Thesis 1624

The Utilisation of Artificial and Natural Food Sources by First Feeding Fry and Small Parr of Atlantic Salmon (Salmo salar) Reared in Freshwater Cages

bу

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

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ABSTRACT

A study was conducted to evaluate the use of freshwater cages to rear small Atlantic salmon, <u>Salmo salar</u>. In particular, the study almed to describe the feeding behaviour of such stocks with respect to artificial and natural food sources.

Results showed that cage systems can be successfully used to first feed Atlantic salmon, or to maintain stocks transferred from tank systems after first feeding. The limiting factor to the method was considered to be the water quality conditions in the cages, which in turn are related to the physio-chemical characteristics of the cage site. Highly productive sites were considered unsuitable for salmon cage culture. Providing environmental conditions remain suitable, and proper mesh sizes and stocking densities are utilised, growth of caged fish can be comparable with those reared in tank systems.

Except for a limited period after first feeding, the main food source of caged Atlantic salmon was determined to be the artificial diets. Crustacean zooplankton were the main food source at and immediately after first feeding, due mainly to their suitable particle size and mobile qualities. However, caged fish generally switched their diet choice to the artificial feed soon after first feeding; this was attributed to a recognition of this as the optimal

feed choice. A proportion of the stock were observed to feed on the zooplankton until it became unsuitable with respect to particle size. Upon switching, these fish were unable to feed on the artificial diets as effectively as those which switched earlier. The late-switchers were considered subordinates, and did not grow as well as the dominants.

The degree of utilisation of natural feeds by fish introduced to cage systems after first feeding was determined to be dependent upon the size of individual zooplankton in relation to the preferred particle size of the fish stock (PFR). As the latter equals and becomes larger than the former, natural feed importance in the diet decreases. It was not determined if this feeding behaviour had effects on the growth of the fish; however, it may have relevance in controlling cestode parasite infections.

An evaluation of the feeding behaviour of the fish in the trials with respect to the concepts of an optimal foraging theory was undertaken.

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1.1 LIFE HISTORY OF ATLANTIC SALMON

1.1.1 Classification and Overview

The Atlantic Salmon (<u>Salmo salar</u> L.) belongs to the family of bony fishes which also includes Pacific salmon (<u>Oncorhynchus</u> spp.), trouts and chars (<u>Salmo</u> and <u>Salvelinus</u> spp.), whitefishes (<u>Coregonus</u> spp.), and grayling (<u>Thymailus</u> spp.). These fish are all native to the northern hemisphere. They are basically cold water species, although they occupy a wide range of habitats. They are generally carnivorous (Bond 1979).

Atlantic salmon are a much written about species; many reviews of their biology have been published (<u>e.g.</u>, Netboy 1980, Went 1980, Sedgewick 1982). They are distributed throughout most countries which border the north Atlantic Ocean. Generally Atlantic salmon follow an anadromous life history pattern. They breed and spend the juvenile stages of their lives in fresh water. Toward the end of this period (between 1 and 6 years) they undergo a physiological transformation known as smoltification, and subsequently migrate to salt water. The salmon then spend a period of 1 or more years feeding at sea before returning to their river of origin to breed and spawn. Atlantic salmon may also spend their entire lives in freshwater.

1.1.2 Life in Fresh Water

1

Atlantic salmon cover an extremely wide geographic range, and the seasonality of their life histories varies much between locations. This account of the freshwater phase of the Atlantic salmon's life history is given primarily with reference to wild stocks in Scotland. Much of the behaviour and ecology was determined using North American stocks, however, the principles are considered applicable to the species as a whole.

In Scotland the Atlantic salmon spawning season occurs between October and January. Spawning takes place in rivers and streams having loose gravel substrates and supplied by clean, well-oxygenated water. Fertilised eggs are deposited in nests, or redds, excavated in the gravel bottom of the stream. The development time of the eggs is highly temperature dependent, but hatching generally occurs in March or April. After hatching the young salmon, known as alevins, remain in the redd for a period of about 6 weeks. During this time, the alevins obtain their nourishment endogenously <u>via</u> a yolk sac.

Upon leaving the redd, salmon alevins begin to feed exogenously. This period is often described as being the most critical in the entire life history of the fish. Certainly the ecology and behaviour of young Atlantic salmon in freshwater is a much researched topic. The following is a

synopsis of this information, particularly with reference to aspects applicable to Atlantic salmon culture.

After the onset of exogenous feeding, young Atlantic salmon are known as fry. Soon after this time they develop characteristic vertical markings on their sides and are known as parr. With the onset of smoltification they are termed smolt (Netboy 1980).

Allen (1941) gives one of the earilest detailed accounts of the feeding behaviour of Atlantic salmon fry and parr in the wild. He, and subsequently several other authors (e.g. Rimmer and Power 1978, Wankowski and Thorpe 1979a) describe the prey of fry and parr in three categories: substrate associated, surface drift, and suspended drift. It has been shown that prey must be in motion to elicit a feeding response by the fish (Rimmer and Power 1978). This motion may be caused by water currents or by the prey themselves. The fish are visual predators, and are highly size selective (Allen 1941, Wankowski 1979, Wankowski and Thorpe 1979b).

Atlantic salmon fry and parr in the wild generally reside in areas of appreciable water current, although any habitat providing suitable water quality conditions may be occupied. Gibson and Keenleyside (1966) report that fry and parr generally remain in unshaded riffle areas during daylight hours. At this time the fish hold station on the

substrate using their pectoral fins and feed upon drifting organisms (Wankowski and Thorpe 1979a). Gibson (1978) showed that young salmon prefer shaded to unshaded areas given a constant depth, but will move to deeper, unshaded water if provided with a choice.

Young salmon in fresh water are naturally a territorial fish (Kalleberg 1958). Many factors affect this behaviour. including prey abundance, water flow, light levels, and fish abundance. Symons (1968) described the development of social hierarchies in populations of young Atlantic salmon and defines members of these hierarchies as either dominant or subordinate in their behaviour. Further, Symons (1971) noted that when food abundance was decreased, aggression by . dominant fish increased, feeding ranges or territories of the dominant fish increased, and subordinate fish were forced into new feeding areas. Subordinate fish generally were significantly smaller than dominant ones. Social hierarchies were reduced when fish density was increased (also reported by Refstie and Kittelsen 1976). Aggression in young salmon is reported to decrease when water flow decreases (Gibson 1978, Wankowski and Thorpe 1979a), and to decrease at night (Keenleyside and Yamamoto 1961).

The combination of feeding and territorial behaviour exhibited by young Atlantic saimon in fresh water provides a mechanism by which individuals may effectively compete for food in the relatively sterile environment they

inhabit. The freshwater phase of the life history is often regarded as a growth period; indeed growth must be maximised so that parr may reach the critical size required for smoitification (Thorpe <u>et al</u> 1980, Wedermeyer <u>et al</u> 1980). It can be concluded that the feeding and territorial behaviour exhibited by individual fish is of paramount importance in reducing the length of the freshwater phase of an Atlantic salmon's life history.

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1.2 ATLANTIC SALMON CULTURE

1

1.2.1 The Decline of Fisheries and the Development of Aquaculture

Atlantic salmon have long been a source of food throughout their native ranges, and many methods for their capture have been devised (Went 1980). These can be divided into two main categories: commercial methods, including various forms of nets and traps; and sport methods, using various forms of rod and line. Commercial and sport catches of Atlantic salmon have been declining over the past century (Netboy 1980, Went 1980).

Initial efforts to culture Atlantic salmon were made in the 1800's by keen anglers to supplement natural stocks and thus ensure the future of their sport (Netboy 1980). The first reported success at rearing Atlantic salmon to smolt from eggs was made in the late 1880's (Maitland 1887). After this time developments were made in the rearing of Atlantic salmon in seawater to supplement commercial fishing. Most recent advances in the freshwater culture have come about in response to the increasing requirements of seawater farmers.

1.2.2 Atlantic Salmon Smolt Production Methods

Present day techniques for culturing Atlantic salmon from egg to smolt encompass a vast amount of knowledge collected over the past century. However, all farming practices are determined by and generally follow the natural life history and behavioural patterns of the fish (Edwards 1978).

Reviews of the current technology of Atlantic salmon smolt production are given by Edwards (1978), Sedgewick (1982), Beveridge (1987), and Laird and Needham (1988). A description of what has become known as standard tank culture is given here.

Fertilised eggs are incubated in a trough or tray system supplied with a steady flow of clean, well-oxygenated water. The incubation period is temperature dependent; hatching generally occurs after about 500 degree-days (calculated as the product of the temperature and the number of days). The eggs are maintained in near darkness throughout this period. This method of incubation closely simulates the conditions present in the egg-containing redds of the wild fish.

The newly hatched alevins are held in the hatchery troughs until the yolk sacs are nearly absorbed (a period of about 300 degree-days). They are then transferred to tanks designed to maximize first feeding success. initially fish

are stocked highly (>25 kg.m⁻³) and water flows are adjusted to the maximum that does not displace the fry from their stations in the tank. Commercially prepared diets are supplied to the tanks, utilizing the water currents to distribute the feed throughout the tank and elicit the feeding behaviour of the fry.

Once the fry have been successfully start-fed they are transferred to larger tanks or raceways. Stocking densities in these tanks are lower than in the first feeding tanks (<20 kg.m⁻³). Water flows are kept at a minimum to provide a continuous flow of clean well-oxygenated water and adequately distribute the diets. The particle size of the prepared diets is periodically increased as the fish grow. The stock is size graded at least once, often in the autumn of the first year of growth. All of these husbandry practices are meant to provide an equal feeding environment to all stock and reduce the effects of social hierarchy formation.

Despite these intensive efforts to reduce behavioural influences, salmon parr stocks will exhibit a degree of differential growth. This results in a proportion of the stock achieving a level of growth to allow them to undergo smoltification after only one year in fresh water. The remainder of the stock does not reach the critical size to smolt until the following year.

This differential growth was first described in the literature by Thorpe (1977) who termed the respective proportions of the stock the upper and lower modal groups (UMG or S1 and LMG or S2 respectively). Recent work indicates the development of a differential level of appetite in a stock of salmon parr in the autumn after first feeding. Potential S1's continue to feed and grow into the winter, while potential S2's show a reduced appetite and thus slow in their growth (Metcaife <u>et al</u> 1986, 1988). 1.3 CAGE CULTURE AND ATLANTIC SALMON SHOLT PRODUCTION

1.3.1 History and Principles of Cage Aquaculture

1

Cage and cage-type structures have been used throughout the world to culture many species of fish for many years. Coche (1983) gives a bibliography of over 900 references on the subject. Beveridge (1987) gives a thorough review of the principles of cage aquaculture and their application to the culture of many different species (unless otherwise indicated this is the source of information provided in this section).

Cage-type structures have been used as holding facilities for fish before taking them to markets for many years. True cage culture, in which stock is held for an extended period over which appreciable growth is achieved, is a recent development.

Modern cage structures encompass a wide variety of designs, each with its own inherent advantages and disadvantages. The most popular type is composed of a floating collar suspending a net mesh bag containing the stock. The entire structure can be moored as a single unit or in rafts to a permanent structure such as the shoreline, a dock, or directly to the sea or lake bed.

Cage culture systems have one primary difference from tank or raceway systems. This is that the culture environment within the cage is not as isolated from the surroundings. The stocks held in culture cages interact closely with the water body containing the cage. In particular, a natural source of food is often available in addition to the prepared diets supplied by the farmer. Indeed, cages are often utilised for this; the high levels of natural food available may mean the farmer can reduce the levels of supplemental feeding.

Cage systems have many practical advantages over tank or raceway type ones. These include low capital costs, simple construction, adaptability, ease of management and harvesting, and they make use of existing water bodies.

Cage systems have few characteristics which can be considered as disadvantages. They are very vulnerable to damage, both intentional and unintentional, and their close interaction with the environment raises concerns over the pollution of the water body they are sited in.

1.3.2 The Use of Cages in Atlantic Salmon Smolt Production

The use of cage systems in Atlantic salmon smolt production has been an increasingly popular practice in Scotland. This has occurred largely because suitable running water

sites are becoming scarcer, and development plans often meet planning problems concerning effluent discharge into rivers and streams with wild salmonid populations Beveridge 1987). In 1985, the Department of Agriculture and Fisheries of Scotland reported that 33 percent of the sites producing smolts in Scotland utilised cages, accounting for 30 percent of the production in that year.

Cages are typically used to rear large parr graded from tank systems. Smaller fish, however, are often reared in the cages and several farms have undertaken trials first feeding alevins in these systems (R. Baillie pers. comm., T. O'Hara pers. comm.).

Not surprisingly, many of the initial trials using freshwater based cages to rear Atlantic salmon smolts have been conducted by commercial companies, and accounts in the scientific literature are scarce (Holm and Moller 1984, Holm 1986, Pepper and Oliver 1986, Pepper <u>et al</u> 1987). These studies made some effort to describe the suitability of cages as smolt production systems. Most importantly, however, they have identified the significance of crustacean zooplankton as a natural food source and investigated its advantages and disadvantages. Few studies were primarily concerned with first feeding alevins and small fry of Atlantic salmon; little emphasis was placed on studying the feeding behaviour of larger fry and parr transferred to and reared in freshwater-based cages.

1.4 OBJECTIVES AND SYNOPSIS OF THIS STUDY

In view of its increasing popularity and the lack of published information on the subject, a study was planned to evaluate the use of cage systems in Atlantic salmon smolt production. In particular, the utilisation of artificial and natural food sources by fish transferred to the cages at different times post first feeding, and the effect of varying natural species compositions on the feeding behaviour of the fish was investigated.

The remainder of this thesis is set out in the following manner.

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Chapter 2 presents the results of a preliminary trial conducted to evaluate the use of a freshwater-based cage to rear Atlantic salmon from first feeding through the first summer of growth, with respect to the environmental conditions within the cage and the growth and mortality of the fish. The feeding behaviour of the stock over the course of the trial is described and discussed in relation to theoretical factors that should affect intensity of feeding and prey selection.

Chapter 3 presents results from a trial in which Atlantic salmon alevins were first fed in cages exposed to a natural food supply differing in composition to that of the preliminary trial. Additionally, the effects of moving

stock to cages at various times post first feeding on feeding behaviour is described.

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CHAPTER 2: THE PRELIMINARY TRIAL

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2.1 INTRODUCTION

2.1.1 Successful Atlantic Salmon Smolt Production

Atlantic salmon smolts are produced in Scotland generally for supply to seawater farmers. As such, smolt producers aim to operate as commercially viable enterprises. They must minimise expenditure by reduction of capital and operational costs while maximizing sales by producing suitable numbers of quality smolts (Shaw and Muir 1987, Eadle in press).

The use of freshwater-based cage systems in Atlantic salmon production can represent a significant reduction of expenditures by the farm. For a production of 100,000 smolts per annum, the use of cages over tanks can reduce the capital costs by as much as 17 percent (Shaw and Muir 1987).

The growth of the stock contributes much to the operational costs and sales revenues of a smolt production unit. As discussed in Chapter 1, slow growth may lead to a high percentage of S2's. The extra costs associated with maintaining these fish for an extra year makes them very expensive to keep (Shaw and Muir 1987, Eadle in press). Indeed, many smolt producers give away or sell S2's for restocking purposes rather than incur the extra expenditures they generate (Beveridge 1987). Economically, then, smolt production in freshwater-based cages can only be considered successful if the reductions in expenditures are not outweighed by poor growth of the stock.

The following sections address the basic environmental parameters and characteristics of the available feed which may affect the growth of juvenile Atlantic salmon reared in freshwater-based cages.

2.1.2 Environmental Factors Affecting Smolt Growth

The environmental parameters relevant to freshwater cage culture are reviewed by Beveridge (1987); included are chemical and physical aspects of water quality, and meteorological aspects such as the velocity of prevailing winds. Reviews of the harmful effects of poor water quality are given by many authors ($\underline{e},\underline{a}$, Alabaster and Lloyd 1980). The principles outlined here are described fully by these authors, and unless otherwise indicated they are the source of the information. Specific criteria are referenced to the reviews they were obtained from.

2.1.2:1 Water Quality

Atlantic salmon are thermal conformers (as are most fish) and as such rely on the ambient water temperature to regulate their physiological processes. With relevance to aquaculture this includes processes such as oxygen con-
sumption, waste production, feeding behaviour, food conversion, and growth. Deviations from near optimal temperatures can cause stress and increase susceptibility to disease and parasite infections. The tolerable temperature range for juvenile salmonids is $3-28^{\circ}$ C; optimum temperature for first feeding, and fry and parr growth are about 10° C and 15° C, respectively (Alabaster and Lloyd 1980).

Oxygen is essential to all higher organisms for the production of energy required for food digestion and assimilation, and activity. Salmonids are very active fish, and thus their oxygen demands require water that is close to 100% saturated (Alabaster and Lloyd 1980).

The acidity or alkalinity of water (pH) can directly damage the gill surfaces of fish, thereby reducing oxygen uptake and thus growth, or even causing death. The pH also affects the health of freshwater fish indirectly by altering the toxicity of pollutants and heavy metals (see Exley and Phillips 1988 for review). These authors give the optimal range for salmonids in freshwater to be pH 6.5-7.5, although values outside this range may be tolerated.

The concentration of several nutrients in freshwater are considered to be relevant to a good culture environment for salmonids. These include nitrogenous (ammonia, nitrite, and nitrate) and phosphorous compounds (various phosphates).

Ammonia is a major metabolic by-product of most aquatic consumers. It is present in water in two forms: ionised (NH_4^+) and un-ionised (NH_3) . Their relative concentrations are dependent upon the temperature and pH of the water. The un-ionised form is generally considered to be much more toxic to freshwater fish. The mode of toxicity of ammonia is complex and variable, but generally a stressed condition results. Maximum permissible levels for salmonids are $2\mu g \cdot l^{-1}$ for the un-ionised and 1000 $\mu g \cdot l^{-1}$ for the ionised species (Haywood 1983).

Nitrite and nitrate are the intermediate and end products, respectively, of the nitrification (degradation) of ammonia. Nitrite is more toxic than nitrate, and as ammonia, exposure to high levels generally results in a stressed condition. The maximum permissible nitrite level is given as 15 μ g.i⁻¹ (EIFAC 1984), while Wickens (1980); noted that a nitrate limit of 5000 μ g.i⁻¹ should be followed. More importantly, however, their levels are also an indication of the ability of an environment to degrade the more toxic ammonia compounds (Wickens 1980).

Phosphorus compounds in general have no direct toxic effects on fish. However, phosphorous is usually the limiting nutrient to phytoplankton growth in freshwater. its measurement can indicate the onset of phytoplankton blooms in the water body (Phillips 1985) and as such-is a predictor of changing dissolved oxygen and suspended solids

levels in the water.

Turbidity of water is caused by suspended organic and inorganic solids. These may have toxic effects, cause depletion of dissolved oxygen from the water, or cause physical damage to fish gills. Turbid waters are often associated with high pathogen levels, with the result that stressed fish are even more susceptible to infection. Maximum permissible suspended solids levels for salmonids are given as 25 mg.1⁻¹ (Alabaster and Lloyd 1980).

2.1.2:2 Meteorology

Wind forces directly affect many of the practical aspects of cage culture by exerting a strain on any structure above the water line, and making husbandry practices difficult. Wind forces are responsible for generating surface waves and currents in freshwater lochs, and as such are indirectly very significant to cage operations. Waves and currents exert a physical force on cages and moorings. Currents can displace cage bags and thereby reduce the volume available to the fish. Wave action generally reduces the clarity of water by increasing suspended solids levels, and reducing the amount of sunlight transmitted into the water column (Beveridge 1987).

The direct and indirect effects of wind on cage systems are dependent upon its speed and direction, and the distance it

travels over the water before reaching the site. These factors are reviewed extensively by the U.S. Army Corps of Engineers (1984).

The level of cloud cover on any given day can affect the amount of sunlight transmitted into the water column. As Atlantic salmon are visual feeders, this and wave action might affect the feeding behaviour of caged stock.

2.1.3 Zooplankton as a Food Source

2.1.3:1 Nutritional Content

Reviews of the proximate composition and energy content of freshwater zooplankton are given, among others, by Vijverberg and Frank (1976), Watanabe <u>et al</u> (1978), and Yurkowski and Tabachek (1979). All note variations in proximate composition with geographical location and trophic status of the water body, and with season. Proximate composition does not generally vary greatly between species within the same water body sampled at similar times.

A natural mixture of zooplankton types from temperate freshwater lakes has a proximate dry matter composition which differs slightly from that of commercially prepared Atlantic salmon diets (see Tables 3 and 25 for proximate compositions of salmon starter and fry diets). By comparison, zooplankton generally have higher protein levels (50-70% versus 54%), similar carbohydrate levels (5-13% versus 7-12%), similar lipid levels (13-24% versus 15-19%), and higher levels of indigestibles (chitin, fibre, and ash; 13-25% versus 11%). Zooplankton of temperate freshwater lochs in summer generally have calorific values in the range of 3.5-6.5 kcal.g⁻¹, while the artificial diets are in the range of 3.5-5.0 kcal.g⁻¹. Zooplankton are generally composed of 90-94% moisture, compared to 9% moisture in artificial diets. Thus, an individual zooplankton does not provide the same levels of nutrients and energy that an individual artificial diet particle does, and in this sense the latter can be considered superior as a food source.

The nutrients present in natural foods (<u>1.e.</u>, zooplankton) are said to be of high quality with respect to the requirements of juvenile fish. Watanabe <u>et al</u> (1978) and Dabrowski and Ruslecki (1983) report that zooplankton proteins have a high ratio of free to total amino acids, thereby making them more available for absorption and assimilation. The lipids of most aquatic organisms provide a good source of linolenic (omega -3) fatty acids which are essential to fish (Yurkowski and Tabachek 1979). Zooplankton often contain significant levels of carotenoid pigments (Simpson <u>et al</u> 1981). Studies have shown these pigments to have functions as vitamin A precursors and in the perception of light, and to aid in decreasing sensitivities to adverse environmental conditions such as high

ammonia and low oxygen levels (see Mikulin and Soin 1975 for review). In a study with Atlantic salmon, Torrissen (1984) showed that diets supplemented with carotenoids promoted growth during the start feed period.

2.1.3:2 Non-nutritional Factors

Zooplankton have been implicated in causing mortalities of early feeding larvae of white fish, <u>Coregonus</u> sp. (Eckmann 1985) and grayling, <u>Thymailus</u> thymailus (Eckmann 1987). The mechanism of this mortality is not fully explained, however, physical damage to the intestinal epithelium and/or the presence of a toxin are suggested.

Copepod zooplankton may act as the host to the procercoid stage of the cestode parasite <u>Diphyllobothrium</u> spp. (Vik 1957). Feeding on infected zooplankton by caged salmonids can lead to the development of the pierocercoid stage of the parasite in the fishes' peritoneal cavity. The resultant stress to the fish may lead to reduced growth, but rarely mortality.

Motion of the prey is vital to stimulating the feeding behaviour of the Atlantic salmon fry (as discussed in Chapter 1). In the low current environment of a freshwater cage, live Zooplankton may provide this stimulus more effectively than artificial diets.

2.1.4 Factors Affecting Feed Selection

2.1.4:1 The Feed Selection Process

The process of prey selection by planktivorous and particulate feeding fish is highly complex, and has been investigated by many authors (<u>e.g.</u> Eggers 1977, O'Brien 1979, O'Brien <u>et al</u> 1986). These and other authors give many formulae for determining the probability of selection of a given prey type; generally this can be described as:

 $P_{\text{molection}} = P_1 \cdot P_2 \cdot P_3 \cdot P_4$

where P_1 = the probability of encounter;

P₂ = the probability of the prey being identified as food and the decision to pursue being made;

 P_3 = the probability of capture; and P_4 = the probability of ingestion.

All components of this equation are affected by the behavioural and morphological characteristics of the predator and the prev.

The probability of a prey type being encountered is partly dependent upon the foraging behaviour of the predator. At one extreme are the ambush predators which wait for the prey to come to them, while at the other extreme are the true planktivores which continually cruise in search of food (O'Brien <u>et al</u> 1986). Atlantic salmon fry occupy an intermediate position on this scale, perhaps toward the ambush end.

The probability of encounter is also affected by the abundance and distribution of the prey, as a more abundant, evenly distributed type will have a higher chance of being encountered than a scarce, patchy distributed type (this has been reported by many authors <u>e.g.</u> Eggers 1977, O'Brien 1979). The abundance of a prey type relative to other types is often considered more important than absolute abundance (Ohguchi 1981, Visser 1982).

Marcotte and Browman (1986) describe the fishes' role in prey identification by identifying perception and cognition as key steps. Perception is limited by the physical constraints of the predators' vision. Cognition (learning and memory) controls the decision making.

Characteristics of the prey will affect perception and subsequent decision making by the predator. Movement of the prey will make it more visible to a predator (Drenner <u>et al</u> 1978, Holmes and Gibson 1986, Marcotte and Browman 1986). Juvenile Atlantic salmon have been shown to select for particles greater than 1.1% for fish less than 40mm fork length, and 2.2% for fish greater than 40mm fork length (Wankowski and Thorpe 1979b). As the cognitive abilities of fish are limited, it is not surprising that many prey characteristics function to confuse the predator. For example, swarming prey confuse by providing so many equal choices that the predator cannot make a decision; prey types which differ from the majority in colour or movement patterns are thus more susceptible to predation (Milinski and Lowenstein 1980, Ohguchi 1981, Marcotte and Browman 1986).

