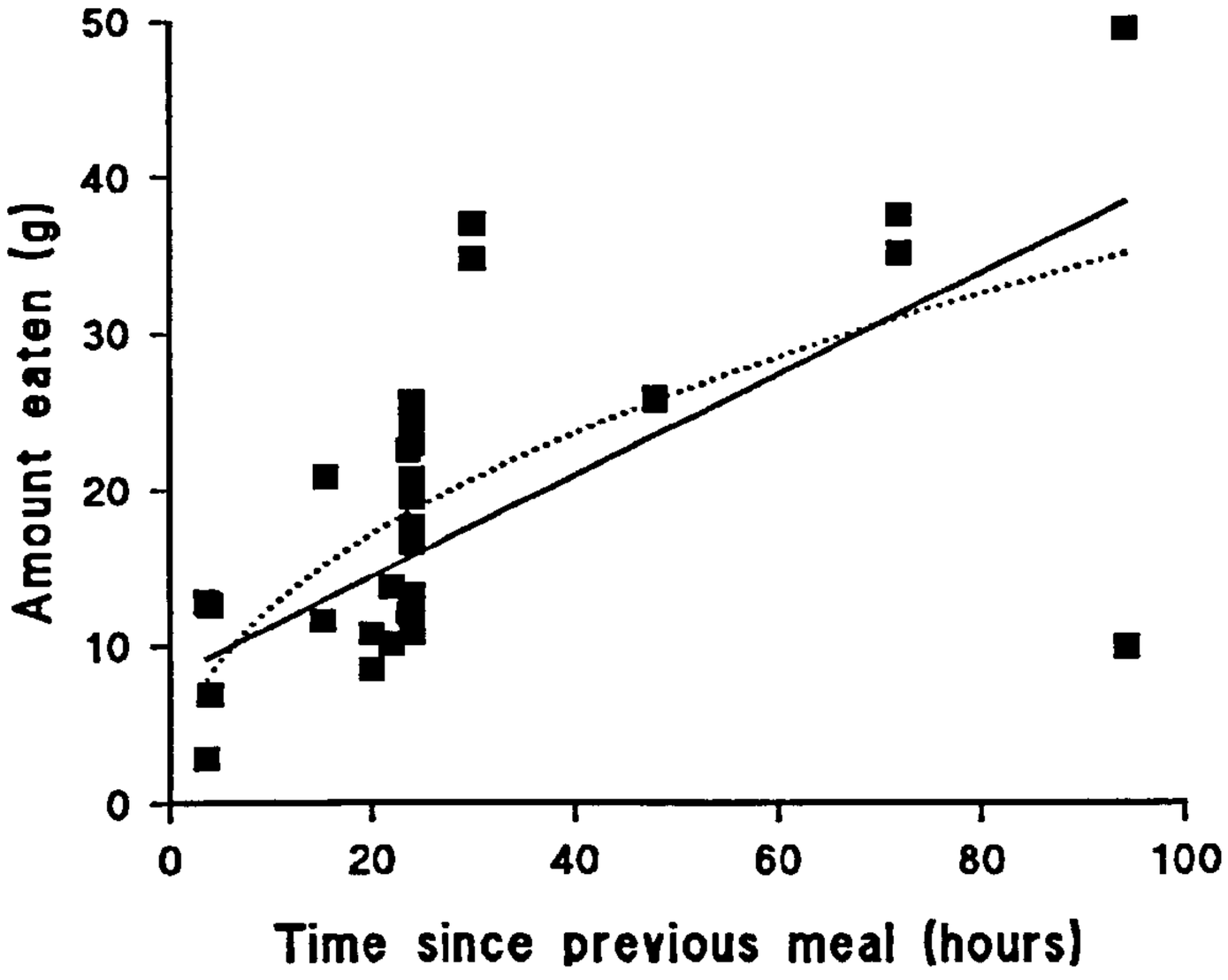


Figure 2.8 Appetite return plot for the squid-fed fish, showing linear regression and fitted curve ($Y = mX^c$).

The appetite return for the pellet-fed group (Figure 2.9), showed no significant correlation to either shaped curve (linear regression $r^2 = 0.2$, $P > 0.05$; exponential $r^2 = 0.2$,$P > 0.05$). The lack of correlation is probably due to the unexpectedly small data set and the evident large scatter of the data (this is illustrated by the large variation of the data at t = 24 hours where amount eaten = 3.14g $\pm$ 1.44 SD (V = 45%).

Fish fed on pellets did not eat a great deal over the course of the trial; intake when expressed as dry weight was lower than that of the squid-fed groups, unlike the results from Chapter 2. The maximum meal size was 4.8g (0.51% body weight) and the average meal size was 2.55g (0.25% body weight).

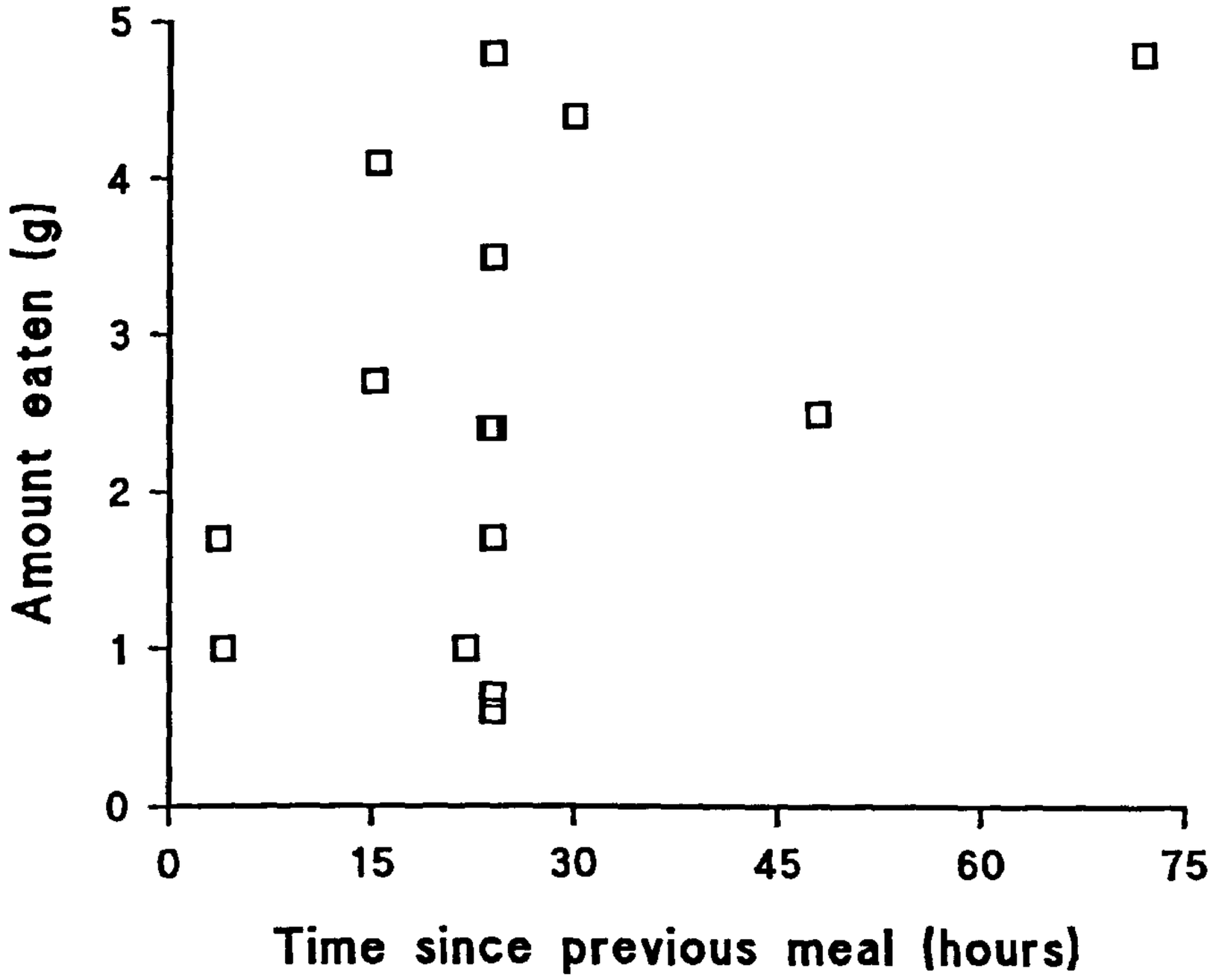


Figure 2.9 Appetite return plot, pellet-fed fish ($Y = mX^c$; linear regression not significant).

Experiment 2.2.1 (b) The relationship between meal size and size of the previous meal.

This experiment was designed so that when the return map, m against m-1, was plotted, the interval between m and m-1 was random, but the preceding interval, between m-1 and m-2, was 24 h (Table 2.2). In this way each trial was preceded by a standard time interval to reduce any long term deprivation influences.

In the squid-fed group a pattern emerged similar to that of model 2.3 above (Figure 2.10); a line of points on the top-right of the graph suggesting that food intake is sometimes limited by stomach volume. For all of the points on the limiting line, meal interval was $\geqq 30$ h, whereas all of the points to the lower-left of the line had a meal interval of < 25 h (by chance there were no meal intervals of 25 - 29h). Thus it would seem that stomach volume was not limiting up to somewhere between 25 h -30 h, and for longer intervals stomach volume was the limiting factor. Along the limiting line the sum of X plus Y was always similar

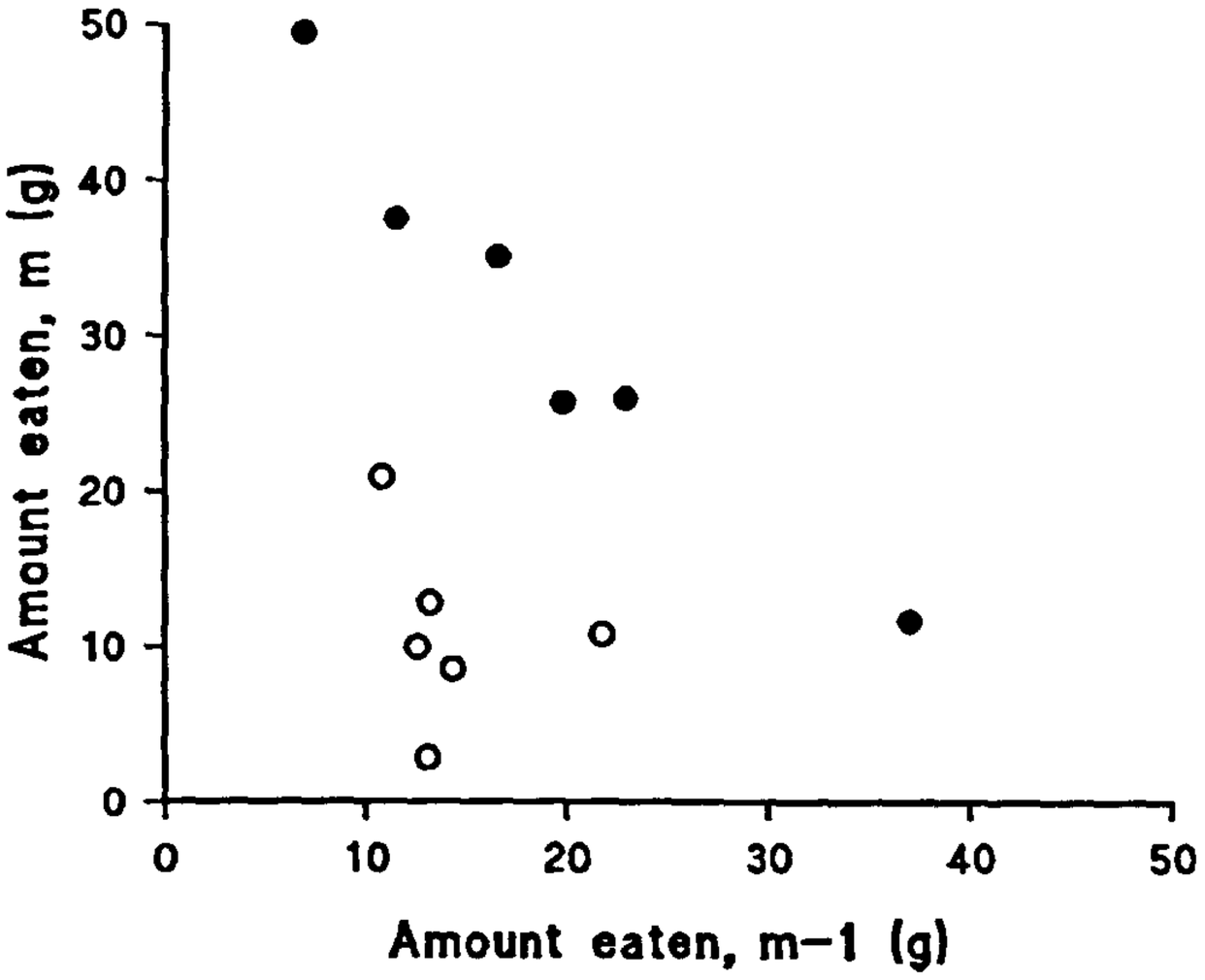


Figure 2.10 Return map of data from appetite return study, where time interval between m and m-1 was random and m-2 preceded m-1 by 24 h. Those meals (m) which were preceded by long inter-meal intervals fell on a single locus. (● = meals where time interval between m and m-1 was ≧ 30 h, O =meals where time interval between m and m-1 was <25 h)

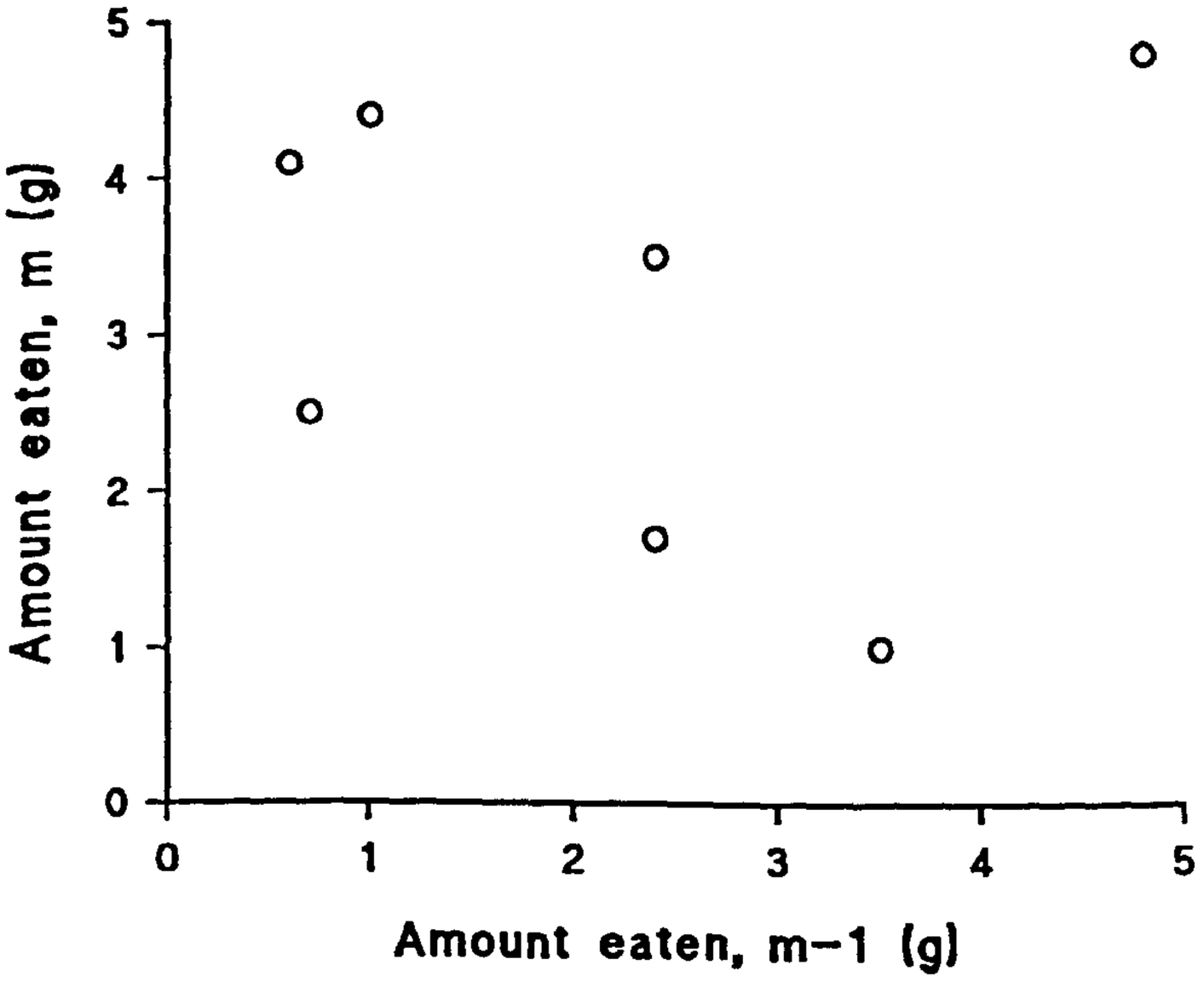


Figure 2.11 Return map of food intake for the pellet-fed group, constructed in similar way to Figure 2.10. There is no evidence for the stomach volume limiting intake.

(mean of X + Y = 50.92 g $\pm$ 3.31 SD). For the remainder of the points the sum of X plus Y was less than 40. There was no statistically significant relationship between the two meals (linear regression, r = -0.31, r^2 = 0.10, P>0.05) presumably because of the latter points.

The pellet-fed fish did not have a limiting line (Figure 2.11), with the sum of (m) and (m-1) being very variable and well below the observed maximum for two consecutive meals, 9.6g. There was no significant relationship between the two meals (r^2 = 0.03, P > 0.05)

Experiment 2.2.1 (c) The effect of time of day on meal size

Multiple regression was used to test whether the time of day of the meal and previous meal size had a significant effect on food intake. Factors included were time of day, the amount eaten in the last meal and time interval. The amount eaten in the last meal had no significant effect, therefore it was eliminated and the test repeated. This showed that time of day had a statistically significant effect on food intake (T = 2.37, P = 0.024) as, unsurprisingly, did the interval since the last meal (T = 4.56, P < 0.001).

2.2.2 Preliminary trials for studies using X-radiography

a) serial slaughter

The results of the serial slaughter are shown in Figure 2.12 (Appendix 2.3). The $BaSO_4$ spheroids in diet one passed out of the gut at a slower rate than the diet from the earliest stage of gastric emptying. This is quite obvious simply from looking at the graph; a linear regression model estimated that only 0.7 % of the spheroids were evacuated per hour, clearly indicating retention (Table 2.5). Unlike the $BaSO_4$ spheroids, where a linear regression was used for simplicity even though the curvilinear fit used for the diet proved as good (residuals: linear regression = 151.09, curvilinear fit = 150.76), the relationship for the proportion of food remaining in the stomach the best fit for was curvilinear (residuals: linear regression = 416.64, curvilinear fit = 255.71).

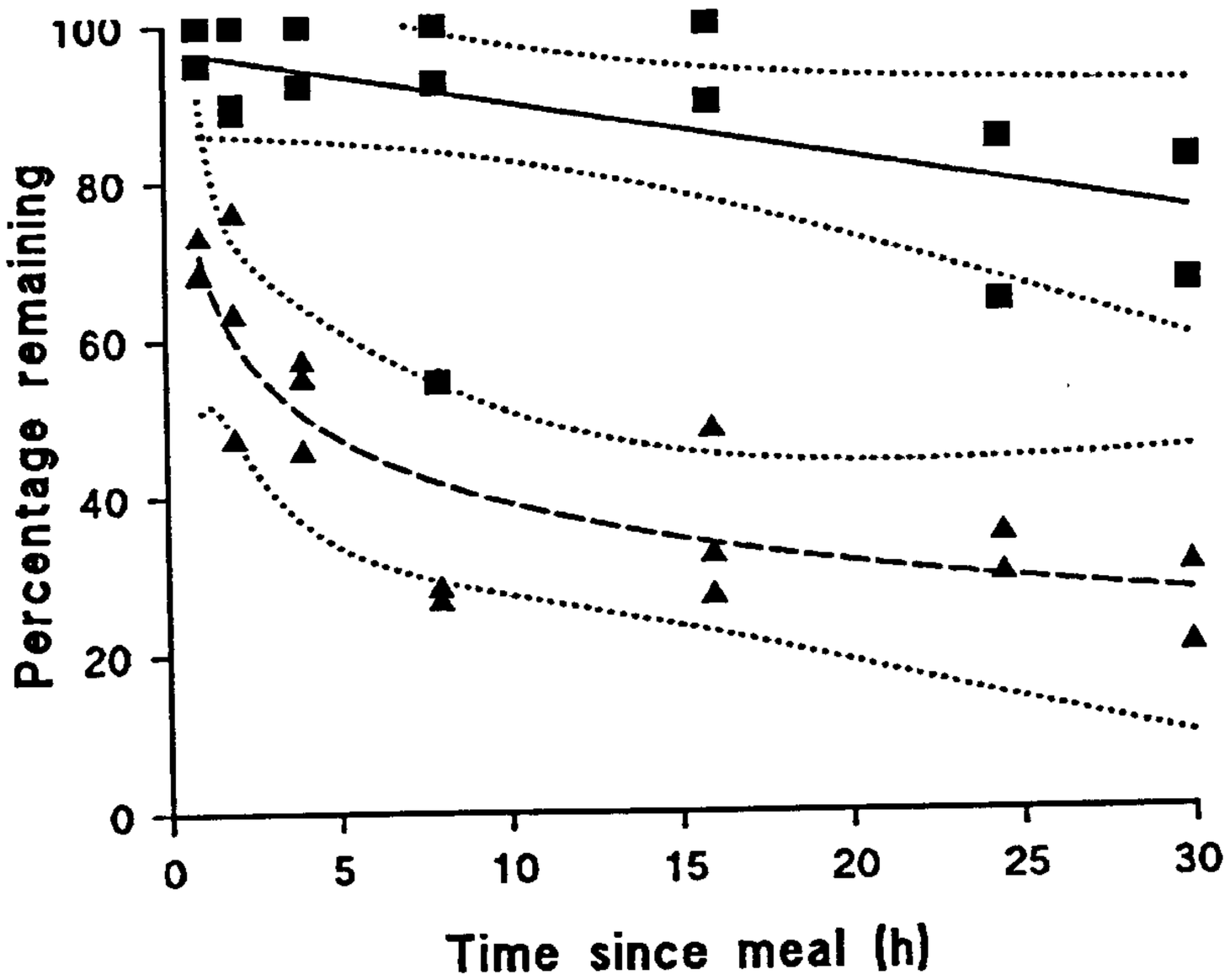


Figure 2.12 Plot of gastric emptying rate of food (▲), estimated using serial-slaughter, compared with the rate of evacuation of barium sulphate spheroids (■). 95% confidence limits are shown.

	Food	Spheroids		Food	Spheroids
r^2	0.74	0.28	m	-37	-0.7
P	<0.01	0.02	b	107.8	97.1
			c	-0.15	-

Table 2.5 Statistical significance and values of constants for fitted curves

(Spheroids = linear regression $Y = b + mX$, Food = curvilinear fit: $Y = b + mX^c$)

b X-radiography

In the case of the ballotini (diet two), 100% retention occurred, with the ballotini being expelled at the very end of gastric evacuation. This was clearly observable from the X-ray plates of all individuals tested (Figure 2.13 is an example of these findings). Retention of barium sulphate spheroids also occurred for diets 3 to 6 (for

example see Figure 2.14), in all of the individuals used. Thus retention of the radio-opaque markers occurred in all fish, for all diet types, moisture contents and binder levels.

Two incidental observations from the X-ray study are pertinent to the question of whether stomach volume could ever limits food intake. Figure 2.15 is an X-ray taken at t = 10 minutes after a prandium, and clearly shows a single pellet which has passed into the intestine immediately after it was eaten. Secondly, Figure 2.16 shows an example where all of the pellets eaten have passed into the intestine whilst still intact. This information needs to be reconciled with the findings above that stomach volume can be limiting under certain circumstances.

Figure 2.13 X-ray of *L. limanda* (105 g), 22 h after a 1 % body weight meal of diet 1. The ballotini are clearly retained in the stomach, whilst a large portion of the diet, marked with $BaSO_4$ powder, has passed into the intestine.

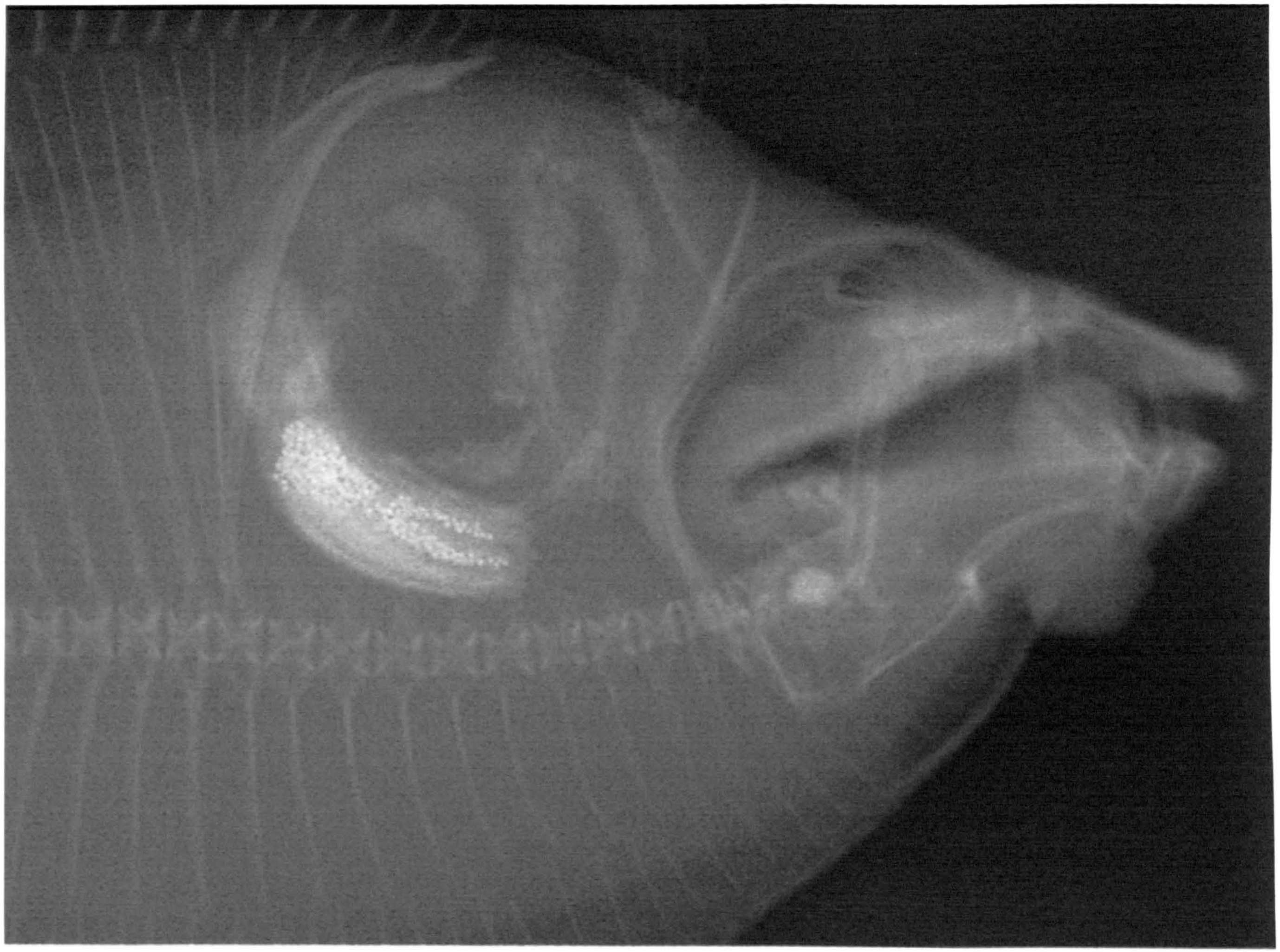


Figure 2.14 Retention of barium sulphate spheroids in diet 3, two hours after the meal was eaten. Clumping is occurring towards the posterior end of the stomach, whilst the diet can be seen in the intestine. The image is not as clear as Figure 2.13, because the fish was X-rayed *in situ*, through 10 cm of water, with a resulting loss of contrast.

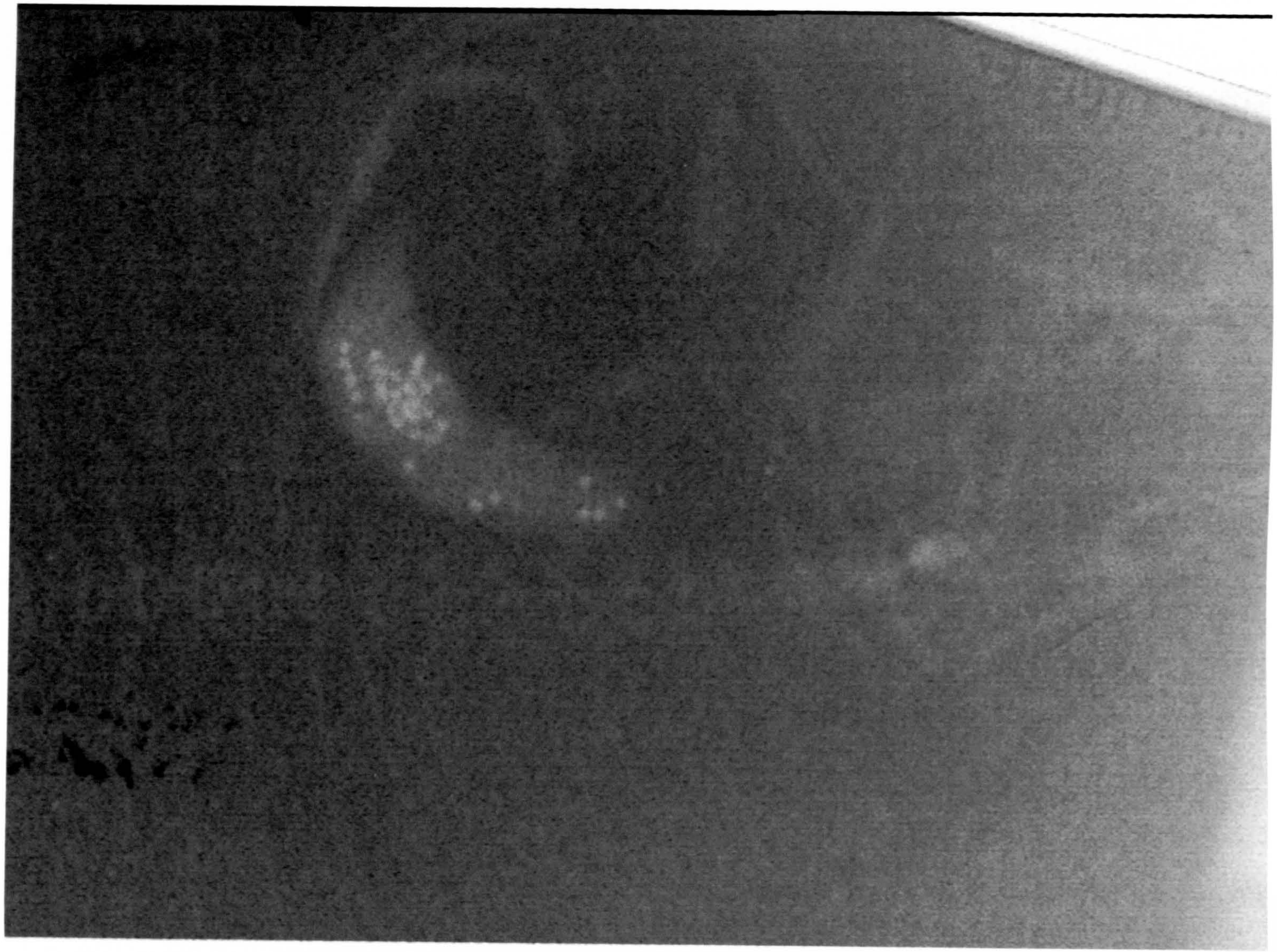


Figure 2.15 ***In situ*** **X-ray of gastrointestinal tract of dab, ten minutes after eating a 1 % BW meal. There is clear evidence that food can pass straight into intestine straight after it is eaten.**

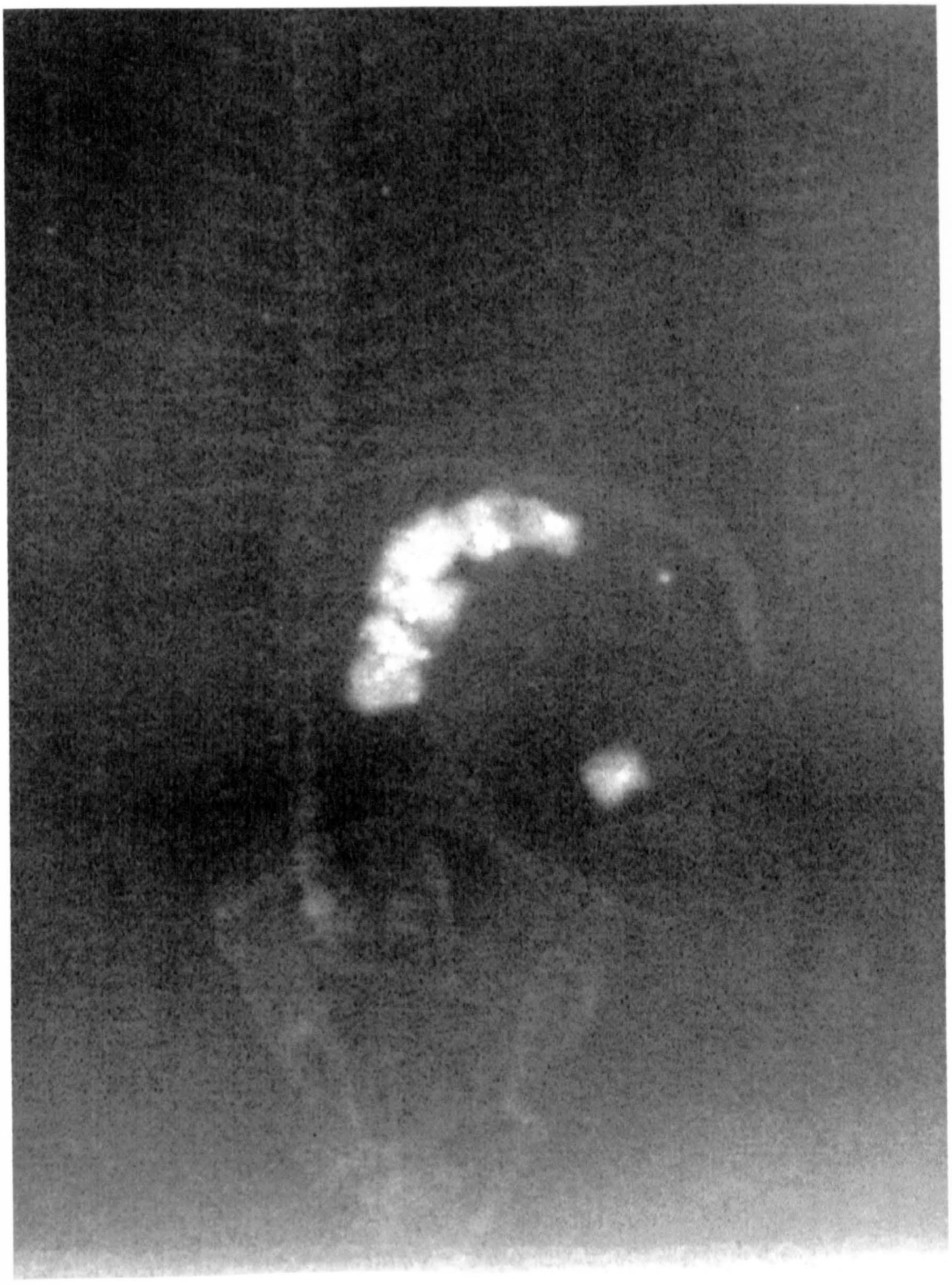
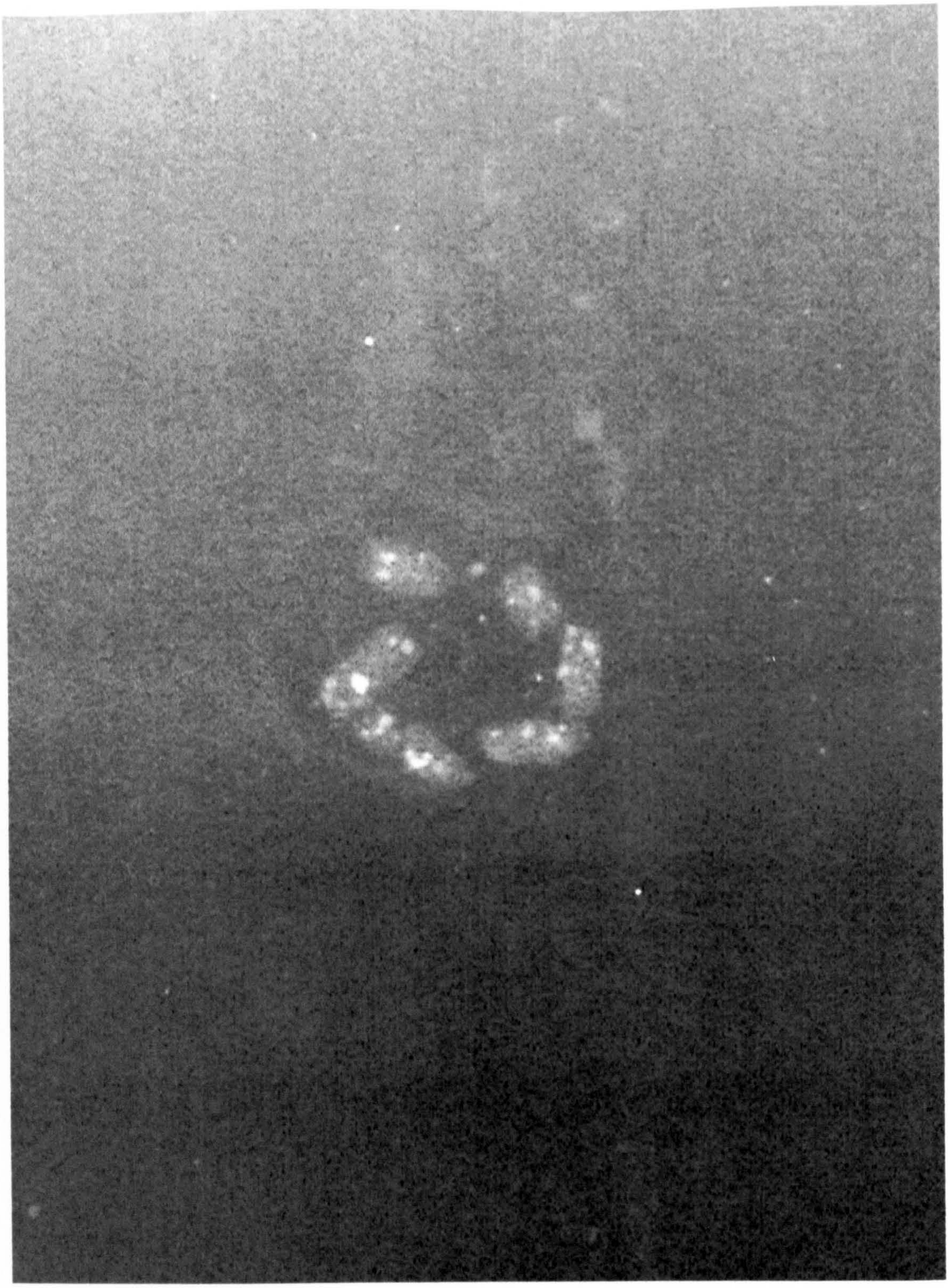


Figure 2.16 In situ X-ray of pelleted food passing from the stomach whilst still intact.



2.3 Determination of the relationship between stomach content and food intake in dab using serial slaughter.

After 10 h an average of 41.4 % $\pm$ 28 SD of the first meal remained. The squid pieces were found to be practically intact in both the stomach and the upper intestine; it was only when they reached the latter half of the intestine that they became unrecognisable as squid. This observation is comparable with the observation with pellets in Figure 2.16, above.

Figure 2.17 is a plot of the amount taken in the second meal as a function of the stomach content. There is clearly no negative correlation present (linear regression $r^2 = 0.002$, $P = 0.95$, appendix 2.4), with the points seemingly falling on three lines:

1) The fish that ate little or nothing in meal 1 but ate well in meal 2
2) The fish that ate well in the first meal but ate little or nothing in the second
3) The fish that ate well in both meals

If the fish that did not eat a significant amount in one of the two meals are ignored, then there appears to be a positive relationship between food remaining in the stomach and food intake in the second meal. This is certainly a result of the *post priori* separation of points, and for this reason no tests of significance were attempted.

The results were not an artefact of differing fish sizes; when the data was transformed with an allometric function (equation 2.2; $b = 0.58$, Jobling 1974) the pattern was still apparent (Figure 2.18).

Regression analysis showed that there was no significant effect of first meal size on the second meal size after the data was transformed ($r^2 = 0.01$, $P = 0.954$).

By 20:30h there was a significant difference between the percentage dry matter content of the squid for the two meals (single factor ANOVA, $F = 53.33$, $P < 0.001$) . Mean values were; meal one 16.1% $\pm$ 1.6 SD, meal two 20.7% $\pm$ 2.1 SD. Prior to the experiment the freshly thawed squid had a dry matter content of 23.8 $\pm$ 0.9 SD.

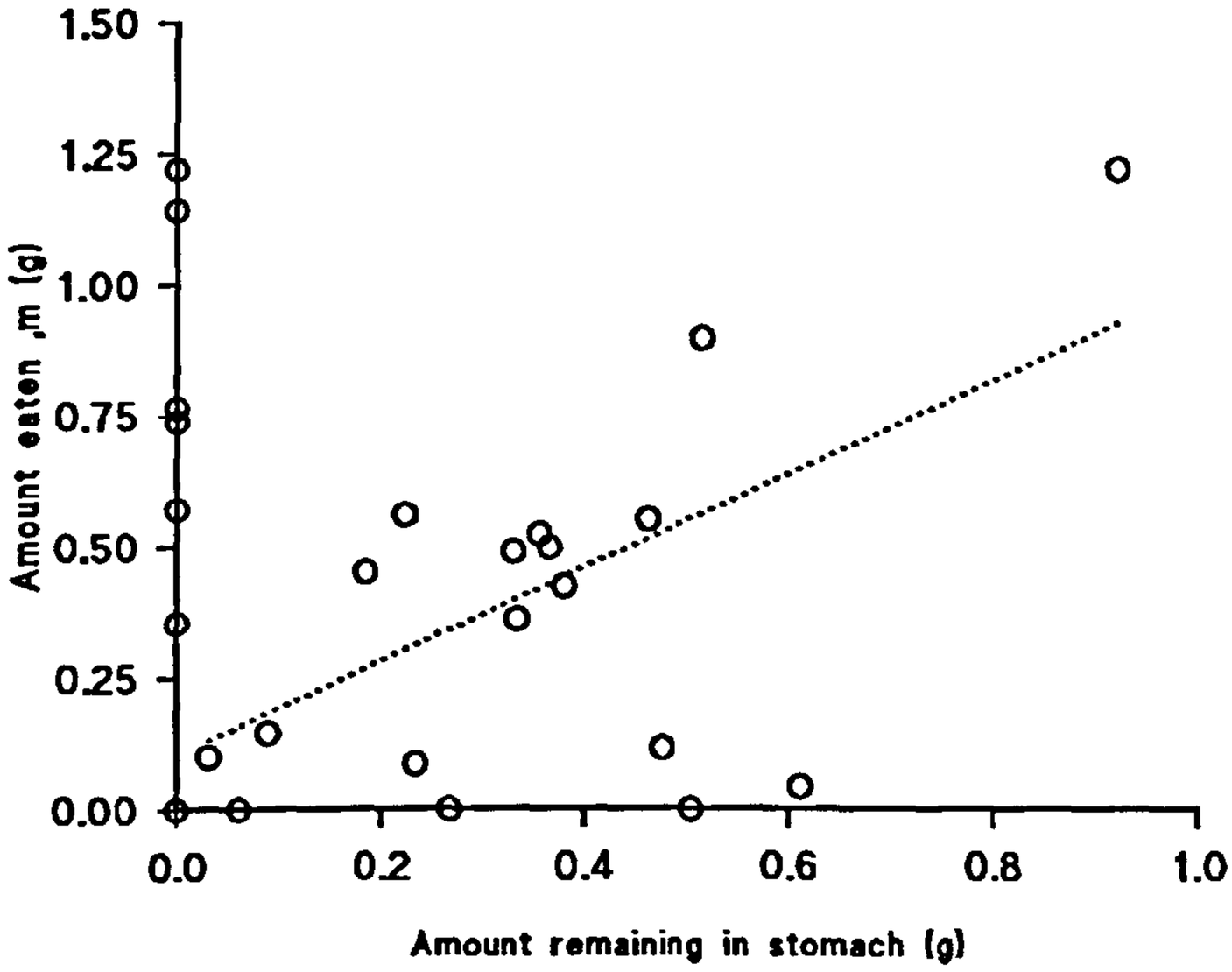


Figure 2.17 Plot of amount eaten in meal m (g) against amount remaining from meal m-1 (g). There is no significant relationship between the two values, although if fish which do not eat much in one meal are discounted a positive relationship correlation is observed (dotted line)

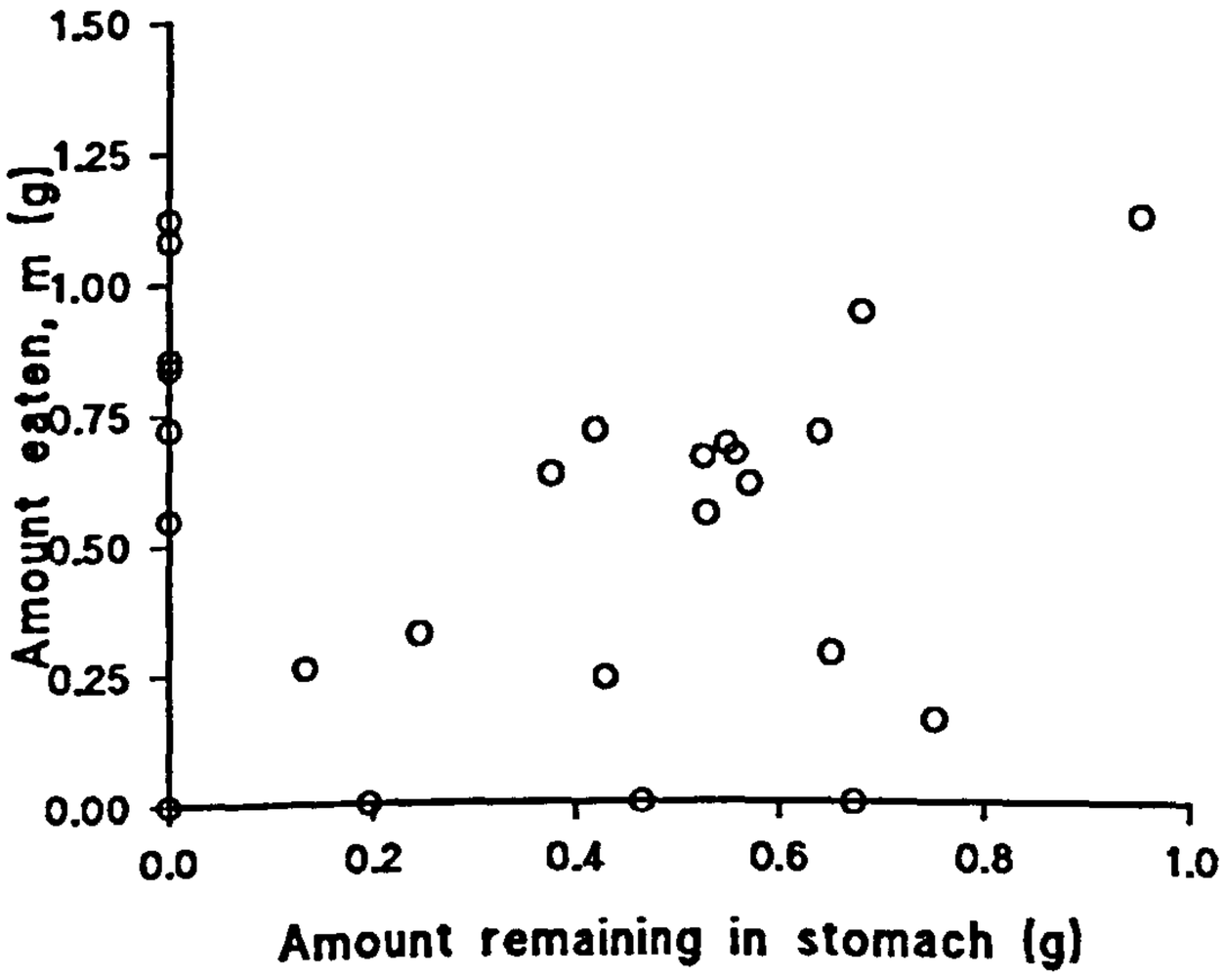


Figure 2.18 Transformed serial slaughter data, (equation 2.2,b = 0.58) showing that the lack of a significant correlation is not an artefact of fish size.

