DOCTOR OF PHILOSOPHY

Food consumption patterns and dietary digestibility of whiting (Merlangius merlangus L.) fed in laboratory conditions

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Award date:
2001

Awarding institution:
University of Wales, Bangor

Food Consumption Patterns and Dietary Digestibility of Whiting (*Merlangius merlangus* L.) Fed in Laboratory Conditions

Thesis submitted for the degree of

Doctor of Philosophy

By

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January 2001

Dedicated

To

My Parents

Wife Norisah

Daughters Athirah, Iffah and Nadia

Sons Naqib and Ikhram

Catatan:

- Allahyarhamah ibunda-ku (Zaimah Abdullah) pastinya tersenyum apabila melihat benih semaian sewaktu hayatnya telah menjadi pokok kejayaan yang tinggi memuncak. Tesis ini juga akan lebih bermakna apabila Allahyarham Adinda Halim Abd. Ghaffar berkesempatan menatapnya bersama seisi keluarga. Semuga Allah mencucuri rahmah ke atas roh mereka berdua -

In the name of Allah, the most compassionate and the merciful

ACKNOWLEDGEMENTS

I am sincerely indebted and thankful to my supervisor Dr. David J. Grove for his patience, his untiring efforts on my behalf throughout my study period and being a fatherly figure to me. His continuous encouragement, help, enthusiasm and advice made it possible for me to present this form of thesis. For these reasons, I must express my sincere apology to his wife Valerie and `Daisy' for stealing his time from them.

My heartiest thanks to Ministry of Science and Technology, Malaysia (Human Resource Development Section) and Universiti Kebangsaan Malaysia for being able to fully sponsor my postgraduate research during the country's serious economic turmoils.

My heartiest thanks are due to my wonderful wife Norisah Abd. Rahman, and my children, Siti Nur Athirah, Iffah Rashida, Mohd. Naqib, Aaina Nadia and Mohd. Hafizhat Ikhram for their understanding as well as being patient to be here with me all through the hard times. I love you all very much. To all my family in Malaysia, Bab, Ayah and Ma on both side, who were always very concerned, *terima kasih* for all the prayers.

I would like to extend my sincere thanks to Mr. Gwyn Parry-Jones, Mr. Berwyn Roberts, Mr Gwyn Hughes, and all staffs onboard of R.V Prince Madog for ceaseless support during the whiting collection. Many thanks to all Fish Lab. Colleagues, Dr. Jon King and to Dr. Andy Owen from Department of Environmental Science UWB for assisting me in carbon and Nitrogen analyses. My heartfelt thanks are also due to many of my social colleagues; Mr. Lokmal, Aziz, Roslan, Jumat, Pak Hamdan, Pak Danni and Mr. Mohammed Seisay, for their continuous hand in hand support and invaluable exchange of ideas of various interest in the many occasions and gatherings in Bangor.

Lastly, to my dear wife and my children, " *tibalah masanya untuk kita balik Malaysia*".

ABSTRACT

Food consumption patterns, digestion processes and growth performance of whiting (Merlangius merlangus L.) in captivity were investigated to clarify the feeding biology of this scarcely researched species. The study began by investigating the gastric emptying process of whiting fed to satiation with fresh sprats containing radio-opaque $BaSO_4$ paste. The movement of chyme along the alimentary tract was traced to estimate gastric emptying time by X-radiography. The results showed that fish ingesting increasing meal sizes (up to 8–10% b.w) required much longer gastric emptying times. Preliminary investigation revealed that emptying follows a curve and not a straight line. The estimation of maximum stomach capacity using an isometric relationship seems to underestimate the satiation meals, especially in smaller fish. In the present study stomach volume (SV) increases allometrically with body weight (W); $SV = 0.438W^{0.662}$. It was found that the power value was constant but changes in the constant denoted differences in packing factors of various prey types (0.39: sprats, 0.61: squid pieces and 0.26: shrimp). Food intake studies also revealed that return of appetite with time was not linear. Indeed, the observations suggested that there were coherent relationships between X-ray studies, return of appetite and gastric emptying. The underlying curves were best described by Andersen's power model ($S_t = S_{max}(1 - S_{max}^{-0.5}\rho.\ 0.5\ .\ t)^2$) for the gastric emptying process and $S_t = S_{max} - (S_{max}(1 - S_{max}^{-0.5}\rho.\ 0.5\ .\ t)^2)$ for return of appetite of whiting fed with squid pieces, sprats and brown shrimp, where S_t = stomach contents at time t, S_{max} = satiation meals, ρ = rate parameter and t = time after feeding.

In addition, a `physiological' power model (meal size: Stimulus $\alpha\ W^{0.33}$ followed by secretion: Response $\alpha\ W^{0.67}$) was used as a basis to model the gastric secretions of hydrochloric acid and pepsin in the stomach of whiting. The results showed that soon after ingestion, pepsin and gastric acid were secreted into the gastric lumen and peaked 2 – 3h after feeding. Fish size had a significant effect on the amount of gastric acid and pepsin secreted; digestive power increased $\alpha\ W^{0.67}$ as predicted by the `physiological model'. However there were no clear effects of meal size (stimulus or distension volume DV) but other factors such as muscular contractions (mixing mechanism) were related to $DV^{0.33}$ in partial support of the model.

Digestibility (absorption) studies after ingestion of the main prey, sprats was efficient; 60 – 68% of moisture, 80 – 94% of protein, 90 – 97% of lipids, 80.5% of carbohydrate and 85 – 96% of energy were actively absorbed as food passes along the gut sections. However, estimated values of nutrient absorption in similar gut sections for wild whiting were slightly lower because of unknown mixture of prey species in the intestinal samples. Proximate analysis of the digesta showed that sprats and brown shrimps probably dominated the main diets in the wild with minor contributions from other crustaceans and polychaetes.

Finally, monitoring of whiting fed on artificial feeds from a demand feeder showed that whiting fed well during the autumn-winter (15°C) seasons. Peak feeding activities occurred between `dawn' and `noon' despite continuous 24h lighting. Overall growth performance was poor, suggesting other factors such as dietary formulation or stress was diverting energy from the growth processes.

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3 Mid. Intestine (Mid. Int.)
4 Rectum (Rect.)

Chapter 1

Literature Review

1.1 General introduction

The whiting, *Merlangius merlangus* (Linnaeus 1758) is an important commercial gadoid species, which is widely distributed around the shallow Atlantic coastal waters of Western Europe (Wheeler, 1969). It is abundant in all British waters, including the Irish Sea. Commercial catch statistics published by FAO (1950–1994) for the four main fishing countries locally (United Kingdom, Ireland, Denmark and France) revealed that nominal catches of whiting progressively increased from the 1950's, reaching a peak in the 1970's (214,000 mt), declined in 1980's with minimum landing at 83,000 mt and showed little recovery in the first quarter of 1990's (90,000 mt). The overall levels of exploitation were approaching optimal yield and no further expansion should be allowed (Anon, 1997). Meanwhile, recent estimated commercial landings for whiting on the west and southwest coasts of the Irish Sea have increased steadily from just 2,000 tonnes in 1988 to just under 8000 tonnes in 1996 (Wheatley *et al.*, 1999). A recent study in the Eastern English Channel showed the whiting to be dominant in the French inshore fishery catches in October (Carpentier, 1998).

Apart from its direct commercial importance, the whiting is an important predator in the coastal and estuarine ecosystem (Henderson and Holmes, 1989; Hislop *et al.*, 1991b; Hislop, 1996; Bax, 1998). Models to estimate predation rates in the field have been developed. Daan (1973) and Hislop *et al.* (1991b) developed a North Sea predation model, suggesting that food intake by opportunistic feeding fish species such as whiting and cod is estimated sufficiently by: Food intake ($g\ d^{-1}$) = (S*2)/A, where S is average stomach content (g), A is the gastric emptying time (GET) in days and was taken as 2.5 for all prey species observed in the stomach (Hislop *et al.*, 1991b). Later, Seyhan (1994) suggested another model for predation of whiting in the Irish Sea based on his findings that the relationship for gastric emptying of prey was linear. His model to estimate food consumption was given by: $F = (S_2 - S_1) + 1.5\ K\ T$, where S_1 and S_2 are average stomach contents at time t_1 and t_2, K is the gastric emptying rate ($g\ h^{-1}$) and T is temperature (^{o}C).
Stomach content analysis of whiting shows that it primarily consumes small pelagic fish and various benthic invertebrates (Jones, 1954; Nagabushanam, 1964; Hislop *et al.*, 1991b; Carpentier, 1998). Larval whiting primarily eat copepod nauplii (Last, 1980). Immature whiting (below 12 cm total length) mainly feed on polychaetes and crustaceans. As they grow, fish form an increasing part of the diet and especially includes herring and sprat juveniles, and sandeels (Jones, 1954; Nagabushanam, 1964) although swimming crabs can be a significant part of the diet (Seyhan, 1994).

In recent years, studies of fish feeding have been extended to assess productivity, based on feeding and predation rates of the animals in the food web of commercially-exploited areas such as the North Sea. This is because of the failure of simple "unit stock" fisheries management models to sustain the local fisheries. These latter models ignore ecological relations among fish, their food and predators. Multi-species fisheries assessment and management for the North Sea is founded on feeding rates of the animals in the food web (Fjøsne and Gjøsæter, 1996; Bromley *et al.*, 1997; Fowler, 1999). Research has included field observations to assess feeding and digestion rates in the major species around Britain including cod (Daan, 1973), sole (Lagardere, 1987) and whiting (Hislop *et al.*, 1991b; Mergardt and Temming, 1997; Seyhan and Grove, 1998). However, more detailed understanding of factors which affect the rates of food intake and digestion is needed for the species under a range of controlled, laboratory-based conditions; difficulties nevertheless arise when applying the results to the animals in their complex, natural environment.

At present, detailed studies on how food is processed in the alimentary canals of fish are scarce. The majority of studies have concentrated on factors affecting feeding rate, gastric emptying time and rate, and overall digestion efficiency (Table 1.1). However, studies on factors affecting especially gastric acid secretion and enzymatic activity in the stomach are limited (Smit, 1967; Holstein and Cederberg, 1986) and little is known about where nutrients and energy are absorbed as food passes thorough the alimentary canal.

Table 1.1. Selected references for appetite, gastric emptying and digestive physiology in fish.

Topics and Authors	Species	Common name	Meal Types	Feeding Mechanism
GET and Food Consumption Model				
Medved, 1985	*Carcharhinus plumbeus*	Sandbar shark	Natural	Voluntary
Hossain *et al.*, 1998, 2000	*Clarius gariepinus*	African catfish	Artificial	Voluntary
Bagge, 1977	*Gadus morhua*	Atlantic cod	Natural	Voluntary
dos Santos and Jobling, 1988, 1990, 1991a,b, 1992	*Gadus morhua*	Atlantic cod	Natural /artificial	Voluntary
Boyce *et al.*, 2000	*Harpagifer antarcticus*	Antarctic spiny plunderfish	Natural	Voluntary
Kitchell and Windell, 1968	*Lepomis gibbosus*	Pumpkinseed sunfish	Natural	Voluntary
Fletcher *et al.*, 1984	*Limanda limanda*	Dab	Natural /artificial	Voluntary
Jones, 1974	*M. aeglefinus/ Gadus morhua/ Merlangius merlangus*	Haddock/ Atlantic cod/ Whiting	Natural	Voluntary
Andersen, 1998, 1999	*Merlangius merlangus*	Whiting	Natural	Voluntary
Bromley, 1988	*Merlangius merlangus*	Whiting	Natural	Voluntary
Mergardt and Temming, 1997	*Merlangius merlangus*	Whiting	Natural	Voluntary
Robb, 1990	*Merlangius merlangus*	Whiting	Natural	Voluntary
Seyhan and Grove, 1998	*Merlangius merlangus*	Whiting	Natural(field)	Voluntary
Seyhan *et al.*, 1998	*Merlangius merlangus*	Whiting	Natural/ artificial	Voluntary
Singh-Renton and Bromley, 1996	*Merlangius merlangus/ Gadus morhua*	Whiting/ Atlantic cod	Natural	Voluntary
Singh-Renton, 1990	*Merlangius merlangus/Gadus morhua*	Whiting/ Atlantic cod	Natural/ artificial	Voluntary
Essington *et al.*, 2000	*Micropterus salmoides*	Largemouth bass	Natural(field)	Voluntary
Ruggerone, 1989	*Oncorhynchus kisutch*	Coho salmon	Natural	Voluntary
Grove *et al.*, 1978	*Oncorhynchus mykiss*	Rainbow trout	Artificial	Voluntary
Windell *et al.*, 1976	*Oncorhynchus mykiss*	Rainbow trout	Natural	Voluntary
Basimi and Grove, 1985a	*Pleuronectes platessa*	Plaice	Artificial	Voluntary
Edwards, 1971	*Pleuronectes platessa*	Plaice	Natural	Force feeding
Jobling, 1980a,b,c, 1981a	*Pleuronectes platessa*	Plaice	Artificial	Voluntary
Nelson and Ross, 1995	*Raja erinacea*	Little skate	Natural	Voluntary
Talbot *et al.*, 1984	*Salmo salar*	Atlantic salmon	Natural	Voluntary

Cont...

Jobling, 1983	*Salvelinus alpinus*	Arctic charr	Artificial	Voluntary
Flowerdew and Grove, 1979	*Scophthalmus maximus*	Turbot	Artificial	Voluntary
Grove *et al.,* 1985	*Scophthalmus maximus*	Turbot	Artificial	Voluntary
Macpherson *et al.,* 1989	*Scyliorhinus canicula*	Lesser spotted dogfish	Natural	Voluntary
Koed, 2001	*Stizostedion lucioperca*	Pikeperch	Natural	Voluntary
Dietary Digestion & Physiology				
Ferraris *et al.*, 1986	*Chanos chanos*	Milkfish	Artificial	Voluntary
Grove and Holmgren, 1992b	*Gadus morhua*	Atlantic cod	Artificial	Voluntary
Holstein and Cederberg, 1980, 1984, 1986.	*Gadus morhua*	Atlantic cod	Simulated	Administered (*in-vivo*)
Holstein, 1976, 1977, 1983	*Gadus morhua*	Atlantic cod	Simulated	Administered (*in-vivo*)
Lied and Solbakken, 1984	*Gadus morhua*	Atlantic cod	Artificial	Force feeding
Smit, 1967	*Ictalurus nebulosus*	Brown bullhead	artificial	Force feeding
Hossain *et al.*, 1997	*Labio rohita*	Rohu	Artificial	Voluntary
Norris *et al.,* 1973	*Lepomis macrochirus*	Bluegill sunfish	Simulated	Force feeding
Dabrowski *et al.,* 1986	*Oncorhynchus mykiss*	Rainbow trout	Artificial	Voluntary
Dimes and Haard, 1994	*Oncorhynchus mykiss*	Rainbow trout	Artificial	Voluntary
Grove and Holmgren, 1992a	*Oncorhynchus mykiss*	Rainbow trout	Artificial	Voluntary
Holmgren *et al.*, 1985	*Oncorhynchus mykiss*	Rainbow trout	Simulated	Administered (*in-vivo*)
Rodenhutscord *et al.,* 1997	*Oncorhynchus mykiss*	Rainbow trout	Artificial	voluntary
Ruohonen and Grove, 1996,	*Oncorhynchus mykiss*	Rainbow trout	Natural/artificial	Voluntary
Ruohonen *et al.,* 1997a,b	*Oncorhynchus mykiss*	Rainbow trout	Natural/artificial	Voluntary
Espe *et al.,* 1999	*Salmo salar*	Atlantic salmon	Artificial	Voluntary
Røsjø *et al.*, 2000	*Salmo salar*	Atlantic salmon	Artificial	Voluntary
Sveier *et al.*, 1999	*Salmo salar*	Atlantic salmon	Artificial	Voluntary
Fernández *et al.*, 1998	*Sparus aurata*	Sea bream	Artificial	Voluntary
Holmgren and Nilsson, 1983	*Squalus acanthias*	Spiny dogfish	Simulated	Administered (*in-vivo*)

1.2 Methods of measurement

Gastric evacuation (or emptying, **GE**) has been measured in several ways. It is not always easy to compare the results from different authors who used different methods because of the differences in specific objectives and fish used in the experiments. Even different methods of handling have shown different results. In the laboratory, GE is often estimated by giving a group of experimental fish a meal of known weight or volume and measuring the amount remaining in the stomach of individual fish by serial slaughter at successive intervals after feeding (Swenson and Smith, 1973; Jobling and Spencer Davies, 1979; Fletcher, 1984; Fletcher *et al.*, 1984; Basimi and Grove, 1985a). This method has certain advantages since it provides direct observation of food items in the stomach. However, large numbers of fish are required in the experiment, and the described GE process only represents the population average, largely ignoring individual variation. An increasingly popular method is to use gastric lavage; a technique of removing stomach contents from live fish at different times after feeding (Foster, 1977; Andersen, 1998; Kristiansen, 1998; Andersen, 1999). This allows each fish to be used several times and individual variation can be assessed. Both methods allow estimation of Gastric Emptying Time (**GET,** usually in hours) and also Gastric Emptying Rate (**GER**, usually in grams per hour); if the gastric emptying curve is not linear, GER varies as digestion proceeds. Direct observations in the field have been attempted in a similar way by observing the stomach contents of sampled fish at different times after capture (Elliott, 1973; Thorpe, 1977; Elliott and Persson, 1978; Basimi and Grove, 1985b ; Seyhan and Grove, 1998).

When the numbers of experimental fish are limited, many workers prefer to use an X-ray technique to facilitate direct observation without killing the fish. This method has been applied regularly in laboratory studies in the last three decades and is widely practiced but with some modifications of equipment and processing between authors (Molnár and Tölg, 1960,1962; Talbot, 1985; Hossain *et al.*, 1998; Hossain *et al.*, 2000). Originally, the process of digestion was monitored by using X-ray-dense structures in the food (otoliths, bones, chitin) (Molnár and Tölg, 1960, 1962) but inclusion of exogenous marker (e.g. barium sulphate) allowed digestion of soft-bodied prey such as polychaetes to be monitored (Edwards 1971). Barium sulphate as a dispersed contrast medium has been widely used (Seaburg and Moyle, 1964; Elliott, 1972; Edwards, 1973; Jobling *et al.,* 1977, Ross and Jauncey, 1981). This was done by mixing radio-opaque $BaS0_4$ at a concentration around 20–25% w/w with the experimental food, to produce a high resolution X-ray images (Edwards, 1971; Edwards, 1973; Jobling *et al.*, 1977; Grove *et al.*, 1978; Grove *et al.*, 1985). Such methods usually allow only GET to be estimated.

An improvement saw the adoption of radio-opaque particulate markers, especially in artificial (pelleted diets). Knowledge of the ratio of marker count to nutrient content in the food allows the nutrient content at later stages of gastric emptying to be estimated from counts of the marker, provided the marker and nutrient are emptied at the same rate. Particulate markers such as ballotini (lead glass beads), electrolytic or metallic iron powder ("filings") and lead 'shot' have been used. The marker should be chosen cautiously since its suitability may vary with species. Iron particles worked well in feeding studies of the Atlantic salmon (*Salmo salar*, Talbot and Higgins, 1983) but not in similar studies for Arctic charr (*Salvelinus alpinus*, Jorgensen and Jobling, 1988). In charr, and in unpublished studies on turbot (Al-Aradi, 1986), retention of the marker relative to food led to overestimates of stomach contents where iron particles were believed to be trapped by gastric mucus. Metallic iron may react in other ways to form ionic ligands or even react with HCl in the gastric secretions.

Ballotini have been widely used to estimate GET and GER. Hossain *et al.* (1998) used them in an X-ray study of African catfish fingerlings and found that they did not affect feed preference and gastric emptying rate. Sims *et al.* (1996) successfully estimated the rates of gastric emptying and return of appetite using formulated diet containing radio-opaque glass beads in lesser spotted dogfish (*Scyliorhinus caniculus*). In contrast, several studies carried out to test the validity of particulate markers indicated that there were significant effects, depending on particle size and density, on estimates of GER and GET caused by retention of the marker (Talbot and Higgins, 1983; dos Santos and Jobling, 1990; Jobling *et al.*, 1995a; Jobling *et al.*, 1995b).

Radioactive substances have also been used as markers. They include chromium (^{51}Cr) (Storebakken *et al.*, 1981), cerium (^{144} Ce) (Cowey and Sargeant, 1972; Peters and Hoss, 1974), caesium (^{137} Cs) (Kolehmainen, 1974) and iodine (^{131}I). These substances must be incorporated in the test meal with appropriate precautions and require safe disposal of materials afterward. Results typically confirm X-radiographic methods except the latter are conveniently presented photographically. An exception was the study in rainbow trout where Aldman and Larsson (1994) used a scintigraphic gamma camera to localise technetium-labelled food (^{99} T ^{m}) in the stomach and as it emptied into the anterior intestine.

Whichever monitoring method is used to study gastric digestion, the meal size is an important variable. It can be quantified as wet weight (Magnusson, 1969; Jones, 1974), volume (Hunt and MacDonald, 1954; Seaburg and Moyle, 1964), dry weight (Windell *et al.*,

1969; Tyler, 1970), and even dried weight of digestible organic matter or calorific content (Kitchell and Windell, 1968; Windell *et al.*, 1969; Machiels and Henken, 1985).

Several methods of presenting the experimental meal to the fish have been used. Force-feeding is usually applied to get relatively large numbers of fish to consume a meal of predetermined size at a particular time. However, force-feeding has also been reported to depress the evacuation rate (Windell, 1966; Swenson and Smith, 1973; Jones, 1974). The simpler or even more natural method is to allow fish to ingest voluntarily a given meal of known size (Elliott, 1972; Elliott, 1975a,b; Jobling and Spencer Davies, 1979). If the amount eaten by each fish cannot be observed (*e.g.* when fish only feed well in groups) particulate markers can be used - followed by X-rays – to assess the amount eaten. Meal size can be offered as a stated percentage of the body weight of fish (Jobling *et al.*, 1977; Flowerdew and Grove, 1979; Basimi and Grove, 1985a). This method may provide an equal stimulus regardless of fish size if the stomach volume increases in proportion to fish weight. In a fish of stated size, meal size could determine the size of the stimulus for acid and enzyme secretion in the stomach, as well as for stimulating muscular contractions (motility). However other scaling factors may come into play. For example, larger *Limanda* took longer to empty a meal of equal *relative* size (*e.g.* 1% bw) than smaller fish (Jobling *et al.*, 1977); GET increased in proportion to body weight $^{0.3856}$. They reported that GER also increased with fish size (since the absolute meal size was larger) but not sufficiently to compensate for meal size difference. Similar results have been reported for walleye, *Stizostedion* (Swenson and Smith, 1973) and whiting, *Merlangius merlangus* (Jones, 1974), turbot, *Scophthalmus maximus* (Flowerdew and Grove, 1979) and northern squawfish, *Ptychocheilus oregonensis* (Bayer *et al.*, 1988). However, similar work on squawfish by Vondracek (1987) found no consistent changes in evacuation rate with increase in fish size using a test meal based on predator weight. Mills *et al.* (1984) found that GET was limited by fish size. Different sizes of young yellow perch fed continuously with zooplankton had GET initially increasing with fish size up to a maximum fish length of 36 mm (TL); further increase in fish length (TL > 36 mm) did not induce any additional increase in GET.

In the present study, it was found that training is usually needed to make the fish ready to take the offered experimental meal voluntarily within a relatively short time. Deprivation of food is usually carried out prior to the start of an experiment in order to ensure complete stomach emptiness (Windell, 1966). However, the period of deprivation should not be too long because the consequent starvation can decrease evacuation rate, metabolic rate and protein synthesis in fish, and can also result in hyperphagia in fish (Windell, 1966; Tyler,

1970; Elliott, 1972; Jobling, 1980a; Jobling, 1981c; Russell and Wootton, 1993; Bull and Metcalfe, 1997). Windell (1966) and Elliott (1972) found that deprivation for less than 7 days has no substantial effect on the digestion rate. There are also workers who used dyed or marked food in feeding experiments. This is to avoid the consequences of food deprivation whilst the test meal can be readily distinguished from other meals (Mills *et al.*, 1984).

A different approach to feeding involves the laboratory use of either automated feeders (for growth studies) or "on-demand" feeding equipment (for appetite studies). Automated equipment is widely used in aquaculture, particularly in developed countries, since it reduces labour and time costs (Wright and Eastcott, 1982; Majid, 1982). Demand feeders have been used quite extensively in the study of rainbow trout (*Oncorhynchus mykiss*) (Grove *et al.,* 1978; Boujard and Leatherland, 1992a,b; Alanärä, 1992a,b; Brännäs and Alanärä, 1994; Sanchez-Vazquez *et al.*, 1995; Wagner *et al.*, 1996; Sanchez-Vazquez and Tabata, 1998). Other studied species include Arctic charr (*Salvelinus alpinus*) (Brännäs and Alanärä, 1993; Brännäs and Alanärä, 1994; Linner and Brännäs, 1994; Alanärä and Kiessling, 1996), sea bass (*Dicentrarchus labrax*) (Begout Anras, 1995; Madrid *et al.*, 1997; Azzaydi *et al.*, 1998; Aranda *et al.*, 1999) and salmon (*Salmo salar* L.) (Juell *et al.*,1994). Adoption of this technique allows longer periods of food intake to be monitored, often with interesting new insights. For example, studies on rainbow trout demonstrated that the use of demand feeders resulted in good growth rate since the fish were allowed full access to the food in comparison to restricted feeding regimen (Alanärä, 1992b). However, studies on Arctic charr found that demand feeding activity gave no significant difference in intake between food of low *versus* high energy density; fish taking the higher energy meal grew at a significantly faster rate (Alanärä and Kiessling, 1996). Demand-feeding sea bass showed prominent feeding rhythms (dual phased) that changed according to the water temperature and seasonal photoperiod (Sanchez-Vazquez *et al.*, 1998).

1.3 Appetite and its control

The study of voluntary food intake in healthy animals is taken as a measure of appetite for the types of food offered. Voluntary food intake is measured as the amount of nutrient consumed in a given period of time, while appetite is the behavioural drive to eat that food, or a specific nutrient therein (Forbes, 1988). Detailed understanding of the control of appetite in even a single species of fish is still poor and the subject undoubtedly requires much research. Physiological factors affecting control of appetite

in fish were reviewed by Fletcher (1984) and more recently by Le Bail and Boeuf (1997). Much of the work on the control of feeding in vertebrates has been carried out on mammals. Endogenous factors controlling eating are complex and involve specific areas of the central nervous system (such as "satiety" and "hunger" centres in the hypothalamus) which are affected by afferent nerves from the alimentary canal, circulating nutrients (*e.g.* blood glucose concentration) and several hormones such as insulin, glucagons, catecholamines, leptin and gut peptides (Louis-Sylvestre and Le Magnen, 1980; Smith *et al.*, 1981; Russek, 1981; Fletcher, 1984; Campfield *et al.*, 1985; Campfield and Smith, 1986; Vanderweele *et al.*, 1986; Stricker and Verballis, 1990). The balance between nutrient contents and the various hormones is envisaged as a sign of the "systemic need" of the animal for food to replenish any deficits. Similar factors are likely to be involved in the control of appetite in fish even though the role of many factors is still unclear (Le Bail and Boeuf, 1997). Many workers report that stomach fullness or emptiness and the density of major macronutrient contents (such as protein, lipids, glucose and dietary energy) in the food are known to influence voluntary food intake (Rozin and Mayer, 1961; Rozin and Mayer, 1964; Grove *et al.,* 1978; Gwyther and Grove, 1981; Jobling, 1981c; Ince *et al.*, 1982; Jobling and Wandsvik, 1983; Grove *et al.*, 1985; Jobling *et al.*, 1995a,b; Valente *et al.*, 1998). The peripheral nervous system and various hormones co-ordinate alimentary function to cope with the consequences of ingesting food. For example, the endocrine and nervous systems control secretion of mucus and digestive enzymes, and also muscular activity of the stomach and sphincters (Fänge and Grove, 1979).

1.4 Factors affecting gastric emptying and feeding rate in fish

The rate at which a fish can consume food is limited by the rate at which the anterior regions of the alimentary canal can digest and move material to more posterior regions. The relationships between the amount of food consumed due to return of appetite and the gastric emptying rate (GER) and time (GET) are complex because the physiology of a fish is affected by both external factors (*e.g.* temperature, oxygen availability, food quality) and also internal factors (*e.g.* fish size, systemic demand). In general, there are four main factors, which affect the gastric evacuation rate and emptying time.

1.4.1 Temperature

Gastric emptying times in many species have been reported to show a negative correlation with temperature (Brett and Higgs, 1970; Flowerdew and Grove, 1979;

Jobling and Spencer Davies, 1979; Fänge and Grove, 1979). Increase of temperature will reduce the GET, by increasing the GER. The trend most usually follows Krogh's curve with Q_{10} values close to 2 (Fabian *et al.*, 1963; Brett and Higgs, 1970; Edwards, 1971; Jones, 1974; Windell *et al.*, 1976; Persson, 1979; dos Santos and Jobling, 1991a; Elliott, 1991) since the exponential curve becomes linear when log (GET) is plotted against temperature and has a slope close to magnitude 0.07-0.08 (Molnár and Tölg, 1962; Jobling and Spencer Davies, 1979; Bromley, 1988; Robb, 1990). In an unpublished study on seven fish species (Grove, pers comm), the temperature coefficient averaged 0.066 ($Q_{10} \approx 1.93$). However, this relationship fails as the thermal tolerance limit of the species is approached. Exceptions to this general pattern have been reported. A linear rather than curved change in GER with temperature has been reported in bluegill sunfish, *Lepomis macrochirus* Rafinesque (Kitchell and Windell, 1968; Kitchell, 1970), channel catfish, *Ictalurus punctatus* (Shrable *et al.*, 1969), cod and whiting, (Singh-Renton, 1990; Singh-Renton and Bromley, 1996) and in whiting (Robb, 1990). Tyler (1970) working on cod and Seyhan (1994) working on whiting reported a sudden change of GET (as GER changed from low to higher rates) which did not fit Krogh's curve.

1.4.2 Fish size and stomach volume

In theory, the volume of the stomach might be expected to increase in proportion to fish weight. Seyhan (1994) found a linear relationship between stomach volume and body weight (n = 42 fish; 48 – 695 g) in whiting:
Stomach Volume (ml) = 0.067*W, where W is fish weight (g).
Jobling *et al.* (1977) also found that the stomach volume of *Limanda limanda* increases in direct proportion to body weight (Vol. = 0.081W – 0.392, r = 0.974, n = 30) and the same is true of *Scophthalmus*, in which the relationship between stomach volume and fish size (g) of freshly killed turbot was given as Vol. = 0.112W – 10.16, r = 0.975, N = 14, P>0.001 (Flowerdew and Grove, 1979). They suggested that digestion experiments should be based on similar *relative* meal sizes (*e.g.* 2% of body weight) in animals of different sizes to give similar stimuli to the gastro-intestinal tract. If satiation meals were proportional to body weight, each fish may receive the equivalent stimulus when fed. However, for the majority of fish species that have been studied, maximum rates of ingestion (satiation) have been found to scale according to (body weight) $^{0.75}$ (Jobling, 1993).

After feeding, meals of the same relative size (as % bw) had longer gastric emptying times in larger *Limanda*, showing that smaller fish evacuate at a relatively faster rate, and this has been confirmed for snakehead *Ophiocephalus striatus* (Pandian, 1967). It is likely that the surface area to volume ratio of food, which decreases for larger meals of the same shape (*e.g.* a food bolus) and also for the available area of gastric epithelium relative to body weight (which also decreases relatively), both limit the relative digestion rate (Grove *et al.*, 1985). In a series of unpublished studies on seven species, Grove (pers comm) found GER varied on average as $W^{0.26}$ (powers were: *Scyliorhinus 0.23; Taurulus 0.53; Anguilla 0.20; Pholis 0.17; Scophthalmus 0.29; Limanda 0.29; Pleuronectes 0.13*). In contrast, Elliott (1972) fed brown trout (*Salmo trutta* L.) with absolute amounts of food organisms of similar size and taxon (where he used seven taxa) and found no effect of fish size on gastric emptying rate.

When turbot of different sizes were fed the same absolute amount of food, emptying was faster in larger fish (Flowerdew and Grove, 1979). However this may not be a universal rule. When fish of different sizes feed to satiation, the complex balance between the amount of food taken, the stomach size, the rate of muscular contractions and the amount of gastric secretion could lead to similar evacuation times. A recent study on the extremely cold water species Antarctic spiny plunderfish (*Harpagifer antarcticus*) found that the effect of fish size on absolute GER was only significant at a fixed ration level of 0.3% wet fish weight (Boyce *et al.*, 2000).

1.4.3 Meal size

Most workers agree that larger meals take a longer time to evacuate in fish of fixed size at stated temperature. The amount of food (g) evacuated in unit time however increases with the size of the meal (Kitchell and Windell, 1968; Elliott, 1972; Bagge, 1977). In addition, meal particle size has been shown to effect the gastric emptying time. Hossain *et al.* (2000) described an exponential gastric evacuation rate in African catfish (*Clarias gariepinus* Burchell) fed to satiation, by which the feed particle size of 1 mm pellets was emptied faster than 1.5, 2 and 3 mm pellets.

However there are conflicting claims as to the relationship between meal size and gastric evacuation rate. A few studies showed that meal size and evacuation rate are either negatively correlated (Ruggerone, 1989) or uncorrelated (Tyler, 1970; Bromley, 1988). Several workers reported that when large food items are consumed an " initial delay" in the gastric evacuation is observed (Macdonald *et al.*, 1982; Medved, 1985). In flatfish, a larger

meal appears to be evacuated at a faster rate (Jobling *et al.*, 1977; Flowerdew and Grove, 1979; Basimi and Grove, 1985a). Meanwhile a study on Antarctic spiny plunderfish (*Harpagifer antarcticus*) revealed that absolute gastric evacuation rate (GER: g day^{-1}) increased with increasing ration mass (Boyce *et al.*, 2000).

Jobling (1987) found in his mathematical analysis of 83 data sets that an exponential regression model gave the best fit for the emptying of small prey and artificial diets from the stomach. Conversely, a better fit was obtained when a linear regression model was applied when large food organisms/ particles were supplied. Elashoff *et al.* (1982) disagreed with the use of an exponential regression model while He and Wurtsbaugh (1993) concluded that the best-fitted model varied between linear, square root and exponential forms. Olsson *et al.* (1999) also reported large individual differences in gastric emptying pattern.

The mathematical model of Fänge and Grove (1979) predicted a curve - which was like that found in dab, plaice and turbot - by assuming that the digestion rate is a surface area phenomenon. This power model was extended (Grove *et al.*, 1985) to describe the observed gastric emptying curves, at least in flatfish, which again were more appropriately described by a power curve rather than an exponential curve. This better fit to observed data had the extra advantage that the emptying curve finished within finite time (rather than infinite time for the exponential model). In a series of unpublished experiments on seven species, Grove (pers comm) found that GET increased approximately as a "square root" model : $(\text{meal size})^{0.52}$. The power terms for the species were:
Scyliorhinus 0.57; Taurulus 0.61; Anguilla 0.32; Pholis 0.36; Scophthalmus 0.58; Limanda 0.68; Pleuronectes 0.49). Andersen (1998,1999) used simple and expanded power models to describe gastric evacuation in whiting. The estimated power values were within the range of 0.36 - 0.77 for his expanded model and closer to 0.5 (square root) from his simple power model. Power values of close to 0.5 are in accordance with earlier findings by Jones (1974) and dos Santos and Jobling (1995) or even with a very recent finding by Koed (2001).

1.4.4 Food types and quality

Differences in prey items are reported to affect the GER markedly. Elliott (1972) found that brown trout, *S. trutta*, completed 90% evacuation of *Gammarus* in 22h at 12°C, whilst the same weight of *Protonemura* needs 26h and *Hydropsyche* 30h. This phenomenon has also been reported by several workers such as Jones (1974), Macdonald *et al.* (1982),

Singh-Renton and Bromley (1996). Nevertheless, an earlier study on sunfish by Windell (1966) and on perch by Persson (1979) found relatively little difference in GER with food type. Separate fractions of a meal (such as digestible organic matter, indigestible chitin or plant material) exhibit differential movement through the stomach (Windell *et al.*, 1972; Kionka and Windell, 1972; Windell, 1978; Macdonald *et al.*, 1982; Mergardt and Temming, 1997) also affect the initial period of delay before digestion starts. Darnell and Meierotto (1962) and Windell (1978) reported that indigestible remnants of chitin from aquatic invertebrates were left in the stomach of bluegill sunfish (*Lepomis macrochirus*) and black bullhead, (*Ictalurus melas*) after the digestible material had been evacuated.

The energetic value of the food also appears to be important since dilution of food with a non-nutritive bulk results in enhanced GER (Grove *et al.*, 1978; Flowerdew and Grove, 1979; Jobling, 1980a,b). It therefore appears that fish eat to satisfy their energy needs (Cowey *et al.*, 1972; Cui and Wootton, 1988). There is considerable evidence to support the hypothesis that gastric evacuation is regulated by the energy or nutrient content of the food. It is most likely that the greater the nutrient density of the food, the slower the gastric evacuation rate (Windell, 1966; Elliott, 1979; dos Santos and Jobling, 1988; Jobling, 1988). Jobling (1988) found that enriched minced herring diet with high-energy content fed to cod *Gadus morhua* increased gastric emptying time. Similar results were obtained with rainbow trout; GET was reduced when the energy density of the diets were reduced by dilution with kaolin (Grove *et al.*, 1978) or *vice versa*, increased GET in plaice with increase of dietary energy content (Jobling, 1980b). However, Gwyther (1978) and Gwyther and Grove (1981) failed to find such a response to dietary dilution in *Limanda limanda* L.

Early workers (*e.g.* Windell, 1966) reported that increased lipid in diets decreases GER. Lipid concentration in excess of 15% of the food dry weight has this effect and Hunt and Knox (1968) suggested the fat stimulated release of hormone similarly to "enterogastrone" in mammals that inhibits gastric mortility. Windell *et al.* (1972) reported that pelleted diets adjusted to three different lipid levels (6.5, 10.5 and 14.5%) were found to pass through the stomachs of rainbow trout at the same rate. However, conflicting results were obtained in their later study on diets with increased fat levels that showed a marked decrease in GER in rainbow trout (Windell *et al.*, 1976).

1.5 Modelling the digestive function of the stomach: the `Physiological Model'

Grove and co-workers have carried out quantitative studies on the gastrointestinal processing rate in several species, including flatfish, and have suggested how the digestion and emptying process might lead to the power equations described in Section 1.4. The developments to date are summarised in this Section.

The major functions of the vertebrate stomach are to receive the ingested food items, to kill them and start the digestion process. In order to achieve this, the gastric epithelium secretes mucus, hydrochloric acid (HCl) and releases the zymogen pepsinogen to produce the proteinase pepsin. The presence of food leads to reflex muscular contractions which mix the luminal contents and also lead to transfer of the acid chyme (fine food particles) to the intestine *via* the pyloric sphincter where the alkaline medium promotes the next phases of digestion. The presence of partially digested food in the anterior intestine leads to the release of hormones stimulating the release of alkaline bile ($NaHCO_3$) and emulsifying agents from the gall bladder as well as pancreatic and intestinal enzymes such as lipase, amylase, and the alkaline proteases (trypsin, chymotrypsin). Some of the hormones, such as cholecystokinin (CCK) and "enterogastrone", exert negative feedback effects on the stomach to inhibit further gastric emptying until the intestine has processed the current mass of chyme. As a result, the stomach is compelled to act as an intermittent pump. Finally, less digestible fractions of the food may be held back and discharged in a final, accelerated phase of gastric emptying.

It is possible to construct a simple, predictive, quantitative model of the process of digestion or gastric emptying in fish. During the main phase, in which the digestible nutrients are processed, digestion rate at any moment should depend on the detection of food in the stomach. The taste and chemistry of food will stimulate release of gastric secretions acting on the stomach content. To model this, a number of simplifying assumptions can be made:

1) The stimulus: In many post-juvenile fish, maximum stomach volume (S_{max}, ml) is directly proportional to the body weight (W, g) so that S_{max} = a W. Current stomach content (S g) can not exceed S_{max} for most foods. Changes in stomach contents (S) induce stretch in the stomach wall, which is detected as a linear stretch of branches of sensory cells (stretch receptors) in the gut wall, that can be oriented in any direction. The stimulus should therefore be given by:

Stimulus = a' $(S/W)^{1/3}$

2) The response: Despite the action of the muscular coats, which mainly promote mixing the gastric contents, the limit to the digestion rate is the secretory activity (acid, enzyme pepsin, mucus) of the gastric epithelium. Given the limited degree of folding of the epithelium, and the uniform distribution in a stated species of oxynticopeptic cells by area in fish of different sizes, the secretory (response) rate of fish at stated temperature must (following from 1 above) be related to stomach surface area. Thus the secretory rate (E) could be given as:

$E = b W^{2/3}$

3) The effective food surface: Digestive secretions released as a result of stimulus (1) and response (2) above act on the surface area of the food bolus. It can be imagined, for simplicity, that a fish chooses similarly shaped single items of food of a size proportional to its body weight (optimal foraging) or conglomerates multiple small items of food taken over a short time period into a bolus of food, which is compacted in the stomach.

Therefore the food mass can only be digested at its surface and it is described by the area formula:

$A = c S^{2/3}$

The digestion rate (dS/dt; GER) at a given temperature is therefore likely to be a combination of the stimulus (1), linked to the response (2) but modified by the effective surface (3). Using these equations the following multiplication equation is derived:

$$\frac{ds}{dt} = -K\left(\frac{S}{W}\right)^{1/3} .W^{2/3} .S^{2/3} \text{ ---------- (1)}$$

The constant, K, combines the constants of proportionality in the earlier equations.

This can be simplified as:

$$\frac{ds}{dt} = K.W^{1/3} .S \text{ ---------- (2)}$$

The predicted power term for weight (0.33) is close to the average found for seven fish species described earlier. At a given temperature, the digestion rate of a given mass of food increases with fish size, that it to say a large fish will digest a given mass of food faster than a small fish.

It is also clear since:

$$\frac{1}{S}ds = -K.W^{1/3}.dt \quad \text{----------(3)}$$

for a given fish size, the emptying of a single meal will follow an exponential curve with time:

$$S_t = S_0 e^{(-Dt)} \quad \text{----------(4)}$$

Where S_t is the residuum of the initial meal (S_o, g) at a stated time (t hours) after feeding, and D = K.W $^{1/3}$. This basic formula is unlikely to apply in practice unless the ingested food is of such low nutrient content that gastric emptying proceeds without an inhibitory feedback from the release of intestinal hormones mentioned earlier. It is much more likely that this "basic" emptying curve is modified; emptying may be constrained into a series of "pulses" so that the anterior intestine is not overloaded with excess chyme which would decrease the efficiency of intestinal digestion. Alternatively, the inhibitory feedback may be more continuous, simply causing a decrease in emptying rate from that predicted by the exponential model. In this model, the stomach reserves to itself the role of the food reservoir and supplies the intestine with chyme at a suitable rate for processing.

The next step in developing the model is to introduce appropriate restriction of gastric emptying. A simple model assumes that intermittent feed-back from the intestinal hormones simply interrupts this exponential emptying rate at intervals. It appears reasonable to assume that pulses of chyme are released, of an equal size, which maximizes intestinal digestion rate without over-loading the capacity to release bile, pancreatic and intestinal secretions. Such a model is developed in Fig. 1.1. In this example, the original meal is emptied in five "pulses" (P) of equal mass. In addition, the last part of the meal, which would typically comprise indigestible residues, is emptied rapidly by true peristalsis (F: "the house keeping of the gut"); this ensures that (unlike an exponential curve) the stomach empties in a finite time. Examination of Fig. 1.1 shows that, if sporadic observations from a fish (or individuals from synchronously-fed group of fish) are taken, then an "emptying curve" will be obtained which varies according to when samples are taken in relation to the occurrence of the "pulses" of emptying.

Observations on the curve 1 happen to fall at times when a pulse is just completed, curve 2 values are obtained in "mid-pause" and curve 3 values apply to the end of pauses. Of course other combinations of sampling occurrences are possible. It is clear that the

"pulsed" emptying curve is no longer exponential.
It is common in the literature to find that collected data are reasonably well described by a power equation:

$$\frac{ds}{dt} = -K.S^b \quad \text{----------(5)}$$

Which, on integration, gives a linear relationship:

$$S_t^a = S_t^a - aK.t \quad \text{----------(6)}$$

Where a = 1- b

In effect, the simplifying assumption was made that gastric emptying approximates a monotonic curve of the form:

$$S_t = \left[S_0^a - aK.t\right]^{\left(\frac{1}{a}\right)} \quad \text{----------(7)}$$

Curves 1 to 3 in Fig. 1.1 were therefore fitted using a search routine (written "QuattroPro" spreadsheet) to select the best power function (a). In this example, the best fits (judged by minimizing the sum of the squares of the deviations of the fitted curve (equation 6) from the observed values) were 0.466 (curve 1), 0.523 (curve 2) and, 0.594 (curve 3). These values happen to be close to the value of 0.5 suggested by Jobling (1981b) and recently Andersen (1998), in their "square root" models although based here on a very different derivation. Jobling achieved this predicted value for "a" simply by assuming that *perimeter* stretch of a tube of fixed length is proportional to the square root of the enclosed volume. This latter model does not easily represent the known sequence by which the stomach and intestine of a fish process food. It is however clear that the results obtained by fitting the power curve to the emptying process of fish of known size, fed a stated meal at stated temperature need not necessarily fit a convenient power function. The value of "a" may vary according to the time samples are taken, the frequency and duration of pauses in emptying, the size of the fish, the temperature and even individual variation in gastrointestinal processing rates.

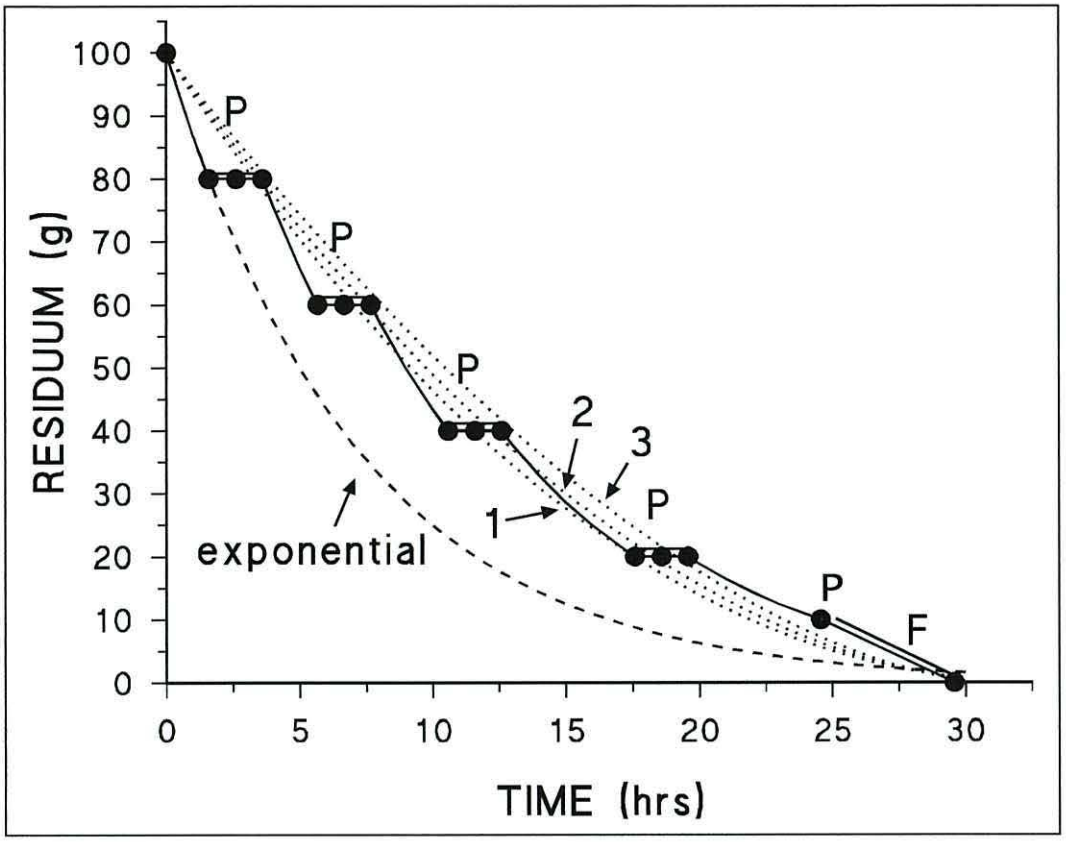


Fig. 1.1. The exponential gastric emptying curve is delayed by four delays caused by inhibitory feedback from the intestine. Five emptying "pulses" (P) of equal size are shown, followed by a terminal clearance of indigestible wastes (F). If stomach samples were taken at the beginning (1), middle (2) or end (3) of the static times, fitted power curves approximate the "square root" model.

Fig. 1.2 examines the consequence of feeding sub-maximal meals to a fish of known size at stated temperature. If a reduced meal is given, it can be expected to follow the curve for a maximum meal but starting at a lower point on the trajectory. Reducing the meal by 40% or 60% of the maximum decreases the initial gastric emptying rate; gastric emptying times however are reduced by only 26% and 43% respectively. Nevertheless, careful experimentation can derive population averages for a species under stated conditions, where appropriate values "a" and "K" are obtained. They enable the likely course of gastric emptying to be predicted. Such prediction can then be used to suggest the shape of the "return of appetite" curve for fish in captivity or for converting sequential field observations of stomach contents into estimates of feeding rate.

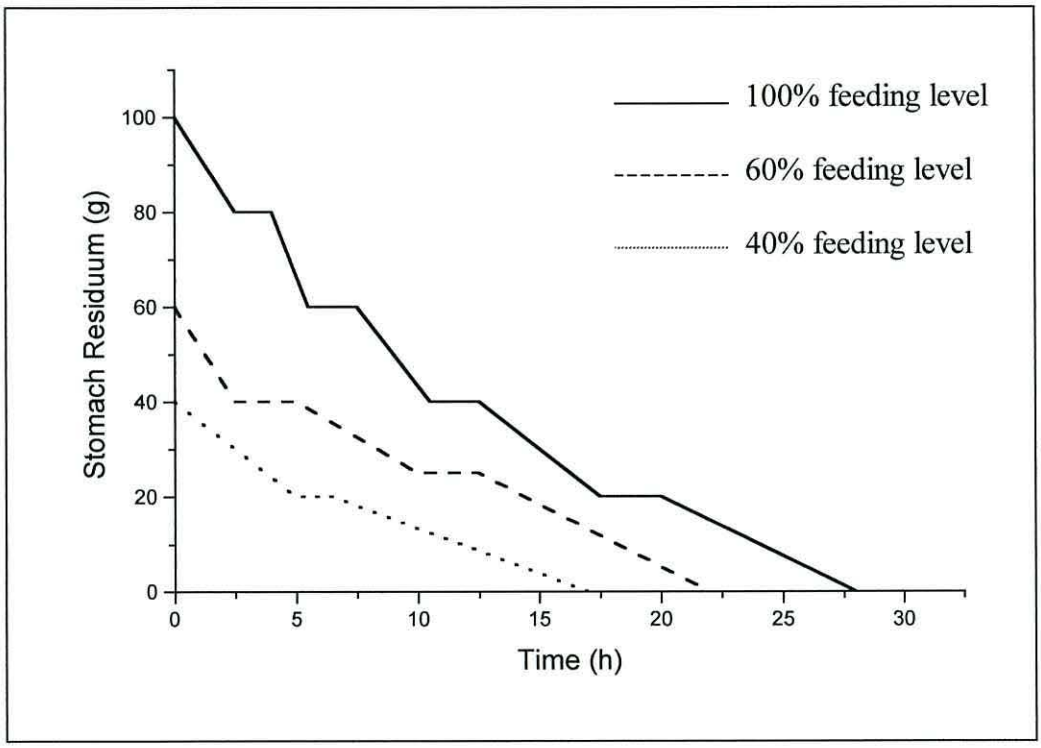


Fig. 1.2. Sub-maximal meals empty along the equivalent, later section of the curve for a maximum meal. Emptying pulses are drawn as irregular steps for clarity.

Grove *et al.* (1985) extended Jobling's power model to include changes in the rate of gastric emptying when several assumptions of the above model are relaxed. Equation (6) was taken to describe the digestion of a single item food of a standard shape and nutrient quality. Three further conditions were examined *viz.*

1) The consequences of eating several of the standard food items in a meal, where the items are conglomerated into a bolus subject to gastric digestion on the surface.
2) The consequences of the items remaining separate, thereby retaining a larger surface area than in (1), with no limitation of available gastric secretions.
3) Conditions as in (2) but where available gastric secretion is limited, and food items must share the available secretions.

The overall emptying rates of combined stomach contents, when "n" food items of similar size are ingested by a fish, given as "aK" in the linear form of the basic model (eq.6), became:

GER:

Condition 1: aK	(unchanged)
Condition 2: $aK.n^{a}$	(increased)
Condition 3: $aK.n^{-1}$	(decreased)

and the gastric emptying times for a meal size, So, originally given as :

$$\frac{1}{aK}S_0 \quad \text{----------(8)}$$

became:

$$\frac{1}{aK}(nS_0)^a \ldots\ldots \frac{1}{aK}n^{-a}(nS_0)^a \ldots\ldots \frac{1}{aK}n^b(nS_0)^a \quad \text{----------(9)}$$

for conditions 1, 2 and 3 respectively. This model predicts that, as meal size increases, gastric emptying time (in comparison with the basic model) should become:

GET:

Increase	(condition 1)
Remain unchanged	(condition 2)
Increase	(condition 3)

The digestion rate of a single item, given as "aK" in the basic model, should become:

aKn^{-a}, aK, aKn^{-1} ---------- (10)

So that the individual item digestion rates should:

Decrease	(condition 1)
Remain unchanged	(condition 2)
Decrease	(condition 3)

This model has been tested for a number of diets in different fish species. Condition 1 (fusion of multiple items into a single bolus) was detected in Dab *Limanda limanda* (Fletcher *et al.*, 1984), whilst competition for gastric juices (condition 3) was found in *Scophthalmus maximus* (Grove *et al.*, 1985).

The studies by Andersen (1998,1999) demonstrated that gastric evacuation in whiting *Merlangius merlangus* could be also described as a simple power model with an exponent close to 0.5, which is unaffected by the meal size.

He also described that the gastric emptying formula using this simple power model can be expanded as a function of many variables such as effects of prey size, prey energy density and prey species on the gastric evacuation. In this model, the gastric evacuation model was expressed according to equation (5), which is written as;

$$\frac{ds}{dt} = -\rho S^{\alpha} \quad \text{----------} \quad (11)$$

Where S is the total weight of stomach contents, and α and ρ are the parameters to be estimated. ρ was expanded as a function of explanatory variables, and the effect of each variable was analyzed separately. For example, the effect of predator size on gastric evacuation is often expressed as a power function of predator size (Jobling, 1981b). Andersen further expanded the model as;

$\rho = \rho_w . W^{\gamma}$ ---------- (12a)

and $\rho = \rho_L L^{\lambda}$ ---------- (12b)

where W and L are wet weight and total length of whiting, respectively and γ and λ are the exponents to be estimated.

The effect of temperature which is described mostly by an exponential function (Bromley, 1994) on gastric evacuation can be expanded as,

$\rho = \rho_T e^{\delta T}$ ---------- (13)

where T is temperature and δ is the coefficient to be estimated.

The prey characteristics also can be included in the model, provided the meal size was held constant. In theory, an increased number of food particles will result in a larger surface exposed to digestion process. Andersen (1999) expanded the rate constant ρ as a

function of prey size;

$$\rho = \rho_V V^{\xi} \quad \text{---------- (14)}$$

where V is the prey weight and ξ is the exponent to be estimated.

Differences in gastric evacuation among different prey items may result from differences in chemical composition, texture and prey morphology. The energy density can be estimated by relating the rate constant ρ as a linear function to energy density

$$\rho = \rho_E (1 - \xi E) \quad \text{---------- (15)}$$

The overall model parameters can be estimated by nonlinear regression using equation (1), integrated over time from 0 to t and expressed as;

$$S_t = S_o(1 - S_o^{(\alpha-1)} \rho(1 - \alpha)\, t)^{1/(1-\alpha)} + \varepsilon \quad \text{---------- (16)}$$

Where S_t is the stomach content at time t after ingestion of meal size So and ε is the random error term.

