EVALUATION OF DIETARY CARBOHYDRATE UTILIZATION BY CAPTIVE SABLEFISH (*Anoplopoma fimbria*)

by

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ABSTRACT

Carbohydrates have variable digestibilities and metabolizable energy values in carnivorous fish. Simple sugars are generally more digestible than complex polysaccharides, and low levels of dietary carbohydrate may contribute more metabolizable energy than high levels. Two experiments were conducted to study the effects of dietary level and processing treatment of wheat starch on the digestibility of diets fed to sablefish (*Anoplopoma fimbria*), in different regions of the digestive tract. Moreover, an experiment was undertaken to determine if the dietary concentration of an indigestible external marker (chromic oxide) influenced its motility, relative to other ingredients in the ingesta, as it passed through the digestive tract. Lastly, a growth experiment was undertaken to compare the performance of sablefish fed formulated diets containing one of two levels of carbohydrate to that of fish fed a natural fish diet. The gastric evacuation of a formulated and a natural diet were also investigated.

Apparent digestibility values for the nutrients in a formulated diet (containing 44.4% cooked wheat and 0.1% chromic oxide) fed to sablefish were noted to increase progressively from the anterior to the posterior regions of the intestinal tract. Within each region of the gut, the apparent digestibility values for most nutrients declined over three sample periods. Carbohydrate (nitrogen-free extract) digestibility down to the distal section of the intestine ranged from 51.0 to 82.8%.

An experiment designed to assess the effect of carbohydrate treatment on the digestibility of four isonitrogenous, isocaloric diets met with partial failure. It was determined that the 1.0% chromic oxide marker flowed at a differential rate to the rest of the ingesta in the digestive tract, which violated the criteria for an effective marker. Consequently, diet digestibility was not determined in this experiment. Hepatic glycogen levels in fish receiving the dietary treatments

were compared, and these values were used to estimate the relative availability (digestibility) of carbohydrate from the respective diets. According to this index, the sequence for digestibility was as follows: pregelatinized starch > cooked wheat > pregelatinized starch/cooked wheat > unprocessed wheat.

Differential movement of chromic oxide relative to other ingesta was observed in sablefish fed on alternate days regardless of the dietary concentration of the indigestible marker (0.1 or 1.0%). It was surmised that the feeding protocol established the circumstances from which marker 'streaming' was observed in the results. Differential transport of Cr_2O_3 through the gut by ingested seawater was suggested as a possible mechanism for the phenomenon.

Sablefish fed a natural fish diet had the highest growth rates, condition factors, liver lipid levels and the lowest feed conversion ratios and liver glycogen levels. Sablefish fed a diet containing 22.2% cooked wheat had a higher growth rate, condition factor, and a lower food conversion ratio, than those fed a diet containing 44.4% cooked wheat. Ingestion of the 44.4% cooked wheat diet resulted in the highest values for liver glycogen and hepatosomatic index.

It was concluded that sablefish have a limited ability to metabolically utilize digestible carbohydrate and that most of the dietary non-protein energy should originate from high quality lipid for maximum protein sparing.

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CHAPTER 1

1.0 INTRODUCTION

A small number of fish species are cultured commercially in the world's temperate marine waters, and in British Columbia finfish mariculture is currently limited to salmonid species. A variety of coldwater species are cultured, or are considered to have good commercial culture potential, in northern Europe and Japan. In British Columbia research is being conducted to assess the potential for the cultivation of commercially valuable sablefish, *Anoplopoma fimbria.*

Short-term studies of impounded wild sablefish have determined that they are unusually hardy, disease-resistant, and fast growing in confinement. High growth rates were produced in sablefish fed chopped fish and invertebrates at densities ≤ 70 kg/m^3, with minimal losses (Kennedy, 1972).

Diets for commercial fish culture have high concentrations of expensive animal proteins. It is recognized that carnivorous fish derive most of their energy requirements from protein and lipid catabolism, and they have a limited ability to digest and utilize carbohydrates. Starches are the least costly source of dietary energy for animal culture, and a definition of optimum treatment and concentration in fish diets, for producing protein-sparing effects and metabolizable energy, has significant economic importance.

Carbohydrates have been shown to improve protein utilization while maintaining high growth rates when used at appropriate concentrations in formulated diets for a variety of fish. The digestibility of simple sugars is superior to that of more complex polysaccharides, and gelatinization improves the digestibility of starches. High levels of available carbohydrate can

depress growth performance in fish, and increase liver glycogen concentration and liver weight, which may adversely affect liver function.

This study was conducted to examine the sites of digestion and absorption of nutrients, and the utilization of formulated diets by sablefish. The digestion and utilization of dietary carbohydrates was measured, and the hepatic storage of glycogen and lipid was monitored.

Rates of gastric evacuation for different diets were studied and the efficacy of chromic oxide as an indigestible external marker for determining nutrient digestibility was explored.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Life history of sablefish

The sablefish, *Anoplopoma fimbria*, also called blackcod, is a commercially important bathypelagic species which inhabits the continental slope of the North Pacific Ocean. The bathometric distribution of adult sablefish extends from surface waters in the northern extremes of their range to depths greater than 2500 m (Sasaki, 1985). Adult sablefish are most abundant between 600 m and 800 m in depth off the British Columbia coast (McFarlane and Beamish, 1983a).

Spawning occurs below 300 m in early spring. Eggs and sperm are broadcast and embryos develop over a two to three-week period. Hatched larvae swim to the surface and begin to feed, developing rapidly as part of the neuston. Epipelagic juveniles drift with currents to inshore waters and can grow to 28 cm by late November (McFarlane and Beamish, 1983b). In British Columbian waters, juvenile sablefish remain in the inshore waters of Hecate Strait, Queen Charlotte Sound, the Strait of Georgia and mainland inlets until age 3 + (mean length = 50 cm), when they migrate offshore to a semi-demersal habitat. Growth rates are reduced in the colder, deeper water, and little growth is recorded for long-lived fish (up to 55 years and 100 cm in length). Sablefish are carnivores whose diet consists of a large variety of fish, crustacea and other invertebrates.

The majority of the sablefish captured in the commercial fishery range from 4 to 35 years of age. Mean annual growth rates range from 0.17 cm to 0.26 cm for adult males, and 0.55 cm to 0.66 cm for females, in studies conducted off the British Columbia coast (McFarlane and Beamish, 1983a).

Periodic strong year-class recruitment is important to the maintenance of sablefish stocks, and this occurred in the early 1950's and 1960's and in 1977. Year-class strength is thought to be determined by the pelagic egg and larvae survival, which is generally influenced by the degree of predation, the availability of appropriate food, and the speed or direction of currents carrying larvae to nursery areas (Kendall and Matarese, 1987). The strength of a year-class is reflected by the recruitment to the fishery. A strong year-class may predominate in the fishery for many years (McFarlane and Beamish, 1983c). Landed values to fishermen can be variable, but are generally high (approximately $4.85 per kg (Can.) dressed, head off, Spring 1990, to approximately $7.70 per kg (Can.), Fall, 1990).

Managers of sablefish stocks use many of the same tools used by salmon managers to maintain stock integrity. These include yearly quotas geared to stock recruitment, limited entry licensing, gear restrictions and restricted openings in discrete oceanographic areas. Sablefish managers do not, however, share the salmon managers' tool of stock enhancement of wild populations through controlled propagation to increase survival of eggs and fry, for mitigating the effects of over-fishing and habitat destruction. Wild sablefish stocks are unlikely to support a larger sustainable fishery than exists at present, and no feasible "enhancement" technologies for wild populations appear to be viable in the immediate future.

2.2 Research in Sablefish Culture

Several researchers have investigated the growth and metabolism of captive larval and adult sablefish (Kennedy, 1969, 1974; Sullivan and Smith, 1982; Gores and Prentice, 1983; Sullivan and Somero, 1983; Shenker and Olla, 1986; Furnell, 1987). A collaborative project is in progress to develop methods for the induced spawning of captive adult sablefish (Solar *et al.*, 1987) and the incubation of fertilized eggs (Alderdice *et al.*, 1988) with the aim of determining a protocol for providing seedstock for commercial aquaculture operations.

Captive adult sablefish (0.5 kg to 4.0 kg) reared in circular tanks were found to gain up to 150 g per month when fed chopped fish ad libitum (Kennedy, 1974). Mortalities of less than 1% per month (except for one group which suffered an epizootic outbreak of furunculosis) were noted. Moreover, sablefish growth performance was found to be unaffected by densities as high as 43 kg per m^2. Summer water temperatures (>10°C) were observed to adversely affect the growth of animals greater than 1.8 kg, but they had no effect on those of lower weight. The dissimilar response may have been due to the acclimation of the larger fish to the cooler waters of their natural deep water habitat.

Sullivan and Smith (1982) reported growth rates of 0.07 kg to 0.085 kg per month and food conversion efficiencies of 10% to 15% for sablefish (average weight 1.8 kg) fed 6-7% of their body weight of chopped squid and mackerel at 8-10 day intervals. Fish fed restricted rations of mackerel (6% of body weight) had approximately one-third of the growth rate and feed conversion efficiency of fish fed mackerel rations of 9-10% of body weight.

Sullivan and Somero (1983) determined that chopped squid and mackerel rations of 0%, 5-7% and 15% of wet body weight fed once per week to sablefish (1.12 kg to 1.35 kg) produced growth of -10%, -11% and 23% of initial weight, respectively, over a 24-month period.

Gores and Prentice (1984) determined the feasibility of rearing injured or undersized commercially captured sablefish to favourable condition and commercial size in floating seapens. Maximum monthly growth in mean weight and length was 100 g and 3.1 mm for juvenile sablefish (approximately 225 g initial weight) fed chopped fish (salmon and herring) ad libitum daily. No reduction in growth was observed during periods of warm water temperatures (up to 13°C).

Growth studies of young sablefish are limited. Shenker and Olla (1983) monitored diurnal prey consumption by wild juvenile sablefish (14 to 88 mm). The consumption rate of live brine shrimp (*Artemia salina*) was 20% of wet body weight per day and a similar high percentage of food intake was observed when larger mysid prey were offered. The smallest juveniles exhibited growth rates as high as 9.45% of body weight per day. A further change of diet to minced fish and squid resulted in body lengths of 220 to 240 mm after 5 months.

2.3 Nutrition

Fish reared in intensive culture systems must receive cost effective formulated diets and optimal culture conditions for maximum growth, food (protein) utilization and good health. A species such as sablefish has the potential for high growth rates in (relatively) warm surface waters, given that the limitations of the culture conditions can be overcome. Laboratory growth studies have indicated that juvenile sablefish adapt well to captivity and that they are capable of growing three to four times faster than their wild counterparts (McFarlane, 1989). Adult sablefish normally grow slowly in the cold waters of their semi-demersal environment. Laboratory studies indicate that the presumed pre-adaptations of 3+ sablefish to cold, deep water are of little consequence in the context of the surface conditions of cage aquaculture, and that the relatively

high growth rates of juvenile sablefish can be sustained in culture conditions relative to the situation in the wild (Kennedy, 1972; Gores and Prentice, 1984).

Formulated pelleted diets must satisfy the nutrient requirements of cultured species in the most economical manner. Their development and continuous refinement as more nutritional knowledge becomes available plays a pivotal role in the success of salmon, rainbow trout and catfish culture. Pelleted diets provide the means for feeding fish controlled rations of uniform, water-stable, nutritionally balanced food. Both feeding frequency and the method of food delivery can be varied. The risk of disease transmission from wild fish, which is high when unprocessed fish are fed, is also minimized when pelleted diets are employed.

Sablefish can maintain high growth rates in captivity on a diet of chopped marine fish. The use of formulated diets, however, has been less successful, since dry diet ($\leq$ 10% moisture) pellets are too hard, and consequently the pellets are rejected by the fish (Kennedy, 1970; Higgs, pers. comm.). Oregon Moist Pellets (OMP) which have a softer texture have been used effectively as a maintenance diet for sablefish (Kennedy and Smith, 1971; Smith, pers. comm.). Economical sablefish culture requires a clear understanding of the nutrient requirements of this species. Moreover, knowledge of feedstuff digestibility, acceptability and nutritional value is essential for the formulation of high performance sablefish diets.

The nutritional requirements of a species at a particular life-history stage are ultimately determined by a biochemical approach utilizing purified diets (Cowey, 1976). General principles of nutrition, digestion and metabolism in carnivorous fishes have been developed over the last 45 years. Specific variations, particularly those reflecting environmental adaptations, have been identified for some commercially important species. For marine species such as sablefish, whose basic requirements are unknown, general principles must be employed as broad guidelines in the formulation of practical diets. The refinement of sablefish nutritional requirements may take

decades, and the use of relatively crude diets, which anticipate their needs will be necessary for commercial culture to proceed.

2.3.1 Carbohydrates

Carnivorous fish require diets with a high content of digestible protein (35-50% of diet). They preferentially derive energy from the catabolism of proteins and lipids for their metabolic functions, but a substantial portion of protein energy can be spared for growth by optimizing the balance between protein and non-protein (lipid and carbohydrate) energy in the diet. Lipids are the main sources of non-protein energy used in commercial feeds and they may form a large part of the natural diet of salmonids. The inclusion of lipids in formulated diets has been shown to contribute to protein-sparing effects in several fish species (Lee and Putnam, 1973; Watanabe *et al.*, 1979; Bromley, 1980; Shimeno *et al.*, 1980).

Carbohydrates are found in insignificant amounts in the natural diets of carnivorous fish. Like lipids, carbohydrates in the form of simple sugars and complex polysaccharides have produced protein-sparing effects in several carnivorous species fed formulated diets (Buhler and Halver, 1961; Ringrose, 1971; Pieper and Pfeffer, 1979; Shimeno *et al.*, 1979; Hilton *et al.*, 1982; Degani *et al.*, 1986). However, carnivorous fish have poor ability to utilize high dietary levels of carbohydrates (Singh and Nose, 1967; Palmer and Ryman, 1972; Bergot, 1979; Furuichi and Yone, 1981) and they oxidize glucose relatively slowly (Cowey *et al.*, 1975).

Carbohydrates are the least expensive source of energy for animal nutrition. Consequently, the physiological and economic importance of energy in fish feeds compels an evaluation of the productive value of carbohydrate sources for cultivated species. It is important

to determine the level of digestible carbohydrate that can be included in diets for various carnivorous fish species without impairing their performance.

All fish which have been so far studied have some ability to utilize dietary carbohydrates (Cowey and Sargent, 1972). In general, the limited utility of carbohydrates in formulated diets can be attributed to deficiencies within the digestive and metabolic processes of carnivorous fish. Incomplete enzymatic breakdown of polysaccharides, and limited absorption of the end products of polysaccharide digestion restricts the nutritive value of carbohydrates. Moreover, poor hormonal control over plasma glucose levels retards their use as immediate energy sources (Palmer and Ryman, 1972; Bergot, 1979; Furiuchi and Yone, 1981). Nonetheless, carbohydrates are incorporated as lipid substitutes as energy sources, in catfish and salmonid diets.

There is no fundamental inability of carnivorous fish to utilize dietary carbohydrates (Pieper and Pfeffer, 1979). The means and mechanisms of digestive and metabolic pathways may, however, be genetically restricted, and inducible enzymatic control mechanisms to accommodate abnormal glucose loads appear to be absent (Walton and Cowey, 1982). No phenotypic adaptation of the digestive system was evident in experiments conducted on rainbow trout (*Oncorhynchus mykiss*) fed high dietary levels of carbohydrate (Buddington and Hilton, 1987; Buddington *et al.* 1987), and no specific adaptive regulatory control of plasma glucose level was apparent for trout after long-term feeding of diets with high levels of either native or processed (extruded) starch (Kaushik *et al.*, 1989). However, studies by Hilton and coworkers (1987b) found significantly higher plasma insulin levels in rainbow trout fed a high carbohydrate diet (25% glucose) compared with those fed a carbohydrate-free diet. Mazur (1990) reported a significantly reduced hyperglycaemic response to an oral glucose tolerance test in chinook salmon (*Oncorhynchus tshawytscha*) acclimated to a high carbohydrate diet prior to testing, which indicated an adaptation response.

The efficacy of carbohydrates as a source of non-protein energy depends on an optimum, species/growth stage-specific ratio of protein to energy, beyond which protein sparing effects decline (Phillips, 1972). Information regarding the digestion and utilization of carbohydrates will be useful in formulating diets producing the most economic feed-to-biomass conversion in sablefish.

2.3.2 Starch

Plant feedstuffs contain simple sugars but most of their carbohydrate is in the form of starch. The latter is the energy source most readily and economically available for animal diets. Starches are polymers of D-glucose residues composed of amylose and amylopectin granules. The residues are bound by α-1,4-linkages. In amylose these are arranged in unbranched chains, while in amylopectin they are arranged in polymers which branch between approximately 24-linked glucose units, at α-1,6-linkages.

Starch products differ essentially in the quality and proportion of their amylopectin content. Most starch granules contain 12-35% amylose and 65-88% amylopectin, depending on the feedstuff source (Vonk and Westem, 1984). The conformation of the "limit dextrin", characterized by the core of the polymers contained within the boundaries of the α-1,6-linkages of amylopectin, differs with each starch. The ratio of amylose to amylopectin and the structure of the limit dextrins can influence the digestibility of starch from different sources (Bergot and Breque, 1983).

Starch granules tend to have a radial symmetry developed in a layered structure. When the granules are heated in the presence of water, the forces binding the structure are broken down irreversibly. Subsequently, the granule swells and the viscosity of the mixture increases, in the

process of gelatinization. In this colloidal state, amylose and amylopectin form hydrated micelles in which the polysaccharide chains are twisted in loose helical coils (Banks *et al.*, 1973).

The physical properties of gelatinized starch are important in the processing of animal feeds. Technological treatments affecting starch gelatization include heating, heating under pressure, and extrusion (essentially the same thing), in an aqueous mixture. Starch may be further processed by recrystallizing the hydrated product in a reconstituted pre-gelatinized dried soluble powder. Extruded pellets for salmonid diets have been demonstrated to have superior durability, water stability and absorptive power compared to steam pellets (Hilton *et al.*, 1981).

2.3.3 Carbohydrate Digestion

The utilization of carbohydrates is regulated by three main factors. First, digestive enzymes hydrolyse complex carbohydrates to monosaccharides. Second, monosaccharides are transported across the mucosal membrane of the intestine, and last, they are intracellularly phosphorylated for entry into the glycolytic, glycogenic, pentose-phosphate, or biosynthetic pathways.

There is a great variation in the digestibility of carbohydrates, and this may be due, in part, to the source of the sugar, dextrin, or starch incorporated into diets, its treatment, and the methodology used to determine digestibility (Bergot and Breque, 1983). In salmonids, the relative efficiency of absorption is as follows: monosaccharides> disaccharides> starch (Phillips *et al.*, 1948; Singh and Nose, 1967; Smith, 1971; Bergot, 1979).

Starches are digested in the intestine by the hydrolytic action of α-amylase enzyme secreted by the exocrine pancreas. The pancreas is diffuse in most teleosts. Omnivores such as the common carp (*Cyprinus carpio*) may secrete 10 - 30 times more amylase than in the

carnivorous rainbow trout to accommodate the high carbohydrate level in its natural diet (Hofer and Sturmbauer, 1985). The optimum pH range for α-amylase activity in teleost fishes is 6.8 - 8.5, which is similar to intestinal pH values (Vonk and Western, 1984).

The α-1,4-linkages of starch are attacked by α-amylase, yielding disaccharide residues. Glycosidases hydrolyse the α-1,6-linkages of dextrins, and the starch is eventually degraded to a mixture of glucose and maltose. Maltase enzyme situated on the brush border of the intestinal wall further degrades maltose to glucose.

Active transport of glucose takes place at the intestinal epithelium. Absorption occurs mostly at the proximal (anterior) intestine at a constant rate (Fange and Grove, 1979).

2.3.4 Carbohydrate Metabolism

Carnivorous fish have a limited ability to regulate plasma glucose levels. Oral glucose tolerance tests result in hyperglycaemia in salmonids which persists 24 hours after glucose administration (Palmer and Ryman, 1972; Bergot, 1979; Hilton *et al.*, 1987a; Mazur, 1990). Furuichi and Yone (1981) observed elevated blood sugar levels five hours after oral glucose administration in the semi-carnivorous red sea bream (*Chrysophrys major*) and the carnivorous yellowtail (*Seriola quinqeradiata*). Glucose intolerance in fish is reminiscent of the diabetic condition in mammals.

Fish have lower glucose requirements and utilization rates than homoiotherms. The rate of glucose oxidation in salmonids is slow (Cowey *et al.*, 1977a, b), and is approximately one tenth that of mammals, in part due to their lower basal metabolic rate (Lin *et al.*, 1978). The glucose diffusion space of rainbow trout has been calculated to be 13.7% of the body weight (Cowey *et al.*, 1977a) compared with 30% for that of the rat (Friedmann *et al.*, 1965). This limitation is

thought to be related to a lower permeability of glucose in carnivorous fish (Christianson and Klungsoyr, 1987).

Intracellular phosphorylation of glucose is catalyzed by hexokinase, whose enzymatic actions are inhibited by glucose-6-phosphate. Shibata (1977, cited in Fideu *et al.*, 1983) reported higher activity of hexokinase in muscle tissue than in liver tissue in rainbow trout. An inducible glucokinase found in most mammals, which acts as an enzymatic control mechanism for abnormal glucose loads, has not been found in rainbow trout (Cowey *et al.*, 1977b) or yellowtail (Nagayama and Ohshima, 1974). The activity of hexokinase increases in rainbow trout fed a high carbohydrate diet and decreases in those fed a high protein diet, or during starvation (Fideu *et al.*, 1983). Hexokinase is regarded as the rate limiting enzyme of glucose metabolism in fish, and in the absence of inducible glucokinase, quickly becomes saturated (Walton and Cowey, 1982). The activity of hexokinase and other glycolytic enzymes plays a role in the regulation of glycolysis, but the role of nervous and hormonal regulation is unclear (Christiansen and Klungsoyr, 1987).

Glycogen formation from glucose-6-phosphate is catalysed by the actions of several enzymes, all of which have been identified in fish tissues. Fish liver glycogen is relatively difficult to mobilize, whereas muscle glycogen is quickly utilized as an energy source. Considering the scarcity of carbohydrate sources in the natural diets of carnivorous fish, it is likely that the central biosynthetic pathway of gluconeogensis is responsible for meeting most of their free glucose requirements, and under natural conditions, for the relative stability of hepatic glycogen levels (Saurez and Mommsen, 1987).

Rainbow trout and yellowtail have shown an ability to regulate gluconeogenesis according to dietary composition. The activities of gluconeogenic enzymes are reduced in fish fed a carbohydrate-rich diet (Cowey *et al.*, 1977b; Shimeno *et al.*, 1979; Higuera and Cardenas, 1985) while glycolytic enzyme activity is increased (Shimeno *et al.*, 1979; Cowey *et al.*, 1981).