(iv) DISCUSSION

The results presented in this Chapter provide evidence that the stomach volume of the dab limits food intake under certain circumstances. Experiment 2.1 proves that, whereas stomach volume is never limiting in pellet-fed fish - who maintain a similar daily intake for all feeding frequencies - dab cannot adjust their intake of squid mantle to maintain average daily energy intake when they are offered food once every three days. This is in contrast to those fed once per day and three times per day, where average daily intakes were similar. Thus dab are able to adapt their food intake to different feeding frequencies, unless the stomach volume is a limiting factor. This finding could be interpreted as evidence supporting the hypothesis of Jobling (1986a, discussed above), where fish fed the low-energy diet would not switch to volume based regulation until feeding was at the lowest frequency studied.

Examination of the return maps for dab fed three times per day and those fed once per day, shows that in all cases a similar pattern is observed; food intake appears to be a noisy periodic (or possibly chaotic) feeding rhythm oscillating around a fixed average point for short periods and then taking excursions before settling around another fixed point. The models demonstrate that the experimental return maps for these two feeding frequencies are not a result of a system which is solely regulated by available stomach volume (model 2.1) as was suggested may be the case in Grove *et al.* (1978), but in these cases stomach volume appears to be unimportant (as in model 2.2). A trial to provide a long enough data set to produce a return map for fish fed every three days was not attempted, as it was thought that the fish would develop a nutritional deficit in the time required.

It proved impossible to determine whether the appetite return curve for squid-fed fish was linear or curved (as observed by Fletcher 1982), as statistical fits explained a similar amount of the variation. In both cases no plateau (indicating M_{max}) was observed, so it could not be said from the appetite return curves themselves whether stomach volume was limiting. However a return map of the appetite return data for fish fed squid mantle, suggests that stomach volume is limiting when fish have not been fed for 30 h or more on this diet, as the behaviour of the

data is very similar to model 2.3. This suggests that when feeding interval is above a certain duration, fish want to eat more than they can fit in their available stomach volume, and volume-based regulation of food intake occurs (Jobling 1986a).

The method of generating a systemic need in models 2.2 and 2.3, effectively a noisy quasi-periodic data set with one frequency very much longer than the other (the non-stationarity), was based purely on qualitative observations of actual data sets. It does not share all the characteristics of the real data however, and more quantitative data about the dynamics driving food-intake are needed before a model can be said to be functionally realistic (see Chapters 5 and 6).

In the case of the unexpectedly limited data for the appetite return curve of the pellet-fed fish, no significant relationships were observed at all. This is certainly due to the short data set, as not observing an increase in appetite as the interval since the previous meal increases would have been unique. The lack of a relationship between (m) and (m-1) in the pellet-fed fish is again due to the small data set, which really only allows qualitative assessment. From what can be seen it does not seem that any kind of limit to food intake was reached.

It is not clear why the pellet-fed fish ate a lower amount than the squid-fed fish, in terms of dry weight and energy (given the results of Chapter 3, below, this result is unexpected). Differences in dry weight of food intake have been observed in *S. gairdneri* (=*O. mykiss*) fed diets of differing water content, formulated diets and semi-frozen mixed sprat (Bromley and Smart 1981). And this has also been found when diets were diluted with binders, for example Hilton *et al.*(1983) in *O. mykiss* but in those cases the bulkier diets had the lower dry weight intake. The low intake may have been a chance result, due to the small data set, or some other (unmeasured) factor was influencing feeding levels

The use of ballotini and Barium-sulphate spheroids as radio-opaque markers has proved impractical in *L. limanda*, when used in the required diets, due to their disproportionate retention; the markers in the first meal were not a true reflection of the proportion of that meal remaining at the time of the second meal. This has been found in other species/diet/marker combinations (Al Aradi 1986, Jørgensen and Jobling 1988, dos Santos and Jobling 1991).

These results differed from those reported by Hailstone (1984), also working with *L. limanda*, who incorporated the dietary ingredients with gelatine, to make a very hard pellet. Using Hailstone's diet in these experiments would not have allowed the results to have been compared with other findings in chapters 2 and 3 and so this diet was not used. It was hoped that increasing the level of methyl-cellulose binder would have caused the pellets to be broken down gradually from the outside first, so that spheroids were released gradually into the chyme, as is presumed to happen in the rectilinear model of GER, where GER is surface dependant (see dos Santos 1990 for a discussion). Clearly this did not happen. Once again the importance of preliminary experiments such as those described above is shown to be an important step in any study of GER that includes the use of radio-opaque markers.

The serial slaughter, carried out to test the suitability of the radio-opaque markers, found that the gastric emptying curve for diet 1 was curvilinear. The hypothesis of Jobling (1986a) that high energy diets would have a linear evacuation rate does not seem to fit this result. For diet 1, used in this trial, the Trouw Aquaculture diet (Table 2.1) was diluted with other ingredients to give a final energy value of approximately 12.5 $kJ.g^{-1}$, this is still considerably higher than the energy content of natural foods. However Jobling (*loc. cit.*) also argued that small pellets such as diet 1 would have a curvilinear evacuation curve.

During the course of the X-ray trials, some unusual observations were made; on occasion food was observed to pass into the intestine within ten minutes of a meal, and pellets were observed, again occasionally, to be intact in the intestine. Furthermore in experiment 2.3 squid pieces were easily recognisable and almost their original shape when they are passed out of the stomach and into the upper intestine. This has been found in both dab and plaice in the wild (Edwards 1971, Kuipers 1975, Gwyther 1978, Basimi and Grove 1985b)

The observation that pellets can pass straight into the gut contradicts the earlier conclusion that stomach volume can have a limiting role in short term food intake in certain circumstances. This is because, if a fish was 'required' to eat more than a stomach-full, then it could simply pass food straight into the intestine. The event however was very rare. Furthermore it is possible that there is some compromise

between digestive efficiency and partially by-passing the stomach (this would be a 'bulk-overload'; analogous to the nutrient overload hypothesis of Jobling 1986b). Because of the above findings, instead of the X-ray method, a serial slaughter was used to test for a correlation between stomach content and food intake. In this experiment, the lack of a negative relationship between the stomach content and food intake proves that GER is not correlated with food intake level in individual fish. This does not preclude some other role of the stomach in the short term regulation of appetite, in addition to the volume being limiting under certain circumstances. Gwyther and Grove (1981) observed a correlation between GET and meal interval in the dab; whether this is a causative relationship has yet to be discovered. What is more, the evidence that GER is correlated with food intake in **groups** of fish in several species still stands, and needs to be reconsidered in the light of these findings.

CHAPTER THREE: LONG-TERM GASTROINTESTINAL RESPONSES TO DIET VOLUME

(i) INTRODUCTION

There is considerable evidence that fish adapt their food intake when offered a diet of novel nutritional quality (see Chapter 1, part vii) including the dilution of dry diets with kaolin. Hilton *et al.* (1983) and Bromley and Adkins (1984) observed that salmonids responded to such dietary dilution with an increase in the relative weight of their stomachs. *O. mykiss* are also able to compensate for diets containing higher water content by increasing their food intake (Bromley and Smart 1981, Ruohonen and Grove 1996). When rainbow trout were offered two diets; [pellets (dry, 22.3 kJ g^{-1}) and herring (wet, 4.5 kJ g^{-1})], the resultant difference in food intake was again mirrored by a change in stomach volume caused by growth of the stomach (Ruohonen and Grove 1996). They concluded that fish could not respond to dietary dilution by simply increasing their GET, but had to also increase their meal size.

Bromley and Smart (1981) found that compensation only occurred up to 35 % water in a moist pellet. When fish were fed minced sprat, *Spattus sprattus*, with a water content of 67 % (typical of natural prey), compensation was incomplete. The level of food intake of a wet diet depended on the meal history of *O. mykiss*; fish which had been fed for a long period on pellets and then offered herring did not eat as much as fish which were already acclimated to herring. On the other hand, fish which were adapted to herring and then switched to dry pellets almost immediately adjusted their intake to a level of intake similar to fish which were already adapted to pellets. Thus it would seem in the former case that stomach volume could be a limiting factor in food intake but in the latter food intake must have been independent of stomach capacity and fullness (Ruohonen and Grove 1996, Ruohonen *et al.* 1997). This finding is comparable with the results of Chapter 2, where stomach volume was found to be limiting in dab when a high level of hunger was combined with a low-energy (bulky) diet. As discussed above, Jobling (1986a), argued that the intake of low-energy food would be regulated by stomach fullness, whereas in high-energy diets regulation would be energy based. The

results of Ruohonen *et al.* (*loc. cit.*) would suggest that volume based regulation would only be short-term, until the stomach adapted its volume to the bulkier diet. In wild dab, the choice of prey of flatfish varies over the year (Basimi and Grove 1985b, Knust 1987) and the amount of food eaten also varies with season, with more fish having empty stomachs in the winter (Jobling 1974). It is possible therefore that at certain times, in the natural course of feeding, dab will increase the bulk of their food intake, and as a result stomach volume would limit food intake. (The laboratory results of Fletcher, 1982, suggest that dab can compensate for changes in prey type by both changing their feeding frequency and the meal size).

The aim in this Chapter was to ascertain whether dab can adjust their stomach volumes to accommodate bulkier diets, and thus reduce the chance of stomach fullness limiting meal size in the long term. Experiments were carried out between 1995 and 1997 investigating gastrointestinal responses to dietary volume **(experiments 3.2.1a to 2.2.4c)**. Prior to these experiments, methodological trials **(experiments 3.1.1 and 3.1.2)** were carried out for a number of reasons:

Grove and Holmgren (1992a) reported that, in *O. mykiss*, inflation of the cardiac stomach induced reflex muscular contractions. The stomach then slowly relaxed to its full volume. In the case of *L. limanda* (Grove and Holmgren 1992b), when stomachs were examined *in vivo*, resistance to gastric distension occurred during the first distension, but in subsequent distensions, compliance was observed, which lasted for at least one hour (Figure 3.1). Thus the shape of the distension curve, when plotted against time, exhibited almost a linear response to the first distension (0.5 kPa distension pressure), in which gastric volume continued to increase gently for the full ten minutes of the distension period and did not reach a maximum plateau. After three or four distensions the curve had changed, with a sharp increase in gastric volume observed immediately a head of pressure was applied, followed by a slow phase further relaxation.

It is clear from this that the number and duration of distensions will effect the recorded value of stomach volume.

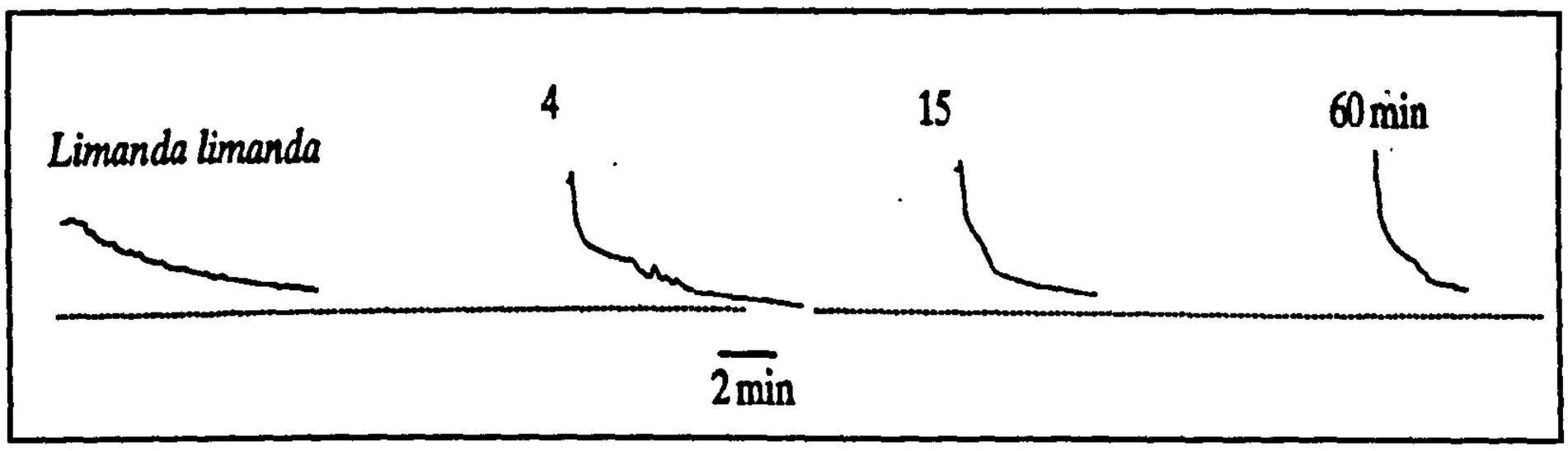


Figure 3.1 *In vivo* responses of *L. limanda* to gastric distension (Figure taken from Grove and Holmgren 1992b). Horizontal broken lines indicate maximum stomach volume (V_{max}) Numbers indicate deflation time between distensions. Distension pressure 0.5 kPa, temperature 14 ^{0}C.

When measuring the volume of a stomach, a commonly used technique is to place the stomach under a burette and to dilate the stomach with a 50 cm (5 kPa) head of pressure (Jobling *et al.* 1977). Holmgren and Grove (1992b) observed that increasing the distension pressure on the stomach caused the volume of the stomach of *Gadus morhua* to increase. It is by no means clear what the optimum pressure head is for the dab; it should be high enough to distend even a robust stomach, yet low enough not to burst the stomach or to stretch it. Thus the relationship between distension pressure and gastric volume needs to be investigated, to discover the best distension pressure to use in subsequent experiments.

Flowerdew and Grove (1979) found that, in *S. maximus* (L)., freezing the stomach prior to measuring the volume resulted in an increased gastric distension as well as giving the stomach wall a tendency to rupture. If this were the case in *L. limanda*, it would be necessary to use only fresh stomachs.

Flowerdew and Grove (*loc. cit.*) also examined the effect of starving *S. maximus* for ten days and found that the stomach volume of starved fish was smaller than fish that had been regularly fed. This finding, if applicable to the dab, has a bearing on the choice of the interval between the previous meal and the measurement of stomach volume.

Following the methodological trials, tests were carried out to determine whether dab in captivity are able to adapt their food intake, when offered discrete meals, as

their diet is switched from a low-energy wet diet (squid mantle) to a high-energy dry diet (Trouw aquaculture commercial pellets). Whether any observed change in food intake is reflected in a change in stomach volume was also investigated, with measurements taken to determine whether any change in stomach volume was due to the stomach growing bigger or simply becoming more easily distensible.

Out of curiosity, in spite of the fact that Jobling (1974) reported that once food has started to enter the intestine the amount of food there remains roughly constant, the weight of the pyloric caecae and intestine were recorded to see if they respond to dietary differences.

The *in vivo* hydration rates of the diets were examined to judge the effect that this would have on the volume of the resulting chyme. These findings were compared with an X-ray study of *in vivo* relaxation rates of stomachs containing food, to see whether the latter compensated for the increase in volume of food with hydration.

(ii) MATERIALS AND METHODS

Because of the lack of basic information about causes of intra-specific variation in stomach volume, a series of novel tests were designed to examine such variation in the dab.

(ii) a General methods

Fish were housed in 250 l tanks, except for the continuously fed group, which were kept in a 3,000 l Fasttank Raceway, (Fast Engineering Ltd, Ireland). Water was treated as described in Chapter 2(ii). The fish in the 250 l tanks were fed by hand, as in Chapter 2(ii). The continuously fed group were kept in a 24 h daylight regime and trained to feed using demand feeders; this data was primarily generated for analyses carried out in Chapter 5 and details of the methodology can be found in that Chapter.

Once the fish had been fed on their experimental diet for the required duration, they were slaughtered with a sharp blow to the head and the brain was destroyed, after which their gastrointestinal tract was carefully dissected out and a piece of thread tied tightly around their pyloric sphincter. The intestine was then cut just above the posterior-most pyloric caecum and, when required, the intestine was

placed to one side for subsequent measurements to be taken. A 1 mm diameter 'Nalgene' tube, attached to a reservoir (Figure 3.2), was inserted into the stomach through the oesophagus and thread was used to securely attach it. The stomach was then placed in a water bath at room temperature in a teleost Ringer solution (House and Green, 1965)

A suitable reservoir, made from the barrel of a syringe (10 ml) , was slung from a 'Bell and Howell' physiological pressure transducer mounted on a clamp stand, which was in turn placed on a 'Lab Jack' for vertical displacement. The pressure transducer was connected to a Devices 3559 transducer pre-amplifier (Settings: 10 mV, +, 000, HF cut = 5, gain = 11.5, range = 0.1 mV) and a 'Lectromed' chart recorder (Figure 3.2).

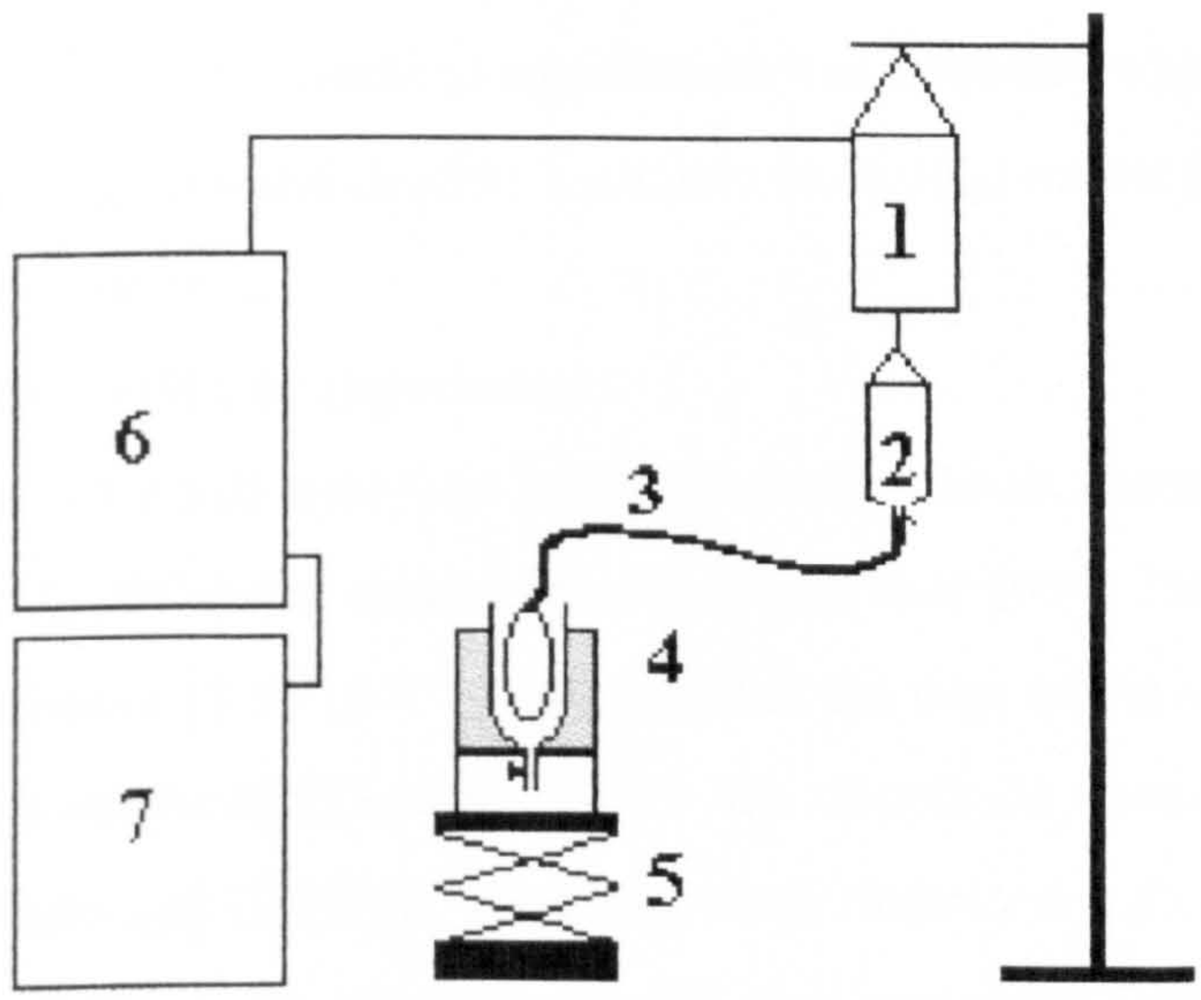


Figure 3.2 Equipment used for measuring the volume of stomachs. A reservoir (2) is hung from a pressure transducer (1) , so that its weight is recorded as an electrical signal by the pre-amplifier (6) and the chart recorder (7). The stomach is situated in a water bath (4) on a laboratory jack (5). As the water bath is lowered to provide the required intra-gastric pressure, Ringer passes into the stomach *via* the nalgene tubing (3) . The weight of the Ringer leaving the reservoir is translated into a stomach distension volume.

Once in place the water bath could be lifted or lowered relative to the height of the reservoir, which was also filled with House and Green Ringer solution, and a tap allowing the Ringer to flow into the stomach was then opened. As the Ringer solution flowed out of the reservoir and into the stomach, the fall in the combined weight of the reservoir and the ringer solution within it was recorded on the chart recorder. The deflection of the pen was then compared against a calibration curve and converted into the volume of water (ml) entering the stomach. A relative stomach volume, SV_r was calculated as the volume of the stomach per 100 g of fish weight

After the gastric distension had been measured, in some trials the remaining two pyloric caeca were removed at the pyloric sphincter and weighed (see below). Statistics were carried out with MINITAB 3.11 software (MINITAB 1996), except for linear regressions and curve fits, which used BIOSOFT FIG-P version 6.0c (BIOSOFT 1991). Statistical significance was accepted when $P \leqq 0.05$. Anderson- Darling tests and Bartlett's statistic were used to test for normality and heterogeneity in the data.

(ii) b Methods specific to experiments

In **experiment 3.1.1** fish were kept in the laboratory for six months and fed on dry pellets once daily, following their capture in September 1994. They were kept at ambient temperature (7.5 -15 ^{0}C = range) and at the time of the analysis the fish had an average weight of 225.00 g $\pm$ 134.7 SD. Stomachs were examined *in vitro* between 09/03/95 and 13/03/95, when they were distended with an intra-gastric pressure of 2 kPa for a period of 10 minutes, then rested for 5 minutes before undergoing a repeat distension. This process was repeated three times, after which a 1 % solution of KCl was added to the Ringer solution to relax the smooth muscle and the stomach was distended once more, this time for 30 minutes. The stomach volume was plotted against the distension number and a linear regression used to test if the slope was significantly different from zero.

Experiment 3.1.2 examined the effect of three factors on stomach volume measurement; distension pressure, freezing stomach samples prior to measurement and the pre-sampling starvation period. Fresh stomachs from six fish (average

weight = 87.5 g + 41.8 SD) were distended under a steadily increasing head of pressure (1 kPa, 2 kPa, 3 kPa and 5 kPa) during December 1996, having been fed on pellets for six weeks after capture. At the time of capture the sea temperature was 16 °C, fish were kept at ambient temperature which had dropped to 10 °C by the time the stomachs were sampled. These were compared, in January 1997, with the stomachs of 22 fish caught at the same time as those above (average weight = 121.14 g ± 51.2 SD) which had been slaughtered and then frozen after being fed on pellets for eight weeks after capture; temperature at the time of sampling was also 10 °C. A third group of fish was starved for two weeks before having their SV_r recorded at the same range of pressure as the pellet-fed fish (n = 5, mean weight = 102.50 g ± 45.6 SD, sampled at 10 °C). Linear regression was used to examine the effect of distension pressure for the three groups.

Experiment 3.2.1 compared the stomach volume of fish kept in the laboratory and fed on different diets, squid mantle and commercial dry pellets (Trouw Aquaculture), once daily. The compositions of the diets are given in table 2.1. Freshly trawled fish, all female (sexual maturity stage III), were placed randomly in a system of six 250 l tanks and the SV_r of the newly caught fish was recorded (n = 16, average weight = 88.18 g ± 22.8 SD). During the first two weeks all fish were fed on squid mantle. Following this period three tanks, chosen randomly, were switched to the commercial pellet and the fish were fed once daily for a further six weeks. Small samples were taken to determine SV_r after two weeks (n = 3, 93.66 g ± 23.5 SD) and four weeks (pellet-fed; n = 5, 112.6 g ± 41.7 SD, squid-fed; n = 3, 94.33 g ± 30.6 SD).

After six weeks in the laboratory on the different diets, SV_r was measured at different pressures as in the methodology trial (experiment 3.1.2) above, but with 1% KCl added to the Ringer solution at the beginning (pellet-fed n = 6, average weight = 123.2 ± 39.9 g; squid-fed n = 9, average weight = 97.5 ± 42.0 g). During this period, the amount of food voluntarily accepted for each meal by fish in each tank was carefully recorded, as described in Chapter 2, section (ii) a.

Once the SV_r had been recorded, the stomach was removed from the reservoir and two cores were drilled from the stomach wall using a 10 mm diameter cork-borer, one from the anterior region and another from the posterior, and weighed. Differences in food intake for the two diets, expressed as wet weight (% BW) dry weight (% BW) energy intake (KJ. g fish^{-1}) and volume (ml.100 g fish^{-1}) were tested using two tailed t-tests, assuming unequal variances and having 95 % confidence limits.

ANOVA was used to test for significant differences in stomach volumes. T-tests were used to examine differences in stomach weight and stomach section weights.

The surface area of the stomach was calculated as:

(weight of stomach/weight of section) x area of section .....(equation 3.1)

In **experiment 3.2.2,** the pyloric caeca and the intestine were taken from the fish examined in experiment 3.2.1 and weighed. ANOVA was used to see if dietary differences had an effect.

Experiment 3.2.3 examined the difference in SV_r between fish that had been demand fed (n = 6, average weight 68.50g $\pm$ 31.2 SD) and those fed once daily on squid and pellets (from experiment 3.2.1). Periodogram analysis (Enright 1965, further developed by Williams and Naylor 1967) was carried out on the food-intake data collected from the demand-fed fish, in order to determine what periodicity was present. In this method, the number of actuations in succesive hours (X_1, X_2, X_3 ....) is scanned for possible rhythms. To test for feeding every *f* h, the data are arrayed as:

1	X_1	X_2	....X_f	
2	X_{f+1}	X_{f+2}	...X_{2f}	
...*x*	$\Sigma X_{x-1(f+1)}$	$\Sigma X_{x-1(f+2)}$	ΣX_{xf}	.....(equation 3.2)

From this array (form estimate) the mean of column sums (X):

$$\frac{\Sigma X_{x\text{-}1(f+1)}+\Sigma X_{x\text{-}1(f+2)}+\Sigma X_{xf}}{f} \qquad \text{.....(equation 3.3)}$$

was calculated. Calculations are made of the variance of ΣX_i around X for a stated length of time or period. A function of this variance (usually standard deviation) is then plotted against period length to produce a graphical periodogram. High values of the periodogram statistics occur when the period under test approximates to the periodicity inherent in the raw data. The original data set is then randomised and new periodogram statistics calculated for each period tested. The periodogram of the randomised data approximates a straight line as all inherent periodicity is lost. By calculation of the least squares regression line for all the randomised periodogram statistics in the range, a straight line with upper and lower 95 % confidence limits is produced. Significant periodicity is assumed at a given period when the true periodogram statistic is greater than the 95 % confidence limit to the randomised data regression line at that point. The program PERIO (version 1.0) was provided by the University of Odense, Denmark, and was used to analyse the data collected in 60-minute intervals.

The data from the demand-fed fish was principally collected for experiments described in Chapter five, and the equipment used is described there.

Experiment 3.2.4 examined diet hydration rates and looked at stomach relaxation rates *in vivo*, after fish had taken a meal.

3.2.4 a Pellet hydration rates were examined *in vivo*. Fish were taken from Red Wharf Bay (n = 32, 61.2 g $\pm$ 16.2 SD), kept in groups in 250 l tanks and acclimated at 16 °C for three weeks. Following a period of 48 h during which they were unfed, fish were offered a 1 % meal, at time t = 0, of Trouw pellets (Table 2.1). At t = 1 h, 2 h, 4 h, 8 h and 12 h a random sample of fish were slaughtered (n = 4), and the stomach dissected out. The stomach content was removed, weighed and then dried at 60 °C until at constant weight. The wet weight/dry weight ratio of the bolus was calculated.

3.2.4 b The rate of hydration of squid was observed in Chapter 2 (t = 0.5 h, 10 h). The methodology is described in that Chapter.

3.2.4 c Two groups of fish (120.7 g ± 28.0, n = 4; 118.7 g ± 20.0, n = 4) were acclimatised to 250 l tanks at 8 °C for three weeks, being fed on Trouw aquaculture pellets once daily. Before the trial, the fish were starved for 48 h to ensure the gut was empty. During the following procedures, fish were anaesthetised using 2-phenoxyethanol (2.5 ml.20 l seawater^{-1}) at each emersion. The first group were removed from their tank and force-fed diet 2 (Table 2.4 and section 2.2.2) The meal was administered as a paste so that, in the early stages of digestion at least, the food will have filled the available stomach volume. This was administered, at time t = 0, from a syringe with a short length of 'NALGENE' tubing (Rochester, NY) on the end, which was placed into the oesophagus of the subject. The fish were immediately X -rayed as described in section 2.2.2.b This process was repeated at t = 1 h, 2 h, 3 h, 5 h, 8 h, 12 h, and 16 h. After this time some of the fish had empty stomachs, so sampling ceased in order to avoid any averaging bias in estimating gastric emptying rates, especially false indication or exaggeration of curvilinearity (Olson and Mullen 1986, Bromley 1988). The second group were treated in the same way, except that 5 h prior to the prandium they were given a 2 % BW (dry weight) meal in the same manner.

Once the above procedure was complete, the X-ray plates were developed and the 2-dimensional area of food in the stomach was measured. These values were converted to an estimated stomach volume by assuming that the stomach was a perfect ellipsiod. The average radius, r, of the 2-dimensional image:

$$(r_W + r_L)/2 \quad \text{.....(equation 3.4)}$$

where r_W is the radius of the width and r_L is the radius of the length of the idealised ellipsoid, was calculated as:

$$_2\sqrt{(\text{area of } BaSO_4 / \pi)} \quad \text{.....(equation 3.5)}$$

The value of r was then used to calculate the volume of an ellipsoid, with an average radius:

$$(r_W + r_L + r_H)/3 \quad \text{.....(equation 3.6)}$$

where r_H is the radius of the height of the ellipsoid. This could not be measured on a 2-dimensional image, and so it was assumed that $r_H = r_W$.

Thus the volume of the ellipsoid was calculated as a sphere:

$$^4/_3 \pi r^3 \text{ .....(equation 3.7)}$$

where:

$$r = (2r_W + r_L)/3. \text{ .....(equation 3.8)}$$

There are dangers associated with assuming that r_H and r_W are equal, as when the stomach relaxes there may not be enough intra-gastric pressure for this to be the case. However examination of dissected, full stomachs revealed that the two values are similar, even when meal size is small.

(iii) RESULTS

Experiment 3.1.1 The effect of repeated distension on recorded stomach volume.

After the first distension at fixed hydrostatic pressure, each subsequent distension caused an increase in the recorded SV_r (Figure 3.3, appendix 3.1); the slope of the SV_r plotted against the sequential distension number is significantly different from zero (linear regression; slope = 1.35, $F = 32.96$, $P < 0.001$).

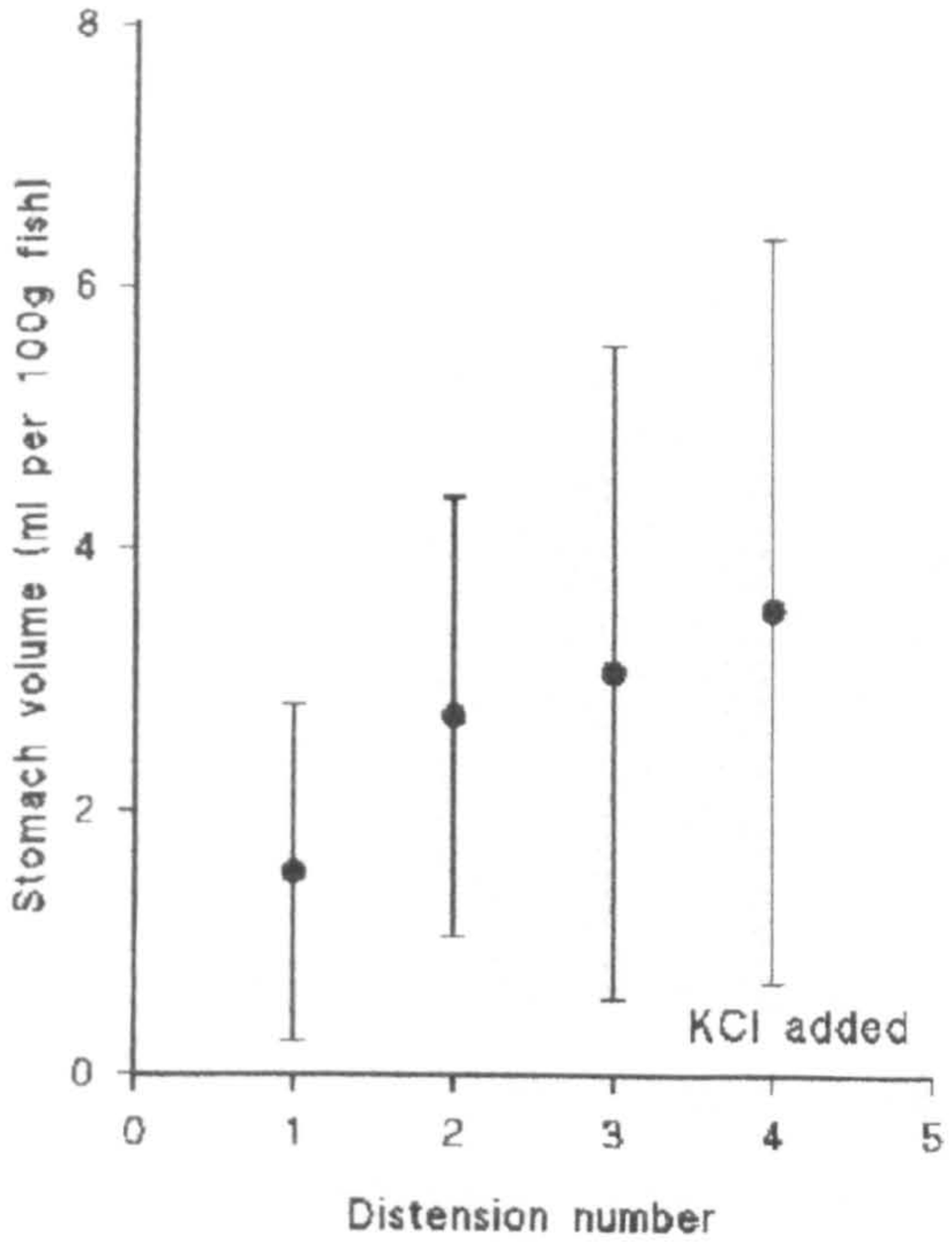


Figure 3.3 The effect of repeated stomach distension on relative stomach volumes (Mean values ± SD, pressure = 2 kPa). The slope of the SV_r plotted against the sequential distension number is significantly different from zero ($F = 32.96$, $P < 0.001$).

In each case, at the time when the pressure was taken off of the stomach, SV_r was still gently increasing. When further distensions were carried out, the SV_r was usually found to stabilise after about the fourth distension (Figure 3.4).

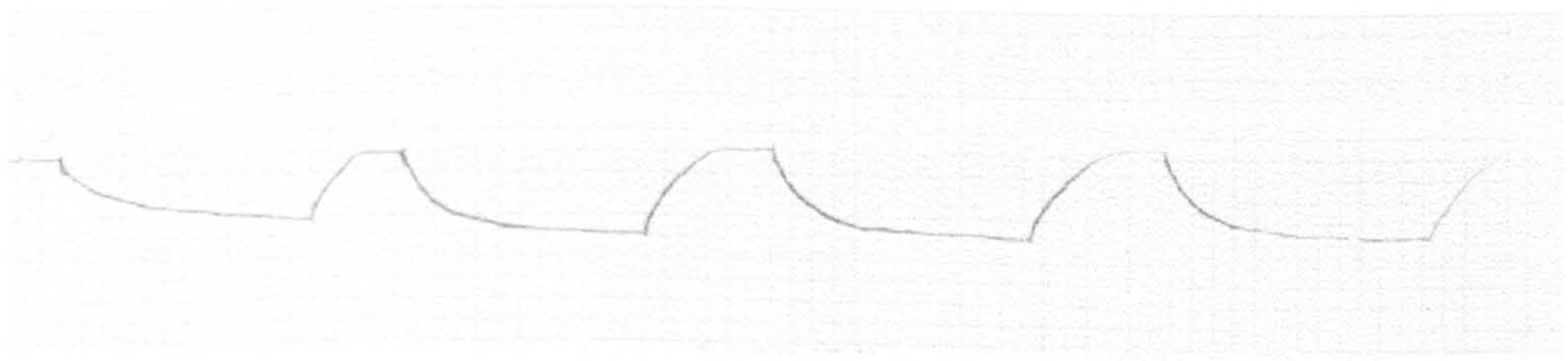


Figure 3.4 Chart record of four repeated distensions on *in vitro* stomach volume (distension pressure = 2 kPa), showing the gradual increase in volume as the stomach wall relaxes. The fourth distension is very similar to the third. The chart flows from left to right, with recorded stomach volume increasing towards the bottom of the chart.

Figure 3.5 Chart record of a live stomach *in vitro*. Contractions of the stomach can be seen to reduce the volume of the stomach. Once again, the chart flows from left to right, with recorded stomach volume increasing towards the bottom of the chart.

The addition of a 1% KCl solution resulted in a further increase in SV_r, caused by the complete loss of stomach tone. This appeared to stabilise and reach a maximum volume after about 0.5 hours. The potential problem that could occur if KCl was not applied is demonstrated in Figure 3.5, where the contractions of the live stomach can be seen to reduce the SV_r quite significantly over the course of a distension.

Experiment 3.1.2 The effect of intra-gastric pressure, freezing of samples and starvation on relative stomach volume

An increase in intra-gastric pressure resulted in an increase in stomach distension in freshly prepared stomachs (Figure 3.6, Appendix 3.2 i). The slope of the linear regression was significantly different from zero (slope = 1.11, $F = 17$, $P < 0.001$). Frozen stomachs distend more at lower pressures than fresh stomachs, although the mean SV_r for frozen and fresh stomachs is similar at 5Kpa (Figure 3.6, appendix 3.2 ii). The overall slope of the change in SV_r of frozen stomachs with increasing pressure was actually lower than that of fresh stomachs (linear regression slope 0.882, $F = 4.41$, $P = 0.044$). This can be explained by differences in the constants, which were higher for the frozen samples and not significantly different from zero for the fresh stomachs (fresh stomachs constant = -0.602, $P = 0.481$; frozen stomachs constant = 2.47, $P = 0.005$). In other words, the frozen samples distend much more readily at lower pressures, and this is reflected in the fact that the curve is not linear for this group. It should be noted that frozen stomachs burst very easily and at 5 kPa pressure, all but one stomach had burst.

The percentage of stomachs that burst at each head of pressure is illustrated in Figure 3.7.

Fish that had been starved for two weeks had very similar average SV_r's (at all pressures) to the fish that had been fed daily on pellets for six weeks (Figure 3.6, appendix 3.2 iii). A linear regression showed that the slope was significantly different from zero (slope = 1.12, $F = 114.15$, $P < 0.001$) and almost identical to the stomach volume for the fish that had been fed daily on pellets.

Experiment 3.2.1 a The effect of diet on food intake

This experiment was not designed to determine which element(s) of diets determine daily food intake (this problem is partly addressed in Chapter 4), rather the aim was to see if stomach volume increases when offered a diet that the fish eat a greater volume of, for whatever reason. The difference in average food intake, expressed as relative dry weight, relative wet weight, energy intake and volume of intake are given in Table 3.1, the raw data is presented in Appendix 3.3.

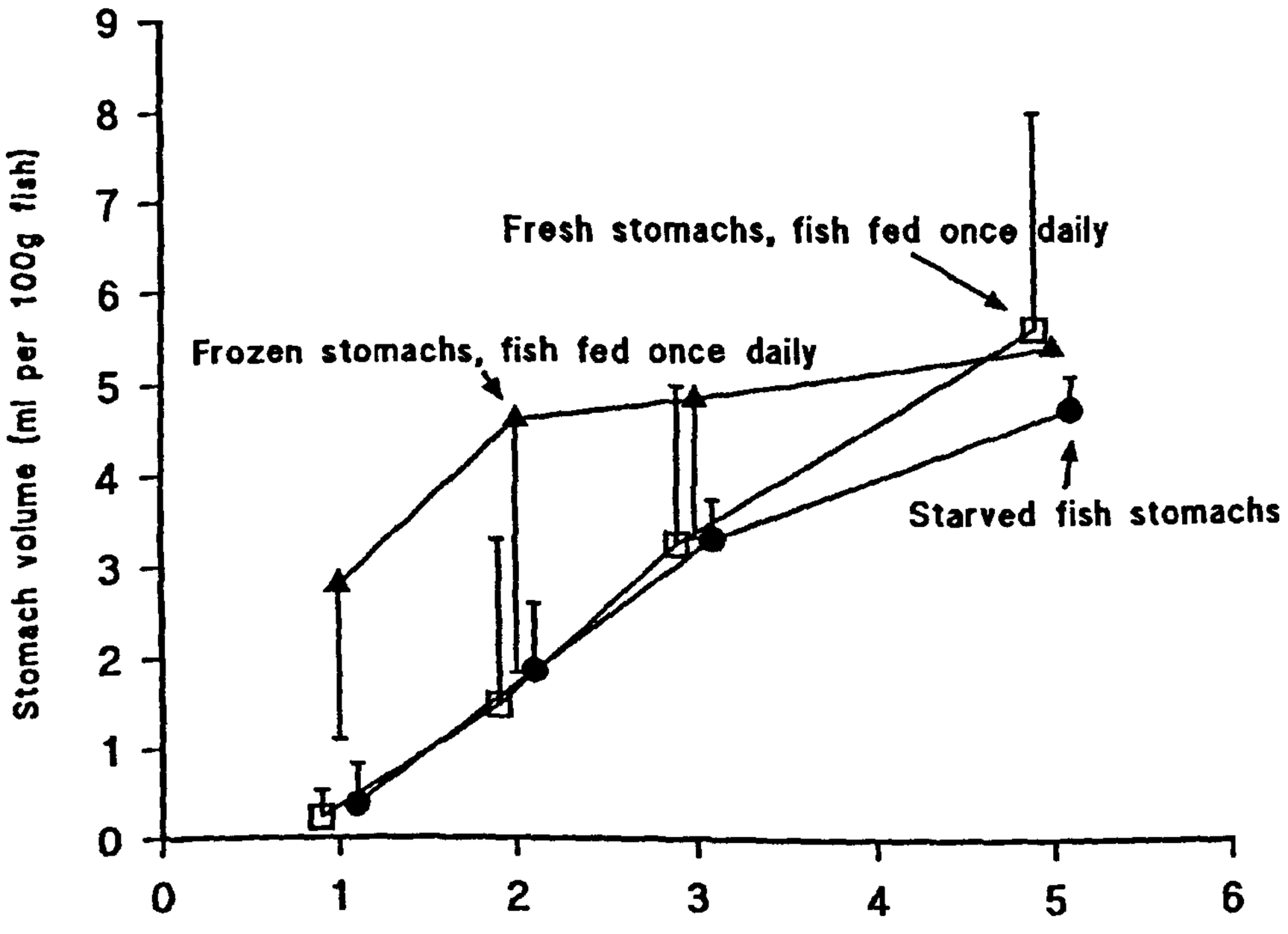


Figure 3.6 Change in relative stomach volume with increase in distension pressure (Error bars represent one standard deviation)

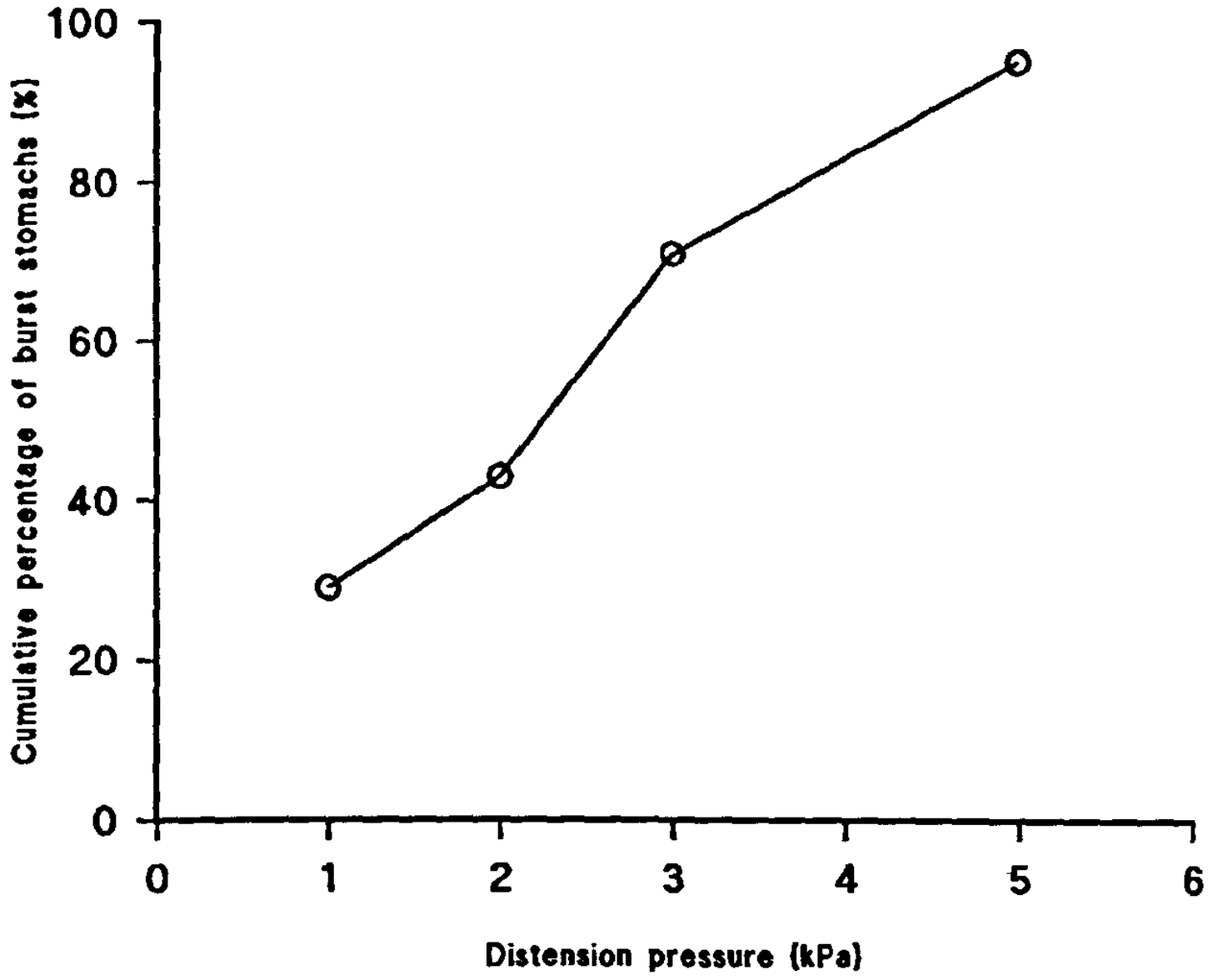


Figure 3.7 Cumulative percentage of burst stomachs with increase in distension pressure

	Squid	Pellet	t-test
Wet weight (% BW)	4.97 (1.8)	1.01 (0.2)	T=6.1, P=<0.001
Dry weight (% BW)	1.19 (0.4)	0.93 (0.2)	T=1.34, P=0.22
Energy intake (KJ.g fish^{-1})	19.86 (7.2)	18.86 (4.0)	T=0.29, P=0.78
Volume (ml.100gfish^{-1})	4.18 (1.5)	0.94 (0.2)	T=5.91, P<0.001

Table 3.1 Average food intakes of squid-fed and pellet-fed fish (standard deviations in brackets). T-tests show that the wet weight and volume taken are significantly different for the two diets, but the difference in dry weight and energy intake are not significant.

There was a significant difference between food intake for the two diets when it was expressed as wet weight and as volume. The difference was not significant when it was expressed as dry weight or energy intake (Table 3.1). The ratio of the average volume taken per meal was 4.5 : 1 (squid : pellets).