The prey capture mechanism of salmonids is described by Wankowski (1979) as a suction action caused by rapidly expanding the buccal and opercular cavitles, resulting in a flow of water containing the prey passing into the mouth. The effectiveness of this type of capture mechanism has been examined by Drenner <u>et al</u> (1978). Air bubbles, inanimate prey, and dead zooplankton are unable to evade capture, and are almost always ingested. Different types of live zooplankton have different swimming speeds and patterns and thus differ in evasion abilities. Cladocerans generally have predictable movement patterns and low swimming speeds and are theoretically easier to capture than copepods, which swim in jerky, faster movements.

Once captured, the probability of a prey being ingested depends on the ability of the predator to manipulate the prey into a position conducive to swallowing. For salmonids, this primarily depends on physical characteristics of the prey such as size and shape (Wankowski 1979).

2.1.4:2 Optimal Foraging

Optimal foraging theory states that the predators should (1) prefer more profitable prey (defined as the difference between the energy value of the prey and the energy which must be expended in capture and handling), (11) be more selective when profitable prey are common, and (111) ignore unprofitable prey which are outside the optimal range regardless of how common they are (Krebs and Davies 1984). The optimal foraging theory is a general summation of feeding behaviours and its application to specific species is therefore highly variable.

Ringler (1983) reviewed the variations in foraging tactics employed by fish, and noted that deviations from optimal foraging may be the result of the status of the predator (e.g. satiated versus hungry, dominant versus subordinate). This author and Marcotte and Browman (1986), who studied Atlantic salmon fry, showed that foraging can vary amongst individual fish, and in this context is dependent upon cognitive traits such as previous experience and motivation. Marcotte and Browman (1986) also noted that foraging abilities increase from first feeding to fry and parr stages of smolt production.

2.1.5 The Preliminary Trial

A preliminary trial was planned to evaluate the use of a freshwater-based cage system to first feed and rear through the first summer of growth a stock of Atlantic salmon. Evaluation was limited to sampling the environmental variables and characteristics of the available feed which were considered most relevant to fish growth. Growth of the trial fish was compared with that of a similar stock reared using standard tank techniques.

Meteorological data were collected mainly as background to aid in elucidating water quality, available feed, and/or fish sampling results.

Various water quality parameters were sampled. Temperature and dissolved oxygen data were collected to compare conditions inside and outside the culture cage, and to compare levels between the trial cage and the control tank. Nutrient, pH, and suspended solids data were collected from inside the trial cage and compared with data from a control area in the loch to further evaluate cage culture conditions.

Crustacean zooplankton were sampled inside the cage, around the cage area, and at the control location to describe the abundance, blomass, and size of the main groups which comprised this natural food source. These data were

compared with similar characteristics of the artificial diets supplied to the trial cage.

Caged fish were sampled regularly and their growth was compared with that of the control fish. Differences were related to the environmental conditions of both systems. Stomach contents analyses were also performed on the cage fish samples to describe their feeding behaviour with respect to theoretical considerations, and to relate this behaviour to their growth pattern. 2.2 MATERIALS AND METHODS

2.2.1 Cage Site Description

2.2.1:1 General Information

Loch Scasiavat was chosen as the site for the preliminary cage trial. This loch is located on the west coast of the isle of Lewis, Outer Hebrides. A commercially-operated, cage-based Atlantic salmon smolt unit was established at this site. Suitable culture conditions, access to laboratory and tank rearing facilities, and good communications with the institute of Aquaculture were the main criteria in choosing this site. This site will be referred to hereafter as site 1.

Figure 1 shows the approximate locations of cage raft and the control sampling area at site 1. The loch was fed by several small burns and direct runoff from the surrounding hills. Drainage from the loch was via one stream connected through an impassable fails to the sea. The surrounding hills were covered with rough grazing and peat bog. The loch was orientated along a south west north east axis.

Table 1 summarises the morphological, hydrological, and ecological characteristics of site 1. The characteristics illustrated are some of those considered relevant to the assessment of a site for freshwater cage culture (Beveridge

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Figure 1. Site 1.

A. Trial cage in the raft
B. Control sampling area
C. Inlet burns

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D. Outlet stream



	Characteristic	Value
	Surface area (x 10 ⁶ m ²)	0.2072
Morphological and Hydrological	Mean depth (m)	10.57
	Mean volume (x 10 ⁶ m ³)	2.068
	Catchmont area (x 10 ⁸ m ³)	1.114
	Annual water input (x 10 ⁶ m ³)	1.379
Q.T	Exchange rate (times . year ⁻¹)	0.667
-	Location (latitude, longitude)	58°10'30"N, 7°3'45"W
	Data source	Murray and Pullar 1910
Morphological and Hydrological Hydrological	Alkalinity (meq.1 ⁻¹)	0.10-0.2
	рн	5.9-6.0
	Temperature (°C)	7.0-20.0
cological	Dissolved oxygen (mg.1 ⁻¹)	9.5-12.0
	Total ammonia (µg.l ⁻¹)	20.0-35.0
	Nitrite (µg.i ⁻¹)	1.0
	Nitrate (µg.1 ¹¹)	100.0-250.0
	Total phosphorus (mg.1 ⁻¹)	5.0-14.0
	Secchi depth (m)	1.0-2.0
	Data source	M. Philips pers. comm.

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Table 1. Morphological, hydrological, and ecological 1 characteristics of site 1.

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Ecological data shown are minimum and maximum values obtained by sampling between April and October and includes data from up to 3 years before the trial began.

1987).

2.2.1:2 Farming Practices

On 12 May 1986 (day 0) 21,800 late yolk sac Atlantic salmon fry were introduced to the trial cage. The mean weight of individual fry was 0.2g. All stock was from the same Conon River parentage (P. Featherstone pers. comm.).

The cage collar used at site 1 was a wooden Crook-type. The initial bag utilised was sectioned into quarters by suspending ropes from midpoint to midpoint of opposing walkways underneath the cage. By raising these ropes, four separate chambers were created. The depth of each chamber was determined by the height of the ropes. Adjacent sections of the cage were used in succession to contain the stock as the mesh became fouled. As the fish were transferred to a new section, the old section was heid out of the water to allow fouling organisms to be washed clean by the weather. All sections of the cage not being used to hold stock were suspended clear of the water in this way. Records were kept of the dimensions of the sections used and the dates of the section changes.

Two other cage bags were utilised during this triai. At 98 and 112 days after first feeding, changes were made to the second and third bag, respectively.

The overall dimensions of the cage bags, dimensions of cage sections, and netting materials used are summarised in Table 2.

Commercially prepared diets were fed to the stock in the trial cage at site 1 using methods established for Atlantic salmon fry culture in tank systems. The amount of diet and the pellet size fed to the stock varied according to water temperature and fish size; these followed guidelines recommended by the feed manufacturer. The proximate composition of the prepared diet and the feeding regimes followed at site 1 are shown in Tables 3 and 4 respectively.

Prepared diets were delivered to the stock using a commercial automatic feeder. This consisted of a feed hopper equipped with a basal vibrating discharge disk. A motordriven, spinning disk was incorporated into the feeder in order to spread the feed over a greater area of the cage.

The feeder was coupled to an electronic control unit which delivered food to the cage at approximately 15-20 minute intervals. Food delivery began around 30 minutes before sunset and stopped around 30 minutes after sunset. This was controlled by light sensing cells on the control unit. One feeder was placed centrally over the cage section or full bag containing the stock.

Day	Full cage bag (m)	Netting Mesh size (mm)	Section dimensions (m)	Comments
0	6.5 x 5.5 x 1.0	4	2.1 x 2.9 x 0.4	
16			2.3 x 2.9 x 0.5	Fish rotated
24			3.5 x 3.5 x 0.6	sections of
43			3.0 x 3.0 x 0.6	the cage as the mesh
55			2.0 x 2.4 x 0.8	became fouled
64			2.7 x 2.7 x 0.9	
74			2.8 x 5.5 x 1.0	
89			2.7 x 5.5 x 1.0	
98	6.5 x 5.5 x 1.0	4	-	Full cage used
112	6.5 x 5.5 x 1.0	10	-	Full cage used

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Table 2. Summary of cage bag and section dimensions and changes, and netting materials used, site 1, 1986. $^{\rm 1}$

Full cage bag and section dimensions are given as length x width x submerged depth. Mesh sizes given are the stretched dimension. All netting was of knotless nylon construction. 1.

Component	Proximate composition
Protein (%)	54
Carbohydrate (%)	7
DII (%)	19
Ash (%)	10
Fibre (%)	1
loisture (%)	9
Metabolizable energy (Kcal.g ⁻¹)	3.77

Table 3. Proximate composition¹ of the artificial diet used at site 1, 1988.

manufacturer, Ewos-Baker.

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Day	Feeding Rate (% body weight. day")	Peilet type	Pellet type		
7	5.0	0			
15	5.0	0			
23	5.5	0			
38	6.0	0			
44	6.0	0			
51	6.0	0			
58	5.0	t			
65	5.0	1			
72	4.5	1	1		
79	4.5	1			
85	4.5	1			
119	3.0	2			

Table 4. Feeding regimes¹ of artificial diets for the cage trial and the control tank, site 1, 1988.

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Mortalities were removed from the trial cage at site 1 on alternate days. Accurate records were kept.

No chemical treatments were given to the stock at site 1 at any time during the trial period.

The cage trial at site 1 was terminated 121 days after first feeding, prior to the autumn size grade by the farm management.

2.2.2 Control Tank Description

2.2.2:1 General Information

To act as a control to the cage trial Atlantic salmon fry of the same parentage to those reared in the cage were raised using standard tank culture methods.

The control fish were reared in a circular tank constructed of moulded glass fibre (Figure 2). Its radius was 2.5m. The depth of the water was about 1.0m (this varied from day to day depending on the cleanliness of the outlet filter). Water was supplied to the control tank via an inlet pipe having an internal diameter of 15.0cm. A top hat type filter was employed to prevent the stock from being swept out through the tank's central outlet. Figure 2. Top (A) and side (B) views of the control tank.

- 1. Inlet pipe
- 2. Central outlet filter
- 3. Feeder

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4. Water level



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Flow rates through the control tank were adjusted continually throughout the trial. Generally, a flow regime was maintained that was slightly lower than that which would have displaced the fry from their stations in the tank (i.e. on the bottom or in the water column).

2.2.2:2 Farming Practices

On 12 May 1986 (day 0) 71,950 late yolk sac Atlantic salmon fry were transferred from hatchery troughs to the control tank at site 1. The fry transferred to the tank were of similar size and stock as those transferred to the trial cage on the same day (<u>i.e.</u> 0.2g mean individual weight, and Conon River parentage).

Fish in the control tank were fed the same commercially prepared diet as those in the trial cage; that is, the diet given to both were of the same production lot and therefore had similar proximate compositions. Similarly, the same feeding regimes were followed for the cage trial and the control.

Prepared diets were delivered to the control fish using an automatic feeder similar to that used in the cage trial. However, the control feeder had no spinning disk to distribute the food. Instead the feeder was situated directly downstream from the tank inlet. Feed was distributed throughout the tank by the water currents. The

control feeder operated on the same intervals and daily schedule as the cage feeder.

The outlet filter of the control tank was cleaned on alternate days. At this time mortalities were removed from the tank. Accurate records of mortality were maintained.

The fish in the control tank were treated for costia (<u>lcythyobodo</u> <u>necatar</u>) on day 32 and day 42. On each occasion a treatment of 200ppm formalin solution was administered to the stock.

As with the cage trial, the control experiment of site 1 was terminated 121 days after first feeding.

2.2.3 Sampling Methods

2.2.3:1 Meteorology

Wind Speed and Direction

At the beginning of the trial at site 1, a cup anemometer and wind vane were coupled to a continuous chart recorder and secured to the cage raft. Charts were removed daily from the recorder. The output was calibrated periodically throughout the trial using a hand-held anenometer and a compass to establish the wind speed and direction.

Cloud Cover

Subjective judgements of percentage cloud cover were made on the same days as zooplankton and fish samples were obtained.

2.2.3:2 Water Quality

Temperature

Water temperature was measured at the trial cage and the control tank of site 1 using a PHOX temperature-oxygen meter. Readings were recorded to the nearest 0.1° C. Measurements were made inside the trial cage 40-50cm below the surface. Measurements were made in the control tank at approximately the same depth as those in the trial cage.

From day 2 to day 42 measurements were taken at 9:00 and 22:00 each day from both cage and control tank. After this, one measurement was taken per day at each location. These were taken between 15:00-16:00.

Dissolved Oxygen

At site 1 dissolved oxygen was measured at the trial cage using the same PHOX meter as for temperature. Readings were recorded to the nearest $0.1mg.1^{-1}$. The meter was calibrated before each sample using loch water poured into a bucket from 1.0-1.5m in height. Loch readings were taken inside the trial cage (40-50cm below the surface, 1.5m away from the leeward cage wall) and from the cage area (40-50cm below the surface, 1.0m away from the windward side of the raft).

Dissolved oxygen measurements were made concurrently with temperature.

Nutrients, Suspended Solids, and pH

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Water samples for analysis of nutrients, suspended solids, and pH were collected from the cage trial at site 1. No water samples were collected from the control tank.

For each sample approximately 2.0 I of surface water was collected in a wide-mouthed polyethylene jar. Upon arrival on shore, 1.0 I of the sample was measured into a rinsed graduated cylinder. The sample was filtered through a field filtration apparatus (Stiriing 1986) containing a preweighed Whatman GF/C filter paper. Approximately 500ml of the filtrate was retained in a polyethylene bottle and frozen for later analysis of nutrients and pH. Individual filter papers were folded in half, wrapped in aluminium foil and frozen for future suspended solids analysis.

Water and filter paper samples were collected on a weekly basis between day 2 and day 85, and on day 119. Samples were obtained from inside the cage and the control area of the cage trial site.

2.2.3:3 Zooplankton

Theoretical Problems of Zooplankton Sampling

The problems associated with the sampling of zooplankton have recently been reviewed by De Bernardi (1984) and Vijverberg (1988). Both authors note that the complexity of the zooplankton community structure and the objectives of a particular study make selection of a sampling method very difficult.

Quantitative zooplankton sampling can be accomplished by the use of devices based on either water collection or filtration (De Bernardi 1984, Vijverberg 1988).

Water collection is generally the better method for small environments where the use of a net is impractical or in productive environments where a net may become easily clogged (De Bernardi 1984). Water samples collected must be large enough to ensure that the water organisms are adequately sampled. Hrbácek (1966, cited in De Bernardi 1984) states that combining water samples together may help in this respect, and may reduce the effect of patchy zooplankton distribution on sampling. For storage purposes, zooplankton in water samples must be either removed by filtration or sedimentation from the sample.

Water flitration methods of zooplankton sampling have the advantage of facilitating the sampling of large volumes of water, thereby reducing the effects of patchy zooplankton distribution. The main problem with this method is that the efficiency of the filtering gear varies greatly with net fabric and mesh size, sampling speed, avoidance by target organisms, escape, and mesh clogging (De Bernardi 1984). Plankton nets vary greatly in their structure and design, and in their filtering efficiencies. Vijverberg (1988) evaluated the efficiencies of plankton nets with mesh sizes of 80 and 335µm. His work showed efficiencies can range between 30 and 70%, depending on the size of the target organisms, degree of clogging of the mesh and net towing speed. The effects of varying filtering efficiencies can, however, be ignored in comparative studies where only relative and not absolute values are required. although the same net should be used for all samplings (De Bernardi 1984).

Sampling Methods Utilised

In view of these considerations, zooplankton were sampled at the cage and cage area locations using a pooled water sample method. These samples were filtered through a conical plankton net for storage purposes. The same net was used to sample the zooplankton at the control area

location using a direct filtration method. These methods are described in the following sections. No zooplankton were collected from the control tank at site 1.

The same conical plankton net was used for all zooplankton sampling at site 1. The net was 1.5m in length with a circular mouth of radius 0.25m. The radius of the net decreased towards the cod-end, which was fitted with an 800ml collection vessel. The vessel had a mouth of radius 3.5cm. The main body of the net was constructed of 250µm nylon mesh. The mouth of the net was held open by a wire support ring, to which a towing harness was attached.

For samples from the cages and cage areas, five 8.0 i buckets of surface loch water were poured through the plankton net suspended vertically over the water surface (totalling 40.0 i filtered). Inside the cage, the five bucket samples were taken from randomly chosen sites. Around the cage area the five bucket samples were taken from the midway point of each side of the cage raft, and from a fifth randomly selected point within the raft.

Zooplankton samples from the control site were obtained by casting the net from the side of a boat. A length of line was attached to the towing harness so that the total length of harness and line was 6.0m. A line and float were attached to the support ring so that the net mouth was suspended approximately 0.75m below the surface. The net

was cast to the windward side of the boat. The boat was allowed to drift downwind until the towing line became taut. The net was then retrieved at a speed of approximately $0.5m.sec^{-1}$, using the boat's outboard motor to hold a steady position. The volume of water sampled (1.18m³) was determined by the formula:

Volume sampled (m ³)	•	Π.	radius of net mouth opening (m)	2	length of tow line and harness (m)	
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! (Severidge 1985). Filtering efficiency was assumed to be 100%.

Sample Preservation

After the required amount of water was filtered, the sides of the net were carefully washed into the collection vessel and its contents were then emptied into a 1.0 i, widemouthed polyethylene bottle. Small amounts of loch water were used to rinse the collection vessel clean. Upon arrival on shore, 2.0ml of Lugol's iodine solution (Beveridge 1985) were added to the samples. After allowing to settle for approximately 6 hours, the supernatant was decanted and the resultant concentrated samples were retained in labelled, wide-mouthed polyethylene bottles for future laboratory analysis. Sampling Schedules

Zooplankton samples were obtained concurrently from the cage and control locations weekly between day 2 and day 85, and again on day 119. Zooplankton samples from the cage area location were obtained weekly between day 23 and day 85, and again on day 119. Cage area samples were taken concurrently with cage and control samples. All zooplankton samples were obtained between 15:00 and 16:00 on the sampling days.

2.2.3:4 Artificial Diets

Throughout the course of the trial at site 1 samples of the prepared diet were obtained. These samples were kept in air-tight containers and retained for future laboratory analysis.

2.2.3:5 Fish

Growth

Samples of fish from the trial cage and the control tank of site 1 were periodically taken to evaluate growth of the stocks. At the trial cage the fish were crowded into a small area by raising portions of the net. Five dip net samples were taken and their catches pooled in an 8.0 i bucket (about 250 fish). A small net was then used to

randomly subsample 50 fish from the pooled cage sample. The remainder were returned to the cage. In the control tank a pooled sample of about 250 fish was obtained by combining the catches of 5 dip net sweeps made across different areas of the tank. A random sample of 50 fish from this pooled sample was also obtained. The remainder were returned to the tank.

The subsamples of fish from the trial cage and the control tank were measured on site to determine fork length (distance from tip of the snout to tip of the tail fork). Readings were recorded to the nearest 0.5mm. After the first two samples fish from the trial cage were also subjectively judged to be red or green in colour. This judgement was based upon the degree of redness in the fins and body of the fish (Figure 3). The decision was recorded with the corresponding fork length data. The minimum preferred particle sizes of individual fish were calculated as 1.1% of fork length for fish < 40mm and 2.2% fork length for fish > 40mm (Wankowski and Thorpe, 1979b).

Stomach Contents

Of the 50 fish sampled from the trial cage of site 1, 20 were preserved in 5% buffered formalin for future stomach contents analysis. After the first two samples 10 red and 10 green fish from each trial cage sample were preserved. No fish from the control tank were preserved for stomach

Figure 3. Red (A) and green (B) fish

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from the trial cage at site 1.

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contents analyses.

Fish not preserved for stomach contents analysis were returned to the trial cage, or the control tank.

Distribution in the Cage

Periodic samples were taken to determine the distribution of fish in the trial cage at site 1. At these sampling times three separate passes of a dip net were made through the cage. These were made along the eastern wall, along the western wall, and through the centre. Great care was taken during passes not to disturb other areas of the cage. The catches from these passes were kept separately in three 8.0 | buckets.

Fork length measurements and a subjective judgement of colour was obtained from 30 fish randomly selected from each bucket. The results were recorded to the nearest 0.5mm, and the fish returned to the cage.

Sampling Schedules

Fish samples were obtained from the trial cage and the control tank at site 1 for growth weekly from 7 to 85 days after first feeding, and again on day 119.

Subjective judgements for colour of fish from the trial cage began on day 23 and continued to be made concurrently with growth measurements.

Preserved samples for stomach contents analyses were obtained from the trial cage on the same days as growth samples.

Sampling to investigate fish distribution was performed in the trial cage only on days 53, 54, 55, 56, 57, 59, 80, 85, 66, 69, 81, and 89. All distribution sampling was done between 13:00 and 19:00 on the day.

2.2.4 Laboratory Analyses of Samples

2.2.4:1 Water Quality

Sample Preparation and pH Measurement

Samples for water quality analyses were removed from the deep freeze and allowed to thaw at room temperature.

Upon thawing, samples were shaken thoroughly and approximately 100ml was poured into a clean glass beaker. The pH of the sample was measured using a Phillips meter and glass electrode. The meter had previously been calibrated with pH4 and pH7 buffer solutions.

Analysis of Nutrients

The phenol-hypochiorite method of total ammonia analysis (Golterman <u>et al</u> 1978, cited in Phillips 1986) was performed using the Technicon Sampler IV auto-analyzer at the institute of Aquaculture.

The percentage of un-ionised ammonia in the samples was determined using the formula:

% un-ionised = <u>100</u> ammonia <u>1 + antilog (pKa-pH)</u>

where pKa is the temperature dependent negative logarithm of the ionisation constant of ammonia (Phillips 1985).

Analyses for nitrite and nitrate were also performed using the auto-analyzer. For nitrite the methods of Strickland and Parsons (1972) and Mackereth <u>et al</u> (1978) were followed. For nitrate the method was that of APHA (1980). All of these methods were slightly modified for use with the auto-analyzer and are cited in Phillips (1985).

The method of Golterman <u>et al</u> (1978, cited in Phillips 1985) was used to measure dissolved reactive phosphorus in the samples.

Suspended Solids Analysis

Filter papers collected from the field were analysed for suspended solids according to the method of Stirling (1985). The filters were thawed for 14 hours and then placed in a drying oven at 75°C for a further 10 hours. The filters were then weighed to the nearest 0.1mg. Suspended solid levels were calculated as:

Suspended Solids = $(W_2 - W_1)$ $(mg.1^{-1})$ V

where W_2 and W_1 = final and initial filter weights (mg) and V = volume of sample filtered (1).

2.2.4:2 Zooplankton

Preserved zooplankton samples were analysed in the laboratory to determine the abundance, species composition, size composition, and blomass of the crustacean zooplankton population at each of the sampling sites and times.

Abundance and Species Composition

The problems and methods of estimating zooplankton abundance are reviewed by Bottrell <u>et al</u> (1976) and McCauley (1984). The methods used in this study to estimate abundance are based on the guidelines suggested by these authors. Each preserved zooplankton sample was allowed to settle for 2-3 hours. A small amount of the supernatant solution was poured away and the volume of the concentrated sample was measured to the nearest 1.0ml in a graduated cylinder. Ten successive 3.0ml subsamples were removed, without replacement, from the well-shaken concentrated sample. The subsamples were removed via a graduated pipette with a tip opening of 4mm. Each subsample was placed in a counting tray and examined under a dissecting microscope.

Individual crustacean zooplankton were identified and allocated to one of the following groups:

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- (1) Cyclops spp., (2) Diaptomus spp., (3) Daphnia spp.,
- (4) <u>Bosmina</u> spp., (5) <u>Chydorus</u> spp., (6) <u>Dlaphanosoma</u> spp.,
- (7) Polyphemus spp., (8) Leptodora spp., (9) nauplil.

The total number per subsample was determined as the sum of the numbers of individuals in each genus group. The total number of individuals per sample was calculated as the sum of subsample totals. Similarly, the number of individuals in each genus group per sample was calculated as the sum of the genus group subsample values.

The total abundance of crustacean zooplankton for each sample was calculated according to the formula:

Abundance $(\bullet,m^{-3}) = \frac{A \cdot B}{C \cdot D}$

Where A - the overall sample total;

- B = the volume of the concentrated sample (ml);
- C = the total volume of all subsamples counted (ml):
- and D = the volume of water flitered (m³).

Species composition (<u>1.e.</u> group composition) was expressed as percent of the total abundance.

Size Composition

The remains of the zooplankton samples from the abundance and species composition analyses were pooled for size composition analysis. Pooling of samples was considered necessary to increase the numbers of some of the genus groups. Observations suggested that the size of individuals did not vary greatly between areas on a given date, or between dates close to each other. On this basis, samples from site 1 were pooled into four groups: samples obtained between days 0 and 44; samples obtained between days 51 and 79; the day 85 samples; and the day 119 samples.

The problems of measuring the length of crustacean zooplankton are discussed by many authors. McCauley (1984) gives a concise review, and identifies inaccuracy of measuring devices, parallax, fatigue of the worker, and variation among anatomical characteristics, as the major sources of measurement error. Accuracy of length measurements is very important, as these values are often used to calculate biomass values and production rates of zooplankton communities (McCauley 1984).

For this study, the method used to measure length of the different zooplankton genus groups was as follows.

A subsample of the pooled concentrated sample was removed using a wide bore pipette (4mm tip aperture) and placed in a counting tray so that individuals were not overlapping one another. The tray was placed under a dissecting microscope equipped with a drawing tube and examined.

The image of the individual was transposed via the drawing tube onto a sheet of paper and the length margins marked. A separate sheet was used for each genus group in each pooled sample. This process continued until 50 individuals of two of the most common groups (generally <u>Cyclops</u> spp., <u>Diaptomus</u> spp., <u>Daphnia</u> spp.) had been observed, or the sample was exhausted. For the Cladocera genus groups, total length was measured as the distance from the tip of the crest to the base of the shell spine. Copepod genus groups were measured from the anterior end of the cephalothorax to the posterior end of the furcal rami, excluding furcal setae. Nauplii were measured from the anterior to the posterior margins of the cephalothorax. A Houston instruments HIPAD digitising pad coupled to a BBC microcomputer was used to measure the length of the individual images recorded on the genus/sample data sheets. A magnification factor (described below) was used to convert the image lengths to actual size of the zooplankton.

