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PHYSIOLOGICAL FACTORS REGULATING APPETITE IN THE LESSER SPOTTED DOGFISH SHARK, SCYLIORHINUS CANICULA (L.)

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PHYSIOLOGICAL FACTORS REGULATING APPETITE IN THE LESSER SPOTTED DOGFISH SHARK, *SCYLIORHINUS CANICULA* (L.)

by

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ABSTRACT

Physiological factors regulating appetite in the lesser spotted dogfish shark, *Scyliorhinus canicula* (L.)

David William Sims

Some aspects of digestive and systemic function were investigated in relation to their role in the peripheral regulation of appetite in juvenile and adult, *S. canicula*. This study represents the first evaluation of the physiological factors that contribute to the control of shark appetite. Daily food intake trials on both juvenile and adult dogfish showed repeatable, self-regulated feeding rhythms indicating the existence of an endogenous component to food intake control. After dogfish consumed satiation meals of 7% wet body weight (wbw) the appetite return increased at constant rates as deprivation time increased. The relative rate of food processing was 50% faster in juveniles than adults. The pattern of gastric emptying of squid diet was exponential and dependent on the degree of stomach fullness, with meals of different size being emptied at different relative rates. It was also shown that dogfish were capable of shunting undigested food into the intestine soon after consumption of large meals. There was inverse proportionality between rate of gastric evacuation and appetite return rate indicating the importance of the physiological perception of relative stomach emptiness in the establishment of appetite. Gastric emptying rates were not influenced by changes in the digestible energy level of the diet, which suggests this shark exhibits a predominantly bulk dependent feeding pattern. Increases in post-prandial metabolism or specific dynamic action (SDA) did not seemingly alter the rate of appetite return in dogfish, though SDA and appetite return were shown to be closely linked metabolic processes. The SDA process in dogfish may have a saturation level determined by cellular metabolism rather than by the respiratory system. The levels of plasma glucose remained uniform after food consumption. The concentrations of triglycerides and protein in plasma were closely controlled post-prandially, suggesting a possible role for these metabolites as systemic signals of metabolic satiety. The results of this investigation are discussed with regard to the multifactorial control of appetite in sharks and the possible use of physiological studies of appetite in the further understanding of fish feeding strategies.

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AUTHOR'S DECLARATION

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award.

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Signed.....

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CHAPTER ONE

GENERAL INTRODUCTION TO PHYSIOLOGICAL REGULATION OF APPETITE

"The importance of intake of materials and digestion for maintenance of living organization needs no emphasis. Yet it is interesting to recall how little we understand about the system that controls the rate at which food is taken and hence growth and replacement proceed, although these processes limit the size and many other aspects of the organism." J.Z. Young (1975).

1.1 INTRODUCTION

Regular selection of food and the quantity of the food ingested will be of the utmost importance in the life of animals and will be the result of decisions that ultimately serve to determine the health and survival of the species (Thomas, 1987). An animal's capability for selection of nutritionally adequate food and the physiological processes involved in digestion will be controlled ultimately by hereditary instructions, though many aspects of food-gathering behaviour will be acquired during the individual's lifetime and influence inherited processes through the higher cerebral centres (Young, 1975). Animals must be able to consume enough food of the type necessary to satisfy the energy demands of metabolism, such as basic bodily functions, activity and food processing, and still partition energy for somatic and gonadal growth. Those individuals that are unable to obtain sufficient nutrients due to inadequate food selection and digestion processes, will not grow or survive. The feeding strategies that different species employ (based upon inherited and learned processes) will depend to a large extent on the manner in which the physiological mechanisms of digestion and metabolism determine the rate of food processing and thus,

the times when more food can be consumed.

Food must be available before it can be consumed and it is well documented that food items will not always occur in equal abundance in the environment throughout an annual cycle (Adams *et al*, 1982). Accordingly, constant levels of food consumption will not be maintained during the course of the year, but rather feeding rates will change concomitantly with the availability of food items (Thorpe, 1977; Adams *et al*, 1982; Lyle, 1983; Kean-Howie *et al*, 1988). However, opportunistic feeding in the environment will not be the only manner in which food intake is controlled. If an animal is recognised to be adapted to its environment then logically it must also be assumed that it has undergone adaptation of its life cycle to the normal cycles of food availability (Peter, 1979). Therefore, daily food consumption is a process by which animals maintain levels of energy intake in the short term, from one day to the next, but in proportion to and within the bounds of long term natural oscillations in the presence of those food items.

A possible basis for regulated acquisition of food by an animal is demonstrated by their ability to compensate for food deprivation by subsequent increases in food consumption. This hypothesis has been well understood in mammals for some considerable time (Hoebel and Teitebeim, 1966; Hoebel, 1971), although it has not been widely applied in studies on lower vertebrates (Peter, 1979; Fletcher, 1984). In studies on mammals, stabilisation of food consumption (and hence body weight) between certain levels depends on the amount and quality of food consumed. If the caloric intake is increased by force feeding and the animal becomes obese, when the normal feeding regime returns the animal will feed hypophagically and a decrease in body weight will be induced. Similarly if the animal is starved and a net reduction in body weight occurs, maintenance of the normal feeding regime will result in hyperphagy followed by a rapid increase in body weight up to the level that occurred before starvation (Peter, 1979).

Clearly compensatory responses of this type indicate that mammals are capable of a short term regulation of food intake. There is some evidence that certain species of fish can regulate daily food consumption (*e.g.* Rozin and Mayer, 1961, 1964), but the investigations are few. The more established view for fish is that the minimum and maximum levels between which body weight may fluctuate at any given period in the annual cycle, will be controlled by a combination of physiological and non-physiological factors (Brett and Groves, 1979; Fletcher, 1984).

The process of feeding not only incorporates what we understand as feeding behaviour, that is, obtaining, handling and ingesting food (McFarland, 1987), but also the physiological events associated with digestion, absorption and assimilation of the dietary items. The relationship between the different facets of feeding and their importance in its control are poorly understood. This can be partly attributed to the biological complexity and interaction of the processes that can influence the behaviour of an animal and will encompass not just physiological, but also ecological and environmental factors. All these factors act on the animal and in some way initiate, modify and inhibit feeding. Separation of the individual components of the feeding process that are likely to influence when further feeding bouts occur, would enable the various factors to be evaluated. Investigations of this type are rare in fish (Fletcher, 1984). Indeed, the quote from Elliott (1975) seems appropriate testimony to the problems facing investigators interested in finding out what factors are important in regulating food intake.

"...food consumption of a brown trout, Salmo trutta L., is affected by a large number of factors which include the size of the fish, the amount of food eaten in a meal, the number of meals in a day, the rate of gastric evacuation, water temperature, activity of the fish, the type of food eaten and the availability of the food organisms. As there is also interaction between some of these factors, it is not surprising that few workers have studied this complex subject."

1.1.1 Non-Physiological Factors Regulating Food Consumption

Certain non-physiological factors help determine the rate of food consumption and they can be principally divided into two broad categories, those that are ecological and those that alter the immediate environment surrounding the animal.

The ecological factors primarily constitute the effects of prey, conspecifics and predators (Wootton, 1990). As already stated, one of the most important ecological factors governing food consumption is the abundance and density of food items. Numerous studies on teleost fish have elucidated various feeding behaviours in relation to prey density and have frequently shown that the fish adopt a feeding strategy that usually maximises gross energy intake when the availability of prey is high (*e.g.* Kean-Howie *et al*, 1988; Batty *et al*, 1990). In addition much research has been undertaken to investigate the effects of prey size and prey type on the energetic costs of capture, handling and ingestion, and this has resulted in hypotheses being formed regarding the adaptive capacity of fish feeding behaviour (for general review see Dill, 1983; for review on sharks see Bres, 1993; Mookerji and Rao, 1994). Opportunism partly determines feeding behaviour in animals including fish and it is generally regarded that through opportunistic feeding animals learn what type of food to consume, and when and where food is likely to be found (McFarland, 1987). Behavioural mechanisms of this kind enable animals to feed most profitably with the minimum of energy wastage as their feeding effort becomes more concentrated (McFarland, 1987).

Intraspecific and interspecific effects are also likely to play a major role in food intake regulation. Some species of fish form large aggregations (*i.e. shoals*, social assembly; *schools*, synchronised swimming movements; Pitcher and Parrish, 1993) partly in order to gain protection from other individuals. It has been shown that this behaviour allows more time to be spent attending to various feeding related activities (Pitcher and

Parrish, 1993). Additionally, there is evidence that shoaling fish can locate food much faster in the group than when solitary (Pitcher *et al*, 1982). The advantages of grouping to individual animals remains quite clear (though for a critical review see Pitcher and Parrish, 1993), however the very effect of grouping, which on the positive side affords protection and presumably other behavioural advantages, may also confer limitations on certain individuals within the group. The existence of social hierarchies, where the animal group may be dominated by a number of individuals, could lead to limitations in food consumption by the subordinate or least competitive members of the group (Braddock, 1945). Hierarchical behaviours of this nature have been reported in studies on captive fish fed a fixed amount of food and where a small number of dominant individuals (that are usually larger) out compete the other fish for this resource (Christiansen *et al*, 1991; Christiansen *et al*, 1992; D.W. Sims, unpublished observations).

The role of predators in helping to regulate the food intake of the prey species is also well established (Wootton, 1990). Not only might the presence of a predator decrease the foraging time of prey individuals (*e.g.* Nordeide and Svåsand, 1990), but the predator may also reduce the activity space within which the prey species could possibly forage (*e.g.* Metcalfe *et al*, 1987). Both effects may alter the feeding behaviour of fish prey to the extent that food consumption rates become reduced. Many species of fish only forage at discrete periods during each day (for review see Helfman, 1993), and so any perturbations to the feeding periodicity as a result of predator presence may have far reaching consequences in the efficiency of food acquisition and day-to-day survival of the prey species.

Environmental factors can determine the rate of food consumption in addition to ecological interactions. Temperature governs the rate of metabolic reactions and is the overriding controlling factor of the plane of metabolism in ectothermic fish (Brett and

Groves, 1979). It has been demonstrated frequently that greater rates of food intake occur at higher water temperatures (*e.g.* Elliott, 1982; Hidalgo *et al*, 1987). At lower temperatures the fish may not feed, but as the temperature increases so the rate of food consumption also increases up to an optimum, before it declines at the highest temperatures (Elliott, 1975; Wootton, 1990). Length of photoperiod as well as light intensity have been shown to influence the nature of foraging periods in many different species of fish (*e.g.* Batty *et al*, 1990; Helfman, 1993). Daily light cycles affect endocrine activity and so may indirectly cause changes in level of food consumption by altering the physiological status of the animal (Brett and Groves, 1979). Water quality parameters have also been shown to affect feeding as they impose a metabolic load on internal regulation. The effects of abnormal pH and salinity levels on fresh and saltwater fish have usually been manifested in lower food intake and poor growth rates (*e.g.* Planichemy *et al*, 1985; Li and Yamada, 1992).

Clearly both ecological and environmental factors contribute to the regulation of food consumption, however the time scale over which these biotic and abiotic parameters exert their control can be considered to be more long term. The water temperature experienced by fish for example, will change quite slowly throughout the annual cycle in contrast to the rapid fluctuations in air temperature that are characteristic of the terrestrial environment. Similarly, the adaptive flexibility in feeding behaviours (Dill, 1983) will probably occur gradually over the time course of a fish's life (Magurran, 1993) and from one generation to another (Rose, 1993), rather than abruptly switch on any particular day to a completely new repertoire of behaviours. Ecological and environmental factors affect food consumption by evolutionary entrainment of the physiological processes associated with feeding regulation. On a daily basis however, the physiological and biochemical parameters associated with nutritional status of the animal are more likely to regulate the

occurrence of feeding bouts. By the establishment of a physiologically driven motivational state (which is in dynamic flux), feeding can become a high-priority activity, if indeed imbalances occur in the levels of nutrients within the animal's internal environment.

1.1.2 Physiological Factors Regulating Appetite

The motivation for feeding behaviour is partly based on the homeostatic principle, inherent in the competent functioning of all biological systems (McFarland, 1987). Peter (1979) stated that food intake in fish was just perhaps another example of regulated homeostasis. From research on higher vertebrates, most notably the laboratory rat (Fletcher, 1984), it is clear that food intake may indeed be closely controlled in a homeostatic manner. The basis for an extension of this hypothesis to lower vertebrates is not aided by the lack of information on physiological control of appetite in fish.

The clear understanding of how an animal's desire for food, or feeding potential, is controlled in the short-term requires an outline of each operating component that defines the "feeding" state. It should then be possible to obtain an objective approach describing the contribution such physiological factors may have in changing or modifying the motivational feeding state and the short-term pattern of subsequent feeding events.

The ability or degree to which any animal consumes food depends on a number of ecological and environmental factors (Fletcher, 1984) in addition to the interaction of physiological processes. Together these factors produce an internally driven motivational "feeding" state (Colgan, 1973; Novin & VanderWeele, 1977; Booth, 1979; Colgan, 1993). The physiological factors play the central role in establishing the motivational state because as the animal uses up energy and nutrients, various imbalances occur in the animal's physiological status and these are registered by the brain (McFarland, 1987).

Deprivation of food results in a state known as hunger. The motivational state leading to feeding behaviour was described as hunger in Colgan's interpretation, but this component of feeding is clearly not the same as appetite itself. Rather, the appetite of an animal is determined by the physiological mechanisms relating to satiety and its control, entrained by the environment, and functionally released through a behavioural response known as hunger. It is the ecological factors pertaining to food consumption that modify the efficacy of the behavioural response (Pitcher & Hart, 1982). The aim of the response behaviour must therefore be towards food acquisition, and the propensity to consume food is the subjective estimation used to describe the level of hunger experienced by the animal. Appetite is therefore pivotal to the very efficiency of feeding, characteristically selective and arguably the physiological protagonist to hunger (Brett, 1971) (or alternatively described as the congenial companion of hunger by Thomas, 1987). A clear definition is provided by Wootton (1990), who generally described that hunger was the tendency or inclination of an animal to feed, whereas appetite was analogous to the quantity of food consumed until cessation of the active event, *e.g.* voluntary food consumption. Therefore we might say that hunger can be characterized as a sequence of physiological changes and cognitive events which are an inevitable consequence of prolonged fasting, whereas appetite will refer to those physiological changes and cognitive events that are evoked by the stimulus properties of foods (Thomas, 1987). Feeding is clearly a broad term given to include not just the motivational state of hunger and its associated behaviours, but the actual magnitude of the animal's appetite at any one time. Very little information exists on control of voluntary food consumption from food stimuli in fish, especially from a physiological standpoint (Peter, 1979; Grove *et al*, 1983; Fletcher, 1984; Colgan, 1993). In the context of this study it is the appetite of fish, within the broadly described feeding event that will be studied in order to determine the effect of physiological factors on the

short-term control of actual food intake (energy acquisition) in fish and any resultant patterns in feeding.

Godin (1981) and Russell and Wootton (1993) both noted that fish will reach maximum food intake (satiation) more rapidly with greater appetite. A fish's return to maximum appetite (or close to it), in order to make maximal use of the available food, would thus appear to be a vital process for its day-to-day survival. Of prime interest therefore, is the identification of the physiological events integral to the establishment of the degree of appetitive status, and secondly the pathways by which appetite might then be regulated.

1.1.2.1 Neural and hormonal control of food intake

The basis for neural control of food intake has been well studied, mostly from investigations using the laboratory rat (Andersson, 1972). The early mammalian research concentrated on establishing which specific brain regions were involved in feeding behaviour. These studies usually lesioned certain parts of the brain and the alterations in feeding behaviours that were observed resulted in those areas of the brain being described as either a 'feeding' or 'satiety' centre (Peter, 1979). This dual centre theory was first proposed by Stellar (1954), though in more recent times it is regarded as somewhat simplistic due to emerging evidence that certain areas of the brain, such as the telencephalon can play an integrative role associating input with output programs (Roberts, 1988). The earlier studies have shown that the ventromedial hypothalamus (VMH) and lateral hypothalamus (LH) were involved in feeding control. It was suggested that the LH can be involved in the organization and activation of feeding behaviour and food intake regulation, whilst the VMH has involvement in food intake regulation in relation to satiation. It is now generally accepted that the hypothalamus, in particular the VMH and

LH are the regions of the brain that are focal to the control of the ingestive behaviours and the vegetative function (Peter, 1979). However, it is not entirely clear how well integrated these regions are, or indeed what other functions might be carried out by these areas of the brain. More recently, Roberts (1988) noted that in mammals the hypothalamus was central to the homeostatic control of bodily functions and regulated behaviour such as feeding, escape, attack, aggression and sex. From the few studies that exist for fish it would appear that the LH has some function as a feeding centre in a manner similar to that of mammals (Peter, 1979; Fletcher, 1984). Although these regions of the brain may be involved in the regulation of food intake in fish (Savage and Roberts, 1975), it is clear they might not solely be responsible for the mediation of feeding behaviours (Fletcher, 1984). It has been suggested that the telencephalon may play a role in feeding behaviour, however there is some doubt as to whether it is important in the overall balance of food intake regulation.

The autonomic nervous system and the action of hormones are important components in mediating the physiological control of feeding in fish (Matty and Lone, 1985). Many hormones are secreted in direct response to the ingestion of food, *e.g.* insulin (Murat *et al*, 1981) and the gastric peptide, cholecystokinin (CCK) (McCaleb and Myers, 1980), indicating their close involvement with digestive function. The role of these hormones in the control of food intake in fish is incompletely known, but it has been demonstrated that CCK is secreted by the duodenal receptors, stimulates gall bladder contraction and pancreatic enzyme secretion leading to a reduction in the flow of chyme into the duodenum (for review see Jobling, 1986). More recent studies on the action of CCK on the visceral organs of fish by Aldman (1994), strongly indicates that one or several CCK-like substances may be involved in gallbladder and gastric emptying control. Hence, hormones may be substantially involved in regulating gut motility, in addition

perhaps to mediating the effect of other physiological factors important in appetite control in fish.

Even though neural and hormonal actions are central components in the instantaneous modifications that must occur to 'update' the motivational feeding state, appetite will not be regulated by these processes alone. Early research on higher vertebrates concentrated mainly on either the 'centralist' or 'peripheralist' theories governing food consumption (Fletcher, 1984). Centralists maintained that food intake was principally controlled by specific neural areas of the central nervous system, while peripheralists contended that the animal's physiological status resulted in the control of appetite. It is now generally accepted that appetite is not regulated by any single process, rather that appetitive status is achieved through integration of both central and visceral nervous systems with the physiological processes of food consumption, digestion, absorption and assimilation (Novin and VanderWeele, 1977).

1.1.2.2 Chemosensory control of appetite in fish

The senses of olfaction and gustation are integral parts of the general appetitive behaviour of fish and are concerned with the initial detection and subsequent localisation of food. The ability of fish to detect food is present from very early times. For example, a study has shown that planktonic yolk-sac larvae of red-sea bream, *Pagrus major* were capable of detecting food using nostril receptors with radially arranged cilia (Tanaka *et al*, 1991). The study also suggested that the larvae were capable of detecting and remaining within the food layers even before the onset of feeding. Much work has been undertaken to elucidate the processes involved in fish chemoreception (for reviews see Hara, 1993). Recent work has also centred on the effect of food attractants as appetite stimulants with the aim of increasing food consumption rates in order to enhance survival

rates and shorten production intervals in fish for aquaculture (Ward, 1991). In particular, L-amino acids have been shown to have positive effects on the feeding behaviour of fish and it is without doubt these chemical cues can influence rates of food intake (Ward, 1991; Heinsbroek and Kruger, 1992).

In addition to teleost fish, a considerable number of studies exist with regard to the anatomy and physiology of chemosensory structures in elasmobranchs (for review see Montgomery, 1988). The majority of the early investigations on sharks had the objective of assessing the behavioural responses of sharks to olfactory stimuli (usually produced by offering dead fish), *e.g.* the work of Hobson (1963) and Tester (1963). However, more recent studies have attempted to quantify the feeding behaviour in response to exposure to different concentrations of different chemicals, *e.g.* in *Sphyrna tiburo* (Johnsen and Teeter, 1985). Clearly the fact that chemical cues will elicit pronounced feeding responses in sharks (Roberts, 1988) indicates their importance as factors regulating appetite. After all, as stated previously appetite can be considered the eventuality of physiological changes and cognitive events that are evoked by the stimulus properties of foods. Clearly, the necessary stimuli can arise from the properties of food both from within the animal and from the environment surrounding the individual. The effect of chemosensory stimuli on shark feeding behaviour has received some considerable attention in comparison to other physiological factors and further research on this topic remains outside the scope of the present investigation.

1.1.2.3 Physiological factors of digestive and systemic function

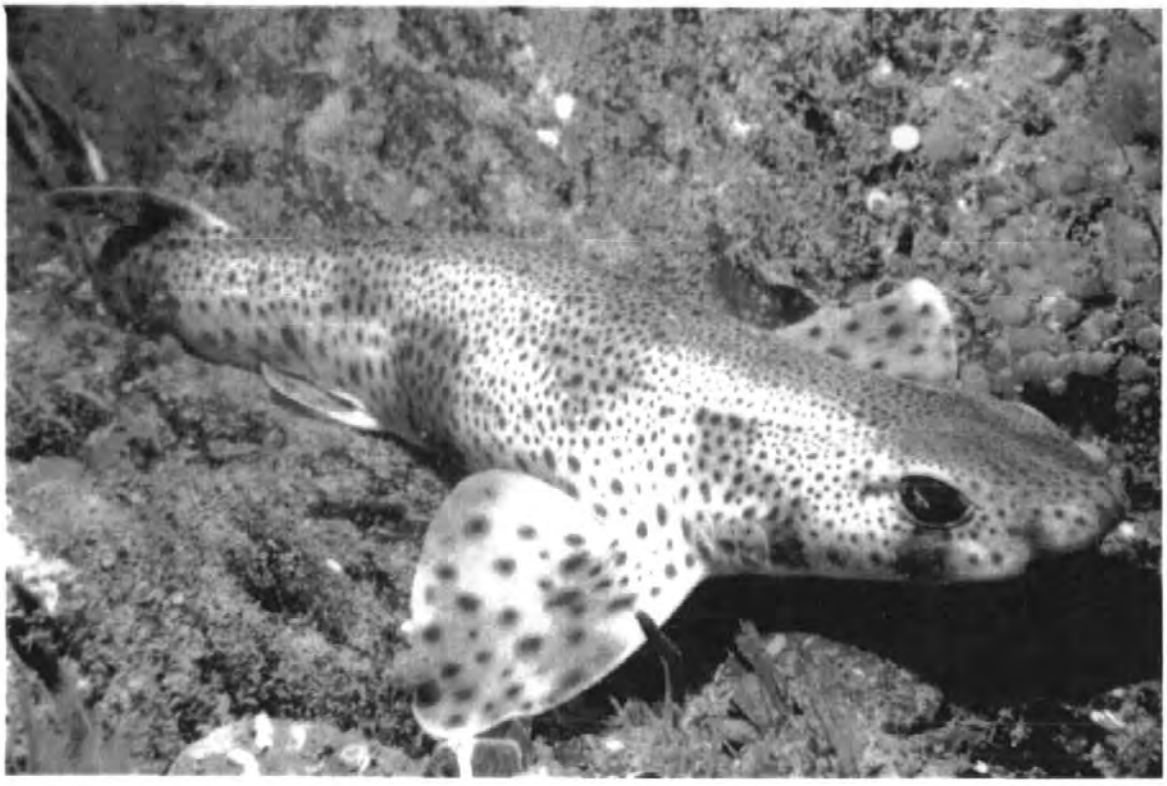
Those physiological factors involved in digestive processes and systemic control of appetite have not been widely studied in fish, despite the fact that various authors have stated their undoubted importance (Brett, 1971; Colgan, 1973, 1993). Few studies have

attempted to find the role of the stomach in appetite control, or the effect of diet quality, post-prandial metabolism or plasma metabolite concentrations on food intake. Specific investigations with the aforementioned objectives have not been applied to any of the 700 or so species of elasmobranchs. The last comprehensive investigation of physiological control of appetite in fish was undertaken by Fletcher (1982) on the teleost, *Limanda limanda*.

There is no information currently available on the role of physiological factors (other than chemosensory stimuli) in the appetite regulation of sharks. This is surprising considering the scientific interest that has surrounded studies that have identified what different shark species actually feed on in the wild (for review see Wetherbee *et al*, 1990). Investigations of that type point to the different feeding strategies that may be operating to determine the feeding habits of that particular species of shark (for review see Bres, 1993), but seemingly do so without reference to the basic biological characteristics of the animal. Until researchers begin to address the physiological basis for the establishment of those feeding strategies it will not be possible to fully appreciate the trophic interactions of the shark with other species in the aquatic environment. Therefore, the assessment of some physiological factors regulating appetite will provide information regarding the mechanisms operating to control bouts of feeding in sharks.

Some authors have appraised anecdotal information from the field and suggested that sharks always have a high propensity to feed (*e.g.* Springer, 1967). From the studies that exist on hunger and appetite in teleosts it is open to speculation whether such claims can be substantiated. It is not known how the processes of appetite regulation might differ in sharks, so it is not appropriate to suggest how potential differences could be manifested as alternative feeding strategies. The comprehensive field sampling of lesser spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758) (plate 1), shark stomachs suggest

Plate 1. The lesser spotted dogfish shark, *Scyliorhinus canicula* (Linnaeus, 1758) pictured in its natural habitat off the Mewstone Ledges, Plymouth. (Photo courtesy of D. Burton)



interesting connotations for the study of appetite in this particular species. Lyle's (1983) investigation showed that only 1% of over two thousand *S. canicula* stomachs were empty of food items, whereas the mean value from 15 other shark species showed over 40% of sampled stomachs were empty (Wetherbee *et al*, 1990). This evidence could indicate that *S. canicula* may have evolved appetite control mechanisms that enable the maintenance of high levels of food consumption. On the basis of such field observations, knowledge of the physiological factors involved in appetite regulation in *S. canicula* would be of interest. In addition, much supportive literature is available regarding the main physiological systems of *S. canicula* (Shuttleworth, 1988), which is probably as a consequence of its relative abundance in the shallow waters of the northeast Atlantic, small size and its ability to adapt favourably to captivity (Wheeler, 1978; Compagno, 1984b). Additionally, *S. canicula* belongs to the shark family (Scyliorhinidae) within the order (Carcharhiniformes), each of which contains the most member species compared with the other families and seven other orders of shark respectively (Compagno, 1984). These characteristics make it a useful model for general concepts in elasmobranch physiology, and so information on the regulation of appetite in this species could be applicable to some other sharks.

1.1.2.4 Objectives and aims of the present study

The primary objective of the current study was to provide information on the physiological factors that contribute to appetite regulation in the lesser spotted dogfish shark, *S. canicula* over the size range 2g-1kg.

The specific aim of the research was to evaluate the effect of the physiological processes associated with digestive and systemic function on the food intake patterns and

appetite revival of *S. canicula*.

The specific objectives of the study were:

1. To investigate the existence of daily food intake patterns in dogfish that may suggest a basis for the endogenous regulation of appetite.
2. To quantify the rates of appetite return in juvenile and adult *S. canicula*.
3. To examine the importance of gastrointestinal emptying on the revival of appetite.
4. To assess what effect diet quality may have on gastric evacuation and food intake patterns.
5. To evaluate the influence of post-prandial metabolism on subsequent food consumption at varying levels of food intake.
6. And finally, a preliminary study into the role of plasma metabolites as possible systemic signals of metabolic satiety.

1.2 GENERAL MATERIALS AND METHODS

1.2.1 Experimental Fish

The group of juvenile dogfish, *Scyliorhinus canicula* (Linnaeus, 1758) used in the experiments of chapter II were purchased from Weymouth Sea Life Centre, Lodmoor Country Park, Dorset. The fish had hatched from eggs laid in the Centre's aquaria by captive females and were kept at a seawater temperature of approximately 15°C. The dogfish were transferred to a 300L seawater tank (90cm diam. x 55cm deep) at the University of Plymouth which received cooled, recirculated water of 34°/$_{oo}$ and 15.0 ± 0.5°C. This tank was one of two of 300L capacity for holding fish in a purpose-built 1200L seawater aquarium, devoted entirely to dogfish feeding and metabolism experiments. On arrival at the University of Plymouth facilities the mean weight of the juvenile dogfish group was 9.38g ± 0.63 S.E. (n=13, approximately 4 months post-hatching). Throughout the studies the dogfish were all weighed in vessels containing seawater on a top-pan balance, the juveniles on a Mettler BB2440 balance and the adults on a Precisa 6000D.

The aquaria were maintained on a 12 h light (L): 12 h dark (D) regime, the light phase illumination at the water surface being 480 lux. Daily measurements of salinity and temperature, and weekly measurements of seawater nitrite concentration were also taken when experimental fish were in the University of Plymouth facilities. When nitrite levels increased a seawater change was facilitated to restore normal levels, however imbalances in water quality of this type were not a common feature of the aquaria.

The seven hatchling *S. canicula* of weight range 2.76-10.61g (the largest was no more than a few months post-hatching) used in chapter V experiments hatched asynchronously from eggs laid in the aquarium of the laboratory of the Marine Biological

Association (MBA), Plymouth. The water temperature at the MBA aquaria was above the set experimental temperature of 15°C, so for eight weeks prior to the commencement of the investigations the dogfish were acclimated in a 300L tank of the 1200L aquarium at the University. The light regime at the MBA laboratory was approximately 12h L:12h D and this was continued at the experimental facilities.

All adult dogfish used in this study were caught in short hauls of an otter trawl from the MBA ship, *RV Sepia* over grounds in the approaches to Plymouth Sound. The dogfish, usually between 500-1000g were recovered in large outside tanks at the MBA laboratory. The dogfish were left in these outside tanks for at least one month before being moved to the experimental tanks.

The adult dogfish utilised for chapter II and in part chapter IV investigations, were re-located from the outside tanks of the MBA to two indoor tanks at the laboratory, each of approximately 300L capacity. The seawater flowing through the tanks was recirculated via a large underground reservoir, the temperature of which was relatively stable at 14-16°C. The fish were also maintained on a 12h L: 12h D light cycle.

After transfer from the MBA laboratory, adult dogfish used in chapter III, IV, V, VI studies were kept in two tanks (1 x 1.5 x 1m) of a 12,000L recirculating water aquarium in the basement at the University of Plymouth. The fish were used in experiments when the four week acclimation and quarantine period was at an end. The seawater in this system was maintained at 14°C throughout the year with weekly checks on salinity, pH, ammonia, nitrite and nitrate. From this University-based stock of adult dogfish several individuals were periodically relocated in one of the tanks of the aforementioned 1200L recirculating system. After one month of acclimation these *S. canicula* were used in the experiments of chapter V.

1.2.2 Maintenance rations and satiation feeding

During acclimation to the surroundings of the experimental aquaria at the University and MBA laboratories, and when between actual experiments, the dogfish were fed on maintenance rations. The meals consisted of chopped gadoid fillets and *Loligo* spp. (Cephalopoda: Loliginidae) muscle and tentacle tissue mixed together for adult dogfish, but solely chopped squid was given to the juvenile fish. The squid flesh was obtained from the Barbican Fish market, Plymouth and subsequently cut into approximately equal sized pieces according to the minimum mouth gape of each of the two size groups of dogfish. The squid was stored frozen at -25°C and thawed for feeding under cold running tapwater. A manufactured pellet (Ewos Ltd., Scotland) was given to juvenile dogfish along with the normal ration of squid for a period of a few days, but the practice was abandoned when observation indicated that consumption of the pellet was suboptimal. All fish were usually fed between 0.5 and 1.0% of their wet body weight (wbw) every second day (juvenile fish), and every third day (adult fish). In the week directly preceding the starvation periods of the experiments both adult and juvenile dogfish were fed to satiation every second (juv.) or third (adult) day. In the large outdoor tanks of the MBA laboratory adult dogfish were fed solely on chopped gadoid fish to satiation.

For experimental purposes it was necessary for dogfish to consume a maximum amount of food at any particular discrete time. This type of feeding was termed satiation feeding and was achieved for all fish, whether singular or in groups for all experiments of this study in the following manner. The experimental fish were fed preweighed amounts of chopped squid or manufactured moist pellet (*cf.* chapter III) liberally until the dogfish's activity was observed to slow down. Close observation was always made during the feeding period to ensure the food was actually ingested by the fish or available to the fish and not tail-flicked into the tank's wastepipe. When the feeding activity stopped the

remaining food was removed and weighed, and fresh squid of known weight was again presented to the dogfish. When there had been no further activity for 15 minutes the food was removed and the fish assumed to be satiated. This point of satiation time was regarded as the feeding time zero and all further measurements over the time course of an experiment were graduated from this reference point. For some investigations re-feeding to satiation was undertaken by feeding dogfish in the same way, though only after the predetermined time had elapsed.

CHAPTER TWO

DAILY FEEDING LEVEL AND APPETITE RETURN

2.1 INTRODUCTION

The daily level of feeding in sharks has not been well studied (Wetherbee *et al.*, 1990). Many investigations throughout the world have catalogued the stomach contents of various species of wild caught sharks, as well as their dietary preferences during the changing seasons and through their development from juvenile to adult (*Squalus acanthias, Scyliorhinus canicula, Scyliorhinus stellaris, Mustelus vulgaris, Galeus canis*, Ford, 1921; *Scyliorhinus canicula*, Eales, 1949; *Heterodontus portusjacksoni*, M°Laughlin and O'Gower, 1971; *Prionace glauca*, Stevens, 1976; *Squalus acanthias*, Jones and Geen, 1977; *Scyliorhinus canicula*, Lyle, 1983; *Carcharhinus leucas*, Snelson *et al.*, 1984; *Carcharhinus plumbeus*, Medved *et al.*, 1985; *Negaprion brevirostris*, Schmidt, 1986; *Sphyrna lewini, Sphyrna zygaena, Mustelus lunulatus*, Galvan-Magana *et al.*, 1989; *N. brevirostris*, Cortes and Gruber, 1990). Few studies however, have addressed the physiological implications of feeding with a view to attempt to quantify the daily ration of sharks. A dichotomy in purpose of the investigations that have been completed is apparent. The majority of the work has centred on the need to estimate the diel food consumption of sharks in order to assess the extent of predation on commercial stocks of teleost fish, their role in the ecosystem or for comparative studies with other fish groups (*S. acanthias*, Jones and Geen, 1977; *S. acanthias*, Brett and Blackburn, 1978; *Isurus oxyrinchus*, Stillwell and Kohler, 1982; *C. plumbeus*, Medved *et al.*, 1988; *N. brevirostris*, Cortes and Gruber, 1990). Most of the more accurate methods used to calculate ration size require controlled laboratory conditions in order to obtain measurements of some of the parameters (Elliott and Persson,

1978). Information on the rate of gastric digestion and subsequent evacuation, the level of routine and active metabolic rate as well as the stomach contents of wild caught sharks at various times during the 24 hour cycle are all necessary for accurate daily ration estimates. Due to the large size of most sharks that are thought to be of greatest importance with regard to commercial fish consumption, it is not surprising that there is a paucity of comprehensive feeding studies in this area. Clearly, calculating the amount of food that a predator ingests in the wild is useful in an ecological context, for the production of fishery management models to predict energy flow between different trophic levels. But investigations of this type are of limited use when trying to ascertain the role of the shark's physiological status in regulating appetite, and thus further feeding bouts. Indeed, because of an abundance of field studies on feeding and not enough basal laboratory studies considering appetite and feeding, appetite was not thought to play a role in the control of feeding in sharks (Springer, 1967). Since then, other scientists working with sharks have acknowledged the probable existence of appetitive feeding regulation mechanisms (Wetherbee *et al.*, 1990) because of the work that has been completed on teleostean fish.

However, laboratory measurements of daily food intake level can give evidence of the existence of feeding regulation mechanisms (Rozin and Mayer, 1961). In two separate, controlled studies on lemon sharks, *N. brevirostris*, cyclical patterns in food intake over longer periods (about 3 months) were evident from the day-to-day levels of food consumption (Graeber, 1974; Longval *et al.*, 1982). Some factor (or factors) controlling appetite was postulated to account for the feeding patterns in these studies other than temperature, light:dark cycle or effect of fish size (Graeber, 1974). The differences in daily food intake that lead to the variations in consumption observed in the latter investigations suggest different magnitudes of appetite at the same times each day.

Therefore, when contemplating the control of appetite in relation to the fish's physiological processes, it does not suffice to measure the quantity of food consumed without reference to the time of last feeding. Many investigations regarding teleost fish have documented that appetite increases with increased food deprivation time (*e.g.* Gwyther and Grove, 1981; Russell and Wootton, 1992, 1993). It has also been shown that in addition to appetite increasing after progressive periods of starvation, the actual rate of feeding is also increased (Godin, 1981). In teleost fish studies the greatest increase in appetite and rate of feeding has been shown to coincide with the fish's natural feeding periodicity (Elliott, 1975; Grove *et al.*, 1978; Kadri *et al.*, 1991). Therefore the temporal aspect of feeding relating to an instantaneous magnitude of fish appetite, that is, what can be described as the appetite revival or return, may be synchronised with physiological processes concerned with food digestion and processing and may ultimately control short-term feeding.

The artificial culture of teleost fish has accelerated the need to understand the physiological energetics of appetite, digestion and growth in captive situations (Jobling, 1993). This industry has fuelled studies on the importance of appetite revival in successful feeding regimes for promoting optimal growth in producing marketable fish. Consequently, the role of physiological parameters in controlling appetite have also been examined in this context (Vahl, 1979; Grove *et al.*, 1985). By quantitative estimates of appetite return under controlled conditions it is possible to assess the role that certain physiological factors may have in regulating further feeding by evaluating their change in relation to appetite return. There are no previous studies that have attempted to quantify the appetite return of elasmobranch fish in order to investigate appetite regulation, with a view to explain any possible feeding strategies operating in sharks. Some early investigations with teleost fish showed the effect of fish size on the level of feeding and appetite return (Grove *et al.*, 1978; Jacob and Balakrishnan, 1981), though no efforts in this area have

been directed towards elasmobranchs. The purpose of this chapter is therefore to characterize the nature of maximum daily food consumption that may imply feeding regulation, and to determine the rates of appetite return for both juvenile and adult dogfish, *Scyliorhinus canicula* implicit to the process of feeding. The aim of this section in the wider context of the work as a whole is to use the appetite measurements to facilitate comparisons with post-prandial physiological processes that will be examined in detail in subsequent chapters of this thesis. When the influence of such processes on appetite revival are evaluated, then possible mechanisms of regulation of appetite and thus further feeding bouts can be suggested.

2.2 MATERIALS AND METHODS

2.2.1 Daily Food Consumption

A group of thirteen juvenile dogfish (trial period mean weight 12.18 g ± 0.53 S.E.) and two groups of four adult *S. canicula* (mean weight 883.3 g ± 62.2 S.E.) were each kept and fed together for a period of 4 weeks prior to the commencement of the experiments. All the experimental fish were starved before the feeding trials were initiated, juveniles for 7 days and adult *S. canicula* for 14 days, so that any residual food from previous meals could be fully evacuated.

On the first day of the feeding trial and at the same time each day thereafter, all fish were fed to satiation on chopped squid. The daily feeding level of juvenile dogfish was monitored for 6 days in three trials and for 8 days in two further trials. The between trial interval was 7 days and the experiments with the juvenile fish were completed over a period of 93 days. The dogfish were individually weighed at the start of the experiment and every second week during the experimental period. The two groups of adult dogfish were fed chopped squid over a period of 15 days. Satiation feeding took place every day except days four, five and twelve to observe initially if the subsequent level of food consumption was increased. The 15 day feeding trials on each adult group were carried out simultaneously.

2.2.2 Statistical Analysis of Feeding Level

Two-tailed Student's t-tests were used to determine any significant diel variations in the feeding level of juvenile dogfish by comparison of the grouped daily observations of food consumption from each trial. One group of food intake observations was compared for significant differences with the group from the preceding day.

The cumulative mean food consumption for juvenile and adult *S. canicula* over the entire feeding trials were calculated and linear models were fitted to these data.

2.2.3 Rates of Appetite Return (AR): Groups

The group of juvenile dogfish (mean wt. 12.92 g ± 0.95 S.E.) and the two groups of adult dogfish (mean wt. 876.79 g ± 34.23 S.E.) were deprived of food for the same period as before and at the start of the experiment were fed chopped squid to satiation. The fish were then not fed until a predetermined time had elapsed. The re-feeding times were selected to occur at intervals of 6-12 hours in order to provide a wide time spread of food intake determinations. At these intervals the fish were re-fed to satiation on chopped squid. When a re-feeding satiation level was recorded the groups of fish were subsequently starved for the usual period in readiness for the next appetite return (AR) determination.

The rate of AR for juvenile dogfish was determined over a period of two months, spaced between the trial periods of the daily food consumption measurements. The dogfish were routinely weighed every two weeks during the trial period. All feeding trials were conducted at 15°C.

2.2.4 Measurements of AR: Individuals

Individual juvenile and adult dogfish previously fed to satiation in the groups stated above were randomly selected from the feeding groups and were isolated in seawater aquaria. They were then deprived of food for 168 h (juveniles) and 240 h (adults) before being re-fed to satiation. Each estimate of appetite was carried out three times at each dogfish size with different fish being used for each determination.

2.2.5 Statistical Analysis and Modelling of Appetite Return

Both linear and sigmoidal models were fitted to the AR observations for juvenile and adult dogfish. A logistic (sigmoidal) function of the form:

$$Y = 1/(a + b\, e^{-kx})$$

was used for modelling the AR data where a, b and k are constants. This type of equation describes a curve with an initial lag phase, a period of maximum rate and a phase where the curve approaches a limit or asymptote equivalent to Y= 1/a. When t=0 then Y=1/a+b (Gilbert, 1989). The rate differences of the standard linear models of juvenile and adult AR were compared by analysis of covariance (ANCOVA) from multiple linear regressions. Sigmoidal models for both dogfish sizes were fitted from the observed data and statistically compared by nonlinear regression analysis of covariance using the Marquardt algorithm (Marquardt, 1963) of StatGraphics Version 6.0 and validated with the Newtonian algorithm of Maximum Likelihood Program (MLP).

2.3 RESULTS

2.3.1 Daily Food Consumption

The results clearly show that after a satiation meal (juveniles 7.44 ± 0.76 % wbw; adults 6.39 % wbw) subsequent diel food consumption was reduced but maintained at a relatively constant lower level. Figures 1 and 2 show the variations in the individual dogfish food consumption rate over the study periods. The voluntary food intake of juvenile dogfish (figure 1) decreased from 7.44 % wbw to approximately 4.5 % wbw after 24 hours ($P<0.05$), and remained at about this level until day 5 of the investigation. After 96 hours, consumption decreased further to about 3 % wbw ($P<0.05$) before rising back to the 4.5 % wbw level on days 7 and 8. The measurements on these days were however, the product of two determinations and not five as was the case for the estimations on the previous six days of the trial. Figure 2 indicates that adult dogfish showed similar trends to the juveniles in food intake pattern after a satiation meal. The consumption of the two groups of adults decreased rapidly after the first day and then oscillated at about 1 % wbw for a further 15 days. The magnitude and direction of the feeding oscillations was generally similar for both groups of adult *S. canicula*. The difference in food intake between adult and juvenile dogfish was evident from the results in table I. The absolute amount of food consumed by adult dogfish each day was approximately 16.5 times that of the juveniles (even though the adults were 73 times larger). In relative terms however, the juvenile fish consumed over four times as much per day as the adult fish (table I). The variability in food intake over the study period was more marked in juvenile dogfish than in the adults (table I), although this was probably due to their small size leading to proportionately larger relative discrepancies in the actual meal size consumed. The cumulative mean food intake of adult and juvenile dogfish over the whole study periods

Figure 1. Daily food consumption of 13 juvenile *S. canicula* in five trials lasting 7 days. Bars represent ± 1 S.E. (n=5). Asterisks above bars indicate significant difference in level of food consumption from the previous day ($P<0.05$). Arrowheads on the abscissa denote the days where the mean is derived from only two group consumption determinations.

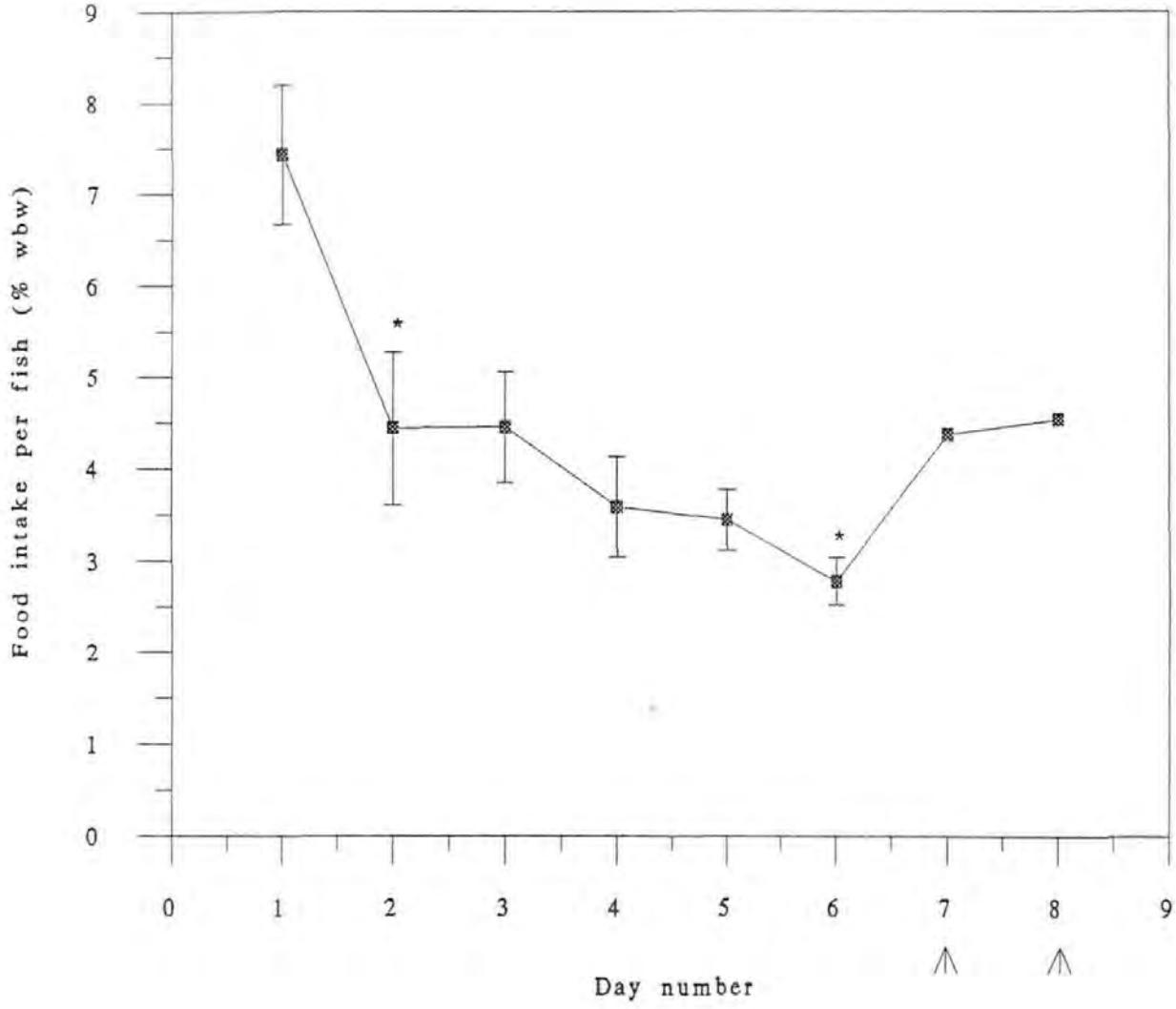

Food intake per fish (% wbw)
0
1
2
3
4
5
6
7
8
9
*
*
0
1
2
3
4
5
6
7
8
9
Day number

Figure 2. Daily food consumption of two groups of four adult *S. canicula* over a period of 15 days. Bar on the abscissa denotes the starvation time before re-satiation.

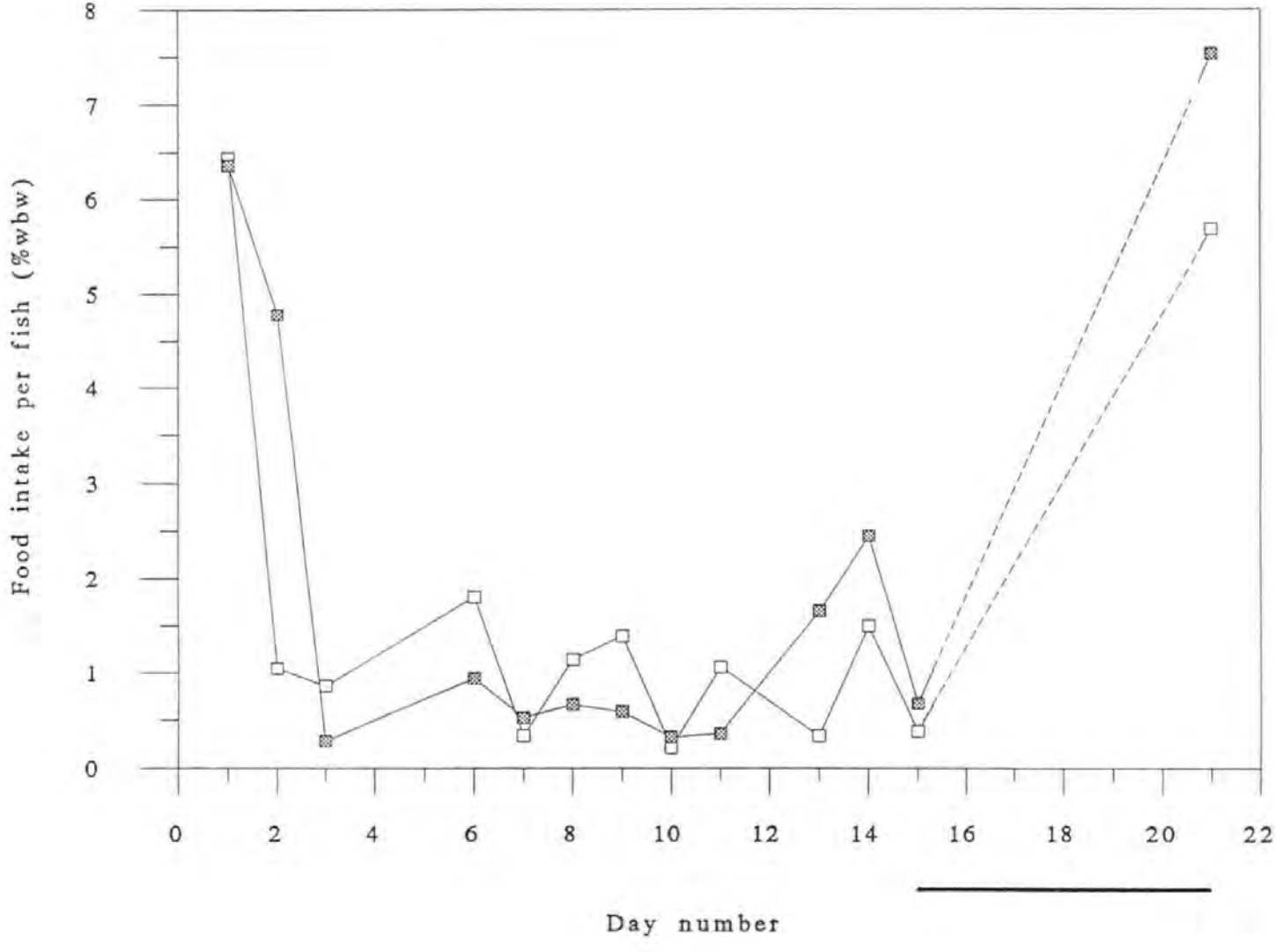

Food intake per fish (%wbw)
0
1
2
3
4
5
6
7
8
0
2
4
6
8
10
12
14
16
18
20
22
Day number

Table 1. Comparative rates of food consumption of juvenile and adult *S. canicula* over the periods of the daily feeding trials.