Insulin and glucagon control the rates at which fuels enter and leave the extracellular space, and also their concentration in transit (Matty and Lone, 1985). Their roles in nutrient homeostasis in fish are, however, different than in mammals. Insulin is an anabolic hormone in teleost fish. Prolonged glucose intolerance and slow, long lasting hypoglycaemic reaction to (usually mammalian) insulin injection indicates that insulin is not relied on to rapidly modulate a metabolic response to high plasma glucose levels in fish (Christiansen and Klungsoyr, 1987). The induced release of insulin has been shown to be dose-dependent at a low range of plasma glucose levels in the silver eel (*Anguilla anguilla*) but higher doses produce no further response (Ince and Thorpe, 1977a). In some species an inverse correlation exists between dietary carbohydrate level and plasma insulin (Yone, 1978; cited in Ince, 1983; Furuichi and Yone, 1981). Under natural conditions, changes in the plasma glucose level probably stimulates insulin release, which effects the transport of low levels of glucose into the cells (Ince, 1983), but the strongest stimulators of insulin secretion in fish are amino acids (Epple and Brinn, 1987).

Recent studies on rainbow trout have shown a positive correlation between plasma insulin levels and dietary carbohydrate concentration (Hilton *et al.*, 1987b). In another study, chinook salmon acclimated to a high carbohydrate diet showed a reduced hyperglycaemic response in glucose tolerance tests, although plasma glucose and plasma insulin concentrations were poorly correlated (Mazur, 1990). Despite increased insulin secretion and adaptation to high dietary carbohydrate levels, salmonids remain intolerant to high glucose loads. This may be due to deficiency in hormone-receptor interactions in skeletal muscles for mediating glucose permeability (Plisetskaya, 1989).

Insulin induces glycogen formation in mammals, but that function is variable in fish. Injections of bovine insulin (Tashima and Cahill, 1968) and codfish insulin (Ince and Thorpe, 1976) have been shown to stimulate the incorporation of ^{14}C-glucose carbon into tail muscle

glycogen and protein in toadfish (*Opsanus tau*) and northern pike (*Esox lucius*), respectively. In rainbow trout, insulin promoted oxygen clearance of labelled plasma glucose to oxidative pathways, with little directed to carbohydrate (glycogen) storage localities (Ablett *et al.*, 1981b). Prolonged insulin administration produced significant body weight increase, and an increase in body protein and lipid, while no increase in liver or muscle glycogen was observed. The role of insulin in carbohydrate metabolism in fish appears to be that of regulating oxidative clearance of glucose rather than its storage as glycogen (Ince, 1983). The major role of insulin in fish is the regulation of protein metabolism (Murat *et al.*, 1981). Insulin enhances the incorporation of amino acids into liver and muscle protein (Ince and Thorpe, 1976; Ablett *et al.*, 1981b) while suppressing gluconeogensis from amino acids (Cowey *et al.*, 1977a; Ablett *et al.*, 1981a).

The pancreatic hormone glucagon induces liver glycogenolysis in teleost fish, resulting in elevated blood glucose levels (Ince and Thorpe, 1977b; Chan and Woo, 1978; Plisetskaya *et al.*, 1989). Exogenous administration of glucagon also stimulates gluconeogensis in several species, therefore opposing the proteogenic function of insulin (Inui and Yokote, 1977; Walton and Cowey, 1979; Mommsen and Moon, 1989). The effects of dietary nutrients on glucagon secretion in fish are undocumented.

2.3.5 Carbohydrate Utilization

Reports in the literature on the optimum quality and level of carbohydrate in formulated diets for fish often appear to be contradictory and confusing. This may be due to variable diet composition, fish species and size, environmental conditions and different digestibilities of carbohydrate feedstuffs. The diets employed in many studies have not been isonitrogenous or isocaloric and in some experiments the dietary concentration of carbohydrates has been increased

at the expense of other nutrients, possibly causing some nutrient deficiency (Pieper and Pfeffer, 1980a). Furthermore, "optimum" levels may be regarded in terms of their protein-sparing effect, the feed conversion ratio, growth performance, and fish health.

The treatment of starch and its level of incorporation into the diet are the most important factors affecting starch digestibility and utilization. The limited digestibility of native starch in salmonids was predicted in early work by Phillips *et al.* (1948). Poor growth rates, pathological liver glycogen deposition and increased mortality were produced by high carbohydrate levels in the diets of brook trout (*Salvelinus fontenalis*). Buhler and Halver (1961) reported similar growth rates in chinook salmon fed low carbohydrate (dextrin) /high protein diets and high carbohydrate/ low protein diets, with an increased protein efficiency ratio (PER) in the latter. They suggested that the earlier negative results in salmonids fed high carbohydrate diets were influenced by nutrient deficiencies and dietary imbalance. They also reported the superior digestibility of low molecular weight sugars, compared to (potato) starch. The high digestibility of low molecular weight sugars, compared with dextrins and starch was confirmed by Singh and Nose (1967). Simple sugars had high digestibility (95-99%) in rainbow trout, over a wide range of levels (20-60%) in the diet, while the digestibility of dextrin and potato starch declined in trout with increasing level in the diet. Other work has confirmed the observation that the digestibility of simple sugars is superior to that of starch. Moreover, rainbow trout ingesting similar amounts of protein and fed diets containing high levels of digestible carbohydrate were noted to have higher growth rates and protein efficiency than those fed diets with lower levels (Bergot, 1979b). Further, weight gain, protein efficiency ratio and net protein utilization in plaice (*Pleuronectes platessa*) fed diets of approximately equal total energy were found to be higher when the diets contained 10-20% of dry weight from glucose and dextrin rather than no carbohydrate (Cowey *et al.*, 1975). By contrast Hilton and Atkinson (1982) reported that a depressed growth rate and

elevated feed conversion in rainbow trout resulted from a high level of dietary glucose (18%). In subsequent experiments, Hilton *et al.* (1987a) determined net energy values of glucose (cerelose) and raw corn starch, that were 24.6% and 12.6% of their respective gross energy values, when they were present at 25% of dry matter in the diets of trout. Several investigators have observed a negative correlation between dietary carbohydrate content and diet digestibility in various species fed diets containing a variety of treated and untreated starches (Edwards *et al.*, 1977; Shimeno *et al.*, 1979; Bergot and Breque, 1983). Austreng *et al.* (1977) found dressed carcass weights (as a percentage of body weight) to be similar in rainbow trout fingerlings fed diets containing 17, 25 and 38% of metabolizable energy as carbohydrate (sucrose, native and cooked wheat). Liver weights were higher in fish fed diets with elevated levels of carbohydrate. In another study, rainbow trout fed diets containing higher metabolizable energy in the form of carbohydrates (glucose, raw wheat starch) had depressed growth rates and condition factors, but significantly improved apparent digestibility of energy and protein (Refstie and Austreng, 1981).

Gelatinization of starch can increase the bioavailability of the dietary carbohydrate. Inaba *et al.* (1963) reported that, at a similar level of incorporation in the diet (about 40%), the digestibility of cooked wheat was higher (48%) than that of uncooked wheat (22%) in rainbow trout. This enhanced digestibility by starch gelatinization was confirmed (in trout) for potato starch (Singh and Nose, 1967) and corn starch (Smith, 1971; Bergot and Breque, 1983; Boccignone *et al.*, 1989). Rainbow trout fed extruded or steam-pelleted diets containing approximately 14% starch (from wheat middlings) showed lower feed consumption, and weight gain, and higher feed efficiency when fed the extruded diet (Hilton *et al.*, 1981).

Kaushik *et al.* (1989) reported that the inclusion of extruded cereal (corn or wheat) or extruded starch in isonitrogenous diets improves the availability of dietary energy in long-term feeding trials (30 weeks) with rainbow trout. Growth and nutrient retention efficiencies in trout

ingesting the extruded diets (except for extruded wheat) were higher that noted in trout fed diets containing raw corn starch, while the protein efficiency ratio and protein retention efficiency were unaffected by treatment.

High carbohydrate levels in salmonid diets have been associated with enlarged livers and high liver glycogen levels (Philips *et al.*, 1948; Buhler and Halver, 1961; Austreng *et al.*, 1977; Bergot, 1979; Hilton *et al.*, 1981; Refstie and Austreng, 1981; Hilton and Dixon, 1982). These effects have also been observed in plaice (Cowey *et al.*, 1975) and yellowtail (Shimeno *et al.*, 1979). Hilton and Dixon (1981) reported sub-lethal liver dysfunction in rainbow trout fed high dietary levels of carbohydrate.

Several other factors besides fish species and the source of starch may affect starch digestibility in fish. These include fish size, feeding regime, water temperature, and the presence of other dietary ingredients (Pfeffer, 1977; Dabrowska and Wojno, 1977; Fange and Grove, 1979). Through their confounding influences, these factors make the prediction and determination of carbohydrate digestibility difficult.

Environmental temperature significantly affects the speed of passage of ingesta through the alimentary canal and its consequent exposure time to hydrolytic enzymes. In general, passage time increases with decreasing temperature and increased fish size. Warmer temperatures have been shown to increase the activity of α-amylase in several species of flatfish (Yasunaga, 1972).

Wheat and some other grains contain albumins which can inhibit the action of α-amylase and thereby reduce starch utilization. In the omnivorous, stomachless carp, amylase inhibition is counteracted by an increase in α-amylase secretion (Sturmbauer and Hofer, 1986). Pepsins secreted by the stomach, and pancreatic proteinases may hydrolyse amylase inhibitors in the digestive tract of rainbow trout, essentially neutralizing them. The temperature employed in extrusion (gelatinization) may also denature albumin inhibitors in feedstuffs (Hofer and

Sturmbauer, 1986). It has been speculated that the denaturation of amylase inhibitors may be partly responsible for the improved digestibility of gelatinized starches in fish (Sturmbauer and Hofer, 1985).

2.3.6 Gastric Evacuation and Intestinal Transit

The rate of daily food consumption and the return to appetite of teleost fish are highly correlated with gastric (stomach) evacuation. The gastric evacuation (emptying) rate (GER; g/h) has been employed to form the basis for the prediction of rates of food consumption of wild (Elliott and Persson, 1978) and cultured fish (Vahl, 1979). The time required for the return to appetite is determined by the relationship between the duration of food deprivation and the food intake (Elliott, 1975; Grove *et al.*, 1978) and appetite is closely related to stomach fullness (Brett and Higgs, 1970; Brett, 1971; Ware, 1972). Gastric evacuation time (GET; h) is used as the basis for gauging effective feeding frequency of cultured species (Fange and Grove, 1979).

In studies with fish, mathematical expressions of gastric emptying emphasize linear, square root or exponential equations. The efficacy of these equations have been inconsistent, since those expressions having the best fit, and therefore the best predictive value, vary with species, food type and experimental conditions (Elliott, 1972; Flowerdew and Grove, 1979; Jobling, 1981a; MacDonald *et al.*, 1982; Faucouneau *et al.*, 1983; Brodeur, 1984; Fletcher *et al.*, 1984; From and Rasmussen, 1984). These factors relate to the physiological control of gastric motility and may be responsible for the inconsistent predictive value of mathematical expressions (Jobling, 1986a).

Curvilinear gastric emptying patterns are characteristic of liquid or well homogenized non-nutrient bulk diets. Muscular contractions propel the stomach contents, at relatively constant-

frequency regardless of volume, while the amplitude of the contractions is volume-dependent, producing an intrinsic emptying pattern (Hunt and Knox, 1968; Jobling, 1986a).

A diet which replaces inert bulk with increased energy density and biochemical (nutrient) constituents results in a departure from the intrinsic emptying pattern observed in mammals (Hunt, 1980) and fish (Windell, 1967; Elliott, 1972; Flowerdew and Grove, 1979; Jobling, 1980; Fletcher *et al.*, 1984). Studies with mammals suggest that volume-dependent mechanisms are overridden, at least to some extent, in response to a feedback inhibition signalled by duodenal or upper intestinal receptors (Meeroff *et al.*, 1975; Stephens *et al.*, 1976; Hunt, 1980). In fish species, gastric motility is reduced with increased dietary energy (Grove *et al.*, 1978; Flowerdew and Grove, 1979; Jobling, 1980; Hofer *et al.*, 1982). Receptors may be tuned to variations in pH, fatty acid anions, and amino acids, as evidenced in duodenal infusion trials with mammals (Cook, 1974; Barker *et al.*, 1978).

A reduction in gastric motility may be a function of a decline in the force of contraction of the stomach musculature (Bell and Grivel, 1975) and/or constriction at the duodenum, resulting in a reduced flow of ingesta (Weisbrodt *et al.*, 1969). Signals affecting feedback inhibition may be under nervous or hormonal control (Bell and Grivel, 1975). The importance of specific receptors or interactions in the regulatory response to dietary stimuli in fish is poorly understood. Gastrointestinal peptide hormones affecting gall bladder (Vigna and Gorbman, 1977) and pancreatic activity during digestion are also related to the regulation of gastric emptying (Yamagishi and Debas, 1978; Moran and McHugh, 1982) and substances known to reduce gastric motility, such as amino acids and fatty acid anions, trigger the release of these gastrointestinal hormones (Dockray, 1982).

Food particles must be reduced by chemical and mechanical processes to a size small enough to pass through the pylorus. Liquid or liquified particles pass quickly, while flesh and

bound homogenates (pellets) require varying degrees of digestion to reduce their size, retarding the intrinsic emptying pattern. The emptying delay resulting from both the integrity of large food particles and the regulatory consequences of receptor stimulants is characteristic of emptying curves in studies where large food items are used (Jones, 1974; MacDonald *et al.*, 1982). Differential (slower) movement of the indigestible fraction (chitin) of the diet has also been observed by Kionka and Windell (1972). Time lags affected by particle size and integrity, and peristaltic inhibition by receptor stimulation and feedback regulation serve to "flatten" the intrinsic curvilinear emptying curve by modifying its volume-dependent actions or by changing the emptying pattern (Fletcher *et al.*, 1984; Jobling, 1986a).

The gastric emptying time (GET) of a fish of a given size may remain the same as real size increases (Windell, 1966; Brett and Higgs, 1970; Elliott, 1972; Possompes *et al.*, 1975) or increase with fish size (Beamish, 1972; Jobling *et al.*, 1977; Garber, 1983). In general, larger meals increase the gastric evacuation rate (GER), though not in direct proportion to the increase in meal size (Beamish, 1972; Jones, 1974; Jobling *et al.*, 1977; Flowerdew and Grove, 1979; Garber, 1983).

Gastric evacuation rates have been observed to vary exponentially with fish weight, and large fish have been observed to evacuate a meal, expressed as a percentage of body weight, more slowly than smaller fish (Jobling *et al.*, 1977; Flowerdew and Grove, 1979; Garber, 1983; Talbot *et al.*, 1984).

Multiple meals may affect digestion and evacuation rate. The rate of emptying increases directly in relation to feeding frequency in a variety of species (Tyler, 1970; Noble, 1973; Jones, 1974; Hofer *et al.*, 1982). Meal size is relevant to the feeding frequency/evacuation relationship. Goldfish (*Carassius auratus*) consume less than their normal daily food intake when the duration of feeding is restricted to one hour. However, they eat more than they would normally consume

in one hour when they are maintained on a 24-hour demand-feeding apparatus (Rozin and Mayer, 1964). Godin (1981) observed a return to appetite in juvenile pink salmon (*Oncorhynchus gorbuscha*) when less than 15% of their stomach capacity had been evacuated. After pink salmon initially filled their stomachs, they kept the stomach full by feeding at a rate which balanced the emptying rate. Starved fish were noted to gorge to the point that the stomach bulged.

Possompes *et al.* (1975), utilizing a chromic oxide-marked meal, determined that a plateau of indicator concentration appeared in the faeces of rainbow trout 14 hours after food ingestion, and this continued for a period of 24 hours. By contrast, a test meal followed by two or three unmarked meals the same day (trout fed to satiety) resulted in distinct bell curves of Cr_2O_3 concentration in the faeces which peaked at 18 and 13 hours, respectively. This suggests increased gastric motility as a consequence of multiple meals. Retention of the marked meal was longer and (perhaps) greater food utilization occurred following a single meal.

In another study using Cr_2O_3 marker, a marked pellet lacking a binding agent was force-fed to dab (*Limanda limanda*), and this was followed three hours later by an unmarked pellet (Fletcher *et al.*, 1984). The transit rate of the ingesta, as determined by Cr_2O_3 in the faeces, was the same as that for two similar pellets fed simultaneously, indicating that mixing had occurred. When the experiment was repeated using pellets containing a binding agent, the transit rate of the marked pellet fed three hours before the second pellet was 35% faster than that of the marked pellet fed simultaneously with the second pellet. This signified that no mixing had occurred between the first and second meal, and that the first meal was emptied faster than the second when a binder was used.

Pre-prandial and post-prandial starvation are common features of many experiments of fish feeding, allowing the examination of a discrete meal. Other studies, particularly those

investigating gastric evacuation, subject fish only to post-prandial starvation. The effects of starvation can influence subsequent meal size and gastric motility (Fange and Grove, 1979).

Long periods of pre-prandial starvation (7+ days) can significantly reduce the emptying rate of fish (Windell, 1966; Elliott, 1972) but this may be due to degenerative processes in the gut which are unlikely after short term starvation (Talbot *et al.*, 1984). An increase in food consumption of 23.6% to 39.8% of dry body weight by juvenile pink salmon starved for 24 and 72 hours, respectively, was noted by Godin (1981). In studies with Atlantic salmon (*Salmo salar*), Talbot *et al.* (1984) reported that pre-prandial starvation prompts higher test meal consumption (than that with daily feeding), and post-prandial starvation decreases the evacuation rate to a degree that is inversely related to the size of a preceding test meal. In fish fed after a test meal, the evacuation rate was not significantly different between pre-prandially fed and starved fish. These observations concur with Godin's (1981) suggestion that, above a minimum threshold of stomach fullness, the evacuation rate is proportional to post-prandial feeding and independent of the size of the test meal.

The environmental temperature has been universally demonstrated to influence gastric motility. Within the physiological limits of a species' tolerance, the gastric evacuation rate is positively correlated with the temperature and negatively correlated with the evacuation time (Fange and Grove, 1979, and papers cited therein).

Possompes *et al.* (1975), using chromic oxide-marked diets, demonstrated that the mean transit time of a marked meal fed to rainbow trout diminishes as the acclimation temperature increases, (35 h at 9°C, 22.4 h at 14°C, and 17 h at 18°C), and so does the transit rate.

The mean transit time of Cr_2O_3 - marked diets decreased from 34 h to 26 h in rainbow trout when temperatures increased from 10°C to 18°C in one day (Fauconneau *et al.*, 1983). After 4 to 7 days, transit time at 18°C water temperature had dropped to 21 h.

2.3.7 Pellet Characteristics and Feeding Response

Wild prey, or parts thereof, are preferred to formulated pellets by salmonids (Paszkowski and Olla, 1985; Stradmeyer *et al.*, 1988) and sablefish (Kennedy, 1970; Smith, pers. comm.). The appetizing qualities of natural food may be due to visual signals such as shape, size, colour and movement (Wankowski and Thorpe, 1979; Irvine and Northcote, 1983; Jakobsen *et al.*, 1987; Stradmeyer *et al.*, 1988) or gustatory responses to taste and texture (Sutterlin and Sutterlin, 1970; Adron and Mackie, 1978; Stradmeyer *et al.*, 1988). A determination of a palatable pellet type has an important function in food consumption and growth rates in cultivated fish species.

2.3.8 Chromic oxide indicator

The quantitative estimation of nutrient digestibility can be accomplished with an indirect method using a solid phase, external dietary indicator, or marker. An effective indicator should be inert, and neither digested, absorbed nor metabolized in the alimentary tract. It should have no appreciable bulk, mix homogeneously with the diet, and move through the tract at the same rate as the rest of the dietary constituents. It should be non-toxic and have no physiological effects, and should have qualities that allow for precise quantitative measurements (Kotb and Luckey, 1972). External indicators used for feed digestibility studies in fish include chromic oxide, polyethylene, titanium oxide, celite (externally incorporated acid - insoluble ash) and bacterial spores.

Furakawa and Tsukahara (1966) reported that the inert markers polyethylene and acid-washed sand appeared to move through the digestive tract more slowly than the rest of the ingesta, and that chromic oxide was a superior reference substance. Buddington (1980)

determined that chromic oxide and hydrolysis-resistant organic matter produced similar results in determining digestive efficiency in rainbow trout and three tilapia (*Tilapia sp.*) species. Titanium oxide and Cr_2O_3 were also found to be equivalent indicators for determining apparent digestibility in Atlantic cod (*Gadus morhua*) by Lied and coworkers (1982), and similar estimates of apparent digestibility were observed with the use of acid-insoluble ash and Cr_2O_3 in rainbow trout (Atkinson *et al.*, 1984).

Tacon and Rodrigues (1984) observed that, in terms of reproducibility, Cr_2O_3 and crude fiber were more reliable than polyethylene and acid-insoluble ash as indicators for the determination of nutrient digestibility coefficients in rainbow trout. This study also determined that at a 2% level, Cr_2O_3-marked diets provided higher digestibility estimates than at lower levels (0.5% - 1%). This indicated a differential (faster) rate of flow of Cr_2O_3 at the higher level, although Furukawa and Tsukahara (1966) suggested that levels of 1% to 6% Cr_2O_3 could be used with confidence for digestibility determinations in fish.

Chromic oxide is routinely incorporated into fish diets for determining nutrient digestibility (Inaba *et al.*, 1963; Cho *et al.*, 1974; Austreng, 1978; Lied *et al.*, 1982; Kaushik and Teles, 1985) and gastric motility (Possompes *et al.*, 1975; Lied *et al.*, 1982; Fauconneau *et al.*, 1983; Fletcher *et al.*, 1984; Lesel *et al.*, 1986). The marker is mixed with the diet and its concentration is quantified in the feed and the ingesta and/or faeces. The digestibility of a given nutrient can be calculated by a ratio method (Maynard and Loosli, 1969) and the faeces concentration of Cr_2O_3 indicates the stage of passage of a test meal.

Differential flow rates of chromic oxide have been observed in dietary studies of such disparate species as burros (*Equus asinus*; Knapka *et al.*, 1967) and American lobsters (*Homerus americanus*; Leavitt, 1985). Bowen (1978) reported "selective rejection" of Cr_2O_3 marker in a detrital aggregate diet fed to juvenile tilapia (*Sarotherodon mossambicus*) which resulted in low

assimilation estimates of nutrients. His observations were challenged by Foltz (1979), particularly with regard to the high (6%) Cr_2O_3 concentration in the test diet. Digestibility experiments with Atlantic cod failed due to an apparent retention of Cr_2O_3 in the proximal intestine (Tonnessen, 1979; cited in Lied *et al.*, 1982).