Experiment 3.2.1 b The effect of diet on stomach volume

It was initially intended to carry out an ANCOVA, comparing the regression lines for SV_r against the distension pressure for the two diets. This was not done because there was significant heterogeneity between samples measured at different pressures (Bartlett's statistic for pellet-fed fish stomachs = 12.677, P = 0.005, Bartlett's statistic for squid-fed fish stomachs = 21.028, P < 0.001). Because of this a one way ANOVA was carried out on the relative stomach volumes (ml.100g $fish^{-1}$) recorded at 3 kPa distension pressure. A significant difference was found between stomach volumes of fish fed on squid mantle and fish fed on pellets (ANOVA F = 11.11, P = 0.005). The mean SV_r for the two groups are given in table 3.2. The ratio of $SV_{r\,squid}$: $SV_{r\,pellets}$ was 2.57 at 3 kPa and 2.35 at 5 kPa, just over half of the ratio (volume of squid : volume of pellets), which was 4.5:1 (Appendix 3.4).

A plot of the change of the stomach volume (ml/100g of fish) with time shows that, whilst the fish were kept on the same diet for the first two weeks the stomach volumes remained similar. When the two groups were subsequently offered different diets, a difference in stomach volume, at a distension pressure of 1 kPa, occurred within two weeks (Figure 3.8, Appendix 3.5). For both groups relative stomach volume decreased, this is probably due to the fact that the energy per unit volume of squid is greater than that of their natural diet (squid = 4.75 $kJ.ml^{-1}$ natural prey = approximately 1 $kJ.ml^{-1}$).

Experiment 3.2.1 c Stomach weight, stomach section weight and area of stomach wall

There was little change in relative stomach weight for either group throughout the experiment (Figure 3.8); the relative weights of the stomachs of fish fed on the two diets were not significantly different (single factor ANOVA, F = 0.46, P = 0.510, Table 3.2, Appendix 3.4.). There was a significant difference in weight of the 10 mm diameter circular sections of stomach wall between the two groups (Kruskal-Wallis H = 5.43, P = 0.02, Table 3.2, Appendix 3.4). *i.e.* after distension at 5 kPa, the stomach wall was thicker for the pellet fed fish. These results indicate that the observed difference in stomach volume can almost entirely be explained by

the stomach not expanding as much at a given pressure for the pellet-fed fish when compared to the squid-fed fish. In other words, the observed difference in stomach volume is due to the stomach developing an ability to stretch more when fed a diet which requires a greater volume to be taken.

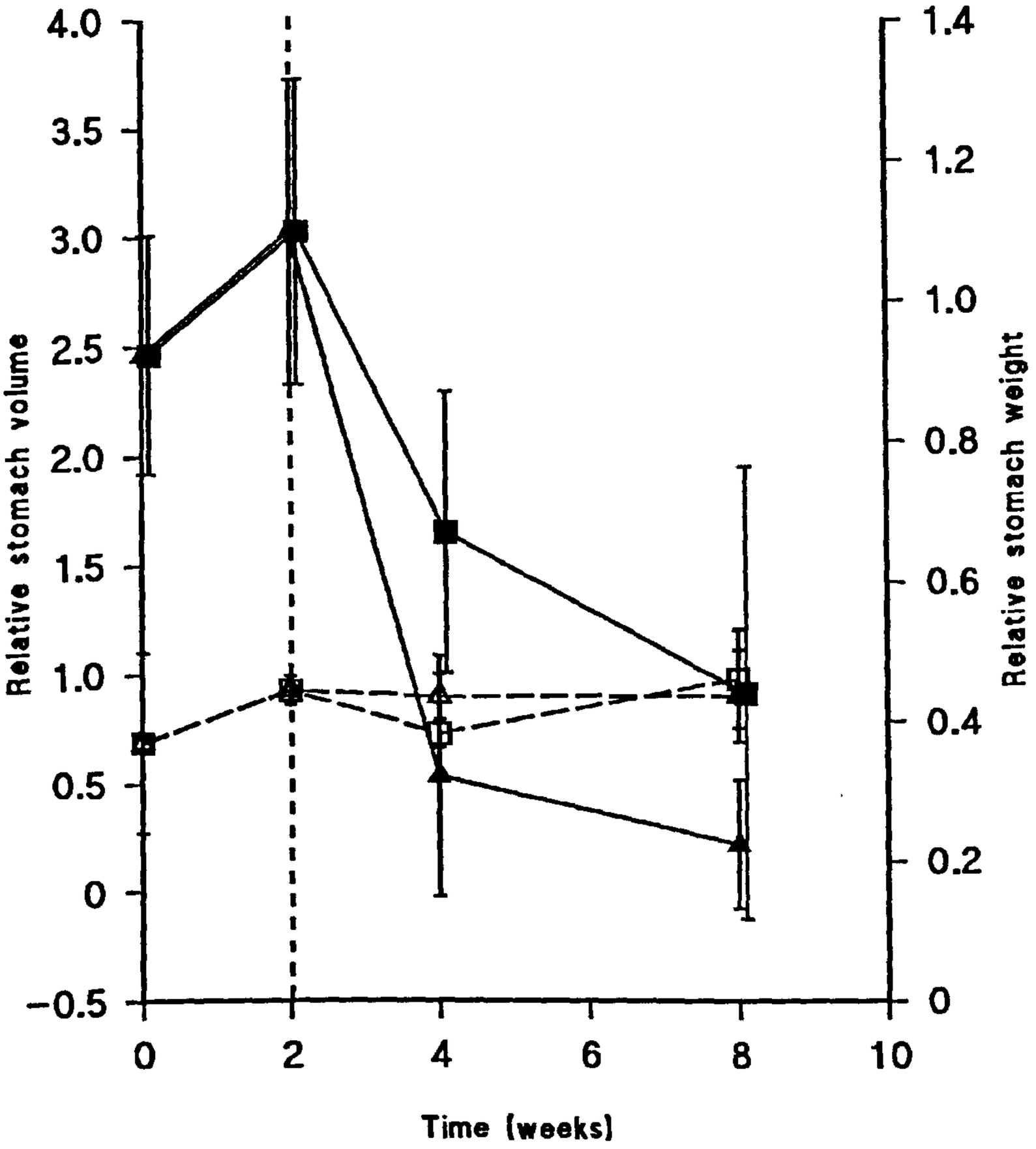


Figure 3.8 Change in relative stomach volume and weight with time.
■ = Average stomach volumes of squid–fed fish
▲ = Average stomach volume of pellet–fed fish
□ = average stomach weight of squid–fed fish
△ = average stomach weight of pellet–fed fish
All fish were fed on squid for the first two weeks

	Pellets	Squid
Relative stomach volume at 3kPa ($ml.100g^{-1}$)	3.24 (1.7)	8.33 (3.4)
Relative stomach volume at 5kPa ($ml.100g^{-1}$)	4.66 (3.1)	10.97 (7.2)
Stomach weight ($g.100g\ fish^{-1}$)	0.90 (0.2)	0.98 (0.2)
Stomach section weight ($g.100g\ fish^{-1}$)	0.033 (0.01)	0.024 (0.01)
Weight of fish (g)	87.5 (42.0)	123.2 (39.9)
Area of stomach wall (sq cm $.100g\ fish^{-1}$)	13.54	21.29

Table 3.2 Average relative stomach volume and weight, fish weight and relative surface area for pellet-fed and squid-fed fish after distension.
(Standard deviations are given in brackets)

Experiment 3.2.2 The effect of diet on the weight of the intestine and the pyloric caeca

Diet has no effect on either the relative weight of the intestine (ANOVA $F = 0.46$, $P = 0.51$) or on the relative weight of the pyloric caecae (ANOVA $F = 0.01$, $P = 0.905$; Table 3.3, Appendix 3.6).

The pyloric caeca from the fish that had been starved for two weeks (experiment 3.1.2) were smaller than those of the pellet-fed fish, although ANOVA found that the difference was not quite statistically significant (ANOVA $F = 4.24$, $P = 0.07$, Table 3.4).

The relative weight of the intestine of starved fish was not significantly different from the fed fish (ANOVA $F<0.01$, $P=0.97$).

	Squid-fed	Pellet-fed	Starved
Weight of intestine ($g.100g\ fish^{-1}$)	0.95 (0.2)	0.95 (0.3)	1.03 (0.4)
Weight of pyloric caeca ($g.100g\ fish^{-1}$)	0.32 (0.1)	0.31 (0.1)	0.19 (0.07)

Table 3.3 Average relative weights of whole intestine and pyloric caecae for squid-fed and pellet-fed fish.
(Pellet-fed n=6, squid-fed n=9. Standard deviations are given in brackets)

Experiment 3.2.3 Comparison of the stomach volume of demand-fed fish and fish fed a 1% BW meal.

The demand-fed fish had similar SV_r to the fish fed a discrete meal of pellets once a day (ANOVA @ 3 kPa; $F < 0.01$, $P = 0.948$, Table 3.4, Appendix 3.7). This is not surprising given that fish that had been starved for two weeks also had similar sized stomachs to the pellet-fed fish (experiment 3.2.2). They also had stomachs of similar weight to the group fed discrete meals (Kruskal Wallis $H = 2.08$, $P = 0.15$, Table 3.4, Appendix 3.7).

Periodogram analysis of the demand-fed fish records shows that the fish had fed with a significant 24 h rhythm, *i.e. o*nce a day, with an harmonic at 48 h (Figure 3.9) . Examination of the average number of actuations per day revealed that the dab (kept under constant illumination) fed more between 19:00 and 07:00 hours, although they fed during most hours of the day (Figure 3.10). Simple examination of the raw data (Appendix 3.8) reveals a peak every 21 hours for the first several days, before general night feeding became established. The daily ration of demand-fed fish was 0.83 % BW as against the pellet-fed fish which took 1.01 % BW.

	Discrete meal-fed fish	Demand-fed fish
Stomach volume @ 3KPa (ml.100g fish weight^{-1})	3.25 (1.7)	2.33 (1.7)
Stomach weight (g.100g fish weight^{-1})	0.90 (0.2)	0.77 (0.2)
Mean fish weight (g)	87.5 (42)	68.50 (31.2)
Daily ration (% body weight)	1.01 (0.2)	0.83 (0.3)

Table 3.4 Average relative stomach volume, relative stomach weight, fish weight and ration of fish fed discrete meals compared with fish fed using demand feeders. (Standard deviations are given in brackets).

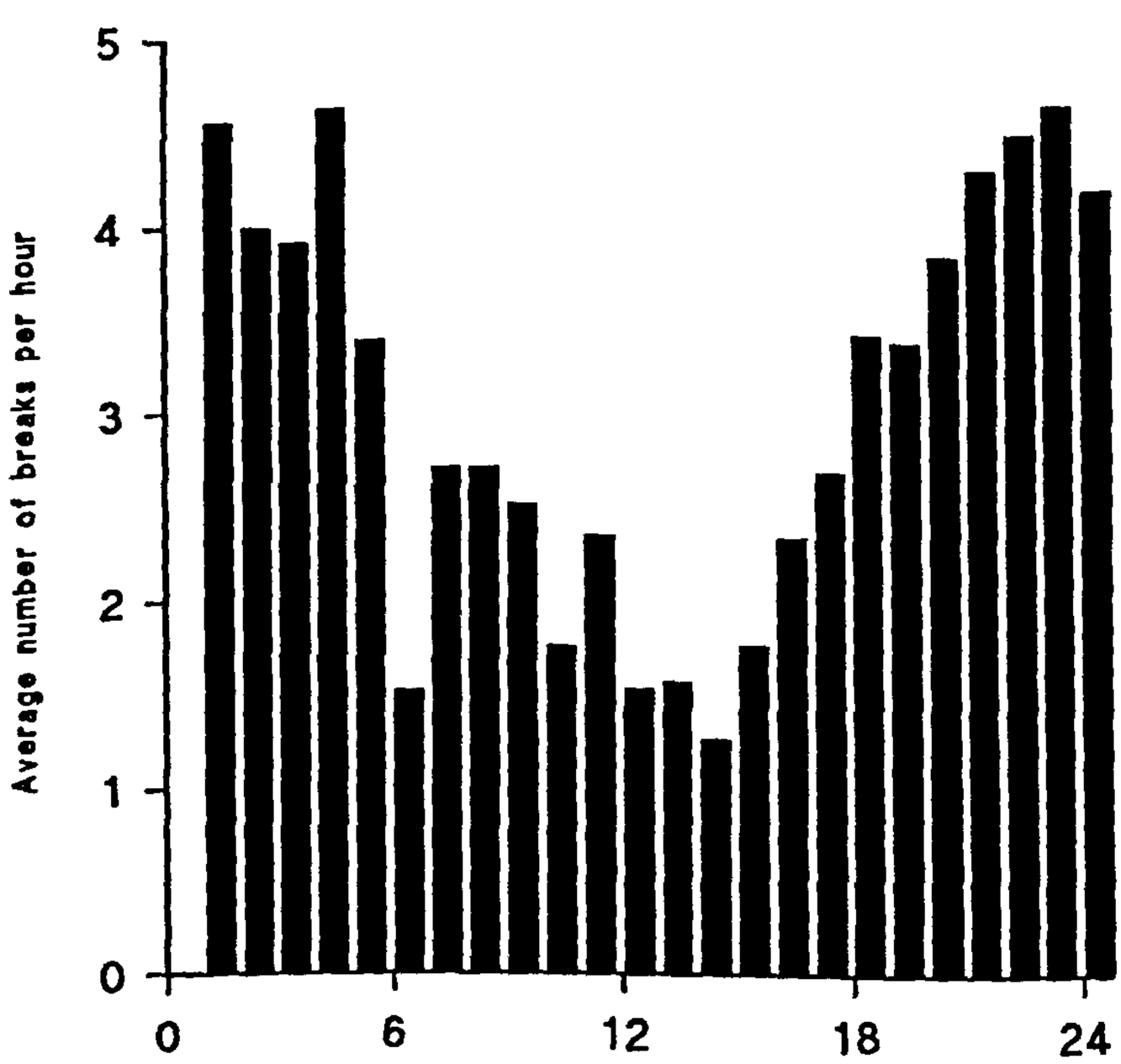


Figure 3.9 Average number of times per hour infra-red beam was broken by fish

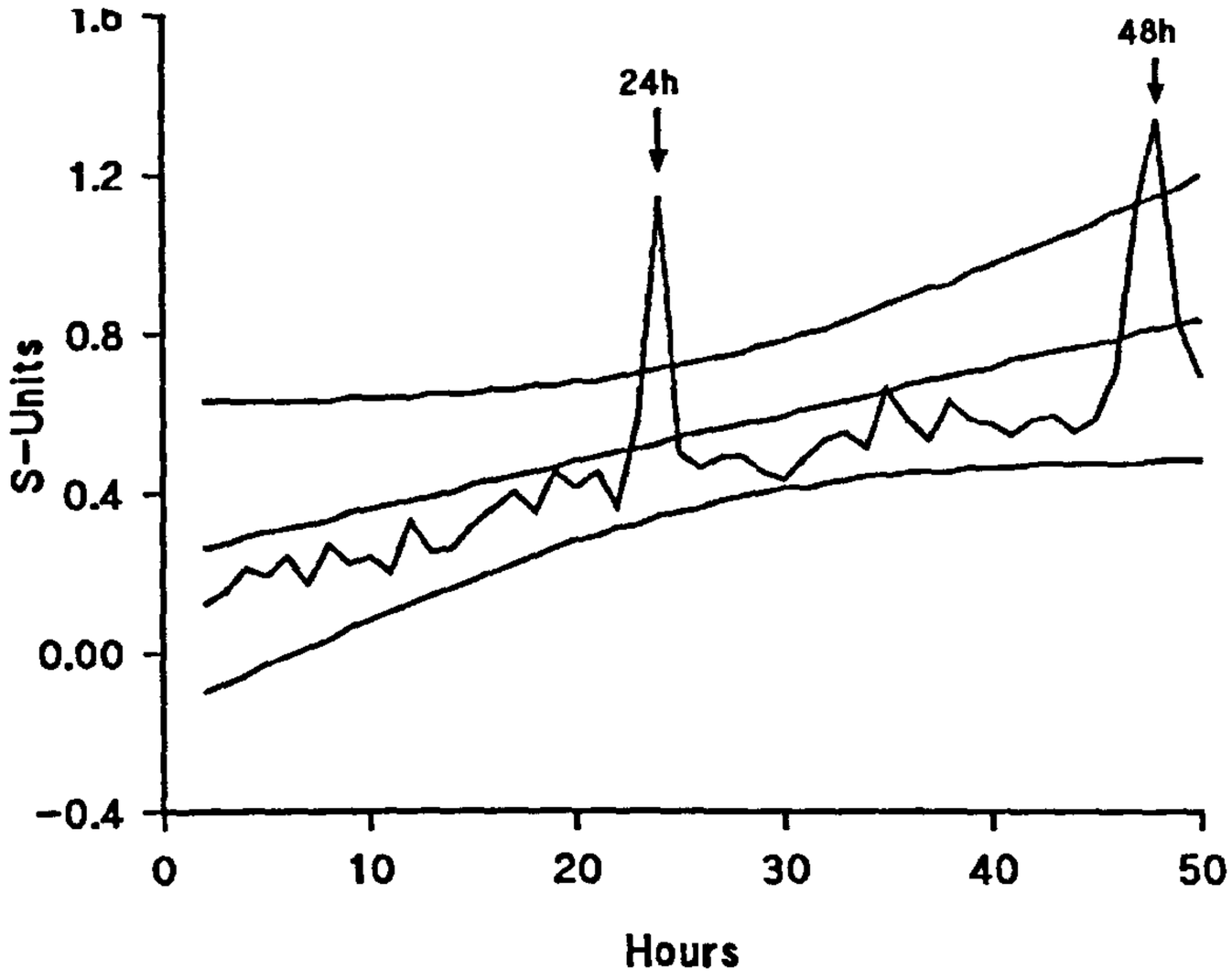


Figure 3.10 Periodogram of feeding rhythm of dab fed using demand feeders.

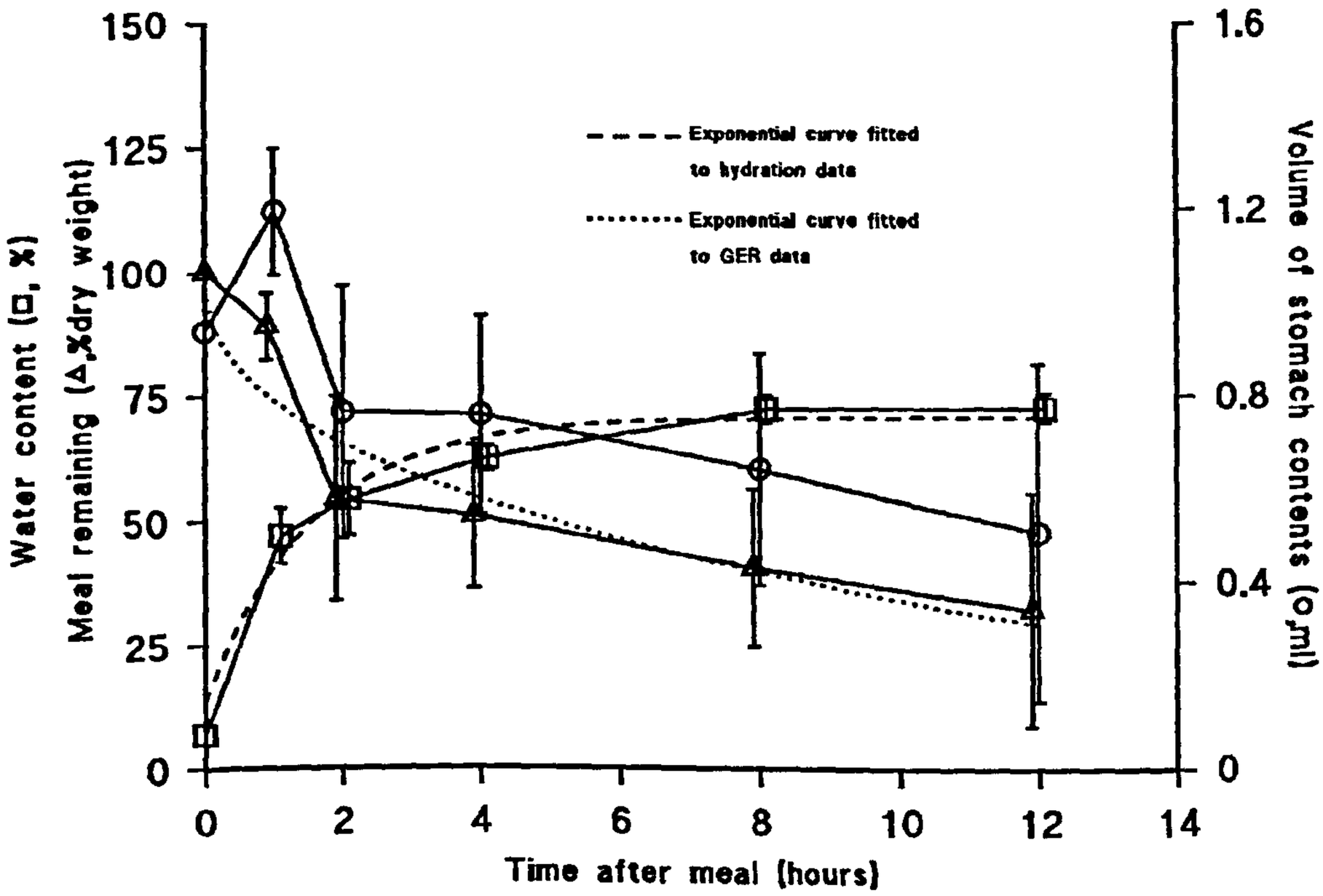


Figure 3.11 Pellet hydration rate (□), gastric evacuation rate (Δ) and volume of stomach contents (o). Exponential curves are fitted to hydration rate (dashed line) and GER (dotted line). Maximum stomach-content volume is reached after one hour, after two hours it is less than at the time of the meal.

Experiment 3.2.4(a) ***In vivo*** **hydration rates of pellets in fish given a 1% body weight meal**

Figure 3.11 shows the hydration rate, gastric emptying and effective volume of pellets in fish fed a 1 % diet. Hydration of the diet occurs rapidly with most taking place in the first hour; after 12 h the water content was 72.5 %, slightly less than the moisture content of squid mantle at t = 0 h. The data was fitted with an exponential model ($r^2 = 0.836$, $P < 0.01$).

Food empties from the stomach rapidly at first, and is followed by a slower evacuation rate which appears linear; the was fitted with an exponential model of GER (P-FIT; $r^2 = 0.949$, $P < 0.05$) in agreement with Jobling *et al.* (1977) and Fletcher (1982), (*n.b.* linear regression of t = 2 onwards is also significant; F = 5.29, P = 0.032). After 12 h, 32 % (dry weight) of the meal remains in the stomach (Appendix 3.9).

The combined hydration rate and gastric evacuation rate lead to a net increase in stomach content volume at t = 1 h of 26 %, after which gastric evacuation cancels out the effect of hydration, and the volume of the stomach contents falls.

Experiment 3.2.4(b) Hydration rates of squid mantle

In vivo hydration rates (Appendix 3.10) show that the amount of hydration in squid, which initially had 76.17 % moisture is much lower than with the pellets; after 0.5h the moisture content of the diet only increased by 5%, and after 10h by 12.5%. After half an hour gastric emptying had not yet commenced, this fact combined with the hydration of the diet, will have meant that the volume peaked around this time, with the 5% increase in moisture corresponding to a 6% increase in volume (from table 3.1).

Experiment 3.2.4(c) Relaxation rates of stomachs

The 2-dimensional X-ray image of the stomachs of fish fed a single meal of diet 2 increased over a period of three hours, by an average of 60 % (Figure 3.12), the estimated stomach volume therefore reached a maximum of 100 % more than at time t = 0 at this time (Figure 3.13). In fish fed a pre-prandial meal the stomach also distended, with the volume increasing by a further 12 % (Figure 3.13).

In the group fed a single prandium, the estimated stomach volume (Figure 3.13) had increased by 44 % after t = 1. This compares with an increase of 26 % (t = 1h) in the volume of the diet due to hydration (see experiment 3.2.4 a, Figure 3.11). Therefore the stomachs relaxation rate is more than sufficient to accommodate the increased volume of chyme due to hydration.

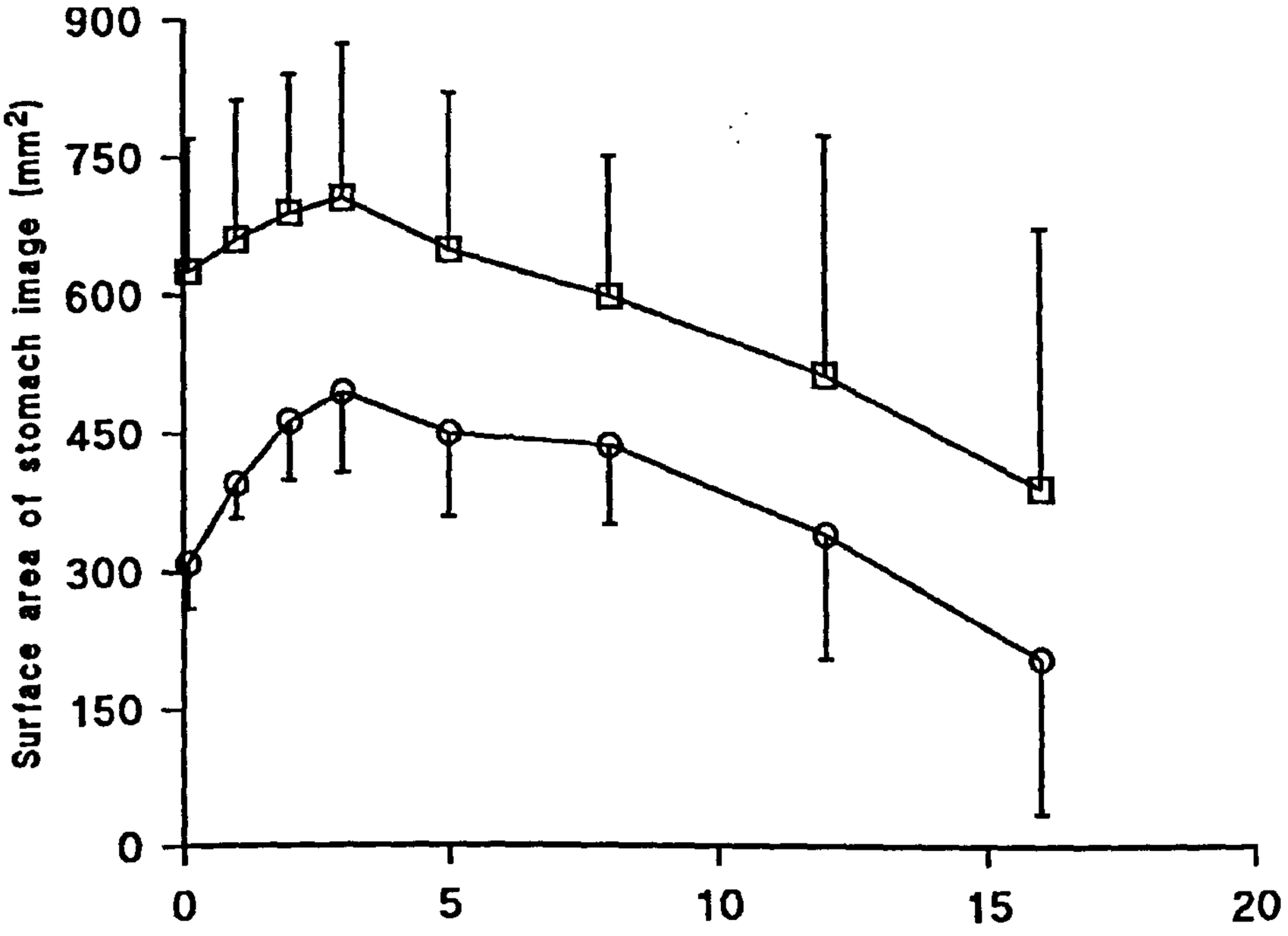


Figure 3.12 Increase in surface area of X-ray image of stomach with time. A maximum is reached after three hours

o = single 1 % BW prandium, □= additional 2 % pre-prandial meal. Error bars are standard deviations

This *in vivo* relaxation rate (where the presence of food may have led to an additional physiological response) can be compared with the increase in stomach volume with repeated *in vitro* distensions in section 3.1. The latter led to an increase of 117 % before stabilising after 0.5 h of the fourth distension (in 1 % KCl solution). The fish fed a single meal gave comparable results (although it took longer), however in the fish given a pre-prandial meal stomach volume was

considerably bigger than observed for a dead stomach distended at 5 kPa *in vitro*, suggesting that the stomach may have responded to some quality of the diet other than its volume.

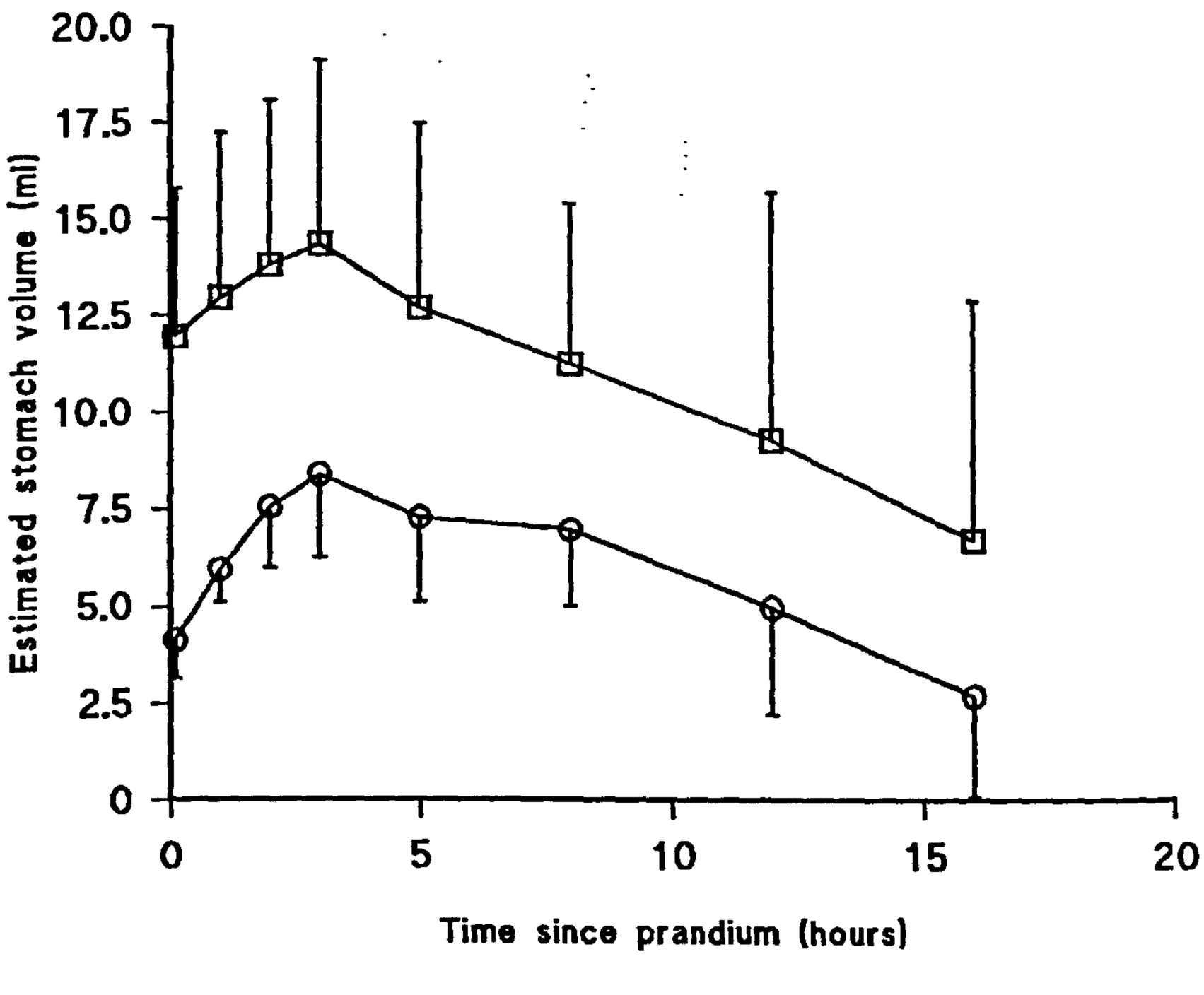


Figure 3.13 Estimated stomach volume based on data presented in Figure 3.12, showing *in vivo* relaxation rate
o = single 1% BW prandium, □= additional 2 % pre-prandial meal Error bars are standard deviations

(iv) DISCUSSION

It is apparent from the methodology trials (section 3.1) that careful consideration is needed when designing experiments to record stomach volumes. The number, pressure and duration of the distensions have an effect up to the maximum stomach volume, so that unless stomachs are distended for sufficiently long to reach this volume, it is necessary to distend every stomach for a fixed time and pressure to get comparable results between treatments. As found in *S. maximus* (Flowerdew and Grove 1979), stomachs distend more at a given pressure when they have

been frozen; they also develop a tendency to burst. Flowerdew and Grove (1979) found that starving *S.maximus* for 10 days reduced the recorded stomach distension when compared with the stomachs of fed fish. This differed from the above results, which showed that starved *L. limanda* have similar gastric distensions to fed fish, at all pressures tested.

As a result of the above trials the following recommendations can be made when measuring the stomach volume of the dab:

1) stomachs should be freshly dissected and not frozen.

2) treat with a 1% KCl solution to avoid error caused by reflex contractions.

3) when comparing stomachs from two different treatments, the head of pressure (or the series of pressures) should be the same for all individuals, as should the duration(s) of the distension(s).

4) when deciding on the pre-sampling food deprivation period, it is more important to wait long enough for the stomach tone to return to that of an empty stomach (to reduce variation due to relaxation of the stomach wall caused by previous meals) than it is to consider the physiological impact of starvation.

It is clear that the recorded volume of a stomach is partly a reflection of the measuring technique, as well as the dietary history of the individual.

In section 3.2.1 a, dab appeared to adapt their food intake to the diets offered; both calorific intake and dry matter intake were similar for the two groups. This is as observed by Poston (1974) and Bromley (1980a), however Ruohonen and Grove (1996) found that *O.* mykiss could not fully compensate for a herring diet; their energy intake was reduced compared to a pelleted diet. It is not possible therefore to determine to which of these qualities of the diet the dab were responding to when they adjusted their food intake. There is evidence that several species can adapt to both water content and energy level of the diet (see above).

In order to determine whether *O. mykiss* were able to adapt to differing dietary water content, Ruohonen *et al.* (1997) placed diets in gelatine capsules. Water was injected into some of the capsules, resulting in two diets which differed only in water content. They proved that *O. mykiss* could adapt to dietary water content and thus maintain a constant dry weight of feed intake. In the case of the dab, the

smallest size of gelatine capsule available was relatively too big and this meant that the decision to reject or accept a single pellet at the end of a meal had a significant impact on the result. This, combined with the expected variation in food intake, meant a large number of replicates would have been required which, when combined with problems getting fish to take the dry capsules before water soaked into them, made this experiment impossible in the dab. Perhaps spraying a thin gelatine coat might prove successful.

What is certain is that dab ate 4.5 times the volume of squid than pellets, which allowed the intended examination of the effect of diet volume on gastric volume. In the laboratory dab are able, within quite a short period, to change the volume of their stomachs when taking diets with different volumes. The fact that there was a significant difference in the weights of the stomach sections indicates that this is due to the stomach becoming more distensible rather than the stomach growing, unlike the results of Hilton *et al.* (1983), Bromley and Adkins (1984) and Ruohonen and Grove (1996). In the study of Ruohonen and Grove (1996), stomach volume increased when a bulkier diet was offered, however when a diet was offered that the fish took less of, stomach volume did not decrease, but stayed the same as at the beginning of the experiment. This was observed in experiment 3.2.1 c, in which fish were switched to diets of higher energy density. It would be of interest to see if the dab stomach increases in volume when the fish are switched to squid after a long period being fed on pellets (*i.e.* if they were switched to diets of lower energy density).

Results from the previous Chapter suggest that in some cases the stomach volume can be a limiting factor in food intake. The fact that the fish can adapt the distensibility of their stomachs to different diets means that such an effect may not be long term. Because fish in the appetite return study (Chapter 2) were fed at random time intervals, they would not have been able to adapt their stomach volumes to the larger meals required after a long period without food. For the model of Jobling (1986a) to occur in the wild for more than the short term, a similar mechanism preventing the stomach volume adapting to large meals would be required.

The demand-fed fish fed with a significant rhythm of 24 h (with an additional harmonic at 48 h), suggesting the existence of a circadian rhythm. However, Gwyther and Grove (1981) found that appetite return coincided closely with GET, when dab demand-fed under continuous illumination. Extrapolation of the fitted curve in section 3.2.4 *a* does in fact give a GET of approximately 24 h, so it is possible that this data does not indicate a circadian rhythm after all (exponential model GET = 27 h, linear approximation of slow phase GET =24 h). The pattern of feeding differed from that of fish fed discrete meals, because it was spread over several hours, indicating either that less food is present in the stomach at any one time or that individual fish were feeding at different times. The average stomach distension in the demand-fed group was similar to fish fed discrete meals, perhaps not surprising given that starved fish also had similar sized stomachs to fish fed pellets once daily. It is possible that below a certain meal volume the degree of stomach distension does not vary, as the stomach wall is not significantly stretched *i.e.* there might be a minimum stomach volume.

The ratio of the difference in relative stomach volume for the two groups (squid : pellets = 2.5 : 1) was not a full reflection of the size of the difference in diet volume (squid : pellets = 4.5 : 1). Examination of the effect of the hydration rates of the two diets and the GER of the pellet diet showed that volume alone would not explain the discrepancy. The volume of the pellet-meal chyme reached a peak after one hour. The ratio between the volume of the stomach contents at this time (squid : pellets) was 3.75 : 1. After 12 hours the pellets in the stomach had a similar water content to the squid mantle, however by this time, sufficient gastric evacuation had taken place to make it unimportant when considering the effect of hydration on increasing the volume of stomach contents.

Gastric emptying (section 3.2.4 a) followed an exponential pattern, as found in section 2.2.2, although it would also fit a model with a rapid emptying phase followed by a slower linear evacuation (as suggested by Grove, unpublished). This again contradicts Jobling (1986a) who suggested that high energy diets such as these should follow a strictly linear evacuation. Jobling (*loc. cit.*) pointed out that

emptying was likely to be a stepped curve and that any monotonic curve or linear models were only approximations for modelling purposes.

The post-prandial *in vivo* relaxation rate of the stomach, estimated in section 3.2.4 c, showed that the stomachs slowly relaxed, with both groups taking 3 h to reach a maximum distension. In the group fed a single meal the degree of distension was similar to that found in the *in vitro* study (section 3.1) Those fed the pre-prandial meal had a greater distension than found in the *in vitro* study suggesting either greater intra-gastric pressure, or a physiological effect of diet leading to stomach distension.

Wild fish are not limited by experimental design to discrete meals at fixed times, and so they are potentially able to adjust their food intake by changing their meal frequency, meal size or average stomach content / meal duration. With regard to changing meal frequency, it may be that the fish in the wild were in fact feeding only once a day; dab have been observed to feed once a day in the Winter, around noon (Gwyther and Grove 1981). Factors such as the effect of sea temperatures, day length and light intensity (dab are mainly visual feeders; De Groot 1969, Carter *et al.* 1991), are important in determining feeding rhythms in the wild. This may not be the case, since feeding times are variable in dab. Carter *et al.* (1991) observed in a Summer survey that feeding time varied with fish size and the type of substrate they were feeding on, and could be crepuscular, nocturnal or diurnal. A significant proportion of fish on sand had food in their stomachs for much of the day, unlike those on mud where most fish had empty stomachs during the night. Nocturnal feeding was observed to occur in nights with strong moonlight. Considering the weight of evidence in the literature (see introduction) it is probable that similar variability also occurs in the Winter. However if dab do feed once a day during the Winter (Gwyther and Grove 1981), the difference in food volume would have to be accommodated by the fish taking a larger meal, or eating over a longer period.

Is the stomach volume of the dab ever a limiting factor in food intake? There is evidence that other species have been unable to compensate their food intake when switched to diets of lower energy density. Ruohonen and Grove (1996) found that

O. mykiss were unable to maintain their dry weight intake when switched from pellets to herring pieces. Yamamoto *et al.* (1995) found that Japanese flounder were unable to increase feeding sufficiently for low nutrient density diets over a four-week period. Ogata and Shearer (2000) found that *Pagrus major* were unable to adapt fully to lower energy diets for three weeks, after which the stomach volume increased and the difference in wet-weight intake of high- and low-nutrient diets became significantly different. Given that the stomach of the dab changes its volume in response to diet quality, it is unlikely that any long-term reduction in food intake of bulky diets would be observed, though in the short-term feeding may be suppressed, as found by Ogata and Shearer (2000).

The dab did not adapt their stomach size to fully accommodate the difference in food volume. The difference in the ratio of food volumes and stomach volumes does not indicate that stomach volume was limiting in this case, as the fish maintained dry weight and energy intake during the trial.

The question remains whether the stomachs become more distensible because stomach fullness is a limiting factor for low-energy diets. There are other possibilities; *e.g.* the relaxation of the stomach may be part of a mechanism for short-term appetite control regulated by a function of the tension of the stomach wall (proportional to the square root of the volume). As the stomach changes its distensibility when lower-energy diets are offered, the tension of the stomach wall in response to the greater volume of food will presumably be reduced, so that tension is not related to the volume of the diet. Instead its energy content or nutrient value may be important and therefore it might be useful in regulation of food intake. (The square root relationship would mean that the discrepancy between food volume difference and stomach volume difference commented on above is much reduced). A third possibility is suggested by the observation that in the plaice, *P. platessa*, the size of stomach contractions is highly correlated to the volume of food in the stomach and this has been proposed to be a kind of constant frequency pump with a variable stroke volume (Hunt and Knox 1968, Jobling 1986a). It is possible therefore that the change in stomach volume relative to the diet volume may simply be an aspect of this mechanism. If stomach volume does

have a role in food intake control, it would be *via* changes in GER due to changes in the behaviour of this pump.

Interestingly if the tension of the stomach wall was important in food-intake regulation, it would have an effect on the model of Hunt and Knox (1968), which would in turn change the GER. Thus GER and food intake could be correlated for this reason, without a direct causal link.

CHAPTER FOUR: MODELLING THE REGULATION OF FOOD INTAKE IN *LIMANDA LIMANDA* (L.) USING PATH ANALYSIS

(i) INTRODUCTION

Several studies have examined the effect of changing dietary composition and energy on voluntary food intake, attempting to determine whether fish are able to adapt their intake to diets of differing quality. These studies have found that fish can compensate for diets of differing energy (*C. auratus*, Rozin and Mayer 1961; *O. mykiss*, Grove *et al.* 1978, Ruohonen *et al.* 1997; *S. maximus*, Al-Aradi 1986; *S. salar*, Paspati and Boujard 1996;). Sveier *et al.* (1999) found that *S. salar* could adapt to low protein diets. Jantrarotai *et al.* (1998), using a factorial design, found that a hybrid *Clarius* catfish adapted to changes in protein level but not to changes in the amount of energy.

In the case of the dab Gwyther and Grove (1981), using on-demand feeders, found that simple dietary dilution (19 - 9 $kJ.g^{-1}$) was not compensated for. However, Fletcher (1982), again using on-demand feeders, found that dab changed their food intake to respond to changes in dietary energy (19 - 13.5 $kJ.g^{-1}$) when the ratios of lipid and carbohydrates were also adjusted. In this case the fish responded to a decrease in lipid by increasing their meal frequency, which led to a significant increase in daily food intake. Reducing the dextrin levels resulted in a small increase in meal sizes, as opposed to meal frequencies, leading to a slight increase in daily feeding. Dab have been also been observed to maintain dry weight and calorific intake when offered diets of differing water content (Chapter 3).

In experiments such as that of Fletcher (1982), it is not possible to change one ingredient, without simultaneously changing either another ingredient and/or the total calorific value of the diet. Clearly, although an adaptation to the novel test diets was statistically apparent, it was not possible to determine whether the fish responded to the change in a particular ingredient, or to the resulting change in

other ingredient(s), the ratios of the ingredients and/or the calorific value of the diet.

One method that allows inferences to be made about causal relationships is path analysis (Wright 1921, briefly introduced in Sokal and Rohlf 1995). This method decomposes observed correlations into "path diagrams" (figures 4.1 to 4.3 are path diagrams for the models tested in this paper) which, together with their associated equations (see methods, below), describe a hypothetical causal scheme (Shipley 1999). It should be emphasised that path analysis is an inferential method and not a **test** of causality, however if the model equations derived from the path diagram do not balance it is possible to discount that particular scheme.

Three hypotheses are proposed to explain the results of Fletcher (1982):

Model 4.1; The differences in the amounts of lipid and dextrin *per se* account for the difference in food intake (figure 4.1)

Model 4.2; The fish 'use' the change in the calorific value of the diet, as well as the levels of the ingredients to determine their feeding levels (Figure 4.2)

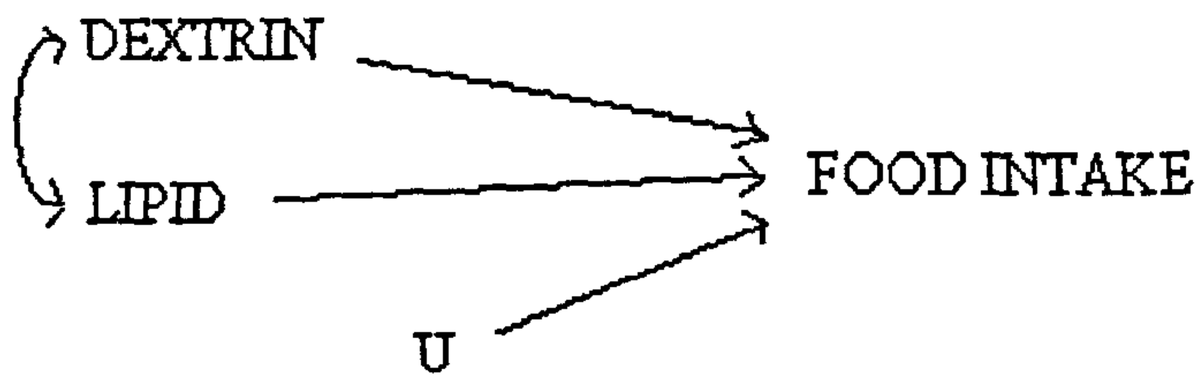


Figure 4.1 Path Diagram of hypothesis 4.1. The change in food intake in response to changing the relative proportion of nutrients in a diet is due to that change *per se*. In this case the ingredients are correlated. U = the total unexplained variables

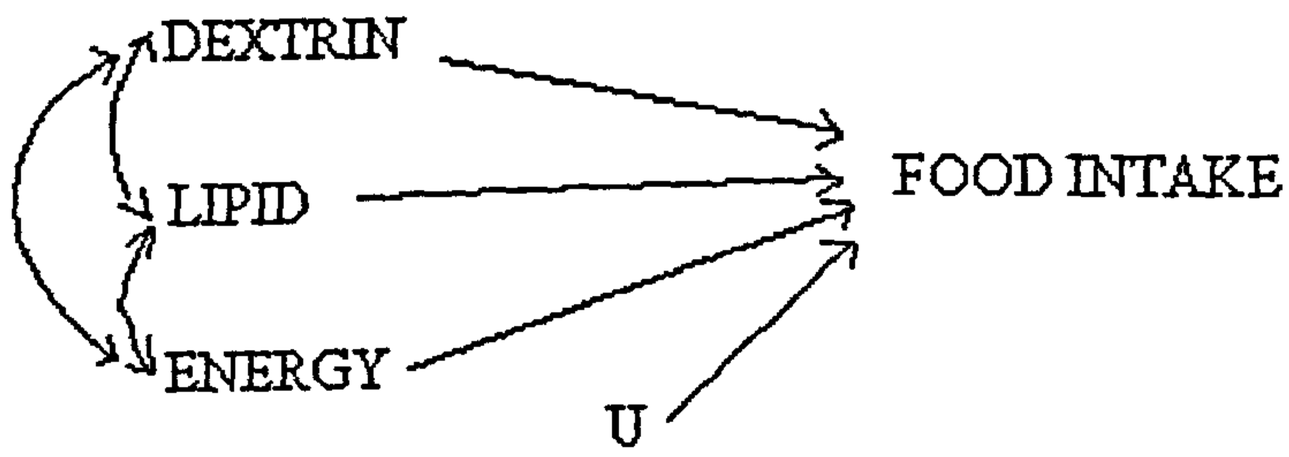


Figure 4.2 Food intake changes in response to both the amount of each ingredient as well as the energy of the diet (hypothesis 4.2). As in hypothesis 4.1 (Figure 4.1) lipid and dextrin are related. Energy is quite reasonably assumed to be correlated to the amount of each ingredient.

Model 4.3; Fish are not feeding for energy itself, rather the level of food intake and the calorific value of the diet are only correlated because they have common causal variables, the nutrients (Figure 4.3). That is, the animals feed for the nutrients and the energy of the food intake is coincidental.