1.6. Gastric emptying in whiting

It has often been assumed that, on average, food intake is limited by the rates of gastric emptying (= evacuation) (Tyler, 1970; Basimi and Grove, 1985a; Talbot, 1985; Bromley, 1988). Gastric processing rate varies with such factors as temperature, food type, meal size or food particle size but especially species (Fänge and Grove, 1979). At present, there is considerable argument about the process of gastric emptying in the whiting. For Example: Jones (1974) claimed that the emptying curve (*i.e.* the decrease in stomach contents with time after a meal) is linear (Fig 1.3a,b). In addition, the gadoid GET increased as $W^{0.44}$ (Fig1.4a,b) and with temperature.

Bromley (1988) also claimed that the emptying curve is linear (Fig 1.5a) but, unlike Jones, claimed GER was independent of the meal size:

S = M - 0.31t , where S = stomach contents (g), M = meal size (g), t = h after feeding. When S = 0 then T = M/0.31. This implies that if larger meals of similar-sized particles are taken, the slower will be the rate of evacuation of the individual prey items comprising the meal. Seyhan (1994) also reported that small meals were emptied linearly but that emptying rate was directly proportional to meal size so that stomach emptying time was constant (Fig. 1.5b). In contrast, Mergardt and Temming (1997) and Andersen (1998) found that large meals clearly followed a curved emptying pattern, which are best described by a power model (Fig 1.6).

$$\frac{dS}{dt} = -kS^a, Where\ a \cong 0.33 - 0.5$$

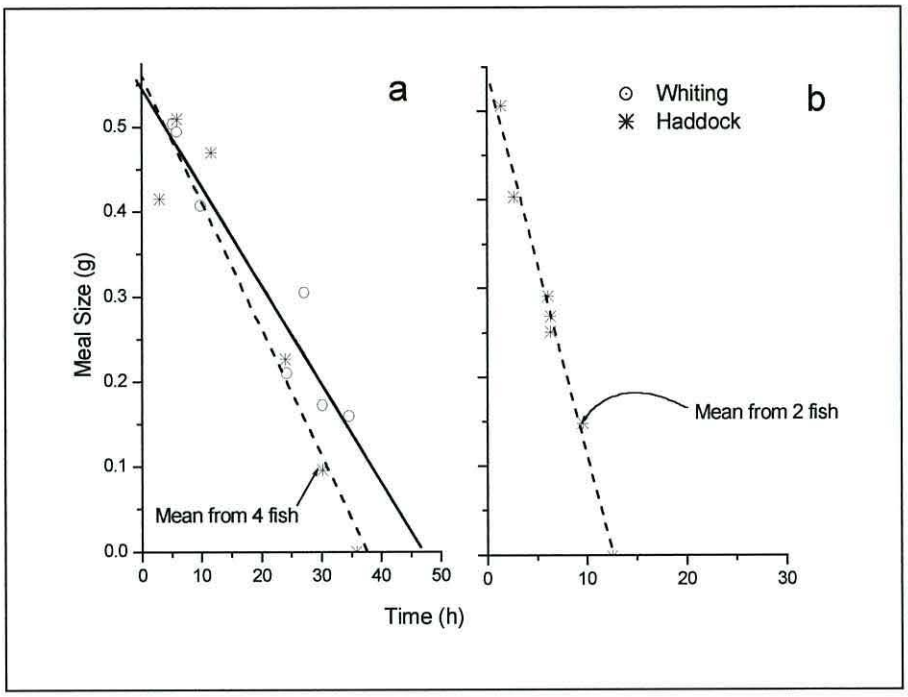


Fig. 1.3(a,b). Gastric emptying curves of pieces of saithe (0.5g) fed to young gadoids were considered to be linear (Jones, 1974).

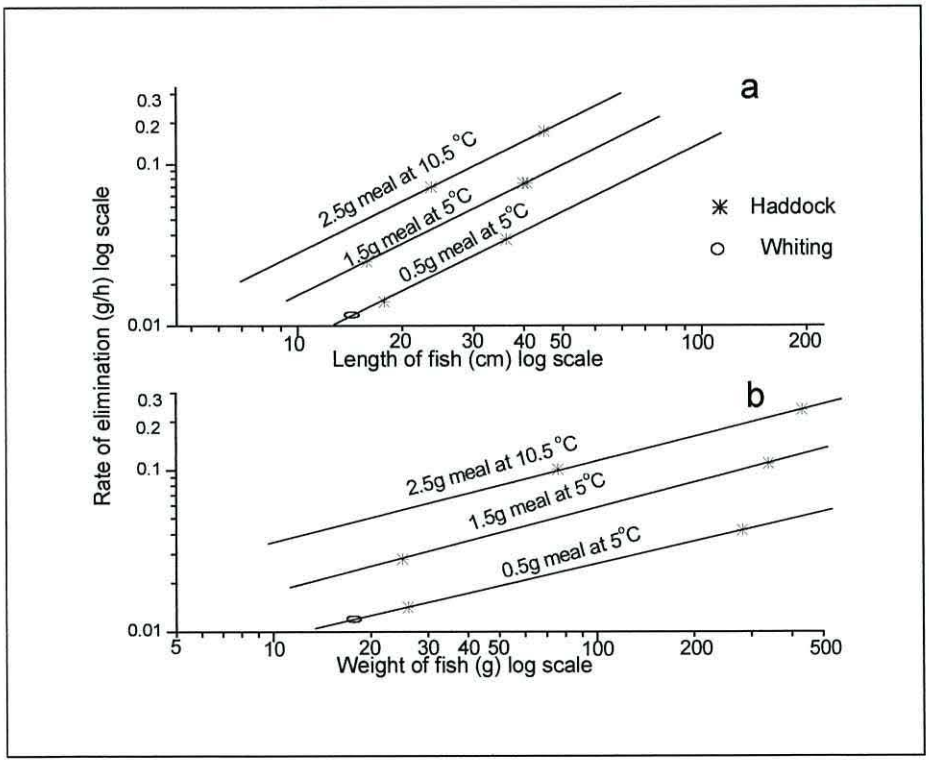


Fig. 1.4(a,b). Gastric emptying rates in young gadoids increased with fish size, meal size and temperature (Jones, 1974).

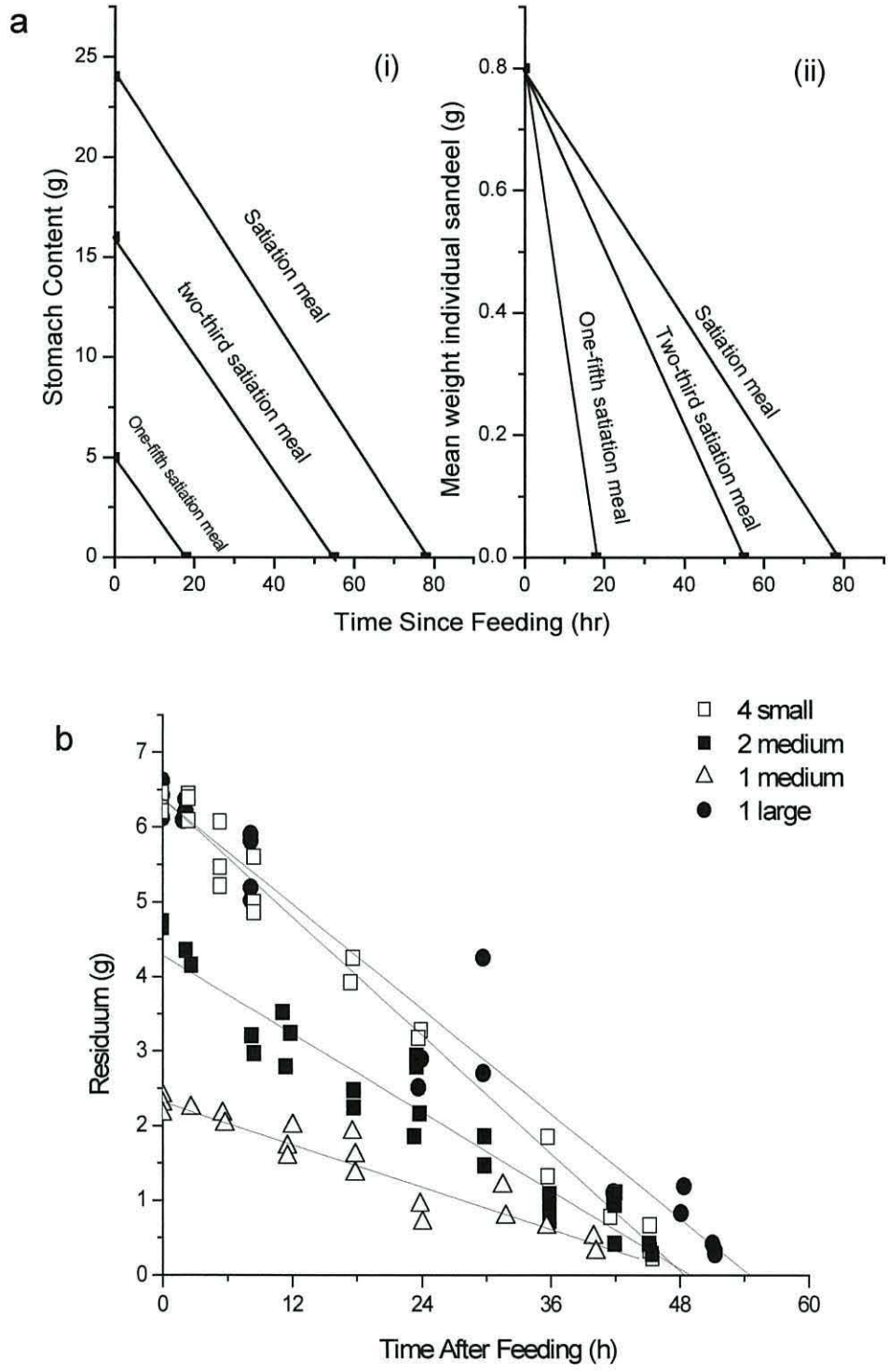


Fig. 1.5(a,b). a) According to Bromley (1988) Gastric emptying rates were independent of total meal size (i) so that individual sandeels in the meal were emptied slower when other items were present (ii).
b) According to Seyhan (1994) Gastric emptying rate increased in proportion to total meal size (whether composed of large or small individual sprat) such that gastric emptying time remained constant.

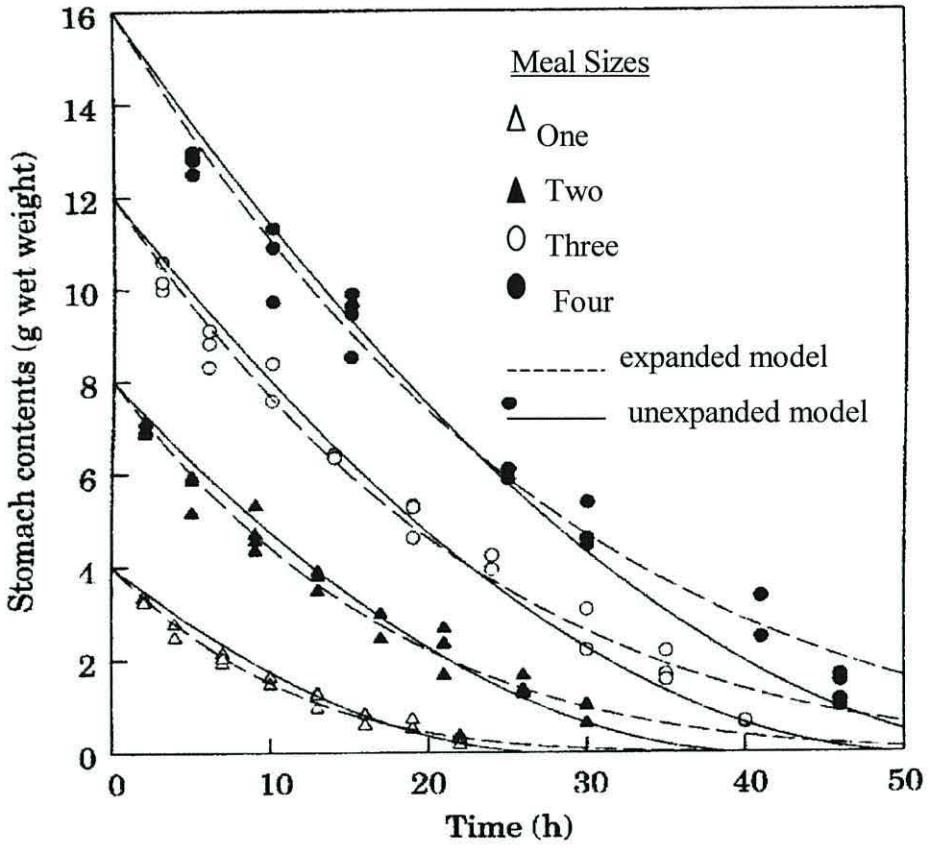


Fig. 1.6. According to Andersen (1998) initial gastric emptying rate increased with meal size but not in direct proportion; larger meals prolonged gastric emptying time.

The curves in Fig 1.6 for small meals of the type studied by Seyhan (1994) are almost linear and the curved emptying process is not apparent until much larger meals are considered. Such meals also have longer GET values. It might be concluded that this change from a " compensatory" digestion rate with small meal size (Seyhan, 1994) where GET is unchanged to " incomplete compensation" of larger meal sizes, where GET is prolonged (Fig 1.6) reflects limits on the capacity of the stomach to digest the food. This topic has not been previously researched. There is also disagreement about the effect of body size on digestion rate of meals of stated size in different species. Some authors conclude that there is no effect of fish size (e.g. Tyler, 1970; Elliott, 1972; Lambert, 1985; Elliott, 1991). Others find that predator size does affect GET (e.g. Swenson and Smith, 1973; Flowerdew and Grove, 1979; dos Santos and Jobling, 1991a). Studies on the effects of meal size and fish size on gastric emptying time were as shown in Table 1.2

Table 1.2. Studies of the effects of meal size ($GET = -k S^{b}_{1}$) and fish size ($GET = kW^{-b}_{2}$) on gastric emptying time (GET), where, S = meal size (g), W = fish weight (g), b = gastric emptying rate (GER) and k = instantaneous emptying rate coefficient.

Sources	b_1	b_2	Species
dos Santos and Jobling, 1991a	0.39	0.39	*G. morhua*
Jones, 1974	0.54	0.44	Gadoids
Fletcher *et al.*, 1984	0.75	0.43	*Limanda limanda*
Gwyther and Grove, 1981	0.67	0.33	*Limanda limanda*
Jobling *et al.*, 1977	(0.89)	0.5	*Limanda limanda*
Andersen, 1998; Andersen, 1999	0.5	0.46	*Merlangius merlangus*
Basimi and Grove, 1985a	0.49	-	*Pleuronectes platessa*
Flowerdew and Grove, 1979	0.613-0.788	0.364	*S. maximus*
Grove *et al.*, 1985	0.448	0.266	*Scophthalmus maximus*
Smith *et al.*, 1989	0.58	-	*Theragra chalcogramma*
Jobling, 1981b	0.57	0.40	Various species

In addition, the effect of temperature may be complex. Figure 1.7 depicts the effect of two different temperature regimes, within the normal physiological range, on the instantaneous digestion rate of meals of varying size in whiting, redrawn from available data in Seyhan (1994) and Bromley (1988). Digestion rate in each group rose as meal size raised to the power 0.77 (compared with the more general value of 0.5).

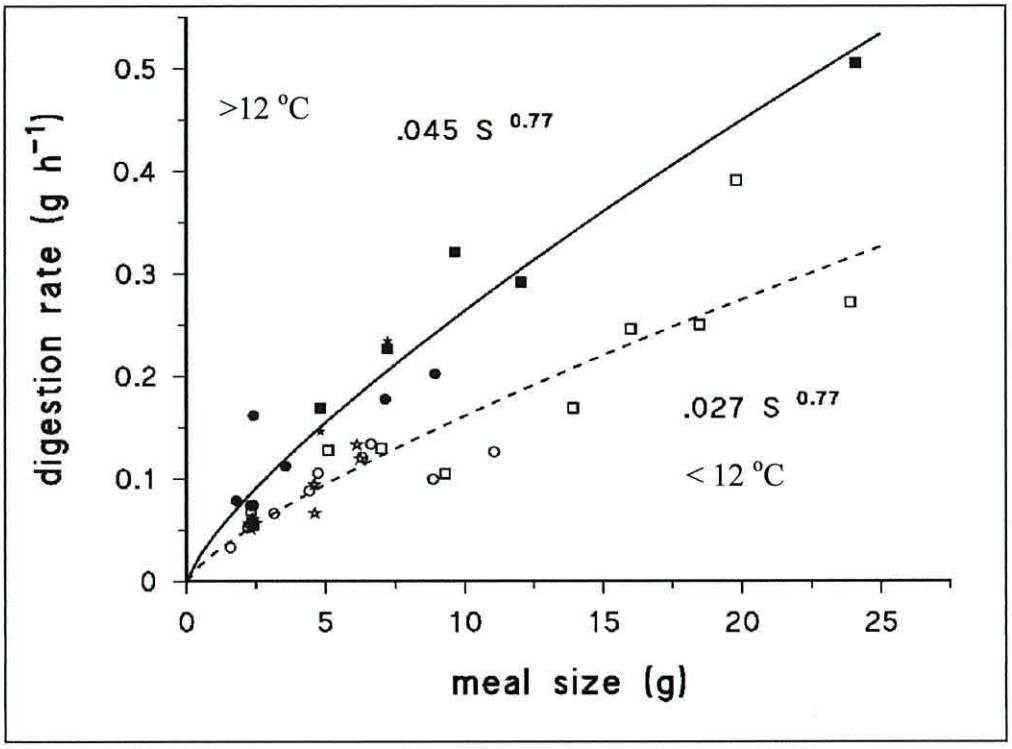


Fig. 1.7. Effect of temperature on the instantaneous digestion rate for whiting obtained by fitting a power curve (see Fig. 1.1) to original data from Bromley (1988) and Seyhan (1994) for whiting fed different meal sizes of sprat. The rate increased rapidly at *ca.* 12°C, near the centre of the physiological temperature range for whiting.(closed >12°C, open <12°C)

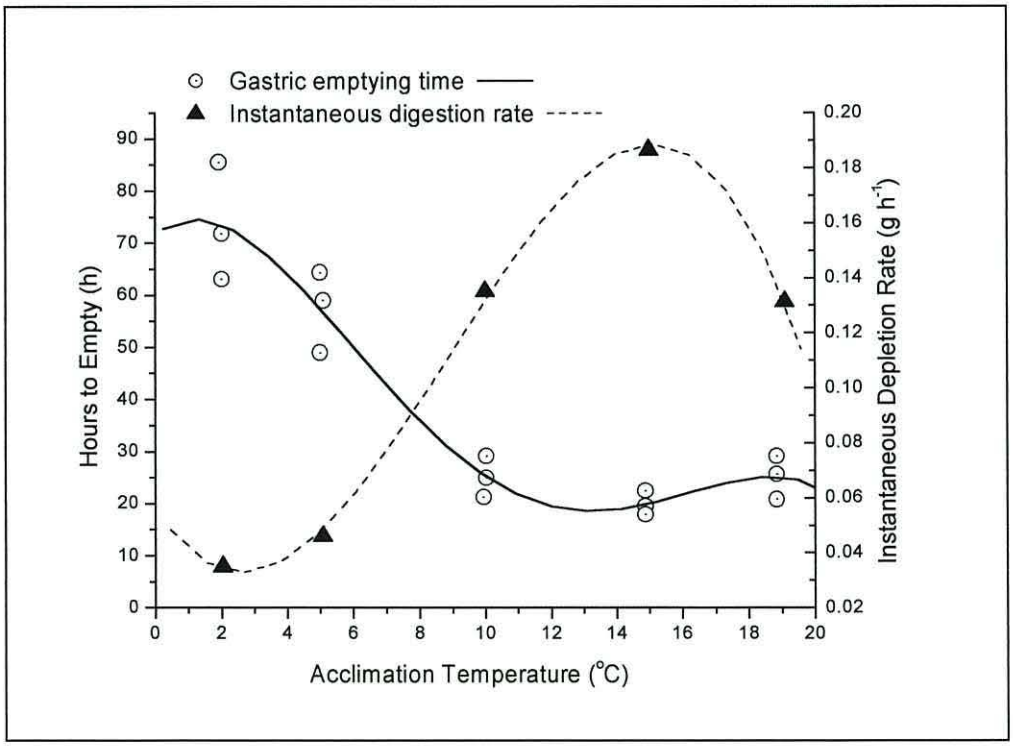


Fig. 1.8. Effects of acclimation temperature on Gastric emptying time and on underlying digestion rate in young cod (Tyler, 1970). Emptying rate in this species increased rapidly near the centre of the cod physiological temperature range.

However, the average temperature difference between the two groups was only 5-7°C whilst digestion rate increased 1.7-fold ($Q_{10} \approx 2.4$, coefficient = 0.088). This is higher than that typically found in fish studies within the normal physiological temperature range. Tyler (1970) studied another gadoid, young cod, and found that the digestion rate increased more than 3-fold between 5 and10°C ($Q_{10} \approx 8$) (Fig 1.8). After this rapid rise digestion rate reached a peak at about 15°C then decreased above this (Fig. 1.8).

1.7 Digestion and absorption processes in fish

1.7.1 General morphology and structure of the alimentary canal of fish.

Detailed descriptions of the morphology & histology of the alimentary canal and digestion processes in fish have been well documented by Kapoor *et al.* (1975), Fänge and Grove (1979), Smith (1989) and Jobling (1995). The alimentary canal is divided into different functional parts, namely the mouth and buccal cavity, the pharynx, the oesophagus, the stomach, the intestine and its associated organs, and the rectum (Fig. 1.9). Most post-larval fish develop a stomach but stomachless fish have evolved in separate families. The alimentary canal structure within a family is strongly associated with feeding habits, mode of feeding and the ecological niche of the species.

In stomachless fish ingested food enters the elongated intestine for the "alkaline" phase of digestion; this condition is frequent in herbivorous and detritophagous species. On the other hand, fish with a well-developed stomach have a relatively short gut length with a unique structure of folded epithelium lining the wall of the stomach; these species are usually carnivorous. There are various types of stomach in fishes. Kapoor *et al.* (1975) described in detail the various forms, from a simple tube to "J" or "U" shapes to expanded pouches, and included histological and ultrastructural features. The stomach wall consists of a number of layers which are protected, supported and strengthened by the *stratum compactum*. The flexibility of this muscle keeps the distension of stomach wall within bounds and is regarded as one of the important adaptive characteristics in many carnivorous fishes (Bucke, 1971; Kapoor *et al.*, 1975).

The most distinct layers in the stomach are the *muscularis mucosa* and smooth (with occasionally striated) muscles lining the stomach wall. The gastric epithelium is associated with secretion of mucus and juices; the layer varies in thickness in different parts of the stomach depending on the degree of gastric gland development.

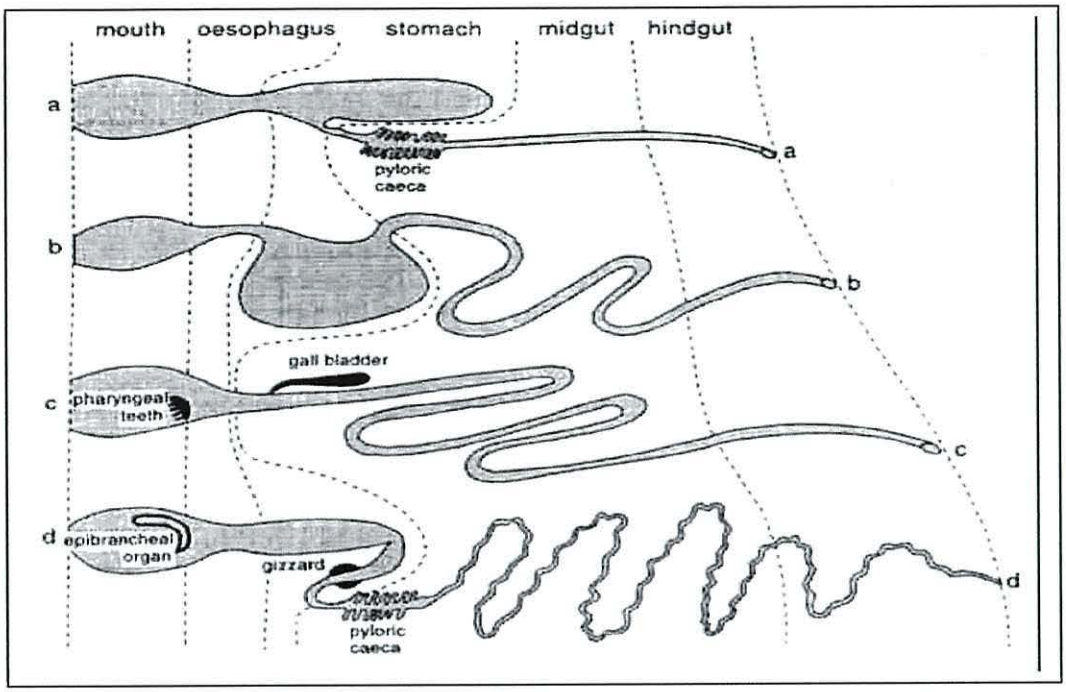


Fig. 1.9. General morphology of the alimentary canal in fish. The whiting has a structure like "a" but the intestine after the pyloric caeca more closely resembles "b". (Adapted from Smith, 1989)

The stomach wall in most carnivorous fish is usually folded, which acts as an adaptive characteristic (to compensate for short overall gut length) allowing appropriate secretion of digestive juices and subsequent absorption of nutrients.

Herbivorous fish have longer intestines to facilitate absorption of protein and associated nutrients present in the debris or indigestible plant materials passing through the alimentary canal. These fish contain high levels of trypsin and chymotrypsin but very low peptic enzyme activities (if at all present). Carnivorous fish typically possess a stomach with a thickly folded wall to provide a larger 'surface area to volume ratio' allowing copious peptic enzyme and acid secretion to digest large volumes of food material. Jobling (1995) postulated that proteolytic enzyme activity in carnivorous fish appears to correlate with feeding habits and relative intestinal length. Carnivores tended to have high pepsin levels but lower activities of pancreatic protease. Insectivorous fish show high levels of intestinal chitinase whilst planktivorous fish tend to produce relatively large quantities of esterase and non-specific lipase. When the food material enters the mouth and oesophagus of bony fish, transfer to the gut takes place rapidly aided by the teeth. Teeth may occur in two general areas apart from the jaws (premaxillae/ maxillae & mandibles): buccal teeth (vomerine, lingual) and pharyngeal (palatine, gill arches).

Many workers have reported that enzyme activity occurs in the buccal cavity and oesophagus of carnivorous fishes (*e.g.* channel catfish and perch: Sis *et al.*, 1979; Hirji, 1983). In contrast, Sarbahi (1951) reported that enzyme activity may occur in the oesophagus of the stomachless goldfish - based on the presence of several alkaline carbohydrase in this region. However, this early finding is questionable since it may have resulted of regurgitation from the intestine and absorption of the enzymes onto the oesophageal epithelium (Smith, 1989). The stomach can disappear in groups of primarily carnivorous fish, even within the same species or genus. This has been related to the alkalification of acidic gastric juice by seawater or food material (*e.g.* crustacean shell) which results in inhibition of peptic activity and decrease in gastric digestion efficiency (Barrington, 1957; Kapoor *et al.*, 1975).

1.7.2 Gastric acid and enzymes (pepsin) activities in the stomach

In mammals, gastric acid release increases when food is sensed, and when food (containing secretagogues such as amino acids) distends the stomach. Acid secretion from parietal (oxyntic cells) is stimulated by the neurotransmitter acetylcholine, by histamine and by gastrin. Gastrin itself is released by gastrin-releasing peptide (GRP) and, like acetylcholine, exerts part of its action indirectly by releasing histamine from enterochromaffin-like cells in the stomach wall. In later stages of digestion, output is inhibited when nutrients in the intestine activate a variety of agents including cholecystokinin (CCK), enteroglucagon, galanin, gastric inhibitory peptide (GIP), neurotensin, pancreatic polypeptide (PP), secretin, tachychinins (such as Substance P [SP]) and somatostatin.

Pepsinogen is released from peptic ("chief") cells after feeding and under the influence of the regulatory peptides Gastrin releasing peptide, bombesin (its non-mammalian counterpart), CCK, SP and secretin. Acetylcholine can augment the response but somatostatin and adrenaline are inhibitory. Pepsinogen is autolytically converted to pepsin under acid conditions in the stomach lumen (Twining *et al.*, 1983).

The mechanisms of control of gastric secretion in fish are not well studied and information is fragmentary. Secretion of hydrochloric acid (HCl) occurs in most fish, except for those without a stomach (Vonk, 1937). This acid is part of the gastric secretion that contains water, salts, mucus and several proteases (especially pepsin) that act on the surface of the food during digestion. A slow, basal release of HCl (100-

150 μmol kg^{-1} h^{-1} was detected in cod (*Gadus morhua*) which was maintained by activity in the vagal nerves (Holstein and Cederberg, 1980; Holstein and Cederberg, 1984). When the stomach is distended, acid secretion increases and the pH of the gastric contents falls. Smit (1967) used a constant stimulus to bullhead stomachs by inserting shaped sponge pieces at 500mg kg^{-1} b wt. He found that the amount of secreted gastric juice (measured after 2 hours) increased about 8-fold with acclimation temperature (from 10 to 30°C) and that acid output rose 1000-fold. Change in pepsin production was less marked (*ca.* 2-fold). He concluded that the increase in the bullhead's gastric digestive power with temperature was primarily due to increase in acidity which enhanced the activity of pepsin (while pepsin concentration itself remained fairly constant - although its activity should increase). Peak digestive power occurred at about 25°C for this species. Even short-term increases in water temperature (*e.g.* 5°C above acclimation temperature) enhanced acid and pepsin output while decreases had the opposite effect.

In the bluegill, the pH of gastric juice depended on "meal size" (distension) since insertion of one glass bead into the stomach produced more acid – to lower pH - than two beads (Norris *et al.,* 1973). The pH began to rise again after 6 hours.

The complex synchronisation of pepsin and acid secretion, with associated changes in muscular activity and flow of blood through the gut, are presumably under neuronal and hormonal control (Holmgren, 1993). For example, gut motility is affected by a large number of substances. When the stomach fills, reflex contractions (which depend on acetylcholine and 5-hydroxytryptamine from nerve cells in the enteric plexus) start mixing the food. Vasoactive Intestinal polypeptide (VIP) inhibits both these neurogenic- as well as myogenic-contractions of the stomach muscles. Cholecystokinin (CCK) contracts the gallbladder, and also inhibits emptying of food from the stomach. The secretion of acid involves cholinergic nerves which act to release the secretagogue histamine near the gastric epithelium (Smit, 1968; Holstein, 1976; Holstein, 1977). This release of acid may be augmented by local release of 5-hydroxytryptamine (5HT, Holstein and Cederberg, 1984), which also causes secretion of pepsin. Gastric acid secretion is stimulated by pentagastrin and histamine in small mammals and in teleosts (Otake *et al.,* 1977; Bomgren *et al.*, 1998; Eno *et al.,* 1998; Trischitta *et al.,* 1998) but gastrin, which also augments the release of acid in elasmobranchs, amphibia and birds has not been detected in teleosts (Jönsson and Holmgren, 1989). Bombesin (the counterpart to GRP) treatment leads to acid release, probably by antagonising the inhibitory effect of vasointestinal polypeptide, VIP (Holstein and Humphrey, 1980). Immuno-histochemical

tests have localised bombesin, gastrin/cholecystokinin, substance P and somatostatin in endocrine cells of the stomach wall (Holstein and Haux, 1982; Holstein, 1983; Holstein and Cederberg, 1986; Aldman *et al.,* 1989; Holmgren, 1993).

Studies by Aldman *et al.* (1989) reported that the gastrin/CCK – like immunoreactive material was present in both muscle and mucosal layers of the whole gut except in the cardiac stomach region of spiny dogfish *Squalus acanthias.* However, the cholecystokinin and gastrin gene in elasmobranchs may be different from that of other vertebrates (Johnsen *et al.,* 1997). Interestingly, it was reported that intestinal absorption of L-Amino acids and increase in luminal osmolarity were both found to affect the secretion of gastric acid in the stomach of fish (Holstein and Haux, 1982; Kidder, 1991).

The localisation of pepsin and its homologues in the stomach of teleosts was described for cod (Arunchalam and Haard, 1985), sturgeon (Buddington and Doroshov, 1986a,b), Atlantic salmon (Einarsson and Davies, 1996), a silurid (Feng *et al.,* 1999) and flounder (Douglas *et al.,* 1999). The zymogens (pepsinogens), the precursors of pepsins, are stored in the same cells (oxynticopeptic cells) which secrete HCl (Smit, 1968; Yasugi *et al.,* 1988); these cells are absent in stomachless fish. The presence of gastric acid lowers the pH to facilitate the activation of pepsin whose endopeptidase action begins breakdown of the food item(s) to form chyme. Optimum proteolysis occurs within the pH range 2.0 – 3.0 in most carnivorous fish (Kapoor *et al.*, 1975; Twining *et al.*, 1983).

Detailed quantification of gastric acid secretion in fish is still rare. Most of the earlier studies on teleosts were reported by Smit (1967), Western and Jennings (1970), Norris *et al.* (1973) and by Holstein and colleagues (*loc. cit* and see Kapoor *et al.*, 1975; Fänge and Grove, 1979; de Silva and Anderson, 1995; Bomgren *et al.*, 1998).

A study of digestive enzymes in fish is important for understanding both their digestion and their nutritional requirements, particularly for cultured fish. Pepsin characteristics and protein digestion rates in the stomachs of teleostei are well established, for example for tuna (Tanji *et al.,* 1996), capelin (Gildberg and Raa, 1983), rainbow trout (Dabrowski *et al.,* 1986; Bassompierre *et al.,* 1998), Atlantic salmon (Einarsson *et al.,* 1996; Einarsson and Davies, 1996), Atlantic cod (Lied and Njaa, 1982; Holstein and Cederberg, 1986), flat fish (Clark *et al.,* 1985; Glass *et al.,* 1987) and in Southern sheatfish, *Silurus meriodionalis* Chen (Feng *et al.,* 1999). A particular digestive enzyme from fish may exhibit distinct differences in physico-chemical properties from homologous

enzymes of mammals. The differences may include catalytic or hydrolytic activity, pH and temperature optima, and different specificity. For example, enzymes in marine fish have higher pH optimum, a lower temperature optimum with a greater stability at low pH than homologues in mammals (Squires *et al.*, 1986; Glass *et al.*, 1989; Haard, 1992; Dimes and Haard, 1994).

The sites of action of pepsin, and of other proteases, are indicated in Fig 1.10. Pepsin is an endopeptidase attacking bonds on the amino- side of tyrosine or phenylalanine under acid conditions. Chymotrypsin acts on the carboxy- side of the same aromatic amino acids but under alkaline conditions whilst trypsin acts on the carboxy- side of arginine or lysine. Amino- and carboxy- exopeptidases act on peptide bonds at appropriate terminal sites.

Amongst different fish, the degree of protein digestion (for example within the stomach) varies depending on factors such as the secretory rate of acid and pepsin, specific activity of pepsin, meal size and retention time, many of which are interactive and dependent on temperature (Smit, 1967). Accordingly each species must be examined independently to assess its ability to digest and absorb nutrients from the diet.

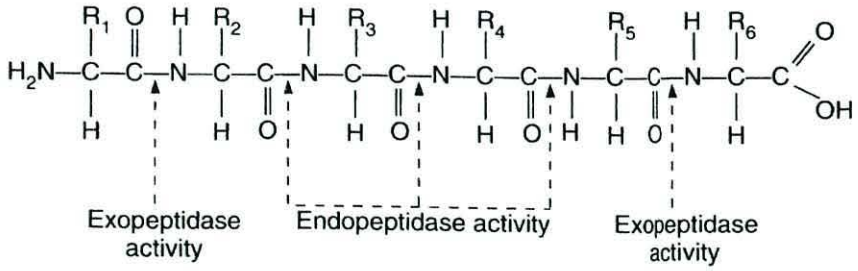


Fig. 1.10. Generalised sites of action of exo- and endo-peptidases on proteins (adapted from de Silva and Anderson, 1985).

1.7.3 Nutrient absorption along the alimentary canal

Food material is usually broken down in the stomach of carnivorous fish through a combination of muscular contractions and enzymatic action in the acidic medium. The breakdown products are expelled from the stomach through the pyloric sphincter as chyme into the anterior intestine through the process known as gastric evacuation. Many species have prominent appendages on the anterior intestine; the finger-like pyloric caeca (intestinal caeca), which are of various types depending on the fish species. These blind-ended tubes serve to increase intestinal absorptive surface area without increasing the length or thickness of the digestive tract (Buddington *et al.*, 1987; Buddington and Diamond, 1987). They can be considered as an extension of the small intestine with similar digestive and absorptive functions (Boge *et al.*, 1979; Bergot *et al.*, 1981; Ash, 1985, Infante and Cahu, 1994).

Dimes and Haard (1994) used pyloric caeca of salmonid (*S. gairdneri*) to monitor protein digestion and absorption ("digestibility") and noted that pH-static methods provide good correlates of protein digestibility found by conventional methods (analysis of food and of the faeces). More recently, Rønnestad *et al.* (2000) found that retrograde peristaltic contractile activity in the pyloric region provides a mechanism by which the fish can fill the caeca with chyme. Observations on whiting digestive tracts show densely-packed pyloric caeca which are likely to be an important site for nutrient absorption.

The intestine of both gastric and stomachless fish shows a variety of mucosal ingrowths but with no villi. In *Salmo*, *Salvelinus* and *Stenodus*, the intestine wall contains muscularis mucosae, stratum compactum and granulosum and muscle coats resembling that of the stomach (Catton, 1948). Generally, the intestine has a simple absorbing columnar epithelium lined with brush borders of various thickness (sometimes known as the striated cuticular border). Other important common constituents are goblet cells, lymphocytes, and various types of granulocytes concerned with the production of zymogen granules (Kapoor *et al.*, 1975). The goblet cells are mainly mucus producers. Amino peptidases were found in abundance in the epithelium throughout the intestine (Western, 1971,Smith, 1980; Dabrowski *et al.*, 1986). In the intestine, the alkaline medium promotes the continuing phases of digestion. The presence of digested food in the anterior intestine releases hormones (*e.g.* CCK, secretin) stimulating the release of alkaline bile containing emulsifying agents from the gall bladder, as well as pancreatic and intestinal enzymes (lipase, amylase, other alkaline proteases). Digestion and food absorption are completed in the small intestine and rectum before residues (plant fibre, bone

fragments, dead cells) are discarded as faeces.

Dabrowski (1983) analysed amino acid concentrations in segments of the alimentary tract of rainbow trout and common carp. He reported that the ratio of free amino acids in intestinal contents to that in the blood of carp most commonly was between 10 and 20, and decreased as digestion proceeded. He also found that the release rate of amino acids from dietary protein was most intense in the second segment (anterior intestine) where most of amino acids were also absorbed. *In vitro* study of the intestinal absorption of amino acids in rainbow trout showed that there was active transmural transport of ^{3}H-labelled glycine, l-leucine, l-isoleucine, l-methionine and l-lysine against a concentration gradient. This study also examined the active transport of l-lysine through the brush border membrane of the mucosal cells using an *in vitro* transport model and found that the intracellular uptake of l-lysine occurred by a saturable transport process which was sensitive to temperature (Gropp *et al.*, 1990).

The intestinal absorption (= digestibility) of macronutrients such as protein, lipid and starch has been well documented (Austreng, 1978; Andrews, 1979; Buddington *et al.*, 1993; Fernández *et al.*, 1998; Sveier *et al.*, 1999; Espe *et al.*, 1999). Most of these workers reported a progressive increase in absorption/ digestibility as the diet passes along the alimentary tract. Fernández *et al.* (1998) found that most absorption of macronutrients occurred in the anterior intestine of the sea bream. Krogdahl *et al.* (1999) found that more than 50% of the absorption of amino acids, starch and lipids took place in the pyloric region of the Atlantic salmon. The pyloric caeca and the distal part of the mid-intestine are the main sites of absorption of ordinary dietary lipids in Atlantic salmon according to Røsjø *et al.* (2000). However, Hernández *et al.* (1994) found decreasing values of starch digestibility (ADC) along the gut of common carp when samples were taken simultaneously at a given time after feeding (although they found an increase in apparent digestibility (ADC) with time after feeding, for samples taken from a given region of the gut.

de Silva and Perera (1984), Ferraris *et al.* (1986) and Anderson *et al.* (1992), confirmed that the observed ADCs in the intestine depended on the region. Digestibility of the foodstuffs was less in the anterior than posterior part of the intestine and tended to be lower in seawater than freshwater.

Nutrient digestibility from natural and artificial feed varies with feeding level and meal size (Henken *et al.*, 1985; Gongnet *et al.*, 1987) as well as with fish size, age and stocking density (Hastings, 1969; Windell *et al.*, 1978), salinity and temperature (Windell

et al., 1978; Pandey and Singh, 1980; de Silva and Perera, 1984) and particularly with dietary components (de Silva and Perera, 1984; Beamish and Thomas, 1984; Hanley, 1987; Krogdahl *et al.*, 1999). Lipids have been reported to depress the rate of gastric evacuation significantly and may have high seasonal variability in natural diets (Kitchell and Windell, 1968; Windell *et al.*, 1969; Elliott, 1972; Grove *et al.*, 1978; Jobling, 1981a; Hofer, 1982; Jobling, 1987).

It is difficult to conduct similar studies on digestibility in the wild but laboratory study using fish fed on their natural food (prey) is possible provided differences in proximate contents of different prey types are reported with the results. These including water content, protein, lipid and carbohydrate levels (Hislop *et al.*, 1991a; Arrhenius, 1998).

1.8 Objectives of the present work

The main objectives of the present study are to establish;

a) Feeding rate of whiting.

The upper limit to feeding can be assessed as the satiation amount that the fish consumes voluntarily. For natural foods, this is usually set by the capacity of the stomach (S_{max} or S_o) although fish fed dry pelleted foods do not fill their stomach fully (Ruohonen *et al.*, 1997a,b) and, at least in the plaice (*Pleuronectes platessa*), some newly-ingested food may pass immediately into the anterior intestine (Basimi and Grove, 1985a). Further food intake depends on the time for appetite to return after a meal. For browsing or grazing fish, such as goldfish (*Carassius*) or tilapia *(Sarotherodon*) feeding may be continuous (Rozin and Mayer, 1961; Rozin and Mayer, 1964; Moriarty, 1973) and feeding rate may match gastric digestion rate. Most other fish take discrete meals and further feeding resumes when the stomach nears or reaches emptiness. Direct observations of meal size and frequency, based on the fish feeding voluntarily on offered feed, can be quantified and compared with gastric processing rates. X-radiography will be used to monitor the motility of digesta in the alimentary tract by incorporating a radio-opaque compound Barium Sulphate ($BaSO_4$) paste in the prey body cavity.

Aims: to measure satiation amount with fish size for different diets to monitor return of appetite and compare with fitted gastric emptying curves.

b) Digestive secretions.

The observed relation between feeding and digestion rates for fish given meals of different quality and quantity need to be linked to the rates of secretion of digestive juices. Quantification of the output of hydrochloric acid and pepsin in the stomach in the presence of food has rarely been attempted. Smit (1967) described the influence of temperature on the rate of gastric juice secretion in catfish (*Ictalurus nebulosus*) but the development of an acceptable standard method for comparative studies (between diets and between fish species) is crucial.

Aims: to measure gastric secretion in relation to meal size and fish weight
to test whether secretions reflect the prediction of equation 1 (page 16).

c) Movement and absorption of macronutrients along the rest of the alimentary canal.

Absorption of macronutrient from the chyme as it passes through the alimentary canal has rarely been reported (Espe *et al.*, 1999). Since each fish can only be sampled once and fish may differ in food intake, an indirect method using internal marker from the food will be adopted (de Silva, 1985; de Silva and Anderson, 1995).

Aims: to localise the main sites of absorption of protein, lipids, carbohydrate and energy.

d) Monitoring of continuous feeding using infrared beams to trigger computer controlled demand feeders.

This is the continuation of the food consumption study in whiting, which deploys a demand feeder unit to allow continuous monitoring of feeding activity of fish in captivity over longer periods. One study of demand-feeding activity of whiting has been reported (Seyhan *et al.*, 1998). The results from this study will be used to measure feeding frequency, including voluntary control of dry matter or nutrient intake, and return of appetite over longer periods. Commercial artificial pellets of known proximate constituents are to be used throughout this part of the study.

Aims: to compare inter-meal intervals with earlier return of appetite studies.

Chapter 2

General Materials and Methods

2.1 Fish maintainance

2.1.1 The fish

Whiting (35–550g body weight) were captured live, from the nearby Menai Strait using hook and line or fish trap, or by trawl net using the R/V "Prince Madog" in the coastal waters mainly east of Anglesey (mainly 53°18'-53°26' N; 3°45'-4°15'W). They were transported to the Fish Laboratory where they were acclimatised at ambient temperature in 4000L aerated holding tanks for at least 4 weeks prior to the start of experiments. Before each experiment, appropriate numbers of healthy fish were transferred into 250L raceway tanks and acclimatised for 14 days prior to the start of the particular study.

2.1.2 The holding system

The 4000 litres stock-holding tank (Fast Tanks, Ireland) (Plate 2.1) was equipped with circular air stones (18 cm diam.). Seawater was recirculated through a biofilter column at 15 L min $^{-1}$ with replacement of new seawater from the settling tanks at 1.1 L min $^{-1}$. The effluent water was pumped to the top of a cylindrical tank (177 cm height x 40 cm diam.) and passed through the biofilter (fibre) and polypropylene ring (2.5 cm thick x 4.5 cm diam.), which provided a substratum for the growth of microflora and macroflora and filter feeder fauna, essential for the functioning of the biological filter. Ammonia levels were < 0.2mg NH_3–N L^{-1} : Nitrite levels were <0.02mg N–L^{-1}. There were no temperature control units available in the stocking system, therefore water temperature followed ambient laboratory water temperature fluctuation. Fish caught in good condition fed voluntarily within 2-4 days after capture. In the holding tanks, fish were fed natural food such as fresh squid (*Illex, Loligo* spp.), sandeel (*Ammodytes* spp.) and sprat (*Sprattus sprattus*). Occasionally, artificial pellets (*e.g.* BOCM Pauls turbot 50, Trouw High Performance trout pellets 5mm) were also given to familiarise fish with artificial diets.

Plate 2.1. The stocking tank system (4000L) with food hopper and supports in place (part of the demand-feeder system).

Plate 2.2. One of the three experimental systems. Each of the six linked tanks was 250L.

2.1.3 The experimental system

The experimental system consisted of 18 rectangular grey fibreglass tanks (each sized 95 x 65 x 40 cm) of 250 litres capacity arranged in two tiers (Plate 2.2). Seawater was recirculated through a biofilter column at 9 L min^{-1} with replacement of new seawater from the settling tanks at 0.8 L min^{-1}. Each of the tanks was connected with a flow through pipe and the end tanks had down pipes, to maintain the water level. The effluent water was pumped up to a similar biofilter as used in the stock system. Each of the tanks was aerated with one or two 15 cm air stones and temperatures were recorded at least weekly. Occasionally fish showed symptoms of stress or illness during summer months when the temperature ranged between 20–23°C. Feeding was reduced or stopped at such time to minimise stress and experiments postponed.

2.2 Feeds

During the study, three types of test meal were used. Frozen squid (*Illex*, *Loligo* spp.) was thawed and cut into small pieces of 0.8–1.8g. The other diets were whole sprat (*Sprattus sprattus* ; 0.7–2 g) and brown shrimp (*Crangon crangon* ; 0.8–3.5g). Frozen squid (*e.g.* QUALY-PAK, Wilmington, Ca.) were bought from the local fish shop, while fresh whole sprats and brown shrimp were caught as needed from Menai Straits using push or seine nets. Batches of these test meals were stored in small freezer bags and kept frozen at -20°C for later use.

2.3 Modelling the return of appetite and gastric evacuation

Collected data on the return of appetite and of gastric evacuation of different types of test meal may be usefully described by a mathematical model, which may itself be useful for making predictions. The model may have to cope with meals that differ in prey species, and also prey size and number (which determine meal size). When offered food, the stomach contents at time zero (S_0) are considered equal to the meal consumed, which cannot be greater than the maximum satiation amount (S_{max}) observed for this diet when offered to healthy, food-deprived fish under the stated conditions. In this study, a non-linear regression was used to estimate parameters of a non-linear model using MicroCalc. Origin™ solfware programme. [A non-linear regression equation was written and the parameters to be estimated were named and declared. The non-linear procedure first examines the starting value specifications of the parameters and evaluates the Chi square value at each combination of values to determine the best set of values to start the iterative

algorithm. The programme uses a choice of two iterative methods; the Levenburg-Marquardt and the Simplex algorithms].

The basic equation was derived from the following power equation model based on Jones (1974);
Gastric evacuation rate of a single particle (dS/dt ; g h^{-1}) is:

$$\frac{dS}{dt} = -\rho S^{\alpha} \quad \text{----------(1)}$$

where S is the present weight of the stomach contents, and α and ρ are the parameters to be estimated.

Grove *et al.* (1985) used this approach to describe the complete emptying curve for a whole meal which forms a simple bolus or for a single item of similar shape but varying size:

$$S_t = (S_0^{\,b} - kt)^{b^{-1}} \quad \text{----------(2)}$$

Where $b = 1 - \alpha$, $k = \rho * b$ and $0 \leq S_0 \leq S_{max}$

Prediction of appetite return over time, based on the amount of space currently unfilled in the stomach, can be derived from equation 2;

$$S_0 - S_t = S_0 - (S_0^{\,b} - kt)^{b^{-1}} \quad \text{----------(3)}$$

Different fish do not always eat the same S_{max}. The amount eaten at a later, stated time can be expressed as a percentage of the average S_{max} for that fish under the stated conditions and the results of different fish combined:

$$100\left(\frac{S_{max} - S_t}{S_{max}}\right) = 100\left[\frac{S_{max}}{S_{max}} - \left[\frac{S_{max}^{\,b}}{S_{max}} - \frac{(k * t)}{S_{max}}\right]^{\frac{1}{b}}\right]$$

$$\%\, S_t = 100\left[1 - \left[S_{max}^{\,(b-1)} - \frac{(k * t)}{S_{max}}\right]^{\frac{1}{b}}\right] \quad \text{-------(4)}$$

Data from Seyhan (1994) estimated b for whiting as 0.77. "k" can be estimated from observed food intake at different times after a satiation meal, by a non-linear regression

of equation 2. Instead, if k is known, the percentage return of appetite at stated times can be predicted.

A related return of appetite model for whiting was also tested, based on the gastric evacuation model described by Andersen (1998; 1999). This was derived by complete integration of equation (1) from time 0 to t and expressed as;

$$S_t = S_0(1 - S_0{}^{(\alpha-1)}\rho(1-\alpha)t)^{(1-\alpha)^{-1}}) + \xi \text{ ----------(5)}$$

where S_t is the total stomach content at time t after ingestion of a meal of size S_o and ξ is the random error term. In their study, the square root version of the evacuation model (α = β = 0.5) is incorporated in equation 5. Model parameters for a maximum satiation meal (when $S_o = S_{max}$) and the rate constant, ρ (GER) can be estimated by non-linear regression of equation 5.

A prediction of the amount eaten (S_t) at time (t) after a previous meal is obtained by reversing the above equation, assuming that the rate of food emptying (GER) is presumed to be determine the rate of return of appetite;

$$S_t = S_{\max} - (S_{\max}(1 - S_{\max}{}^{(\alpha-1)}\rho(1-\alpha)t)^{(1-\alpha)^{-1}}) \text{ ----------(6)}$$

And when expressed as relative percentage of the observed maximum meal eaten, the equation is written as;

$$\%S_t = 100(\frac{S_{\max}}{S_{\max}} - (\frac{S_{\max}}{S_{\max}}(1 - S_{\max}{}^{(\alpha-1)}\rho(1-\alpha)t)^{(1-\alpha)^{-1}})) \text{ ------(7)}$$

2.4 Biochemical determination techniques and procedure

The diets tested in this study were analysed to detect whether noticeable differences in chemical composition occurred and which might explain differences in digestion rates.

2.4.1 Preparation of dry samples

Samples of diets and gastrointestinal contents were weighed to the nearest mg and kept frozen at –18°C prior to freeze-drying. The samples were transferred to an Edwards Super-Modulyo freeze-drying system at -40 to -60°C for 3–4 days depending on the size of the samples. The dry samples were ground to homogenous powder using pestle & mortar and kept in capped soda glass tubes in the freezer or desiccator for further biochemical analysis.

2.4.2 Ash determination

Ash is a heterogeneous group of materials, which includes the non-combustible inorganic components of meal and diets. The experimental meal or practical diets usually contain a small proportion of ash, normally less than 12% of dry weight, but in the bony fish the proportion of ash can be 14–19%. The ash contents of the samples were procured by weighing amounts of precisely weighed, powdered dried samples on pre-weighed aluminium foil and burnt in the muffle furnace for 3.5h at 450 °C.

Calculation:

$$\%\, Ash = \frac{Ash\ \ wt}{Sample\ \ wt} x100 \quad ----------(8)$$

2.4.3 Crude nitrogen and protein

2.4.3.1 MacroKjeldahl methods of crude nitrogen determination

The MacroKjeldahl determination of organic nitrogen was used to calculate the total protein content of experimental meals, digesta and faeces (for samples > 75 mg). The MacroKjeldahl method initially converts amino nitrogen into ammonia, which is then quantified by an acid-base titration.

a) *Digestion process*

Three replicates of each sample (100-250mg) were weighed to the nearest 0.01 mg and placed into a labelled clean digestion tubes. Three replicates of ammonium chloride powder were used on each occasion as the nitrogen standard to measure the recovery (usually between 98 and 101%). The amount of standard was chosen to match the expected nitrogen in samples, based on the assumption that at least 50% of the dry weight is protein and the expected nitrogen content 16% of this (conversion factor is 6.25). Five digestion tubes were used as blanks. To each of the tubes, 2 "Kjeltab" tablets (catalyst containing copper and potassium) and 12 ml concentrated sulphuric acid were added. The digestion tubes were placed in a 1026 Tecator digester hot block at 420°C for 55 minutes. The samples were cooled (20-30 min) prior to the distillation process.

b) *Distillation process*

A stock solution (10 L) of 4% boric acid with dissolved indicator (Methyl red, 10mg L^{-1} and Bromocresol Green 7mg L^{-1}) was prepared in advance. The sample distiller (Tecator Kjeltek model 1026) was warmed up ready to receive the sample tubes. A receiver flask containing 25 ml of 4% boric acid with indicator was placed on the unit to collect the distillate. The level of boric acid must be above the end of the glass receiver tube to avoid losing of ammonia in to the air from the distillate. When the unit door was closed, the automatic distillation began by adding 80 ml of distilled water followed by 3 x 25 ml of 10 M sodium hydroxide. Steam was generated and distillation continued for 3.6 min. As the distillate collected, the grey boric acid solution gradually changed to green. The distillation cycle and drainage were automatically stopped after approximately 4-5 minutes.

c) Titration process

The green sample solutions were titrated using an Jencons "Digitrate" electronic titrator containing 0.1 Molar hydrochloric acid (HCl) prepared from Fisher Chemicals "Solutrate" standards. The end point of the titration was when the green solution turned grey. The acid titres for samples were corrected by subtraction of the average blank titre and the observed crude nitrogen contents calculated;

$$\%N = \frac{14.01 \times \text{ml of titrant (spl)} - \text{ml of titrant (blks)} \times \text{Mol. of acid} \times 100}{\text{g of samples} \times 10 \times 101} \quad \text{-------- (9)}$$

where average recovery of ammonium chloride standard was 101%; % protein can be estimated by multiplying %N with a factor of 6.25 as a recommended factor for animal product.