Regardless of the causes of the discrepancies, ("selective rejection", "retention", "streaming"), the phenomenon of the differential movement of Cr_2O_3 indicator in widely diverse species (while appearing to be an acceptable marker for most diets and fish species) suggests that a measure of caution must be exercised when employing this marker for investigating the nutritional requirements of species new to aquaculture, and when assessing the information it helps to provide.

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Introduction

Five experiments were conducted at the West Vancouver Laboratory of the Department of Fisheries and Oceans, to investigate the utilization of formulated diets, and the sites of digestion and absorption in sablefish. Also investigated were rates of food passage of different diets. In particular, the utilization of dietary carbohydrate was studied as a matter of practical interest for the culture of sablefish. The utility of chromic oxide as an inert external indicator was also assessed.

The feeding experiments were conducted on wild-captured sablefish previously employed in feeding experiments, and obtained from the Pacific Biological Station, Department of Fisheries and Oceans, Nanaimo, B.C. All fish were contained in 3.05 meter diameter, 6000 litre fibreglass circular tanks with a radial seawater discharge of 42 litres per minute. Each tank was covered with plywood sheets or plastic covers, permitting a minimum of direct sunlight to strike the water surface. The fish were acclimatized and maintained between experiments on a diet of chopped herring and/or Oregon Moist Pellets (OMP).

3.2 Experiment 1: Digestibility of cooked wheat, and determination of α-amylase activity.

A high carbohydrate diet was used to investigate the process of digestion in sablefish.

Thirty-seven sablefish ranging in length from 53.7 cm to 61.4 cm (mean = 57.1 cm), and in weight from 1.866 kg to 2.774 kg (mean = 2.142 kg) were hand-fed daily rations to satiation. The test diet (diet 1), contained 44.5% of dry matter as finely ground wheat (Table 1) and it had a nitrogen-free extract (NFE) level of 35.5% (Table 2). Crude protein content was 38.0% and crude lipid, 14.2%. Water was added to produce a moisture content of 40%. The high dietary moisture content was chosen to accommodate the observations that sablefish reject test diets of less than 30% moisture (Higgs, pers. comm.) and that they accept OMP (30% moisture). Chromic oxide (0.1%) was employed in Diet 1 as an inert external marker.

The method used to calculate the gross energy (G.E.) and the metabolizable energy (M.E.) values of the diet was based on the gross chemical composition of the diets using assumed digestibility values for the different chemical components. The M.E. of protein (4.5 kcal/g) was derived by subtracting the heat of combustion of excreted ammonia nitrogen (0.95 kcal/g) from the G.E. of protein (5.66 kcal/g) (Brett and Groves, 1979), and applying a digestibility coefficient of 95% for all sources of protein. Lipids and nitrogen-free extract were assumed to be 100% digestible and were assigned energy values of 9.5 kcal/g and 4.0 kcal/g, respectively (Table 2). The more commonly applied digestibility coefficient of 40% (1.6 kcal/g) for starch was not used since the digestibility of carbohydrates varies widely in fishes, and nothing is known of that in sablefish.

Herring meal and wheat were finely ground (U.S. 40 sieve) and dry-blended with other ingredients in a Hobart mixer. Water was added in a ribbon mixer. The diet was wrapped in aluminum foil and autoclaved at 120°C (1 atmosphere) for one hour. Pellets of 25 - 40 mm in length were manufactured through a 12.7 mm (1/2 inch) die with a moist-feed pelleter, and then were immediately frozen in plastic bags at -20°C. The pellets were thawed before feeding the sablefish.

Table 1 - Composition of Diets 1 to 7 : g/kg on a dry matter basis

Ingredients	Diet 1	Diet 2 & 3[1]	Diet 4	Diet 5	Diet 6	Diet 7
Ground wheat	444.5	444.5	222.4	-	222.2	444.4
Pregelatinized wheat starch[2]	-	-	181.0	362.2	-	-
Herring meal	280.5	280.5	321.6	362.8	272.3	272.3
Condensed herring solubles	75.0	75.0	75.0	75.0	75.0	75.0
Herring oil[3]	100.0	100.0	100.0	100.0	175.4	100.0
Gelatin	50.0	50.0	50.0	50.0	50.0	50.0
Wheat gluten meal[4]	-	-	-	-	36.9	-
α-cellulose	-	-	-	-	118.2	8.7
Wheat middlings	34.0	25.0	25.0	25.0	34.9	34.6
Vitamin premix[5]	2.4	2.4	2.4	2.4	2.4	2.4
Mineral premix[6]	7.0	7.0	7.0	7.0	7.0	7.0
Ascorbic acid	1.6	1.6	1.6	1.6	1.6	1.6
Choline chloride (60%)	4.0	4.0	4.0	4.0	4.0	4.0
Chromic oxide	1.0	10.0	10.0	10.0	-	-

[1] All diets except Diet 2 were autoclaved.

[2] Redigel-11; Ogilvie Mills, Montreal, Quebec.

[3] Stabilized with BHA-BHT (1:1) 0.33%.

[4] Whetpro 80 (80% protein), Ogilvie Mills, Montreal, Quebec.

[5] Vitamin premix to supply (mg/kg of dry diet unless otherwise indicated): Ca-pantothenate 155.1; pyridoxine HCL 34.5; riboflavin 56.7; niacin 283.65; folic acid 18.9; thiamin mononitrate 32.15; biotin 2.84; heterazeen (22.5% menadione) 110.2; vitamin B12 0.057; vitamin E 567.3 IU; vitamin D3 2269 IU; vitamin A 9455 IU; inositol 378.

[6] Mineral premix to supply (mg/kg dry diet): Mg 141.8, Mn 69.2, Zn 28.36, Co .94, Cu 3.29, Fe 47.3, I 5.1, Na 1950.6. Sources of minerals were magnesium sulphate, manganese sulphate, zinc sulphate, cobalt chloride, copper sulphate, ferrous sulphate, potassium iodide, sodium chloride,respectively.

Table 2 - Calculated proximate composition and energy values for the formulated diets, and whole herring (% dry matter)

Analysis[1]	Diet 1	Diet 2 & 3	Diet 4	Diet 5	Diet 6	Diet 7	Herring[2]
Crude protein (%N x 6.25)	38.00	38.00	38.00	38.00	38.00	38.00	60.5
Crude lipid	14.21	14.21	14.18	14.15	21.77	14.30	28.1
Ash	5.75	5.74	5.68	5.63	6.86	7.32	8.7
Crude fibre	1.56	1.55	0.95	0.35	13.04	2.69	1.7
NFE	35.52	35.50	36.19	36.87	20.38	37.69	1.0
Energy (kcal/kg)							
Crude protein:							
M.E. (4.5 kcal/g)	1710	1710	1710	1710	1710	1710	2723
G.E. (5.7 kcal/g)	2166	2166	2166	2166	2166	2166	3449
Lipid:							
M.E. & G.E. (9.5 kcal/g)	1350	1350	1347	1344	2068	1360	2670
Carbohydrate:							
M.E. & G.E. (4.0 kcal/g)	1421	1420	1448	1475	815	1508	68
TOTAL M.E.	4483	4480	4505	4529	4593	4578	5461
TOTAL G.E. (digestible energy)	4937	4936	4961	4985	5049	5034	6187

[1]Totals exclude vitamin and mineral premix, and chromic oxide. All formulated diets contained 40% moisture.

[2] Hart *et al.*, 1940; proximate analysis from ripe herring averaged.

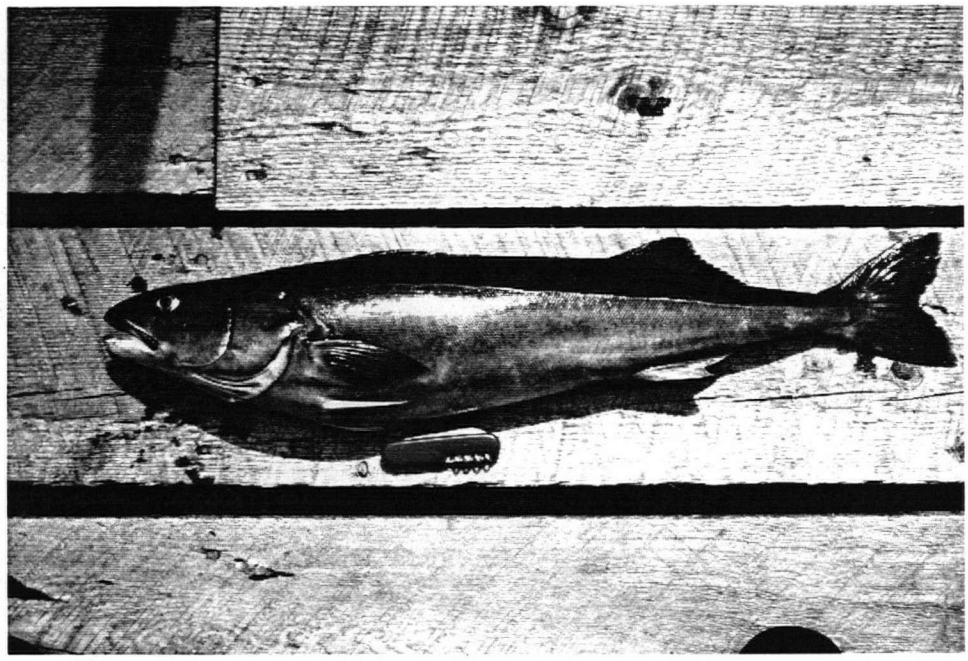
The fish were fed the formulated diet daily for 10 days prior to sampling, and then they were sampled three times over a two-week period, with intervals of 6 and 8 days between samplings. The sea water temperature in the experimental tanks, ranged from 11.1 to 13.5°C (mean = 12.5°C) in the feeding period prior to the first sample, 10.9 to 11.9°C (mean = 11.6°C) between samples 1 and 2, and 10.5°C to 11.6°C (mean = 11.0°C) between samples 2 and 3. Feeding protocol was consistent between sampling times. Fish were sampled approximately 18 hours after feeding. At each sample time, five fish were anaesthetized with tricane methanesulphonate (MS 222) and then killed by cutting the gills. Each fish was wiped with a damp cloth, measured to the nearest millimetre (fork length) and weighed on an electronic balance to the nearest gram.

The abdomen was opened and the gastrointestinal tract (GI) was divided into three segments by ligations at the oesophagus, the pylorus, the caudal bend of the intestine, and the anus (Fig. 1). The GI sections were numbered 1, 2 and 3, beginning with the distal intestine and the proximal intestine (section 2) included the pyloric caeca. The contents of the segments was washed into plastic containers, immediately frozen, and freeze-dried.

Gastrointestinal mucosal moisture, and the bile of samples 2 and 3 were measured for pH with a Cole-Parmer Model 5985-80 pH meter. The proximal and distal intestines and the caeca from the five fish in sample 3 were frozen and assayed for α-amylase activity. The sections were rinsed with distilled water, cut into small pieces and homogenized with a Potter-Elvehjem homogenizer. The homogenate was mixed with distilled water and centrifuged for 20 minutes at 1000 x g. The supernatant was diluted with distilled water, and analyzed for α-amylase activity by the method of Dahlqvist (1962).

Dried samples of feed and ingesta were blended and stored in acid-washed glass vials. Replicate measures (100 g - 300 g) of the diet and ingesta samples were weighed into acid-

A



B

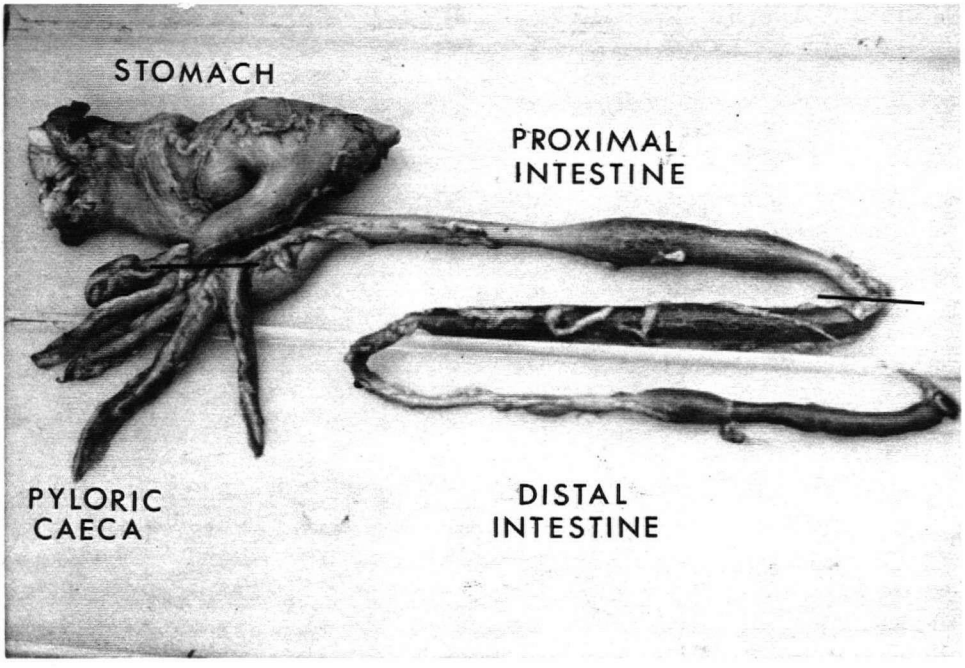


Fig. 1 - **A: Sablefish (Anoplopoma fimbria), of approximately 2.5 kg in weight and 60 cm in length.**

B: Gastrointestinal tract, indicating the stomach, and proximal and distal sections of the intestine. The three sections of the alimentary tract which were used for digestibility determinations are indicated.

washed petri dishes and prepared and analyzed for minerals on an Inductively Coupled Argon Plasma-Atomic Emission Spectrometer (ICAP-AES) Jarrell-Ash Model 975 Plasma Autocomp.

Samples from each sample period and G.I. section were pooled. Cr_2O_3 concentration of pooled samples was derived from weighed chromium concentrations of individual samples. Pooled ingesta and diet samples were subjected to analysis as follows:

1) Total nitrogen was measured as Total Kjeldahl Nitrogen (TKN) using the micro-Kjeldahl method, on a Technicon Autoanalyser II (Technicon Instrument Co. Ltd., Industrial Methods 369-75 A/A and 334-74 W/B). Percent nitrogen was multiplied by 6.25 to estimate protein content.
2) Lipid was determined by the method of Bligh and Dyer (1959).
3) Moisture and ash were determined by AOAC (1975) methods.
4) Crude fiber was determined by the method of McQueen and Nicholson (1977).
5) Nitrogen-free extract (NFE) was determined by difference:

   %NFE = 100% - % protein - % lipid - % ash - % crude fibre.
6) Organic matter was determined from dry matter digestibility corrected for ash content.

The digestibility co-efficients for the various nutrients were determined as follows:

Apparent Digestibility Coefficient (%) =

$$100 - 100 \times \frac{(Cr_2O_3 \text{ in feed (\%)}}{(Cr_2O_3 \text{ in ingesta (\%)}} \times \frac{\text{nutrient in ingesta (\%))}}{\text{nutrient in feed (\%))}}$$

3.3 Experiment 2: Digestion and utilization of diets containing variously treated wheat starch

Four high-carbohydrate, isocaloric and isonitrogenous diets were prepared to determine

their digestibility in sablefish. The diets were formulated to contain approximately the same energy, in the form of NFE, from ground wheat and pregelatinized wheat starch (Tables 1 and 2; diets 2 - 5). Concentrations of herring meal were altered to adjust for the protein present in ground wheat which was restricted or absent in diets 4 and 5. All diets were mixed as described previously for diet 1, and chromic oxide was added at a level of 1.0%. Three diets were vacuum sealed in aluminum packages and autoclaved, while diet 2 was untreated. Pelleting and storage was similar to Experiment 1. The gross and metabolizable energy contents of diets 2 and 5 were calculated as per Experiment 1 (Table 2).

One hundred forty-eight sablefish ranging in length from 56.5 cm to 68.2 cm (mean = 62.8 cm), and in weight from 2.187 kg to 3.836 kg (mean = 2.971 kg) were evenly and randomly distributed into four 6000 litre tanks (n = 37 fish per group). One of the formulated diets was assigned to each tank of fish. The fish were fed on alternate days for eight days before sampling. The feeding frequency was restricted to once every two days to ensure that all fish exhibited good appetite before sampling. This was not the case in Experiment 1, when several sacrificed fish had empty stomachs (see Results, 4.1). The fish were fed their prescribed diet slowly to satiation , over the course of approximately one hour.

The texture of diet 5 (high pregelatinized starch content) and to a lesser extent diet 4 (low pregelatinized starch content) was too soft to maintain pellet integrity, and these diets were dispensed, and after thawing, as small moulded balls.

Five sablefish were sampled from each tank at two times, each 16 days apart. Feeding protocol was maintained between sample times, and the sea water temperature ranged from 7.9 to 9.5°C (mean = 8.14°C). Fish were anaesthetized and slaughtered 18 hours following their last meal. Each fish was measured and weighed, and the gastrointestinal sections were sampled, as described for Experiment 1. The pH values of the gastrointestinal section contents and the bile

were determined for fish of the first sample period with a pH meter, and the livers from the fish of the second sample were removed, weighed, bagged and frozen at -20°C for future analysis of glycogen and lipid content.

Mineral and nutrient analyses were conducted as outlined in Experiment 1, as was the weighing and pooling of the ingesta samples from the different gastrointestinal sections. Liver glycogen was assayed by the method of Hassid and Abraham (1957), and liver lipid concentration was determined according to the procedure of Folch *et al.* (1957). The hepatosomatic indices of fish in each sample were calculated as: (wet weight of liver (g)/wet weight of fish (g)) x 100. Arcsine transformations of data pertaining to liver glycogen and lipid, and hepatosomatic index, were made to achieve homogeneity of variance. Thereafter the data were subjected to one-way analysis of variance without replication, to determine the significance of dietary effects (SAS version 6.03, 1988). Treatment means were compared using Duncan's New Multiple Range Test (maximum comparisonwise error = 0.05).

3.4 Experiment 3: Influence of chromic oxide concentration on the digestibility of formulated diets in sablefish.

To test the hypothesis that the different levels of chromic oxide indicator used in Experiments 1 and 2 influenced the accuracy of the results, two diets supplemented with either 0.1 or 1.0% Cr_2O_3 were fed to sablefish. The test diets were diets 1 and 3, which had been employed in Experiments 1 and 2, (Table 1). Aquarium conditions were identical to those in the preceding experiments. Water temperature averaged 10.6°C during the feeding of the 0.1% Cr_2O_3 diet, and 13.4°C during the feeding of the 1.0% Cr_2O_3 diet.

Thirty sablefish, ranging from 57.4 to 69.1 cm (mean = 61.9 cm) in length and 2.056 to 4.158 kg (mean = 2.886 kg) in weight, were fed diet 1(supplemented with 0.1% chromic oxide) to

satiation every second day for a total of five times (10-day study). Seven fish were anaesthetized and killed 18 hours following the final meal, and the ingesta of the gastrointestinal sections were sampled as previously described in Experiment 1. The remaining fish were fed diet 3(supplemented with 1.0% chromic oxide) a further five times, on alternate days, and the sampling procedure was repeated.

The contents of the ingesta and samples of the diets were freeze-dried, homogenized and stored in acid-washed vials. Replicate sub-samples were solubilized to H_2CrO_4 (chromic acid) as per Perkin Elmer Analytical Methods for Atomic Absorption Spectroscopy (Anon, 1976) and the samples were analyzed on a Perkin Elmer Atomic Absorption Spectrometer, Model 560, for chromium content.

The derived concentrations of chromium in replicate sub-samples were averaged and the means of the chromium concentrations in the feed and stomach contents were compared, for each diet treatment, using the Student's 't' distribution.

3.5 Experiment 4: Gastric evacuation of formulated and natural diets.

A formulated diet and a chopped herring diet were fed to sablefish to observe the characteristics of the diets' gastric motility. The formulated diet was similar to that described for diet 3, Experiment 2 (Table 1) but lacking Cr_2O_3 marker. The herring was chopped into chunks of approximately 3 cm square. Each diet was fed to one of two groups of 32 fish. Sablefish lengths ranged from 52.3 cm to 69.8 cm (mean = 64.9 cm) and weights ranged from 1.507 kg to 4.117 kg (mean = 2.8 kg). The temperature of the seawater was 10.1 to 11.2°C during the course of the sampling.

Each group of fish was fed their respective diet, to satiation every second day for a total of three times (6-day period) before sampling. After the final feeding, six fish were immediately captured from each tank, anaesthetized with MS 222 and killed by a blow to the head. The carcasses were quickly strung, head up, on a pole through the gills, to prevent the loss of fluid stomach contents, and then immediately frozen at -20°C. The process was repeated at 6, 12, 24 and 36 hours after the completion of feeding.

The frozen fish were partially thawed and the still-frozen ingesta were removed from the stomach. Samples were stored frozen in plastic bags. The fish were then measured and weighed on an electronic balance.

The frozen stomach samples were weighed, thawed, and homogenized. Replicate sub-samples were weighed, freeze-dried, and once again weighed. The calculated moisture was averaged from the replicate sub-samples and the organic matter content for the whole sample was determined from dry matter corrected for ash content. The geometric mean (G.M.; calculated to ensure that the variances were homogeneous) of the ratios of organic matter of ingesta to wet body weight were calculated for fish fed each diet for each sampling time. The G.M. $\pm$ 2S.E. of the ratios for fish fed diet 3 were regressed as the natural logarithm, logarithm (base 10), arcsine and square root transformations, against time, in order to determine the transformation which best linearized the data over the period of measurement.

3.6 Experiment 5: Influence of dietary carbohydrate concentration on carbohydrate utilization by sablefish

Two isonitrogenous and isocaloric diets were formulated to investigate the effect of dietary carbohydrate concentration on utilization of carbohydrate by sablefish. The compositions of the experimental diets are provided in Table 1 (diets 6 and 7). Two levels of cooked wheat (22.2%

and 44.4%) were incorporated into diets fed to two groups of sablefish over a 14-week period. NFE levels were 20.4 and 37.7% (Table 2), and wheat gluten meal was added to adjust the protein content of the diet containing the lower level of ground wheat (diet 6). Indigestible α-cellulose was used as a filler for this diet. The proximate compositions and calculated energy contents of the formulated diets are presented in Table 2. A third (control) diet of previously frozen chopped whole herring was fed to a third group of sablefish. The herring were mainly ripe prespawning males.

The diets were mixed and manufactured according to procedures described in Experiments 2 through 4. Aquarium facilities were also the same. Thirty-four sablefish in each of three 6000 litre tanks were fed one of the test diets to satiation on alternate days. Sablefish lengths ranged from 50.7 to 62.0 cm (mean = 56.8 cm) and weights from 1.450 to 3.269 kg (mean = 2.132 kg). Bags containing feed were weighed before and after feeding and each ration was recorded. The experiment was conducted during a period of falling water temperatures (Fig. 10).