Path analysis was employed to model the correlations and path coefficients, and is illustrated in the methods, below.

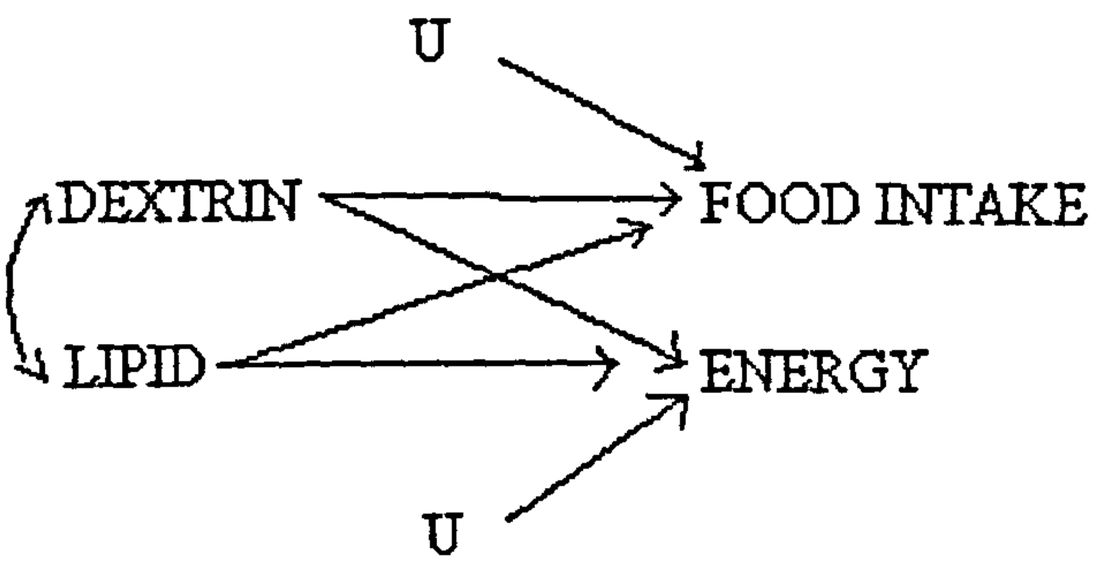


Figure 4.3 Hypothesis 4.3 considers the possibility that energy and food intake are correlated because they have common causes; the ingredients.

(ii) METHODS

Data was taken from Fletcher (1982), in which records of food intake were generated from dab that had been trained to self-feed using demand feeders. Each actuation of the demand feeder trigger was rewarded with a portion of diet and logged, allowing the feeding pattern and total amount of food taken daily to be calculated.

	Diet 1	Diet 2	Diet 3	Diet 4
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
Energy $KJ.g^{-1}$	19.26	15.86	16.22	13.56

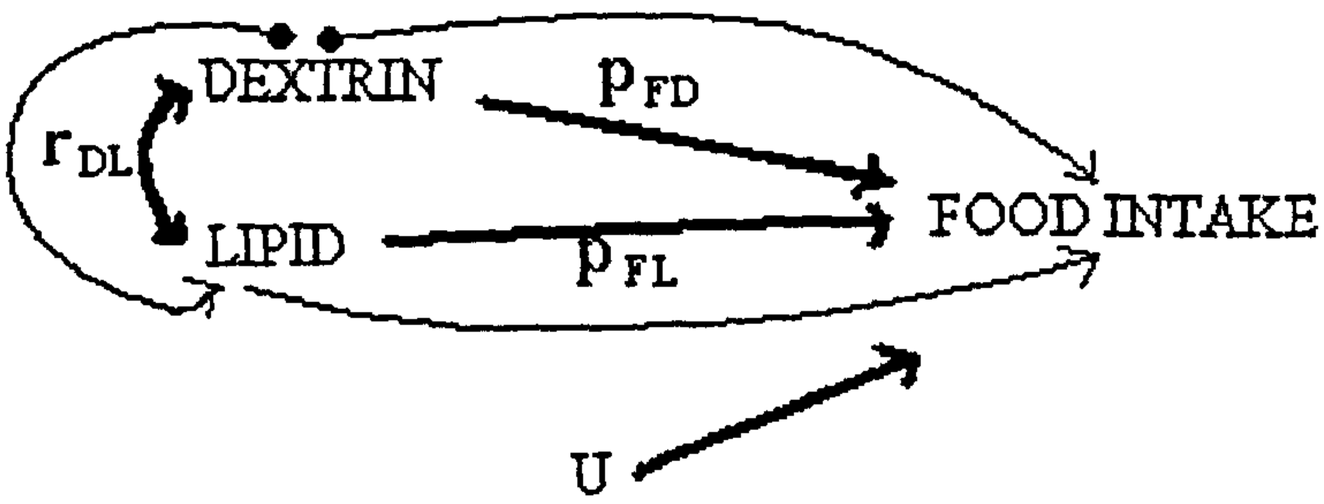
Table 4.1 Composition of diets (percentages) used in demand feeding experiments (Fletcher 1982).

Four diets were used; diet compositions are given in table 4.1. The ingredients that were manipulated were dextrin and lipid, with kaolin added as a filler in varying amounts as the sum of the weight of dextrin and lipid differed. Further details of the experiment are detailed in Fletcher (1982).

The path diagrams given in figures 4.1, 4.2 and 4.3 (where U represents the total of the unexplained variables) were translated into their associated equations, and these were solved to see if the two sides of the equation balanced.

A brief description of how this was done, given below, is largely drawn from Sokal and Rohlf (1981) and the interested reader should refer to this text for more detail.

Figure 4.4 shows detail of how model one is tested. Food intake, the criterion variable (F), is affected by two predictor variables, dextrin (D) and lipid (L). The single headed arrows indicate cause and effect relationships, or path coefficients, (P_{DF}, P_{LF}). The double-headed arrow indicates the correlation between the predictor variables (r_{DL}) (Figure 4.4). In this case the levels of lipid and dextrin were chosen by the experimenter, so this correlation has no real biological meaning, however it is essential to include r_{DL} when testing the model.



$$r_{DF} = p_{FD} + r_{DL} p_{FL}$$

Figure 4.4 The correlation between dextrin and food intake is the result of a direct route, the path coefficient p_{FD}, and an indirect route, the sum of the correlation between dextrin and lipid (r_{DL}) and the path coefficient (p_{FL}).

The correlation between the criterion variable F and the predictor variable D is the sum of the products of the chain of path coefficients and correlations by which they are connected. In general terms this rule is expressed as:

$$r_{jY} = p_{Yj} + \sum_{i \neq j} r_{ij} p_{Yi} \quad \text{.....(equation 4.1)}$$

where r_{jY} = the correlation coefficient between the predictor variable j and the criterion variable Y, p_{Yj} = the direct path coefficient between j and Y, r_{ij} is the correlation between i and j, p_{Yi} is the path coefficient between i and Y.

In our case, the equation for the correlation between dextrin and food intake is:

$$r_{DF} = p_{FD} + r_{DL}\, p_{FL} \quad \text{.....(equation 4.2)}$$

In other words dextrin has an effect on food intake; (a) directly (p_{FL}) and (b) indirectly *via* the sum of the correlation between lipid and dextrin (r_{LD})and the path coefficient between lipid and food intake (p_{FL}) (Figure 4.4). If the model gives a good explanation of the causal relationship between lipid, dextrin and food intake, the value of r_{DF} derived from equation 4.2 above, should equal the correlation coefficient calculated in the standard way. This should be exact, barring rounding errors.

The formula for the determination of food intake is taken from the general formula:

$$1 = \sum_i p^2_{Yi} + 2 \sum_{ij} p_{Yi}\, p_{Yj}\, r_{ij} + r^2_{UY} \quad \text{.....(equation 4.3)}$$

(The total variance of Y must be a function of all of the variances of the predictor variables and their causal pathways, if the model is correct this must be equal to 1).

That is, the total variance of Y is the sum of the squares of the path coefficients along all paths leading to Y, added to the sum of the product of the path coefficients along all paths leading to Y multiplied by their correlations for all possible pairs of predictor variables, added to the total unknown variables (Sokal and Rohlf 1981).

The unknown variable, r^2_{UY}, is the total of the unexplained variables influencing food intake:

$$r^2_{UY} = 1\text{-}R^2_Y \quad \text{.....(equation 4.4)}$$

In the case of model 4.1, equation (4.3) translates to:

$$1= p^2_{FD} + p^2_{FL} +2(p_{FD}\, p_{FL}\, r_{DL}) + r^2_{UY} \text{ .....(equation 4.5)}$$

For model 4.2 (Figure 4.2), fish are assumed to be able to adapt to the energy value of the diet as well as to the amount of lipid and dextrin present. This model is very similar to the first, except that the calorific value of the diet is added as a third predictor variable. There are clearly correlations between both lipid and energy, as well as between dextrin and energy, so these are connected with double headed arrows.

In this case equation (4.3) translates to:

$$1= p^2_{FD} + p^2_{FL} + p^2_{FE}$$
$$+2(p_{FD}\, p_{FL}\, r_{DL}+ p_{FD}\, p_{FE}\, r_{DE}+ p_{FE}\, p_{FL}\, r_{LE}) + r^2_{UY} \text{ .....(equation 4.6)}$$

In model 4.3 (Figure 4.3), the changes in food intake and energy levels are assumed to be correlated only because they have a common cause; the ingredients. In this case, if the sum of all the pathways connecting energy and food intake is equal to the direct correlation coefficient between energy and food intake then hypothesis 4.3 is supported.

This is expressed by:

$$r_{YZ} = \sum_i p_{Yi}\, p_{Zi} + \sum_{ij} (p_{Yi}\, p_{Zj} + p_{Yj}\, p_{Zi})\, r_{ij} \quad \text{.....(equation 4.7)}$$

or specifically to model 4.3:

$$r_{FE} = p_{FL}\, p_{EL} + p_{FD}\, p_{ED} + p_{FL}\, p_{ED} r_{LD} + p_{EL}\, p_{FD} r_{LD} \text{ .....(equation 4.8)}$$

To obtain values for the functions in the above equations, the **average** daily food intake was used in model 4.1. In models 4.2 and 4.3 the **daily** feed values were used. This is because, as a short cut, path coefficients can quickly be derived by taking their component functions from statistical packages. In models 4.2 and 4.3 this short cut was not possible using the average daily food intake, due to problems when attempting to carry out an ANOVA on highly correlated data (for the purposes of path analysis however, these correlations are unimportant). By using the data in these two different ways, the values of correlation coefficients and path coefficients were changed, making them biologically meaningless (which they were anyway, as the levels of lipid and dextrin where chosen by the experimenter). However this too made no difference to the analyses carried out in this paper.

(iii) RESULTS

Of the three models being tested model 4.1 (selecting nutrients only) and model 4.2 (controlling energy intake as well as selecting nutrients) were shown to be valid. However model 4.3 did not stand up to analysis. The reason that the results inferred that two of the models were correct and not just one are discussed below. The values for each parameter in the models are given below:

Model 4.1

Using equation 4.5 (Figure 4.1):

$0.0061168 + 0.8384126 + 2(-0.0825328 \times -0.9156487 \times -0.5303144) + 0.235$

$= 1.0000713893$

Model 4.2

Using equation 4.6 (Figure 4.2):

$$1.0927028 + 0.1203451 + 0.0000075 + 2\{(1.0453243 \times -0.002748 \times 0.283187) + (1.0453243 \times 0.3469079 \times -0.291329) + (0.3469079 \times -0.002748 \times 0.72786)\} + 0 = 0.999$$

Model 4.3

Using equation 4.8 (Figure 4.3):

$$0.03846 \neq (-0.002748 \times -0.000325) + (1.0453243 \times 0.1235437) + (-0.002748 \times 0.1235437 \times 0.283187) + (-0.000325 \times 1.0453243 \times 0.283187) = 0.12914$$

This is not the correlation coefficient of food intake and energy level in the diet and so this model is disproved.

(iv) DISCUSSION

In this chapter path analysis has been demonstrated to be useful as a tool to study models of causation in studies of regulation of food intake.

The fact that model 4.1 shows that the nutrients alone explain the change in food intake, whereas model 4.2 shows that both energy and nutrients work well can be explained by the fact that in model 4.1 the effect of energy on intake becomes hidden in the unknown variable, r_{UY}, a composite of all the sources of unexplained variation. The models tested indicate that dab can adapt their food intake to both changes in the nutrients, dextrin and lipid, and to the change in calorific value of the diet. Therefore any attempt to explain the mechanism by which dab adapt their food intake to the quality of the diet should take both these factors into account.

The results presented above cannot determine the relative importance of energy

and nutrients; Jobling (1981b) and Jobling and Wadvick (1983) suggest that the energy level of a diet has more influence than the nutrients in appetite regulation. The data used here was generated by an experiment that was not intended for this purpose, so the statistical values generated for use in path analysis have no real biological meaning. For example the correlation between lipid and dextrin is meaningless because the two were under the complete control of the experimenter. Thus, whilst it is has proven possible to test models of causation, no conclusions can be drawn about the actual strengths of these causations.

The method can however be used to demonstrate the difficulty in designing experiments testing the effects of dietary ingredients on food intake.

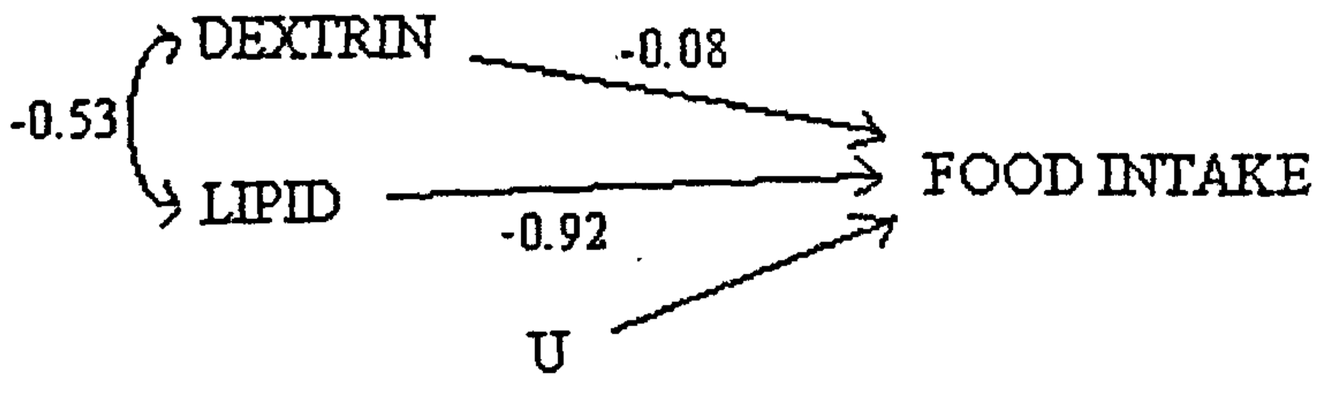


Figure 4.5 Correlation coefficient and path coefficients for model 4.1. The direct effect of increasing lipid is to decrease food intake, whilst the indirect effect, *via* its correlation with dextrin is to increase food intake.

Figure 4.5 shows the path diagram of model 4.1 with values for path coefficients (single headed arrows) and the correlation coefficients (double headed arrow) included. It can be seen that lipid and dextrin are negatively correlated, (correlation coefficient, r_{LD}, = -0.53) so that as lipid is increased, dextrin decreases.

If we were able to increase only the lipid levels, the direct effect on food intake would be strong and negative; the path coefficient, p_{LF}, was found to be -0.92. In this experiment though, we also have to take into account the indirect effect of

increasing lipid on food intake *via* its relationship with dextrin. The **indirect** effect of increasing lipid content, *via* its negative correlation with dextrin, is to **increase** food intake. This is because the increase in the lipid content was accompanied by a decrease in the dextrin content. The resulting overall correlation between lipid level and food intake, r_{FL}, which takes into account both the direct and indirect pathways is:

$-0.92 + (-0.53 \times -0.08) = -0.87$ (from equation 4. 3).

OF course, correlation is not an appropriate measure in such a trial, but the problem illustrated is well worth considering when designing experiments of this type; if increasing dextrin had had a stronger negative effect on food intake, the decrease in food intake (g) resulting from increasing lipid would perhaps not have been picked up by Fletcher (1982).

CHAPTER FIVE: LOW DIMENSIONAL DYNAMICS OF FOOD INTAKE IN THE DAB, *LIMANDA LIMANDA* (L.).

(i) INTRODUCTION

In nature, fish face constantly-changing food availability, with different prey species having differences in nutritional value. It is also necessary to adapt to intra-specific nutritional variation, for example with season. The quality of each prey species will vary for a large number of other reasons, for example with environmental variables such as temperature or the quality and availability of their own food. Fish adapt to these changes whilst their systemic need is also continuously changing due to growth, breeding cycle as well as seasonal and environmental variability. In the case of the sand dab, *Limanda limanda* (L.), Gwyther *et al.* (1981) found that they did not respond to a dilution of the diet. However, Fletcher (1982) found that dab changed adjusted their food intake when offered diets of different nutritional value, specifically responding to changes in the concentrations of carbohydrate and lipid. The previous chapter inferred that *L. limanda* can adapt to differences in the energy density of the diet as well as to the concentrations of dextrin and lipid. Despite a considerable amount of research into the mechanisms that control food intake in fish, it is unclear how fish are able to adapt to a changing diet so rapidly and successfully.

Day to day food intake generally exhibits large variation (figure 5.1a), this is particularly the case in flatfish (Pandian 1970). The study of this variation *per se*, by looking at food intake as a time series, offers a new approach in the study of food intake. Here, food intake is looked at as a dynamical process (*i.e.* food intake is changed by a number of 'inputs'). By identifying the types of dynamics that are driving the time series, it is hoped insights will be gained into the regulation of food intake.

This Chapter uses non-linear analyses to examine qualitatively the dynamics behind food intake in the dab, and to estimate how many variables regulate the process. This approach was in part determined by evidence suggesting that the dynamics determining food intake could be either a non-stationary periodic or a chaotic

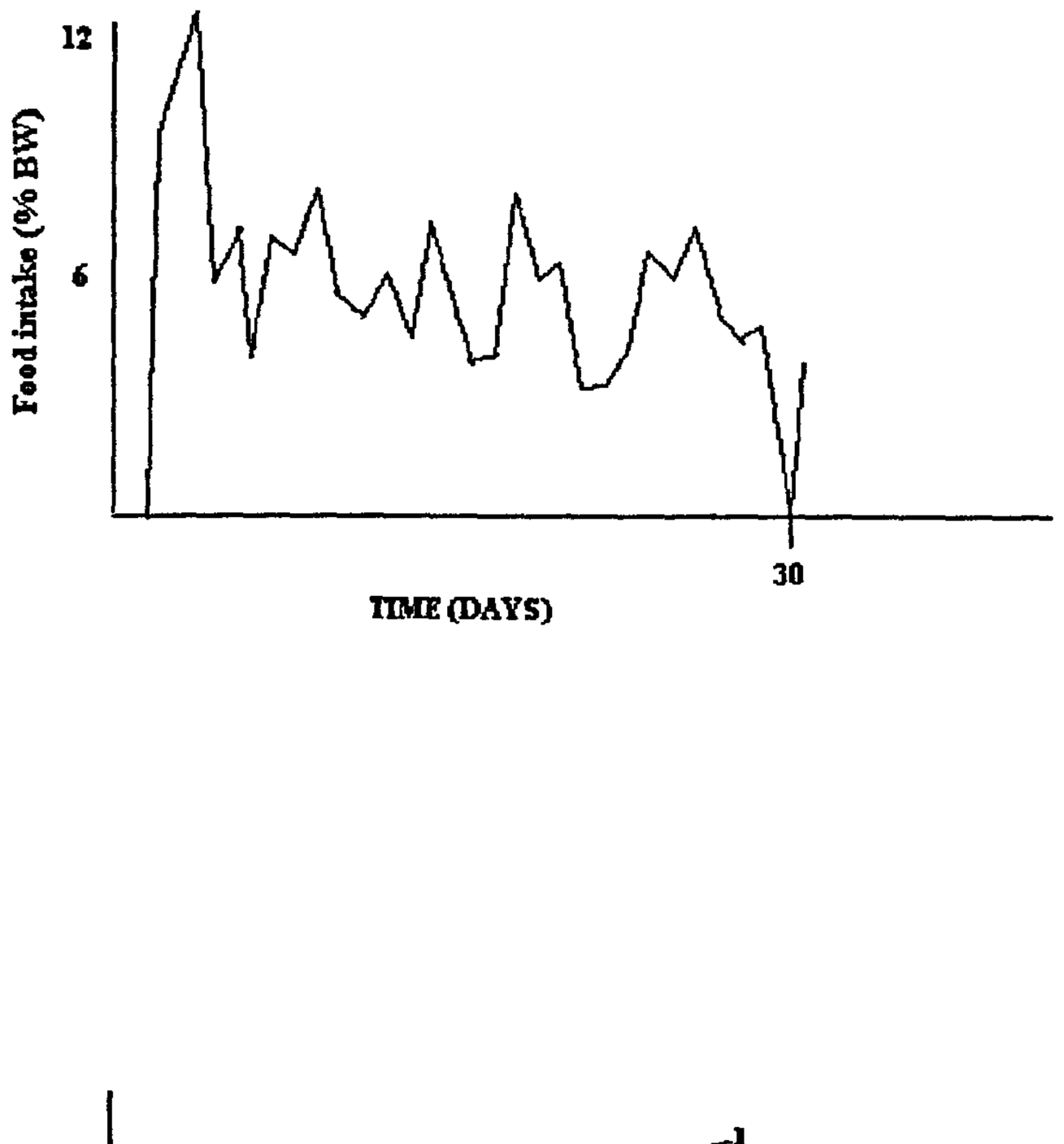


Figure 5.1a ***(top)*** **A real time plot of a food intake time series for a group of** ***L. limanda*** **fed on squid mantle at 15^{0}C. Variation once again is seen to be great (V = 40% for a tank of six fish with an average start weight of 120g over 30 days).**

Figure 5.1b ***(bottom)*** **Food intake (from Figure 5.1a) at time t is plotted against food intake at time t-1 to give a return map. It can clearly be seen that the pattern repeats itself roughly every three meals; that is the data is period-three. The data here oscillates around one mean intake (or attractor, A) for a few days, before unpredictably and quickly changing to another (B).**

system (see Dunstan 1993, May 1976 and Cvitanovic 1989 for an explanation of these terms).

Two factors infer that the latter is the case: Firstly time series of food intake, where the fish were fed discrete meals once daily, have been found to be approximately 'period three' (*e.g.* Figure 5.1b), particularly when novel diets were offered. Li and Yorke (1975) suggest that period three infers chaos. Secondly, although examination of the power spectra of short pieces of our time series appeared to be roughly periodic (figures 5.1b and 5. 2), they are in fact unpredictable

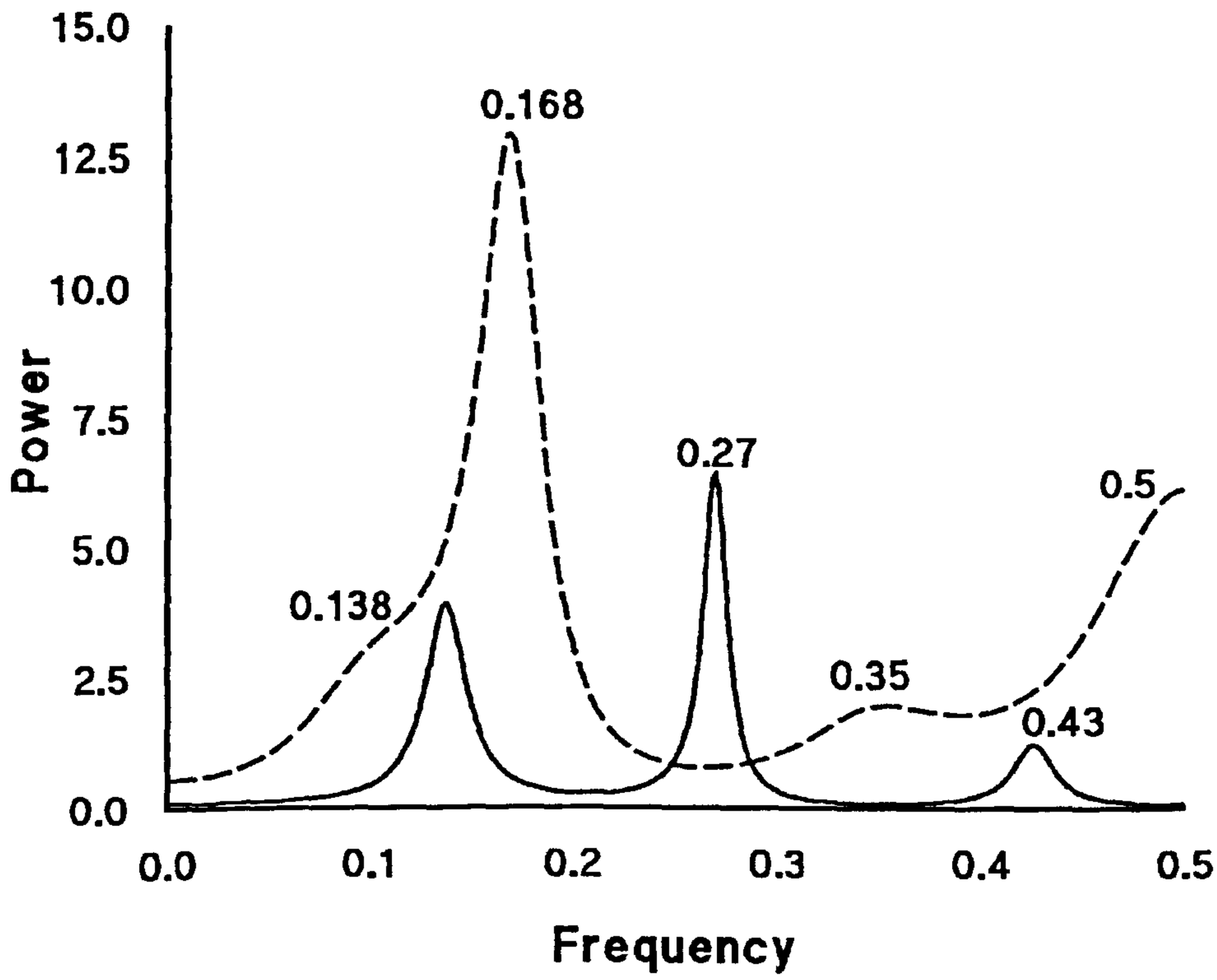


Figure 5.2 Frequency spectrum for dab fed squid mantle at 15^{0}C (dashed line) and 8^{0}C (full line), showing apparent periodicity with harmonics.

This became apparent from examination of the time series from a demand-fed population when the probability of the group actuating the hoppers X times in an hour was examined. The relationship was found to be a power curve:

$$p(n) = 1/(n+1)^{1.8} \qquad \text{.....(equation 5.1)}$$

where n is the number of actuations per hour and p(n) (= 0.0-1.0) is the probability of there being n actuations in a particular hour (curve fit; n = 19, P<0.01; BIOSOFT FIG-P, Cambridge). The fact that this is a power law, means that it is impossible to predict the value of n at any one time from the preceding points. Furthermore power curves have a similar shape at all scales (a "scale invariance"), this implies self-similarity which is also a characteristic of chaotic systems (Casti 1997).

These results also indicate Lévy distribution, with a greater variance than a normal distribution, and the distribution curve has much wider 'tails' (Casti 1997). This would explain the high variation in food intake frequently observed in flatfish (Pandian 1970). As a result of this finding food intake can be considered a Lévy random walk (Casti 1997), where typically there is no characteristic size for the length of each step (in this case each 'step' would be equivalent to amount eaten per hour).

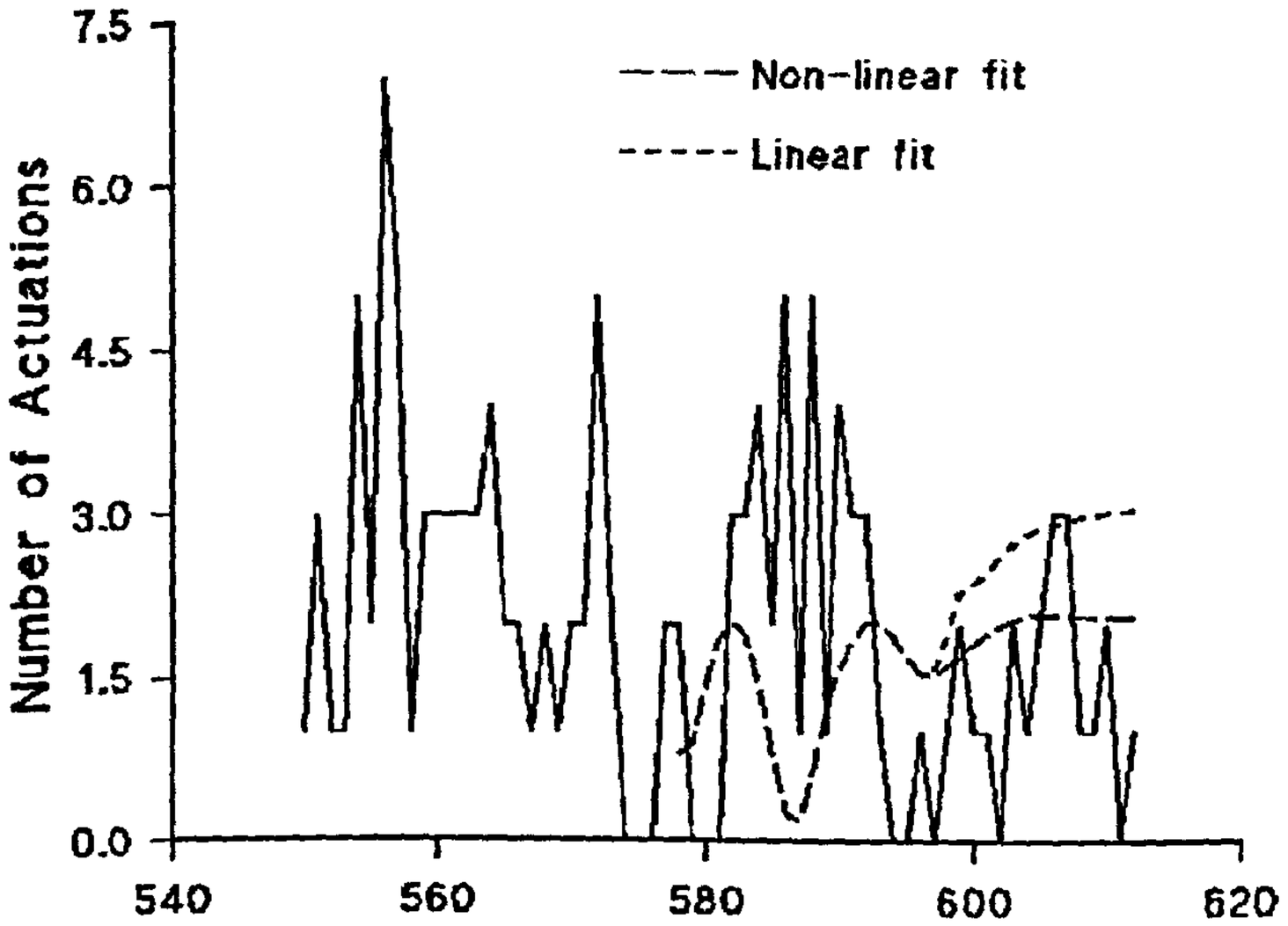


Figure 5.3 Attempts to model a time series of food intake in the dab have proved unsuccessful. Analyses were carried out using the Chaos Data Analyser Software and methodologies are described in Sprott and Rowlands (1992)

Attempts to predict a food intake time series more than one meal ahead have so far met with little success, as demonstrated in Figure 5.3.

Ruohonen (1999) used a neural network, that had been 'trained' on a previous season's feeding data, to predict one meal ahead. He found that the model worked reasonably well, but failed to predict the extreme values. Unpredictability in a time series is considered an indicator of chaotic dynamics (May 1976).

Although Figure 5.1b does appear to be roughly periodic, this does not rule out chaos, as a **short** time series may appear periodic for a chaotic system too. This is because chaotic systems are a sequence of unstable periodic orbits (Spano and Ditto 1994).

Chaotic systems have certain universal properties which could, if detected in food intake data, prove chaos (see introduction).

A simple function described by May (1976) provides a good example of a system that is capable of chaos:

$$X_{t+1} = aX_t(1-X_t) \quad \text{.....(equation 5.2)}$$

where X_{t+1} is the value of X at time t+1, X_t is the value of X at time t and a is a constant. Figure 5.4 shows a plot of this equation at five different values of a; 1, 1.5, 2, 3.1 and 3.57. The system behaves very differently in each case; when a =2 the time series is one dimensional with all values of X the same, when $a = 3.1$, X oscillates between two similar points and finally when $a = 3.57$ the system becomes chaotic and therefore aperiodic.

Skinner *et al.* (1994) pointed out that biological chaos allows low-energy control with complex deterministic consequences, as is evident in Figure 5.4. Chaos-based models have been presented which, when compared with non-chaotic systems, are able to adapt more quickly to novel stimuli (Skarda and Freeman, 1987, on neural activity; Chen and Aihara, 1995, using transient chaos in neurodynamics; Mitra and Skinner, 1992, transient chaos in the olfactory bulb.)

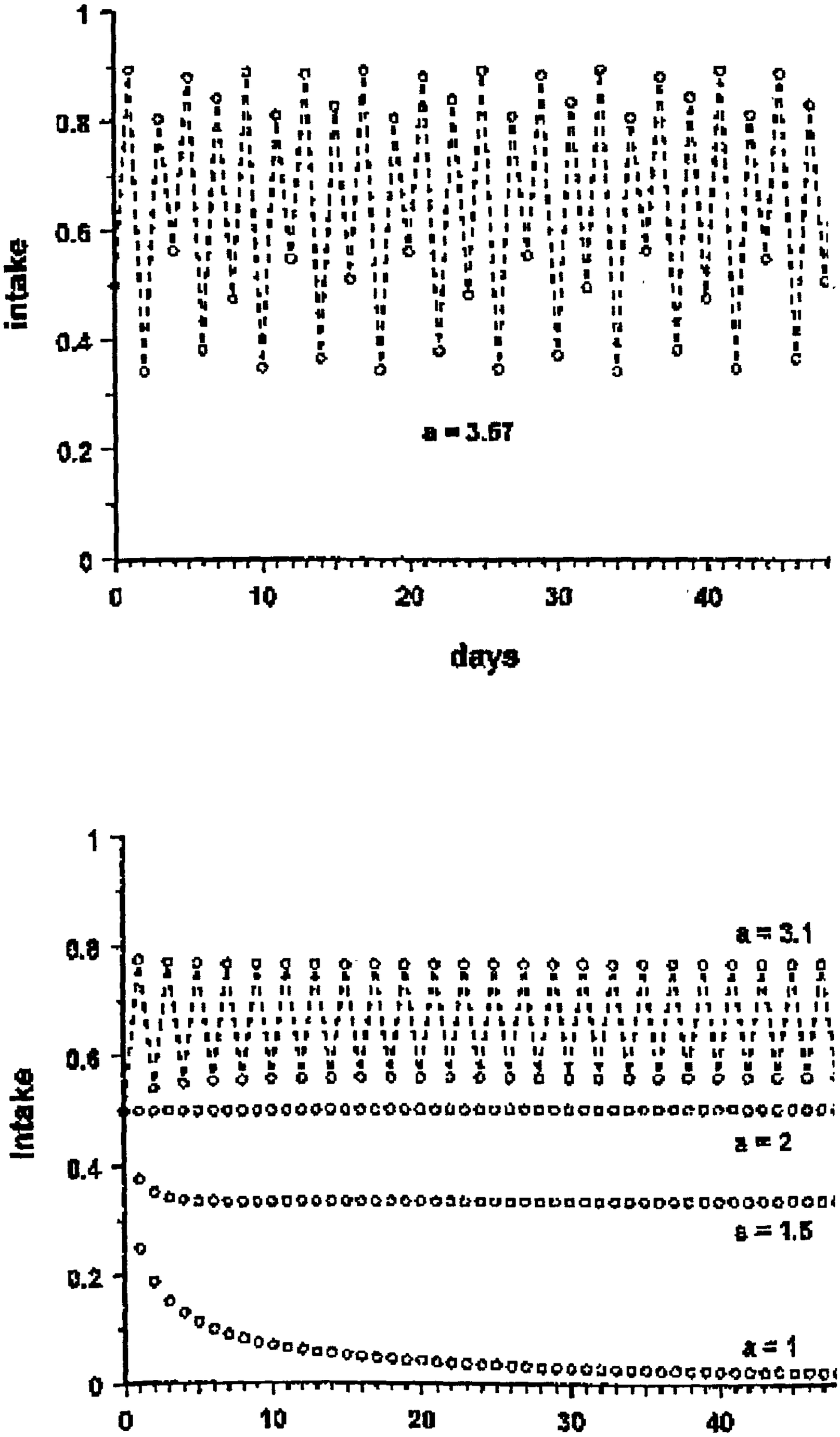


Figure 5.4 Plots of the function Xt+1 = *a*Xt (1-Xt), with various values of *a*. At *a* = 1 the time series tends to zero, for *a* = 1.5 to *a* = 2.0 the system stabilises at a fixed value. For *a* = 3.1 the system is periodic and when a is 3.57 (top) the sytem becomes chaotic

This study asks several questions about the nature of food intake dynamics in *L. limanda*; is variation in food intake deterministic or influenced by so many variables that it is essentially random? If the system is deterministic, is it nonlinear and what dimension is it (*ie* how many variables govern it). Finally, is it possible to determine if it is chaotic or periodic? It is hoped that the results of these analyses will give the initial information required to develop a future model that offers an explanation of the adaptive nature of food intake.

(ii) MATERIALS AND METHODS

Generating time series.

Time series were generated in two ways; firstly by providing demand feeders to allow fish constant access to a pelleted diet (Table 2.1). Fish were allowed access to food whenever they required it, having been trained to demand-feed by a process of positive reinforcement. To receive a portion of pellets, the fish had to break an infra-red (IR) beam by swimming through it. They were trained to do this by a process of positive reinforcement, being offered pellets at meal times with a rod with a red-painted tip placed in the tank close to the feeding location. After a few days they were only fed (or rewarded) if they approached the red tip of the rod, with time the fish were expected to touch the rod before receiving their reward. Once they were touching the rod, it was placed in the tank in such a way that the fish had to break the IR beam to touch it. Eventually it was found that the fish would break the IR beam without the presence of a rod. This process took about four weeks, and fish were left for another four weeks prior to data collection. Each actuation of the IR beam sent a 5V DC signal to a PC, where it was recorded and which resulted in the delivery of approximately 2.2g of pellets from a hopper (Figure 5.5, Appendix 5.1). Fish that were demand fed were kept in a 3,000 l 'Fasttank' during November 1996 (Appendix 3.7), with the temperature range 13-15 °C.

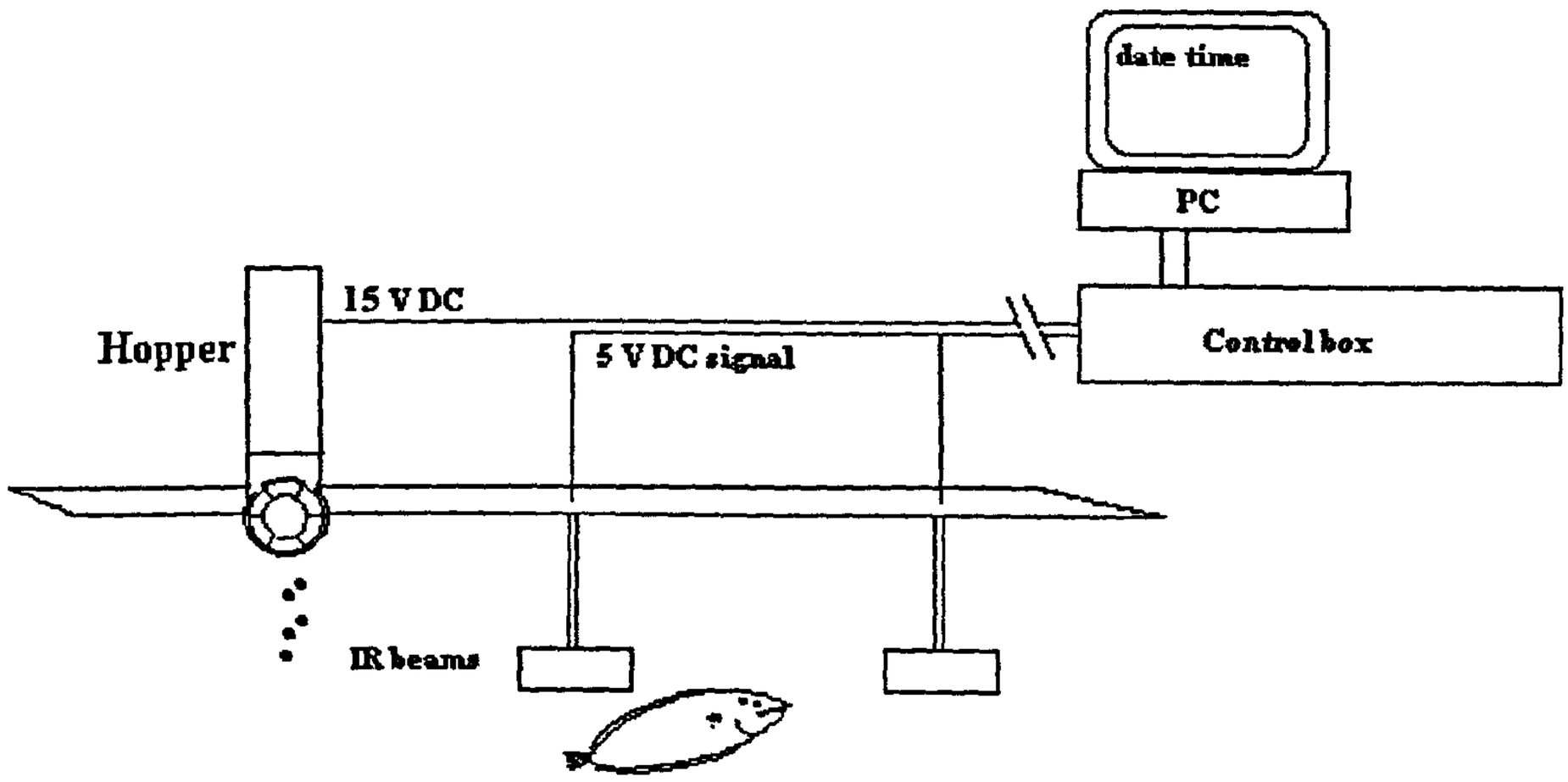


Figure 5.5 The infra-red demand feeding system. When a fish swims through the infra-red beam, a 5V DC signal is sent to the control box, which then sends a return 15V DC signal to a hopper, driving a stepper motor. The delivery is recorded on a PC as an ascii file, giving the date, time, which beam was broken and amount delivered. There is also a feedback loop built into the software that detects whether the hopper is blocked and employs various strategies to unblock it.

A second group were offered discrete meals by hand, once daily (Appendix 5.2). These were fed squid mantle, which was offered a fixed weight at a time and replaced with more as soon as it was finished. This was to keep the feeding stimulus as constant as possible, since Colgan (1972) found that, in the pumpkinseed fish, the amount eaten was proportional to the amount of food offered. Fish were fed to satiation, the uneaten diet removed and the amount remaining recorded. This group were kept in 0.25 m^3 tanks, with temperatures ranging from 7 to 9 °C, during January / February 1995.

Both trials took place under constant illumination. Water was partially recirculated through a biological filter, with a trickle feed through-flow to flush out soluble wastes. Tanks were kept well oxygenated and cleaned daily.

Preparation of data

The data sets from the demand feeding experiment were analysed in two forms; The first examined was food delivered per unit time. The choice of the time units here was the smallest interval that did not give falsely low dimensions due to over-sampling (Rapp 1994). This would occur because, when too small time intervals are examined, successive data points would be similar and as a result the data set can appear to be one-dimensional. Therefore analyses were carried out for food eaten every two hours rather than hourly, as originally planned. This solved the over-sampling problem, but halved the number of data points. Secondly, results were analysed as the interval between actuations of the hopper. This has the advantage of giving a long data set, as every actuation of the hopper provided a point.

Data from the discrete meal experiments was too short to carry out all of the analyses described below. However its dimension could be estimated using the PD2i algorithm when it had been enveloped between a one-dimensional data set, *i.e.* a straight line (Skinner *et al.* 1991).

Examination of the topography of time series for evidence of determinism and chaos

The first question asked of the data set was whether the time series is deterministic or stochastic (Kaplan and Glass 1992). Mpitsos (1994) stated that

"....the best evidence for a given dynamics...is from the examination of phase space itself using phase portraits, Poincaré sections and return maps."

Therefore data from the demand-fed group was differenced and plotted three-dimensionally with the axes representing meal m, meal m-1 and meal m-2 (a three-dimensional return map). If the data was random the plot would appear as randomly scattered points, however if the data is deterministic some form, or pattern, would be evident.

Recurrence Diagrams as a test for determinism.

Another way of examining a time series for determinism, which can overcome the problem of aspect, is the method of recurrence diagrams (Haken, 1983, Skinner 1994). Ekmann *et al.* (1987) pointed out that if a time series is not stationary, the

embedded data (*ie* the data in phase space, where phase space is the spatial dimensions plus their accompanying momentum dimensions) will not be either, and thus the stationarity of the geometry of that set can be tested. A recurrence diagram makes a picture in which the vector lengths between the 12-dimensional vectors are plotted if they are small. To produce a diagram, one chooses the percentage of the dynamic range of the data to use (ε, at the smallest end of the range). This is a subjective choice; Skinner (1994) recommends starting at 20% of the data range, although if this portion of the data consists entirely of very short vectors this value should be increased. Lengths between ε and zero are plotted in a field V_{i+1} *vs* V_i, where 1 is a lag in the data series between the two vectors whose distance is being plotted. Vector differences are given a colour between white (zero distance) and dark grey (distance at ε). If large enough data sets are examined, and if enough nearest neighbours are plotted then, in **deterministic** data sets, the individual points merge together to form patterns. Subtle departures from stationarity, which cannot be picked up from a changing value of the moving average, will produce readily discernible distortions in these patterns.

These patterns can infer other qualities of a data set, as well as determinism and stationarity; namely the dimension (see below) of the time series and whether the system may be chaotic. One-dimensional systems will produce horizontal lines, two-dimensional systems will produce a 45^0 slope, data higher than three dimensions will produce only complex patterns of recurrence points. Vertical columns of points represent 'recurrence clustering', typical of stationary chaotic data (Skinner 1994).

The recurrence diagram for *L. limanda* was produced from the interval data set, as this method gives better results with a large number of data points.

Correlation dimension (D2) and the Point correlation Dimension (PD2i)

The number of dimensions necessary to explain the total behaviour of a system can be reconstructed from a sample of a time series of any one of the underlying variables (Packard *et al.* 1980, Takens 1981). Grassberger and Procaccia (1983) developed an algorithm for this reconstruction; the correlation dimension (D2). D2 requires a long, stationary data set, which is seldom, if ever, found in biology,

(Theiler 1988). This method (Figure 5.6, largely from Skinner *et al.*, 1994) requires a time series to be either discrete or a continuous signal needs to be digitised. (fig 5.6a). First, the value of tau (τ) is estimated (this is the first zero autocorrelation of the time series (fig 5.6b) .

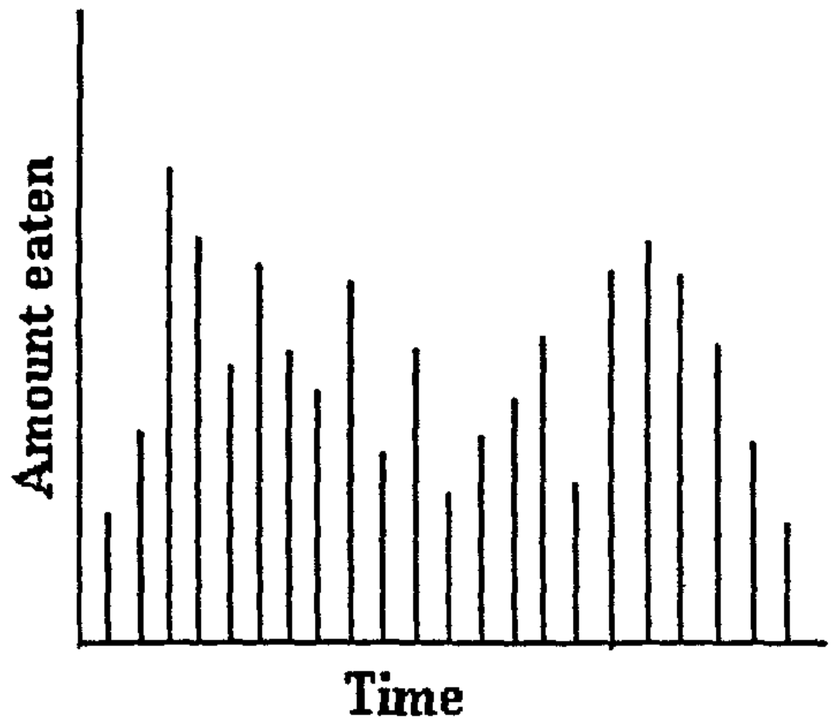


Figure 5.6a Discrete time series of feed intake.