Before any measurements were taken, however, the method was evaluated for its precision. This was done in a three-step process.

First, the precision of the image drawing via the drawing tube-dissecting microscope system was evaluated by repeated drawing and measurement of a standard interval. In this case, measurements were taken to the nearest 0.5mm with a ruler. The coefficient of variation of these measurements was determined to be 1.72% of the mean image length. This precision suggested the drawing method was accurate.

Second, the precision and accuracy of the HiPAD measurements were evaluated. To accomplish this, intervals of 10, 20, 30, 50 and 80mm were marked onto a drawing sheet. Each interval was measured 50 times on the HIPAD. Mean lengths, standard deviations, coefficients of variation, and magnification factors (actual length divided by mean HIPAD length) were calculated for each group of 50 measurements. These results are shown in Table 5. They show an excellent series of coefficients of variation. The measurement technique was therefore considered precise. The set of

Table 5. Results of repeated measurements to determine the precision of the MIPAD measuring system.

:

ctual interval length (mm)	Number of measurements	Mean HIPAD Length (mm)	Standard deviation (m)	Coefficient ¹ of variation (X)	Magnification ² factor
10.0	8.	9.962	0.098	0.978	1.004
20.0	20	19.870	0.122	0.614	1.007
30.0	20	29.954	0.151	0.503	1.002
50.0	20	50.020	0.196	0.392	0.099
80.0	20	80.108	0.158	0.196	0.899

Coefficient of variation was calculated as the standard deviation divided by the mean and expressed as a percent. -

Magnification factor was calculated as the actual divided by the mean HIPAD length. ~

magnification factors shown in Table 5 have a mean value not significantly different than a hypothetical value of 1.0 (t= 1.438, d.f. = 4, p > 0.05). This method of measurement was therefore considered both precise and accurate.

Finally, a series of intervals was drawn using the microscope/drawing tube system, and these drawings were measured on the HIPAD. This was done to determine the coefficient of variation (that is, precision) of the method from actual specimen to final measurement, and to determine the total magnification of the system. Standard intervals of 0.2, 0.4, 0.8, 1.0 and 2.0mm were drawn 50 times and the measurements taken on the HiPAD. The entire procedure was repeated a second time. Mean HIPAD measurements, standard deviations, coefficients of variation, and magnification factors were calculated for each group of 50 measurements. These results are shown in Table 6. Several important trends emerge from this data. A correlation analysis of measured HIPAD length against actual interval length shows a significant positive correlation (r = 0.8799, d.f. = 9, p < 0.001). The set of coefficients of variation shows good overall precision in the technique, with increased precision for the longer intervals. The set of magnification factors are in close agreement, themselves having a coefficient variation of 2.43%.

Table 6. Results of repeated measurements to determine the precision and magnification from actual specimen to final HIPAD measurement.

tual interval length (mm)	Number of measurements	Mean HIPAD length (mm)	Standard deviation (mm)	Coefficient of variation (X)	Magnification factor
0.2	8	5.547	0.2970	4.9	0.0366
	8	446.0	0.3180	16 .0	0.0374
0.4	20	10.434	0.3558	3.41	0.0383
	80	10.361	0.4025	3.88	0.0386
0.8	99	20.684	0.3334	1.61	0.0387
	20	20.612	0.3913	1.90	0.0388
1.0	. 05	25.344	0.6668	2.63	0.0395
	20	25.431	0.7051	2.77	0.0393
2.0	99	50.697	0.4814	0.95	0.0395
	50	50.708	0.4879	0.96	0.0394

A mean magnification factor of 0.0386 (95% confidence limits \pm 0.0007) was used to calculate actual zooplankton lengths from the image lengths measured by the HIPAD system.

Blomass Estimation

Methods for the estimation of zooplankton blomass have been critically reviewed by many authors (<u>e.g.</u> Dumont <u>et al</u> 1975, Bottrell <u>et al</u> 1976, Culver <u>et al</u> 1985). Perhaps the most comprehensive examination of the subject is given by McCauley (1984). He considered the blomass of a population or portion of the population (<u>e.g.</u> species or genus groups) to be the product of the abundance and the mean weight of individuals, or:

B = N.W

The precision of the biomass estimate depends partly upon the precision of the estimation of abundance of zooplankton. Precision of abundance estimates depends upon many characteristics of both the zooplankton themselves and the sampling programme. Repeated samplings for abundance were not taken in this study and so no measure of precision of this factor was available. The assumption was made that the field methods used in this study were precise and that repeated samplings would have shown minimal variations. The precision of the blomass estimate also depends partly upon the estimation of the mean weight of individuals. Estimations of the mean weight of zooplankton individuals should be performed directly for each study to maximise precision (Lawrence <u>et al</u> 1987). However, this is often not practical, as measurements of weight of individual zooplankton is a very time consuming and costly task.

Length-weight regressions are the most common method of estimating mean weight. These may be calculated directly as part of the study, or published regressions may be utilised if resources do not permit the direct approach. In any case, the regressions are derived from a series of paired weight and length measurements. Estimation of mean weight of individuals can then be calculated easily by estimation of mean length of a group of individuals (<u>e.g.</u> population, species) and substituting this value into the regression equation.

Regression equations can take many forms, but the form utilised by McCauley (1984) is perhaps the most common:

InW = Ina + b InL
where InW = natural logarithms of the dry weight (µg);
Ina = the intercept of the regression line;
b = the slope of the regression line;
and InL = the mean of natural logarithms of length
measurement (mm).

Lawrence et al (1987) and McCauley (1984) cite Persson and Ekbohm (1980) who give the following formula to calculate variances of mean weights derived from length-weight regressions:

$$s_{inil}^2 = RMS$$
, $n^{-1} + (inL - x) - s_L^2 + b^2$, s_L^2

where S² = variance of InW;

RMS = residual mean square of the regression;

- n = number of paired observations used to derive the regression;
- x = mean of the inL measurements from the regression;
- s_{-}^2 = variance of x;
- b = slope of the regression;
- inL = the transformed mean length substituted into the regression;

and

 $S_1^2 = variance of InL.$

W - e'nil

Natural logarithmic transformations of the length and weight data used in the regressions is necessary to normalise the distributions and reduce inequality among variances (Lawrence et al 1987). Conversion of calculated InW values back to arithmetic form is therefore:

When the variance of InW can be calculated, the conversion must be of the form:

$$W = e^{(IIII + S^2_{1III})/2}$$

This is to correct for the skewed distribution created when converting logarithmic to arithmetic values (Lawrence <u>et al</u> 1987).

Based on the recommendations of Bottrell et al (1976), McCauley (1984), and Lawrence et al (1987) published regression equations were used to estimate mean weight of individuals using the mean length data derived directly from this study. These weights were then used to calculate the blomass of the zooplankton genus groups for all sampling times and days, using the abundance data also directly derived from this study. The regression equations were chosen for each genus group first upon the similarities between the sampling methods and ecological conditions of the study used to derive the regression and this study. if this produced a choice of regressions, the one which was derived using a range of lengths most similar to the measured lengths of this study was chosen. If a choice was still available, the availability of data for calculating the variance of the calculated mean weight was used as the deciding characteristic.

The regression equations used in this study and their associated statistics are shown in Table 7.

Table 7. Regression equations¹ and their associated statistics that were used to calculate the mean weight² of individual zooplankton from directly measured mean lengths.

Genus group ⁴	Regression equation	Range in length (mm)	c		×	s ²	RMS	Reference		1
Cyclops	0.8370 + 2.6688 InL	0.272-1.238	201	0.98	-0.4569	0.1341	0.0386	Lawrence	i al	587
Diaptomus	1.0542 + 2.7482 lnL	0.384-1.140	262	0.98	-0.2767	0.0679	0.0178		•	•
Daphnia	0.9455 + 3.1108 InL	0.560-1.542	58	0.93	-0.1599	0.0833	0.1130	•		•
Bosmina	2.4751 + 3.3614 InL	0.204-0.492	88	0.99	-1.2210	0.0413	0.0082			•
Chydorus	2.6447 + 1.9796 InL	0.219-0.310	60	0.99	N/A	N/A	NIA	Culver et		985
Diaphanosoma	1.2740 + 3.2454 InL	0.390-1.318	85	0.96	-0.2940	0.1026	0.0800	Lawrence	t al 1	687
Polyphemus	12.9153 + 2.15 InL	N/A	12	NVA	N/A	NIA	N/A	Dumont et	-	975
Leptodora	0.4450 + 1.8730 lnL	2.268-6.804	17	0.99	N/A	N/A	N/A	Culver et	=	985
Nauplii	1.2461 + 2.2650 lnL	0.128-0.300	8	0.99	-1.6053	0.0793	0.0061	Lawrence		687
										1

Regression equations are of the form InW = Ina + bInL.² 2. Weight units are µg. 3. Length units are mm, except <u>Polyphemus</u> which are µm. 4. <u>Cyclops</u> and <u>Diaptomus</u> regressions are for copepodid and adult stages. Nauplii regression is that for <u>Diaptomus</u> nauplii which was very similar to that for <u>Cyclops</u> nauplii.

2.2.4:3 Artificial Diets

Particle Size

A sample of each of the pellet types utilised during the trial at site 1 was analysed to determine the mean particle size of each. Size was taken as the maximum dimension of the particle. Particles were measured using the same procedure as for zooplankton length (section 2.2.4:2). Fifty particles of each pellet type were measured.

Blomass and Abundance

The blomass and abundance of artificial diets in the trial cage on fish/food sampling days was determined by the following calculations, based on a number of assumptions.

The values obtained by biomass and abundance calculations were considered to represent the biomass and abundance of artificial diets in the trial cage at any minute on the sampling day. To justify this consideration, several assumptions were made. First, it was assumed the feed was spread homogeneously over the cage surface area. Second, it was assumed the feed was delivered constantly throughout the period the feeder was active. Finally, it was assumed that sinking rates obtained in the laboratory were representative of those of particles in the cage. The implications of making these assumptions are discussed in

section 2.4.

Biomass of artificial feed was calculated as:

51	W _{art}	$= \frac{1 \times 10^8 \cdot A \cdot B}{C \cdot D \cdot E}$
where	8M _{art}	= biomass of artificial feed (mg.m ⁻³);
		<pre>= feeding rate (% body weight.day⁻¹);</pre>
	B	= total fish weight in the cage (kg);
	С	= time feeder was active (min.day ⁻¹);
	D	= cage surface area (m ²);
and	E	= sinking rate of particles $(m.min^{-1})$.

Particle sinking rates were determined in the laboratory. Thirty particles of each pellet type were individually released into a graduated cylinder containing de-ionised water at 12°C. The time for each particle to pass through a 30cm interval was recorded. The mean time of each particle type was calculated, and sinking rates expressed in terms of meters per minute.

Abundance of artificial feed was calculated as:

ABert	= F.BM _{ert}
where	$AB_{art} = abundance of artificial feed (* .m-3);$
	<pre>F = number of particles per unit weight (* .mg⁻¹);</pre>
and	BM_{art} = biomass of artificial feed (mg.m ⁻³).

The number of particles per unit weight was also determined in the laboratory. Three random samples of each of the pellet types were obtained. From each sample a 100.0mg subsample was weighed into a petri dish. The number of particles in each dish was determined with the aid of a dissecting microscope. The mean number of particles of each pellet type was calculated and expressed in terms of number per milligram.

It was not possible to obtain estimates of variance of the calculated abundances and biomasses of artificial feed.

Volume to Weight Conversion Factors

Volume to wet and dry weight conversion factors for each pellet type utilised at site 1 were determined for use in the stomach contents analysis of the fish (section 2.2.4:4).

Three 1.0ml replicate samples of each pellet type were measured into pre-weighed graduated cylinders. The diets were packed tightly into the cylinders with a glass plunger to eliminate air spaces as much as possible. Each cylinder was weighed to the nearest 0.1mg on a Mettler balance. The weight of the samples was determined as the before and after difference in cylinder weight. The mean of each triplicate sample was calculated and considered as the volume to wet weight conversion factor. The dry weight content of the artificial diets used at site 1 is reported to be 91% (Ewos-Baker). The volume to dry weight conversion factor for each pellet type was thus calculated as the product of the wet weight conversion factor and the dry weight content.

2.2.4:4 Fish

Stomach Contents Analyses

Fish samples collected from the trial cage at site 1 were subjected to stomach contents analyses in the laboratory. The results of these analyses were subsequently used to calculate selection indices for the fish.

Eight fish from each sample were prepared for analysis by soaking for 12 hours in distilled water (to reduce the effect of formalin fumes). Four of these fish were randomly selected and each measured for fork length to the nearest 0.5mm. These four fish from each sample were then analysed for stomach contents. The remaining fish were retained for future reference.

Review papers on the subject of stomach contents analysis methods used in fish ecology studies have been published by several authors (Hynes 1950, Pillay 1952, Langler 1956, Windeli 1970, Windell and Bowen 1978, Hyslop 1980). The most recent of these is perhaps the most useful in the context of this particular study.

Hysiop (1980) identifies two types of fish studies utilising stomach contents analysis. The first type is one which assesses the fishes' nutritional standing in the ecological community. It is mainly concerned with comparisons of the study fish diet, with the food available and with other fish species or groups. The second type of study is one in which an attempt is made to estimate the total food consumption of the study fish population. Studies of this type involve a calculation of dally ration from laboratory or field estimates of physiological constraints such as the gastric evacuation pattern. It is unfortunate that gastric evacuation data are not available for this particular study. Stomach contents analyses were performed only for type 1, or comparative purposes.

Hysiop (1980) noted that stomach contents may be examined for individual fish, but it is best to consider all the fish in one sample together. Further, he considered three methods of analysis used in conjunction the best way to judge the importance of a food category. First, the numerical method involves counting the number of a food item in the stomach, totalling for all stomachs in the sample, and expressing the result as a percent of the total number of all the food items of all categories. Second, the occurrence (or frequency) method involves counting the number of stomachs in the sample that contain one or more

individuals of the food item category. The result of this method is expressed as percent of the number of stomachs sampled. Finally, a gravimetric or volumetric method involves some technique of measuring the weight or volume of a food category in the stomach, totalling these values for all stomachs and expressing the result as percent of the total of all stomachs in the sample.

in his review Hyslop (1980) notes that by considering number and bulk (or volume) together, an accurate picture of the dietary importance of a food type can be gained. He describes two methods for combining the results of the numerical, volumetric, and frequency analyses to obtain an index of relative importance of a food type. Hyslop (1980) concludes his discussion of these methods by saying that neither is more accurate than the other.

One method of obtaining an index of relative importance (IRI) is described by Hyslop (1980) as:

IRI = (SN + SV). SF

where

%V = percent composition by the volumetric method:

%N = percent composition by the numeric method;

and %F = percent composition by the frequency method (all percents expressed as decimals).

Each of the components of this IRI is simply the probabillty of a particular food type to be part of the fishes' diet as determined by that method. The IRI, therefore, is

the combined probability of all three methods. The iRi given above and described by Hyslop (1980) puts more importance on the numeric and volumetric methods than the frequency method (by summing their probabilities before multiplying with the frequency probability). As all three methods have variation caused by both differences in feeding behaviour between fish and errors in analysis, it seems unrealistic to put more weight on the results of some methods than others. A more suitable calculation of an IRI would be as:

IRI = %N . %V . %F

where %N, %V and %F are expressed as decimals.

This puts equal weight on the results of all three methods. It also results in a possible range of values of 0 to 1. This is in fitting with the nature of an IRI, that is, expressing the importance of a particular food type in the diet as the probability of its occurrence determined by three discrete analyses. Calculation of an IRI by this method does not, however, include any estimate of variance or precision of the index. This makes statistical comparability invalid, as the worker must assume minimum variation.

Based on the guidelines described by Hyslop (1980), the following methodology was used for stomach contents analysis of fish samples.

The stomach contents were considered to be those contained between the oesophagus opening and the pyloric sphincter. This was obtained by cutting the head off anterioriy to the insertion of the pectoral fins, making an incision along the ventral line and carefully removing the entire alimentary canal. The stomach was cut along its longitudinal axis, and the contents removed with forceps and placed on a clean, calibrated well slide (see description below).

The contents of the stomach were separated into zooplankton, artificial food, non-zooplankton natural, and mucus categories. Where possible, the latter two categories were removed from the sample and not considered in the stomach contents analysis. Complete separation of these contents from the rest of the contents was generally impossible, and was thus a source of error in the analysis.

The remaining stomach contents were then analysed with respect to the frequency of occurrence, number, and weight (indirectly via a volumetric method) of the components.

The presence or absence of the artificial and zooplankton food categories in the stomach was recorded for the frequency of occurrence method.

individual zooplankton were separated into genus groups and counted. When only fragments of individuals were present,

the number of eye spots (Cladocera) or anterior sections of the cephalothorax (Copepoda) were counted. The total numbers for each genus group were added together to obtain a total zooplankton value for the numerical method. Numerical values for the artificial food category were not obtainable at this stage of the analysis.

The volumetric method used was an adaptation of that originally described by Hellawell and Abel (1971). In this method the stomach contents are squashed to a known depth and volume determined by measurement of the area of the squash. Hellawell and Abel (1971) thoroughly evaluated the method and determined its precision as about 3.5% error for soft contents and about 8.0% error for hard bodied/contents. As the stomach contents of this study were predominantly soft, this method was considered adequate for estimating stomach contents volume.

Weil sildes were fabricated to measure contents volume using the squash method from the basic design of Hellawell and Abel (1971). Six different sildes of varying well depths were made by the following method. Polyvinyl lactophenol was used to cement an equal number of glass coverslips on either side of a standard microscope slide. The stomach contents were placed in the centre of the well slide and a second slide was used to squash the contents to the depth of the coverslips. The mean depth of the well slides was determined by taking ten measurements of the gap

between the well slide and the cover-slide using Vernier calipers. These data are shown in Table 8.

The areas of the stomach contents squashes were determined in two steps. First, a trace was made around the image of the squash projected onto a drawing sheet through a drawing tube/dissecting microscope system. The area of this image was then determined using a planimeter, and the area of the actual squash was calculated using a pre-determined magnification factor (see below). The calculated squash area was then multiplied by the slide depth to give the volume of the food category under examination.

The precision and accuracy of the area measurement using the planimeter was evaluated. To accomplish this, circles of radii 1.0, 2.0, 3.0; 4.0, 5.0, and 6.0cm were accurately drawn on a sheet of drawing paper. Each circle was measured 30 times with the planimeter. The mean measured area was compared with the actual area. These results are shown in Table 9. They show good coefficients of variation of the measured areas and thus good precision. Highly significant agreement between measured areas and actual areas for circles of radius 1.0-6.0cm indicate good accuracy of the method.

A final test of precision of the stomach contents volume measurement method is described as follows. Four small circles were drawn accurately on a sheet of drawing paper.

Table 8. Mean depth and associated statistics of well slides used in stomach contents volume analyses.

Slide	Number of coverslips	Number of measurements	Mean depth (mm)	Standard deviation (mm)	Coefficient of variation (X)
-	•	0	0.60	0.00	0.0
~	v	10	0.85	0.04	4.7
м	7	10	1.03	0.02	1.9
•	0	0	1.31	0.02	1.5
S	=	10	1.68	0.02	1.2
6	13	0	2.16	0.06	2.8

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Table 9. Comparison of measured areas with actual areas to determine the precision of the planimeter.

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aniher lenta	Actual	Mumber	Maan	Standard	Coefficient		
of circle (cm)	arga (cm)	of measurements	area (cm)	deviation (cm ⁾	of variation (%)	t-1 value	Significance ²
1.0	3.14	90	3.16	. 0.156	4.94	0.702	p > 0.05
2.0	12.57	30	12.72	0.591	4.65	1.39	p > 0.05
3.0	28.27	90	28.43	0.844	2.97	1.04	p > 0.05
4.0	50.26	30	50.57	1.084	2.14	1.57	p > 0.05
5.0	78.54	30	78.54	0.941	1.20	•	p > 0.05
6.0	113.10	30	112.51	1.975	1.76	1.64	p > 0.05

t-value: students t-yalue to test the null hypothesis that the mean measured area was not different than the actual area. :

2. Significance: probability of getting a larger t-value.

Each circle was projected through the microscope/drawing tube system and the image traced 30 times. The area of each trace was determined with the planimeter. The mean of the measured areas and associated statistics were calulated. A magnification factor was calculated as the ratio of mean image circle area to actual area. These results are shown in Table 10. The precision of the method was determined by the combined mean coefficients of variation of slide depths and image area measurements (after McCauley 1984):

C.V._t = $(C.V._d)^2 + (C.V._a)^2$ 0.5 where C.V._t = total coefficient of variation; C.V._d = mean depth coefficient of variation; and C.V._a = mean area coefficient of variation.

A result of 2.88% was obtained, and the method was considered to be precise.

The coefficients of variation shown in Table 10 indicate that the combination drawing tube and planimeter method of measuring the area of stomach squashes is very precise, and the magnification factors calculated were therefore considered accurate. A mean magnification factor (\pm 95% c.i.) was calculated and used for all subsequent conversions (160.22 \pm 5.75). The combined coefficient of variation of slide depth and measured area also indicates good precision, and therefore results obtained by this

Table 10. Mean measured areas, associated statistics, and calculated magnification factors of standard circle images drawn through the dissecting microscope/drawing tube apparatus and measured with the planimeter.

	Moan areg (cm ²)	. Standard deviațion (cm ²)	Coefficient of variation (X)	Magnification factor	
30	29.16	.8076	2.77	164.56	
30	56.08	1.0456	1.86	161.47	
30	88.88	, 1.7358	1.95	158.69	
90	126.36	2.0954	1.66	156.17	

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method can be considered accurate.

Calculation of wet and dry weights was necessary to allow comparison between zooplankton and artificial feed biomass and the amounts of these food types in the fishes' stomachs. Direct measurement of contents weight was not considered a suitable method. Complete separation of stomach contents components proved to be a near impossible task. In the measurement of stomach contents volume this was not an important problem, as visual examination allowed compensations to be made in the tracing. However, in the measurement of weight, incomplete separation would result in errors.

Artificial food category volumes were converted to wet and dry weights using the appropriate conversion factors (determined in section 2.2.4:3). An estimate of the number of artificial food particles comprising the stomach contents of this category was then calculated by multiplication of the calculated wet weight by the appropriate value of number of particles per unit weight (also determined in section 2.2.4:3).

Zooplankton food category volumes were converted to dry weights using a constant conversion factor. Bottreil <u>et al</u> (1976) considered zooplankton to be neutrally buoyant in fresh water and thus have a density of 1.0mg.mm⁻³. A survey of the literature revealed that freshwater zooplankton

generally comprise 8% dry matter. When combined, these two factors give a zooplankton volume to dry weight conversion factor of 0.08mg.mm^{-3} .

The results of the frequency, numerical, and gravimetric analyses were combined for all the fish examined from one sample, as prescribed by Hyslop (1980).

indices of relative importance (IRI) for total zooplankton and artificial diet food categories were calculated for each sample using the combined data and the formula:

IRI = %N . %V . %F

where all percents are expressed as decimals.

The relationship between the indices of relative importance of each fish group and time were determined. No statistical comparison on a day by day basis was possible.

Selection Indices

Many different methods for the calculation of selection indices are reported in the literature. These methods have been reviewed by many authors (<u>e.g.</u> Cock 1978, Straus 1979, Kohler and Ney 1982, Pearre 1982). Most agree that the choice of a selection index depends greatly upon the aims of the particular study, the data available, and the statistical relevance of the resultant index. All selection indices are the result of some method of comparison between the proportions of a particular food component in the stomach contents samples and the environmental samples. The precision and accuracy of the calculated index is thus a function of the precision and accuracy of the stomach contents and environmental samples (Straus 1979).

Two commonly used selection indices are the forage ratio, originally described by Savage (1931, cited in Cock 1978) and lyley's index of electivity (lyley 1961). These indices are described, respectively, by the formulae:

$$FR = \frac{r_1}{p_1}$$
and
$$E = \frac{r_1 - p_1}{r_1 + p_1}$$

where r_i and p_i are the proportions of the ith food component in the stomach contents and the environmental samples, respectively.

The main disadvantage of these indices is that they are a ratio of two continuous variables expressed as proportions, and thus the variance expressions for the indices cannot be described accurately (Straus 1979). This makes statistical comparison of results difficult.

Straus (1979) suggested an alternative index of selectivity. He termed this the linear index of food selection.

It is described by the formula:

As this index is a linear combination, its variance can be accurately described by the summation of the variances of the proportions r_1 and p_1 . Straus (1979) gave the following expression to calculate the variance of L_1 when only single predator and prey samples were available:

 $S^{2}_{exp}(L_{1}) = S^{2}_{exp}(r_{1}) + S^{2}_{exp}(p_{1})$ $= \frac{r_{1}(1 - r_{1})}{n_{r}} + \frac{p_{1}(1 - p_{1})}{n_{p}}$

where S^2_{pp} is the expected variance of L_i , r_i and p_i , and n_r and n_p are the total number of prey organisms in the stomach contents and environmental samples.

The calculated variances can then be used to calculate confidence intervals or to statistically test the difference between values of L_i , r_i , or p_i and hypothetical values, or values calculated using different data sets.

Straus (1979) also noted that various measures of r_i and p_i may be used in the calculation of L_i . Numbers, weights, or volumes can be used in the calculations. Results of stomach contents of individual fish may be used to calculate r_i , or combined fish samples may be considered as one in the calculation of r_i . Straus' linear index was chosen as the method to evaluate feed selection by the cage trial fish of site 1. Indices were calculated using contents data combined for each sample. Indices of the cage red and cage green fish groups were statistically compared separately for each sampling date.