Sablefish were sampled prior to the commencement of feeding. Fish were anaesthetized with MS222, weighed and measured, and returned to their tanks. Two fish from each tank were killed, weighed and measured, and the livers were removed, weighed, and frozen in plastic bags. A piece of muscle approximately 20 cm^3 was cut from the left flank below the posterior end of the dorsal fin and similarly stored. Further samples were taken at 30, 56 and 96 days after the initiation of the study. Weights and lengths were recorded and five fish from each group were killed and sampled for liver and muscle, as described above.

Liver and muscle samples were analyzed for glycogen concentration by the method of Hassid and Abraham (1957). Hepatic lipid concentrations were measured using the method of Folch *et al.* (1957).

The total food intake of each group was expressed on a moisture-free basis by using 30

and 60% for the percentages of dry matter in whole herring and each of the formulated diets, respectively.

The mean weights determined for the final sample time were subjected to analysis of variance, without replication. Weight gains (g/fish) were obtained from total biomass gain. Dry food intake (DFI) was calculated as grams per fish per day, adjusted for mortalities.

The feed conversion ratio,[1] protein efficiency ratio,[2] and specific growth rate[3] were calculated for each diet and sample period.

Arcsine (angular) transformations of liver glycogen, liver lipid, muscle glycogen, hepatosomatic index[4] and condition factor[5] measurements, for each sample from each sample period, were subjected to two-way analysis of variance (ANOVA) without replication (SAS, version 6.03, 1983).

Treatment means were compared using Duncan's New Multiple Range Test (maximum comparisonwise error = 0.05).

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[1] Feed conversion ratio was calculated as: dry weight of feed fed(g)/wet weight gain of fish (g).

[2] Protein efficiency ratio was calculated as: gain in wet body weight(g)/protein intake(g).

[3] Specific growth rate was calculated as: 100 x (ln (body weight) $_{t1}$ - ln (body weight) $_{t0}$ / t1 - t0).

[4] Hepatosomatic index was calculated as: wet weight of liver (g)/wet weight of fish (g) x 100

[5] Condition factor was calculated as: (fish weight (g)/fish length $(cm)^3$) x 10^2 (Anderson and Gutrenter, 1983).

CHAPTER 4

4.0 RESULTS

4.1 The digestibility of nutrients from a high carbohydrate diet

The apparent digestibility coefficients (ADC) for the nutrients in diet 1 in relation to three sections of the gastrointestinal tract, at three sample times, are presented in Table 3.

The concentration of chromic oxide in the ingesta, relative to that in the feed, increased in each gastrointestinal section at each sample time except for the last, where the concentration was slightly lower in the distal than in the proximal intestine (Fig. 2).

The apparent digestibility coefficients for the nutrients in diet 1 in the three gastrointestinal sections, at three sample times, are listed in Table 3. The stomach contents of three of five sablefish were contaminated with partially digested small fish of unproved origin, and all stomach samples from this period were discarded as a precaution against error. One fish from the second sample and one from the third contained no stomach contents, and these were discarded and replaced with other fish whose stomachs contained ingesta.

In general, the digestibility of proteins, lipids and carbohydrates (nitrogen-free extract) increased as the ingesta moved down the digestive tract, at most sample times (Fig. 3). An exception to this was the drop in the digestibility of nitrogen-free extract (NFE) from the proximal to the distal intestine (67.9 to 51.0) at the third sample time (Fig. 3C). The digestibility of ash was erratic with respect to both gastrointestinal section and sample time (Figs. 3D and 4D).

Table 3 - Apparent digestibility coefficients (ADC) for Diet 1 in three gastrointestinal sections for three sample periods

	SAMPLE #1 (day 10)			SAMPLE #2 (day 16)			SAMPLE # 3 (day 24)		
	Stomach	Proximal intestine	Distal intestine	Stomach	Proximal intestine	Distal intestine	Stomach	Proximal intestine	Distal intestine
Organic matter (%)	68.1	81.7	87.8	-	81.8	86.6	54.4	67.9	63.2
Protein (%)	56.5	84.3	92.8	-	88.0	89.1	56.2	63.7	73.8
Lipid (%)	63.3	78.9	93.3	-	77.4	96.0	50.9	71.2	89.1
Ash (%)	69.5	61.6	69.0	-	57.4	77.9	52.2	37.0	22.7
NFE (%)	47.2	80.0	82.8	-	77.4	82.4	55.7	67.9	51.0

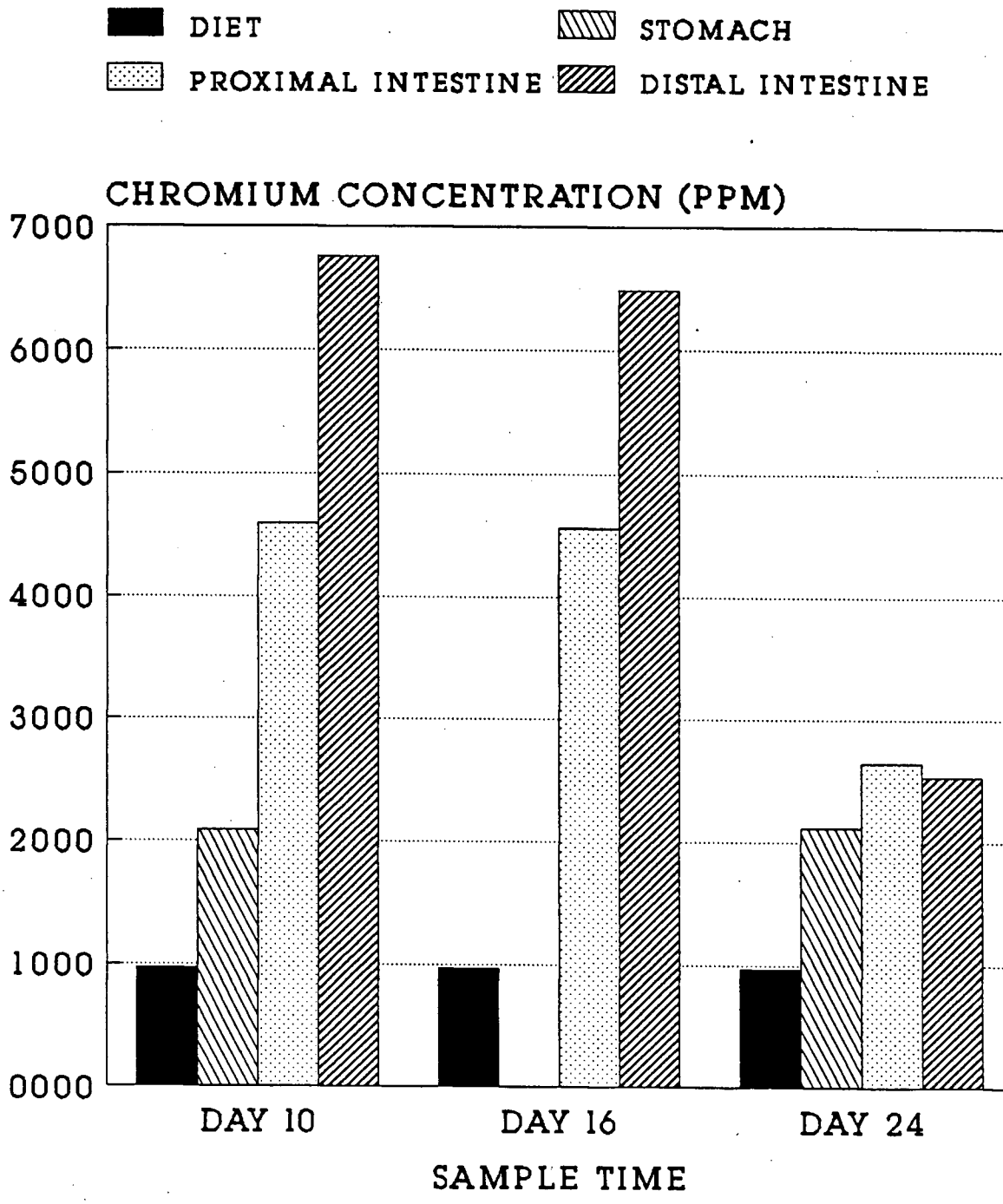


Fig. 2 - Chromium concentration in diet 1 and in the ingesta from three gastrointestinal sections of sablefish.

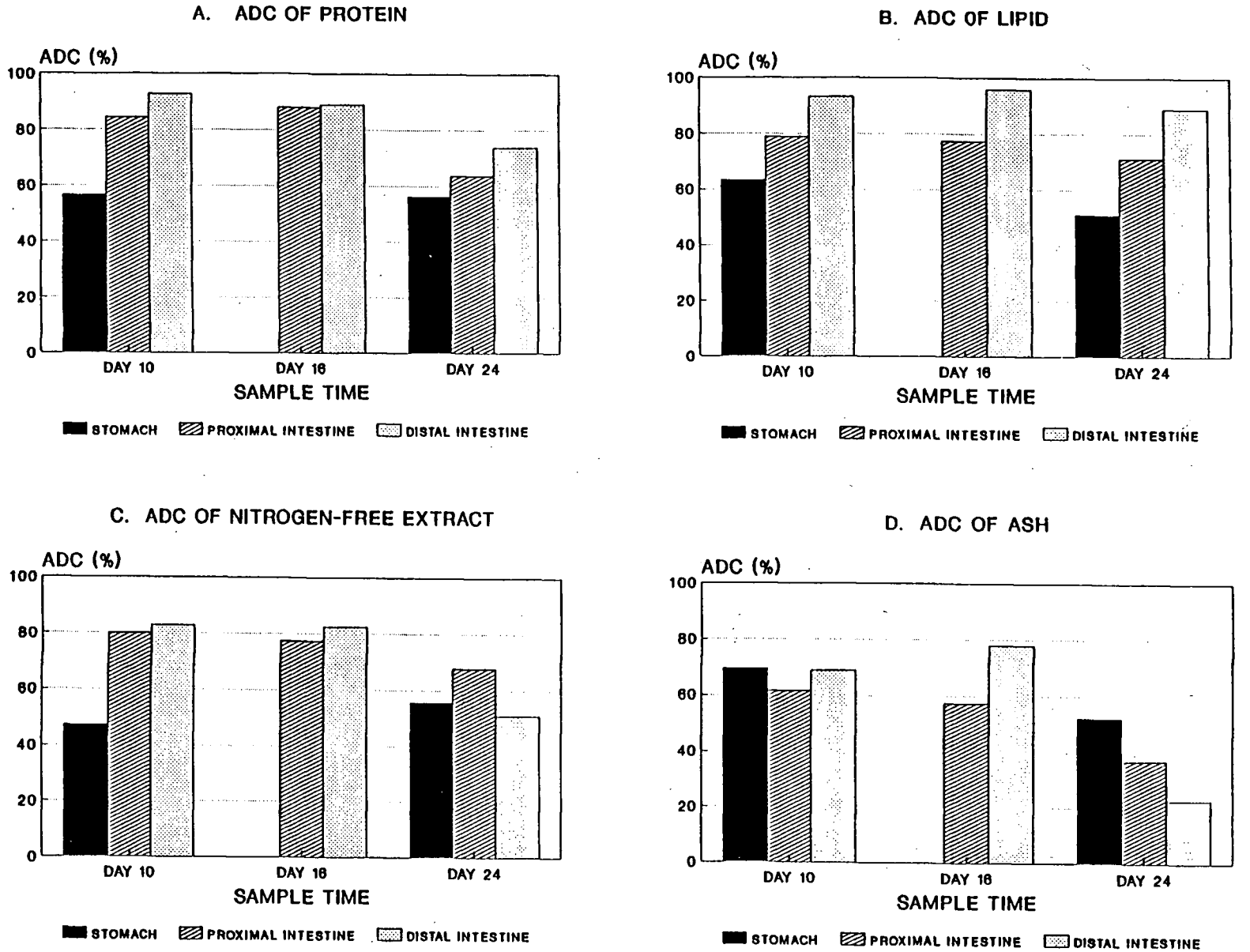


Fig. 3 - Temporal changes in apparent digestibility coefficients (ADC) for diet 1, in three gastrointestinal sections.

Overall digestibility of organic matter ranged from 63.2 to 87.8 in the distal intestine (Table 3). The apparent digestibility of NFE ranged from 51.0 to 82.8. The former value emphasizes that there was general decline in digestibility values for most nutrients in most digestive tract sections at successive sampling times, and in particular, in the final sample (Fig. 4).

The apparent digestibility of protein in the distal intestine dropped from 92.8% at the first sampling time to 73.8% at the third, while that of lipid ranged from 89.1 to 96.0%, and showed no apparent trend over time, and no significant differences between samples (Figs. 4A and 4B).

The pH values of the mucosal fluid of the digestive tract and the bile are listed in Table 4. These values were measured after the feeding trial had commenced, and no pre-trial measurements were made. The stomach fluid showed high acidity (pH 1.7 - 4.1) while the intestinal sections and bile were generally basic.

The amylase activities of the homogenized intestinal sections are presented in Table 5. The distal intestine had the highest activity (15.3 units per gram), but in general, the level of α-amylase was low in all sections.

The sablefish were at first enthusiastic consumers of the pellets, but daily consumption became variable and declined as a whole through the course of the experiment. No record of food consumption by sablefish was kept in Experiment 1.

Stomach contents were generally fluid on the surface of the food mass, whereas the centre of the mass more closely resembled the consistency of the feed. The intestinal contents were, by comparison, uniformly fluid, and most of the contents of the intestinal sections could be poured into sample containers for processing and analysis. Despite this fluidity, no loss of ingesta was observed during sampling, and careful handling prevented regurgitation of feed from the stomach.

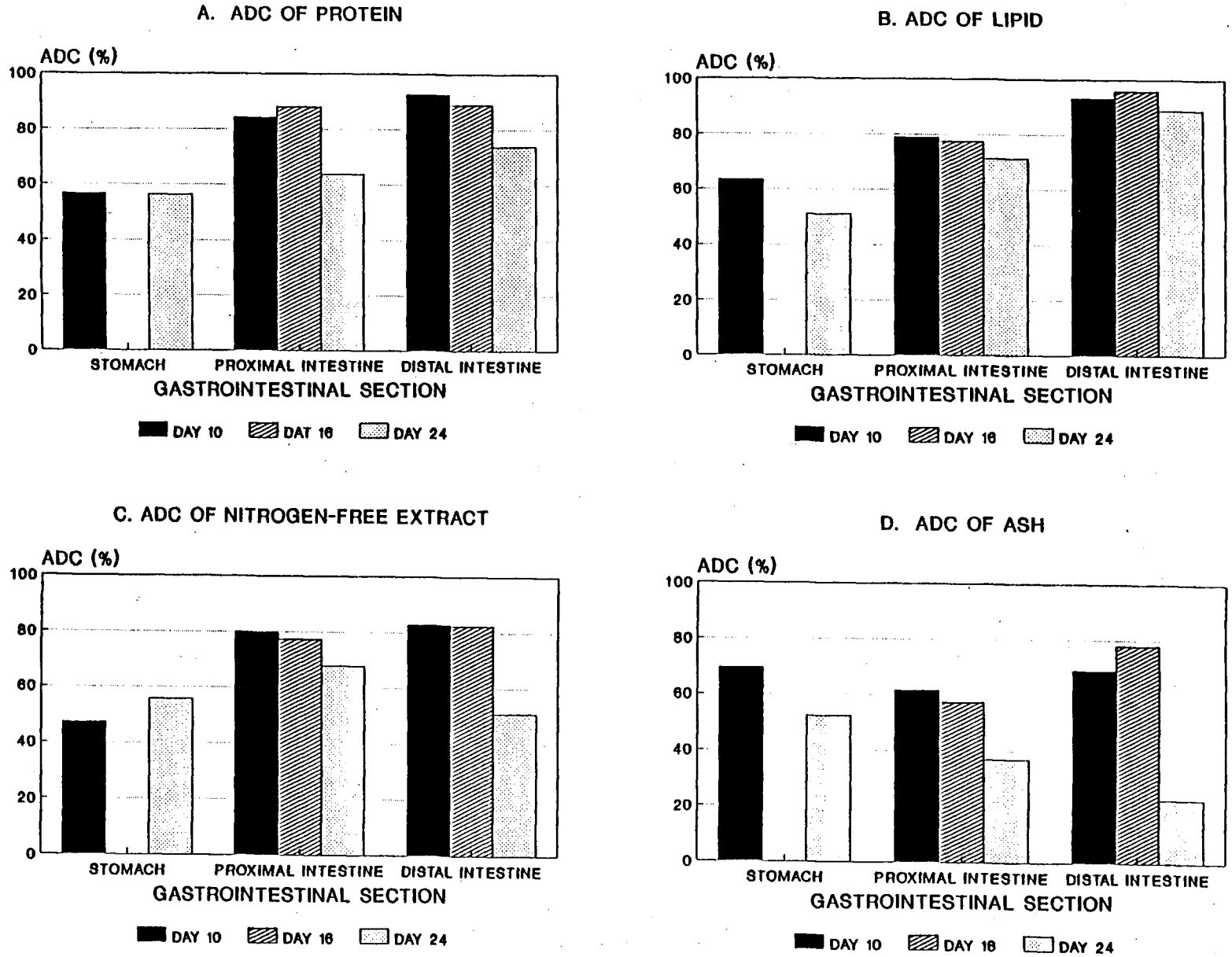


Fig. 4 - Apparent digestibility coefficients (ADC) for nutrients in diet 1 in relation to section of the gastrointestinal tract, and sample time.

Table 4 - pH Values of the gastrointestinal tract and the bile of sablefish

Section	Number Sampled	Range in pH	Mean pH
Stomach	14	1.7 - 4.1	3.0
Proximal intestine	19	6.7 - 7.7	7.3
Distal intestine	19	7.4 - 8.0	7.7
Bile	6	7.0 - 7.9	7.5

Table 5 - Amylase activity in the intestinal tract of sablefish

Intestinal section	Amylase activity (units)[1]
Pyloric caeca	Negligible
Proximal section	3.5
Distal section	15.3

[1]The unit of amylase activity is defined as the activity which liberates reducing groups at the rate corresponding to 1 micromole of substrate per minute at 25°C.

4.2 The digestibility and utilization of carbohydrates from four dietary treatments containing high levels of carbohydrate

Mineral analyses of diet and digestive tract samples revealed that for each dietary treatment the concentration of chromium in the diet was higher than that in the ingesta of the stomach and proximal intestine (Fig. 5). These results contradict criteria for effective external markers. The concentration of an effective marker must remain the same in the ingesta as in the feed, in the absence of digestion, or be elevated by the digestion and absorption of nutrients from the ingesta. The data suggest that the Cr_2O_3 marker was moving at a differential rate (faster) through the digestive tract relative to that of the other ingredients (nutrients) of the ingesta.

The concentration of Cr_2O_3 in the G.I. tract increased from the stomach contents through the intestinal sections, in accordance with the results of Experiment 1. However, the fact that Cr_2O_3 was either absorbed at the stomach and intestinal walls, or was transported at a faster rate than the rest of the ingesta, precluded its use as a means for determining nutrient digestibility in Experiment 2. Therefore, relative comparisons of nutrient digestibility between dietary treatments from measurements of the distal intestinal ingesta, whose Cr_2O_3 levels were higher than those of the diets, (and whose digestibility could therefore be calculated) would have no veracity. Moreover, the relative movement of Cr_2O_3 in a given G.I. tract section, or from one section to the next, could not be assumed to be consistent for each diet. Consequently, no estimates of apparent digestibility of nutrients were made for sablefish fed diets 2 to 5.

Alternately, data from liver analysis can be used to shed some light on the digestibility and utilization of carbohydrates in sablefish. Figure 6A illustrates the liver glycogen levels of sablefish fed the four diet treatments. The diets had a significant effect on the concentration of liver glycogen (ANOVA, $p < 0.01$). Sablefish fed diet 2 (raw wheat) had the lowest level of liver

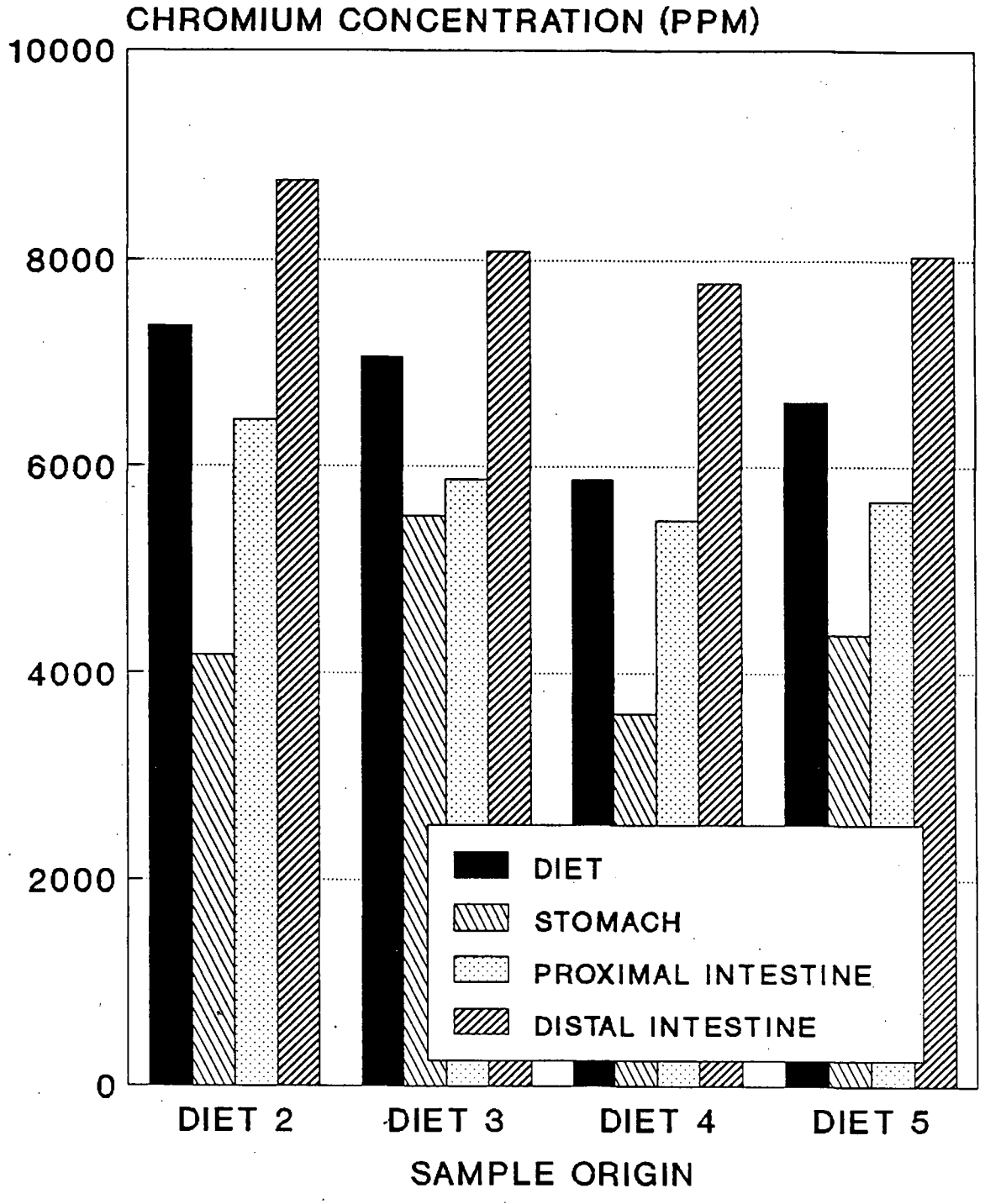
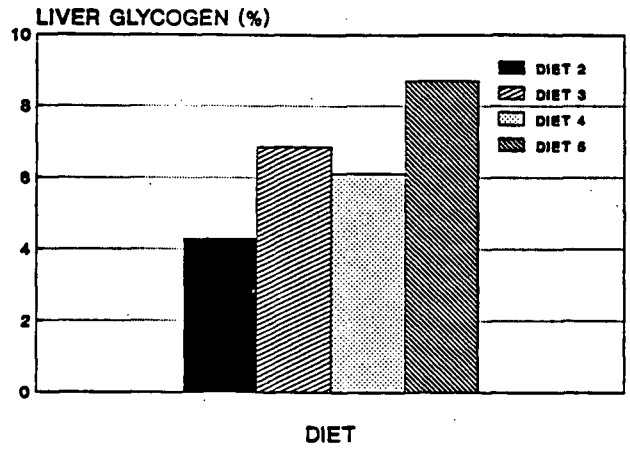


Fig. 5 - **Chromium concentrations in the test diets and in the respective contents of three gastrointestinal sections sampled from sablefish.**

A.