2.4.4 Carbon Hydrogen and Nitrogen Analysis (C:H:N)

In addition to the Macro-Kjeldahl method of determining crude nitrogen, further determination of crude nitrogen on small samples of digesta (ca. 10 mg) were performed using a non-dispersive infrared microcomputer-based instrument : Carbon, Hydrogen and Nitrogen Analyser (LECO® CHN2000) assisted by Dr. Andrew Owen (Department of Environmental Sciences, School of Biological Sciences, UWB). This instrument can produce very precise measures of the organic carbon and nitrogen in small samples. Prior to samples analysis, the machine was first calibrated with EDTA, which contains a known proportion of carbon (40.9%) and nitrogen (9.5%) as a standard. Details of the procedure are described in Appendix 2.1.

2.4.5 Total lipid contents

Determination of lipid contents in the test meal and the digesta were carried out using a modified Charring method originally described by Marsh and Weinstein (1966). Three replicates of each sample were precisely weighed (2 mg) and were placed into 15 ml graduated centrifugal conical tubes. Standards were prepared using 2 ml of 20, 40, 60, 80 and 100 μg cholesterol ml^{-1} dissolved in 2:1 chloroform/methanol and treated like the samples except no further chloroform/ methanol was added. To each of the samples and a blank, 2 ml of 2:1 chloroform/methanol was added and were left in the dark for 30 minutes to extract the lipids from the samples.

To each sample, 0.4 ml of 0.7% sodium chloride solution was added to purify the lipid extract (removing the impurities) and the samples were then centrifuged at 3000 rpm for 5 minutes. The volume of the lower phase was recorded, then 1 ml of this phase was removed carefully using a clean pipette and placed in labelled thick walled digestion tubes. These tubes were placed in a water bath at 80°C for 30 minutes to evaporate off the solvent.

After cooling, 2 ml of concentrated sulphuric acid was added to each tube. All tubes were carefully placed in the hot block at 200°C for 15 minutes. The tubes were then placed in a cold water bath (approx. 10°C) to cool, 3 ml of distilled water was then added and the contents were vortex mixed and left for 10 minutes in a cold water bath. Approximately 2 ml from each tube was then transferred to 10 mm light path plastic cuvette for spectrophotometry reading. A CE303 grating spectrophotometer (Cecil Instrument) was used and set at 375 nm wavelength. A linear regression was calculated from the cholesterol standards to estimate the cholesterol equivalent in the samples. The total lipid content was calculated by multiplying these values by the volume of the solvent recorded from the lower phase; a correction factor of 1.25 was used as recommended by Barnes and Blackstock (1973). The total lipid contents were expressed as % dry weight of the samples.

Calculation:

Let regression equation obtained from the cholesterol standard be,

$Y = a + b.X$, where Y is an absorbance reading, a is an intercept value and b is a slope. The estimation of cholesterol equivalents of the samples were then obtained from X value obtained from the equation.

Total lipids were then estimated;

$$\text{Total lipid (\%dry wt)} = \frac{\text{X value x Vol. of solvent x 1.25}}{\text{Dry weight of sample}} \times 100 \quad \text{---------- (10)}$$

2.4.6 Carbohydrate contents

Determination of carbohydrate content in the test meal and digesta were carried out using the Modified Anthrone Colorimetric Method as reviewed by Dubois *et al.* (1956).

Reagent:

a) Anthrone mixture

The anthrone mixture was prepared by adding 0.2g of anthrone to 8 ml of ethanol and 30 ml of distilled water. The mixture was completed by gradually stirring in 100 ml of

concentrated sulphuric acid to form a yellowish colour complex. The mixtures are stable for use within 3 days at room temperature.

b) 5%Trichloroacetic acid reagent (TCA)

The solvent solutions were prepared by dissolving 5g trichloroacetic acid in 100 ml of distilled water.

Procedure

Three replicates for each sample were precisely weighed within the range of 5–10 mg and placed into 15 ml graduated conical glass centrifuge tubes. To each of the sample tubes, 2 ml of 5% trichloroacetic acid was added. The tubes were capped with aluminium foil before standing in a hot water bath at 90°C for an hour. The tubes were air cooled for 10 minutes and centrifuged at 3000 rpm for 5 minutes. The volume in each tube was recorded and a sub-sample of exactly 0.5ml of each supernatant was pipetted into labelled thick glass boiling tubes. Standard solutions were prepared using 100 μg, 80 μg, 60 μg, 40 μg and 20 μg D-glucose ml $^{-1}$. 0.5 ml of each glucose standard solution were pipetted into new boiling tubes and 0.5 ml distilled water to serve as reagent blanks.

To each of the sample, standard and blank tubes, 4 ml of Anthrone mixture was added and then vortex mixed. All the boiling tubes were placed in a hot water bath at 100 °C for exactly 7 minutes. The tubes were cooled at room temperature for 10 minutes. The absorbance readings were obtained using a 1 cm light path plastic cuvette at 620 nm. The total carbohydrate present in the sample can be estimated based on the standard curve of D-glucose where the μg/ml equivalent of glucose can be obtained. The estimated equivalent concentration of total carbohydrate for each sample was multiplied by the volume recorded earlier to obtain total carbohydrate in whole samples.

2.4.7 Energy contents

The gross energy of larger samples (*e.g.* food 0.25-1g) was determined by combustion using a Gallenkamp CBB-330-O1OL ballistic bomb calorimeter. Temperature changes were calibrated using benzoic acid (British Chemical Standards, 26.45 kJ [6.319 kcal] g^{-1}) as the primary standard and domestic sugar (17 kJ [4,06kcal] g^{-1}) as a secondary standard. Typically, fat/lipid upon complete oxidation yields approximately 38.9 kJ [9.3 kcal] g^{-1}, carbohydrates near 16.7 kJ [4 kcal] g^{-1} and protein approximately 23.4 kJ [5.6 kcal] g^{-1}. For smaller samples (0.5-3mg), energy contents were calculated using a wet oxidation method using chromic acid. However, this method is claimed to completely oxidise the carbohydrate and lipid of digestible food with incomplete digestion of protein

material (approx. 60% oxidation, Foster and Gabbott, 1971). Results derived from parallel micro- or macro-Kjeldahl analysis for protein can be used instead to correct wet oxidation energy estimates by calculating the remaining protein energy.

In principle, the wet oxidation method relies on the spectrophotometric determination of the amount of unreduced dichromate remaining after an oxidation reaction between the sample and a wet oxidation mixture containing a known amount of potassium dichromate. The digestion mixture was prepared by precisely weighing 2.5 g of potassium dichromate, initially mixed with 10 ml of distilled water and gradually made up to 500 ml with concentrated sulphuric acid. Three replicates of each sample (0.5 to 3 mg depending on expected energy content) were precisely weighed out using an analytical balance (Ohaus Analytical Plus) and added to 12 ml digestion tubes and 2 ml of digestion mixture added. 2 ml of digestion mixture were added to three empty tubes to serve as controls. The sample and control tubes were then placed in a hot block at 100°C for 1 hour. After cooling, the samples were diluted with 50 ml of distilled water and the absorbency was read at 347 nm wavelengths, using a CE303 grating spectrophotometer (Cecil Instruments), in 5 mm path length quartz glass cuvettes. Blank readings were procured by preparing sulphuric acid solution (2 ml of concentrated acid made up to 50 ml with distilled water). Standard solutions (without heating in the hot block) were 2 ml of digestion mixture made up to 50 ml with distilled water.

Calculation of energy content:

There is 10 mg of potassium dichromate present in the 2 ml of reagent; the amount reduced by the sample is therefore calculated as follows:

The standard solution of diluted digestion mixture usually gives an absorption reading of 0.75, relative to the blank. Initially assume that the heated control tubes give the same reading. If the sample gave a reading of E = 0.4, then the fraction of dichromate that has disappeared is;

Reduced $K_2Cr_2O_7 = (0.75 - 0.4)/0.75 * 10 = 4.67$ mg

3 mg of dichromate is the equivalent of 0.489 mg of oxygen, thus 4.67 mg is equivalent to 0.761 mg of oxygen consumed. An oxycalorific coefficient (for protein/fat/carbohydrate mixtures in animal prey) is 14.15 J [3.38 cal] mg^{-1} 0_2. Then 1 mg of dichromate is equivalent to 0.163 mg oxygen and 2.31 Joules [0.551 cal]. Thus, 10 mg of the dichromate is equivalent to 23.06J [5.51 cal], which is the maximum energy capacity of 2 ml of the digestion mixture.

In normal circumstances, the control tubes in the hot block will lose a little colour due the presence of impurities; typically this absorbance reading is 0.7. Thus, the maximum

energy capacity 23.06 J [5.51 cal] can no longer be used and should be replaced by; (0.7 /0.75)*23.06 = 21.53 J [or 5.14 cal]. This is the energy capacity that should be used to calculate energy in the sample. Since only 60% of the dietary protein may react with potassium dichromate, a correction for the remaining 40% of protein content is required.

This is found from the protein content of the same sample obtained from Kjeldahl methods. In summary, the estimated total energy of the sample in Joules can be calculated using the following formulae;

Energy of the sample (J) = ((X –E)/X) * 2.31J + (9.48 * P) ----------(11)

Where,

X = Absorbance of the Standard

E = Absorbance of the Sample

P = Protein value of sample from a Kjeldahl method

2.5 X-Radiography studies of gastric processing of diets.

The aim will be to compare the "return of appetite" curves between different diets and to establish whether this is caused by differences in gastric emptying rates.

2.5.1 Experimental design

X-radiography was used to observe images of food in the alimentary canal of fish at stated times after feeding. A small amount of barium sulphate paste (*ca* 0.1-0.15 ml) was injected into the dorsal muscles of whiting common prey, fresh whole sprats using a hypodermic syringe (1ml). This radio opaque substance produces clear X-ray images of the stomach contents. The results can also be used to validate the gastric emptying models of the various prey types obtained separately. In a serial slaughter study, 5-10 fish at each selected time were killed by stunning and destroying the brain. Each fish was X-rayed and the films immediately processed in the adjacent dark room. The X-rayed fish were kept frozen in the freezer for less than 24 h for the following experiment in section 2.6.

2.5.2 The X-Ray technique and the film processing protocol

A portable X-Ray machine (Model: PRI 10/III) was used. The objects (i.e. live or dead fish) were placed on a cassette (Agfa) containing a screen (Curix Blue C2) and film (Curix RP1 Plus 100 NIF or Curix Blue HC-S Plus 100 NIF, 18 x 24cm) 30-31cm from the X-ray source. Exposure times of 0.2-0.3 second produced satisfactory images of

food and indigestible solids in the stomach and intestine of the whiting.

Films were developed as follows:

i) G150 Agfa developer (1.1 litre diluted in 5.6 litre of distilled water).

-immersion and agitation time 50 sec to 60 sec under red light

ii) Kodak Max Stop Bath solution (1.3 litre diluted in 5.4 litre of distilled water.

- immersion and agitation time 15 sec under red light

iii) Agfa G350 fixer solution (1.3 litre diluted in 5.4 litre of distilled water)

- immersion and agitation time 1 minute under red light
- immersion and agitation time for 15 minutes in the normal light

iv) Rinse bath and air dried.

X-Ray negatives were examined using a fluorescent-light viewing screen and the X-ray images were captured digitally (a camcorder Sony TRV66E Hi8XR was used). The captured images were downloaded to a personal computer and further enhanced using Paint Shop Pro (Ver. 4) programme software.

2.6 Digestion and absorption of nutrient along the gut

2.6.1 Experimental design

The purpose of this experiment was to study the changes due to macronutrient absorption as food passes through different sections of the alimentary tract. Five fish were transferred into each of 250L experimental tanks and acclimatised for about 1-2 weeks feeding with fresh whole sprats. It is important to note that, due to limited healthy whiting supply, this experiment was related to the X-ray experiment in section 2.5 by which, the sprats used as test meal containing (*ca* 0.1-0.15 ml) barium sulphate paste. Preliminary observations showed that the appetite of fish was remarkably increased as the whiting became accustomed to the diet (s). The fish were deprived of food for 3-4 days prior to the start of the experiment. The ambient temperature ranges during the course of experiment were between 11.1–15.6°C. After feeding a known quantity of fresh whole sprats, fish were killed at pre-determined times like those in the return of appetite and gastric emptying time experiments. At each sampling time, the selected fish were killed and their length and weight measured. The alimentary canal of each individual fish was carefully extracted from the carcass, then cut into four sections:

A: stomach, B: pyloric caeca or anterior intestine, C: middle intestine and D: rectum leading to anal opening (Fig 2.1). The contents (digesta) were extruded from each of the sections and placed on a labelled aluminium cup and weighed to the nearest mg. The stomach contents (digesta in section A) were recorded for their wet weight and the

data (wet weight data) were used to estimate the gastric emptying model in section 2.3. The cups were kept frozen at –18°C in sealed containers and then freeze-dried for 3-4 days depending on the amount of sample material. The dried samples were weighed to the nearest mg and ground to a fine, homogeneous powder using a pestle & mortar and a Glen Creston ball mill. Samples were then transferred into labelled soda glass tubes for analysis of their macronutrient contents. In addition, stomach and intestine samples from whiting obtained from the wild were also analysed in the same manner for comparison.

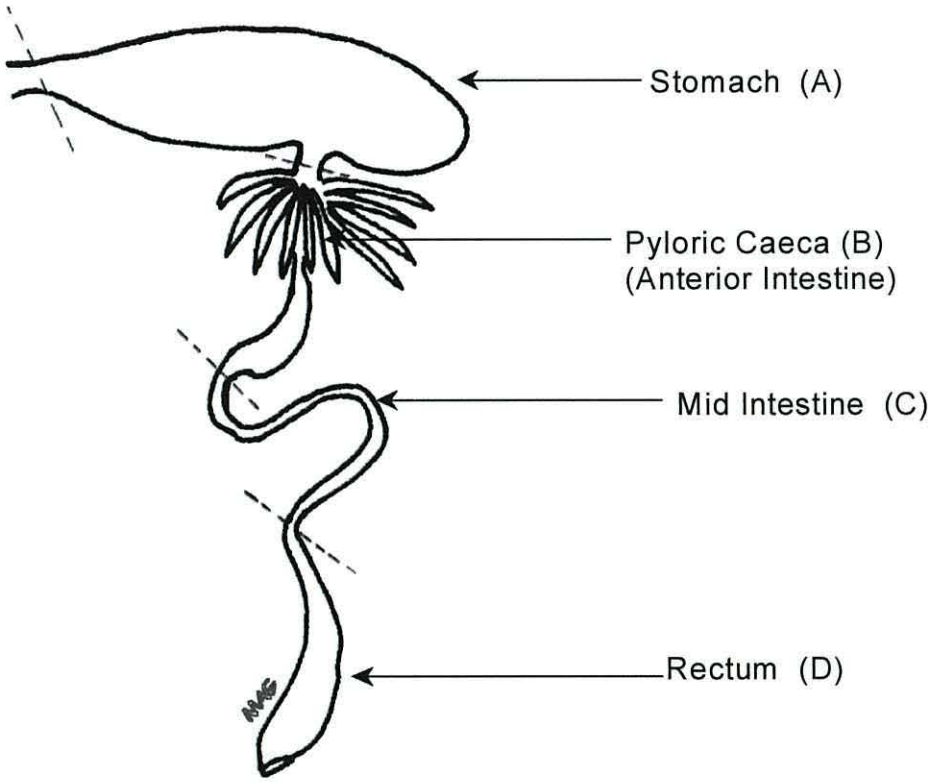


Fig. 2.1. Diagram of the whiting alimentary canal (not to scale). The sections (A-D) indicate zones from which contents (digesta) were removed. The narrow mid intestine (C) is *ca.* five or six times longer than B or D.

2.6.2 Estimation of macronutrient absorption

The site of absorption of macronutrients (*i.e.* Protein, Total Lipids and Total Carbohydrate) from the digesta as food passed along the different sections of the alimentary tract was estimated from ash and nutrient contents in samples from adjacent regions. The formula to calculate absorption is based on earlier concept of Conover Ash Ratio Technique (Conover, 1966), which assumed that aquatic animals absorb relatively little mineral in their natural diet such that ash could serve as an internal marker for food and feces. The digestibility equation was later modified by Kolb and Luckey (1972) and Maynard *et al.* (1979) as follows;

$$\%\ Absorption = 100*\left(1-\frac{Ash_a : Nutrient_a}{Ash_b : Nutrient_b}\right) \text{----------(12)}$$

Where suffix **a** refers to the proximal and **b** refers to the distal samples of adjacent gut segments.

2.7 Gastric juice extraction technique and measurement of total gastric acid production in the stomach of live whiting

The method was modified and developed based on original methods described by Smit 1967.

2.7.1 Experimental design

Eight whiting (size range 150–500 g) were transferred from the holding tank to be held individually in 250L experimental tanks and acclimatised for 4 weeks before the start of experiment. Each fish was fed chopped sprat every alternate day but deprived of food for at least 3-4 days prior to the start of each feeding trial. Three meal sizes were used, i.e. 1.5 g, 2.5 g and 4 g. Plastic sponges (Jumbo^{tm} Super Absorbent) were shaped to fit the narrow oesophagus opening and elongated stomach and the size was set in accordance to the weight of the fish. Approximately 0.035 g dry weight of soft high absorbent plastic sponges were used per 100 g of fish. A fine thread was attached to the anterior end of each sponge and slightly moistened with deionised neutral distilled water pH 6.7–7.1 (see plate 2.3E) and weighed. Each fish was anaesthetised in fully-aerated seawater containing 0.11–0.3 ml L^{-1} of 2-Phenoxy-ethanol (Sigma) for 5–10 min. The tranquillised fish was carefully placed in a plastic tray lined with blotting paper moistened with a stress coat liquid (Animal House).

A preliminary test was carried out to detect acid and pepsin in unfed fish which have been quickly killed. Base levels of acid and pepsin were similar to those recovered in the *in-vivo* sponge method which is described below.

A lightly moistened sponge with string attached was gently pushed into the PVC tube (10 mm diam. x 150 mm length) (plate 2.3A), and the tube carefully inserted down to the distal end of the oesophagus. A glass rod (plate 2.3B) was then used to push the sponge through the PVC tube so that it was placed in the stomach without contamination with seawater. The fish was immediately returned to the tank where they recovered after 10–15 min. At later selected sampling times, the fish were again tranquillised. The thread attached to the sponge was drawn through a larger PVC tube (bore diam. 12 mm; plate 2.3F) and the tube inserted into the anterior oesophagus to provide smooth passage for withdrawing the sponge. This prevented the anterior oesophagus from squeezing out gastric juices from the sponge and to avoid contact with seawater. Occasionally, the fish swallowed the attached string and created

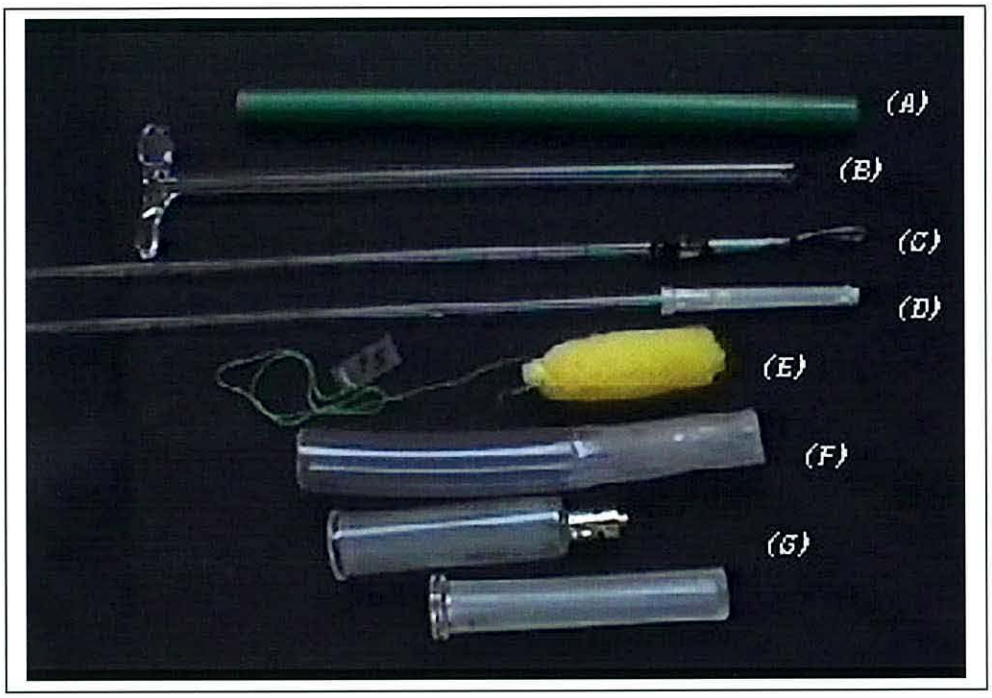


Plate 2.3(A-G). Tools used to recover gastric juice. A: PVC tube (10mm diam. x 150 L). B: solid glass rod (5mm diam. x 150mm L). C & D: hollow glass tube with attached steel wire and plastic cap. E: sponge with labelled thread attached. F: large PVC tube (12mm bore x 80mm L). G: glass syringe and plunger (20mm diam.).

difficulties for sponge retrieval. Special recovery tools were devised to overcome the problem; they consisted of a hollow glass rod tipped with a steel wire loop (Plate 2.3C, D). The 10 mm diam. PVC tube was again inserted into the oesophagus to allow access to the stomach cavity. The recovery tool was inserted through the tube, rotatedaround its long axis to hook the string and the string and sponge carefully withdrawn.

The sponge was quickly transferred to clean and dry, tared glass vials (25 ml) and weighed to the nearest 0.01mg on an analytical balance; the difference in sponge weight is the amount of gastric juice collected. The sponge was immediately placed into a 20 mm diameter glass syringe and the syringe rod (plate 2.3G) depressed to squeeze the gastric juice into a graduated conical centrifuge tube to confirm the volume of undiluted gastric juice. 50 μl of the undiluted gastric juices were pipette into an Eppendorff tube containing 0.95 ml of 0.01N HCl for subsequent enzyme activity determination. The sponge was squeezed repeatedly and washed with de-ionised neutral distilled water to make a final dilution volume between 2–4 ml. The final dilution volumes were recorded.

In order to remove turbidity, the milky gastric juice solution was centrifuged at 10000 rpm for 15-20 min and the clear supernatant poured into a 50 ml conical flask. A few drops of indicator solutions (Bromocresol green and Methyl red (2:1)) were added to form a pink solution in the acidic extract. Measurements of gastric acid were carried out by titration to a colourless end-point (pH 6.2-6.6) with 0.001N NaOH ; a pale green colour confirmed the end-point was passed.

The acid titre was recorded and converted into milliequivalents of acid (mequiv). Initial experiments had shown that insertion of sponge into an empty stomach of starved fish failed to detect traces of gastric acid secretion in the stomach.

Example:

0.001 M NaOH were used for titration.

Let the volume of titre = 1.2 ml

1 N of NaOH = 40 g/l

0.001 N = 0.04 g/l (0.001 moles/1000ml)

= 0.04 mg/ml

1.2 ml = 1.2 x 0.04 = 0.048 mg equivalent wt.

$$\text{Equivalent wt acid} = \frac{0.048}{0.04} \times \frac{0.001}{1000} \text{ mole HCl}$$

$$= 0.0000012 \text{ equivalent wt acid}$$

$$= 0.0012 \text{ milliequivalent wt. acid}$$

2.7.2 Determination of pepsin activity in the gastric juice

Measurements of pepsin activities were based on the method described by Rick and Fritsch (1974). The proteolytic enzyme pepsin is secreted from the gastric mucosa of the stomach as an inactive zymogen, pepsinogen, which is converted to active pepsin by cleavage of a peptide. Pepsin is an endopeptidase, which shows low substrate specificity. Methods of determining pepsin activity include measurement of changing viscosity of protein solutions, determining the nitrogen content in liberated trichloroacetic acid-soluble products, determining the tyrosine and tryptophan content of the hydrolysis product with the phenol reagent of Folin and Ciocalteu, or by direct measurement of the UV absorption of the peptides formed after action of enzyme on the selected substrate such as haemoglobin. In the present study, pepsin in the gastric juice was determined and assayed using the Phenol Reagent technique of Folin and Ciocalteu (1927) (see also Lowry *et al.*, 1951).

Measurements with haemoglobin as substrate

Assay with Phenol Reagent of Folin and Ciocalteu

The method adopted here is as suggested originally by Anson (1938) and later averaged by Rick and Fritsch (1974).

Principle

Pepsin splits off peptides from haemoglobin, which are soluble in trichloroacetic acid. The tyrosine and tryptophan contents of these compounds can be determined by spectrometric measurement of the extinction at 578 nm.

Equipment

Spectrophotometer for measurement at 578 nm, Temperature controlled water bath (25°C) and stopwatch.

Reagents

Hydrochloric acid, A.R., 0.2 N
Hydrochloric acid, A.R., 0.06 N
Hydrochloric acid, A.R. 0.01 N
Sodium hydroxide, A.R., 0.5 N
Trichloroacetic acid
Phenol Reagent of Folin and Ciocalteu (Merck)
Bovine haemoglobin (Dry powder)
L-(--)-Tyrosine (chromatographically pure)

Preparation of solution

Substrate solution: 2g. dry haemoglobin powder were dissolved in 0.06 N HCl and made up to 100 ml gradually in 250ml conical flask (pH ca 1.8). Stir the solution until all the haemoglobin powder completely dissolved. Centrifuge off any stromata, which may be present at 4000 rpm for 15 min (^ 2300 x g).

Tyrosine standard solution (0.001 M tyrosine): A tyrosine standard solution was prepared by dissolving 181.9 mg. L-(--)- tyrosine powder in 0.2 N HCl and made up to 1000 ml in an Erlenmeyer flask.

Trichloroacetic acid (5% w/v TCA): TCA solution was prepared by dissolving 5 g. trichloroacetic acid crystal in distilled water and made up to 100 ml.

Pepsin standard stock solution: Pepsin standard solutions were prepared by dissolving 10 mg pure pepsin crystal in 100 ml of 0.01 M HCl. This stock solution is equivalent to 100 μg pepsin/ml 0.01M HCl. This stock solution is diluted in series to make 80 μg/ml, 60 μg/ml, 40 μg/ml and 20 μg/ml respectively. These solutions were treated in the same manner as the gastric juice samples and the hydrolysis products (absorbance reading) were plotted and regressed against the μg pepsin/ml equivalent.

Stability of solutions

The substrate and tyrosine solutions are stable in a refrigerator at 0-4°C. To prevent bacterial contamination 2.5mg thimerosal (merthiolate (Lilly)) can be added per 100 ml substrate solution and formaldehyde (0.5% final concentration) in the tyrosine solution.

Collection, treatment and stability of samples.

Collection: 50μL of undiluted gastric juice (saved from the earlier gastric acid determination) were dissolved in 0.01M HCl and kept in the freezer for further analysis within 24h.

Stability of enzyme in sample.

The enzymes in samples or pepsin solutions are stable for about 8 days at 4°C. The proteolytic activity of gastric juice initially increases after collection, reaches a maximum at about 9h (+15%) and then slowly falls. The initial value is reached after about 24h and activity decreases by about 2% in the next 24h.

Construction of the Tyrosine Standard Curve.

Pipette into 50 ml Erlenmeyer flasks:

Tube #	Tyrosine Stand. Sol. (ml)	0.2 N HCl (ml)	0.5 N NaOH (ml)
1	0.2	4.8	10
2	0.4	4.6	10
3	0.6	4.4	10
4	0.8	4.2	10
5	1	4	10
Add with continual shaking 3 ml dilute phenol reagent (Sol. II)			
Measure the extinction against a blank containing:			
5 ml 0.2 N HCl 10 ml 0.5 N NaOH 3 ml Phenol reagent. (sol II)			

The tyrosine standard curve is as shown in Fig. 2.2.

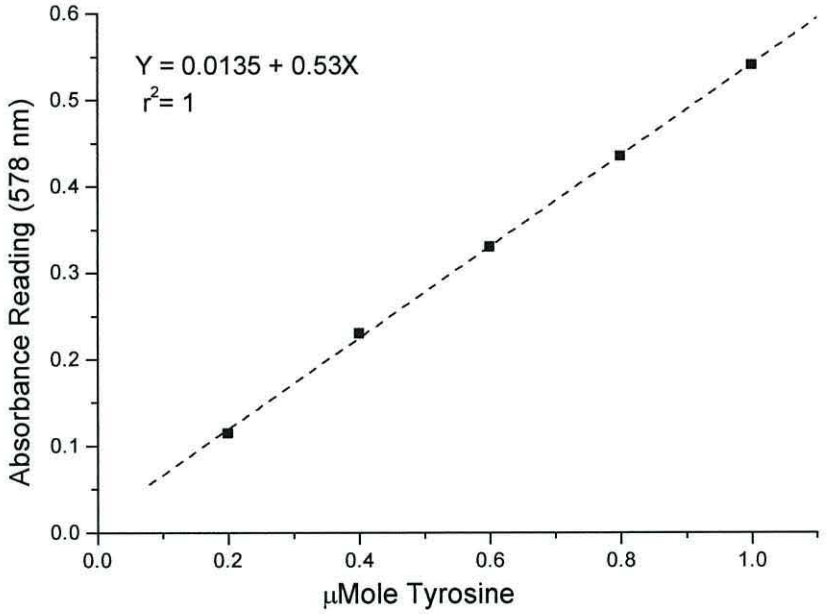


Fig. 2.2. Calibration curve for tyrosine standards.

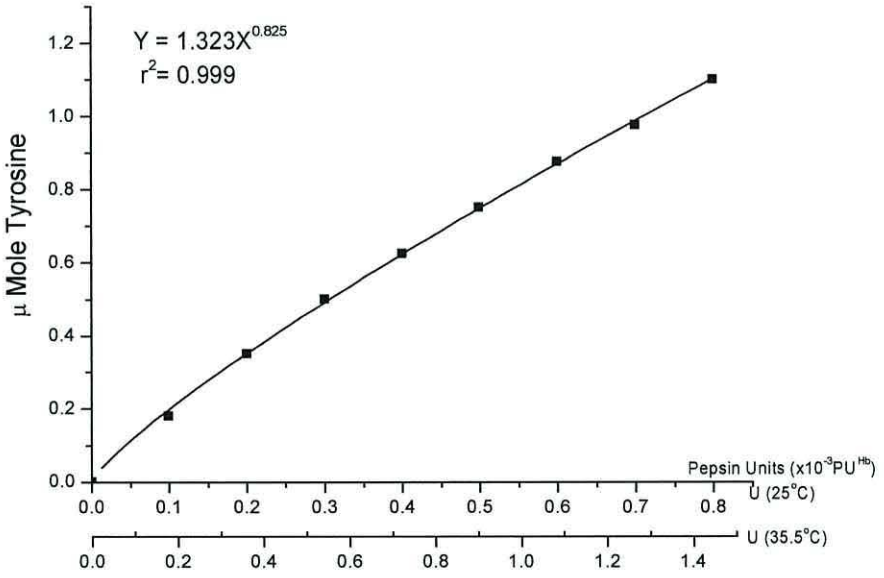


Fig. 2.3. Conversion curve relating tyrosine content to enzyme units based on the hydrolysis of haemoglobin by pepsin [modification of the method of Anson (1938) and Rick & Fritsch (1974)]. Upper abscissa scale: pepsin units according to Anson (1938) – PU^{Hb} and International Units, 25°C (U 25°C). Lower abscissa scale: International Units 35.5°C. Ordinate: tyrosine equivalent (μ mole) of the hydrolysis product in 5ml filtrate. Enzymatic reaction was 10 min at 25°C.

Assay System

Wavelength: 578, 691 or 750 nm; light path: 1 cm: volume of incubation mixture: 6 ml. ; incubation temperature: 25°C. Read against blank. Bring substrate solution to 25°C before start of assay.

Enzymatic Reaction

Pipette into 20 ml centrifuge tubes:		Concentration in assay mixture
Substrate solution (1)	5.0 ml	16.7 mg/ml 0.05 N HCl
Equilibrate to 25 °C (water bath)		
Gastric juice 0.01 N HCl Or enzyme sol.	50µl 0.95ml 1 ml	
Mix and incubate for **exactly** 10 min at 25 °C		
Trichloroacetic acid solution (IV)	10 ml	
Shake, filter or centrifuge off precipitate (20 min at 4000 g)		
Colorimetric Reaction		
Pipette into 50 ml Erlenmeyer flask:		
Filtrate or supernatant fluid 0.5 N NaOH	5 ml 10 ml	0.278 N
Add with constant shaking:		
Dilute Phenol reagent (sol. II)	3.0 ml	

A blank was prepared for every assay by adding 10 ml Trichloroacetic acid solution (IV) to substrate (I), then add sample or enzyme (1ml), mix and filter or centrifuge off the precipitate. Take filtrate or supernatant fluid for the colorimetric reaction. The absorbance reading from the gastric juices sample were plotted against pepsin standard curve and pepsin equivalent (µg pepsin ml^{-1}) were determined from the samples following the regression equation **$y = 0.0035x + 0.0652$.**

The absorbance readings from the gastric juice sample were also read against the µmole tyrosine standard in order to determine the enzyme activity, expressed as pepsin units (PU^{anson}) at 25°C or 35°C. The reading were made based on the modified Tyrosine-Enzyme Unit conversion curve originally provided by Anson (1938) as shown in Fig. 2.3.

2.8 Demand feeder studies

Aims: This is a long-term appetite monitoring study where the fish were trained to use the infra-red beam demand feeders. The long-term feeding patterns were monitored using periodogram software.

2.8.1 Development of demand feeding unit

An automated demand-feeding unit was used to provide a continuous monitoring of fish feeding activity in the experimental tank. The unit consists of up to twelve infra-red beams each with its own hopper. These are connected by shielded cables to a PC *via* a control box. The control box is designed so that additional hoppers/beams can be added simply be sliding in another circuit board. The IR beams are mounted in a waterproof casing, which is suspended from a wooden crossbar over the tank. Both the beams and the hoppers plug into waterproof sockets on the wall near the experimental tank. The system is based on the actuations that are received by the computer as a result of fish passing or breaking through the infrared beam emitted between a Transmitter and Receiver unit. The moment of fish breaks the beam, a 5V DC signal is sensed by the control box, which in turn generates a 12 volts DC signal to operate the hoppers. The hoppers are powered by a stepper motor, which also turns a wheel, which has a groove cut into it every 60°. A mechanical arm runs along the edge of the wheel, and sends signals back to the computer every time it falls into the groove. When the arm falls back into a groove it indicates the hopper has rotated enough and the hopper is switched off. Between each groove, a fixed amount of pellets is released into the tank. The average weight of pellets recorded after each release was 2.223 ± SE, 0.072g. Each operation of a hopper is recorded on a hopper- specific ASCII file, detailing the date, time, number of breaks in the current hour, time taken for delivery, and amount of food delivered.

2.8.2 Experimental procedure

Over a period of six months, fish were caught locally from the Menai Strait. Prior to the start of the experiment, the fish were trained to demand food over 3–4 weeks, based on a respond and reward technique. A glass rod with a red tip was placed near the water surface. The fish were deprived of food for 72h, the rod was put into the tank and when a fish touched the red tip, a few pellets were offered to the fish. The fish training was carried out twice daily until fish were accustomed to demand feeding. When the

demand-feeding unit was set up, a reinforcement training was carried out. A shorter rod with a red tip was placed into the water above the infra-red light path between transmitter and receiver to attract the fish as before. Fish were removed from the experimental tanks and were weighed individually. A total of 20 whiting of closely similar size (average 129.5 ± 5.203 g) were selected and replaced in the experimental tank for the actual demand feeding experiment. All demand feeding experiments were carried out under a 24h lighting period.

2.8.3 Analysis of demand feeding data

Analysis of the recorded actuations to determine feeding rhythms was carried out using the periodogram analysis method using "perio" software (ver. 1.0) developed by Alf Aagaard (courtesy of Alf Aagaard, Institute of Biology, University of Odense, Denmark, 1993). This is an extension of the earlier method developed by Enright (1965) and later adapted by Williams and Naylor (1967). In this method the number of actuations in each succeeding hour ($X_1, X_2, X_3, \ldots\ldots$) is scanned for possible rhythms. To test for feeding every f h, the data are arrayed as:

$X_1\ X_2, \ldots\ldots X_f$

$X_{f+1}\ X_{f+2} \ldots\ldots X_{2f}$ ---------- (a)

From this array (form estimate) the mean (X) for each column is calculated:

$$\frac{\sum X_1 + \sum X_2 + \sum X_f}{f} \quad \text{----------} \quad \text{(b)}$$

Although many statistical techniques can be employed in the analysis of data collected in a time series, the periodogram is particularly well suited to biological data (Williams and Naylor, 1978). Essentially the variance of the $\sum X_i$ values around X for a stated period are calculated. A function of this variance (in the form of standard deviation or coefficient of variance) is then plotted against period length to produce a graphical periodogram. High values of the periodogram statistic occur when the period under test approximates the periodicity inherent in the raw data.

To decide whether the high values are significant, the original data set is then randomised and new periodogram statistics value calculated for each period tested. The periodogram of the randomised data approximates a straight line and upper and lower 95% confidence limits are produced. Significant periodicity is assumed when the true periodogram statistic for a given period is greater than the upper 95% confidence limit to the randomised data regression line at that point. The periodogram analysis is regarded as a descriptive statistic rather than one giving a very critical significance value.

Chapter 3

Estimation of gastric emptying in whiting (*Merlangius merlangus* L.) using X-radiography

3.1 Introduction

Seyhan (1994) studied the gastric emptying of natural food (*Sprattus*) by whiting (40-400g bw) over the temperature range 8 to 16°C and reported that gastric emptying of meals up to 4% of body weight was usually complete within 50 hours, independent of meal size. In contrast, Bromley (1988) found much longer gastric emptying times (GET) – up to 100 hours - under similar conditions when his fish consumed voluntary meals which were up to five-fold larger.

To resolve this disagreement, a short study repeating Seyhan's X-radiography technique was carried out using larger meal sizes. The work was carried out using the radio- opaque marker, barium sulphate paste ($BaSO_4$), injected into whole sprat to act as a contrast medium. The marker was held back relative to the food in Arctic charr *Salvelinus alpinus* (Jorgensen and Jobling, 1988), and in Atlantic cod, *Gadus morhua* (dos Santos and Jobling, 1991b) but Seyhan (1994) found that the injected marker remained mixed with the food. He also reported that the relationship between stomach volume (SV, ml) and body weight (W) for whiting (48 to 695 g, n = 42) was:

$$SV = 0.067\ (SE,\ 0.0027)\ W$$

which may be used as a useful predictor of satiation meal size for natural food items.

3.2 Material and Methods

X-Radiography

In this experiment, at least five experimental fish of approximately similar size were transferred to each experimental tank and accustomed to accept whole sprats meal over a two-week period. Sprats were injected with barium sulphate paste (Conc. 1g $BaSO_4$: 5 ml distilled water) at approximately 0.1-0.15 ml g^{-1} wet weight and kept frozen prior to the start of the experiment. It is important to use amounts of barium sulphate, in each sprat, which are proportional to its weight to produce similar image densities as the food breaks up. Fig. 3.1 shows the relationship between the amount of barium sulphate paste that could easily be injected and the sprat's body wet weight (Prey size, g); each 1 g of sprat contained 0.110 ± 0.002 ml barium sulphate paste ($r = 0.8856$; $N = 40$; $P < 0.0001$).

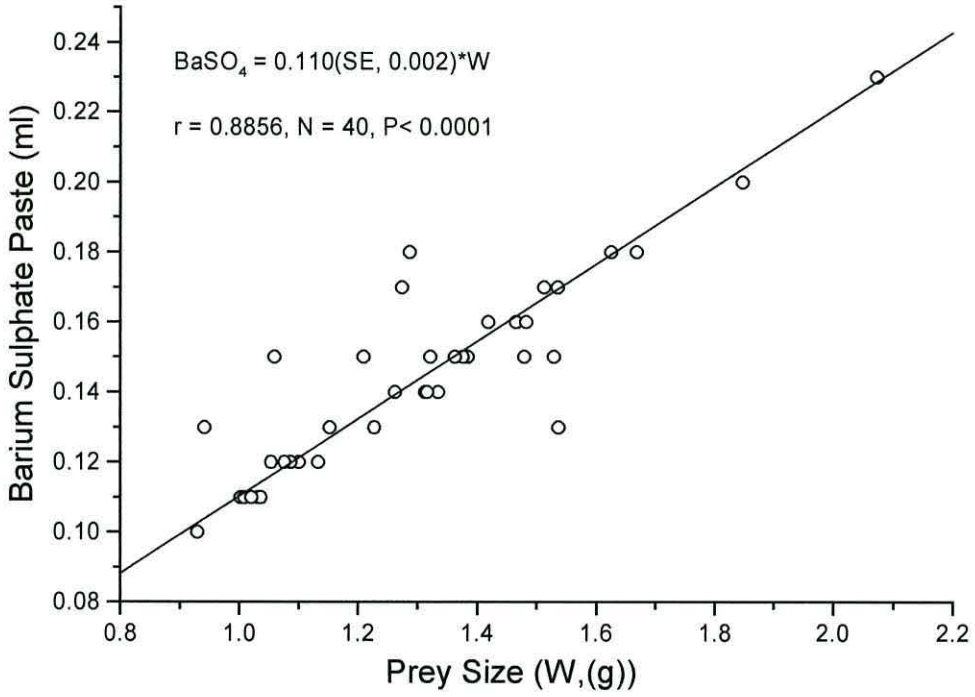


Fig. 3.1. Relationship between amount of barium sulphate paste inclusion (ml) and prey size (fresh whole sprats, g) used for X-radiography study.

All fish were deprived of food for 96h and then offered pre-weighed labelled sprats to satiation. The exact amount eaten was recorded for each fish. At a selected time after feeding, stunning and immediately destroying the brain killed each fish. X-ray films were taken and the alimentary tract opened to remove contents. This allowed comparison between the location of the food and the barium meal image from the X-rays. The digesta in each section of the alimentary tract of each fish were retained to be used for further analysis after the X-radiography. Details of the X-ray method and film processing procedure were as described in Chapter 2.

3.3 Results

The large ellipsoid stomach of whiting can be easily distinguished from the mass of pyloric caeca, intestine and rectum after dissection (Plate.3.1) and in the X-ray films. The stomach is separated from the intestine by the pyloric sphincter which is encircled by the pyloric caeca, and is followed by a narrow, thin-walled intestine leading to an expanded rectum (Plate 3.1). The alimentary tract contained digested sprats and the associated $BaS0_4$ paste; comparison of stomach contents and X-ray images showed the marker component ($BaS0_4$) moved along with the test meal digesta and showed similar gastric emptying times. When filled with digesta, the intestinal wall expanded forming a very delicate thin walled tube. Special care needs to be taken especially during extrusion of the digesta from different parts of the intestine avoiding displacement of digesta contents to different sections of the alimentary canal. Dissecting a semi-frozen alimentary tract is recommended so that accurate digesta position in the alimentary tract section is procured.

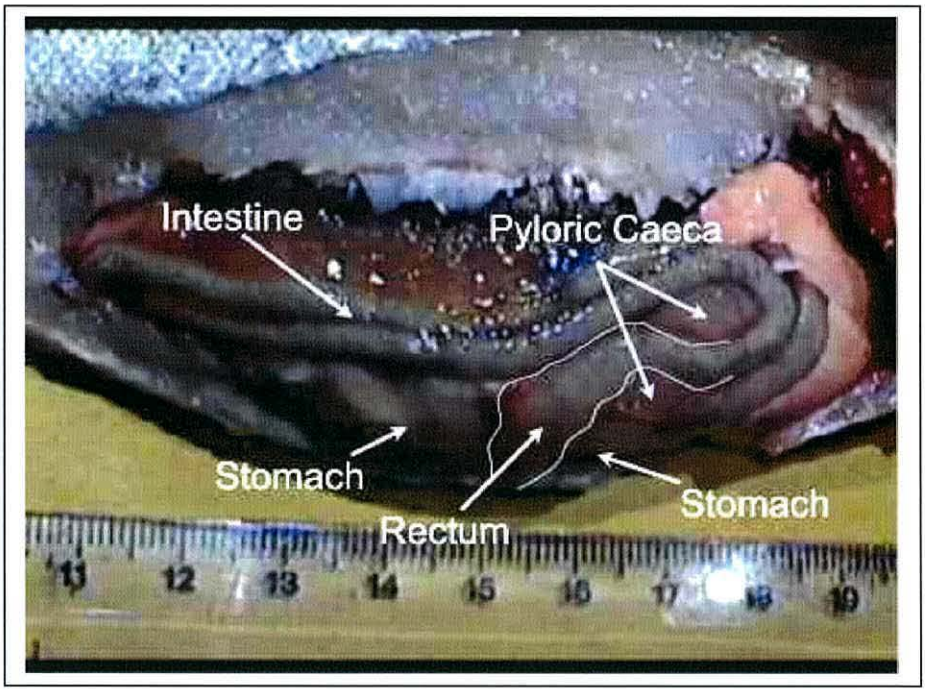


Plate 3.1. Close up view of partially frozen fresh whiting alimentary tract showing details of the sections containing whole sprats mixed with radio-opaque $BaS0_4$.

Plates 3.2 – 3.5 show the X-ray image of small and medium sized whiting 12h after ingesting meals of 8-10% of their body weight (8 to 19 g) of labelled sprats. The stomachs are full, food has already entered the proximal and distal arms of the intestine loop but has not reached the rectum. In Plates 3.4 and especially 3.5, labelled chyme can be seen within parts of the pyloric caeca.

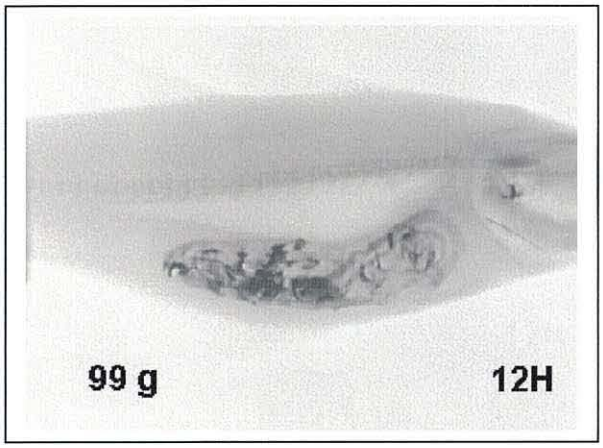
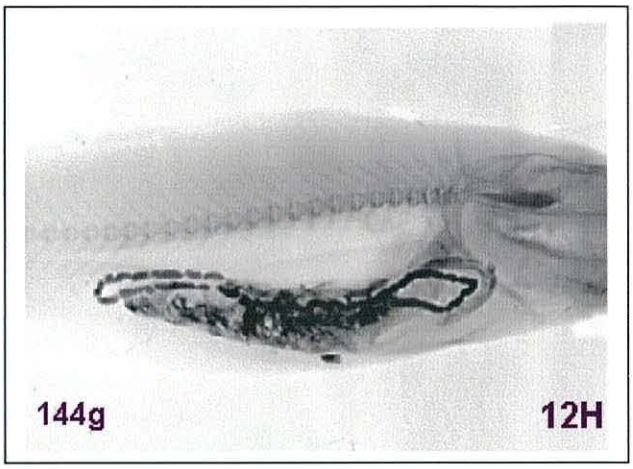


Plate 3.2

Plate 3.3



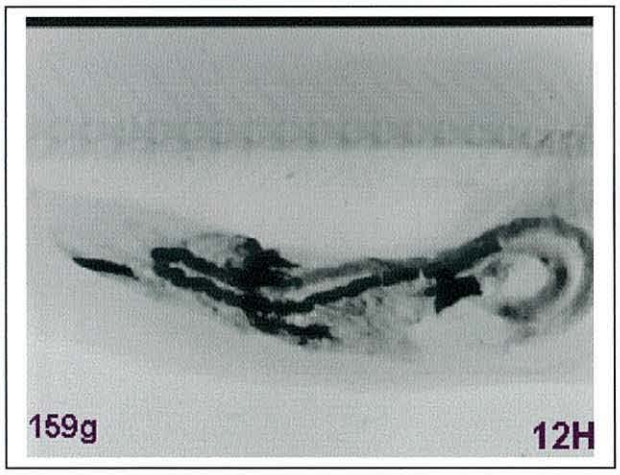
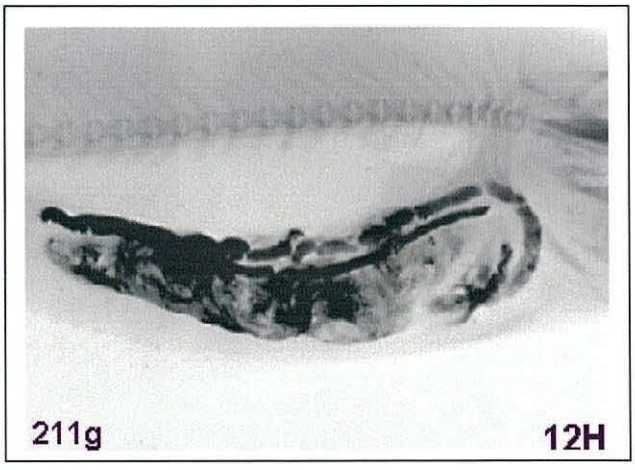


Plate 3.4

Plate 3.5



Plates 3.6 – 3.9 are images taken 24h after feeding. Labelled material has reached the rectum in smaller fish (Plates 3.6 and 3.7) but food is still present in the stomach (see especially Plate 3.8). Dissection showed that 35-55% of the original meal was still present at this location in these fish. One fish (189g) ate approximately 75% (9g; *ca* 5% of body weight) of its estimated maximum capacity (13g).

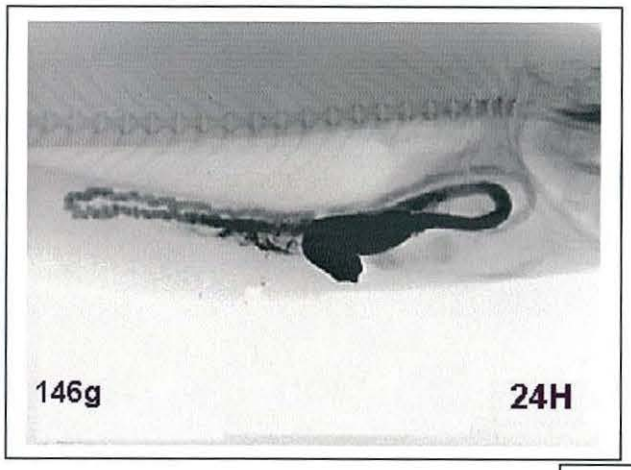
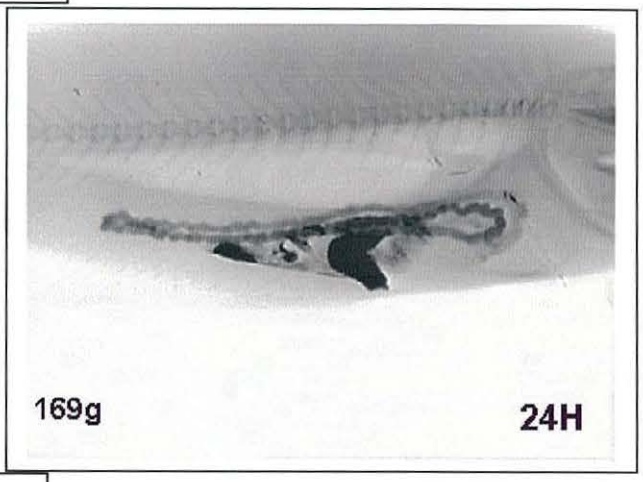


Plate 3.6

Plate 3.7



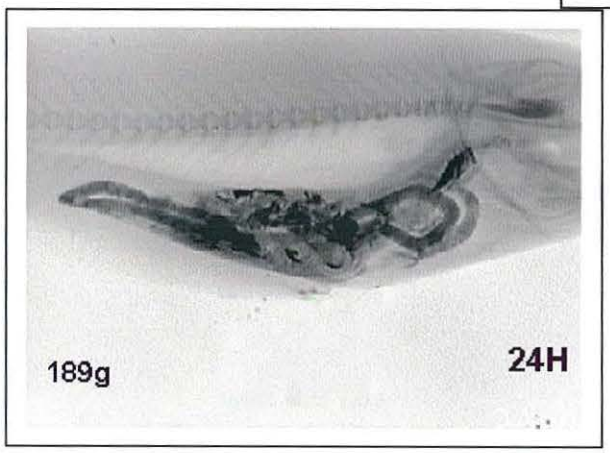


Plate 3.8

Plate 3.9

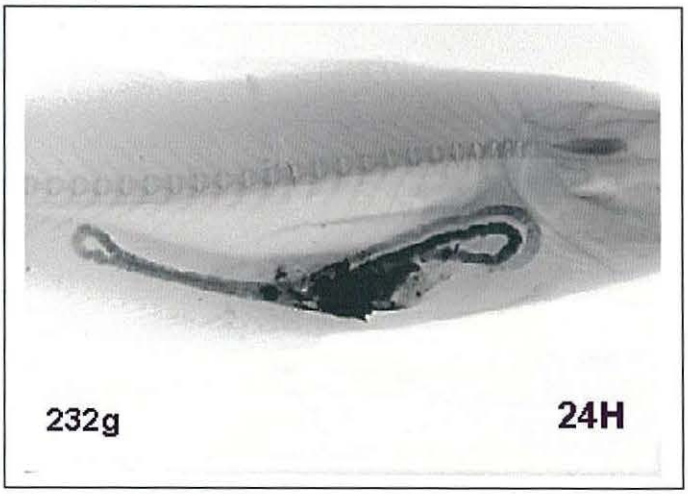


Plate 3.10 – 3.13 show images after 36h. Stomachs were only partially empty (70 – 80% of the meal had moved on) and the labelled digesta fully filled the intestinal tracts with most of it concentrated in the rectal region. The smallest fish in the group (156g) ingested 16g of fresh whole sprats comprised more than 100% of the predicted maximum capacity of stomach (11g) from Seyhan's (1994) formula. After 36h only 31% (5g) of the meal was left in the stomach. Similar excessive ingestion was also displayed by the fish in Plate 3.11 (197g), which consumed 16g of sprats leaving 5.4g (34%) in the stomach after 36h. Larger fish (Plates 3.12, 3.13) retained 20-30% of the original meal at this time.