	Mean Individual Food Consumption ($g.fish.day^{-1}$)	Coefficient of Variation of Diel Food Consumption[a] (% of mean wt.)	Relative Consumption Rate (g.10g wet $fish.day^{-1}$)
Juveniles	0.47 ± 0.03	1.437	0.38 ± 0.03
Adults	7.76 ± 1.15	0.602	0.09 ± 0.01

[a] *calculated from (standard deviation/ mean) x 100*

(figure 3) shows the very strong linear relationship for the total amount of food consumed. The regression coefficient (r^2) was 0.97 for adults and 1.00 for juvenile dogfish. Additionally, figure 2 demonstrates that a starvation period of 144 hours after day 15 of the investigation increased the food consumption of the two groups of adult dogfish from about 1 % wbw preceding starvation to about 5.5 % and 7.5 % wbw, values close to the initial satiation meal taken by the fish at the beginning of the investigation.

2.3.2 Appetite Return Measurements

In order to quantify and compare the rates of AR, a best fitting model of the observed appetite revival was needed. Figures 4 and 5 show rates of AR fitted by linear and nonlinear logistic (sigmoidal) models for juvenile and adult dogfish respectively. Both models were shown to be good representations of the data with regression coefficients (r^2) above 0.83 (figures 4 and 5) for both sizes of fish. Statistical comparison of the sigmoid models of adult and juvenile dogfish AR by ANCOVA was not possible however. Use of the Marquardt algorithm, which fits the estimated and calculated linear and non-linear coefficients of the sigmoid model (a, b and k) simultaneously, showed large inaccuracies in the determination of the actual equation coefficients. In order to compare the shapes of the juvenile and adult dogfish sigmoid models an ANCOVA was attempted in which a six parameter (coefficients) additive model (one for juvenile data, one for adult data) was fitted to the grouped AR data. This model works in six dimensional space to calculate a mathematical relationship for the juvenile and adult food intake data points in the group. Even though there were 22 AR determinations for juvenile dogfish and 24 for the adults, the second set of three fitted model coefficients (a, b and k) was not accurate. The calculated coefficients of the six parameter sigmoid model for adult and juvenile dogfish did not correlate with the individual sigmoid model coefficients already calculated from

Figure 3. The cumulative mean percentage food intake of juvenile (■) and adult (□) *S. canicula* over the trial periods. Regression equations; juvenile, % wbw = 4.21 + 3.81d, r^2 = 1.00; adult, % wbw = 7.12 + 0.75d, r^2 = 0.97.

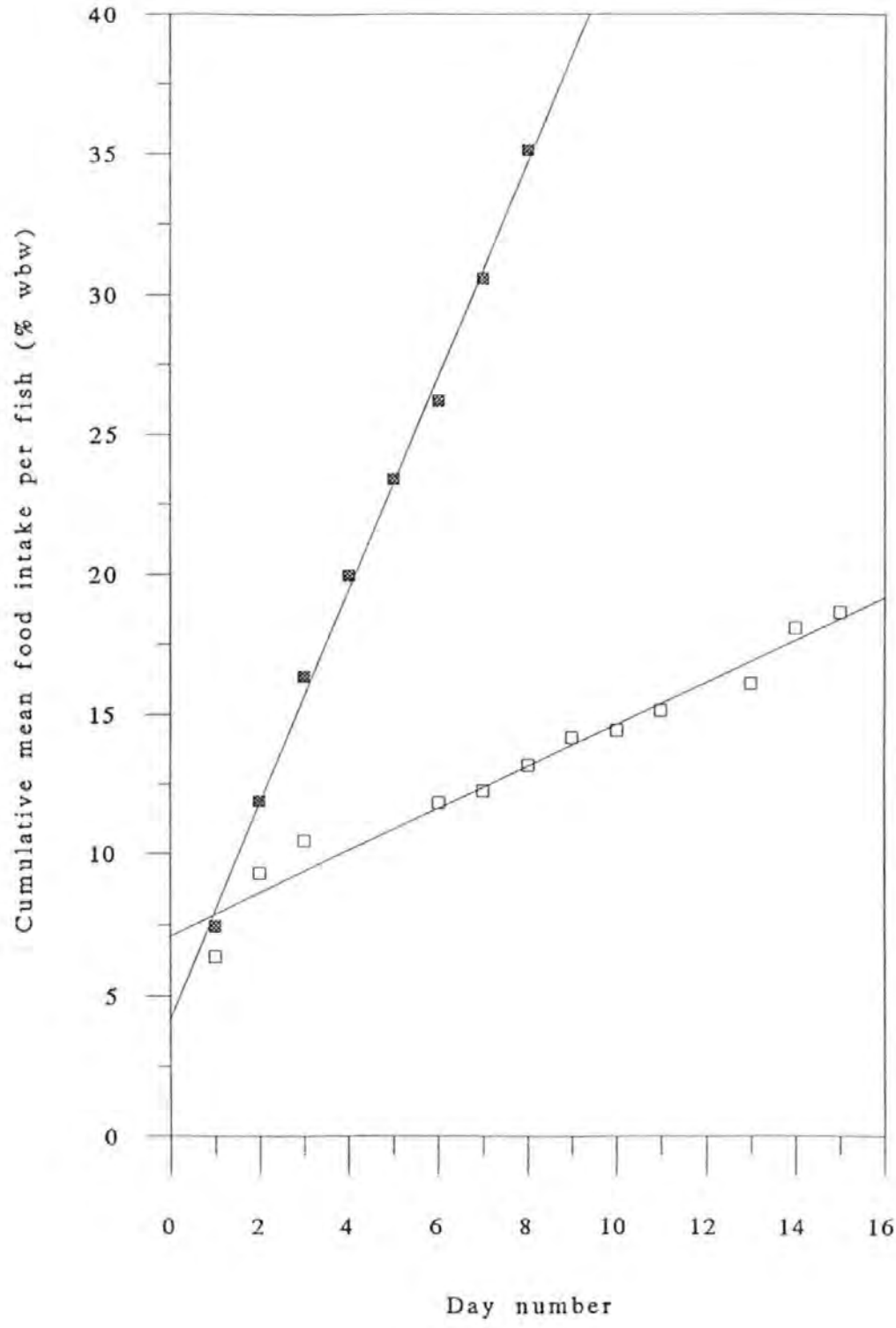

Cumulative mean food intake per fish (% wbw)
40
35
30
25
20
15
10
5
0
0
2
4
6
8
10
12
14
16
Day number

Figure 4. Appetite return of a group of 13 juvenile *S. canicula* fitted with linear (solid line) and sigmoid (dotted line) models. Fitted regression equations; linear, AR = 12.08 + 0.93t, $r^2 = 0.83$; sigmoid, AR = $1/ (0.009 + 0.072\ e^{-0.048\ t})$, $r^2 = 0.85$.

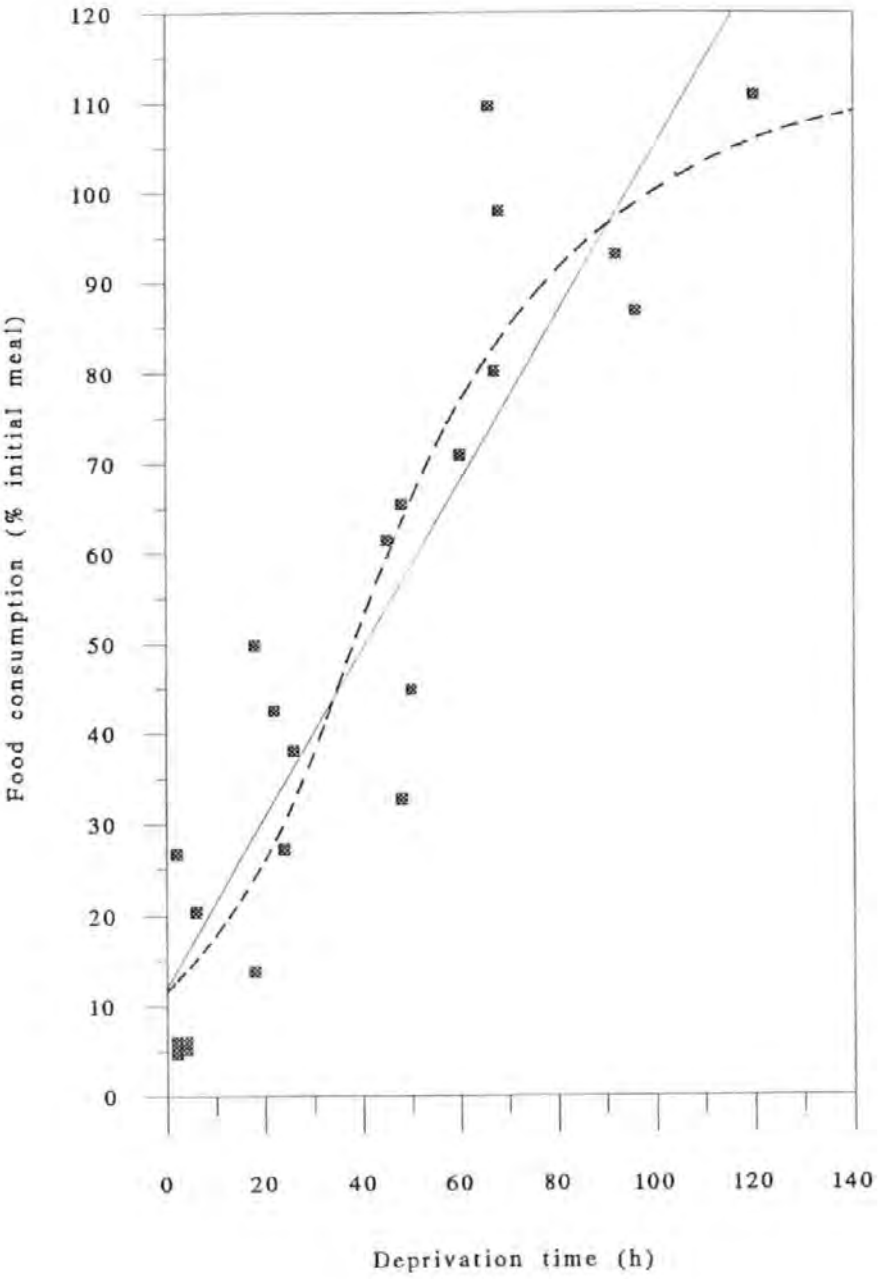
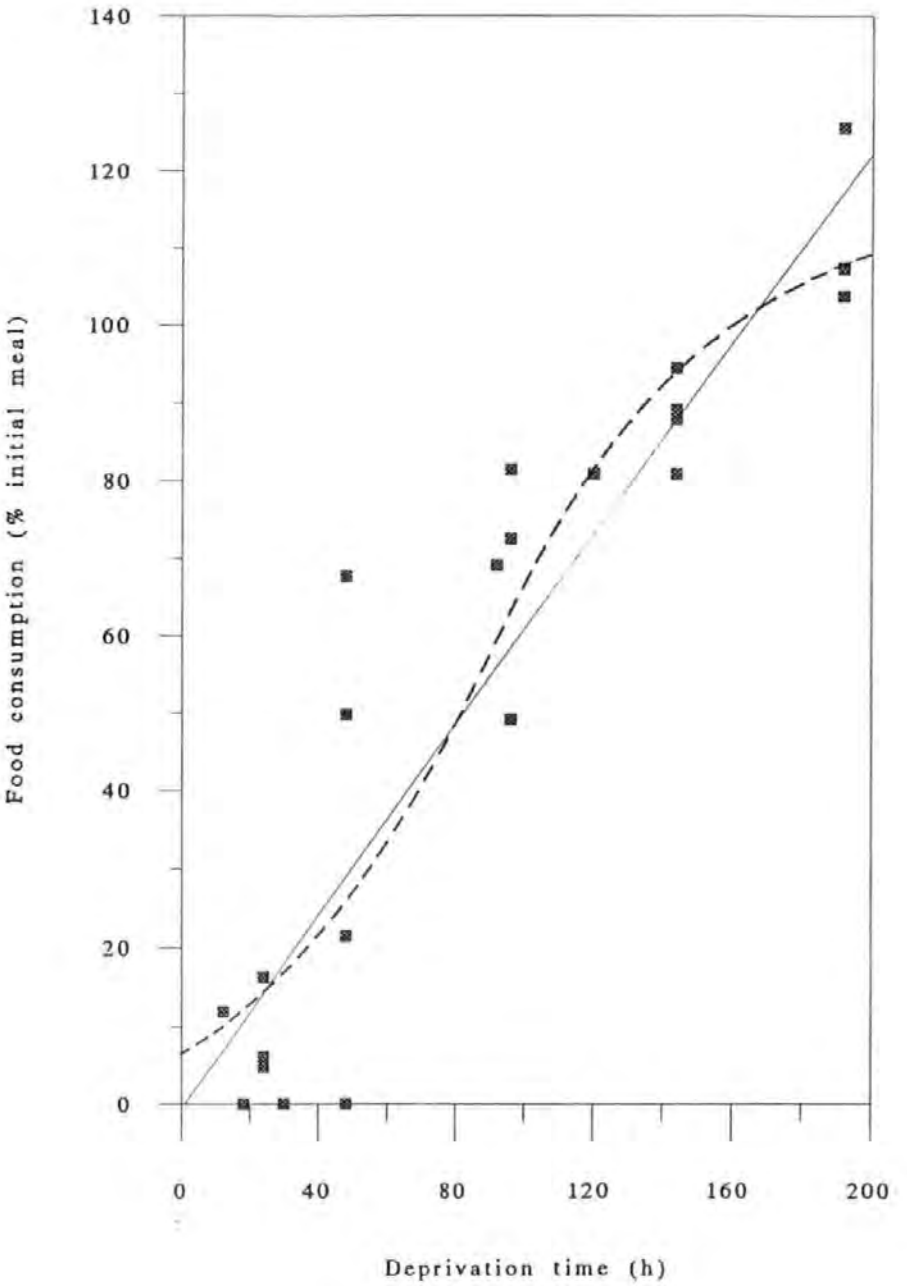

Food consumption (% initial meal)
120
110
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Deprivation time (h)

Figure 5. Appetite return of two groups of four adult *S. canicula* fitted with linear (solid line) and sigmoid (dotted line) models. Fitted regression equations; linear, AR = -0.53 + 0.61t, $r^2 = 0.87$; sigmoid, AR = $1/ (0.009 + 0.105\ e^{-0.028t})$, $r^2 = 0.87$, where t = time in hours.


140
120
100
80
60
40
20
0
Food consumption (% initial meal)
0
40
80
120
160
200
Deprivation time (h)

the individually fitted models (figures 4 and 5). This indicates that the data set was not adequate for statistical comparison of fitted sigmoid models. Replication of the ANCOVA test but using the more accurate Newtonian algorithm, capable of fitting linear and non-linear components independently was not successful. These results indicate that although it was possible to fit sigmoid curves to the data, the comparison of shape of curve by ANCOVA was not possible due to the spacing and number of the return food intake determinations.

Table 2 shows the rate of AR in juvenile fish was 1.52 (linear) and 1.67 (sigmoid) times greater than the adult AR. In addition, the time taken for a return meal of comparable size to the initial meal to be consumed again was quite long, approximately 165 hours in the adult dogfish compared to 95 hours in the juveniles. Absence of a delay in appetite return after meal consumption was noted and suggested that iniation of appetite recovery occurred quite rapidly after meal ingestion. The predicted AR values calculated for the actual time period over which AR measurements were taken in the current study, suggests that both sizes of fish will consume more food than that taken in the initial meal after 96 hours (juveniles) and 168 hours (adults) (table 3). Finally, figure 6 demonstrates that with the precise food consumption measurements obtained from individual dogfish shown against the predicted linear AR's obtained from grouped dogfish AR determinations, it is clear that the linear model may explain the AR of dogfish with some accuracy and indicate the level of a limit to AR.

Table 2. Instantaneous rates of AR from the fitted linear and sigmoid models and the predicted times for 100% initial meal consumption for juvenile and adult *S. canicula*.

	Slope/Shape of Fitted Model (h^{-1})		Predicted Time for 100% Initial Meal to be Consumed (h)	
	Linear	Sigmoid	Linear	Sigmoid
Juvenile	0.93[a]	-0.05[b]	94.54	96.71
Adult	0.61[a]	-0.03[b]	164.80	157.50

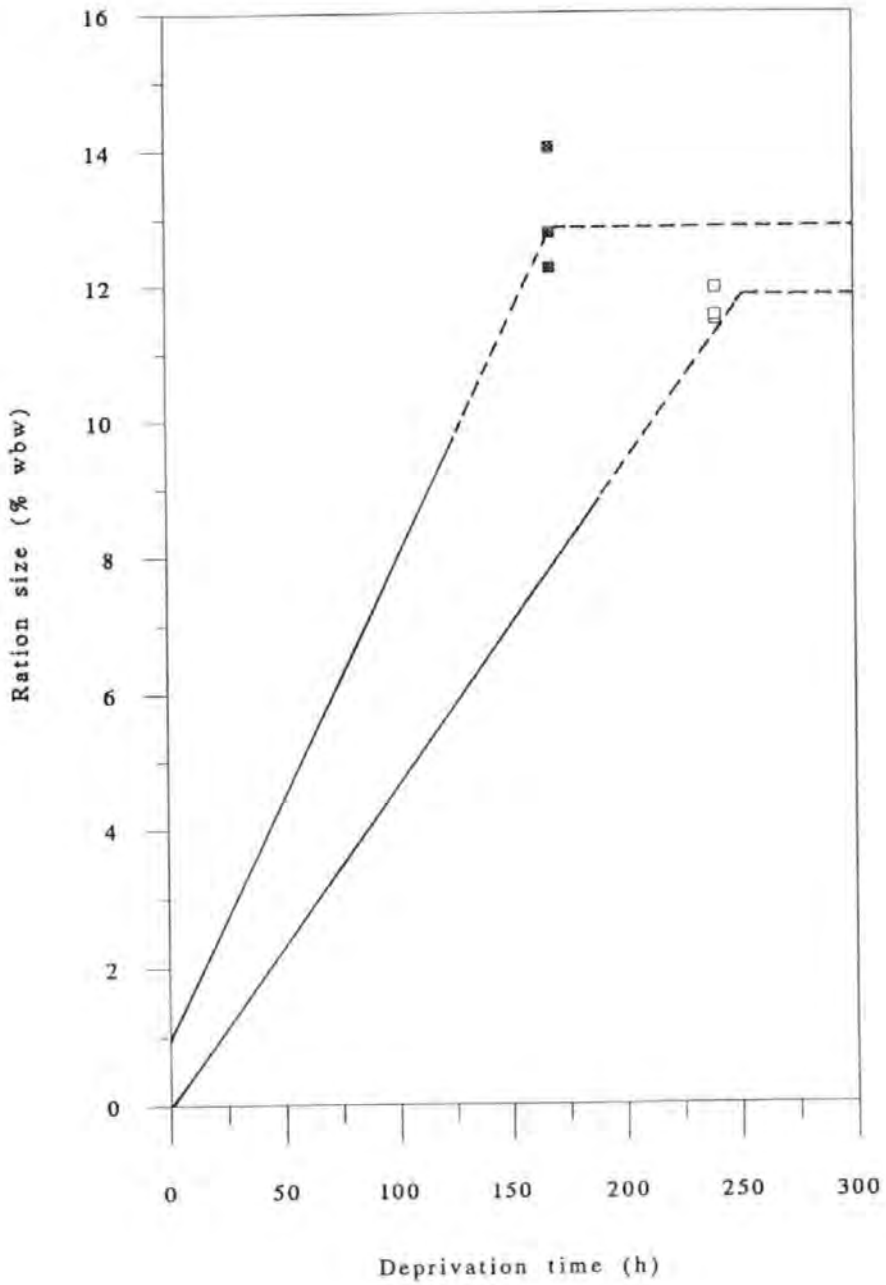
[a] *Significant difference between juvenile and adult AR, $P<0.001$*
[b] *Analysis of covariance inconclusive due to the scattered data points*

Table 3. Predicted AR using the linear model for both juvenile and adult *S. canicula* at daily intervals.

Time (h)	Predicted Appetite Return using Linear Models[a] (% initial meal)	
	Juveniles	Adults
1	13.01	0.08
24	34.40	14.11
48	56.72	28.75
72	79.04	43.39
96	101.36	58.03
120	123.68	72.67
144	*	87.31
168	*	101.95
192	*	116.59

[a] *Juvenile dogfish, $AR=12.08+0.93t$, $r^2=0.83$; Adults, $AR=-0.53+0.61t$, $r^2=0.87$*

Figure 6. Return of appetite estimations for grouped juvenile (uppermost line) and adult (lower line) *S. canicula* as represented by the linear models and extrapolated (-----) to include the individual dogfish AR determinations (juvenile ■; adult □) to an estimated limit.


16
14
12
10
8
6
4
2
0
Ration size (% wbw)
0
50
100
150
200
250
300
Deprivation time (h)

2.4 DISCUSSION

The internal drive or motivational feeding state known as hunger (Colgan, 1973; Tinbergen, 1989), ultimately brought about by the physiological mechanisms relating to appetite and feeding control (Brett, 1971), has been stated as being absent in sharks (Springer, 1967). The results of the investigations of this chapter immediately bring Springer's (1967) *ad hoc* suggestions in question. Generally, the present studies have demonstrated that the daily level of food consumption of dogfish oscillates about a relatively constant amount. In addition, the appetite of these scyliorhinid elasmobranchs recovers very rapidly within the first 24 h after meal ingestion with no observed delay in appetite initiation. The asymptote or limit to this consumption was variable (though consistent with their daily intake pattern) and perhaps therefore difficult to establish. These findings would therefore firstly suggest that appetite regulation is in operation in both juvenile and adult *S. canicula*. Secondly, that increasing deprivation time raises the level of food intake, and that the profile of appetite return might not be of the type usually associated with elasmobranch feeding strategy, that is, of a slow recovery of appetite after a large meal.

After a satiation meal the juvenile dogfishs' level of food consumption was relatively constant when offered food items at 23 hour intervals. Likewise, the food intake of adults oscillated, though remained fairly constant at a much lower level than the initial satiation meal. The daily ration of adult dogfish (1.1% wbw) is similar to that estimated from wild shark studies in water temperatures similar to that experienced by *S. canicula*, *e.g.* 1.3% wbw for *S. acanthias* (Jones and Geen, 1977) and 1.1% wbw for *C. plumbeus* (Medved *et al.*, 1988). Oscillations in food consumption about a relatively constant level have been demonstrated in sharks in both laboratory and field situations. In a careful study

on two groups of juvenile lemon sharks, *N. brevirostris* under controlled conditions, Graeber (1974) showed quite wide food intake oscillations for sharks over a three month period and postulated cyclical patterns in food intake. Similarly, another study of *N. brevirostris* noted variable levels in food ingestion (Longval *et al.*, 1982). The patterns in consumption of the latter investigation implied that these sharks may have four day cycles in food intake resulting, presumably, from variable daily feeding rates. Whilst the aim of the present study was not to elucidate any specific cycles in food intake, the variability about a constant level observed in daily food consumption of juvenile and adult dogfish does suggest some regulatory mechanisms controlling feeding level. Some field investigations on sharks have also shown little variability in stomach contents over a 24 hour period. Cortes (1987, cited in Wetherbee *et al.*, 1990) working on *N. brevirostris* found little diel variation in the mean ratio of dry weight of stomach contents to wet body weight of shark. Analysis of the sandbar shark, *C. plumbeus* stomach contents from the wild also gave no significant differences in gross stomach content over 24 hours (Medved *et al.*, 1985). The relatively constant stomach content of these sharks however, does not necessarily corroborate the laboratory observations in daily food consumption made during the present study. The stomach contents of sharks in the previous wild studies showed prey in various states of digestion and may indicate that these sharks feed asynchronously and not necessarily on a daily basis or in a manner similar to sharks under controlled conditions. Clearly though the *S. canicula* of the present study, when fed daily under controlled conditions were able to maintain their food consumption within quite narrow bounds. Smagula and Adelman (1982) suggested a self-regulated day-to-day food consumption for the teleost, *Micropterus salmoides* even though the intake levels were quite variable. Rozin and Mayer (1961, 1964) working on the goldfish, *Carassius auratus* showed that individual fish were able to maintain their intake about a certain level with

single daily feeds and proposed this was some evidence towards the notion that short-term food intake was regulated in some manner. The pink salmon, *Oncorhynchus gorbuscha* is not agastric as is *C. auratus*, but was able to maintain relatively constant feeding levels for 11 hours after the initial high feeding rates (to satiation) (Godin, 1981). This author thus suggested that after the salmon initially filled their stomachs, the stomach was kept full by the constant feeding rate balancing the rate of gastric evacuation.

The existence of short-term regulation of appetite from daily feeding level experiments can further be postulated for dogfish by comparison with the lemon shark studies of Graeber (1974). As well as monitoring daily food consumption these studies used duplicate tanks of lemon sharks which were fed simultaneously. Both groups of *N. brevirostris* exhibited similar patterns of food intake over the three month period of the trial. Graeber (1974) suggested that because of these similar, (though independent) oscillations in group food consumption, some common factors must be controlling appetitive behaviour in a similar way for all the sharks. The two groups of adult dogfish used in the present study also showed these simultaneous increases and decreases in food consumption, thus supporting the findings of the *N. brevirostris* study. The cumulative food intake responses of *S. canicula* in this study also suggest the existence of feeding regulation determined by the level (magnitude) of appetite as the rates of consumption were highly linear for the periods over which the measurements were taken. This indicates that a seemingly repeatable, self-regulated daily feeding level occurs for dogfish fed to satiation under controlled conditions, and it is therefore quite evident that some appetite regulation factors are operating in dogfish.

From the initial daily feeding studies with *S. canicula* it was clear that food deprivation time was important in determining the level of subsequent food consumption. Several other shark studies have shown that the level of voluntary food intake (appetite)

is dependent on deprivation time (Graeber, 1974; Longval *et al.*, 1982; Casey *et al.*, 1985). Casey *et al* noted for captive *C. plumbeus* that their peak in food consumption occurred after a few days of deprivation. From the subsequent timed re-feedings with progressive deprivation time accounting for the appetite return curve of the dogfish in this study, the rate of appetite revival after a known meal size was quite predictable. The appetite return was equally possible to model with both linear and sigmoid mathematical relationships. The sigmoid model however, did not withstand comparative statistical analysis and so cannot be considered or relied upon as a strong model to best represent the return of appetite of dogfish. Some teleost investigations have been able to successfully model appetite return using sigmoidal relationships. Appetite increased sigmoidally with deprivation time in the silurid catfish, *Heteropneustes fossilis* (Singh and Srivastava, 1985). These authors stated that the sigmoidal rate of appetite return, incorporating a lag phase soon after the satiation meal and an asymptotic phase as food consumption reaches a maximum, was regulated by gastric emptying. They maintained that food intake was solely dependent on the available space in the stomach and generally increased with time and the gastric evacuation process. Sigmoidal models of appetite return were also found to explain the appetitive processes operating after a satiation meal in the minnow, *Phoxinus phoxinus* (Russell and Wootton, 1993). Again the decline in gut contents after a meal was highly correlated with the return of minnow appetite. For the dogfish appetite return it seems most appropriate to use linear models as there was no obvious (or evident) initial lag or final asymptotic phase to appetite revival. The lag phase in the return of appetite is generally thought to represent the time for liquefaction of the meal in the stomach before the commencement of gastric emptying (Grove *et al.*, 1978), whilst the asymptote of the sigmoid curve is equal to the amount of food that produces maximum stomach fullness (1.73% wbw for *H. fossilis*, Singh and Srivastava, 1985; approx. 10%

wbw for the mako shark, *I. oxyrinchus*, Stillwell and Kohler, 1985). Linear models, however are logically inadequate for representing appetite return because the model implies that there is no limit to stomach (or foregut) capacity. This cannot be true for any fish and so the linear model must be regarded with some reserve in this respect.

The rates of appetite return shown for dogfish in this study indicates that they rapidly recover appetitive status after a satiation meal by having a minimal lag period in appetite return. Minimisation of this lag period may perhaps be achieved by moving undigested food rapidly from the stomach into the anterior intestine. Such intragastrointestinal movements may be a strategy of opportunistic predators, such as the dogfish (Lyle, 1983) and has been shown to be the maximal appetite return strategy of the sand dab, *Limanda limanda*, whose lag phase of appetite return is short due to transferance of undigested food from the stomach straight to the intestine (Grove *et al.*, 1985). This mechanism presumably facilitates faster initial stomach clearance of food and leads to a greater appetite much sooner after a satiation meal than might necessarily be expected. Similarly, dogfish may use this mechanism to rapidly recover appetite after a large meal, as would be strategically useful in times when prey was relatively abundant.

The absence of an asymptote in the appetite return of both juvenile and adult dogfish in this study may indicate that the size of a satiation meal shows extreme variability after any period of food deprivation, and perhaps especially so when predicting appetite revival from groups of fish. Elliott (1975) and Grove *et al* (1978) used individual brown trout, *Salmo trutta* and rainbow trout, *Salmo gairdneri* (*Oncorhynchus mykiss*) respectively, for studies on rates of appetite return and were able to accurately measure the maximum meal that could be ingested after varying deprivation times. Incorporation of appetite determinations from individual dogfish indicated higher levels of food consumption than for grouped dogfish and so might more clearly represent a true

asymptote to meal ingestion. Grouped adult dogfish that initially took a 7% wbw meal (to satiation) were then individually re-fed after 240 hours and consumed a 12% wbw meal. It is clear that interactions within the groups of *S. canicula* may indeed have been responsible for a lower level of satiation food consumption at the start of the experiments. Approximately 7% wbw was the satiation ration for both grouped juvenile and adult fish. The maximum individual food intake was about 11-14% wbw for both juvenile and adult dogfish compared to an estimated maximum ration (stomach capacity) of 10% wbw in *L.oxyrinchus* (Stillwell and Kohler, 1985). Fänge and Grove (1979) in their review of digestion processes in fishes stated that *L. limanda* has a stomach volume equivalent to 10% wbw, *C. auratus* up to 21% wbw whereas sculpins generally (Scorpaenidae and Cottidae: Scorpaeniformes) have been noted to ingest 30-50% wbw at a single feeding. In the present investigation 11-14% wbw was the highest ration size recorded when dogfish were individually fed to satiation and may represent an appetite return limit for dogfish in captivity.

For the determination of dogfish AR, linear models were considered the most appropriate and have been used before to best represent a correlate of appetite revival against time. Elliott (1975) used a linear model to best represent the relationship between cumulative weight of food consumed by *S. trutta* (a function of appetite) over time, even though there was some evidence of a sigmoidal correlation. Food consumption in the latter study commenced rapidly with little lag in the return of appetite and stopped abruptly at satiation within each feeding period. Therefore Elliott (1975) concluded that this relationship was sigmoidal but with very short "tails" at the beginning and end of the feeding period. According to Elliott (1975) the linear model was thus " a good compromise" to explain the consumption of food when the use of a sigmoidal curve was not entirely appropriate. For the present study on dogfish, the linear model can be

considered quite accurately as representing the appetite return of both juvenile and adult *S. canicula* if the level of maximum individual fish realimentation is regarded as a possible asymptote of appetite return. With variable levels of food intake it is clear that an asymptote would be difficult to establish and even then, not be entirely accurate.

Obviously, the purpose of these first investigations of daily feeding and appetite return was to establish if any regulatory mechanisms may be in evidence in dogfish and to quantify the appetite return of dogfish with a view to investigating the effect of certain physiological processes on the recovery of appetite. What can be said of dogfish appetite at this stage is that revival is rapid after satiation compared to the traces obtained from experiments on teleosts, even if the overall appetite return time is quite prolonged compared to other fish. It appears for dogfish that their appetite returns at a constant rate as the deprivation time increases. In addition it is clear that the general forms of daily feeding and appetite return are similar for both juvenile and adult *S. canicula*. This similarity in appetitive strategy points to common factors influencing appetite for both adults and juveniles. The juvenile dogfish consume more food relatively after a satiation meal than the adults, indicating faster rates of food processing rather than suggesting that appetite might be regulated through development in some quite different manner.

As some evidence for appetite regulation can be postulated for dogfish it is now necessary to ask what factors might be pivotal in controlling the ability of a fish to feed. Working on *S. gairdneri* (*O. mykiss*), Grove *et al* (1978) showed that appetite was related to stomach fullness and that the fishes feeding rhythms closely paralleled gastric emptying. Gwyther and Grove (1981) demonstrated for *L. limanda* that stomach fullness was the major factor in the control of appetite. In later studies on the turbot, *Scophthalmus maximus*, Grove *et al* (1985) noted that the return of appetite was dependent on the degree of stomach emptiness in this species. Other studies on teleostean fish appetite that have

already been mentioned (Brett, 1971; Colgan, 1973; Fletcher, 1982, 1984), suggested that other factors such as post-prandial metabolic rate, energy content of the diet and systemic factors all play a part in appetite regulation as well as stomach emptying. The appetite return of dogfish in this study was interesting in two distinct ways and provide avenues for further investigation. Firstly, the shark's lack of any appreciable lag phase in appetite return may be brought about by newly ingested food moving undigested into the intestine soon after the meal is ingested, thus facilitating the rapid initial revival rates observed in both juvenile and adult dogfish. This suggests that the stomach may be of prime importance in the short-term regulation of appetite. In addition, although the initial recovery of appetite is quite rapid (*i.e.* no observable lag phase), the time of appetite return as a whole is long in comparison to the teleost studies. It has been well documented in the literature that elasmobranch gastric evacuation is quite slow compared to teleostean fish (Jones and Geen, 1977; Medved, 1985; Wetherbee *et al.*, 1990), so the overall period of appetite return might be closely linked with the long emptying time observed for the elasmobranch stomach. Thus, studies of dogfish gastric evacuation patterns may elucidate the importance of the stomach in the appetite regulation of elasmobranch fish.

CHAPTER THREE

GASTROINTESTINAL EVACUATION AND APPETITE

3.1 INTRODUCTION

The role of the stomach as a prime regulator of appetite has been the subject of a number of recent studies on a wide range of teleostean fish species (Brett, 1971; Grove *et al.*, 1978; Flowerdew and Grove, 1979; Grove and Crawford, 1980; Gwyther and Grove, 1981; Fletcher, 1982; Grove *et al.*, 1985; Singh and Srivastava, 1985; Russell and Wootton, 1992, 1993). Generally these investigations have all demonstrated that the return of fish appetite was dependent on the rate and time of decrease in stomach or foregut contents. In the stomach, ingested food is degraded by the combination of enzymatic action in an acidic environment and rhythmic contractions of smooth muscle in the stomach wall (Grove, 1986; Bromley, 1994). Digesta then passes from the stomach through the pyloric sphincter into the intestine where nutrient absorption takes place. The rate at which the digesta leaves the stomach constitutes a gastric emptying pattern. The physiological mechanisms of food emptying and how they might influence gastric emptying patterns are central to the understanding of how appetite may be physiologically regulated in fish (Jobling, 1986).

Stomach emptying rate and voluntary feeding level (appetite) have been termed as being analogous to input rate=output rate (Bromley, 1994). Gastric evacuation rate as a physiological factor governing appetite revival and regulation is only valid however, if the variables that may influence the rate are also considered. The time required and the rate at which fish empty their stomachs has been shown to depend on water temperature and the type of food consumed, which will in turn be affected by the size of the meal and the

size of fish that consumed the meal. In addition, the actual stomach emptying phase will be dependent on the degree of distension of the sac-like stomach, the secretory surface area of the stomach and the surface area of the meal (Grove, 1986). These parameters all serve to affect the nature in which the stomach undertakes two important functions; optimization of the ratio of nutrients: digestive juices entering the intestine; and the grinding of food into small particles ready for absorption and selectively retaining larger particles until sufficiently reduced (dos Santos, 1990). Variables such as these will affect the gastric emptying pattern. Investigations on stomachless fish however, have indicated that satiation and appetite were controlled even without the fish's possession of a true stomach (Grove and Crawford, 1980). Other studies that closely relate appetite return with gastric evacuation demonstrated that the stomachs of experimental teleost fish were nearly empty before voluntary feeding resumed (Steigenberger and Larkin, 1974; Grove *et al.*, 1978). Therefore, the assumption that the level of stomach fullness may solely regulate appetite should not be considered in isolation from the physiological influences of the post-absorptive factors resultant from entire gastrointestinal evacuation.

That stomach fullness is fundamental in modifying the potential for food intake in fish has been widely established (for reviews see Fänge and Grove, 1979; Fletcher, 1984), though to what extent the actual dynamics of gastric evacuation may influence appetite control, in conjunction with subsequent passage and absorption of food through other regions of the gut is not well understood. Gastrointestinal evacuation is mediated by autonomic neural (Andrews and Young, 1993) and hormonal control (Matty and Lone, 1985), including certain feedback mechanisms that have generally been inferred for fish from mammalian studies (Jobling, 1986; dos Santos, 1990). The basis for appetite regulation in fish may ultimately depend on the physiological mechanisms involved with food processing and subsequent physiological status (Grove, 1986). These will therefore

be dependent to a large degree on the factors that influence the rate of gastrointestinal evacuation (Bromley, 1987). The rate at which food leaves the stomach and passes through the gastrointestinal tract of fishes will ultimately determine the degree of absorption of nutrients by the gut (Bromley, 1994). The consequences of these post-absorptive metabolic processes have been postulated in some studies to play a major role in controlling subsequent food consumption (Brett, 1971; Beamish, 1972; Fletcher, 1982; Wetherbee *et al.*, 1987).

Notwithstanding the central role of the alimentary tract in the regulation of appetite revival, post-absorptive factors such as plasma metabolite level have been considered to be of variable importance (Brett, 1971; Fletcher, 1982). This is mainly due to whether the fish species were capable of adjusting food intake according to the nutrient density of the meal (Flowerdew and Grove, 1979; Gwyther and Grove, 1981; Grove *et al.*, 1985). The involvement in appetite control of various systemic factors, *e.g.* changes in levels of circulating glucose, fatty acid, glycerol or amino acids (Smith, 1989) has indicated that the gastrointestinal tract itself may be controlled from certain receptors, whether associated with the gut (Leek, 1972; Jobling, 1986; P.L.R. Andrews, pers. comm.) or other visceral organs (Smith, 1989), and thus be sensitive to changes in physiological nutritional status. The rate of appetite return therefore, may be modified or controlled by chemical factors relating to the rate of food processing and physiological status of the fish, which by direct or indirect action influences the neural and hormonal mediation implicit to the mechanical functioning of gut evacuation.

The actual factors controlling the physiological mechanisms of food emptying are incompletely known for fish (Jobling, 1986). Few studies have attempted to experimentally unite observations of the dynamics of stomach emptying with that of intestinal evacuation with a view to investigating the appetitive control processes that may

influence short-term feeding regulation. Recently, a number of authors have provided possible explanations regarding the involvement of the intestinal region of the gut in food intake control of fish, by drawing on the mammalian literature for comparison (Grove, 1986; Jobling, 1986; Bromley, 1987; dos Santos, 1990). Therefore, for studies on physiological control of appetite it is an oversight not to investigate some aspects of the function of the whole gut in relation to food consumption and appetite.

Smith (1989) noted in his review that the subject of gastric evacuation in fishes was discussed in more papers than any other single digestive function. Experiments that have profiled the decrease in stomach contents of fish over time have been primarily undertaken in order to attempt to quantify feeding rates of natural fish populations (Bromley, 1994). The purpose of assessing natural feeding rates of fish is to estimate their predation and feeding interactions. Food consumption models have been constructed from gastric evacuation studies in conjunction with quantitative observations on the feeding behaviour of fish in the wild (*e.g.* Thorpe, 1977; Elliott and Persson, 1978; Jobling, 1981b; Bromley, 1987). The driving force of such investigations has enabled the construction of trophic webs with an ultimate view to enhancement of fish stock management (Bromley, 1994). It has been within this general context that studies of gastric evacuation in elasmobranch fishes have been completed.

Gastric evacuation has been investigated in several species of elasmobranch (dogfish, presumed *S. acanthias*, Van Slyke and White, 1911; *S. acanthias*, Jones & Geen, 1977; *I. oxyrinchus*, Stillwell and Kohler, 1982; *C. plumbeus*, Medved, 1985; *N. brevirostris*, Cortes, 1987 cited in Wetherbee *et al.*, 1990; Wetherbee *et al.*, 1987; Schurdak and Gruber, 1989; *S. canicula*, Macpherson *et al.*, 1989; *N. brevirostris*, Wetherbee and Gruber, 1990; *Mustelus californicus*, San Filippo, 1993). All of these authors, except Van Slyke and White (1911) fitted an empirical mathematical model to

their observed gastric emptying data. Bromley (1994) reviewed the mathematical relationships that have been used to model gastric evacuation. Linear, exponential, surface dependent and growth models (namely Gompertz growth function, Winsor, 1932) have all been used to describe the gastric evacuation rate of different elasmobranch species (Medved, 1985; Cortes, 1987 cited in Wetherbee, 1990; Macpherson *et al.*, 1989; San Filippo, 1993). None of these studies however, have evaluated the role of gastric emptying patterns as a component part of metabolism integral to the physiological control of appetite.

Gastrointestinal evacuation rates have not been well researched in elasmobranch fish. Van Slyke and White (1911) working on dogfish, Wetherbee *et al* (1987) and Wetherbee and Gruber (1990) on *N. brevirostris* are the only investigations that have investigated the evacuation of food through the gut of sharks. Some unpublished studies have also investigated the gastrointestinal emptying rates of elasmobranch fish (*S. canicula*, D.J. Grove and co-workers, pers. comm.; *Raja clavata*, de Souza cited in Flowerdew and Grove, 1979). The purpose of the former authors' work was to improve the overall understanding of shark digestive physiology, with a view to probing the relationships between rate of food consumption, rate of digestion and rate of growth. Clearly, these aspects of elasmobranch physiology are of prime importance to our understanding of how appetite might be regulated, but these investigations were not designed to be sufficiently wide ranging to represent a synthesis of the possible gut related mechanisms influencing appetite control. Therefore, the objective of this part of the present study was to evaluate the role of gastrointestinal evacuation in the physiological regulation of appetite in the elasmobranch, *S. canicula*. In previous studies the rate of gut evacuation was found to change predictably with fish size (Flowerdew and Grove, 1979; Grove, 1986), so only adult and not juvenile dogfish were used for these sets of

experiments.

Discrete sampling of alimentary tract content has been the only method used in gastrointestinal evacuation rate trials, as continuous direct observation is not practicable (Bromley, 1994). However, continuous measurements of gastrointestinal evacuation rates in *Oncorhynchus mykiss* have recently been made using gamma scintigraphy (Aldman, 1994). X-radiography has been utilised for studying gut evacuation rates of fish since the early work of Molnár and Tölg (1962) and Edwards (1971). Before the early 1980's barium sulphate ($BaSO_4$) was used as a contrast medium for following the passage of food through the gut of fish (Edwards, 1973; Jobling *et al.*, 1977; Grove *et al.*, 1978; Ross and Jauncey, 1981). During the early 1980's Talbot and Higgins (1983) developed a quantitative X-radiographic method that tracked the progress of small, indigestible iron particles through the gut of fish. Although juvenile individuals of a single shark species have been X-radiographed in a previous study (*N. brevirostris*, Wetherbee *et al.*, 1987), a quantitative X-radiographic method for use in gastrointestinal evacuation experiments on elasmobranchs has not previously been described.

The specific aims of this part of the synthesis of physiological control of appetite in sharks were to quantify the gut evacuation rates of adult *S. canicula* using X-radiography and to validate the applicability of the method by serial samples of dogfish gastrointestinal tracts. Rigorous validation of the technique has been previously shown to be necessary as some species of fish show a tendency for selective retention of the marker particles (Grove, 1986; Jorgensen and Jobling, 1988; Jobling *et al.*, 1993). By quantification of the rates and times of gut evacuation in conjunction with simultaneous measurements of absorption efficiency and level of crude protein in the digesta, it was hoped to ascertain the relative importance of the gut emptying patterns in appetite return. This would then suggest how appetite might in part be regulated and indicate the existence

of any feeding strategies relating to appetite revival operating in sharks.

3.2 MATERIALS AND METHODS

3.2.1 Formulation of a Moist Pellet

A diet was formulated to incorporate indigestible dietary markers enabling X-radiographic measurement of gastric evacuation in fish. It was important that the components of this manufactured diet were kept as close to the energy profile of squid muscle as possible (chapter IV), because squid muscle was utilised in other investigations of this study and diet homogeniety was essential. A moist diet promoting palatability was of prime consideration to the diet formulation as the *S. canicula* of this study had already demonstrated they would not consume dry pellets (Ewos Ltd., Scotland) to satiation (chapter II). In addition it was important that the pellet sank as the dogfish fed almost entirely on the bottom of aquaria, presumably a persistence of their wild feeding behaviour. A ratio of 60% wet squid to 40% dry diet component was fixed upon. This formulation was based on 50% protein for the dry mass equivalent of the final diet. The dry component of the diet consisted of a white fishmeal (Provimi 66), vitamin premix, binder (Protanal) and cornstarch (Sigma Chemicals Ltd.) (Table 4).

Wet squid (%)	Overall proportion of dry ingredients (%)	Proportion of dry ingredients (%)	Type of ingredient
60	40		
	28.4	71.0	Fishmeal
	2.4	6.0	Vitamin premix
	1.2	3.0	Binder
	8.0	20.0	Cornstarch

Table 4. Diet formulation for the moist dogfish pellets used in the gastric evacuation studies.

The cornstarch was included in the dry component of the diet so that the energy profile of the diet could be altered if necessary, by 100% substitution of cornstarch with a pure marine oil source (cod liver oil).

3.2.2 Manufacture of the Moist Pellet

Fresh squid (*Loligo vulgaris*; Loliginidae) were obtained from the Barbican Fish Market, Plymouth during winter months and gutted, cleaned and chopped and then frozen down into hand-sized blocks. Each block of squid that was needed was further grated into small thin slivers using a standard cheese grater and these slivers were collected, weighed and left to stand whilst the dry ingredients were weighed out. The amount of each dry ingredient weighed was in proportion (3:2) to the amount of squid grated. The dry components were placed in the bowl of the food processor (Hobart A120) and mixed for approximately 30 minutes. The indigestible dietary markers, glass beads of diameter 0.40-0.52mm (Ballotini beads, Jencons, U.K.) were incorporated into the mixed dry ingredients at this stage. The beads were scattered uniformly over the surface of the dry dietary components and were then mixed into them for a further 30 minutes.

The defrosted squid puree was added to the uniform dry portion of the diet very slowly to avoid premature coagulation of the diet and this was blended on the slowest speed setting. When half of the grated squid had been added, approximately 20ml of water was added to the remaining squid and turned over with a hand spatula. This wet portion of squid was then slowly added to the rest of the coagulating diet. With further mixing the diet coagulated into a single, large moist ball. The ball of diet containing the glass beads was removed from the mixer and pressed flat with a stiff board until approximately 1.5cm in thickness. The flat block of moist squid diet was then frozen to -25°C until required.

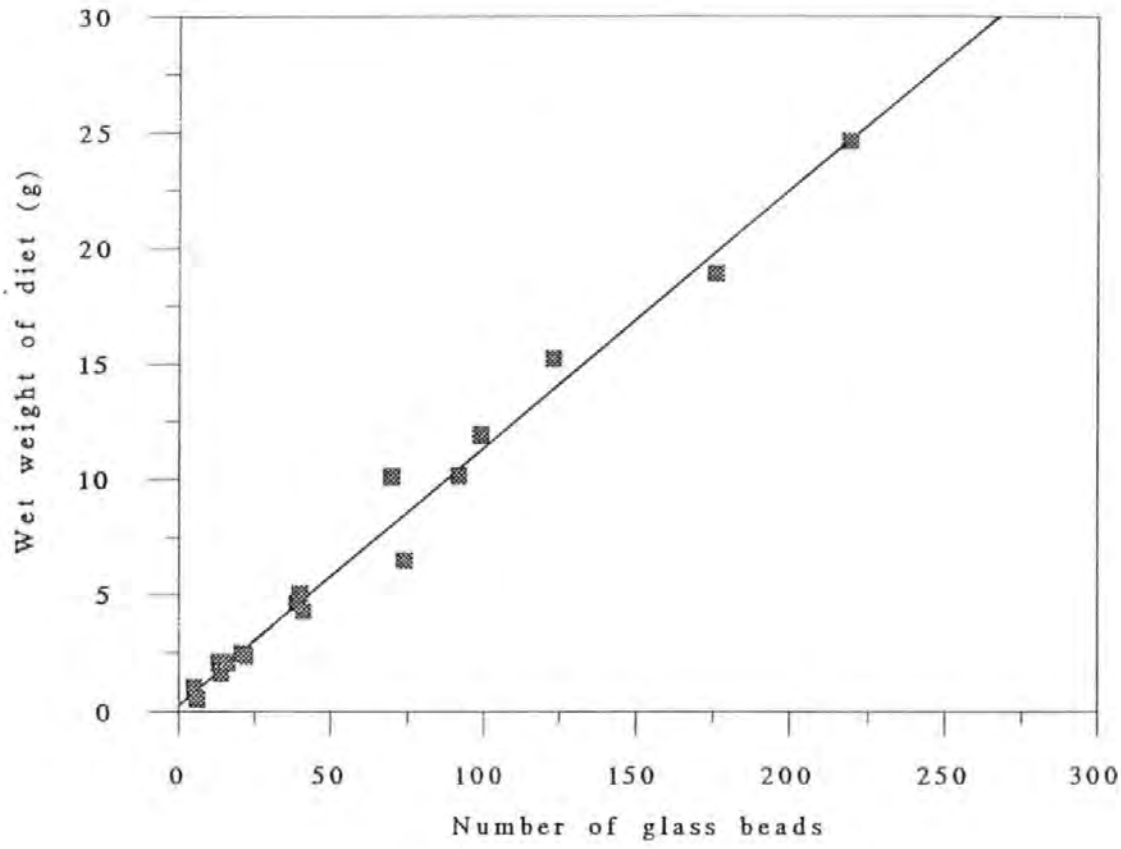
The diet was calibrated by X-radiography (section 3.2.3) to ensure that the glass beads were evenly distributed throughout the diet preparation. Uniform bead distribution was signified by a linear relationship between the number of beads and the weight of food. It was necessary to produce a regression equation for the number of beads in relation to a given weight of diet so that in further investigations the weight of food present in the stomach of *S. canicula* could be estimated from the serial X-radiographs. Figure 7 shows the highly linear relationship obtained for the number of glass beads against weight of diet. Therefore the diet manufacturing method described above was sufficient to ensure uniform distribution of markers within the diet and this protocol was strictly adhered to whenever the experimental diet was made.