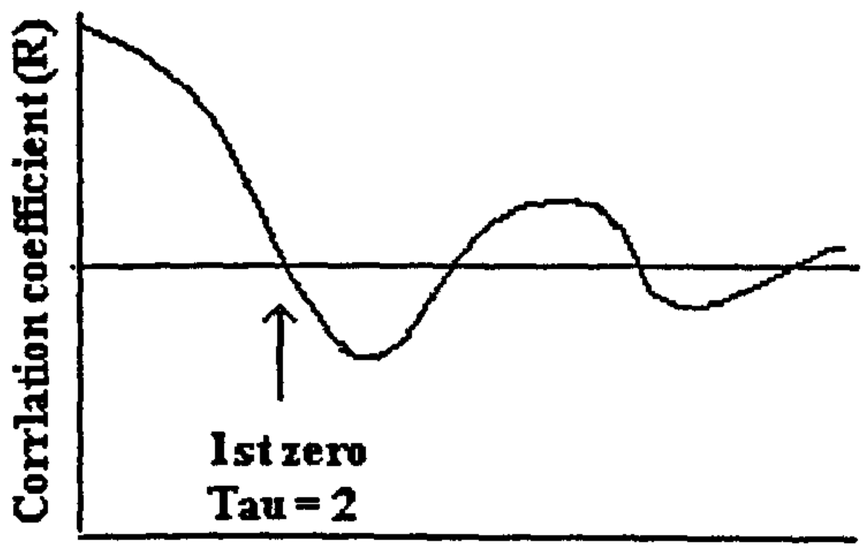
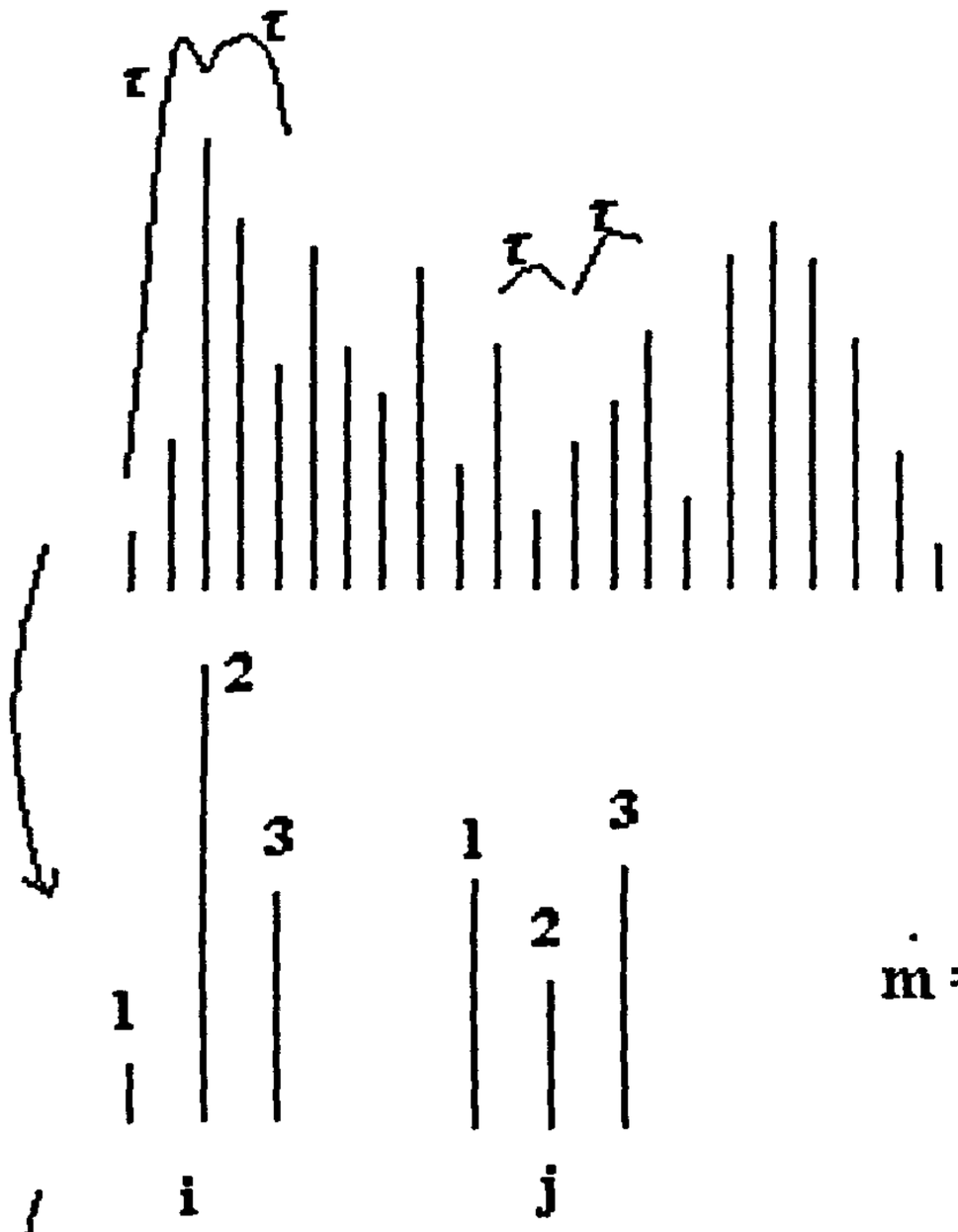


Figure 5.6b The first zero autocorrelation is found at a time step of $\tau = 2$.



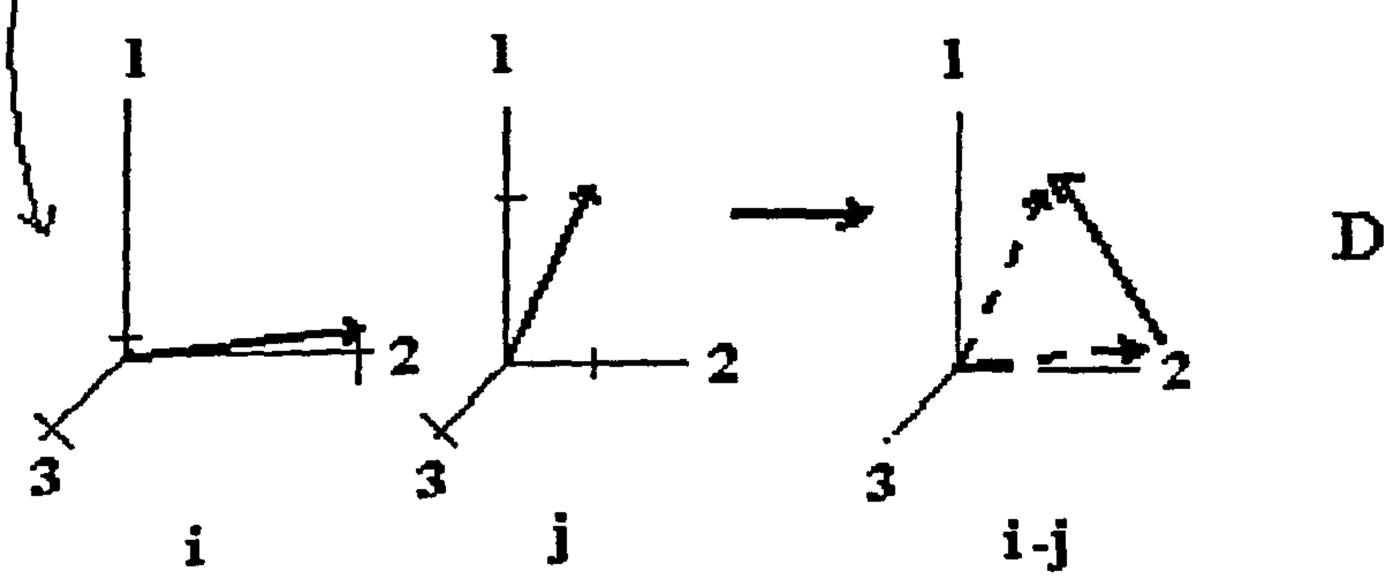


Figure 5.6c For all required embedding dimensions, m (in this case m = 3), and a delay time of τ (2 in this example) the first reference vector, i, is taken and a j-vector is taken by starting at another point.

Figure 5.6d A difference vector is constructed, which is the difference between the i-vector and the j-vector.
The reference vector is kept in the same place whilst it is compared with all j-vectors. After this a new reference vector is taken and this in turn is compared against all j-vectors

The time series is examined using various embedding dimensions, up to:

$$(2 \times CD_{expected}) + 1 \quad \text{.....(equation 5.3)}$$

Figure 5.6c continues the example using an embedding dimension of three. Here, a reference vector is taken at a specific point, i, (the i-vector) another vector is then made by starting at a different point (the j- vector). A difference vector is then made by subtracting the i-vector from the j- vector (fig 5.6d). The reference vector is kept in the same place whilst it is compared with all j- vectors, one of which is constructed from each data point in the time series, which is its first co-ordinate. The starting vector is then moved to the next point in the time series and this new i-vector is compared against all j- vectors. This is done for all i points.

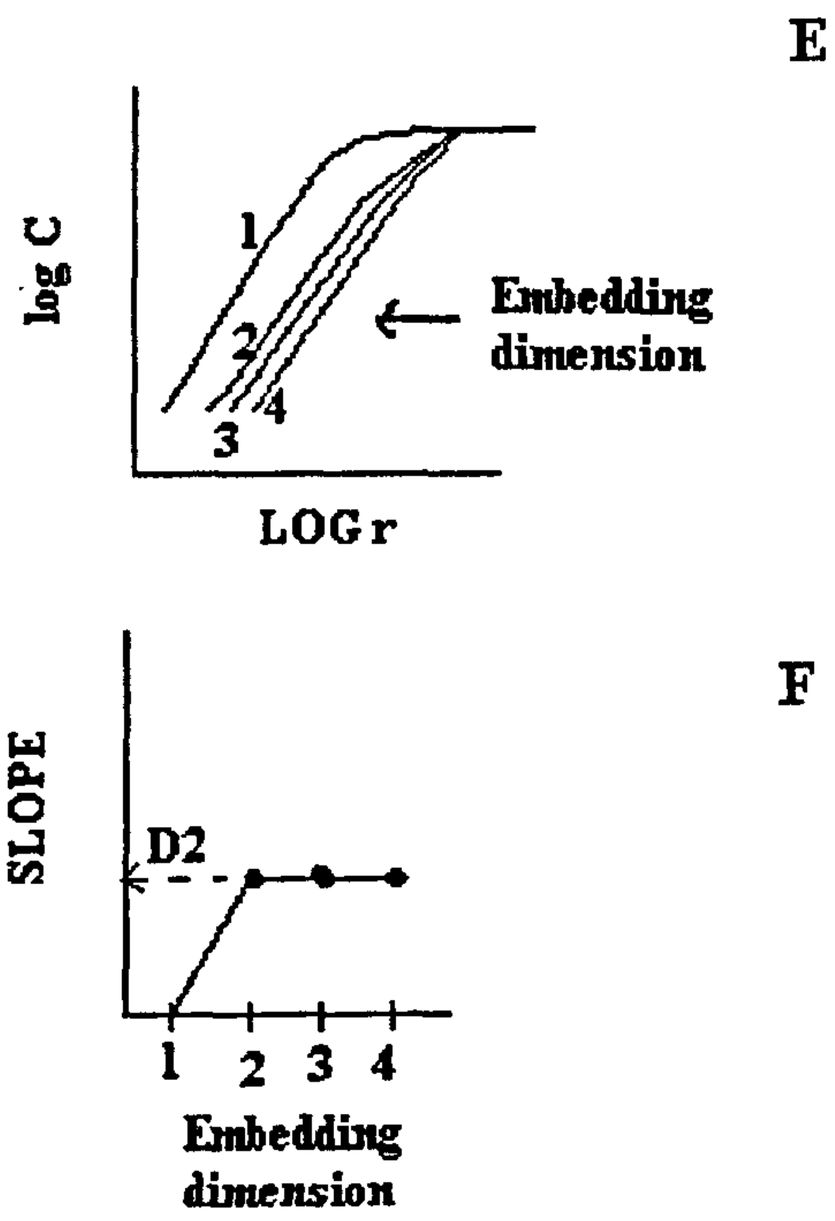


Figure 5.6e The number of vector differences smaller than each range value r are plotted as a log-log cumulative histogram for all embedding dimensions.

Figure 5.6f Plot of the slope in the linear region of the cumulative histograms for each embedding dimension. The value stabilises at an embedding dimension of 2 and above, giving the value of D2.

Next the number of the vector differences smaller than each range value, r, are plotted in a cumulative histogram (fig 5.6e). This is carried out for all embedding dimensions being examined. Part of the resulting slope for each embedding dimension is linear and, if the data set is sufficiently long and stationary, the value of the slope stabilises (fig 5.6f), and this is the dimension of the time series.

The pointwise scaling dimension, D2i developed by Farmer *et al.* (1983), estimated D2 as a function of time. This is because each reference vector, i, is compared with all the j-vectors, and the resultant CD **for each reference vector** is included as a point in a time series. Mayer-Kress *et al* (1988) suggested that this method may be less prone to errors caused by non-stationarities in the data, although stationarity was still an assumption of the method.

Skinner *et al.* (1990a, 1990b, 1991) then developed the point correlation dimension (PD2i). The method is similar to the D2i method. In this algorithm however, each reference vector seeks only its own stationary 'sub-epochs' to determine dimension. That is, the j- vectors that will contribute most to the dimension estimate must arise from a sub-epoch that manifests scaling characteristics similar to those surrounding the i-vector itself. It does this by including or rejecting vector differences from the dimension estimate, depending on the plot length of the small log-r values of the cumulative histogram (in the linear scaling region) and whether or not it meets linearity and convergence criteria. This means that stationarity is no longer a requirement. Skinner *et al.* (1994) describe these methods.

The number of dimensions controlling the behaviour of a system is not necessarily an integer. It is argued that a fractional value indicates that at least one dimension has an exclusion of points within part of its range, and therefore a strange attractor exists, *i.e.* the system is chaotic (Sprott and Rowlands 1992). In practice analysis of 'noisy' biological data is unlikely to detect values of PD2i that are *significantly* non-integer even when the generator is. However the method can usefully tell us whether the generator of the time series is of low dimension or not.

The PD2i algorithm was estimated for the two-hourly data set and from the discrete data sets which had similar values of τ as described above.

The estimation of D2, which requires a long data set, was carried out for *L. limanda* on the demand feeder interval data, to allow a comparison with the PD2i algorithm.

Surrogate data sets

This method (Theiler *et al* 1992) is a statistical approach in which some linear process is specified as a null hypothesis. Because all that is available for analysis is a single time series, surrogate data sets are then generated that fit the null hypothesis. Several replicates of these surrogates are produced, allowing error bars to be fitted; the real data can then be compared with the surrogates using a discriminating statistic.

Theiler *et al.* (1992) describe four null hypotheses against which the real data can be tested:

1) The observed data is fully described by independent and identically distributed (IID) random variables.

This surrogate is simply generated by temporally randomising the real data and has an identical mean and variance, however any temporal correlations are destroyed.

2) The data is non-IID uncorrelated normally-distributed noise.

This is generated by the Ornstein-Uhlenbeck process. A discrete sampling of this process yields a model of the form:

$$x_t = a_0 + a_1 x_{t-1} + \sigma e_t \qquad \text{.....(equation 5.4)}$$

where e_t is uncorrelated gaussian noise of unit variance. a_0 is the mean, a_1 the variance and σ the autocorrelation time of the time series. These are estimated from the original time series, with the first autocorrelation time being used (Theiler *et al.* 1992). The equation above is iterated, using a pseudorandom number generator for the unit variance gaussian *et* to generate the surrogates.

3) The data is linearly autocorrelated, normally-distributed noise.

This model mimics the original time series in terms of mean, variance and the autocorrelation function for delays of $\tau = 1,.....,q$ This is an auto regressive (AR) model and the null hypothesis in this case is that all the structure in the time

series is given by the autocorrelation function, or equivalently, by the fourier spectrum.

4) The data is a static nonlinear transform of linear, normally-distributed noise.

Here the null hypothesis is that the dynamics are linear, but that the observation function is nonlinear.

The algorithms used to generate the surrogate data to test these null hypotheses are described in more detail in Theiler *et al.* (1992); software developed by Rosenstein (1993) provides such surrogates from a real data set.

In this study we chose D2, carried out on the *L. limanda* interval data, as the discriminating statistic. This was a difficult choice, given the problems associated with applying the D2 algorithm to biological data, but moving averages of this time series suggested that the interval data is stationary, one of the requirements of the D2 technique. Another requirement is a long data set; because every actuation of the hopper provides a data point, the time series of interval between actuations was the longest data set available. Further, the comparison of surrogate data with the real data tests for *relative* differences; we are not hoping to ascertain the real dimension of the time series. As Theiler and colleagues state, all that is required to reject a null hypothesis is that the statistic have a significantly different distribution for the data than for the surrogates (Theiler *et al.* 1992).

Lyapunov exponent.

One can determine whether or not a system is chaotic by measuring the dispersion of data points plotted in phase space. A two dimensional phase plot is made by plotting each data point against another data point located in the series,τ number of data points away (τ; Figure 5.1b is a phase space plot with $\tau = 1$). This will produce a trajectory, bounded by the upper and lower limits of the data.

Non-random data will appear as loops. If two adjacent points on different loops are marked, in a chaotic system their distance from each other will increase as one runs through the time series. This divergence can be calculated using the Lyapunov exponent, which is the average exponential ratio of divergence or convergence of nearby orbits in phase space, there are as many Lyapunov exponents as there are

dynamical equations, however only the largest exponent is used and if this is positive then the system is chaotic. (A value of one indicates that the separation of nearby trajectories doubles, on average, in the time interval between the data samples). A negative value indicates a periodic data set and zero values indicate that the data set is close to a bifurcation (Wolf *et al.* 1985, Sprott and Rowlands 1992). One problem with this technique is that a noisy periodic data set can also give a positive value, giving a false impression of chaos. Given that all biological data sets are, compared with those from physico-chemical experiments, usually short and noisy, it is not possible to conclude that a positive Lyapunov exponent indicates chaos. However it is possible to prove that a system is periodic if the Lyapunov exponent is negative.

Rosenstein *et al.* (1993) have produced a version of the Lyapunov exponent for shorter, more noisy data sets, such as biological time series. In this paper we analysed the data using the method of Rosenstein *et al.* (1993) using software by Rosenstein (1993). This was carried out on both the 2 hourly data set, as well as the Poincaré section derived by taking the maxima from this data set. This is because Byrant (1992) points out that for data sets taken at regular time intervals there will be always be a zero exponent, corresponding to displacement along the orbit, and that this problem is not present in Poincaré sectioned data. Choosing the maxima of each cycle is a good choice, as this may reduce measurement error (Byrant 1992). The Lyapunov exponent (Wolf *et al.* 1985) requires a long, stationary data set, probably longer than we have available, however, as the embedding dimension and/or the number of points are increased a stabilisation of the results may indicate that the value is reliable. This was attempted on the interval data set using software commercially available through Physics Academic Software (Sprott and Rowlands 1992).

(iii) RESULTS

Figure 5.7 shows the demand-fed interval data differenced and plotted in a three-dimensional return map. Examination of its topography shows a clearly deterministic structure, with the points arranged around a six-armed cross.

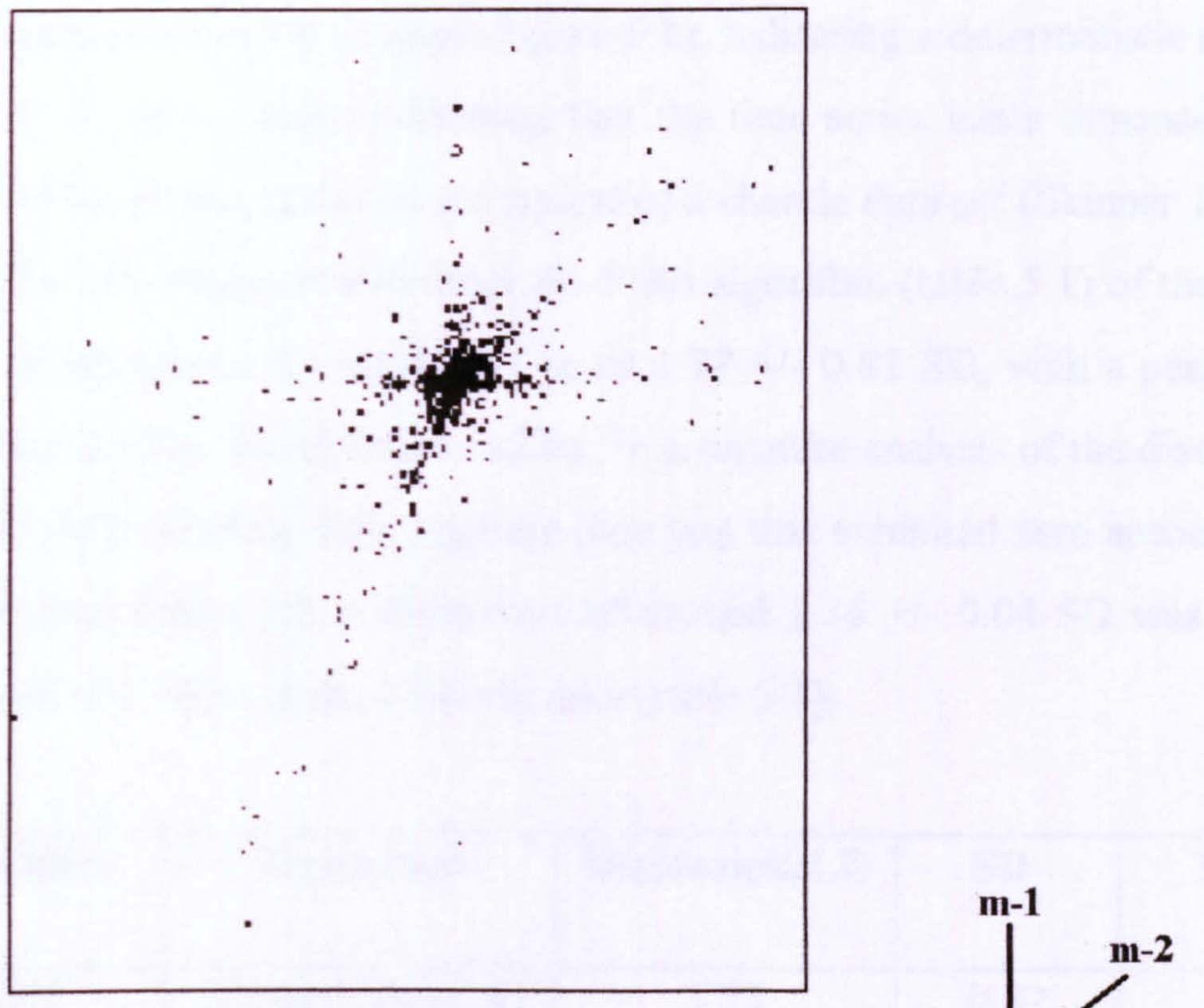


Figure 5.7 Three dimensional plot of differenced interval data, showing a clearly deterministic structure. The points fall on to an attractor that is shaped like a six armed cross.

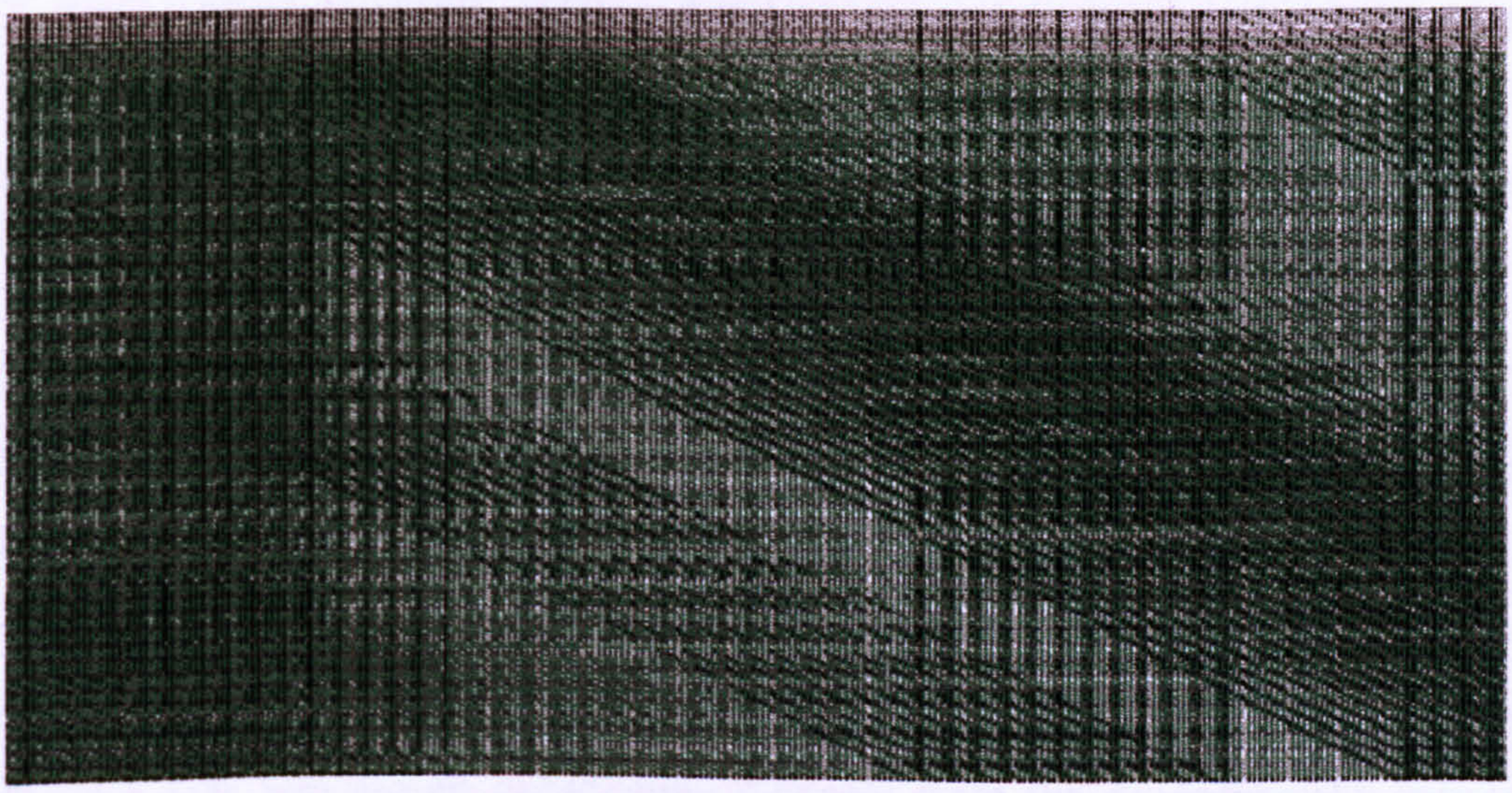


Figure 5.8. Recurrence diagram of the 2 hourly data set. The diagonal lines of <45° indicate a dimension of less than two. The vertical breaks are typical of chaotic data

The recurrence diagrams for both interval and 2 hour data sets shows clear diagonal patterns (see for example Figure 5.8), indicating a deterministic system, with the <45° slant pattern indicating that the time series has a dimension of less than two. The vertical columns are typical of a chaotic data set (Skinner 1994) Support for this finding comes from the PD2i algorithm (table 5.1) of the 2-hourly data set which shows the dimension to be 1.73 +/- 0.82 SD, with a peak at 1.40. This indicates a low dimensional system. In a separate analysis of the discrete data, calculated by combining three separate data sets that exhibited zero autocorrelation at similar time delays (τ), a dimension of around 1.36 +/- 0.04 SD was observed with a peak at 1.40 as in the 2-hourly data (table 5.1).

Algorithm	Treatment	Dimension/LE	SD	Peak
PD2i	2 hourly, demand fed	1.73	0.82	1.40
PD2i	Discrete , one meal per day	1.36	0.04	1.40
LE	Interval, demand fed	0.000		
LE	Maxima, 2 hourly, demand fed	0.000		

Table 5.1 PD2i and Rosenstein's Lyapunov exponent for different treatments of the time series.

When comparing the time series with surrogate data, the correlation dimension (D2) of the interval data set shows that the value saturates at an embedding dimension of around 6 (Figure 5.9) and that dimension is approximately 3.0 to 3.5. This is somewhat higher than the PD2i results, which is indicative of the inability of the D2 algorithm to deal with non-stationary or noisy data. The D2 values of the surrogate data sets are all significantly different from the experimentally-derived data set above an embedding dimension of 2-3, showing that the data is generated by a deterministic, non-linear system.

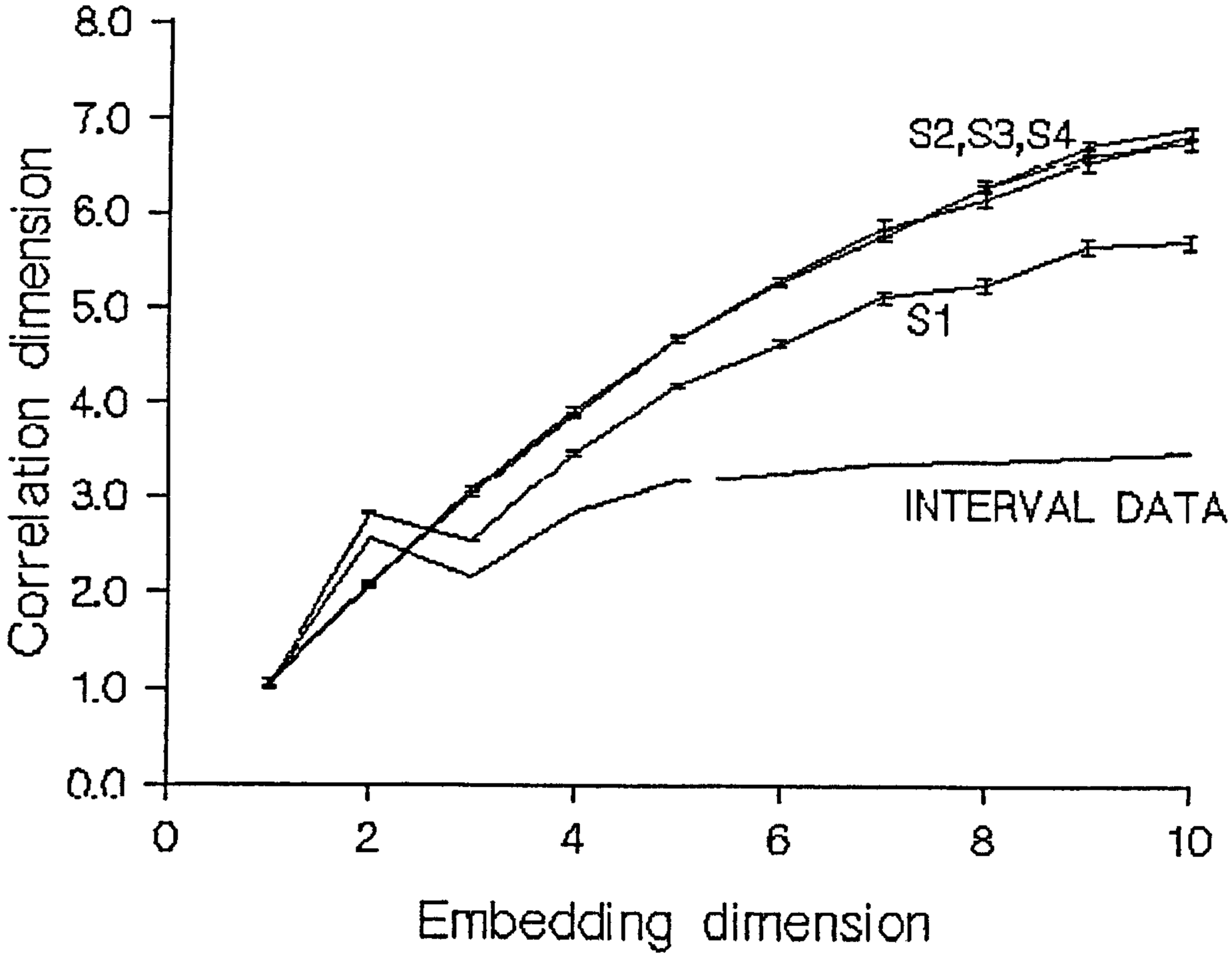


Figure 5.9 Correlation dimension and surrogates of interval data. All surrogates are significantly different from the experimental data. n=4 for all surrogates.

S1= Randomised phase data.
S2= Independent and identically distributed (IID) random variables.
S3= Non-IID uncorrelated gaussian noise.
S4= Static nonlinear transform of linear gaussian noise.

The Lyapunov Exponent of the interval data gives a positive value, though it fails to stabilise with increasing embedding dimension or increasing number of data points (figures 5.10a and 5.10b). It cannot therefore be decided whether the data is chaotic or periodic using this analysis, unless a greater number of data points is available. Rosenstein's Lyapunov Exponent algorithm, which gives better results for shorter, noisier data sets, gives a value of approximately zero ($R^2 = 0.89$, Table 5.1).

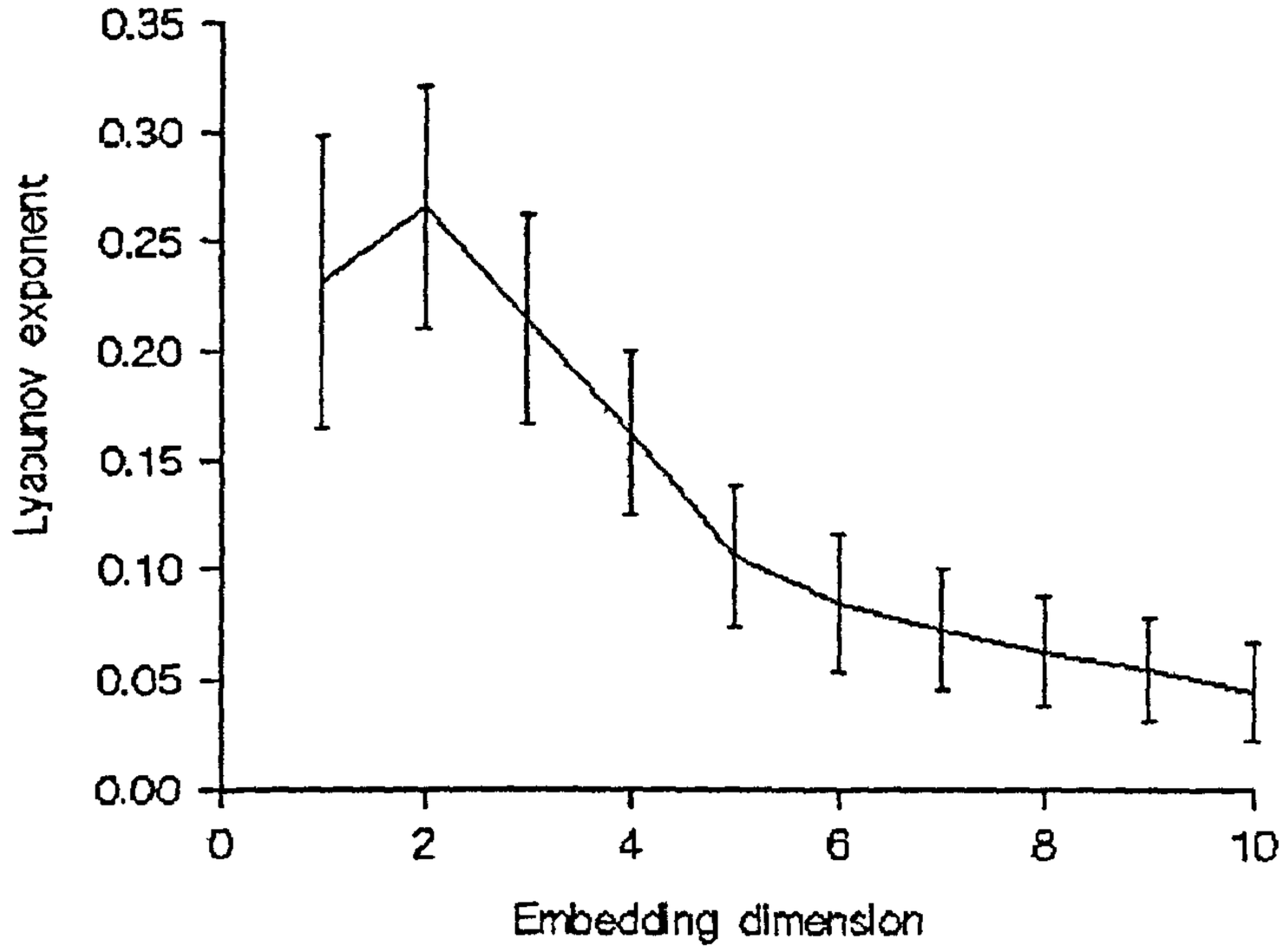


Figure 5.10a The Lyapunov exponent fails to stabilise with increase in embedding dimension. Error bars are calculated from 2.5 times the standard deviation of the slope of separation of trajectories divided by the square root of the number of trajectories followed.

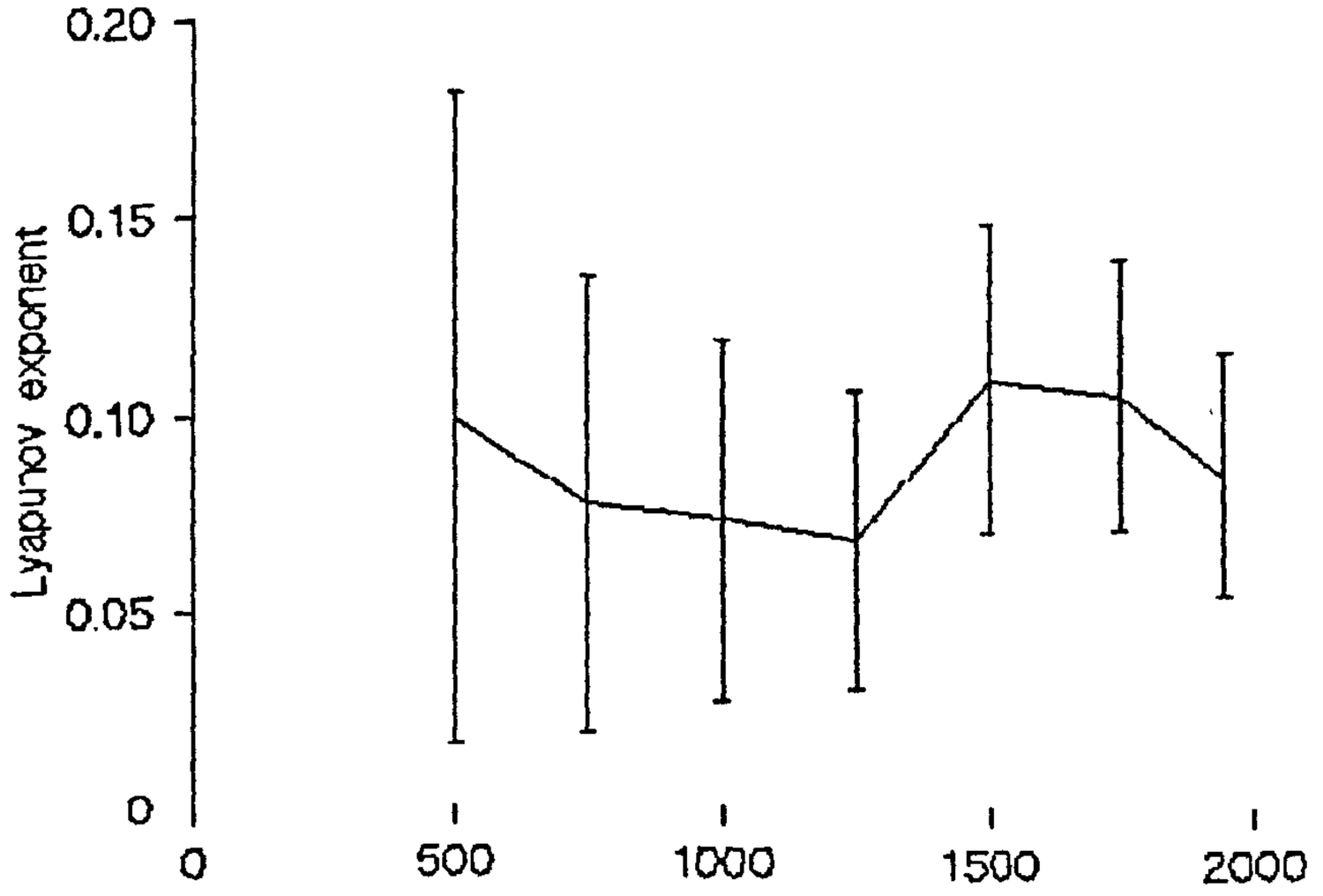


Figure 5.10b. The Lyapunov exponent fails to stabilise with increase in number of data points. Error bars are calculated as for Figure 10a.

The value remains zero for the Poincaré section derived from the maxima of the bi-hourly data ($R^2 = 0.61$, table 5.1), so this value is not an error caused by the data being sampled in discrete time intervals (Byrant 1992).

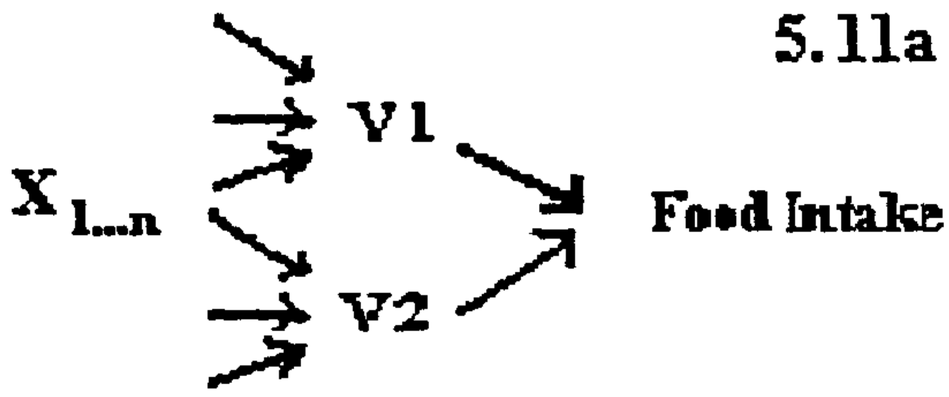
(iv) DISCUSSION

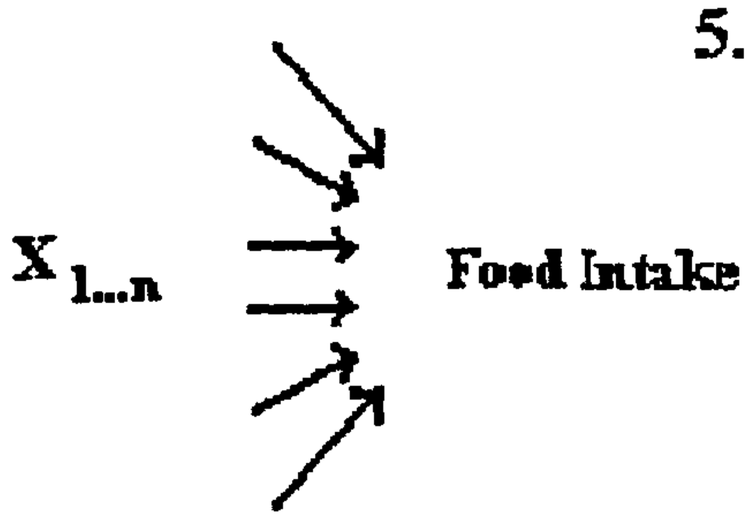
The results presented above show that food intake in groups of dab is generated by a deterministic, non-linear, low-dimension system where food intake is directly regulated by only about two variables (Figure 5.11a). This rules out direct multi-factorial control of food intake (Figure 5.11b). It also seems unlikely that a single drive is in direct control of food intake, (Figure 5.11c), although a model is possible in which the two variables acted on the drive which was **completely** correlated with food intake (correlation coefficient = 1.00), as shown in Figure 5.11c. The transfer of information from the drive to food intake would have to be very simple and linear.

Colgan (1972) suggested that food intake is controlled by a systemic need, as well as a gastric factor. He argued that these two variables acted on food intake *via* a single drive. Our findings do not fit with this hypothesis, instead systemic need (possibly a central drive) would have to act in conjunction with at least one other independent factor. One possibility was presented by Jobling, (1986), who argued that gastric or intestinal factors may act as a peripheral regulator of food intake.

The analyses provided a great deal of information regarding food intake dynamics, but did not achieve the our original aim of determining whether food intake is chaotic, non-linear but periodic, or on the edge of chaos. However, when combined with the previously observed unpredictability within time series of food intake and excursions into period three behaviour, the fact that the Lyapunov exponent is close to zero may indicate a dynamic that is chaotic or on the edge of chaos. (Wolf *et al.*, 1985, give an example of a Belousov-Zhabotinskii reaction that was in a chaotic regime but on the edge of a period three window, which they found had a largest Lyapunov exponent of 0.0054 ± 0.0005). If it is tentatively speculated that the food intake time series of *L. limanda* is on the edge of chaos, then food intake could be a complex adaptive system and may demonstrate

properties found in these systems, such as adaptivity and the very interesting prospect that it could have emergent properties (Mitchel-Waldrop 1992).





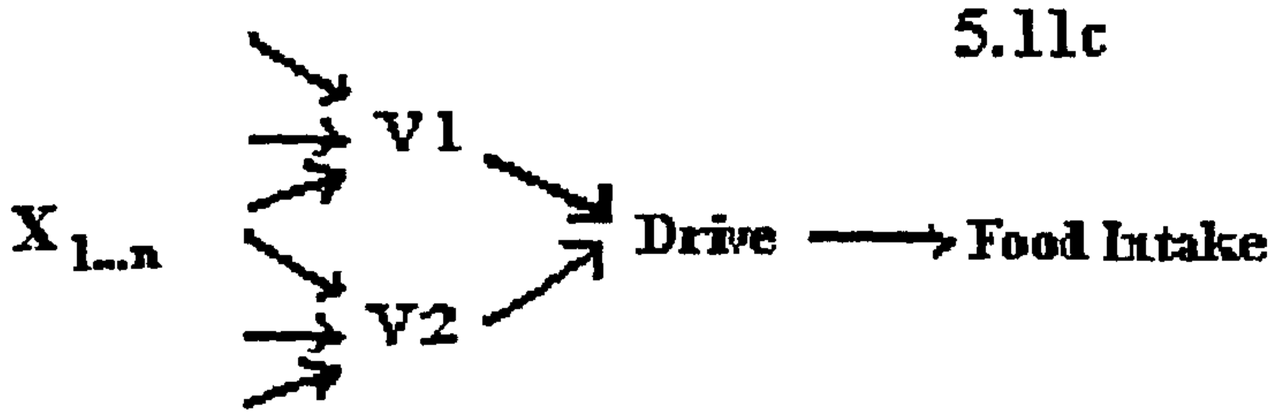


Figure 5.11a The model suggested by the results, where two variables (V1 and V2) are in direct control of food intake. Factors (X1...n) which are known to affect food intake such as temperature act *via* these two variables.

Figure 5.11b The concept of multi-factorial control of food intake, in which all variables are able to directly affect food intake is unlikely according to our findings

Figure 5.11c If there was a 'drive' which regulated food intake our model suggests it would have to be completely correlated to food intake (correlation coefficient =1) and the relationship would have to be linear.

Another possible mechanism for adaptive feeding behaviour can be drawn from the results of Viswanathan *et al.* (1996), when looking at flight patterns in albatross. They found that the probability of a particular flight time was proportional to:

$$1/(t+1)^2 \quad \text{.....(equation 5.5)}$$

where t = flight time. This has been interpreted as mostly short random flights, with occasional excursions to new areas, which require much longer flights. This allows the albatross to find new food sources more effectively; making its feeding strategy more adaptable.