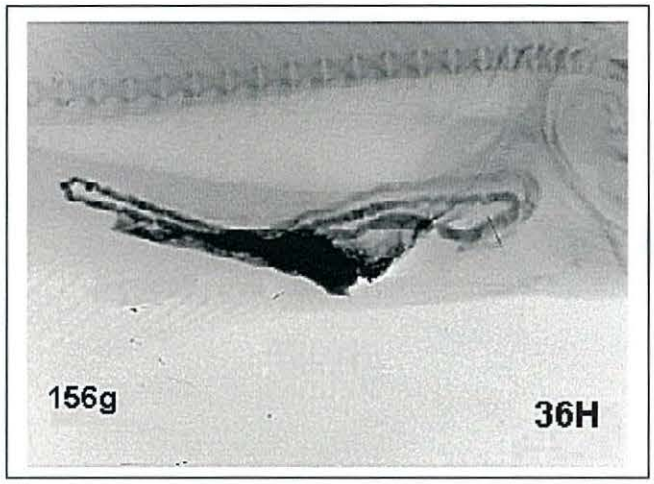
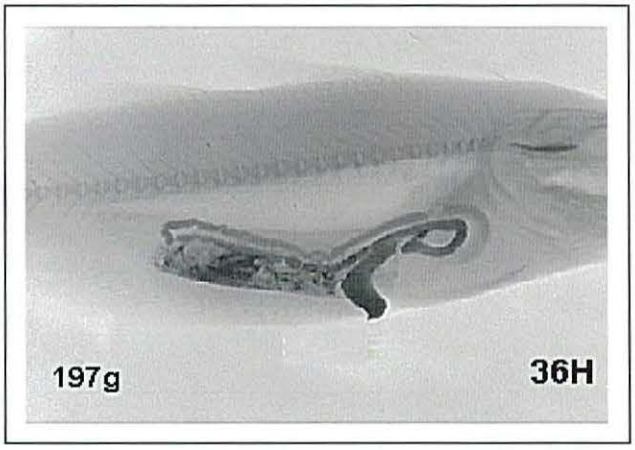


Plate 3.10

Plate 3.11



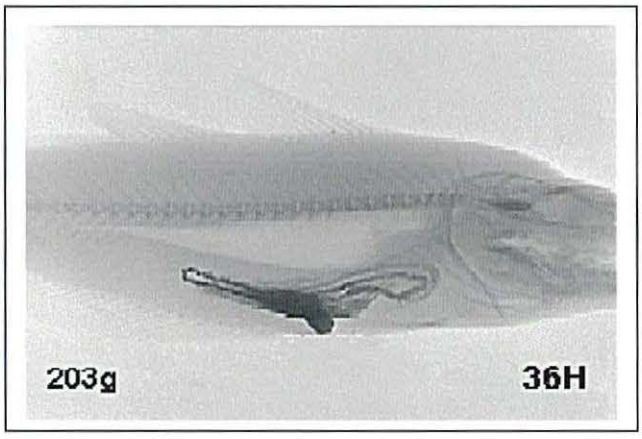
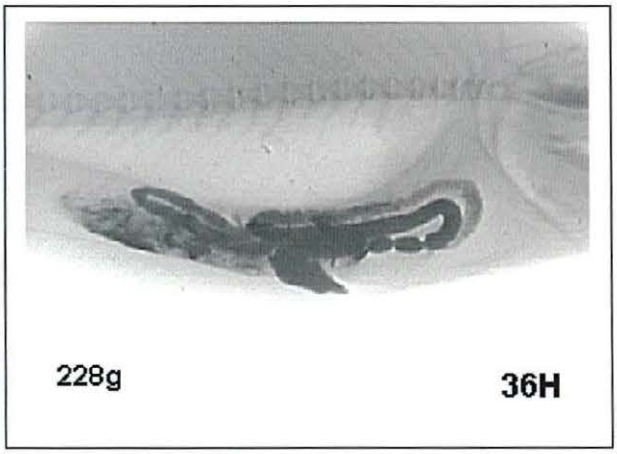


Plate 3.12

Plate 3.13



Plates 3.14 – 3.17 show that two fish had emptied their stomach within 48h as expected from Seyhan's (1994) study. These fish (134g and 163g) had ingested only 4g and 5g of sprats respectively and are comparable with the experiments that Seyhan conducted. However, the whiting in Plates 3.14 and 3.17 had not fully emptied their stomach; 23 – 25% of the meal remained. These whiting accepted large meals - *ca* 6 – 10% on their body weight - and required more than 48h to completely empty their stomach.

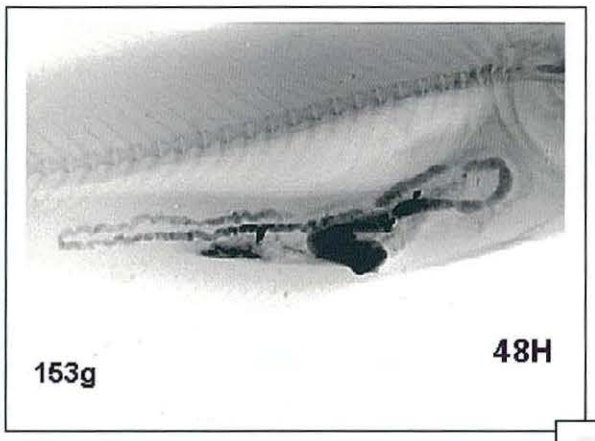
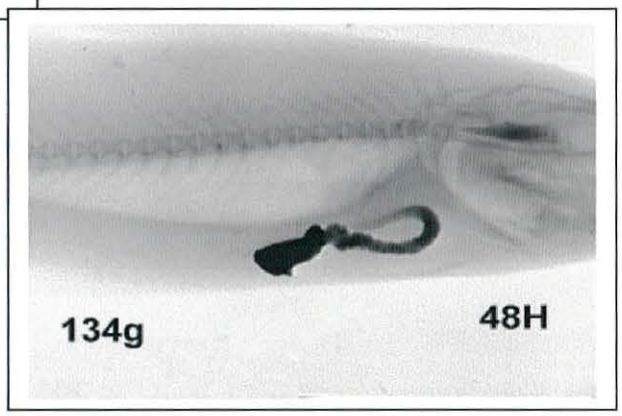


Plate 3.14

Plate 3.15



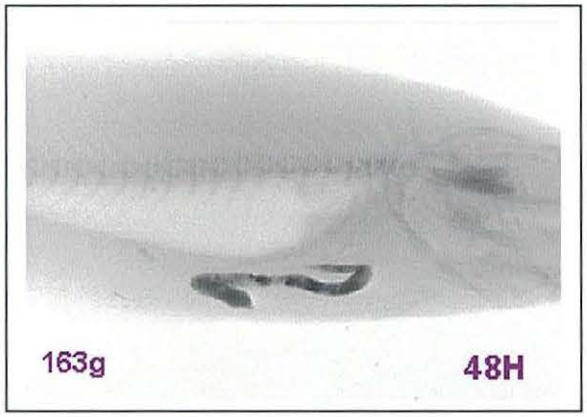
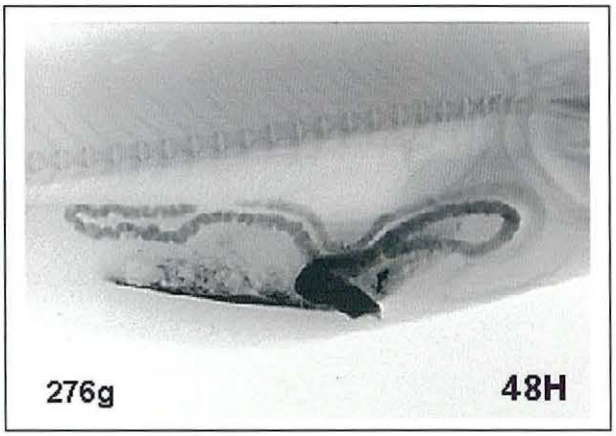


Plate 3.16

Plate 3.17



Plates 3.18 – 3.21 showed that there were still some digesta left in the stomach of several fish even after 60h. Those fish with empty stomachs had ingested around 30–45% of their expected maximum stomach capacities, only *ca* 3% of their body weight. The larger fish in Plate 3.21 (288g) ingested 25g of sprats (9% bw) and still retained 3g in the stomach after 60h. The fish which took small meals had digesta concentrated mostly in the posterior intestine and rectal regions. Those taking large meals had digesta in all sections of the alimentary. Interestingly, Plate 3.19 showed a 159g fish that ingested around 5g of sprats (3% of body weight), which had completely emptied the stomach, defecated all the digesta, but left residues of label in the pyloric caeca.

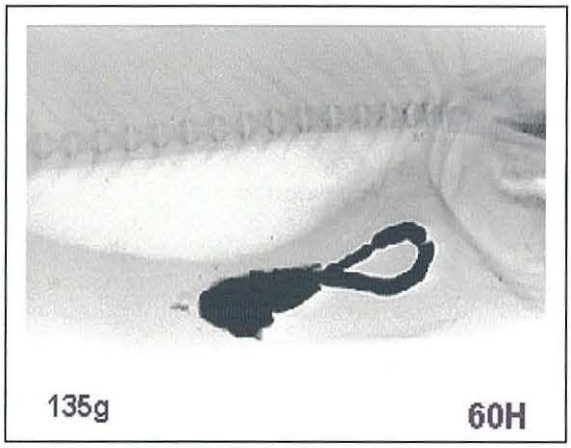
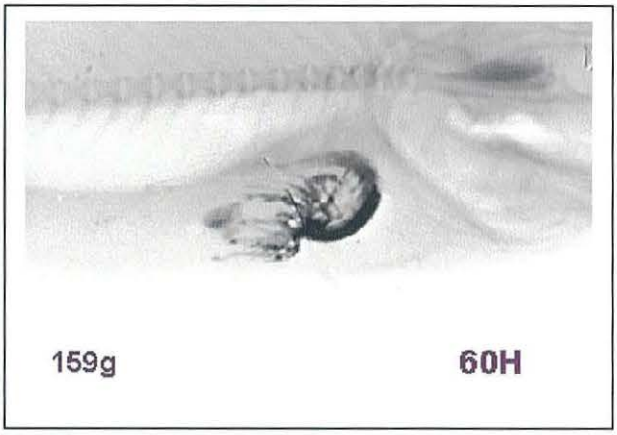


Plate 3.18

Plate 3.19



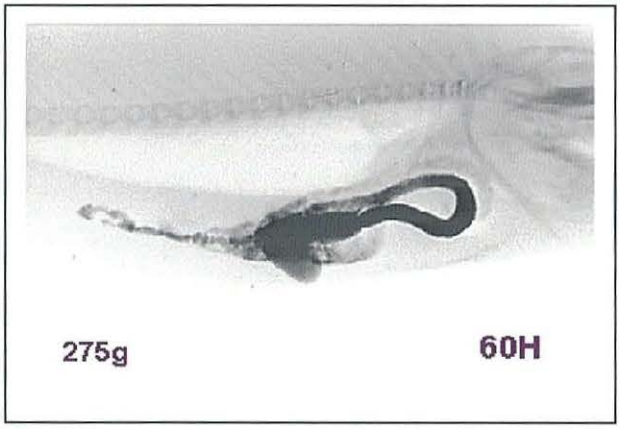
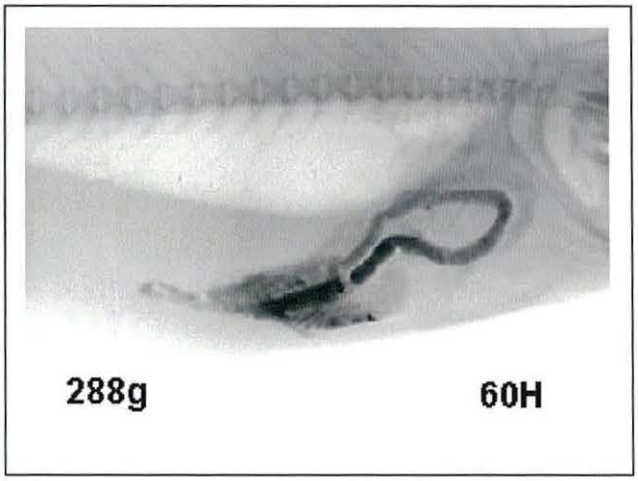


Plate 3.20

Plate 3.21



Plates 3.22 – 3.25 show the status of digesta in the alimentary tracts after 72h. Small fish (140g, 157g) that had ingested more than 100% of their estimated maximum stomach capacity (9 – 10.5g, 7 – 9% bw) had apparently emptied their stomach, leaving most of the digesta concentrated in the posterior intestine and rectal region. Similar fish (173g, 183g) that ingested more that 10% of their body weight (19g) or 158% of the estimated maximum stomach capacities, retained small amount of digesta in the stomach (4 – 5g, 20 – 25% of the total amount ingested). Whiting which consumed more than 10% bw meals had gastric emptying times greater than 72h.

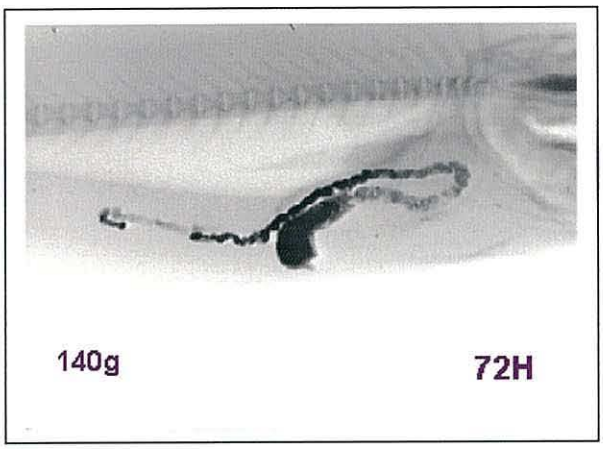
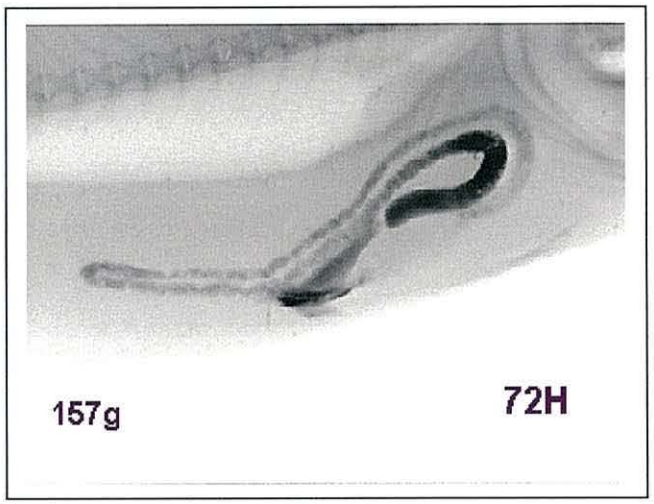


Plate 3.22

Plate 3.23



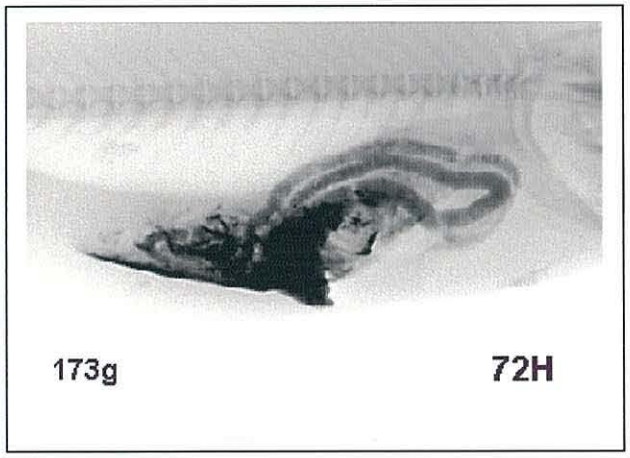
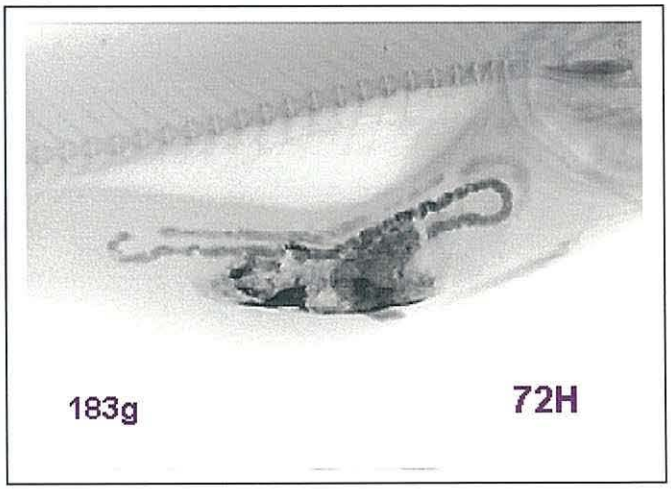


Plate 3.24

Plate 3.25



The collected data on fish size, voluntary meal size and stomach contents at stated times after feeding are analysed and discussed in Chapter 4.

3.4 Discussion

In the present study, the disappearance from the X-ray images of the whiting of the radio-opaque marker ($BaSO_4$) injected into the prey closely followed the change in stomach contents obtained by dissection. This indicates that this technique is useful as a tool to observe both food intake and the gastric emptying process in the whiting. However, the use of barium sulphate paste within the natural food may itself affect the digestion rate of the item. Results obtained in this section must be compared with studies of gastric emptying of sprat in the absence of the marker (Chapter 4). Great care is required during injection of the sprat to retain the measured amount of paste in the sprat body cavity due to its delicate tissues. The problem was minimised by injecting an amount in proportion to the sprat weight.

During feeding, the experimental fish were hand fed (*ad libitum*) to satiation with partially frozen sprats and, as long as the fish quickly swallow the whole sprats, this helps minimise loss of the marker in the surrounding water. Once the semi-frozen sprat is in the stomach, digestion begins with secretion of acidic gastric juices; the marker mixes well with the sprat tissue to form radio-opaque chyme.

The estimation of maximum stomach capacity using the linear relationship suggested by Seyhan (1994) based on distension seems to underestimate the satiation amounts for whiting in the present study. Several whiting exceeded the predicted maximum by up to 60%. This may suggest individual variation in stomach size or perhaps an allometric rather than isometric relation between volume and fish weight. Jobling (1981b) reviewed evidence that satiation amount increases with body weight raised to a power (0.7-0.8), indicating that smaller fish eat relatively more ($g\ g^{-1}$ bw) than larger fish.

One of the aims of this study was to examine the claim by Seyhan (1994) that gastric emptying time remained constant as meal size increased, since the work of Bromley (1988) contradicts this. Both Bromley's fish, and those in the present study, ate much larger voluntary meals than those of Seyhan and in both cases gastric emptying time was prolonged well beyond that reported by Seyhan.

Small meals were processed at the rate that Seyhan predicted. His finding that gastric emptying rate (GER) is directly proportional to meal size, thereby maintaining constant GET, may only apply to small meals (<3% bw) for whiting.

Chapter 4

Satiation amount, the return of appetite and gastric emptying of whiting fed on natural diets

4.1 Introduction

Quantifying food consumption in fish populations is essential to the study of fish productivity in the wild and fisheries management (Pope and Macer, 1996; Bax, 1998; Patterson, 1998; Fowler, 1999). Appetite studies are also important to construct feeding protocols for novel species in aquaculture, since developments in fishing technology have resulted in depletion of commercial marine resources (de Silva and Anderson, 1995; Jobling *et al.,* 1995a,b).

The amount of food eaten in single meals and over longer time periods has been widely studied in fish. Studies on salmonid fish were reported by Brett and Higgs (1970), Brett (1971) on *Oncorhynchus nerka*; Elliott (1975b) on *Salmo trutta;* Adron *et al.* (1973), Grove *et al.* (1978), Ruohonen *et al.* (1997a,b) with *Oncorhynchus mykiss* (= *Salmo gairdneri*) and similar work has been done on marine species. These include:
gadoids:- *Melanogrammus aeglefinus* (Jones, 1954; Jones, 1974; Hall, 1987), *Gadus morhua* (Jones, 1974; dos Santos and Jobling, 1988; dos Santos and Jobling, 1990; dos Santos and Jobling, 1991a,b; dos Santos and Jobling, 1992; dos Santos and Jobling, 1995) and *Merlangius merlangus* (Jones, 1974; Bromley, 1988; Robb, 1990; Seyhan, 1994; Seyhan *et al.*, 1998; Andersen, 1998,1999).
flatfish:- *Limanda limanda* (Jobling *et al.,* 1977; Gwyther and Grove, 1981, Fletcher *et al.,* 1984), *Pleuronectes platessa* (Jobling and Spencer Davies, 1979; Jobling, 1980c) and *Scophthalmus maximus* (Flowerdew and Grove, 1979; Grove *et al.,* 1985; Bromley, 1987) and perciformes:- *Dicentrarchus labrax* (Santulli *et al.*, 1993; Azzaydi *et al.,* 1998; Sanchez-Vazquez *et al.,* 1998; Aranda *et al.*, 1999) and *Sparus aurata* (Andrade *et al.,* 1996; Fernández *et al.,* 1998).

Under similar conditions, most workers agree that the maximum amount eaten in a meal (S_{max}) increases with body weight (W) in the relationship:

$$S_{max} = a\ W^{b}$$

Where b = 0.7-0.8 (Jobling, 1993). However, Seyhan (1994) suggested that the stomach capacity of whiting increases linearly with body weight (b = 1) and that S_{max} may do so too. This contradiction needs to be resolved.

The return of appetite after a meal is usually closely related to the rate at which a meal is processed by the stomach; either food is consumed to fill the newly-created "space" or appetite does not return until at least part of the previous meal is processed (Gwyther and Grove, 1981; Fletcher *et al.,* 1984; Grove *et al.,* 1985; Bromley, 1994). The rate at which gastric contents decrease with time has been described using a variety of

empirical mathematical expressions which are chosen to best describe the pattern for the species under study. Exponential, linear and square-root models - which usually assume stomach emptying begins immediately after ingestion of a meal - are most frequently used to describe the relationship between the food remaining in the stomach and the time elapsed after feeding (Tyler, 1970; Elliott, 1972; Elliott and Persson, 1978; Jobling, 1981b; Lambert, 1985). There is also evidence that lags or delays may occur at the beginning of the digestion process. These are most evident in elasmobranch species (Medved, 1985; Macpherson *et al.,* 1989) and suggest that more complex models (such as the power exponential formula of Elashoff *et al.,* 1982) and surface dependent models as reviewed by Persson (1986) may be more suited to describe gastric emptying data especially in elasmobranchs.

Jones (1954) reported a linear gastric emptying curve for whiting & other gadoids. Similar findings were also reported by Bromley (1988), Robb (1990), Seyhan (1994) and Singh-Renton and Bromley (1996). Jones (1974) suggested a more general differential equation based on a power model (see page 23-24 in Chapter 1) should represent change in stomach content with time, and that this could be adapted for different species. Depending on the value of the power term (α) the emptying curve could be linear ($\alpha = 0$), exponential ($\alpha = 1$) or a power curve ($\alpha \neq 1$). Two decades later, the same model was further developed into a useful power model by Temming and Andersen (1994) to describe gastric emptying rate in cod *Gadus morhua* (L.) and by Andersen (1998,1999) for whiting. They concluded, in contrast to previous workers, that gastric emptying in whiting followed a curve.

This chapter describes food consumption of whiting fed with different prey types (natural diets): squid pieces (*Illex*, *Loligo* spp), sprats (*Sprattus sprattus*) and brown shrimps (*Crangon crangon*) under laboratory conditions. The aim of this chapter is to determine the factors affecting satiation amount, based on single meals offered *ad libitum* to individual fish and to quantify the rate of appetite return. This should allow the shape of the underlying curve (describing how food empties from the stomach) to be inferred. Direct observations on gastric emptying obtained by dissection (gastrectomy) or gastric lavage can test whether the inferred curve shape from appetite studies is appropriate and whether Andersen's recent model is more appropriate.

4.2 Material and Methods

4.2.1 Satiation amount

The aim of this experiment was to estimate the maximum feeding capacity of the whiting (S_{max}) fed on test diets, squid pieces, whole sprats and whole brown shrimps. After a week in the holding tank, whiting (35–500g) were individually transferred into 250L tanks. They were acclimatised for 7 days with intermittent feeding to satiety with squid pieces (0.8–1.8g), sprats (0.7–2g) or brown shrimps (0.8–3.5g). Sufficient amounts of food items were weighed and test meals offered to the fish for about 15–20 minutes until the fish showed signs of satiety (when offered food was no longer eaten by fish). Uneaten items were recovered, weighed and the amounts of food consumed by each fish calculated. Tests were repeated every 96h over the following days until satiation amounts stabilised. Since no temperature control was available, the temperature during the course of this long satiation experiment (6 months) varied between 9.7-19.3°C.

4.2.2 Return of appetite following a satiation meal

A preliminary experiment to monitor the return of appetite was carried out using six whiting of various sizes fed with squid pieces and twelve whiting fed with sprat at temperatures between 14–16.2°C. The maximum satiation amount (S_{max}) of prey eaten (to the nearest 0.001g) by each fish was recorded after prior food deprivation for 84 – 96h to ensure complete stomach emptiness. This experiment followed the feeding schedule described in Appendix 4.1 over a period of 5 weeks. The curves used to fit the return of appetite data were based on the non-linear functions derived from Grove *et al.* (1985: Eq 3) and Andersen (1998: Eq 6) described in Chapter 2 (page 43-44). The data obtained from this experiment were also used to estimate the likely gastric emptying rate (GER) and time (GET) of the different prey by inverting the graphs.

4.2.3 Gastric emptying study based on single meals.

4.2.3.1 Gastrectomy method

An initial study of gastric emptying was carried out using the information obtained by dissecting the fish used in the X-ray study of Chapter 3. The stomach contents, obtained from serial slaughter of whiting at different times after feeding the sprat, were weighed (wet weight) to the nearest 0.001g. Curves of the change in contents with time were

fitted to the data using the maximum voluntary meal size as a standard, as used by Fletcher *et al.* (1984). The models used to describe gastric emptying of the test meal were as described in equation 2 (Grove model) and 5 (Andersen model; Chapter 2, page 43-44).

4.2.3.2 Gastric lavage method

a) Experiment I

The next gastric emptying experiments were conducted on a group of whiting (n = 57; wt range = 62–317g) in the 4000L holding tank, partitioned into 3 compartments, using single meals. Temperature ranged between 11–13°C. Fish were deprived of food for 96h before the start of the experiment since preliminary experiments indicated that this was close to the GET for larger meals. Fish were fed to satiation with squid pieces (Mean wt. = 0.676 ± 0.227g). Immediately after feeding 3 fish were taken and anaesthetised with 0.3 ml/litre 2-Phenoxyethanol for about 5 min. The stomach contents were removed by gastric lavage using a polythene tube of 0.4 cm diameter fitted with a tap, connected to a seawater container (4.3L), which was inserted *via* the oesophagus into the stomach. The container was placed 2 meters above the fish and a gentle flow of water (5-8 ml/sec) flushed out the stomach contents into a fine mesh sieve (100 µm). The recovered items were blotted and their wet weights recorded to the nearest 0.001g. Further samples were taken from the remaining fish after 4, 8, 12, 24, 48 or 60h. When the first fish with empty stomachs were encountered, sampling was halted to avoid distortion in the shape of the fitted emptying curve (Olsen and Mullen, 1986).

The initial stomach contents (S_0) for each fish were estimated using the equation;

$$S_0 = N_t \times W_o$$

where N_t, is the number of partly-digested food items (squid pieces) observed in the stomach and W_o is the average original weight (g) of the pieces before feeding.

b) Experiment II

The first experiment revealed noticeable individual variation between fish for stomach contents at a stated time. Contributory factors were differences in the actual amount of food (S_0) taken by each of fish of different size at time zero (t_o) and some ambiguity in determining the amount of food emptied from the stomach towards the end of gastric

emptying (*i.e.* >60h). A second series of experiments were designed to investigate the problem further. Each of six 250L tanks was stocked with 3 whiting of closely similar size (179.8 ± SD,11.4g), each marked with a unique combination of red, yellow and/or white coloured cotton threads at the base of the first dorsal fin. In addition, each piece of squid was also marked using red coloured cotton thread so that the actual amount of food remaining in the stomach at a given time could be related to the accurately-known, original meal size by counting the threads.

The fish were fed to satiation and the amount of food eaten was recorded. After a predetermined time (t_t), all fish in the related tank were subjected to gastric lavage, the threads counted and the amount of food weighed and recorded. The same equations (2 and 5, Chapter 2) were used to describe the data. Predicted emptying curves were also constructed as the inverse of the return of appetite curves for different prey types for comparison with the direct observations.

4.2.4 Statistical analysis

The parameters from the return of appetite models of Grove *et al.* (1985) and Andersen (1998) were estimated by a non-linear regression of equation (4) and equation (7) when expressed as percentage satiation amount, and equation (3) and equation (6) when expressed as absolute satiation amount (Chapter 2, page 43 - 44) using the Levenberg-Marquardt Method in MicroCalc^{tm} Origin^{tm} software. S_{max} values were estimated from the Y intercept on graphs as maximum initial or satiation meal size, while the rate parameters, K and ρ, and their confidence limits were estimated by the best-fit procedures in the software. The adequacy of the models was evaluated by comparison of the total data variance and the non-linear coefficient of determination r^2, which was adjusted to account for the number of estimated parameters according to Sokal and Rohlf (1981). Other basic statistical information on the estimated parameters in the model were acquired from the software package itself (e.g. MicroCalc^{tm} Origin^{tm}). Multiple linear regression analyses were performed to test the effect of temperature, meal size and fish size on the rate of appetite return or gastric emptying rate using Minitab Statistical Programme (Rel. 12.23: Minitab Inc., 1999).

4.3 Results

4.3.1 Satiation amount

A constant deprivation time (96h) was chosen as a standard since preliminary observations, by dissection, on 10 small and medium size whiting fed to satiation with the test meals showed all stomachs were emptied within 96h. Seyhan (1994) reported that the relationship between stomach volume and fish size (48 to 695g bw) was best described by a linear function constrained through the origin:

Stomach Volume (ml) = 0.067(SE, 0.0027) W, r = 0.878, n = 42,P<0.0001.

where W is fish weight (g). However, re-examination of his data showed considerable scatter around the line with 95% confidence limits close to ± 50% of the prediction. The first question is how much of the available stomach volume is filled with their natural prey when the whiting feeds to satiation. As a preliminary test, the density of the prey was found by the volume displacement of water by known weights of natural prey. These were best described by linear functions:

Squid pieces: Volume (ml) = 1.04901(SE,0.01202)*W, r = 0.9785, n = 30, P<0.0001.

Whole sprats: Volume (ml) = 1.04721(SE,0.00806)*W, r = 0.9871, n = 30, P<0.0001.

Whole brown shrimps: Volume (ml) = 1.01237(SE,0.00597)*W, r = 0.9954, n = 30, P<0.0001.

The results indicated that 1g wet weight of prey is almost equivalent to 1 ml volume (P<0.0001) for all prey types. The maximum stomach volume suggested by Seyhan can predict S_{max} in the present study based on assumption that 1 ml of stomach volume is equivalent to 1 g meal size (prey). Deviations from this prediction may reflect differences in the degree of packing due to shape differences among the prey items.

Squid pieces of uniform size (0.888 ± 0.021g, n = 60) were prepared which should enable whiting to pack the maximum space in the stomach efficiently since the pieces are small and flexible. Results from fresh whole Sprats (*Sprattus sprattus*) (1.547 ± 0.090g n = 60) and fresh brown shrimps (*Crangon-crangon*) (1.306 ± 0.060g n = 60) indicate how efficiently these natural preys may pack into the stomach of voluntary-feeding fish.

The results of the satiation feeding experiments are shown in Table 4.1 and Fig. 4.1. For whiting of a given size, the largest weight and volume ingested occurs when they are fed on squid pieces, followed closely by sprats whilst ingestion of brown shrimps was lower. Satiation amount (S_{max}) increased allometrically with fish weight (W) as:

$$S_{max} = a\ W^{b}$$

where b = 0.64–0.7. The variations in "a" indicated that different packing factors do exist for different prey species when fed to whiting (Fig 4.1 and Table 4.1). A whiting of 500g, which Seyhan considered should have a stomach volume of 33.5 ml, ingested 32.5g of squid, 29.4g of sprat but only 14.7g of brown shrimp. However, smaller whiting ate larger meals than predicted from Seyhan's model; a 50g fish typically eats 7g of squid but has a predicted stomach volume of only half this size. The results suggest that the stomach increases in volume allometrically with fish size rather than the linear relationship suggested by Seyhan. His original data set can be re-described, by fitting a power equation but fixing the average value of b (= 0.662 found from the present feeding study) as:

$$SV = 0.438\ W^{0.662}$$

and the 95% confidence limits of the fit reduce to ± 31%. Whiting of 50g bw would have on average a stomach volume of 6 ± 1.8ml and 500g fish 27 ± 8.1 ml.

Table. 4.1. Comparison of allometric parameters a and b to indicate the variation of satiation meal (S_0) of different prey types eaten by whiting of various sizes ($S_o = a*W^b$). (Temp. range: 9.7 – 19.3 °C).

Prey (meal) Types	**Estimated Parameters**		**r**	**n(df)**
	a±SE	b±SE		
i) Squid pieces	0.608±0.143	0.640±0.042	0.814***	147(145)
ii) Sprats	0.389±0.212	0.696±0.095	0.790***	24(22)
iii) Brown shrimp	0.257±0.147	0.651±0.096	0.726***	41(39)

*** significant at P<0.001

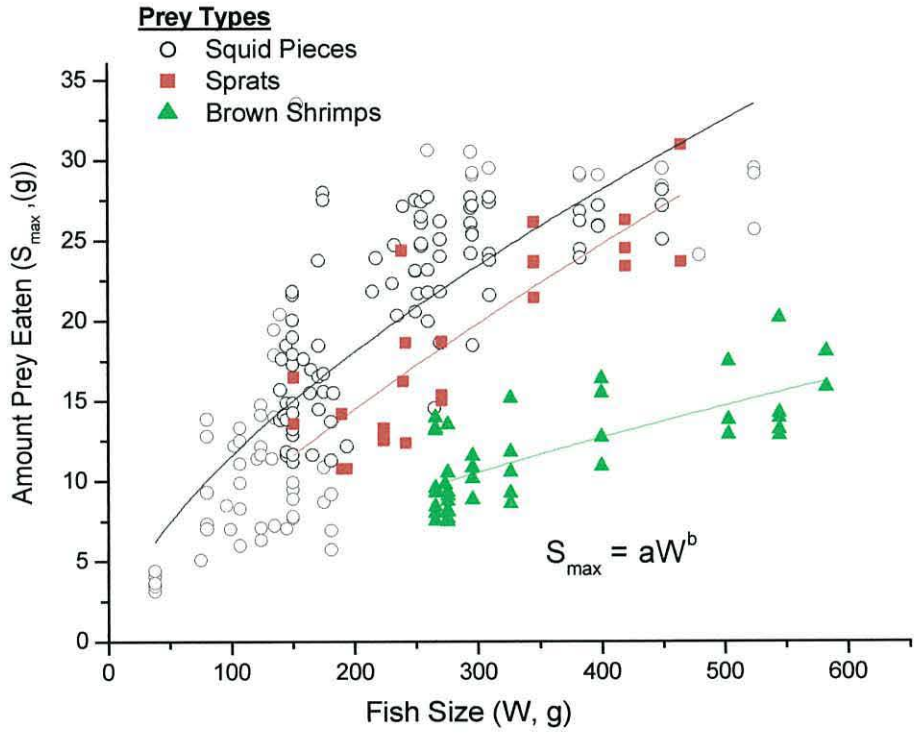


Fig. 4.1. Relation between size of satiation meal (S_{max}, g wet wt) and fish size (W, g wet wt) of whiting feeding on different prey types (temperature range 9.7-19.3°C: see Appendix 4.2).

4.3.2 Return of appetite

4.3.2.1 Effects of fish size

The return of appetite experiments were carried out in several stages. In a preliminary study, six whiting of mainly medium size (100-200g) were fed to satiation with squid pieces (each 0.888g ± 0.021g from the same batch) at temperatures between 14 & 19°C with a modal temperature of 14.5°C. When expressed in relative terms (*i.e.* as percentage of satiation amounts) voluntary intake returned more rapidly with time for smaller fish (Figs 4.2a,b). However, when expressed in terms of absolute weight (g) the instantaneous digestion rates were similar whether fitted by the Grove's model (K), and especially when fitted to Andersen's model (ρ) (Table 4.2). Figures 4.2a,b show the curves for appetite return estimated by applying both the Grove and the Andersen Model to the data sets. A significant finding from this analysis is that the values of K and ρ are independent of meal size (and fish weight). With such a small data set and its variability it was difficult to decide whether appetite return was linear or curved over deprivation time. Because Andersen's power model gave more consistent fits to the raw data and was developed for gadoids rather than flatfish, this model was tentatively adopted for subsequent analyses.

Table 4.2. Estimates of the rate parameter k using equation 4 (Grove's model) and ρ using equation 7 (Andersen's model) on return of appetite of whiting of various sizes fed squid pieces.

Fish no. (wt g) Estimated So (g)	Estimated K or $\rho \pm$ 95%CL(g/hr)	Temp. °C	Statistical summary
Equation # 4	**b = 0.77**		r = 0.916 r^2 = 0.840 N* = 35 df = 30 $r_{0.05(2),30}$ = 0.349 P< 0.001 (*number of data entering in regression)
Fish 1 (38 g)			
Observed S_{max} = 4.436	0.039±0.009	15.6	
Fish 3 (171 g)			
Observed S_{max} = 23.750	0.070±0.024	16.1	
Fish 4 (145g)			
Observed S_{max} = 18.603	0.078±0.022	16.3	
Fish 5 (124g)			
Observed S_{max} = 14.735	0.064±0.018	16.1	
Fish 6 (107g)			
Observed S_{max} = 13.324	0.087±0.020	16.2	
Equation # 7	**α = 0.5**		r = 0.912 r^2 = 0.832 N* = 35 df = 30 $r_{0.05(2),30}$ = 0.349 P<0.001 (*number of data entering in regression)
Fish 1 (38 g)			
Observed S_{max} = 4.436	0.039±0.011	15.6	
Fish 3 (171 g)			
Observed S_{max} = 23.750	0.041±0.016	16.1	
Fish 4 (145g)			
Observed S_{max} = 18.603	0.050±0.016	16.3	
Fish 5 (124g)			
Observed S_{max} = 14.735	0.044±0.014	16.1	
Fish 6 (107g)			
Observed S_{max} = 13.324	0.065±0.018	16.2	

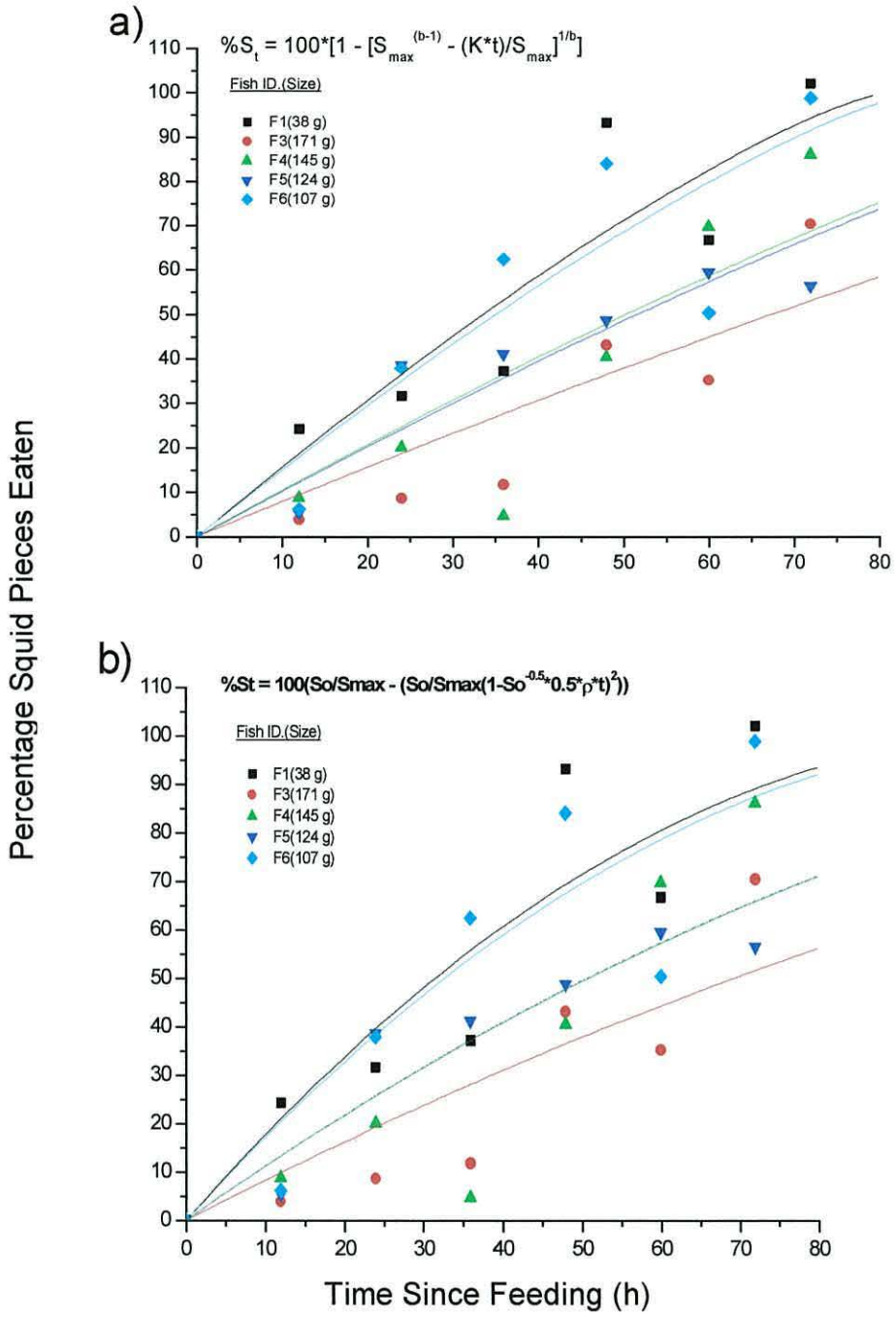


Fig. 4.2. Trends in return of appetite after satiation feeding for six whiting of various sizes fed on pieces of squid. Curves were fitted using inverted forms of two gastric emptying equations:
a) Grove's model (equation 4 in text) and
b) Andersen's model (equation 7) [see Appendix 4.3].

The average for our values of ρ (= 0.048) is included in Andersen's equation to predict gastric emptying which would accompany ingestion of different meal sizes (Fig 4.3). The predicted gastric emptying curves for small meals (up to 6g) agree well with the data reported by Seyhan (1994). He measured stomach contents using gastric lavage after feeding whole sprats (as shown in Fig. 1.5b in Chapter 1) but considered the trend was linear.

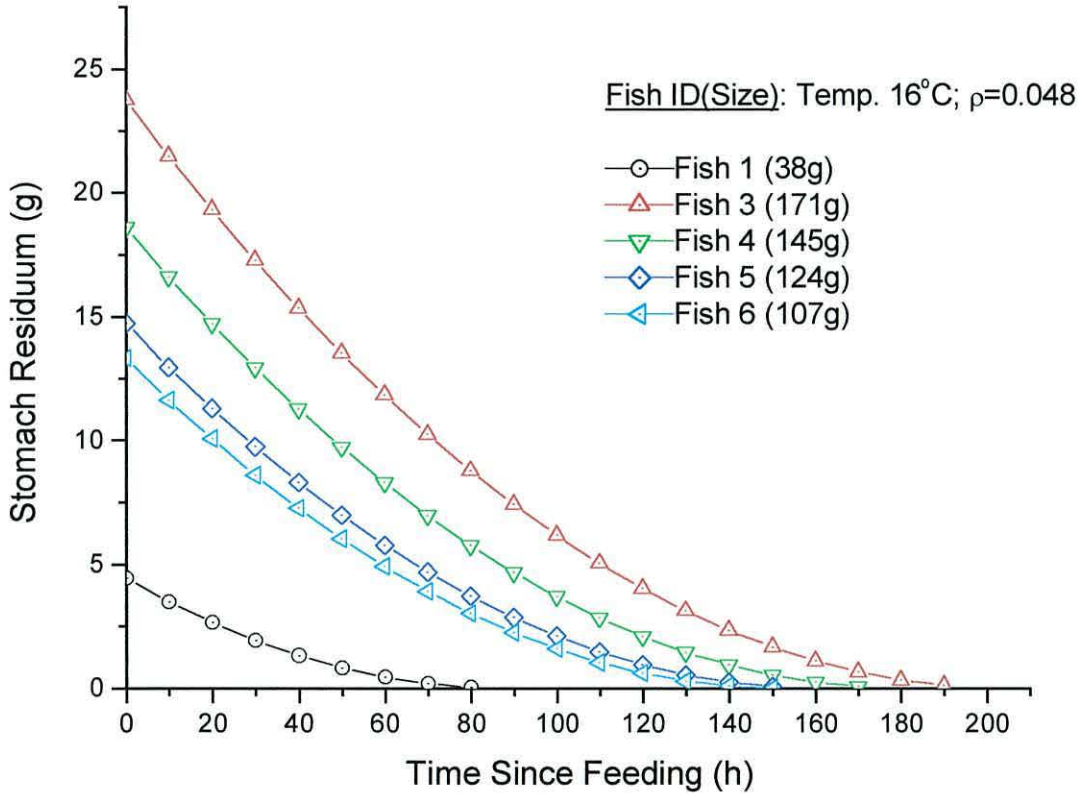


Fig. 4.3. Predicted gastric emptying curves calculated from the appetite-return study in Fig. 4.2 using Andersen's model for a diet of squid pieces. Instantaneous digestion rate (ρ) was constant and initial gastric emptying rate (GER) increases with meal size but without full compensation so that gastric emptying time (GET) increased. Emptying of small meals follows a curve close to the linear model suggested by Seyhan for sprat (1994).

4.3.2.2 Effects of prey type

Different species of prey may be digested at different rates and consequently affect the return of appetite in whiting. Such effects need to be quantified if feeding rates in the wild are to be estimated from stomach contents (Bromley, 1994). The return of appetite of whiting after feeding on different prey - whole sprats or whole brown shrimps - can be compared with a more extended study using squid pieces. The more detailed study of appetite return of 12 whiting (246-571g) fed on squid at *ca.* 12°C, was carried out in which every deprivation time was preceded by the same fasting period (96h). These curves were clearly not linear. The amounts eaten (g) after different deprivation times are shown in Fig. 4.4(a-f) and values for S_{max} and ρ in Table 4.3a. Curves were fitted based on the combined data from the two studies using squid as food (17 whiting, 38-571g) using Andersen's model and confirmed the previous finding that S_{max} was dependent on fish size (Fig. 4.7a,b) but that ρ (0.125$\pm$ SD 0.002) was not.

Figure 4.5(a-f) and Table 4.3b show the return of appetite when whiting were instead fed with sprats. The results showed that, for 151–465g whiting, the satiation amount (S_{max}) changed in similar fashion with body weight as for squid but with a slightly smaller packing factor a = 0.464 (Fig 4.7a). The return of appetite trend was again curved and the digestion rate parameter (ρ) was also similar to the squid value for most of the fish.

Table. 4.3(a,b,c). Estimates of the rate parameter (ρ) from return of appetite data for whiting fed on squid pieces, brown shrimp and sprats using equation 6 (Andersen's model).

Fish no (wt g) Estimated S_{max} ±95%CL (g)	Estimated ρ ±95%CL (g/h)	Temp. °C	Statistical Remarks
	α = 0.5		r^2
a) *Prey Type1: Squid pieces*			
F1(271g); Est. S_{max} = 29.687±3.910	0.104±0.015	12.4	0.953***
F2(265g); Est. S_{max} = 22.635±2.732	0.131±0.028	12.4	0.963***
F3(346g); Est. S_{max} = 20.760±3.405	0.107±0.025	12.6	0.914***
F4(360); Est. S_{max} = 23.927±3.067	0.128±0.027	12.6	0.957***
F5(295g); Est. S_{max} = 24.526±3.628	0.159±0.048	12.8	0.899***
F6(271g); Est. S_{max} = 19.277±5.008	0.102±0.036	12.8	0.846***
F7(246); Est. S_{max} = 19.818±2.899	0.134±0.038	12.2	0.912***
F8(472g); Est. S_{max} = 24.766±3.276	0.133±0.029	12.2	0.940***
F9(256g); Est. S_{max} = 23.210±2.850	0.120±0.023	10.8	0.937***
F10(571g); Est. S_{max} = 27.039±3.137	0.120±0.019	10.8	0.973***
F11(353g); Est. S_{max} = 26.792±5.56	0.111±0.026	11.0	0.986***
F12(295g); Est. S_{max} = 24.194±2.173	0.164±0.030	11.0	0.947***
b) *Prey Type 2: Sprats*			
F1 (224 g); Est. S_{max} = 13.629±4.661	0.087±0.041	14.4	0.939***
F2 (218 g); Est. S_{max} = 7±1.735	0.081±0.044	14.4	0.616**
F3 (346 g); Est. S_{max} = 26.393±2.060	0.176±0.027	14.7	0.934***
F4 (194 g); Est. S_{max} = 14.369±4.326	0.059±0.016	14.7	0.898***
F5 (420 g); Est. S_{max} = 24.291±4.747	0.095±0.014	14.4	0.979***
F6 (271 g); Est. S_{max} = 22.158±3.580	0.158±0.027	14.3	0.931***
F7 (240 g); Est. S_{max} = 11.10±1.913	0.102±0.035	14.9	0.933***
F8 (465 g); Est. S_{max} = 27.787±2.020	0.159±0.023	14.9	0.981***
F9 (242 g); Est. S_{max} = 21.001±2.350	0.132±0.028	14.5	0.970***
F10 (239 g); Est. S_{max} = 21.990±5.230	0.061±0.011	14.5	0.883***
F11 (151 g); Est. S_{max} = 14.362±0.614	0.120±0.010	14	0.995***
F12 (190 g); Est. S_{max} = 16.299±5.379	0.087±0.036	14.6	0.905***
c) *Prey Type 3: Brown Shrimps*			
F1 (276 g); Est. S_{max} = 17.748±4.218	0.073±0.019	12.4	0.854***
F2 (266 g); Est. S_{max} = 14.403±2.404	0.072±0.013	12.4	0.950***
F3 (504 g); Est. S_{max} = 33.244±7.084	0.088±0.017	12.6	0.694**
F4 (274 g); Est. S_{max} = 11.560±3.572	0.098±0.021	12.6	0.694**
F5 (266 g); Est. S_{max} = 15.803±5.283	0.079±0.029	12.8	0.788**
F6 (296 g); Est. S_{max} = 10.191±2.558	0.074±0.024	12.8	0.833***
F7(276 g); Est. S_{max} = 12.117±6.902	0.046±0.017	12.2	0.746**
F8 (583 g); Est. S_{max} = 16.752±2.278	0.182±0.028	12.2	0.906***
F9(250 g);Est. S_{max} = 12(fixed)	0.074±0.029	10.8	0.849***
F10 (327 g); Est. S_{max} = 14.184±3.401	0.092±0.033	10.8	0.804**
F11 (545 g); Est. S_{max} = 19.429±2.954	0.127±0.036	11.0	0.962***
F12 (400 g); Est. So= 18.226±6.585	0.098±0.047	11.0	0.941***

** significant at P< 0.05,
*** significant at P< 0.001

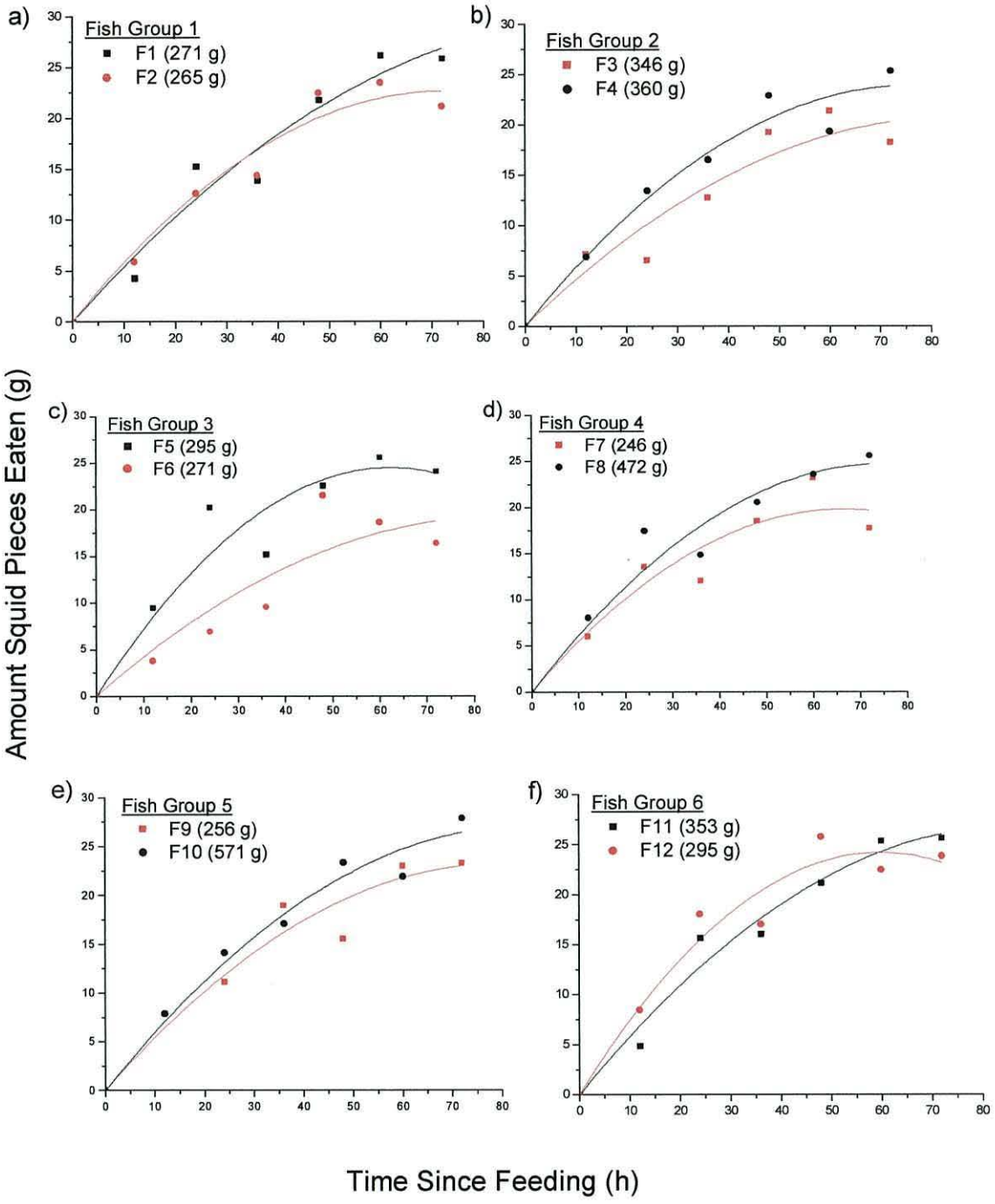


Fig. 4.4 (a-f). Return of appetite curves for 12 individual whiting fed squid pieces. Each meal was offered under a fixed cycle: 96h food deprivation, a satiation meal and then the test meal at the stated time since satiation. Curves were fitted using Andersen's model (Equation 6: see Appendix 4.4).

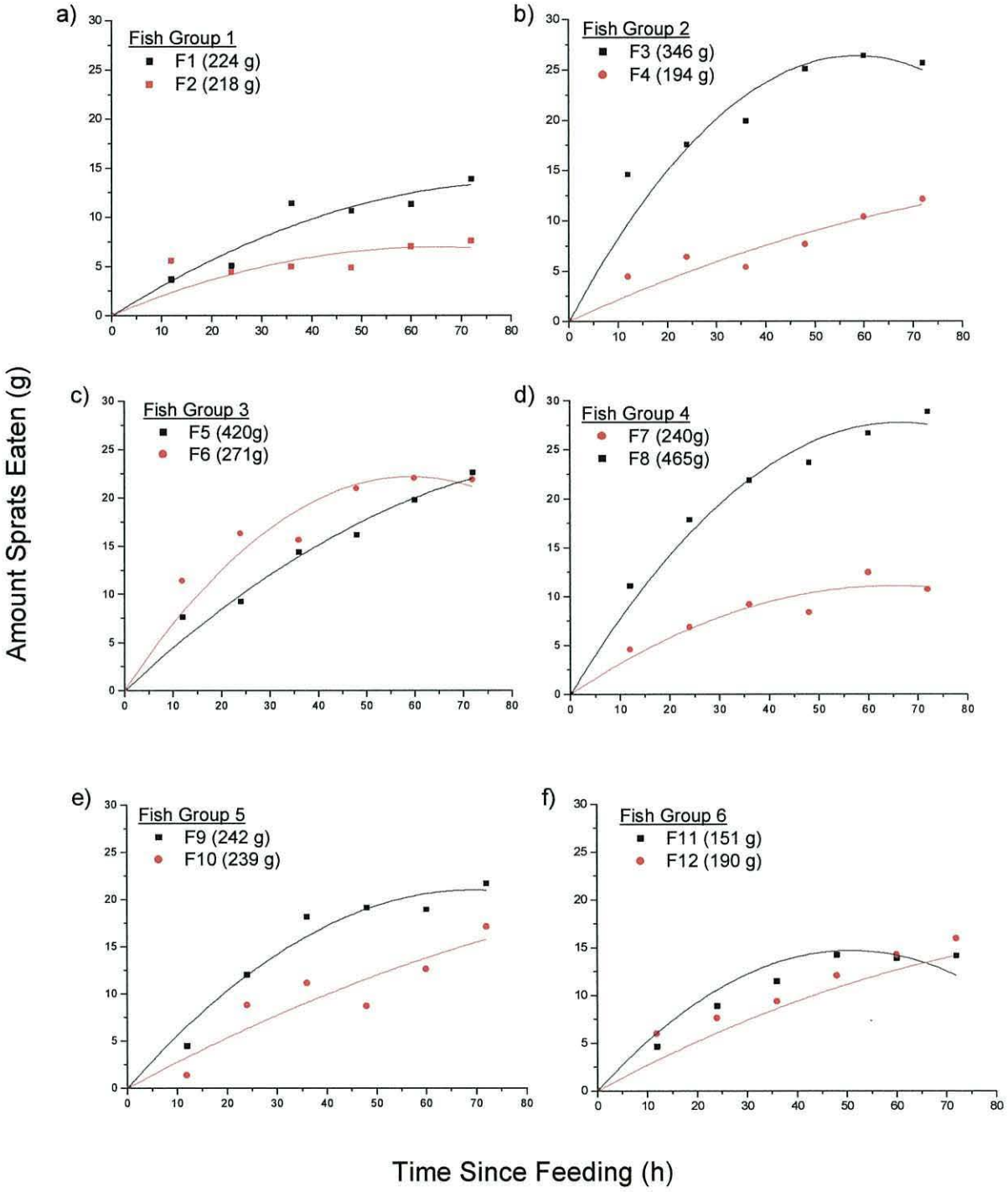


Fig. 4.5. (a-f). Return of appetite curves for 12 individual whiting fed whole sprats. Experimental design and curve fitting as in Fig. 4.4.