3.2.3 General X-Radiography Technique

All X-radiographs were taken using a portable Philips Practix variable power output (kV) X-ray unit with light beam diaphragm attachment. This unit was situated in a basement cellar of the University in close proximity to the aquaria facilities. All X-radiographs were taken by the author whilst wearing a thermo-luminescent detector (TLD) badge which monitored the cumulative X-ray dose obtained when exposing the *S. canicula* to X-rays. The TLD insert was supplied and monitored by the National Radiological Protection Board (NRPB, Didcot, Oxon.). Cumulative dose readings were supplied every 13 weeks by NRPB and by the end of the period over which X-radiographs had been taken, the dose acquired by the author was equivalent to 0mSv (milliSieverts).

Blue sensitive film (RP1, 24 x 30cm, AGFA-Gevaert N.V., Belgium) was used for all X-radiographs and when an X-radiograph was to be taken a sheet of film was placed in a rigid plastic cassette (AGFA Blue R4, Curix screens, 24 x 30cm). The X-ray film was sensitive to light and so the X-ray cassette not only protected the sheet of film from

Figure 7. The uniform relationship between the number of indigestible glass marker beads (N_m) and the wet weight (WW) of the diet. Regression equation, $WW = 0.27 + 0.11N_m$ $r^2 = 0.98$, $n = 17$.


Wet weight of diet (g)
30
25
20
15
10
5
0
0
50
100
150
200
250
300
Number of glass beads

incident light, but also allowed an accurate X-ray image to be recorded on the film. Within the cassette were two AGFA Curix rare earth screens and the sheet of film was held tight against, and firmly between these two screens. When struck by X-rays (that passed through the subject) the screens fluoresce in proportion to the number of actual X-rays and strength of the incident X-radiation. The film was sensitive to the intensity of the screens fluorescence and thus produced an image of the subject on the film.

The dogfish or excised alimentary tracts to be X-radiographed were placed directly on a plastic bag covering a loaded cassette. The X-ray cassette was put on a 3mm lead sheet (80 x 80cm) situated on the ground, with the X-ray generator head exactly 1m above the subject. The light beam diaphragm unit enabled the subject(s) to be "framed" so that the area in which X-rays would strike the subjects, cassette and lead sheet, was exactly known. At no time during any of the X-radiographs was the path of the X-rays allowed to overlap the edge of the lead safety sheet. An exposure time of 0.1 or 0.2 seconds at an X-ray penetrating power of 40-50kV (fixed 0.2mA) depending on the size of the subject was selected on the control panel of the X-ray unit. The control panel was about 2m distant from the X-ray generator head and the subject, and separated from this exposure area by a 30cm thick concrete wall. When the X-ray generator head had preheated, the area of exposure was set and the exposure time and power of the X-ray beam was selected, the X-ray unit was activated via a remote button by the author at a further distance of 3m from the control panel. When exposure of the subject to X-radiation was completed and no further X-radiographs were needed, the X-ray unit was switched off and isolated from the mains power supply.

The film sheets were manually developed under brown light (Kodak filter #67) in the photographic darkroom of the Department of Biological Sciences, University of Plymouth by the author. The sheets of film were removed from the cassettes and clipped

into metal frames which were immersed in developer (G150, AGFA-Gevaert N.V., Belgium) for 3 minutes. After exactly 3 minutes the framed film was removed from the developer and dipped through fresh tapwater before being submerged in a tank of fixative agent (G350, AGFA-Gevaert N.V., Belgium) for approximately 3 further minutes. When fixed, the films were rinsed under running tapwater and dried in a cabinet at 70°F for a couple of hours. The dried films were analysed.

3.2.4 Validation of the X-radiographic Technique for Estimating Gastric Evacuation

3.2.4.1 Serial samples of dogfish

A group of 20 adult *S. canicula* (mean weight 697.5 ± 23.1g S.E.) were fed a satiation meal over a period of one hour such that each fish was assumed to have received a meal of the experimental diet equivalent to 7.84% wbw. They were fed with known weights of thawed diet containing glass beads that had been cut into small cubes whilst still frozen (approx. 1 x 1 x 1.5cm dimension). The experimental diet for this particular investigation also contained 1.25% chromic oxide (Cr_2O_3) by weight of the dry component of the diet, substituted at the expense of cornstarch. The group of adult dogfish were accustomed to the moist experimental pellets as they had been presented light rations of diet twice in the two weeks preceding the experimental period. The fish exhibited normal feeding behaviour and consumed a satiation meal of similar magnitude to that achieved when chopped squid was fed to groups of adult dogfish (chapter II).

When the dogfish had reached satiation, three of them were randomly chosen and each killed with a blow to the head. The fish were weighed intact, before the gastrointestinal (GI) tract of each dogfish was carefully dissected out so that the number of beads in each region of the gut could be accurately determined. The GI tracts were removed from the dogfish in the following manner. An initial incision was made into the

ventral surface of the fish just anterior to the ischiopubic bar of the pelvic girdle and the skin and muscle surrounding the viscera was cut into. This cut was continued anteriorly until the pectoral girdle was reached. In order to extract the entire gastrointestinal tract, the pectoral girdle was cut through, to expose the pericardial cavity so that full access to the oesophagus was possible. The large liver of the dogfish was removed and four ligatures (thin braided cord) were tied tightly around the gut wall to separate the three portions of the gut, namely (i) cardiac stomach, (ii) pyloric stomach and (iii) the intestine region (duodenum, ileum and rectal regions). The role of the ligatures was to stop any migration of the gut contents between the main regions of the gut whilst X-radiography was undertaken. The anterior ligation was tied as close to the beginning of the oesophagus, while the posterior ligature was tied below the rectal gland, where the rectum opens into the dogfish's cloaca. The second ligature was tied around the narrow region of the alimentary canal where the cardiac stomach joins the pyloric stomach. The third ligature was tied close to the pyloric sphincter which was easily located from a visual inspection of the surface of the gut. When the gut of each of the dogfish had been extracted from the visceral cavity the gut lengths were X-radiographed.

Four more adult dogfish from the experimental group were sampled at 24 h, and another four at 72 h. Three more of the group were serially sacrificed at 120 h and another three at 192 h, whilst the three remaining fish were caught and killed at 288 h in the same way as the first fish. All dead dogfish were weighed, their GI tracts dissected out and subsequently X-radiographed.

3.2.4.2 Analysis of actual gut contents

After the GI tracts were X-radiographed the contents of each ligatured section of the gut (cardiac, pyloric, intestine) were emptied into preweighed aluminium foil dishes.

The contents of the intestine (duodenum, ileum and rectal regions) usually also contained some faecal material. It was possible to extract the faeces from the posterior end of this gut length (*i.e.* from the very short large intestine) as there was clear definition between faecal material, usually in the form of a pellet, and the thick liquid chyme of the ileum region. The faeces from each dogfish gut were emptied into a separate foil dish. The contents in each of the four foil dishes for each fish was weighed and then the digesta samples were placed in a preheated oven at 110°C for 24h or until constant dry weight of sample was achieved. The dried digesta samples from each gut region for each fish were again weighed.

The moisture content of digesta in each region of each dogfish gut was calculated using the relationship:

$$\text{Moisture content (\%)} = \frac{\text{wet wt. digesta - dry wt. digesta}}{\text{wet wt. digesta}} \times 100$$

3.2.4.3 Analysis of X-radiographs

The X-radiographs of dogfish GI tracts were viewed on a standard light table (PLH Scientific Ltd.). The radiopaque glass beads were clearly visible in the gut of the individual dogfish (plate 2, p. 91). Standard acetate sheets for use on an overhead projector were laid over the X-radiographs and the outline of the gut and the number of beads in each region of the gut were counted. The ligatures marked the boundaries of each region of the gut so it was very clear how many beads were in, for example the intestine as opposed to the pyloric stomach as the outline of the soft tissue of the gut was visible. Counting the beads was done by marking the position of the bead on the acetate sheet with a permanent ink pen. The number of beads in each region of the gut was counted. The beads in the cardiac stomach were always well separated and every bead could be

counted without error or discrepancy. The beads that clumped together in other sections of the gut were counted twice and if the two determinations were not in agreement, then a third determination was undertaken. If the number counts of beads were still at odds but within 1-5 beads of the other counts, then a mean was taken of the total bead counts.

The number of beads in each portion of the dogfish gut was converted to the estimated dry weight of food present in the tract using the following relationship:

$$\text{Est. dry weight} = \frac{\text{number of beads} \times 0.111 - 0.266}{100} \times (100 - \text{digesta moisture content})$$

where N 0.111 - 0.266 = estimated wet weight of food (section 3.2.2) and digesta moisture content was calculated as given in section 3.2.4.2. The purpose of the conversion was so the estimated stomach contents determined by X-radiography could be compared to the actual stomach contents of the experimental fish.

3.2.5 Determination of Food and Crude Protein Absorption Efficiency by Dogfish

3.2.5.1 Chromic oxide method for absorption efficiency estimations

The experimental test diet containing inert chromic oxide (Cr_2O_3) and the digesta collected as part of the investigations of section 3.2.4.1 were all assayed for chromium. This was achieved by flame atomic absorption spectroscopy of the experimental diet and the digesta samples after an initial wet acid digestion phase first described by Furukawa and Tsukahara (1966). The acid digestion procedure was necessary because of the inert nature of chromic oxide.

Dried digesta samples obtained from dogfish GI tracts taken over 288 h (section 3.2.4.2) and dried diet samples were weighed (50mg) into warm, dry 250ml borosilicate

digestion tubes. Concentrated nitric acid (Analar grade) was added (5ml) to each tube and all tubes were placed in a preheated digestion block (Gerhardt-Kjeldatherm KT-20) and heated to 120°C for approximately 1 hour. Fumes from this stage of the acid digestion were bubbled through 15% sodium hydroxide (Gerhardt Turbosog). The liquid samples were clear of organic matter after 1.5 hours although varying amounts of a green precipitate was present in all the tubes. When the tubes were cool, 3ml of concentrated sulphuric acid and 2ml of perchloric acid were added to each of the tubes. The digestion block was preheated to 240°C and the tubes were heated for a further 1.5 hours. A dull yellow solution in the tubes was obtained on completion of the digestion phase and when cool, 50ml of deionised water was added to each tube. Each sample was poured into a separate volumetric flask and made up to 100ml with deionised water. A subsample of these final volumes were stored in small plastic bottles in the dark at -2°C until they were analysed for chromium.

The analysis for chromium was undertaken using a Varian AA-975 series Flame Atomic Absorption Spectrophotometer fitted with a chromium lamp of wavelength 357.9nm. The lamp current was set at 7mA and the spectral band pass setting was 0.2nm.

The apparent dry matter digestibility of the experimental diet was calculated using the following formula:

$$\text{Apparent Absorption Efficiency (\%)} = 100 - \left(100 \times \frac{\%\ Cr_2O_3 \text{ in food}}{\%\ Cr_2O_3 \text{ in digesta}} \right)$$

3.2.5.2 Kjeldahl method for estimation of nitrogen (crude protein)

For both dietary and digesta protein content determination, 500mg samples of dried digesta (obtained from serially slaughtered dogfish (section 3.2.4.2)) and dried experimental diet were each carefully weighed into separate 250ml borosilicate digestion

tubes. Two "Kjeltabs tct" catalyst tablets were added to each tube prior to 20 ml of concentrated sulphuric acid. The tubes were then heated at 200°C on a preheated digestion block for approximately 45 minutes before being heated at 380°C for a further 45 minutes. When the liquid samples in the tubes were a translucent emerald green colour with no precipitate they were allowed to cool before the distillation phase of the procedure. Three tubes containing no organic matter were run through the same procedure and treated as blank controls.

Each digestion tube in turn was placed into the distillation unit (Gerhardt Vapodest 3S Automatic) where 40% sodium hydroxide and hot distilled water was added to the acid digested sample. Individual conical flasks each containing 25ml saturated boric acid and 15 drops of BDH "4.5" Indicator was positioned in the distillation unit to collect the ammonia distillate from each acid digested sample tube.

The ammonium borate solution and indicator in each flask for each original sample tube was titrated against 0.25M hydrochloric acid. The endpoint of the dynamic flux of alkaline ammonium borate solution to boric acid and ammonium sulphate salts was characterised by the colour change from a blue solution through clear to pale pink. The amount of 0.25M HCl delivered was recorded for each sample.

The amount of % crude protein (CP) in each sample was calculated as follows:

$$\% \text{ CP} = \frac{(V_2 - V_1) \times 14 \times 6.25 \times 100 \times 0.25M}{W}$$

where V_2 was the volume of 0.25M HCl delivered to each of the samples, V_1 was the volume of acid delivered to the blank tubes and W was the weight of the organic sample in milligrams.

Combination of the latter equation with that used to obtain estimates of food absorption efficiency (AE) in section 3.2.5.1 gives the relationship:

$$\text{Apparent CP AE (\%)} = 100 - \left(100 \times \frac{\%\ Cr_2O_3 \text{ in food}}{\%\ Cr_2O_3 \text{ in digesta}} \times \frac{\%\ \text{nutrient in digesta}}{\%\ \text{nutrient in food}}\right)$$

and was used to calculate the apparent nutrient AE of the experimental diet from the digesta samples taken from serially slaughtered dogfish.

Crude protein coefficients were calculated in the following way:

$$\text{CP Coefficient} = \frac{\text{dry wt. CP in total digesta (g)}}{\text{dry wt. of total digesta (g)}}$$

3.2.6 Serial X-Radiography of Dogfish to Determine Rates of Gastric Evacuation

3.2.6.1 Number tagging of experimental fish

A group of 25 adult dogfish *S. canicula* (mean wt. 742.5 ± 16.4 g S.E.) were individually caught, weighed and marked with a numbered tag. All the tags of dimension 14 x 5mm were of one colour and were each threaded onto a separate length of nylon line which was pushed through the base of the leading edge of the first dorsal fin of each dogfish. The line was tied off in a generous loop so that the buoyant plastic tags could float free and above the body of the fish, thereby preventing any skin irritation. The needle and the fin area that was to be penetrated were cleaned with 60% ethanol before the fish was actually tagged. The dogfish were left to equilibrate after the tagging procedure for two weeks. During the two weeks the dogfish did not exhibit any unusual behaviour and continued to feed quite normally. The dogfish were tagged so that they could be identified for serial X-radiography, to avoid the same individual fish being consecutively X-radiographed.

3.2.6.2 Gastric evacuation studies

The group of number tagged adult dogfish were fed a 7% wbw meal of the experimental diet (exact formulation given in 3.2.1) to correspond with the satiation level that was obtained for dogfish in the return of appetite investigations (chapter II). This known weight of thawed experimental diet containing glass beads was given to the dogfish over a period of 1 hour. The end of the feeding period was noted as time zero and from this point, anaesthetised dogfish were serially X-radiographed. Anaesthetised fish were used because the unanaesthetised dogfish exhibited active swimming movements when out of water that were shown to persist for some considerable time. Immobile dogfish were needed so that the X-radiograph image was not distorted by any sudden movements.

The anaesthesia used was ethyl p-Amino benzoate (benzocaine) (Sigma Chemical Co. Ltd., Poole, Dorset) and was prepared by dissolving 1g of the white powder in 100ml of 70% ethanol. A 10L seawater anaesthetic bath was made up by adding 5ml of benzocaine in alcohol for each litre of seawater. Immediately after feeding (t=0) two random dogfish were removed from the holding tank by hand and transferred to the anaesthetic bath. Initially, the dogfish thrashed their bodies quite violently to escape the bath but after a few seconds slumped back into the anaesthetic. The dogfish were immobilised quite quickly due to their raised ventilation rate from being active, and with a tight cover on the anaesthetic bath were taken to the X-ray unit whilst still becoming anaesthetised. Long forearm rubber gloves were used to remove each dogfish from the anaesthetic bath and they were each placed in turn, ventral surface down on the plastic bag surrounding the X-ray film cassette. From the dorso-ventral aspect the entire dogfish gut was observable in a single plane. The dogfish were then X-radiographed as detailed in section 3.2.4.2. The completion time of the X-ray procedure, from the start of

anaesthetisation to the point of recovery initiation was not longer than 10 minutes.

The two dogfish were taken back to the holding facilities in the anaesthetic bath and recovered by holding them, one in each hand, just below the surface of the water in a current of aerated seawater. Each fish was assumed to have recovered fully when they exhibited swimming movements and they were then allowed to descend freely to the bottom of the aquaria. After recovery, the dogfish were observed for another 15 minutes to ascertain whether they were able to ventilate their gills and swim properly. During the X-radiographic studies all dogfish that were anaesthetised were recovered successfully.

In the 7% wbw study, two different dogfish were anaesthetised and X-radiographed every 24 hours until the X-radiographs showed the stomach to be empty. At the end of the trial the dogfish were all weighed and allowed to equilibrate for another two to three days. After this time the dogfish group were fed a meal assumed to be equivalent to 3.5% wbw per fish. As before, two dogfish were anaesthetised and X-radiographed every 24 h until the stomachs of the fish were observed to be empty.

The investigations were repeated at both ration levels with weighings of the dogfish at the end of each trial followed by at least three days of equilibration. For all the trials each dogfish was X-radiographed not more than once in any seven day period so that they were not adversely affected by more frequent anaesthetisation. At no time during the gastric evacuation trial period were there any mortalities of the fish due to the anaesthetisation or X-radiograph regime.

The live dogfish X-radiographs were analysed in the same way as those of the dogfish GI tracts. At least three counts were made of the number of beads in each region of the gut and with further experience in observing the gut and in the quality of the X-radiographs, the accuracy of bead counts for each gut region was increased. The estimated dry weight of food remaining in each dogfish stomach was calculated as given

in section 3.2.4.3 and the values were then re-expressed in terms of percentage food remaining in the stomach. The percentage of food remaining was used as the measure of gastric evacuation so accurate comparisons in GER could be made to take into account small variations in the meal size originally given.

3.2.7 Cardiac Stomach Dimensions

The cardiac stomach dimensions (length and diameter) were measured to the nearest 0.1mm in 34 adult dogfish fed different quantities of the experimental diet containing glass beads. The dimensions were measured by direct determination from dead fish (n=11) and by separate (from previous studies) X-radiographs of living dogfish (n=23). The length and diameter of the cardiac stomach of X-radiographed dogfish was estimated by measuring the greatest distance between the glass beads on the film, both anteriorly/posteriorly (to give length of stomach) and laterally (to give diameter of stomach). Three of the dogfish had not been fed any experimental diet before they were killed with a blow to the head, and their stomachs served as controls for the measurements from other dogfish.

3.2.8 Modelling Gastric Evacuation Rate (GER) and Statistical Analysis

The decrease in cardiac stomach contents of adult *S. canicula* was modelled using exponential and square root functions. The exponential model used was the generalized relationship given in Bromley (1994), such that,

$$S_t = S_0 \ e^{-Bt}$$

where S_0 represents the meal size consumed and S_t the stomach contents at the given time t, with t being the time in hours and B denoting the instantaneous rate of gastric evacuation. The square root model used was the one given in Jobling (1981), such that,

$$S_t^{0.5} = S_0^{0.5} - kt$$

where k is a constant and $S_0 = a^2$ where a is the intercept to the y axis. A variation on Jobling's (1981) square root model from Bromley's (1994) review was also fitted to the experimental data, with a function of the form,

$$S_t = S_0 - 2\sqrt{S_0}\ B\ t + (B\ t)^2$$

The three equations were modelled by nonlinear regression to obtain least squares estimates of the parameters in the model. The Marquardt search algorithm (Marquardt, 1963) of Statgraphics Version 6.0 determined the estimates to minimise the residual sum of squares of the user defined functions (the equations). The exponential curve shapes fitted by the algorithm at each ration level (3.5% and 7% wbw) were statistically compared by analysis of covariance (ANCOVA).

The changes in cardiac stomach dimensions with increase in food intake were modelled using the generalized power function for length change of the form,

$$L_w = L_0\ W^B$$

where L_0 was the length of the stomach when no food was present, L_w the length of the stomach containing W dry weight grams of food and W represents the dry weight grams of food present in the cardiac stomach.

3.3 RESULTS

3.3.1 Gastric Evacuation

The experimental diet fed to the adult dogfish throughout the investigations was uniform in glass bead marker content. The number of glass beads increased in direct proportion to the wet weight of diet and was best represented by a linear regression (regression coefficient , r^2=0.98; figure 7, p. 77).

Twenty adult *S. canicula* were each fed a 7% wbw meal of the experimental diet and the gut contents of individual fish were serially sampled by direct weight measurement and by X-radiographic analysis. Figure 8 shows the correlation between the actual stomach contents of dogfish and the predicted stomach contents estimated from X-radiographic analysis of the number of beads actually present from stomachs sampled from 0-72 h. The linear relationship between actual and estimated stomach contents on a dry weight basis remained close to the equilateral line (where actual contents=estimated contents), though deviation from this line was more marked at the higher levels of actual stomach content. This indicates that the X-radiographic method of stomach content prediction may have marginally underestimated the actual stomach contents when digestion of the larger meals was taking place. Generally though, the good agreement found between the actual weight measurements of cardiac stomach contents and that from X-radiographic estimations of the stomach content was sufficient validation, and thus the basis for utilising X-radiography in further gastric evacuation studies.

In further gastric evacuation studies 25 adult dogfish were fed the experimental diet and serially X-radiographed. Plates 2-8 illustrate the passage of a 7% wbw meal through the gut of different pairs of experimental fish. After only 1.5 h some beads had already left the cardiac stomach, which suggests that initiation of gastric evacuation began

Figure 8. Estimated dry weight stomach contents as determined by X-radiographic measurements from 10 serially sampled dogfish guts correlated with actual dry weight stomach contents of the same fish. Regression equation, Actual DW = -0.41 + 1.07Est. DW, $r^2 = 0.95$; n = 10.

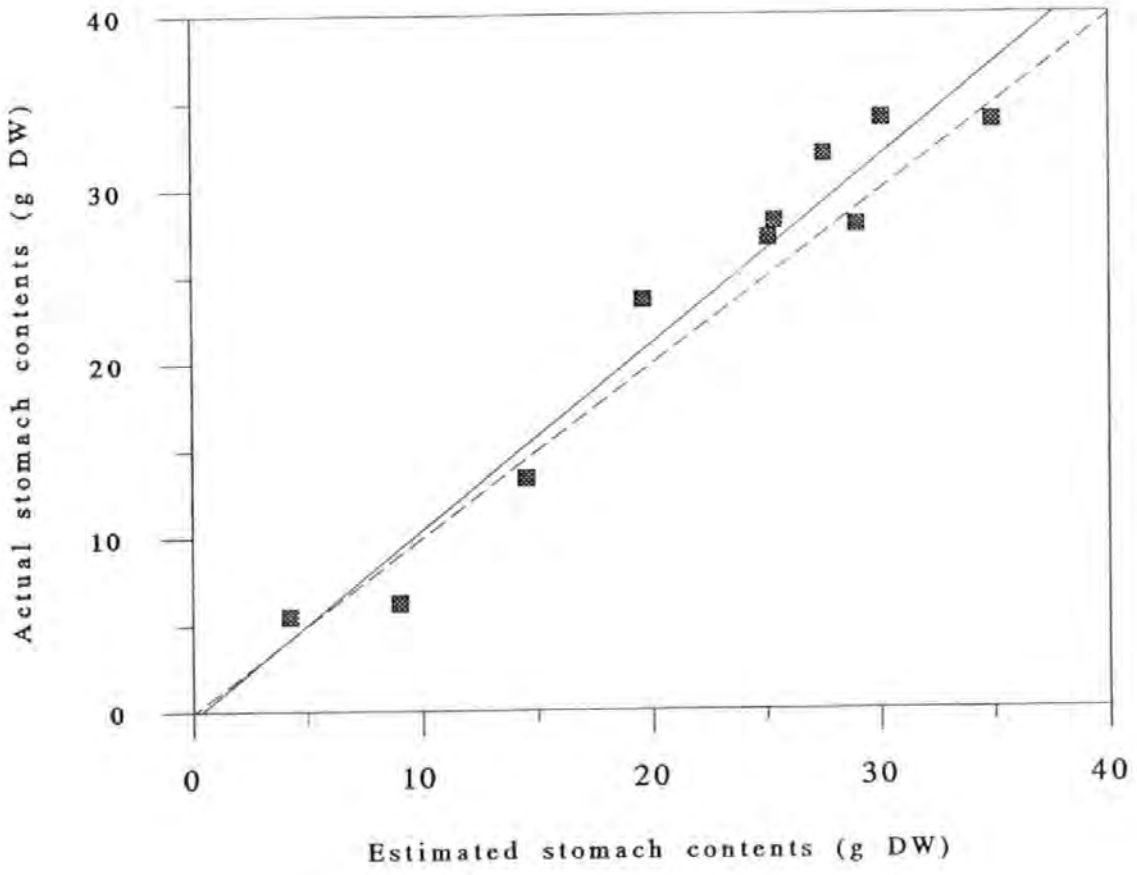
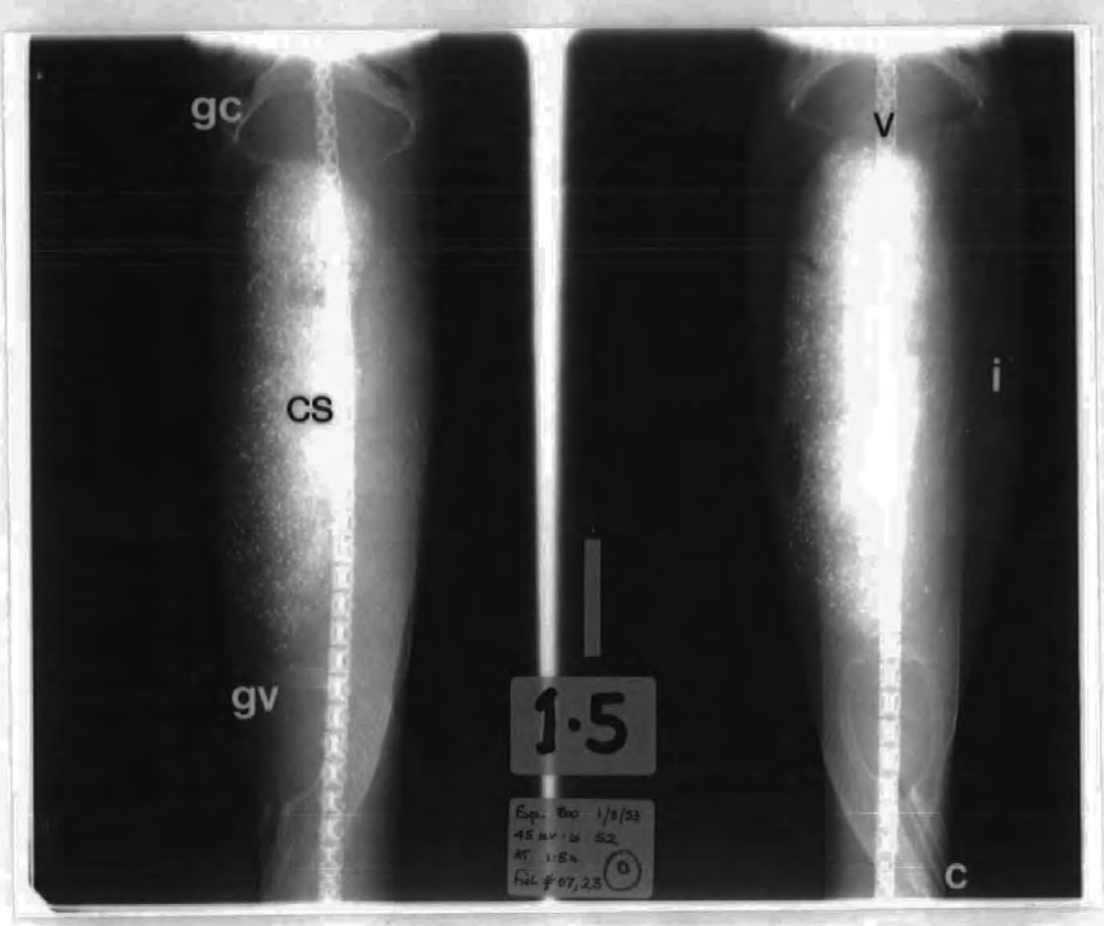

Actual stomach contents (g DW)
Estimated stomach contents (g DW)
0
10
20
30
40
0
10
20
30
40

Plate 2. X-radiograph of the stomach contents of two adult *S. canicula* 1.5 hours after being fed 7% wbw meals of squid pellets marked with radiopaque glass microspheres (0.40-0.52mm diameter). The small white dots represent the glass beads. The X-radiograph was taken with the dogfish lying dorso-ventral and at right-angles to the x-ray beam. Heads of the dogfish point towards the top of the page. Vertical scale bar represents 3cm. Key to abbreviations: *c*, claspers; *cs*, cardiac stomach; *gc*, pectoral girdle; *gv*, pelvic girdle; *i*, intestine; *v*, vertebrae; *p*, pyloric stomach; *rc*, rectum.

Plate 3. X-radiograph of two adult *S. canicula* 24 hours after consumption of 7% wbw meals of marked squid diet. Key to abbreviations: *cl*, cloaca; *i*, intestine; *p*, pyloric stomach.


gc
cs
gv
v
i
c
1·5

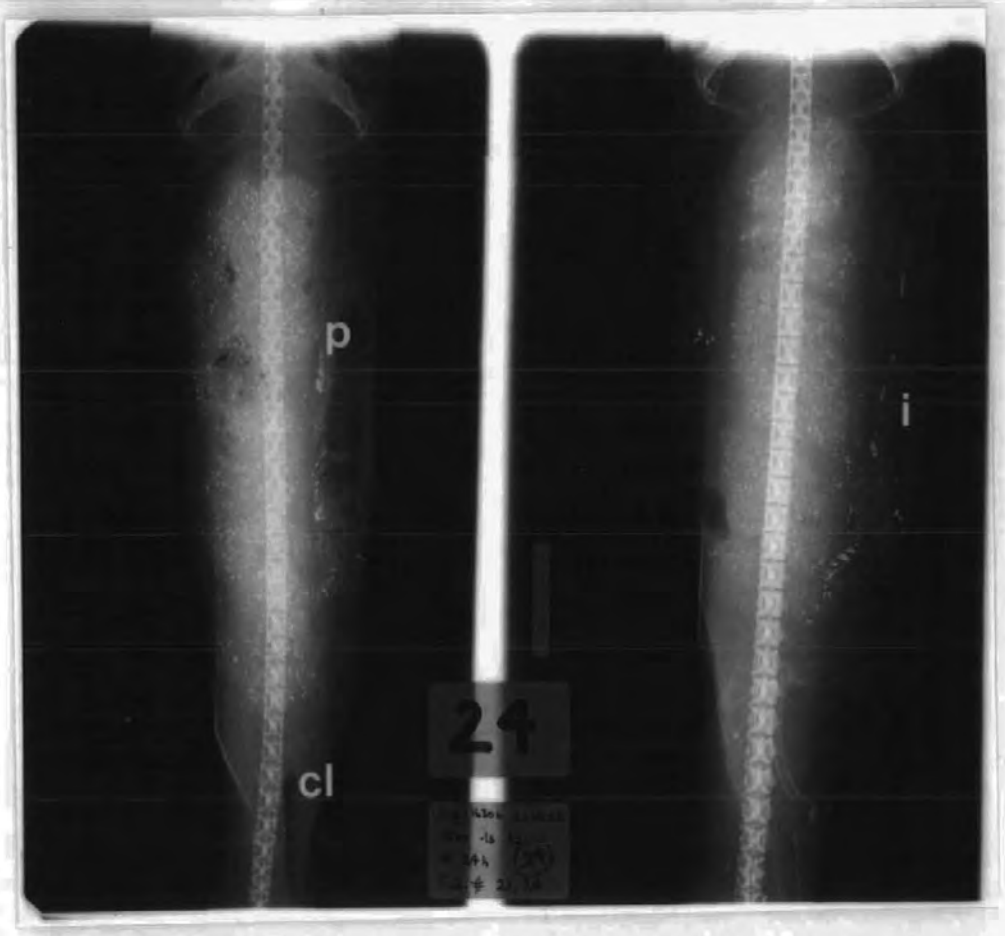
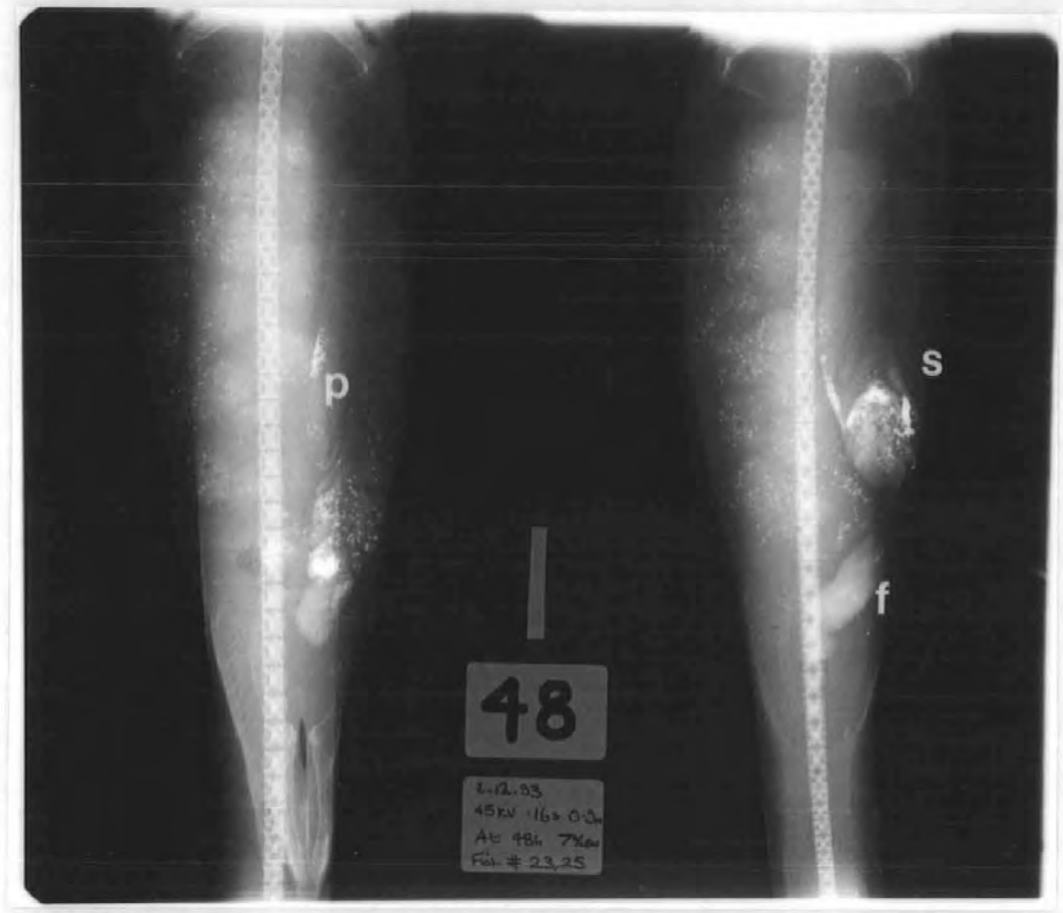

p
cl
i
24

Plate 4. X-radiograph of two adult *S. canicula* 48 hours after consumption of 7% wbw meals of marked squid diet. Key to abbreviations: *f*, faecal pellet; *p*, pyloric stomach; *s*, spiral valve.

Plate 5. X-radiograph of two adult *S. canicula* 120 hours after being fed marked diets. Key to abbreviations: *r*, bead retention in pyloric stomach.


p
s
f
48

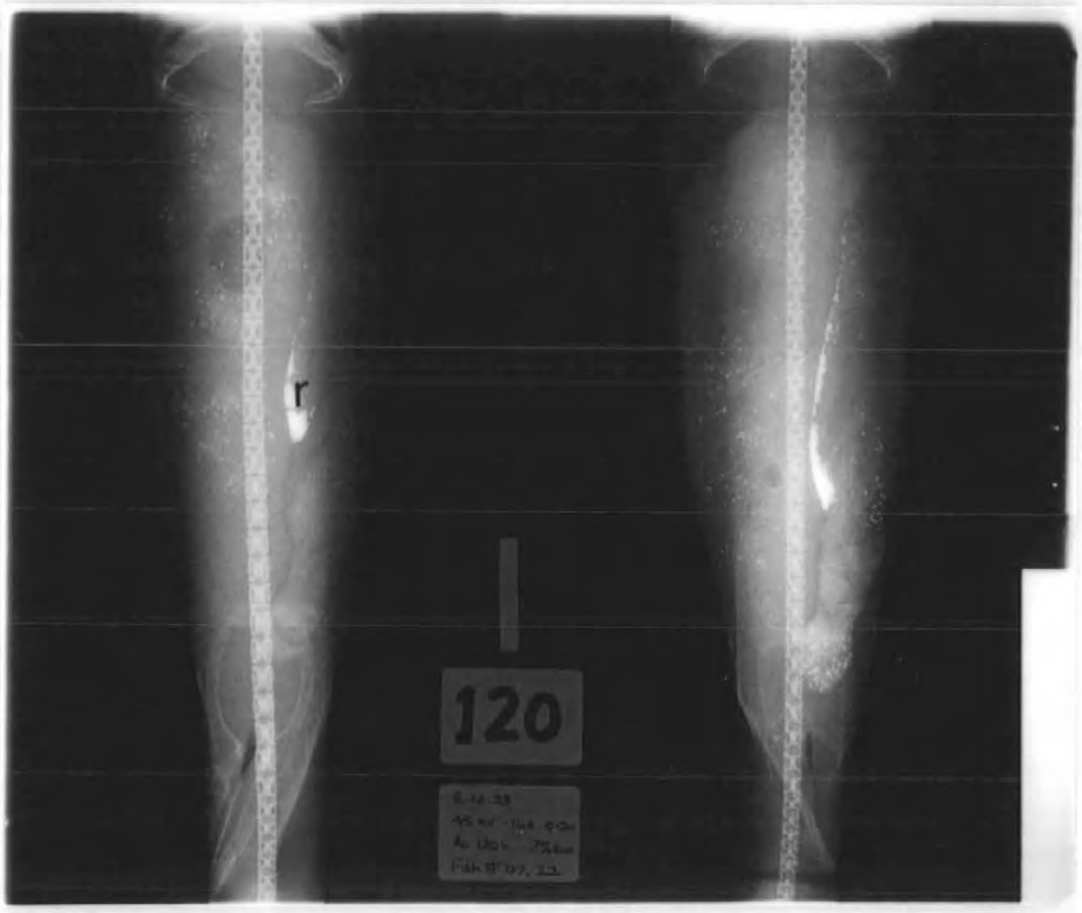
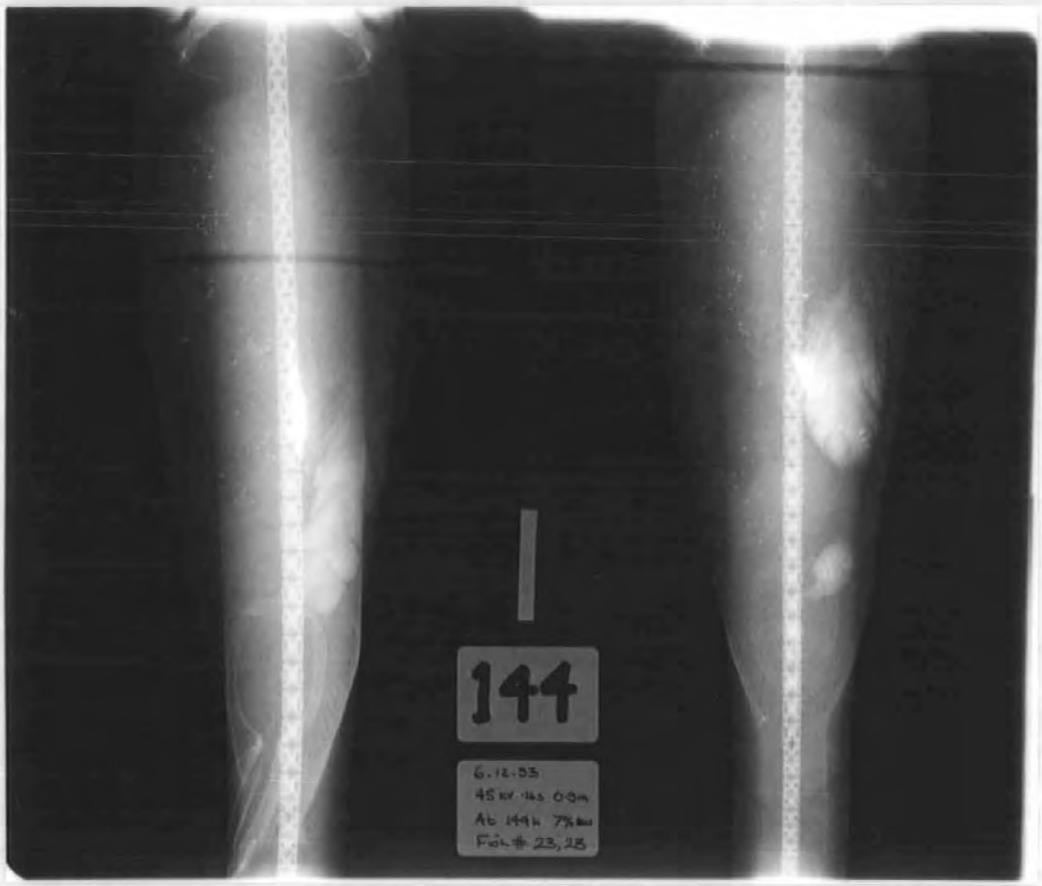

r
120

Plate 6. X-radiograph of two adult *S. canicula* 144 hours after consumption of a 7% wbw meal of marked squid diet.

Plate 7. X-radiograph of two adult *S. canicula* 192 hours after consumption of a 7% wbw meal of marked squid diet. Key to abbreviation: *s*, spiral valve.


144
6.12.55
45 kV 4s 0.8m
Ab 144h 75 kV
Fol. 23,25

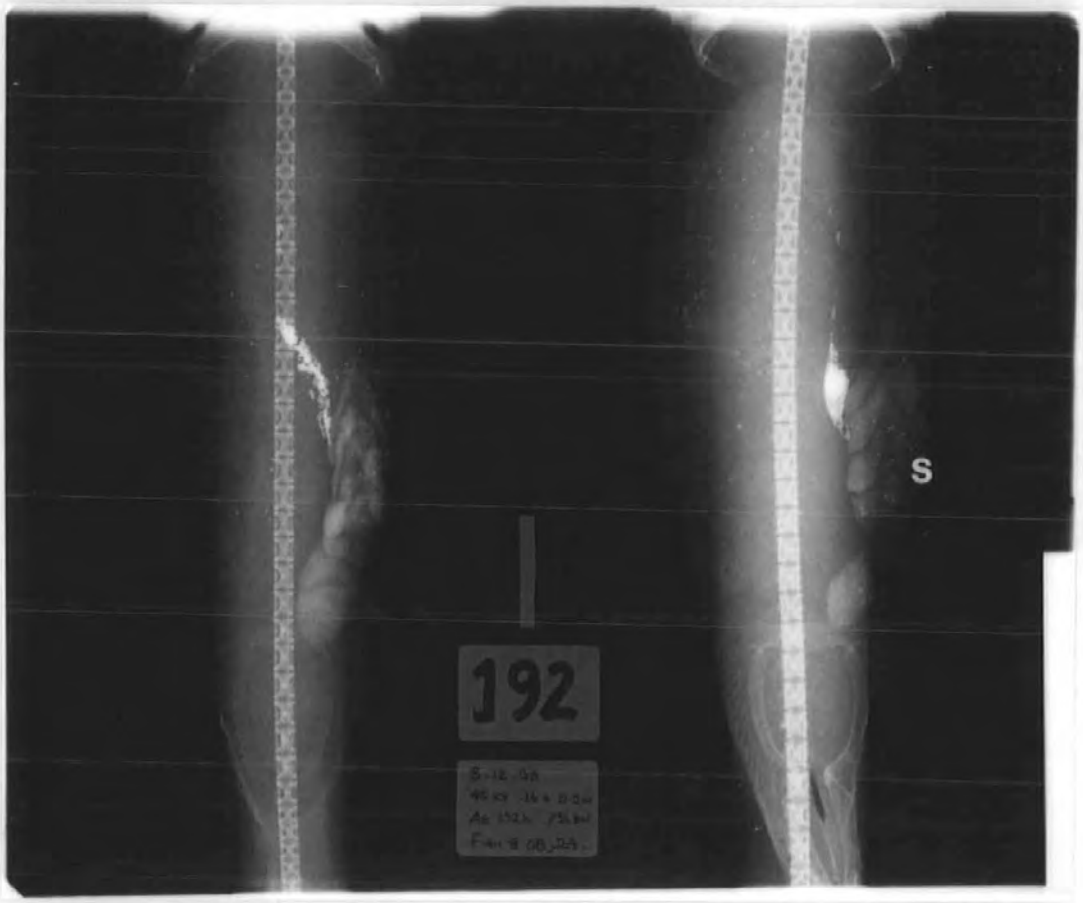
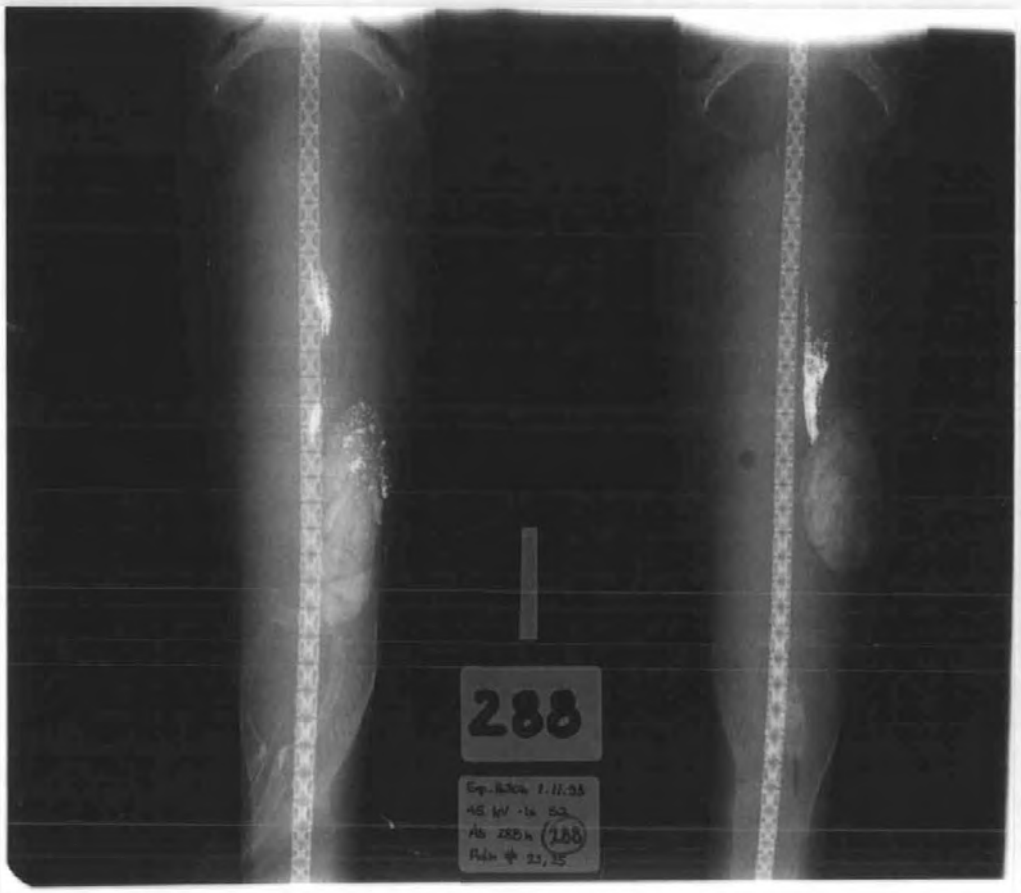
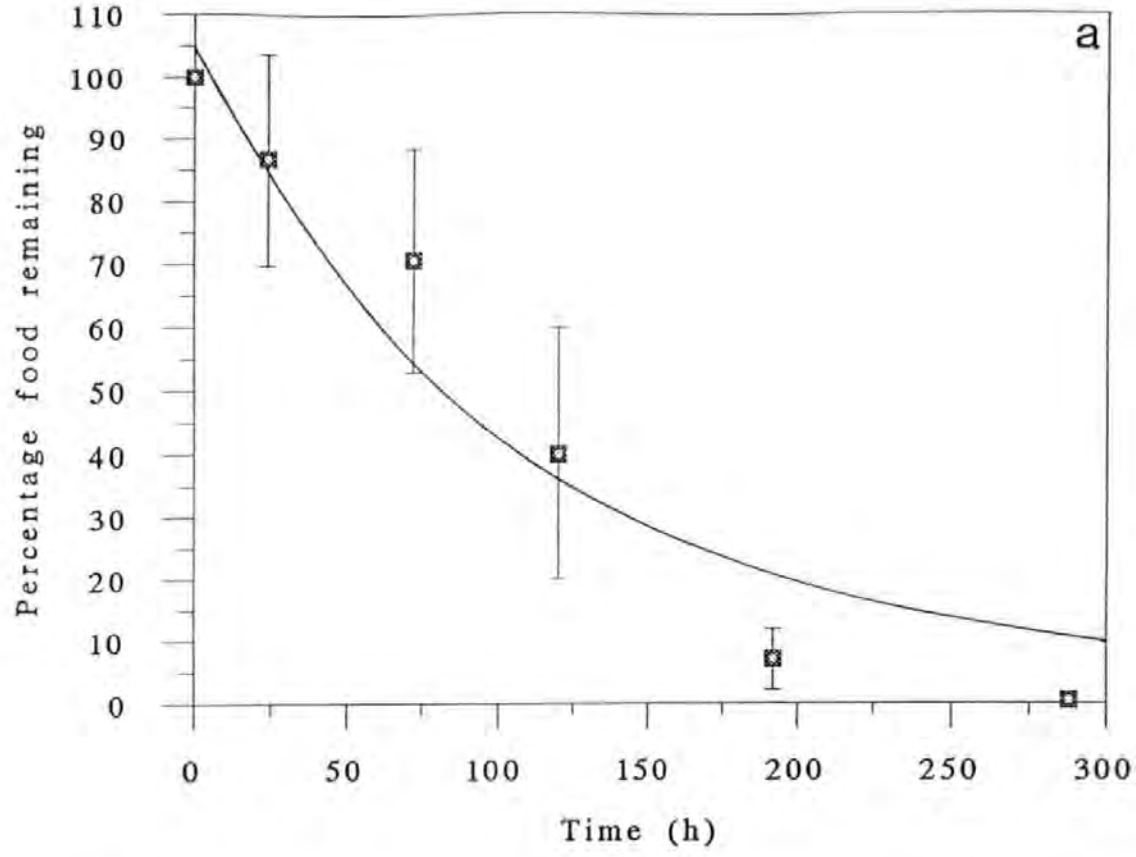

S
192

Plate 8. X-radiograph of two adult *S. canicula* 288 hours after consumption of a 7% wbw meal of marked squid diet.