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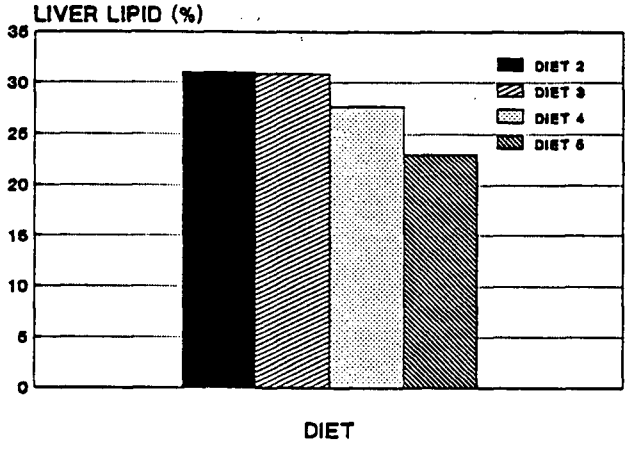


C.

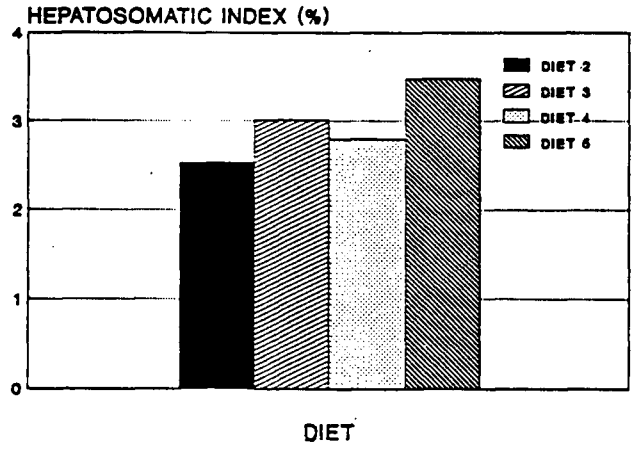


Fig. 6 - Liver glycogen and lipid content, and hepatosomatic indices in sablefish fed four diets varying in the source and level of carbohydrate.

Table 6 - Effect of four diet treatments on the liver glycogen, liver lipid, and hepatosomatic index of sablefish.

Diet	Liver[1,2] glycogen (%)	Liver lipid (%)	Hepatosomatic index (%)
Diet 2	4.29[a]	31.04[a]	2.53[a]
Diet 3	6.85[b]	30.88[a]	3.01[ab]
Diet 4	6.10[ab]	27.68[a]	2.79[a]
Diet 5	8.71[b]	23.06[a]	3.48[b]

[1] Values in columns with the same superscript are not significantly different (Duncan's Multiple Range Test; p = 0.05).

[2] Mean values were obtained from analysis of variance.

glycogen, and this amount was significantly lower than the levels of liver glycogen noted in sablefish fed diets 3 (cooked wheat) and 5 (pregelatinized starch) (Table 6).

The concentration of liver lipid was lower in fish fed Diets 4 and 5 than in those fed Diets 2 and 3 (Fig. 6B), but there were no statistically significant differences (ANOVA, $p>0.05$).

The hepatosomatic indices (HSI) of the groups fed the test diets were calculated to determine the relative utilization of nutrients from each treatment. In this regard, the trend for HSI values was similar to that described for liver glycogen levels (Fig. 6C). There was a significant added component due to treatment effects in the mean square between Diets 2 to 5 (ANOVA, $p < 0.05$). Fish fed diet 5 had the highest hepatosomatic index, and the value for the HSI of these fish was significantly higher than those fish fed diets 2 and 4 (cooked wheat; pregelatinized starch) (Table 6).

Statistical comparison of hepatic indices are not truly valid unless similar amounts of food were ingested by each group. The food consumption by the sablefish in each group was not, unfortunately, recorded in Experiment 2. Comparisons between the hepatic conditions produced in sablefish by the dietary treatments should, strictly speaking, be subjective.

All diets were palatable to the sablefish, but the consumption of diet 2, and to a lesser extent diet 3, was lower that of diets 4 and 5, towards the end of the experiment. All stomachs sampled contained ingesta, unlike several samples in Experiment 1. The feed most enthusiastically and consistently accepted by the sablefish was the soft and amorphous diet 5 (pregelatinized starch), but unlike the slightly erratic feeding behaviour observed for fish in Experiment 1, the alternate-day feeding protocol in Experiment 2 elicited an eager feeding response in fish receiving all of the dietary treatments.

4.3 Concentrations of chromium in the digestive tract of sablefish fed diets supplemented with 0.1% or 1.0% of Cr_2O_3

Figure 7 illustrates the concentrations of chromium found in the gastrointestinal contents of sablefish fed diets supplemented with 0.1 or 1.0% of Cr_2O_3. In each case the chromium concentration of stomach ingesta was noted to be lower than that of the feed, and hence the use of Cr_2O_3 marker for digestibility determination was questionable. The concentration of Cr_2O_3 rose in the proximal and distal sections of the intestine of fish receiving both treatments, in a similar manner to the results found in Experiment 2. The results differed in that the chromium concentrations in fish given both diets were higher in the proximal intestine than in the feed, unlike the situation in Experiment 2.

The concentration of chromium in the distal intestine ingesta of fish fed the 1.0% Cr_2O_3 diet was notably higher than in the diet. This was not true for fish fed the 0.1% Cr_2O_3 diet.

The chromium concentrations in the feed and stomach samples within each diet treatment were compared using the Student's 't' distribution. The chromium concentration of the 0.1% Cr_2O_3 feed was significantly higher than in the stomach ingesta ($p<0.01$). The 1.0% Cr_2O_3 feed was also significantly higher in chromium concentration than that in stomach contents ($p<0.05$).

4.4 Gastric evacuation of a formulated and a natural diet by sablefish

The stomach contents of sablefish fed diet 3 were found to decrease in a curvilinear fashion (Fig. 8A). The digestion of chopped herring appeared to be delayed for many hours, and the sample period was consequently too short to provide enough data points to establish a specific relationship between time and gastric evacuation (Fig. 8B).

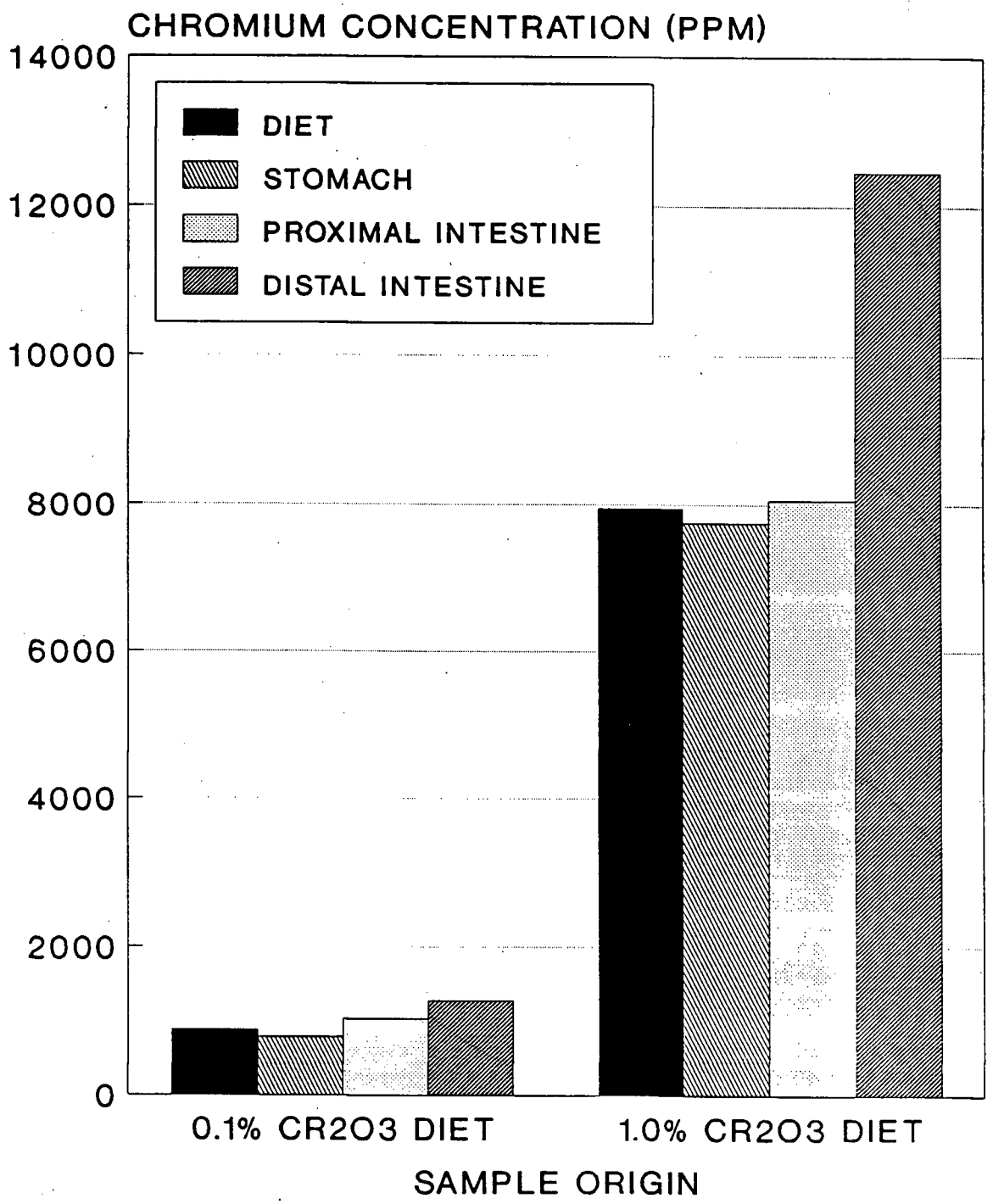


Fig. 7 - **Chromium concentration in the feed and the ingesta from three gastrointestinal sections, for diets containing either 0.1% or 1.0% chromic oxide.**

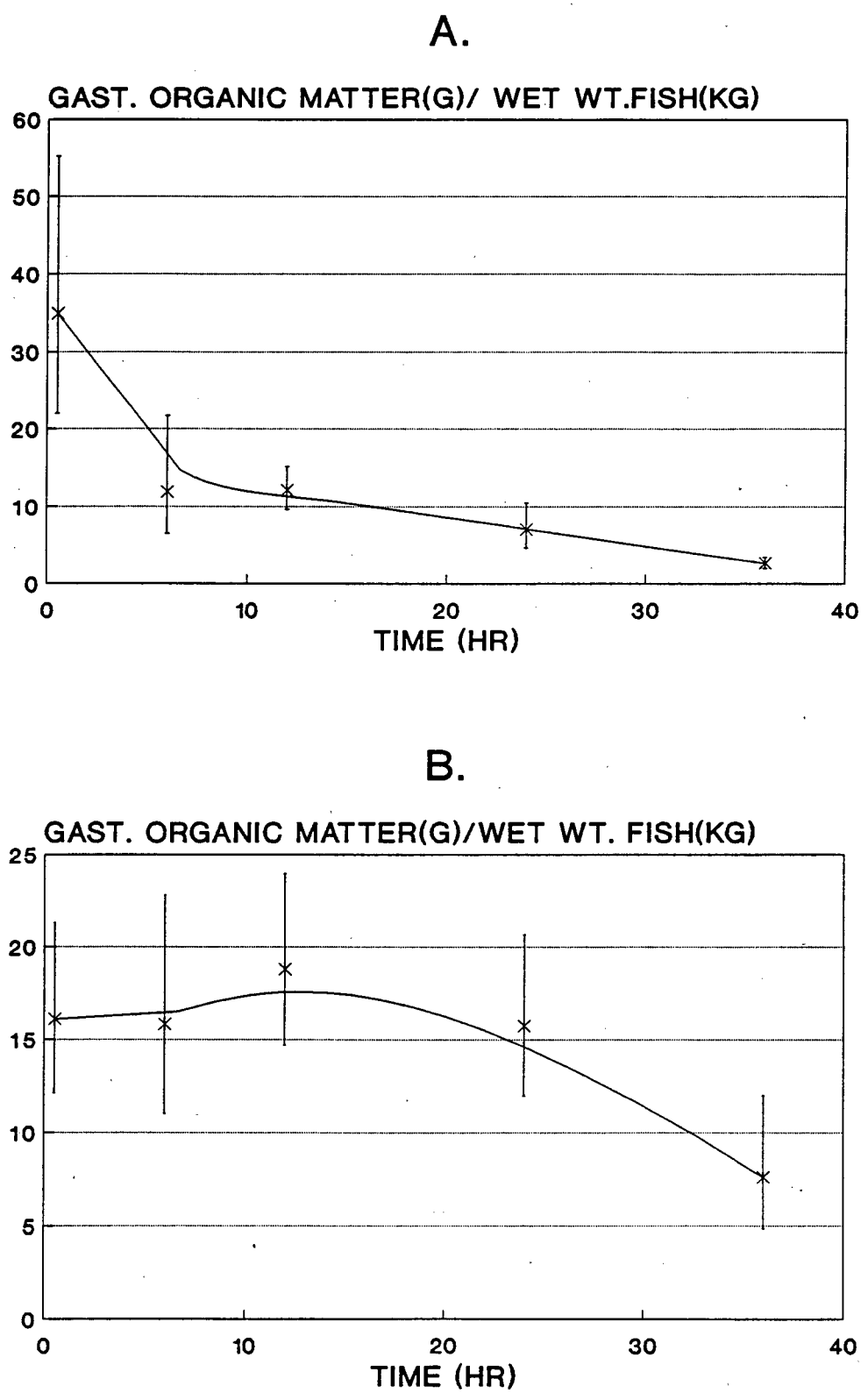


Fig. 8 - Gastric evacuation of organic matter in sablefish fed diet 3 (A) or herring (B) (G.M. ± 2 S.E.) The curves describe the lines of best fit, and the error limits were obtained from transformed data.

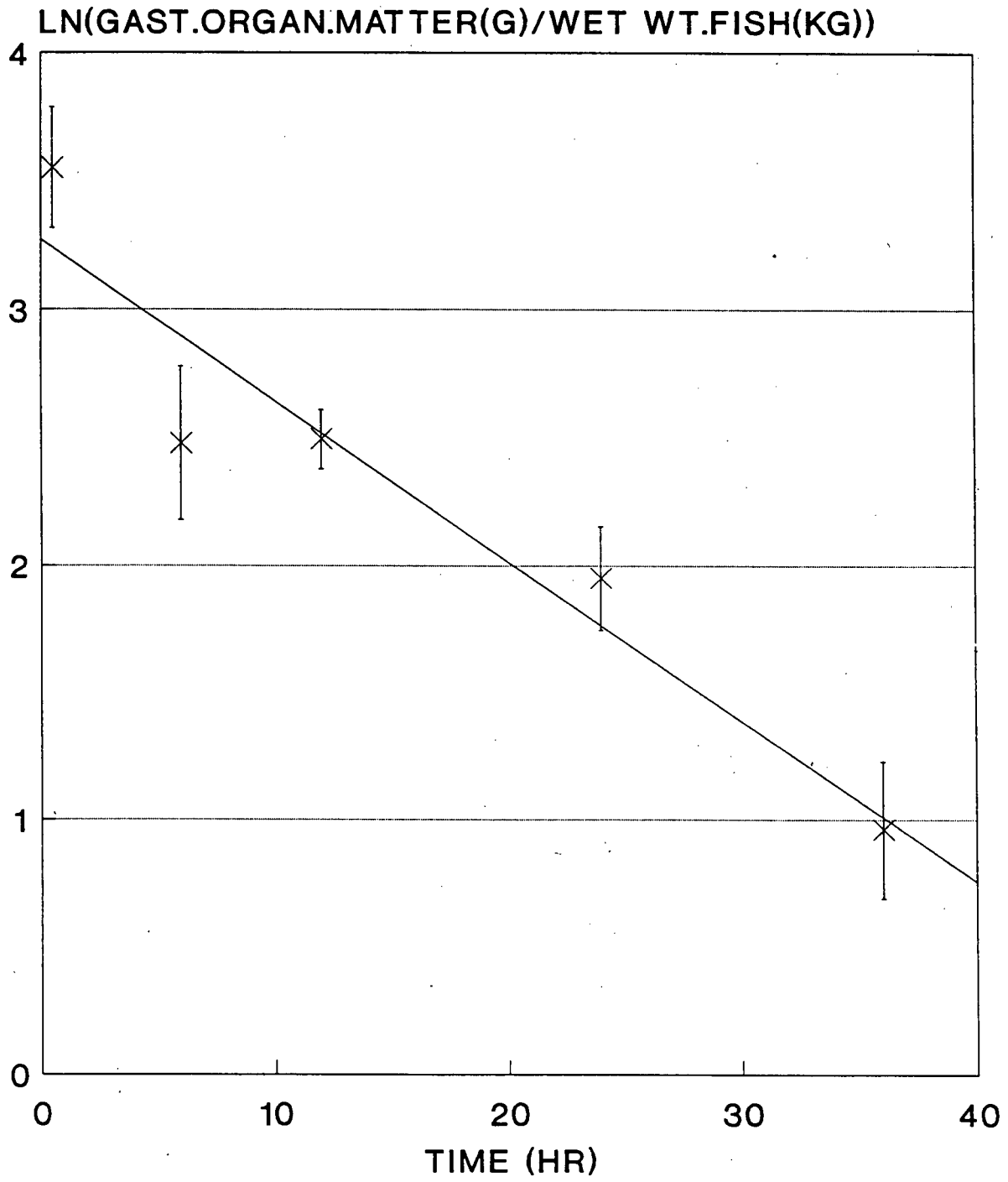


Fig. 9 - Gastric evacuation rate of organic matter from sablefish fed diet 3. Data points and error limits are natural logarithms (Ln) of G.M. ± 2 - SE (Fig. 7A). The line represents the regression of Y on X.

Table 7 - Coefficients of determination (R^2) of the gastric evacuation of Diet 3, as expressed by five mathematical transformations of the geometric mean values of the organic matter of stomach contents/wet weight of fish (g/kg)

Mathematical Transformation	Coefficient of Determination (R^2)
Linear	0.659
Square Root	0.803
Arcsine	0.740
Logarithm (base 10)	0.910
Natural logarithm (base e)	0.913

Table 8 - Calculated gastric evacuation times for Diet 3 at a temperature range of 10.1 to 11.2°C

Time to % Gastric evacuation (h)		
50%	75%	100%[1]
26.2	39.0	53.0

[1] The theoretical time value at 100% evacuation discounts sloughed epithelial cells and exuded fluids.

The coefficients of determination (R^2) for the regression of five mathematical transformations of the geometric means of the organic matter of stomach contents/wet weight of fish, for each sample time, are listed in Table 7. The natural logarithm best linearized the gastric emptying curve over the scale for meal size/wet weight of fish fed diet 3. The regression equation derived from the transformed data is as follows:

Eq. 1 $\quad \text{Ln } Y = 3.2735 - 0.0629\ X$

The linearized gastric emptying curve is presented in Fig. 9. The error ranges perhaps reflect the small sample size for each sample time, and demonstrate the wide range of individual food intake for fish of this size and species.

Estimates of times (hr) for 50, 75 and 100% gastric evacuation of diet 3 are listed in Table 8. These were respectively, 26.2, 39.0 and 53.0 hours.

4.5 The utilization of nutrients from three diet treatments over time

Several parameters were measured to provide insight into the utilization of the three dietary treatments by sablefish. The changes in the mean body weights of sablefish fed diet 6 (22.2% cooked wheat), diet 7 (44.4% cooked wheat) and chopped herring, over time are provided in Table 9 and Fig. 10. The seawater temperature dropped sharply during the first 20 days of the feeding trial (13.7°C to 10.4°C, mean daily temperature), and then declined more gradually for the duration of the experiment.

The pattern of growth for sablefish fed the herring diet was generally linear for the 96-day feeding trial. Fish fed the formulated diets, however, grew quickly during the first (30 day) feeding period, and growth declined during the second and third periods. Diet 6 supported a higher rate of growth than diet 7.

Table 9 - Weight gain, specific growth rate (SGR), dry food intake (DFI), feed conversion ratio (FCR) and protein efficiency ratio (PER) of sablefish fed diets containing one of two levels of cooked wheat or a reference diet of chopped herring for 96 days.