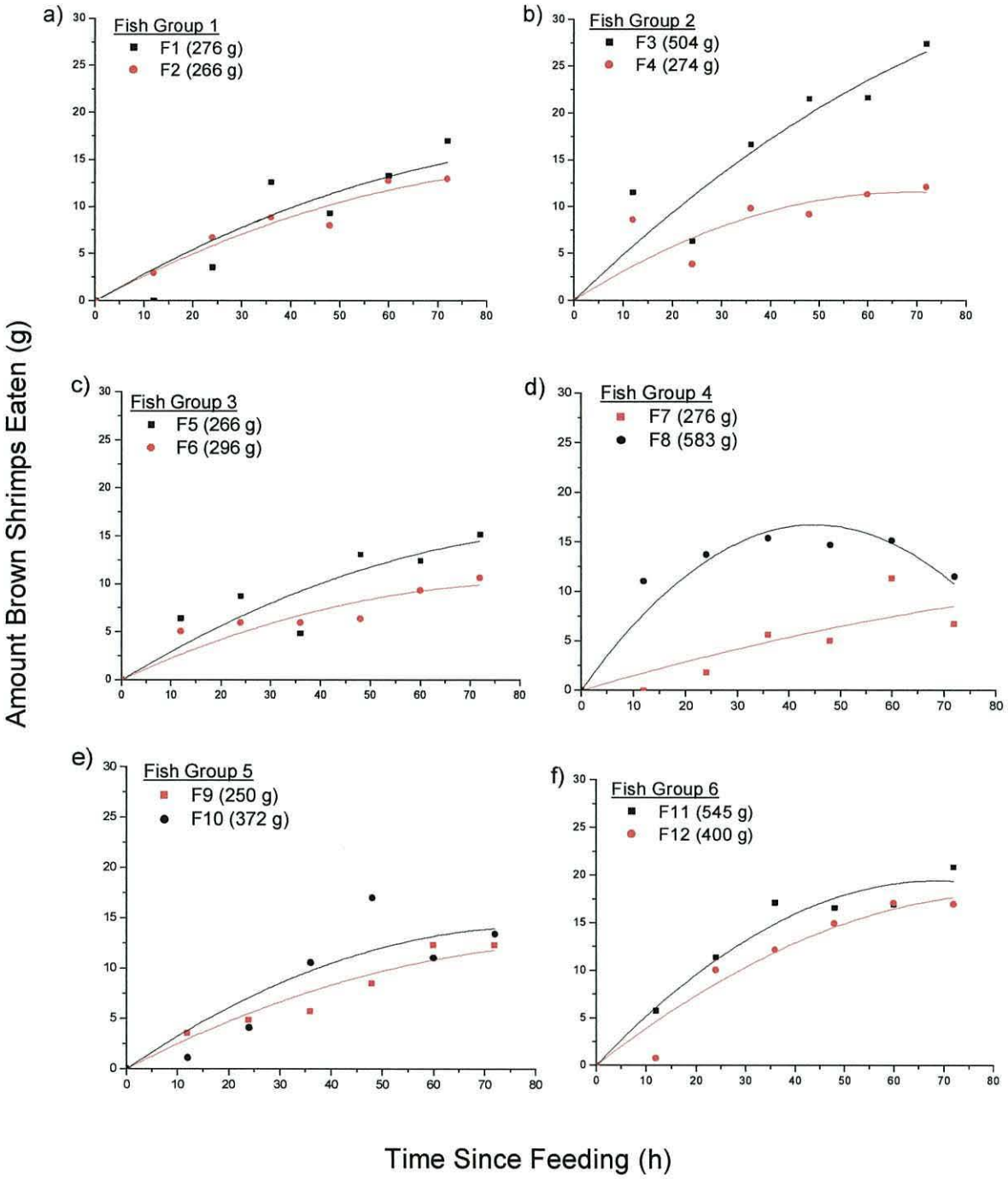


Fig. 4.6. (a-f). Return of appetite curves for 12 individual whiting fed whole brown shrimps. Experimental design and curve fitting as in Fig. 4.4.

When shrimps were offered (Figure 4.6 and Table 4.3c), satiation meal size was smaller (Fig.4.7a) as was expected from preliminary studies (Fig 4.1). The trend lines were again non-linear. However, ρ was no longer constant but increased significantly (from 0.07 to 0.15) over the size range studied (Fig. 4.7b; Table 4.4c(ii)).

The absolute rate of appetite return for whiting fed brown shrimps was relatively low (0.046–0.182g h^{-1}) when compared with squid or sprat (0.2-0.55g h^{-1}). Large whiting were able to consume their satiation meal within 15-20 minutes, a shorter time than the 25-30 minutes required by smaller fish.

4.3.2.3 Effects of temperature

The results described in Table 4.2 and Table 4.3a,b,c show that temperature had varied between 9.5 to 17°C (Median range:10.8–16.3°C) over the several months of the study. Temperature is an important abiotic factor, which affects both food consumption and digestion rate in fish. The next analysis re-investigated its effect on appetite return. The data-set for squid covered a sufficient temperature range to allow a multiple regression analysis of the relations among body size, satiation amount and temperature on the digestion parameter ρ. The results (Fig. 4.7a) and in Table 4.4(a, b, c) had demonstrated strong correlations (r^2> 85%) between satiation meals and fish size. In this extended analysis, neither body weight (w) nor its correlate, satiation amount (S_{max}), had significant effects on ρ but its value *decreased* significantly with temperature ($\rho = A - 0.0173T$; $P<0.0001$; Table 4.4 a(ii)). Despite the restricted data-set, after small corrections for ambient temperature, ρ for sprat became similar to that for squid and was still independent of body size whereas for shrimp ρ increased with body size ($\rho = A + 0.00024W$; $P<0.006$; Table 4.4c(ii); Fig 4.7b).

Table 4.4a(i, ii), b, c(i, ii). Predicted variables and analysis of variance of rate parameters (ρ) for whiting fed to satiation with; a) squid pieces, b) fresh whole sprats c) fresh brown shrimp.

a) Squid pieces					
i) Predictor	Coef.	SE Coef.	T-ratio	P	
Constant	0.2851	0.05891	4.84	0.0001	
Temp.	-0.0155	0.00324	-4.78	0.0001	
S_{max}	0.00106	0.00110	0.96	0.354	
		ANOVA			
Source	DF	SS	MS	F	P
Regression	2	0.01993	0.00997	21.38	0.0001
Error	14	0.00653	0.00047		
Total	16	0.02646			
ii) Predictor	Coef.	SE Coef.	T-ratio	P	
Constant	0.33019	0.03541	9.33	0.000	
Temp.	-0.0173	0.002661	-6.49	0.000	
		ANOVA			
Source	DF	SS	MS	F	P
Regression	1	0.019506	0.019506	42.07	0.0001
Error	15	0.006955	0.000464		
Total	16	0.026461			
b) Fresh whole sprats					
Predictor	Coef.	SE Coef.	T-ratio	P	
Constant	0.0962	0.6494	0.15	0.886	
Temp.	-0.00366	0.04508	-0.08	0.937	
S_{max}	0.003634	0.001636	2.22	0.053	
		ANOVA			
Source	DF	SS	MS	F	P
Regression	2	0.006047	0.003024	2.52	0.135
Error	9	0.010787	0.001199		
Total	11	0.016834			
c) Fresh brown shrimps					
i) Predictor	Coef.	SE Coef.	T-ratio	P	
Constant	0.2216	0.1941	1.14	0.287	
Temp.	-0.01191	0.01575	-0.76	0.471	
S_{max}	0.000936	0.001934	0.48	0.641	
		ANOVA			
Source	DF	SS	MS	F	P
Regression	2	0.001195	0.000598	0.41	0.675
Error	8	0.011561	0.001445		
Total	10	0.012757			
ii) Predictor	Coef.	SE Coef.	T-ratio	P	
Constant	-0.0012	0.1354	-0.01	0.993	
Temp.	0.00071	0.01039	0.07	0.947	
Weight	0.0002364	0.0000647	3.65	0.006	
		ANOVA			
Source	DF	SS	MS	F	P
Regression	2	0.0082996	0.0041498	7.45	0.015
Error	8	0.0044572	0.0005571		
Total	10	0.0127567			

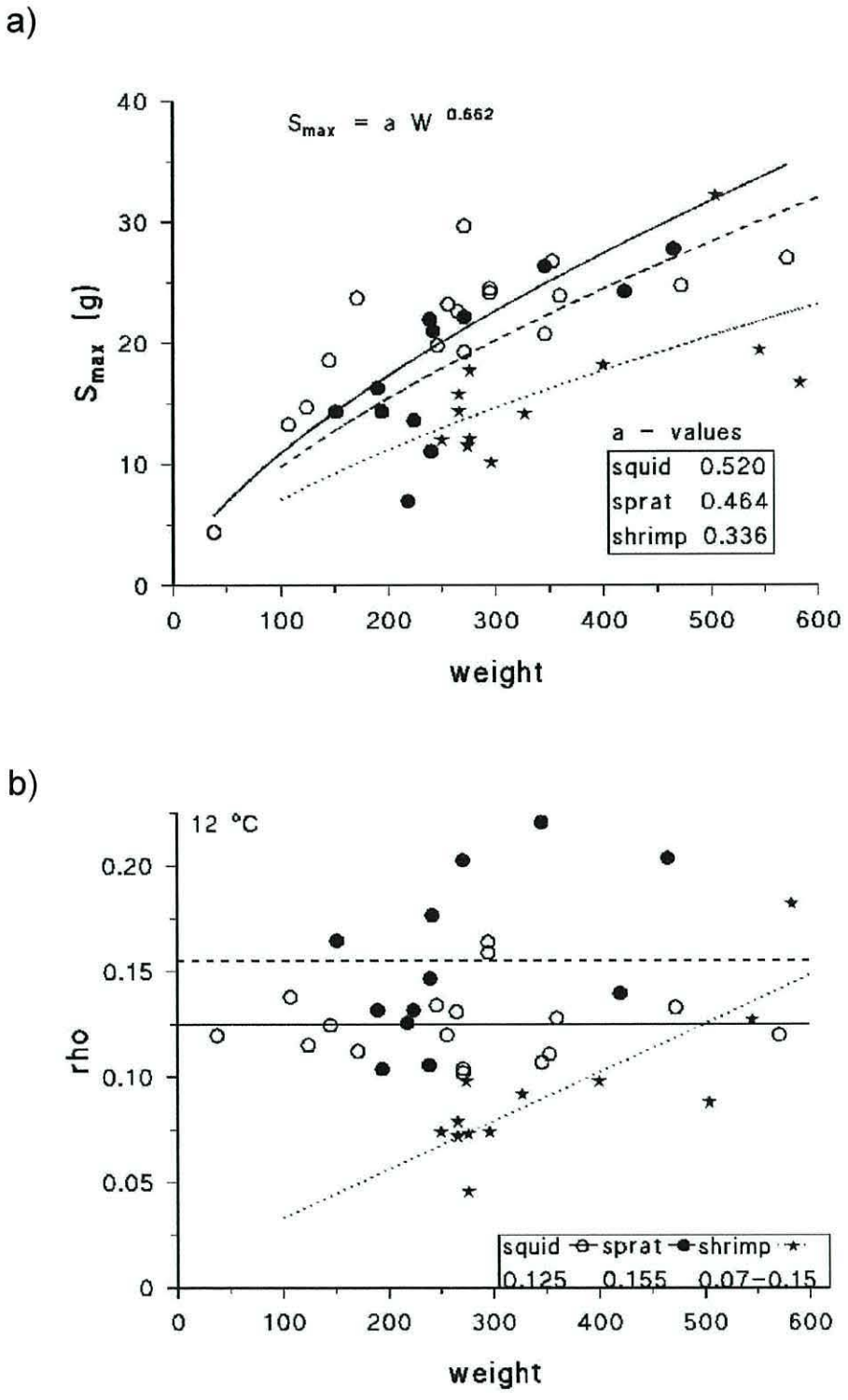


Fig. 4.7. (a) Allometric relationship between maximum meal size (S_{max}, g) and body weight (g) for whiting fed three diets (squid pieces, whole sprats, whole brown shrimp) (b) Rate parameters (ρ) for whiting of different sizes fed the same three diets.

4.3.3 Gastric emptying

In the above studies, the parameters of the gastric emptying process in Andersen's model were inferred by assuming that appetite return simply reflects the amount of space available in the whiting stomach. Direct observations of Andersen's ρ-values were attempted for sprat and for squid from gastrectomy and lavage respectively.

In Chapter 3, serial slaughter (gastrectomy) was used to determine the amount of voluntarily-ingested sprat left in the stomach at selected times after feeding. These data were important to relate the X-ray images to actual gastrointestinal contents and are shown in Table 4.5. However, the fish did not all eat the same amount. To combine the results, the two points representing the largest voluntary maximum meal size of a whiting at time zero (S_o) were taken as the best estimate of S_{max} and its stomach residuum (S_t) at stated time (t) after feeding was plotted graphically and a reference line was drawn. Smaller meals taken at time zero (S_o) and their stomach residua at known later times were plotted sequentially on the same graph by shifting the initial S_o value parallel to the X-axis to join the reference line. This produced a "pooled" emptying curve, which was then fitted by non-linear regression using Andersen's (Eq. 5) and Grove's models (Eq. 2). The estimated rate parameters (gastric emptying rates) are shown in Fig. 4.8 and in Tables 4.5 and 4.6(II). The average maximum meal size ($S_{o/max}$) for whiting (100–300g) used in the present study was 27g and the gastric emptying rate parameter (ρ) was 0.09 ± 0.011 (95%CL) h^{-1}. Inferred values (Table 4.3b) were 0.06 to 0.18 from the appetite return curves of 12 individual fish. The amount of stomach residuum 12h after feeding was approximately 23g and this value dropped steadily with time up to 48h. The emptying process predicted using Grove's model was closely similar to that of Andersen's model except for the last stages of gastric emptying; evacuation rate of the last 10% of the meal was over-estimated by the former model.

Table 4.5. Amount fed (S_0) and residuum in stomach after gastrectomy at stated time since feeding for whiting fed with whole sprats containing barium sulphate ($BaSO_4$) paste.

Fish ID	Fish Wt. (g)	S_o (t = 0) (g)	Time Since Feeding (h)	Stomach Residuum (g)	Temp. °C
1	211	14.589	12	11.314	15.6
2	159	6.965	12	4.551	15.6
3	144	10.864	12	8.372	15.6
4	115	5.911	12	4.023	15.6
5	180	9.779	12	5.696	15.6
6	264	19.962	12	16.476	15.6
7	99	10.4	12	7.428	15.6
8	257	9.584	24	5.113	15.6
9	232	11.175	24	3.555	15.6
10	169	6.016	24	2.513	15.6
11	189	8.505	24	4.017	15.6
12	137	5.585	24	2.248	15.6
13	146	8.76	24	3.904	15.6
14	124	4.834	24	1.879	15.6
15	203	6.09	36	1.196	15
16	197	15.76	36	5.406	15
17	228	17.1	36	5.185	15
18	156	15.939	36	5.147	15
19	276	27.564	48	6.662	14.4
20	153	9.638	48	2.244	14.4
21	163	4.889	48	0	14.4
22	134	4.013	48	0	14.4
23	277	5.54	60	0	13.9
24	159	4.77	60	0	13.9
25	275	8.25	60	1.047	13.9
26	135	3.375	60	0.03	13.9
27	135	5.447	60	1.475	13.9
28	112	2.475	60	0.027	13.9
29	167	5.01	60	0.046	13.9
30	156	10.92	60	0.136	13.9
31	288	25.235	60	3.061	13.9
32	157	13.215	72	0.156	11.1
33	173	18.622	72	4.716	11.1
34	183	18.918	72	3.853	11.1
35	152	10.424	72	0.011	11.1
36	207	9.326	72	0	11.1
37	140	13.202	72	0	11.1

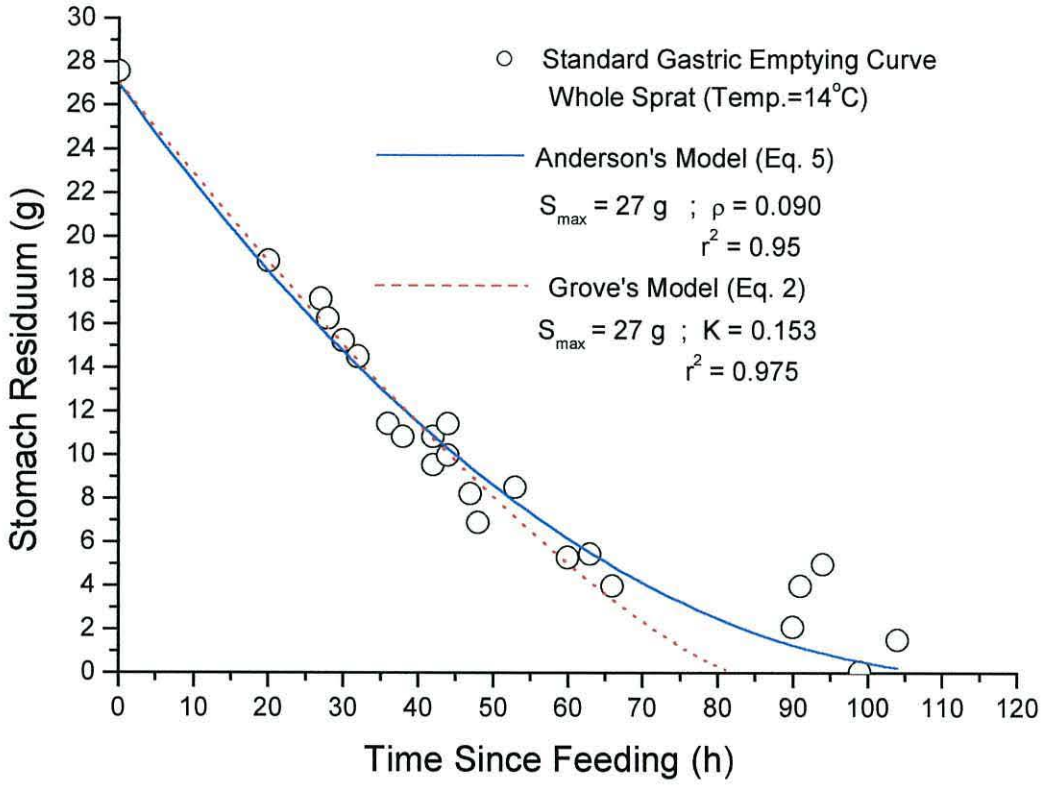


Fig. 4.8. "Standard" or `pooled' Gastric emptying curve obtained by super-imposing results from individual whiting fed different meal sizes of sprat in the X-Ray study followed by gastrectomy.

For squid, results obtained from the first gastric lavage experiment were subject to possible error. Variation in initial meal size (S_0) between fish was back-calculated from the number of incompletely digested squid pieces recovered from the stomach and their original weights. It was possible that some squid pieces had left the stomach at later stages of gastric digestion. The data from the whiting were categorised into three size groups, small (< 100g), medium (101–200g) and large (>200g) and data sets within each were "pooled" graphically as before. The summarised results are shown in Table 4.6 (Ia) and Fig. 4.9; values of ρ varied between 0.08 to 0.13 (*cf* 0.10 to 0.16 in Table 4.3a).

To avoid the problems caused by estimating original meal size from back-calculation, a further gastric lavage experiment was carried out using individually-marked whiting feeding on tagged squid pieces so that the absolute amount of food intake and the amount left (incompletely digested pieces) in the stomach were precisely known by counting the number of threads recovered from the stomach. Observations were again combined into a "pooled" emptying curve (Table 4.6(Ib) and Fig. 4.10). For this experiment, based on single observations from 16 medium fish, ρ was 0.07 ± 0.04(95%CL) h^{-1}. This was intermediate between the small study of appetite return using 5 medium fish (0.04 to 0.07; Table 4.2) and the extended study using 12 medium to large fish (0.10 to 0.16 h^{-1}).

Table 4.6(I(a,b),II,III): Experimental data for gastric emptying in whiting fed on squid pieces, whole sprats and whole brown shrimp to estimate satiation meals (S_{max}), rate parameters k of Grove's model (Eq. 2) and ρ of Andersen's model (Eq. 5) using fixed power factors b = 0.77 of Grove's model and α = 0.5 of Andersen's model.

Meal Types	**Fish Size**	**Temp**	**Eq. No.**	**Estimated Parameters**			r^2	**n**	**df**	**Statistical Summary**
I a) Squid pieces	Wt(g) ± sd	Avg. T°C		S_{max} ±95%CL (g)	b or α (fixed)	k or ρ ±95%CL(g/hr)				
i) Small	89.4±17.2	12	2	16.78 ± 1.728	0.77	0.173±0.030	0.974	4	2	$r_{0.05(2),2}$ = 0.950; P<0.001
	89.4±17.2		5	18.412±2.249	0.5	0.127±0.101	0.968	4	2	$r_{0.05(2),2}$ = 0.950;P<0.001
ii) Medium	147.7±21.2	12	2	24.231±1.846	0.77	0.105±0.014	0.971	6	4	$r_{0.05(2),4}$ = 0.811;P<0.001
			5	27.596±2.507	0.5	0.080±0.030	0.971	6	4	$r_{0.05(2),4}$ = 0.811;P<0.001
iii) Large	228±33.1	12	2	35.322±1.866	0.77	0.126±0.010	0.968	8	6	$r_{0.05(2),6}$ = 0.707; P<0.001
	228±33.1		5	37.033±2.934	0.5	0.079±0.021	0.937	8	6	$r_{0.05(2),6}$ = 0.707; P<0.001
b)Tagged squids										
max. meal size	179.6±47.0	16	2	34.73±0.945	0.77	0.120±0.005	0.978	16	14	$r_{0.05(2),14}$ = 0.497;P<0.001
(pooled/standard)	179.6±47.0		5	35.913±1.298	0.5	0.072±0.004	0.967	16	14	$r_{0.05(2),14}$ = 0.497;P<0.001
II. Sprats										
Gastrectomy	179.8±51.4	14	5	27±1.100	0.5	0.090±0.011	0.950	23	21	$r_{0.05(2)21}$ = 0.413;P<0.001
III.Brown shrimps										
Return of appetite	355±117.1	12	5	16.087±1.089	0.5	0.079±0.01	0.992	7	5	$r_{0.05(2),5}$ = 0.755; P<0.001

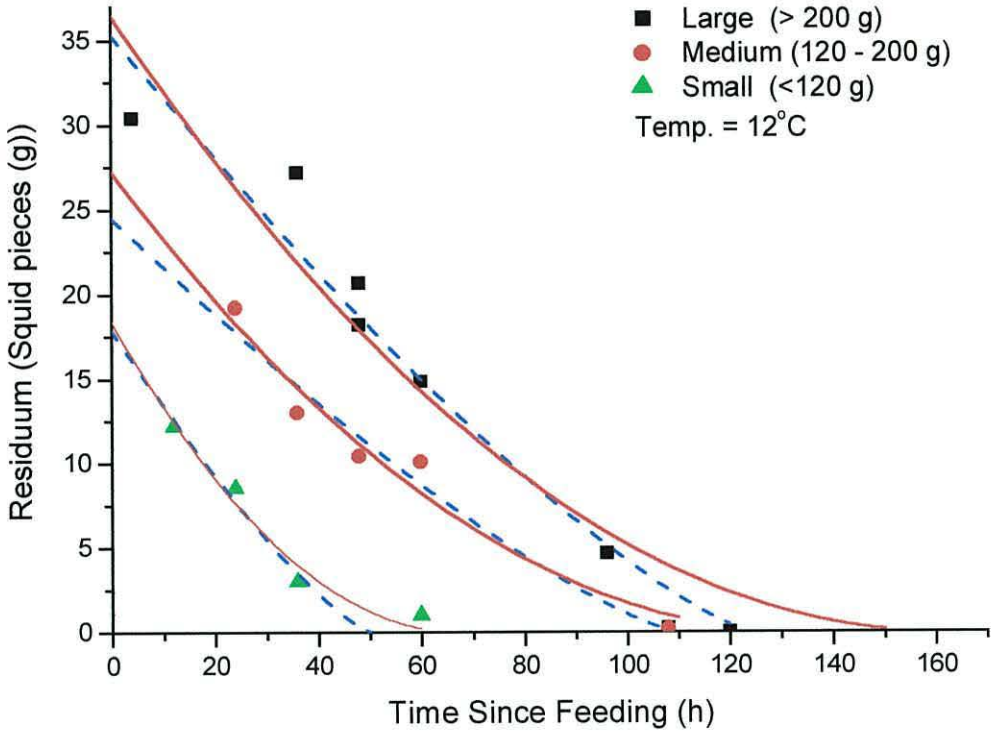


Fig. 4.9. Gastric emptying trends for large (■), medium (●) and small (▲) whiting subjected to gastric lavage at various times after feeding squid pieces. Curves were fitted using Grove's model (Equation 2, dashed lines) and Andersen's model (Equation 5, solid lines).

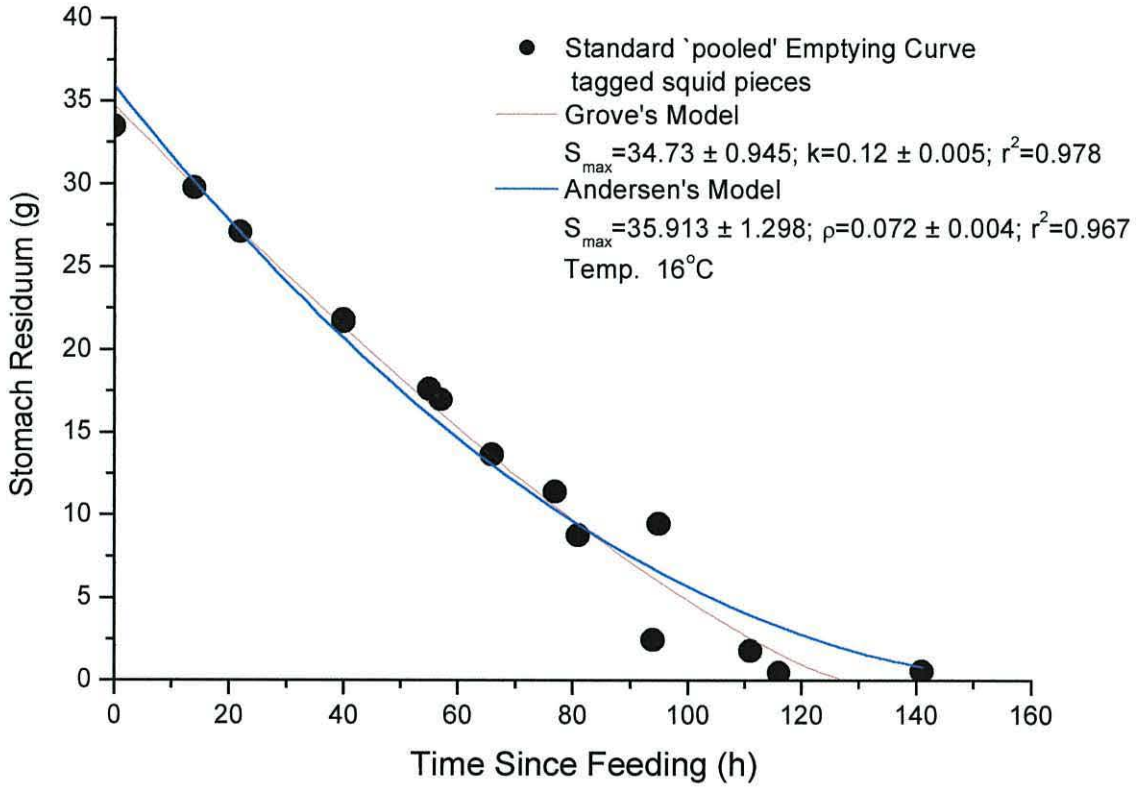


Fig. 4.10. Gastric emptying trends for whiting fed tagged squid pieces to avoid errors due to complete digestion or "other loss". Curves were fitted as in Fig. 4.9.

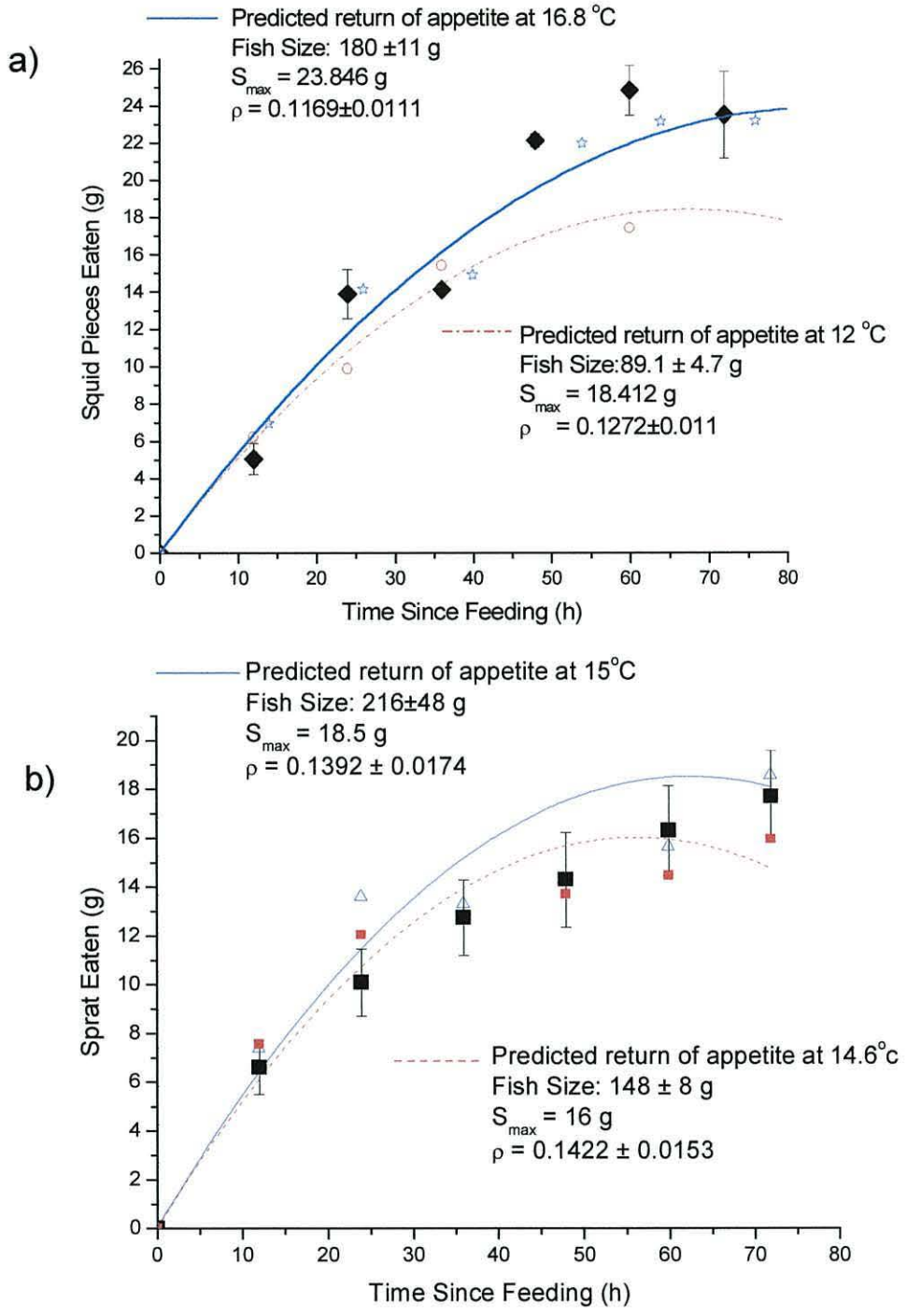


Fig. 4.11(a,b). Observed food intake in (a) whiting (333 ± SD, 98g) fed on squid pieces (0.888 ± SE, 0.021 g) at 12.3°C (♦) and (b) whiting (267 ± SD, 95g) fed on fresh whole sprats (1.547 ± SE, 0.09g) at 14.4°C (■) related to time after satiation meal. Vertical bars are the standard errors. The predicted return of appetite curves fitting are derived from the observed gastric emptying experiments at the stated temperature using Andersen's Model (Eq. 6).

4.4 Discussion

Satiation feeding experiments showed that there were clear allometric relations of the form $S = a W^{b}$ between the amount of prey ingested and the predator size. Similar trends were found by Waiwood *et al.* (1991); Ruohonen *et al.* (1997a); Ruohonen and Grove (1996). The weight exponent for whiting (b = 0.64 to 0.70) is close to that suggested (0.7–0.8) as a "rule of thumb" by Jobling (1993). The results also showed that the coefficient (a) varied between different prey types (Fig 4.7a). It is likely that whiting were able to `pack' squid pieces at optimum capacities (a = 0.520) in the stomach, leading to voluntary meal sizes close to 20% of body weight after a period of food deprivation. Maximum meal sizes for sprats (a = 0.464) and brown shrimps (a = 0.336) were smaller.

If there is a delay, after feeding the fish to satiation, before appetite begins to return it appears to be short. Previous studies have found that appetite returns in inverse relationship with gastric emptying in many species of fishes (Brett, 1971; Elliott, 1975a,b; Grove *et al.,* 1978; Grove *et al.,* 1985; Sims *et al.*, 1996; Paeaekkoenen *et al.,* 1999). Results from Seyhan (1994), Andersen (1998,1999) and the present study (*e.g.* Fig 4.8) confirm that a relatively simple model can be used to describe gastric emptying in whiting. Any delay before gastric emptying begins is too short to warrant the use of more complex models, such as the Power exponential model (Elashoff *et al.*, 1982). The observed "return of appetite" curves in the present study agree quite closely with the independent, direct observations of gastric emptying. For example, whiting fed to satiation on 25-30g of squid pieces required 70-80h for appetite to return (Fig 4.4) by which time at least 80% of the original meal would have emptied from the stomach (Fig 4.10, 4.11). More detailed comparisons are shown in Figure 4.12 and Table 4.7(a-f). Appetite for sprat returned a little slower than predicted from GER (Fig 4.11). This may reflect the larger energy content (oil) in the fish – which may require longer for the body to process – and/or the increased content of ash (mainly bone). dos Santos and Jobling (1988) pointed out that food with low energy density may emptied exponentially over time but energy-rich items cause delays in gastric emptying. For whiting fed with brown shrimps, the estimated digestion rate parameter (ρ) was found to increase with fish size. Possibly this is related to the greater amount of gastric juice secretion from the larger stomach of bigger fish required to cope with the shrimp exoskeleton. Similar decrease in GER for shrimps were reported by Bromley (1991); Hopkins and Larson (1990); Macdonald *et al.* (1982); Singh-Renton (1990) for whiting and redfish.

Table 4.7. Comparison of gastric emptying rates ρ between the present study and the data published by Andersen (1998) for whiting of various sizes fed different meal sizes.

Meal types and sizes (g) (±95%CL)	Fish size g (SD)	Estimated parameters		Temperature (T °C)
		ρ ±95%CL	r^2	
a) Prey species: Herring				Andersen data
Fixed S_{max}	373(42)	α = 0.5		(9.8)
4.0		0.148±0.009	0.962	
8.0		0.134±0.007	0.966	
12.0		0.128±0.006	0.979	
16.0		0.123±0.006	0.969	
b) Prey species: Sandeel				Andersen data
Fixed S_{max}	517(74)	α = 0.5		(9.8)
4.5		0.109±0.006	0.949	
9.0		0.095±0.004	0.976	
18.0		0.098±0.004	0.976	
c) Prey species: Goby	84(12)			Andersen data
Fixed S_{max}		α = 0.5		(6.0)
1.0		0.049±0.002	0.963	
5.0		0.050±0.002	0.965	
d) Prey species: Brown shrimp				Andersen data
Fixed S_{max}	414(39)	α = 0.5		(12.5)
1.0		0.082±0.004	0.956	
5.0		0.106±0.006	0.954	
Fed to satiation;				This study
Est. S_{max} = 16.087±1.089	355(117)	0.079±0.010	0.992	
e) Prey species: Sprats				
Fed to satiation		α = 0.5		This study
i) Est. form appetite expt.				(14)
Est. S_{max} = 18.961±2.188	267(92)	0.083±0.013	0.987	
ii) Gastrectomy				(14)
Est. S_{max} = 27±1.100 g	180(51)	0.090±0.011	0.950	
f) Prey species: Squid pieces				This study
Fed to satiation		α = 0.5		(12)
i) Gastric lavage				
Est. S_{max}	173(63)			
18.412± 2.249		0.127± 0.101	0.968	
27.596±2.507		0.080±0.030	0.971	
37.033±2.934		0.079±0.021	0.937	
35.913±1.298 (Standardized)	180(47)	0.072±0.004	0.967	(16)
ii) Est. from appetite expt.				
Est. S_{max} = 26.634±3.291	333(95)	0.100±0.022	0.972	(12)

A number of factors have been reported to influence the gastric emptying process and these, too, should influence the rate of appetite return. Most workers agree that fish fed with larger meals will initially empty their stomachs at a faster rate (g h^{-1}) as reported for whiting by Robb (1990) and for turbot by Bromley (1987). This observation is implicit in the gastric emptying curves referred to here as the Grove (Equation 2) and Andersen (Equation 5) models. For example, Basimi and Grove (1985a) summarised results for turbot (*Scophthalmus maximus*), dab (*Limanda limanda*) and plaice (*Pleuronectes platessa*) and reported that the gastric emptying time for different species varies; GER is related to exponents 0.45, 0.25 and 0.49 of the meal size respectively. The exponent values may also vary between prey types. Andersen (1998) found additional "prey" exponents at fixed meal size of 0.55 for herring and sandeel, 0.46 for common goby and 0.36 for brown shrimps. Meanwhile a recent study by Koed (2001) found that values were between 0.51-0.53 for pikeperch fed with rainbow trout and roach.

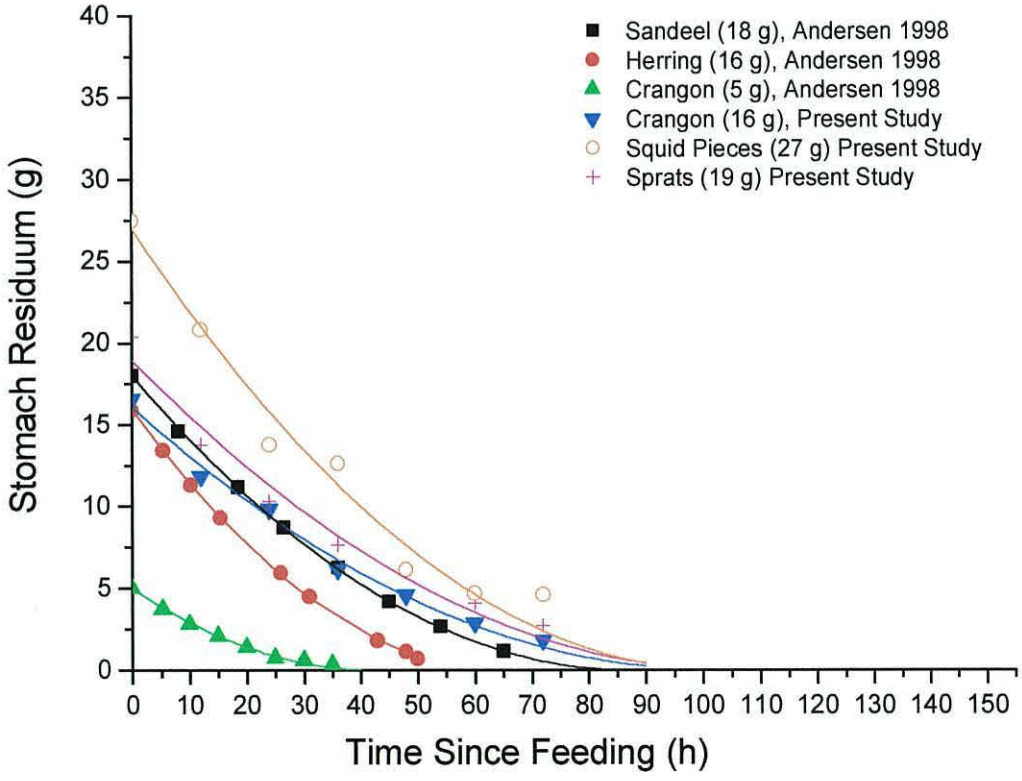


Fig. 4.12 Variations in gastric emptying time estimated from whiting of various sizes fed on different prey types and meal sizes from the present study and the data redrawn from Andersen (1998). (see details in Table 4.7)

In addition, the effects of predator size (fish size) on gastric emptying of fish have been subject to much discussion, and there is no clear consensus concerning the influence of predator size on gastric emptying. Several previous workers concluded that there were no effects of fish size on gastric emptying rate (Tyler, 1970; Elliott, 1972; Lambert, 1985; Elliott, 1991) whereas others reported that predator size does affect gastric emptying (Jones, 1974; Flowerdew and Grove, 1979; dos Santos and Jobling, 1995); instantaneous gastric emptying rate is frequently found to vary as (body weight)^b where b is in the range 0.2-0.4.

The results from the present studies of both return of appetite and gastric emptying indicated that the rate parameter ρ was probably independent of maximum meal size (S_{max}). The consequent non-linear emptying curve found here and by Andersen (*loc. cit.*) contradicts Seyhan's (1994) finding that small meals were emptied linearly. Linear emptying of natural prey items in whiting had also been reported by Robb (1990); Bromley (1988); Bromley (1991); Singh-Renton and Bromley (1996). Seyhan also found that GET was constant for meals of sprat between 1 and 7g, indicating that GER increased in direct proportion to meal size. This was not tested directly in the present study. However the larger fish took bigger satiation meals and took longer times to empty their stomach. It is a consequence of the consistent values of ρ found here for a wide range of meal sizes that larger meals lead to extended GET values. Gastric emptying seems best described by a power function: $dS/dt = -\rho S^{\alpha}$ where S is the current weight of stomach contents, ρ and α are the parameters to be estimated. This description was originally developed by Jones (1974) and recently used by dos Santos and Jobling (1992); Temming and Andersen (1994); Andersen (1998, 1999). Integration of this differential equation (see equation 5 in Chapter 2) produced non-linear emptying curves which fitted the current data sets well. In the present study, the value of α was fixed at 0.5 which, according to Andersen, best described gastric emptying when compared with an earlier suggestion of 0.77 (Grove pers. comm).

Jobling (1981b) reviewed data from a number of studies and suggested that an exponent 0.5 (a square root function) would be appropriate to describe the relationship between meal size and gastric evacuation rate and time. Unpublished data from seven species supplied by Grove found values of α between 0.32 and 0.68 (average 0.52). The "square root" effect of meal size on gastric emptying may be a useful "rule of thumb" for other species whose digestion has not been studied. In a recent study Boyce *et al.* (2000) also reported that gastric emptying rate (GER) increased with ration mass in the cold water Antarctic plunderfish but that the non-linear gastric emptying curves

were best fitted using Gompertz curves. Relatively few workers have directly tested the effect of fish size on gastric processing rate when meals of *identical* size (g) are offered. In flatfish, GET decreases with fish size under these conditions presumably because GER increases (Jobling *et al.*, 1977; Flowerdew and Grove 1979). Larger fish should have greater digestive capacity so that maximum GER (g h^{-1}) should increase with body weight: GER = a W^{b}. Where large partial-factorial studies have been made, values of **b** have ranged between 0.17 and 0.53 (average = 0.26) close to the value b = 0.33 predicted in Chapter 1 of this work. The value of **a** varies with dietary niche, being highest in particulate feeders (microphags) and lowest in predators of large prey (macrophags; Fänge and Grove, 1979). In practice, GER is probably determined by a combination of the stimulus of food, the potential secretion from the gastric epithelium, the surface area of the prey item and its digestibility (see Holmgren *et al.*, 1983; Chapters 1 & 6 of this thesis). The results obtained in the present study agree with Andersen's findings that instantaneous gastric emptying rate (ρ) does not change with meal or predator sizes but does change with prey type. This change may be due to differences in surface area (form and shape of the body) and the integument and exoskeleton of the prey species, which in turn influence the packing of the prey item in the stomach.

Salvanes *et al.* (1995) discussed in detail the surface-dependent gastric evacuation model and suggested that four parameters should be considered to affect the gastric emptying process: digestion velocity (enzyme breakdown reaction depending on prey geometry and temperature), prey length, initial prey radius and the density of the prey. Surprisingly, no clear effect of temperature on digestion rate was detected in the present study. Typically, Q_{10} values of *ca* 2-fold have been described and Seyhan (1994) reported a value of 1.8. However, as described for cod in Fig 1.8 (Chapter 1) changes in "instantaneous depletion rate" over the temperature range 10-19°C were difficult to detect. Fish acclimated to different ambient temperatures may partially compensate their digestive physiology and mask responses which are easily seen with acute temperature changes (Smit, 1967). This study was not designed to investigate temperature and further study would be required to examine its effects.

Chapter 5

Nutrient absorption along the alimentary tract of whiting (*Merlangius merlangus* L.)

5.1 Introduction

Carnivorous fish, such as the whiting, generally require diets high in protein and energy (Zeitoun *et al.*, 1976; Cho *et al.*, 1985) and this is important when making artificial diets (Cowey and Sargeant, 1972; Cowey *et al.*, 1972; Cowey *et al.*, 1974; Cowey *et al.*, 1975; Bromley and Adkins, 1984). To be acceptable, the food must also be palatable and of appropriate size if optimal digestion, feed utilisation and growth is to be achieved (Sveier *et al.*, 1999). The rate of energy supply from all nutrients is related to the animal's ability to digest the food ration at appropriate rates, and to extract the maximum possible levels of energy and nutrients from the food for its metabolic requirements.

The efficiency of nutrient extraction or absorption, termed `Nutrient Digestibility', has been measured in different ways. Most authors use inert marker(s) to calculate apparent digestibility (absorption) coefficients (ADCs) by monitoring relative changes in inert marker and nutrient concentration in faeces relative to diet fed (Maynard and Loosli, 1969). The most popular exogenous inert marker has been chromic oxide but micro-tracers such as ferro-nickel-magnetic alloy powder (F-Ni) (Kabir *et al.*, 1998) or n-alkanes (Thorrson *et al.*, 1997) and recently β-carotene (Boyce *et al.*, 2000) have also been used. For more sensitive and delicate determination of macro and micronutrients, radioactive compounds such as ^{14}C labelled substrates and ^{15}N labelled protein have been used especially to study detailed lipid (Koven *et al.*, 1997) or protein (Campbell *et al.*, 1997) absorption and subsequent utilisation in fish. Endogenous markers, such as lignin (Law *et al.*, 1985), hydrolysis resistant ash or organic matter, or even crude fibre (de Silva, 1985), have also been used.

Laboratory studies on artificial feeds have shown differences in digestibility depending on feeding level and meal size (Henken *et al.*, 1985; Gongnet *et al.*, 1987) as well as with size, age and holding density of the fish (Hastings, 1969; Windell *et al.*, 1978). Salinity and temperature (de Silva and Perera, 1984; Windell *et al.*, 1978; Pandey and Singh, 1980; de Silva and Perera, 1984) and particularly the dietary components also affect digestibility (Beamish and Thomas, 1984; de Silva and Perera, 1984; Hanley, 1987; Krogdahl *et al.*, 1999). However, it is extremely difficult to conduct studies on gastrointestinal contents of wild fish since the composition of the original meal is rarely known with sufficient accuracy. As a compromise, laboratory study using fish fed on their natural food (prey) is possible although it must be remembered that the nutrient content of any natural food may vary with season or location (Hislop *et al.*, 1991a; Arrhenius, 1998). The most widely reported seasonal macronutrient variation in prey is

for lipid, which has incidentally been reported to depress the rate of gastric evacuation (Kitchell and Windell, 1968; Windell *et al.*, 1969; Elliott, 1972; Grove *et al.*, 1978; Jobling, 1981a; Hofer, 1982; Jobling, 1987).

The present study represents an attempt to locate the site of absorption, and its efficiency, for macronutrients passing through the alimentary tract of wild whiting caught in the Irish Sea. Data was also collected from laboratory whiting conditioned to feed on whole sprat, which is a major part of the diet of local whiting (Seyhan and Grove, 1998). The sprats used in this experiment were injected with radio-opaque barium sulphate paste as part of the X-ray experiment for validating the gastric emptying model in Chapter 3.

5.2 Material and Methods

5.2.1 Whiting from the wild

Samples of wild whiting were collected from North-East Anglesey by RV "Prince Madog" during September – October 1998 using a beam trawl net and then kept in the freezer at – 20°C for further analysis. 250 whiting of various sizes were sampled by removing the alimentary tracts from the carcasses. The tracts were gently cut into four sections as described in detail in Chapter 2 and their contents extruded. Due to the small size of the intestine and the several analyses required, the samples from each part of the canal; Stomach (A) (n = 23; 760 mg dry wt.), Anterior Intestine (B) (n = 38; 354 mg dry wt.), Middle Intestine (C) (n = 47; 537 mg dry wt) and rectum sections (D) (n = 63; 612 mg dry wt.) were each pooled into labelled aluminium cups to give sufficient amount of dried material. These were frozen and then freeze-dried for three days. The dried samples were homogenised using pestle and mortar and further ground to a fine powder using a ball-mill. The homogenised samples were then kept in labelled borosilicate tubes for proximate analyses of nitrogen (by Kjeldahl analysis), total lipid, carbohydrate, ash and total energy as described in Chapter 2 (page 45-51). Since sprat and, to a lesser extent crustacea, dominate the diet of local whiting, samples of intact prey items were also analysed.

5.2.2 Laboratory whiting

Fish were fed to satiation every alternate day with sprats and then deprived of food for four days to empty the stomach. At the start of the experiment, the fish were fed again to satiation with whole sprats containing 0.1-0.15 ml $BaSO_4$ g^{-1} (see Chapter 3) and left for pre-selected periods before sampling (12, 24, 36, 48, 60, and 72h). Although $BaS0_4$ is chemically inert, it is assumed here that it does not interfere with nutrient absorption Results in Chapter 3 showed that the fish that ingested more than 10% of their body weight required 72h to empty their stomach; such fish still retained 20-25% of the meal. On the other hand, fish that ingested less than 10% bw had empty stomachs within 72h. Fish were killed by a blow to the head, the brain was destroyed by pithing and the fish immediately X-rayed. The bodies were then kept in the freezer prior to dissection and removal of alimentary tract contents.

The experiment was "two-tiered" so that the digesta samples for biochemical analyses were obtained at the same time that X-raying traced the movement of food along the gut. Dried samples were analysed as described above and in Chapter 2 for ash, lipid, carbohydrate and energy. Moisture content was also measurable (unlike that for the frozen wild whiting samples). Because of limitations in sample size, carbon and nitrogen analysis was carried out using a C:H:N Analyser rather than the Kjeldahl method. Faecal materials from whiting fed on the sprats were collected by carefully siphoning the material into collector flask using a plastic tube (2 mm diam.) connected to the collector flask *via* rubber tubing (3 mm diam.). The collected materials were gently washed using distilled water, pooled into borosilicate tubes and kept frozen at –18°C. Later the frozen faecal materials were freeze dried for 2 days and the dried samples transferred into labelled borosilicate tubes ready for biochemical analysis.

The nutrient contents in the digesta were used to determine the relative percentage absorption gradient (using Apparent Digestibility Coefficients; ADCs) along the alimentary tract, based on ash contents of adjacent samples (Eq.12, Chapter 2, page 54). For laboratory-fed whiting, it was assumed that the barium sulphate moved together with the food, as found for turbot by Al-Aradi (1986).

5.2.3 Statistical analysis

Differences between samples were first presented based on sample means and standard errors. All data sets to be compared were tested for normality (Anderson-

Darling test) and homogeneity of variance (Levene test) using Minitab statistical package (Minitab Inc., 1999). Data were found not normally distributed and variances were equal so the Kruskal-Wallis test was employed to compare differences in concentrations of nutrients and dietary energy contents in the gut sections at different times after feeding. Where significant differences were found to exist, Dunn's procedure (*post hoc* test) for multiple comparisons was used to identify which samples were different.

5.3 Results

Table 5.1 shows the summary of proximate nutrient analyses of two prey species, sprat and brown shrimps, which are frequently found in the stomach of wild-caught fish. Squid pieces were also analysed since these have frequently been used in feeding studies, including Chapter 4, but the composition has rarely been reported. Brown shrimps contained the highest ash contents (18%) followed by sprats (13%) and squid pieces (7%). Higher ash contents in brown shrimps were due to the presence of chitinous exoskeleton or carapace and appendages whereas in sprats it is due to the bony endoskeleton. Squid pieces contained the highest moisture content (81%), followed by sprat (73%) and brown shrimp (69%). The squid contained the highest protein content by dry weight (78%) followed by brown shrimp (66%) and sprat (59%). The highest total lipid was found in our sample of sprat (16-17%), followed by squid (6.8%) and brown shrimp (4-4.5%). The total carbohydrate content in all test meals was generally low (1-2%).

Table 5.1 Proximate analyses of experimental meals (%dry wt ± S.E.).

Samples	Ash	Moisture	Protein	Total Lipid	Carbo-hydrate	Energy (KJ/g)
Test Meal						
Whole Sprat (1.05 ± 0.08g dry wt., n = 20)	13.025 ±1.280 (n = 8)	73.210 ±0.876 (n = 20)	59.095 ± 1.910 (n = 12)	16.736 ±2.138 (n = 8)	1.223 ±0.272 (n = 7)	21.152 ±1.458 (n = 17)
Squid pieces (42.93 ± 2.37g dry wt, n = 6)	6.896 ±0.902 (n = 2)	81.145 ±0.541 (n = 6)	78.284 ±1.205 (n = 9)	6.789 ±0.536 (n = 3)	1.296 ±0.234 (n = 7)	17.601 ±0.487 (n = 9)
Brown Shrimps (0.38±0.016g dry wt, n = 20)	18.020 ±0.123 (n = 3)	69.162 ±0.528 (n = 20)	66.072 ±1.590 (n = 3)	4.336 ±0.184 (n = 3)	1.579 ±0.110 (n = 7)	15.634 ±1.021 (n = 3)
Faeces						
Sprats $BaSO_4$-Labelled	74.860 ±0.163 (n = 2)	-	19.947 ±0.778 (n = 2)	3.230 ±0.699 (n = 5)	1.37 ±0.08 (n = 2)	5.122 ±0.537 (n = 8)

The results also indicated that sprats contained the highest energy content by dry weight (21 ± 1.5 KJ/g) followed by squid pieces (17.6 ± 0.5 KJ/g) and brown shrimps (15.6 ± 1.0 KJ/g); these dietary differences were mainly due to lipid contents.

The stomach contents of the wild whiting were intermediate in nutrient content between those of intact sprats and shrimps (Table 5.1 & 5.3). The composition suggests that the diet in these fish had been approximately 70% sprat and 30% shrimp. The diet was rich in protein (64% by dry weight) and lipids (13%) but carbohydrate content was low (<2%). Also shown are the estimates of protein, lipid and carbohydrate from the other segments of the gut (Table 5.3a). The ash content increased by the time digesta had reached the anterior intestine and remained similar thereafter. Both protein and lipid concentrations decreased in successive gut regions but carbohydrate concentrations remained similar.