Selection indices (L_i) were calculated using numeric (artificial, total zooplankton, and zooplankton groups) and gravimetric (artificial and total zooplankton) data to represent r_i . Abundance (artificial, total zooplankton and zooplankton groups) and blomass (artificial and total zooplankton) data were used to represent p_i in the calculation of individual and sample selection indices.

2.2.5 Statistical Analyses

Unless otherwise indicated, all statistical analyses were based on methods described by Sokal and Rohlf (1987).

Where possible, statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS inc. 1986) package on the VAX-A mainframe computer at the University of Stirling.

2.3 RESULTS

2.3.1 Meteorology

No consistent trend was seen in the meteorological data collected from site 1 (Table 11). These data are given only as an aid in the description of the environmental conditions on the available food and fish sampling days.

2.3.2 Water Quality

2.3.2:1 Water Temperature

Water temperature data from site 1 are presented in Figure 4. Morning (9:00) and evening (22:00) readings showed a trend of warming over the day. These and daily (15:00) readings showed a general increase until peak temperatures (18.5 and 14.2°C for cage and control tank respectively) were observed about 50 days after first feeding. Values thereafter declined until the end of the trial. Control tank water temperatures were generally 1-2°C lower than trial cage ones.

2.3.2:2 Dissolved Oxygen

Figure 5 shows dissolved oxygen data obtained from site 1. Levels increased both inside and outside the cage on the day (9:00 to 22:00). These and daily (15:00) values showed
Day	Wind speed (km.h)	Wind direction ²	Cloud cover (%)*
7	22-25	SW	100
15	20-30	E	100
23	12-14	SW	75
38	7-9	NE	50
44	9– 12	E	75
51	12-15	SW	50
58	12-15	SW	50
65	12-15	W	75
72	6-7	N	100
79	7-9	NE	100
85	2–3	NW	50
119	7 -9	NE	75

Table 11. Wind speeds and directions and percentage cloud cover on selected dates at site 1, 1988.

1. Wind speeds shown are the minimum and maximum on the day.

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2. Wind directions and cloud cover are given as the approximate average over the day.

Figure 4. Water temperatures within the trial cage and the control tank, site 1, 1986.

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Figure 5. Dissolved oxygen inside and outside the trial cage, site 1, 1986.

Readings taken at:

▲ 9:00
⊽ 22:00
+ 15:00

A. Inside

B. Outside



a general decline in dissolved oxygen levels as the trial progressed to about 50 days after first feeding, then a general increase to the trial end. Levels outside the cage were consistently higher than inside, both over the day and over the trial.

2.3.2:3 pH, Suspended Solids and Nutrients

The pH dld not vary greatly over the course of the trial at site 1 (Figure 6A).

Great variations in suspended solids levels were seen inside the trial cage (Figure 6B.). From the start until about mid-trial, levels rose and fell at times which coincided closely with dates of net changes. After this suspended solids levels in the cage generally increased toward the trial end. In comparison, suspended solids at the control area steadily increased until about 25 days after first feeding then decreased to the trial end.

Cage and control levels of dissolved reactive phosphorus (DRP) were low from the start of the trial until about day 25, corresponding with high suspended solids levels at both locations during this period (Figure 6C). Between this time and about day 60 cage and control DRP levels often rose and fell concurrently with net changes. After this time, DRP levels at both locations steadily increased to the trial end. Figure 6. pH (A), suspended solids (B), and dissolved reactive phosphorus (C), site 1, 1986.

Cage
 Control
 Net changes







Un-ionised and ionised ammoria levels were seen to follow similar trends (Figures 7A and 7B, respectively). This is not surprising as both were calculated directly from the measured total ammonia concentration. Un-ionised ammonia levels remained a small fraction of the ionised levels throughout the trial.

Cage un-ionised and ionised ammonia, nitrite, and nitrate (Figures 7A, 7B, 7C and 7D, respectively) all generally increased over the trial, often fluctuating coincidentally with net changes. Control levels of these parameters also steadily increased over the trial period. However, ammonia and nitrite control levels did not become as high as those recorded in the cage, while control levels of nitrate did.

2.3.3 Fish Mortality and Growth

2.3.3:1 Mortality

The initial mortality of the fish heid in the trial cage was higher than that of the fish heid in the control tank (Figure 8). The mortality rate was similar for both cage and control fish during the first 15-20 days. For the next 30 days the mortality of the cage fish increased substantially, while the rate in the control tank only slightly increased. After this period to the trial end the rate of mortality of the cage fish decreased to a level close to that of the control stock. Figure 7. Unionised ammonia (A), ionised ammonia (B), nitrite (C), nitrate (D), site 1, 1986.

Cage
 Control
 Net changes

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Figure 8. Fish cumulative mortality,

site 1, 1986.

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A. Cage

B. Control tank



2.3.3:2 Growth

Each of the fish groups showed a significant positive growth trend over the course of the trial (Figure 9 and Table 12). Further, Table 13 indicates that there was no significant difference between the over-trial growth trends of control (CON) and cage overall (CO), CON and cage red (CR), CON and cage green (CG), or CR and CG fish groups.

While these results indicate no differences in the overall trends, Table 14 shows that the size of the fish groups differed on a day by day basis. The CO fish were generally of similar size to those of the CON group. The exception to this was between 38 and 51 days after first feeding, when the CON group were significantly larger. CON fish were generally larger than CR fish. The CG group often was seen to be larger than the CON group, particularly toward the end of the trial. Finally, the CR fish were observed to be initially larger than the CG fish; however, by day 58 this trend had reversed and remained so until the end of the trial.

2.3.4 Fish Distribution in the Cage

On many days significant differences in the colour proportions (<u>i.e.</u> number of red fish : number of green fish) between east, central and west areas of the trial cage were observed (Table 15). However, on some days a non-sig-

Figure 9. Fish growth, site 1, 1986

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- A. Cage (overall)
- B. Cage red

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- C. Cage green
 - D. Control tank

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Table 12. Results of statistical tests to determine the relationship between size and time after first feeding for cage overall, cage red, cage green, and control fish groups, site 1, 1986.

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1.	2
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tis	end
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3.235 + 0.0070x 1962 *** 3.170 + 0.0077x 2565 *** 3.209 + 0.0066x 1619 *** 3.182 + 0.0081x 2029 ***	lny = a + b x'	^r regression ²	Significance ³	
3.170 + 0.0077x 2565 ••• 3.209 + 0.0066x 1619 ••• 3.182 + 0.0081x 2029 •••	3.235 + 0.0070×	1962	I	
3.209 + 0.0066x 1619 *** 3.182 + 0.0081x 2029 ***	3.170 + 0.0077x	2565	:	
3.182 + 0.0081x 2029 2029	3.209 + 0.0066x	1619	:	
	3.182 + 0.0081x	2029	1	ì

lny = a + bx: the regression equation where lny is the dependent variable, a is the y-intercept, b is the the regression coefficient, and x is the independent variable. 1.

^fregression: the variance ratio to test the null nypothesis that the variance of the dependent variable could not be explained by the regression on the independent variable. ~

Significance codes: *** = p < 0.001, ** = p < 0.01, * = p < 0.05, n.s. = not significant. e.

Table 13. Results of statistical tests to compare the growth regressions of control, cage overall, cage red, and cage green fish groups, site 1, 1986.

Comparison	F-value ^l	Significance ²
ntrol - cage overall	0.1789	
ntrol - cage red	0.0829	n.s.
ntrol – cage green	0.2993	n.s.
de red - cage green	0.7684	л. в.

1. F-value: variance ratio to test the null hypothesis that $b_1 = b_2$.

2. See Table 12 for significance codes.

Table 14. Results¹ of statistical tests to compare the mean fork length of control, cage overall, cage red and cage green fish groups, site 1, 1986.

Day	Control - cage overall	Control – cage red	Control – cage green	Cage red - cage green
7	0 n.s.	0 n.s.	0 n.s.	0 n.s.
15	ŧ	ŧ	+	ŧ
23	+ n.s.	+ I	+	+ n.s.
38	+ ***	+ ***	+ ***	+ ***
44	+ ***	+ ***	+ n.s.	- *
51	+ ***	+ ***	+ ***	+ n.s.
58	+ ñ.s.	+ •	- n.s.	- n.s.
65	+ n.s.	+ **	_ ==	_ ***
72	• •	+ ***	+ n.s.	_ **
79	- n.s.	+ n.s.	- **	_ ***
85	- n.s.	+ n.s.	- •	
119	- n.s.	+ •	_ ***	

Statistical test: student's t-test.

 Results show sign of the t-value and its significance. t-value: for testing the null hypothesis that there was no significant difference between the mean fork lengths.

See Table 12 for significance codes.

Stati: requi	red)	test:	Chi-square	analysis (Ya	tes correc	tion where
	c	age Area ¹		Com	bar i son ²	
Day 	East	Central	West	E-C-W	E-C	w-c
53	<u>29</u> 1	<u>22</u> 8	2 <u>3</u> 7	6.53 (*,2)	4.71 (*,1)	0.00 (n. s. ,1)
54	2 <u>4</u> 6	22 8	<u>22</u> 8	0. 48 (n.s.,2)	0.09 (n.s.,1)	0.00 (n.s.,1)
55	19	<u>18</u> 12	<u>24</u> 6	3.15 (n. s., 2)	0.00 (n.s.,1)	1.98 (n.s.,1)
58	2 <u>1</u> 9	15 15	<u>24</u> 6	8.30 (*,2)	1.74 (n.s.,1)	4.69 (*,1)
57	20 10	<u>11</u> 19	<u>19</u> 11	6.57 (*,2)	4.27 (*,1)	3.27 (n.s.,1)
59	1 <u>6</u> 10	<u>12</u> 18	<u>18</u> 12	2.49 (n.s.,2)	0.60 (n.s.,1)	1.67 (n.s.,1)
60	19	11 19	2 <u>3</u> 7	10.28 (**,2)	3.27 (n.s.1)	8.21 (**,1)
65	29	9 21	<u>25</u> 5	35.56 (***,2)	25.91 (***,1)	15.27 (***,1)
66	<u>28</u> 4	1 <u>5</u> 5	no sample	7.70 (**,1)	7.70 (**,1)	-
69	17 13	10 20	no sample	2.42 (n.s.,1)	2.42 (n.s.,1)	
81	18 12	<u>8</u> 22	1 <u>3</u> 17	6.79 (*,2)	5.50 (*,1)	1.17 (n.s.,1)
89	<u>16</u> 14	<u>11</u> 19	<u>11</u> 19	2.28 (n.s.,2)	1.08 (n.s.,1)	0.00 (n.s.,1)

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Table 15. Results of statistical analyses to determine differences in the proportion of red and green fish in different areas of the trial cage, site 1, 1986.

1. Cage area results show number red over number green fish in each of the cage areas.

 Comparison results show the chi-square value to test the null hypothesis of no difference (significance and degrees of freedom in brackets).

See Table 12 for significance codes.

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nificant difference was obtained, and rarely was the difference significant at the p < 0.001 level. This trend became weaker when only east and central, and west and central areas were compared. That is, fewer dates showing significant differences in the colour proportions between areas were seen.

The size frequency distributions of the red and green fish groups (pooled from all areas) were significantly different on most sampling days (Table 16). Generally there were higher proportions of red fish in the smaller size classes and green fish in the larger size classes on each day.

When data were pooled over all sampling days, significantly different size frequency distributions between red and green fish were shown in each of the three cage areas (Table 17). Again, these differences were caused by higher proportions of red fish in the smaller and green fish in the larger size classes.

Finally, each area of the cage showed significantly different size frequency distributions over the course of the sampling period, disregarding any separation of red and green fish groups (Table 18). This difference is a result of there being more fish in the extreme size-classes in the peripheral areas than in the centre of the cage.

Table 16. Results of statistical tests to determine differences in the size frequency of red and green fish over all areas of the trial cage, site 1, 1986.

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Statistical test: Calculation of G-statistic (size classes combined where required)

			Cla		(mm)1				
Day	<30	31-33	34-38	37-39	40-42	43-45	46-48	>49	GZ
53	31	23 3	<u>30</u> 5	17 6	1	-	-	-	0.63 (n.s.,1)
54	5 T	<u>11</u> 2	27 3	<u>19</u> 11	<u>5</u> 4	1	-	-	8.83 (**,1)
55	<u>3</u> 1	<u>9</u> 1	22 4	<u>25</u> 12	<u>2</u> 10	<u>0</u> 1	-	-	10.32 (**,1)
58	6 1	<u>6</u> 0	<u>26</u> 12	<u>19</u> 13	3 4	-	-	-	3.25 (n.s.,1)
57	$\frac{1}{1}$	<u>13</u> 4	1 <u>4</u> 4	<u>21</u> 17	$\frac{1}{13}$	<u>0</u> 1	-	-	23.08 (****,3)
59	-'	<u>8</u> 1	<u>11</u> 8	<u>21</u> 15	5 18	1 <u>2</u>	-	-	62.16 (***,2)
60	<u>6</u> 1	<u>7</u> 2	17 2	<u>18</u> 18	5 13	<u>0</u> 1	-	-	21.87 (**,3)
65	ł	18 0	<u>27</u> 2	1 <u>3</u> 7	<u>5</u> 10	1_ 8	-	-	41.85 (**,2)
66	10	<u>5</u> 1	10 0	18 7	<u>3</u> 10	<u>3</u> 4	<u>0</u> 1	-	20.31 (****,2)
69	1 6	3	10 3	7 10	4	<u>0</u> 4	ę	-	8.77 (n.s.,3)
81	-	\$	ŧ	10 1	12	8 20	3	1 9	34.52 (****,3)
89	-	-	30	1 <u>0</u> 2	10 5	9 13	17	2 15	29.17 (****,4)

1. Size class results show number of red over number of green fish; numbers combined from all cage areas.

 G: G-statistic value to test the null hypothesis that red and green fish had similar size frequency compositions (significance and degrees of freedom in brackets).

See Table 12 for significance codes.

Table 17. Results of statistical tests to determine differences in the size frequency distributions of red and green fish groups in different areas of the trial cage, over all sampling dates, site 1, 1986.

				Size cla	ss (mm) ¹				
Area	8	31-33	34-36	37-30	40-42	43-45	46-48	6¥.	75
ast	=	2	102	1	22	1	m	~	88.36
	•	•	æ	35	28	17	12	-	(5'***)
Sentral	•	1	52	69	23	10	0	1	64.23
	•	6	21	73	58	31	=	13	(5'***)
fest		42	67	68	18	7	4	0	80.33
	4	\$	16	21	30	=	13	10	(9'***)

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Table 18. Results of statistical analysis to evaluate the difference in the size frequency analysis of fish (combined red and green) between areas of the trial cage, site 1, 1986.

				Size cla	1 (m) ss					
	8	31-33	34-36	37-39	40-42	43-45	46-48	6 7	er	
ŧ	15	99		112	20	24	15	e		
ntral	60	20	£	142	81	Ŧ	=	z	176.2 (***,14)	
t	18	14	63	68	48	18	17	2		

Size class results show the total (combined red and green) number of fish in each size class; numbers combined from all sampling dates. -

G-statistic value to test the null hypothesis that the size frequency compositions of the fish in each cage area were similar (significance and degrees of freedom in brackets). ö ~

See Table 12 for significance codes.

2.3.5 Characteristics of the Available Feed

2.3.5:1 Abundance

The total zooplankton abundance within the cage, around the cage area, and at the control area generally increased from the beginning of the trial, peaked about 80 days after first feeding and then declined to the trial end (Figure 10). The total zooplankton abundance around the cage area was usually higher than within the cage or at the control site. The control area usually had the lowest total zooplankton abundance.

Within the cage the artificial diet contributed less to the total available food abundance as the trial progressed, and was always a small fraction of the total food abundance (Table 19).

Within the cage, copepod genus groups always dominated the zooplankton community in terms of abundance (Figure 11A). Cladoceran genus groups (<u>Bosmina</u>, <u>Diaphanosoma</u>, and <u>Polyphemus</u>) varied in abundance in the cage, but were never dominant. <u>Daphnia</u> did not appear in these samples until day 119. Nauplii were conspicuous early in the trial but became much less common later.

At the control area copepod genus groups dominated the zooplankton abundance for most of the trial (Figure 11B).

Figure 10. Total zooplankton abundance at the cage (A), control (B), and cage area (C) locations, site 1, 1986.

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Day	% Abundance	X Biomass
7	3.6	69.3
15	2.5	56.1
23	3.7	67.9
38	12.2	88.0
44	1.3	41.1
51	1.6	45.9
58	3.1	75.2
65	0.2	25.3
72	0.8	54.7
79	0.1	14.7
85	0.1	9.3
119	0.1	11.2

Table 19. Amounts of the total available feed comprised by artificial diet with respect to abundance and biomass, site 1, 1988.

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Amounts of artificial diets are presented as percent of the total available feed. Total available feed was considered as the sum of the artificial diet and total zooplankton abundances and biomasses in the cage on the sampling days.

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Figure 11. Zooplankton abundance group composition at the cage (A), control (B), and cage area (C), site 1, 1986.

- 1. Cyclops
- 2. Diaptomus
- 3. Daphnia
- 4. Bosmina
- 5. Diaphanosoma
- 6. Polyphemus
- 7. Leptodora
- 8. Chydorus
- 9. Nauplii















DAY 51





DAY 65



DAY 72



DAY 79



DAY 85





DAY 15



DẠY 23



DAY 38



B





B

DAY 58

DAY 65



DAY 72



DAY 79







BE YAC

DAY 44







DAY 58















DAY 119

The exceptions to this were on day 72, day 79, and day 85 when the cladocerans <u>Bosmina</u> and <u>Diaphanosoma</u> dominated. Daphnia first appeared at the control area in the final sample. Nauplil were always of minimal importance to the total zooplankton abundance of the control area.

Copepod genus groups dominated the zooplankton community abundance around the cage area on all days except day 51, 68, 65, 72 and 79 (Figure 11C). On these days the community around the cage was dominated by cladocerans. This dominance began with an increase in the abundance of <u>Polyphemus</u>: <u>Bosmina</u> and <u>Diaphanosoma</u> also showed importance during this period. As at the other areas, <u>Daphnia</u> did not contribute to the total zooplankton abundance until the final sample. Naupili were never important in the abundance of zooplankton around the cage area.

2.3.5:2 Size

No significant changes in size over the course of the trial at site 1 were seen for <u>Cyclops</u>. <u>Diaptomus</u>. <u>Bosmina</u>, or <u>Diaphanosoma</u> genus groups (Figure 12 and Table 20). <u>Polyphemus</u> showed a slight, though significant, increase in size over the trial. <u>Daphnia</u> were only seen on the samples on day 119. At this time this group was determined to have a size of 0.929 ± 0.085 mm (mean \pm S.E.). No <u>Leptodora</u> were measured. Nauplii were too rare to be considered in the size composition analysis.
Figure 12. Particle sizes of the zooplankton groups and the artificial diets, and red and green fish group PFR's, site 1, 1986.

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- 1. Cyclops
- 2. Diaptomus
- 3. Daphnia
- 4. Bosmina
- 5. Diaphanosoma
- 6. Polyphemus
- 7. Leptodora
- 8. Chydorus
- 9. Nauplii
- A. Artificial diets
- B. Red fish group PFR
- G. Green fish group PFR



Table 20. Results of statistical tests to determine the relationship between size and time affer first feeding for zooplankton genus groups, and cage red and cage green fish minimum PFR's, site 1, 1986.

Statistical test: least squares regression analysis; independent variable - days after first feeding;

Significance³ Fregression² y = a + bx dependent variable - mean size. Dependent parameter

Cyclops	0.850-0.001x	4.632	n.s.	
Diaptomus	0.858-0.001x	0.658	n.s.	
<u>Bosmina</u>	0.474+0.001x	3.119	n.s.	•
<u>Diaphanosoma</u>	0.521-0.001x	1.471	n.e.	
<u>Polyphemus</u>	0.600+0.002x	22.708	:	
Cage red PFR	0.058+0.009x	29.325	:	
Cage green PFR	0.024+0.011x	60.705	:	

y = a + bx: the regression equation where y is the dependent variable, a is the y-intercept. b is the regression coefficient, and x is the independent variable. .

See Table 12 for descriptions of footnotes 2 and 3.

The artificial diet's particle size was seen to increase over the course of the trial (Figure 12). Due to the nature of this data set, no regression of artificial diet particle size against time was valid.

The minimum acceptable particle size (PFR) of the cage red (CR) and cage green (CG) fish groups showed significant increases over the trial at site 1 (Figure 12 and Table 20).

Figure 12 shows that up until about 70 days after first feeding all zooplankton groups measured and the artificial diets were of a size greater than both fish groups' PFR's. After this time the fishes' PFR's increased to become greater than the zooplankton groups' sizes. The artificial diets' size increased with the fishes' PFR's at this time. The CR fish group's PFR remained smaller than the size of most zooplankton groups for a longer time than the CG group's PFR.

2.3.5:3 Biomass

Within the cage, around the cage area, and at the control area, the total zooplankton blomass generally increased from the beginning of the trial to a peak at about 80 days after first feeding and then declined to the trial end (Figure 13). As with zooplankton abundance, the highest total zooplankton blomass was usually around the cage area; the control area was usually the lowest.

The artificial diet contributed much more to the total available food blomass in the cage than did artificial diet abundance to its total (Table 19). The proportion of artificial diet in the total food blomass in the cage generally decreased over the trial.

Within the cage, copepod genus groups generally dominated the total zooplankton biomass (Figure 14A). The exceptions to this were on day 51 and day 58 when the ciadocerans (mostly <u>Polyphemus</u>) dominated. <u>Daphnia</u> did not contribute to the total zooplankton biomass in this area until the final sample. Nauplil played only a minor role.

At the control area copepod genus groups dominated the total zooplankton blomass for all but days 65, 72, and 79 (Figure 14B). At that time cladocerans were most prevalent with the dominant genus changing among <u>Polyphemus</u>. <u>Bosmina</u>. and <u>Diaphanosoma</u>. <u>Daphnia</u> did not contribute until day 119 and nauplil were unimportant.

Around the cage area copepod genus groups only dominated the total zooplankton biomass on days 38, 44, and 85. For the rest of the trial, <u>Polyphemus</u> and occasionally <u>Bosmina</u> were most common (Figure 14C). <u>Daphnia</u> contributed to the overall cladoceran dominance of the zooplankton biomass until day 119. Nauplil were unimportant to the total

Figure 13. Total zooplankton biomass at the cage (A), control (B), and cage area (C) locations, site 1, 1986.

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Figure 14. Zooplankton blomass group composition at the cage (A), control (B), and cage area (C) locations, site 1, 1986.

See Figure 11 for key.

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DAY 15



DAY 23



DAY 38



DAY 44

DAY 51



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DAY ?9









DAY 65





DAY 72

DAY 79



B









c

zooplankton blomass around the cage area of site 1.

2.3.6 The Importance and Selection of Artificial and Natural Foods

2.3.6:1 Indices of Relative Importance

The index of relative importance of artificial diet (IRI_{art}) showed a positive significant regression with time for the cage red (CR) and cage green (CG) fish groups (Figure 15 and Table 21). The index of relative importance of total zooplankton (IRI_{200}) did not regress significantly over the course of the trial for either fish group (Table 21). However, zooplankton were seen to be relatively unimportant in the diets of CR and CG fish throughout the trial (Figure 15).

There was no significant difference between the IRI_{art} against time regressions of CR and CG fish groups (analysis of variance of regression coefficients, F = 0.428, n.s.). Figure 15 suggests a slight difference in these regressions, with CG fish showing a greater rate of increase, and reaching a higher level than CR fish. The scatter of points around these trends may have contributed to the obtained non-significant difference. Figure 15. Indices of relative importance, site 1, 1986.

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RA. Red fish group, artificial feed
RZ. Red fish group, total zooplankton
GA. Green fish group, artificial feed
GZ. Green fish group, total zooplankton

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DAYS AFTER FIRST FEEDING

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Table 21. Results of statistical tests to determine the relationship between the indices of relative importance of artificial feed and zooplankton and time after first feeding for cage red and cage green fish groups, site 1, 1986.

least squares regression analysis; independent variable - days after first feeding; dependent variable - index of relative importance. Statistical test:

ish group	Food type	$y = a + bx^{1}$	^F regression ²	Significance ³
be red	Artificial	-0.0520 + 0.0072x	8.011	•
	Zooplankton	0.1704 - 0.0016x	3.351	
nge green	Artificial	0.2247 + 0.0095x	15.911	:
	Zooplankton	0.0679 - 0.0009x	1.739	n.s.

See Table 20 for the description of footnote 1 and Table 12 for descriptions of footnotes 2 and 3.

2.3.6:2 Linear Indices of Selection

The linear indices of selection for artificial diet and total zooplankton by abundance and biomass are shown in Figures 16A, B, C, and D.

The linear indices of selection for the artificial diets by abundance and blomass (L_{aa} and L_{ab}, respectively) indicated negative selection on both sampling days before separation of the colour groups. After separation, the CG group showed positive selection with respect to both abundance and blomass on all sampling days. The CR group, however, maintained negative selection for the artificial diet for one more sampling day with respect to abundance, and two more sampling days with respect to biomass. After this time the CR fish positively selected the artificial diets with respect to both abundance and biomass.

The CG group's selection for the artificial diets was significantly greater than the CR group's on all sampling days with respect to abundance (p < 0.001), and on all except day 72 with respect to biomass (p < 0.05).