288

soon after meal ingestion (plate 2). Plate 2 also illustrates that the glass beads can be clearly seen within the individually visible experimental diet pellets. After 24 h (plate 3) some digesta had moved from the cardiac stomach and was present in the pyloric region in some quantity. At the end of 48 h individual pellets in the stomach were still present though it was obvious that they were more diffuse and were being broken down, as they appeared quite pitted on the X-radiograph (plate 4). The experimental diet pellets were fully digested between 120-144 h (plates 5 and 6) and the flow of beads from the stomach was quite uniform even though clumping of some beads occurred in the pyloric region of the gut during this time. Few beads, thus little food remained in the cardiac stomach after 192 h (plate 7) and they were completely evacuated after 288 h (plate 8). The decline in the contents of the cardiac stomach was estimated on a dry weight basis in the same way as in the validation study. Figures 9a and b show the gastric evacuation profiles from both the validation study and from an X-radiographic study. Exponential models were fitted to these experimental data and from table 5 it is clear that the curve shapes, or slopes representing gastric evacuation of the 7% wbw meals determined by X-radiography and actual stomach contents weighings were not significantly different (P>0.25). The regression coefficients (r^2) for the gastric evacuation of 7% wbw meals were both above 0.95 and the y intercept parameters, representing the amount of food present in the stomach at time zero (t=0) were both close to the expected 100%. The close agreement of these two fitted models to the actual and estimated gastric emptying data suggests that X-radiographic methods utilising the rate of disappearance of glass beads was accurate in representing actual stomach evacuation of adult dogfish. Table 6 however, indicates the slight underestimation of the X-radiographic prediction of gastric evacuation at the 7% feeding level compared with the model predictions from actual stomach content weight. For example, the time required for 75% gastric evacuation was 61 h from the X-

Figure 9. Stomach emptying rates of adult *S. canicula* determined by (a) actual weighings of stomach contents and (b) X-radiography after an initial meal of 7% wbw. The ordinate expressed as the percentage of food remaining (S_t) on a dry matter basis. The fitted curve to the upper panel is exponential and the fitted curves to the lower panel are exponential (solid line) and square root (dashed line). Nonlinear regression equations; (a) $S_t = 105.5\ e^{-0.009\ t}$, where t is time in hours, $r^2 = 0.95$, each data point represents the mean of either 3 or 4 determinations; (b) $S_t = 102.2\ e^{-0.010\ t}$, $r^2 = 0.96$, each data point represents the mean of 4 determinations (solid line); $S_t = (9.80 - (0.03\ t))^2$, $r^2 = 0.97$ (dotted line). Bars denote ± 1 standard error of the mean.


a
Percentage food remaining
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (h)

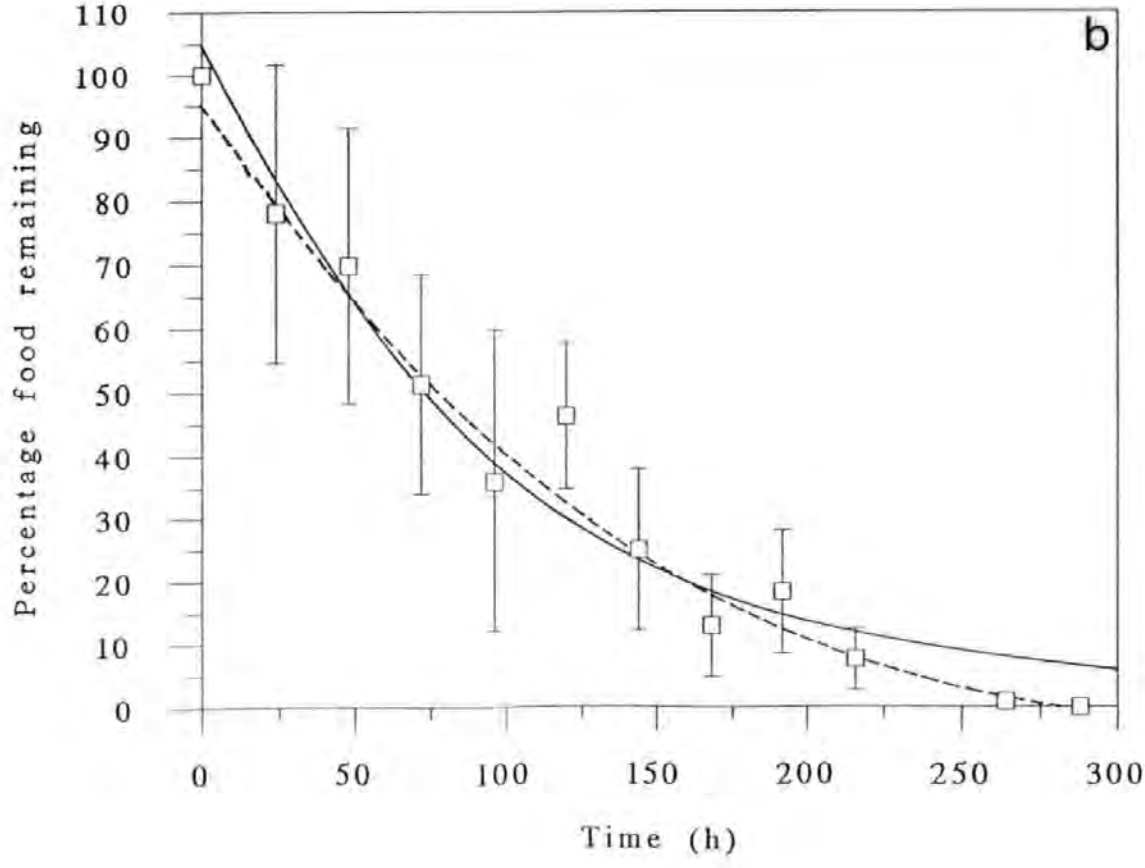

b
Percentage food remaining
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (h)

Table 5. Statistical analysis summary of the fitted exponential functions at each meal size.

	Regression[1]			ANCOVA[2]		
				Curve shape (slope)		
Meal size (% wbw)	a	b	r^2	F	d.f.	*P*
3.5	97.4	-0.024	0.97			
				11.59	1:20	<0.005
7	102.2	-0.010	0.96			
				0.51	1:15	>0.250
7[v]	105.5	-0.009	0.95			

[1] *Coefficients obtained from the fitted exponential function* $y=ae^{-bx}$, *where y represents the percentage food remaining in the cardiac stomach and x signifies the time course.*

[2] *Significant differences in curve shape shown by analysis of covariance and significance assumed at* $P<0.05$.

Table 6. Comparison of gastric evacuation times determined from the fitted exponential models of each ration level and of the validation study.

	Predicted times (h) for gastric evacuation (%)					
Meal size (% wbw)	3%	25%	50%	75%	97%	99%
3.5	0.07	4.73	12.07	24.61	62.98	82.86
7	2.27	13.44	31.05	61.15	153.23	200.95
7[v]	4.05	16.47	36.03	69.48	171.79	224.81

[v] *From fitted exponential model of actual cardiac stomach content decline*

radiographic prediction compared to 70 h from the actual weight estimates. Clearly, the X-radiographic model for gastric evacuation of adult dogfish was very similar to that produced from actual serial stomach weights, but underestimated food presence at the higher levels of feeding.

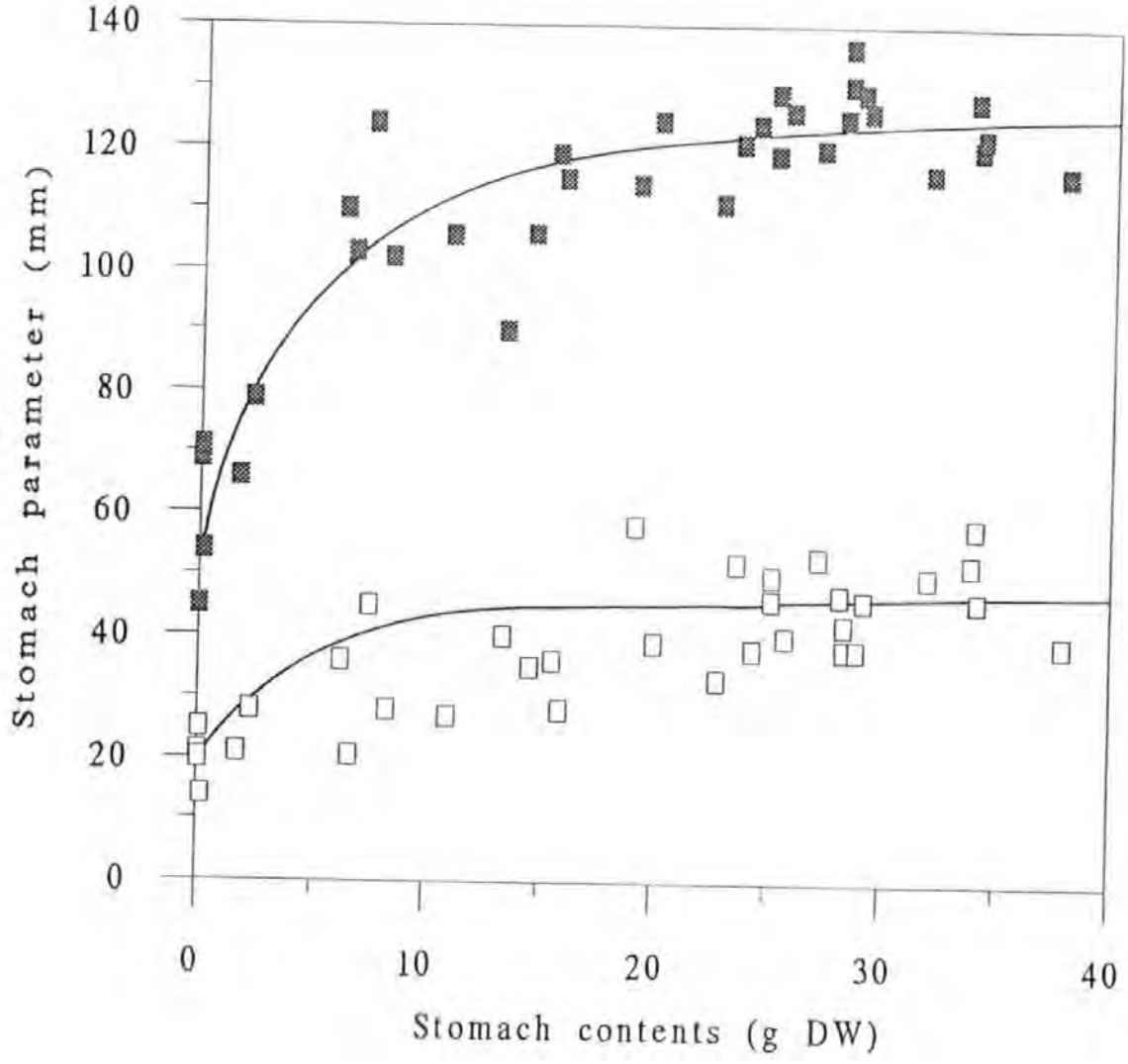
Figure 9b shows the Jobling (1981) square root model fitted to the 7% wbw X-radiographic determinations of stomach emptying in addition to the fitted exponential function. At the higher ration level the r^2 of the square root model was similar to that of the exponential model, 0.97 and 0.96 respectively, which indicated little difference in the prediction accuracy of dogfish gastric evacuation between either model. However, the residual mean sum of squares (RMS) was higher in the exponential model (44.5) than in the Jobling square root model (32.6), which indicated the square root model was a marginally better fit. The fitted Bromley (1994) square root model of the form,

$$S_t = 90.7 - 2\sqrt{90.7 \times 0.097\, t + (0.097\, t)^2}$$

was not a good explanation of the stomach emptying data as the RMS was quite high (77.4) and the estimation of *a* (the ordinate intercept) was not as accurate as the exponential or Jobling square root models. From figure 10 however, it is clear the length and diameter of the cardiac stomach of adult dogfish increased in a nonlinear manner with a concomitant increase in the amount of food present in the stomach. The assumption of square root models in general, maintains that the rate of evacuation is proportional to the square root of the weight (volume) of stomach contents, if the length of the stomach remains constant. Thus, the efficacy of applying the square root model to accurately predict the gastric evacuation rate of adult dogfish was not appropriate in view of the change in the length of the dogfish stomach (best described by a power function) with an increased level of feeding.

The effect of meal size on the gastric evacuation rate of adult *S. canicula* was also

Figure 10. The change in length (■) and diameter (□) of the cardiac stomach of adult *S. canicula* at varying levels of food intake. Nonlinear regression equations (power functions); length $L = 4.39\ DW^{0.12}$, $r^2 = 0.85$, $n = 34$; diameter $D = 3.29\ DW^{0.13}$, $r^2 = 0.59$, $n = 34$.


140
120
100
80
60
40
20
0
Stomach parameter (mm)
0
10
20
30
40
Stomach contents (g DW)

investigated. Figure 11 demonstrates that an exponential function best represented not only GE at the 7% wbw feeding level, but also the GE of a 3.5% wbw meal (regression coefficients of 0.96 and 0.97 respectively). The 3.5% wbw meal was evacuated at over twice the rate of the larger meal (table 5) and the analysis of covariance of the fitted model curve shapes indicated significant differences in instantaneous rate of gastric evacuation between the two meal sizes ($P<0.005$; table5). The predicted times for percentage gastric evacuation in table 6 indicate that 99% gastric evacuation of a 3.5% wbw meal was evacuated after 83 h compared to 201 h for the 99% gastric evacuation time of a 7% wbw meal.

From figure 12 it was evident there was inverse proportionality between gastric evacuation (percentage food remaining) and the return of appetite of adult *S. canicula* (chapter II) after a 7% wbw meal. The rate of appetite return increased rapidly as the rate of gastric emptying was maximally decreasing, which continued until 250 h when the stomach was relatively empty and the rate of appetite return was assumed to have reached an upper limit.

3.3.2 Gastrointestinal Evacuation

The passage of food through the gastrointestinal tract was followed from X-radiographs of anaesthetised dogfish, but uniform bead flow past the pyloric sphincter was not observed and so quantitative estimates of gastrointestinal evacuation from X-radiography was not feasible. From the X-radiographs however (plates 2-8), the actual presence of digesta was apparent and so the X-radiographs were useful for estimates of intestinal fullness and faecal pellet production rate. Some of the experimental diet appeared in the intestine after only 1.5 h (plate 2). After 24 h, digesta was clearly visible in the intestine along with clumped patches of glass beads (plate 3). The first evidence of faecal pellet

Figure 11. Exponential stomach emptying rates of adult *S. canicula* fed 3.5% wbw (□) and 7% wbw (■) of an experimental diet. Nonlinear regression equations; for a 3.5% wbw meal, $S_t = 97.4\ e^{-0.024\ t}$, $r^2 = 0.97$, each data point represents the mean of 4 determinations; caption of figure 9 for 7% wbw meal. Bars represent ± 1 standard error of the mean.

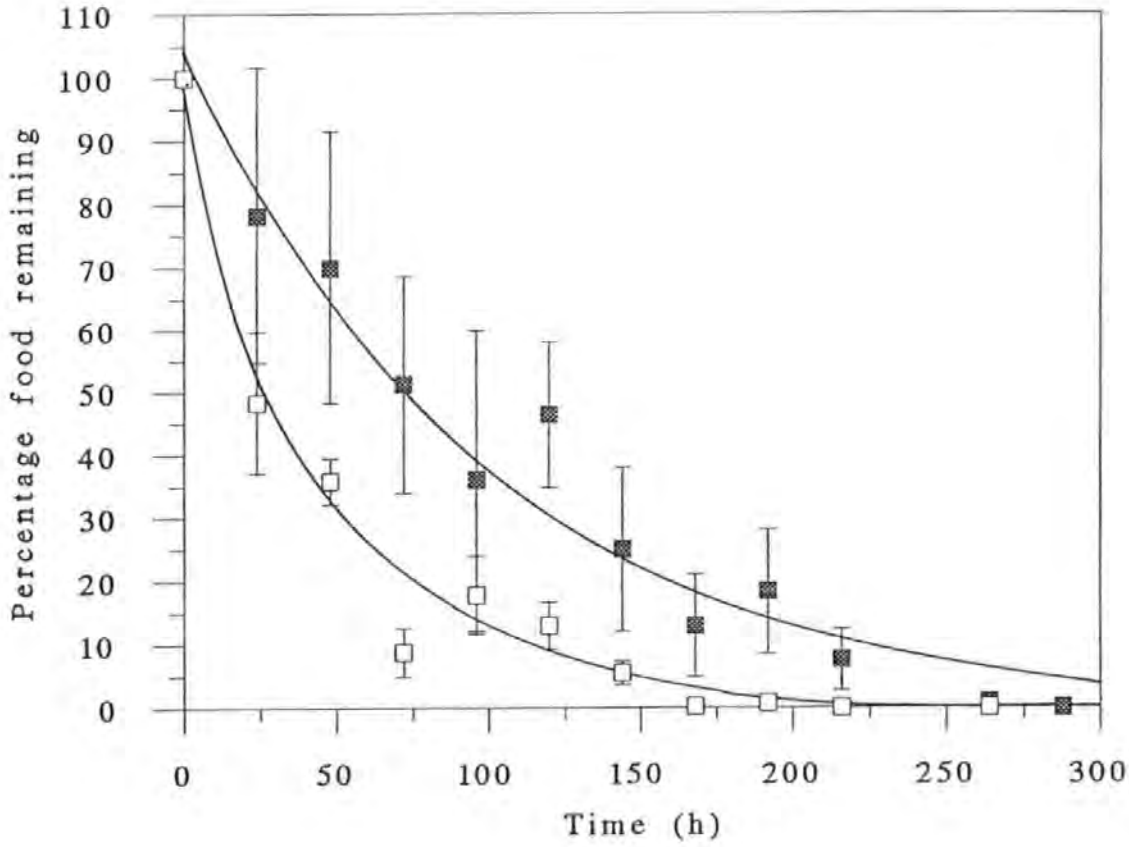
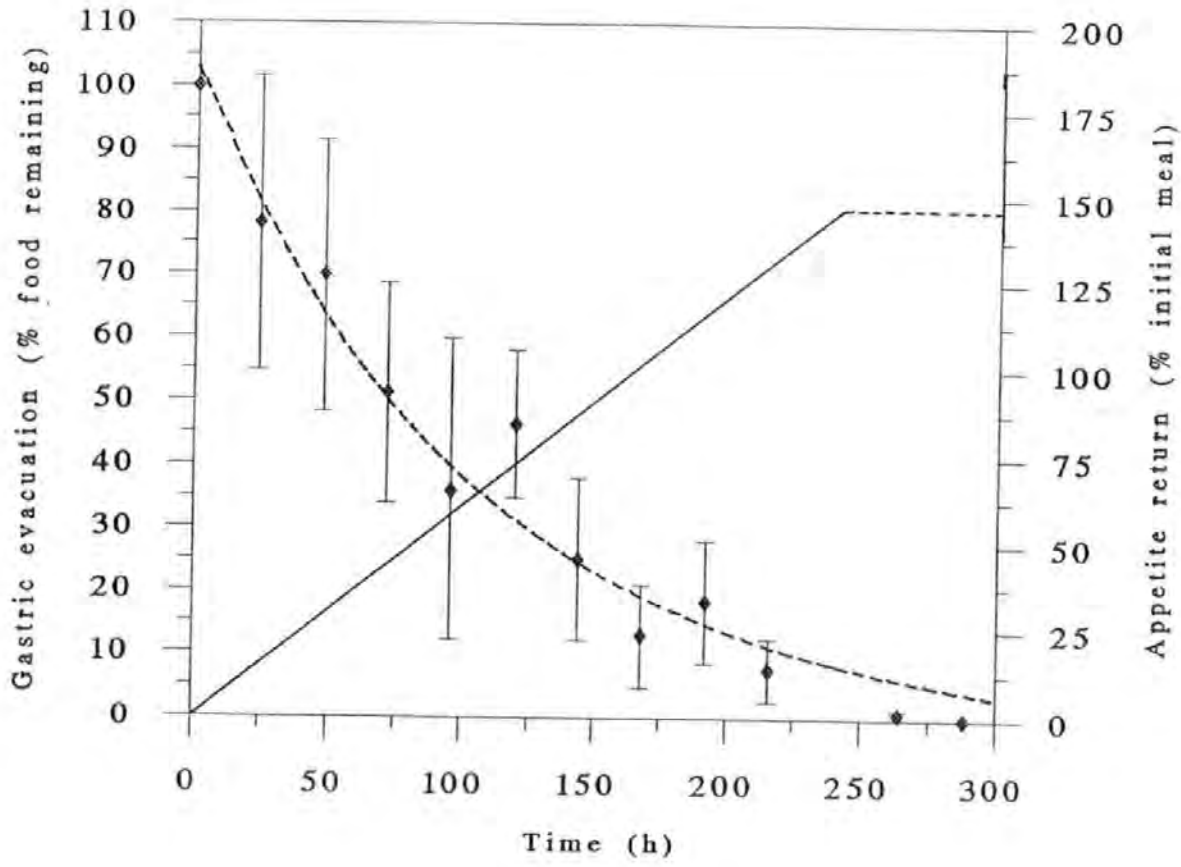

Percentage food remaining
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (h)

Figure 12. Gastric evacuation (dotted line) and appetite return (solid line) rates of adult *S. canicula* after an initial meal of approximately 7% wbw. The appetite return rate was taken from the regression equation given in figure 5 (chapter II).

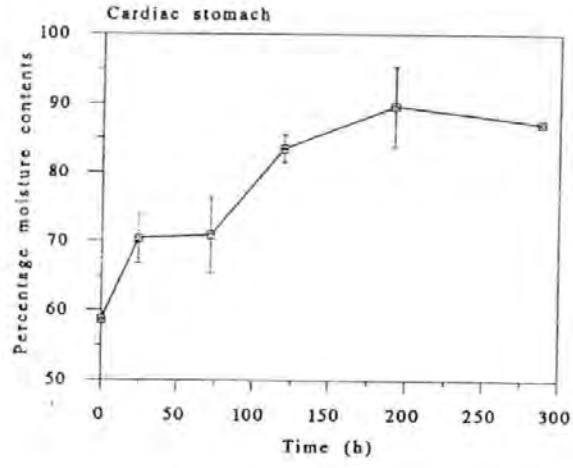

Gastric evacuation (% food remaining)
110
100
90
80
70
60
50
40
30
20
10
0
Appetite return (% initial meal)
200
175
150
125
100
75
50
25
0
0
50
100
150
200
250
300
Time (h)

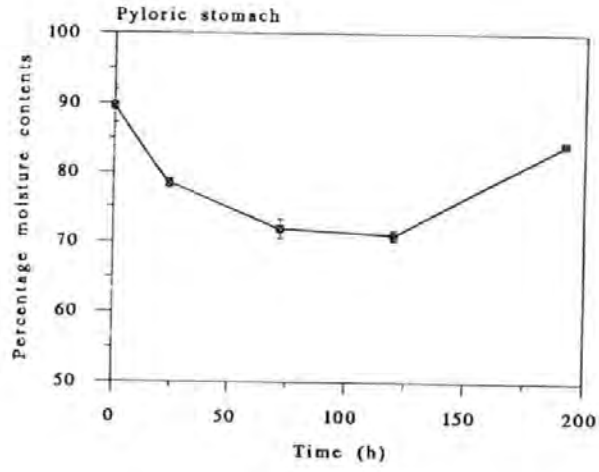
formation occurred at 48 h post feeding (plate 4) and continued throughout the period of 48-288 h (plates 4-8), during which time the spiral intestine was always full of digesta.

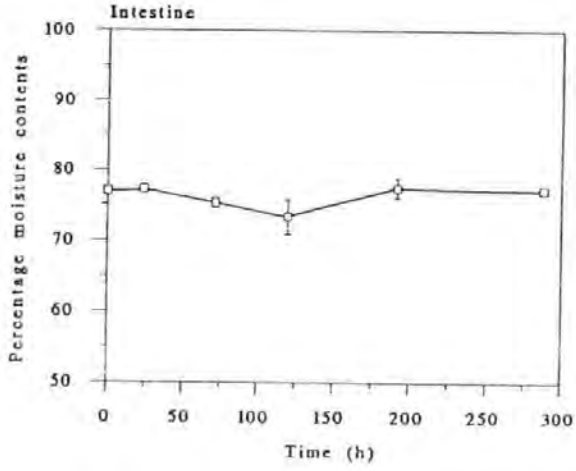
The gut moisture contents were monitored of the twenty adult *S. canicula* serially killed over the time course of gastric evacuation. The gradual increase in the liquefaction of the meal in the cardiac stomach of the dogfish is shown in figure 13a. The cardiac stomach contained approximately 58% moisture at t=0, which rose gradually to about 90% after 200 h. In contrast, the profile of pyloric stomach moisture content was generally downward from 90% at t=0 to the plateau of 70% after 125 h, before it rose again at the end of the period from 125-192 h to 85% (fig. 13b). The increase in moisture content of the cardiac stomach digesta was clearly due to the liquefaction of the meal as digestion proceeded, whilst the lowering of pyloric stomach moisture contents was the direct result of a higher proportion of dry matter entering the pyloric region. The moisture contents of the intestine digesta remained at a constant level (~76%) as did the moisture content of the faecal material in the rectum (~70%) (figs. 13 c and d).

Figures 14a-d illustrate the changes in quantity of dry matter digesta in each of the four regions of the dogfish gut over time. Also shown on each of the figures is the proportion of the total digesta content that was attributable to crude protein on a dry matter basis (determined by Kjeldahl analysis). The decrease in total dry matter contents of the cardiac stomach (fig.14a) was the same as that shown in figure 9 and so decreased exponentially. The proportion of crude protein present in the digesta decreased but not as rapidly as the total quantity of digesta. This implies that the amount of digesta crude protein increased in relation to total amount of digesta present in the cardiac stomach as time progressed. This trend is shown graphically in figure 15, where the crude protein (CP) coefficient (g dry wt. CP/ g dry wt. total) for the cardiac stomach rose steadily from about 0.53 at t=0 to 0.63 after 120 h. Figures 14 b-d all show similar traces of increases

Figure 13. Moisture contents of adult *S. canicula* from digesta recovered at known time intervals from (a) cardiac stomach (b) pyloric stomach (c) intestine and (d) rectal regions of the gastrointestinal tract.


Cardiac stomach
Percentage moisture contents
100
90
80
70
60
50
0
50
100
150
200
250
300
Time (h)


Pyloric stomach
Percentage moisture contents
100
90
80
70
60
50
0
50
100
150
200
Time (h)


Intestine
Percentage moisture contents
100
90
80
70
60
50
0
50
100
150
200
250
300
Time (h)

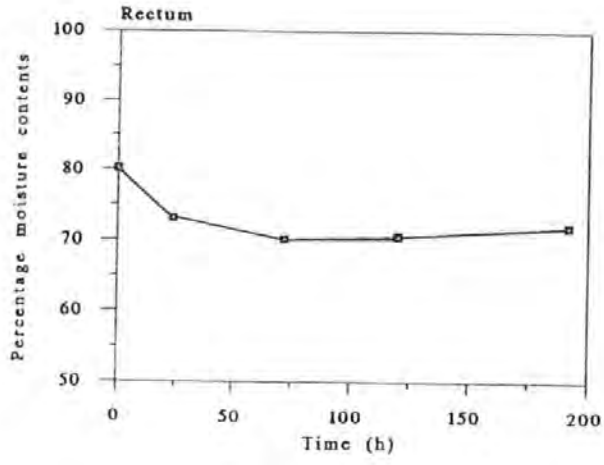
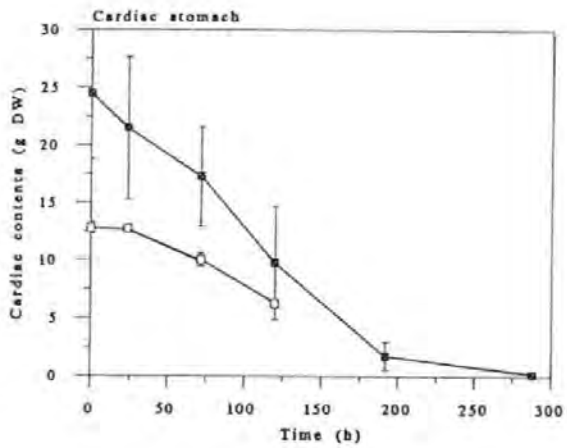
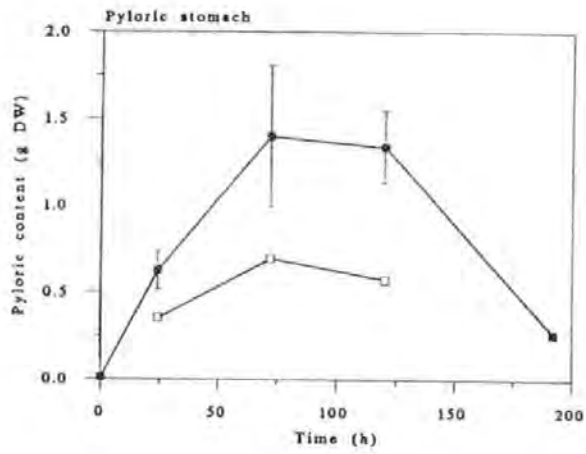
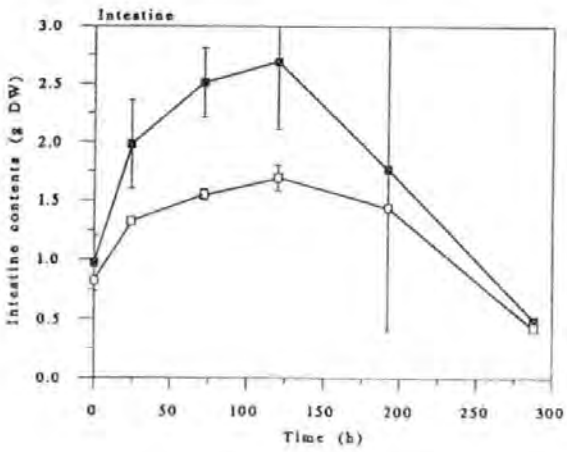

Rectum
Percentage moisture contents
100
90
80
70
60
50
0
50
100
150
200
Time (h)

Figure 14. Variation in total dry weight gut contents (■) with dry weight crude protein content (□) of adult *S. canicula* after a 7% wbw meal for the (a) cardiac, (b) pyloric, (c) intestine and (d) rectal regions of the gastrointestinal tract. Bars denote ± 1 standard error of the mean.


Cardiac stomach
Cardiac contents (g DW)
Time (h)


Pyloric stomach
Pyloric content (g DW)
Time (h)


Intestine
Intestine contents (g DW)
Time (h)

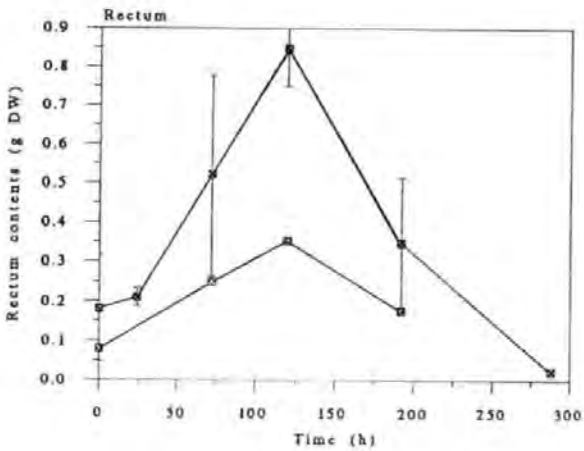
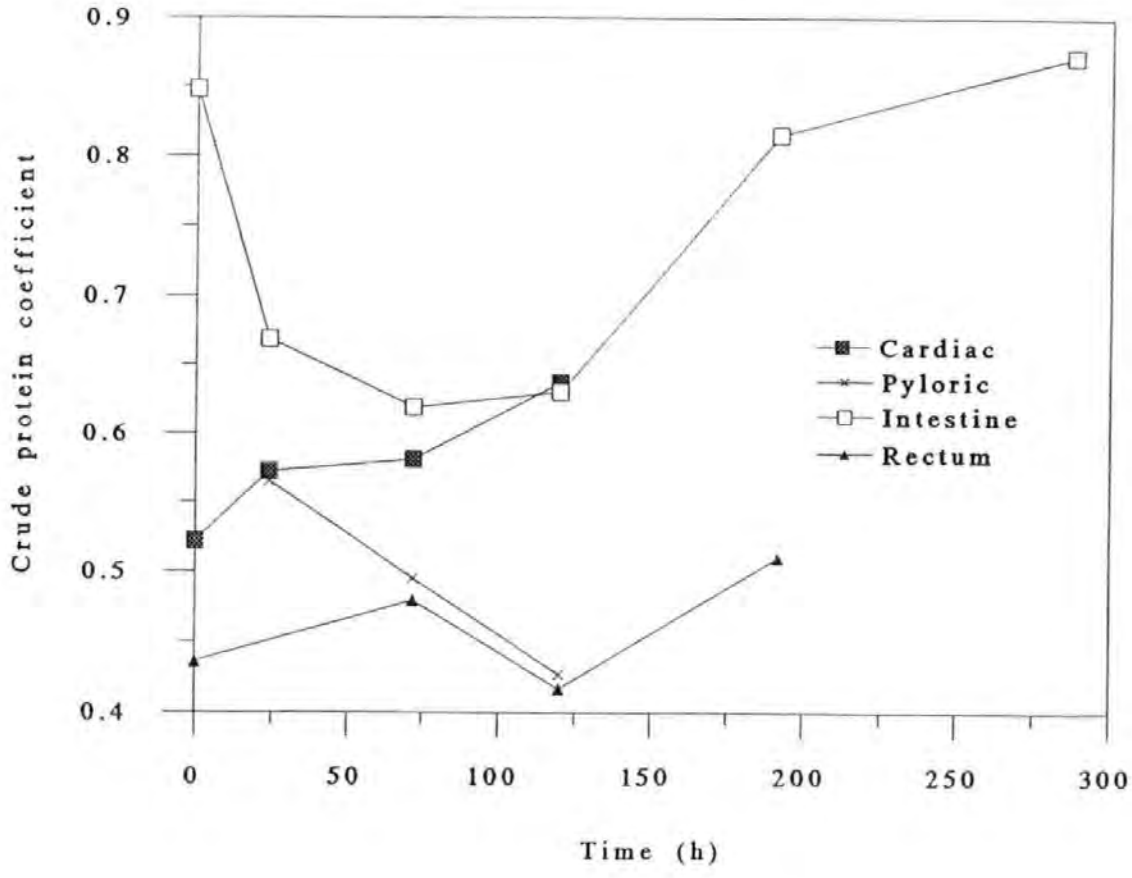

Rectum
Rectum contents (g DW)
Time (h)

Figure 15. Crude protein coefficient variation in the four regions of the gut of adult *S. canicula* after a 7% wbw meal.


0.9
0.8
0.7
0.6
0.5
0.4
Crude protein coefficient
0
50
100
150
200
250
300
Time (h)
Cardiac
Pyloric
Intestine
Rectum

in gut content. The trends were signified by a rapid rise in quantity of digesta entering the specific region of the gut, up to a maximum level between 100-125 h, followed by a gradual relative decrease in the amount of digesta flowing into each portion of the gut. The CP coefficient remained relatively constant for the faecal material recovered from the rectal region of the dogfish, however the CP coefficient in the pyloric region of the gut slowly decreased over the time course. The CP coefficient of the intestinal region showed a decrease soon after feeding and fell approximately 1.5 times the initial value after 75-125 h, before rising to the original level after complete gastric evacuation (fig. 15c). This result suggests that the crude protein component of the total digesta that was present in the intestine was progressively lowered throughout the period of gastrointestinal evacuation. A maximum difference occurred during the time directly following the period of greatest absolute stomach digesta emptying. In addition, it can be seen from figures 12 and 14c that the stomach content empties exponentially, as the amount of digesta entering the intestine increased in an exponential manner. These results suggest that the rates of digesta emptying from either portion of the gut may be interdependent.

From the chromic oxide analysis of food and faecal matter (extracted from the rectum between 72 and 120 h after feeding) the mean absorption efficiency (AE) was calculated at 42.4% ± 6.3 S.E. In addition, the apparent crude protein AE was estimated to be 99.7% between 72 and 120 h post-feeding.

3.4 DISCUSSION

Analysis of the stomach contents of over two thousand *S. canicula* during an 18 month period in a study by Lyle (1983), showed that only 1% of the stomachs were devoid of food items. This population percentage of stomachs containing food represents an extremely high value in comparison to the many other stomachs sampled from various shark species (for review see Wetherbee *et al*, 1990). The observed maintenance in dogfish stomach volume from the former study may indicate slow rates of digestion, but equally may also suggest that dogfish have evolved useful strategies relating to appetite control and subsequent food consumption. From this section of the present study on dogfish, it is clear that the pattern of gastrointestinal tract emptying is fundamental to the regulation of appetite in this shark species, and may provide evidence of appetite control strategies that might facilitate dogfish to consume food on a relatively continual basis. Generally the results indicate that gastric evacuation in dogfish is a lengthy process, though inception of these processes occurs comparatively rapidly after food consumption. Greater instantaneous gastric evacuation rates occurred after consumption of smaller meals, indicating that the pattern of emptying was dependent on the degree of stomach fullness. Over the time period there was inverse proportionality between gastric evacuation rate and rate of appetite return, as well as with the rate of increase in intestinal fullness. These overall observations along with the methods used to obtain the information will now be discussed in more detail.

The quantitative X-ray method has had variable success in accurately determining rates and times of stomach and intestinal emptying. The first study to use countable dietary markers showed uniform passage of indigestible iron particles through the digestive tract of Atlantic salmon, *Salmo salar*. For this species of fish the technique was

considered a precise, non-invasive way of estimating food consumption and subsequent evacuation rates (Talbot and Higgins, 1983). These authors validated their observations by extensive serial samples of fish gut contents and showed that particles were not selectively retained in any part of the gut, and that digesta flow was aptly estimated by profiling bead flow. Similarly, the use of polystyrene spheroids incorporated into the food given to *Limanda limanda* enabled accurate estimates of gut evacuation to be made as selective retention of beads was not demonstrated from serial X-radiography (Grove, 1986). However, Grove (1986) also showed that juvenile *Scophthalmus maximus* did selectively retain particles within their gut, meaning that the X-ray method was not directly applicable to this species. In a similar vein, Jørgensen and Jobling (1988) published "....a cautionary note" concerning the applicability of using radiopaque particles to quantify stomach contents for gastric evacuation experiments. Using Arctic charr, *Salvelinus alpinus*, these workers experienced problems in estimation of stomach contents as particles remained in the fish's stomach long after the bulk of the digesta had been evacuated.

For adult dogfish in this study, gastric evacuation rates could be quite accurately estimated from serial X-radiographs as there was no difference in digesta content of actual stomachs obtained from serially killed fish and the estimated contents from X-radiograph bead counts. Validity of estimating stomach emptying using X-radiography was also shown from there being no difference between the modelled evacuation rates calculated from actual stomach content weights and from X-ray marker estimations. The increase in relative evacuation rate with halving of meal size as was demonstrated in this study, further suggests that bead movement from the stomach was dependent on digesta flow rather than being due to other factors, such as selective or preferential retention of beads in the stomach. The X-ray method did however, lead to some underestimation of the

actual stomach contents at the higher levels of food consumption. These more marked differences obtained at greater meal sizes were not significant and so did not lead to discrepencies between the two modelled gastric evacuation rates.

Although the gastric emptying pattern was accurately monitored by bead counts from serial X-radiographs, gastrointestinal evacuation rates could not be estimated from these same X-radiographs. The glass beads did not flow uniformly past the pyloric sphincter and aggregations of beads occurred in the pyloric portion of the stomach. The beads were initially evacuated in conjunction with digesta flow, but most of the indigestible markers were retained until the digesta had fully passed into the intestine of the dogfish. Similar observations were made by Grove (1986) and Jørgensen and Jobling (1988) in that the food in the alimentary tracts of *Scophthalmus maximus* and *Salvelinus alpinus* were fully evacuated before the radiopaque markers moved through the gut. Clearly there is no scope to use measurements of differential bead flow through the intestinal region as an accurate representation of the rates of gastrointestinal evacuation.

The pattern of gastric emptying has been postulated to be of prime importance for the control of appetite in fishes (Grove *et al*, 1978; Grove and Crawford, 1980; Gwyther and Grove, 1981; Grove *et al*, 1985; Singh and Srivastava, 1985; Russell and Wootton, 1993). Hence, the model used to describe the gastric evacuation patterns is also important for interpretation of such rates with respect to appetite regulation and further food consumption. An exponential decline in stomach contents best represented the gastric evacuation of adult dogfish in the present study. The selection of a suitable model to accurately predict rates of gastric evacuation is a prime consideration towards our understanding of physiological mechanisms governing appetite regulation. Grove (1986) in his lucid discussion of gastrointestinal physiology indicated that two distinct models, both theoretically volume dependent, may result in a differing conjecture relating to the

physiological bases of appetite regulation. The current part of the present study appraised two volume dependent models (exponential and Hopkins (1966) square root) and it was evident that the allometric assumptions inherent to the square root model could not be applied to *S. canicula*. Persson (1986) demonstrated the inadequacies of Jobling's (1981) reworking of Hopkins' original model by measuring the stomach dimensions of individual perch, *Perca fluviatilis* after they had been fed different amounts of food. For the application of the square root model, the length of the stomach is assumed to remain constant so that the rate of evacuation is solely proportional to the square root of the volume of a radially distending cylinder with constant length. In both this study on dogfish and the Persson study on perch, the length of stomachs varied with the weight of food consumed according to a power relationship. Thus, the square root model was not applicable to describe gastric evacuation for dogfish in the present study as the assumption of the model was unjustifiable.

Three previous investigations on elasmobranchs have also applied exponential relationships to model gastric emptying. Schurdak and Gruber (1989) showed that *N. brevirostris* exhibited exponential gastric evacuation when fed on white fish fillets of blue runner, *Caranx chrysos*. It has been noted that exponential declines in stomach content are more likely to occur when easily digestible, small prey items are ingested (Jobling, 1986). Linear or curvilinear relationships (usually incorporating a lag phase) (dos Santos and Jobling, 1992) have mostly been shown to best represent the emptying rate of proportionately larger prey items that might be more difficult to digest. This viewpoint has been put forward and used to substantiate the linear gastric evacuation models applied to the lemon shark study of Cortes (1987, cited in Wetherbee *et al*, 1990). Cortes explained (E. Cortes, pers. comm.), that the particle sizes used in his experiments were larger than in the study of Schurdak and Gruber (1989), thus the evacuation rate was more likely to

be linear. However, it becomes apparent that the Cortes data was rather scant towards the end of the sharks' stomach emptying period and so a number of pertinent models could have been adequately fitted, including an exponential decay pattern. San Filippo (1993) also demonstrated that gray smoothhound sharks, *M. californicus* evacuated ingested crabs from their stomachs in an exponential manner. In a study that appraised the validity of several volume and surface dependent models to best describe the evacuation rate of sub-adult *S. canicula*, Macpherson *et al* (1989) showed the exponential model was adequate to describe the evacuation characteristics of small easily digested prey items (as originally stated by Jobling, 1986). These researchers added, however, that the surface dependent model was more appropriate for this species when two or three larger items, more impervious to digestion occurred in the stomach. Therefore, application of an exponential model to predict dogfish gastric evacuation when fed a squid based diet is supported by previous studies.

The total times for gastric evacuation in the dogfish at the higher ration levels were very long in comparison to other elasmobranchs, but are important to consider in conjunction with appetite return times, because they may indicate whether the period of gastric evacuation is common with that of appetite return and therefore perhaps suggest some regulatory interdependence. Temperature influences gastric evacuation rate and time to a high degree *e.g.* Jobling and Davies (1979) and Ross and Jauncey (1981), so only investigations undertaken at an experimental temperature similar to this study (15°C), will be considered further. The spurdog, *S. acanthias* when force fed herring took 124 h to reduce the food to a fluid state at 10°C (Jones and Geen, 1977). In an earlier investigation on the stomach emptying rates of *S. acanthias* Van Slyke and White (1911) noted that the time for 100% stomach evacuation was greater than 48 h when the fish were fed chopped beef at 15°C. Smoothhound sharks, *M. californicus* held at 15°C cleared their stomachs

of blue crabs after approximately 60-70 h and the guts of the sharks were observed to be completely empty after 95 h (San Filippo, 1993). The adult sharks in the present study took 83 and 201 h to clear their stomachs of the squid based diet at ration levels of 3.5 and 7% wbw respectively. From investigations listed in table II of Fänge and Grove (1979), the mean gastric evacuation time for 18 different species of teleost fish fed variable meal sizes was only 28 h at 15°C. Clearly then, the time for complete stomach emptying was much greater in the elasmobranchs compared to the teleosts, but dogfish values were quite lengthy in comparison to other studies on sharks carried out at a similar temperature.

The total gastric evacuation time of dogfish given 7% wbw meals correlated well with the cessation period of appetite return after a similar sized meal. Thus, the appetite of the dogfish was judged to be greatest when the stomach was nearly empty. Other studies using teleost fish have showed that appetite was greatest when the stomach was nearly empty (Grove *et al*, 1978; Grove *et al*, 1985), and emphasised the importance that the stomach evacuation pattern itself may have on the regulation of appetite. Dogfish appetite return may be governed primarily by the emptiness of the stomach (to whatever degree), and evidence for this is obtained from total gastric emptying times coinciding with the levels of greatest appetite return (food intake). Although determination of evacuation times is important to elucidate correlation with appetite return measurements, it is the *pattern* of gastric and gastrointestinal emptying that might be most significant for investigating the role of gut emptying in appetite control.

The form of the gastric emptying curves obtained for adult dogfish suggest certain physiological mechanisms that may be operating in this shark to regulate appetite in such a way as to maximise the potential for future food consumption. A previous study demonstrated time lags preceding the start of gastric evacuation in juvenile sandbar

sharks, *C. plumbeus* (Medved, 1985). Other studies on various teleost species have also shown the existence of such time delays to stomach emptying, *e.g. Stizostedion vitreum vitreum* (Swenson and Smith, 1973), *S. maximus* (Grove *et al*, 1985) and *Coregonus lavaretus* (Rösch, 1987). Medved (1985) stated that the lag phase to gastric evacuation represented the time needed for the digestive juices to attack and begin to break down the resistent integument of the prey. In addition it was conjectured that enzyme reactions were exponential in nature and might therefore be expected to start slowly. The latter supposition may not be entirely accurate in the majority of instances, as most gastric evacuation studies on fish have not observed long lag periods preluding stomach emptying, so curvilinear models of evacuation have usually been fitted that do not incorporate a gastric delay period, *e.g. Merlangius merlangus* (Bromley, 1988), *Salmo trutta* (Elliott, 1991). In fact, Jobling (1986) suggests that these lag phases have only generally been shown to occur when extremely large meal sizes have been administered to experimental fish.

In adult dogfish, food was seen to enter the intestine after as little time as 1.5 h when fed at the higher ration level of 7% wbw. In the Medved (1985) study on sandbar sharks, the lag phase was seen to last for approximately one tenth of the overall evacuation time compared to under one hundredth of the total time for dogfish stomach emptying in this study. In addition, *S. canicula* of the present study (which were similar in size to the sandbar sharks) were fed meals seven times larger than the sharks in Medved's study. These differences in initial gastric evacuation time illustrate that the dogfish are able to mobilize the stomach contents relatively quickly. The rate of initial appetite return at high levels of feeding is very rapid in the dogfish. This rapid appetite recovery may be achieved by shunting (presumably) undigested food into the intestine quite soon after initial food consumption so that some appetitive response returns. The

absence of a lag phase was observed in the rate of appetite return in the dogfish (chapter III) and no concomitant lag phase was observed in the rate of gastric evacuation at higher ration levels. These results suggest that dogfish recover appetite by rapid commencement of gastric emptying when the stomachs were quite full of food. Similar observations, of shunting undigested food into the intestine without prior disintegration in the stomach were noted for *L. limanda* (Gwyther and Grove, 1981; Grove *et al*, 1985). I suggest that this shunting may occur in dogfish as a physiological response to high levels of food intake, leading to appetite recovery so that more food could be rapidly consumed. Shunting undigested food into the intestine soon after consumption may be more likely to occur as a direct stimulus of food bulk. Greater distension of the stomach wall may initiate stimulation of gastric stretch receptors resulting in greater muscle contraction amplitude (Jobling, 1986). With ingestion of large meals it might therefore be likely that shunting would occur, but possibly not at smaller meal volumes where the stomach is distended to a lesser degree. In the present study, no shunting of undigested food appeared to occur when dogfish consumed meals of 3.5% wbw. This implies that the faster emptying response that results in a rapid appetite recovery, is a weight dependent response, and so the regulatory stimulus may originate from greater stomach distension and the influence of gastric mechanoreceptors (Leek, 1972).

The overall form of the dogfish gastric emptying curve is exponential, with the greatest relative rates of evacuation occurring in the first half of the evacuation period (0-100 h at 7% wbw), followed by a slowing of digesta output in the latter half of the evacuation period. Bromley (1994) states that for an exponential decay pattern the instantaneous evacuation rate is proportional to the amount remaining in the stomach. Therefore, the exponential rates of dogfish gastric emptying combined with the increase in relative evacuation rate of smaller meals indicates that dogfish have a weight dependent

gastric emptying pattern related to the degree of stomach fullness. The results of this study showed an inverse proportionality between the rate of gastric evacuation and the rate of appetite return. As the stomach of the dogfish emptied the appetite returned concomitantly. Similar findings to those of the present study have been obtained for some teleost species, namely *S. trutta* (Elliott, 1975), *S. gairdneri* (*O. mykiss*) (Grove *et al*, 1978), *S. maximus* (Grove *et al*, 1985), *H. fossilis* (Singh and Srivastava, 1985) and *P. phoxinus* (Russell and Wootton, 1993). These authors all showed that appetite return was proportional to the degree of stomach emptiness. From these investigations and for dogfish in the current study, it is suggested that the amount of food in the stomach itself controls the pattern of emptying, thereby directly affecting appetite and further food consumption. The main physiological basis leading to these processes of appetite recovery may be the action of gastric stretch receptors (Leek, 1972; Jobling, 1986). The importance of gastric emptying patterns to the feeding regulation of an opportunist predator, such as the dogfish (Lyle, 1983), which maintains a high level of stomach fullness, might be expected. The present study represents the first proposal however, that such patterns in gastric emptying may itself influence the regulation of appetite in an elasmobranch species.