Because of limited numbers of whiting and time constraints, the laboratory study was limited to the main natural diet, sprat. Table 5.2 shows the results of carbon and nitrogen analyses from different regions of the alimentary tract at different times after feeding. The nitrogen amounts were used to estimate the protein contents in these regions. The contents of section A (stomach) showed no consistent change over the 72h, suggesting that there was little selective separation of nutrients (protein and lipid) from food which had not yet left the stomach. The expected decrease of both nitrogen and carbon concentration along the gut was seen but these concentrations were similar for a stated zone throughout the 72h digestion period (Fig. 5.1). Estimation of moisture,

ash, lipid, carbohydrate and energy were carried out on samples independently of the C:N analysis.

Table 5.3 summarises the average composition of the digesta in different sections of the alimentary tracts (gut sections) of these whiting, independent of time after feeding. Also included are observations for wild fish.

In Figures 5.2(a-f), the concentrations of nutrients, ash and energy at various times after feeding with sprat in the laboratory confirms that these were remarkably constant for a stated region. Water content decreased from *ca.* 77% to 54% along the gut. The sprat nutrients had been diluted by the presence of injected $BaSO_4$.; ash content was 47%. In wild whiting, the ash content had risen from 13 to 28% of the sample dry weight by the time food reached the anterior intestine and remained near this level thereafter (Table 5.1 and 5.3). In contrast, ash concentration in laboratory fish rose steadily toward posterior regions of the gut to reach *ca* 80%.

The concentration of protein, lipid and energy all decreased along the gut for both wild and laboratory fish in a similar pattern. Interestingly, carbohydrate concentration in both groups rose between the stomach and anterior intestine before declining and there was some indication that this localised increase was maximal toward the end of the 72h study. The concentrations of each nutrient within a stated gut section showed that there were no significant differences over time (Kruskal-Wallis: $P>0.05$; see Appendix 5.2(I-IV)).

However, nutrient contents were found to decrease steadily along the gut at all times. These declines were significant for protein (Kruskal-Wallis: $H = 41$, $df = 3$, $P<0.0001$), for total lipids ($H = 35.34$, $df = 3$, $P<0.0001$), digestible energy ($H = 38.63$, $df = 3$, $P<0.0001$) and carbohydrate ($H = 32.98$, $df = 3$, $P<0.0001$). Dunn's procedure for multiple comparisons was used to locate where changes in content occurred. For protein, contents of *adjacent* sections of the gut were not significantly different but the fall in protein was real by the time food reached the middle intestine and rectum. The same was true for the fall between anterior intestine and rectum. Similar results were also observed for total lipids and digestible energy contents (Appendix 5.2II & III). Carbohydrate contents changed in a different manner along the gut. A significant increase occurred between stomach and anterior intestine, followed by a clear decrease in the middle intestine and rectum (see Appendix 5.2 (IV)).

Table 5.2. Summary of carbon and nitrogen (C:N) analysed from digesta collected from stated alimentary tract sections at different times since feeding laboratory-reared whiting on whole sprat.

Time Since Feeding	Alimentary Tract Section	n	Carbon contents (%dry wt)	Standard deviation	Nitrogen contents (%dry wt)	Standard deviation	C:N Ratios
12H	A	2	32.05	0.356	8.430	0.141	3.802
	B	2	19.4	0.141	4.425	0.035	4.384
	C	2	16.2	0.707	3.935	0.332	4.117
	D	2	9.44	0.057	2.490	0.071	3.791
24H	A	2	26.3	0	6.925	0.035	3.798
	B	2	19	0.141	4.385	0.120	4.333
	C	2	12.5	0.141	3.540	0.028	3.531
	D	2	6.58	0.156	2.200	0.014	2.991
36H	A	2	21.1	0.283	5.605	0.191	3.764
	B	2	18.6	0.283	3.930	0.042	4.733
	C	2	11.95	0.071	3.215	0.064	3.717
	D	2	8.08	0.170	2.610	0.000	3.096
48H	A	2	25.75	0.212	6.800	0.113	3.787
	B	2	26.45	0.071	6.085	0.134	4.347
	C	2	12.95	0.212	4.025	0.035	3.217
	D	2	8.475	0.007	3.265	0.021	2.596
60H	A	2	32.2	0.283	7.100	0.113	4.535
	B	2	17.4	0.283	3.770	0.042	4.615
	C	2	15.15	0.071	3.825	0.064	3.961
	D	2	8.47	0.014	2.755	0.092	3.074
72H	A	2	22.55	0.071	6.070	0.014	3.715
	B	2	25.55	0.071	5.185	0.120	4.928
	C	2	14.2	0.283	3.205	0.163	4.431
	D	2	7.885	0.035	2.585	0.078	3.050

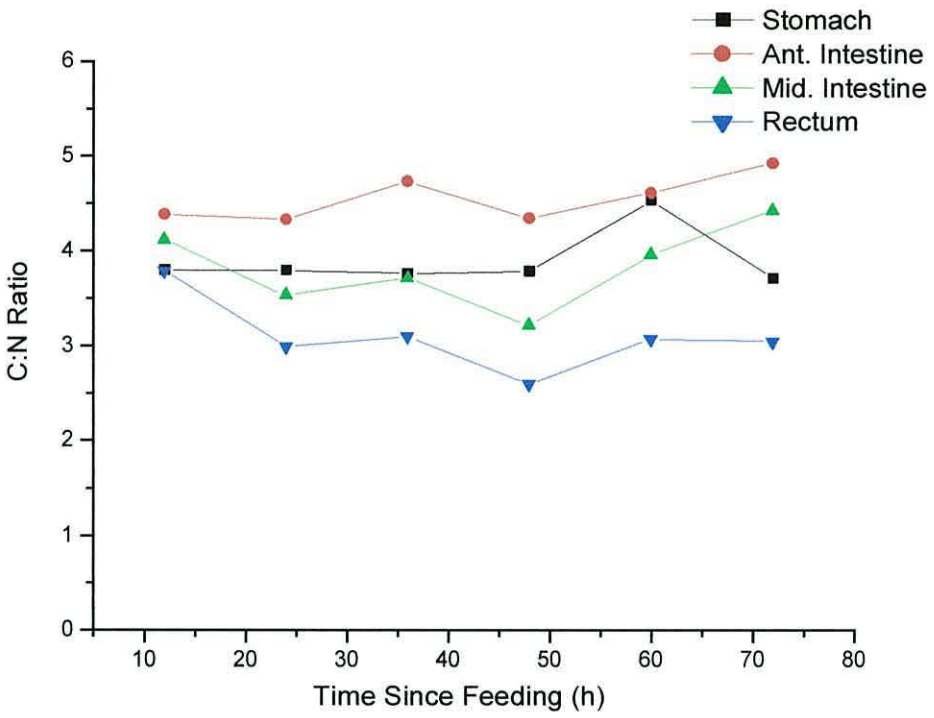


Fig 5.1. C:N ratios in different gut sections at different times after feeding fresh whole sprats containing $BaSO_4$ in laboratory-held whiting.

Table 5.3. Proximate analysis of digesta (% dry wt ± S.E.) from stated alimentary tract sections of (a) whiting collected from the wild and (b) laboratory-held whiting fed on whole sprats containing Barium Sulphate paste (0.1-0.15 ml/gram sprat wet weight).[n=number of replicates analysed].

	a) Proximate analysis of digesta (Wild Whiting)					**b) Proximate analysis of digesta (Laboratory reared whiting)**					
	Inorg. Comp.	**Organic Component**			**Total Energy (KJ/g)**	**Inorg. Comp.**	**Organic Component**				**Total Energy (KJ/g)**
	Ash	Protein	Total Lipids	Total CHO		Ash	Moisture	Protein	Total Lipids	Total CHO	
Test meal (Sprat +$BaS0_4$)						46.525 ±0.185 n = 4	78.533 ±1.925 n = 5	44.200 ±0.443 n = 5	9.296 ±0.244 n = 4	0.676 ±0.045 n = 4	11.518 ±0.714 n = 4
Stomach	13.196 ±0.0125 n = 2	64.381 ±0.643 n = 7	12.620 ±0.867 n = 7	1.712 ±0.108 n = 4	17.739 ±0.539 n = 6	43.074 ±2.038 n = 12	77.407 ±1.832 n = 6	42.636 ±1.679 n = 12	10.124 ±2.305 n = 12	0.954 ±0.091 n = 12	11.872 ±0.833 n = 12
Py. Caeca (Ant. Int.)	27.924 ±0.535 n = 2	57.650 ±1.150 n = 4	7.844 ±0.350 n = 5	2.050 ±0.064 n = 4	15.515 ±0.453 n = 4	56.679 ±1.906 n = 12	71.948 ±1.648 n = 6	28.938 ±1.496 n = 12	6.308 ±0.516 n = 12	1.713 ±0.166 n = 12	8.824 ±0.460 n = 12
Intestine (Mid. Int.)	24.425 ±0.475 n = 2	53.740 ±1.958 n = 5	7.233 ±0.242 n = 5	2.367 ±0.064 n = 5	14.377 ±0.652 n = 3	69.424 ±0.926 n = 12	62.919 ±1.725 n = 6	22.651 ±0.653 n = 12	3.692 ±0.723 n = 12	0.926 ±0.102 n = 12	5.739 ±0.509 n = 12
Rectum	28.641 ±0.012 n = 2	49.136 ±1.053 n = 5	5.313 ±0.254 n = 5	2.028 ±0.118 n = 3	13.171 ±0.214 n = 5	80.376 ±0.557 n = 12	53.738 ±1.534 n = 6	16.568 ±0.612 n = 12	1.505 ±0.225 n = 12	0.605 ±0.047 n = 12	2.827 ±0.321 n = 12

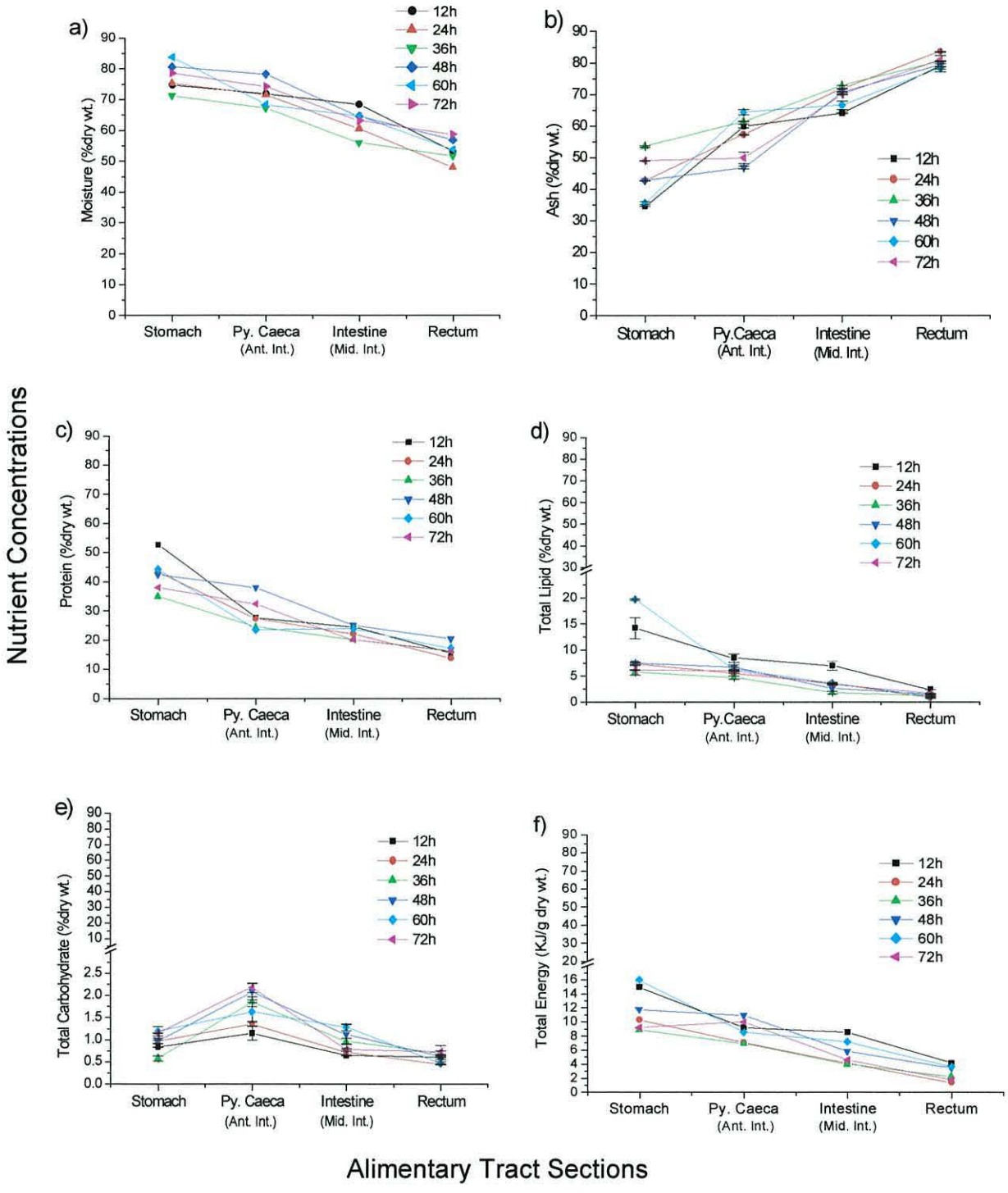


Fig. 5.2 (a-f). Macronutrient concentrations in digesta from different gut sections at different times after feeding fresh whole sprats containing $BaSO_4$ in laboratory-held whiting.

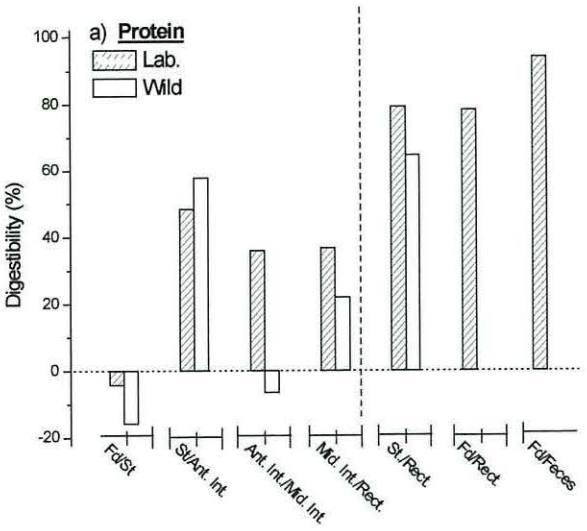
Fig. 5.3(a-d) shows estimates of apparent digestibility coefficients (% ADCs) for macronutrients and energy as food passes through the different gut sections independent of time since feeding. For wild whiting, whatever the original diet mix was, approximately 58% of protein, 71% of total lipids and 43% of carbohydrate appear to have been absorbed in passage between the stomach and the anterior intestine (including the pyloric caeca). There was an unexpected negative apparent "absorption" between samples from the anterior- and mid-intestine for all nutrients analysed. In the passage of the remaining nutrients from mid-intestine to rectum, approximately 22% of protein, 37% of total lipids and 27% of carbohydrate were absorbed.

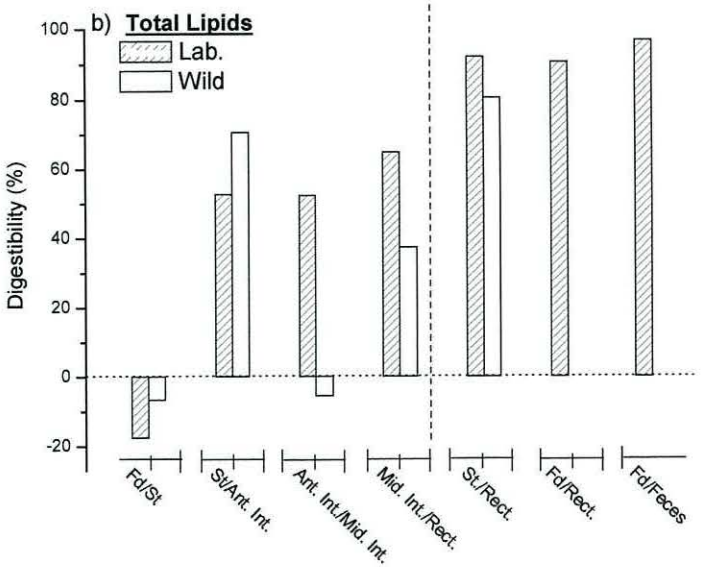
Despite the presence of quantities of barium sulphate in the sprat fed to whiting in the laboratory, which diluted the concentration of nutrients, similar patterns of apparent absorption efficiencies for the major nutrients were found when compared with wild fish (Fig. 5.3(a-d); see also Appendix 5.1). The major difference from wild samples was that active absorption of protein, lipid and energy was detected in all parts of the intestine, including the region between anterior- and mid-intestine. In both studies, net absorption had begun as food passed from the stomach into the anterior intestine. The pattern for the small content of carbohydrate however was again different. Net addition of carbohydrate to gastrointestinal contents occurred in the stomach, which persisted in the anterior intestine for laboratory fish (Fig. 5.3c).

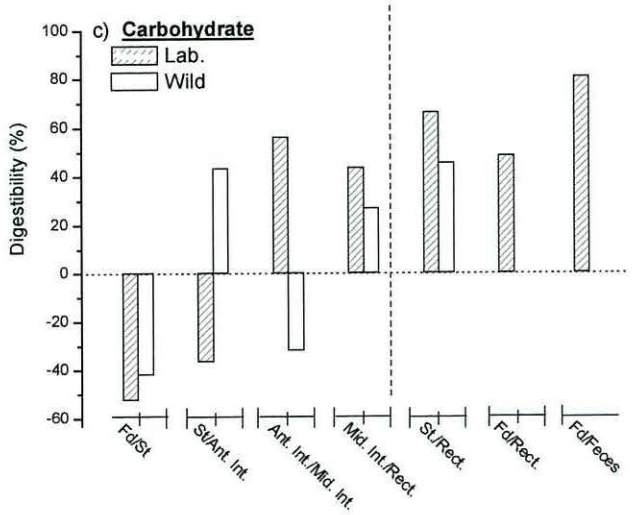
Between the stomach and rectum in laboratory whiting fed with sprat, the overall effect of absorption in the different zones meant that 79% of protein, 92% of lipids, 66% of carbohydrate and 87% of energy had been removed. Comparison of food with faeces in these fish suggested that a further small absorption of all nutrients occurred in the rectum. It is equally likely however that this apparent increase in ADC could have been caused at least in part by leaching of nutrients from the faeces prior to collection.

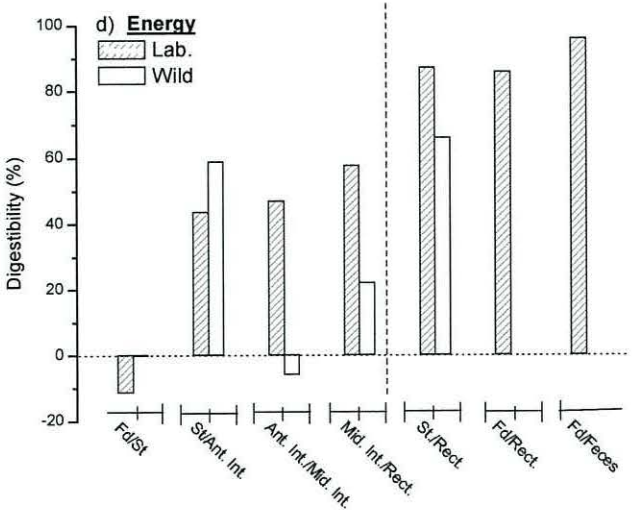
Fig. 5.3 (a-d). Apparent digestibility coefficients for a) protein, b) lipids, c) carbohydrate and d) energy between adjacent regions of the whiting alimentary tract. Hatched bars are values for laboratory-held whiting fed known meals of sprat whilst open bars are for samples from wild whiting. Columns to the right of the vertical dashed line are values for non-adjacent gut sections. For the food/stomach estimate of wild whiting, the fish were assumed to have eaten a diet of 70% sprat/30% shrimp.

Key: **Fd** food; St stomach (Section A); **Ant.Int**. anterior intestine with pyloric caeca (Section B); **mid. Int** middle intestine (Section C); **Rect** posterior intestine or rectum (Section D); **Feces** for laboratory-held whiting may have lost some soluble nutrient by leaching and ADCs may be over-estimated.


a) Protein
Lab.
Wild
Digestibility (%)
100
80
60
40
20
0
-20
Fd/St
St/Ant. Int.
Ant. Int./Mid. Int.
Mid. Int./Rect.
St./Rect.
Fd/Rect.
Fd/Feces


b) Total Lipids
Lab.
Wild
Digestibility (%)
100
80
60
40
20
0
-20
Fd/St
St/Ant. Int.
Ant. Int./Mid. Int.
Mid. Int./Rect.
St./Rect.
Fd/Rect.
Fd/Feces


c) Carbohydrate
Lab.
Wild
Digestibility (%)
100
80
60
40
20
0
-20
-40
-60
Fd/St
St/Ant. Int.
Ant. Int./Mid. Int.
Mid. Int./Rect.
St./Rect.
Fd/Rect.
Fd/Feces


d) Energy
Lab.
Wild
Digestibility (%)
100
80
60
40
20
0
-20
Fd/St
St/Ant. Int.
Ant. Int./Mid. Int.
Mid. Int./Rect.
St./Rect.
Fd/Rect.
Fd/Feces

5.4 Discussion

Whiting is a carnivorous species and little information is available about its digestive physiology or absorption of nutrients. A general description of the anatomy and histology of the alimentary canal of closely related fish species (family Gadidae) was given in Chapter 1 (page 29-30). The whiting, like the cod, has a well-developed stomach and a large number of pyloric caeca (comparable to Fig 1.9a) and a moderately long intestine (like Fig 1.9b). Its gastrointestinal epithelium is rich in goblet cells which secrete copious mucus which overlies the apical borders of the absorptive cells. The anterior intestine exhibits extensive mucosal folding. With the pyloric caeca, this region has a large secretory and absorptive area for a given intestinal length when compared to the lower intestine and rectal regions which have a less developed mucosal folding (Ferraris and Ahearn, 1984). It was expected that the stomach would act as a reservoir which holds newly-ingested food and begins digestion by the action of pepsin on the contents in an acid medium. The short anterior intestine and associated pyloric caeca should act as a major site for further digestion and absorption which is completed in the longer middle and posterior intestine (= "rectum") before food is extruded as faeces.
The natural diet in local waters is dominated by sprat (Seyhan, 1994), samples of which contained mainly protein (59 ± 2% dry wt.) and also lipids (17 ± 2% dry wt.) and traces of carbohydrate (1± 0.2% dry wt.). This composition may vary seasonally (Arrhenius, 1998; Hislop *et al.*, 1991a). Wild fish in this study had mainly eaten such fish as well as some crustaceans. Laboratory-held whiting were fed with sprat, labelled with barium sulphate, at meal sizes of 6 – 10% bw.

As the $BaSO_4$-labelled sprat passed along the gastrointestinal tract its ash content rose (from 46.5 to 80.4% of dry matter) whilst water content (moisture) in samples decreased from 79% to 54%. Using Ash content as an internal marker, these figures give a "compacting factor" (Conover, 1966) which suggests that about 60% of the water in the food was absorbed during transit to the rectum. For wild whiting the ash content doubled by the time food reached the anterior intestine but did not increase thereafter. These fish probably ingested several types of prey in the wild and the contents in different gut regions may come from different mixtures of species. Nevertheless, the contents of natural food (13.2% in stomach and 28.6% in rectum) indicate that 68% of dietary water is absorbed.

As expected from similar studies (Austreng, 1978; Lied *et al.*, 1982; Fernández *et al.*, 1998), concentrations of protein and lipid, as well as energy content, decreased in

similar fashion as transit of food along the gut proceeded in the laboratory-fed whiting. There was no indication that any nutrient was selectively retained in or expelled from the stomach relative to others during the 72h duration of the study. Although the carbohydrate level in the sprat diet was low, this component increased in the anterior intestine presumably reflecting secretion of mucus.

Ash contents (% dry wt.) were used as an internal marker based on the assumption that aquatic animals absorb relatively little of the mineral (mainly fish bone fragments) in their natural diet nor any barium sulphate (when present) (Conover, 1966). This allowed ADC values to be calculated between adjacent regions of the gut for each nutrient.

For protein, the small negative ADC shown in Fig 5.3a between original food and stomach contents is most likely due to release of enzymes from the secretory `oxynticopeptic' gland cells of the stomach wall. 50% or more of the protein leaving the stomach had been absorbed in the short anterior intestine which carries the numerous pyloric caeca. These results agree with those for rainbow trout (Austreng, 1978; Dabrowski and Dabrowska, 1981; Dabrowski *et al.*, 1986). Krogdahl *et al.* (1999) found that more than 50% of amino acid absorption took place in the pyloric region of salmon.

Between the stomach and rectum, about 80% of the protein had been removed but the estimate from faecal analysis (94%) may include errors caused by nutrient leaching into the water. Protein ADCs usually lie in the region 82-93% although occasional values as low as 70% have been recorded (Table 5.4).

Some lipid was apparently "added" after food entered the stomach. Contents in distal parts of the guts probably come from different meals because of the long digestion time established in Chapter 4 and may explained this anomalies. After reaching the anterior intestine, 50-70% had been absorbed. Lipid digestion and absorption takes place mainly in the anterior intestine where the pyloric caeca are situated and pancreatic lipases are secreted (Fänge and Grove, 1979; Buddington and Doroshov, 1986a,b; Borlongan, 1990). Unlike protein, notable amounts of lipid continued to be removed between the middle intestine and rectal sections. Other workers have reported that lipid digestion and absorption continues in the posterior intestine / rectal region, particularly in carnivorous fish with short digestive tracts (Smith and Lovell, 1973; Hofer, 1982; Ferraris and Ahearn, 1984; Smith, 1989; Koven *et al.*, 1997; Krogdahl *et al.*, 1999). The absorption of total lipids between stomach and rectum was ca. 92% and, between food and faeces, 97% although the latter figure might be increased by nutrient leaching. Typical ADCs for lipid are in the range 86-97.5%, although values as low as 82% have

been reported (Table 5.4).

The patterns for removal of energy between the gut sections closely followed that of the high-energy lipids. More than 85% of food energy had been removed on reaching the rectum and faecal contents suggested maximal ADC of 96%. Typical expected values are in the range 85-95% (Table 5.4).

Absorption of carbohydrate was poor in comparison to protein and lipid, which probably reflects the low carbohydrate content present in the natural prey. ADC values indicate that considerable addition of carbohydrate occurred in the stomach, as well as the anterior intestine, probably as mucus (Kapoor *et al.*, 1975; Ferraris and Ahearn, 1984). Absorption continued in the posterior regions of the gut leading to overall ADC values of 50 –70% (rectum) or 80.5% (faeces). Typical values are about 82% (Table 5.4)

The ADC values obtained here are likely to vary under different conditions. Although the data from wild fish give generally similar patterns to that from laboratory fish, the uncontrolled diet led to anomalies (*e.g.* negative ADC values in the mid intestine) which suggest the field method is less trustworthy. ADC values may be affected by ration size as well as diet type (Fernández *et al.*, 1998).

Similar studies have produced contradictory conclusions, ranging from those that have found a clear effect of ration size on ADCs of several diet components to other studies that have not found such a decline. Windell (1978) did not find any effect of ration size on the ADCs of protein and lipids but did observe a reduction for carbohydrate energy increased ration size in rainbow trout. In contrast, Cui *et al.* (1994), using a faeces collection method from grass carp, did not find changes in ADCs with ration for energy, although they did find increased ADC for dry matter at the highest (*ad-libitum*) ration and lower ADC for protein at the lowest ration used. β-carotene was used as exogenous marker to study absorption efficiency in Antarctic spiny plunderfish fed on their natural diets (Antarctic krill and amphipods) at very low temperatures (0.5–1.5°C); increased ration size resulted in higher absorption efficiency despite increased GER (Boyce *et al.*, 2000).

Table 5.4. Comparisons of the nutrient apparent digestibility (%dry weight) in various carnivorous fish species.

Species	Diets	Sample Acquisitions	Nutrient Digestibility (% dry wt)				References
			Protein	Lipids	Carbohydrate	Gr.Energy	
Anguilla rostrata	Fish meal (herring)	Food -faeces	90.7	-	-	90.3	Tibbetts *et al.*, 2000
Clarias gariepinus	Fish meal	Food -faeces	-	-	-	90	Hossain, 1998
Dicentrarchus labrax	Fish meal	Food -faeces	93	-	-	91	Gomes Da Silva and Olivia-Teles, 1998
Gadus morhua	Fish meal (herring)	Food - stomach		-	-	85-95	dos Santos and Jobling, 1988
Gadus morhua	Fish meal (Saithe)	Ant. Int -mid.Intestine	91	82	-	-	Lied *et al.*, 1982,Lied and Njaa, 1982 Lied and Lambertsen, 1985
Hippoglossus hippoglossus	Fish meal	Food -faeces	84.1	97.5	82.7	88.4	Grisdale-Helland and Helland, 1998
Merlangius merlangus	Fresh Sprats	Food -faeces	94.1	96.6	80.5	95.8	Present study
Oncorhynchus mykiss	Fish Meal (herring)	Food - faeces	92	97	-	91	Cho *et al.*, 1985
Oncorhynchus mykiss	Meat + bone meal	Food -faeces	70	-	-	-	Dimes and Haard, 1994
Pleuronectes platessa	Cod muscle	Food -faeces	91	-	-	-	Cowey *et al.*, 1974
Salmo salar	Fish meal (herring)	Food -faeces	82	97	-	-	Espe *et al.*, 1999
Salmo salar	Fish meal	Stomach -ant.Intestine	-	94	-	-	Røsjø *et al.*, 2000
Salmo salar	Fish meal	Food -faeces	82.5	94	-	-	Sveier *et al.*, 1999
Salvelinus alpinus	Isoenergetic diet	Food -hindgut	-	88	-	-	Olsen *et al.*, 1998
Scienop ocellatus	Fish meal (menhaden)	Food -faeces	77	88	-	95	Gaylord and Gatlin III, 1996
Sparus aurata	Brown fish meal	Food -faeces	90.8	-	-	-	Fernández *et al.*, 1998
Sparus aurata	Fish meal	Food -faeces	83	86	82	-	Nengas *et al.*, 1997

Chapter 6

Gastric secretion of hydrochloric acid and pepsin in whiting *Merlangius merlangus* L.

6.1 Introduction

No information on acid or pepsin secretion has so far been reported for whiting. In Chapter 1, the work of Seyhan (1994) on gastric emptying rates was compared with that of Andersen (1998). Seyhan used small meals and found that digestion rate increased in direct proportion to meal size so that gastric emptying time (GET) remained constant. In contrast, Andersen reported that larger meals were not fully compensated by increase in digestion rate so that GET was prolonged as meal size increased.

Gastric digestion and emptying depends on two main processes. On one hand, stomach distension causes reflex muscular contractions (Grove and Holmgren, 1992a,b) whilst simultaneously stimulating gastric secretion. It was suggested that stomach distension should produce a stimulus proportional to the cube root of meal volume and that secretion amount should be limited by the surface area of the gastric epithelium (or $BW^{2/3}$; see Chapter 1). This Chapter investigates gastric secretion in the stomach of whiting fed with pieces of sprat to test if acid and enzyme outputs support either of the contrasting conclusions of Seyhan and of Andersen.

6.2 Materials and methods

This experiment utilised four 250L tanks with constant seawater flow and aeration. The range of ambient temperature during the course of experiment was between 11.1–16.9°C. Each tank contained two fish, with a plastic mesh partition to accommodate one fish in each half. Fish were selected so that each tank contained whiting of similar size in the range 150–250g. They were acclimatised in the tanks for a week prior to the start of the experiment. During this period, the fish were offered a few pieces of chopped sprat every alternate day. For the main experiments, an initial plan was to take gastric samples 1, 2, 3 and 4h after feeding. Three meal sizes of sprat were chosen: 1, 2.5 or 4g. The fish were deprived of food for 3 days prior to a test feeding to allow stomachs to empty. Subsequently the same meal sizes were used in an extended experiment to take samples 12h and 24h after feeding. Despite careful handling, it was not possible to keep the same individual experimental fish for use throughout the long experimental period. There were several cases where fish became stressed by repetitive handling. Such fish were discarded and replaced by other acclimatised fish.

6.2.1 Feeding and gastric acid sampling

The test meal was given to the free-swimming fish and, a few minutes later, the fish were anaesthetised following the procedure described in section 2.7.1, Chapter 2 (page 55). A slightly moist, plastic sponge of known weight was inserted 5 min later and the fish returned to the tank. The sponge containing gastric juice was retrieved at the selected time by re-anaesthetising the fish, the juice were squeezed out and a sample immediately titrated to determine acid content (section 2.7.1, Chapter 2). The experiments were repeated for different meal sizes.

6.2.2 Pepsin enzyme assay

When each sample of undiluted gastric juices was squeezed from a sponge, 50μl was pipetted into 1.5 ml plastic Eppendorff tube containing 0.95 ml of 0.01 N HCl. These solutions were immediately frozen at -18°C and analysed within 24h. Pepsin determination was carried out based on the protocol described in section 2.7.2 in Chapter 2 (page 58). The method used haemoglobin as substrate and end products were assayed with Phenol Reagent (Folin and Ciocalteu). The standard curve of Absorption (y) based on known concentration of pepsin (x) was **y = 0.0035x + 0.0652** which was used to calculate the μg pepsin/ml equivalent from the gastric samples.

6.3 Data Analysis

6.3.1 Data presentation and interpretation

In Chapter 1 (Section 1.5, page 15-22) a simple model of the process of gastric digestion was proposed whereby an underlying exponential decrease in stomach contents after a meal was interrupted by inhibitory feedback by nerves and/or hormones from the anterior intestine to produce a "power curve". The stimulus for gastric secretion was proportional to the cube root of the fullness of the stomach:

$$\frac{dS}{dt} \quad \alpha \quad \left(\frac{S_o}{S_{\max}}\right)^{0.333}$$

and the secretory response was proportional to the surface area of the gastric epithelium. These two assumptions can be combined using a simple model of the stomach geometry to suggest the relative levels of gastric secretion (acid, pepsin) that

might be expected when fish of different body weight (100-700g) ingest meals of known size (from 1 to 4g). The stomach was considered to be an open cylinder completed at one end (the pyloric sphincter) by a hemisphere. The stomach expanded isometrically as meal size increased to a maximum volume of 8 ml for each 100g of body weight. The calculation details are shown in Appendix 6.3 whilst Fig 6.1 predicts the relative amounts of acid or pepsin that might be released. This model can be used as a template for comparison with observed measurements of acid or pepsin from the whiting.

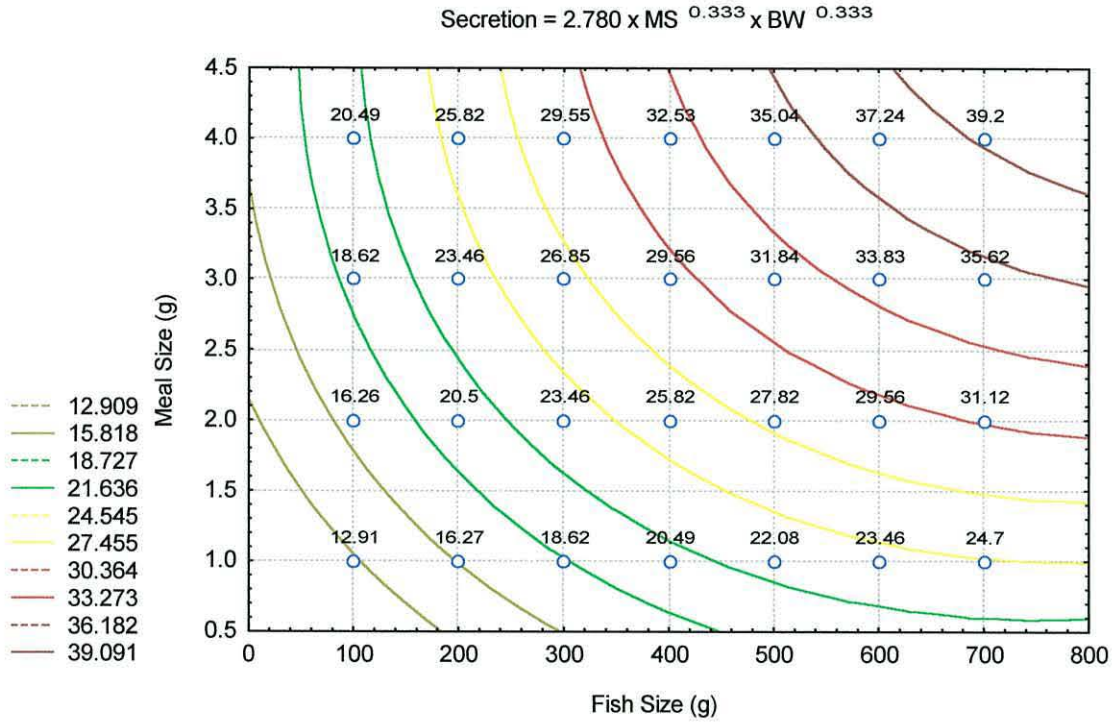


Fig. 6.1. Predicted pattern of relative secretion levels of gastric juice components (acid, pepsin) if the response is caused by the stimulus (Meal sizes, MS) of linear stretch acting on available surface area of gastric mucosa (as a function of fish body weight, BW) (described in Appendix 6.3).

Pepsin and acid measurements were stored using a Corel Quattro Pro (Ver. 8) spreadsheet and classified by fish size, meal size and time since feeding together with the measurements of gastric acid and enzyme production (Appendix 6.1). Contours of either gastric acid (Mequiv. wt. Total HCL x 10^3) or pepsin (µg/ml equiv. min^{-1}) (represented by Z) secreted by fish of weight (X, grams) fed with fixed meal size (Y, grams) for each selected time since feeding were fitted and drawn using the surface area model $Z = b1 . b2 (Y^{0.333}) . b3 (X^{0.333})$. The graphic program Statistica (StatSoft, 1995) was used to obtain non-linear estimations of b1, b2 and b3 values followed by plotting a response surface with contours, so that the effects of fish size and meal size on acid or enzyme production could be visualised and compared with the model predictions. Empirical description of the pattern of responses was obtained using a quadratic model $Z = A + BX + CY + DX^2 + EXY + FY^2$, from which surface response contours were calculated and superimposed on the predictions of the fullness/surface area equations.

6.3.2 Statistical analysis

For statistical analysis, it was appropriate to collect the data into three size groups of fish: small (150–249g), medium (250–379g) and large (380–550g). Pearson's correlation coefficient was used to analyse the relation between gastric acid and enzyme pepsin secretion by different size groups of fish. Raw data sets for gastric acid and pepsin enzyme production were first tested for normality (Anderson-Darling test) and homogeneity of variance (Levene's test). Data were found not normally distributed even after transformation. This meant that a sound statistical inference - that simultaneously accounted for the effects of fish size and meal size on gastric acid and pepsin production - was not possible. All basic statistical analyses were performed using Minitab Ver. 12.23 statistical package (Minitab Inc., 1999).

6.4 Results

6.4.1 General findings

The amount of gastric juices (ml) extracted from the whiting of various sizes, fed on different fixed meal sizes and sampled at different times since feeding, were as shown in Table 6.1. The volume of juice which could be recovered from small fish (150 – 250g: 202 ± 8g; n = 13) was on average 1.43 ± 0.33 ml, from medium fish (251–379g; 300 ± 17g; n = 7) 1.50 ± 0.08 ml, and from large fish (380 – 550g; 470 ± 29g; n = 5) 2.28 ± 0.03 ml. A progressive increase in the amount of gastric secretion was observed during the first two to four hours after feeding for all size groups: the amount of gastric juice recovered was from 0.3 ml up to 3.55 ml. The amount of juice recovered from the small fish after 12 to 24h was usually less (Table 6.1).

Table 6.1 Volume of gastric juices (ml) extracted from fish of various sizes fed with stated meal sizes collected at different times since feeding. Small: 150-250g, Medium 251-379g, Large 380-550g.

Time Since Feeding (h)	**1**			**2**			**3**			**4**			**12**			**24**		
Meal size (g)	1	2.5	4	1	2.5	4	1	2.5	4	1	2.5	4	1	2.5	4	1	2.5	4
Fish Size (g)	0.70	0.55	1.00	0.45	0.85	1.10	2.40	2.00	0.80	1.35	2.10	1.35	1.50	1.40	1.80	1.30	1.40	2.00
Small	1.60		1.10	1.40	0.80			0.85	0.60			2.40	1.90	2.30	1.40	1.30	2.00	2.00
	0.30				1.90				2.40			0.80	0.80	1.00	1.30	2.40	2.10	1.60
	1.00													1.90				
Mean	**0.90**	**0.55**	**1.05**	**0.93**	**1.18**	**1.10**	**2.40**	**1.43**	**1.27**	**1.35**	**2.10**	**1.52**	**1.40**	**1.65**	**1.50**	**1.67**	**1.83**	**1.87**
SE	**0.27**		**0.05**	**0.47**	**0.36**			**0.58**	**0.57**			**0.47**	**0.32**	**0.28**	**0.15**	**0.37**	**0.22**	**0.13**
	1.70	1.40	2.90	2.20	1.20	1.00	1.70	0.90	0.40	1.05	1.40	2.60						
Medium		1.20	0.75	0.95	1.10	1.10	1.80	1.35	1.90	1.00	2.10	1.20						
		1.00	1.20	0.75		1.10	1.20	3.00		1.10	2.70							
		1.60				1.70	0.80			2.50	2.40							
Mean	**1.70**	**1.30**	**1.62**	**1.30**	**1.15**	**1.23**	**1.38**	**1.75**	**1.15**	**1.41**	**2.15**	**1.90**						
SE		**0.13**	**0.65**	**0.45**	**0.05**	**0.16**	**0.23**	**0.64**	**0.75**	**0.36**	**0.28**	**0.70**						
	3.20	2.00	2.40	2.00	3.30	2.70	1.70	1.50	3.20	1.90	1.60	3.10						
Large	2.00	1.70	1.40	2.00	2.80	2.00	2.35	1.25	3.55	3.00	2.30	2.35						
	1.80	3.00	1.60	3.50	2.50	1.40	1.75	2.50	2.25	1.30	2.05	3.00						
Mean	**2.33**	**2.23**	**1.80**	**2.50**	**2.87**	**2.03**	**1.93**	**1.75**	**3.00**	**2.07**	**1.98**	**2.82**						
SE	**0.44**	**0.39**	**0.31**	**0.50**	**0.23**	**0.38**	**0.21**	**0.38**	**0.39**	**0.50**	**0.20**	**0.24**						

Gross Secretions: Mean ± SE

Small: 1.43 ± 0.33

Medium: 1.50 ± 0.08

Large: 2.28 ± 0.03

If the stimuli which cause gastric secretion apply equally to release of acid and of pepsin, then the observed levels of these two components might be correlated. Smit (1967) however reported that release of these two components was independent.

Graphs of the amounts of acid secreted and pepsin concentration in the same samples are shown in Figs. 6.2(a-e); the raw data is in Appendix 6.1 and 6.2. One hour after feeding (Fig 6.2a), the overall pattern of gastric acid output against pepsin activity was erratic. The small fish produced small amounts of acid and pepsin, which were not related to meal size. Indeed, there were no significant correlations between gastric acid secreted and pepsin concentration. Similar responses were observed in the medium and large groups of fish.

After 2h (Fig 6.2b), fish of all size groups started to show good responses; the levels of gastric acid and pepsin secretion were increased. A significant correlation of gastric acid production per-unit pepsin concentration was observed in medium size fish ($P<0.05$) but no significant correlations were observed amongst the small and larger fish. There was some indication for small fish that secretion levels (acid, pepsin) increased with meal size at this time but this was not apparent for larger fish.

At 3h after feeding (Fig. 6.2c), levels of acid and pepsin increased further. The generally erratic correlation between gastric acid and pepsin secretion was maintained although the results for the large fish exhibited a significant correlation ($p<0.05$). Again, no regular relation between meal size and secretion levels of acid or pepsin could be detected.

After 4h, the levels of acid and pepsin had increased further but there was still poor correlation between these components, save for that of small fish ($p<0.05$). However, meal size did not clearly affect the amount of these that were secreted.

Samples were taken from small fish at 12 and 24h after feeding all three meal sizes (Fig 6.2e). There was no correlation between the two components of gastric secretion and neither was related to meal size. However by this stage of the experiment the fish may have become stressed by the continued handling, which might affect the findings.

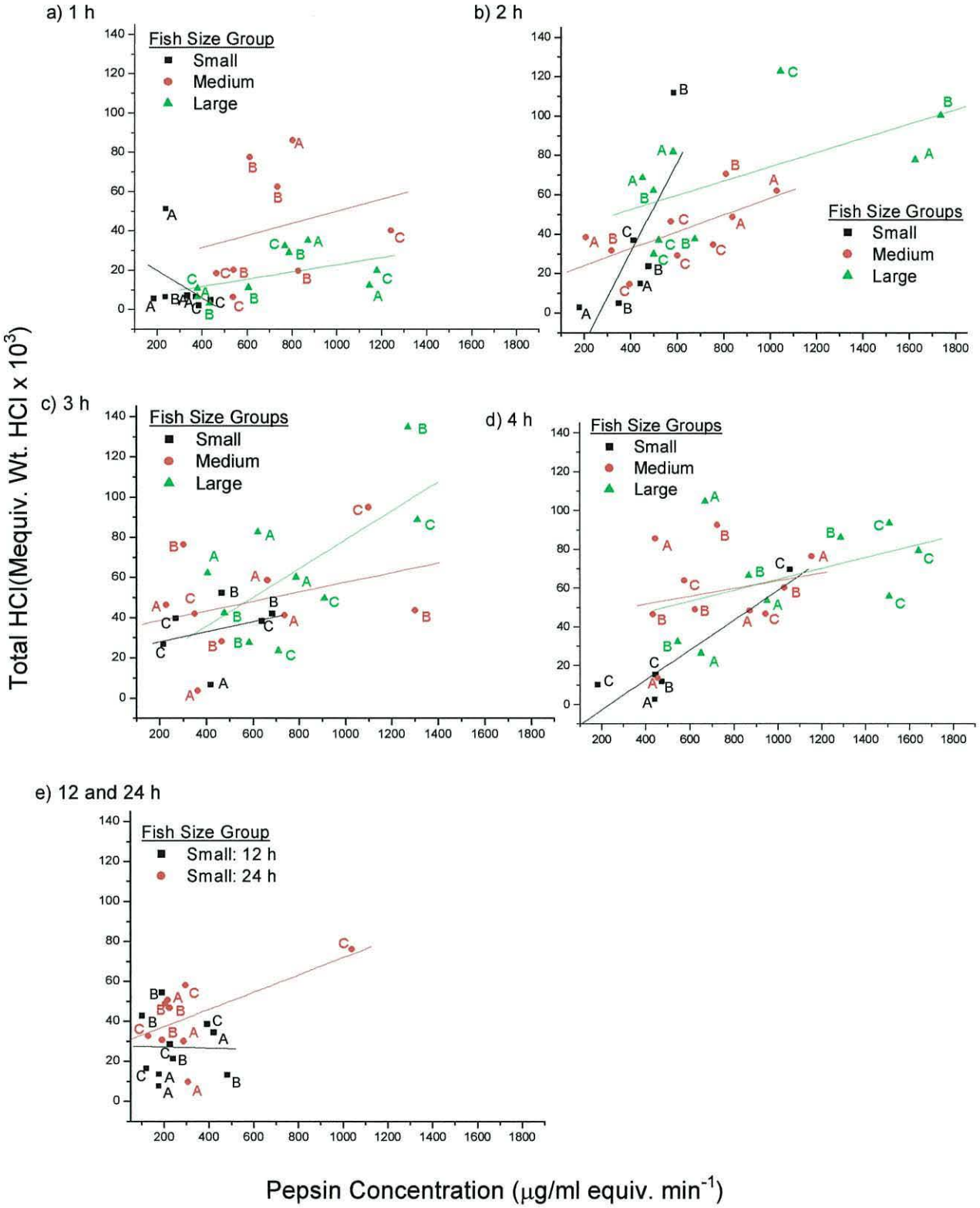


Fig. 6.2. Correlations between hydrochloric acid and pepsin concentrations in individual samples of gastric juice taken from whiting of various sizes (small = 150 - 249g, medium = 250-379g, large = 380-550g), fed different meal sizes (A = 1g; B = 2.5g; C = 4g) at different times after feeding.

6.4.2 Modelling Gastric acid and pepsin production in relation to fish size and meal size at different time since feeding.

Because there was little correlation between acid output and pepsin content, these two components of the gastric juice will be treated separately. The data presented in Fig 6.2 a-e can be presented to compare the patterns of gastric acid production (Mequiv. wt. acid x 10^3) and pepsin production (µg/ml equiv. min^{-1}) with the relative predictions made by the fullness/surface area model introduced in the Methods section (Figures 6.3a-d and 6.4a-d).

One hour after feeding, the highest acid secretion responses were found mostly in medium size fish (86 Mequiv. wt. acid x 10^3) in comparison to small and large fish with a gross mean around 24 Mequiv. wt. acid x10^3. Meanwhile, active pepsin production was already shown by fish of all size groups but particularly among the medium and large whiting (737–1242 µg pepsin/ml equiv. min^{-1}).

At 2h since feeding (Fig. 6.3b; Fig.6.4b), production of gastric acid (49 Mequiv. wt. acid x10^3) and pepsin (652 µg pepsin/ml equiv. min^{-1}) was higher especially among the larger fish (for acid production) and among the medium and larger fish (for pepsin production). Greatest increases in pepsin production (>1500 units) were observed in larger fish fed 1 to 2.5 g of sprat.

After 3h (Fig 6.3c; Fig. 6.4c), the mean amount of gastric acid and pepsin secretions had apparently stabilised for fish of all size groups with gross mean production around 50 Mequiv. wt. acid (x10^3) for acid and 638 µg pepsin/ml equiv. min^{-1} for pepsin.

This pattern persisted at 4h since feeding (Figs 6.3d; 6.4d), mean production of acid was 53.8 Mequiv. wt. (x10^3) and similar patterns of pepsin production were also observed (813 µg/ml equiv. min^{-1}).

Close inspection of Figs 6.3 b-d shows that acid contents of the gastric juice generally increased, apparently with both meal size and with fish size, although some aberrant data points existed. For example small fish after 2h had 3 units after a 1g meal size, increasing to 24 (at 2.5g) and 37 (at 4g). The values for medium fish were 38, 70 & 46 and for large fish 78,100 and 123 units respectively. A similar trend was seen between meal size, fish size and pepsin content (Fig 6.4 b-d).

Fig. 6.3 (a-d). Total HCl content (Mequiv. wt. x 10^3) of the recovered gastric juice from whiting of different sizes fed varying meal sizes of sprat. Coloured contours are the predicted pattern (scaled from Fig. 6.1) and black contours show the observed responses fitted to a descriptive quadratic model.