Before separation of the fish colour groups, the linear indices of selection for natural feed (total zooplankton) by abundance and blomass (L_{zta} and L_{zb} , respectively) indicated negative selection on day 7 and positive selection on day 23. After separation, the CR group showed

Figure 16. Linear indices of selection for artificial diet by abundance (A) and biomass (B), and total zooplankton by abundance (C) and biomass (D), site 1, 1986.

- R. Red fish group
- G. Green fish group

X. Significant difference between points







DAYS AFTER FIRST FEEDING

positive selection with respect to biomass for two more sampling days and for one more sampling day with respect to abundance. The CR group showed negative selection of natural feed for the remainder of the trial. The CG group, however, always showed negative selection for natural feed with respect to both abundance and biomass (all significant at p < 0.05).

The CR group's selection for natural feed was significantly greater than the CG group's on all sampling days with respect to abundance (p < 0.001), and on all except day 72 with respect to biomass (p < 0.05). It should be noted that this result is the opposite of that obtained for the artificial feed selection by the cage fish groups.

Selection indices for the <u>Cyclops</u>. <u>Diaptomus</u>, <u>Bosmina</u>, <u>Polyphemus</u>, and nauplii zooplankton groups (with respect to abundance) are shown in Figures 17A, B, C, and D, respectively.

Before separation of colour groups, the cage fish only showed positive selection for <u>Diaptomus</u> and <u>Bosmina</u> on day 15; no other groups were positively selected. After separation, selection by the CR group was positive for <u>Cyclops</u> on days 23, 51, and 79, and for <u>Bosmina</u> on days 44, 51, 72, and 79. Selection by the CG fish group was positive on day 44 (<u>Polyphemus</u>). On all other days selection by the CR and CG groups was negative for all Figure 17. Linear indices of selection for <u>Cyclops</u> (A), <u>Diaptomus</u> (B), <u>Bosmina</u> (C), and <u>Polyphemus</u> (D) zooplankton groups, site 1, 1986.

See Figure 16 for key.



DAYS AFTER FIRST FEEDING

zooplankton groups.

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Comparison between the selection indices for the zooplankton groups by the fish groups indicated that the CR fish showed greater selection than the CG fish for <u>Cyclops</u> on all days except 38 and 44, for <u>Diaptomus</u> on days 38 and 58, and for <u>Bosmina</u> on days 44, 51, 58, 79, and 85. Conversely, the CG fish only showed greater selection than the CR fish for <u>Cyclops</u> on day 44, <u>Bosmina</u> on day 65, and <u>Polyphemus</u> on days 44 and 58. All differences reported were significant at the p < 0.001 level.

The zooplankton groups <u>Leptodora</u> and <u>Chydorus</u> were not encountered at site 1, and therefore no selection indices are shown for these groups. <u>Diaphanosoma</u> were always negatively selected, no differences were observed between fish groups. <u>Daphnia</u> were only seen on the final sampling day (119). At this time both fish groups showed negative selection, with no statistical difference between the CR and CG groups.

All selection indices shown were significantly different from zero, at least at the p < 0.05 level.

2.4 DISCUSSION

2.4.1 Water Quality and Meteorology

The environmental parameters measured during the preliminary trial at site 1 were seen to interact closely with one another. This section will discuss these interactions, relating them to the success of the trial.

in tank systems the high water flows utilised generally mean that the fish have a continuous supply of clean oxygen saturated water. Wastes (ammonia, faeces) and uneaten feed are removed from the fishes' environment. Providing exit screens are kept clean, suspended solids are generally very low in tanks.

By the nature of their environment, freshwater cage systems are not provided with the same water flows that tank systems enjoy. Further, studies have shown that the supply of water can be reduced by reductions in the size and type of net mesh, by a decrease in caged fish activity, and by reductions in incident water currents. Flows can also be reduced by increases in the degree of fouling on the mesh (Wee 1979, Torres 1984). These have been recognised as limiting factors in the use of freshwater cages to rear salmonid species (Beveridge 1987). They were also observed to affect many of the water quality parameters measured during this study.

Water temperatures recorded-at site 1 were favourable for the culture of Atlantic salmon, with respect to seasonal trends and diel changes. Similar seasonal trends were observed at the cage site and the control tank, and so it is unlikely that water temperature contributed to any growth differences in the fish held in these systems.

Seasonal changes in the dissolved oxygen content of the water at site 1 were, as expected, inversely proportional to temperature changes. A reduction in oxygen levels inside the cage compared with outside can be accounted for by respiratory activities, mainly of the fish but also of other forms of aquatic life. This result itself indicates that the flow of water through the cage was insufficient to maintain the near saturated dissolved oxygen levels preferred by salmonids (Alabaster and Lloyd 1980). The effect of low wind speeds (and thus incident water current strength) and fouling on the flow of water in the cage was demonstrated on and around day 38. During this period wind speeds were very low, fouling was heavy (a net change was actually made on day 43), and dissolved oxygen differences inside and outside the cage were at a maximum (approximately 10.5 and 11.5 mg.1⁻¹, respectively).

Suspended solids levels in the trial cage at site 1 were generally much higher than at the control site. It is suggested that most of the suspended material in the cage were a result of the culture activities (<u>i.e.</u> fish facees

and uneaten food). Early in the trial some of the suspended material was probably planktonic algae and/or algae dislodged from the cage walls (where it grew as fouling). This is suggested as suspended solids levels at the control area (considered to be mostly planktonic algae) during this period were at a peak. Also, dissolved reactive phosphorus levels were low at both locations during this period. Phosphorus is generally the limiting nutrient to algal production in freshwater, and low dissolved phosphorus levels often indicate a high algal biomass (Beveridge 1984). Towards the end of the trial the contribution to cage suspended solids made by planktonic algae appeared to decrease, as control suspended solids levels decreased, and cage and control dissolved reactive phosphorus levels increased. Regardless, cage suspended solids were seen to increase to the trial end (after a net change associated drop). It can therefore be assumed that most of this suspended material resulted from the accumulation of fish particulate wastes in the cage.

The fluctuation of suspended solids levels with net changes and the steady accumulation toward the trial end indicate that particulate wastes were not being removed from the cage. As with cage oxygen reductions, these results show that flow rates of water through the cage were insufficient to maintain optimum culture conditions for Atlantic salmon.

A further example of inadequate waste removal from the fish cage is demonstrated by the results of the ammonia. nitrite, and nitrate analyses. These results show that all three nitrogenous compounds increased in concentrations at both cage and control locations over the trial. However, toward the end of the trial control ammonia levels were not as high as those in the cage; nitrite and nitrate control levels were as high as those in the cage. This suggests that ammonia was accumulating in the trial cage. Ammonia is the chief excretory product of aquatic animais, and in ! the ionised form is considered very toxic to salmonids (Haywood 1983). It is usually rapidly degraded to nitrite and nitrate by an assemblage of nitrifying bacteria, and only accumulates when this degradation system becomes overloaded or dies, or flushing rates through the environment are low (Wickens 1980, Colt and Armstrong 1981).

None of the water quality parameters discussed here reached toxic levels during the preliminary cage trial. However, many reached levels much higher than measured at the site control area, and therefore sublethal effects on the stock cannot be discounted. Indeed, the mortality of the cage fish increased, and the growth of the cage fish was reduced (with respect to that of the control tank fish) during the period around day 38 when oxygen depletion and suspended solids levels were elevated.

As discussed, much of the sub-optimal water quality recorded in the trial cage at site 1 can be attributed to poor flows of water through the system. The results also show many of the parameters (suspended solids and ammonia, in particular) to vary in relation to net changes. For these reasons it is suggested that optimal culture conditions could be maintained by the introduction of a vigilant husbandry programme. The cage bag should be inspected regularly for fouling, particularly during times of increased algal growth and low wind speeds at the site. Net changes should be made efficiently to reduce stress on the fish (who may already be in a stressed condition due to poor water quality). Care must be taken not to pass accumulated suspended solids from the old cage bag to the new. Finally, cage bags constructed of the largest available mesh size capable of holding the stock should be utilised to maximise the supply of clean, well-oxygenated water to the stock.

2.4.2 Characteristics of the Available Feed and Feeding Behaviour

2.4.2:1 The influence of the Cage Raft on the Distribution of Zooplankton

Results of the zooplankton sampling at site 1 indicated that the abundance and blomass were much higher around the cage raft than at the control area. In a similar study, Dey (1984) found no significant differences between cage and Control Zooplankton abundances but suggested that predation by wild fish around the cage raft may have influenced his results. No wild fish population was present at site 1 (Murray and Pullar 1910, P. Featherstone pers. comm.).

Two explanations for the observed zooplankton distribution are suggested. As discussed earlier, cages act as a barrier to water currents and wave action. Zooplankton may seek shelter there for this reason. However, no data concerning zooplankton distribution around the cage raft with respect to the direction of incident water currents and waves was available and therefore this can be questioned. Studies have shown that levels of primary production are often elevated around cage rafts due to increased levels of phosphorus resulting from culture activities (a.g., Phillips et al 1985). Zooplankton may be attracted to this food source.

The relative blomass and abundance of the zooplankton groups was also influenced by the cage rafts. Cladoceran types were more prevalent in this area than at the control area, while the converse was observed for copepod types. Cladoceran species of zooplankton have been shown to predominate in areas of high productivity, while copepods prefer more infertile environments (Morgan <u>et al</u> 1980). Also, cladoceran species are generally considered to be poorer swimmers than copepods (see section 2.1.4:1), and cladocerans have been shown to rely on vegetation for shelter from rough conditions more than copepods do (Malone and McQueen 1983). Often large predatory cladoceran species (<u>Polyphemus</u>, <u>Leptodora</u>) were more common around the cage area than at the control area; no doubt they were attracted by the overall higher zooplankton numbers.

Differences were also seen between the absolute and relative blomass and abundance of zooplankton groups around the cage area and within the cage (absolute levels were lower, and generally copepods dominated in the cage). This may have been caused by the cage itself, acting as a physical (individuals unable to pass through the mesh) or a behavioural barrier. Although this was probably a contributing factor, a more likely explanation is that predation by the fish in the cage largely resulted in the differences observed. Predation by planktivorous fish has been shown to reduce absolute abundances, and the relative abundance of easily captured zooplankton types (Brooks and Dodson 1965). This predation was impossible to quantify, therefore the zooplankton within the cage must still be considered to be generally copepod dominated.

The increase in zooplankton abundance around the cage raft and within the cage (relative to the control area) means that the probability of a cage fish encountering an individual zooplankton is increased. Similarly, the increase in the relative abundance of cladoceran types

implies that once encountered, the probability of an individual zooplankton being captured is increased. According to the probability of selection equation (described in section 2.1.4:1), the chance of an individual zooplankton being selected is thereby increased, relative to the probability of selecting an artificial diet particle. It should be noted at this point that the results indicate that although crustacean zooplankton were seen to dominate the total available feed with respect to particle abundance, their increase in blomass was not enough to the total available feed blomass.

2.4.2:2 General Growth and the Development of Colour Groups in the Cage Fish

The growth trend of the fish heid in the control tank (CON) was not significantly different than that of the cage fish, overall (CO). On a day by day basis, the CON fish were significantly different in size only at times of poor cage water quality (as discussed in section 2.4.1). The results of the preliminary trial therefore suggest that the water quality conditions in the cage affected growth, but exposure to a natural food source had no effect on the growth of the cage fish population. However, the results also indicate different feeding behaviours and different growth patterns were present amongst the cage fish, and that those differences were related to fish colour. The decision to distinguish between different coloured individuals when measuring the growth and analysing the stomach contents of the cage fish was based on the following observations. There appeared to be two distinct groups of fish developing in the cage, one group consisting of individuals with a predominant red colour to their skin and fins (CR), and one group of individuals without the red tint (CG). Further, the two groups seemed to occupy different areas of the trial cage, with the CR fish distributed mostly around the perimeter and the CG fish mostly in the central area. Preliminary examinations of fishes' stomachs revealed those in the CR group to have fed significantly on crustacean zooplankton, while those in the CG group had not. Simpson et al (1981) reported that crustacean zooplankton are generally a good source of carotenoid pigments (usually orange-red in colour), which are deposited in the skin and fins of fish that feed heavily upon them. It followed that the fishes' colour could serve as a biological marker to monitor the growth of the fish with respect to their feeding behaviour.

2.4.2:3 Distribution of Fish in the Trial Cage

The results of the distribution sampling indicated that during this period the CR fish were smaller than the CG fish. The CR fish were also determined to be distributed more around the perimeter of the cage, while the CG fish were found more in the central area (confirming the original observation). Finally, it was shown that the peripheral areas often contained fish of extreme size (both small and large) regardless of colour.

It is suggested that a dominance hierarchy had been established in the cage, based upon the availability of the natural and artificial food types. The feeder supplying the artificial diets was situated over the centre of the cage. Aithough for abundance and biomass calculations it was assumed the artificial feed was spread homogeneously over the cage area, it is likely that the bulk of it entered the water in a small area underneath the feeder. The natural feed (crustacean zooplankton) most certainly entered the cage through the cage walls; thus the distribution of this food was probably greatest around this area. By optimal foraging rules (Krebs and Davles 1984) and factors governing dominant/subordinate relations in. Juvenile salmonids (Symons 1971, and others) the following can be postulated. The primary food choice of the caged fry should have been the more profitable (with respect to energy per particle) artificial diet. However, due to the limited distribution of this feed, some of the stock were unable to compete (subordinates) and were forced to forage elsewhere for food. Although energetically less profitable, the crustacean zooplanktons' particle size and inherent motility during this time made them an attractive alternate food source.
It is interesting to note that the observed distribution patterns of fish broke down after about day 80. This was soon after the time when the minimum preferred particle size of the CR fish (PFR) increased to a level greater than the particle size of most zooplankton groups. These fish may have switched to feeding on artificial diets at this time, as this food source continued to provide particles of mean optimum size. To obtain this feed, the CR fish would have to move toward the centre of the cage, thus resulting in a breakdown of the distribution pattern. This observation is supported by the indices of relative importance data, and is discussed further in the following section.

Finally, the presence of extremely large fish in the peripheral areas of the cage could be attributed to these being dominant fish which, having been successful feeding on artificial diets, began to forage on the zooplankton, perhaps to fulfil a nutritional requirement not available from the artificial diets (linolenic fatty acids or carotenoids, for example). Krebs and Davies (1984) noted that prey quality is often a complicating factor in optimal foraging, and a predator may deviate from the most energy profitable feed source to a sub-optimal one in order to obtain a nutritionally balanced diet.

Pepper <u>et al</u> (1987) found similar distribution and feeding differences in a stock of juvenile Atlantic salmon reared in a freshwater cage to those observed in the preliminary

trial at site 1. These authors also suggested a dominant/ subordinate relationship governing distribution and feeding behaviour. Holm (1986) also reported similar results.

2.4.2:4 Differential Feeding and Growth

The calculated indices of relative importance (IRI and IRI____) showed many differences between the cage red and cage green fish groups. Both groups increased their utilisation of the artificial diets over the trial, and, although no statistical comparison was possible, the results suggested that the CG fish utilised this feed source to a higher degree than the CR fish. From the time the fish groups were differentiated, the CG fish were seen to only minimally utilise crustacean zooplankton as a food source, while the CR group was seen to forage on this food source until some time after the sample on day 51. Indeed. the indices of relative importance of artificial and natural feeds were very similar in the CR fish for about the first half of the trial. After this time the index of relative importance of artificial feed increased rapidly in the CR fish, but not to the level observed in the CG fish.

The decrease in the utilisation of natural food (and increase in the utilisation of the artificial diets) of the CR fish occurred at approximately the same time as the PFR of this group increased above the levels of most zooplankton groups' size. This lends support to the suggestion

made in the previous section-that these fish switched their feeding behaviour in order to obtain a suitable feed particle at this time. The fact that CR IRI_{at} levels did not reach these observed in the CG fish suggests that, upon switching, the CR fish were not as effective foraging on the artificial diets as the CG group.

The differential utilisation of artificial and natural food sources by the CR and CG fish was manifested in the growth of these groups. Generally the CG fish were larger than the CR fish (on a day to day basis), reflecting the overall higher utilisation of the optimal artificial diet. Koebele (1985), investigating the causes of differential growth in <u>Tilapia zillii</u>, also attributed growth differences to a disproportional acquisition of food, and noted that dominant/subordinate relationships were the cause of this.

The CR fish were observed to be greater in size than the CG fish on the second sampling day in which colours were distinguished (day 38), even though the CG group showed higher utilisation of artificial feeds and lower utilisation of natural feeds up until that time. This suggests that the zooplankton may have held a nutritional advantage over the artificial diet (e.g. carotenoids. linoleic fatty acids) which aided growth in the smaller fish. This advantage, however, was not suitable to sustain a higher growth rate throughout the trial. Similar findings have been reported by Holm (1986) and Pepper <u>et al</u> (1987), who note that while zooplankton -may be an adequate food source for start-feeding, it must be supplemented with artificial diets to maintain the high growth rates required by commercial smolt producers.

The differential growth observed in the CR and CG fish groups could be attributed to the separation of S1 and S2 fish inherent in any stock of Atlantic salmon. However, in a recent study assessing the factors associated in this separation, Metcalfe et al (1988) noted that significant separation in salmon parr stocks does not generally occur until September or October of the first year of growth. After this time, the appetite and growth of potential S2 fish is severely reduced, while potential S1 fish continue to feed and grow, albeit at a slightly lower level. The results from the preliminary cage trial at site 1 suggest that the differential feeding behaviours of the cage fry resulted in a lower overall feeding intensity by the CR (subordinate) fish with respect to the CG (dominant) fish throughout the triai. Therefore, while some of the differential growth can be attributed to intra-stock differences, the feeding behaviour of the CR and the CG fish may have exacerbated the effect.

It should be noted that relations between the indices of relative importance of the different feed types between the cage fish groups discussed here are subjective; that is, they have no statistical confidence. However, many of the selection, which have statistical relevance. These are discussed in the following section.

2.4.2:5 Factors Affecting Feed Selection

The calculated linear indices of selection for the cage fish at site 1 show trends and differences closely related to changes in fish distribution, growth, and the indices of relative importance. These, in turn, can be related to changes in the characteristics of the available feed and the behaviour of the fish.

On the first sampling day, negative selection was observed for both the artificial and natural feeds by the cage fry. The indices of relative importance were also low at this time. The fish were transferred to the trial cage from the hatchery as late yolk sac fry. It is not surprising that no appreciable exogenous feeding was observed in the stock at this time.

On day 15 the cage fish were observed to positively select zooplankton, and negatively select the artificial diets. Zooplankton dominated the available feed particle abundance at this time. It is possible that the fry simply encountered this feed more often than the artificial feed, thus the probability of selection of zooplankton would be higher (refer to section 2.1.4:1 for the probability of selection

equation). Straus (1979) noted that a negative selection index may indicate avoidance of a prey type, or that the prey was inaccessible to the predator. It is possible that inadequate distribution of the artificial diet on day 15 made it relatively unavailable to most of the cage fish, thereby increasing the probability of zooplankton selection. This could also represent the beginnings of the separation of fish colour groups. As both the zooplankton and artificial diet particles were of similar size at this time, it is doubtful this played a role in the differential feed selection observed. Rimmer and Power (1978) noted that small Atlantic salmon fry preferred moving to nonmoving prey. It is suggested that this contributed greatly to the differential feeding of the cage fry, as the live zooplankton most certainly exhibited a higher degree of movement than the artificial diets did.

The cage fish in general were observed to switch their selection of Zooplankton to the artificial diets over a period between days 23 and 44. This selection switch was accompanied by a similar switch in the indices of relative importance and later a breakdown of the observed fish distribution pattern in the cage, and could be attributed to either the fishes' size selective behaviour or to the fish recognising which feed type is the better energy source (<u>i.e.</u> optimal foraging). These possibilities are discussed below.

Optimal foraging theory states that a predator should select the feed type that provides the most energy-profitable prey (Krebs and Davies 1984). Further to this, Marcotte and Browman (1986) suggested that juvenile Atlantic salmon, at the onset of exogenous feeding, have limited cognitive abilities, and therefore are not capable of recognising the optimal feed at this time.

The artificial diets dominated the available feed biomass throughout the trial, and as both feed types probably had similar energy content per unit dry weight, the artificial feed could be considered the optimal feed source. It is suggested that as the cage fish grew, they recognised this and switched their diet accordingly.

As discussed earlier, the limited distribution of the artificial diets may have resulted in a dominant/subordinate hierarchy developing in the cage, and only the CG fish were able to make this switch effectively. The CR fish continued to select the natural feed for a short time after the CG fish switched. The fish distribution, indices of relative importance, and growth results all support this hypothesis. Both CR and CG fish groups positively selected the artificial diet and negatively selected zooplankton after day 44, which indicated that the former was the preferred feed type (<u>i.e.</u> they were optimally foraging). The fact that the CR group's selection for the artificial diet was generally lower than the CG group's suggested that

the CR fish were not as effective foraging on this feed source (as did the indices of relative importance); that is, they were subordinates.

Many authors have reported Atlantic salmon to be size selective predators. Wankowski and Thorpe (1979b), however, quantified this relationship by describing a minimum preferred particle size (PFR) that could be calculated directly from the fish fork length. These authors noted that the young salmon followed this selection very strictly, and optimum growth was only achieved when optimally sized particles were supplied.

The cage fish were observed to switch selection from zooplankton to artificial feed before the sample on day 51. However, comparison of the zooplankton size groups and the fishes' PFR's showed that the latter did not increase above the size range of the zooplankton until around day 70. It is therefore suggested that the optimal foraging relationship described above had a greater influence on prey switching than did size differences in the prey. However, the size of the zooplankton probably contributed much to the dominant/subordinate relationship by providing a suitably sized feed to those fish which could not successfully compete for the optimal feed. The observed breakdown of the fish distribution pattern soon after day 70 supports this theory (as discussed in section 2.4.2:3). Over the course of the trial,-cladoceran zooplankton groups were observed to be positively selected more than copepod groups, even though the cage zooplankton was generally considered to be copepod dominated. Drenner <u>et al</u> (1978) reported that cladocerans are theoretically easier to capture than copepods. Reports by Morrison (1983), Holm and Moller (1984), Holm (1986), and Pepper <u>et al</u> (1987) all showed that, given a choice, salmonids will select a cladoceran zooplankton type over a copepod. Ease of capture was always given as the reason for this selection.

With respect to total zooplankton, the CR and CG fish groups generally showed negative selection. However, the CR group showed positive selection for <u>Cyclops</u> on three sampling days and <u>Bosmina</u> on six, while the CG group only showed positive selection for <u>Polyphemus</u> on one day. This result again shows the differential feeding of the fish groups, and also shows that when forced to take zooplankton, the CR fish selected the most common, and the easiest captured types.

2.4.2:6 Sources of Error

It is somewhat unrealistic to assume that the stomach contents observed in any one sample of fish were an accurate indication of overall feeding behaviour on that day. A fish's stomach contents varies over time as a function of the rate of gastric evacuation and feeding

activity (Eiliot and Persson 1978). Indeed, several authors ($\underline{e.g.}$ Straus 1979 and Hysiop 1980) note that failure to consider these factors is a major source of error in the application of analytic methods.

Fish gastric evacuation rates have been the subject of Intense study in recent years. The factors cited as controlling gastric evacuation are highly controversial. and as such many models describing the function have been proposed. Kionka and Windell (1972) noted that artificial diets required a longer time to be passed from the stomachs of rainbow trout than natural feeds did. They attributed this to the fact that the artificial diets contained a higher energy level (per unit wet weight) than did the natural feed, and therefore would be passed to the intestime at a slower rate to maximise energy absorption (i.e. not overload the system). A similar relationship between energy content and gastric evacuation rate has been described by Jobling (1984), and Kolok and Rondorf (1987). The work of Kionka and Windell (1972) also showed that indigestible portions of the natural feed (i.e. exoskeletons) were retained longer in fish stomachs than the digestible organic portions. Gannon (1976) reported cladoceran zooplankton to be evacuated faster from the stomachs of alewife (Alosa pseudoharengus) than codepods.

At present no study has been conducted to evaluate the gastric evacuation rates of mixed artificial diet and Zooplankton meals in juvenile salmon, or indeed any fish. It is therefore impossible to predict with any degree of certainty the effects of differential digestion rates on the stomach samples obtained during the preliminary trial. It may be suggested that both the artificial diets and ZOOplankton were over-represented in the stomach samples and thereby over-represented in the indices of relative importance; however, relative to each other there would be little change. The exception to this may have occurred in the first feeding alevins, whose limited digestive capacity may have resulted in an over-estimation of zooplankton with respect to artificial diets. Throughout the trial the relative importance of cladocerans may have been underestimated with respect to codepod types.

Atlantic salmon fry and parr generally exhibit a definite diurnal pattern of feeding activity (Pinskii 1987, Browman and Marcotte 1986). As the fish are visual predators, no feeding occurs at night. A peak in activity in the early morning is therefore not surprising; subsequent feeding peaks have been observed at mid-day and late in the evening. The lack of available data preciudes any accurate prediction on the effects of the periodic feeding behaviour in the stomach contents data obtained from site 1. Samples were taken around mid-afternoon, possibly during a luii in feeding activity. The data may therefore under-estimate

the true importance and selection of the available feeds. However, slow gastric evacuation rates could have caused a gradual accumulation of food over the day, thereby negating the aforementioned under-estimation.

Crustacean zooplankton are known to exhibit diurnal patterns of vertical migrations within water bodies (Zaret and Suffern 1976, Stich and Lampert 1981). Generally these migrations involve a downward movement in the daylight hours, accompanied by an upward movement or random distribution at night. This behaviour is considered mostly to be a mechanism of predator avoidance; however, other factors such as phytoplankton distribution are also important. As the zooplankton samples were obtained in mid-afternoon. they may have under-estimated the abundance of this food source available to the stock, particularly in the morning when feeding activity may have been at a peak. The effect of diurnal zooplankton migration on the results obtained from the preliminary trial was thought to be minimal for two reasons. First, site 1 contained no natural fish population, and second, increased production levels around the cage may have meant that phytoplankton numbers were always high around this area.