Diet and Feeding Interval	Mean weight gain (g/fish)	SGR[1] (%/day)	DFI[2] (g/fish/day)	FCR[3]	PER[4]
Diet 6					
day 1-30	342	0.49	18.20	1.60	1.65
day 31-58	54	0.29	16.17	6.24	0.42
day 59-96	146	0.23	15.59	4.92	0.53
Diet 7					
day 1-30	322	0.47	20.83	1.94	1.36
day 31-58	30	0.26	19.43	9.70	0.27
day 59-96	53	0.18	20.63	16.54	0.16
Herring Diet					
day 1-30	367	0.55	17.16	1.36	0.94
day 31-58	304	0.49	15.15	4.60	0.50
day 59-96	255	0.39	14.26	4.84	0.50

[1] Specific growth rate = $100 \times (\ln W_2 - \ln W_1 / t_2 - t_1)$

[2] Dry feed intake = total dry feed intake (g)/# fish/feeding interval (days)

[3] Feed conversion ratio = dry feed intake (g) /wet weight gain of fish (g)

[4] Protein efficiency ratio = gain in body weight (g) /protein intake (g)

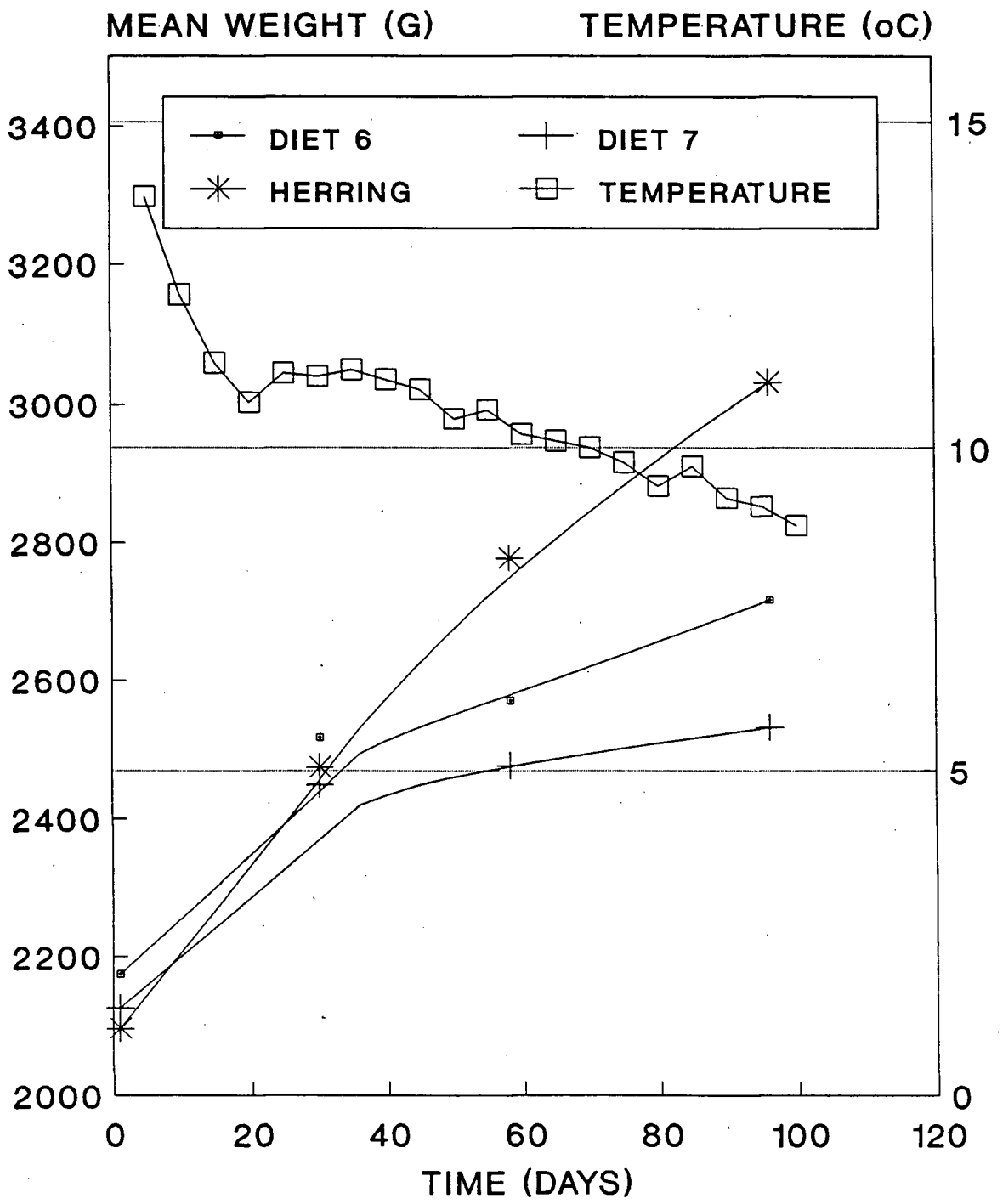


Fig. 10 - Effect of three diet treatments on the mean weight of sablefish over time, in relation to water temperature. Curves describe lines of best fit.

Analysis of variance revealed highly significant differences ($p < 0.01$) in body weight due to diet treatment. The mean weight of sablefish fed the herring diet was significantly greater than those of fish fed diets 6 and 7. The mean weight of diet 6-fed fish was significantly greater than that of fish fed diet 7 (Table 10).

Mean weight gains by sablefish were highest during the first feeding period for all test diets, and thereafter declined (Table 9). The weight gains for fish fed both formulated diets was lowest during the second feeding period (day 31-58), and those for both groups increased slightly during the final feeding period. Sablefish fed the herring diet had high but declining weight gains throughout the experiment.

The specific growth rates (SGR) of the groups declined over the course of the feeding trial, regardless of diet treatment. Herring-fed sablefish maintained the highest SGR throughout the experiment, and the SGR for this group declined in linear fashion (Table 9; Fig. 11A). The growth rates of fish fed diets 6 and 7 declined most rapidly between day 31 and 58, after which the rate of decline was similar to that observed for fish fed the herring diet.

Dry food intake was highest in sablefish fed diet 7, throughout the experiment. (Table 9, Fig. 11B). While sablefish fed diet 6 and herring consumed less food as the experiment progressed, the DFI of sablefish fed diet 7 remained high and relatively constant. The DFI of diet 6 by sablefish was consistently higher than that of herring.

Fish fed diet 6 and the herring diet had similar feed conversion ratios (FCR), whereas those fed diet 7 had comparatively poor food utilization. The FCR's were relatively low for the first (30 day) feeding period for fish fed all diets, but they increased for all groups during the second feeding period (Fig. 12A). Food utilization in fish fed diet 6 and chopped herring changed little in the third feeding period. By contrast, the FCR for fish fed diet 7 rose to 16.5 during the final feeding period.

Table 10 - Effect of diet treatment on mean weight and condition factors, liver and muscle glycogen levels, liver lipid content and hepatosomatic index of sablefish.[1]

Diet	Mean[2] weight (g)	Condition factor ($g/cm^3 \times 10^2$)	Liver glycogen (%)	Liver lipid (%)	Muscle glycogen (%)	Hepatosomatic index (%)
Diet 6	2716^{b}	1.18^{a}	6.26^{a}	25.58^{ab}	0.035^{a}	3.24^{ab}
Diet 7	2531^{a}	1.16^{a}	7.10^{a}	22.73^{a}	0.016^{a}	2.73^{a}
Herring	3032^{c}	1.28^{b}	4.50^{b}	28.52^{b}	0.015^{a}	3.34^{b}

[1] Mean weights from the final sample, and overall values for liver glycogen and lipid, muscle glycogen, HSI and CF were obtained from analysis of variance.

[2] Values in columns with the same superscript are not significantly different (Duncan's Multiple Range Test; p = 0.05.)

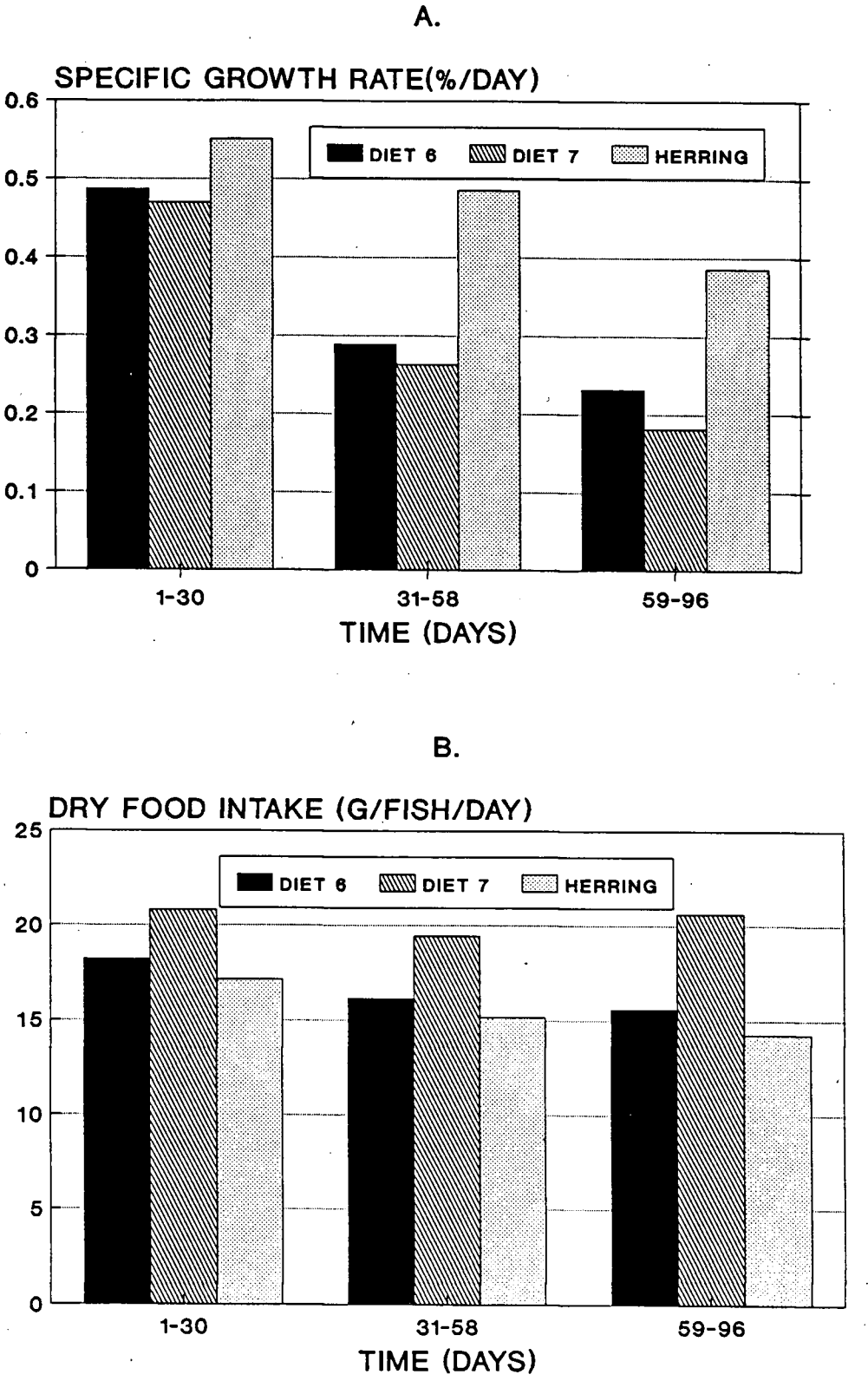


Fig. 11 - Effect of three diet treatments on the specific growth rates (SGR) and dry food intake (DFI) of sablefish for three time periods.

The protein efficiency ratios (PER) for the fish fed the test diets declined between the first and second feeding periods and subsequently there was little change during the remainder of the experiment (Fig. 12B). Fish fed diet 6 and the herring diet exhibited the best utilization of dietary protein for growth between day 30 and day 96. Those fed diet 7 had the poorest protein utilization during this interval.

The condition factors of sablefish fed the test diets for 96 days are presented in Fig. 13A. Herring-fed fish showed an initial improvement in their condition factors, and this continued to improve, at a decreasing rate for the rest of the trial period.

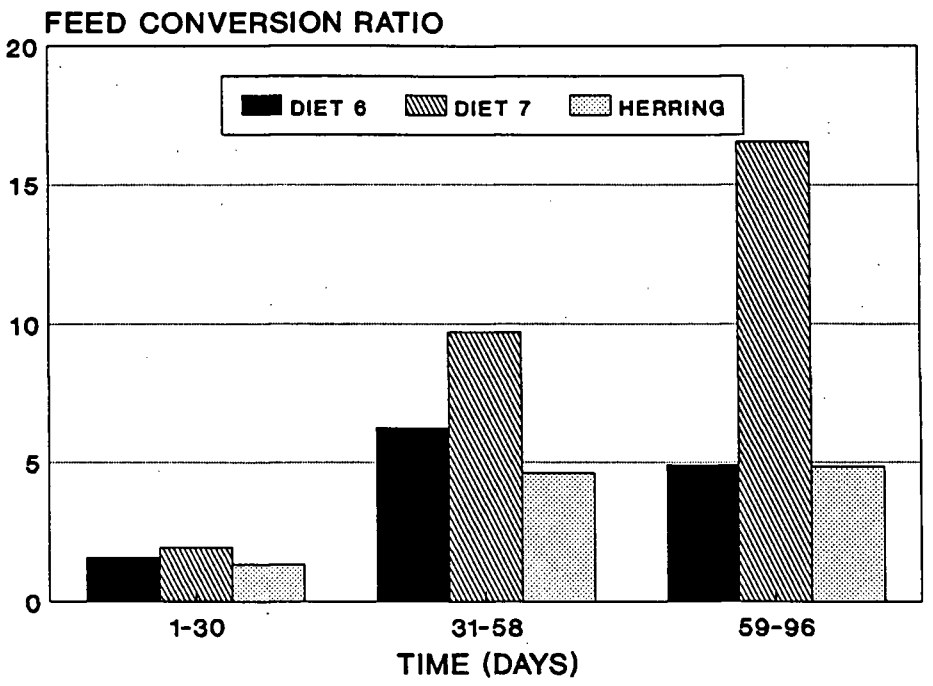
The condition factors of fish fed diets 6 and 7 both rose during the first feeding period. Thereafter they declined to levels slightly greater than the values observed at the beginning of the feeding trial.

The differences in mean condition factor were significant between dietary treatments (ANOVA; $p<0.05$), but were not significant between sample times. Sablefish fed the herring diet had significantly higher condition factors than those fed diets 6 and 7, while the condition factors of the fish fed the formulated diets did not differ (Table 10).

The hepatosomatic index (HSI) values of sablefish fed the three diets are recorded in Fig. 13B. The HSI values for fish fed all diets rose from the first to the second sample time, and this was most evident in the herring-fed fish. At the third sampling time the HSI value for fish fed diet 7 was lower than those of the other groups, and between the third and fourth sample there was an increase in HSI for these fish. HSI values varied little for fish fed the herring diet between the second and fourth sample times, while fish fed diet 6 had the highest HSI values by the end of the study.

The differences in hepatosomatic index values between groups fed the test diets, and between sample times, were significant (ANOVA; $p<0.05$). Fish fed diet 6 had a significantly

A.



B.

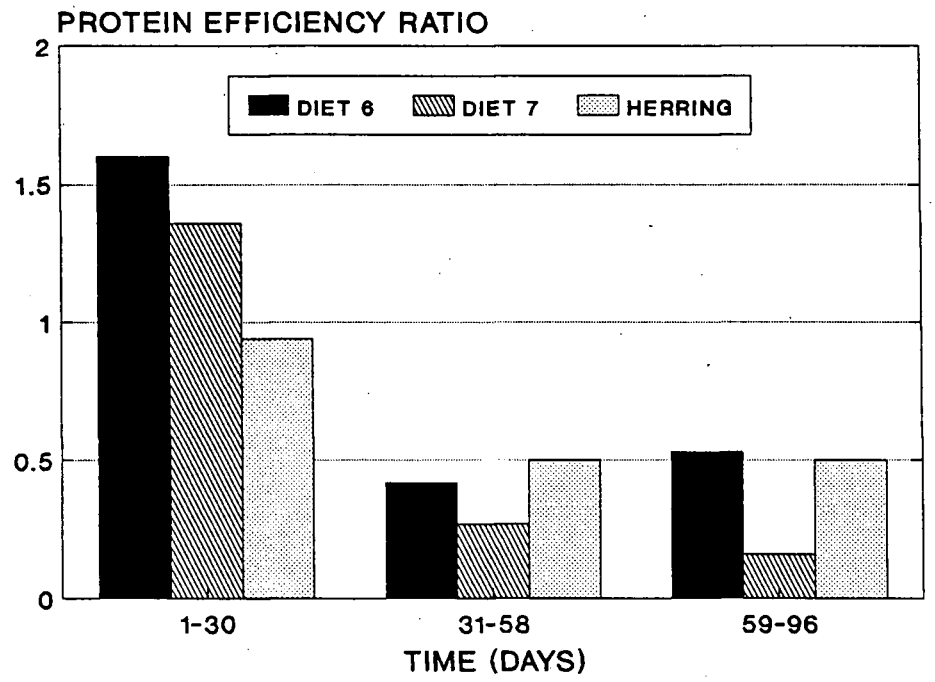
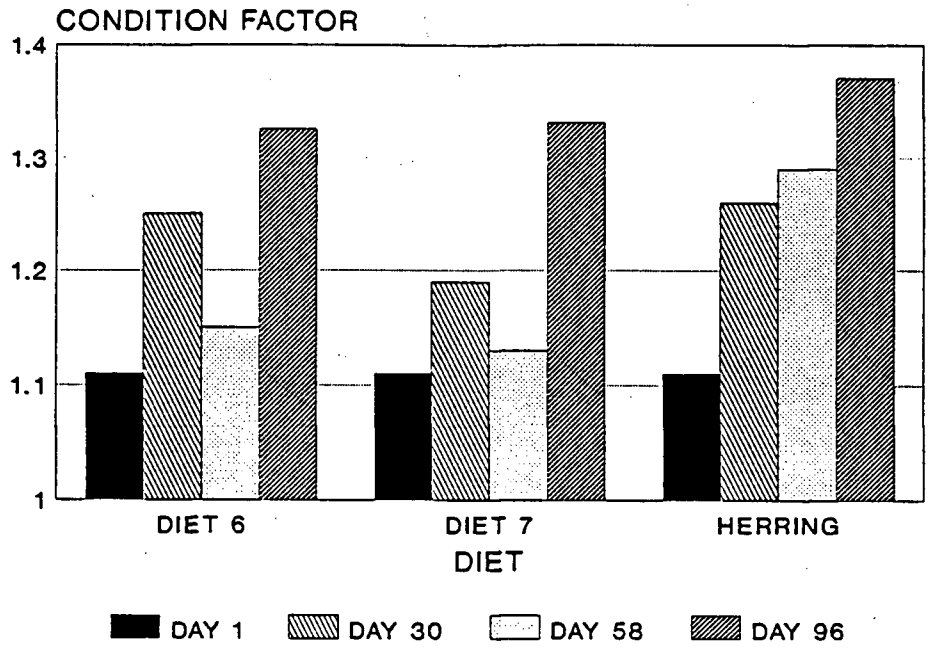


Fig. 12 - Effect of three diet treatments on the feed conversion ratio (FCR) and protein efficiency ratio (PER) of sablefish for three time periods.

A.



B.

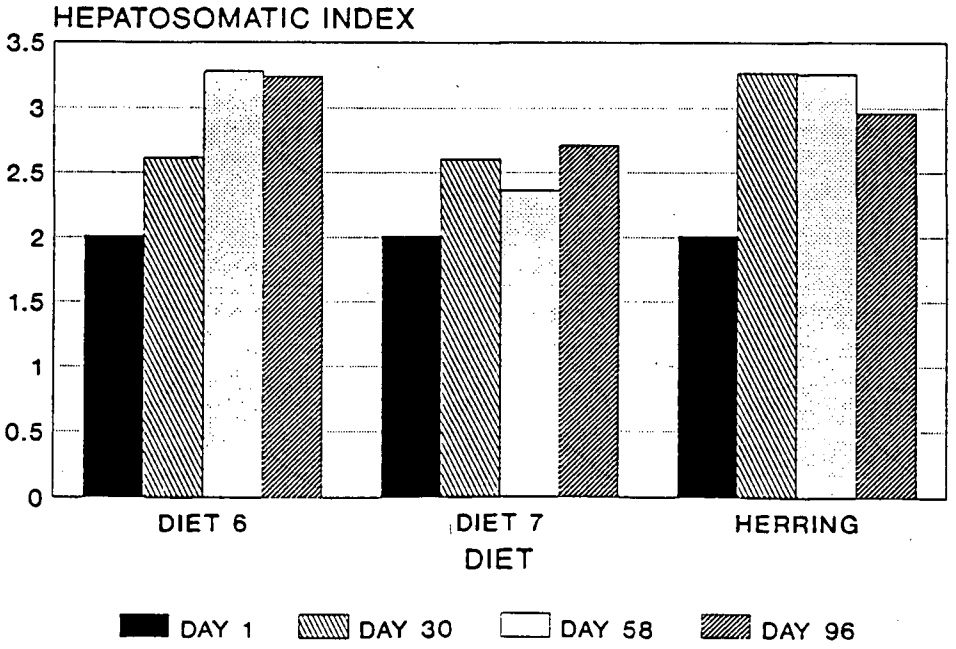


Fig. 13 - Effect of three diet treatments on the condition factor values and hepatosomatic indices in sablefish over four sample times.

higher HSI value than those fed diet 7, but the HSI of fish fed diet 7 did not differ significantly from that of fish fed the herring diet (Table 10). The mean HSI value after 96 days was significantly higher than that after 30 days, but did not differ significantly from that after 58 days. The mean HSI values after 58 and 30 days were not significantly different.

The livers of sablefish examined at each sample time showed little external evidence of liver dysfunction. An occasional mottling of the external surface of the liver was never associated with a particular diet treatment or sampling time.

The concentration of liver glycogen rose substantially in sablefish fed each of the test diets, then dropped, and finally rose again by day 96 (Fig. 14A) The liver glycogen concentrations of herring-fed fish were consistently lower than those in fish fed the formulated diets at each sample time following the start of the feeding trial, and those in diet 7-fed fish were highest. Diet treatment had a significant effect on mean liver glycogen levels of fish in the three feeding periods (30, 58 and 96 days; ANOVA indicated $p < 0.01$)) but there were no significant differences due to sampling time. Liver glycogen levels in herring-fed sablefish were significantly lower than in fish fed both formulated diets (Table 10). Fish fed diets 6 and 7 had similar liver glycogen levels.

Liver lipid concentrations were, in general, negatively correlated with those of glycogen (Figs. 14A and B). Moreover, all groups showed a decline in liver lipid content between day 1 and 30. The reduced levels of liver lipid were for the most part maintained throughout the experiment. Herring-fed fish maintained a higher hepatic lipid level relative to fish fed diets 6 and 7, and those fed diet 7 consistently had the lowest levels of lipid in their liver.