It has been shown for the teleost *C. lavaretus*, that as the ration size given to the fish was increased the food utilization efficiency decreased (Rösch, 1987). This implies that the greater absolute evacuation rates (g h^{-1}), that occur when larger meals are emptied from fish stomachs may lower the efficiency with which the nutrients can be digested and absorbed. The intestine region of the gut enables the absorption of nutrients and so therefore may possibly be co-factorial in regulating the rate at which the stomach is emptied. Such an interplay between different portions of the gut has been postulated as occurring via feedback loops (Vahl, 1979; Jobling, 1986). As a consequence of this, the intestine could be considered integral to the functioning and possible regulation of gastric

emptying, and thus by causation, in the processes leading to appetite control.

The transit of digesta from the cardiac stomach of dogfish was exponential in nature and showed inverse proportionality with the magnitude of appetite return. During the first half of the total gastric evacuation period, when the relative rate of stomach contents emptying was greatest, the increase in intestinal fullness was also exponential. The profile of intestinal filling increased exponentially however, and in direct proportion to the manner of decrease in dogfish stomach contents. When the rate of gastric evacuation slowed, an expected decrease in the amount of digesta entering the intestine was observed. It may be possible to postulate that just as the rate of appetite return can be linked to stomach emptying, so might the influence of intestinal fullness in part regulate appetite by a reflex inhibition of gastric emptying (Daniel and Wiebe, 1966). The intestine may also be important to food intake control by regulating the movement of digesta from the stomach. Jobling (1986) was able to confirm the effect of gastric distension on the amplitude of gastric musculature contractions of *Pleuronectes platessa*. This author demonstrated that the contraction amplitude increased in relation to stomach volume in this species. Jobling suggested that for fish, the pattern of gastric emptying would be affected to a great extent by gastrointestinal receptors that might be responsible for the feedback control of gastrointestinal emptying, and thus the regulation of feeding itself. In the present study on dogfish, the suggestion that the mechanical characteristics of both the stomach and the intestine regulate appetite and further feeding bouts, accords with earlier work on mammal gut mechanoreceptors.

In mammals, it is known that gastric emptying is regulated by feedback mechanisms dependent to a varying degree on (1) smooth musculature contractions of the stomach wall and (2) contractions of the pyloric and intestinal musculature (Jobling, 1986). Daniel and Wiebe (1966) used anaesthetised dogs to investigate the transmission

of reflexes across the gastroduodenal junction. They demonstrated that gastric distension had a reflex inhibitory effect on the electrical and mechanical activity of the duodenum. Further to this they showed that duodenal distension caused a decrease in tone and peristaltic activity of the stomach, including the pyloric antrum and sphincter (Daniel and Wiebe, 1966). The responses were abolished by chemical sympathectomy and bilateral vagotomy, causing Leek (1972) to describe the stomach-intestine inhibitory reflex as an extrinsic one. Studies of this kind on mammals provided strong evidence for the involvement of *in series* tension receptors in the control of gastrointestinal emptying (Iggo, 1957; Leek, 1972), even though the precise mechanisms by which these effects were mediated were little understood (Jobling, 1986). Both hormonal and extrinsic nerve pathways have also been implicated as possible effectors (Jobling, 1986).

Inhibitory reflexes may constitute the mechanical feedback loops necessary for gastrointestinal emptying regulation in dogfish. Control of gastric emptying leading to regulation of appetite and further food consumption may be brought about by a gastric-intestine distension antagonism as has previously been demonstrated for mammals. Although inhibitory reflexes occur in mammals, and despite the organization of the gut of *S. canicula* having certain similarities to that of carnivorous mammals (Andrews and Young, 1993), the same mechanisms governing gastro-intestinal interplay cannot be assumed for dogfish. The gastric motility patterns of adult *S. canicula* were studied *in situ* by Andrews and Young (1993). They observed very little spontaneous activity of the gut and noted (somewhat to their surprise) that no peristaltic contractions passed over the stomach and continued to the spiral intestine. Similar observations were made for *M. canis* by Alvarez (1927). It is possible that the sphincter located between the stomach and the spiral intestine is paramount to the regulation of gastric emptying and its function may be influenced by tension mechanoreceptors located in the spiral intestine.

From the results of this chapter I have proposed that appetite is regulated by the stomach emptying of digesta in a weight dependent manner, possibly by the action of gastric tension mechanoreceptors. The exponential decrease in stomach contents of dogfish was mirrored by a consequential exponential increase in intestinal contents, so it may equally be possible that the gastric emptying pattern of dogfish is affected by the weight dependent rate of intestinal filling. Although interactions of this kind may not represent a strict inhibitory reflex pattern and certainly no such claims have been reported for *S. canicula* (Andrews and Young, 1993), the spiral intestine may influence the motor activity patterns of the stomach through regulation of the smooth muscle constriction known as the pylorus. Further research on the motor activity patterns of the spiral intestine in conjunction with gastric motility studies might explain how appetite may, in part be regulated.

The absorption efficiency of dogfish (on a dry matter basis) was low in comparison to values obtained from studies on the lemon shark, *N. brevirostris*. Wetherbee and Gruber (1993) noted that lemon sharks were as efficient as teleosts in absorbing nutrients from the food, with values ranging from 76 to 87% on a dry matter basis. The absorption efficiency of dogfish was approximately half the upper value obtained for lemon sharks. An explanation for this may be afforded by Cortes and Gruber (1994), who suggest that at high levels of feeding the absorption efficiency of the lemon shark will decrease. It is likely that the feeding level in the present study (7% wbw) was high enough and gastric evacuation relatively too rapid for efficient absorption of nutrients from the digesta. However, it is also likely that the absorption efficiency of a weight dependent feeder (as proposed here) would in fact be low in comparison to shark species that are known to feed intermittently (Wetherbee *et al*, 1990). Some teleost fish species, *e.g. S. maximus* have been shown to adjust their level of intake according to the energy density of the food

(Flowerdew and Grove, 1979; Grove *et al*, 1985). The current study provides some evidence that more easily digested components of the diet were digested first, because the crude protein coefficient was seen to increase in the stomach throughout the period of gastric emptying. Therefore, does this indicate that the regulation of dogfish appetite may be influenced by the energy density of the food rather than solely by its physical presence? Adjustment of food intake according to energy value of the food would perhaps, not be expected in a weight dependent feeding strategy, as I have so far suggested for dogfish appetite regulation. If the dogfish was able to elevate or decrease food consumption depending on the quality of the meal consumed, then the physiological mechanisms relating to this energostatic response may have important connotations in assessment of the physiological factors regulating appetite.

CHAPTER FOUR

DIETARY DIGESTIBLE ENERGY AND APPETITE

4.1 INTRODUCTION

A large number of biotic and abiotic factors interact to alter the rate at which food is processed by animals. As well as the main controlling factors such as body size and temperature affecting gastric emptying rate in fishes (Brett and Groves, 1979; Fänge and Grove, 1979), the physical and chemical characteristics of the ingested meal also affect alimentary tract emptying rates (Jobling, 1987). The overall meal size, number of individual items, quantity of food and the interaction of multiple meals are important factors that may modify food consumption regulation (Fletcher *et al*, 1984; Jobling, 1987). The effects of meal size and number of individual food items consumed by fish have received the most attention from the literature in this context. The capacity for meal quality to modify gastric evacuation was first suggested for fish by Hess and Rainwater (1939), and more recently its role in determining food consumption rates of a variety of species has been the subject of more detailed research, *e.g. Sarotherodon mossambicus* (*Oreochromis mossambicus*) (De Silva and Owoyemi, 1983), *Salvelinus alpinus* (Jobling and Wandsvik, 1983) and *Sebastes melanops* (Brodeur, 1984).

In mammals, infusion of specific nutrients, for example glucose, into the stomach have been associated with subsequent reductions in food intake by an equicalorific amount (Fletcher, 1984). Additionally, the energy density of meals given to mammals have been directly linked to the degree of slowing of the gastric evacuation process (Hunt, 1980). Nutrient dense meals have been demonstrated to take longer to evacuate from the gastrointestinal tract of mammals, as high energy value components of the meal take

longer to digest and absorb than meals containing non-nutrient bulk (Jobling, 1986). Many fish studies exist that have investigated dietary formulations for the optimal balance of specific nutrients, *e.g.* macronutrients (for reviews see Wilson, 1989; Sargent *et al*, 1989; Cowey and Walton, 1989) and micronutrients (for reviews see Halver, 1989; Lall, 1989), with a view to improving fish growth for aquaculture. Investigations of this kind have indirectly given evidence of food intake control by occasionally stating that food consumption was increased when fish were given diets with varying ratios of specific nutrients. Few studies have been completed on fish however, where the aims were to investigate directly the role of total dietary energy on the regulation of food intake.

From the investigations on teleosts that have been undertaken, most studies used food mixed with non-nutrient diluents (*e.g.* kaolin) and noted that in some species the level of food intake increased when food of lower total nutrient energy content was consumed (Rozin and Mayer, 1961; Grove *et al*, 1978). In contrast, another study indicated that dietary energy dilution did not change the level of food consumption (Gwyther and Grove, 1981). These authors interpreted the food intake adjustments as being due to whether the fish species under examination were able to compensate their consumption rates according to the total energy content of the meal. The main criticisms levelled at these interpretations have been that the actual ratio of dietary nutrients remains unchanged by dilution, so in addition to the effect of total dietary energy on food intake, other factors important in food intake control may have been operating (Jobling and Wandsvik, 1983). The digestibilities of protein, lipid and carbohydrate are known to change depending on level of feeding and inclusion in the diet (Windell *et al*, 1978; Ellis and Smith, 1984). Therefore the importance of total available dietary energy in appetite regulation has been questioned (Jobling and Wandsvik, 1983). It has been postulated that physiological detection of the digested components of the meal is a more likely pathway

for possible regulation of appetite. This is logical if the systems that may enable such reception are considered. Mammalian research in this area has led to the hypotheses that digestion products are detected by receptors located in the upper intestine (Hunt, 1980). Several authors have proposed that similar systems of food intake regulation may operate in fish (Jobling and Wandsvik, 1983; Bromley, 1987). In these cases the digestible energy, that portion of ingested energy actually digested and absorbed by the fish, may therefore be more likely to influence gastrointestinal evacuation if actual levels of digestion products were detectable.

The importance of digestible energy on food intake has been indirectly investigated in teleosts by aquaculturists who wished to assess the digestibility of various components of the diet in order to achieve better growth performances (Tabachek, 1986; Hemre *et al*, 1990). The effect of varying digestible energy levels on the food consumption characteristics of an elasmobranch have not previously been examined. Therefore, the purpose of these investigations was to evaluate how different dietary levels of digestible energy may affect the rate of gastric emptying and how possible changes in gastrointestinal physiology may manifest as overall adjustments in the amount of food consumed. It was hoped that by investigating the effect of digestible energy on food intake and gastric emptying, other possible pathways for appetite regulation in sharks may become evident.

4.2 MATERIALS AND METHODS

4.2.1 Manufacture of High Energy Diet (HE)

The formulation of the HE diet was the same as that described in section 3.2.1 and 3.2.2, except that 20% of the dry component made up from cornstarch (8% of the total composition), was directly substituted with marine oil (cod liver oil). The diet containing 8% cornstarch was termed "low energy" (LE) and the diet with 8% oil was referred to as "high energy" (HE). The comparison in composition of the two diets is given in the first part of table 7 (p. 144).

4.2.2 Gastric Evacuation Studies

The group of number tagged adult dogfish used to determine the gastrointestinal emptying patterns of the LE diet (details given in section 3.2.6) were also given the HE diet in subsequent trials at 3.5 and 7% wbw ration levels. The procedure of anaesthetisation and X-radiography used previously when these fish were fed meals of the LE diet, was followed for the trials with HE diet. Two trials were conducted at each ration level, giving four determinations at each 24 h time interval.

The percentage of food remaining in the stomach of each dogfish was calculated in the same way as described previously, and statistical interpretation was similar for the investigation outlined in section 3.2.8.

4.2.3 Feeding Trials

The groups of thirteen juvenile and eight adult dogfish used in the daily food intake investigations of chapter II (described in section 2.2.1) were used for two further feeding trials. A few days after the dogfish had last been fed on chopped squid they were fed

moist pellets of the LE diet to satiation every second day for approximately 30 days. After 3-4 further days the dogfish were given the HE diet to satiation every second day, again for a period of about 30 days. Both size groups of *S. canicula* were each fed at the same time of day throughout the feeding trials and the fish were routinely weighed during the non-feeding days between each trial. The moist pellets of the two experimental diets were chopped and thawed in the usual way and soaked in squid juice before being presented to the dogfish. Some faecal pellets were collected from the bottom of the tanks during each trial. The faecal samples were dried to constant weight in an oven at 110°C and subsequently ground to a fine powder with a pestle and mortar. The faecal powder samples were stored in glass vials within a dessicator prior to chemical analysis.

4.2.4 Analysis of Diets and Faeces

4.2.4.1 Estimation of dietary nitrogen and crude protein (CP)

The percentage nitrogen (N) or crude protein (N x 6.25) present in the HE experimental diet was determined by the classical Kjeldahl method according to the procedure used previously in section 3.2.5.2 to estimate the nitrogen present in the LE diet.

4.2.4.2 Calorimetry of diet and faecal samples

The energetic value of dried samples of the LE and HE diet as well as dogfish faecal samples from both dietary treatments were estimated by adiabatic bomb calorimetry. A Gallenkamp automatic adiabatic bomb calorimeter was used for all the energy estimations and benzoic acid was used as the standard. Approximately 1g of diet powder was pressed into a pellet and suspended by gun cotton from a platinum wire connecting the anode and cathode inside the bomb. Absorption of the combustion gases was achieved

by inclusion of 1ml of water in the bottom of the bomb. The bomb was then charged with pure oxygen to 30 bar pressure and immersed in a water jacket of known temperature. The bomb was fired and the maximum temperature reached by the water jacket was recorded.

The energetic value of the benzoic acid standard and the diet and faecal samples was calculated using the following formula,

$$E_S = \frac{\Delta_t S \,/\, \Delta_{t/g} B \times E_B}{W}$$

where E_S represents the energy value of the sample in kJ g^{-1}, $\Delta_t S$ is the temperature change in °C due to combustion of the sample, $\Delta_{t/g} B$ denotes the temperature change due to the combustion of 1g of benzoic acid while E_B is the energy value of 1g of benzoic acid standard in kJ g^{-1}. W is the weight of the sample.

Dried faecal samples were not large enough for a suitable pellet (~1g) to be made for combustion. In these instances benzoic acid was added to the powdered faecal material and a composite pellet was pressed. Thus, the energetic value of the faecal material was obtained by difference between the temperature rise due to the proportion of benzoic acid combusted and the overall temperature rise observed. The difference was attributable to the combustion of the faecal material present in the composite pellet.

Five sample replicates of each diet type and two faecal samples from each dietary treatment at the 7% wbw feeding level were completed.

4.2.5 Energy Calculations

4.2.5.1 Digestible energy, D_E

The energy actually absorbed by the dogfish, termed the digestible energy D_E, was calculated from the relationship,

$$D_E = I_E - F_E$$

where I_E is the gross ingested energy and F_E the energy content of the faeces produced.

The amount of faecal material produced was established from section 3.3 (figure 14d) for a 7% wbw meal of the LE diet. Thus, the value for the dry weight of faeces produced was taken from the previous chapter (2.13g dry wt.) and by multiplication by the energetic value for 1g dry weight of the faeces, D_E was established. The assumption was made that the amount of faeces produced by the adult dogfish was the same for a 7% wbw meal of both LE and HE diets.

4.2.5.2 Empirical derivation of D_E

In addition to the calculation of D_E from actual energy values of dry weight samples of diet and faeces, D_E was also calculated from stated values of the digestibility of the macronutrients (protein, lipid and carbohydrate). The calculation of D_E by this method for dogfish that consumed 7% wbw meals of LE and HE diets is given in appendix I.

4.2.6 Statistical Analysis

The levels of food intake of the two diet types by adult and juvenile dogfish were compared within each size group by Student's t-test.

4.3 RESULTS

4.3.1 Diet Composition and Utilisation

The percentage moisture and crude protein components of the LE and HE diets were very similar (table 7). However, due to 100% substitution of cornstarch with marine oil the energy content of the HE diet was 1.92 kJ g^{-1} dry weight higher than the LE diet (table 7). Table 7 also shows the utilisation characteristics of the two diets and it is clear that the level of gross ingested energy was approximately 20 kJ $fish^{-1}$ higher in the dogfish fed the HE compared to the LE diet. The calculated values for the total energy ingested for fish on each diet type (appendix 1) were in close agreement with the actual energy contents determined by calorimetry (table 7). The amount of energy theoretically available in the non-protein portion of the HE diet was approximately twice the level in the LE diet. From bomb calorimetry of faecal samples, the digestible energy of the LE diet was 64%, whereas 91% of the energy in the consumed HE meal was apparently digested by the dogfish. Empirical calculation of the digestible energy of the LE and HE diets from stated macronutrient digestibility values (from other fish species), gave D_E values in the same order of magnitude as those calculated from diet and faecal samples (table 7). However, probably not all the faecal pellets were collected in the serial slaughter study of chapter III, therefore these apparent levels of digested energy may be higher than actually occurred. Even so, the relative difference in D_E between the diets was essential, and the D_E levels were clearly substantially different.

4.3.2 Gastric Emptying Patterns

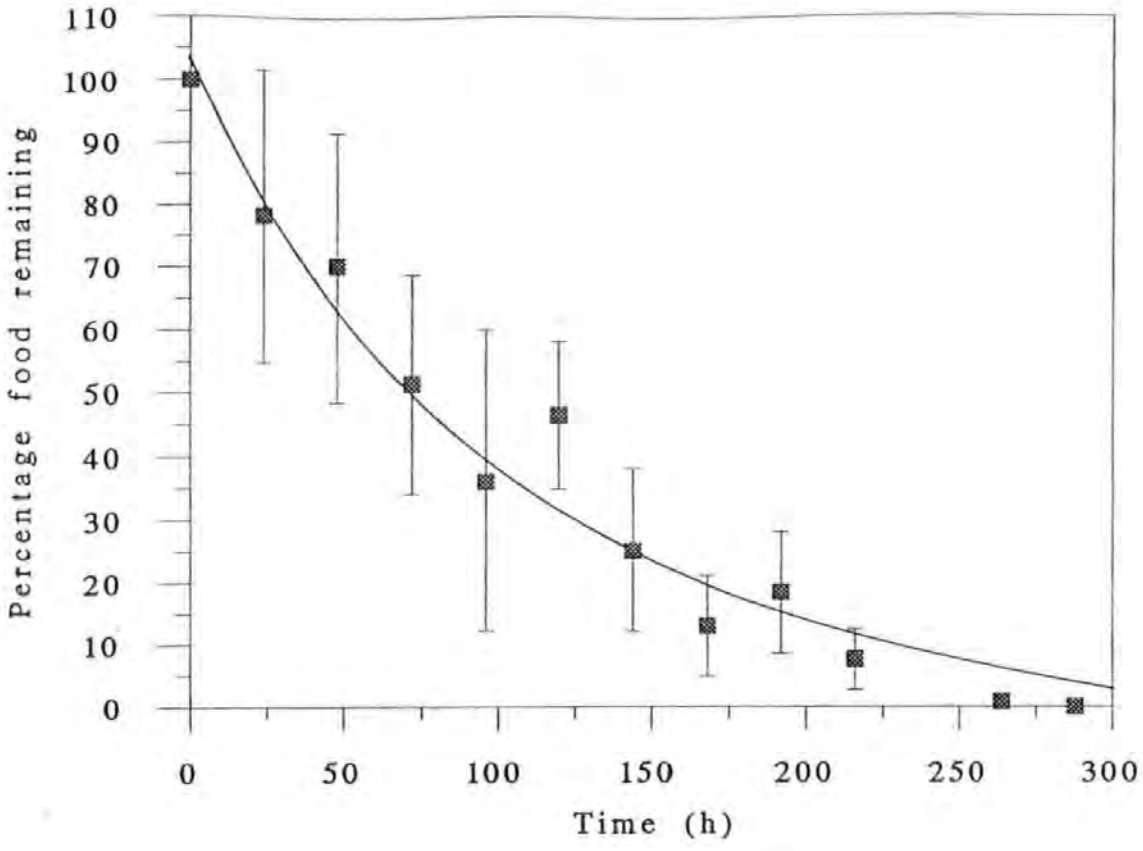
The exponential rates of decline in stomach contents of adult dogfish when fed LE and HE pellets (in separate trials) (figures 16 and 17) were not significantly different at either

Table 7. Composition and utilisation characteristics of low and high energy diets given to adult dogfish during the food intake trials.

	Composition of experimental diets	
	Low energy (LE)	High energy (HE)
Moisture (%)	52.0	52.1
Crude protein (%)	55.5	55.2
Marine oil inclusion (%)	-	8.0
Cornstarch inclusion (%)	8.0	-
Energy (kJ g^{-1} dry wt.)	21.54	23.46
	Utilization of energy (kJ $fish^{-1}$) *	
Total ingested energy	527.95	547.56
Total ingested energy (by empirical derivation)	499.82	551.14
Gross ingested energy: non-protein (by empirical derivation)	88.49	164.87
Digestible energy, D_E	338.51	499.38
D_E (by empirical derivation)	398.65	464.15

**Represents the utilisation characteristics after a 7% wbw meal.*

Figure 16. Gastric evacuation rate of adult dogfish fed a 7% wbw meal of low (upper panel, LE) and high energy (lower panel, HE) diets. Regression equation: LE, $S_t=102.2e^{-0.010t}$, $r^2=0.96$; HE, $S_t=102.2e^{-0.009t}$, $r^2=0.95$. Number of determinations for each point, 4. Bars represent ± 1 S.E.M.


Percentage food remaining
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (h)

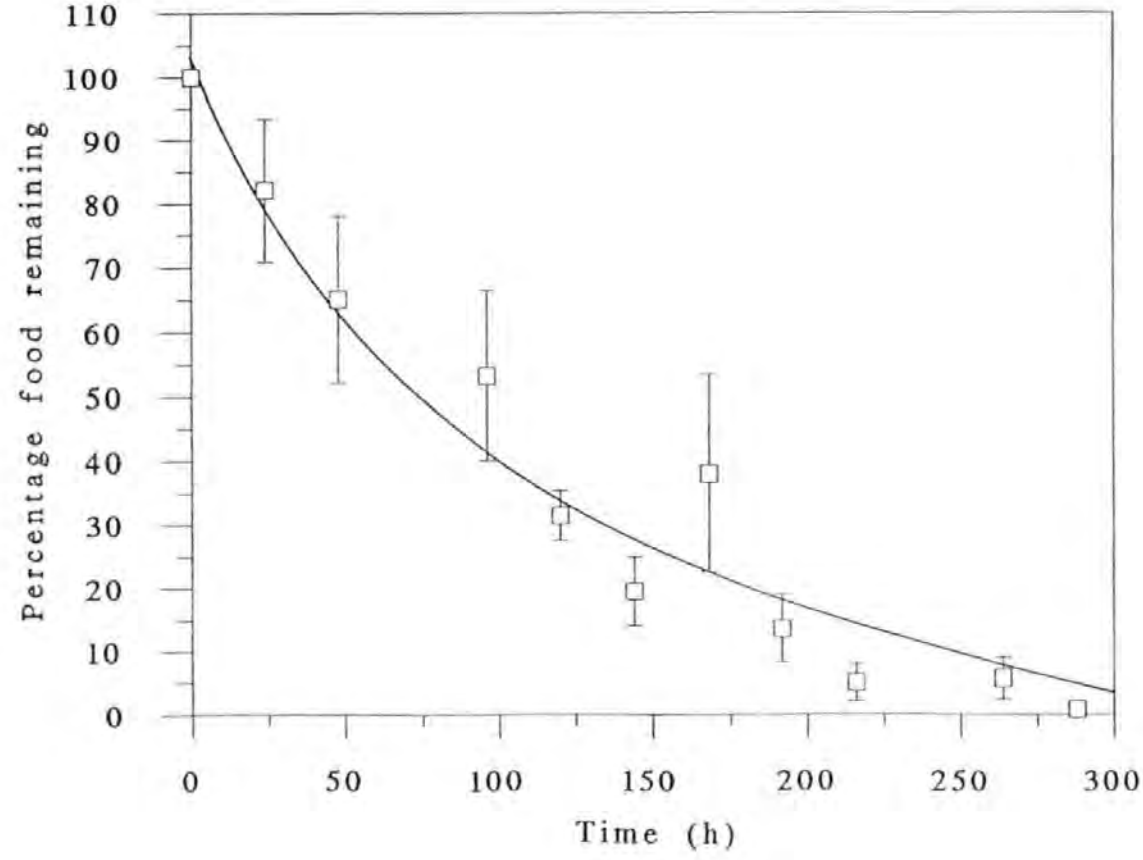
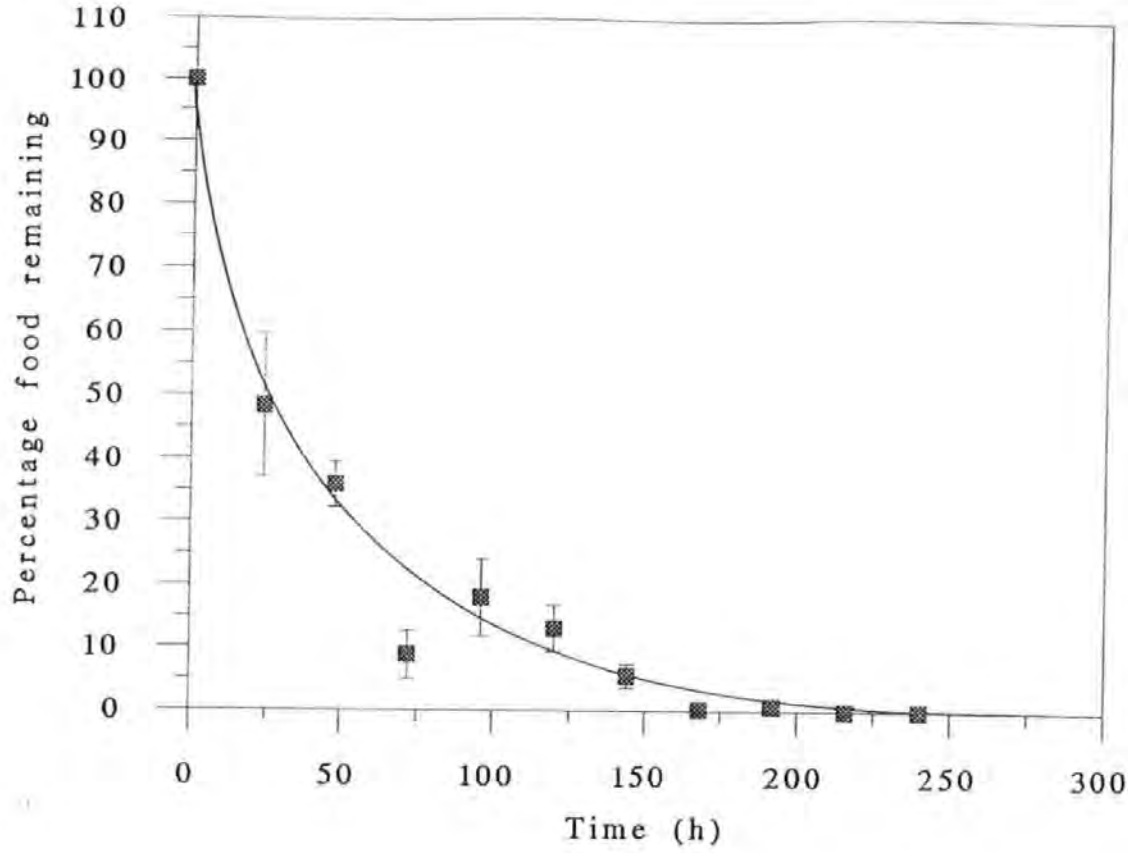
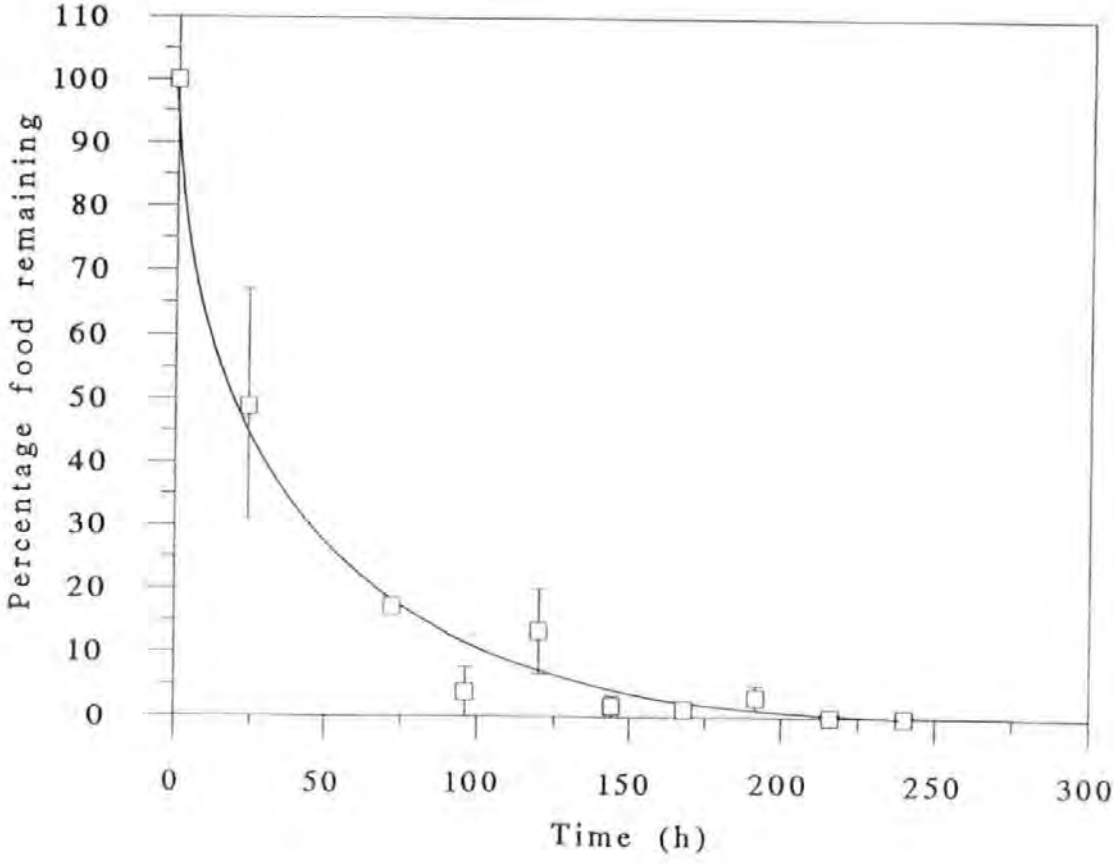

Percentage food remaining
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (h)

Figure 17. Gastric evacuation rate of adult dogfish fed a 3.5% wbw meal of low (upper panel, LE) and high energy (lower panel, HE) diets. Regression equations: LE, $S_t=97.4e^{-0.024t}$, $r^2=0.97$; HE, $S_t=98.6e^{-0.026t}$, $r^2=0.99$. Number of determinations for each point, 4. Bars represent ± 1 S.E.M.


Percentage food remaining
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (h)


Percentage food remaining
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300
Time (h)

3.5 and 7% wbw ration levels (table 8). The instantaneous relative rate of gastric evacuation increased with a decrease in the meal size of the HE diet given. These results were the same as those observed with LE diet (chapter III). The six potential models fitted to both diet treatments at 3.5% wbw ration level, slightly underestimated *a*, the intercept on the *y* axis, whilst the models fitted to the higher ration levels, slightly overestimated the intercept (table 8). The overall times for gastric emptying were similar for both LE and HE treatments at both ration levels (figures 16 and 17). From the X-radiographs, there was no observable difference in the total gastrointestinal evacuation time of dogfish fed the LE and HE diets.

4.3.3 Food Intake Patterns

The level of food intake of juvenile dogfish on a wet matter basis decreased by about 1.5% wbw when the diet of the fish was changed from chopped squid to moist LE pellets (figure 18). The food intake patterns of juvenile dogfish fed LE pellets, and subsequently HE pellets were similar in that they fluctuated quite widely about a common level (figure 18). The mean food intake level for LE and HE diets given to juveniles was 2.4 and 2.3% wbw respectively. These levels of feeding were not significantly different ($P>0.2$, table 9). From figure 18, the lowest levels of food intake usually followed the highest levels of food intake and this pattern was similar for juveniles fed squid, LE and HE diets. Although the levels of juvenile dogfish food consumption were different between squid and LE/HE diets on a wet matter basis, the dry matter daily food intakes for all three diets were not different ($P>0.5$, table 9) and ranged from 0.06-0.07 g food dry wt. 10g wet fish^{-1} d^{-1} (table 9).

Food consumption of adult dogfish was maintained at a relatively constant level when the food was changed from squid to LE to HE pellets (figure 19). Wide fluctuations

Table 8. Fitted exponential model parameters for adult dogfish gastric evacuation when fed low and high energy diets at two ration levels.

	Meal size: 3.5% wbw		7% wbw	
	LE	HE	LE	HE
y intercept	97.4	98.6	102.2	102.2
Instantaneous rate of gastric evacuation	-0.024*	-0.026*	-0.010†	-0.009†
Regression coefficient (r^2)	0.97	0.99	0.96	0.95
RMS	30.6	16.0	44.5	66.5

**denotes values not significantly different (ANCOVA), P>0.75*

†denotes values not significantly different (ANCOVA), P>0.50

Figure 18. Juvenile dogfish daily food intake of squid (■) and the food intake patterns when fed every two days on low (□) and high (◆) energy experimental diets.

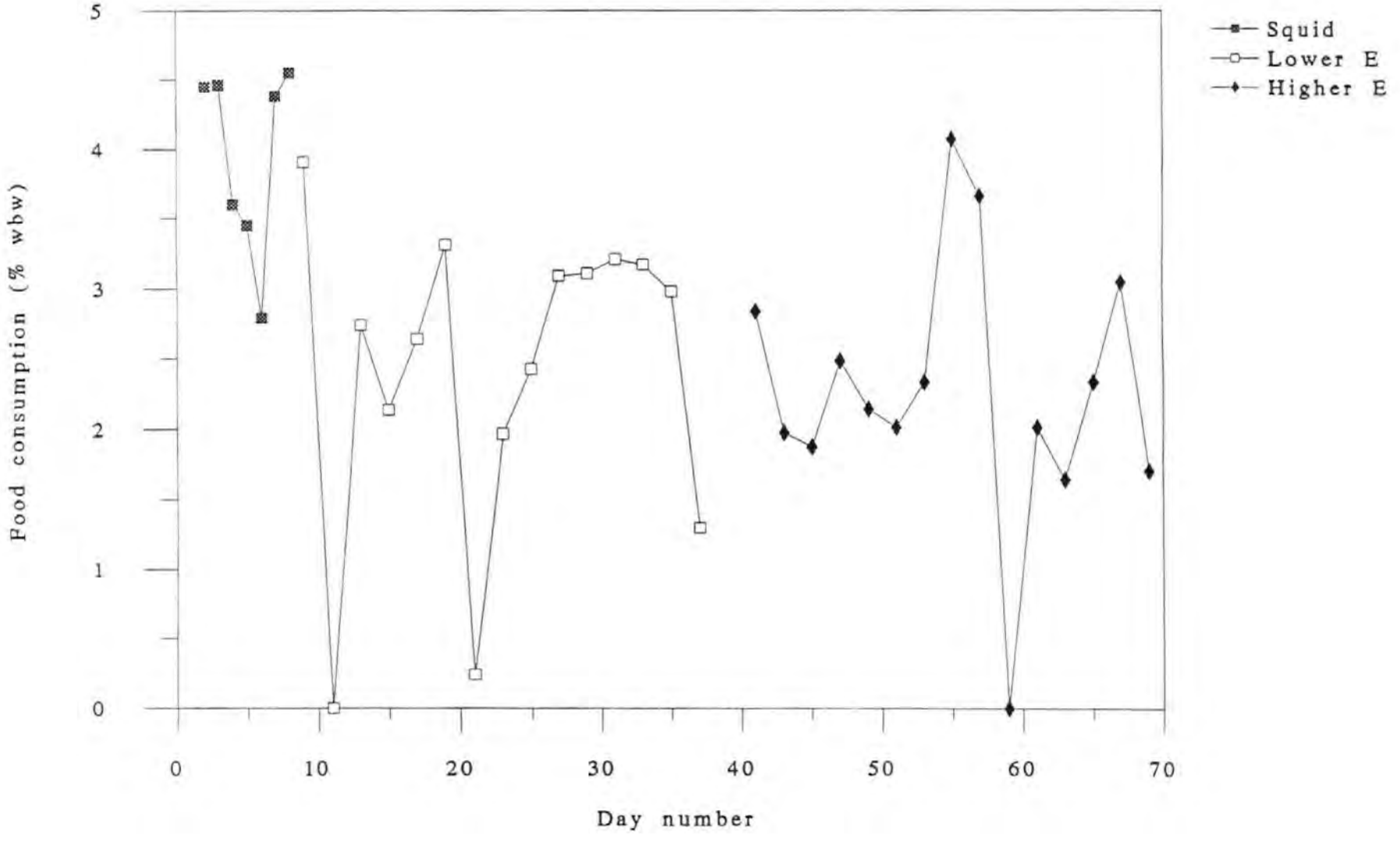

Squid
Lower E
Higher E
Food consumption (% wbw)
Day number
0
1
2
3
4
5
0
10
20
30
40
50
60
70

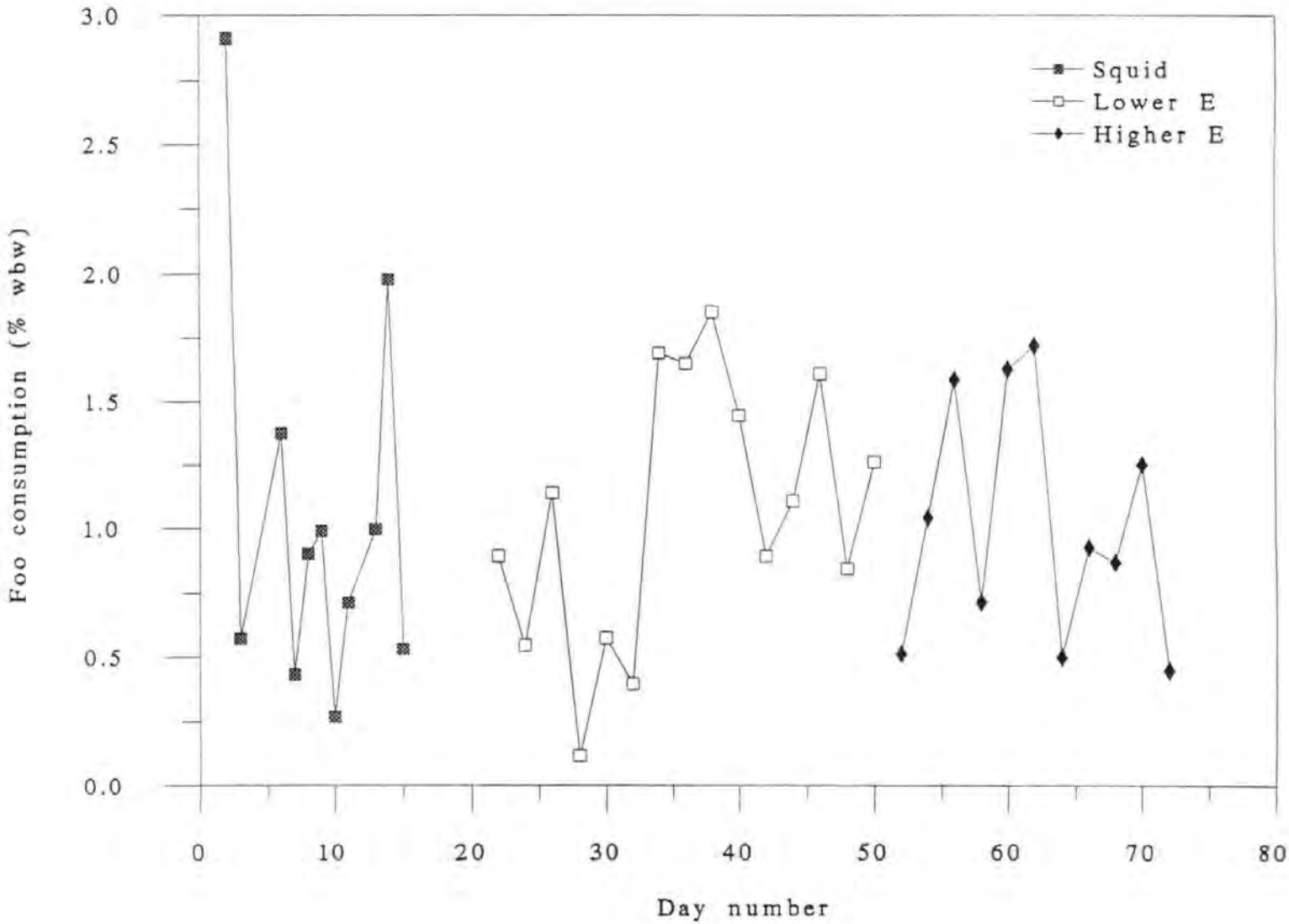
Table 9. Summary of the food intake characteristics of adult and juvenile *S. canicula* when fed on three diets (LE: lower energy pellet, HE: higher energy pellet, SQ: fresh squid) in trials lasting one to four weeks.

	Diet	Juvenile	Adult
Food intake (% wbw)	LE	2.417 ± 0.291*	1.069 ± 0.135†
	HE	2.280 ± 0.243*	1.018 ± 0.142†
Food intake (g dw.10g wet fish^{-1}d^{-1})	LE	0.060 ± 0.010	0.027 ± 0.005§
	HE	0.057 ± 0.090‡	0.026 ± 0.005
	SQ	0.069 ± 0.005‡	0.022 ± 0.008§
Gross ingested energy (kJ g dw^{-1} 10g wet fish^{-1}d^{-1})	LE	1.292 ± 0.217	0.583 ± 0.102
	HE	1.326 ± 0.197	0.613 ± 0.118
	SQ	1.681 ± 0.122	0.526 ± 0.191

**No significant difference (t-test), P>0.2*

†‡§*No significant difference (t-test), P>0.5*

Figure 19. Adult dogfish daily food intake of fresh squid (■) and the food intake patterns when fed every two days on low (□) and high (◆) energy experimental diets.


Squid
Lower E
Higher E
Foo consumption (% wbw)
Day number
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80

in food intake about a common level were observed for adult dogfish (figure 19) and showed similar traits to the food intake patterns already observed for juvenile dogfish. As in the juveniles, the lowest levels of food intake exhibited by adult fish were usually followed by the highest peaks. The mean food consumption levels of adult dogfish fed on LE and HE pellets were not significantly different ($P>0.5$, table 9) and were 1.07 and 1.02% wbw respectively (table 9).

4.4 DISCUSSION

From the results it is clear that a change in dietary energy content and in the amount of energy digested and absorbed does not affect the rates of gastric evacuation or the consequent levels of food consumption in dogfish. The study showed that digestible energy values were 1.5 times greater for dogfish fed high energy meals than when fed low energy meals, but these changes were not manifested in noticeable compensations in food intake and digestion rate. The rate of gastric emptying and the level of food consumption were not different between dogfish fed LE and subsequently HE diets, despite the energy digestibility being much higher in the HE diet. These results suggest that dietary digestible energy level was not a factor in the regulation of gastrointestinal evacuation and food consumption of dogfish.

Some investigations on teleosts have demonstrated that adjustment in food consumption to maintain energy intake does occur when nutrient-rich and nutrient-deficient meals were given. The present study maintained juvenile and adult dogfish firstly on chopped squid for approximately 1-2 weeks, then on the LE diet for 30 days followed by the HE diet for 30 days. Similar diet switching procedure, but on four day cycles between normal and kaolin-diluted normal food was used by Rozin and Mayer (1961) to investigate food intake regulation of *Carassius auratus*. The goldfish learned to press levers for food pellets and readily accepted pellets of normal and kaolin-diluted normal food. In trials undertaken by these authors, food consumption of goldfish was elevated by a factor of nearly two each time the kaolin-diluted food was substituted with the normal food. Clearly, *C. auratus* elevated food intake to maintain a relatively constant total energy consumption. Although Rozin and Mayer (1961) did not investigate the effect of digestible energy on the regulation of appetite and food consumption of *C. auratus*, their findings

indicate that goldfish compensate for nutrient deficiency by increasing food consumption, presumably by an increase in the rate of food evacuation. These results were not similar to the observations made for dogfish of the present study.

The levels of food intake of *Salmo gairdneri* (*Oncorhynchus mykiss*) (Grove *et al*, 1978), *Scophthalmus maximus* (Flowerdew and Grove, 1979; Grove *et al*, 1985) and *Pleuronectes platessa* (Jobling, 1981c) have also been shown to increase in response to consumption of nutrient-deficient meals. Compensatory adjustments of these kinds have been interpreted generally as indicating that the total dietary energy level is of prime importance in regulating food intake. Usually, consumption of high energy meals is thought to lead to decreases in gastrointestinal evacuation rates, presumably in response to longer digestion times of high energy nutrients. These delays in digestion time lower food consumption rates (Grove, 1986; Jobling, 1986). From the present study on dogfish it is clear that the importance of dietary energy level (whether the ingested or digested amount) in the control of appetite, suggested by previous investigators may not be equal for all species. Gwyther and Grove (1981) demonstrated *Limanda limanda* were not able to elevate their plane of food consumption when fed diets of low energy density. However, in another study *L. limanda* were found to respond very rapidly to changes in dietary energy value (Fletcher, 1984). Other investigations also provide contradictory evidence for an energetic basis for physiological control of appetite. Increase in the dietary lipid content did not influence the gastric evacuation time of *Sarotherodon mossambicus* (*Oreochromis mossambicus*), in fact it was observed that the diet higher in lipid was evacuated more quickly (De Silva and Owoyemi, 1983). In contrast, a study of two populations of *O. niloticus* showed that the population consuming most carbohydrate in their diet had greater growth rates and better condition than the population that generally consumed more lipid (Getachew, 1987). The digestibility of lipid is higher than that of

carbohydrate (Bergot and Breque, 1983; Ellis and Smith, 1984) and the energy content of a gram of lipid is about twice that of carbohydrate (Jobling, 1983). The results of Getachew's study may therefore indicate that gastric evacuation in *O. niloticus* consuming lipid may have been prolonged and the food consumption times less frequent. Consequently, it was suggested that more food was consumed and processed with greater efficiency into somatic growth by the *O. niloticus* consuming mostly carbohydrate.

Despite the contradictory studies, the few remaining investigations undertaken to elucidate the role of dietary energy on appetite regulation generally show that total dietary energy content does influence gastric evacuation and food consumption rates. The dogfish showed dietary insensitivity with respect to appetite regulation when compared to the species already mentioned, in that the role of digestible energy on appetite and food consumption appears to be of little importance. As aforementioned, the sand dab, *L. limanda* has sometimes been shown to regulate food intake according to dietary energy level (Fletcher, 1982, 1984), but has also been shown not to (Gwyther and Grove, 1981). Although Fletcher (1982, 1984) found evidence of an energostatic basis for food intake control in *L. limanda*, it has been stated that *L. limanda* was much less sensitive to dietary food quality than *S .gairdneri* (*O. mykiss*) (Fletcher *et al*, 1984). The latter author suggests that such dietary insensitivity may result from a wide variety of prey being taken by *L. limanda* in nature. Indeed, Gwyther and Grove (1981) also suggested that the reason *L. limanda* may not regulate their daily energy intake was due to their widely varying diet. Wild dogfish also consume a very wide range of organisms of very different energetic value, from crabs and small fish to the foot of the whelk, *Buccinum undatum* (Lyle, 1983). A catholic feeding strategy such as this may help explain why the stomach emptying and food intake patterns of dogfish originally wild-caught for this study, were not sensitive to differences in dietary digestible energy.

Food intake in dogfish fluctuated quite widely about a relatively constant level, indicating that appetite was closely related to the degree of stomach emptiness. As previously noted for LE diets in chapter III, the instantaneous relative rate of gastric emptying increased with a decrease in HE meal size. The pattern of gastric evacuation was suggested in chapter III to be regulated in a weight dependent manner. Therefore, the same emptying pattern was in operation when HE diets were also fed to dogfish. The food intake rates of dogfish fed squid, LE and HE diets can be considered to be as a consequence of the control of the gastric emptying patterns. Thus, what is of interest in this section of the present study is that dogfish appear to maintain a constant rate of dry matter food intake, irrespective of whether squid (80% moisture), LE or HE diets (50% moisture) were consumed. This suggests a mechanism whereby dogfish can physiologically "perceive" the amount of dry matter ingested and maintain food intake with respect to this information. It is not known how fish could perceive such dietary parameters, whether based on the quality of the diet or the quantity. It has been hypothesised that functional duodenal receptors in fish could respond to variations in pH, osmotic pressure, fatty acid anions and certain amino acids (Jobling, 1986). Or the stomach may release, via nervous or hormonal feedback mechanisms, varying volumes and concentrations of digesta such that the intestine receives a constant mass of nutrient, dry weight or energy (Grove, 1986). It must be open to speculation, but from past studies it would appear that some fish species are capable of assessing the amount of energy in chyme, whether total energy (Grove *et al*, 1985; Bromley, 1987) or the portion known as digestible energy (Jobling and Wandsvik, 1983). Certainly from this study, the dogfish cannot be considered to be one of these "energy monitoring" species.