Many of the described characteristics of the cage fish feeding behaviour were based on the Wankowski and Thorpe (1979b) preferred particle ratio (PFR). For the preliminary trial, the minimum PFR values were utilised; that is,

1.1% fork length for fish <-40mm and 2.2% fork length for fish > 40mm. However, the authors of the ratio note that optimal growth was obtained for the PFR ranges of 1.1-9.0% and 2.2-2.6% fork length for fish < 40mm and > 40mm, respectively. Some variation around the observed trends must therefore be expected. Indeed, the slight discrepancy in the timing of the observed switch in CR fish feeding behaviour and stock distribution in the cage could be accounted for in this manner. It was for this reason (<u>1.e.</u> the range of PFR) that no statistical comparisons between the fish PFR's and artificial and natural feed particle sizes on a day by day basis was attempted. However, the trends observed have good foundations in the scientific literature, and were therefore considered relevant.

Finally, the differential feeding behaviour and the distribution of the cage fish described from the preliminary trial were based upon a non-quantitative (<u>1.e.</u> subjective) determination of colour. Although colour was closely associated with other, more quantitative traits, no doubt it was estimated with a high degree of variability.

Straus (1979), in describing the limitations of his selection index, noted that many factors in the sampling programmes, the available feed, and the fish themselves, contribute to the variability of the calculations. It was also noted that many of these factors work in opposition to one another. This author concluded that the researcher and

the reader must accept this variation, and treat the results with as much contempt as is required by the study objectives.

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2.5 SUGGESTIONS FOR FUTURE-INVESTIGATION

Results from the preliminary trial showed that mortality and growth of the cage fish was affected by poor water quality, and that these conditions were partially caused by algal growth on the cage mesh reducing the flow of water through the system. This was considered a limiting factor, even though the low phosphorus levels measured at site 1 and the dominance of copepod zooplankton indicate this site was one of low productivity. It follows that cage fouling should be much greater at a site of higher productivity. Results from a trial conducted at such a site would provide valuable information to assess the overall usage of cage systems for Atlantic salmon smolt production.

The Zooplankton population was dominated throughout the trial at site 1 by copepod species. In theory, copepod Zooplankton are more difficult to capture than cladocerans, largely because of their quick and unpredictable movements. To fully evaluate the feeding behaviour of Atlantic salmon fry in freshwater cage systems, it would be useful to conduct trials at a site where the Zooplankton population was dominated by cladocerans.

The trial at site 1 evaluated the use of freshwater cages to first feed Atlantic salmon fry. However, most smolt producers in Scotland who utilise cage systems transfer fish from tank systems after this time. By conducting a

series of trials in which fish were transferred to cage systems at increasing times post first feeding, the optimum time of transfer could be determined, particularly with reference to utilisation of the optimal (artificial diet) and suboptimal (crustacean zooplankton) feed sources.

These suggestions served as the basis of the second year's field trials. They are described in the following chapter.

CHAPTER 3: THE SECOND YEAR'S FIELD TRIALS

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3.1 INTRODUCTION

The cage trial at site 1 was performed under very suitable environmental conditions, particularly with respect to water quality parameters, the species composition of the available natural feed, and the absence of major disease and parasite pathogens. Sites such as this are probably not common in Scotland. Morgan <u>et al</u> (1980) noted that as the productivity of freshwater bodies increase, the species composition of the crustacean zooplankton generally shifts from copepod to cladoceran types. As discussed in the previous chapter, cladoceran zooplankton are easier to capture than copepods. in addition, a higher overall productivity would produce water quality conditions much less suitable for salmonid culture than at site 1.

Many smolt producers in Scotland who utilise cage systems do not transfer stock from the hatcheries until some time after first feeding, as the fish begin to increase their growth rate and running water resources become limiting (Beveridge 1987). The results of the preliminary trial suggested that most of the fishes' behaviour governing the utilisation of artificial and natural food sources is established soon after first feeding and that natural food utilisation is markedly reduced soon after the preferred particle size of the fish increases above the size range of the crustacean zooplankton.

A set of trials was planned to further evaluate the use of freshwater based cages to culture Atlantic salmon smolts, based upon the suggestions from the preliminary trial. First, a trial was planned to first-feed a stock in a site believed to be much more productive than site 1. Second, a series of trials was planned to investigate the effect of moving fish to cages from tank systems at various times post first feeding on the utilisation of natural and artificial food sources.

The trials described in this chapter were planned after considering the results and the sources of error associated with the preliminary trial. Diurnal sampling of the crustacean zooplankton and the cage fish was undertaken wherever possible to quantify this source of variation. Feeding behaviour was not associated with fish colour, only with size. A laboratory experiment was planned to evaluate the effects of differential gastric evacuation on observed stomach contents. Unfortunately, this experiment was not successful, and this factor remains an unquantified source of error in the study. Similarly, minimum preferred particle sizes were calculated to evaluate the size selective feeding behaviour, and as such no statistical comparisons between feed size and PFR were attempted, only subjective comparisons. This, as well, remains a source of variation.

Apart from the changes mentioned above, the second year's field trials were conducted in a very similar manner to the

preliminary trial. No new principles were employed in these trials, and the reader is advised to refer to the preliminary trial for explanations or references. 3.2 MATERIALS AND METHODS -

3.2.1 Cage Site Descriptions

3.2.1:1 General Information

The locations of the cage trials conducted during the second year of the project will be referred to hereafter as sites 2, 3, 4, and 5. Event days referred to in this section are given as days after the first feeding of the stock.

Site 2 was a small freshwater pond at the Howletoun Fish Farm, part of the institute of Aquaculture, University of Stirling (Figure 18). Water was supplied to the pond primarily through a small channel at one end. The pond was drained by a single outlet. Flow rates remained fairly constant to the pond.

Site 3, Loch Glashan, was located near Lochgliphead in Argyll (Figure 19). Water was supplied to the loch by several large streams and burns. Drainage of water was controlled by a hydro-electric dam on the eastern shore. The hills surrounding the loch were covered mostly by coniferous forestry and peat bogs. The loch had a general north-south trend.

Figure 18. Site 2.

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A. Trial cage

B. Inlet

:

C. Outlet



Figure 19. Site 3.

- A. Trial cage in the raft
- B. Control sampling area
- C. Inlet streams
- D. Hydro-electric dam



Site 4, Loch Leathan and the Storr Lochs (considered here as one water body), was situated north of Portree on the Isle of Skye (Figure 20). As at site 3, the drainage of site 4 was controlled by a hydro-electric dam located at the north west end. Input of water to the site was via a large burn connected to a nearby loch and several insignificant streams. The surrounding hills comprised largely of rough grazing and peat. The loch had a general south east-north west trend.

Site 5, Loch Tralaig, was located near Kilmelford, Argyll (Figure 21). Inflow of water came from a large stream at the eastern end and several minor streams. Again, this site's outflow was controlled by a hydro-electric dam. The hills around the loch contained some forestry, but were mostly covered in grasses, scrub bushes and peat.

The morphological and hydrological characteristics of sites 2, 3, 4, and 5 are summarised in Table 22.

The ecological characteristics of sites 2, 4, and 5 are summarised in Table 23. Although no background ecological data were available for site 3, samples were collected during the course of this trial and the results are presented in section 3.3.1.

Figure 20. Site 4.

See Figure 19 for key.

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Figure 21. Site 5.

See Figure 19 for key.



Table 22. Morphological and hydrological characteristics of sites 2, 3, 4, and 5.

Characteristic	2	e	4	5	
Surface area (x10 ^{6,2})	0.0000	2.30	1.16	0.59	
Mean depth (m)	2.00	18.00	4.49	12.51	
Mean volume (x10 ^{6,3})	0.00018	21.20	2.60	7.56	
Catchment area (x10 ⁶ 2)	N/A	10.00	13.25	11.45	
Annual water input (x10 ^{6,3})	0.158	15.83	19.30	13.74	
Exchange rate (times.year ⁻¹)	876	0.747	7.70	1.82	
Location (latitude, longitude)	56°6°28"N 3°58°22"W	56°6'1"N 5°20'33"W	57°28'37"N 6'9'25"W	56°17'30"W 5°25'30"W	
Data source ²	-	2	e	•	

1. Data for the catchment area were not applicable to site 2.

Data sources: 1. P. Featherstone pers. comm.; 2. North of Scotland Hydro-Electricity Board; 3. M. Phillips pers. comm.; 4. Murray and Pullar 1910. ~

Table 23. Ecological characteristics of sites 2, 4, and 5. 1

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		Site		
Characteristic	2	•	S	
<pre>ilkalinity (meq.l ')</pre>	0.2 - 0.5	0.4	0.2 - 0.3	
	6.5 - 7.0	6.8 - 7.3	7.2 - 7.7	
emperature (°C)	10.0 - 19.0	7.0 - 19.0	7.0 - 18.0	
issolved oxygen (mg.l])	8.0 - 13.0	10.0 - 12.0	10.0 - 12.0	
otal ammonia (µg.1 ⁷)	100.0 - 550.0	3.0 - 30.0	20.0 - 130.0	
litrite (µg.1.)	4.0 - 19.0	1.0	1.0 - 6.0	
litrate (µg.l ⁻¹)	200.0 - 500.0	1.0 - 20.0	10.0 - 80.0	
otal phosphorus (mg.1,)	. 20.0 - 100.0	5.0 - 15.0	1.0 - 10.0	
uspended solids (mg.l ⁻¹)	5.0 - 9.0		1.0 - 3.0	
ecchi depth (m)	•	2.0 - 2.5		,
lata source	-	. 2	~	

Ecological data shown are minimum and maximum values obtained by sampling between April and October and includes data from up to 3 years before the trials. 1.

2. Data sources: 1. Wahab 1986; 2. M. Phillips pers. comm.

3.2.1:2 Farming Practices -

At site 2 a wooden Kames-type cage collar was utilised. The collar was placed in the pond approximately mid-way between the inlet and the outlet. The cage bag was constructed of 4mm stretched mesh knotless netting. The bag's overall dimensions were 7.32m x 7.32m x 1.0m deep (submerged). A divisional panel constructed of the same knotless netting created two equal sections to the cage. The bag was positioned on the collar so that the divisional panel ran parailel with the long axis of the pond.

A rope was suspended under the cage perpendicular to the divisional panel in order to divide the cage into equal quarters. When this section became fouled with algae, uneaten food and faeces, the fish were transferred to a full haif cage section. Similarly, the stock was moved to the other half of the cage when the current half became fouled. The previously utilized half was then raised from the water and left to be weather cleaned.

At site 3, a wooden cage collar was used to suspend a cage bag initially having dimensions of $6.5m \times 5.5m \times 1.5m$ deep (submerged). This bag was constructed of 4mm stretched mesh knotless nylon netting. Net changes were made by the farm management throughout the course of the trial. On day 83 the initial bag was replaced by a 10mm stretched mesh net of dimensions $6.5m \times 5.5m \times 3.5m$ (submerged). This was

replaced by a bag constructed of 15mm stretched mesh of dimensions 6.5m \times 6.5m \times 3.0m (submerged) on day 127.

A wooden cage collar was also used at site 4. Initially the collar supported a cage bag of 6.5m \times 6.5m \times 2.0m deep (submerged). This was replaced by the farm management on day 120 by a bag 6.5m \times 6.5m \times 3.0m deep. Both bags were constructed from 10mm stretched mesh knotless netting.

At site 5 a Kames-type wooden collar was used to support a bag of dimensions 5.8m x 4.6m x 2.0m (submerged). On day 161 this was changed by the farm management to a bag 5.8m x 4.6m x 4.0m (submerged). Both bags were constructed of 12mm stretched mesh knotless netting.

As at site 1, the culture cages at sites 2 and 3 were partially covered by green netion shading screens. In each case the screens were placed centrally over the cage. The screens covered approximately 65% of the surface area of the cages.

information on the dates of introduction, relative ages, mean weights, numbers, and origins of the fish introduced in the trial cages at sites 2, 3, 4, and 5 are summarised in Table 24.

Two types of commercially prepared diet were fed to the stock held at sites 2, 3, 4, and 5, with proximate composi-

Table 24. Characteristics of the fish stocks introduced to the trial cages at sites 2, 3, 4, and 5, 1987.

		Si	•	
Characteristic	2	e	•	S
Date of introduction	23 April	16 June	3 July	30 July
Relative age (days after first feeding)	•	2	71	8
Mean weight (g)	0.19	0.43	0.75	2.90
Number	35,000	28,900	64,100	35,000
Stock origin	Landcatch	Landcatch	Wester Ross	Kanes

The actual dates of first feeding of the fish stock used at these sites occurred in the week 19-25 April. For convenience, all stocks were said to be first fed on 23 April, the actual date at site 2. ..

tions varying slightly between them (Table 25). Ewos-Baker feed was used at sites 2, 3, and 5. BP Nutrition feed was used at site 4.

The feeding regimes followed were those recommended by the feed manufacturers. The amount of diet and the pellet size fed varied according to water temperature and fish size and are shown in Table 26.

At sites 3, 4, and 5 prepared diets were delivered to the trial cages using similar feeders. These were similar in construction to those used at site 1; each was equipped with a spinning disk to distribute the food over a greater area of the cage. The feeders' activity was controlled using similar units to those utilised at site 1. Feed was delivered at approximately 15-20 minute intervals. Delivery began about 30 minutes before sunrise and ended about 30 minutes after sunset. One feeder was placed centrally over the cages at each of these sites.

At site 2 prepared diets were delivered to the cage using a conveyor belt type feeder. The daily ration was placed on the belt and was dropped to the cage as the belt slowly wound onto a spool. A spring-loaded clockwork mechanism controlled the winding of the belt. No spreader mechanism was utilised. One feeder was placed centrally over the cage at site 2.
-	Manufacturer's proximate composit	
Component	Ewos-Baker	SP Nutritio
Protein (%)	54	54
Carbohydrate (%)	7	12
) (%)	19	15
Ash (%)	10	10
ibre (%)	1	1
bisture (%)	9	9
letabolizable energy (kcal.g ^{**})	3.77	4.93

Table 25. Proximate compositions 1 of the artificial dists used at sites 2, 3, 4, and 5, 1987.

1. Proximate compositions are those supplied by the feed manufacturers, Ewos-Baker and BP Nutrition.

Table 26. Feeding regimes¹ of artificial diets for the cage trials at sites 2, 3, 4, and 5, 1987.

2			9	1		4	1		2	1
۵.	-0	•	۵	•	-	۵	•	-	٩	v
5.0	0	55	4.5	-	76	4.5	1	101	3.0	~
5.5	•	74	4.5	1	67	3.5	2	111	3.0	~
5.5	•	95	3.5	8	118	3.0	2	145	2.5	e
5.5	•	116	3.5	~	146	2.5	9	175	2.5	m
4.5	1	144	3.5	e	176	2.0	e			
4.5	1	174	3.0	•						
4.5	1									
4.5	1									
3.5	2									
3.5	8									

Feeding regimes followed are those recommended by the feed manufacturers: Ewos-Baker (sites 2, 3, and 5) and BP Nutrition (site 4). ..

a - Day. ~ ~ +

b = Feeding rate (% body weight.day⁻¹). c = Pellet type.

Mortalities were removed from the trial cage at site 2 on alternate days. Accurate records were kept. However, at sites 3, 4, and 5 mortalities were removed from the cages by the farm staff on an irregular basis and only estimates of mortality were obtainable from these trials.

Various chemical treatments were administered to the stocks at sites 2, 3, and 5. On day 103 the fish at site 2 were treated for a heavy infection of the parasite <u>ichthyoph-</u> <u>therius multifilis</u> (white spot) using malachite green and formalin mixtures. At site 3 a similar treatment was given for an infection of the parasite <u>ichthyobodo necatar</u> (costia) on day 91. Also at site 3, on day 157, a prophylactic treatment of oxylinic acid was administered in preparation for grading. Formalin and malachite treatments for <u>1. multifilis</u> and <u>1. necatar</u> were given to the fish at site 5 on days 139 and 146, and on 4 October. No chemical treatments were administered to the stock held at site 4.

Termination days of the second year's field trials were as follows: day 103 at site 2 (when the fish became heavily parasitised); day 174 at site 3; day 178 at site 4; day 175 at site 5 (after the last sampling day at each of these sites).

3.2.2 Sampling Methods

3.2.2:1 Water Quality

Temperature

Water temperature was measured within the trial cages at sites 2, 3, 4, and 5. A PHOX temperature oxygen meter was used to obtain readings, which were recorded to the nearest 0.1° C. Measurements were made 40-50cm below the surface.

Nutrients and pH

Water samples were collected from the cage trials at sites 2, 3, 4, and 5 for later analyses of nutrients and pH. The sampling method followed was similar to that described in section 2.2.3:2, however, no filter papers were retained for suspended solids analyses in the second year's sampling.

In lieu of suspended solids measurements, Secchi depth was measured at the cage areas and control area locations of sites 3, 4, and 5. It was measured using an aluminium disk painted black and white in opposing quarters suspended by a line marked at 0.5m intervals. Secchi depth was determined as the average of the depths at which the disk disappeared from view on descent in the water and reappeared on lifting (Stirling 1985).

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Sampling Schedules

Temperature measurements were made at sites 2, 3, 4, and 5 once per sampling day from these sites.

Water samples were collected from site 2 concurrently with the weekly samples and the 16:00 sample on day 89. Water samples were collected from sites 3, 4, and 5 with the 16:00 fish and zooplankton samples on each day.

At site 2, water samples were collected only from within the cage, while at sites 3, 4, and 5 they were collected from within the cage and the control area.

Secchi depth measurements were made at the cage area and control locations of sites 3, 4, and 5 with the 16:00 fish and zooplankton samples.

3.2.2:2 Zooplankton

Sampling Methods Used

Zooplankton were sampled at sites 2, 3, 4, and 5, using the same method as at site 1 (section 2.2.3:3). Identical conical plankton nets were used at all five cage sites. The same pooled water sample methods were followed at all cage and cage area locations, and the same direct filtration method was followed for all control area locations. All samples were preserved using Lugol's lodine solution (Beveridge 1985) following the same method as outlined for site 1.

Sampling Schedules

Zooplankton samples were obtained from site 2 only from within the trial cage. Sampling began on day 18 and continued on an almost weekly basis until day 94. A diurnal zooplankton sample was taken from site 2 on day 89. For this samples were obtained at 30 minutes before sunrise, 10:00, 13:00, 16:00, 19:00, and 30 minutes after sunset.

Zooplankton samples were obtained from inside the trial cages at sites 3, 4, and 5 on a diurnal basis. The cage area and control area locations of these sites were sampled for zooplankton once each sampling day at around 15:00. Table 27 summarizes the dates and times of zooplankton samplings at sites 3, 4, and 5.

3.2.2:3 Artificial Diets

Samples of all types and sizes of artificial diets utilised at sites 2, 3, 4, and 5 were obtained. These were retained in airtight containers for future laboratory analyses.

Site	Location ¹	Day	Time schedule ²
3		55	a
		74	8
		95	b
		116	b
		144	b
		174	c ·
	þ,c	All days	d
4	a	76	a 🖉 🖉
		97	b
	4	118 .	b
		146	b
		176	c
	þ,c	All days	d
5	a	104	b
		117	b
		145	b
		175	c
	b,c	All days	đ

Table 27. Locations, dates, and times of zooplankton sampling at sites 3, 4, and 5, 1987.

1. Locations: a. cage; b. cage area; c. control area.

Time schedules: a. 07:00, 10:00, 13:00, 16:00, 19:00, 22:00;
 b. 30 min. before sunrise, 10:00, 13:00, 16:00, 19:00, 30 min. after sunset; c. 30 min. before sunrise, 10:00, 13:00, 16:00, 19:00; d. one sample per day at 16:00.

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3.2.2:4 Fish

Growth and Stomach Contents

Fish samples were obtained from the trial cages at sites 2, 3, 4, and 5 using the same method as for the site 1 cage trial (section 2.2.3:5). Approximately 15 fish from each of the samples were preserved in 5% buffered formalin for future length measurements and stomach contents analysis. Fish collected in the samples and not preserved were immediately returned to the cages. No differentiations between red and green fish were made.

3.2.3 Laboratory Analyses of Samples

3.2.3:1 Water Quality

Samples for water quality analysis from the second year's field trials were treated and analysed using similar methods to those described in section 2.2.4:1. No suspended solids analysis was undertaken for these samples.

3.2.3:2 Zooplankton

Preserved samples from sites 2, 3, 4, and 5 were analysed to determine the abundance and genus group composition, size composition, and biomass of the crustacean zooplankton population at each of the sample locations and times. These analyses were similar to those described in section 2.2.4:2. The following changes, however, were applied.

For the size composition analyses, samples from site 2 were pooled for samples from: days 18 and 26, days 33 and 41, days 53 and 51, day 58, days 82 and 89, and day 94. Diurnal samples from sites 3, 4, and 5 were pooled on each sampling date for these analyses.

3.2.3:3 Artificial Diets

Samples of the artificial diets used at sites 2, 3, 4, and 5 were analysed in the laboratory to determine particle sizes, biomasses and abundances in the trial cages at the sampling times, and volume to weight conversion factors (for use in stomach contents analyses, section 3.2.3:4).

Methods to determine the mean particle size of each of the pellet types, and biomasses and abundances were the same as those followed for site 1 (section 2.2.4:3).

Methods to determine volume to wet weight conversion factors for each pellet type used in the second year's trials were similar to those used in the first year (section 2.2.4:3). Determination of volume to dry weight conversion factors were also similar; however, for samples from site 4 a dry weight content of 92% (BP Nutrition) was used. 3.2.3:4 Fish

Growth and Stomach Contents Analyses

Eight fish from each sample from sites 2, 3, 4, and 5 were prepared for analyses by soaking in distilled water for 12 hours to reduce the effect of formalin fumes. These fish were measured for fork length to the nearest 0.6mm. These data were used to determine the mean size of individuals on each sampling date. Data from the diurnal samples were pooled to obtain an overall mean fish size. Minimum preferred particle sizes were calculated as in section 2.2.3:5.

Four of the fish measured for growth were randomly selected from each sample and analysed for stomach contents. The remainder of the fish were retained for future reference.

1

The stomach contents of the fish samples from sites 2, 3, 4, and 5 were analysed according to the methods given in section 2.2.4:4 (with the exception of numbers of individuals in each zooplankton group; only total zooplankton was determined).

Results of the stomach contents analysis were recorded. Results were grouped together for each sample. Data from individual fish of the diurnal samples were pooled to obtain an overall daily sample (OD). This daily sample was

then divided into two groups. Data from fish whose fork length was less than or equal to the median fork length of the daily sample were combined to comprise the lower daily group (LD). The upper group contained data from fish whose fork length was greater than the pooled daily sample median (UD).

Indices of relative importance (IRI) were calculated separately for each sample, combined diurnal sample (OD), and combined lower and upper group samples (LD and UD, respectively).

Selection Indices

2

Straus' linear indices were calculated to evaluate the feed selection by the cage trial fish at sites 2, 3, 4, and 5 using the methods described in section 2.2.4:4. Indices were calculated using the combined sample, OD, LD, and UD data.

3.2.4 Statistical Analyses

Unless otherwise indicated, all statistical analyses were based on methods described by Sokal and Rohif (1987). Where possible, statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc. 1986) facility on the VAX-A mainframe computer at the University of Stirling.

3.3 RESULTS

3.3.1 Water Quality

Temperature data obtained from sites 2, 3, 4, and 5 are shown in Figure 22. Site 2 showed an increasing trend over the duration of the triai. Sites 3 and 4 showed slight rises in temperature early in the trials, but generally a declining trend was observed. Site 5 exhibited a declining temperature trend.

Site 2 was a more nutrient rich culture environment than sites 3, 4, and 5 (Table 28). Sites 3 and 5 were similar in their nutrient status over the courses of the trials. Site 4 was the most nutrient poor of the sites studied during 1987.

3.3.2 Fish Mortality and Growth

With the exception of site 3, the mortalities of the fish held at the cage sites in the second year's experiments were low (Table 29).

At site 2 the mortality shown was that over the period of sampling. However, shortly after the last sample of this trial an outbreak of white spot (<u>ichthyophtherius multi-filis</u>) resulted in the mortality of over 40% of the stock (P. Featherstone pers. comm.).

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Figure 22. Water temperatures within the trial cages, sites 2, 3, 4, and 5, 1987.

Α.	Site 2
8.	Site 3
c.	Site 4
D.	Site 5

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Table 28. Results of analyses of water samples taken from sites 2, 3.4, and 5, 1987.¹

		2			1
Parameter	2	'n	4	5	1
H	6.5-6.7	6.8-6.9 (6.8-6.9)	6.7 (6.7)	6.7-6.8 (6.7-6.8)	
Un-ionised ammoni <u>p</u> (µg.1 ⁻¹)	0.06-0.46	0.05-0.16 (0.05-0.13)	0.02-0.07 (0.08-0.10)	0.06-0.15 (0.07-0.11)	
Ionised amonia (µg.1 ⁻)	67.21-495.97	29.17 -68.16 (27.96-112.62)	26.69-61.13 (51.20-74.93)	43.71-131.17 (60.81-72.50)	
Nitrițe (µg.1 ⁻)	5.00-14.91	0.94-3.21	0.31-1.40	0.48-2.71	
Nitrate (µg.1 ⁻¹)	210.14-552.99	174.27-418.12 (180.33-531.24)	100-04-181.23 (112.28-168.56)	163.23-277.04 (155.78-259.87)	
Dissolved reactive phosphorus (mg.l ⁻)	1.48-8.08	0-1.24 (0-0.67)	0-0.72 (0-0.94)	0-1.54 (0-0.80)	
Secchi depth (m)	•	1.2-2.4 (1.4-2.4)	1.1-1.9 (1.3-1.9)	1.3-1.9 (1.5-1.9)	

Results show the range of values obtained over the course of the trials. Unbracketed results are from the cage samples; bracketed results are from the control area. :

	Site	Total mortality (%)
÷	2	2.53
	3	17.19
	4	0.94
	5	0.29

Table 29. Total mortality 1 of the fish stocks held at sites 2, 3, 4, and 5, 1987.