Diet treatment significantly affected the overall mean liver lipid concentrations (ANOVA indicated $p< 0.05$), while sample time was without effect. Hepatic lipid levels in herring-fed fish

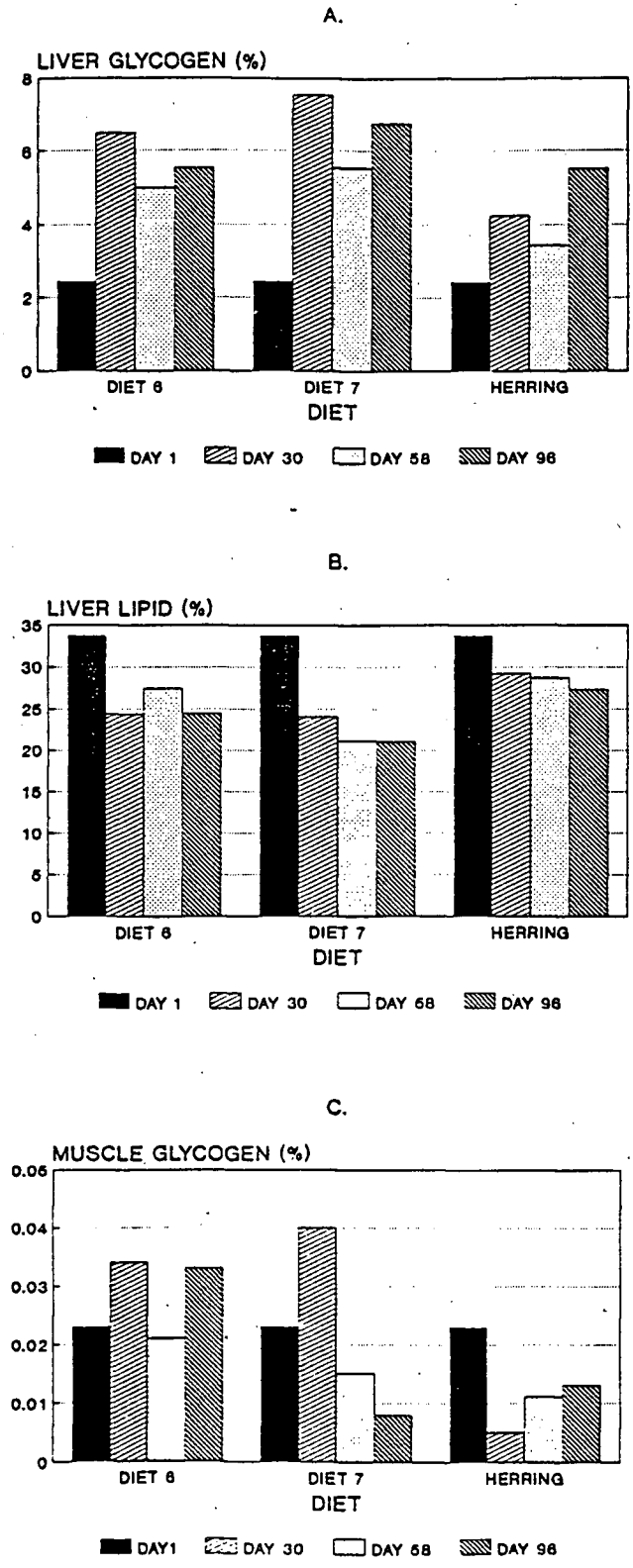


Fig. 14 - Effect of three diet treatments on the liver glycogen and lipid concentrations, and muscle glycogen concentrations, in sablefish over four sample times.

were significantly higher than those of fish fed diet 7 (Table 10). There were no significant differences in liver lipid concentrations of fish fed diet 6 and diet 7.

Muscle glycogen concentrations were erratic with respect to both dietary treatment and sample time. Muscle glycogen levels in herring-fed fish were generally lower than in the fish fed the formulated diets (Fig. 14C). Fish fed diet 7 had a sharp rise in muscle glycogen content on day 30, but this level dropped well below pre-feeding trial levels at subsequent sampling times. Muscle glycogen concentrations in fish fed diet 6 showed no consistent trend with time.

No significant differences in mean muscle glycogen levels were found with respect to either diet treatment or sample time (ANOVA; $p>0.05$) (Table 10).

The experiment was conducted in the autumn under conditions of declining sea water temperatures and photoperiod. The experimental animals had been fed maintenance diets of chopped herring and OMP prior to pre-experimental food withdrawal, and many of the parameters of nutrient utilization responded quickly and substantially to the increases in feeding level and frequency (Figs. 13 and 14).

Three fish died during the feeding trial; one each in tanks fed diet 7 and herring, and one from the former group which jumped from the tank during the evening. The deceased fish found in the tanks were thin, and no evidence of ingesta was observed in the digestive tract. No other outward signs of disease were observed. Many fish were abraded on the left side of their heads and caudal fins, presumably as a result of swimming clockwise against the (mild) current, and rubbing against the tank walls. None, however, appeared to be infected by pathogenic organisms.

CHAPTER 5

5.0 DISCUSSION

5.1 Digestibility of carbohydrates

Many recent studies have sought to evaluate and quantify the protein-sparing effects of energy-yielding nutrients in the diets of cultured fish. The utility of using carbohydrates as protein-sparing energy sources is not well understood, although the addition of low levels of carbohydrates has been demonstrated to have a sparing effect in several species (Cowey et al, 1975; Austreng, *et al.* 1977; Pieper and Pfeffer, 1979; Shimeno *et al.*, 1979). High levels of dietary carbohydrate have been shown to be poorly digested and to have low metabolizable energy values. The usefulness of carbohydrates in fish diets appears to depend to a large extent on their complexity, treatment and dietary level (Ringrose, 1971; Edwards *et al.*, 1977; Pieper and Pfeffer, 1979; Kaushik *et al.*, 1989).

The limited value of carbohydrates in the nutrition of carnivorous fish has been attributed to insufficient enzymatic breakdown of carbohydrates in the digestive tract (Singh and Nose, 1967; Bergot 1979; Furuichi and Yone, 1982) and to the low rate of aerobic oxidation of glucose (Cowey and Sargent, 1979). The poor metabolizable energy value of glucose is related to an inability to control blood glucose levels (Palmer and Ryman, 1972), which limits the tolerable level of available carbohydrate in the diet.

Some fish species have a limited ability to adapt to elevated dietary carbohydrate (Cowey *et al.*, 1977 a, b; Furuichi and Yone, 1980; Hilton and Atkinson, 1982; Mazur, 1990) but this is

circumscribed over a narrow range of blood glucose levels. Hilton *et al.* (1987b) reported significantly higher plasma insulin levels in rainbow trout fed a high carbohydrate diet than in trout fed a carbohydrate diet, but the higher insulin levels did not prevent hyperglycaemia.

The results of Experiment 1 (diet 1) indicate that sablefish are able to digest a relatively high proportion of dietary carbohydrate in a high (35.5% NFE) carbohydrate diet, under the conditions and protocol employed in the experiment (Figs. 3C and 4C). The digestibility coefficients are, it should be noted, "apparent", and they more accurately signify absorption or disappearance of nutrients relative to the Cr_2O_3 marker. Considering the substantial length of the distal section of the intestine, it would be rash to consider the high digestibility values of this section as reliable estimates of nutrient absorption. Only analysis of faecal samples would provide a credible estimate of digestibility.

Disappearance, as measured by apparent digestibility coefficients, occurred in all sections of the digestive tract, and except for the erratic estimates of ash digestion, and the anomalous drop in NFE digestion in the distal intestine in the third sample time, the digestibility of nutrients increased as the ingesta passed through the digestive system (Figs. 3 and 4). The results are generally consistent with those of previous studies which have charted nutrient digestibility through sections of the digestive tract (Phillips *et al.*, 1948; Austreng, 1978; Lied *et al.*, 1982).

Apparent digestibility coefficients of most nutrients declined in each digestive tract section, at each sample period. Within the time frame of the experiment, the sablefish showed no adaptive ability to increase carbohydrate digestion or glucose absorption, but rather lost some of their digestive and absorptive capacity for most nutrients. Studies with rainbow trout have demonstrated a similar inability to adapt to a high carbohydrate diet in a long-term feeding trial, and their disaccharide enzyme activity and glucose absorption rate actually decreased over time (Buddington and Hilton, 1987).

The chromium analyses for Experiment 1 indicated that the Cr_2O_3 marker conformed to the criteria by which an effective marker is judged. The exceptional drop in Cr_2O_3 concentration from the proximal to the distal intestine in the third sample (Fig. 2) appears to be correlated only with sea water temperatures (sample 2 mean temperature = 11.6°C; sample 3 mean temperature = 11.0°C) as regards experimental conditions. This result serves to emphasize the long recognized difficulty in estimating the progress of digestion, and the well-documented influence that water temperature exerts on the gastric motility of ingesta in fish. The chromium concentrations measured in the third sample suggest that the digestive process was incomplete for the meal preceding sampling, and that consequent calculations for nutrient digestibility for this sample may be unreliable.

The external indicator method of determining nutrient digestibility in fish, using Cr_2O_3 marker, is a widely accepted procedure. The behaviour of the marker, relative to the rest of the ingesta, is rarely examined or questioned, however, and reference to experimental conditions, feeding protocol, the influence of dietary composition, and the length of the experiment are often partly or completely ignored when nutrient digestibility is calculated. Researchers normally regard faecal concentrations of marker as representing the completed digestive process, and if test meals containing an external indicator are used, precautions against precocious and late sampling are usually taken to assure that samples represent the maximum concentration of marker, and, it is assumed, maximum nutrient digestion. The results of sample 3, in this light, represent an incompletely digested meal. Research with large Atlantic cod (approximately 1.5 kg) using 0.1% of dietary Cr_2O_3 in digestibility studies revealed similar results at the terminal sample (Lied *et al.*, 1982). The concentration of Cr_2O_3 declined from the adjacent intestinal section suggesting that inappropriate sampling times can distort the accuracy of nutrient digestibility estimates from faecal samples.

The analysis for α-amylase confirmed its presence and potential for hydrolytic activity in the sablefish gut, and the pH levels of the intestine conform to appropriate conditions for amylase activity in other animals (Tables 4 and 5) (Vonk and Western, 1984). The levels of α-amylase in the intestinal mucosa suggests that the capacity for sablefish to hydrolyse polysaccharides is relatively low, although comparable analyses for other species have not been found (analyses of intestinal contents with different methods are the norm). Furthermore, the results provide no information regarding α-amylase secretion as a function of time, but they merely record its presence in secretary (pancreatic) cells. The relatively high ADC values calculated for carbohydrate may imply that α-amylase secretion in sablefish is sufficient to accommodate relatively high levels of carbohydrate (NFE). These speculations have to be considered in light of the distinction between "digestibility" and "disappearance".

Chromic oxide clearly departed from the stomach faster than the rest of the constituents of the ingesta for each dietary treatment in Experiment 2 (Fig. 5). Concentrations of Cr_2O_3, which were lower in the stomach contents than in the feed, produced negative digestibility (or disappearance) estimates in the apparent digestibility equation. The dilution of Cr_2O_3 can be attributed to either an endogenous discharge of material (sloughed mucosal cells, enzymes, mucus), or a differential movement of Cr_2O_3 from the gut, relative to the rest of the ingesta. The latter circumstance could have involved the absorption of Cr_2O_3 by the digestive tract, or the "streaming" of the marker through the digestive tract, leaving a diluted concentration of marker in the ingesta. Endogenous infusion of nutrients, and absorption of Cr_2O_3 through the gut are unlikely, and without precedent, while evidence of "streaming", "selective rejection" and "retention" of the Cr_2O_3 marker has been documented for several species (Knapka *et al.*, 1967; Bowen, 1978; Tonnessen, cited by Lied *et al.*, 1982; Leavitt, 1985). Lied *et al.* (1982) also present data which demonstrate streaming of Cr_2O_3 in Atlantic cod, although this was not

commented upon. Whatever the reason for the differential movement of the Cr_2O_3, the criteria for the qualities of an effective marker were violated, and calculations of nutrient digestibility were invalid.

Some indication of the relative digestibility and utilization of diets 2 to 5 in sablefish may be found in each group's levels of liver glycogen and lipid, and in the values for the hepatosomatic index (Fig. 6).

The digestibility of complex carbohydrates in fish has been repeatedly shown to improve with treatments such as gelatinization, which hydrates and denatures starch granules (Bergot, 1979; Shimeno *et al.*, 1979; Bergot and Breque, 1983; Boccignone *et al.*, 1989). Improved digestibility may not be followed by an increase in productivity when levels of available carbohydrate are high. The liver functions as a dynamic storehouse of glycogen, and glycogen levels are sensitive to the rate of feeding, and dietary content. Storage of glucose as liver glycogen effectively removes it as an immediate energy source, and over short time periods the liver can provide an index of the nutritional state of a fish (Tyler and Dunn, 1976).

Liver glycogen levels and hepatosomatic index values have been observed to increase in response to elevated available dietary carbohydrate levels in fish (Austreng *et al.*, 1977; Hilton and Atkinson, 1982; Hilton and Slinger, 1982; Kaushik and de Oliva Teles, 1985). By inference, the diets which produce the highest liver glycogen levels and HSI values are those with the highest carbohydrate digestibility, dietary energy and protein levels being equal. In this context, the ingestion of diet 5 (pregelatinized starch) resulted in the highest liver glycogen and HSI values and therefore it may have had the highest available carbohydrate content (Figs. 6A and 6C). Hence, the sequence for carbohydrate digestibility was: diet 5 (pregelatinized starch) > diet 3 (cooked wheat) > diet 4 (cooked wheat: pregelatinized starch) > diet 2 (raw wheat). Liver glycogen and HSI values were closely correlated in fish fed each diet.

The raw and cooked wheat diets produced the highest liver lipid levels in sablefish, and the pregelatinized wheat the lowest. Austreng *et al.* (1977) observed that both liver glycogen and liver lipid levels increase with increased levels of metabolizable energy in rainbow trout diets. The reason for the negative correlation between hepatic glycogen and lipid levels in the present study cannot be explained clearly.

5.2 Gastric evacuation of formulated and natural diets

The gastric emptying curve for fish fed diet 3 most closely resembles the volume-dependent curvilinear emptying patterns of liquids or well-homogenized bulk (Fig. 8A). The long delay preceding measurable evacuation of chopped herring precludes a meaningful interpretation of the results (Fig. 8B). It could be surmised, however, that the emptying curve may resemble a more linear emptying pattern, where higher energy-density nutrients result in volume-dependent mechanisms being overridden in response to feedback inhibition signalled by duodenal or intestinal receptors (Jobling, 1986a).

The time lag in the digestion and evacuation of chopped herring can be attributed to the physical size and integrity of the pieces, and this should be contrasted with the swift disintegration and movement of the diet 3 pellets. Mechanical breakdown of natural or formulated feeds may be highly relevant to the establishment of feeding rates and schedules for high moisture or high bulk diets.

Appetite is closely correlated with gastric emptying, and the feeding frequency of carnivorous fish with short intestines can be scheduled according to their gastric evacuation rate of flesh or formulated pellets (Brett and Higgs, 1970; Grove *et al.*, 1978). The emptying rate and

return to appetite will therefore be important factors for establishing feeding practices for sablefish, considering their marked preference for moist, soft- textured, and therefore bulky diets.

Detailed investigations are necessary to relate feeding rate, maximum feed intake, satiation time, and temperature to determine optimum conditions for maximum growth using a particular diet. The gastric evacuation rate is a useful tool for relating appetite to feeding rate since appetite and gastric evacuation are so intimately related.

5.3 Growth performance and utilization of carbohydrates at two dietary concentrations

Experiment 5 was designed to investigate the growth performance and energy utilization of two levels of carbohydrate in sablefish. Chopped herring served as a reference diet which enabled comparisons of sablefish performance with respect to growth, feed conversion, protein utilization, condition factor and liver composition.

The depressed growth rates of sablefish fed the formulated diets suggests that the available levels and balance of nutrients in these diets were not comparable to those in the herring diet and, in particular, that the energy from gelatinized wheat starch had little if any effect on sparing protein for growth. The apparent digestibility (or disappearance) of diet 1 NFE from the distal intestine samples, and the liver glycogen deposition observed in fish from Experiment 2, suggest that carbohydrate digestion occurs in sablefish, although no quantitative data for completed digestion were generated.

The differences in the growth rates of sablefish related to the three diet treatments provide more concrete data for estimating the relative utility of the different carbohydrate levels. The low growth rate of fish fed diet 7 (high carbohydrate level) relative to the growth rates of fish fed diet 6 (lower carbohydrate level) and chopped herring, is reminiscent of several studies investigating

carbohydrate utilization in rainbow trout. Refstie and Austreng (1981) reported that growth rates of trout increased as the available carbohydrate level in the diet was decreased from 45 to 15%. Likewise, Hilton and Atkinson (1982) observed depressed growth in trout fed a high dietary level of carbohydrate (21% cerelose), and they estimated that a digestible carbohydrate level in excess of 14% is not efficiently utilized. By contrast, Austreng *et al.*, (1977) reported that rainbow trout fingerlings fed isonitrogenous diets containing 17%, 25% and 38% of metabolizable energy as carbohydrate (raw wheat, cooked wheat and sucrose) grew at similar rates, and had equal dressed carcass weights. Also, plaice fed isonitrogenous diets containing 10%-20% carbohydrate (glucose and dextrin) had higher weight gains than fish fed equal amounts of protein and energy, but no dietary carbohydrate (Cowey et al, 1975).

Thus, it appears from the literature that carbohydrates may serve as sources of metabolizable energy when they are included in the diets of a variety of carnivorous fish at appropriate levels. At a given level of incorporation, the protein-sparing effects of carbohydrates are dependent on the digestibility of the carbohydrate, and the protein/energy balance in the diet. The growth rates supported by the formulated diets in the present study on sablefish suggest that a higher protein/energy balance and a lower level of available carbohydrate would improve growth performance, and perhaps produce the protein-sparing effects of carbohydrates observed from diets fed to other species.

The specific growth rates supported by the herring diet were considerably higher than those noted for fish fed the formulated diets, and there appeared to be no significant difference in growth rates between fish fed diets 6 and 7 (Fig. 11A). SGR values and water temperature were positively correlated, and the decline in values was surprisingly steady considering the wide fluctuations found in the groups for FCR, PER condition factor and HSI values.

The dry feed intake of sablefish fed diet 7 (37.7% NFE) was consistently high during the course of the study but their growth performance and feed efficiency was low (Table 9). By contrast, fish fed diet 6 (20.4% NFE) consumed less feed, at a declining rate, but sustained better growth performance and conversion efficiency, particularly during the final two feeding periods (Fig.11B).

The herring diet derived approximately 50% of its metabolizable energy (calculated) from protein, while in the formulated diets, 37% of the M.E. came from the protein fraction (Table 2). Furthermore, much of the protein content of the formulated diets (approximately 17%) was derived from wheat protein. Lipid supplied 45% of the M.E. in diet 6, and only 30% of the M.E. in diet 7. The high contribution of wheat starch to the M.E. of diet 7 (33%) compared with diet 6 (18%) may account for the high feed consumption and poor growth performance of sablefish fed the former diet. Although the estimated energy density of the diets was equal, the energy efficiency of fish fed diet 7 was inferior to those of fish fed the other diets, and was reflected in the lower growth rates, and higher DFI. The artificial diets in this study were formulated to be isonitrogenous and isocaloric on the basis of M.E. values for digestible protein, lipid and carbohydrate (NRC, 1981). The increase in the level of herring oil in diet 6 may be partially responsible for the increase in sablefish growth rates, and this implies that the diets were not isoenergetic, and that the M.E. values for digestible carbohydrates used for the formulations were inaccurate. This has been suggested as the case in high carbohydrate diets fed to rainbow trout (Hilton *et al.*, 1987a), and other species (Jobling, 1983).

The dietary treatments resulted in similar feed conversion ratios during the initial feeding period (Fig. 12A). While the FCR values for fish fed diet 6 and the herring diet rose somewhat in the second feeding period, they remained relatively constant during the third. FCR values for fish fed diet 7 rose throughout the feeding trial as water temperatures declined. The dramatic

drop in the productive efficiency of diet 7 cannot be attributed (solely) to overfeeding, since all groups were fed their prescribed diet to satiation. There were no inconsistencies in alternate-day feeding rates, nor were the sablefish observed to frequently reject pellets after mouthing them. Therefore, it appears that a large proportion of diet 7 passed undigested through the digestive tract during the latter two-thirds of the experiment.

The decline in protein utilization in all groups during the second feeding period was substantial, and like the trend for growth rate and food utilization this may be related to declining water temperatures (Fig. 12B). The difference between the PER values for fish fed the herring diet and diet 6, and the lower value of PER for fish fed diet 7, may involve the physical isolation of wheat protein in a manner similar to that of carbohydrate, described above, or to a lower digestibility of wheat protein. Since the protein utilization of diet 6 and the herring diet were comparable, a protein-sparing effect of carbohydrate is indicated. Protein utilization has been unaffected or improved in fish fed diets with high levels of available carbohydrates (Cowey *et al.*, 1975; Bergot, 1979; Shimeno *et al.*, 1979; Kaushik and de Oliva Teles, 1985; Kaushik *et al.*, 1989).

The condition factor values of sablefish arising from the dietary treatments can be examined with respect to pre-experimental conditions, and to the utilization of absorbed glucose over time. The experimental animals were fed maintenance diets of chopped herring and OMP prior to the feeding trial, and condition factor and HSI values were low relative to those values calculated after the first 30 days of the experiment (Figs. 13A and 13B). The initial rise in condition factor values for sablefish fed the test diets reflect to some degree an increase in liver weight, relative to body weight (Fig. 13A). The HSI values themselves responded to changes in liver glycogen and lipid concentration, for all treatments (Fig. 13B). The increase in the condition factor of fish fed the herring diet is positively correlated with the highest HSI value, and the

lowest increase in liver glycogen concentration. Fish fed diet 6 had a condition factor value similar to that of fish fed the herring diet, but without the large apparent increase in HSI value. The liver lipid concentrations, which fell with all diet treatments, were negatively correlated with increasing dietary carbohydrate level. Liver lipid levels in the diet 6-fed fish fell 9% in the first feeding period, while liver glycogen rose 4%. The level of liver lipid produced by the herring diet fell only 3%, and glycogen concentrations rose only 2%. It is difficult to account for the high HSI value produced by the herring diet at the first sampling. The data indicate that the increase in the weight/length relationship in the herring-fed fish was derived more from an increase in liver weight and less from an increase in the weight of somatic tissue. By comparison, diet 6 supported higher somatic gains relative to increased liver weight.

It is necessary to note that, while there were no significant differences in the mean weights of sablefish between tanks prior to sampling and after the first feeding period (ANOVA; $p = 0.05$), the mean weight of sablefish sampled for liver analysis after the first feeding period was considerably lower for fish fed the herring diet (approximately 2.2 kg) than for fish fed the formulated diets (approximately 2.6 kg). The respective mean liver weights, however, were nearly equal. Samples were collected randomly, but by virtue of the small sample size (5) and the relatively wide range of fish weights, the improbable discrepancy occurred. The low mean weight of herring-fed fish samples probably distorted the HSI value from the population mean, inflating the liver weight to body weight ratio. The high liver weights themselves, relative to body weights, cannot be explained.