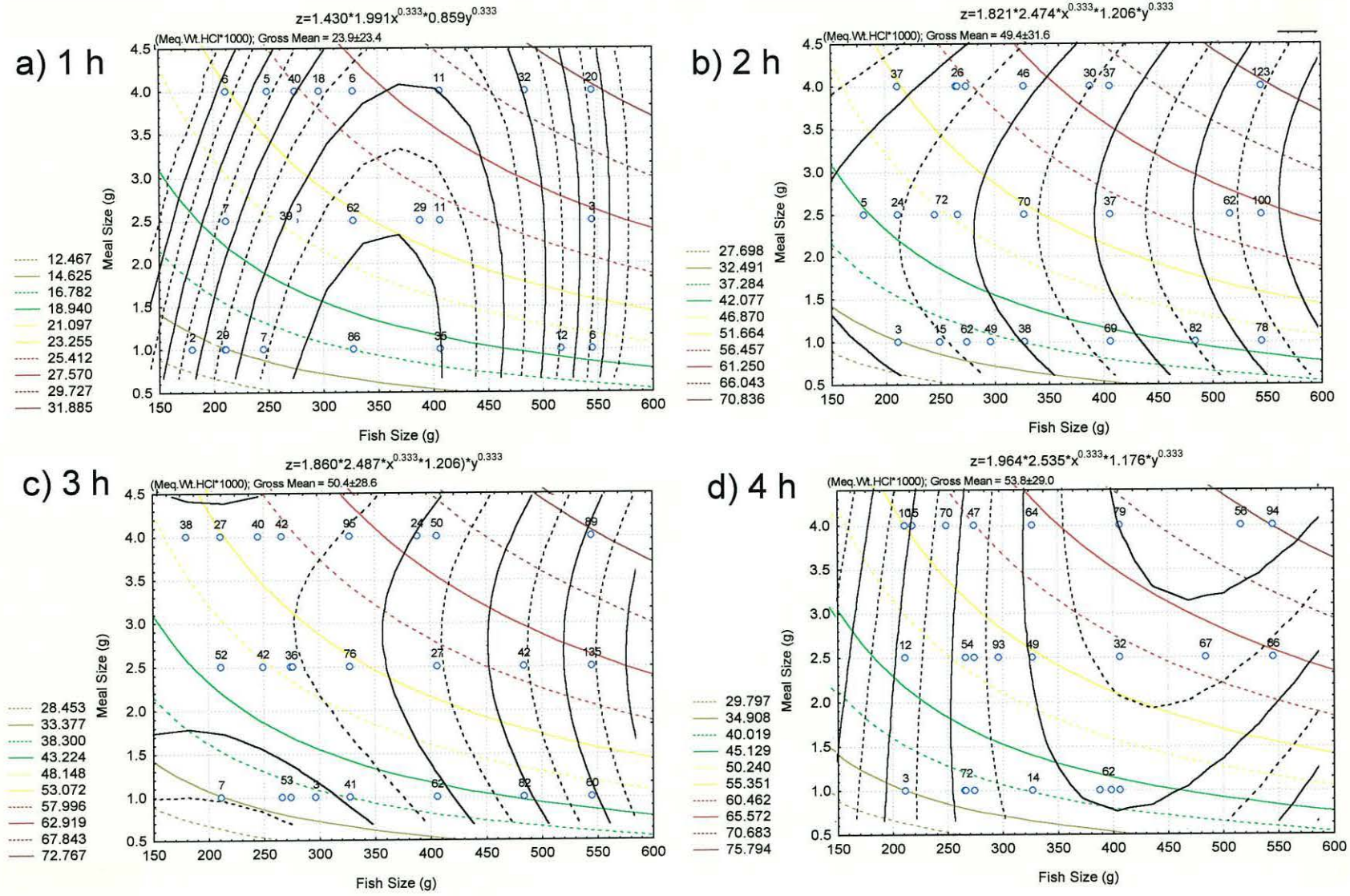
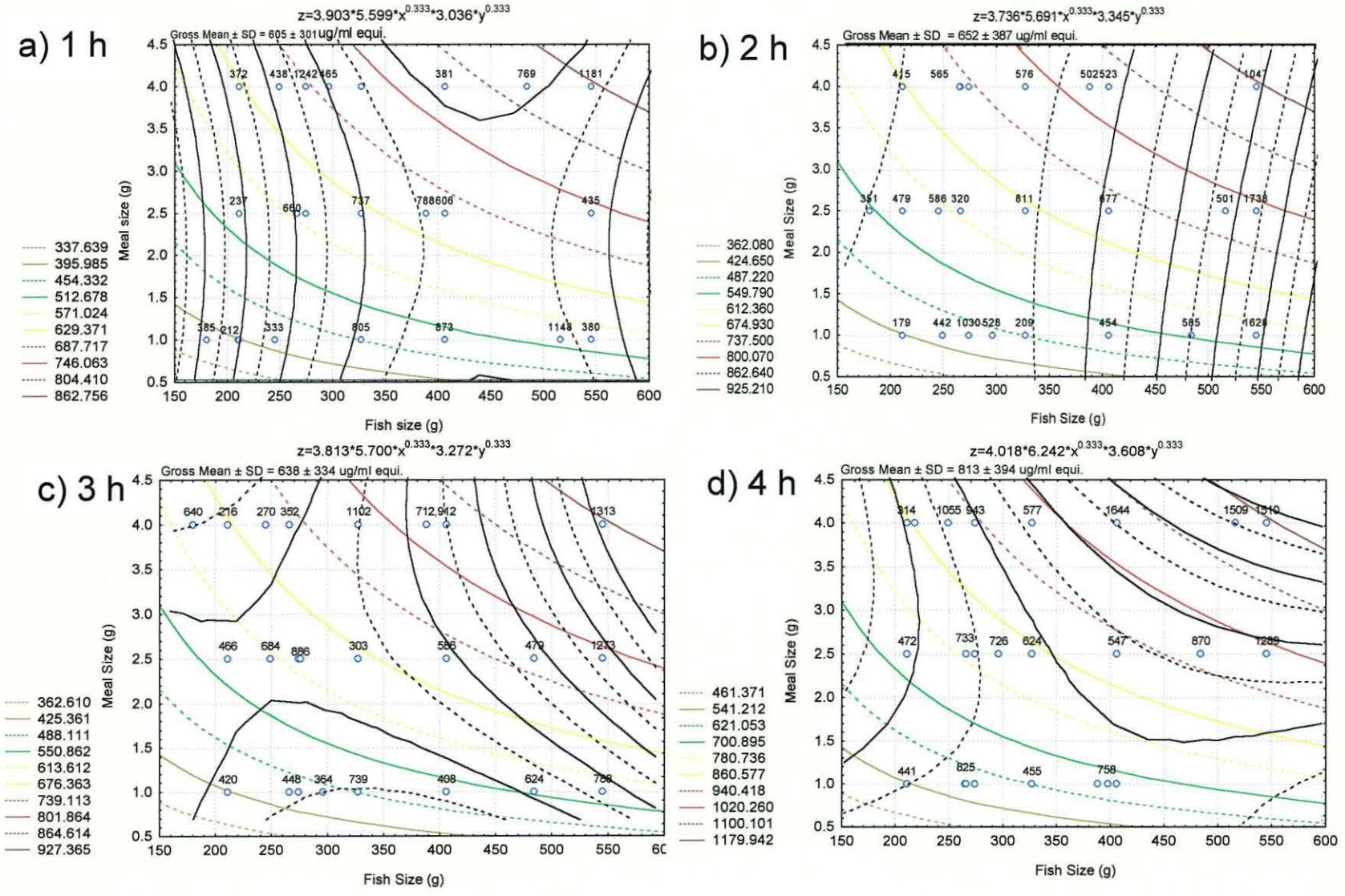

a) 1 h
z=1.430*1.991x^0.333*0.859y^0.333
(Meq.Wt.HCl*1000); Gross Mean = 23.9±23.4
12.467
14.625
16.782
18.940
21.097
23.255
25.412
27.570
29.727
31.885
Meal Size (g)
Fish Size (g)
b) 2 h
z=1.821*2.474*x^0.333*1.206*y^0.333
(Meq.Wt.HCl*1000); Gross Mean = 49.4±31.6
27.698
32.491
37.284
42.077
46.870
51.664
56.457
61.250
66.043
70.836
Meal Size (g)
Fish Size (g)
c) 3 h
z=1.860*2.487*x^0.333*1.206)*y^0.333
(Meq.Wt.HCl*1000); Gross Mean = 50.4±28.6
28.453
33.377
38.300
43.224
48.148
53.072
57.996
62.919
67.843
72.767
Meal Size (g)
Fish Size (g)
d) 4 h
z=1.964*2.535*x^0.333*1.176*y^0.333
(Meq.Wt.HCl*1000); Gross Mean = 53.8±29.0
29.797
34.908
40.019
45.129
50.240
55.351
60.462
65.572
70.683
75.794
Meal Size (g)
Fish Size (g)

Fig. 6.4 (a-d). Pepsin content (μg pepsin/ml equiv. min^{-1}) of the recovered gastric juice from whiting of different sizes fed varying meal sizes of sprat. Coloured contours are the predicted pattern (scaled from Fig. 6.1) and black contours show the observed responses fitted to a descriptive quadratic model.


a) 1 h
z=3.903*5.599*x^0.333*3.036*y^0.333
Gross Mean ± SD = 605 ± 301 ug/ml equi.
Meal size (g)
Fish size (g)
337.639
395.985
454.332
512.678
571.024
629.371
687.717
746.063
804.410
862.756
b) 2 h
z=3.736*5.691*x^0.333*3.345*y^0.333
Gross Mean ± SD = 652 ± 387 ug/ml equi.
Meal Size (g)
Fish Size (g)
362.080
424.650
487.220
549.790
612.360
674.930
737.500
800.070
862.640
925.210
c) 3 h
z=3.813*5.700*x^0.333*3.272*y^0.333
Gross Mean ± SD = 638 ± 334 ug/ml equi.
Meal Size (g)
Fish Size (g)
362.610
425.361
488.111
550.862
613.612
676.363
739.113
801.864
864.614
927.365
d) 4 h
z=4.018*6.242*x^0.333*3.608*y^0.333
Gross Mean ± SD = 813 ± 394 ug/ml equi.
Meal Size (g)
Fish Size (g)
461.371
541.212
621.053
700.895
780.736
860.577
940.418
1020.260
1100.101
1179.942

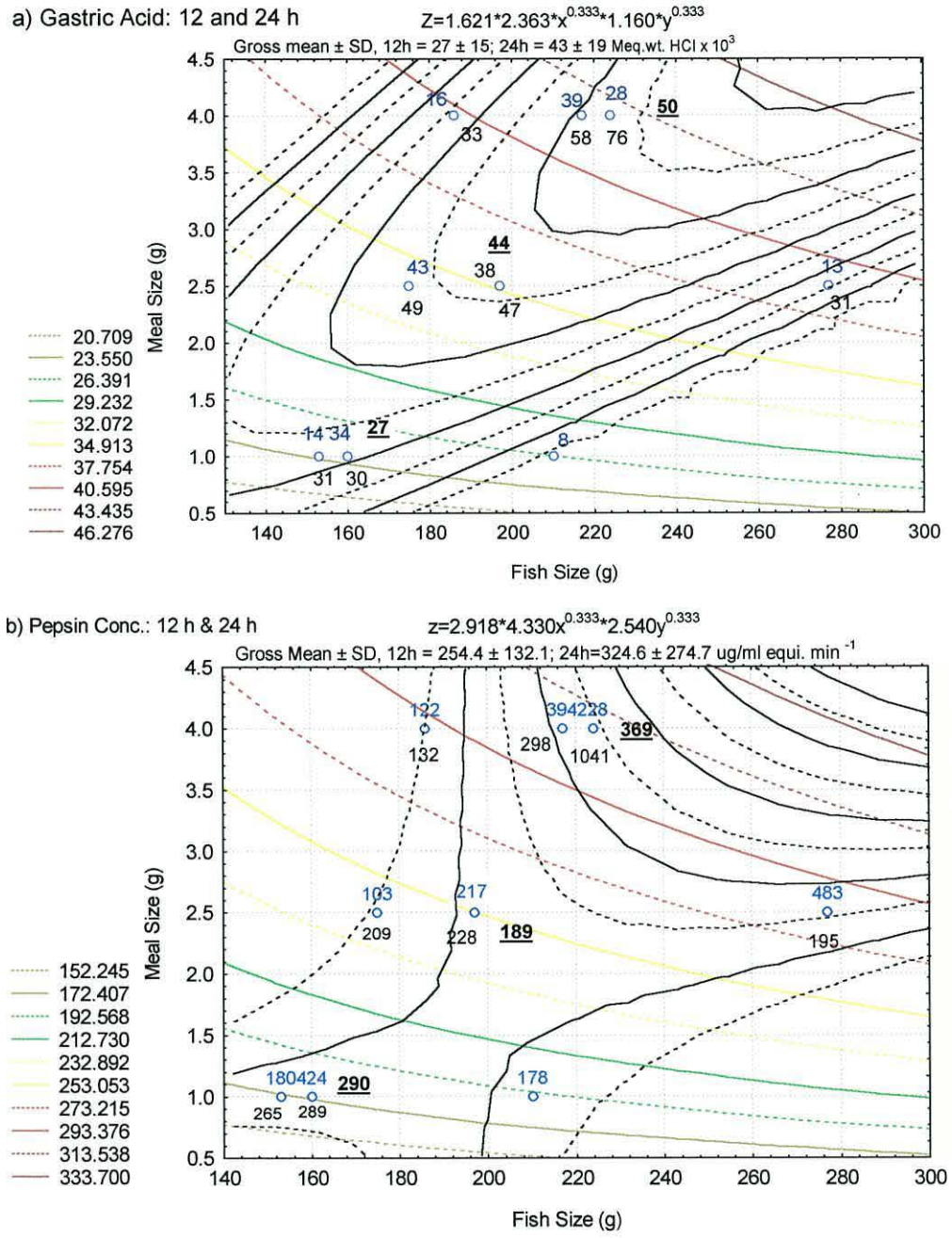


Fig. 6.5. (a,b). Acid and pepsin contents of gastric juice a) 12h and b) 24h after feeding whiting of various sizes with different meal sizes of sprat. Contours constructed as in Figs. 6.3 and 6.4. Levels of both constituents were less that those at 4h after feeding and again the observed patterns – especially for medium and small fish secreting acid – showed only a weak effect of meal size on secretion rates.

Finally, it was possible to obtain gastric juice samples after 12 and 24h from the small size group of whiting. The limited data-set revealed that average acid (27 & 43 units) and pepsin (254 & 325 units) levels had decreased from earlier values. Similar erratic patterns for gastric acid and pepsin against meal size and fish size were found with no clear effects of meal size either on gastric acid or pepsin production (Figs 6.5a,b).

Figures 6.6(a,b) summarise the mean total gastric acid and pepsin production by small, medium and large size groups of whiting fed on the three different meal sizes, independently of the time since feeding. The strong effect of fish size and the limited effect of meal size is clearly shown. However, the available data does allow a test of part of the gastric emptying model which began this chapter. The hypothesis that gastric secretion depends on stomach surface area (proportional to $W^{2/3}$) is confirmed in Figure 6.7.

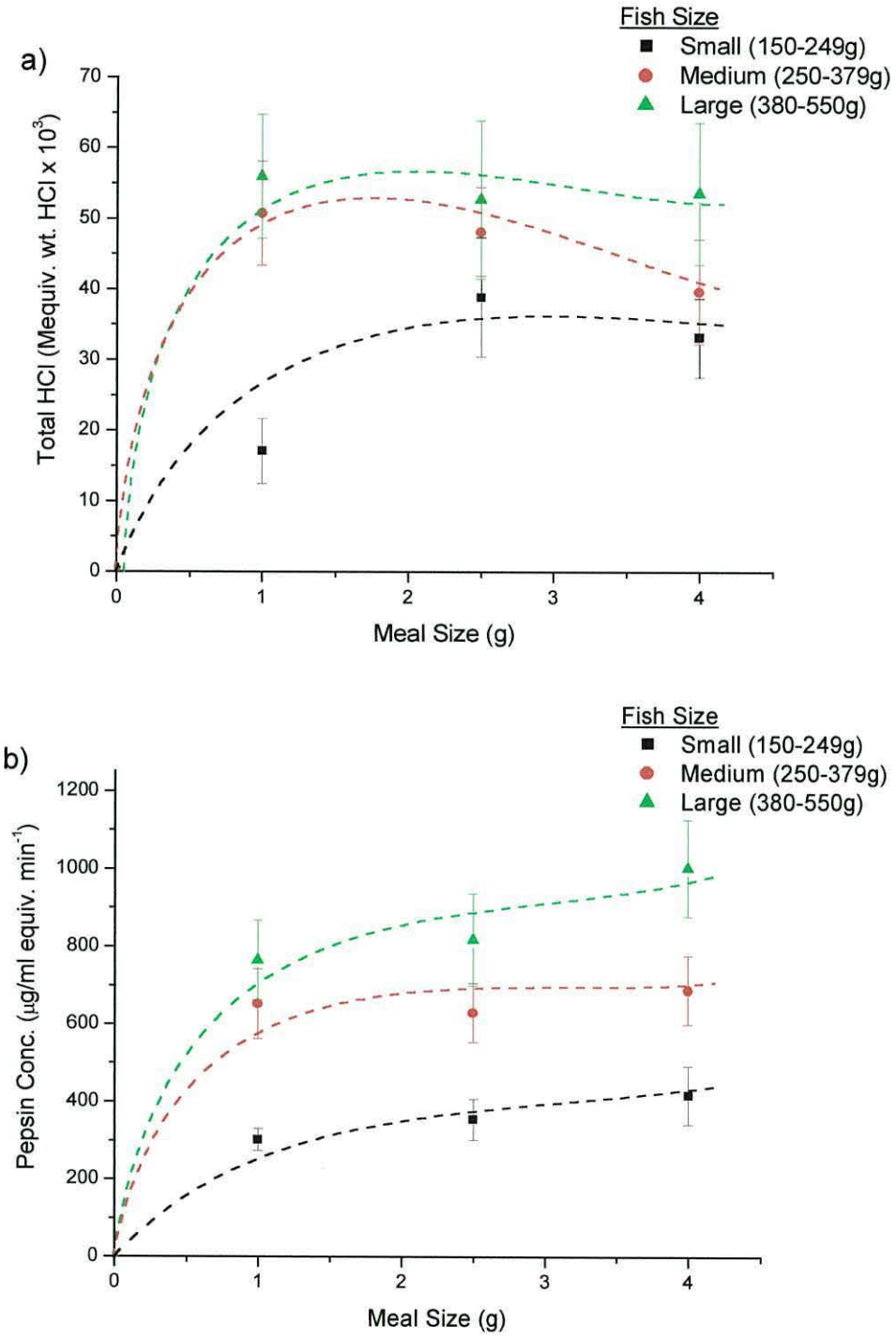


Fig. 6.6 (a,b). Effects of meal size on gastric secretion of (a) HCl and (b) pepsin in small, medium and large whiting. The prediction that the amount of acid and/or pepsin would be proportional to meal size within a size group was not supported. In contrast, the secreted amounts, which rise asymptotically, are more consistent with Andersen's observations on gastric digestion curves (1998) than with Seyhan's (1994). Lines were fitted by eye.

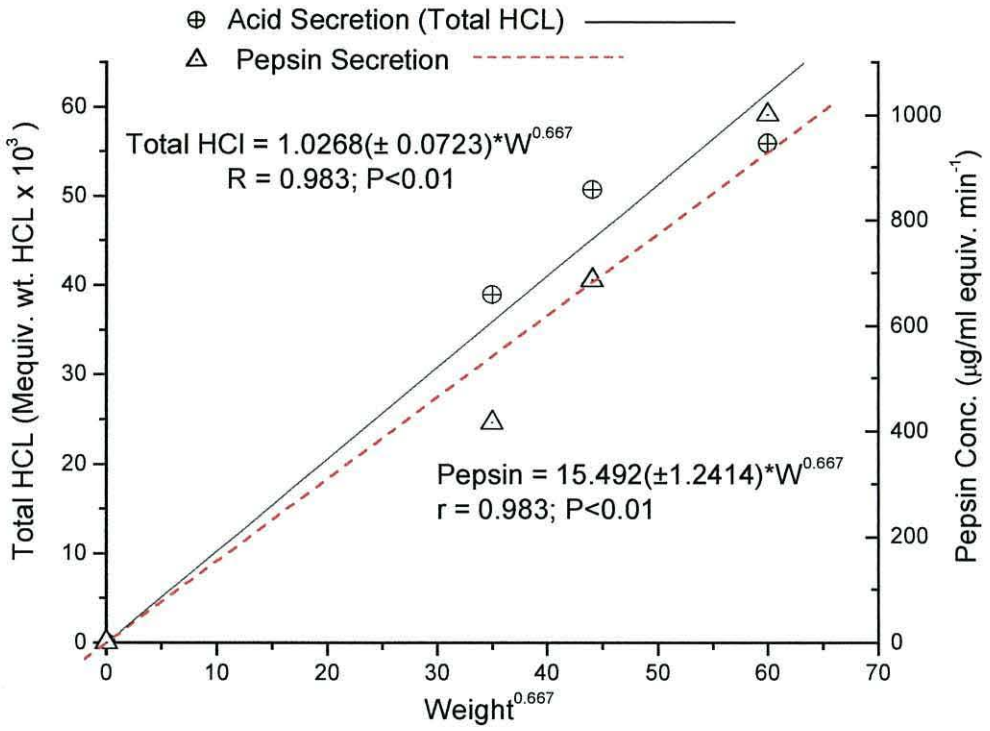


Fig. 6.7. HCl and pepsin production in relation to transformed average fish size. The response is similar to the surface area limitation used in the physiological model for gastric digestion ($Y = aW^{0.667}$).

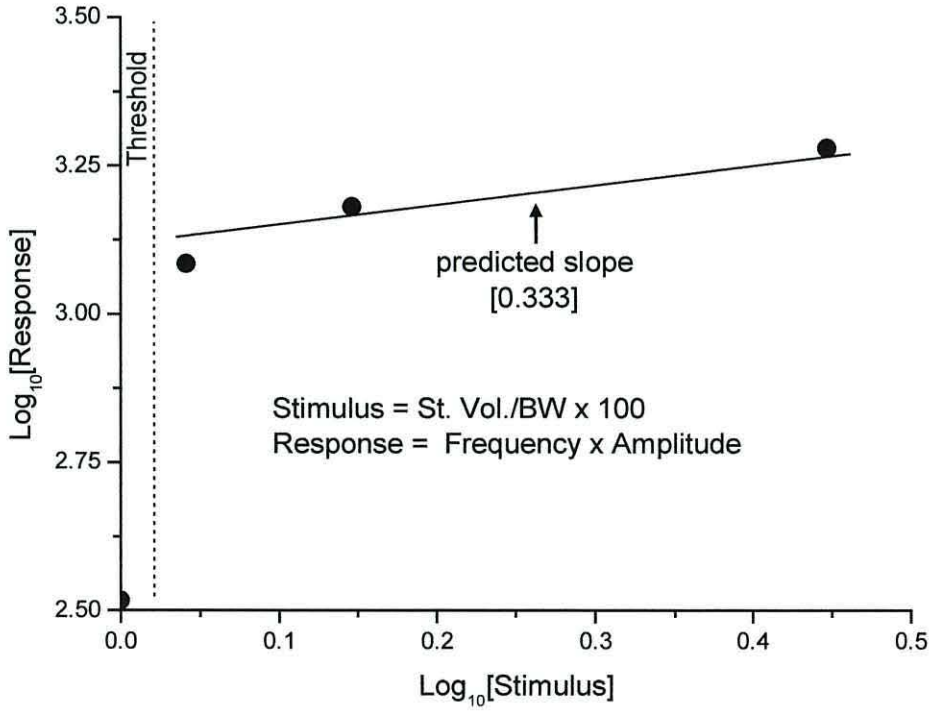


Fig. 6.8. Response of gastric smooth muscle related to gastric distension (ml $100g^{-1}$) in *Limanda limanda* (recalculated from Jobling, 1974). Above a small threshold, the stimulus scales as a cube-root event of the type used in the physiological model for gastric digestion (Stimulus = a(St. Vol./BW)$^{1/3}$.

6.5 Discussion

The present study showed that it took between 2–3h for the whiting to achieve maximum gastric acid and pepsin production within the temperature range 11.1–16.9°C. In addition, it was found that the amount of acid released was not in proportion to the amount of pepsin enzyme secretion although they are both released by the same 'oxynticopeptic' cell. Smit (1964) showed that release of acid and pepsin were not necessarily directly coupled. Information on acid and enzyme releasing mechanisms is scarce although it has been well documented that gastric juice secretion, as well as muscular contractions, occur in fish following distension of the stomach by ingestion of food (see Holmgren *et al.,* 1983; Holmgren, 1989; Jönsson, 1994; Grove and Holmgren, 1992a,b). The food items probably act as multiple stimuli. Increase in stomach contents stretches the stomach wall and activates branches of sensory cells leading to changed or increased muscular contractions. The nutrients of the food may also elicit secretion by activating chemo-sensors. Previous studies to measure gastric acid production in teleosts were based on ingestion of artificial material such as plastic sponges and glass beads which are completely different from their natural prey (Smit, 1967; Norris *et al.,* 1973). The used of artificial materials in such experiments might induce secretion of gastric juices by the "stretch" stimulus without any contribution from bio-chemical factors in the "food". The present study utilised natural prey as the test meal and may reveal more realistic responses of the fish stomach. The method still however required insertion of artificial, soft, highly-absorbent plastic sponges (0.035 g dry wt. $100g^{-1}$ fish wet weight) along with the food, which were used as a gastric juice recovery tool. These pieces were relatively smaller: only 70% the size of the equivalent pieces used by Smit (1967).

The present study revealed that gastric acid production - expressed as total hydrochloric acid strength (Mequiv. wt. acid) - varied depending on fish size and, to a lesser extent, on meal size. The prediction (in Chapter 1) that secretion should vary with body $weight^{2/3}$ has been supported. The results also show that pepsin production reached stable levels earlier than did the acid. In the bullhead at 20°C, after 0.5 hours pepsin had reached 35% of the level observed after 4.5h whilst acid was less than 0.1% of its level (Smit, 1967). The overall pattern of pepsin secretion in the present study is in agreement with the study by Uys *et al.* (1987) who found that protease activity in African catfish *Clarias gariepinus* responded immediately to food intake, reached its maximum 2.5h after feeding and decreased progressively 4h after feeding.

The technique used in this study cannot however be used to monitor the full output of acid and pepsin since some of the stomach contents are emptied soon after feeding (Chapter 3). A fraction of the acid and pepsin will form part of the chyme, will be lost from the stomach and must presumably be replaced by further secretion.

When gastric secretion of acid or pepsin was plotted against meal size (Figs. 6.6a,b), secretion increased asymptotically, not linearly. This means that the present study does not agree with the earlier idea forwarded by Seyhan (1994), at least as far as gastric secretion is concerned. He reported that gastric emptying rate (GER) of small meals (1-6g) is directly proportional to meal size so that gastric emptying time (GET) was constant. It was considered possible that this compensatory response was associated with gastric secretion being directly proportional to meal sizes within this range of meal sizes. This now seems unlikely and, if such compensation for meal size does occur, it must be linked to changes in muscular activity of the gastric smooth muscle and pyloric sphincter which promote more rapid mixing and movement of the food. The work of Norris *et al.* (1973), revealed that the gastric secretions in bluegill (*Lepomis macrochirus*) at time of autopsy (5h after feeding) following ingestion of one glass bead were more acid than found using 2 glass beads. The effect of meal size on the gastric acid secretion in the present study was unclear and could be overshadowed by the strong effect of fish size.

Incidentally, the medium sized fish ingesting 1 and 2.5g sprats showed the highest response of gastric acid production during the first-hour after feeding (36 – 88 Mequiv. wt. acid $x10^3$, Fig 6.3a). This probably reflects an "optimum" state of initial gastric stimulation when compared with small fish (which have a smaller area of secretory epithelium) and large fish (where the a fixed meal is a relatively smaller stimulus). After 3-4h, the large whiting (>450g) – as expected – had released most acid and pepsin following the larger meals but it took time to achieve this. There is also a possibility that decrease of gastric acid secretion over time could be due to gastric secretory reserves becoming exhausted (Norris *et al.*, 1973).

The process of gastric secretion may not be simple. It is possible that the stomach secretes gastric acid in phases during the course of gastric digestion, leading to the observed fluctuation of acid levels detected in the stomach (Figs 6.7a-d). Fluctuations of pepsin production after feeding were observed by Einarsson *et al.* (1996) in the Atlantic salmon. They found that the pepsin activity in both stomach mucosa and digesta of starved and fed fish fluctuated during the course of 20 days. They also found that

ingestion of food caused secretion of pepsin from the stomach mucosa within 1h, which also suggested that pepsinogen was rapidly synthesized and secreted at the onset of feeding.

It was not possible to control water temperature during the six weeks of this study (range between 11.1–16.9°C) and this may also have led to variation in the observed patterns of secretion. Smit (1967) and Phillips (1969) reported that water temperature plays an important role in regulating the amount of gastric juice secretion in teleosts but did not discuss meal size and fish size as controlling variables.

The maximum level of gastric juice secretion in whiting is not known. The present study was specifically designed to test Seyhan's (1994) findings and only utilised maximum meal sizes of 4g of sprat pieces. Determination of the level of gastric secretion when fish of the present size are fed to satiation (*ca* 25-30g) requires further research that would certainly require development of new methods.

Very little work has been attempted to study the reflex motility of the stomach when distended to different degrees. Jobling (1974) attempted to monitor contractile responses of the stomach of dab (*Limanda limanda* L.) to different levels of distension using inflatable intra-gastric balloons and a pressure transducer. His data, although limited, suggests that muscular activity does increase with the cube root of stomach contents but only after a threshold fullness has been reached (Fig 6.8). The findings on the stimulus for motility and the dependence of secretion on gastric surface area support the first two (of the three) terms of the model introduced in Chapter 1 to describe the gastric digestion and emptying process. The third term suggests that gastric juices act on the surface of the food items and gradually erode them to form a chyme (Holmgren *et al.*, 1983). This appears true of the whiting since no partially-digested pieces of food in the anterior intestine were observed in the gastrectomy study (see Chapter 5).

The empirical model presented by Andersen (1998,1999) is closely similar to the present "physiological" model. His work was based on a larger range of meal sizes than Seyhan used. GER did not increase linearly with meal size, suggesting either incomplete compensation for meal size by digestive juice secretion, and perhaps by muscular contractions, which prolonged the gastric emptying time. His model appears more suitable for describing the gastric emptying process and digestion in whiting than the more restricted model of Seyhan.

Chapter 7

Growth performance of whiting (*Merlangius merlangus* L.) during longer-term demand feeding

7.1 Introduction

In this Chapter an attempt is made to study food intake of whiting over longer periods than were tested in Chapter 4. For this study, the whiting needed to adapt to artificial diets (dry pelleted food) to fit requirement of a demand feeding systems. There have been studies on longer-term fish feeding patterns - both in the laboratory and later in aquaculture systems - since the early development of demand-feeders for experimental use in fish (Rozin and Mayer, 1961; Rozin and Mayer, 1964; Adron, 1972; Adron *et al.*, 1973; Landless, 1976; Grove *et al.*, 1978).

The basic principle that is widely adopted in laboratory studies is to train fish to obtain food by pressing a lever, biting a coloured tip rod or entering a selected area of the tank to break a light beam or touch a sensor plate. The "touch and reward" technique proved successful in many species of fish and usually requires 3–4 weeks of intensive training for naïve individuals. Trigger devices coupled to computers have been used to record trout demand-feeding activity (Boujard and Leatherland, 1992b; Cuenca and De La Higuera, 1994), including a combination with PIT-tags to record individual feeding behaviour within a group (Brännäs and Alanärä, 1993,1994). Demand-feeding has also been investigated in large groups of trout and Atlantic salmon kept in full-scale farming conditions, in net cages (Alanärä, 1992a), ponds and tanks (Statler, 1982; Tipping *et al.*, 1986) or in marine net pens (Fernoe *et al.*, 1995).

For aquaculture, the deployment of automatic demand feeders has reduced both labour and feed costs, whilst allowing fish to feed *ad-libitum* with reduced feed wastage. Potential disadvantages of this method include the variation in ability of individual fish to adapt to the equipment as well as the establishment of hierarchies and territorial behaviour around the feeding points. Not all individuals may be able to feed to satiation (McCarthy *et al.*, 1992). Well documented studies for commercially- important fish species exist for rainbow trout *Oncorhynchus mykiss* (Walbaum) (Grove *et al.*, 1978; Alanärä, 1992a; Boujard and Leatherland, 1992a; Alanärä and Brännäs, 1993; Alanärä, 1994), Arctic charr *Salvelinus alpinus* (Christiansen *et al.*, 1992; Brännäs and Alanärä, 1994), sea bass *Dicentrarchus labrax* (Sanchez-Vazquez *et al.*, 1995; Begout Anras, 1995; Madrid *et al.*, 1997; Sanchez-Vazquez *et al.*, 1997; Azzaydi *et al.*, 1998; Aranda *et al.*, 1999) and Atlantic salmon (Juell, 1991; Juell *et al.*, 1993; Juell and Westerberg, 1993; Juell *et al.*, 1994; Juell, 1995a,b; Juell *et al.*, 1995). Investigations have included the influence of time-restriction on demand feeder activation on fish growth (see Alanärä, 1992b). In contrast, the development of hydro-acoustic 'food detectors' – to

detect uneaten pellets at the bottom of tanks or cages - integrated with automated feeders, can limit wastage and improve the apparent growth performance without the need to condition the fish to activate a feeding trigger system (see Juell *et al.*, 1993). There is very little information on longer-term feeding patterns in the whiting. A report by Seyhan *et al.* (1998) compared the dry weight intake of a more natural food (frozen sprat *ca* 75% water, offered continuously) with that for whiting trained to demand pelleted food (5% water) from a newly-designed feeder whose trigger was a narrow beam of infra-red light. The groups of demand-fed fish maintained their daily dry weight intake of pellets (0.8%bw) when compared with the equivalent intake for fish offered frozen sprat manually (0.85%bw). The former group, when held under a 12L:12D regimen at *ca* 11°C, fed rhythmically every 20-22 hours suggesting that appetite returned when average stomach contents had reduced by 60% from a previous meal. These authors did not however report any results for growth of the demand-fed fish.

In the present study, further trials were conducted to monitor voluntary food intake and also growth of whiting in captivity using an improved demand feeder design, again incorporating an infra-red beam as trigger.

7.2 Material and Methods

7.2.1 Experimental procedure

An isolated 4000L experimental tank located in a closed, quiet room was used throughout the experiment. Twenty whiting were transferred into this tank from a laboratory stock, which were caught locally in the coastal waters around Anglesey in the previous six months. Prior to the start, the fish were trained to demand feed for 3 – 4 weeks based on the "response and reward" technique using a red-tipped glass rod (Seyhan *et al.,* 1998). The fish were initially deprived of food for 72 h to maximise hunger, the rod was put into the tank and when a fish touched the red tip, a few pellets were offered to the fish. The fish training was continued twice daily until 50–60% of the whiting stocks were judged to be used to the demand feeding. The diet used throughout the experiment was High Performance trout diet # 50 (Trouw Aquaculture (UK); 5 mm pellets); proximate composition is shown in Table 7.1.

The design of the new automated infrared demand-feeding unit is shown in Fig. 7.1. When the unit was set up, a shorter red-tipped rod was placed into the water between the transmitter and the receiver to attract the fish. Details on the working principle and the development of the demand feeder system were described in Chapter 2.

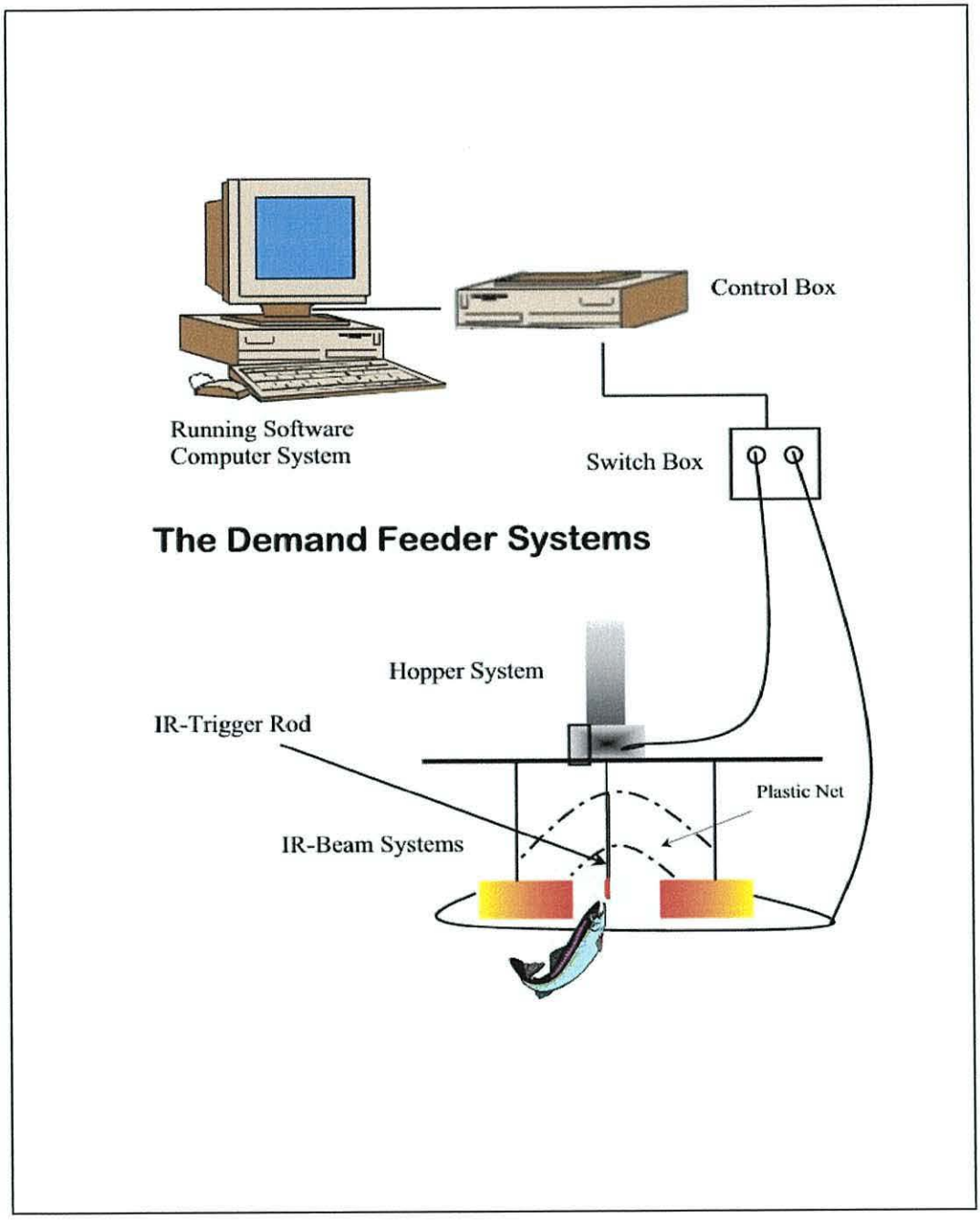


Fig. 7.1. Schematic diagram of infrared demand feeder systems used in the experiment.

Table 7.1. Proximate contents of High Performance trout pellets #50 (Trouw Aquaculture U.K).

Diet Ingredient	Percentage of diet (by weight)
Protein	45
Water	8
Oil	21
Carbohydrate and fibre	16
Ash	10
Phosphate	1.1
Digestible energy	18.88KJ/gram of diet

After the training period all fish were anaesthetised, removed from the experimental tanks and weighed. Subsequent demand feeding experiments were carried out under continuous (24h) lighting; ambient temperature decreased from 21-13.5°C over the 78 days of the subsequent experiment (mid-July 1999 to the first week of November 1999). Although the average delivery of pellets for each actuation of the food hopper was known, unlike the method of Seyhan *et al.* (1998) the amount of food delivered was checked independently. The initial and final weights of pellets stored in the hopper system were recorded at suitable intervals, usually every two days. The weight differences measured the amount of pellets demanded.

Planned trials are not always immediately successful. The first trial in summer was abandoned since there was high mortality of fish because ambient sea-water temperature unexpectedly rose to above 20°C and no suitable cooling systems were available. All dead fish were measured for their length and weight; the durations of their survival since the start of the experiment were also recorded. In addition, an unexpected electronic fault was noticed in early August which led to "dummy triggering" of the hoppers every time certain power plugs were switched on at other points in the laboratory. This was partly caused by imbalance in the loading on the three phases of the electrical supply within the laboratory complex. The demand feeding experiment was temporarily abandoned and the control box system was sent to the electronics department for modification.

As water temperature subsequently declined, training was resumed using the "touch and reward" technique and amounts of pellet consumed recorded. The actual demand feeding experiment was only resumed during the last week of August 1999 with 10 selected, trained fish of known length and weight. To avoid stress and potential injury,

these fish were tranquillised using procedures described in Chapter 2 before measuring them. They were then returned to the main tank and allowed to use the demand feeders for the 78 day period of the study.

Results for growth of whiting in relation to the amount of food eaten were based on the following formulae recently reviewed by Lazo and Davis (2000) unless otherwise stated;

i) Instantaneous growth rate (G, day $^{-1}$)

$$G = \frac{Ln(Wi / Wo)}{ti - to}$$, where W_i = final weight at time t_i and W_o= inital weight at time t_o.

ii) Specific growth rate (SGR, %bw day $^{-1}$) (Houde and Schekter, 1981)

$$SGR = 100 * (e^{G} - 1)$$, where *G* is obtained from (i)

iii) Estimated gross growth efficiency (GGE, %)

$$GGE = 100 * (\frac{Weight\ gained}{Weight of\ food\ eaten})$$

iv) Food conversion ratio (FCR)

$$FCR = \frac{Food\ eaten}{Weight\ gained}$$

7.2.2 Statistical analysis

Results are reported as mean ± standard error (SE) unless otherwise stated. The individual specific growth rates were found to be normally distributed (Anderson-Darling test; Minitab Inc., 1998). The results from the experimental fish were divided into three groups based on body size, so that the means of specific growth rates and their coefficient of variation (CV) could be calculated and compared. The coefficient of variation (CV, Zar, 1984) was used to examine the variability in specific growth rate for individual fish:

$$CV(\%) = \frac{(St.deviation(SGR)\ x\ 100)}{Mean\ (SGR)}$$

A high coefficient of variation for the SGRs among individuals of a group or between groups may mean that factors, such as hierarchical dominance, affect the process of demanding food. Dominant fish could actuate the trigger more frequently compared to others and/or claim the food. Statistical comparisons of mean specific growth rate were made using Scheffe's multiple comparison test (SPSS Inc., 1999).

7.2.3 Analysis of demand feeding data

The following succinct description of our method of periodogram analysis was given by Seyhan *et al.* (1998):

Analysis of the actuations recorded by the recorder to determine feeding rhythms was carried out by the periodogram analysis method using PERIO software (ver. 1.0) developed by A. Aagaard (Institute of Biology, University of Odense, Denmark, 1993). This method is an extension of the earlier method developed by Enright (1965) and later by Williams and Naylor (1967). In this method the number of actuations in each succeeding hour ($X_1, X_2, X_3, \ldots\ldots$) is scanned for possible rhythms. To test for feeding every f h, the data are arrayed as:

$X_1\ X_2, \ldots..X_f$

$X_{f+1}\ X_{f+2} \ldots\ldots X_{2f}$ ---------- (a)

From this array (form estimate) the mean (X):

$$\frac{\sum X_1 + \sum X_2 + \sum X_f}{f} \quad \text{---------- (b)}$$

was calculated. Although many statistical techniques can be employed in the analysis of data collected in a time series, the periodogram is particularly well suited to biological data (Williams and Naylor, 1978). Essentially calculations are made of the variance of $\sum X_i$ around X for a stated length of time or period. A function of this variance in the form of standard deviation is then plotted against period length to produce a graphical periodogram. High values of the periodogram statistics occur when the period under test approximates to the periodicity inherent in the raw data.

The original data set is then randomised and a new periodogram statistic calculated for each period tested. The periodogram of the randomised data approximates a straight line and upper and lower 95% confidence limits are produced. Significant periodicity is assumed when the true periodogram statistic for a given period is greater than the upper 95% confidence limit to the randomised data regression line at that point. The periodogram analysis is regarded as a descriptive statistic rather than one giving a very critical significance value.

7.3 Results

7.3.1 Estimation of the growth performance of whiting fed on High Performance trout diet # 50 (5 mm pellets)

The total amount of pellets consumed by the group of whiting (mean initial wet weight: 155 ± 28g) during the course of the experiment (78 days) was 908g (Table 7.2 and 7.3), although one of the 10 fish died after 58 days. The amount of diet released from the hopper at each actuation was 2.223 ± 0.072(SE) g dry wt. (n=30). Since the total amount of diet consumed was on average 11.641g day $^{-1}$ (Table 7.2), the mean daily food intake for each fish was found to be 1.20g, corresponding to 0.774% body weight.

During the first 46 days of the experiment, temperature fell slowly from 21 to 18.5°C and decreased abruptly to *ca* 15.5°C at the start of October. Total daily intake for the group in this first period averaged only 9.3g day^{-1}. After the sudden temperature decrease and a few days delay (until October 6th) appetite increased dramatically to average 17g day^{-1} despite the loss of one fish on October 18th and a continued decrease of temperature to 14°C.

Table 7.2. Amount of diet consumed by whiting during the demand-feeding experiment. From 21/08/99 – 30/09/99 temperature average 19°C; from 01/10/99 – 3/11/99 it was 14.8°C.

Loading Date	Initial Wt (g)	Retrieval Date	#days Interval	Remaining Weight (dry:-g)	Amount Eaten (dry:-g)
21/08/1999	304.5	27/08/1999	6	230.1	74.4
27/08/1999	206.1	06/09/1999	10	87.5	118.6
06/09/1999	132.2	22/09/1999	16	29.6	102.6
22/09/1999	102.5	30/09/1999	8	31.5	71
30/09/1999	88.6	06/10/1999	6	26.3	62.3
06/10/1999	80.1	11/10/1999	5	24	56.1
11/10/1999	58.6	14/10/1999	3	2.3	56.3
14/10/1999	70.2	16/10/1999	2	18	52.2
16/10/1999	80	18/10/1999	2	26.8	53.2
18/10/1999	76.6	21/10/1999	3	11.6	65
21/10/1999	110	25/10/1999	4	55.1	54.9
25/10/1999	149.2	28/10/1999	3	72	77.2
28/10/1999	148.6	30/10/1999	2	81.3	67.3
30/10/1999	129.2	03/11/1999	4	78.7	50.5
03/11/1999	108.2	07/11/1999	4	87.2	21
Totals			**78**		**908**
Average diet consumption/day				**11.641 g**	

Table 7.3 shows the growth performance of individual whiting in the group (n was initially 10) voluntarily demand-feeding on Trouw High Performance trout diet #50. The observed instantaneous growth rates ranged between 1.366 – 2.629 x 10^{-3}, with a mean value 1.942 ± 0.418 x 10^{-3} day^{-1} and specific growth rates ranged between 0.137–0.263, with a mean value of 0.194 ± 0.042 % wet body weight day^{-1}. The accumulated weight gained among the 10 whiting was 214g.

Table 7.3. Growth performance in a group of 10 whiting during the demand-feeding experiment; ambient temperature ranged between 13.5 – 21°C.

Fish #	init.wt (g)	Final wt (g)	Duration (Days)	Av. Daily growth (g)	G day^{-1} x10^{-3}	SGR (%bw day^{-1})
1	92.3	108.3	78	0.22	2.04950	0.20578
2	107.7	117.6	58	0.17	1.51620	0.15279
3	122	137.4	78	0.19	1.52404	0.14878
4	123.4	147.8	78	0.32	2.31319	0.23750
5	123.1	151	78	0.36	2.61901	0.26329
6	135.8	157.1	78	0.27	1.86794	0.18426
7	151.9	173.9	78	0.28	1.73408	0.17345
8	154	183.4	78	0.37	2.23996	0.22144
9	155.2	184	78	0.37	2.18232	0.22013
10	168.6	187.8	78	0.24	1.38267	0.13669
Total	**1334**	**1548**				
Avg	**155**				1.94289	0.19449
SE	**9**				0.13228	0.01325
Wt. Gained (g)	**214**					
Food eaten(g)	**908**					
Estimated Gross Growth Efficiency (%)	**23.568**					
Food Conversion Ratio	**4.243**					

Based on the amount of weight gained for the total amount of food eaten, the estimated gross growth efficiency was 23.6%. The overall food conversion ratio was 4.243, which implied that the whiting, when fed with high performance trout diets, might not be suitable for aquaculture. However, since food intake increased dramatically after temperature fell below 16°C, it is probable that whiting FCR values under optimum conditions may be lower than this. The FCR value recommended suitable for aquaculture should be lower, between 1–1.5.

Table 7.4. Variation in specific growth rate (SGR) between three size groups (final weight) of whiting.

Size Groups	Wt (g)	SGR (%bw/day)	Mean*	Stdev.	CV(%)
1	108 118	0.20516 0.15173	0.1745 [a]	0.0375	20.897
2	137 148 151 157	0.15252 0.23159 0.26224 0.18697	0.2083 [a]	0.0516	24.768
3	174 183 184 188	0.17356 0.22425 0.21847 0.13836	0.1887 [a]	0.0408	21.713

* means with the same letter superscript are not significantly different from each other (Scheffe's multiple comparison, P >0.05)

There were no significant differences in specific growth rates among the three size groups (P >0.05, Table 7.4). A higher relative coefficient of variation for specific growth rate was found in whiting within size group 2 in comparison to the other two size groups although the differences were small.

7.3.2 Periodogram analysis of demand feeding data

It took 4 – 5 weeks for the whiting to learn how to break the infrared beam to demand food. During the early stages of learning, development of hierarchical dominance was observed among the fish. However, at times when one fish broke the infrared beam, other fish took advantage by competing for the available food. Plate 7.1 shows a whiting breaking the infrared beam to demand food.

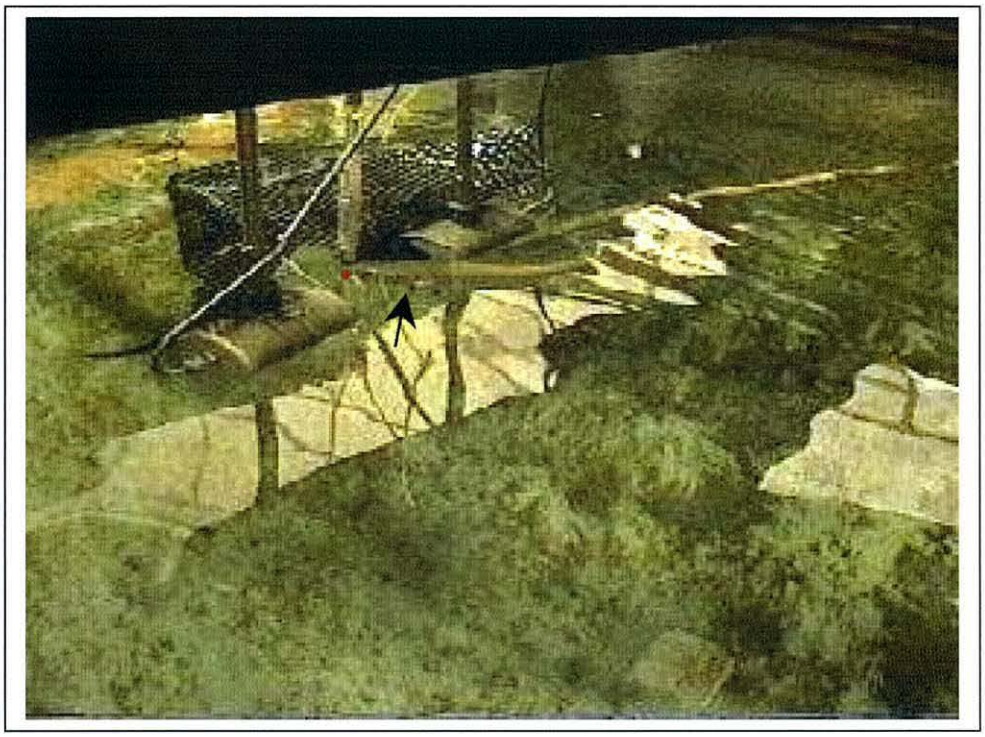


Plate. 7.1. A whiting striking (see arrow marker) the red-tipped rod (breaking infrared beam) on demanding food.

The pattern of actuations and the corresponding estimated amount of diet released from the hopper system over the observation period (1700h) is shown in Fig 7.2. The numbers of actuations were relatively low in August and September, mainly between 3-5 actuations/day (6-12g dry wt. diet day^{-1}), when temperatures were high. Demand feeding increased as the ambient temperature decreased at the start of October. From the middle and toward the end of October, where the ambient temperature ranged between 13.8–15°C (13.98 ± 0.026°C), the number of actuations increased on the average to 10 actuations day^{-1} (22g dry wt. diet) and occasionally as high as 15 actuations (33g dry wt. diet).

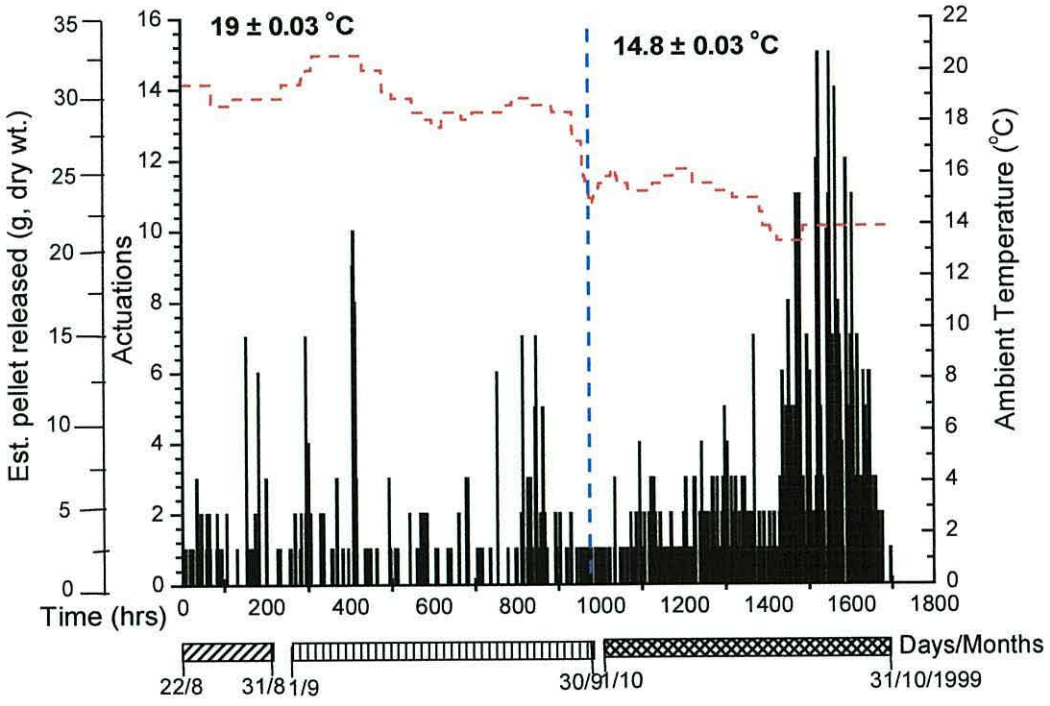
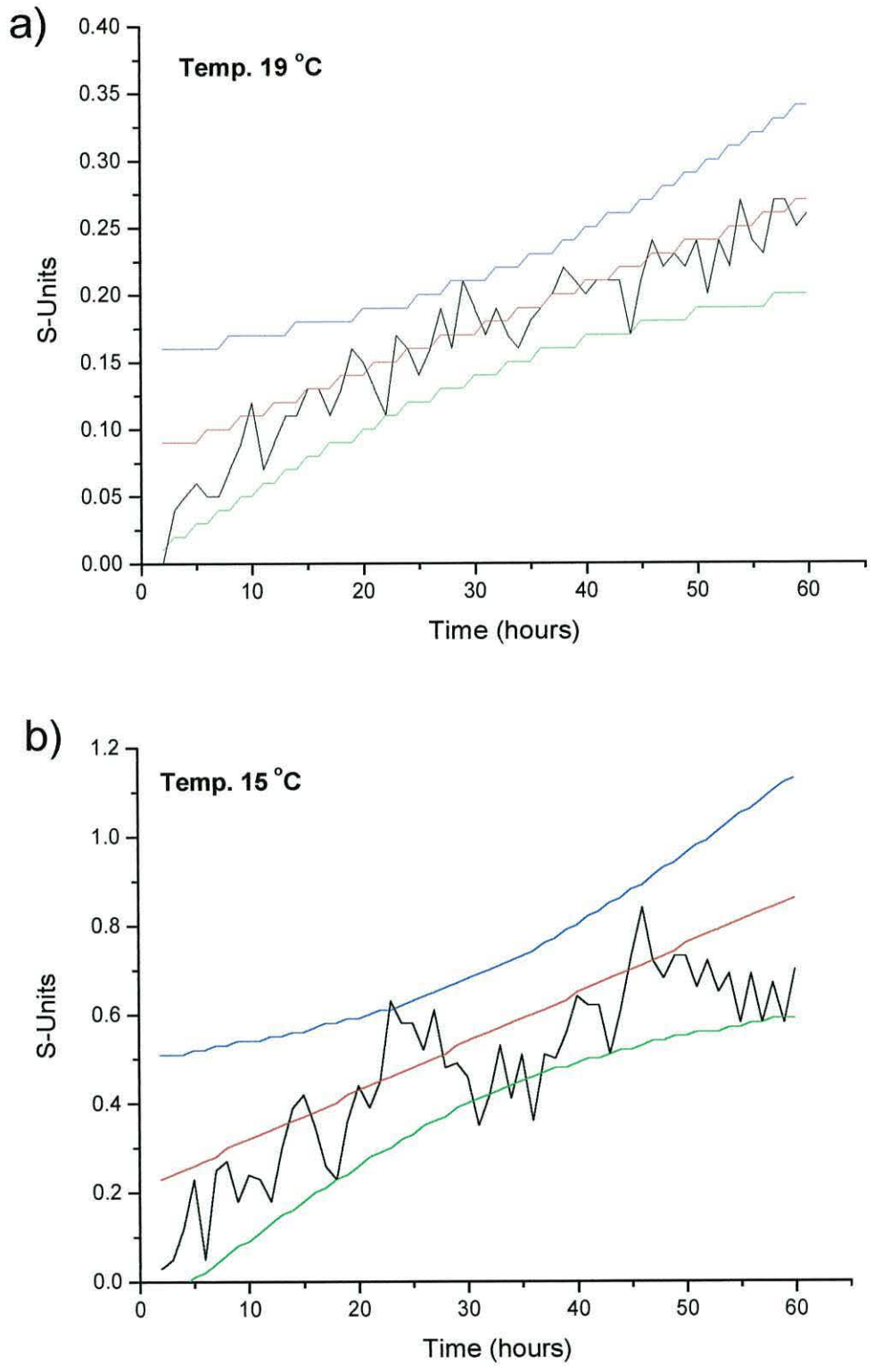


Fig. 7.2. The pattern of hourly actuations (aggregated actuation frequency) recorded during the demand-feeding experiment (August 22nd to October 31st 1999).

Figure 7.3(a-b) shows the results of periodogram analysis - S-units plotted against possible feeding frequency (= period in hours). In the early part of the experiment (Fig. 7.3a) the whiting fed every 30 hours. Once the temperature had decreased and the fish were feeding more intensively, a clear feeding rhythm every 23h was found (Fig 7.3b). It is difficult, in Fig 7.2, to quantify daily intake during the period of heavy feeding in late October. To do this, during the last eleven days of October, when feeding activity was high, 669 actuations were made. The numbers of actuations on each day were expressed as a percentage of the total over this period (Fig 7.4a) to see how variable daily intake was. The whiting daily demand for food varied almost ten-fold.

As a further study, feeding activity was examined over the 24-hour cycle (Fig. 7.4b). The fish preferred to feed during external daylight hours, despite the 24h continuous lighting. Whiting preferred to feed in the morning and in the afternoon followed by a marked decreased in feeding activity at dusk, night and at dawn.



Figs 7.3(a,b). Periodogram analysis for whiting fed on high performance trout 5 mm pellets at different temperatures, a) 19°C b) 15°C. [Blue and green lines denoted upper and lower 95% confidence limits from the reference red lines].