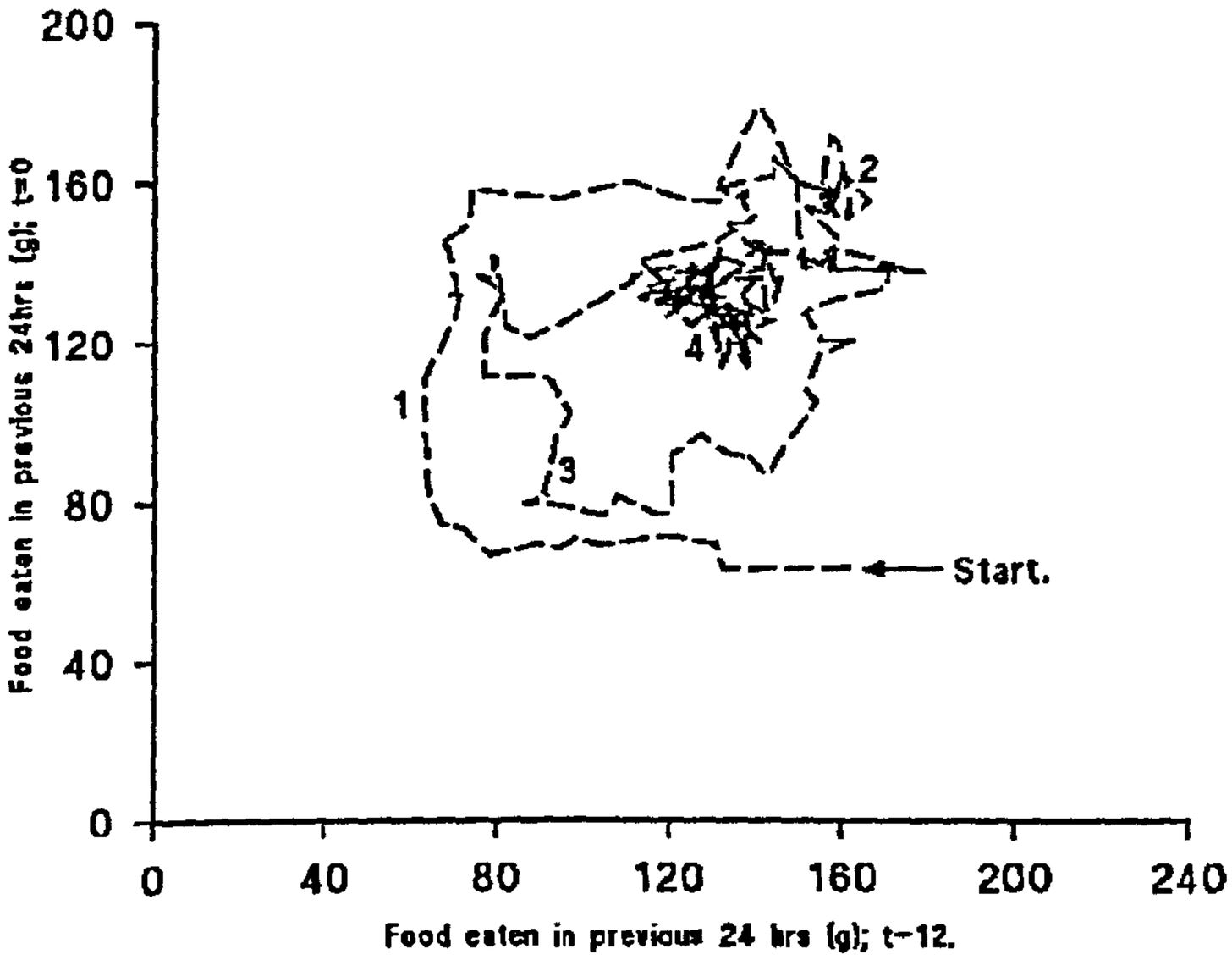


Figure 5.12 A return map of food intake in demand-fed trout, *O. mykiss,* fed a novel diet. Hourly food intake data was smoothed over 24 h, to remove fluctuations caused by regular meal times, and updated once per hour.Initially the amount eaten varied rapidly (1; a long excursion), after which it settled roughly at around 160g.24h^{-1} (2). A further long excursion (3) preceded a period of small changes (4), at around 130g.24h^{-1}, where the feeding levels remained for most of the remainder of the trial. Towards the end of the trial, the average feed intake returned to (2 for a short period. The long excursions resulted in an increase in variance for the data set.

This is remarkably similar to equation 5.1 above, describing the probability of there being n actuations in one hour Interestingly, visual examination of feeding behaviour in trout, where 24 h food intake was adjusted every hour to give a smoothed data set, shows similar patterns (Figure 5.12). It could be that this observed feeding behaviour could allow fish to adapt better to an unpredictable food supply.

The dynamics reported here are of a kind that have been observed in several biological, chemical and physical systems and by analogy several more potentially interesting avenues of research become available.

It is possible that by monitoring the rhythm of food intake it may be possible to detect disease at an early stage. Goldberger and West (1987) argue that the topography of a time series is a useful physiological indicator of good health. Buchanan (1988) points out that the dynamics of several systems become more orderly when a subject is unhealthy, for example in heart beat intervals, and the EEG of hepatic coma. Skinner *et al.* (1993) found that in high risk patients with documented non-sustained ventricular tachycardia, the dimension of heartbeat dynamics took excursions below a dimension of 1.2 in patients at imminent risk of lethal ventricular fibrillation (normally the dimension values ranged between 2 and 5). This measure was found to be specific, with all patients who had these excursions manifesting lethal ventricular fibrillation later the same day. (Clearly the word 'disorder' in these cases is a misnomer, as the onset of disease leads to more orderly, less complex dynamics). In a further development researchers have used methods derived from chaos theory to successfully control ouabain-induced arrhythmia in rabbit heart preparations (Ott *et al.* 1990, Ditto and Pecora 1993).

The original aim was to determine whether the day to day variation in food intake could be not only a result of biological time lags, but also a mechanism for adapting to novel diets. Whilst the precise nature of the dynamics of food intake in *L. limanda* could not be determined, we believe that the results may prove to be the starting point for an adaptive model of food intake, in which day to day variation provides the organism with the mechanism to adapt to changing diets.

CHAPTER SIX: LOW DIMENSIONAL DYNAMICS OF FOOD INTAKE IN INDIVIDUALS AND GROUPS OF *ONCORHYNCHUS MYKISS* (WALBAUM), WITH A COMPARISON WITH *MERLANGIUS MERLANGUS* AND *DICENTRACHUS LABRAX*.

(i) INTRODUCTION

In the previous chapter the variation in food intake in the sand dab, *L. limanda* (L.), was regarded as a time series and its characteristics examined using several non-linear analyses, to determine some of its dynamical features. Food intake in dab was found to be a deterministic system, with suprisingly little noise present, non-linear and low dimensional (*i.e.* it was directly controlled by few variables). Furthermore, the dynamics of food intake were unpredictable and sometimes 'period-three', both of which are characteristics of chaotic systems (Li and Yorke 1975, Lorenz 1963). Although the observed determinism and non-linearity also fit in with the hypothesis that food-intake regulation is a chaotic system, it proved impossible to determine whether this was the case, either from the Lyapunov Exponent (Wolf *et al.* 1985), or from visual examination of phase space diagrams, (Mpitsos 1994). However the findings as they stand provide interesting information for the choice of class of model when modelling food intake and, if similar dynamics are found in other species, provide interesting prospects for further research, particularly into adaptive models of food intake.

The findings described above were for a group of dab, rather than individual fish, and it is quite possible that they could arise from a deterministic hierarchy, rather than from deterministic food intake. This is not necessarily the case, because the universality of scaling found in chaotic systems (if the systems are chaotic, Cvitanovic 1989) would mean that dynamics of the individual could reasonably be expected to be observed in groups. Unfortunately dab kept individually do not feed well, therefore this paper examines time series from both individuals and groups of *O. mykiss* (Walbaum). It is also possible that the observed dynamics are only found

in the dab. To get an idea of whether our previous findings are specific to the dab, the dynamics of food intake are also examined for groups of Sea Bass (*D. labrax*) and Whiting (*M. merlangus*).

(ii) MATERIALS AND METHODS

Data collection

Time series were generated using several different types of feeding apparatus, as data was obtained from several aquaria. However all shared the principal of rewarding fish with a fixed ration of food when the fish actuated hoppers, either by pushing a rod or trigger, or by swimming through an infra red beam. Luckily, for the purposes of time series analyses (and provided that a similar ration is dispensed at each actuation) it is not necessary to know the method of actuation or how much the hopper dispensed at each actuation, merely the number of actuations per unit time or the interval between actuations.

The data for groups of trout were generated at the Marine Science Laboratories, Menai Bridge, UK. In feeding preference trials, using demand feeding equipment described in Chapter 5, a group of thirty-six individuals was offered food from two hoppers, with two diets being alternated between the hoppers every eight days over thirty-two days. For the purposes of this study, this had the effect of increasing the area of any attractor, testing further the results observed above (Byrant 1992). The data for individual trout was generated at the Finnish Game and Fisheries Research Institute, Evo (Ruohonen unpublished). In order that each fish would perform enough actuations each day to allow time series analyses to be carried out, each actuation was rewarded with single, gelatin-encapsulated pellet of food (Ruohonen *et al.* 1997).

The whiting data was collected at Menai Bridge (Zeping unpublished). Fish had previously been trained to demand feed by Seyhan (Seyhan *et al.* 1998), using the equipment described therein (a prototype of the equipment used for the group of trout in this paper). Again, for the purposes of a feeding trial, four different diets were offered to the fish in sequence, all of which were subjected to the analyses.

Finally the sea bass data was donated by the INRA, St Pée, France (Boujard *et al.* 1996). This data was presented to us in the form of amount of food demanded per hour. As the data set had several consecutive hours where no food was demanded each day (because the fish ate fairly discrete meals), the data was clumped into two hour intervals in order to avoid the long strings of zero values (a straight line) being interpreted as one dimensional. This halved the length of the data set. Because the time series were generated for other research purposes, we must assume that environmental conditions were not always kept as constant as they were in the previous Chapter. However this, together with the different techniques for collecting the data, enable a judgement to be made about the robustness of the dynamics observed.

Analyses

Analyses used were described in the previous Chapter.

(iii) RESULTS

Groups of trout.

Data, recorded as the time between each demand for food (interval data) was used in all cases, as this provided longer data sets for the analyses.

Some signs of determinism were observed when the amount eaten (m) was plotted as a function of the amount eaten in the two previous hours (m-1, m-2). This was in the form of a 'crucifix', although the majority of points are found to be in a central core area, due to the scaling (Figure 6.1).

Recurrence diagrams revealed obvious structure, both for each hopper separately and for the two hoppers combined (Figures 6.2 to 6.4). For the individual hoppers a slope of less than 45° was observed, indicating low dimension, with vertical elements of the pattern indicating chaotic behaviour. For two hoppers combined the pattern was slightly more complex, with two separate slopes apparent (hold it at arms length). The phenomenon was not apparent in the individual hoppers, even though two diets were alternated with time in each hopper, therefore the change of diet does not appear to change the topography of the time series.

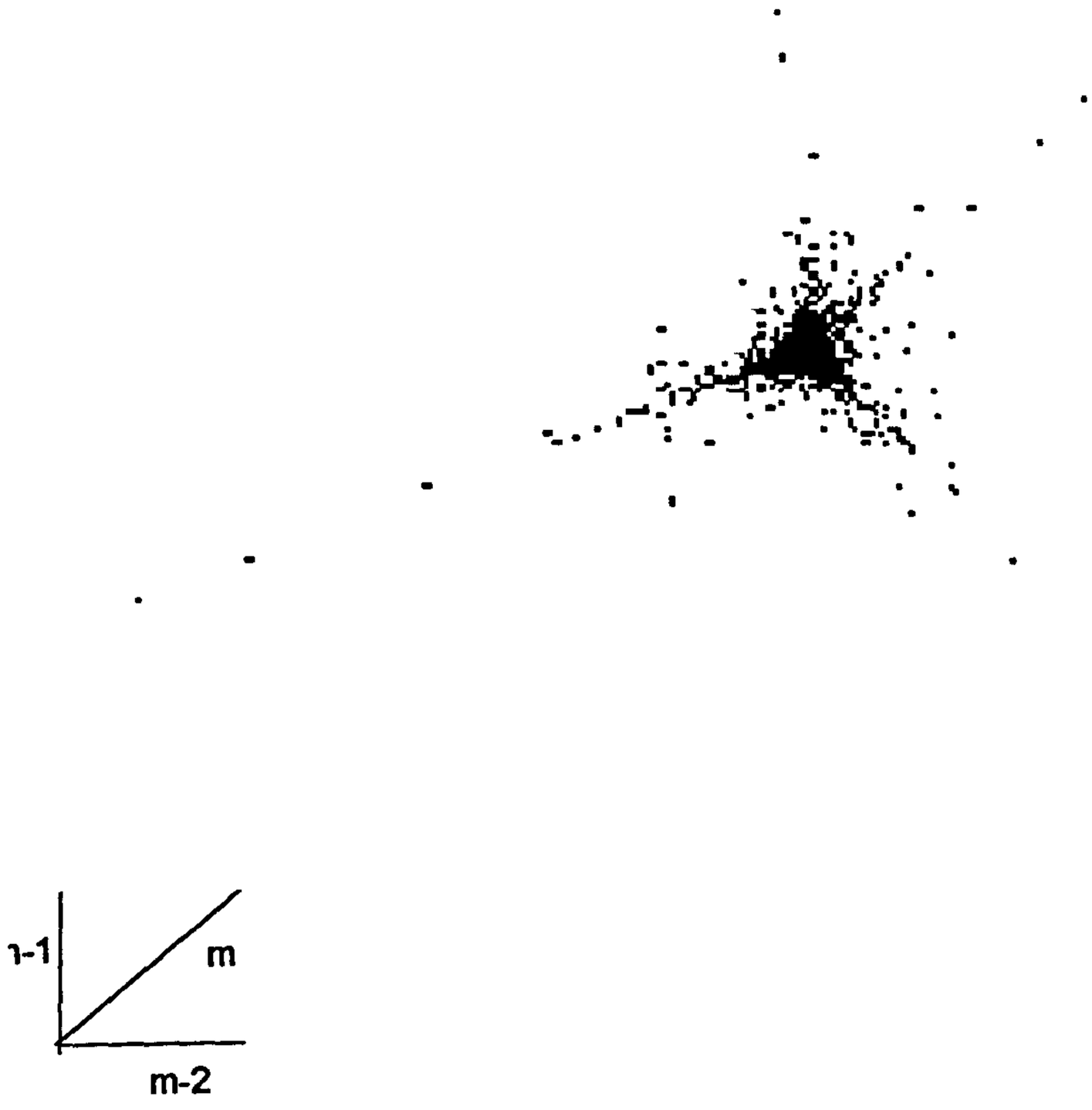


Figure 6.1 3-D plot of differenced data, groups of rainbow trout.

	Interval data	R^2	Maxima	R^2
Group of trout	0.000	0.57	0.000	0.66
Individual trout	0.001	0.45	0.002	0.44

Table 6.1 Rosenstein's Lyapunov exponent for groups and individual trout. The results are strikingly close to zero.

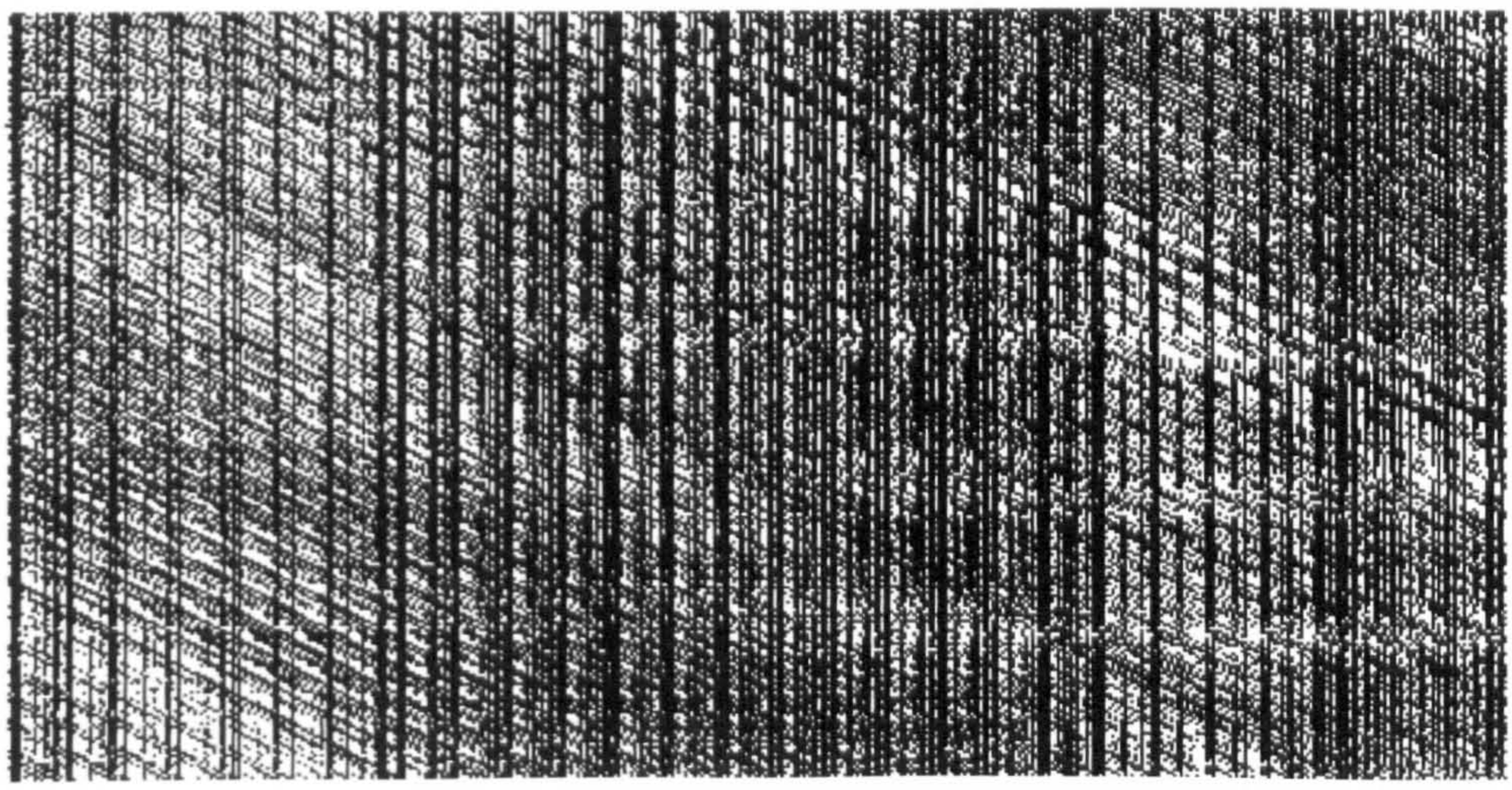


Figure 6.2 Data from hopper one, for the group of trout. Diagonal lines indicate a dimension of approximately two, vertical breaks are typical of chaotic dynamics.

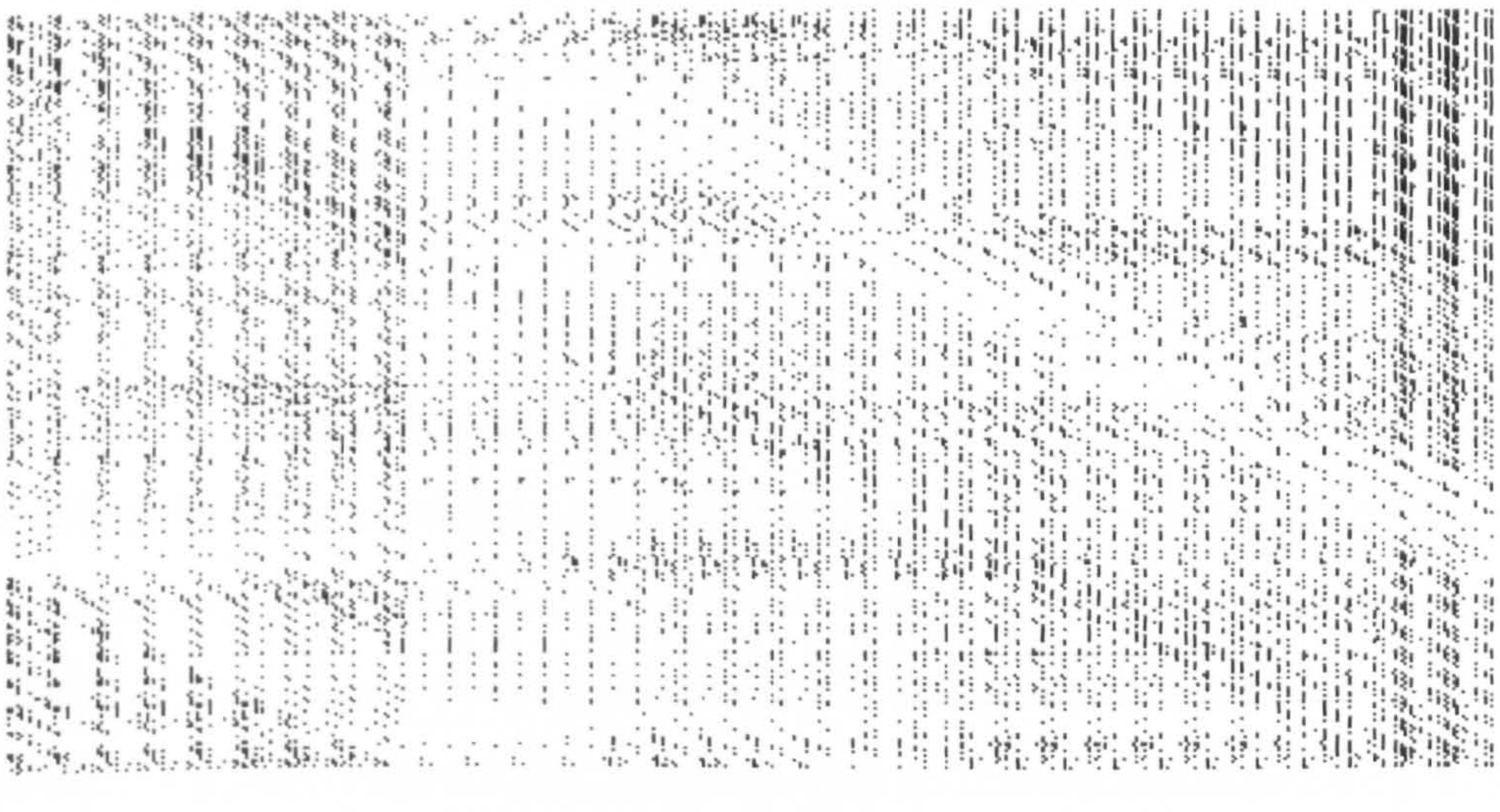


Figure 6.3 Data from hopper two, group of trout, very similar to that of hopper one. The shorter data set is reflected in fewer points in the recurrence plot.

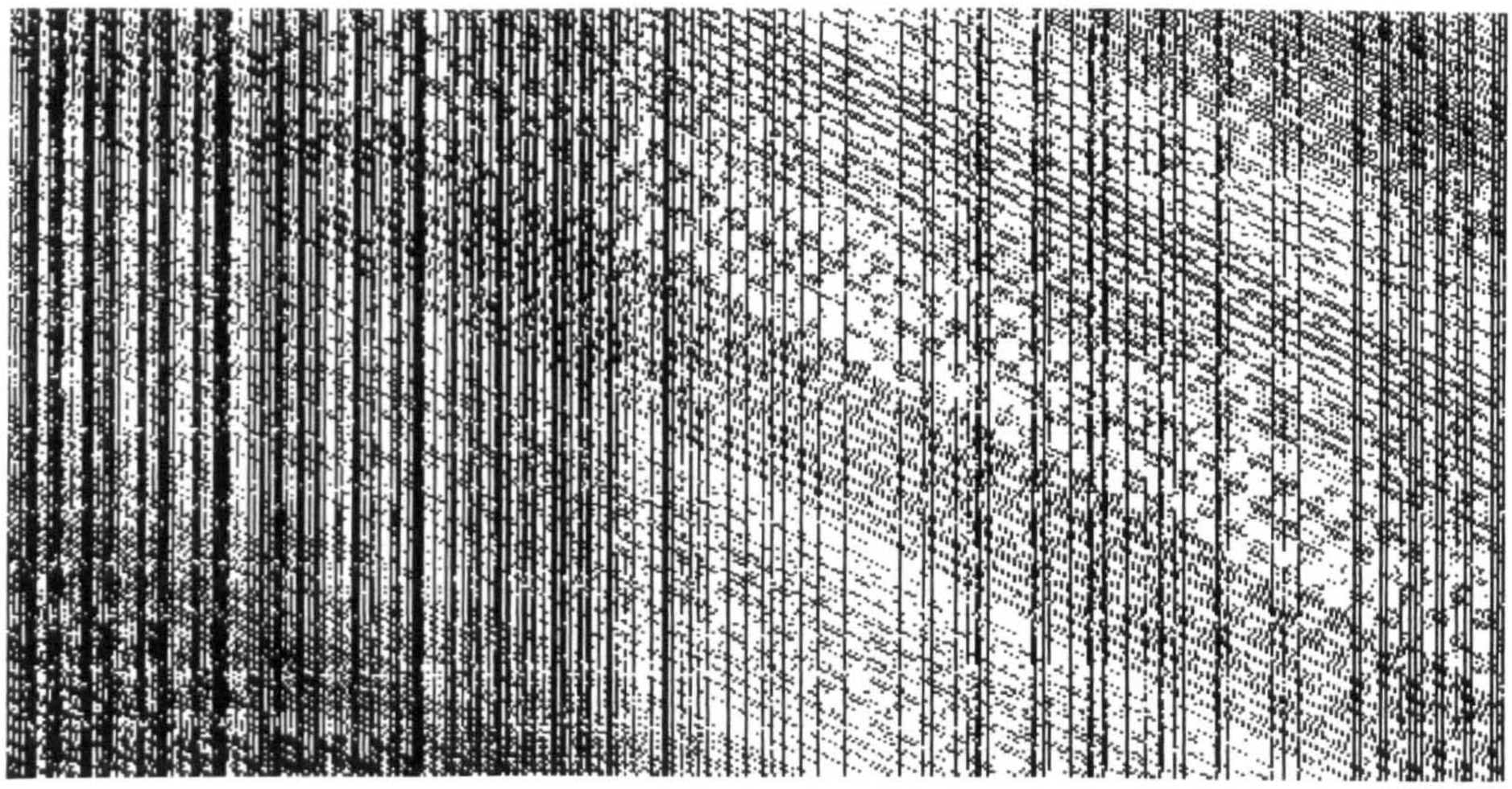


Figure 6.4 Recurrence plot of the two hoppers combined. Here there are two diagonal patterns, the second occurs since two different time series are combined and the resulting dimension is greater.

Using the interval between each actuation, the PD2i was calculated. This was similar for the data recorded from the two individual hoppers (although hopper two had fewer actuations over the course of the experiment) as well as for the data of the hoppers combined. The mean values range from 3.8 to 4.1 (Figures 6.5 to 6.7).

D2 was found to stabilise at a dimension of around 1.5 to 2.0 and surrogates were significantly different, suggesting the dynamics are non-linear (Figure 6.8).

The Lyapunov exponent did not stabilise as the embedding dimension increased, remaining only slightly positive (Figure 6.9). Nor did it stabilise with increasing number of points (Figure 6.10). Unfortunately no definite conclusions could be drawn from this analysis. Rosenstein's Lyapunov exponent, developed to allow examination of shorter, noisier data sets, was found to be close to zero for both hoppers combined, as well as for the maxima data taken from the first data set (Table 6.1). However in both cases R^2 was low, (the method of Rosenstein *et al.*, 1993, does not calculate the coefficient of determination).

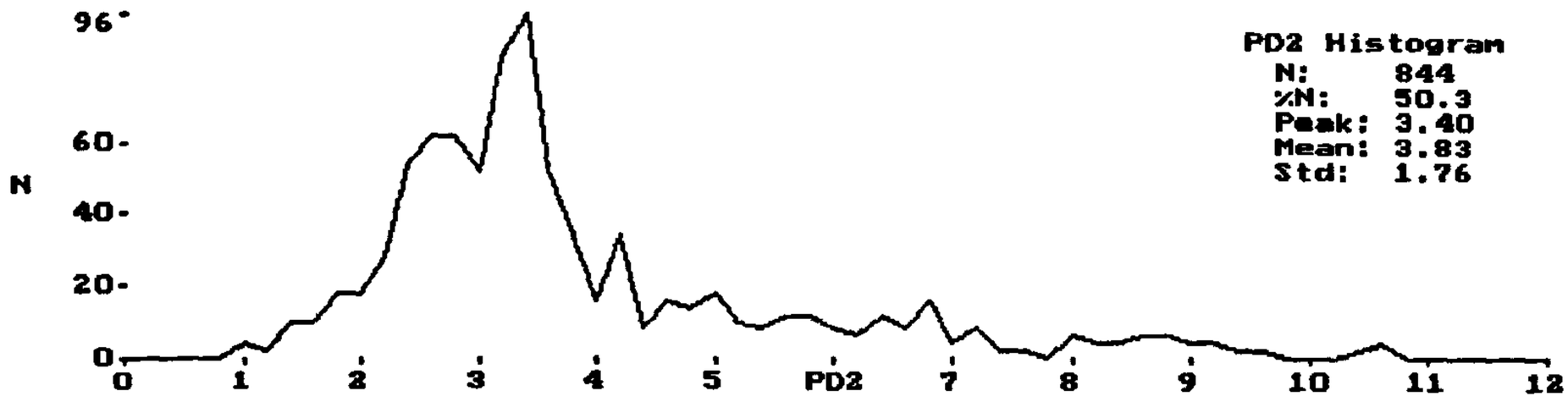


Figure 6.5 PD2i histogram for the time series of actuations for hopper one, clearly showing that the dynamics are low dimensional.

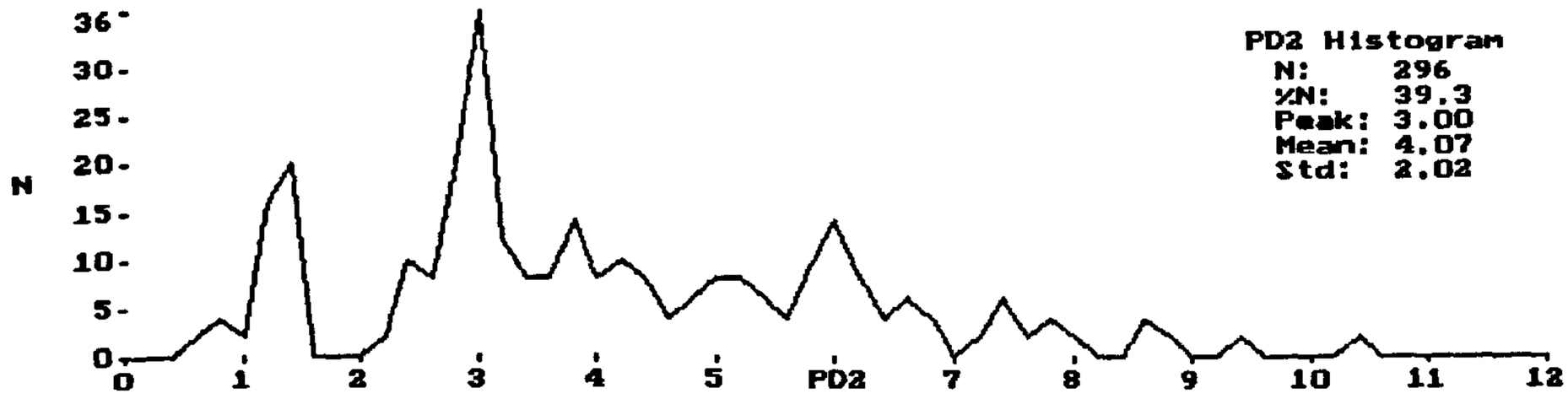


Figure 6.6 PD2i histogram, showing similar results in hopper two to those in hopper one, although the data is more noisy, probably as a result of the fact that the data set is shorter than that for hopper one.

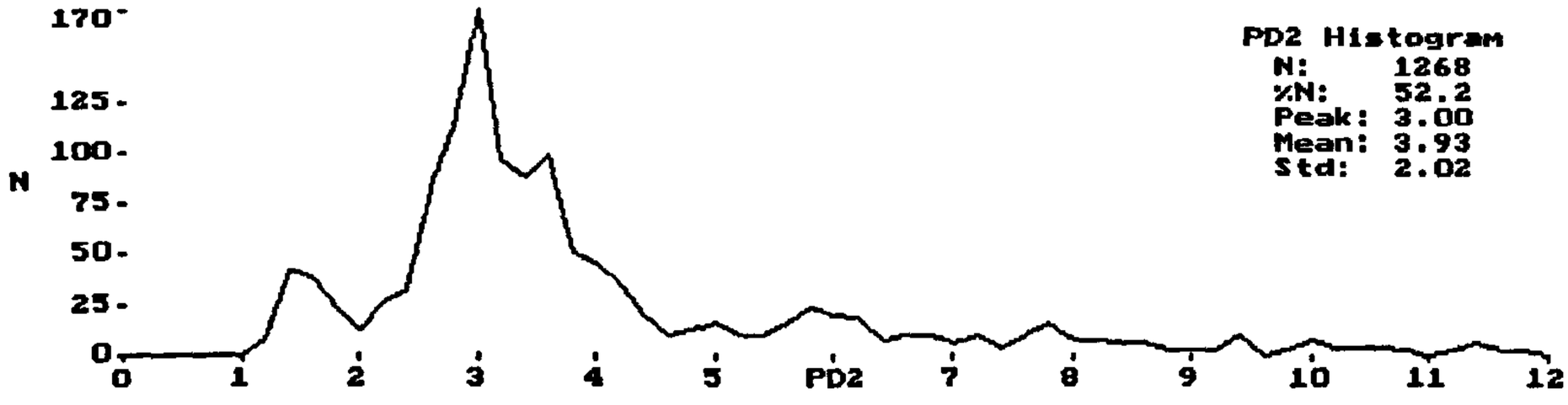


Figure 6.7 PD2i histogram of the two hoppers combined, clearly very similar to the histograms for individual hoppers.

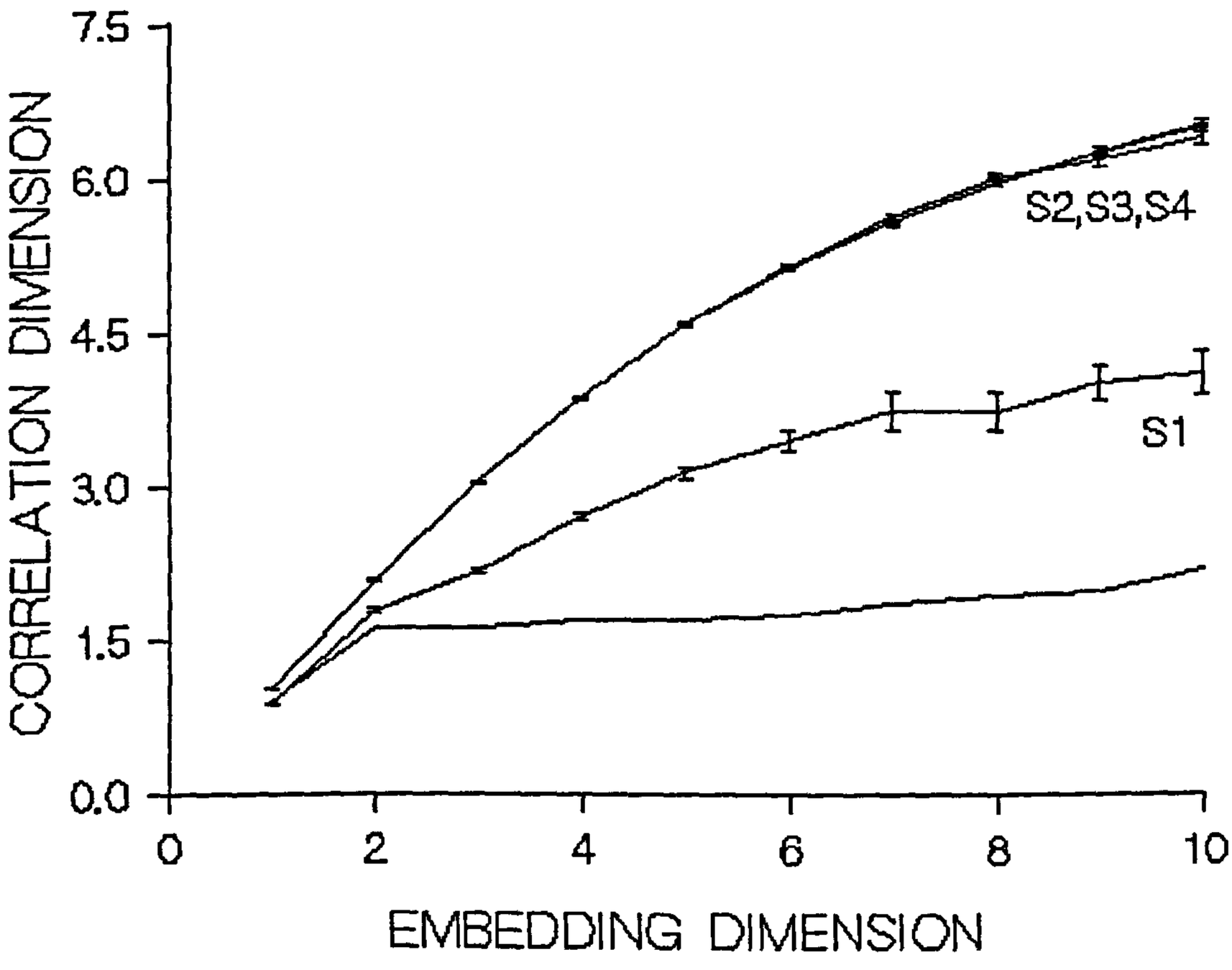


Figure 6.8 Correlation dimension in a group of trout (solid line), with comparisons against surrogates. CD is significantly lower than all surrogates, suggesting non-linearity.

S1= Randomised phase data.
S2= Independent and identically distributed (IID) random variables.
S3= Non-IID uncorrelated gaussian noise.
S4= Static nonlinear transform of linear gaussian noise.

Individual trout

As in the groups of trout, some form was observed for individual trout when the return map was examined. This shape was similar to the portrait found for groups of trout (Figure 6.11). Recurrence diagrams were examined for the four data sets of individual fish placed one after the other (the series data set), this was carried out after the value of the time delay (τ) was found to be similar for all four data sets (Figure 6.12) The single longest data set was also examined alone (Figure 6.13). Both cases showed similar results to groups of trout. The individual fish data was rather short for this analysis, and therefore the recurrence diagram was not as dense as others observed so far.

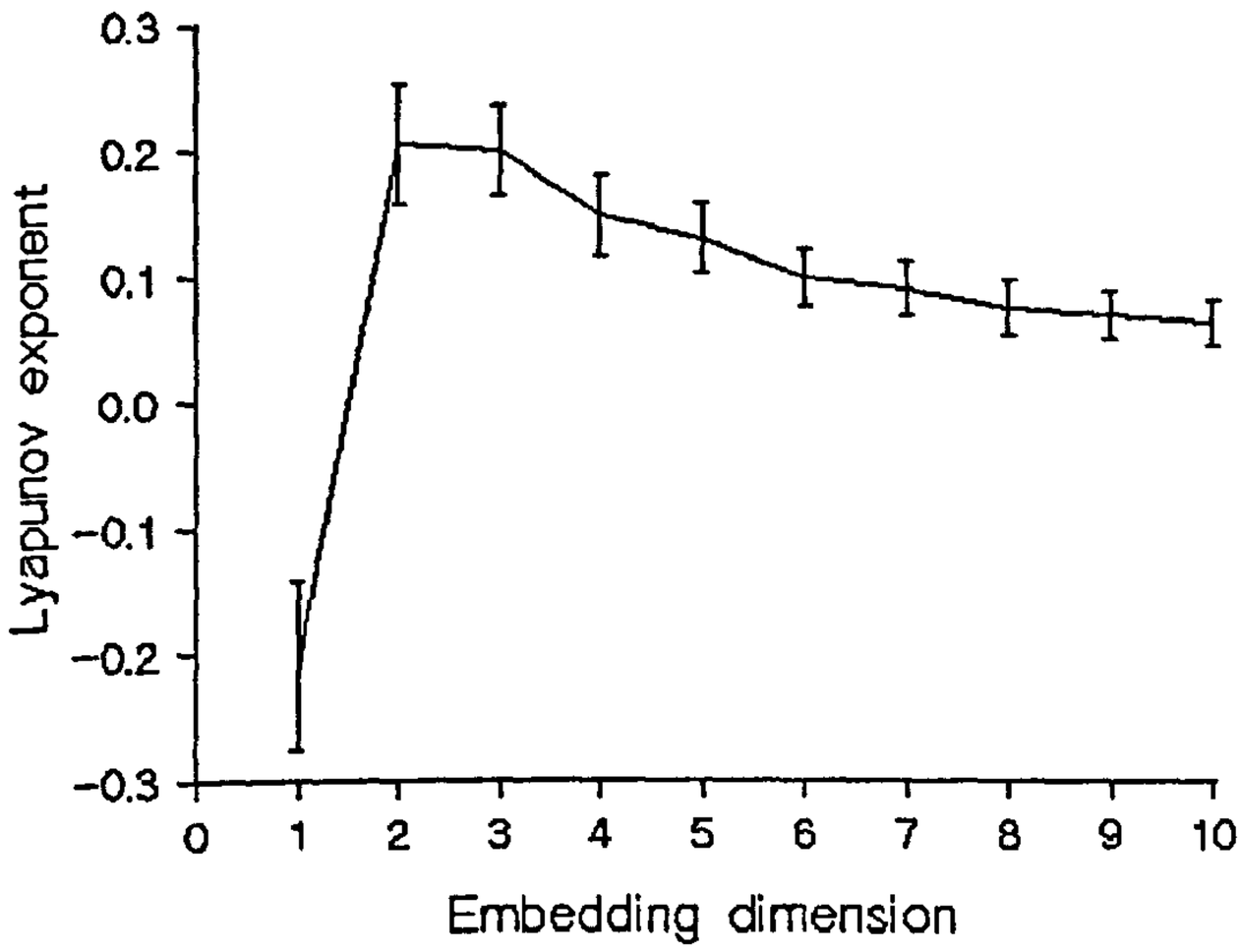


Figure 6.9 Change in Lyapunov exponent with increasing embedding dimension for the group of trout. The value fails to stabilise, so it is not possible to state whether food intake dynamics of trout are chaotic or periodic.

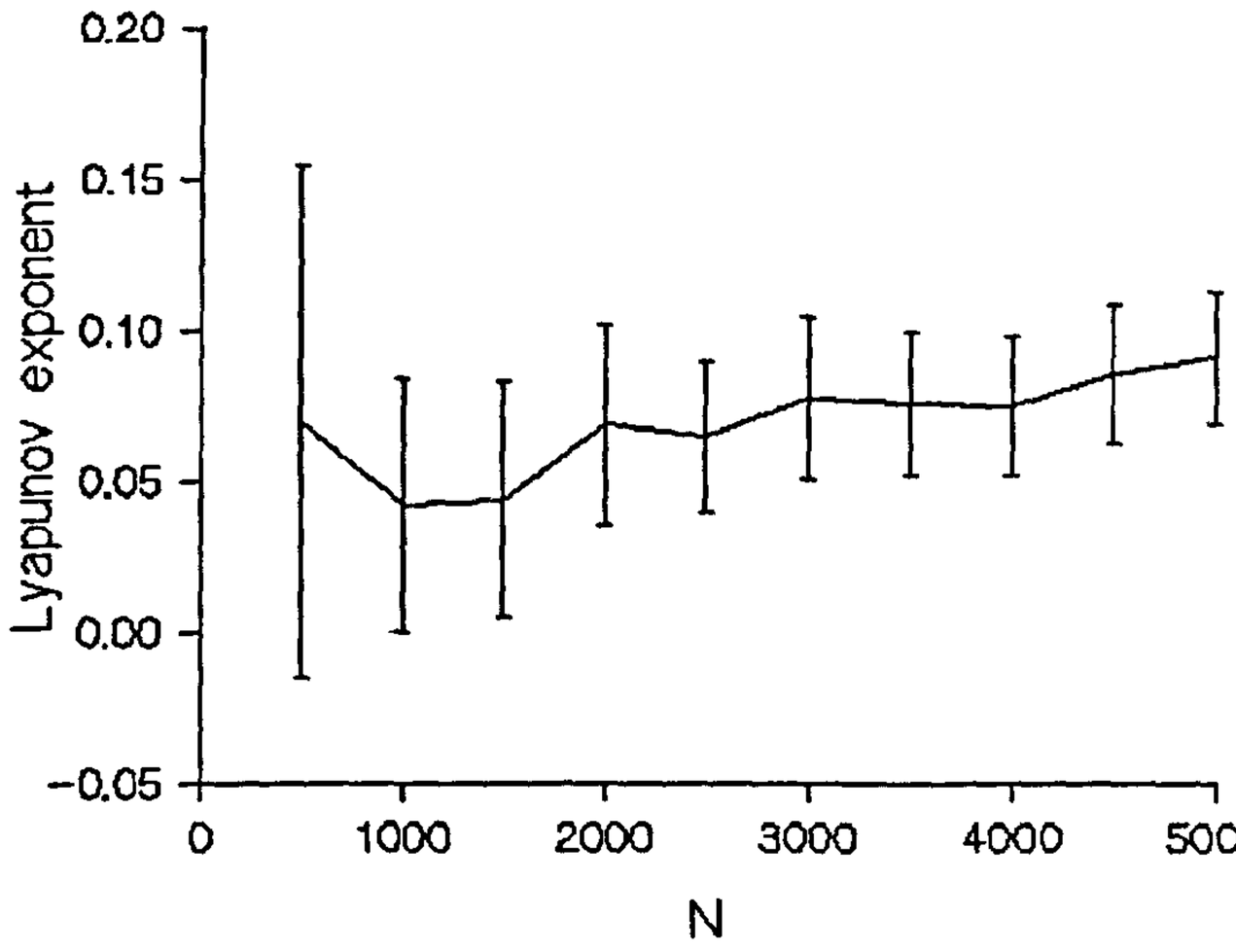


Figure 6.10 Change in the Lyapunov exponent for the group of trout with increasing no. of data points, again the LE fails to stabilise so no conclusions can be drawn.

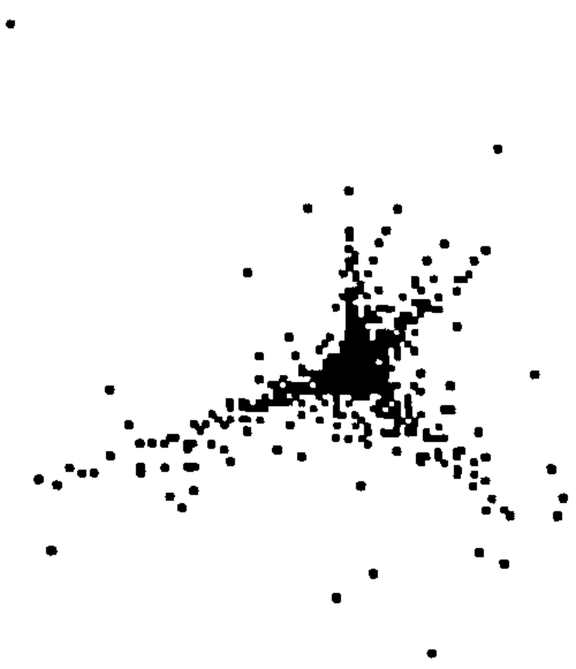


Figure 6.11 3-D image of differenced individual trout data, showing very similar topography to the same image for groups of rainbow trout.