1. Total mortality is given as the mortality of the fish stock held in the trial cages over the sampling periods.

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The mortalities recorded from sites 4 and 5 may underestimate the true mortality of the stock. At these sites mortality was recorded by the farm staff whose husbandry practices may have resulted in inaccurate estimates. Conversely, the high mortality shown at site 3 is probably due to a concerted effort by the farm staff to remove and assess fish losses after an outbreak of costia (<u>ichythyobodo necatar</u>).

The stocks held at sites 2, 3, 4, and 5 showed significant positive growth trends over the course of the trials (Figure 23 and Table 30).

Comparisons of the growth trends of the fish of all cage sites (1, 2, 3, 4, and 5) revealed that they were not significantly different from one another (Table 31).

3.3.3 Characteristics of the Available Feed

3.3.3:1 Abundance

Total Zooplankton Abundance

On comparable days after first feeding, site 4 zooplankton abundances were generally the highest of all the sites studied during the second year's field trials. Site 5 zooplankton abundances were intermediate between those of site 4 and site 3, while site 2 zooplankton abundances were Figure 23. Fish growth, sites 2, 3, 4, and 5, 1987.

A. Site 2
B. Site 3
C. Site 4
D. Site 5

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Table 30. Results of statistical tests to determine the relationship between size and time after first feeding for fish held at sites 2, 3, 4, and 5, 1987.

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Statistical test: least squares regression analysis; independent variable – days after first feeding; dependent variable – natural logarithm of fish fork length

3.241	9 + 0.0063x	· 627.78	:
3.009	0 + 0.0101x	1977.53	1
3.393	0 + 0.0064x	421.21	:
3.46	7 + 0.0058x	231.61	1

Compar i son	F-value ¹	Significance ²
1-2	0.42	n.s.
1-3	1.46	n.s.
1-4	0.36	n.s.
1–5	0.54	n.s.
2–3	2.14	n.s.
2-4	0.01	n.s.
2-5	0.24	n.s.
3-4	1.36	n.s.
3–5	1.31	n.s.
4-5	0.02	n. s.

4

Statistical test: analysis of variance of regression coefficients (b_1, b_2) .

Table 31. Results of statistical tests to compare the growth regressions of fish held in the trial cages of sites 1 (1986) and 2, 3, 4, 5 (1987).

See Table 13 for footnote descriptions.

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the lowest (Figure 24).

At sites 3, 4, and 5, the total zooplankton abundances in the trial cage were generally greater than those from around the cage area. Control area zooplankton abundances were generally the lowest at these sites.

The total zooplankton abundance at site 2 showed an increase from first feeding to day 61. After this time abundance declined sharply. It remained at a comparatively low level until the end of the trial.

The highest zooplankton abundances at site 3 were recorded on the first sampling day after stock introduction. Levels at all locations at this site generally declined over the trial.

Site 4 peak zooplankton abundances were seen mid-way through the trial. Generally, however, abundances at all locations at this site declined over the sampling period.

Cage total zooplankton abundances at site 5 remained fairly consistent over the trial. Cage area samples were seen to increase in abundance, although the differences observed at this location were rather small. For unknown reasons three of the four control samples from site 5 were poorly preserved, and thus no over-trial trends at this location were obtained.

Figure 24. Total zooplankton abundances at site 2(A), site 3(B), site 4(C), site 5(D), 1987.

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The total zooplankton abundances within the cage shown for day 89 at site 2 and all days at sites 3, 4, and 5, are the means of the diurnal values obtained on each day. These mean values were considered relevant as analyses of the diurnal data showed very few significant regressions of abundance against time (6 of 103 regressions using linear, logarithmic, and polynomial models of total and percent group composition values against time of day showed Fvalues significant at p < 0.05).

Zooplankton Group Composition

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The group compositions of the zooplankton abundances at site 2 are shown in Figure 25. The majority of the samples were dominated by the cladocerans <u>Chydorus</u> and <u>Daphnia</u>. The only exception to this was on day 26 when the copepods <u>Cyclops</u> and <u>Diaptomus</u> dominated. Few other groups were represented in the zooplankton at site 2; <u>Bosmina</u> was present on day 18, and nauplii were seen in the samples of day 68.

The cage Zooplankton abundance at site 3 was usually dominated by the copepods <u>Cyclops</u> and <u>Diaptomus</u> (Figure 26A). At these times <u>Daphnia</u> contributed greatly to the Zooplankton abundance, while other cladocerans and nauplii played minor roles. On day 174 <u>Daphnia</u> dominated the zooplankton abundance in the trial cage.

Figure 25. Zooplankton abundance group composition within the trial cage, site 2, 1987.

- 1. Cyclops
- 2. Diaptomus
- 3. Daphnia
- 4. Bosmina
- 5. Diaphanosoma
- 6. Polyphemus
- 7. Leptodora
- 8. Chydorus
- 9. Nauplii





DAY 94

Figure 26. Zooplankton abundance group composition at the cage (A), control area (B), and cage area (C), site 3, 1987.

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See Figure 25 for key.





B



С

At the control area of site 3 copepods dominated the zooplankton abundance on days 55, 74, and 144 (Figure 26B). <u>Daphnia</u> were common in the control samples, dominating in the day 174 sample. Nauplii contributed more to the control area's zooplankton abundance than within the cage.

Copepods dominated the zooplankton around the cage area at site 3 only early in the trial (Figure 26C). Nauplii were common toward the end of the trial, as were <u>Daphnia</u>. The predatory cladocerans <u>Polyphemus</u> and <u>Leptodora</u> were often observed in the cage area samples.

At site 4, the cage zooplankton abundances were dominated by <u>Cyclops</u> on days 76 and 97 (Figure 27A). It is interesting to note that <u>Diaptomus</u> was rarely seen. <u>Daphnia</u> and nauplii were common in these initial samples. The final three cage samples from site 4 were dominated by <u>Daphnia</u>.

The control area zooplankton abundance at site 4 was also dominated by <u>Cyclops</u> on the initial two sampling days (76 and 97) (Figure 278). <u>Cyclops</u> and <u>Daphnia</u> were equiabundant on day 146. Poorly preserved samples from day 116 and day 176 rendered genus group composition analyses impossible.

Site 4 cage area zooplankton abundance was equally dominated by <u>Cyclops</u> and <u>Daphnia</u> (Figure 27C). Nauplii were the only other zooplankton group observed in these samples. Figure 27. Zooplankton abundance group composition at the cage (A), control area (B), and cage area (C), site 4, 1987.

See Figure 25 for key.



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A



в



DAY 176


The zooplankton composition in the cage at site 5 was highly variable over the course of the trial (Figure 28A). Nauplii dominated the abundance on all but the initial sampling day (day 104). On this day the copepods <u>Cyclops</u> and <u>Diaptomus</u> were dominant. The copepods declined as the cladoceran <u>Daphnia</u> increased in abundance over the trial.

Unfortunately, only one of the control area samples of site 5 was preserved suitably for group composition analysis (Figure 28B). On this day (day 117) <u>Diaptomus</u> dominated.

The cage area zooplankton abundances at site 5 also showed variable compositions (Figure 28C). The copepods <u>Cyclops</u> and <u>Diaptomus</u> dominated on day 104 and then declined in Importance over the trial. The cladoceran <u>Daphnia</u> gradually increased in importance at the cage area to dominate on day 175. Nauplii were also prevalent in these samples, dominating on days 117 and 145. <u>Polyphemus</u> was observed only on day 117.

Artificial Diet Abundance

The abundance of artificial diet particles in the trial cages at sites 2, 3, 4, and 5 generally comprised small percentages of the total available feed (Table 32).

At site 2 the relative abundance of artificial diet generally decreased over the course of the trial. Incon-

Figure 28. Zooplankton abundance group composition at the cage (A), control area (B), and cage area (C), site 5, 1987.

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See Figure 25 for key.





A



в



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no sample

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DAY 145



DAY 117



DAY 145

c

			Site				·
			3		4		5
Day	*	Day	x	Day	x	Day	x
18	4.2	55	0.1	76	0.4	104	0.2
26	25.2	74	0.2	97	0.1	117	0.2
33	7.7	95	0.1	118	0.1	145	0.2
41	1.9	116	0.2	146	0.1	175	0.2
53	0.5	144	0.1	176	0.3		
61	0.5	174	0.4				
68	4.6	0.					
82	20.2						
89	0.7						
94	0.4						

Table 32. Amounts of the total available feed comprised by artificial diets (with respect to abundance), sites 2, 3, 4, and 5, 1987.

Amounts of artificial diets are presented as percent of the total available feed. Total available feed was considered as the sum of the artificial diet and the total zooplankton abundances in the trial cages. gruent values obtained on day-26 and day 82 may be a result of an underestimate of the zooplankton abundance on these days caused by inadequacies of the sampling method.

At sites 3, 4, and 5 artificial diet relative abundances remained very low and showed no changing trend over the trials.

3.3.3:2 Size

Changes in the particle sizes of zooplankton and artificial diets at site 2 are shown in Figure 29A. <u>Cyclops</u> showed no significant change, <u>Diaptomus</u> and <u>Chydorus</u> each showed a significant decrease, and <u>Daphnia</u> significantly increased in size over the trial (Table 33). The particle size of the artificial diets was seen to increase over the trial at site 2. (Due to the nature of this data set, no regression analysis was undertaken. A similar situation existed for the artificial diets at sites 3, 4, and 5.)

Examination of Figure 29A shows that all zooplankton groups present (except <u>Chydorus</u>) were larger than the PFR for only approximately the first half of the trial.

Only two of the zooplankton groups measured from site 3 showed significant changes in size over this trial (<u>Diap-</u> <u>tomus</u> and <u>Daphnia</u> decreased in size) (Figure 29B and Table 34). <u>Cyclops</u> and <u>Polyphemus</u> showed no size change over the Figure 29. Particle sizes of the zooplankton groups and the artificial diets, and fish PFR's, site 2(A), site 3(B), site 4(C), site 5(D), 1987.

1. Cyclops

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- 2. Diaptomus
- 3. Daphnia
- 4. Bosmina
- 5. Diaphanosoma
- 6. Polyphemus
- 7. Leptodora
- 8. Chydorus
- 9. Nauplii
- A. Artificial diets
- F. Fish PFR





Table 33. Results of statistical tests to determine the relationship between size and time after first feeding for zooplankton genus groups and fish minimum PFR's, site 2, 1987.

least squares regression analysis; independent variable - days after first feeding. dependent variable - mean size. Statistical test:

Dependent parameter	Y = a + bx ¹	Fregression ²	Significance ³	1
<u>Cyclops</u>	0.627 + 0.002x	0.462		
Diaptomus	0.603 - 0.001x	5.102	•	•
<u>Daphnia</u>	0.638 + 0.003x	53.385	1	
Chydorus	0.413 - 0.002x	19.812	:	
Fish PFR	-0.020 + 0.011x	46.998	:	

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See Table 20 for footnote descriptions.

Table 34. Results of statistical tests to determine the relationship between mean size and time after first feeding of zooplankton genus groups, and fish PFR's, site 3, 1987.

lin	I
first	
after	
days	
variable	and a second sec
independent	
analysis;	
regression	
squares size.	
- mean	
test: riable	
tatistical spendent va	

Dependent parameter	$y = a + bx^{l}$	^f regression ²	Significance ³
Cyclops	0.844 + 0.001x	1.292	
Diaptomus	0.845 - 0.001x	20.392	ł
Daphnia	1.262 - 0.003x	58.259	I
Polyphemus	1.933 - 0.002x	1.049	n.s.
Fish PFR	-0.107 + 0.013x	55.334	:

See Table 20 for footnote descriptions.

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period, while the artificial diets' particle size was seen to increase.

At site 3, crustacean zooplankton were only of suitable size to the fish for a short time after introduction to the cage. The artificial diets increased in size in response to increases in fish PFR, but did not exceed the PFR levels toward the end of the trial (Figure 29B).

At site 4, <u>Cyclops</u> showed no significant change in size over the trial period, while <u>Daphnia</u> significantly decreased in size (Figure 29C and Table 35). The artificial diets increased in size throughout the trial.

Figure 29C indicates that at site 4 the zooplankton genus groups measured were smaller than the minimum PFR virtually from the time of fish introduction.

At site 5, <u>Cyclops</u> and <u>Daphnia</u> showed significant size decreases over the trial. <u>Diaptomus</u> showed no size change (Figure 29D and Table 36). The artificial diets increased in size.

At the time of their introduction to the cage, the fishes' PFR was greater than all zooplankton types' particle size; this difference increased over the course of the trial (Figure 29D). Table 35. Results of statistical tests to determine the relationship between mean size and time after first feeding of zooplankton genus groups, and fish PFR's, site 4, 1987.

least squares regression analysis; independent variable - days after first feeding; dependent variable - mean size. Statistical test:

Dependent parameter	y = a + bx ¹	Fregression ²	Significance ³
Cyclops	0.740 + 0.001x	2.033	
Daphnia	1.172 - 0.002x	26.361	:
Fish PFR	0.342 + 0.009x	111.400	I

See Table 20 for footnote descriptions.

Table 36. Results of statistical tests to determine the relationship between mean size and days after first feeding of zooplankton genus groups, and fish PFR's, site 5, 1987.

: :

feeding:		
first		
after		
days		
variable		
independent		
analysis;		
regression		
squares	size.	
least	- mean	
test:	ariable.	
Statistical	dependent v	
	-	

Dependent parameter	$y = a + bx^{1}$	Fregression ²	Significance ³
Cyclops	1.644 - 0.005x	37.033	:
Diaptomus	0.786 + 0.001x	0.034	
Daphnia	1.369 - 0.003x	13.055	:
Fish PFR	0.264 + 0.10x ·	210.246	•

See Table 20 for footnote descriptions.

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3.3.3:3 Blomass

Total Zooplankton Biomass

Site 4 generally showed the greatest total zooplankton biomass values on comparable days after first feeding (Figure 30). Biomass levels at site 5 were generally intermediate between those of site 4 and site 3. Site 2 showed the lowest total zooplankton biomasses of the sites examined during the second year.

Total zooplankton blomass levels inside the cage were generally greater than those around the cage area at sites 3, 4, and 5. Control area zooplankton blomass values were usually the lowest of all locations at sites 3 and 4, while at site 5 they were similar to those of the cage area location.

Over the trial at site 2 total zooplankton blomass within the cage was seen to increase to a peak at 61 days after first feeding. After this time, the total blomass followed a declining trend to the end of the trial.

At sites 3 and 4 the total zooplankton biomass at all locations was at a maximum on the initial sampling after stock introduction (55 and 76 days after first feeding at sites 3 and 4, respectively). A general decline in total zooplankton biomass at all locations was seen over the

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Figure 30. Total zooplankton biomass at site 2(A), site 3(B), site 4(C), site 5(D), 1987.

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DAYS AFTER FIRST FEEDING

course of the trials at both of these sites.

The total zooplankton biomass at site 5 was also at a site maximum at all locations on the initial sampling day (day 104). After this time the cage total zooplankton biomass was seen to generally decline, while the cage area levels did not appear to follow any changing trend. Poor preservation of all but one of the control area samples at site 5 prevented determination of any over-trial trends in the total zooplankton biomass at this location.

A regression analysis was performed using the cage diurnal total zooplankton blomass data from day 89 at site 2 and all days at sites 3, 4, and 5 in a similar manner to that performed using the total zooplankton abundance data. Of 72 regressions performed, only 2 showed significant Fvalues (p < 0.05). This indicated no appreciable diurnal trend in blomass or zooplankton. The cage levels shown on these days are therefore averages of the diurnal area.

Zooplankton Group Composition

Of the majority of sampling days the zooplankton biomass in the trial cage at site 2 was dominated by the cladoceran <u>Chydorus</u>. <u>Daphnia</u> also contributed much to the total biomass (dominating on day 18) (Figure 31). The copepods <u>Cyclops</u> and <u>Diaptomus</u> were generally a minority in the total zooplankton biomass at site 2.

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Figure 31. Zooplankton biomass group composition within the trial cage, site 2, 1987.

1. Cyclops

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- 2. Diaptomus
- 3. Daphnia
- 4. Bosmina
- 5. Diaphanosoma
- 6. Polyphemus
- 7. Leptodora
- 8. Chydorus
- 9. Nauplii







Within the trial cage at site 3 the total zooplankton biomass was dominated by the copepods <u>Cyclops</u> and <u>Diaptomus</u> on the first four sampling days (55, 74, 95, and 116 days after first feeding) (Figure 32A). <u>Daphnia</u> dominated on the final two sampling days (days 144 and 174).

Dominance of the zooplankton blomass at the control area of site 3 was shared between the copepod <u>Diaptomus</u> and the cladoceran <u>Daphnia</u> on all but day 174 when nauplii dominated (Figure 32B).

Cladoceran types (<u>Chydorus</u> and <u>Daphnia</u>) dominated the zooplankton blomass around the cage area at site 3 on days 74, 116, 144, and 174 (Figure 32C). Copepod types played a lesser role in the zooplankton blomass at this area than within the cage or at the control area, dominating only on days 55 and 95.

Site 4 cage zooplankton biomasses were composed mostly of <u>Daphnia</u> on all but 97 days after first feeding when <u>Cyclops</u> dominated (Figure 33A). No other zooplankton type made an important contribution to the total biomass at this location.

Poor preservation of samples from the control area at site 4 meant that estimates of zooplankton biomass were only possible on 3 of the 5 sampling dates (Figure 338). Dominance was shown by <u>Daphnia</u> on days 76 and 146. <u>Cyclops</u>

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Figure 32. Zooplankton blomass group composition at the cage (A), control area (B), cage area (C), site 3, 1987.

See Figure 31 for key.



DAY 74



DAY 95

1



DAY 116





DAY 144









DAY 95

DAY 116













DAY 95



Figure 33. Zooplankton blomass group composition at the cage (A), control area (B), and cage area (C), site 4, 1987.

See Figure 31 for key.

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DAY 97



DAY 118 .

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DAY 146











no sample





no sample



no sample

С







DAY 118

DAY 146



dominated in the remaining sample, from day 97.

At the cage area of site 4 samples were always dominated by <u>Daphnia</u> with respect to total zooplankton biomass (Figure 33C). <u>Cyclops</u> was the only other zooplankton type to make an appreciable contribution to the zooplankton biomass at this location of site 4.

The cage zooplankton blomass at site 5 was dominated by copepods (particularly <u>Diaptomus</u>) on days 104, 117, and 145 (Figure 34A). <u>Daphnia</u> dominated the final samples on day 175.

The only suitably preserved sample from the control area of site 5 (taken 117 days after first feeding) was dominated by <u>Diaptomus</u> (Figure 348). <u>Cyclops</u>, <u>Daphnia</u>, and nauplil all played minor roles.

The dominance of the cage area zooplankton blomass at site 5 followed a similar trend to that within the cage at this site (Figure 34C).

Biomass of the Artificial Diets

The artificial diets at sites 2, 3, 4, and 5 comprised a much greater percentage of the total available feed by blomass than by abundances (compare Table 37 with Table 32).

Figure 34. Zooplankton biomass group composition at the cage (A), control area (B), and cage area (C), site 5, 1987.

See Figure 31 for key.



DAY 117

A








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DAY 117

DAY 104

no sample

DAY 175

no sample

DAY 145

в



2		3		4		5	
)ay	x	Day	*	Day	x	Day	×
8	83.5	55	5.2	76	25.0	104	42.9
26	96.7	74	17.5	97	43.6	117	62.0
33	77.7	95	35.6	118	24.4	145	85.3
41	43.4	116	71.5	146	44.2	175	86.6
53	. 43.7	144	49.0	176	86.8		
51	41.2	174	75.7				
38	87.1			•			
82	97.6						
89	81.4						
94	60.7						

Table 37. Amounts of the total available feed comprised by artificial diets (with respect to biomass), sites 2, 3, 4, and 5, 1987.

of the artificial dist and total zooplankton biomasses.

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At site 2 the relative blomass of artificial diets decreased from the start of the trial, as the zooplankton blomass increased. A subsequent rise in the relative blomass of artificial diets followed as the zooplankton blomass decreased from day 61 to the trial end.

At sites 3, 4, and 5, the relative blomass of the artificial diets generally increased over the trial periods. These increases corresponded with decreases in zooplankton blomasses at these sites.

3.3.4 The importance and Selection of Artificial and Natural Feeds

3.3.4:1 Indices of Relative Importance

Changes Over the Trial Period

The indices of relative importance of artificial diets (IRI_{art}) were greater than the indices of relative importance of zooplankton (IRI_{zoo}) on all sampling days at sites 2, 3, 4, and 5 (Figure 35A, B, C, D, respectively).

At site 2 IRI_{art} rose quickly after the fish were introduced to the cage. Maximum levels (1.00) were reached only 25 days after first feeding. High levels were maintained over the trial period. Figure 35. Daily indices of relative importance, site 2(A), site 3(B), site 4(C), site 5(D), 1987.

A. Artificial diets

÷

Z. Total zooplankton



Conversely, IRI₂₀₀ values at-site 2 were always very low. Levels had dropped to near the minimum (O) by the second sampling day and remained so over the trial.

At site 3 IRI_{art} levels increased from the sample with stock introduction (day 55) to peak on day 116. A decline in IRI_{art} at this site was then observed to the trial end. Values of IRI_{art} at site 3 never reached the theoretical maximum (1.00).

IRI₂₀₀ levels at site 3 fell to near the minimum value soon after the stock was introduced to the cage.

On the initial sampling day after stock introduction at site 4 (day 76), IRI_{art} was very low. This increased markedly to peak on day 97. Values of IRI_{art} were lower than this on all subsequent sampling days, although a high degree of variability was observed. IRI_{art} values never reached the theoretical maximum at site 4.

A decrease in IRI₂₀₀ levels at site 4 was observed from the beginning of the trial. However, values were often greater than zero during this trial, particularly on sampling days when IRI_{art} was low.

IR1_{art} values at site 5 increased from the beginning of the
trial. A site maximum was reached on the third sampling
day (day 145). The level was lowered on the final sampling

Figure 36. Diurnal indices of relative importance, site 2, 1987.

A. Artificial dietsZ. Total zooplankton



Figure 37. Diurnal indices of relative importance, site 3, 1987.

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A. Artificial dietsZ. Total zooplankton

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on day 97, when IRI_{art} was seen to increase over the morning to near maximum values in the afternoon and then decrease slightly into the evening. On all other diurnal sampling days at site 4 IRI_{art} was seen to be highly variable.

Diurnal sampling at site 4 showed zooplankton to be relatively unimportant in the fishes' diets throughout the day on all sampling days (Figure 38). When marked changes were shown, IRI₂₀₀ was seen to decrease over the morning to near minimum levels by the afternoon (this situation was seen in the initial samples, day 97).

On ail diurnal sampling days at site 5, IRI_{art} was seen to generally increase (Figure 39). Peak daily values were generally reached by mid-day.

The diurnal samples at site 5 showed IR1₂₀₀ to be minimal over the entire day on each occasion (Figure 39).

Differences Between Upper and Lower Fish Groups

On a date by date basis, IRI_{art} values for the upper group fish (UD) were generally greater than those for the lower group fish (LD) at sites 3, 4, and 5 (Figure 40). Conversely, when differences in IRI₂₀₀ were present UD fish generally showed lower values than LD fish (Figure 41). Figure 38. Diurnal indices of relative importance, site 4, 1987.

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A. Artificial diets

Z. Total zooplankton

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IND. OF RELATIVE IMP.

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Figure 39. Diurnal indices of relative importance, site 5, 1987.

- A. Artificial diets
- Z. Total zooplankton

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Figure 40. Daily indices of relative importance for the artificial diets, upper and lower fish groups, site 3(A), site 4(B), and site 5(C), 1987.

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U. Upper fish groupL. Lower fish group



DAYS AFTER FIRST FEEDING

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Figure 41. Daily indices of relative importance for total zooplankton, upper and lower fish groups, site 3(A), site 4(B), and site 5(C), 1987.

U. Upper fish group

L. Lower fish group

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DAYS AFTER FIRST FEEDING

3.3.4:2 Linear Indices of Selection

Changes Over the Trial Periods

The linear indices of selection for artificial diets by biomass and abundance (L_{ab} and L_{aa} , respectively) from all sampling days at sites 2, 3, 4, and 5 are shown in Figure 42. Selection indices for total zooplankton (by blomass, L_{tzb} , and abundance, L_{tza}) are not shown. These were determined to be of equal magnitude to the corresponding selection index for artificial feed, but of opposite sign. In other words, figures of L_{ztb} and L_{zta} would be mirror images to those of L_{ab} and L_{aa} , respectively. All selection indices were significantly different from zero (random feeding) at least at the p < 0.05 level.

At site 2 selection for the artificial diets was always positive. This selection was seen to increase from first feeding and peak in the period between days 41 and 61. After this time artificial diet selection was observed to drop dramatically, but still remain positive. Selection then again increased from the sample on day 82 to the trial end (day 94). L_{aa} was always significantly greater than L_{ab} at site 2 (p < 0.05).

The linear indices of selection for artificial diets at site 3 were positive throughout the trial. However, a general declining trend toward random feeding was observed

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Figure 42. Daily linear indices of selection for artificial diets, site 2(A), site 3(B), site 4(C), site 5(D), 1987.