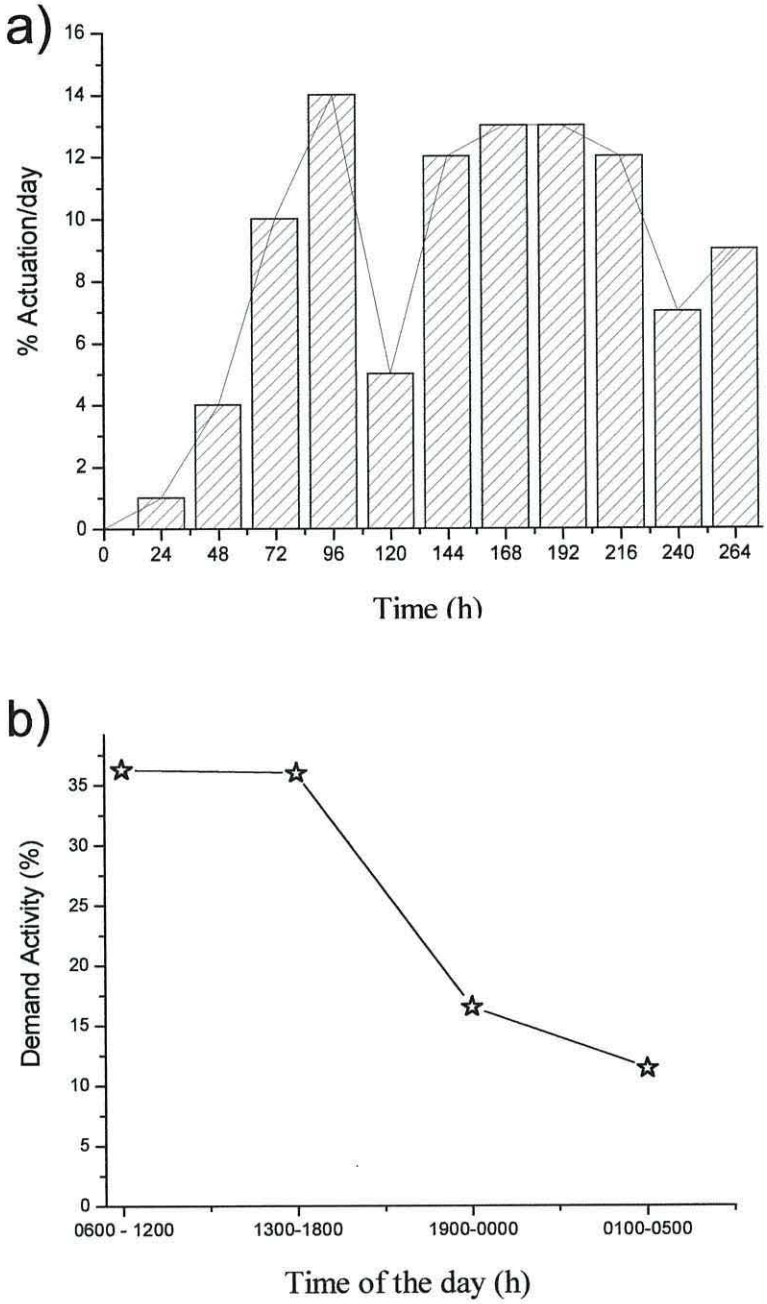


Fig.7.4(a,b). Trend of demand activity over 264h period (20/10 – 1/11/99) and during time of the day by a group of 10 whiting.

7.4 Discussion

Optimisation of growth rates and feed efficiency in fish are potentially influenced by the way in which food is made available, the type of diet, feeding method and frequency, the duration of each feeding period, and the amount of food delivered at the suitable time. In the present study, whiting were self-fed with High Performance (Trouw) 5 mm dry trout diets using an infrared automatic demand feeder over 78 days without limits on available ration or access time.

The 155g whiting responded poorly to this method of dispensing dry diets. Their low intake (0.78% bw day^{-1}) was close to that reported by Seyhan *et al.* (1998). The return of appetite curves for hand-fed whiting offered sprat (80% water; Fig 4.5a-f) can be recalculated to show that the fish (151-465g bw) should be able to consume at least 0.6% bw dry matter every day, values which agree with the demand feeder results.

This intake was reflected in the poor growth performance as indicated by low biomass increase (weight gained), individual growth and specific growth rates and high food conversion ratio (FCR). There were no detectable differences in specific growth rates among different size groups of whiting, but differences in coefficient of variation of the specific growth rates among the size groups might be explained by the hierarchical dominance that does exist among the individuals sharing the diets.

In the present study, it is difficult to estimate the actual amount of diet consumed by each fish since individuals were not tagged and the test diets used were not reformulated with radio-opaque particles, which could be observed using X-radiography. Detailed findings on the variability of food intake and monitoring of individual response to demand feeding have been well documented. Brännäs and Alanärä (1993) used a PIT-tag (Passive Integrated Transponders) system with unique individual codes to monitor individual Arctic charr (*Salvelinus alpinus*) feeding activity with a demand feeding system. They stated that a pronounced shift in bite-number distribution among the individuals was due to the development of a dominance hierarchy, in which the dominant individuals monopolised the trigger. In related studies, artificial diets formulated with radio-opaque ballotini glass beads were used to estimate the amount consumed by the individual fish in different size groups using demand feeders (McCarthy *et al.*, 1992). They suggested that the strength of the feeding hierarchy and the variability in individual consumption decreased as the food availability increased.

A previous study by Seyhan *et al.* (1998) revealed that whiting consumed more control diet - which was closely similar to the diet used in the present study - in comparison to diluted diets (60% kaolin). The mean food intake of dry diets by individual whiting in the present study was estimated at 1.20g day $^{-1}$ (0.774% wet b.w.) in comparison to 1.83 (0.82% wet b.w.) reported by Seyhan on their control diets. The low consumption by mass of dry diet was not surprising, considering their low water content (5-7%) and higher amount of dry matter and energy (18.88KJ/g) in comparison to the high consumption (*ad libitum*) of the diet of sprats which contained at least 75% water and approximately 21KJ/g energy content (see Chapter 5). Many workers postulate that the gross food intake of many fish decreases with decreasing level of moisture and increasing level of dietary energy (Grove *et al.*, 1978; Marais and Kissil, 1979; Kaushik and Olivia-Teles, 1986; Alanärä, 1994; Morales *et al.*, 1994, Ruohonen *et al.,* 1997a). When fish are offered low energy diets, the rate of consumption increases – within limits - to maintain daily energy intake.

Since the diet used in this study was not developed for whiting, dietary moisture level and palatability may have influenced the food intake. Several studies reveal that fish offered wet diet consumed more and grew faster in comparison to dry diet in turbot (*Scophthalmus maximus*) (Grove *et al.,* 2001 Aquaculture Research: in press). However, in Atlantic salmon, there were no significant effects of dietary moisture on the growth in comparison to the dry diet (Hughes, 1989). When dry diet enters the stomach, water is added to moisturise it, which causes the food to expand to fill the available stomach volume. The fish becomes satiated, lowering the appetite of the fish. The ability of fish species to moisturise the dry diet after ingestion varies among different species of fish; water content ranged between 63–75% in salmonid and 78% in largemouth bass (Hughes and Barrows, 1990).

The present study exposed the whiting to unlimited reward level and time access for feeding. Other studies report that there are significant effects of restricted reward level as well as time-restricted access on demand feeding activities in fish. For example, in rainbow trout (*Oncorhynchus mykiss*), voluntary feed intake and specific growth rates were significantly decreased as fish were exposed to reduced reward levels. Time-restricted access has led to a decrease in daily feed intake and, to some extent, low reward level was compensated by increasing the frequency of bite (actuation) activities (Alanärä, 1994; Gélineau *et al.*, 1998).

Ambient temperature, which was 13.8–21°C during this study, probably influenced the food consumption rate. Alanärä (1994) stated that demand feeding activity of rainbow trout was significantly lower at 5°C in comparison to 15°C; he also found no significant relationship between temperature and feed conversion. Similar findings were also reported in dab (*Limanda-limanda*) (Gwyther and Grove, 1981), where they found that *relative* daily food intake increased with temperature but decreased with body size. In sea bass (*Dicentrarchus labrax* L) Azzaydi *et al.* (1998) and Aranda *et al.* (1999) found that daily feeding rhythm varies with temperature and photoperiod. Whiting was more readily available inshore during winter in our local waters and observations throughout the present study showed that it was susceptible to skin haemorrhagic ulceration when exposed to ambient temperature higher than 19°C. Also in the present study, the highest frequencies of actuations were obtained when the temperature ranged between 13.3–13.9°C (Fig. 7.2), which could be their optimal temperature range for feed intake.

Toward the end of the experiment (Fig. 7.4a,b), the whiting established a circadian feeding rhythm, even though they were held under constant 24h lighting. They preferred to feed between dawn and noon of each day. Circadian feeding and diel feeding rhythms were commonly found in many species as parts of their feeding ecology adaptation (Boujard and Leatherland, 1992b). Boujard *et al.* (1991) found marked circadian rhythms of food demand in *Hoplosterum littorale* (Hancock 1828). Feeding activity was mainly nocturnal, with two peaks, and was synchronized with the diel light cycle. In rainbow trout (*Oncorhynchus mykiss*) more than 98% of the feeding demand occurred during the photophase with a main peak at dawn and an occasional peak at dusk (Boujard and Leatherland, 1992a).

In conclusion, the whiting held for longer-term demand feeding trials ate amounts of food (dry weight) at rates comparable to that from hand-fed whiting offered sprat, once water content was allowed for. Since digestion efficiency in whiting is comparable to other fish chosen for aquaculture, the poor growth performance may represent the need to formulate diets specifically for this species, to identify suitable holding temperatures and to minimise stress. These changes might allow more of the absorbed energy to be allocated to growth rather than be expended in respiration.

Chapter 8

General Summary and Conclusions

8.1. Introduction

The thesis describes new aspects of the feeding biology of whiting in captivity and addresses the objectives set out in Chapter 1. Studies were conducted over two years using the available facilities in Nuffield Fish Laboratory, School of Ocean Science, University of Wales Bangor. Live whiting were obtained from the nearby Menai Strait using traps or hook and line, whereas samples of live and freshly-killed whiting from coastal waters of the Irish sea were obtained by trawling on board R.V. "Prince Madog". The aim of this final chapter is to discuss in brief the general outcome of the present study from the practical point of view and also to make suggestions for future research.

8.2. Modelling of food consumption in whiting

A power model to describe gastric emptying was proposed based on the likely physiology of gastric function. With Stimulus α $W^{0.33}$ and Response α $W^{0.67}$ together with negative feedback from the intestine to convert the predicted exponential curve to a "stepped" version which, when smoothed, became a power curve.

In Chapter 3 gastric emptying time in whiting fed on whole sprats containing a radio-opaque Barium Sulphate ($BaSO_4$) paste was monitored using X-radiography.
The inclusion of marker paste in the body of sprats proved it mixed homogeneously with the digesta and passed through the alimentary tract in the form of radio-opaque chyme. The time to empty the stomach (by X-Rays) using larger meals exceeded that reported by Seyhan (1994; *ca* 48hours) for meals <3% bwt. Meal size here was 8-10% bw and GET could easily exceed 72 hours. Full compensation between GER and meal size to maintain constant GET was disproved, at least for larger meal. The results were further checked by dissection (gastrectomy) to determine the amount of digesta left in the stomachs over different time after feeding, and this preliminary data showed that gastric emptying was curved instead of linear.

The estimation of maximum stomach capacity using the linear relationship suggested by Seyhan (1994) based on distension seems to underestimate the satiation amounts for whiting in the present study (Chapter 4). Re-examination gives:
$SV = 0.438\ W^{0.662}$.

Different diets (squid, sprat, shrimp) packed differently so that satiation amounts were: squid = 0.608 $W^{0.640}$ sprat = 0.389 $W^{0.696}$ shrimp = 0.257 $W^{0.651}$.

Return of appetite with time did not follow a straight line as predicted by Seyhan for small meals and others for any meal size. The observations suggested the underlying gastric emptying curve should be instead a power curve without an initial delay period. Of two alternatives designed for whiting, Andersen's model (power = 0.5) was found superior to that of Grove (power = 0.77). This was confirmed by performing gastric lavage experiments in addition to the gastrectomy (Chapter 3). A coherent relationship between X-ray studies, gastric emptying and appetite return was established with Andersen's model as the common foundation:

$S_t = S_{\max}(1 - S_{\max}^{(\alpha-1)}\rho(1-\alpha)t)^{(1-\alpha)^{-1}}) + \xi$, for the gastric emptying process and;

$S_t = S_{\max} - (S_{\max}(1 - S_{\max}^{(\alpha-1)}\rho(1-\alpha)t)^{(1-\alpha)^{-1}})$, for the return of appetite.

where α = 0.5 and ρ varies with prey types (average values 0.16 for sprats, 0.13 for squids and *ca* 0.11 for brown shrimps).

For whiting fed to satiation with 27g of sprats, the GET was 105h in autumn-winter (14 °C). GET for whiting fed on tagged squid pieces (34g) at 16°C was approximately 140h. This was primarily a meal-size difference because the rate parameters (ρ) were remarkably constant (0.08–0.13 h^{-1}) for whiting fed on squid and sprats; for shrimp ρ increased with fish size.

8.3. Digestibility and absorption of macronutrient in whiting

Digestion of sprat, the main prey of whiting in this area, was efficient. 60-68% of prey body water was absorbed along the gut. Whiting fed sprat in the laboratory showed Apparent Digestibility Coefficient (ADC) values for protein (80-94%), lipid (90-97%), carbohydrate (80.5%) and energy (85-96%) were comparable with those for other fish fed suitable diets. A considerable proportion of all nutrients were absorbed in the short region (anterior intestine / pyloric caeca) but active absorption continued for all nutrients in transit along the longer middle and posterior intestine segments.

In wild whiting samples, estimated ADC values were protein (65%), lipids (81%)and energy (65%) in passage from the stomach to the rectal region. It was not possible to ascertain the

prey in intestinal samples which probably contained a mixture of species. Sprats and brown shrimps probably dominated the diet, based on biochemical analysis of the stomach contents but polychaetes and other crustaceans could have made a minor contribution. If nutrient absorption is ongoing in the rectal region then ADCs from extruded faeces could be higher.

8.4. Role of gastric acid and enzyme pepsin in the gastric phase digestion

After ingestion of a meal, first pepsin and (more slowly) acid was secreted into the gastric lumen to peak at 2-4 h (49.4–53.8 Meq. wt. acid x 10^3; 652–813 μg pepsin/ml equiv. min^{-1}). Fish size had a significant affect on the amount of gastric acid and pepsin secreted; digestive power increased $\alpha W^{0.67}$, as predicted by the "physiological" model (Chapter 1 pp 15 – 23) but the predicted effects of meal size (stimulus, distension volume DV) were not clear. Instead, results for flatfish from Jobling were cited which showed muscular contractions (mixing mechanism) were related to $DV^{0.33}$ in partial support of the model.

8.5. Growth performance under continuous demand feeding of artificial diets in whiting

Monitoring of growth performances using various formulations of diet for potentially commercial fish is essential when developing aquaculture practices; it combines cost reduction with optimal production and profit. In addition, it widens our understanding of the range of feeding behaviour and requirements of fish.

Whiting adapted well to using demand feeders. They fed approximately every 29-30h at 19 °C during August – September and every 23h at 15°C in October. In the latter period, peak feeding activities occurred between "dawn" and "noon" despite continuous 24h lighting. During this period they ate similar daily amounts of dry weight to that predicted by the voluntarily feeding experiments using natural prey (0.78 %bw day^{-1}). However growth performance was poor (FCR ≈ 4.2), suggesting that digestibility was poor or that holding conditions/stress was diverting energy from the growth processes. Hierarchical dominance among the individual fish might exist among the experimental fish.

8.6. General conclusions and future research

Whiting proved to be sensitive and vulnerable, especially at the higher ambient seawater temperatures found during summer. Careful husbandry was required to obtain good observations from healthy fish. The underlying patterns of feeding and digestion rates found here, as well as digestion efficiencies, can be improved to predict feeding and digestion rates of whiting in the wild. The extensive stomach content collected by Seyhan could be re-analysed to improve the estimated food intakes through the seasons of the year. At present the work of Andersen on North Sea whiting, which primarily eat sand-eels, seems equally applicable to the Central and Eastern Irish Sea where the diet is predominantly sprats. The measures of digestibility obtained using natural prey items are comparable with those typical of commercially cultures species. The poor growth of our fish, which were feeding adequately, using demand feeders, suggests more detailed study of essential nutrients and vitamins - including amino acid and polyunsaturated fatty acid (PUFA) requirements – is likely to improve growth rate and efficiency.

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10. Appendices

Appendix 2.1 Operation procedure of C:H:N Analyzer

a) Procedure and theory of operation (refer to Fig. A)

Analysis begins by weighing a sample and placing it into the sample holder. When 'analyse' is selected, the sample falls into the combustion chamber where the temperature of the furnace and flow of oxygen gas, cause the sample to combust. The combustion process will convert the carbon, hydrogen and nitrogen into CO_2, H_2O and $N0_x$. These gases are then passed through the IR (infrared) cells to determine the carbon and hydrogen content and a TC (thermal conductivity) cell to determine N_2.

Combustion gases are swept through the combustion tube. After exiting the furnace, the gases pass through a filter and collect in the ballast tank. To control combustion, the flow of oxygen to the combustion chamber is varied by the selection of the proper solenoid valves and their associated restrictors. Oxygen flow is measured by the Oxygen Flow Rotameter before it enters the combustion chamber. Additionally, the furnace temperature can be varied to control combustion.

After the ballast tank is filled with sample gas, it is permitted to equilibrate before being released through the IR cells and the aliquot loop. During the time ballast tank gases equilibrate, valve SV14 is open, permitting the pressure transducer to measure ballast tank pressure. The valve V8 and SV14 close and V7 and V9 open, permitting sample gas to exhaust through the hydrogen and carbon infrared cells and fill the aliquot doser. Shortly after, when the ballast tank pressure falls, valves V6, V8 and V9 close, trapping gas in the IR cells and aliquot doser.

While sample gas is trapped, valve V5 opens, allowing the combustion chamber to be purged. This prepares the system to analyse another sample. The voltage output from the IR cells is also being read and processed by the computer. This produces the analysis results for hydrogen and carbon.

Sample gas in the aliquot doser, is swept by the carrier gas to catalyst heater where $N0_x$ gases are reduced to N_2. Then removal process is done by Lecosorb® to remove CO_2 and anhydrone to remove H_20. This leaves N_2 and helium to flow through one side of the TC cell. The other side of the TC cell receives carrier gas from open valve SV15, after it is filtered by the carrier gas scrubber. The gases in both sides of the TC cell are

compared, and an output voltage results. This voltage is feed to the computer where it processes, displayed and stored as the nitrogen measurement result. After a nitrogen measurement is made by TC cell, the system is purged. Exhaust valves V5, V8, V9 and SV17 open, relieving the system sample gas and preparing it for another analysis.

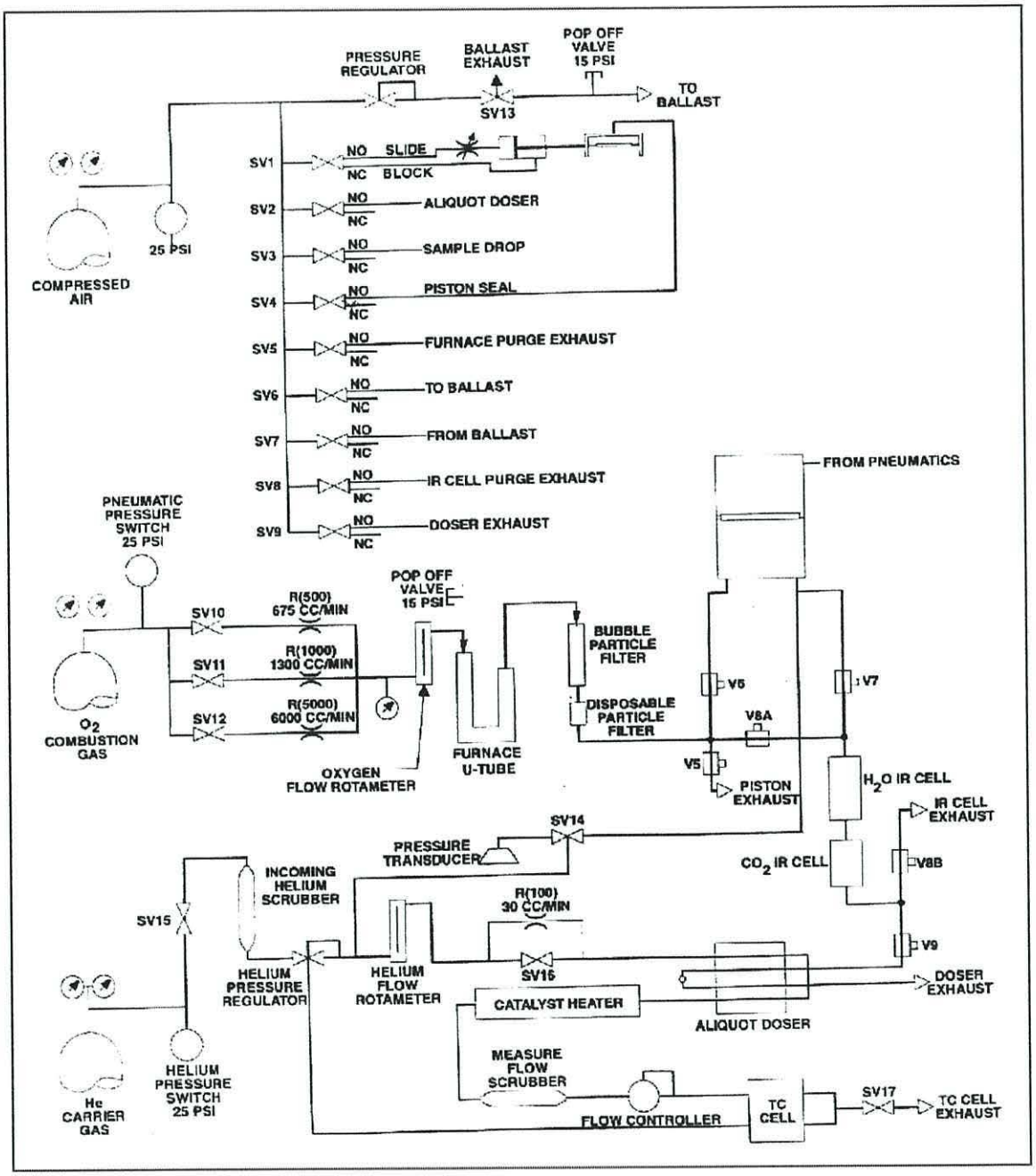

Fig. A. Flow diagram of C:H:N Analyzer operation.

Appendix 4.1: Feeding Contingency Table for Return of Appetite Study: Prey Type: _____

Fish #	F1/F2		F3/F4		F5/F6		F7/F8		F9/F10		F11/F12	
	S;d;t	meal(g)	S;d;t	meal(g)	S;d;t	meal(g)	S;d;t	meal(g)	S;d;t	meal(g)	S;d;t	meal(g)
t=0 84-96h	10/02 10am		10/02 10am		15/02 10am		10/02 10am		11/02 10am		11/02 10am	
T1	12h 10/02 10pm		24h 11/02 10am		36h 16/02 10pm		48h 12/02 10am		60h 13/02 10pm		72h 14/02 10pm	
t=0 84-96h	14/02 10am		15/02 10am		20/02 10am		16/02 10am		17/02 10am		18/02 10am	
T2	24h 15/02 10am		36h 16/02 10pm		48h 22/02 10am		60h 18/02 10pm		72h 20/02 10am		12h 18/02 10pm	
t=0 84-96h	19/02 10am		20/02 10am		26/02 10am		22/02 10am		24/02 10am		22/02 10am	
T3	36h 20/02 10pm		48h 22/02 10am		60h 28/02 10pm		72h 25/02 10m		12h 24/02 10pm		24h 23/02 10am	
t=0 84-96h	24/02 10am		26/02 10am		04/03 10am		01/03 10am		28/02 10am		27/02 10am	
T4	48h 26/02 10am		60h 28/02 10pm		72h 07/03 10am		12h 01/03 10pm		24h 01/03 10am		36h 28/02 10pm	
t=0 84-96h	02/03 10am		02/03 10am		11/03 10am		05/03 10am		05/03 10am		04/03 10am	
T5	60h 04/03 10pm		72h 05/03 10am		12h 11/03 10pm		24h 06/03 10am		36h 06/03 10pm		48h 06/03 10am	
t=0 84-96h	08/03 10am		09/03 10am		15/03 10am		10/03 10am		10/03 10am		10/03 10am	
T6	72h 11/03 10am		12h 09/03 10pm		24h 16/03 10am		36h 11/03 10pm		48h 12/03 10am		60h 12/03 10pm	

Appendix 4.2. Satiation meal (S_{max}) of whiting fed with different prey types[1] at the ambient temperature range between 9.7 -19.3 °C.

Fwt(g)	S_{max}	PT	Fwt	S_{max}	PT	Fwt(g)	S_{max}	PT	Fwt(g)	S_{max}	PT	Fwt(g)	S_{max}	PT	Fwt(g)	S_{max}	PT
38	3.494	1	145	13.837	1	181	13.734	1	296	29.178	1	151	16.46	2	266	13.301	3
38	4.087	1	145	14.888	1	181	11.31	1	296	25.334	1	151	13.567	2	266	13.163	3
38	3.133	1	145	11.833	1	183	15.5	1	310	27.334	1	190	10.769	2	266	13.964	3
38	4.436	1	145	18.456	1	194	12.195	1	310	24.133	1	190	14.185	2	266	7.529	3
38	3.478	1	150	11.21	1	215	21.816	1	310	27.661	1	194	10.762	2	266	8	3
38	3.66	1	150	11.702	1	218	23.9	1	310	29.475	1	224	12.54	2	266	8.396	3
75	5.1	1	150	14.899	1	231	22.3	1	310	21.578	1	224	13.262	2	266	9.302	3
80	7.337	1	150	11.662	1	233	24.7	1	310	23.75	1	239	24.326	2	266	9.589	3
80	7.025	1	150	18.988	1	235	20.304	1	383	29.014	1	240	16.199	2	274	9.811	3
80	13.87	1	150	17.267	1	240	27.1	1	383	26.778	1	242	12.384	2	276	8.085	3
80	12.81	1	150	21.624	1	250	23.078	1	383	24.427	1	242	18.599	2	276	13.535	3
80	9.303	1	150	12.895	1	250	27.482	1	383	26.189	1	271	15.032	2	276	7.47	3
96	8.484	1	150	7.688	1	250	23.113	1	383	23.901	1	271	18.646	2	276	9.325	3
99	7	1	150	9.491	1	250	20.557	1	383	29.122	1	271	15.341	2	276	8.166	3
102	12.2	1	150	14.266	1	252	21.68	1	398	27.145	1	346	21.383	2	276	7.666	3
107	8.291	1	150	13.234	1	255	26.1	1	398	29.047	1	346	23.677	2	276	10.54	3
107	6.006	1	150	17.934	1	255	27.378	1	398	25.897	1	346	23.557	2	276	9.083	3
107	13.324	1	150	7.796	1	255	26.473	1	398	25.861	1	346	26.064	2	276	8.79	3
107	9.882	1	150	20.033	1	255	24.662	1	450	28.347	1	346	26.103	2	296	8.841	3
107	12.502	1	150	21.816	1	255	24.771	1	450	25.045	1	420	23.368	2	296	10.161	3
107	11.096	1	150	8.9	1	260	19.968	1	450	27.119	1	420	26.234	2	296	10.849	3
121	11.421	1	150	9.935	1	260	23.147	1	450	29.444	1	420	24.478	2	296	11.56	3
124	7.082	1	154	33.488	1	260	27.679	1	450	28.114	1	465	23.617	2	327	8.616	3
124	6.34	1	159	17.615	1	260	30.574	1	480	24.056	1	465	30.913	2	327	10.562	3
124	12.138	1	164	15.5	1	260	21.742	1	525	29.443	1				327	11.795	3
124	11.64	1	165	16.968	1	265	14.56	1	525	25.633	1				327	15.145	3
124	14.735	1	166	11.648	1	270	21.783	1	525	29.11	1				327	9.254	3
124	14.117	1	171	14.484	1	270	26.171	1							400	16.379	3
133	11.421	1	171	18.456	1	270	25.068	1							400	15.477	3
135	19.428	1	171	23.75	1	270	23.224	1							400	12.713	3
135	13.985	1	171	16.535	1	270	18.603	1							400	10.933	3
135	17.852	1	175	10.867	1	295	27.014	1							504	12.879	3
135	21.221	1	175	9.333	1	295	27.66	1							504	13.815	3
140	13.83	1	175	15.585	1	295	24.178	1							504	17.443	3
140	20.395	1	175	27.969	1	295	26.056	1							545	12.862	3
140	15.706	1	175	27.512	1	296	23.172	1							545	13.333	3
141	17.615	1	175	16.695	1	296	27.147	1							545	14.252	3
143	14.1	1	181	9.193	1	296	29.045	1							545	13.194	3
145	7.213	1	181	9.037	1	296	18.456	1							545	20.167	3
145	11.635	1	181	5.748	1	296	25.478	1							583	18.061	3
															583	15.84	3

[1] PT 1 = Squid pieces
PT 2 = Whole sprats
PT 3 = Whole brown shrimps

Appendix 4.3. Preliminary return of appetite data from whiting feeding on squid pieces

Fish #	Fish wt.(g)	S_{max}	Deprivation time (hrs)						T°C
		t=0	12	24	36	48	60	72	range
1	38	4.436	1.074	1.4	1.649	4.134	2.958	4.523	14.5-18.8
2	181	13.734	1.362	5.495	5.677	4.855	9.183	5.077	14.5-19.3
3	171	23.75	0	2.042	2.795	10.236	8.363	16.715	14.5-19.2
4	145	18.603	1.621	3.742	0.863	7.524	12.977	16.011	14.5-19.3
5	124	14.735	9.794	5.688	6.064	7.178	8.769	8.323	14.4-19.3
6	107	13.324	0.822	5.056	8.321	11.203	6.713	13.167	14.5-19.3

Appendix 4.4. Return of appetite data (St) for whiting fed on different prey types at different time since feeding (TSF) (Amb. Temp. : 9.7 – 16.9°C)

FID	FGrp	Fwt	TSF	St	PT	FID	FGrp	Fwt	TSF	St	PT	FID	FGrp	Fwt	TSF	St	PT
1	1	271	0	0	1	1	1	224	0	0	2	1	1	276	0	0	3
1	1	271	12	4.205	1	1	1	224	12	3.676	2	1	1	276	12	0	3
1	1	271	24	15.22	1	1	1	224	24	5.074	2	1	1	276	24	3.529	3
1	1	271	36	13.87	1	1	1	224	36	11.41	2	1	1	276	36	12.6	3
1	1	271	48	21.76	1	1	1	224	48	10.64	2	1	1	276	48	9.262	3
1	1	271	60	26.14	1	1	1	224	60	11.31	2	1	1	276	60	13.29	3
1	1	271	72	25.82	1	1	1	224	72	13.88	2	1	1	276	72	16.97	3
2	1	265	0	0	1	2	1	218	0	0	2	2	1	266	0	0	3
2	1	265	12	5.873	1	2	1	218	12	5.555	2	2	1	266	12	2.913	3
2	1	265	24	12.57	1	2	1	218	24	4.383	2	2	1	266	24	6.685	3
2	1	265	36	14.36	1	2	1	218	36	4.958	2	2	1	266	36	8.828	3
2	1	265	48	22.48	1	2	1	218	48	4.857	2	2	1	266	48	7.994	3
2	1	265	60	23.48	1	2	1	218	60	7.01	2	2	1	266	60	12.73	3
2	1	265	72	21.14	1	2	1	218	72	7.558	2	2	1	266	72	12.95	3
3	2	346	0	0	1	3	2	346	0	0	2	3	2	504	0	0	3
3	2	346	12	7.125	1	3	2	346	12	14.59	2	3	2	504	12	11.5	3
3	2	346	24	6.5	1	3	2	346	24	17.56	2	3	2	504	24	6.309	3
3	2	346	36	12.74	1	3	2	346	36	19.9	2	3	2	504	36	16.61	3
3	2	346	48	19.24	1	3	2	346	48	25.07	2	3	2	504	48	21.51	3
3	2	346	60	21.36	1	3	2	346	60	26.38	2	3	2	504	60	21.64	3
3	2	346	72	18.23	1	3	2	346	72	25.66	2	3	2	504	72	27.38	3
4	2	360	0	0	1	4	2	194	0	0	2	4	2	274	0	0	3
4	2	360	12	6.872	1	4	2	194	12	4.446	2	4	2	274	12	8.601	3
4	2	360	24	13.44	1	4	2	194	24	6.391	2	4	2	274	24	3.844	3
4	2	360	36	16.53	1	4	2	194	36	5.377	2	4	2	274	36	9.807	3
4	2	360	48	22.9	1	4	2	194	48	7.637	2	4	2	274	48	9.188	3
4	2	360	60	19.33	1	4	2	194	60	10.37	2	4	2	274	60	11.31	3
4	2	360	72	25.37	1	4	2	194	72	12.13	2	4	2	274	72	12.09	3
5	3	295	0	0	1	5	3	420	0	0	2	5	3	266	0	0	3
5	3	295	12	9.478	1	5	3	420	12	7.644	2	5	3	266	12	6.407	3
5	3	295	24	20.23	1	5	3	420	24	9.273	2	5	3	266	24	8.714	3
5	3	295	36	15.2	1	5	3	420	36	14.37	2	5	3	266	36	4.832	3
5	3	295	48	22.57	1	5	3	420	48	16.17	2	5	3	266	48	13.06	3
5	3	295	60	25.6	1	5	3	420	60	19.77	2	5	3	266	60	12.4	3
5	3	295	72	24.12	1	5	3	420	72	22.59	2	5	3	266	72	15.15	3
6	3	271	0	0	1	6	3	271	0	0	2	6	3	296	0	0	3
6	3	271	12	3.756	1	6	3	271	12	11.38	2	6	3	296	12	5.078	3
6	3	271	24	6.933	1	6	3	271	24	16.3	2	6	3	296	24	6.002	3
6	3	271	36	9.555	1	6	3	271	36	15.61	2	6	3	296	36	5.978	3
6	3	271	48	21.52	1	6	3	271	48	20.95	2	6	3	296	48	6.389	3
6	3	271	60	18.66	1	6	3	271	60	22.03	2	6	3	296	60	9.336	3
6	3	271	72	16.4	1	6	3	271	72	21.9	2	6	3	296	72	10.67	3
7	4	246	0	0	1	7	4	240	0	0	2	7	4	276	0	0	3
7	4	246	12	6.024	1	7	4	240	12	4.573	2	7	4	276	12	0	3
7	4	246	24	13.56	1	7	4	240	24	6.862	2	7	4	276	24	1.853	3
7	4	246	36	12.06	1	7	4	240	36	9.2	2	7	4	276	36	5.622	3
7	4	246	48	18.56	1	7	4	240	48	8.345	2	7	4	276	48	5.038	3
7	4	246	60	23.23	1	7	4	240	60	12.46	2	7	4	276	60	11.32	3
7	4	246	72	17.77	1	7	4	240	72	10.74	2	7	4	276	72	6.764	3
8	4	472	0	0	1	8	4	465	0	0	2	8	4	583	0	0	3
8	4	472	12	8.045	1	8	4	465	12	11.06	2	8	4	583	12	11.05	3
8	4	472	24	17.46	1	8	4	465	24	17.84	2	8	4	583	24	13.71	3
8	4	472	36	14.87	1	8	4	465	36	21.87	2	8	4	583	36	15.37	3
8	4	472	48	20.6	1	8	4	465	48	23.68	2	8	4	583	48	14.67	3
8	4	472	60	23.59	1	8	4	465	60	26.7	2	8	4	583	60	15.11	3
8	4	472	72	25.58	1	8	4	465	72	28.86	2	8	4	583	72	11.5	3
9	5	256	0	0	1	9	5	242	0	0	2	9	5	250	0	0	3
9	5	256	12	7.855	1	9	5	242	12	4.44	2	9	5	250	12	3.534	3
9	5	256	24	11.12	1	9	5	242	24	12.04	2	9	5	250	24	4.871	3
9	5	256	36	18.92	1	9	5	242	36	18.16	2	9	5	250	36	5.71	3
9	5	256	48	15.5	1	9	5	242	48	19.12	2	9	5	250	48	8.507	3
9	5	256	60	22.99	1	9	5	242	60	18.91	2	9	5	250	60	12.29	3
9	5	256	72	23.27	1	9	5	242	72	21.66	2	9	5	250	72	12.32	3
10	5	571	0	0	1	10	5	239	0	0	2	10	5	327	0	0	3
10	5	571	12	7.867	1	10	5	239	12	1.381	2	10	5	327	12	1.099	3
10	5	571	24	14.12	1	10	5	239	24	8.81	2	10	5	327	24	4.097	3
10	5	571	36	17.11	1	10	5	239	36	11.14	2	10	5	327	36	10.57	3

Cont....

10	5	571	48	23.36	1	10	5	239	48	8.663	2	10	5	327	48	16.97	3
10	5	571	60	21.92	1	10	5	239	60	12.62	2	10	5	327	60	11.01	3
10	5	571	72	27.87	1	10	5	239	72	17.1	2	10	5	327	72	13.4	3
11	6	353	0	0	1	11	6	151	0	0	2	11	6	545	0	0	3
11	6	353	12	4.866	1	11	6	151	12	4.622	2	11	6	545	12	5.751	3
11	6	353	24	15.67	1	11	6	151	24	8.892	2	11	6	545	24	11.36	3
11	6	353	36	16.07	1	11	6	151	36	11.5	2	11	6	545	36	17.08	3
11	6	353	48	22.12	1	11	6	151	48	14.22	2	11	6	545	48	16.56	3
11	6	353	60	25.34	1	11	6	151	60	13.86	2	11	6	545	60	16.93	3
11	6	353	72	25.65	1	11	6	151	72	14.15	2	11	6	545	72	20.81	3
12	6	295	0	0	1	12	6	190	0	0	2	12	6	400	0	0	3
12	6	295	12	8.455	1	12	6	190	12	6.01	2	12	6	400	12	0.75	3
12	6	295	24	18.02	1	12	6	190	24	7.641	2	12	6	400	24	10.04	3
12	6	295	36	17.01	1	12	6	190	36	9.345	2	12	6	400	36	12.17	3
12	6	295	48	25.76	1	12	6	190	48	12.1	2	12	6	400	48	14.92	3
12	6	295	60	22.47	1	12	6	190	60	14.23	2	12	6	400	60	17.07	3
12	6	295	72	23.84	1	12	6	190	72	15.92	2	12	6	400	72	16.95	3

Appendix 5.1. Estimate of apparent macronutrient digestibility coefficients or absorption (% dry wt) as food passes through the alimentary tract based on ash content and macronutrient concentration in each of the alimentary sections

	Wild Whiting				Laboratory-reared Whiting					
Alimentary Tract Sections	**Protein**	**Total Lipid**	**Total CHO**	**Total Energy (KJ/g)**	**Protein**	**Total Lipid**	**Total CHO**	**Carbon**	**Nitrogen**	**Total Energy (KJ/g)**
Food -Stomach	-15.8	-6.7	-41.7	-0.1	-4.2	-17.6	-52.4	-	-	-11.3
Stomach -Ant. Int.	57.7	70.6	43	58.7	48.4	52.6	-36.5	33.2	44.4	43.5
Ant. Int. - Mid. Int.	-6.6	-5.4	-32	5.9	36.1	52.2	55.9	43.2	32.9	46.9
Mid. Int. - Rectum	22.0	37.4	26.9	22.9	36.8	64.8	43.0	48.7	36.4	57.5
Overall Stomach - Rectum	64.8	80.6	45.4	65.8	79.2	92.0	66.0	83	78.4	87.2
Food -Rectum	-	-	-	-	78.3	90.6	48.2	-	-	85.8
Food -Faeces	-	-	-	-	94.1	96.6	80.5	-	-	95.8

Appendix 5.2(I-IV). Results of Kruskall-Wallis tests and Dunn's Procedure for multiple comparisons of nutrients in different gut sections[2] at different time after feeding.

I) Protein

a) Time since feeding

Time	N	Median	Ave Rank	Z
12	8	26.78	26.4	0.41
24	8	24.56	23.3	-0.28
36	8	22.37	19.9	-1.02
48	8	31.38	30.1	1.24
60	8	23.69	24.5	0.00
72	8	26.31	22.9	-0.36
Overall	48		24.5	

H = 2.48 DF = 5 P = 0.780

b) Gut Sections

Gut Sec.	N	Median	Ave Rank	Z
1	12	43.06	42.0	5.00
2	12	27.66	29.8	1.50
3	12	22.69	19.3	-1.48
4	12	16.31	6.9	-5.02
Overall	48		24.5	

H = 41.00 DF = 3 P = 0.0001

Dunn's Procedure for multiple comparisons of differences of proteins concentration (% dry wt.) in different gut sections.

Row	COMPARING		ABS DIFF	ST DEV	DIFF/SD
1	1	2	12.2	5.71548	2.13456ns
2	1	3	22.7	5.71548	3.97167***
3	1	4	35.1	5.71548	6.14122***
4	2	3	10.5	5.71548	1.83712ns
5	2	4	22.9	5.71548	4.00667***
6	3	4	12.4	5.71548	2.16955ns

SE of difference = 2.63826

ns, no significant difference (P>0.05); ***, Significant difference at P<0.05

II) Total Lipids

a) Time since feeding

[2] 1 Stomach (St)
2 Ant. Intestine (Ant. Int.)
3 Mid. Intestine (Mid. Int.)
4 Rectum (Rect.)

TIME	N	Median	Ave Rank	Z
12	8	7.767	35.2	2.38
24	8	4.650	21.8	-0.61
36	8	3.284	16.5	-1.77
48	8	4.728	24.6	0.03
60	8	4.854	26.0	0.33
72	8	4.797	22.9	-0.36
Overall	48		24.5	

H = 7.84 DF = 5 P = 0.165

b) Gut Sections

Gut Sec.	N	Median	Ave Rank	Z
1	12	7.397	38.7	4.07
2	12	6.064	31.9	2.12
3	12	3.530	20.2	-1.24
4	12	1.479	7.2	-4.95
Overall	48		24.5	

H = 35.34 DF = 3 P = 0.0001

Dunn's procedure for multiple comparisons of differences in total lipids concentration (% dry wt.) in gut sections

Row	COMPARING		ABS DIFF	ST DEV	DIFF/SD
1	1	2	6.8	5.71548	1.18975ns
2	1	3	18.5	5.71548	3.23683***
3	1	4	31.5	5.71548	5.51135***
4	2	3	11.7	5.71548	2.04707ns
5	2	4	24.7	5.71548	4.32160***
6	3	4	13.0	5.71548	2.27453ns

SE of difference = 2.63826

ns, no significant difference (P>0.05); *, Significant difference at P<0.05**

III) Energy

a) Time since feeding

TIME	N	Median	Ave Rank	Z
12	8	8.753	30.2	1.27
24	8	5.597	19.3	-1.16
36	8	5.131	18.9	-1.24
48	8	8.444	28.2	0.83
60	8	7.818	27.7	0.72
72	8	6.734	22.6	-0.41
Overall	48		24.5	

H = 4.91 DF = 5 P = 0.426

b) Gut sections

Gut Sec.	N	Median	Ave Rank	Z
1	12	11.101	40.2	4.50
2	12	8.881	31.4	1.98
3	12	5.246	19.4	-1.45
4	12	2.878	6.9	-5.02
Overall	48		24.5	

H = 38.63 DF = 3 P = 0.0001

Dunn's procedure for multiple comparisons of differences in digestible energy contents (KJ/g digesta) in different gut sections.

Row	COMPARING		ABS DIFF	ST DEV	DIFF/SD
1	1	2	8.8	5.71548	1.53968ns
2	1	3	20.8	5.71548	3.63924***
3	1	4	33.3	5.71548	5.82629***
4	2	3	12.0	5.71548	2.09956ns
5	2	4	24.5	5.71548	4.28661***
6	3	4	12.5	5.71548	2.18704ns

SE of difference = 2.63826

ns, no significant difference (P>0.05); ***, Significant difference at P<0.05

IV) Carbohydrate

a) Time since feeding

TIME	N	Median	Ave Rank	Z
12	8	0.7495	18.5	-1.33
24	8	0.8515	21.0	-0.77
36	8	0.8345	22.6	-0.41
48	8	0.9960	28.1	0.80
60	8	1.2565	28.5	0.89
72	8	0.9930	28.2	0.83
Overall	48		24.5	

H = 3.88 DF = 5 P = 0.567

b) Gut sections

Gut Sec.	N	Median	Ave Rank	Z
1	12	0.9660	24.2	-0.07
2	12	1.7845	41.6	4.88
3	12	0.8695	23.3	-0.33
4	12	0.6325	8.8	-4.48
Overall	48		24.5	

H = 32.98 DF = 3 P = 0.0001

Dunn's Procedure for multiple comparisons of differences in carbohydrate concentration (% dry wt.) in different gut sections

Row	COMPARING		ABS DIFF	ST DEV	DIFF/SD
1	1	2	17.4	5.71548	3.04437***
2	1	3	0.9	5.71548	0.15747ns
3	1	4	15.4	5.71548	2.69444***
4	2	3	18.3	5.71548	3.20183***
5	2	4	32.8	5.71548	5.73880***
6	3	4	14.5	5.71548	2.53697ns

SE of difference = 2.63826

ns, no significant difference (P>0.05); *, Significant difference at P<0.05**

Appendix 6.1. Gastric secretion data

FishID	Wt (g)	TSF (h)	Meal size(g)	Tot. Vol (ml)	Total HCl(x103)	Pep.Conc. µg/ml equiv. min^{-1}	Pep. Act. min^{-1} U (35.5°C)	T°C
1	210	1	1	0.3	5.603	184.573	18.199	13.9
2	245	1	1	1	7.151	333.228	32.319	12.9
3	211	2	1	0.45	2.743	179.109	17.679	12.9
4	327	2	1	0.95	38.259	208.778	19.550	12.9
5	484	3	1	2.35	82.443	623.918	58.852	13.9
6	406	3	1	1.75	62.066	408.278	38.511	13.9
7	398	4	1	1.3	104.663	671.437	69.043	12.9
8	265	4	1	1	85.581	445.124	44.589	12.8
7	545	1	2.5	1.7	3.093	434.750	41.232	12.8
8	265	1	2.5	1.2	77.347	612.468	60.713	12.8
1	266	2	2.5	1.1	31.429	320.353	30.785	14.2
2	245	2	2.5	0.8	111.744	585.928	61.787	13.1
3	211	3	2.5	0.85	52.229	465.844	48.051	13.1
4	327	3	2.5	0.9	76.207	302.592	29.020	13.1
5	484	4	2.5	2.3	66.539	869.502	85.269	14.2
6	406	4	2.5	1.6	32.351	546.996	53.601	14.2
5	484	1	4	1.6	32.244	769.104	77.757	15.6
6	406	1	4	1.4	10.669	381.468	36.370	15.6
7	545	2	4	2	122.627	1047.098	107.671	13.9
8	265	2	4	1.1	14.352	398.419	38.740	13.9
1	266	3	4	0.4	41.900	351.590	35.546	15.6
2	245	3	4	0.8	39.526	269.461	25.894	15.0
3	211	4	4	0.8	10.249	181.626	17.024	15.0
4	327	4	4	1.2	63.885	576.923	57.812	15.0
3	211	1	1	0.7	51.286	240.121	23.303	14.7
4	327	1	1	1.7	86.134	805.145	81.472	14.7
5	484	2	1	2	81.780	584.807	55.866	14.4
6	406	2	1	2	68.597	454.032	42.087	14.4
7	545	3	1	1.7	60.039	788.744	77.954	14.4
8	296	3	1	0.8	3.412	364.797	37.137	14.4
1	266	4	1	1.05	48.337	872.396	96.674	14.4
2	274	4	1	2.5	76.372	1156.571	116.930	14.7
1	266	1	2.5	1	20.064	539.389	56.353	12.2
2	274	1	2.5	1.6	19.702	829.026	85.247	11.9
3	211	2	2.5	0.85	23.657	478.952	48.422	11.9
4	327	2	2.5	1.2	70.414	811.283	85.405	11.9
5	484	3	2.5	1.25	42.135	479.239	46.780	12.2
6	406	3	2.5	1.5	27.423	585.869	56.744	12.2
7	545	4	2.5	2.05	86.014	1289.205	132.349	11.1
8	296	4	2.5	1.4	92.598	725.766	73.504	11.1
7	545	1	4	2.4	19.751	1180.946	118.870	14.2
8	296	1	4	0.75	18.348	465.263	48.230	14.2
1	266	2	4	1	28.889	603.592	62.669	13.1
2	274	2	4	1.7	34.494	755.458	76.042	14.4
3	211	3	4	0.6	26.611	216.410	21.206	13.1
4	327	3	4	1.9	94.722	1101.996	114.235	13.1
5	516	4	4	2.35	55.929	1509.276	157.684	14.4
6	406	4	4	3	79.369	1643.663	166.898	14.4
5	516	1	1	2	12.125	1147.712	118.017	15.3
6	406	1	1	3.2	35.102	872.976	82.275	15.3
7	545	2	1	3.5	77.784	1628.348	164.627	15.3
8	296	2	1	0.75	48.597	838.552	85.366	15.3

Appendix 6.1 cont...

1	266	3	1	1.7	46.251	231.580	21.353	15.3
2	274	3	1	1.2	58.548	664.509	68.884	14.4
3	211	4	1	1.35	2.772	441.286	42.573	14.4
4	327	4	1	1.1	13.603	455.430	45.472	14.4
3	211	1	2.5	0.55	6.471	237.058	23.861	15.6
4	327	1	2.5	1.4	62.419	736.622	76.360	15.6
5	516	2	2.5	2.5	62.052	501.044	46.647	16.4
6	406	2	2.5	2.8	37.492	677.248	63.478	16.4
7	545	3	2.5	2.5	134.591	1273.101	130.911	16.7
8	276	3	2.5	1.35	27.987	468.301	46.065	16.7
1	266	4	2.5	2.7	46.562	434.969	40.152	16.4
2	274	4	2.5	2.4	60.292	1030.129	102.853	15.6
1	249	1	4	1	4.872	438.361	43.927	16.1
2	274	1	4	2.9	40.065	1242.164	125.032	15.6
3	211	2	4	1.1	36.776	414.520	41.083	15.6
4	327	2	4	1.1	46.357	575.878	59.217	15.6
5	388	3	4	2.25	23.464	711.976	68.823	16.1
6	406	3	4	3.55	49.560	912.086	86.287	16.1
7	545	4	4	3.1	93.563	1510.309	154.533	16.1
8	218	4	4	1.35	15.318	444.782	43.333	15.6
7	545	1	1	1.8	6.366	380.182	35.634	16.7
8	180	1	1	1.6	2.142	384.934	36.279	16.7
1	249	2	1	1.4	14.840	442.631	42.619	15.0
2	274	2	1	2.2	61.953	1030.859	105.121	16.7
3	211	3	1	2.4	6.507	420.208	38.789	16.7
4	327	3	1	1.8	40.983	738.999	74.053	16.7
5	388	4	1	1.9	26.372	650.985	63.791	15.0
6	406	4	1	3	53.548	952.988	92.302	15.0
5	388	1	2.5	2	28.892	787.998	77.216	15.6
6	406	1	2.5	3	11.032	605.510	56.287	15.6
7	545	2	2.5	3.3	100.271	1738.988	176.577	16.9
8	180	2	2.5	1.9	4.812	350.597	32.499	16.9
1	249	3	2.5	2	41.937	683.646	66.085	15.6
2	274	3	2.5	3	43.523	1302.768	131.711	15.0
3	211	4	2.5	2.1	11.861	472.017	43.650	15.0
4	327	4	2.5	2.1	48.993	624.422	59.651	15.0
3	211	1	4	1.1	6.445	371.599	36.203	16.9
4	327	1	4	1.2	6.201	537.700	54.362	16.9
5	388	2	4	1.4	29.822	502.351	49.036	15.6
6	406	2	4	2.7	36.846	523.053	48.622	15.6
7	545	3	4	3.2	88.637	1312.859	130.360	15.6
8	180	3	4	2.4	38.281	639.495	60.616	15.6
1	249	4	4	2.4	69.597	1055.173	105.736	15.6
2	274	4	4	2.6	46.619	943.984	92.679	16.9
1	160	12	1	1.9	34.326	423.829	40.020	10.8
2	210	12	1	1.5	7.610	178.062	16.422	10.7
3	153	12	1	0.8	13.579	179.647	16.838	10.8
4	175	12	2.5	1	42.760	102.882	9.584	11.1
5	197	12	2.5	2.3	54.336	192.063	17.707	11.1
6	277	12	2.5	1.9	13.169	482.977	45.112	11.1
5	197	12	2.5	1.4	21.302	241.093	22.229	10.8
7	217	12	4	1.8	38.603	393.466	37.014	10.7
8	186	12	4	1.3	16.368	121.968	11.325	10.7
9	224	12	4	1.4	28.374	228.003	21.064	10.7
1	160	24	1	2.4	29.924	289.305	26.711	11.1
3	153	24	1	1.3	50.468	219.907	20.277	11.1
3	153	24	1	1.3	9.551	309.453	29.192	11.1

Appendix 6.1 cont...

4	175	24	2.5	1.4	48.683	208.511	19.235	11
5	197	24	2.5	2	46.642	227.504	21.033	11
6	277	24	2.5	2.1	30.636	195.029	18.244	11
7	217	24	4	1.6	57.905	298.074	27.668	11
8	186	24	4	2	32.564	132.136	13.005	11
9	224	24	4	2	75.880	1041.304	107.599	11

Appendix 6.2. Correlation between acid and pepsin production by whiting of various size at different time after feeding.

	Pearson Correlations between acid and pepsin production											
	1h			2h			3h			4h		
Fish Size	r	n	Sig.	r	n	Sig.	r	n	Sig.	r	n	Sig.
Small	-0.374	7	no	0.762	6	no	0.301	6	no	0.918	5	yes
Medium	0.253	8	no	0.667	9	yes	0.417	9	no	0.228	10	no
Large	0.480	9	no	0.590	9	no	0.672	9	yes	0.439	9	no
	12h			24h			Significant level: no, P>0.05; yes, P<0.05					
Small	-0.026	10	no	0.615	9	no						

Appendix 6.3. Calculation details of stomach geometry model

Consider a hollow oblong block with ONE END OPEN [a.cm*(a/5)cm*(a/5)cm]

a=	b=	c=
2.5	0.5	0.5

Vol= 0.625 SA=0.84 L^2

SA= 5.25 Vol=0.04L^3

Try a cylinder of length a and RADIUS a/10

a=	b=
5	0.5

Vol= 3.9270 SA=0.6597 L^2

SA= 16.4934 Vol=0.0134 L^3

Cylinder AGAIN but end is a hemisphere (last 1/5th)

a=	b=
20	2

cyl len= spher rad

18 2 SA=.5655 L^2

Vol= 242.9498 Vol=0.02723 L^3

SA= 251.3274

a	b	c	Vol	SA	logL	LogVol	LogSA
2.5	0.5	0.5	0.625	5.25			
5	1	1	5	21			
7.5	1.5	1.5	16.875	47.25			
10	2	2	40	84			
12.5	2.5	2.5	78.125	131.25			
15	3	3	135	189			
17.5	3.5	3.5	214.375	257.25			
20	4	4	320	336			
2.5	0.25		0.4909	4.1233			
5	0.5		3.9260	16.4934			
7.5	0.75		13.2536	37.1101			
10	1		31.4159	65.9735			
12.5	1.25		61.3592	103.0835			
15	1.5		106.0288	148.4403			
17.5	1.75		168.3697	202.0437			
20	2		251.3274	263.8938			
2.5	0.25		0.4745	3.9270	0.3979	-0.3238	0.5941
5	0.5		3.7961	15.7080	0.6990	0.5793	1.1961
7.5	0.75		12.8118	35.3429	0.8751	1.1076	1.5483
10	1		30.3687	62.8319	1.0000	1.4824	1.7982
12.5	1.25		59.3139	98.1748	1.0969	1.7732	1.9920
15	1.5		102.4945	141.3717	1.1761	2.0107	2.1504
17.5	1.75		162.7574	192.4226	1.2430	2.2115	2.2843
20	2		242.9498	251.3274	1.3010	2.3855	2.4002

stom length v volume

Regression Output:

Constant	-1.51757
Std Err of Y Est	1E-15
R Squared	1
No. of Observations	8
Degrees of Freedom	6
X Coefficient(s)	3
Std Err of Coef.	1.3E-15

Vol= 0.030369 *L^ 3

SA= 0.628319 *L^ 2

stom length v SA

Regression Output:

Constant	-0.20182
Std Err of Y Est	7.8E-16
R Squared	1
No. of Observations	8
Degrees of Freedom	6
X Coefficient(s)	2
Std Err of Coef.	9.6E-16