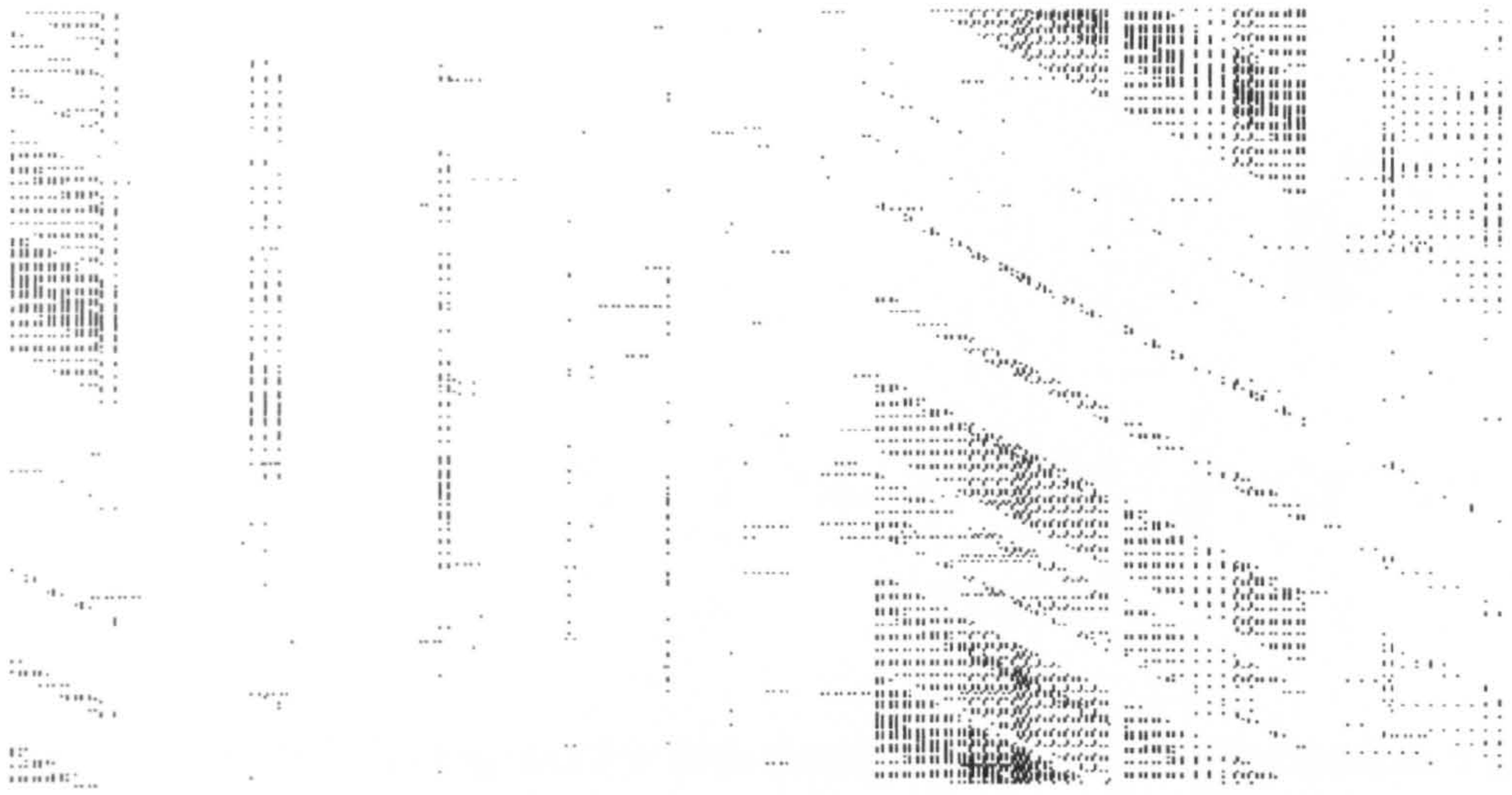


Figure 6.12 Recurrence diagram of four individual data sets of trout, generated from individual fish, pasted sequentially.

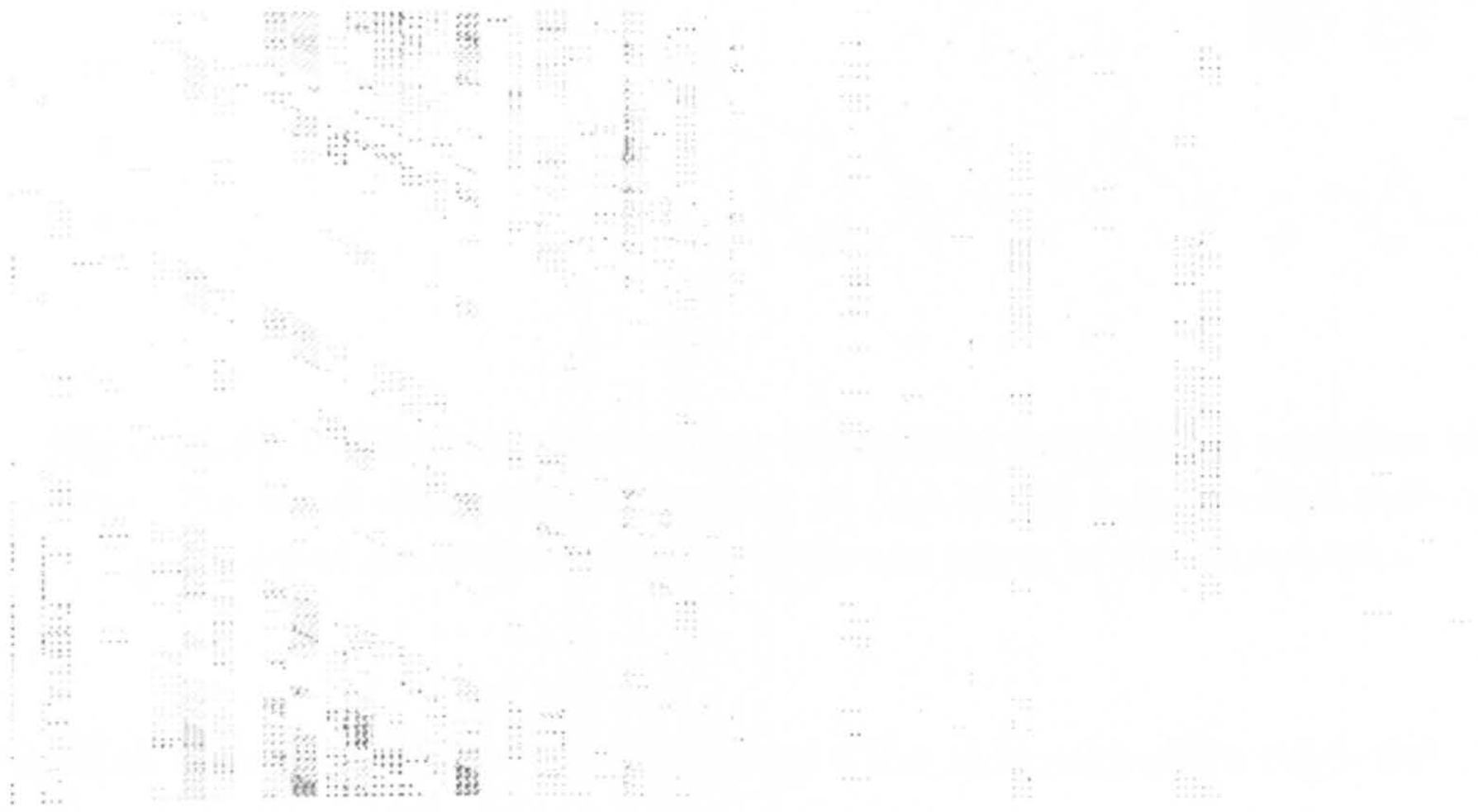


Figure 6.13 Recurrence diagram for a single trout. The short data set leads to a rather sparse pattern, but the topography appears very similar to the time series produced by a group of rainbow trout.

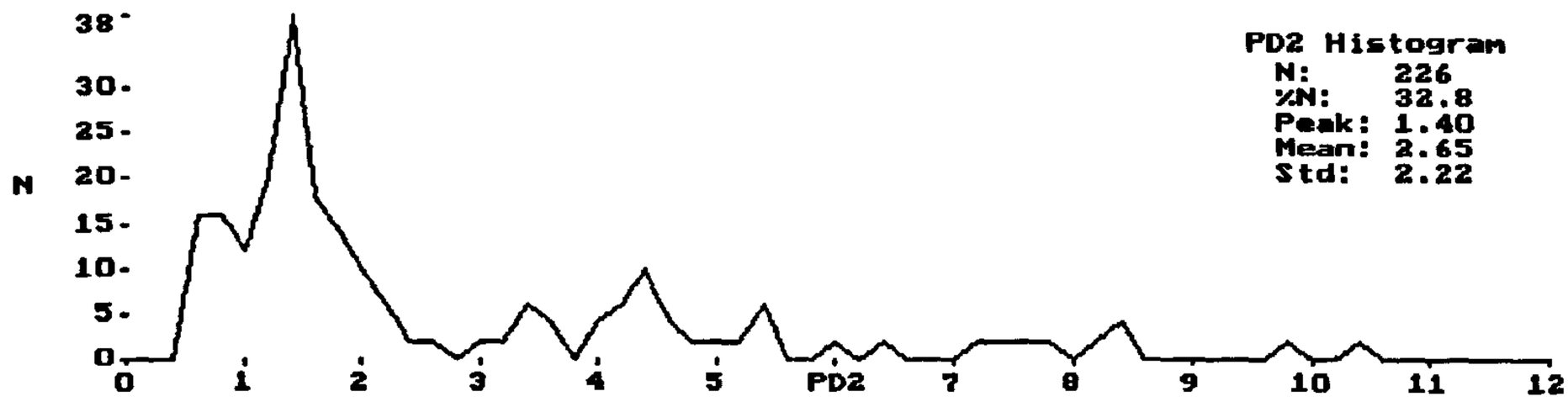


Figure 6.14 PD2i histogram for individual trout data, dimension has a peak remarkably similar to dab, although a few minor peaks at higher dimensions increase the mean value.

Figure 6.15 PD2i histogram of four individual fish, placed one after the other. The mean dimension increases, as one would expect when different generators are responsible for different parts of the time series.

The PD2i histogram (Figure 6.14) indicated a low dimension for a single fish. The series data set had a higher dimension (Figure 6.15), with a tail indicating some noise or randomness, although this was a small fraction of the area under the histogram. This was probably the result of combining data.

D2 was also calculated from the series data set, and stabilised at a dimension of around 2.5. The phase-randomised surrogate data set, generated by randomising the experimental data, was only significantly different from the raw data set at an

embedding dimension of 6 and above, after which they were significantly different (Figure 16). Again this is probably a result of combining data from different individual fish. In spite of the closeness of the two curves at lower embedding dimensions, the apparently structured topography observed in a return map, and the <45° slope in the recurrence diagrams (including that from a single fish) indicate that the system is deterministic and low-dimensional.

Following the failure of the Lyapunov exponent to stabilise for the group of rainbow trout, only Rosenstein's Lyapunov algorithm was carried out on the shorter individual *O. mykiss* data sets; we did this on the series data set, and on the maxima of this data set. Again we found the data to approximate zero in both cases, though the values of R^2 are small (Table 6.1).

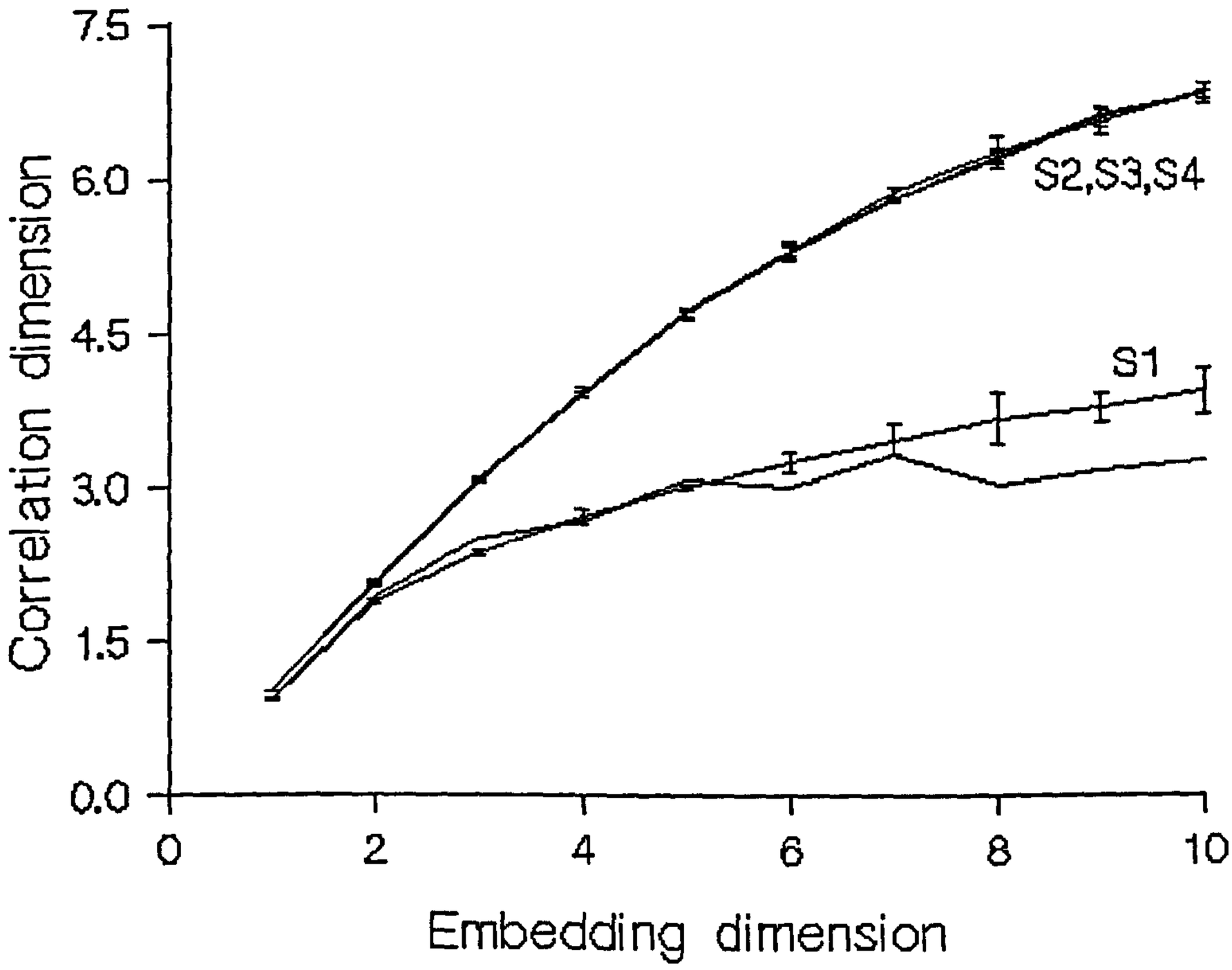


Figure 6.16 Comparison of raw data (solid line) with surrogate data sets in individual trout. Note the similarity between the raw data and the randomised phase data (S1) at low embedding dimension. (not deterministic) The difference between the two only becomes significant above d= 5. This suggests that by combining the four data sets, one produces a much noisier time series. S1= Randomised phase data, S2= Independent and identically distributed (IID) random variables, S3= Non-IID uncorrelated gaussian noise, S4= Static nonlinear transform of linear gaussian noise.

Sea bass

Because the data for sea bass was not available as interval data, and the bi-hourly data sets were rather shorter than one would like for the analyses carried out above, a more limited analysis was carried out.

This data had a rather less obvious shape in phase space (Figure 6.17). Recurrence diagrams again exhibited diagonal patterns, indicating determinism, with a dimension of approximately 2 (Figure 6.18). The mean PD2i was around 5, higher than the other species examined, although a large peak around 3.4 was observed (Figure 6.19). The D2 appears low (Figure 6.20) although it does not stabilise, indicating noise in the data set. Surrogates were all significantly different from the raw data, therefore it would seem that the time series is non-linear.

Whiting

Data for whiting was only available in the form of actuations per hour and was limited in length. As in the sea bass, examination of the time series in phase space revealed no obvious determinism (Figure 6.21), however the recurrence diagram contradicted this, being very similar to those found in hopper one of the group of rainbow trout (Figure 6.22), suggesting low dimensional determinism. The mean PD2i was 2.5 ± 3.1 SD (Figure 6.23), similar to the trout group data sets. The SD was large because of a small amount of noise. The D2 was also of low dimension, although it never quite stabilises. It was significantly different from the linear surrogates against which it was tested, proving that the data is non-linear (Figure 6.24).

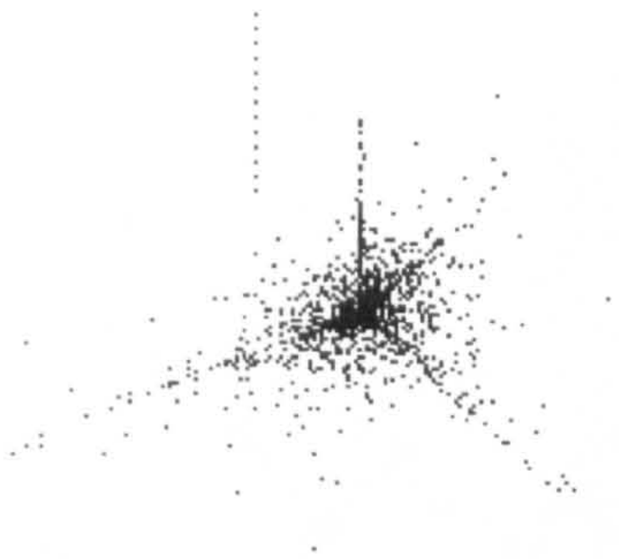


Figure 6.17 Three dimensional return map of food intake in the sea bass, with less obvious determinism than in trout.

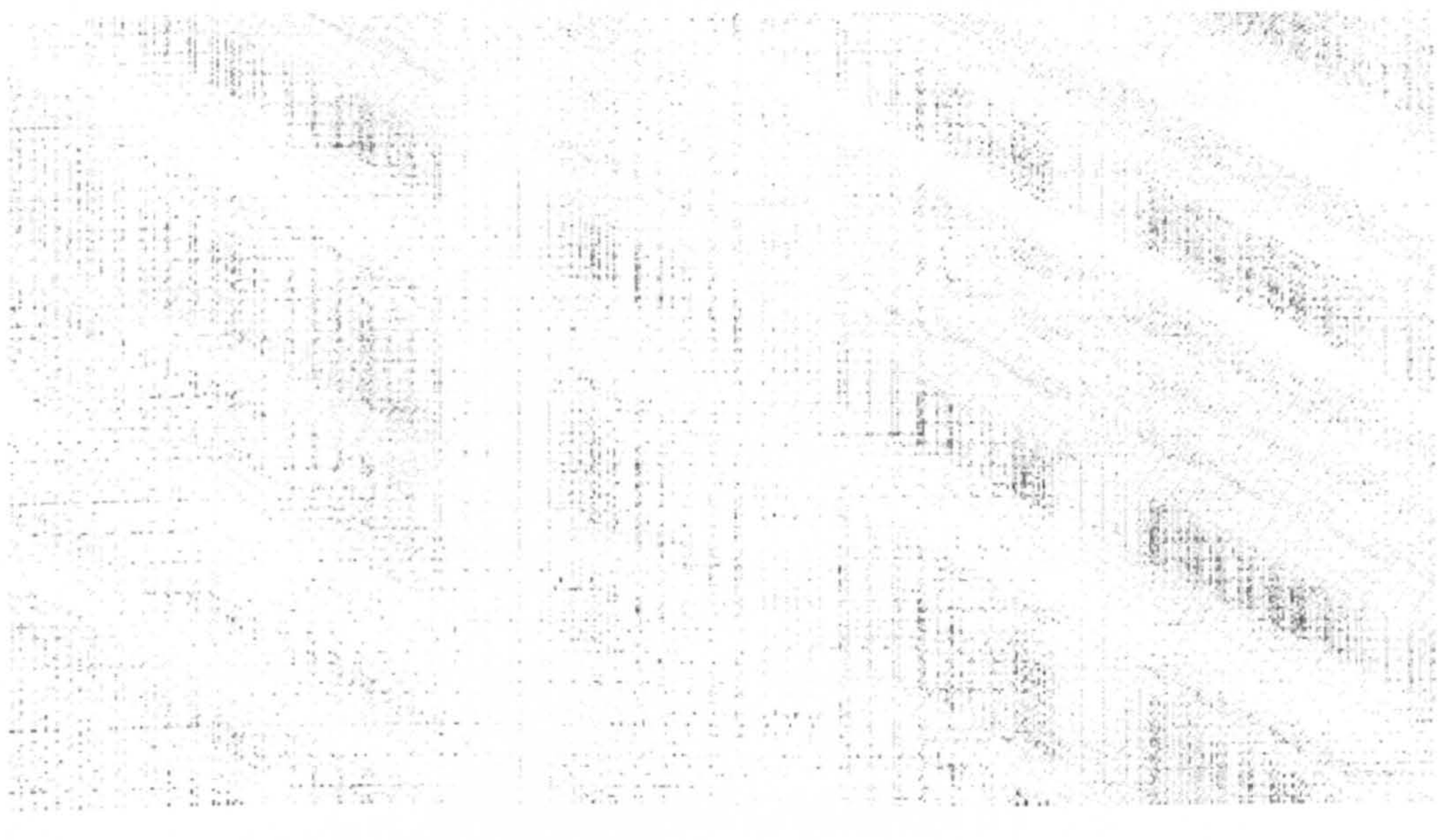


Figure 6.18 Recurrence diagram of sea bass time series, diagonal bands indicate a dimension around 2. Vertical breaks in the pattern are typical of a chaotic time series.

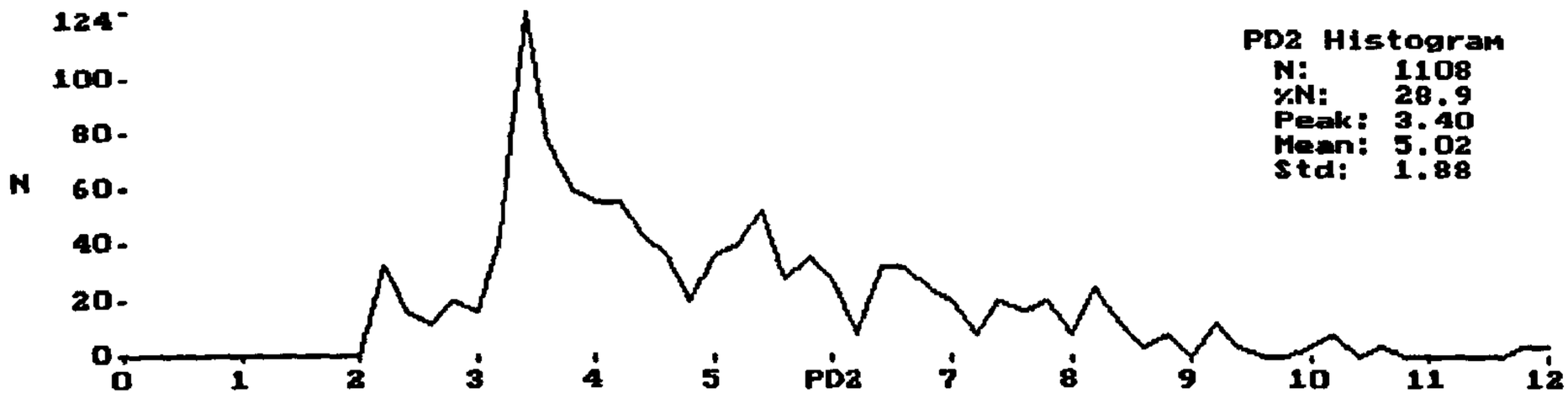


Figure 6.19 Histogram of time series of sea bass, four different data sets added together. The negative binomial distribution indicates that the system has some noise in it, although the main peak is fairly clear, around 3.4.

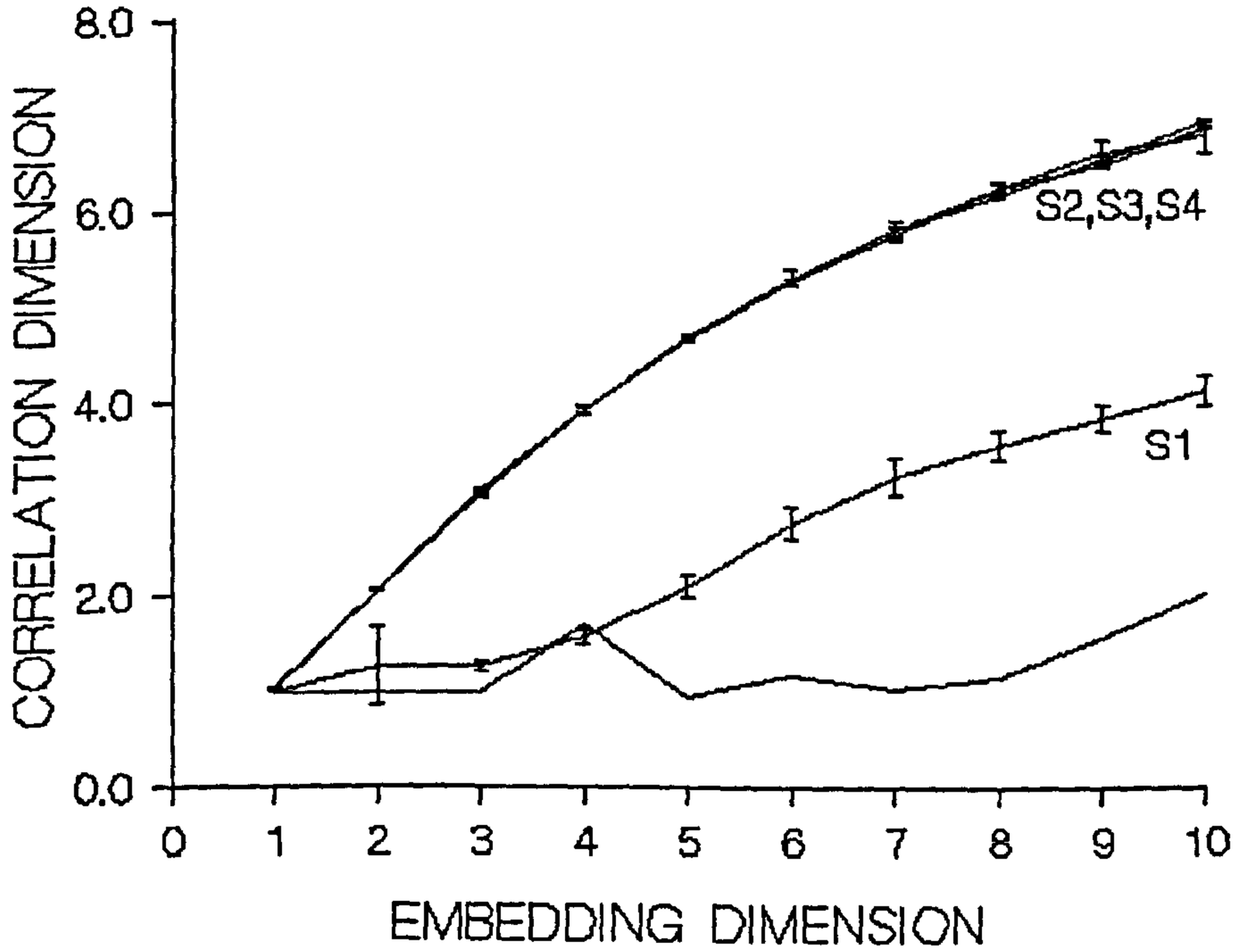


Figure 6.20 Correlation dimension of raw data (solid line) compared with surrogates for sea bass, clearly there is a significant difference, although the failure of the CD to stabilise indicates some noise in the system. S1= Randomised phase data, S2= Independent and identically distributed (IID) random variables, S3= Non-IID uncorrelated gaussian noise, S4= Static nonlinear transform of linear gaussian noise.

Figure 6.21 3-dimensional return map of food intake for whiting, showing no obvious determinism

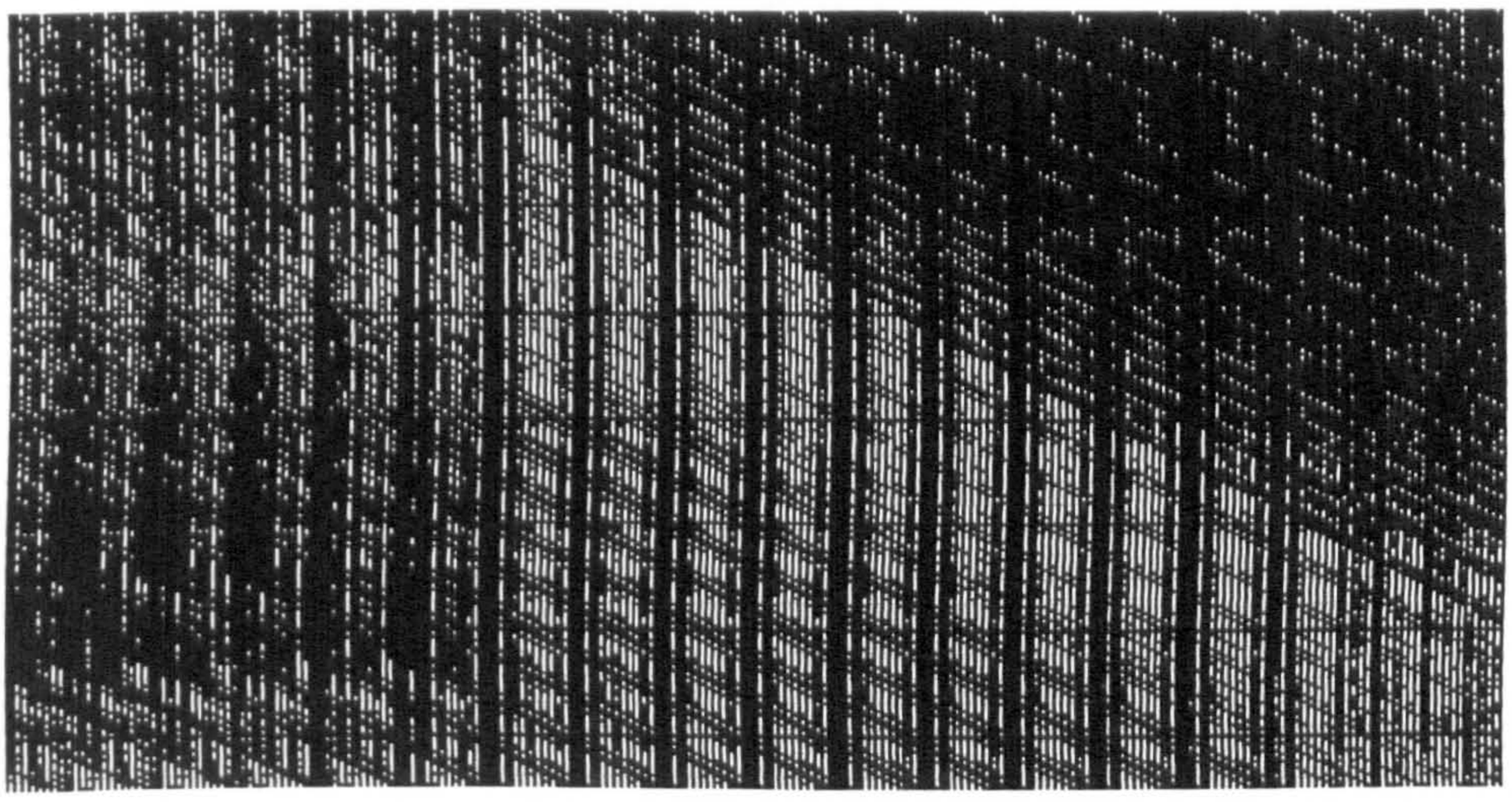


Figure 6.22 Recurrence diagram for the whiting data. diagonal bands with a slope of about 45^0 indicate a dimension around 2. Vertical breaks in the pattern are typical of a chaotic time series.

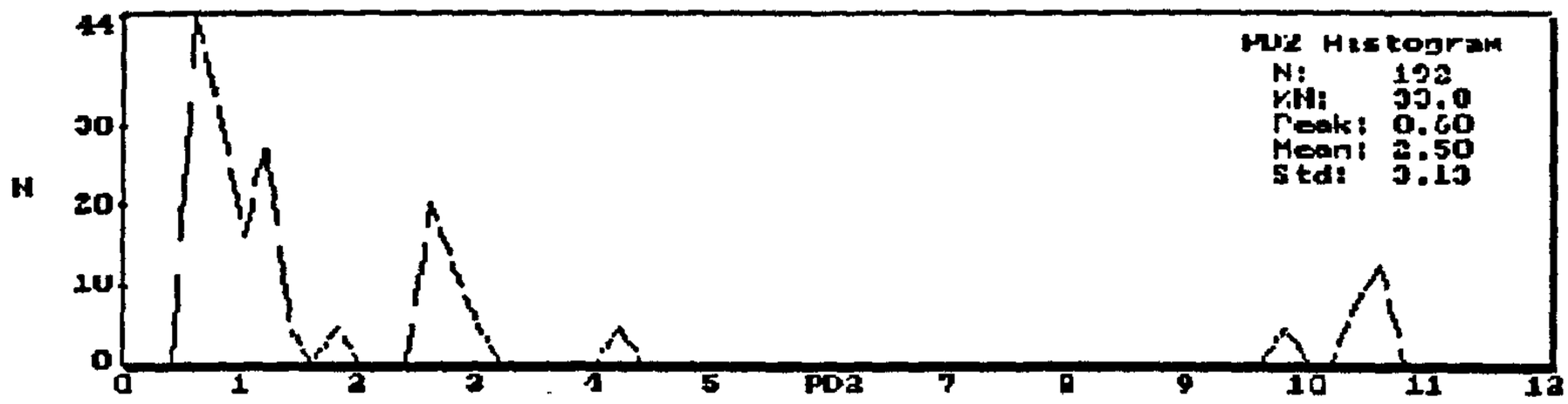


Figure 6.23 PD2i histogram for the whiting data. Most of the data is around 1-dimensional, although a small amount of noise increases the average dimension

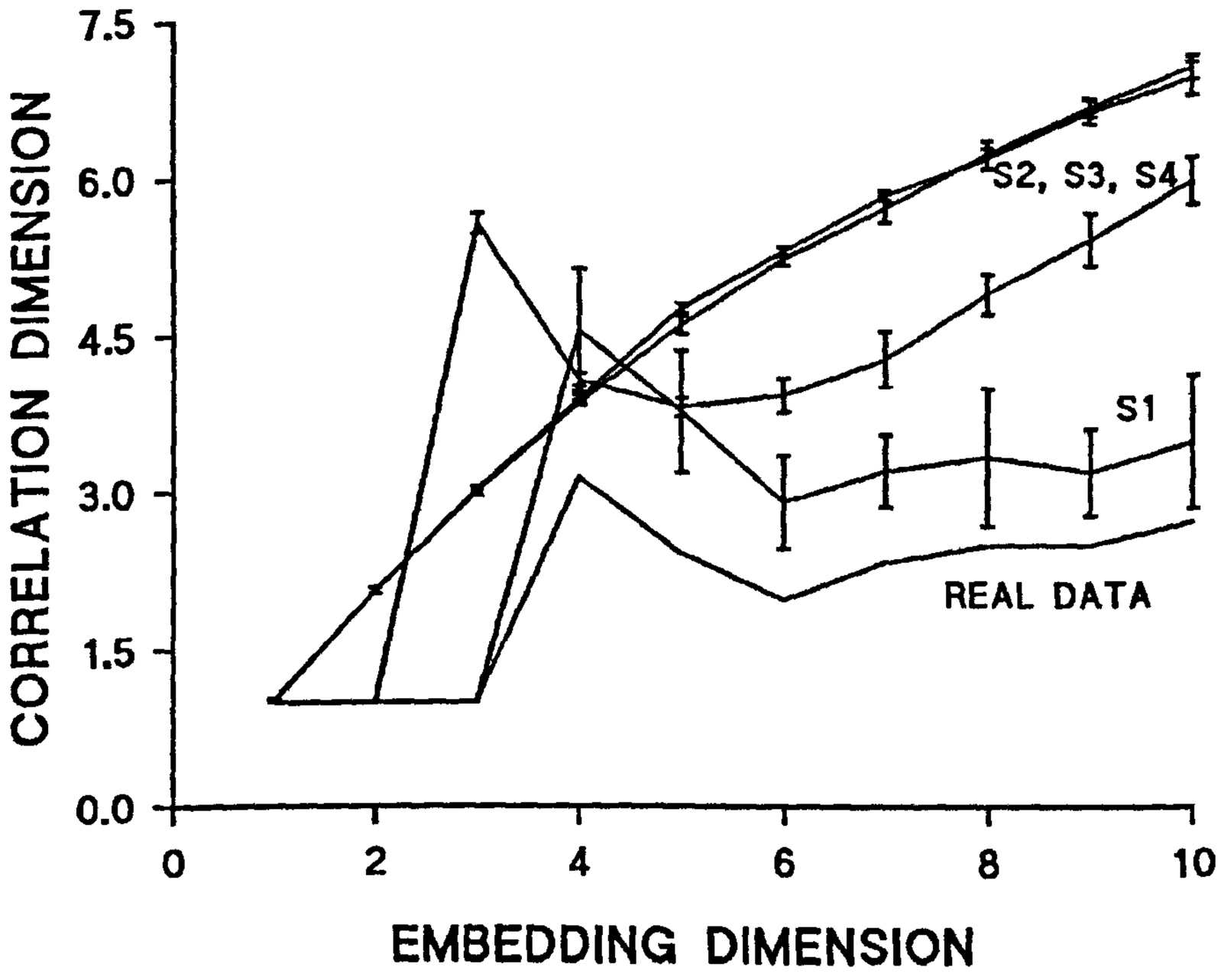


Figure 6.24 Correlation dimension compared with surrogates for whiting. Whilst the randomised data follows the trend of the real data, there is still a significant difference. Thus the data is significantly nonlinear, though noisy. S1= Randomised phase data, S2= Independent and identically distributed (IID) random variables, S3= Non-IID uncorrelated gaussian noise, S4= Static nonlinear transform of linear gaussian noise.

(iv) DISCUSSION

As in the dab, food intake in the trout is a deterministic, low dimensional, non-linear system. There appears to be no difference between the dynamics of food intake in individual fish and in groups of fish, although individual fish time series, when placed in sequence and then analysed, had a higher dimension and appeared more noisy than the data from both a single fish and from the group (as indicated by the tail on the PD2i histogram in Figure 6.14).

The observed time series topography in groups of dab is therefore not necessarily the effect of hierarchical interactions, but could be instead an expression of the principle of universality of scaling, a typical feature of which is self-similarity at different levels of organisation (see Cvitanovic 1989 for a discussion).

In the group of trout the extra, slightly steeper slope observed in the recurrence diagram for the two hoppers combined (Figure 6.4) indicated that summing the data from the hoppers results in a compound dimension (Skinner 1994a), and therefore that the two data sets have different generators. Landless (1976) found that, whilst the dominant fish is able to move freely around a tank the near dominants are not, having instead large territories. Therefore it is conceivable that the two hoppers are actuated at least partly by different fish and that for each hopper the combination of individual fish would result in different attractors. If this were the case then hierarchical interactions may have a modifying effect on the dimension of the time series. (This would also explain the increase in dimension seen when data from four individual fish was placed in series). In contrast changing the diets with time in a single hopper had no effect on the topography of the time series, suggesting that for a single group of fish different diets may all fall on the same attractor.

Similar dynamics were found for sea bass and whiting although the former appeared rather noisy. This is quite remarkable as the data was collected in several different ways, without strict environmental controls. Given that rhythms of food intake can be influenced by exogenous as well as endogenous factors, one would expect that the influence of external variables would be apparent in the data, which is after all the sum of the different rhythms influencing food intake. This suggests

once again that the influence of environmental variables must be expressed *via* a small number of variables which alone have **direct** influence on food intake.

CHAPTER SEVEN: SUMMARY AND CONCLUSIONS

The idea that the stomach volume has a role in the regulation of food-intake has long been established, where stomach fullness leads to meal termination (Vahl 1979, Fletcher 1984, Jobling 1986a). Frequent observations that the gastric emptying curve correlates with appetite return (*e.g.* Grove *et al.* 1977) and that GET is similar to voluntary meal interval in demand-fed fish (*e.g.* Gwyther and Grove 1981) have been cited as evidence that fish feed to stomach fullness. Studies in Chapter 2 demonstrated that in the case of the dab, food intake was only limited by the stomach volume when they were fed on a low-energy diet at low meal frequencies, where the latter resulted in a high systemic need. This was exemplified using three models to simulate food-intake data, with the stomach's role differing in each. In the first, where fish fed to stomach fullness all the time, food-intake rapidly became a constant value. This was clearly not the case in any of the experiments. The second model assumed that stomach volume was never limiting, behaving in a broadly similar manner to the data for fish fed a high-energy diet and to fish fed high meal frequencies. Finally, in the third model, food intake was assumed to be mainly regulated by systemic need, only being limited by the stomach volume when systemic need was high. In this case food intake patterns when the meal sizes were low behaved in the same way as model 2. When food intake was consistently high the third model behaved as in model 1, however when systemic need varied and a mixture of large and small meals were taken, the large meals were constrained to a single (1-dimensional) line, where feeding was a function of stomach volume and stomach content from the previous meal. Thus all three models had specific predictive characteristics which could be looked for in feeding studies. Examination of the real data showed that stomach volume was not limiting in fish fed either a high- or low- energy diet when they were fed every 24 h or less. However when meal interval increased to more than this for the low energy diet, stomach volume was occasionally a regulatory factor limiting intake, as found in model 3. The fact that stomach volume is not limiting in

most cases does not mean that distension signals from the stomach (Jobling 1986a) cannot have a role as a satiety signal.

In Chapter 3, dab were shown to be able to adapt their stomach volume to accommodate the volume of their diet; they did this by changing the distensibility of the stomach. This differed from findings in rainbow trout, whose stomachs grew in response to bulkier diets (Ruohonen and Grove 1996). This suggests that, when fish are feeding on a low-energy diet and have a high systemic need so that intake is regulated as in model 3, the fish will be able to increase their stomach volumes until they are once again regulating intake according to their systemic need only, as in model 2. An experiment to test this hypothesis would be quite straight forward, and is certainly worth attempting.

Stomach volume in the dab shows both inter- and intra- individual variation, the latter occurring in response to dietary volume (which in turn is a response to diet quality in this case, although feeding frequency has been found to have an effect in other species *e.g.* Ruohonen and Grove, 1996). Similar results have been found in other species and therefore it may be that this is a general feature of the teleost stomach.

Another outcome of this work is that stomach volume is a function of the technique used to measure it. It is not possible therefore to make observations such as have been made in the past about the volume of different species of fish, for example that 100g dab have a stomach volume of 8 ml (Jobling *et al.* 1977, Basimi and Grove 1985a).

Very often, in diet manipulation studies, when one ingredient is changed another characteristic of the diet must change to accommodate it, as a result it can prove very difficult to determine which aspect of the diet the fish are adapting to and which are unimportant. In Chapter 4, path analysis showed that dab appear to be able to adapt to both changes in the level of each ingredient *per se*, as well as to the energy content of the diet. This fits with the observation that satiety-inducing CCK is stimulated in the duodenum by nutrients in the diet (Jobling 1986a and references therein) , and satiety is also signalled by neuronal input related to energy metabolism in the liver (Friedman *et al.* 1999).

It would be of great interest to attempt to produce a model of food intake that explained the ability of dab to adapt their food intake to novel diets.
Whilst there is some information in the literature regarding the physiological control of food intake, there is little information on the dynamics of food intake, and an exploratory study of these was carried out on the dab (Chapter 5). Because food intake was highly variable from day-to-day, such data was amenable to time series analysis. Time series were discovered to be unpredictable and sometimes period-3; they were also observed to be deterministic, non-linear and low-dimensional. These results infer that food-intake dynamics are either non-stationary periodic, or are chaotic or on the edge of chaos; a complex dynamical system. It was not possible to determine without a doubt whether food intake in this species was chaotic or not, due to the noisy nature of biological data. However the data was of a type which would allow the future development of an adaptive model of food intake through analogy with models in other fields, for example neurobiology.
It may also be possible, by analogy this time with methods in biomedical research, to develop a method for the early identification of disease in farmed fish. Chapter 6 examined time series of food intake of individuals and groups of *O. mykiss* these were found to be similar to each other and to *L. limanda*. Shorter data sets were briefly examined for *Merlangius merlangus* and *Dicentrachus labrax*. The former showed similar characteristics to *L. limanda* and *O. mykiss*. The latter, although noisier, also proved rather similar. Thus the observed characteristics of food intake are not only to be found in *L. limanda*, although several more species would have to be examined before it would be possible to make a judgement on the universality of such dynamics amongst teleosts.
One clear observation from this study is that, even when fish are fed a single diet, the day-to-day variation in food intake is high. As a result of these findings, (and the observations that natural feeding times are very variable, Spieler 1999) it is clear that strict adherence to fixed feeding charts by fish farmers will on occasion lead to under-feeding, and at other times

over-feeding. One answer to this problem is either to use demand-feeders, or automatic feeders (such as 'Aquasmart', Hobart, Tasmania), which are able to detect whether fish are hungry or sated .

APPENDICES

Appendix 2.1

Effect of meal interval on food intake (%BW)in fish fed on diets of differing water content

Meal Interval	Squid		Pellets	
	Mean	SD	Mean	SD
0.33	1.87	0.92	0.3	0.19
1	5.33	2.14	0.82	0.35
1	7.53	2.5	1.11	0.23
3	7.57	3.63	1.92	1.41

Appendix 2.2

Appetite return data		
	Amount eaten (g)	
Time since previous meal	Squid	Pellets
20	8.5	*
24	22.9	0.7
48	25.9	2.5
22	13.8	1.0
30	34.8	4.4
15.17	0.0	2.7
24	13.1	2.4
3.66	2.8	1.7
24	16.6	4.8
72	35.1	4.8
23.66	12.1	2.4
18.5	*	*
24	25.6	3.5
4	6.9	1.0
94.33	49.5	*
24	10.8	*
15.4	20.9	4.1
24	20.7	1.7
20	10.8	*
24	19.8	*
48	25.7	*
22	10.1	*
30	37.0	*
15.17	11.6	*
24	13.2	*
3.66	12.8	*
24	11.5	*
72	37.6	*
23.66	22.5	*
18.5	*	*
24	24.5	*
4	12.6	*
94.33	9.9	*
24	17.6	*
15.4	*	*
24	19.5	*

Appendix 2.3

Comparison of retention of diet and radio-opaque markers		
time since meal	% food left	% spheroids left
1	96.3	95.0
1	84.6	100.0
1	49.5	100.0
2	77.6	100.0
2	77.5	90.0
2	61.1	88.9
4	74.4	100.0
4	62.8	92.3
4	56.9	100.0
4	31.6	100.0
8	38.7	54.5
8	32.1	100.0
8	66.5	92.6
16	11.4	100.0
16	28.6	90.0
16	42.5	100.0
24.5	22.0	64.3
24.5	32.8	85.0
30	17.0	82.4
30	17.2	66.7

Appendix 2.4 Stomach content at t=10h and amount taken in second meal.