If dogfish do not monitor ingested or digested energy, then how could different amounts of nutrient energy be digested in the same time period without slowing of

gastrointestinal evacuation, as exemplified by the present study? Differences in food consumption rates would not be expected if the diets given to the fish were not sufficiently different in composition. Brodeur (1984) gave chopped squid and fish (*Thaleichthys pacificus*) to the black rock-fish, *Sebastes melanops* and found very similar exponential gastric evacuation rates for both food types expressed as dry weight. The author reasoned that the two food types were not substantially different in proximate composition, and so no differences in emptying rate could be expected. The present study has shown that the energy digested and absorbed by the dogfish was higher for HE compared to LE diet, which was the objective of my original formulations. It could be postulated however, that such levels may not actually be significantly different to elicit a physiological adjustment in gastric evacuation and food consumption rates. Jobling and Wandsvik (1983) showed that the digestibility of dietary energy was lower in diets of low protein content fed to Arctic charr, *Salvelinus alpinus*, and it was this feature of the diets that influenced food intake. The digestibility of energy in the diets of this study were higher for the HE diet than for the LE diet because of the high level of lipid inclusion rather than protein, as protein level was held constant for LE and HE diets. With consideration to the findings of other studies already described, one might have expected a slowing in gastric evacuation and food intake rates when dogfish were fed the HE diet. However, this did not occur for dogfish, suggesting the efficiency of digestion and absorption of nutrient energy from the HE diet was maintained, despite the presence of more high energy components that would be more difficult to digest. In support of the observations for dogfish, the utilisation efficiency and energy retained by *S. alpinus* was shown to be greater with higher dietary lipid inclusion (Tabachek, 1986). Bromley (1987) postulated from gastric evacuation models of *Scophthalmus maximus*, that the high degree of control over the processes of digestion and evacuation stabilised chyme flow into the

intestine. This author also stated that such a fine control mechanism may maintain digestion and nutrient absorption at an optimum. High lipid levels inhibited gastric emptying in *S. maximus*, but the same level of gastrointestinal control in dogfish, though on a weight dependent basis could explain why high energy meals can be digested and retained seemingly without alteration in gastric emptying pattern. The turbot has a constant rate of gastric evacuation, independent of meal size (Bromley, 1987) and therefore a slowing in emptying may be expected with an increase in dietary lipid. In contrast, dogfish have comparatively *long* gastric emptying times with the rate dependent on the degree of stomach fullness and influenced by (though to what extent is not known) intestinal fullness, perhaps via a feedback loop (chapter III). The overall time and weight dependent nature of dogfish gastrointestinal evacuation may suggest that diets of potentially different digestible energy contents could be processed without any increase or reduction in gastric emptying and food consumption patterns, and with no overall slowing effect on the recovery of appetite.

There are no previous studies that have examined the effects of diet quality on the regulation of appetite in sharks. The appetite of dogfish does not appear to be regulated by the level of energy in the food, which queries whether this benthic elasmobranch possesses the receptors that could bring about such food intake control on the basis of digested energy. Or indeed, whether reductions in feeding rates are necessary at all when characteristically long evacuation times, perhaps allowing maximal digestion and absorption of different food types are controlled in a weight dependent, rather than an energy dependent manner. Investigations of teleost fish have demonstrated elevations and reductions in food intake according to dietary energy level and have indicated that certain receptors located in the upper intestine may monitor the total, digested or metabolizable energy level. From this information, it has been postulated, that fish can regulate food

consumption according to diet quality (Jobling and Wandsvik, 1983). As dogfish are unable to adjust intake with respect to energy density (perhaps because they have never needed to benefit from such a feeding strategy), then other regulatory pathways may operate to modify gastric emptying patterns after absorption of the digestible components of the food.

The evacuation of food from the stomach and gastrointestinal tract is not likely to be a smooth process, but rather a pulsed pattern of emptying (Jobling, 1986; Jobling, 1987). Clearly then, the pulsed emptying model will change according to the feedback signals modifying the general pattern of gastrointestinal evacuation. What is not known is how the bulk dependent evacuation process of dogfish may be modified by post-prandial factors other than the level of dietary digestible energy. Appetite regulation in dogfish does not appear to be influenced by diet quality, but is certainly affected by the quantity of food consumed. Hence, if the basis of appetite regulation is dependent on the bulk of food, a post-prandial increase in metabolism related to the amount of food consumed and processed may affect appetite, perhaps through limiting the energy available for other processes, *e.g.* gastric motility or locomotor activity. Soofiani and Hawkins (1985) stated that the high energy demand following feeding may leave little capacity within the metabolic scope for performing any other activity. Therefore, post-prandial and post-absorptive metabolic factors could potentially affect appetite regulation to a greater degree than perhaps the physiological factors already considered.

CHAPTER FIVE

APPETITE RETURN AND POST-PRANDIAL METABOLISM

5.1 INTRODUCTION

Specific dynamic action (SDA) represents the rise in metabolic rate (energy expenditure) associated with the consumption and processing of a meal (Jobling, 1981a). This post-prandial elevation in metabolic rate and the accompanying exothermic heat loss was first demonstrated by Laplace and Lavoisier (cited in Jobling, 1981a) with homeothermic animals and was first termed the "specific dynamic effect" by Rubner (1902, cited in Jobling, 1981a). The effect has been subsequently termed heat increment (Brett and Groves, 1979), calorigenic effect (Nelson *et al*, 1977), the thermic effect of food, specific dynamic action (SDA) and also apparent specific dynamic action (Beamish and Trippel, 1990).

Since those early experiments, many phyla have been investigated with regard to the existence of an SDA response. The effect is known to occur in asteroid echinoderms (Vahl, 1984), brachyuran crustaceans (Wallace, 1973), isopods (Carefoot, 1990 a,b,c), terrestrial (Kreiger, 1966; Ashworth, 1969; Kreiger, 1978) and marine mammals (Costa and Kooyman, 1984; McConnell *et al*, 1992) and in fish. The first experimental determinations of this kind on fish were reported by Warren and Davis (1967) from previously unpublished work. Since then SDA investigations have been undertaken on a variety of species, for example *Gadus morhua* (Saunders, 1963), *Micropterus salmoides* (Beamish, 1974), *Blennius pholis* (Vahl and Davenport, 1979), *Pleuronectes platessa* (Jobling and Davies, 1980), *Limanda limanda* (Fletcher, 1982), *Clupea harengus* (Kiorboe *et al*, 1987), *Ctenopharyngodon idella* (Carter and Brafield, 1992) and *Brachydanio rerio*

(Lucas and Priede, 1992). In the majority of SDA studies conducted with fish the elevation in post-prandial metabolism following a meal has been measured by indirect calorimetry (*eg.* Beamish, 1974; Jobling & Davies, 1980; Lucas & Priede, 1992), where the oxygen consumption of the fish increases after food intake. However, direct calorimetry of fish has been attempted (Smith *et al*, 1978) and more recently, heart rates of captive and wild fish have been monitored by telemetry and enabled field estimates of SDA (Lucas and Armstrong, 1991; Lucas *et al*, 1991).

Fish energy budgets place SDA as a net loss of digestible energy (that portion of energy that is not metabolized) (*e.g.* Solomon and Brafield, 1972; Flowerdew and Grove, 1980; Kerr, 1984; Diana, 1987), though it is not known exactly which of the processes involved in food consumption and digestion constitute the major part of this post-feeding energy loss. Possible causes of the effect have been postulated by several authors. From earlier studies the heat loss following feeding was thought to occur as a consequence of ATP formation arising from the oxidative catabolism of amino acids and not from carbohydrate or fatty acids in the TCA cycle (Krebs, 1964). This author stated that the increase in metabolic rate was due to the work of digesting the protein meal and also the result of the inefficient thermochemical oxidation of protein to urea. However, the post-prandial metabolic rate and the hepatic synthesis of urea are not closely linked and therefore may not adequately explain the existence of SDA (Garrow and Hawes, 1972). Restriction of the heat loss to the specific process of deamination is no longer regarded valid as energy losses, even though smaller, still accompany the catabolism of lipid and carbohydrate (Brett and Groves, 1979). Grasping, chewing and swallowing in addition to the demands of gastrointestinal musculature are not thought to constitute a major part of SDA because no significant increases in metabolic rate of fish have been demonstrated after feeding inert meals of kaolin (Jobling, 1981). However, Tandler and Beamish (1979)

estimated that gastrointestinal work accounted for approximately 10-30% of the total SDA, though other investigations have shown the mechanical portion of SDA to be much lower, between 1 and 5% (Jobling, 1981; Cho *et al*, 1982).

The hypothesis that SDA represents the "energy costs of growth" has been proposed by a number of researchers. In young humans recovering from malnutrition it has been shown that the greatest SDA was during catch-up growth, a period of rapid growth rate following prolonged nutritional deprivation (Kreiger, 1966; Kreiger, 1968; Ashworth, 1969). Similarly it has been demonstrated for fish that a strong correlation exists between SDA and growth (Carter and Brafield, 1992). Houlihan (1991) stated that the rate of protein synthesis can be paralleled by the rate of oxygen consumption, which suggests the post-prandial increases constituting SDA may largely result from the production of new proteins. Clearly SDA cannot be attributed to or explained by a single process, and the components of SDA are now thought primarily to be: gastrointestinal muscular motility, digestive enzyme formation and release, costs associated with digestion and absorption and the assimilation of the digestive products (Carter & Brafield, 1992). Recent studies on fish and reviews of mammalian literature suggest that although SDA is the product of these physiological components, it could largely be considered to be the eventuality of the costs of biosynthesis and tissue protein turnover (Jobling, 1981a; Jobling, 1983; Beamish and Trippel, 1990).

Despite many studies on possible physiological 'causes' of SDA and the existence of various explanations in the literature (Jobling, 1983; Beamish & Trippel, 1990), little has been reported on the role of SDA in food intake control of fish. Fletcher (1984) stated that the elevated oxygen consumption following a meal did not prevent further food intake but some authors have however, indicated that SDA may be of importance in appetite regulation (Beukema, 1968; Muir & Niimi, 1972; Vahl & Davenport, 1979). Little

information referring to SDA as contributing to the processes of appetite regulation in fish exists in the literature and the studies that have been completed are often contradictory. Some fish have eaten little following high levels of feeding which has led investigators to conclude that appetite may return as a consequence of lowered SDA (Beukema, 1968; Muir and Niimi, 1972). Other studies have shown that fish would consume more food before the level of SDA had decreased from the maximum level (Schalles and Wissing, 1976; Fletcher, 1982). The conflicting findings of these investigations do not enable a clear understanding of the role that SDA may have in the regulation of appetite.

Fletcher (1984) also stated that SDA, although not directly controlling food consumption may influence the amount of energy available for other processes, such as activity. Increases in post-prandial metabolic rate attributable to SDA in some fish species have been demonstrated to equal or actually exceed the level of active metabolism measured during maximum sustained swimming (Soofiani and Priede, 1985). Hence SDA can occupy much of the available scope for activity, which could influence appetite regulation through modifications to the plane of metabolism. The cardiorespiratory system of fish such as these has been stated as having evolved to supply the greater oxygen demand following feeding, rather than the metabolic demands of swimming (Butler, 1986). Such evidence suggests that SDA may therefore limit not only the energy that could be partitioned to locomotor activity, but also the amount of energy available for gastrointestinal motility. Thus SDA may impose a significant metabolic cost to the fish by occupying much of the metabolic scope at higher ration levels and this may in turn affect appetite regulation (Niimi & Beamish, 1974; Jobling, 1981a; Lucas & Priede, 1992).

There are no previous studies exploring the role of SDA on appetite regulation of elasmobranchs. The aims of the present investigation were therefore to quantify the SDA effect at different levels of feeding and evaluate the influence of SDA on the return of

appetite (AR) in juvenile and adult dogfish, *Scyliorhinus canicula* (L.). In this way it was hoped to provide information about the possible relationship between post-prandial metabolism and the short-term control of further food consumption.

5.2 MATERIALS AND METHODS

5.2.1 Oxygen Consumption and Activity Measurements

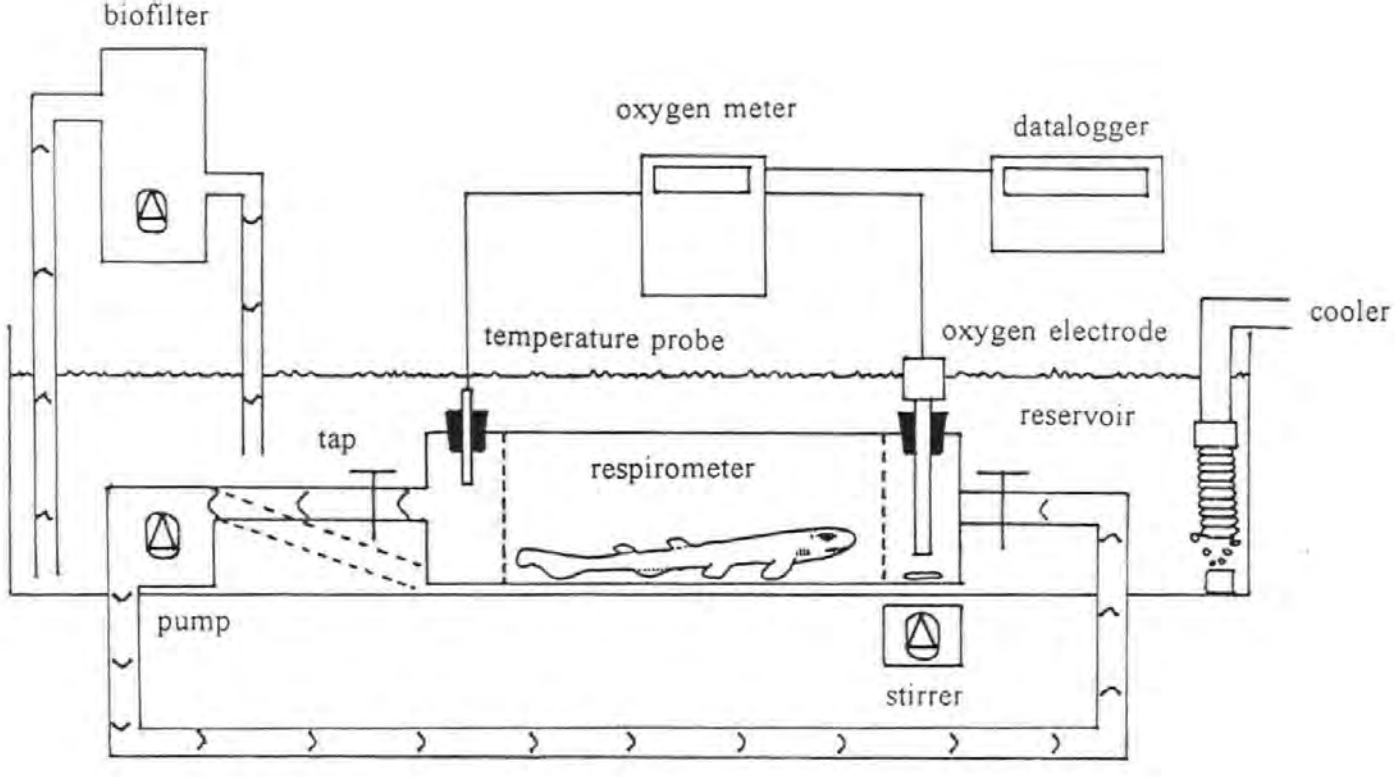
5.2.1.1 Respirometry of juvenile dogfish

A 5.8 l closed respirometer with a single polarographic oxygen electrode (WTW EO96) was used to obtain the oxygen consumption measurements (figure 20). The metabolic rate (M_R) changes of the fish were evaluated using indirect calorimetry, a measurement of the rate of oxygen consumption, $\dot{V}O_2$.

The Perspex respirometer (48 x 11 x 11cm) was fitted with one detachable end enabling the fish to be placed inside. One-way valves on each end-plate of the respirometer, including the detachable plate, were connected to the inlet and outlet hoses of a small pump (Eheim 1250, 16 L min^{-1}) which enabled flushing. A Perspex baffleboard at the inlet end of the respirometer kept the oxygen probe and magnetic stirring 'flea' separate from the experimental fish, whilst a second baffle at the outlet prevented the fish escaping when the chamber was flushed. Two holes in the top of the respirometer allowed insertion of the oxygen electrode and temperature probe. A rubber bung around each probe ensured a tight seal without leakage of the respirometer water to that in the surrounding bath.

The oxygen electrode was connected to an oxygen meter (WTW OXI96) and measurements of dissolved oxygen in the chamber were sampled every 10 minutes from the meter by a data logger (Grant Instruments, Cambridge). The electrode was re-calibrated in water-saturated air every time the respirometer was flushed. The temperature probe was connected to the data logger and concurrent readings were taken with those of oxygen consumption.

Figure 20. Operational diagram of the closed system respirometer for juvenile dogfish. Arrowheads represent the direction of water flow. Dotted lines from the pump denotes that this end-plate of the respirometer was detachable in order for the chamber to be flushed with aerated water from the water reservoir.


biofilter
oxygen meter
datalogger
cooler
temperature probe
oxygen electrode
reservoir
tap
respirometer
pump
stirrer

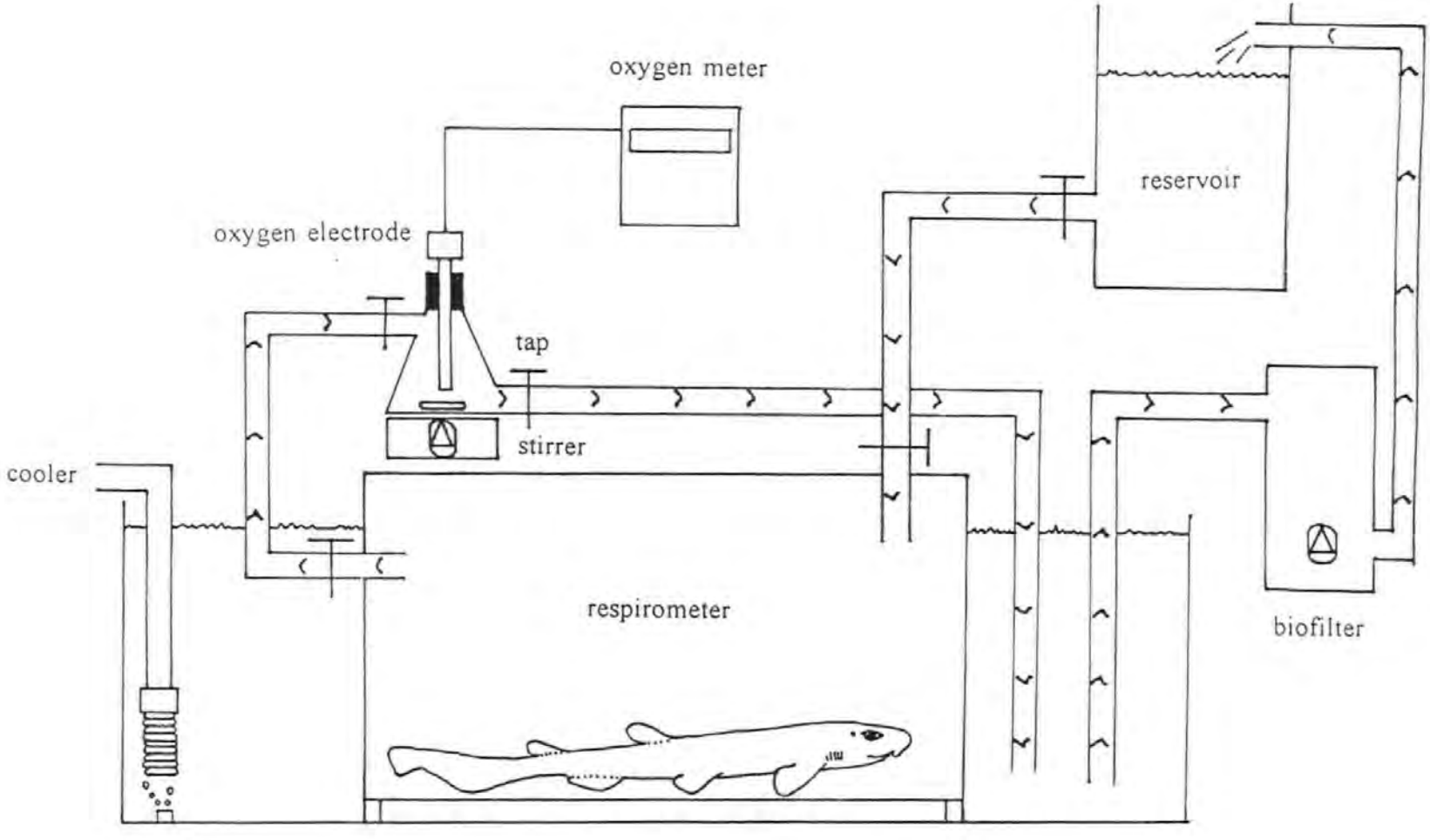
5.2.1.2 Respirometry of adult dogfish

The metabolic rate of the adult fish was also measured by indirect calorimetry, but in a larger closed respirometer (72 x 52 x 30cm, 112L) (figure 21). The adult dogfish could be observed within the respirometry chamber through the Perspex sheet which constituted the removable top plate of the respirometer. An airtight seal was made between the perspex sheet and the rubber edge of the chamber. After the dogfish had been introduced to the respirometer, trapped air was displaced by flooding the chamber with water from an overhead reservoir. Water in this reservoir was continually aerated and pumped from the water bath containing the respirometer through a biological filter (Eheim 2015, 540 L h^{-1}). The influx of water from the reservoir to the respirometer was controlled by means of a one-way tap on the Perspex sheet. Measurements of oxygen saturation were taken every 30 minutes using a polarographic oxygen electrode (WTW EO96) and oxygen meter (WTW OXI96). A sample of respirometer water was taken by allowing water to flow via a second one-way tap to a 250ml conical flask which held the oxygen probe. Water was allowed to flow through the flask and into the bath surrounding the respirometry chamber for approximately 30 seconds thereby ensuring that water remaining from previous samplings was completely flushed from the sample flask. A rubber bung around the probe provided an airtight seal at the top of the flask and the water flow over the electrode membrane was maintained using a magnetic follower and stirrer. The temperature of the water inside the flask was recorded concurrently with that of oxygen saturation and was maintained for all the trials at 15°C.

5.2.1.3 Calculation of oxygen consumption

The oxygen consumption of the juvenile dogfish was calculated using the generalized equation stated in Parsons (1990):

Figure 21. Operational diagram of the closed system respirometer used for adult dogfish. Arrowheads denote direction of water flow.


oxygen meter
reservoir
oxygen electrode
tap
stirrer
cooler
respirometer
biofilter

$$VO_2 = \frac{b\ s\ v}{w}$$

where VO_2 is the rate of oxygen consumption in mg O_2 kg^{-1} h^{-1}, b is the rate of change of partial pressure of oxygen during the test period, s is the solubility of oxygen calculated at the experimental temperature and pressure, v is the volume of the respirometer, and w is the live weight of the fish. Each of the test periods for juvenile and adult dogfish were of three hours duration as the dissolved oxygen decline over two hours, in the case of juvenile dogfish was too slight to provide a representative rate of loss of oxygen for use in calculations. When oxygen consumption measurements were made over long periods, the three hour test periods were not adjacent, but spaced at approximately 6-12 hour intervals. Extensive control trials with each respirometer were completed before introduction of fish and no changes in oxygen content of the respirometers occurred over the test period.

5.2.1.4 Activity measurements

The activity level (A_L) of single juvenile dogfish in the respirometer was recorded by a video camera (Sony CCD-F355E) positioned in front of the respirometer, inclined forwards at an angle of 30° to the horizontal to give maximum coverage of the fish in the chamber against the white background. Video observation of adult dogfish took place using a mirror positioned above the respirometer inclined at an angle of 45° to the horizontal. The reflected image gave full coverage of the area inside the respirometer. The video observation unit was separated from the respiratory area by a black sheet, so that frequent changes of the video cassette did not disturb the fish. Activity was also periodically observed in real-time from a remote camera positioned above either of the respirometers and connected to a television monitor.

The A_L of the dogfish was quantified by determining from the videotapes the number of minutes in each hour that the fish spent in various degrees of activity. The activity was classified into three types: movements of the head, trunk and tail region but with no body re-positioning; re-positioning of the body, and active swimming. Using the clock on the video screen and the frame-by-frame analysis the activity time per hour and the frequency of motion were determined accurately for each of the experimental trials.

5.2.2 Diel Rhythms in Metabolism and Activity

5.2.2.1 Juvenile dogfish

Three dogfish were used in five 72 hour experimental trials during which the metabolic rate and activity level of the fish were monitored. The oxygen consumption measurements were made as in section 5.2.1.1. A single dogfish was starved for 48 hours prior to placing in the respirometer, where it remained for a further 18 hours in order to equilibrate. Throughout the trial the dogfish could move freely within the respirometer. The dissolved oxygen concentration in the respirometer was never allowed to fall below 50% air saturation. Before this level was reached the chamber was flushed through with fully aerated water from the surrounding bath. The respirometer was flushed by displacing the outlet end-plate from the sealed position and turning the valves and the pump on for about three minutes in each case and this was adequate to totally replace all the water in the chamber. Flushing did not affect adversely the activity or oxygen consumption of the fish thereby keeping interruptions to the fish's natural rhythm to a minimum. Each trial was started on day one and finished on day four of the experiment.

5.2.2.2 Adult dogfish

Each of three dogfish were used in a 24 hour study. Oxygen consumption was first

measured at 14.00h on Day 1 and readings were terminated at 15.00h the following day. The adult dogfish were starved for 72 hours before the trial was initiated with a further 18 hours allowed for acclimation to the study environment. As with the juvenile fish, the oxygen saturation of the respirometer was not allowed to fall below 50%. When this level was approached, clean seawater was flowed through the respirometer until the oxygen saturation was closer to 100%.

5.2.2.3 Activity measurements

The activity patterns of the juvenile dogfish in the chamber were recorded continuously for four hours, from 05.00-09.00h, 11.00-15.00h, 17.00-21.00h and 23.00-03.00h throughout 72 hours. The activity of the adult dogfish was continuously measured throughout the 24 hour period in each of the trials both by video and real-time observation. The main illumination for the experimental environment (260 lux) came on at 07.00h and went off at 19.00h. A low level of light (30 lux) was maintained throughout the night time hours so that dogfish activity could be recorded.

5.2.2.4 Statistical analysis

All the means stated are ± the standard error of the mean. One-way analysis of variance was used to test the significance of differences between the means of the $\dot{V}O_2$ and A_L for the juvenile fish over the 12 hours of light compared to the 12 hours of darkness. The same was completed for the $\dot{V}O_2$ and A_L of adult fish and finally the levels of activity of juvenile and adult fish during the dark period were compared.

5.2.3 Standard Metabolic Rate

Individual starved fish from five weight classes (2.86-4.30g; 10.41-16.30g; 56.25g (single

fish); 170-220g; 600-1000g) were weighed before being placed in either the small or large respirometer. This was done 18 hours before any determinations of oxygen consumption took place. During this period of adjustment to the chamber environment, the small respirometer for juveniles was left open to the surrounding water bath, while the large respirometer had a continuous flow of water through it, thereby keeping the important seawater parameters constant (15°C, 35‰). After the 18 hour settlement period (ending at ~10.00h), the respirometers were sealed from the surrounding water and initial measurements of seawater oxygen saturation inside the respirometers were made. All the measurements were obtained in the 10.00 - 18.00h period as this time has been previously shown to be when dogfish exhibit their lowest rates of metabolism (*cf.* results section 5.3.1). Video and real-time observation of dogfish activity meant that only when dogfish were totally inactive for long enough periods could measurements of oxygen consumption be considered to be representative of standard metabolic rate. Hence, only measurements of M_R made during inactive periods were included in the results.

5.2.4 SDA Measurements

5.2.4.1 Juveniles and adults

Juvenile (10.90-16.30g) and adult dogfish (588.8-802.2g) were deprived of food for 7 and 10 days respectively, before a trial commenced. Single fish were placed into the respirometers and allowed to settle down for approximately 18h. After this time the respirometers were closed and for the next 24h the metabolic rate of each fish was measured. When steady resting metabolic rates had been obtained the fish were hand fed squid mantle *in situ* over a period of one hour. The juvenile and adult dogfish were fed squid meals of either approximately 1 and 49g respectively (approx. 7 % wbw, the satiation level of group feeding dogfish) or a meal of approximately 12.5% wbw, before

the respirometers were closed and the oxygen concentration of the water recorded. The oxygen consumption of the fish was monitored until it returned to (and stabilised at) the baseline level obtained pre-feeding. Four trials of each fish size were completed at the 7% wbw feeding level and three at the 12.5% wbw level. Peak levels in oxygen consumption and duration of the SDA effect were recorded and the SDA magnitude, the oxygen consumed above that of the resting metabolism was calculated (with a BASIC computer program) for each trial. The energetic value of the squid was calculated in order to estimate the amount of gross ingested energy (IE) lost as SDA.

5.2.4.2 Calculation of ingested energy (IE) losses

The amount of energy in dried squid was determined by bomb calorimetry (*cf.* section 4.2.4.2). The total energy from the squid meal given to the dogfish (ingested energy, IE) was calculated by multiplication of the amount of energy in one gram of dried squid by the dry weight of the consumed meal.

The amount of IE expended as SDA was calculated from the value of the SDA magnitude in g O_2 multiplied by the oxycalorific coefficient of 13.55 kJ g^{-1} O_2 (Brett & Groves, 1979) and expressed as a percentage of the total IE.

5.2.4.3 Statistical analysis

Two-tailed Student's t-tests were used to compare the means of meal size, peak level in oxygen consumption, SDA duration, SDA magnitude and the ingested energy losses due to SDA, both between and within fish sizes.

5.3 RESULTS

5.3.1 Diel Rhythms in Metabolism and Activity

The $\dot{V}O_2$ and A_L of the juvenile dogfish fluctuated during each diel cycle in a well defined manner (fig. 22) with predictable, repeated diel rhythms over the three day period. Levels of $\dot{V}O_2$ and A_L were relatively high but varied at night. The same general trend was evident for the adult dogfish over 24 hours, with elevations in activity and metabolic rate after the onset of darkness, but returning to baseline levels before the light phase (fig. 23). The main peak in activity and metabolic rate for juvenile and adult dogfish was from approximately 00.00 to 03.00h, though the juvenile fish were active earlier in the dark period also, from about 19.00 to 00.00h.

Both the mean $\dot{V}O_2$ and A_L of the juvenile fish were found to be significantly different in the hours of darkness compared to the rates in daylight hours (F-values 30.73 and 71.68 respectively, $P<0.0001$) (table 10). The mean $\dot{V}O_2$ increased by 37.9% from 62.0 (S.E. 2.9) mg O_2 kg^{-1} h^{-1} in light to 85.5 (S.E. 3.1) mg O_2 kg^{-1} h^{-1} during the dark. Similarly, the mean A_L of juvenile *S. canicula* rose from a daytime level of 0.6 (S.E. 0.2) min h^{-1} to 14.5 (S.E. 1.6) min h^{-1} in the dark. The daytime $\dot{V}O_2$ and A_L of the adult fish was significantly different to the night-time levels (F-values 17.14 and 5.57 respectively, $P<0.029$) with the mean $\dot{V}O_2$ increasing by 166.8% from 32.5 (SE 4.3) mg O_2 kg^{-1} h^{-1} in daylight to 86.7 (SE 8.7) mg O_2 kg^{-1} h^{-1} at night (table 10). The mature dogfish were not observed to be active by day (mean 0 min h^{-1}), but in darkness the activity elevated to 5.3 (SE 1.4) min h^{-1}. Mean night activity of the adult fish were individually compared with each of the three dark period activity levels of juvenile fish, and found to be significantly different on the second and third nights of the juvenile study ($P<0.05$ and 0.02 respectively), though not on the first ($P>0.60$). (When juvenile activity was approximately

Figure 22. Rhythms in activity level (A_L) (upper panel) and rate of oxygen consumption ($\dot{V}O_2$) (lower panel) for five trials on single juvenile dogfish over 72 hours. The variegated bar on upper panel represents the light:dark regime. Bars represent ± 1 S.D. Dotted lines on upper panel denote periods that the dogfish were not video-observed. Small arrows on lower panel indicate the times when the respirometer was flushed.

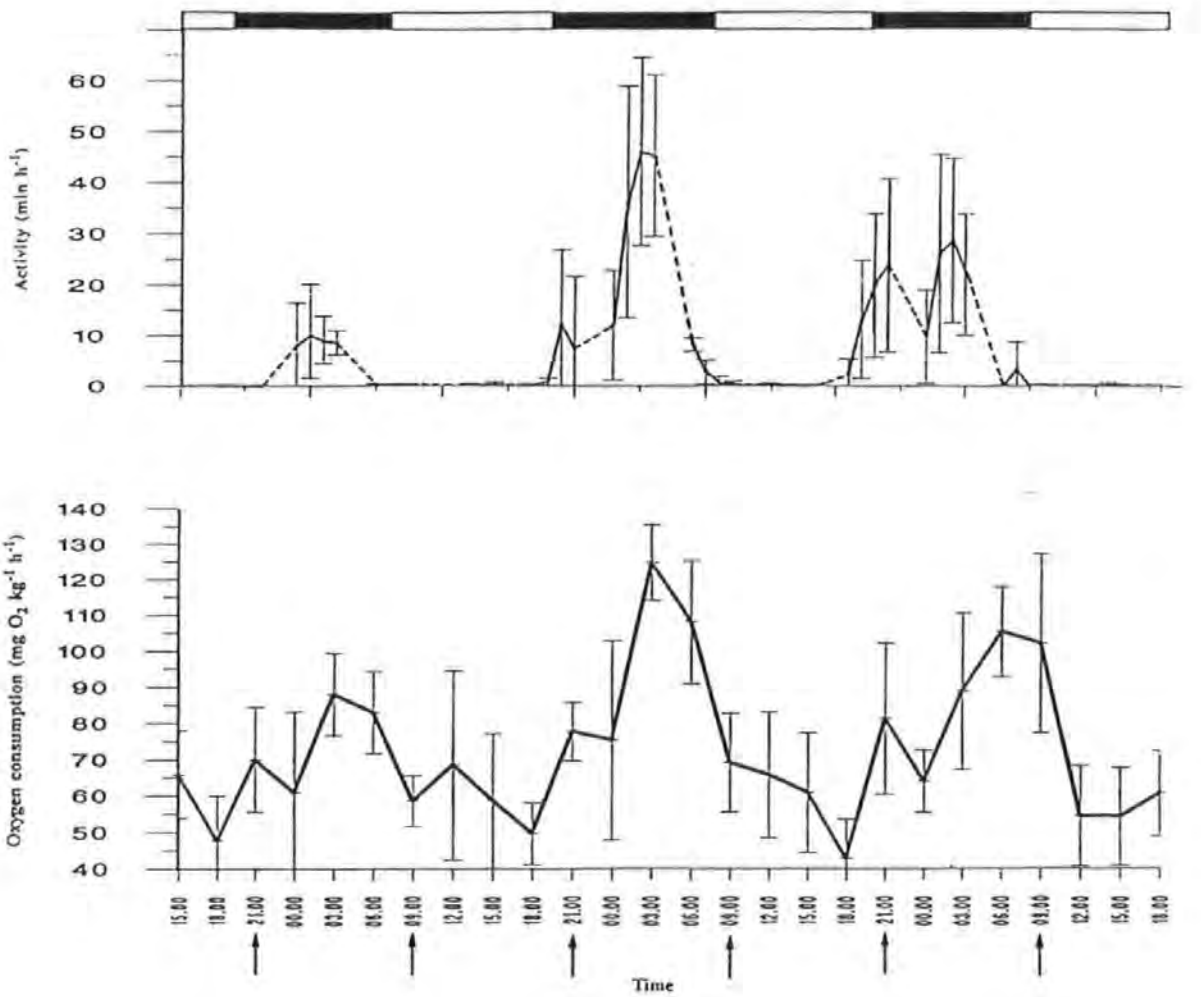
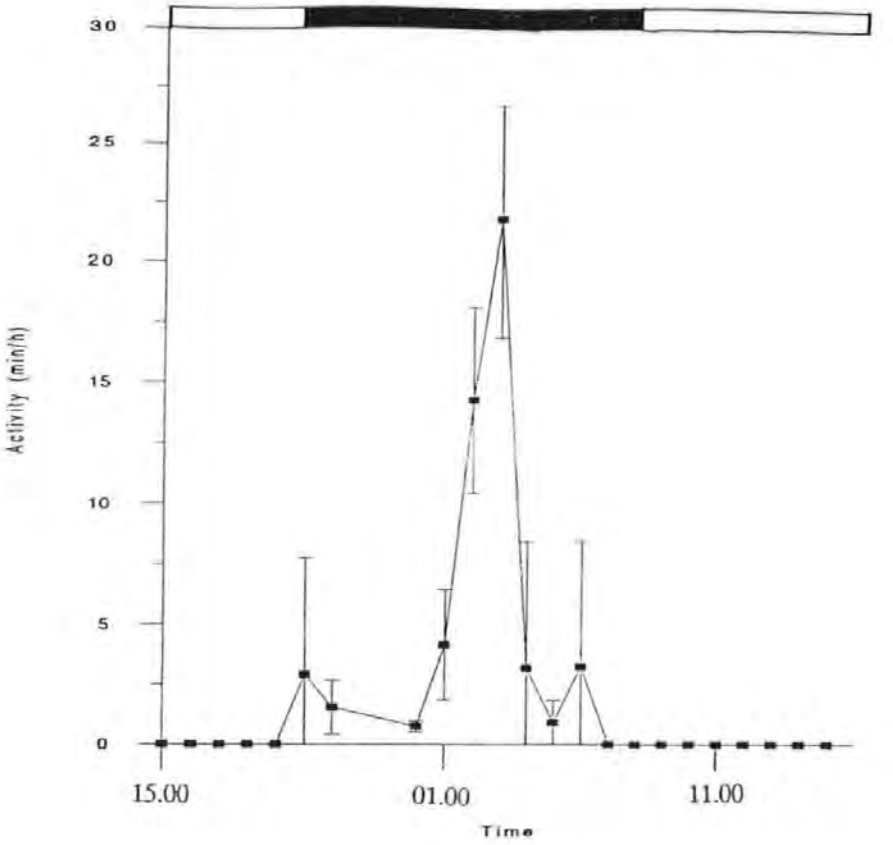

Activity (min h-1)
60
50
40
30
20
10
0
Oxygen consumption (mg O2 kg-1 h-1)
140
130
120
110
100
90
80
70
60
50
40
15.00
18.00
21.00
00.00
03.00
06.00
09.00
12.00
15.00
18.00
21.00
00.00
03.00
06.00
09.00
12.00
15.00
18.00
21.00
00.00
03.00
06.00
09.00
12.00
15.00
18.00
Time

Figure 23. Rhythms in mean activity level (A_{1_L}) (upper panel) and rate of oxygen consumption ($\dot{V}O_2$) for three trials on single adult dogfish over 24 hours. Variegated bar on upper panel represents the light:dark regime. Bars denote ± 1 S.D.


30
25
20
15
10
5
0
Activity (min/h)
15.00
01.00
11.00
Time

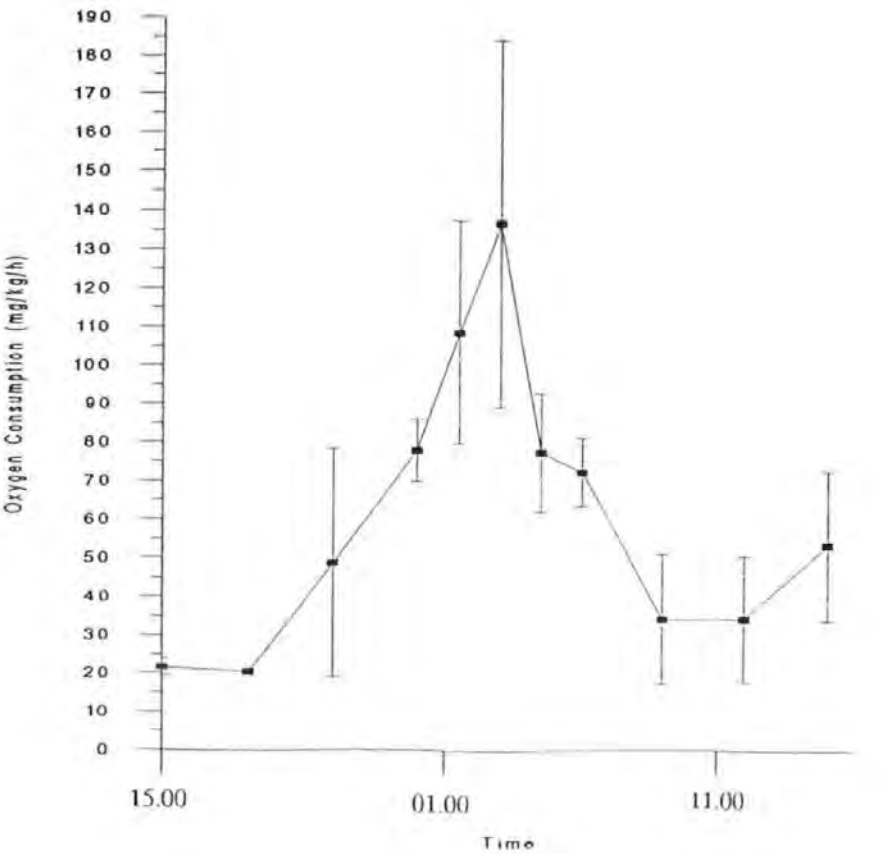

190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Oxygen Consumption (mg/kg/h)
15.00
01.00
11.00
Time

Table 10. The mean rates of oxygen consumption and activity during light and dark periods for three juvenile dogfish in five 72 hour trials and three adults in three 24 hour trials.

		Light (07.00-19.00h)	Dark (19.00-07.00h)
Oxygen consumption ($\dot{V}O_2$) (mg O_2 $kg^{-1}h^{-1}$)	Adult	32.5 ± 4.3	86.7 ± 8.7
	Juvenile	62.0 ± 2.9	85.5 ± 3.1
Activity (A_L) (min h^{-1})	Adult	0	5.3 ± 1.4
	Juvenile	0.6 ± 0.2	14.5 ± 1.6

5 min h^{-1} higher than that of the adults). Generally, the small dogfish were active for longer during the dark period than adult fish and also at two distinct periods compared to the single activity phase of the adult fish.

5.3.2 Standard Metabolic Rate (R_S)

The oxygen consumption of dogfish increased exponentially with an increase in body weight (fig. 24) and the relationship was represented by the equation,

$$R_S = 0.104\ W^{0.855}$$

where R_S was the amount of oxygen consumed by the fish and W represents the live weight of the fish.

5.3.3 Post-prandial Metabolism and Appetite

5.3.3.1 Fish size and ration level

In general the results show that the oxygen consumption of juvenile and adult dogfish increased to maximum levels (2.7 ± 0.1 x resting rate) 4-10 hours post-feeding, before gradually returning to the pre-feeding levels. Figures 25 and 26 show the traces in oxygen consumption following feeding of 7% and 12.5% wbw meals to both juvenile and adult dogfish. Oscillations in the metabolic rates of the juveniles and adults at either ration level may be attributable to spontaneous activity, even though oxygen consumption determinations were only made when the fish were apparently at rest. These increases therefore, probably represent periods of time when oxygen consumption was measured following continuous activity. Table 11 shows the SDA responses to the 6.52 ± 0.73-11.65 ± 0.15 % wbw and 7.25 ± 0.23-13.02 ± 0.53 % wbw meals given to adult and juvenile dogfish respectively. The four parameters used for comparison were peak level in oxygen consumption, duration, magnitude of the SDA effect and percentage of ingested energy

Figure 24. The relationship between standard metabolic rate (R_S) and weight for dogfish of weight 3-1000g at 15°C. Regression equation, $R_S = 0.104\ W^{0.855}$, $r^2 = 0.98$. Number of fish used, 40.

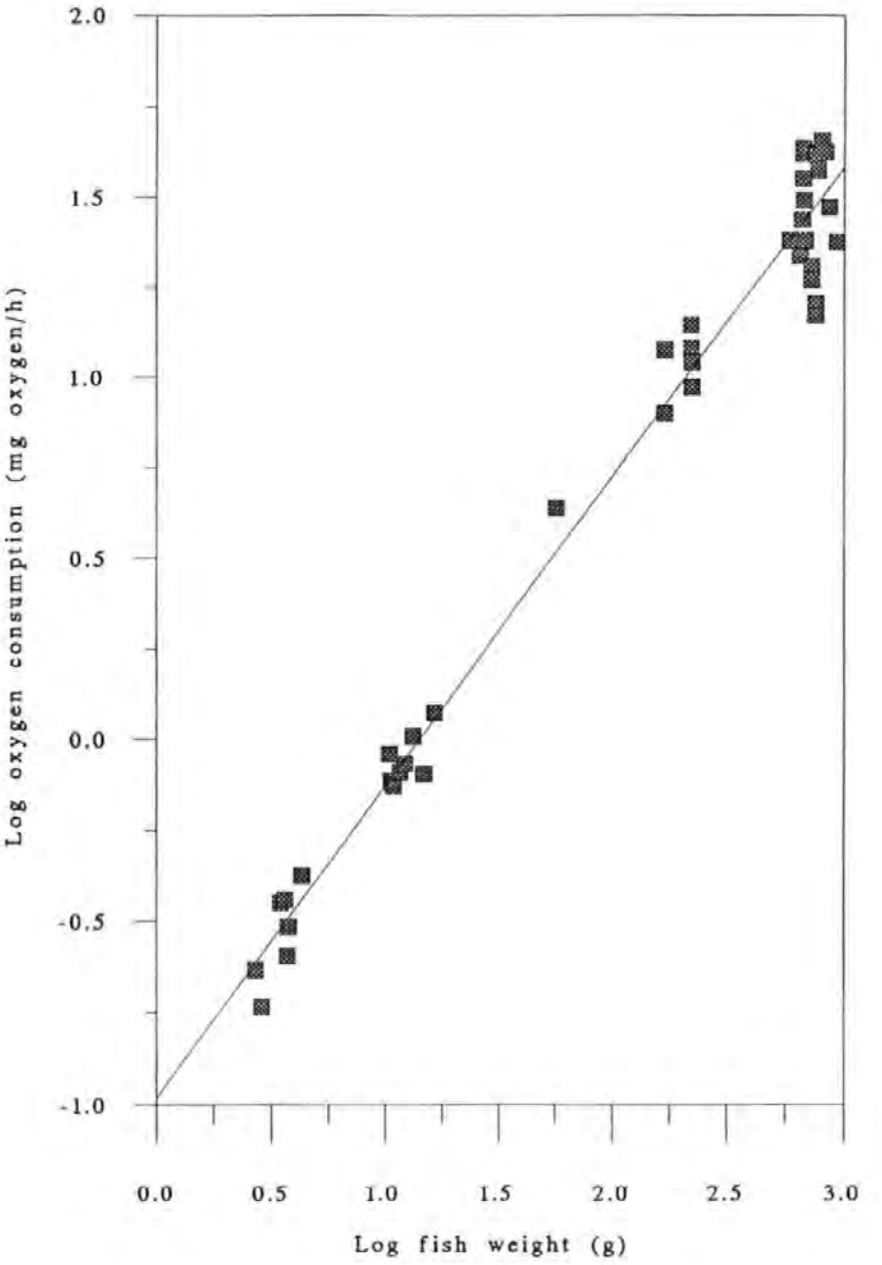
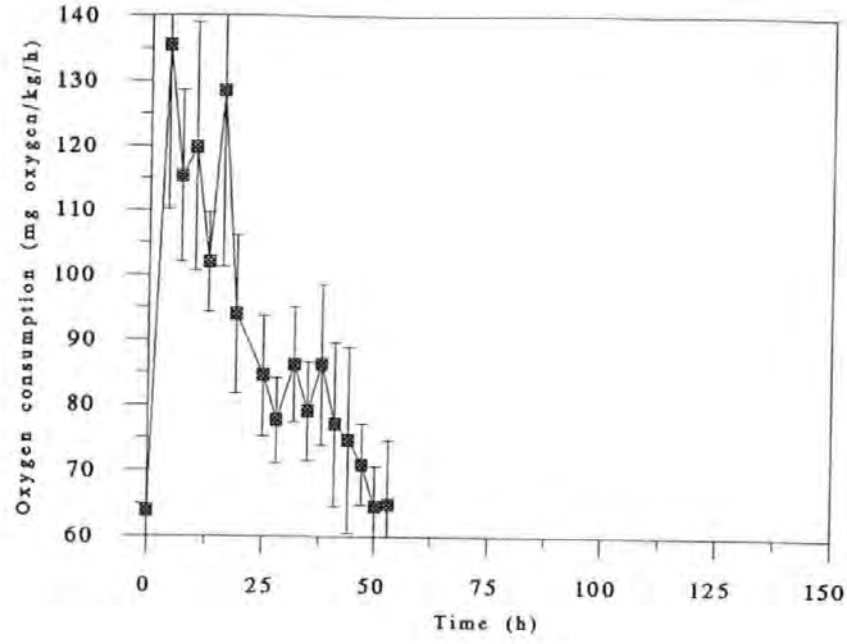

2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
Log oxygen consumption (mg oxygen/h)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Log fish weight (g)

Figure 25. The mean oxygen consumption of juvenile dogfish after being fed meals of 3.5% wbw (■) and 7% wbw (□). Bars represent ± 1 S.E. Number of determinations for each point: upper panel, 4; lower panel, 3.


Oxygen consumption (mg oxygen/kg/h)
140
130
120
110
100
90
80
70
60
0
25
50
75
100
125
150
Time (h)

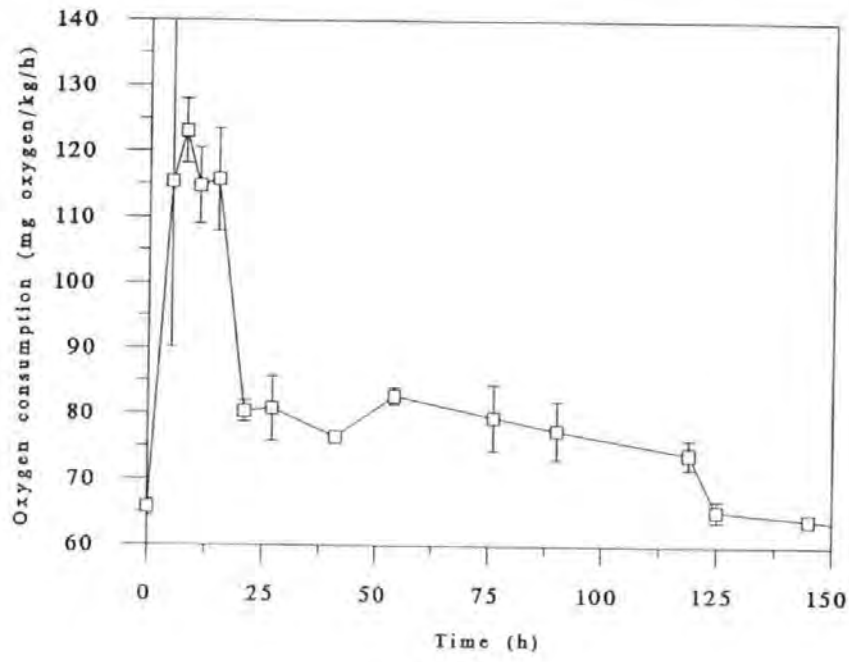
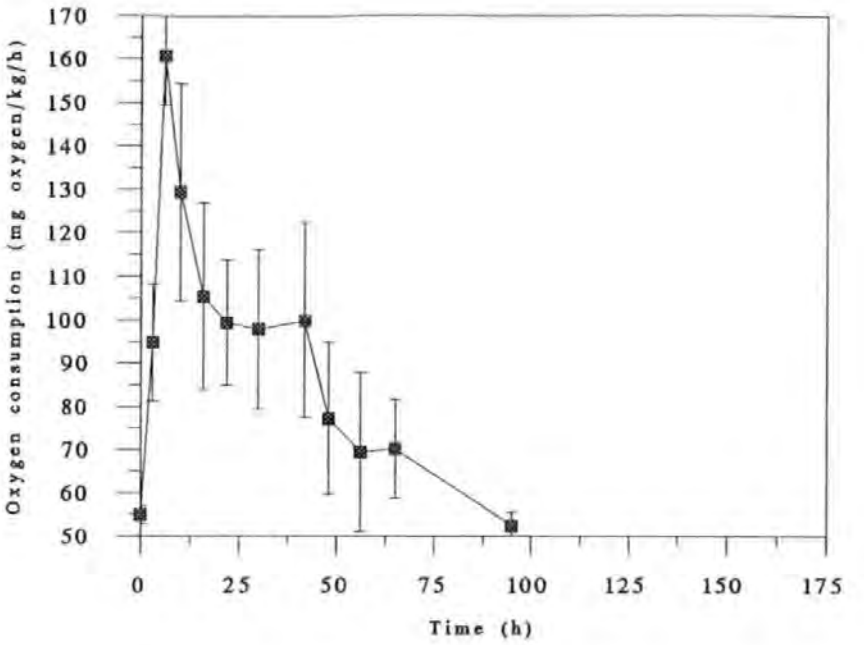

Oxygen consumption (mg oxygen/kg/h)
140
130
120
110
100
90
80
70
60
0
25
50
75
100
125
150
Time (h)

Figure 26. The mean oxygen consumption of adult dogfish after being fed meals of 3.5% wbw (■) and 7% wbw (□). Bars represent ± 1 S.E. Number of determinations for each point: upper panel, 4; lower panel, 3.