- A. Abundance
- B. Biomass
- X. Significant difference between points



DAYS AFTER FIRST FEEDING

In these indices, particularly with L_{ab} after the sample on day 95. As at site 2, L_{aa} was generally significantly greater than L_{ab} at site 3 (p < 0.05); the exception to this was on the second sampling day (74).

Site 4 linear indices of selection were also always positive with respect to artificial diets abundance and blomass. A general declining trend was observed in L_{ab} , particularly after the sample on day 97. L_{aa} was much more variable over the course of the trial at site 4, and often was significantly lower than L_{ab} (p < 0.05).

 L_{ab} and L_{aa} were seen to generally decline after stock introduction at site 5, although values were always significantly greater than zero. L_{aa} was always significantly greater than L_{ab} during this trial (p < 0.05).

Diurnal Changes

The diurnal sample taken on day 89 at site 2 showed the selection for artificial diets to increase dramatically from negative to positive over the morning with respect to both biomass and abundance (Figure 43). Peak levels were generally obtained at the 10:00 sample and maintained throughout the day.

On the first two sampling days at site 3 (days 55 and 74), L_{ab}^{+} was seen to be highly variable over the day (Figure 44). Figure 43. Diurnal linear indices of selection for artificial diets, site 2, 1987.

- A. Abundance
- B. Biomass

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Figure 44. Diurnal linear indices of selection for artificial diets, site 3, 1987.

A. Abundance

B. Biomass



For the remainder of the sampling days a general diurnal increase in the selection of artificial diets was observed at this site. With respect to abundance, the diurnal samples taken at site 3 showed no consistent trend in artificial diet selection. The exception to this was on day 116, when an increase over the day was observed.

The diurnal samples obtained from site 4 showed almost no consistent trend in L_{aa} or L_{ab} (Figure 45). Exceptions to this could be inferred from the data obtained on day 97 (with respect to L_{aa}) and day 176 (with respect to L_{ab}), when general increases in selection of the artificial feed were observed.

At site 5 selection of the artificial diets with respect to blomass generally showed an increasing diurnal trend (Figure 46). Selection of this feed type with respect to abundance was consistently high over all sampling days; when changes were observed an increasing trend over the day was noted.

Differences Between Upper and Lower Fish Groups

Selection for artificial diets by blomass was often not significantly different between the UD and LD fish groups at sites 3 and 5 (p < 0.05) (Figure 47). Significant differences were observed at site 4 on days 76 and 118 (UD greater than LD, p < 0.05); indeed, the LD group at site 4

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Figure 45. Diurnal linear indices of selection for artificial diets, site 4, 1987.

A. Abundance

B. Biomass



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Figure 46. Diurnal linear indices of selection for artificial diets, site 5, 1987.

A. Abundance

B. Biomass

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Figure 47. Daily linear indices of selection for the artificial diets by biomass, upper and lower fish groups, site 3(A), site 4(B), and site 5(C), 1987.

- U. Upper fish group
- L. Lower fish group

X. Significant difference between points



were seen to negatively select the artificial diets by blomass and therefore positively select crustacean zooplankton on the initial sampling day after stock introduction.

With respect to abundance, selection of the artificial diets was generally significantly greater (p < 0.05) for the UD than for the LD fish groups at sites 3 and 5 (Figure 48). At site 4 differences were much more variable, as often the LD fish group showed a higher selection index (p < 0.05) than the UD group did.
Figure 48. Daily linear indices of selection for the artificial diets by abundance, upper and lower fish groups, site 3(A), site 4(B), and site 5(C), 1987.

- U. Upper fish group
- L. Lower fish group
- X. Significant difference between points



3.4 DISCUSSION

3.4.1 Cage First Feeding in a Productive Site

3.4.1:1 The Success of the Trial

The trial conducted at site 2 shows very clearly that productive water bodies are not suitable locations for cage systems to rear juvenile Atlantic salmon. The trial was abandoned when approximately 40% of the stock died as a result of a parasite infection, although mortality was generally low throughout the sample period. No evaluation of the trial with respect to fish growth was possible as temperature and stock origin differences prevented growth comparison between the cage trial sites.

Water quality analysis of site 2 showed it to be the most nutrient rich of all the cage trial sites utilised in this study. None of the parameters measured reached lethal levels. However, values high in the tolerable range were recorded, and therefore sublethal effects on the stock cannot be discounted. The exception to this was water temperature, which showed suitable values throughout the trial.

Suspended solids or primary production were not directly measured at site 2. Highly productive freshwater bodies generally have high standing crops of algae (planktonic or

attached), high zooplankton standing crops dominated mostly by cladocerans, and high levels of parasite and disease pathogen production (Morgan et al 1980). High standing crops of algae and zooplankton suggest that oxygen depletion of the water is likely to occur. A zooplankton community of high standing crop dominated by cladocerans increases the chance that cage fish will encounter and subsequently capture this natural feed. High levels of parasite and disease pathogens increases the chance of infection in the fish. Obviously, these characteristics of highly productive water can be detrimental to cage saimon smolt production. This was demonstrated by the relative success of the preliminary trial (located at a site of low productivity) and failure of the trial at site 2. Indeed. Beveridge (1987) notes that sampling to determine the physical, chemical, and biological characteristics at a site should be undertaken before any decision to establish a cade culture operation is made.

3.4.1:2 Feeding Behaviour

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Throughout the trial at site 2 the index of relative importance of the artificial feed was always higher than that for crustacean zooplankton. The natural feed only showed importance (as IRI_{zoo}) in the fishes' diet on the initial sampling day after first feeding. IRI_{art} was also relatively low on this day, but increased soon thereafter. This same increase in IRI_{art} after first feeding was observed in the preliminary trial, and denotes the development of exogenous feeding behaviour throughout the stock. It was surprising to find IRI₂₀₀ to be so low throughout the trial at site 2, particularly as the available zooplankton was dominated by cladocerans throughout the trial, and that these zooplankton were of a suitable size to the fish (with respect to PFR) for almost half the trial. IRI_{art} showed variability throughout the trial, indicating that not all the stock was maximally feeding on the artificial diets. Those fish that were not feeding maximally on this feed, for some reason, were not taking the natural feed as they were observed to do in the preliminary trial.

These observations are supported by the linear indices of selection calculated from site 2. They show the artificial diets were always selected positively while the natural feed was always avoided (both with respect to abundance and blomass). It should be noted at this point that the fluctuations observed in the linear indices of selection at site 2 (particularly the dramatic decrease in L_{aa} and L_{ab} , and therefore increase in L_{ata} and L_{atb}) are a result of fluctuations in the proportion of these feed types measured in the total available feed, not in the feeding behaviour of the stock. The index of selection utilised was simply the difference between the percentage of a food type in the fishes' stomachs and the percentage of that food type in the available feed (Straus 1979).

A decrease in the zooplankton abundance and blomass, as observed on day 68 (and therefore an increase in artificial diet relative abundance and blomass) could result in the decrease in L_{aa} and L_{ab} observed at site 2 (and increases in L_{ZLA} and L_{ZD}) if the proportion of these food types in the fishes' stomachs remains unchanged.

Several reasons can be given for the virtual exclusion of crustacean zooplankton from diets of the stock at site 2.

The morphological, hydrological, and ecological characteristics of site 2 obtained before the trial began indicated that this would be a very productive site. The crustacean zooplankton at site 2 showed a species composition typical of such sites. However, the abundance and biomass of the zooplankton community was observed to be very low (in relation to the other, less productive sites); this was not expected. Malone and McQueen (1983) noted that cladoceran zooplankton often migrate to littoral zones during the daytime, returning to open water during the night. This behaviour could explain why such low standing crop zooplankton levels were measured. The result of this, with respect to feeding behaviour, is that the crustacean zooplankton comprised only small percentages of the total available feed. Thus, their encounter rate by the foraging fish was probably low, and the probability of being selected was also low.

The feeder utilised at site 2 to supply the artificial diets to the stock operated in a slightly different manner from the feeder utilised in the preliminary trial. At site 2 the artificial feed was delivered to the cage as it dropped from a constantly moving conveyor belt. At the preliminary trial, the artificial feed was delivered to the cage at intervals of 15-20 minutes. It could be suggested that the continuous supply of artificial diets at site 2 allowed the stock to feed on this optimal food source virtually at will and until satiated, and there was no need to feed on zooplankton. In the trial at site 1, the bursts of feed delivery meant that the access time to this feed source was limited, all fish would be competing at the same time, and therefore the chances of achieving satiation would be reduced. As a consequence, subordinate fish who were unable to compete successfully for the artificial diets were forced to feed on the zooplankton.

The effect of diurnal variation in feeding behaviour as a cause of error to the analysis of this study was evaluated on one sampling day during the trial at site 2 (day 89). This sample showed the artificial diets to increase in importance (as IRI_{art}) over the day, while zooplankton remained virtually unutilised by the fish (as IRI_{200}). This result supports the once-daily sample results that the artificial diets were used almost exclusively as a food source. However, the diurnal indices of selection show that early in the morning the fish selected negatively for

the artificial diets (and therefore positively for zooplankton) with respect to blomass, and fed randomly at this time with respect to abundance. This result suggests that zooplankton did have some importance in the diet of the fish; however, this importance can hardly be considered significant.

Finally, differential gastric evacuation may have affected the stomach contents observed from the site 2 fish samples. As noted in the preliminary trial discussion, the high energy content of the artificial diets relative to the zooplankton may have resulted in the former food type being evacuated from the stomachs at a slower rate than the latter food type. The result would be an overestimation of the importance and selection of the artificial diets, and an underestimation of zooplankton. This may have contributed to the observed results. However, as the exoskeleton of zooplankton is also reported to be retained for long periods in the stomachs of small fish, the effect of differential gastric evacuation on the results is believed to be minimal.

3.4.2 Feeding Behaviour and-the Effects of Increasing the Time of Stock Transfer

3.4.2:1 Characteristics of the Available Feed

The zooplankton sampling at sites 3, 4, and 5 supported the observations made from the preliminary trial that the cage raft acts as an attractant to crustacean zooplankton, as cage and cage area levels were consistently higher than control area ones. Cladoceran zooplankton were often more prevalent around the cage areas than the control areas, again supporting the suggestion made from the preliminary trial that these zooplankton are attracted more to the cage raft by higher production levels than copepod types are.

Often at sites 3, 4, and 5 the cage area zooplankton levels were lower than within the cage, while the reverse was generally true in the preliminary trial. At site 1 it was suggested that the cage may have acted as a physical or behavioural barrier. This was not considered important, and alternatively it was suggested that cage fish predation was the main cause of the difference. The results from sites 3, 4, and 5 suggest that perhaps the cage bag was important in affecting zooplankton distribution during the preliminary trial. The cage bags utilised at sites 3, 4, and 5 were constructed of a mesh whose openings were much greater than the average size of most of the crustacean zooplankton, possibly allowing easier movement through the cage than at the preliminary trial where the mesh size was only about twice that of zooplankton size. Once inside the cage, the utilisation of this food source by the cage fish was much lower at sites 3, 4, and 5 than at the preliminary trial (see the next section). The combined effect of these factors thereby resulted in the observed zooplankton distribution. With respect to the operation of cage smolt production systems, this is important as the larger the mesh utilised, the more natural feed will be available to the stock. However, large mesh sizes are only used when the fish are of a size in which zooplankton are not heavily utilised, and the fact that large mesh sizes maximise flow rates and maintain optimal water quality mean that this importance is minimal.

Diurnal sampling of the crustacean zooplankton within the trial cages at sites 3, 4, and 5 showed no consistent trend at one site or between sites on similar days after first feeding. This result confirms the discussion with respect to the preliminary trial that diurnal migrations of crustacean zooplankton had little effect on the results of this study. This is not to say that diurnal variations in zooplankton abundances within the cage did not exist. The sampling programme and methods of this study may not have been sensitive enough to detect such variations.

The relative abundance of the artificial diets was always much lower than the relative zooplankton abundance at sites

3, 4, and 5. Conversely, the artificial diets were always dominant in the available feed with respect to blomass. This is similar to the result obtained from the preliminary trial; again it can be attributed to the fact that the artificial diets contain more dry matter per particle than do the crustacean zooplankton. No over-trial change was observed in the relative importance of the artificial and natural feeds (with respect to abundance or blomass) at sites 3, 4, and 5. This result was obtained because as the zooplankton standing crop decreased over the trials at all sites, so did the number of artificial diet particles (as a function of increasing particle size) and the mass of artificial diet delivered to the cage (as a function of decreasing dally ration size).

Perhaps the most important difference in the available feed at sites 3, 4, and 5 was the size of the crustacean zooplankton with respect to the fishes' minimum preferred particle size (PFR) at the times of stock introduction. At site 3 the average size of most zooplankton groups' individuals was larger than the fishes' PFR for the first two sampling days (56 and 74). This situation was present at site 4 only on the initial sampling day (76). At site 5, this situation was never present. These relationships Correspond closely with the feeding behaviour of the stock held at these sites, described in the following section. It should be noted that no statistical comparison between the mean size of the zooplankton groups and the fish PFR's

on separate sampling days was obtained; the reasoning for this is described in section 2.4.2:6. This is therefore recognised as a source of error in the study; however, the general relationship is still considered important.

3.4.2:2 Feeding Behaviour

The indices of relative importance calculated from sites 3, 4, and 5 all show that maximal feeding was not reached by these fish stocks until some time after they were introduced to the cages. This could be attributed to the stress of transfer, but also to the change of feeding environment. That is, in the tank systems they were accustomed to the high flow rates and having feed particles carried to them in the current. In the cages they would have had to forage for feed failing into and slowly sinking through the water. Some time would be required before their feeding behaviour was adapted.

The effect of time of transfer on the utilisation of natural and artificial food sources is also demonstrated by the indices of relative importance. Zooplankton were observed in the diets of the fish at sites 3 and 4 on the initial sampling days (although to a low degree), while at site 5 zooplankton were relatively unimportant in the fish diets. At sites 3 and 4 the zooplankton were of a sultable particle size to the stock (with respect to PFR) on the initial sampling day, while at site 5 they were too small.

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reduction in temperature (and therefore a reduction in activity) at those sites. Metcaife <u>et ai</u> (1988) suggested that Atlantic salmon parr show a reduced appetite in the autumn of their first year; this may also be the cause of the decreases in the indices of relative importance.

Site 4 showed the greatest variability in indices of relative importance of all the second year's field trials. The zooplankton at this site were also generally dominated by cladocerans. This demonstrates the effect of species composition on the fishes' feeding behaviour; that is, the natural feed was dominated by easily captured individuals which, although of suboptimal size and quality, still may have appeared an attractive food source.

The linear indices of selection calculated from sites 3, 4, and 5 lend statistical support to the observed trends in the indices of relative importance. Artificial diets were always selected for, zooplankton were always avoided. This indicates that although some zooplankton were taken, the fish were generally able to select the optimal food source. It may also be suggested that the fish were selecting this food source as a result of experience; that is, they recognised it as the source of food in the tank systems.

The linear indices of selection generally tended toward random feeding at sites 3, 4, and 5. This corresponds with the decreases in the indices of relative importance

observed, and again may be -attributed to a reduction in temperature and/or reduced appetite.

As the stock at site 4 showed the greatest variability in indices of relative importance, it also showed the greatest variability in selection indices. This, again, is attributed to the dominance of cladocerans in the zooplankton community.

Differences in the linear indices of selection with respect to abundance and biomass can be attributed to their relative differences in importance in the available feed. For example, if a stomach sample was observed to consist completely of artificial diet (100%), a large selection index would be obtained with respect to abundance, as the artificial diets were always a small percent of the available feed by this parameter. However, a smaller index would be obtained with respect to biomass, as the artificial diet comprised a greater amount of the available feed with respect to this parameter. This is recognised as a weakness of the linear index of selection (Straus 1979).

The indices of relative importance and selection indices of the upper and lower fish groups at sites 3, 4, and 5 reinforce the observation made in the preliminary trial that the smaller members of a population rely more on the natural food source than the larger fish do. It is interesting to note that the greatest variability in

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feeding behaviour between these fish groups was generally observed at site 4, where cladocerans dominated the crustacean zooplankton.

The diurnal indices of relative importance and linear indices of selection calculated for sites 3, 4, and 5 showed much variability. Often a trend of increasing importance and selection of artificial diets was observed, while zooplankton decreased in importance and selection. This may indicate that the natural feed source was utilised early in the mornings, before the automatic feeders became active. That is, the feeders were not activated early enough to coincide with the fishes' morning peak in activity. It could also be argued that these results simply represent an accumulation of artificial feed in the fishes' stomachs due to slow gastric evacuation. Regardless, the results indicate that the caged fish did not demonstrate any consistent diurnal pattern in their feeding behaviour (at least within the time of sampling at these sites). This factor can therefore be discounted as having a significant effect on the daily sample trends observed at these sites and at the preliminary trial.

Finally, the effects of gastric evacuation on the results still remains an unquantified source of error in this study. However, the trends observed were fairly consistent, and it is believed they accurately represent the feeding behaviour of the caged fish. 3.4.3 Juvenile Atlantic Salmon as Optimal Foragers

Much of the feeding behaviour described in this study has been related to the concepts of an optimal foraging theory. In brief, an animal whose foraging behaviour is described by this theory will (i) select prey which yields the most amount of energy for a given expenditure, (11) increase in selectivity as the abundance of profitable prey increases. and (iii) not select unprofitable prey regardless of how common they are (Krebs and Davies 1984). Optimal foraging theory has for the most part been developed as a result of laboratory experiments. Variables not directly involved in the foraging process have largely been heavily controlled or excluded. Consequently, the indirect effect on foraging behaviour by such variables is not included in the generalized theory. In field studies, the application of such a theory can be complicated by the presence of variables which were excluded in the laboratory (Mangel and Clark 1986).

The cage trials undertaken in this study can be considered as controlled field experiments. Certain variables could not be completely controlled. These include water temperature and quality, and the abundance, species composition, and nutritional quality of the natural food source. Other variables could be, and were, controlled. These include number and stocking density of the fish, no predation, and all aspects of the artificial food supply.

Perhaps the most obvious observation made from the cage trials was that not all individuals foraged for feed in a similar manner. This was true for individuals within the same cage at any given time or over a period of time, and also between cages and sites at any given time. This observation was manifested in the calculated indices of relative importance and linear indices of selection. They suggested that members of the population foraged at varying levels below the theoretical optimum. In all trials the theoretical optimum feeding strategy was to always select an artificial feed particle. This assumption was considered valid as the artificial diet was more nutrient and energy dense than the natural feed, and the artificial diet was completely non-evasive. Also, the artificial diet was supplied to the cages in quantities that provided an overabundance of particles for the fish. Given this, why did all of the fish in all of the trials not feed at the theoretical optimum level?

Natural environments vary both spatially and temporally, and the foraging behaviour of a fish must be flexible to adapt to these changes (Dill 1983). Similarly, as morphological characteristics vary between individuals, so do behavioural and physiological characteristics. Combined, these can set varying limits on the foraging capabilities of individuals (Ringler 1983). These sources of variation could partially account for the observed feeding behaviour in the cage trials. However, if these were the only

contributing factors, a set of results would have been obtained that showed a mean sub-optimal foraging capability for each population of fish, with a near normal distribution of foraging capabilities above and below this mean. This was not the case. Results from all of the trials suggested that there was a separation in foraging behaviours between large and small members of the population. Therefore, other factors besides natural, random variation in environment and the fish themselves must have been operating.

Juvenile Atlantic salmon are naturally aggressive and territorial (Keenleyside and Yamamoto 1961). This behaviour can lead to the development of social hierarchies within a population, and a disproportionate allocation of food resources can result (Symons 1971). Ringler (1983) suggested that larger or more dominant fish could take an increasingly disproportionate allocation of food and thus grow at a faster rate than subordinates. Further, if the dominant members of a population select the most profitable prey types and thereby reduce the abundance of this prey, subordinates may be forced to adopt a feeding strategy that could be considered optimal for what they have to choose from. This has also been discussed by Dill (1983) who suggested that foraging fish must adopt a strategy of continual decision making. The ultimate goal of these decisions is to forage optimally on the available feed. Metcalfe (1986) reported experiments that suggested the

inherent social behaviour of rainbow trout reduces the potential to predict foraging behaviour from optimal foraging theory. The social hierarchies created result in differential allocation of resources according to status. which in turn produced great variability in the growth and fitness of different members of the population. Metcalfe (1955) suggested that dominant and subordinate subpopulations may adopt different optimal feeding strategies. Dominants take the classic position of maximizing intake with minimal cost. Subordinates, depending on the exact conditions and choices they are left with, may adopt a strategy based on minimising energy expenditure so that a positive difference between energy intake and expenditure can be maintained. This strategy, of course, would not provide the energy surplus for growth that the dominant strategy would. The findings of these authors suggest that all fish in all populations of this study foraged nearoptimally, given the constraints of their intraspecific competition. Dominant fish foraged on the artificial diet in accordance with optimal foraging theory. Rather than waste energy competing with the dominants for the artificial feed remaining, subordinate fish chose instead to forage on the very abundant zooplankton. It is suggested that the subordinate fish switched from the natural to artificial food sources at the point when an individual zooplankton no longer provided a suitable amount of energy. The extra energy gained by obtaining an artificial feed particle was worth the energy spent in obtaining it.

One criticism of the application of optimal foraging theory to fish feeding behaviour studies is that it assumes the predator has perfect knowledge of the characteristics of the available prey at any given moment (Townsend and Winfield 1985). This assumption is usually not valid in the natural environment. It would be more reasonable to expect a fish to learn which prey is the most profitable through a series of decisions (Dill 1983). Ware (1971) showed that rainbow trout develop search images for specific prey, and suggested that the development of these images is a process of learning what prey is most profitable and how to recognise this prey. Marcotte and Browman (1986) suggested that foraging behaviour in juvenile fish developed in accordance with the development of perception. cognition, and neuroethology. That is, feeding behaviour becomes more precise and near-optimal as the animal's learning capabilitles expand.

The inclusion of learning in the optimal feeding process could explain many of the sub-optimal feeding behaviours observed in this study. In the preliminary trial, the first feeding alevins fed only on instinct. Marcotte and Browman (1986) referred to this as following their "hard wiring". The zooplankton provided many stimuli to initiate this feeding behaviour. Therefore, although it was the theoretical sub-optimal food choice, zooplankton was more important in the diets than the artificial feed. As the alevins developed physiologically and gained experience

trying different potential-prey, they learned that the optimal food choice was the artificial diets. As discussed earlier, more dominant members of the population probably made this diet switch sooner than the subordinates (who may have recognised the artificial diets as the optimum, but could not compete without expending considerable amounts of energy). In the second year's trials, fish moved to the cages most certainly were ingrained with a search image (Ware 1971) of an artificial feed article as the optimal prey type. On transfer, if the zooplankton were of a similar size as the artificial food, subordinate members of the population may have chosen to switch to this food type. Most members of the population probably continued to feed on the prey type they recognised as the optimal. Those who switched to zooplankton soon learned that the artificial diet was the better food source. For these reasons, zooplankton was never utilised for long after transfer (if at all) at sites 3, 4, and 5.

3.5 CONCLUSIONS

- 1. Freshwater based cage systems can be successfully utilised to first feed and rear small Atlantic salmon, as an alternative to traditional tank systems. The success of such operations depends largely upon the water quality conditions within the cages, which in turn are related to the physio-chemical characteristics of the cage site. The effects of these factors can be controlled by a strict husbandry programme of careful and frequent net changes. It is also advisable to undertake a thorough sampling programme at a prospective site to evaluate its trophic status.
- 2. Generally, Atlantic salmon reared in freshwater cages will only utilise the natural food source in a limited period after first feeding. A shift to artificial diets can be expected, primarily because this food source offers a continual supply of suitably sized high energy particles.
- 3. Although the use of natural feeds is markedly reduced after first feeding, a proportion of the stock will continue to utilise this food source until it becomes unsuitable with respect to particle size. These fish can be considered as subordinates, as they are unable to compete successfully against the dominants for the

optimal artificial feed. Upon switching, the subordinate fish generally will not feed as effectively as the dominants.

- 4. Differential feeding may result in a differential growth of the cage stock. From this study it was impossible to separate the effects of this from that of inherent S1/S2 differences. Differential feeding may exacerbate the S1/S2 split.
- 5. Much of the differential feeding could be controlled by adequate distribution of the artificial diet to all areas of the cage. This would provide all fish with a near equal access to the optimal food source, thereby reducing competition. Conversely, a continuous rather than burst supply of feed may provide fish which were critically unable to compete an opportunity to feed once those more competitive are satiated.
- 6. The degree of utilisation of natural feed in Atlantic salmon moved to freshwater cages after first feeding depends largely upon the preferred particle size of the fish in relation to the size of individual crustacean zooplankton. As the former equals and becomes greater than the latter natural feed utilisation is markedly reduced. The preferred particle size is easily calculated as a percentage of the fish fork

length.

- 7. From this study it was impossible to determine the effects of varying utilisation of natural feed (in relation to time of transfer) on growth of the fish. However, the relationship has significance to the success of an operation, particularly in controlling infection by parasites transmitted <u>via</u> the crustacean zooplankton.
- 1.8. The abundance and species composition can affect the utilisation of natural feed by caged salmon, no matter when they are transferred from the hatchery. Clado-ceran zooplankton types are ingested more readily than copepods. A high abundance of zooplankton will reduce the relative abundance of the artificial feed, and thereby increase the chance of zooplankton ingestion. It is suggested that a thorough sampling of the zooplankton community would be useful in predicting the feeding behaviour of caged fish. This study also showed that cage rafts attract large numbers of zooplankton, particularly cladocerans; this factor must therefore be considered.

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