Fish weight (g)	amount remaining (g dry wt/100g fish)	amount eaten 20:00 h (g dry wt/100g fish)
109	0.51	0.00
68	0.00	0.00
58	0.38	0.42
76	0.06	0.00
50	0.36	0.52
96	0.48	0.11
99	0.00	0.76
85	0.09	0.14
170	0.61	0.04
59	0.22	0.56
59	0.00	0.57
70	0.37	0.50
94	0.00	0.74
61	0.52	0.90
94	0.19	0.45
53	0.23	0.08
235	0.00	1.14
57	0.46	0.55
134	0.92	1.22
50	0.33	0.36
35	0.00	0.35
68	0.03	0.10
92	0.00	1.22
75	0.33	0.49
50	0.27	0.00

Appendix 3.1

Effect of repeated distensions on recorded stomach volume 02-27-1995,13-03-1995			
Relative stomach volume (ml/100gfish)			
Distension Number			
1st	2nd	3rd	KCl
1.80	1.99	1.95	2.63
0.18	ND	ND	2.02
0.72	ND	ND	ND
1.48	1.55	1.31	1.74
1.54	2.73	3.06	3.53
1.28	1.68	2.49	2.83
Average fish weight = 225g SD = 134.72			

Appendix 3.2

Effect of distension pressure, freezing the sample and a two week pre-prandial starvation period on relative stomach volume

Sampled December 1996/January 1997

1)Pellet-fed once daily, fresh stomach preparations

	ml per 100g			
fish wt (g)	1KPa	2KPa	3KPa	5KPa
62	0.26	0.68	1.92	5.47
92	0.00	0.00	2.17	0.00
44	0.00	0.00	1.70	2.64
82	0.00	1.89	3.46	4.62
166	0.75	4.73	6.23	9.14
79	0.32	2.53	4.00	6.10

2) Pellet-fed once daily, frozen stomach preparations

	ml per 100g			
fish wt (g)	1KPa	2KPa	3KPa	5KPa
125	2.50	4.37	Burst	
100	0.38	1.59	3.18	Burst
162	Burst			
113	Burst			
79	1.28	3.25	Burst	
113	4.83	12.03	Burst	
100	0.56	2.50	4.70	5.38
27	Burst			
187	2.57	Burst		
60	Burst			
171	2.38	3.02	4.54	Burst
129	4.21	Burst		
59	0.00	2.20	3.49	Burst
95	4.54	6.71	Burst	
174	3.74	4.93	Burst	
132	Burst			
80	2.21	4.76	5.83	Burst
110	Burst			
162	4.02	5.62	7.28	Burst
98	5.49	Burst		
119	Burst			
270	Burst			

3) Starved fish, fresh stomach preparations

	ml per 100g			
fish wt (g)	1KPa	2KPa	3KPa	5KPa
168	0.12	1.38	2.76	5.10
73	0.00	2.74	3.90	
100	0.80	2.05	3.13	
69	0.91	2.20	3.49	4.65
52	0.00	0.87	3.21	4.42

Appendix 3.3

Average food intake for fish fed on squid and on pellets, expressed as wet weight, dry weight, energy intake (J) and volume (ml)								
	Wet (g)		Dry (g)		Energy (J)		Volume (ml)	
Diet	Squid	Pellet	Squid	Pellet	Squid	Pellet	Squid	Pellet
	5.32	0.82	1.27	0.75	21.28	15.21	4.48	0.76
	8.75	0.98	2.09	0.90	35.00	18.27	7.37	0.91
	6.30	1.24	1.50	1.14	25.20	23.11	5.30	1.16
	3.75		0.90		15.00		3.16	
	4.36		1.04		17.44		3.67	
	3.61		0.86		14.44		3.04	
	4.04		0.96		16.16		3.40	
	3.59		0.86		14.36		3.02	
Average	**4.97**	**1.01**	**1.19**	**0.93**	**19.86**	**18.86**	**4.18**	**0.94**
SD	**1.80**	**0.21**	**0.43**	**0.20**	**7.20**	**3.99**	**1.51**	**0.20**

Sampled daily food intake (%BW), 16 celcius

Diet	Squid							Pellets	
	3.05	5.68	4.65	5.46	4.16	10.75	7.89	0.93	1.05
	4.30	2.54	3.67	4.58	3.42	10.16	5.68	0.88	1.27
	2.78	4.86	2.53	2.08	3.21	4.71	4.16	1.00	1.60
						9.39	7.50	1.13	1.06
Average	**3.38**	**4.36**	**3.61**	**4.04**	**3.59**	**8.75**	**6.31**	**0.98**	**1.24**
SD	**0.81**	**1.63**	**1.06**	**1.75**	**0.50**	**2.75**	**1.73**	**0.11**	**0.26**
No/mean wt	n=7,79.6g	n=6,82.7g	n=6,82.2g	n=6,80.5g	n=6,85.5g	n=6,80.5g	n=6,85.5g	n=6,82.7g	n=6,82.2g

Daily food intake (%BW) at 15 celcius (Oliva, unpublished)

Pellets	Squid
1.88	8.98
0.21	12.07
0.87	5.5
0.97	6.64
0.96	3.74
1.8	6.62
0.96	6.05
1.09	7.69
0.83	5.18
0.79	4.63
0.3	5.6
0.91	4.11
0.79	6.81
0.82	5.45
0.66	3.56
0.76	3.76
0.37	7.52

continued

pellets	squid	
0.96	5.4	
1.13	5.9	
0.79	2.95	
0.59	2.96	
0.57	3.88	
0.5	6.04	
0.7	5.35	
0.67	6.59	
0.85	4.49	
0.73	3.95	
0.69	4.18	
0.71	0.19	
0.63	4.01	
2.66	**3.68**	**Mean**
2.93	**2.88**	**SD**
n=4,180g	n=4,182g	

Appendix 3.4

Relative stomach volume, whole stomach weight and stomach section weight
Values are per 100g of fish

	Stomach vol @			Stomach section wt			
	Fish wt (g)	3KPa	5KPa	Stom wt	posterior	anterior	Overall
squid, 6 weeks	72	6.49	0.00	1.36	0.05	0.06	
	190	6.14	8.18	0.66	0.01	0.01	
	126	13.04	19.21	1.05	0.01	0.02	
	71	7.69	15.41	1.04	0.03	0.03	
	100	13.79	16.89	1.09	0.02	0.02	
	130	11.15	17.85	1.08	0.01	0.02	
	116	6.75	10.34	1.03	0.03	0.02	
	134	4.50	0.00	0.87	0.03	0.03	
	170	5.45	10.84	0.65	0.02	0.02	
Average	**123.22**	**8.33**	**10.97**	**0.98**	**0.02**	**0.02**	**0.02**
SD	39.94	3.43	7.24	0.22			0.01
pellets, 6 weeks	79	4.00	6.10	1.15	0.05	0.04	
	62	1.92	5.47	0.69			
	92	2.17	0.00	0.91	0.03		
	82	3.46	4.62	0.66	0.02	0.03	
	166	6.23	9.14	1.14	0.05	0.03	
	44	1.70	2.64	0.86	0.04		
Average	**87.50**	**3.25**	**4.66**	**0.90**	**0.04**	**0.03**	**0.03**
SD	42.02	1.72	3.12	0.21			0.01

Appendix 3.5 Change in relative stomach volume (ml/100g fish) and relative stomach weight (g/100g fish) with time.

weeks since capture	wild rel vol	wild rel wt	sq rel vol	sq rel wt	pe rel vol	pe rel wt
0	2.32	0.96				
0	2.00	0.96				
0	3.25	0.75				
0	2.29	0.08				
2			2.67	0.87		
2			3.84	0.92		
2			2.58	1.00		
4			1.80	0.68	1.29	1.04
4			2.21	0.72	0.32	0.79
4			0.95	0.80	0.94	0.91
4					0.00	0.81
4					0.13	0.96
8			0.68	0.66	0.26	0.69
8			0.00	0.87	0.75	1.14
8			0.00	1.03	0.00	0.91
8			2.03	1.36	0.32	1.15
8			0.88	0.65	0.00	0.66
8			2.31	1.05	0.00	0.86
8			0.00	1.04		
8			0.00	1.09		
8			2.35	1.08		

Appendix 3.6

Relative weights of Intestines and Pyloric Caeca			
Treatment	Fish wt (g)	Intestine wt	Pyloric caeca wt
sq for 6 weeks	72	1.32	0.54
	190	0.83	0.15
	126	0.86	0.28
	71	1.17	0.33
	100	0.79	0.35
	130	0.95	0.32
	116	1.06	0.37
	134	0.96	0.28
	170	0.69	0.22
Mean	**123.22**	**0.96**	**0.32**
SD	39.94	0.20	0.11
pe for 6 weeks	79		0.54
	62		0.23
	92	1.10	0.33
	82	1.05	0.17
	166	1.05	0.25
	44	0.57	0.43
Mean	**87.50**	**0.95**	**0.32**
SD	42.02	0.25	0.14
starved for 2 weeks	168.00	1.32	0.23
	73.00	0.74	0.08
	100.00	1.36	0.25
	69.00	0.72	0.20
	52.00	0.34	0.15
Mean	**102.50**	**1.04**	**0.19**
SD	45.63	0.43	0.07

Appendix 3.7 Fish weight (g), relative stomach volume and weight of the demand fed fish.

Fish wt (g)	Rel. Stom. Vol.	Rel. stom. wt
119	4.62	0.86
85	3.73	0.82
60	0.32	0.88
33	1.73	0.85
42	0.90	0.60
72	2.67	0.64
68.50	**2.33**	**0.77**

Appendix 3.8 Number of actuations per day and per hour. The right hand column indicates the average number of actuations for a particular hour of the day, the bottom row indicates the total number of actuations in a day.

	Number of actuations per day																								
h/d	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	
1	11	0	5	6	9	13	8	7	3	1	2	6	4	5	3	2	0	1	6	4	2	5	5	2	4	4.6
2	5	0	6	7	11	6	8	6	2	1	2	4	7	4	3	0	4	1	4	3	5	4	3	1	3	4
3	5	0	7	6	9	7	6	3	0	0	1	6	8	6	4	3	1	6	4	3	2	2	4	2	3	3.9
4	6	0	6	7	5	8	6	5	0	2	9	11	7	8	2	3	3	6	3	3	7	3	4	1	1	4.6
5	1	0	4	11	7	5	5	5	1	0	2	5	5	4	1	3	5	2	5	3	4	4	1	2	0	3.4
6	2	0	1	0	1	1	12	2	2	0	4	1	0	0	0	0	3	0	1	0	1	4	1	2	0	1.5
7	1	0	3	1	7	2	13	4	9	0	3	1	4	4	3	0	0	0	2	2	0	2	1	5	1	2.7
8	0	0	13	1	13	3	11	0	12	1	0	0	3	1	0	2	0	0	1	2	1	0	2	2	0	2.7
9	8	0	1	3	6	3	4	7	4	1	1	2	2	5	4	0	0	0	2	3	3	2	1	0	1	2.5
0	1	0	0	2	6	3	2	0	0	2	2	6	9	1	0	1	0	0	1	0	3	0	3	0	2	1.8
11	3	13	0	5	5	10	3	1	0	1	1	3	1	2	2	0	1	2	0	0	0	4	1	0	1	2.4
12	2	4	6	1	0	2	0	0	1	1	0	1	1	1	1	3	2	2	1	4	1	2	0	1	2	1.6
13	6	1	4	0	1	1	2	2	1	1	0	2	1	2	0	5	1	2	1	0	0	1	0	5	2	1.6
14	4	5	1	0	0	0	1	0	2	1	2	0	1	0	1	0	1	0	0	3	4	2	1	2	0	1.2
15	3	4	1	0	1	1	1	2	2	1	3	0	0	1	4	1	2	0	4	1	1	2	3	7	0	1.8
16	3	3	5	1	2	4	0	2	1	4	2	2	1	5	2	2	1	2	3	3	2	1	3	5	0	2.4
17	6	0	2	3	2	4	1	8	2	2	0	3	2	5	1	1	2	2	5	1	6	3	2	1	3	2.7
18	2	0	3	4	3	5	10	6	4	1	2	3	5	2	6	1	2	1	5	3	3	4	5	3	3	3.4
19	3	0	2	5	6	7	8	6	0	1	1	3	3	2	6	4	4	3	5	3	3	4	1	3	4	3.5
20	2	0	6	6	6	8	5	5	3	3	2	4	7	1	2	2	4	2	8	4	4	3	7	3	2	4
21	4	0	9	4	4	5	7	6	2	1	3	4	6	3	6	2	4	5	10	2	5	6	4	3	5	4.4
22	12	0	8	5	6	4	9	5	2	2	4	5	5	5	6	4	2	5	11	2	4	5	2	3	1	4.7
23	16	0	8	7	6	7	6	7	3	0	4	5	5	5	5	4	3	2	10	1	2	2	3	4	5	4.8
24	9	0	7	8	11	5	5	6	6	1	0	1	10	3	1	4	4	1	6	6	2	4	2	2	1	4.2
Total	115	30	108	93	127	114	133	95	62	28	50	78	97	75	63	47	49	45	98	56	65	69	59	59	44	

Appendix 3.9 *In vivo* hydration rate (expessed as the wet:dry ratio) and GER of pelleted diet

time (h)	wetdry	Fish wt
1	1.68	82
1	1.69	51
1	2.04	80
1	2.01	38
1	2.02	59
1	1.81	45
1	1.92	59
1	1.77	49
1	2.05	59
1	1.96	46
2	2.04	49
2	1.99	82
2	3.04	53
2	2.15	54
2	2.04	46
4	2.43	47
4	2.60	74
4	2.95	95
4	2.83	64
4	2.67	70
4	2.51	65
4	2.77	60
8	4.20	72
8	3.87	80
8	3.48	99
8	3.35	80
8	3.28	48
12	4.07	40
12	3.76	51
12	3.12	44
12	3.86	43
12	3.52	73

time (h)	% meal remaining
0	100.00
1	82.75
1	96.00
1	84.11
1	83.50
1	74.25
1	76.50
1	84.75
1	90.32
1	82.50
1	88.50
2	35.68
2	68.44
2	39.50
2	45.75
2	83.25
4	35.14
4	42.63
4	40.00
4	48.00
4	71.85
4	69.90
4	48.15
8	14.40
8	51.63
8	36.56
8	50.38
8	49.50
12	25.00
12	24.25
12	69.00
12	5.50
12	36.60

Appendix 3.10

Surface area of X-ray plates (mm^2), showing stomach relaxation due to meal

hour	1% bw no previous	1% bw 2% previous
0	247	667
0	368	677
0	304	742
0	318	416
1	421	457
1	340	820
1	406	658
1	414	705
2	395	717
2	425	798
2	536	467
2	496	774
3	374	801
3	530	454
3	499	791
3	579	779
5	448	426
5	334	612
5	462	743
5	556	816
8	315	417
8	440	739
8	501	711
8	493	529
12	170	370
12	485	687
12	405	213
12	303	760
16	402	602
16	160	664
16	260	117
16	0	182

APPENDIX 5.1

INSTRUCTIONS FOR THE USE OF THE DEMAND FEEDER SYSTEM.

DESCRIPTION

An automated system which delivers food when an individual fish actuates the system by breaking an infra red (IR) beam.
The system consists of up to twelve IR beams (currently six are operating) each with its own hopper. These are connected by shielded cables to a PC *via* a control box, which are situated in a dry area. The control box is designed so that additional hoopers/beams can be added simply be sliding in another circuit board. The IR beams are mounted in a waterproof casing, which is suspended from a wooden crossbar over the tank. Both the beams and the hoppers plug into waterproof sockets located in the downstairs temperate wet room of the fish laboratory. Two sockets are located in the small wooden side room and four are above the shallow raceway, the latter sockets enable either four hoppers to be used in the shallow raceway, or two can be placed in the deeper tank by the entrance to the wet lab. (NB Some of the leads are longer than others and not all will reach this tank).
When the beams are broken, 5 volts DC passes to the control box, which in turn generates a 12 V DC signal to operate the hoppers. Each operation of a hopper is recorded on a hopper specific ASCII file, detailing the date, time, number of breaks in the current hour, time taken for delivery and amount of food delivered.

THE HOPPERS

It is possible to control the amount of food delivered from the hoppers by setting the degree of rotation of the impeller. This can be adjusted upon opening the program, when the parameters for each hopper are set (see below). The hoppers are powered by a stepper motor, which also turns a wheel with a groove cut into it every 60°. An spring loaded arm with a wheel, runs along the edge of the wheel, and sends signals back to the computer every time it falls into a groove. When the arm falls back into the groove that indicates the hopper has rotated enough, the hopper is switched off.
The program monitors the hoppers for blocking by timing the interval it takes to pass between two grooves; if it is longer than the expected period, it takes steps to unblock it. This involves rotation of the impeller back and forth for a period, before turning forwards again.
Whilst any hopper is operating, the other hoppers will not work. In order that food is delivered every time that a beam is broken, the software has a queuing system that notes the signal from every beam in order and delivers food from their respective hoppers sequentially.

Maintenance of the hoppers

Apart from general cleaning there is little maintenance required. Be sure to position the hoppers in a dry (*eg* polystyrene) box, preferably with a lid, to reduce diets getting damp and the hoppers getting corroded.
On occasion the impellers start to wear (they go soft), These are impellers from an outboard motor and can be obtained from a chandlers (Dave in the workshop

knows the details, and the packaging from one, with the part number on it is in the 'bit boxes' by the PC).

THE IR BEAMS

These are quite easy to use, provided they are maintained properly. They consist of two circuit boards, a receiver and a transmitter, which are enclosed in a waterproof casing, When the beam is broken a signal passes up the yellow cable of the receiver. There are two small screws on components of the board, one of these adjusts the width of the IR beam, and is occasionally useful.

When setting up the beams, the distance between them is important; too close and the fish will be inhibited from passing between them, too far apart and the beam will break very easily, even for particles of faeces in the water. The best distance is found when the brackets on the wooden cross bar are 13 cm apart.

Maintenance of the IR beams

Provided the beams are sealed properly (see below), there is not too much maintenance. The glass on the front of the casing is permanently sealed and just needs algae removing from it now and then. There is quite often a problem with condensation inside the unit, this can be minimised by sealing it in a cold dry atmosphere. It may be a good idea to put a sachet of silicon inside the unit.

The beams are **sealed** by an 'O' ring on the back plate of the casing, as well as a nipple around the cable at its entry point.

Remove the back plate (do not do this with anything that will dent the plastic; the 'O ring needs to press against a completely flat surface to work, a photocopy card will do the job). The 'O' ring can also be removed by sliding a credit/photocopy/library card down its edge and pinching it out (Note that sometimes the card may snap; use one you don't mind losing. Again, nothing harder than the plastic should be used). Check that the 'O' ring has not perished (are there any tiny cracks in it?). Make sure it is free of dust, hair *etc.* A light coating of silicon grease should be applied. Note that it is not the grease that seals the unit, but the 'O' ring, the grease is to stop the 'O' ring from perishing. If you have no silicon grease Vaseline may be used, but this does not protect the 'O' ring effectively.

The nipple through which the cable enters the unit is sealed with silicon sealant (as used on aquaria), this can fail and will then need replacing. If the rubber seal inside the nipple is perished it is best to fit a new nipple altogether. Unscrew the old nipple by turning it counter-clockwise, clean the back plate thoroughly and remove it. Note where the cables leave the circuit board and remove them with the aid of a soldering iron. (I think maybe students are not supposed to do this any more?) Remove the cables and pull them through the old nipple and feed them through the new one. Solder the wires back on to the circuit board, taking care not to overheat the board.

You will have to Smear a little silicon sealant around the thread of the new nipple and screw it in clockwise. When it is in place start to tighten the thread on the cap of the nipple. The action of tightening the thread presses the rubber seal against the cable. If you are feeling a little nervous about trusting this, a little silicon sealant can be smeared around the cable as well as the outside of the nipple .

Your unit should now be water tight; **test this in fresh water first**. If it leaks you will have less chance of losing your IR beam. After an initial short period check for leaks. If there are none then leave the beams in the water overnight and check again. If it is still OK you can proceed with your experiment.
Spare boards are to be found on the shelves close to the PC, together with some spare parts. It is a good idea to check you have some spares in case of flooding. Contact Ray Wilton / Ann Hammerstein to join the queue for electronics jobs. **Be warned**: they are busy people and this can take time!

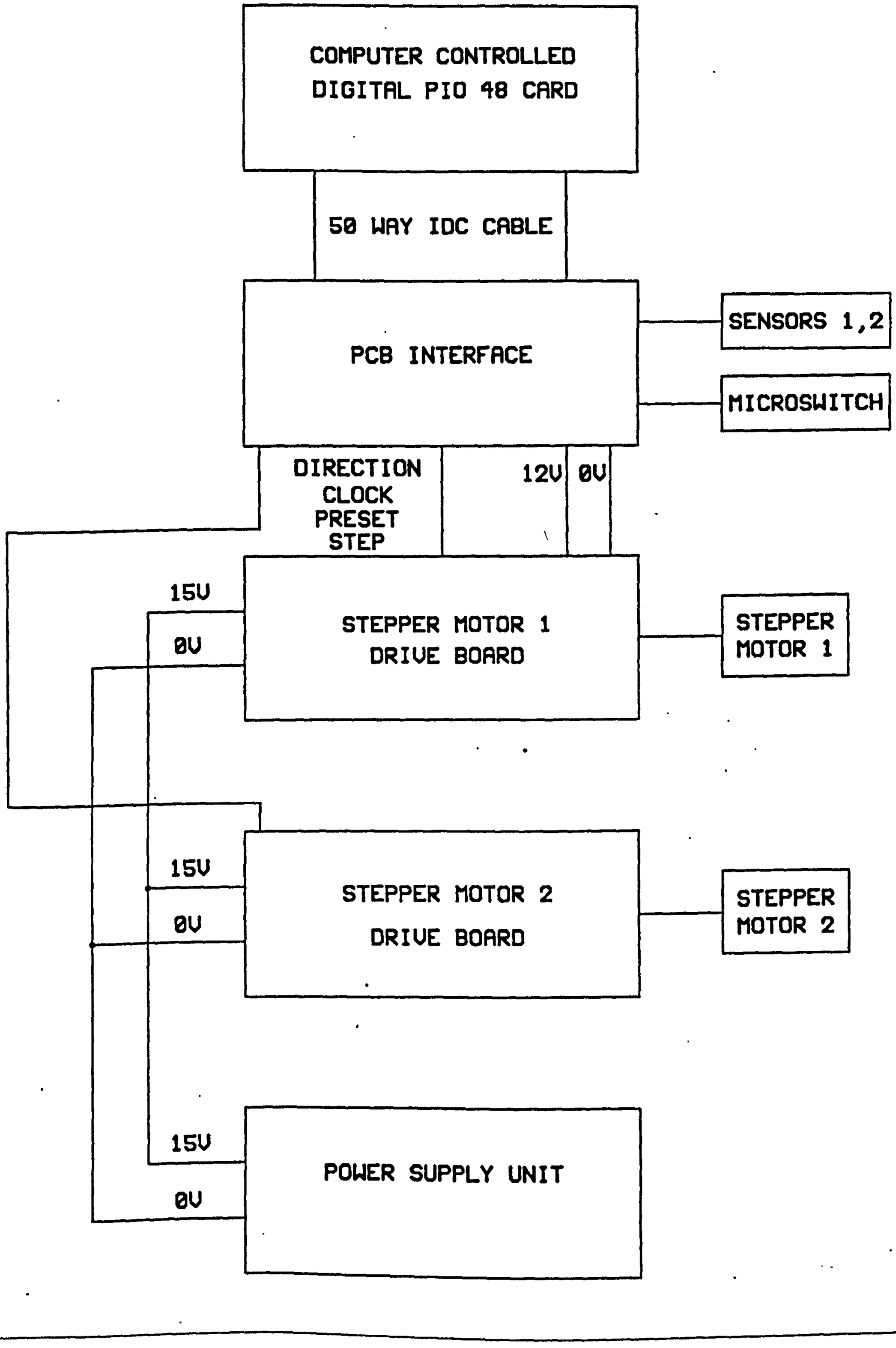
SOCKETS AND PLUGS

It is obvious which sockets are for the beams and which are for the hoppers as they have a different number of pins. The plugs for one combination of hopper and beam are found one above the other.
When not in use replace the caps on both the plugs and the sockets. You will have to find out which plug is which by trial and error (sorry can't remember) If it is not to your liking, you can always swap the plugs at the back of the control box, having first unplugged it.

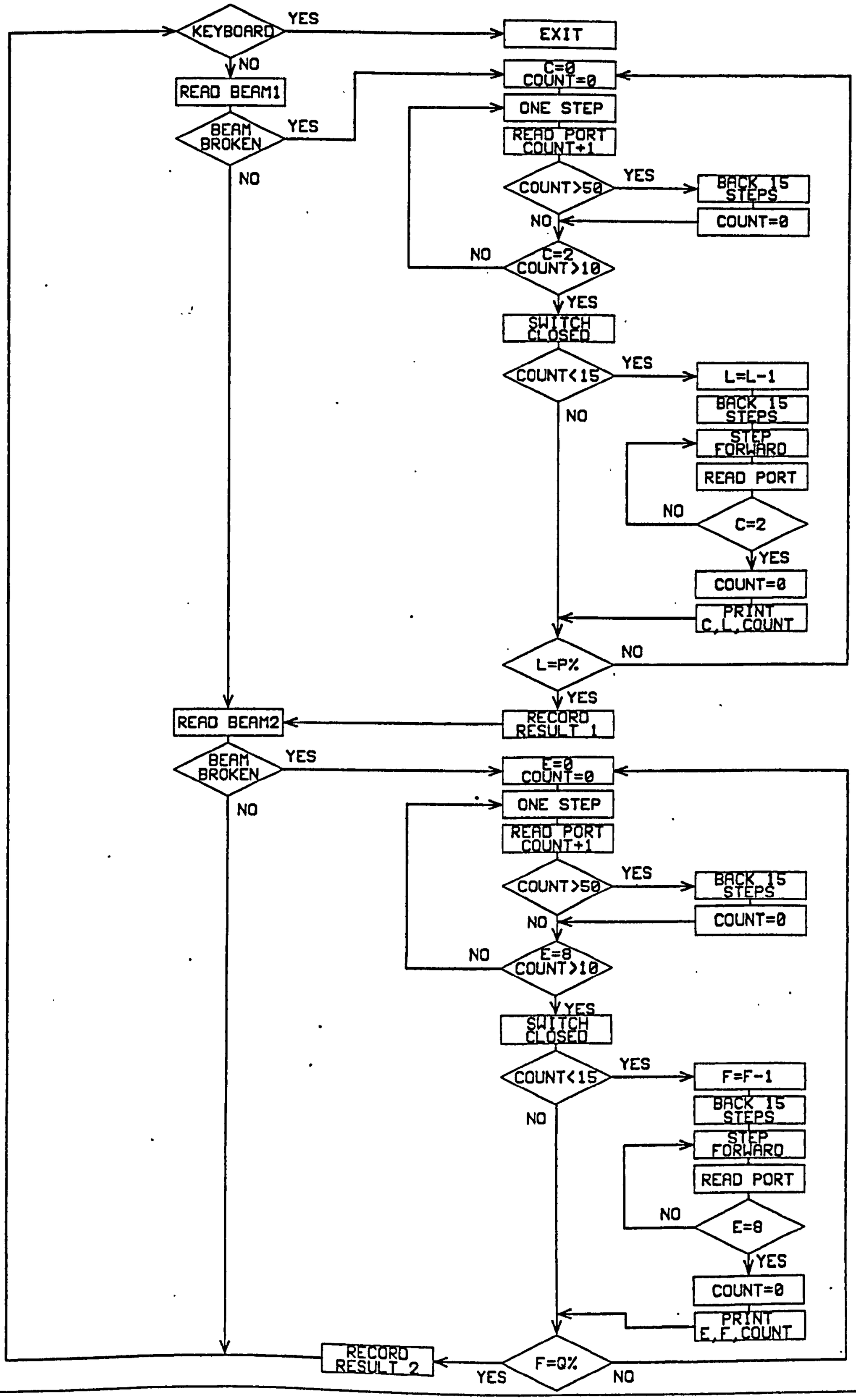
CONTROL BOX

This is the domain of the electronics technicians. There is a switch at the back for on/off.

BLOCK DIAGRAM FOR TWO UNIT FISH FEEDER



FLOW CHART FOR TWO UNIT FISH FEEDER

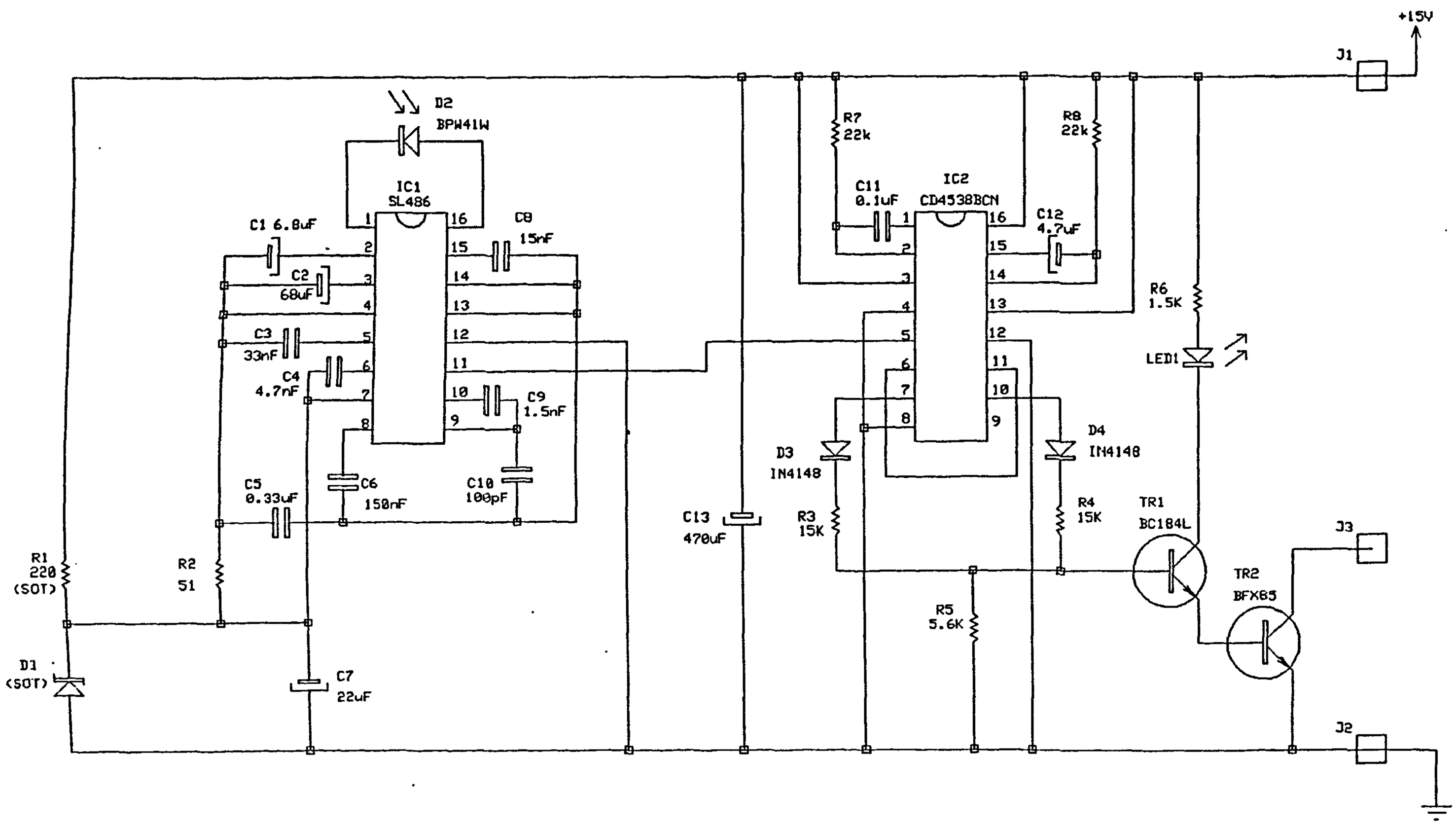


```
CLS
BASEA = 768          ' base address
PORTA1 = BASEA       ' port A1 address
PORTB1 = BASEA+1     ' port B1 address
PORTC1 = BASEA+2     ' port C1 address
PORTA2 = BASEA+4     ' port A2 address
PORTB2 = BASEA+5     ' port B2 address
PORTC2 = BASEA+6     ' port C2 address
P% = 0               ' stepper motor steps unit 1
Q% = 0               ' stepper motor steps unit 2
T% = 0               ' time period
R$ = ""              ' record directory 1
S$ = ""              ' record directory 2
OUT BASEA+3, 128     ' set data direction, output port A1, B1 and C1
OUT BASEA+7, 153     ' set data direction, input port A2 and C2, output B2
A=B=C=D=E=F=G=H=I=J=K=L=0
GOSUB STEPDELAY

PRINT
PRINT "                FISH FEEDING PROGRAM"
PRINT
PRINT " ONE STEP GIVES 60 DEGREES ROTATION OF MOTOR SPINDLE"
PRINT
INPUT " TYPE THE NUMBER OF STEPS FOR EACH FOOD DELIVERY FROM UNIT 1     ", P%
PRINT
INPUT " TYPE REFERENCE FOR DATA FROM UNIT 1 IN THE FORMAT XXXXXXXX.XXX  ", R$
PRINT
INPUT " TYPE THE NUMBER OF STEPS FOR EACH FOOD DELIVERY FROM UNIT 2     ", Q%
PRINT
INPUT " TYPE REFERENCE FOR DATA FROM UNIT 2 IN THE FORMAT XXXXXXXX.XXX  ", S$
PRINT
PRINT " TIME IS INPUT IN SECONDS"
PRINT
INPUT " TYPE THE TIME PERIOD YOU REQUIRE FOR DATA COLLECTION          ", T%
PRINT
PRINT " PRESS ANY KEY TO ABORT"

WHILE NOT INSTAT              ' check keyboard
  A = INP(PORTA2)             ' read port A2
  B = A AND 1          ' check sensor bit unit 1, 0-beam broken, 1-beam in tact
  IF B = 0 THEN               ' if beam broken loop to step motor
  print "beam 1 broken"
  OUT PORTB2, 1               ' switch relay on, power motor
     FOR L = 1 TO P%          ' stepper motor steps indicator
        C = 0                 ' set micoswitch sensor to 0
        COUNT = 0
        DO UNTIL C = 2 AND COUNT > 10
           COUNT = COUNT+1
           GOSUB STEPON1
           GOSUB STEPON1
           GOSUB STEPBACK1
           GOSUB STEPON1
           GOSUB STEPON1
           GOSUB STEPBACK1
           A = INP(PORTA2)      ' read port A2
           C = A AND 2          ' check microswitch loop if not set
           PRINT "STEP=";COUNT
           IF COUNT >50 THEN
           FOR K= 1 TO 15
           GOSUB STEPBACK1
           NEXT K
           COUNT = 0
           END IF
        LOOP
      print "Switch 1 closed"
      IF COUNT < 15 THEN
      L = L - 1
      FOR J = 1 TO 15
      GOSUB STEPBACK1
      PRINT "BACK 1"
      NEXT J
       DO UNTIL C = 2
        GOSUB STEPON1
        A = INP(PORTA2)
        C = A AND 2
       LOOP
      COUNT = 0
      PRINT C;L;COUNT
      END IF
      NEXT L
```

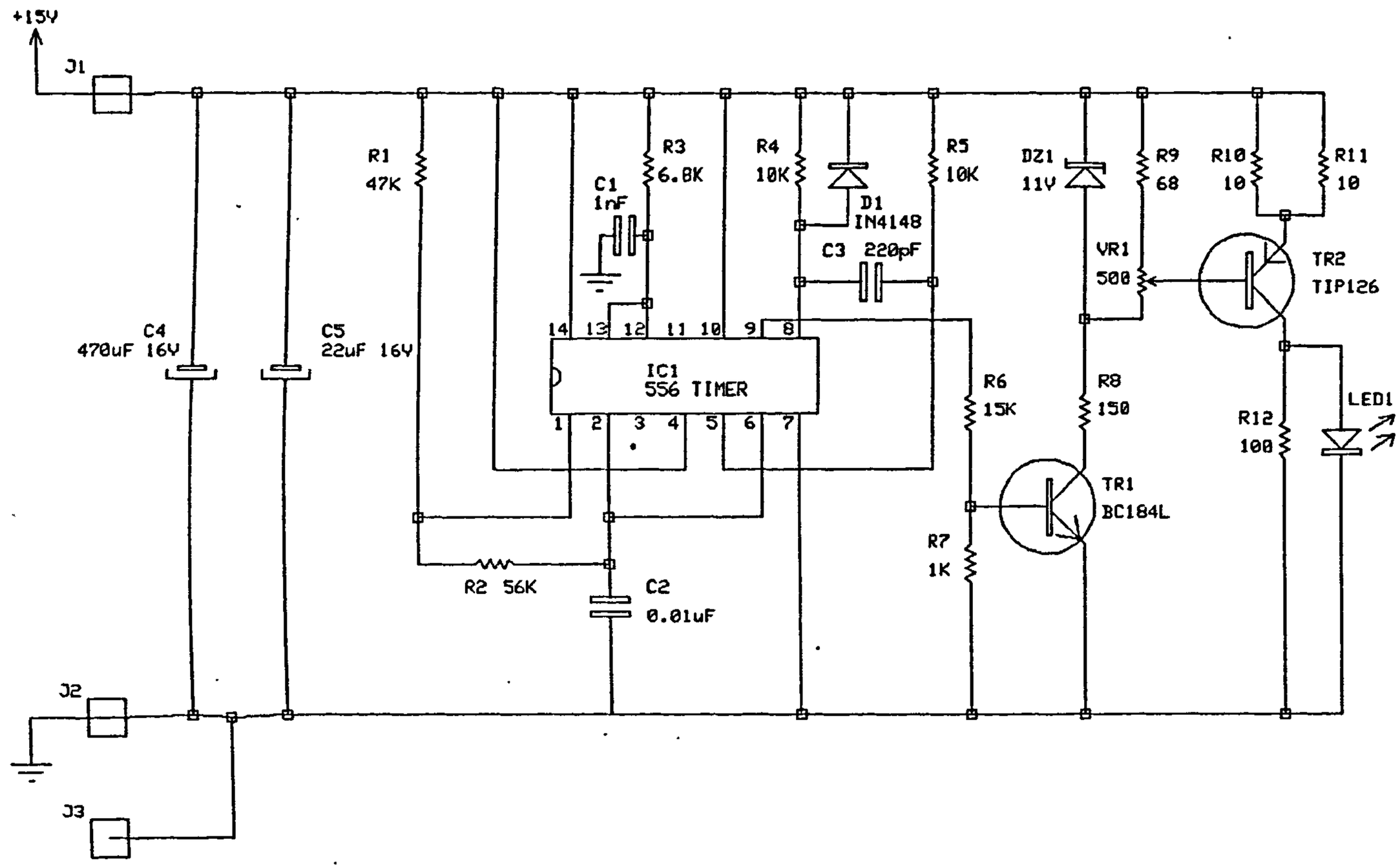
```
81          OUT PORTB2, 0              'switch relay off
82          PRINT "RECORD UNIT 1"
83          GOSUB RECORD1
84       END IF
85      A = INP(PORTA2)
86      D = A AND 4
87       IF D = 0 THEN                 ' if beam broken loop to step motor
88       print "beam 2 broken"
89       OUT PORTB2, 1                 ' switch relay on, power motor
90          FOR F = 1 TO Q%            ' stepper motor steps indicator
91             E = 0                   ' set micoswitch sensor to 0
92             COUNT = 0
93             DO UNTIL E = 8 AND COUNT > 10
94                COUNT = COUNT+1
95                GOSUB STEPON2
96                GOSUB STEPON2
97                GOSUB STEPBACK2
98                GOSUB STEPON2
99                GOSUB STEPON2
100               GOSUB STEPBACK2
101               A = INP(PORTA2)      ' read port A2
102               E = A AND 8          ' check microswitch loop if not set
103               PRINT "STEP=";COUNT
104               IF COUNT >50 THEN
105               FOR G = 1 TO 15
106               GOSUB STEPBACK2
107               NEXT G
108               COUNT = 0
109               END IF
110            LOOP
111         print "Switch 2 closed"
112         IF COUNT < 15 THEN
113         F = F - 1
114         FOR H = 1 TO 15
115         GOSUB STEPBACK2
116         PRINT "BACK 2"
117         NEXT H
118          DO UNTIL E = 8
119           GOSUB STEPON2
120           A = INP(PORTA2)
121           E = A AND 8
122          LOOP
123         COUNT = 0
124         PRINT E;F;COUNT
125         END IF
126         NEXT F
127         OUT PORTB2, 0              'switch relay off
128         PRINT "RECORD UNIT 2"
129         GOSUB RECORD2
130      END IF
131   WEND
132   END
133
134   STEPON1:
135               OUT PORTA1, 1        ' set direction unit 1
136               GOSUB STEPDELAY      ' delay
137               OUT PORTA1, 3        ' set clock and direction to step motor unit 1
138               GOSUB STEPDELAY      ' delay
139   RETURN
140
141   STEPBACK1:
142               OUT PORTA1, 0        ' set direction unit 1
143               GOSUB STEPDELAY      ' delay
144               OUT PORTA1, 2        ' set clock and direction to step motor unit 1
145               GOSUB STEPDELAY      ' delay
146   RETURN
147
148   STEPON2:
149               OUT PORTA1, 4        ' set direction unit 2
150               GOSUB STEPDELAY      ' delay
151               OUT PORTA1, 12       ' set clock and direction to step motor unit 2
152               GOSUB STEPDELAY      ' delay
153   RETURN
154
155   STEPBACK2:
156               OUT PORTA1, 0        ' set direction unit 2
157               GOSUB STEPDELAY      ' delay
158               OUT PORTA1, 8        ' set clock and direction to step motor unit 2
159               GOSUB STEPDELAY      ' delay
160   RETURN
```

```
161
162   STEPDELAY:                        ' delay routine
163      FOR I=1 TO 20
164      NEXT I
165   RETURN
166
167   RECORD1:
168   OPEN "C:\RECORD1.DAT" FOR APPEND AS #1
169   A$ = DATE$
170   B$ = TIME$
171   WRITE #1, A$, B$, P%, R$
172   CLOSE #1
173   RETURN
174
175   RECORD2:
176   OPEN "C:\RECORD2.DAT" FOR APPEND AS #2
177   A$ = DATE$
178   B$ = TIME$
179   WRITE #2, A$, B$, Q%, S$
180   CLOSE #2
```

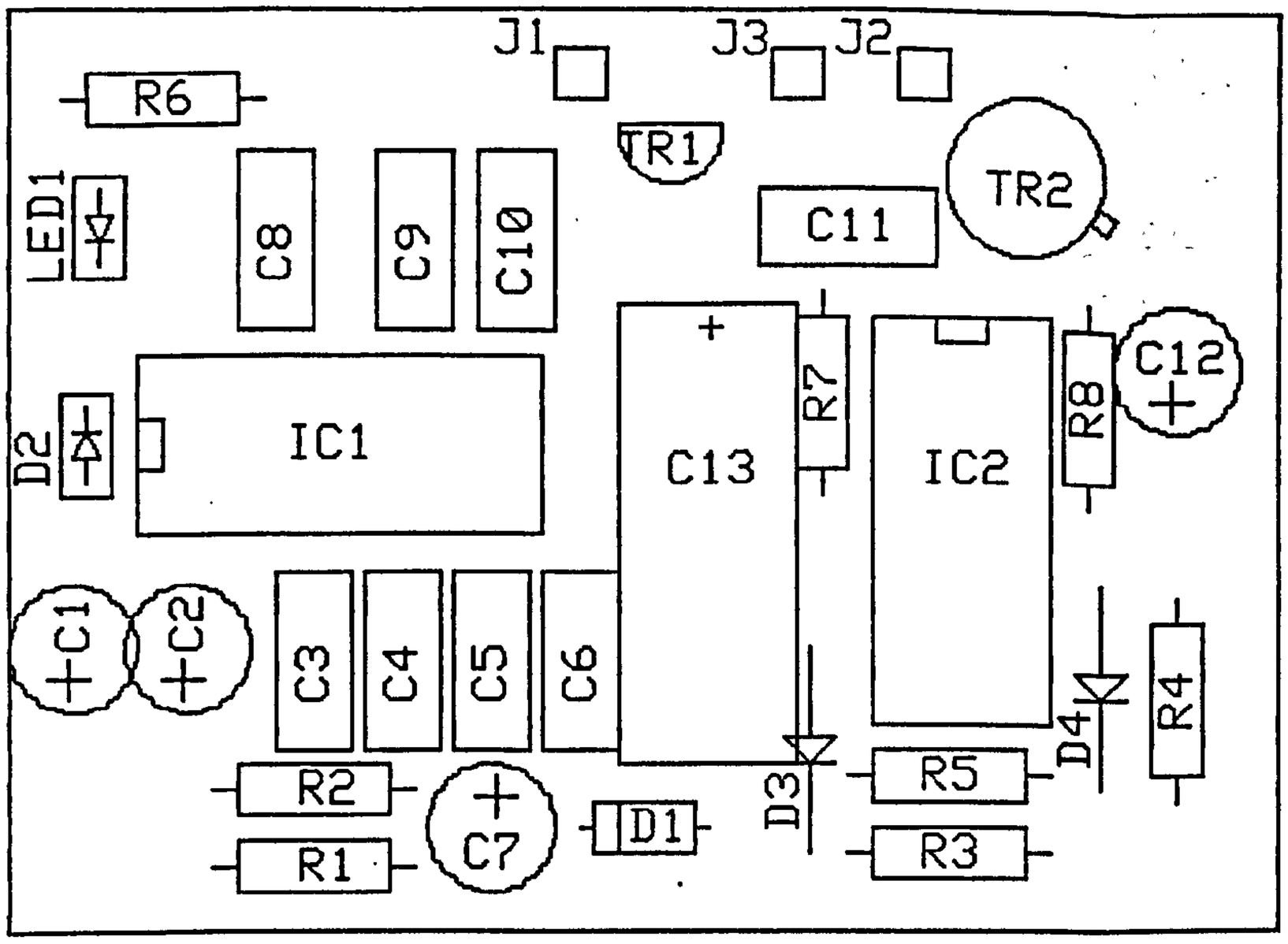
INFRARED RECEIVER P88 MAY 94



Infrared Transmitter



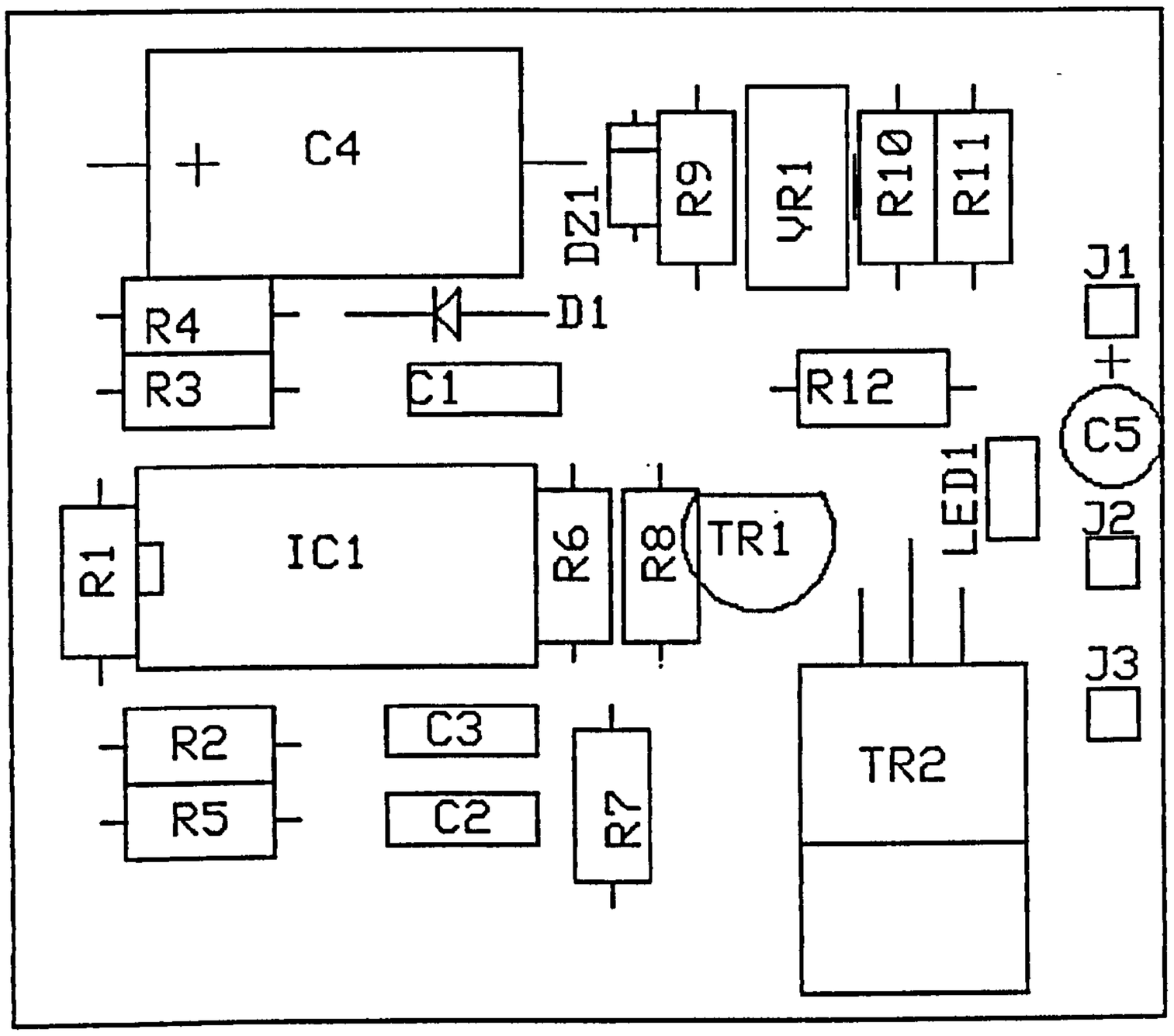
Infrared Receiver **P88.1** **May 1994**



Farnell Electronic Components

R1	220 ohm	MRS25220R
R2	51 ohm	MRS2551R
R3	15K	MRS2515K
R4	15K	MRS2515K
R5	5.6K	MRS255.6K
R6	1.5K	MRS251.5K
R7	22K	MRS2522K
R8	22K	MRS2522K
C1	6.8uF	227-754
C2	68uF	227-810
C3	33nF	143-677
C4	4.7nF	147-669
C5	0.33uF	143-683
C6	150nF	143-681
C7	22uF	227-780
C8	15nF	143-675
C9	1.5nF	147-666
C10	100pF	148-092
C11	0.1uF	143-680
C12	4.7uF	227-742
C13	470uF	294-548
D1	SOT	Only needed if threshold of IC2 needs to be changed
D2	BPW41W	BPW41N
D3	1N4148	1N4148
D4	1N4148	1N4148
LED1	Red LED	HLMP-K150
TR1	BC184L	BC184L
TR2	BFX85	BFX85
IC1	SL486	SL486DP
IC2	CD4538BCN	CD4538BCN

Infrared Transmitter P88.1 May 1994



FARNELL ELECTRONIC COMPONENTS

R1	47K	MRS2547K
R2	56K	MRS2556K
R3	6.8K	MRS256.8K
R4	10K	MRS2510K
R5	10K	MRS2510K
R6	15K	MRS2515K
R7	1K	MRS251K
R8	150 ohm	MRS25150R
R9	68 ohm	MRS2568R
R10	10 ohm	MRS2510R
R11	10 ohm	MRS2510R
R12	100 ohm	MRS25100R
VR1	500 ohm	3386X500R
C1	1nF	147-665
C2	10nF	143-674
C3	220pF	147-661
C4	470uF	294-548
C5	22uF	227-780
D1	1N4148	1N4148
DZ1	11V	BZX85C11
LED1	IR Transmitter	178-301
TR1	BC184L	BC184L
TR2	TIP126	TIP26
IC1	556 Timer	TS556CN

Appendix 5.2 Amount of squid mantle taken in discrete meal, offered once daily.

Daily intake of squid (g)
36
22.3
16.8
7.4
7
5.6
10.8
29.2
10.2
9.68
10.66
15.58
11.12
13.1
12.9
12.32
10.96
5.5
10.96
0
7.72
18.1
4.02
10.34
8.26
9.54
1.84
10.62
0.9
1.04
0.54
0.42
0.32
0
2.02

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