Oxygen consumption (mg oxygen/kg/h)
170
160
150
140
130
120
110
100
90
80
70
60
50
0
25
50
75
100
125
150
175
Time (h)

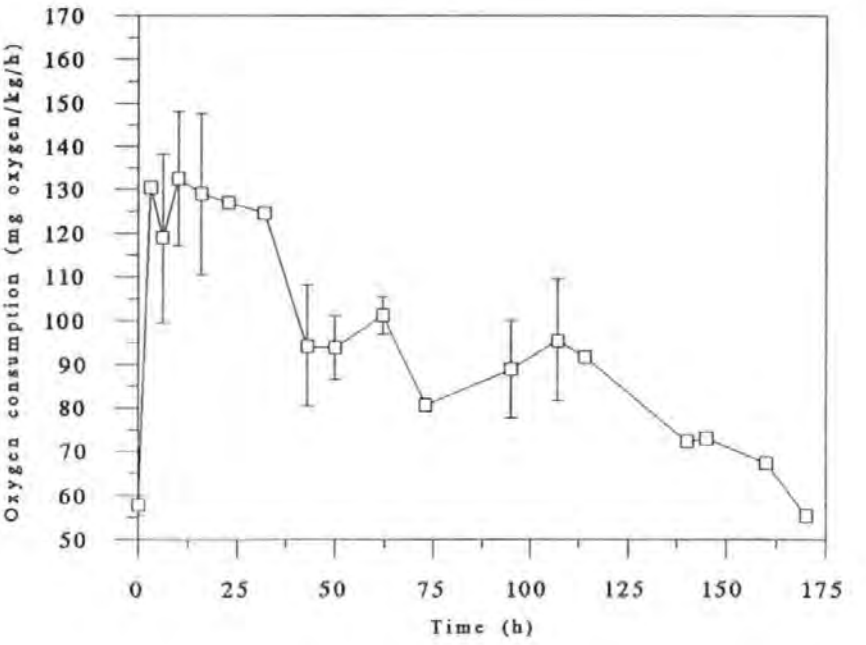

Oxygen consumption (mg oxygen/kg/h)
170
160
150
140
130
120
110
100
90
80
70
60
50
0
25
50
75
100
125
150
175
Time (h)

Table 11. The effect of single meals on the post prandial oxygen consumption of juvenile and adult *S. canicula* (means ± 1 standard error of the mean).

	Juveniles		Adults	
Meal size (% wbw)	7.25 ± 0.23	13.02 ± 0.53	6.52 ± 0.73	11.65 ± 0.15
No. of determination	4	3	4	3
Peak level (% above resting)	159.52 ± 19.85	109.18 ± 13.32	198.34 ± 24.37	171.05 ± 22.42
SDA duration (h)	45.67 ± 8.78	133.50 ± 9.82	83.83 ± 7.03	143.47 ± 16.42
SDA magnitude (mg O_2)	20.08 ± 4.43	25.89 ± 1.32	2177.28 ± 624.59	4247.06 ± 971.16

lost as SDA. The peak levels in metabolic rate occurred within 4-10h for fish in each size class when fed either ration level (table 11). The maximum levels were similar between the two meal sizes for juvenile (P>0.05) and adult dogfish (P>0.25), and were not different between juveniles and adults at the 7% wbw ration level (P>0.1). Although the peak levels in SDA were not significantly different for both juvenile and adult dogfish at either ration level, the peak levels of SDA were consistently lowered at the higher meal size compared to the smaller ration. The duration of the SDA effect, that is the time taken for pre-feeding resting rates of metabolism to resume was nearly twice as long for adult dogfish than for juveniles fed 7% wbw (P<0.01). The duration of the SDA effects associated with consumption of the larger meals were longer than the SDA duration of smaller meals in both juveniles and adults (P<0.0005, table 11). The magnitude of SDA was not different (P>0.05) when the meal given to the juvenile dogfish was approximately doubled (table 11). However, the SDA magnitude of adult dogfish doubled with a concomitant doubling in meal size (P<0.0005). Figure 27 indicates that the percentage of ingested energy lost as SDA was not different between meal sizes for either juvenile (P>0.25) or adult dogfish (P>0.25), but the percentage IE lost by the adults was approximately three times greater than the amount lost by the juveniles (P>0.5).

5.3.3.2 SDA and appetite return

Figure 28 shows the rates of appetite return for juvenile and adult dogfish, when fed similar sized meals to those given to the fish used in the SDA determinations (juvenile 7.58 ± 1.99 %bw; adult 7.68 ± 1.24 %bw) (*cf.* chapter II). It is clear that dogfish were willing to consume more food before the SDA effect had fully ended. The SDA peak occurred approximately 4-6 h after feeding, and food consumption of the adult fish was about 1.5% of the initial meal (IM) at this time. In contrast juveniles were able to

Figure 27. The amount of ingested energy (IE) lost as SDA by juvenile (dark shading) and adult (light shading) dogfish fed meals of approximately 7 and 12.5% wbw. Bars denote ± 1 S.E. Asterisks denote significant difference between SDA losses of juvenile and adult dogfish, $P<0.02$. The same significant difference ($P<0.02$) between SDA losses of juvenile and adult dogfish was also found at the higher ration level. The SDA losses of each size of dogfish between ration levels were not significantly different at the 5% level.

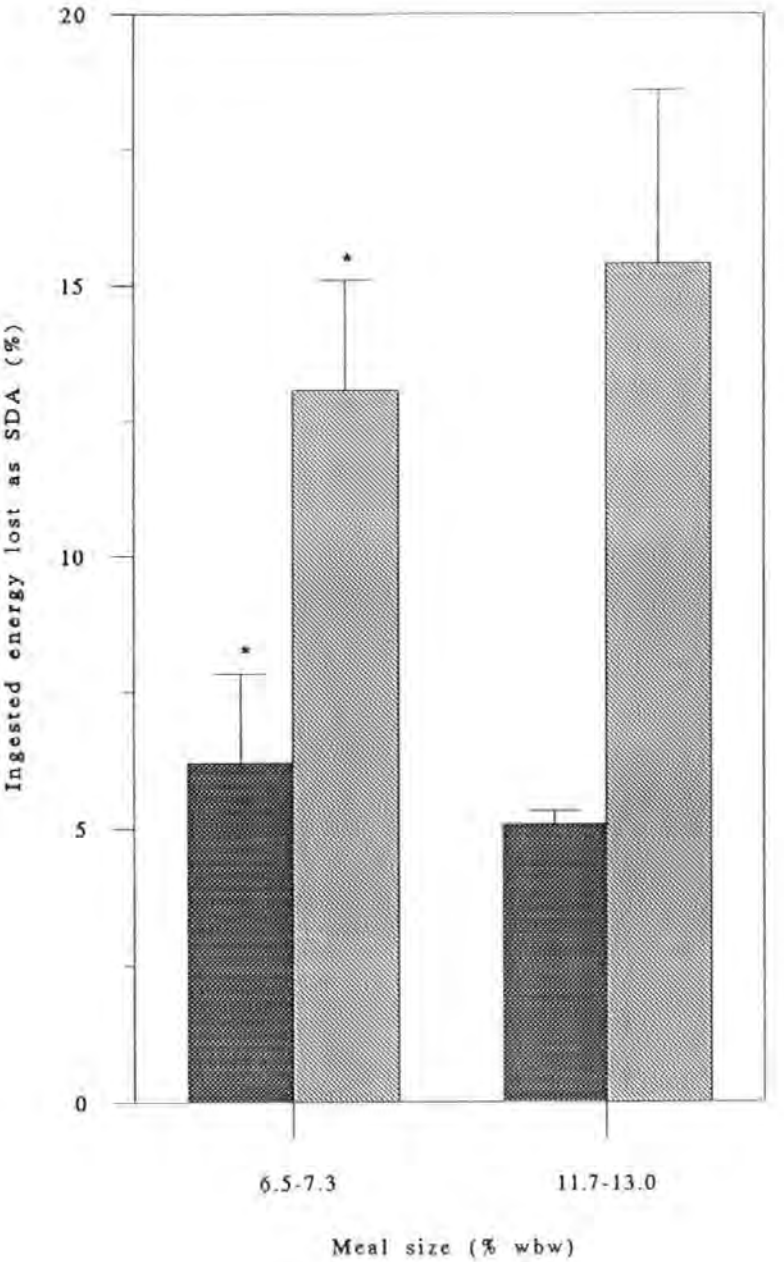
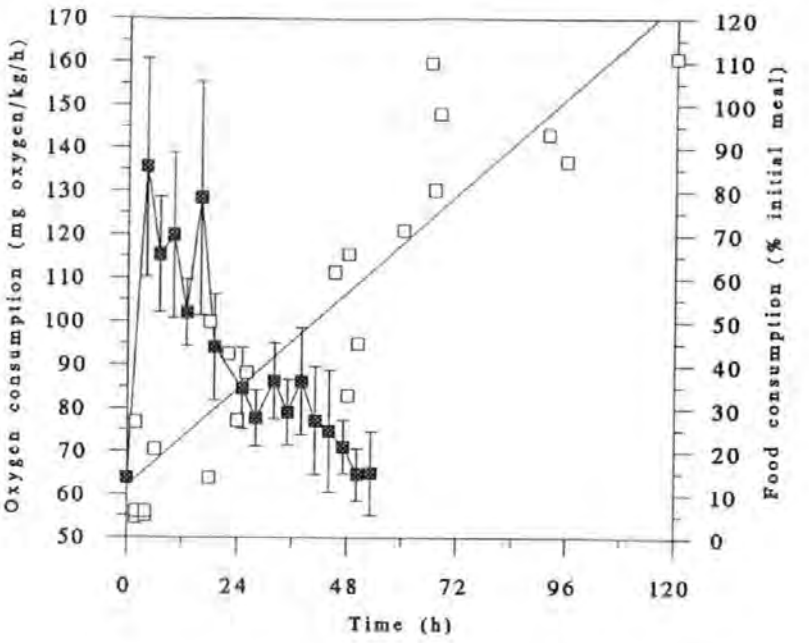
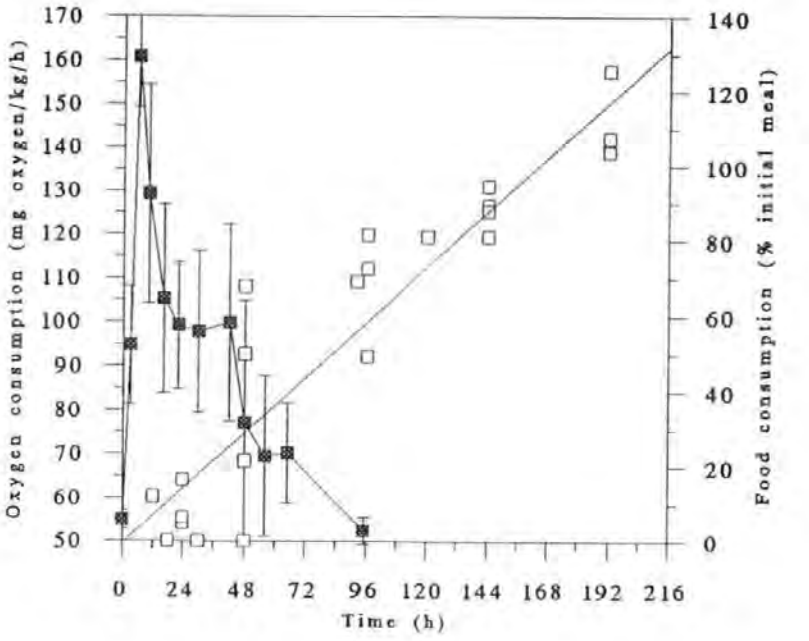

20
15
10
5
0
Ingested energy lost as SDA (%)
*
*
6.5-7.3
11.7-13.0
Meal size (% wbw)

Figure 28. The oxygen consumption (■) and rate of appetite return (□) of juvenile (upper panel) and adult (lower panel) dogfish after being fed meals of 7% wbw. Bars on the solid squares represent ± 1 S.E., number of replicates for each oxygen consumption trace, 4. Appetite return regression equations; upper panel, AR=12.08+0.93t, r^2=0.83, number of determinations, 22; lower panel, AR=-0.53+0.61t, r^2=0.87, number of determinations, 24. Note the difference in *x* axes scale of the two panels.


Oxygen consumption (mg oxygen/kg/h)
Food consumption (% initial meal)
Time (h)
170
160
150
140
130
120
110
100
90
80
70
60
50
120
110
100
90
80
70
60
50
40
30
20
10
0
0
24
48
72
96
120


Oxygen consumption (mg oxygen/kg/h)
Food consumption (% initial meal)
Time (h)
170
160
150
140
130
120
110
100
90
80
70
60
50
140
120
100
80
60
40
20
0
0
24
48
72
96
120
144
168
192
216

consume over 17% IM. When the SDA effect had subsided (after 45 h juven.; 84 h adults) the juveniles were able to consume 58% IM and the adults were consuming about 56% IM. The rates of appetite return were significantly different (P<0.001; table 12), with juvenile fish consuming relatively more food at all times compared to adult dogfish. The time taken for juveniles to consume 100% of the initial meal again was 94h compared to 163h in adults, however the duration coefficients of SDA and AR (table 12) were very similar for both adult and juvenile fish.

Figure 29 illustrates that the maximum peak in SDA after consumption of a 7% wbw meal coincides with the fastest rates of gastric evacuation and intestinal filling. When the SDA effect had ended (after about 100h) the gastric evacuation rate was slowing and consequently, the quantity of digesta arriving in the intestine was also decreasing.

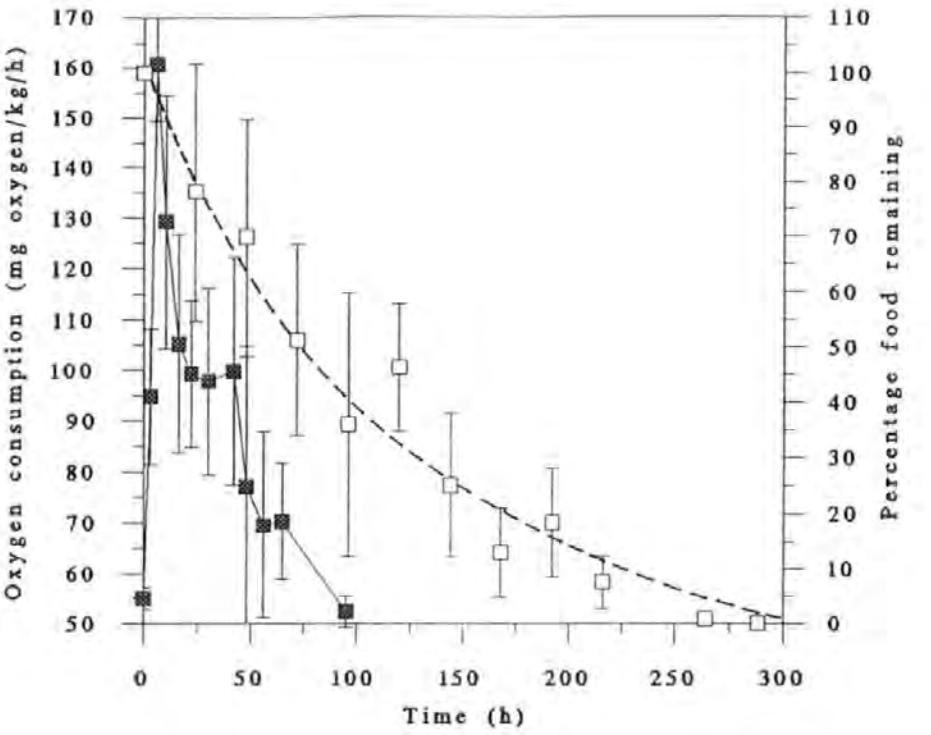
Table 12. The rates of appetite return and duration coefficient of specific dynamic action/return of appetite in juvenile and adult *S. canicula* when fed single meals (means ± 1 standard error of the mean).

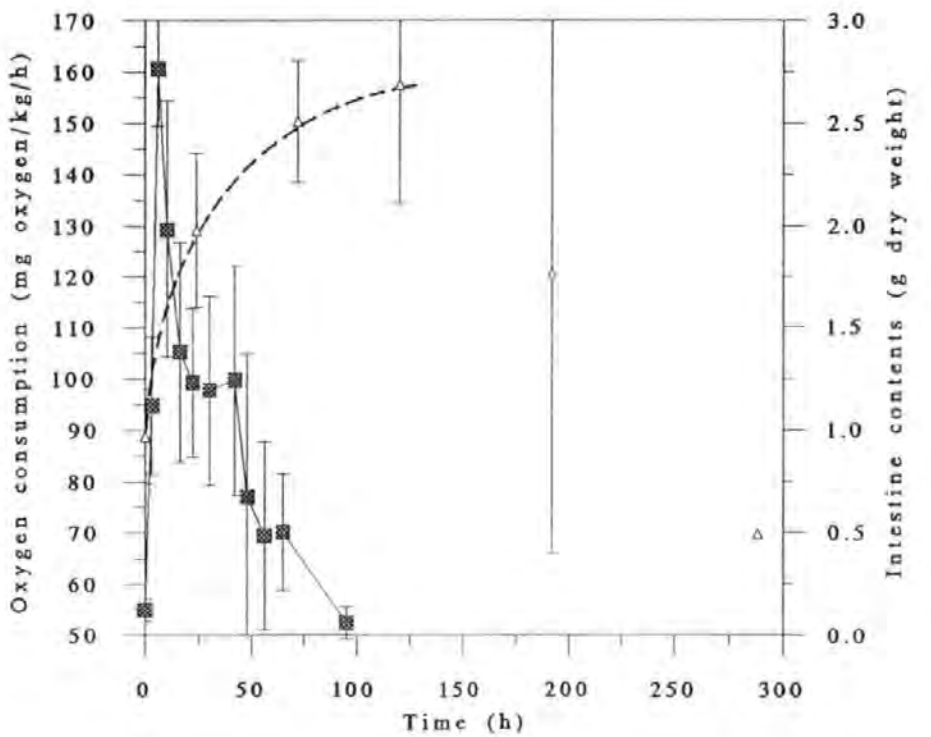
	Meal size (% wbw)	No. of determination	Rate	Duration coefficient (D_{SDA}/D_{AR})
Juveniles	7.58 ± 1.99*	22	0.932†	0.488
Adults	7.68 ± 1.24*	24	0.614†	0.516

**No significant difference between juveniles and adults, $P>0.05$*

†*Significant difference between juveniles and adults, $P<0.001$*

Figure 29. The SDA response of adult dogfish (■) compared with rates of gastric evacuation (□, upper panel) and intestinal filling (▵, lower panel) following a meal of 7% wbw. Bars denote ± 1 S.E.M.


Oxygen consumption (mg oxygen/kg/h)
Percentage food remaining
Time (h)
170
160
150
140
130
120
110
100
90
80
70
60
50
110
100
90
80
70
60
50
40
30
20
10
0
0
50
100
150
200
250
300


Oxygen consumption (mg oxygen/kg/h)
Intestine contents (g dry weight)
Time (h)
170
160
150
140
130
120
110
100
90
80
70
60
50
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
250
300

5.4 DISCUSSION

5.4.1 Activity Patterns and Standard Oxygen Consumption

The metabolic rate and activity of juvenile and adult dogfish increased significantly at night. During daylight hours the dogfish were mostly inactive and the metabolic rates usually decreased steadily over this period, from high night-time levels to low daytime resting rates. Nocturnal activity followed by daytime resting has been demonstrated for adult dogfish in a previous study (Metcalfe and Butler, 1984), as well as for two other species of bottom-dwelling sharks, *Heterodontus francisci* and *Cephaloscyllium ventriosum* (Nelson and Johnson, 1970). Phased bouts of activity over the diel cycle, leading to wide fluctuations in metabolic rate (from near basal to active) characterize the sluggish nature of the bottom-dwelling sharks such as *S. canicula* and do not allow the effects of feeding on metabolism to be easily studied. From the traces in activity and metabolism of juvenile and adult dogfish over 72 and 24 hours respectively, it is clear that prolonged activity occurred during only the dark phase of the diel cycle (up to a maximum of about 50 min h^{-1} in juveniles at night). These large variations in metabolic rate could lead to inaccuracies in quantifying the actual metabolic increment attributable to SDA by erroneous summation of active metabolism and SDA. To avoid these errors post-prandial measurements of oxygen consumption were limited to when the dogfish were inactive. However, oscillations in metabolic rate of *S. canicula* were observed and were probably a result of measurements that followed periods of continuous activity. In an investigation of SDA in plaice, *Pleuronectes platessa*, oxygen consumption measurements were also limited to the times after feeding when the plaice were not active in order to minimise the inclusion of active metabolism with SDA (Jobling and Davies, 1980).

Analysis of diel rhythms in metabolic rates and activity levels of juvenile and adult

dogfish showed that the lowest metabolic rates occurred in daylight between 10.00 and 18.00 h. The lowest resting metabolic rates of fish have been termed standard metabolism (Brett and Groves, 1979). The relationship between standard metabolic rate and fish size was calculated for dogfish in the present study during periods of daytime inactivity and were similar to rates found in previous metabolism studies of benthic elasmobranchs. The standard metabolic rate of a 1kg dogfish at 15°C was 38.2 mg $O_2kg^{-1}h^{-1}$ in this study, whilst the relative rate increased to 74.5 mg $O_2kg^{-1}h^{-1}$ for a 10g juvenile. The standard metabolic rate of *S. canicula* was determined by Hughes and Umezawa (1968) as lying between 28.6-78.7 mg $O_2kg^{-1}h^{-1}$ for fish of 150 to 600g in size. Brett and Blackburn (1978) found that the resting rate of *Squalus acanthias* was 32.4 mg $O_2kg^{-1}h^{-1}$ for a 2kg individual at 10°C, although they noted that the true standard may really lie between 20 and 30 mg $O_2kg^{-1}h^{-1}$. Resting metabolic rates in *S. acanthias* five times higher than Brett and Blackburn's values were recorded by Pritchard *et al* (1958) in fish between 100 and 900g. However, these researchers stated that minimal resting metabolic rates were probably not measured in their study. It is likely that Pritchard *et al*'s level of 'resting' metabolism was excessively high due to the spurdogs being restrained within narrow cylindrical respirometers. The lowest reported standard metabolic rate for an elasmobranch is that of 20.1 mg $O_2kg^{-1}h^{-1}$ for little skates, *Raja erinacea* weighing 0.5kg and kept at 10°C (Hove, 1993). When standardized to 15°C (using a Q_{10} of 2.3, Brett and Groves, 1979) this value becomes 23.1 mg $O_2kg^{-1}h^{-1}$, which is still low in comparison to the standard metabolism of similar sized *S. canicula* from this study. Mean oxygen consumption rates between 20 and 30 mg $O_2kg^{-1}h^{-1}$ were recorded for three adult fish in the 24 hour study of this investigation, however the reductions in metabolic rate of these particular individuals may have been brought about by the period of food deprivation the animals underwent before being placed in the respirometers. It is known that the metabolic

rate of fish will generally decrease as the period of food deprivation increases and the lowest rates observed in these dogfish may be due to such reductions (Jobling, 1982).

The log-log plot of standard metabolic rate against dogfish weight gave a relationship where the power exponent of weight was 0.86. Brett and Groves (1979) showed that this exponent was approximately 0.67 for a wide range of warm-blooded animals and represented the surface area to volume ratio of the animal. These authors found that the mean value for a range of teleost species was also 0.86. In addition Parsons (1990) calculated the weight exponent to be 0.86 when the standard metabolic rate of five species of sharks (*Sphyrna tiburo*, *Negaprion brevirostris*, *Squalus acanthias*, *Scyliorhinus canicula* and *Scyliorhinus catalus*) and a range of teleosts at 15°C were plotted against their weight.

The metabolic rate determinations for juvenile and adult dogfish in this study, during trials over 72 and 24 hours as well as those restricted to periods of inactivity to elucidate standard metabolism, can be considered to be in line with results obtained from other studies on sharks and teleosts. These initial studies have identified that the metabolic rate profiles of sluggish fish, such as dogfish over several days and the accurate determination of standard metabolic rates are necessary studies if estimations of SDA are to be precisely determined.

5.4.2 Important Parameters of the SDA Effect

In general the SDA studies showed the metabolic rate of dogfish increased following consumption of a meal, and from an initial peak decreased quite steadily to pre-feeding levels. It was also evident that the rate of decrease in metabolic rate after meal consumption was generally dependent on the size of the meal. The magnitude of SDA and hence the metabolic load on the animal was dependent on the peak level and duration of

the effect. These two parameters were of prime importance to understanding the role of SDA in appetite regulation and therefore each is discussed here in more detail.

The peak level in oxygen consumption for both juvenile and adult dogfish at both meal sizes occurred 4 to 10 hours after ingestion of a meal and is comparable with other fish studies. The SDA peak of juvenile thick-lipped mullet, *Crenimugil labrosus* (*Chelon labrosus*) occurred after 1-4 hours (Flowerdew and Grove, 1980), whereas the peak in oxygen consumption for the benthic *P. platessa* occurred between 5 and 7 hours post-feeding (Jobling and Davies, 1980). Hamada and Ida (1973) showed two peaks in the post-prandial metabolic rate of *Cyprinus carpio* and *Carassius auratus*, the first after 3-4 hours and the second between 5 and 8 hours. Indeed, Flowerdew and Grove (1980) recorded a second peak in the oxygen consumption of *C. labrosus* 6-11 hours after feeding. A second peak in SDA was not observed in the present study on dogfish, but the timing of the initial increase in metabolism following feeding was quite uniform when dogfish are compared to the aforementioned teleosts. This suggests that the initial peak in metabolism could arise from a physiological process common to all these species. However, the degree to which the metabolic rate increased above standard or resting rates following feeding is not the same in all species, and may serve to illustrate the differences in aerobic capacity of the species concerned, as well as perhaps elucidate the metabolic strategies of these species. The peaks in initial SDA did not differ with dogfish size and were 2.7 times the lowest resting rates. The peak rate in oxygen consumption was 1.8 times resting metabolism in *Lipophrys pholis* (Vahl and Davenport, 1979), 2.0 in *P. platessa* (Jobling and Davies, 1980), 2.6 for *Kuhlia sandvicensis* (Muir and Niimi, 1972) and 1.6 for *Lepomis macrochirus* (Schalles and Wissing, 1976) and *Gadus morhua* (Saunders, 1963). The peak levels of dogfish SDA above resting metabolic rates were slightly greater than most teleosts listed here and implies that SDA in dogfish may occupy

much of the available metabolic scope.

Post-prandial peaks in oxygen consumption attributable to SDA and oxygen uptake due to night-time peaks in dogfish activity were little different. Soofiani and Priede (1985) showed that oxygen consumption of *G. morhua* following meal intake was greater than that of active metabolism. These observations led the authors to state that the cardiorespiratory system had evolved to supply the demands of feeding rather than activity, and that SDA occupied much of the metabolic scope of these fish after feeding. The same could be postulated for dogfish from evidence in the present investigation, as the peak metabolic rates due to SDA matched the rates obtained after continuous night-time swimming. However, from measurements of oxygen consumption made on an adult dogfish agitated to swim faster for a short time within the respirometer, it is possible that maximum metabolic rates recorded during night activity may not represent the maximum sustainable power output of the dogfish within its aerobic metabolic scope (Priede, 1985). The metabolic rates recorded after the forced swimming were approximately 100 mg $O_2 kg^{-1} h^{-1}$ higher than the peak SDA level following feeding, although it may be equally possible that this higher rate of oxygen consumption was partly due to repayment of the oxygen debt following anaerobic metabolism. It is not known, and must not therefore be assumed that the initial peaks in dogfish SDA were elevated to the likely limit of the animal's metabolic scope for aerobic activity between 4-10 hours after feeding. It can be said though, that the SDA incurred after feeding in dogfish occupied a major portion of the scope for energy available for aerobic metabolism.

When the meal size consumed by dogfish was doubled there were no differences in SDA peak level for either juveniles or adults. Jobling and Davies (1980) demonstrated that the SDA peak rates were similar above a threshold level when increasing meal sizes were given to *P. platessa*. Over a range of meal sizes the peak rates of oxygen

consumption were not different, which led the latter authors to suggest the SDA process may have a saturation level determined by cellular metabolism rather than by the respiratory system. This theory could equally be applicable to the SDA peaks observed for dogfish since the peak metabolic rate of SDA did not vary with increasing meal size and the peak may not necessarily equal the metabolic rate obtained by maximum sustained swimming. Therefore, the SDA effect on dogfish metabolism may depend to a large degree on the rate at which food breakdown products can be metabolised as well as to the extent SDA occupies the metabolic scope of dogfish.

The duration of SDA increased with consumption of greater meal sizes in both juvenile and adult dogfish. In previous studies the SDA durations of teleost fish have been shown to be highly dependent on meal size (for reviews see Jobling, 1981a, 1983). The duration of SDA in juveniles was generally shorter at each meal size when compared with the SDA duration of adult *S. canicula*. This may result from the higher relative rates of food consumption and processing rates of the younger dogfish compared to the mature individuals (*cf.* chapter II). Although the peaks in SDA did not vary between dogfish sizes or because larger meals were consumed, the increase in SDA duration with meal size means that the magnitude of the SDA effect generally increased with meal size in dogfish. SDA duration of adult *S. canicula* doubled with a concomitant doubling in the amount of food consumed, which led to a twofold increase in SDA magnitude. Other investigations on teleosts have reported similar findings. The magnitude of SDA was also shown to be proportional to the increase in meal size in *P. platessa* (Jobling and Davies, 1980), *Micropterus salmoides* (Beamish, 1974) and *Lepomis macrochirus* (Pierce and Wissing, 1974). However the SDA magnitude of juvenile dogfish did not increase appreciably when larger amounts of food were consumed. The SDA duration from consumption of a 13% wbw meal by juvenile dogfish was approximately three times that of the 7% wbw meal,

but the SDA magnitude was only marginally higher and found not to be significantly different. At the higher meal size the juvenile SDA rapidly lowered after the initial peaks which did not occur in the SDA of adult fish. The rapid lowering in oxygen consumption rate was responsible for the reduced SDA magnitude in juveniles and may represent a physiological strategy that limits the loss of post-prandial energy.

The ingested energy lost as SDA was three times greater in adults when given either ration size compared to the juveniles. Some workers have demonstrated relationships between SDA and growth in mammals, fish and some invertebrates (Kreiger, 1966, 1978; Ashworth, 1969; Jobling, 1983; Vahl, 1984) and have postulated that SDA is primarily the direct metabolic eventuality of the costs of protein synthesis, protein tissue turnover and growth (for review see Jobling, 1983; Houlihan *et al.*, 1990; Carter & Brafield, 1992). The juvenile fish in the present study had higher levels of food consumption coupled with lower SDA costs when compared to adults, but this may represent efficient conservation of post-prandial metabolic energy rather than implying lower rates of biosynthesis and growth. Juvenile fish have a high potential for fast growth rates resulting from high rates of food turnover (Kiorboe *et al.*, 1987), so the differences in food intake between adult and juvenile fish of this investigation may be primarily a developmental strategy rather than principally a consequence of the SDA effect. The energy lost as SDA in adult dogfish was similar to the levels found for *L. macrochirus* (Schalles and Wissing, 1976) and *Micropterus salmoides* (Beamish, 1974). The cost of growth is known to increase and the efficiency of the process decreases as fish become larger (Kiorboe *et al*, 1987), so the lower ingested energy losses due to SDA in juvenile dogfish may represent a significant strategy for maximising growth whilst keeping the energetic costs of meal processing to a minimum.

5.4.3 SDA and the Regulation of Appetite

Apart from the differences in relative food consumption and processing rates between young and mature dogfish (possibly manifested as different ingested energy losses) it is evident that SDA is closely associated in the processes leading to appetite return. The most important findings of the present study pertaining to SDA and appetite control were that the relationships between SDA and appetite return (AR) were similar for both sizes of *S. canicula*, and that food consumption continued throughout the period of SDA with maximum post-feeding metabolism not completely eliminating further food consumption. Similar results to those of the present study have been demonstrated for different size groups of *Limanda limanda* (Fletcher, 1982), *Lepomis macrochirus* (Schalles & Wissing, 1976) and *Kuhlia sandvicensis* (Muir & Niimi, 1972) and for single size *Blennius pholis* (Vahl & Davenport, 1979). The coefficients of SDA and AR duration were very similar in juvenile and adult dogfish, indicating a close involvement of SDA in the revival of food consumption for both sizes of fish. The relationship however, may not necessarily be one of strict regulation since the amount of food consumed by adult and juvenile dogfish after the SDA effect had subsided, was only 50-60% of a satiation meal. Clearly whilst the SDA effect and AR are closely linked (Jobling, 1981a), it is also evident that SDA is not the regulator of further food intake in either juvenile or adult *S. canicula*, since the existence of any such regulation would be expected to be manifested as greater relative rates of food intake as SDA diminished and a more marked suppression of food consumption due to the SDA effect. Since these influences were not observed in the current investigation the likely role of SDA in food intake control may be that of a component mediator of the plane of metabolism, which in turn influences other physiological processes.

Direct evidence for influence of post-prandial metabolism on food intake is lacking

but it has been suggested that SDA may affect appetite return in the short-term by limiting the rate at which a meal can be processed and so slow the overall clearance of the gastrointestinal tract (Jobling, 1981a; Fletcher, 1984). These conclusions may help explain the findings for adult dogfish, which were that as SDA magnitude increased with increasing meal size (from greater demand for oxygen by metabolic pathways involved in food processing) the instantaneous rate of gastric evacuation decreased resulting in greater overall gut emptying times (*cf.* chapter III). However, this investigation also showed that the gastric evacuation and intestinal filling rates were greatest when the SDA effect was maximally deviated from the pre-feeding levels. If SDA in dogfish was acting to limit the energy available for gastrointestinal activity, thereby altering appetite return directly, then greater rates of gastric evacuation and intestinal filling might be expected in the latter stages of the SDA effect when metabolic rates were decreasing. Such influences were clearly not observed in the present study. This evidence indicates that appetite return may be only indirectly affected by SDA, which could act by limitation of metabolic energy available for the physiological and biochemical processes important in determining the rate of food digestion and assimilation.

Fletcher (1982) stated that *Limanda limanda* was limited by the level to which it could elevate its oxygen uptake after a meal. The Atlantic cod, *G. morhua* was also limited in post-prandial oxygen uptake as their rates of oxygen consumption actually exceeded (for a time) the level of active metabolism (Soofiani and Priede, 1985). Although the SDA of both *P. platessa* (Jobling, 1981a) and *K. sandvicensis* (Muir and Niimi, 1972) occupied approximately 50% of the scope for activity, the oxygen uptake was still also limited because the peak level in SDA did not increase with meal size. Therefore, the limitations to post-feeding oxygen uptake in fish seem to be of two types. Those species whose uptake is limited because much of the available metabolic scope is

occupied by SDA (*e.g. G. morhua*), and those where the level of SDA does not increase above a certain level, though that level may not necessarily be equal or close to the active metabolic rate (*e.g. P. platessa, K. sandvicensis*). On the basis of this study, dogfish may be more similar in physiological strategy to *G. morhua*. In addition Vahl and Davenport (1979) demonstrated for *Blennius pholis* (*Lipophrys pholis*) that the difference between routine and active metabolism was of the same magnitude as maximum SDA and that this would influence food intake. In a similar manner, I suggest that the SDA of dogfish could limit further food intake, though only by perhaps indirect means, when the fish have other physiological demands in addition to SDA.

For adult dogfish the SDA magnitude increased as the meal size consumed also increased. In addition, the peak levels of SDA were of the same magnitude as the peak metabolic rates resulting from continuous swimming activity. Thus, the rate of food processing (digestion, absorption and assimilation) might become limited by the available oxygen that was not required for activity or basal metabolism. If the dogfish were active during the processing of a meal (as could be likely in the wild), then the oxygen demand from locomotor muscles and SDA could potentially exceed the uppermost level of aerobic metabolism. Jobling (1981a) suggested a possible way in which this situation could result in a lowering in food processing rates. If digestion and absorption of nutrients from the high ration levels proceeds unchanged at high levels of activity without further processing, the blood would accumulate metabolites, such as free amino acids perhaps above the maximum carrying capacity and a toxic effect may result. The latter author postulated that to avoid the "toxic" effect, the rate of food processing would be slowed by shunting blood away from the gut. In support of this, reductions in gastric evacuation rate have been observed at high levels of activity (Tyler, 1977). Contrastingly, the gastrointestinal motility of humans has to be shown to actually increase during moderate exercise

(Christiansen, 1991 citing Keeling *et al*, 1990).

Additional locomotor activity could lead to reductions in the rate of food processing as the magnitude of feeding metabolism matches active metabolism. It is interesting to note that in dogfish the rhythmicity of activity during the night followed by inactivity during daylight hours was maintained when they had been deprived of food for some days. Reductions in bouts of activity during the night might therefore be expected after large or multiple meals as the oxygen available for activity becomes more limiting. It was noted for dogfish that activity was often depressed compared to normal rates during the 24 hours following the consumption of the largest meals. Similarly, the scope for activity of *B. pholis* (*L. pholis*) was seen to become greatly reduced (by as much as 60%) following feeding. In the wild a similar strategy may occur in dogfish. Cumulative SDA from multiple meals (Fletcher, 1984) and the low incidence of empty stomachs of wild dogfish (Lyle, 1983) indicates that activity might be reduced so the processing rate of meals could be maintained at an optimum. Recent work on lemon sharks, *N. brevirostris* showed that these sharks were as efficient at absorbing nutrients from ingested meals as teleosts (Wetherbee and Gruber, 1993), so short-term reductions in dogfish activity after feeding may occur in order to maintain rather more rapid (and ideally, just as efficient) meal processing rates. However, the SDA of juvenile dogfish at high ration levels (13% wbw) was no greater than at lower feeding levels. This low SDA magnitude could represent a physiological strategy whereby scope for aerobic activity is maintained without lowering the rate of meal processing at the higher meal intake. Information of gastric evacuation rates of juvenile dogfish would be useful to provide a physiological basis for this possible strategy.

In dogfish, I suggest that SDA could affect short-term appetite regulation if dogfish were active after meal consumption and this high oxygen demand could cause the rates

of food processing to become prolonged. If rates of absorption and assimilation were slowed then the rate of intestinal evacuation may also be reduced. From previous chapters of this thesis (*cf.* chapters II, III and IV) it has been proposed that appetite regulation is maintained in a direct fashion by food bulk dependent mechanisms controlling the pattern of gastric emptying, with possible feedback signals (from mechanoreceptors?) perhaps modifying that pattern by detection of intestinal fullness. The SDA effect may influence the gastric emptying pattern and therefore appetite, albeit indirectly, by further modifying rates of meal processing when the animal's metabolic demands exceed the scope for activity. The mechanisms by which meal processing rates could be monitored are not known in fish, but certain receptors capable of detecting the presence and concentration of nutrient metabolites in the blood could perhaps modify the degree of motility of the gut by efferent nervous and hormonal stimulation of the gut musculature. The physiological regulation of appetite in dogfish would therefore appear to be a multifactorial process with feedback loops connecting the rates of metabolism, gastric and intestinal fullness and food consumption.

CHAPTER SIX

PLASMA METABOLITES AND APPETITE CONTROL: A PRELIMINARY STUDY

6.1 INTRODUCTION

The role of plasma metabolites in the regulation of appetite has been well studied in higher vertebrates, but little research has been directed towards fish. In mammals, plasma metabolites (also termed plasma nutrients) such as glucose, free fatty acids, glycerol and amino acids have all been shown to play some role in modifying food intake (Fletcher, 1984). The degree to which they influence appetite has been shown to be largely dependent on the post-prandial concentration of those nutrients in the plasma. Whilst the biochemical conceptualization of the effects of plasma nutrients in food intake regulation remain vague, it is generally supposed that levels of plasma nutrients are in some way recognised, quantified and continually monitored in organs such as the brain or liver (Booth, 1979). It is clear that an animal must obtain a physiological perception of the levels of circulating metabolites in order for compensatory physiological changes to occur in the rates of food absorption and in the mobilization of existing energy stores. The existence of such systems of post-absorptive control of satiety can be appreciated in higher vertebrates from the evidence that they can closely regulate the carrying capacity of metabolites in the blood by hormonal action (Stryer, 1988; Mathews and Van Holde, 1990).

Much of the higher vertebrate research on plasma metabolites and appetite control has centred on carbohydrate metabolism and the dynamics of glucose utilisation in response to infusion of polypeptide hormones such as insulin (Anika *et al*, 1980; Morley

and Flood, 1991) and neuropeptides (Inui *et al*, 1991; Kalra *et al*, 1991). Infusions of plasma metabolites into the bodies of animals have elucidated the possible effects some nutrients may have on feeding behaviour when they were in excess in the blood (Booth, 1979). Administration of carbohydrate and food-derived glucose by oral, intragastric, duodenal, portal and jugular methods have all induced satiety in mammals (Fletcher, 1984), and thereby the plasma concentration of these nutrients have been regarded as of prime importance in feeding control in omnivorous higher vertebrates. The mammalian investigations reviewed by Fletcher suggests the animal can detect the level of circulating plasma glucose and that maximally deviated concentrations can lead to the induction of satiety.

Equal importance of glucose in the systemic regulation of food intake in carnivorous animals (including fish) may perhaps be unlikely however, considering the difference in natural diets of the two animal groups. The metabolism of omnivores is dominated by the utilization of carbohydrate, as their natural diet probably contains sufficient amounts to satisfy the needs of the whole animal (Cowey *et al*, 1977a). In contrast, the diet of carnivores will contain little carbohydrate and it has been widely demonstrated that cultured fish require high protein diets for optimal growth (Cowey *et al*, 1977a), with complex carbohydrates being poorly assimilated by fish (Cowey and Walton, 1989).

The wild diet of fish will contain little carbohydrate and so teleologically these fish might not be expected to have evolved efficient mechanisms to deal with dietary carbohydrates (Hilton and Atkinson, 1982). Indeed some investigations on fish have demonstrated that glucose is respired much less rapidly than in rats or mice (Cowey *et al*, 1975), that fish have a limited ability to efficiently adapt to increases in dietary carbohydrate (Hilton and Atkinson, 1982) and that fish exert fine metabolic control of

gluconeogenesis in contrast with their inability to regulate blood glucose level (Cowey *et al*, 1977b). In addition, fish do not demonstrate any adaptive capacity to regulate post-prandial glycemia levels when fed for long periods on carbohydrate rich diets (Kaushik *et al*, 1989). Hence, poor metabolic control of blood glucose in fish resulting in low glucose tolerance (Patent, 1970) questions the extent to which plasma glucose level contributes to appetite regulation in fish.

Notwithstanding the teleologic approach to understanding the possible role of plasma nutrients in food intake control in fish, various studies have measured plasma glucose before and after feeding. However the investigations are often contradictory giving evidence that post-prandial fluctuations in plasma glucose may or may not influence further food consumption (Bellamy, 1968; Gwyther, 1978 cited in Fletcher, 1984; Peter, 1979; Fletcher, 1982). The motive for undertaking these investigations perhaps comes from research on higher vertebrates, where close control of glucose metabolism has been shown by rapid secretion of insulin and glucagon by the pancreatic β-cells and α-cells respectively, to counter imbalances in blood glucose concentration. The importance of glucose in appetite regulation of higher vertebrates has been clearly demonstrated (Peter, 1979; Stryer, 1988). The purpose of other studies on plasma metabolites in teleost and elasmobranch fish have been more fundamental in their approach, in that they have been undertaken to further understand the biochemistry of intermediary metabolism (Zammit and Newsholme, 1979) in relation to nutrition and enhancement of fish growth (Cowey *et al*, 1975; Cowey *et al*, 1977a,b; Hilton and Atkinson, 1982). Plasma glucose acting as a possible satiety factor has not been studied in elasmobranchs directly, though some indirect (and contradictory) measurements have been made recently pertaining to seasonal variation in glucose levels (Gutierrez *et al*, 1988; DeRoos, 1994).

The preferential use of protein over carbohydrate as a dietary energy source in the

metabolism of fish has meant the role of amino acids as possible satiety factors have also been investigated (Fletcher, 1984). The post-prandial profile of amino acids in circulating blood have been shown to reflect the dietary levels and in addition serial deletion of each of the ten essential amino acids from diets has been reported to lead to loss of appetite (Fletcher, 1984) and growth (Tacon and Cowey, 1985). Despite these observations the role of amino acids in appetite regulation in fish remains unclear.

The liver of higher vertebrates itself is known to be able to respond to high levels of plasma glucose by increasing phosphorylation and glycogenesis, which provides evidence that the liver can detect the 'fed' state (Mathews and Van Holde, 1990). As has already been stated, the importance of plasma glucose in the food intake control of fish is unclear, hence further research in fish should perhaps centre on the post-prandial level of metabolites derived from lipids and proteins. These macronutrients are present in large quantities in the natural diet of fish and so may potentially exert greater metabolic effect on appetitive status than carbohydrate derived metabolites.

The liver is an important organ for regulating circulating plasma nutrients as most breakdown products from digestion are taken up by the liver for processing and it is in the liver that fuel components are synthesised for utilization by other organs (Mathews and Van Holde, 1990). Clearly the liver is important for the storage and mobilization of fuel molecules and its role in the regulation of plasma metabolites, together with the relative importance of the stored lipid fractions arising from dietary sources can be appreciated from studies of starvation in fish. In recent studies the metabolic response of elasmobranch fish to starvation has been generally to maintain the supply of glucose to the tissues that are absolutely dependent on this fuel and preserve protein by shifting the metabolic fuel from glucose to fatty acids and ketone bodies (Zammit and Newsholme, 1979; DeRoos *et al*, 1985; DeRoos, 1994). In addition, the stored lipid in elasmobranchs

is concentrated in a single organ, the liver and can account for up to 70% of the wet liver weight in some species (Patent, 1970). Clearly the hepatic source of lipid is of major physiological importance to the animal. Thus, the role of metabolites derived from lipid could be central to the animal's physiological perception of its biochemical nutritional status by the metabolites functioning as systemic satiety signals. It is not known exactly which plasma metabolites influence appetite (if not all of them) as few studies have been undertaken which attempt to measure the concentrations of plasma nutrients before and after a meal.

The purpose of the preliminary investigation was to measure four plasma metabolites before and after feeding and during the period of appetite return. From any observed post-prandial changes in metabolite level it was hoped to assess what contribution these systemic factors may have in appetite regulation in sharks.

6.2 MATERIALS AND METHODS

6.2.1 Blood Sampling

Twenty adult dogfish (mean weight 697.5 ± 23.0g) were kept in aquaria for six months before any blood samples were taken. During this time the animals were used in other experiments concerned with gastrointestinal evacuation. When these investigations were not in progress the dogfish were fed maintenance rations. Before blood sampling the dogfish were deprived of food for 14 days to make sure the gastrointestinal tract was empty of food from previous meals. Three days before the fish were fed, blood was taken from three of the tagged fish. After the 14 days starvation, pellets of high energy diet (formulation given in section 3.2.1) were given to the dogfish such that each fish was assumed to have eaten 7% wbw. Immediately following the cessation of feeding, blood samples were taken from three of the dogfish. After 24 hours post-feeding blood was removed from another four fish. This was repeated with four other fish at 72 hours, and again blood was serially removed from three different individuals each at 120, 192 and 288 h. No individual dogfish was sampled more than once during the entire trial.

Samples of blood from each dogfish were taken while the unanaesthetised fish were held firmly down with their ventral surface uppermost. Some 5 ml syringes fitted with sterile hypodermic needles (21 gauge) were used to extract the blood from each fish and were pre-heparinised according to Rowley (1990) (heparin in phosphate buffered saline, 10-20 units ml^{-1}, Sigma Chemical Co. Ltd., Poole, Dorset, U.K.). For each dogfish the needle was pushed into the musculature, in the mid ventral line immediately caudad to the anal fin as far as the vertebral column. When the needle reached the vertebrae it was retracted slightly and a steady vacuum in the syringe was maintained by carefully pulling out the syringe plunger until blood entered. This constant pressure was maintained

until the 5 ml syringe was full. The blood from each fish sampled was decanted into 3 Eppendorf tubes and centrifuged for 5 minutes at 6400 rpm to obtain the plasma. The clear supernatant was carefully pipetted from each tube into a clean, labelled tube and kept frozen at -25°C for 2 days until the plasma was analyzed.

6.2.2 Measurements of Plasma Metabolites

6.2.2.1 Glucose

Glucose reagents (Sigma Diagnostics, Sigma Chemical Co. Ltd., Poole, Dorset, U.K.) were used for the quantitative enzymatic determination of glucose in plasma. The procedure used is given in Sigma Diagnostics pamphlet No. 510.

The frozen plasma samples from each dogfish at each time interval were thawed at room temperature. Stoppered 5 ml plastic tubes were labelled and 1.8 ml of deionised water was added to each. Into five of these tubes 0.2 ml of deionised water was added (blanks) whilst 0.2 ml of a glucose standard (27.78 mmol l^{-1} β-glucose in benzoic acid solution) was added to each of twelve replicate tubes. Dogfish plasma (0.2 ml) was added to the remaining labelled tubes, there being 5 tube replicates per fish. All stoppered tubes were mixed by gentle swirling.

The plasma in each test sample was de-proteinised with a combination of sodium hydroxide and zinc sulphate solution. All the tubes were well mixed by shaking and subsequently centrifuged for 5 minutes at 6400 rpm. The clear supernatant from each tube (0.5 ml) was removed carefully and transferred to a set of clean labelled tubes.

A solution of o-dianisidine dihydrochloride (colour reagent) was mixed with a preparation of glucose oxidase and peroxidase (horseradish) in buffer salts and 5 ml of this combined enzyme-colour reagent was added to each tube. All tubes were incubated at 37°C for 30 minutes in a covered water bath to avoid exposure of the tubes to direct

sunlight.