Elevated liver weight to body weight ratios have been reported for several fish species fed high dietary levels of carbohydrate (Austreng *et al.*, 1977; Shimeno *et al.*, 1979; Hilton and Dixon, 1982; Kaushik and de Oliva Teles, 1985; Buddington and Hilton, 1987). Several studies concluded that condition factor values decrease with increasing carbohydrate level in diets fed to

rainbow trout (Edwards *et al.*, 1977; Refstie and Austreng, 1981).

Liver glycogen levels found in fish fed diets 6 and 7 reached a plateau, perhaps near a saturation equilibrium, after the initial feeding period (Fig. 14A). Liver glycogen levels in herring-fed fish fluctuated somewhat, but generally increased through the feeding periods, while remaining significantly lower than the levels found in fish ingesting the formulated diets. The abrupt rise in liver glycogen levels produced by the formulated diets helps to interpret the results for condition factor, FCR and HSI values. The relatively steady levels of liver glycogen produced by the formulated diets may represent glycogen saturation, restricting glycogenesis, and increasing blood glucose levels. This in turn may restrict the active transport of glucose at the intestinal epithelium, reducing absorption. In these circumstances, carbohydrate digestibility would cease to be a limiting factor and both digested and undigested carbohydrates would pass unutilized through the digestive system.

Liver lipid concentrations were established at more or less steady levels after the first feeding period in sablefish fed all of the test diets (Fig. 14B). The drop in lipid levels contradicts reports from previous studies, where steady or increased concentrations of liver lipid were observed in yellowtail (Shimeno *et al.*, 1979) and rainbow trout (Austreng *et al.*, 1977; Piepper and Pfeffer, 1979) fed high levels of dietary carbohydrate. Since all of the test diets resulted in lower liver lipid levels than found in the low-rationed, pre-experimental fish, the lipids may have been mobilized for energy purposes during the study.

Muscle glycogen levels were not influenced by diet treatment or feeding period, and the values were low in all cases (Fig. 14C).

Reduced water temperatures slow the rate of gastric evacuation and intestinal transit in fish, and gut contents are exposed for longer periods to enzyme activity. Increases in temperature did not affect the digestibility of major nutrients in diets fed to rainbow trout, in studies by

Possompes *et al.* (1975), but Choubert *et al.*(1982) observed an increase in digestion with an 8°C rise in water temperature for the same species. Maintenance energy requirements and energy intake decrease with decreasing ambient temperatures (Fange and Grove, 1979).

Kitamikado and Tachino (1960) reported that starch hydrolytic activities from the combined digestive organs of rainbow trout approximately doubled when the temperature rose from 5°C to 20°C. It can be speculated that an intrinsically low level of carbohydrase activity in sablefish was depressed even further by the drop in water temperature in the latter stages of Experiment 5, and hence proportionally less carbohydrate was digested in sablefish fed diet 7 than in those fed diet 6. Long-term exposure of sablefish to the high level of dietary carbohydrate or prolonged elevation of blood glucose level may have also depressed carbohydrase activity (Buddington and Hilton, 1987). Finally, the geometric relationship between the ingesta and its interface with the intestinal walls may have played a role in reducing the digestibility of diet 7. While the surface area between intestine and ingesta was presumably the same in fish fed diets 6 and 7, the density of the wheat fraction of diet 7 was twice that of diet 6. Under conditions of limited (and perhaps declining) hydrolytic activity, proportionally less of the diet 7 carbohydrate may never have been exposed to starch enzymes, and therefore passed undigested through the system.

5.4 Limitations in the use of chromic oxide marker

The inconsistent behaviour of chromic oxide, in its role as an external marker was apparently the product of procedural and environmental variables imposed on the experimental framework. Evidence of probable differential movement of Cr_2O_3 from the ingesta has been reported in mammals (Knapka *et al.*, 1967), fish (Bowen, 1978) and invertebrates (Leavitt, 1985).

The latter study reported "streaming", or increased relative motility of Cr_2O_3, from diets containing from 0.1% to 1.0% of the marker fed to American lobster. Lied et al., (1982) presented data which can be interpreted as evidence for streaming of Cr_2O_3 in the gut of adult Atlantic cod.

Several variables distinguished Experiment 1 from Experiment 2, and several other variables distinguish both experiments from the majority of digestibility studies in fish. A most obvious distinction between the first two experiments was the different levels of dietary marker employed. Experiment 3 showed that the dietary Cr_2O_3 concentration was an unlikely source of the differences in Cr_2O_3 behaviour between Experiments 1 and 2. Although the distribution of Cr_2O_3 in the digestive tracts of animals from Experiment 3 was somewhat different than that which characterized Experiment 2, the decline in stomach ingesta levels of marker, relative to levels of marker in the respective diets, demonstrated the same differential movement of Cr_2O_3, under the conditions in which the experiments were conducted.

Feeding protocol was changed from one daily satiation meal in Experiment 1, to alternate-day feeding in Experiment 2. The purpose of the change was to ensure that each fish had sufficient appetite to ingest a substantial meal, and to reduce the likelihood of empty stomachs in sampled fish, such as occurred in Experiment 1. The estimated gastric emptying time in fish fed diet 3 at 10-11°C was 53.0 hours (Table 8). It is reasonable to assume that the stomachs of sablefish studied in Experiments 2, 3 and 4 were empty or nearly empty when feed was offered.

Fletcher *et al.*, (1984) described a study which determined that, when two feed pellets containing a binding agent were fed to dab 3 hours apart, the first (marked) pellet was evacuated in the faeces first, separately from the second. When the diet contained no binder, pellets fed 3 hours apart mixed, and the transit of the first (marked) pellet was delayed (35% slower), as if both pellets had been fed simultaneously.

A precept of digestibility studies which employ external markers is that the faecal

concentration of marker represents its maximum concentration in the digestive tract. Possompes *et al.* (1975) reported that the maximum faecal Cr_2O_3 concentration, from a marked meal fed to 100 gram rainbow trout, was obtained 14 hours after feed intake. Post-prandial feeding with unmarked pellets did not influence the time taken for faecal Cr_2O_3 to reach a maximum level, nor was the maximum ratio of Cr_2O_3 to dry matter altered. However, faecal Cr_2O_3 concentration from post-prandially fed fish dropped soon after its maximum concentration was reached, whereas that in fish fed a single meal persisted for 10 to 15 hours. The brief period of maximum Cr_2O_3 concentration in faecal samples from post-prandial fed fish was interpreted as being a consequence of an accelerated transit rate. In both cases, faecal sampling prior to the attainment of the maximum Cr_2O_3 concentration would produce spurious estimates of nutrient digestibility.

Lied *et al.*, (1982) published data describing the distribution of Cr_2O_3 throughout the digestive tract of adult Atlantic cod. Following the feeding of a single marked meal to starved fish, the accumulation of Cr_2O_3 marker in sections of the digestive tract was assessed at seven sample times over a 72 hour period (Fig. 15). A wave of maximum Cr_2O_3 concentration passed through the gut from section to section of the tract, over time, and peaked in the terminal sample after 72 hours. At this time, the stomach, pyloric caeca, and anterior intestinal contents contained lower concentrations of Cr_2O_3 than that in the feed. Stomach concentrations of Cr_2O_3 fell below that of the feed as early as 36 hours post-feeding, and this shows evidence of 'streaming' of the marker. There was no time during the course of the experiment when the digestibility of the nutrients in the diet could have been accurately determined using relative Cr_2O_3 concentrations, according to the criteria for the use of effective markers.

Lied and co-workers also described Cr_2O_3 accumulation in the digestive tracts of cod fed a marked diet three times a day, to satiation (Fig. 16). The highest concentration of Cr_2O_3 was found in the posterior intestine, adjacent to the terminal sample. It can be speculated that the

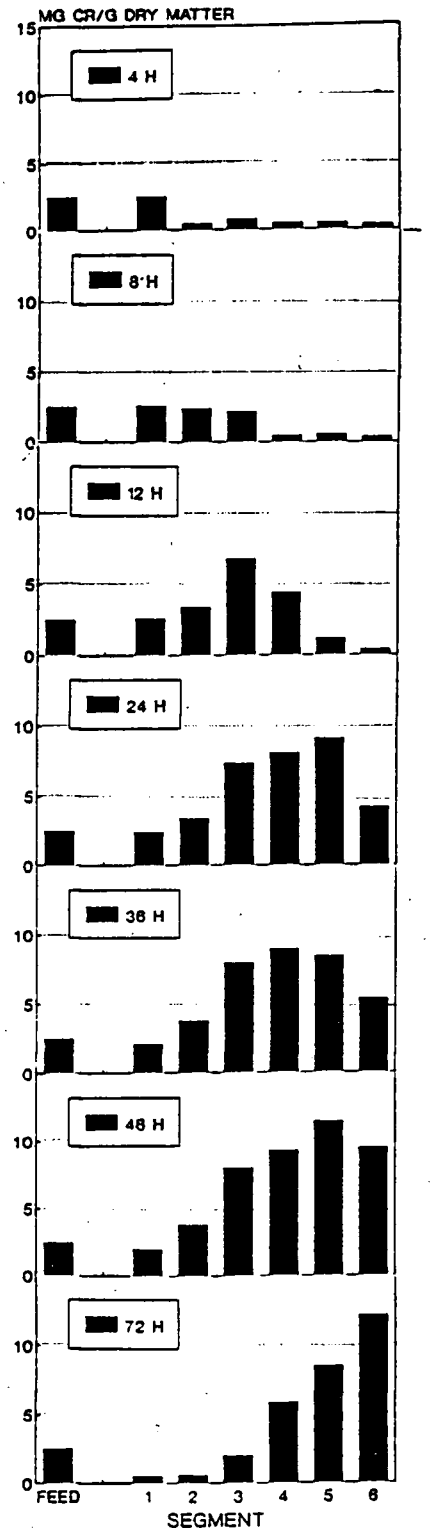


Fig. 15 - Accumulation and flow of chromic oxide in the gastrointestinal tract of Atlantic cod fed minced saithe mixed with 0.1% Cr_2O_3 marker. Fish were force-fed a single meal after a 5-day period of starvation, and sampled at 4, 8, 12, 24, 36, 48 and 72 h after feeding. Chromic oxide concentration in each segment was measured as mg Cr per g dry matter of digesta (or feed). (Transcribed from Lied *et al.*, 1982. Fig. 3, p.857. Segments correspond to: 1 - stomach; 2 - pyloric caecae; 3 - anterior intestine; 4 - mid intestine; 5 - posterior intestine; 6 - anus.)

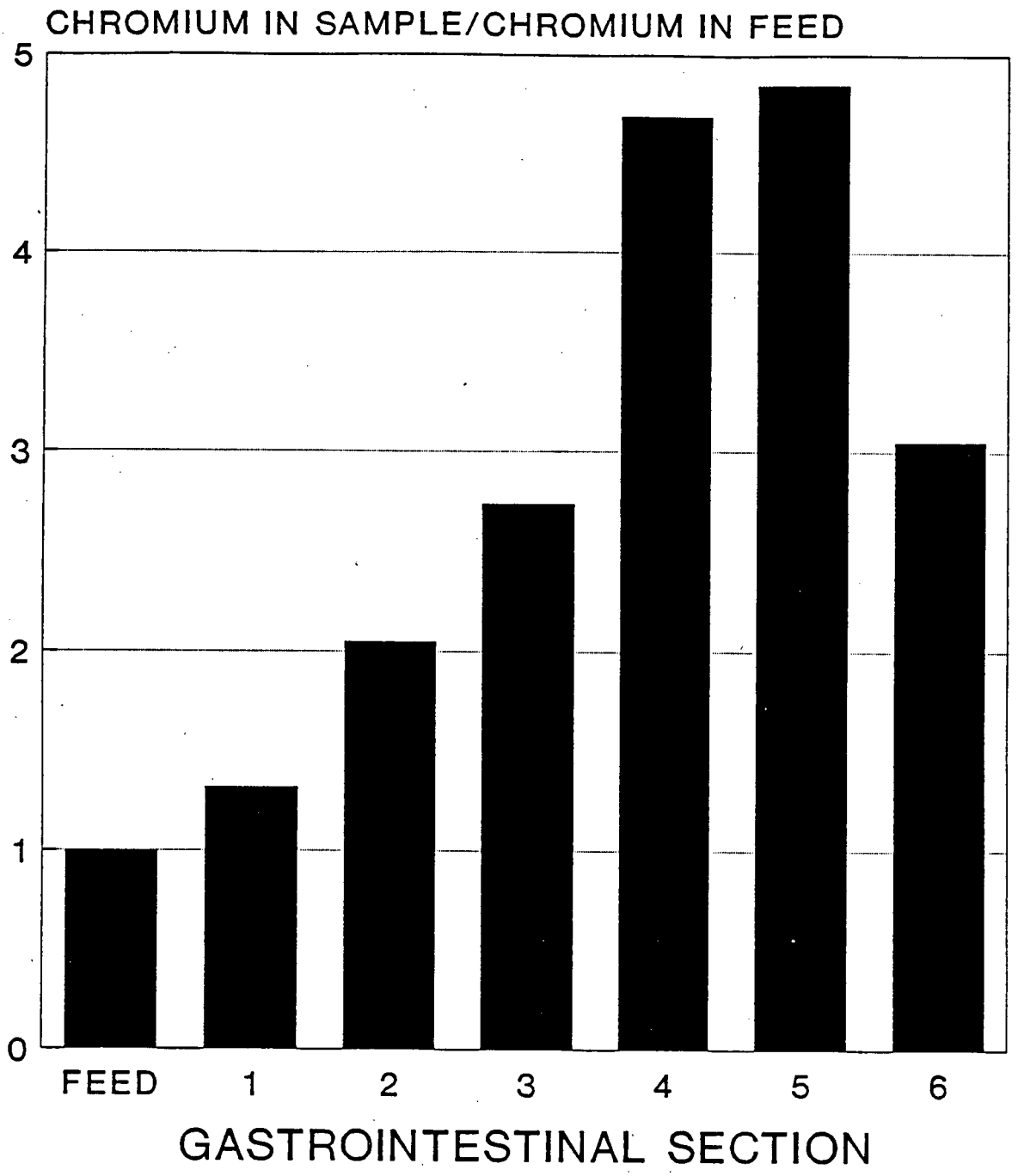


Fig. 16 - Accumulation of chromium in the gastrointestinal tract of Atlantic cod fed minced caplin, mixed with 0.1% Cr_2O_3 marker, to satiation twice daily for five days prior to sampling. The concentration ratios of the indicator are related to the level of marker in the feed. (Transposed from Lied *et al.*, 1982. Table 1, p.857. Segments correspond to: 1 - stomach; 2 - pyloric caecae; 3 - anterior intestine; 4 - mid intestine; 5 - posterior intestine; 6 - anus.)

lower terminal Cr_2O_3 concentration represented a digestive process which was incomplete, since maximum faecal concentration had not been reached at the sampling time. The steady accumulation of Cr_2O_3 through the gut indicates that the marker moved as expected for the digestibility determination of nutrients, but that sampling was premature.

The experiments described above support the theory that feeding protocol is a relevant variable in digestibility determinations, and they help to explain the dubious results that were obtained in Experiment 2 of this study.

The stomach contents of pre- and post-prandially starved sablefish fed diet 3 were estimated, using Equation 1 (Section 4.4), to be 54% of a standard meal after 24 hours and only 10% after 48 hours (notwithstanding temperature effects). The latter estimate describes the pre-feeding gastric status of fish in Experiments 2, 3 and 4.

Each of the diets used in this study, except diet 2 (Experiment 2), contained cooked or pregelatinized starch, which has considerable binding qualities. Considering the relatively small amount of ingesta remaining in the stomach, and the binding properties contributed by gelatinized starch, it is safe to suggest that little mixing occurred between meals in the experiments where feeding was restricted to alternate days. Also, each meal probably emptied independently of the other. The resulting single- meal profiles of Cr_2O_3 concentration in the gut can be found in Figures 5 and 7 for sablefish, and Figure 15 for Atlantic cod.

Over 50% of a previous meal remained in the stomach of sablefish when they were fed 24 hours after their previous meal, and the potential for the mixing of the two meals was higher in this case than in the situation where the meals were spaced 48 hours apart. Referring to Figure 15, the concentration of Cr_2O_3 in the stomach of Atlantic cod 24 hours after feeding was the same as that in the feed. An infusion of feed at this time, and the subsequent mixing of this meal with the remains of the previous meal would counteract a drop in Cr_2O_3 concentration (observed after

36 hours). Evidently, timely replenishment of marked ingesta in the stomach maintains the Cr_2O_3 concentration at the same level as in the feed or, at a sufficient feeding level and frequency and coincident with digestion, maintains a higher stomach concentration of Cr_2O_3 (Figs. 2 and 16). When the feeding protocol is appropriate, a continuity of ingesta can be established, and the chromic oxide profile in the digestive tract can be visualized as being constant, over time.

The process of marker accumulation (or concentration) in the digestive tract reflects the indicator dilution principle described by Zeiler (1958), in which an indicator is introduced continuously into a fluid system. When the level of an indicator in the system reaches a maximum, the overflow of indicator exactly equals its rate of inflow, and the level remains the same until the indicator inflow rate is diminished or increased. In the stomach ingesta of sablefish, Cr_2O_3 levels would represent the maximum level of a fluid system in the absence of digestion, when indicator is replaced at a constant rate. As digestion and absorption remove nutrients from that system, the Cr_2O_3 level will increase, and a concentration profile will be established throughout the digestive tract.

Diurnal feeding schedules interrupt a constant supply of marked ingesta to the stomach, while the quantity of faeces excreted per unit of time is constant throughout a 24-hour cycle (Possompes *et al.*, 1975). Frequent feeding apparently provides enough continuity between meals to provide the basis for estimating nutrient digestibility using relative Cr_2O_3 concentrations, as has been noted in the majority of studies on fish. Inappropriate feeding schedules can, however, undermine the assumptions on which digestibility measurements are based.

The chromic oxide profile in G.I. tract of sablefish at day 24 of Experiment 1 (Fig. 2) closely resembled that for thrice-daily fed Atlantic cod (Fig. 15). Both profiles imply that samples were taken before the digestion of a meal was completed, and that the modified indicator dilution principle described above incompletely describes the digestive process when diurnal

feeding schedules apply. Nightly fasting introduces a discontinuity of indicator input, and a wave of falling and rising Cr_2O_3 concentration caused by 'streaming' passes through the gut with the meal ingested prior to fasting, superimposed on the established concentration profile. From Figures 2 and 15, it can be seen that faecal sampling at an inappropriate time may provide data which do not represent completed digestion.

The experiments conducted in the present investigation differed from most nutritional studies on fish since large fish, held in a sea water environment, were studied. The majority of digestibility studies have involved juvenile fish, whose feeding rates, growth rates and evacuation rates are higher than older, larger animals of the same species. It is clear from the results of this study, however, that the methodology developed for studying feedstuff digestibility in juvenile fish may have to be modified to obtain accurate estimates of diet and feedstuff digestibility in large fish. The change in feeding protocol was likely the cause of the anomalous results in Experiment 2, and there are few precedents for this effect in nutritional studies with fish. The digestibility methods developed for smaller fish were found to be lacking in reliability and convenience when large sablefish were used.

Most of the research on the nutrient requirements of fish has been conducted on freshwater fish, or on the freshwater life-stages of anadromous or catadromous species. Whereas teleosts in freshwater must actively expel water absorbed through the gills into the hypotonic environment to maintain their osmotic balance, marine teleosts continuously drink sea water to replace the osmotic loss of water across the gills and through the kidneys. Salt water is not absorbed directly against an osmotic gradient, but is diluted by the diffusion of salts across the oesophageal and stomach walls, and water is absorbed at the intestine (Rankin *et al.*, 1983). The greater salt load produced by the process is countered by its excretion at the gills (sodium and chloride ions) and in the urine (magnesium and sulphate ions).

Studies have reported measurements of nutrient digestibility, using Cr_2O_3 marker, for several marine species (Shimeno *et al.*, 1977; Lall and Bishop, 1979; Jobling, 1981a). In each of these studies, the experimental animals were juveniles, which were fed frequently, and were sampled only for faeces content. No noteworthy deviations from anticipated results were recorded for these studies. However, in experiments conducted in salt water on larger Atlantic cod, Lied *et al.* (1982) determined that Cr_2O_3 marker displayed minor differential movement in relation to the rest of the ingesta when feeding was frequent and sampling untimely (Fig. 16), and more definitive differences were noted when a single, isolated meal was fed (Fig. 15).

The circumstances in which Cr_2O_3 "streaming" takes place can reasonably be attributed, in adult Atlantic cod, and in sablefish, to the feeding protocol adopted for the experiments. The mechanics of Cr_2O_3 movement may be associated, however, with the movement of sea water through the digestive tract, as water is replenished in the fish.

CHAPTER 6

6.0 CONCLUSIONS

Carbohydrate digestion, or disappearance, was observed to occur in each section of the gut. Indirect evidence (liver glycogen deposition) suggested that the digestibility of the different carbohydrate sources in sablefish was as follows: pregelatinized starch > cooked wheat > pregelatinized starch/cooked wheat > raw wheat. A considerable amount of absorbed glucose was not used immediately as an energy source, but was deposited as liver glycogen.

The growth performance and energy utilization of sablefish fed a formulated diet containing 22.2% of cooked wheat was superior to that for a diet containing 44.4% cooked wheat, and those in both diets were inferior relative to that for a natural reference diet. Sablefish fed both formulated diets had high liver glycogen levels, and low condition factors.

The pattern for gastric evacuation of a formulated diet was curvilinear, and resembled the emptying pattern for low energy, high bulk diets. Chopped fish required too long a period of time to begin evacuation for an accurate description of an emptying pattern to be made.

The levels of carbohydrate, and the protein/energy ratios employed in this study appeared to be inappropriate for promoting sablefish growth. Higher dietary levels of crude protein and lipid, and lower levels of cooked wheat would likely improve the growth performance of sablefish fed formulated diets.

The use of chromic oxide in digestibility studies on sablefish poses a conundrum. The marker has been used effectively in digestibility studies conducted on small fish fed at least once daily. However, the use of the marker for evaluating digestibility in large sablefish was

problematic, especially since preliminary observations suggest that this species, at this size, should be fed on alternate days. The results of this study and a previous study on another marine species, the Atlantic cod, suggest that this feeding protocol is inappropriate for satisfying the criteria for an external marker using chromic oxide.

The recent findings of Hajen (1990), who studied feedstuff digestibility in post juvenile chinook salmon in sea water indicate that the "Guelph System" of faecal collection is appropriate for fish in the marine environment. This may be the case for sablefish provided that attention is paid to variables such as feeding frequency and ration level.

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