The absorbances (A) of the solutions from each tube were read at 442 nm with the blank as reference, on a Cecil Instruments Series 5000 double-beam spectrophotometer. The wavelength of 442 nm was selected by scanning in the range 400-500 nm for the absorbance peak (λ_{max}) of the glucose standard solutions. All readings were completed within 30 minutes.

The concentration of glucose was calculated in the following way,

$$\text{Plasma glucose (mmol } l^{-1}) = \frac{A_{test}}{A_{standard}} \times 27.78 \text{ mmol } l^{-1}$$

6.2.2.2 Pyruvate

Pyruvate reagents (Sigma Diagnostics as before) were used in the quantitative enzymatic determination of pyruvate in plasma at 340 nm. The procedure utilised lactate dehydrogenase catalysis of the conversion of pyruvate to lactate with the oxidation of nicotinamide adenine dinucleotide H (NADH) to NAD. The reduction of absorbance at 340 nm due to the oxidation becomes a measure of the amount of pyruvate originally present.

Individual samples of dogfish plasma at each time interval were deproteinised with cold 8% perchloric acid and decanted into centrifuge tubes and vortex mixed for approximately 30 seconds. As before, there were five replicate tubes per fish plasma sample. These samples were centrifuged at 1500g for 30 mins. Aliquots of the clear supernatant were pipetted into 2 ml cuvettes and equal volumes of Trizma base solution (Sigma Chemicals) and NADH solution were added to each cuvette. The contents were mixed by inversion. The initial absorbance of each sample was taken at 340 nm versus water as reference. Then 0.05 ml of lactate dehydrogenase was quickly added to each cuvette and were then mixed by inversion several times. After 5 minutes the final

absorbances were read and the concentration of pyruvate in plasma was calculated thus,

Pyruvate (mmol l^{-1}) = (Initial Absorbance - Final Absorbance) x 0.723 mmol l^{-1}*

*conversion factor from the Sigma pamphlet no. 726-UV.

6.2.2.3 Triglycerides

Triglyceride reagent (INT) was used in the quantitative enzymatic determination of triglycerides in plasma at 500 nm. The active constituents of the triglyceride reagent are given in Sigma Diagnostics pamphlet No. 336.

Triglyceride reagent (1 ml) was added to five replicate stoppered tubes for each dogfish sampled at each time interval. Deionised water and triglyceride calibrator (0.01 ml) were added to tubes marked blank and standard respectively, whilst 0.01 ml of plasma was pipetted into each of the sample tubes. The contents of the tubes were well mixed by gentle inversions and then incubated in a 30°C water bath for 15 minutes. The absorbance of each solution was read at 500 nm against water as the reference. The concentrations of triglycerides were calculated in the following manner,

$$\text{Triglycerides (mmol } l^{-1}) = \frac{A_{test} - A_{blank}}{A_{calibrator} - A_{blank}} \times 2.83 \text{ mmol } l^{-1}\dagger$$

†conversion factor from Sigma pamphlet no. 336

6.2.2.4 Total Plasma Protein

Sigma Diagnostics Total Protein Reagent (TPR) was used for the quantitative colourimetric determination of total protein concentration in plasma at 540 nm. The active ingredients of TPR are given in the pamphlet No. 541.

Into stoppered tubes labelled blank, standard and test samples was added 1 ml of TPR followed by 0.02 ml aliquots of deionised water, serial dilutions of bovine serum albumin (BSA) stock solution and dogfish plasma respectively. The tubes were incubated

for 10 minutes at ambient temperature and the absorbances of the solution were read at 540 nm versus the reagent blank as reference.

The procedure was calibrated against absorbances of serial dilutions of a BSA stock solution and a linear regression with a coefficient (r^2) of 0.98 was obtained. From this relationship the concentration of total plasma protein in dogfish was calculated thus,

Total plasma protein (mg ml^{-1}) = 1.4 + 190.3 x A_{540}

6.2.3 Statistical Analysis

The post-prandial variations in plasma metabolites were analyzed by one-way analysis of variance (ANOVA) and the within and between group variability compared by means of Duncan's multiple range test (Duncan, 1955).

6.3 RESULTS

6.3.1 Glucose and Pyruvate

Figure 30 shows the traces in plasma glucose and pyruvate before and after a meal of 7% wbw. The concentration of glucose in dogfish plasma showed a sharp decline during the first 24 hours following the meal, before slowly increasing until 120 h, followed by a subsequent decrease from then to 288 h post-feeding. The range of these fluctuations was about 0.1 mmol l^{-1} which represented a 30% reduction in glucose concentration from the highest levels. Although these trends in glucose concentration were evident, none of the levels of plasma glucose were statistically of significant difference ($P<0.05$). The error bars representing the standard error of the mean were quite large for each plasma glucose determination at each time interval, indicating some variability in plasma glucose concentration between individual fish. Thus we can conclude that despite fluctuations in plasma glucose concentration after food consumption the level generally remained constant for the post-prandial duration.

The plasma pyruvate concentration remained constant preceding food consumption, but decreased significantly after feeding to about half the prefeeding level. From 24 to 72 hours the pyruvate concentration stayed the same before decreasing further to about 25% of the initial prefeeding concentrations where it remained. In contrast to the standard error bars of the plasma glucose determinations, there were small variations in pyruvate levels between individual dogfish within each time interval.

6.3.2 Triglycerides and Total Protein

The plasma concentration of triglycerides and total protein fluctuated in a very similar manner (figure 31). The level of triglycerides was constant during the 72 hours preceding

Figure 30. The concentration in adult *S. canicula* blood plasma of glucose (■) and pyruvate (□) before and after consumption of a 7% wbw meal. The dogfish were fed at time zero (t=0). The bars represent ± 1 standard error of the mean. Numbers denote the significant difference between groups at the 5% level. Group means that share the same number are not significantly different.

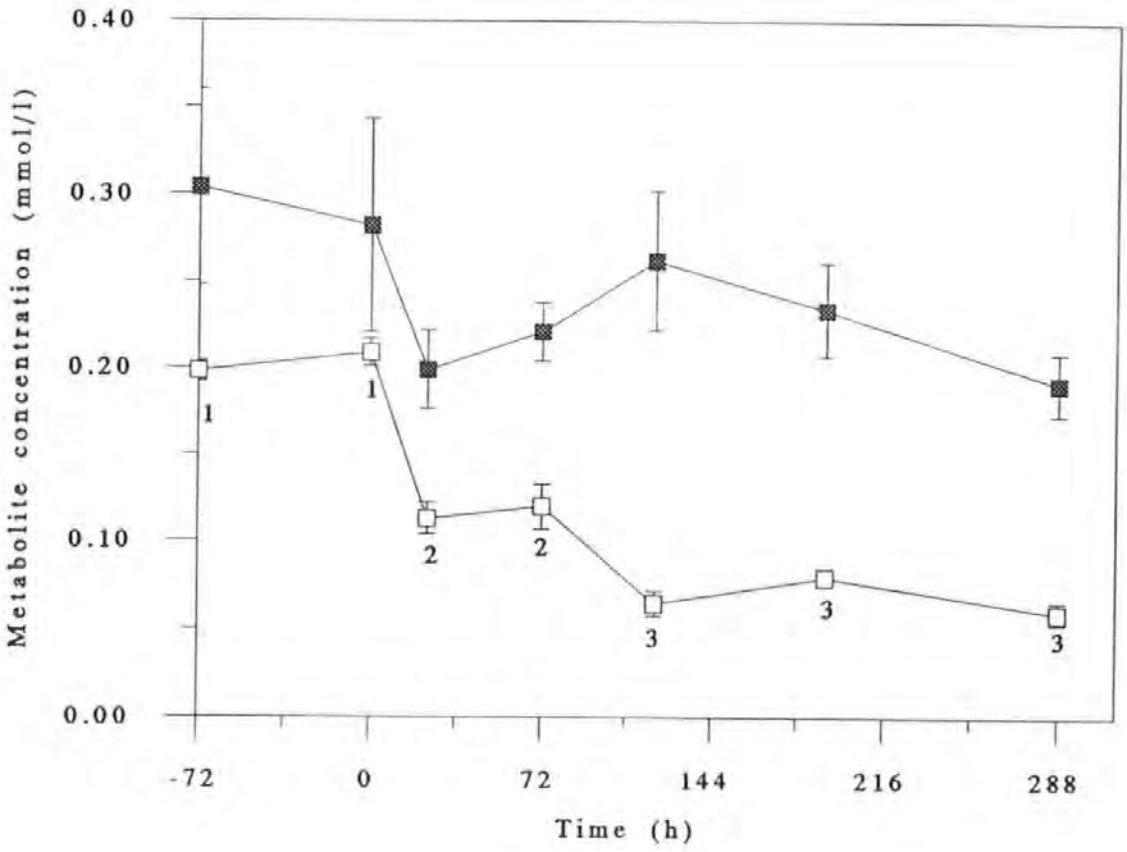
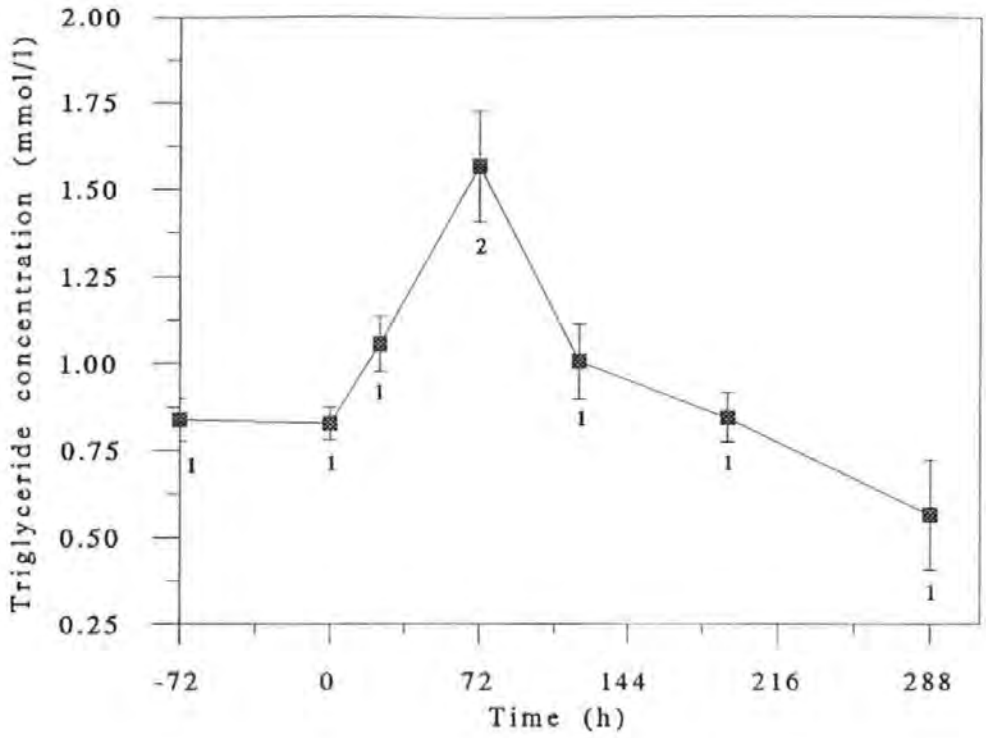
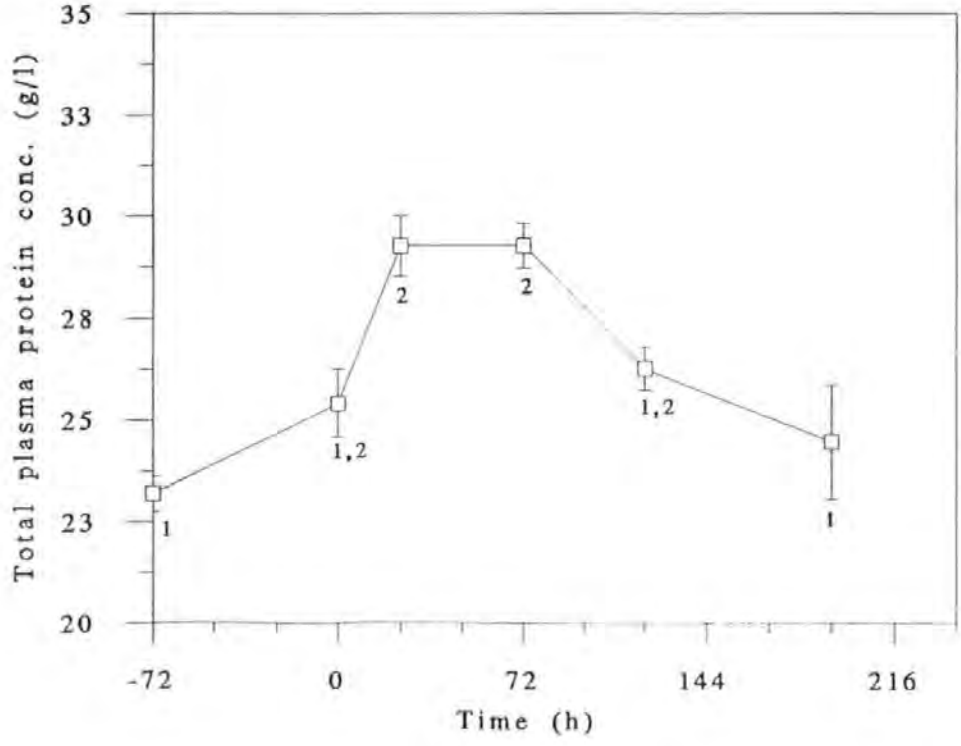

0.40
0.30
0.20
0.10
0.00
Metabolite concentration (mmol/l)
-72
0
72
144
216
288
Time (h)
1
1
2
2
3
3
3

Figure 31. Changes in adult dogfish blood plasma triglycerides (upper panel) and total protein (lower panel) concentrations before and after a meal of 7% wbw. Remainder of caption same as that of figure 30.


Triglyceride concentration (mmol/l)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
1
1
1
2
1
1
1
-72
0
72
144
216
288
Time (h)


Total plasma protein conc. (g/l)
35
33
30
28
25
23
20
1
1,2
2
2
1,2
1
-72
0
72
144
216
Time (h)

food consumption, but after feeding the plasma triglycerides increased over the next 72 hours to approximately double the levels after 12 days starvation. From 72 to 120 h the concentration of plasma triglycerides decreased to normal prefeeding levels where they remained, though there was a general decrease from then until 288 h but this trend was not statistically significant.

Total protein concentration in dogfish plasma was higher immediately after feeding ($P<0.0001$) than the levels measured 72 hours previously (figure 31) and continued to increase during the first 24 h after food consumption. The highest concentrations in total plasma protein were maintained from 24 to 72 hours and were approximately a third greater than the levels obtained 72 h preceding feeding. From 72 to 192 h post-feeding the protein concentrations decreased to the levels that had been measured three days before the dogfish were fed.

6.4 DISCUSSION

The results of this preliminary study demonstrated that following consumption of a 7% wbw meal of HE diet the plasma glucose concentration of adult dogfish did not change appreciably and remained fairly constant at the pre-feeding levels. Additionally, the plasma pyruvate concentration gradually decreased after feeding in contrast to the plasma levels of triglycerides and total protein which rose to a maximum before returning to prefeeding levels.

The plasma glucose level of dogfish in this study fluctuated within narrow bounds (0.19-0.30 mmol l^{-1}) over a 15 day period. In an earlier study Gutierrez *et al* (1988) determined plasma glucose concentrations of adult *S. canicula* on ten occasions throughout the year. The glucose levels they measured were somewhat higher than those of this investigation, ranging from 0.4-1.0 mmol l^{-1}. All the experiments in the current work were undertaken on dogfish kept in seawater at or just below 15°C, whereas in their study the seawater was not maintained at a constant temperature and oscillated between 15 and 21.5°C depending on season. The plasma glucose level in little skates, *Raja erinacea* had been shown to increase by 150% when the water temperature was raised from 13.5 to 25°C (Grant and Simon, 1965). Therefore the glucose concentrations obtained in the study of Gutierrez *et al* (1988) may have been elevated above those found in the present work due to the dogfish being held in seawater that was generally at a higher temperature and was also variable. However, considerably higher glucose levels were measured in a group of spurdogs, *Squalus acanthias* (from 1.8-2.5 mmol l^{-1}) over a period of nine days in seawater kept at 12-14°C (DeRoos *et al*, 1985). Although the spurdogs were kept at lower temperatures than our dogfish, the elevations in plasma glucose level could be due to greater metabolic activity in *S. acanthias* compared to *S. canicula* as previous studies have

shown that glycaemia can be dependent to some degree on the activity of the animals in question (Blasco *et al*, 1992). The spurdog is generally described as an active species which swims constantly at a steady pace and is able to capture fast-swimming fish prey, *e.g. Clupea harengus* (Jones and Geen, 1977; Compagno, 1984a). In contrast, the dogfish is a bottom dwelling shark that will remain quiescent for long periods (this study; Compagno, 1984b). Greater muscle activity, characteristic of a more active species might result in greater metabolic demand for glucose and possibly greater turnover of lactate, which is a substrate for the recycling of glucose in a glucose synthesis pathway (gluconeogenesis). Thus, it might be expected that the concentration of plasma glucose may be higher at any one time in the systemic circulation of an individual spurdog than those measured in the more sluggish dogfish. By comparison to recent studies it would appear that the range of plasma glucose concentrations in dogfish in this study is narrower and fixed at a lower level. Despite this, the glucose levels of dogfish here, may be lower compared to other studies due to generally lower seawater temperature which did not fluctuate, combined with the characteristically inactive nature of the species.

The level of plasma glucose in adult dogfish was not significantly different before or after being fed a 7% wbw meal. Although the concentrations fluctuated they remained at a significantly steady level throughout the 15 day trial. Other studies on fish have noted that the plasma glucose level increased immediately after feeding (*Rooseveltiella nattereri*, Bellamy, 1968; *Squalus acanthias*, Patent, 1970; *Salmo gairdneri* (*Oncorhynchus mykiss*), Cowey *et al*, 1977b), though in two of the investigations this was due to intra-arterial injection of glucose and being fed diets high in carbohydrates. Most studies of elasmobranch blood glucose levels have shown that the concentration remains relatively constant after feeding and also during starvation. Grant *et al* (1969) did not record any significant changes in plasma glucose in three species of ray (*R. erinacea, Raja oscillata*

and *Raja laevis*) after feeding or during the subsequent 40 days of starvation. Relatively uniform levels of plasma glucose were recorded in *S. acanthias* after 3 to 8, 9 and 20, and 29 days of starvation (DeRoos *et al*, 1985; DeRoos, 1994). In plaice, *Pleuronectes platessa*, Cowey *et al* (1975) showed there were no marked effects on plasma glucose level, even when glucose and dextrin were in the food given to the fish. The HE diet consumed by the dogfish in the current study did not contain any carbohydrate, so perhaps no post-prandial increases in plasma glucose would be expected. Despite this, the fact the plasma glucose concentration of the dogfish remained unchanged for 12 days following feeding suggests the dogfish were able to maintain plasma glucose levels over this period. These observations are in agreement with the other studies that have measured plasma glucose concentrations in sharks and rays both after feeding and during the course of starvation.

Even though the plasma glucose levels in dogfish were not statistically significant there was an indication of a slight decrease soon after feeding and again towards the end of the sampling period. Slight decreases in glucose were also noted soon after feeding in *R. erinacea*, but as in the case of dogfish, were not of statistical significance (Grant *et al*, 1969). In addition, downward trends in plasma glucose level were measured in *Salmo trutta fario* after eight days starvation, when during the initial eight days the glucose level had been maintained (Navarro *et al*, 1992). Similar measurements were made by Blasco *et al* (1992) working on *Cyprinus carpio* after 67 days starvation. It was suggested by these authors that the fish had a greater dependence on the process of gluconeogenesis to provide glucose for essential metabolism rather than utilize stored glycogen in the latter stages of starvation. They reasoned that rapid utilisation of glucose produced from the gluconeogenic pathway and exceeding the rate of turnover, could lead to slight decreases in plasma glucose levels. It is possible that progressive necessity for activation of

gluconeogenesis was in operation in dogfish and a significant decrease may have occurred if measurements of plasma glucose could have been taken after 288 hours.

The constant plasma glucose concentrations measured before and after feeding in dogfish suggest that plasma glucose may not be of prime importance in the short-term regulation of appetite. The consistent levels of plasma glucose during feeding and over the time of digestion, absorption and meal assimilation indicated the fish were manufacturing glucose via gluconeogenesis or mobilizing stored glucose by glycogenolysis. During starvation the glycogen in the liver of *C. carpio* actually increased, while the blood glucose levels fluctuated quite widely (3.2-6.6 mmol l^{-1}) (Nagai and Ikeda, 1971, cited in Cowey *et al*, 1977b). From this it was concluded that glycogen was not serving as an immediate glucose source, that there was a very low flux between glycogen and glucose and gluconeogenesis was operating to meet demand. Other studies on teleost fish have shown that liver glycogen stores are protected by an increase in gluconeogenesis (Cowey *et al*, 1977b; Blasco *et al*, 1992; Navarro *et al*, 1992). Indeed, Cowey *et al* (1977b) noted the inability of trout to control blood glucose concentration was partly due to lack of glucose phosphorylating capacity. They also demonstrated that regulatory control over gluconeogenesis was important in trout because the rate and extent of release of glucose from the liver by glycogenolysis was very slow.

The concentration of plasma pyruvate was uniform before feeding, but rapidly decreased soon after food consumption where it stabilised at a lower level. The reduction in plasma pyruvate concentration could indicate progressive activation of the gluconeogenic pathway in dogfish of this study. Glucose and pyruvate are inextricably linked in the biochemical processes of glycolysis and gluconeogenesis, glucose being catabolised in the cytosol of cells whilst pyruvate is converted to glucose (from substrates such as serine and alanine) in the liver. (It should be noted that amino acids contribute the

majority of carbon to glucose in fish (Moon *et al*, 1985).) Although the two pathways are recipricolly regulated, one pathway is not a simple biochemical reversal of the other (Stryer, 1988). Therefore, in the context of the present investigation, a lowering in plasma pyruvate levels, together with maintenance of plasma glucose may indicate that pyruvate was being rapidly utilized and there was biochemical predominance in the gluconeogenic pathway. Another study on an elasmobranch (*R. erinacea*) suggested overall decreases in metabolite level could be explained partly by increased utilization rates exceeding rates of production (Grant *et al*, 1969). It is not possible to conclude which biochemical pathways for plasma glucose maintenance were predominating in dogfish without measurements of liver glycogen levels, in addition to gluconeogenic and glycogenolytic enzyme activity during the process of appetite return.

With regard to the literature available on glucose metabolism and regulation in teleosts, it is clear that blood glucose levels are not well controlled. The enzyme glucokinase, which provides glucose-6-phosphate to promote the storage of glucose as glycogen has been shown to be absent in rainbow trout, thus reducing the phosphorylating capacity of the animal (Cowey *et al*, 1975). The present investigation showed that plasma glucose concentration remained quite uniform during the appetite return. The maintenance in plasma glucose level may have been brought about by the gluconeogenic pathway, which in contrast to glycogenesis is quite finely controlled in fish (Cowey *et al*, 1975; Cowey *et al*, 1977a; Cowey *et al*, 1977b). If gluconeogenesis were not operating in dogfish to maintain the level of plasma glucose (as no carbohydrate was available from the diet), then the fluctuations in glucose concentration would have been expected to be much greater than actually were observed. For plasma glucose to act as a satiety factor, the level would be expected to change as the time after initial feeding increased, and therefore have the potential to act as an instantaneously changing biochemical signal to

bring about the return to appetite. The constant glucose levels observed after feeding and during the appetite return in dogfish indicate that glucose concentration in plasma may not act directly as a satiety factor. It is not easy to understand how a constant background concentration of plasma glucose, that is produced to satisfy the animal's direct metabolic needs and seemingly unrelated to food consumption, could induce the changes in feeding behaviour brought about by satiety and consequently return of appetite.

The appetite of grouped piranhas, *R. nattereri* was greatest when the plasma glucose concentrations were decreasing to their minimum (Bellamy, 1968). That author stated that blood glucose was a sensitive indicator of food intake and postulated that the fish's metabolic processes were related to feeding periodicity. Clearly, metabolic processes are quite likely to be related to the return of appetite and Bellamy's statement is not to be doubted, but other studies on teleosts do not support the latter authors conclusions that blood glucose concentration could indicate appetitive status. In agreement with the present dogfish investigation, Gwyther (1978, cited in Fletcher, 1984) showed for *Limanda limanda* that plasma glucose did not fluctuate post-prandially and was not therefore relevant in the control of food intake. Additionally Fletcher (1982) demonstrated that *L. limanda* would still consume food even when plasma glucose was maximally deviated from pre-feeding levels. Taking into account the constant post-feeding amounts of glucose in plasma of dogfish and the agreement of these observations with other studies on teleosts and elasmobranchs, it is not easy to contemplate the role of plasma glucose in the systemic control of appetite being anything but of minor importance. After all, the natural prey of dogfish is unlikely to contain much carbohydrate (at least probably not enough to satisfy the animals needs) and so teleologically these fish would not rely on plasma glucose to exert an important metabolic contribution to appetite regulation. Thus, it is my suggestion that plasma glucose is of little importance as a systemic satiety signal in the

multifactorial control of food intake.

In contrast to plasma glucose, the plasma concentrations of triglycerides and protein changed during the period of appetite return in a manner that could suggest close involvement with the post-prandial metabolic processes of food intake control. The profile of each metabolite after feeding was similar in that there was an initial increase (within 72 h) before concentrations returned to prefeeding levels. These results suggest that levels of both dietary lipid derived metabolites and protein in the plasma must be quite finely controlled and thus perhaps of some importance in post-prandial metabolism.

Plasma total protein showed similar trends in concentration profile to triglycerides. It was not possible to distinguish from the crude estimations in the current work what proportion of the protein measured resulted from *de novo* protein synthesis in the plasma from absorbed dietary amino acids or from liver secreted albumin whose function would be to solubilize non-esterified fatty acids. It is likely that both sources were measured, hence it is difficult to speculate the importance of protein in appetite return. Fletcher (1984) stated that appetite regulation based upon plasma levels of amino acids would entail complicated biochemical mechanisms of recognition. The importance of amino acids in appetite control could however be based upon their deamination and transamination into α-keto acids, the carbon skeletons that emerge in major intermediary metabolic pathways. The carbon skeletons of twenty amino acids are termed either ketogenic, if they give rise to ketone bodies (via acetyl Co A) or glucogenic, if they give rise to intermediates involved in glucose pathways (Stryer, 1988). Thus, the fate of the ketogenic amino acid carbon skeletons in elasmobranch metabolism may play significant roles as biochemical satiety signals in fish that preferentially metabolize protein and lipids over carbohydrate.

Triglycerides or triacylglycerols are uncharged esters of glycerol and the mode of storage for fatty acid fuel molecules (Stryer, 1988). The pre-feeding levels of triglycerides

(after 14 days starvation) in *S. canicula* were maintained at around 0.8 mmol l^{-1} plasma whilst the maximum level after feeding was approximately 1.5 mmol l^{-1}. Zammit and Newsholme (1979) (who also worked in Plymouth) found the mean concentration of plasma triglycerides to be 0.85 mmol l^{-1} (range 0.6-1.0 mmol l^{-1}) in *S. canicula*, but somewhat lower (0.39 mmol l^{-1}) in *Squalus acanthias*. The value they obtained for *S. canicula* is in good agreement with the pre-feeding triglyceride concentrations found in the current work. The latter authors measured the plasma triglycerides and protein concentration in some teleosts (*Dicentrachus labrax*, *Mullus surmuletus* and *Scomber scombrus*) and it appears these levels were about two to three times lower in elasmobranchs by comparison.

The post-prandial increases in plasma triglycerides and protein were clearly the product of digestion and absorption of the HE diet, which consisted of approximately 50% protein and 8% marine oil on a dry matter basis. Therefore the marked increases in these metabolites after feeding would be expected, but what was of most interest was the way in which the post-prandial increase and subsequent decrease to normal concentrations took place within the time period of gastrointestinal evacuation of the meal and the accompanying appetite return (*i.e.* within about 288 h). Therefore it would appear that the plasma concentrations of protein and triglycerides are quite closely regulated during the period of appetite return of dogfish.

In their reviews of the chemical influences on feeding behaviour, Booth (1979) and Fletcher (1984) both note that plasma fatty acids (PFA) have very little direct effect on appetite, though they each stated that unlike PFA, plasma glycerol was proportional to the rate of triglyceride hydrolysis. These authors suggest that it is by means of the rate of triglyceride hydrolysis that the quantity of energy reserves of a normal (neither an obese or starved) animal could be monitored. The liver plays a central role in lipid based

metabolism and can itself respond to changes in plasma metabolites to maintain a balance by either mobilization of reserves or storage of nutrients (Mathews and Van Holde, 1990). Therefore the post-prandial pattern in plasma triglyceride concentration could act as an important indicator of physiological nutritional status. If the nutrient reserves are monitored in some way by detection of plasma triglyceride concentration, then the profile measured in dogfish in this study may provide useful evidence of a lipid derived basis to systemic regulation of appetite. However it is also evident that appetite return in dogfish was increasing even when the plasma triglyceride level was at its maximum. Hence, it may be equally likely that the concentration of lipid derived metabolites were not an altogether dominating factor in the multifactorial control of dogfish appetite.

Recent investigations on sharks have emphasized the importance of lipid derived metabolites in the metabolic processes operating during starvation (Zammit and Newsholme, 1979; DeRoos *et al*, 1985; DeRoos, 1990). The findings of these studies support the hypothesis that has been suggested here, that close control of lipid derived metabolites could be of prime importance in elasmobranchs' physiological perception of satiety and appetite (released as the behavioural response termed hunger). Zammit and Newsholme (1979) demonstrated that ketone bodies (β-hydroxybutyrate and acetoacetate) were the most important fat fuels in *S. canicula* regardless of whether the animals were starving or not. Ketone bodies are produced in the liver from acetyl Co A when fat breakdown predominates, are known to be normal fuels of respiration and are quantitatively important as sources of energy (Stryer, 1988). In comparison to elasmobranchs, ketone bodies may not be of equal importance as a fuel molecule in teleost metabolism during starvation (Zammit and Newsholme, 1979). Accordingly, DeRoos *et al* (1985) showed that ketone bodies were primary fuel molecules in the metabolism of *Squalus acanthias*. The level of plasma ketone bodies has been shown to increase with

increased food deprivation time (Zammit and Newsholme, 1979; DeRoos, 1994) and so may operate in some way as signals of the 'fed' state. Although ketone bodies were not measured in dogfish in the current study, one can speculate that lipid derived metabolites such as triglycerides and ketone bodies could be important as possible biochemical markers that signal the animal's physiological nutritional state.

From this section of the present investigation it can be suggested that plasma glucose is unlikely to play a major role in the regulation of appetite in *S. canicula* as uniform levels were measured throughout the period of digestion and appetite return. In contrast plasma triglycerides and protein are likely to have much more important functions as systemic signals in appetite regulatory processes. Further work on the involvement of plasma metabolites in food intake control should centre on how the concentrations of ketone bodies and ketogenic amino acids change after food consumption and with the return of appetite.

CHAPTER SEVEN

GENERAL DISCUSSION AND CONCLUSIONS

Some aspects of digestive and systemic function were investigated in relation to the peripheral regulation of appetite (voluntary food intake) in juvenile and adult lesser spotted dogfish sharks, *Scyliorhinus canicula*. The results of the study generally showed that the rate of appetite return was probably dependent, to varying degrees, on a number of factors associated with gastrointestinal and metabolic processes. The peripheral mechanisms of gastrointestinal physiology and systemic metabolism have not been suggested previously as possible operating devices in the multifactorial control of appetite in an elasmobranch species. This study therefore represents the first attempt to identify the important factors in the regulation of shark appetite.

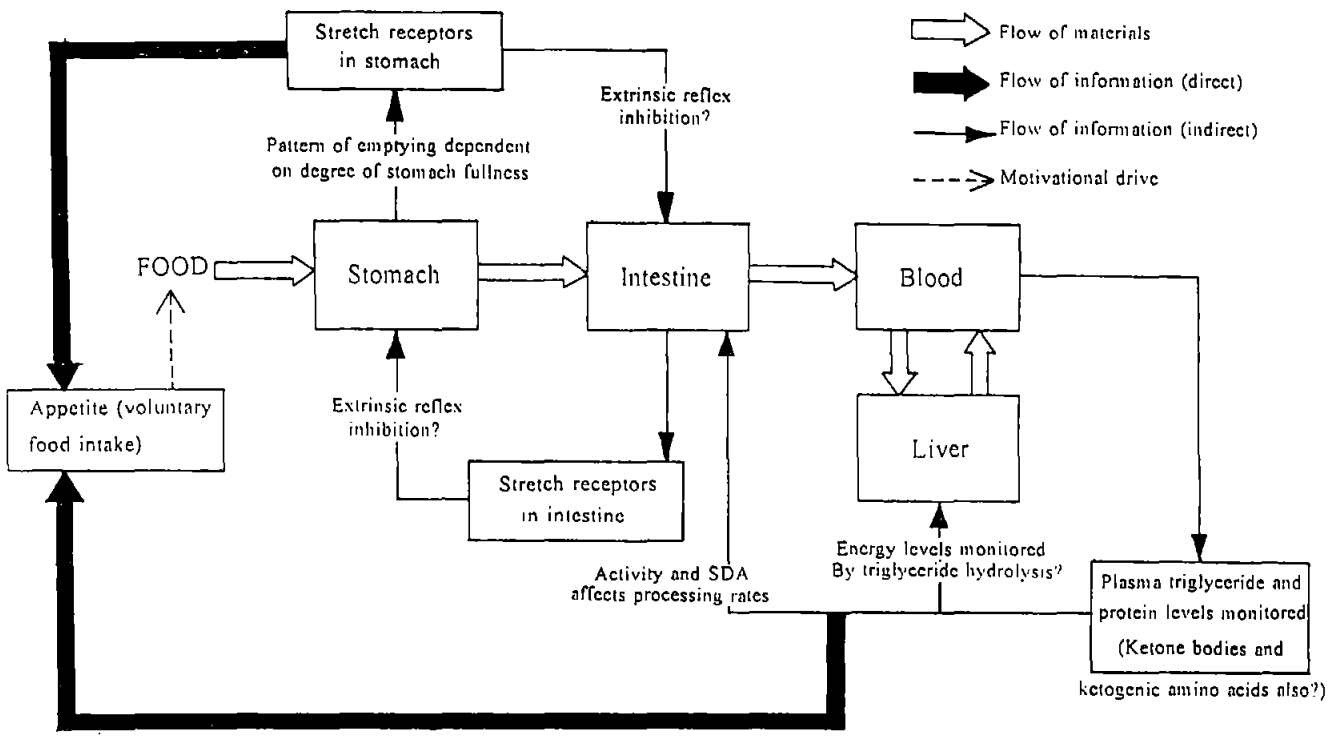
The peripheral control of appetite in mammals has been known for some time to operate multifactorially (Novin and VanderWeele, 1977; Blundell and Latham, 1979; Booth, 1979). Some of the more comprehensive studies on appetite in teleosts have also indicated that a number of factors concerned with digestive and systemic function contribute to the overall short-term regulation of food intake (*Oncorhynchus nerka*, Brett, 1971; *Lepomis gibbosus*, Colgan, 1973; *Limanda limanda*, Fletcher, 1982). However, few fish studies have produced a simplified model as a synthesis for defining the important elements in the regulation of appetite in a particular species. The models that have been constructed for teleosts have usually incorporated information from more than one species and from a variety of articles in the literature. In the tentative model proposed by Colgan (1973) to help explain the basis for motivational drive in fish, information on appetite regulation (*N.B.* Colgan defined this as hunger) was from his own studies on *Lepomis gibbosus* and to a large extent from the studies on *Gasterosteus aculeatus* by Beukema

(1968) and Tugendhat (1963, cited in Colgan, 1973). In another generalized model on the control of food intake, Vahl (1979) put forward a hypothesis based upon the observations reported for *Oncorhynchus kisutch* (Averett, 1969, cited in Vahl, 1979), *Kuhlia sandvicensis* (Muir and Niimi, 1972), *Salmo trutta* (Elliott, 1972) and *Oncorhynchus nerka* (Brett, 1971). In Vahl's model a simplified flow-diagram was used to show the interaction of some of the factors that were explained in the text in mathematical form. Hence, I have used the general framework of Vahl's flowchart to illustrate and summarize the interaction of the different digestive and systemic processes that have been suggested in previous chapters of this thesis to influence the regulation of appetite in dogfish.

Figure 32 illustrates an hypothesis for the regulatory pathways that contribute to the establishment of appetitive status in the shark, *S. canicula.* From the results presented in chapter II, it was evident that appetite was indeed regulated to some degree by dogfish and that there was no appreciable lag phase in the rate of appetite return after food consumption. Even though the period over which appetite returned was quite prolonged (presumably as a consequence of lower rates of food processing and metabolism compared to more active fish species, chapter V), the return of appetite increased at a constant rate as deprivation time increased. Clearly, sharks do not always have the same level of appetite, this level being dependent on the time since consumption of the last meal. The appetite response of this shark appears to be under close control by a number of peripheral physiological factors. From the model (figure 32) it is suggested that relative stomach emptiness and the level of plasma triglycerides were the two main physiological factors directly involved in the manifestation of appetite in dogfish, whereas the other factors investigated in this thesis can be considered to act more indirectly.

As in all fish, if appetite exists food can be ingested, whereupon the stomach will become distended. The degree of distension will depend on the amount of food consumed

Figure 32. An hypothesis for the control of appetite in the lesser spotted dogfish shark, *Scyliorhinus canicula* involving digestive and systemic function. (General format of diagram taken from Vahl (1979) but modified with additions to incorporate the findings of this study on *S. canicula*.)


Flow of materials
Flow of information (direct)
Flow of information (indirect)
Motivational drive
Stretch receptors in stomach
Extrinsic reflex inhibition?
Pattern of emptying dependent on degree of stomach fullness
FOOD
Stomach
Intestine
Blood
Appetite (voluntary food intake)
Extrinsic reflex inhibition?
Liver
Stretch receptors in intestine
Energy levels monitored By triglyceride hydrolysis?
Activity and SDA affects processing rates
Plasma triglyceride and protein levels monitored (Ketone bodies and ketogenic amino acids also?)

and this investigation has shown that it is the regulation of stomach fullness that will directly affect the level of appetite in dogfish. The findings from chapter III indicate the pattern of digesta emptying from the stomach was dependent on the degree of stomach fullness. By means of *in series* stretch receptors in the stomach wall of dogfish the degree of stomach fullness could be closely monitored and relayed to the higher centres by the sympathetic portion of the autonomic nervous system. The physiological perception of the relative emptiness of the stomach would be possible with such mechanoreceptors and could play a direct role in the establishment of appetite by providing a constant stream of information on the quantity of food that could possibly be consumed at any particular time.

Figure 32 indicates the action of a feedback loop between the stomach and intestine and *vice versa*. There were two main reasons why these possible regulatory pathways have been postulated as having a role in the gut emptying process. Firstly, the relative rate of gastric emptying actually decreased when larger meals were consumed by the dogfish. For example, it was evident that when a 7% wbw meal in the stomach was emptied so that the equivalent of a 3.5% wbw meal remained, this amount was not emptied at the same relative rate as a distinct 3.5% wbw meal. Elliott (1991) acknowledged that some investigations found constant rates of gastric evacuation irrespective of volume ingested (*e.g.* in *Pleuronectes platessa*, Jobling and Davies, 1979), whereas some investigations showed decreases in evacuation rate with increasing meal size. The decrease in relative gastric evacuation rate with increasing meal size indicated that the stomach emptying rate of dogfish was perhaps influenced by the amount of digesta that had already been emptied into the intestine. This physiological process was suggested in chapter III and it is possible that when the contents of the spiral intestine reached a threshold level after gradual filling, then feedback inhibition of gastric motility

would occur, logically perhaps, to avoid material being pushed through the intestine too quickly leading to inefficient absorption of nutrients.

In addition to the action of the feedback loop, possibly antagonistically connecting gastric and intestine motility, it is also suggested on figure 32 the mode of mediation of the inhibitory pathways based upon the observations of chapter IV. Previous authors have proposed that control of food intake could be mediated partly by chemoreceptors influencing gastric contractions via feedback loops (Grove, 1986; Jobling, 1986). The current investigation showed that the gastric emptying pattern of dogfish was independent of the level of digestible energy. Hence, a possible regulatory pathway for the control of gastric emptying (already established as of direct importance to the level of appetite) could be via reflex inhibition of motility, possibly mediated by extrinsic afferent nerves from mechanoreceptors located within the smooth musculature of the gut wall. Although such reflex inhibitory mechanisms have not been demonstrated in elasmobranchs it has been shown that vagal stimulation may cause inhibition of a spontaneously active stomach (Nilsson and Holmgren, 1988 and references therein). In contrast, stimulation of the splanchnic nerve has produced excitatory responses in the elasmobranch gut, though at lower stimulation frequencies (<4Hz) the responses have been shown to be inhibitory (Young, 1980, 1983). However, the possible existence of inhibitory innervation between the stomach and intestine and *vice versa* has yet to be investigated in elasmobranchs. Reflex inhibition across the gastroduodenal junction has been variously shown to occur in mammalian systems and was demonstrated to be mediated extrinsically (Daniel and Wiebe, 1966; Leek, 1972). Therefore, it would be interesting in further studies to examine the true nature of the autonomic nerve fibres innervating the elasmobranch gut. Indeed, inhibitory control of motility could come from extrinsic nerve modulation of the ganglion cells within the enteric nerve plexuses (intrinsic) that send axons to all regions of the gut

and will inhibit myogenic activity to produce a normal resting state prior to modulation. Hence, characterising the role of afferent nerve pathways from the spiral intestine and their influence on gastric motility would provide more information on the autonomic regulation of digestive processes. The role of gastric peptides (peptidergic innervation) could also be investigated in this context as the recent work of Aldman (1994) on rainbow trout, *Oncorhynchus mykiss* implicates the action of these hormones with the control of gastric and gallbladder motility. Information on the interaction of neural and hormonal pathways in the regulation of gastric evacuation would therefore be of great help in our understanding of the physiological processes involved in feeding control.

The flow of digesta from the stomach to the intestine and the associated rates of emptying, however controlled and integrated will all be of importance in the dogfish's physiological perception of stomach emptiness. As well as gastric volume directly regulating further food intake, figure 32 shows the probable importance of post-absorptive factors in appetite control.

Nutrients absorbed across the intestine will be assimilated in the liver and it is by monitoring the level of certain plasma metabolites that the liver could also monitor the 'fed' state in dogfish. The concentration of plasma triglycerides and protein were closely controlled post-prandially whilst the plasma levels of glucose remained constant. This suggests that lipid and protein derived metabolites (physiologically detected by the liver?) could be central to the systemic control of appetite in sharks (chapter VI). Whilst it is proposed that triglycerides and protein were perhaps important factors in the perception of metabolic satiety, the current study did not conclusively evaluate their role. Further investigations using different nutrient dense foods and their effect on the rate of appetite return could give information on their relative effect as indicators of metabolic satiety. The function of ketone bodies and the three ketogenic amino acids in appetite control was not

examined in the present study. The role of ketone bodies in the starvation metabolism of sharks is well documented (Zammit and Newsholme, 1979; DeRoos *et al*, 1985; DeRoos, 1994). Due to the undoubted importance of ketone bodies as primary fuel molecules in elasmobranch metabolism, it is likely that their relative presence in the plasma may be of substantial significance in the perception of metabolic satiety. Further investigations of their utilization and mobilization during appetite revival could suggest other ways in which the dogfish could perceive biochemical nutritional status.

The hypothesis of dogfish appetite control (figure 32) also indicates the possible effects of SDA. If dogfish were active after consuming large meals, then the rate of nutrient absorption (*e.g.* amino acids) across the intestine could become *slowed* to avoid a 'toxic' level of amino acids in the blood, as alanine is also produced as a waste product of skeletal muscle metabolism (chapter V). Such a mechanism could occur by blood being shunted away from the tissues of the gut associated with nutrient absorption during locomotor activity. Blood flow through the capillary beds of the different tissues constituting the gut wall is controlled by innervation of the vascular bed and the tissue functions will be affected by a regulation of their blood supply (Nilsson and Holmgren, 1988). Hence, high levels of activity and SDA will represent a substantial metabolic load and may therefore operate indirectly in regulating appetite by reducing the rate of nutrient absorption across the intestine.

Another avenue for further research relating to appetite control has been suggested by the current investigation. Generally the appetitive response and daily food intake levels of juvenile dogfish were similar to those of the adults, reinforcing the known fact that physiological processes emanate from hereditary instructions and are not necessarily linked to the stage of development. The rate of food processing was greater in juveniles and this led to faster rates of appetite return, however this could be predicted considering that rapid

growth is of prime importance in juvenile fish. The most interesting difference between the physiologies of juvenile and adult dogfish was that the metabolic losses due to food processing (SDA) were relatively much lower in juveniles at all ration levels than those of the adults. This physiological characteristic of juveniles could represent a strategy for conservation of metabolic energy and perhaps will result in maximum utilisation of ingested energy being channelled into the processes leading to somatic growth. A bioenergetic study of the post-prandial metabolism of juvenile dogfish would be most interesting in conclusively demonstrating the existence of such a strategy.

The appetite control hypothesis for dogfish attributes the degree of stomach fullness (or relative emptiness) and the level of plasma triglycerides and protein as direct physiological factors in appetite regulation. The role of intestinal fullness, post-prandial metabolism and possibly the energy density of the food after actual absorption can be considered as secondary factors that probably modify the function of the direct physiological processes. Of the few investigations on appetite control that exist in fish, the general findings of the present investigation are supported. Brett (1971) and Colgan (1973) both found that gastric fullness and plasma metabolites were of prime importance. Other authors, who did not examine the role of systemic function on appetite, found that the degree of gastric fullness influenced the level of food intake to a great extent (*e.g.* Grove *et al*, 1985; Singh and Srivastava, 1985; Russell and Wootton, 1993). It would appear that the general physiological bases of appetite regulation in the shark, *S. canicula* were similar to those of teleosts. Monitoring the degree of stomach fullness and the level of certain plasma metabolites (literally, how much room is left in the stomach and what are the current levels of energy reserves), appears to be how fish generally have solved the problem of physiologically regulating their rate of food intake within the ecological and environmental pressures that through the process of evolution have done the same.

The value of investigating the processes involved in the short-term regulation of appetite in fish is to provide a further dimension in our understanding of the feeding strategies that have evolved in certain fish. The same general physiological pathways of appetite regulation may have been selected for in different groups of animals through evolution, but the 'design' details of the systems influencing appetite will be different depending on the feeding habits of the fish species in question. For example, the low incidence of empty stomachs in a population of dogfish from the Irish Sea (Lyle, 1983) together with the general findings of the current work can help explain, in more accurate terms the feeding strategy employed by sharks such as dogfish. From the present study it is clear that dogfish can ingest extremely large amounts of food (up to 14% wbw in this study) within about 30 minutes, with the stomach becoming greatly extended. Food will be evacuated from the dogfish stomach in a weight dependent manner, seemingly irrespective of the level of digestible energy of the food items. Indeed, dogfish consume a wide range of prey in the wild (Lyle, 1983) so it is clear that a feeding strategy based upon energy maximisation would be largely redundant compared to maximisation of the actual number of food items. Gastric evacuation of some digesta will occur quite rapidly after consumption, though the stomach will not be completely empty for about 12 days (after a meal of 7% wbw). There is no lengthy lag period to appetite revival after food consumption and the rate of appetite return remains constant during the first 8-10 days. Some salmonids have been shown to empty their stomachs fully before voluntary feeding was resumed (Steigenberger and Larkin, 1974; Grove *et al*, 1974). This strategy may be linked to the salmonids' feeding periodicity, but dogfish will consume more food before complete evacuation of the initial meal. Hence, if food is readily available in the wild it is easy to understand why a high incidence of food items occur in the stomachs of dogfish. Dogfish are nocturnally active so their strategy will be to 'top-up' their stomachs

with food during the night (though this might not occur every night) and remain inactive during the day to digest the meal. In so doing, the detrimental metabolic effects of activity and SDA (which is greatest 4-10 hours after feeding) will be minimised and consequently the level of some plasma metabolites (which are monitored) can be maintained below their 'toxic' carrying capacity. Clearly, the physiological balance of relative stomach emptiness with the level of certain plasma metabolites will be implicit to the occurrence of further feeding bouts.

Certain life-history characteristics of the species might be reflected in the mechanisms relating to appetite control, so in this respect physiological studies of appetite are of interest. Integration of the physiological processes involved in the peripheral control of appetite together with ecological and environmental observations pertaining to feeding, will allow a more comprehensive perspective of the feeding strategies employed by sharks such as dogfish. It is not until all factors known to influence the appetite of a shark have been carefully examined by physiologists and ecologists, that a synthesis will emerge enabling our greater understanding of the trophic relationships of these ancient, predatory fish with the other species of animals that inhabit the earth's oceans.

APPENDIX ONE

Calculation of digestible energy (D_E)

For dogfish fed 7% wbw of low energy diet (LE)

(1) Amount of diet consumed per fish,

squid	28.26 gWW	5.07 gDW
dry component	18.84 gDW	

(2) Energy contributed by diet (ingested energy, I.E.),

		g DW	kJ
squid	79.27 % protein*	4.02	95.03
	5.18 % lipid*	0.26	10.28
	15.54 % carbohy.*	0.79	13.55
dry component	71.0 % protein	13.38	316.30
	20.0 % carbohy.	3.77	64.66
			499.82

(3) Total ingested energy (kJ),

	protein	lipid	carbohydrate
	411.33	10.28	78.21
Digestibility values (%)	84.40†	86.30‡	54.50§
Energy not digested (kJ)	64.17	1.41	35.59

D_E = 499.92 - 101.17 = 398.65 kJ (~ 76.76% I.E.)

For dogfish fed 7% wbw of high energy diet (HE)

(1) Amount of diet consumed per fish,

squid	26.91 gWW	4.92 gDW
dry component	17.94 gDW	

(2) Energy contributed by diet (ingested energy, I.E.),

		g DW	kJ
squid	79.27 % protein*	3.60	85.10
	5.18 % lipid*	0.25	9.89
	15.54 % carbohy.*	0.76	13.03
dry component	71.0 % protein	12.74	301.17
	20.0 % carbohy.	3.77	141.95
			551.14

(3) Total ingested energy (kJ),

	protein	lipid	carbohydrate
	386.27	151.84	13.03
Digestibility values (%)	84.40†	86.30‡	54.50§
Energy not digested (kJ)	60.26	20.80	5.93

D_E = 551.14 - 86.99 = 464.15 kJ (~ 84.22 % I.E.)

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* From Sidwell *et al* (1974) on a dry matter basis
† From Spyridakis *et al* (1989) for *Dicentrachus labrax*
‡ From Ellis and Smith (1980) for *Salmo gairdneri* (*Oncorhynchus mykiss*)
§ From Bergot and Breque (1983) for *Salmo gairdneri*

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To my family