Factors affecting feed intake, aggression, growth and condition around transfer to sea in Atlantic salmon

(*Salmo salar*)

by

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DECLARATION

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis. To the best of my knowledge and belief this thesis contains no material previously published or written by another person except where due acknowledgement is made in the text.

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"It's the way scientists write up a research report or paper: we follow different avenues of inquiry, going down blind alleys, hitting a fast lane or taking a shortcut, zigzagging along as we probe an interesting observation or phenomenon. Then, when it's time to "write it up," we shuffle through the experiments, tossing some out and organizing the remainder into an order that creates the illusion that a direct path was taken from the initial question to the final results."

David Suzuki (The Autobiography)

Abstract

Atlantic salmon is an anadromous species undergoing smoltification to adapt to the seawater environment. In aquaculture despite being fully smolted when transferred from freshwater hatcheries to sea cages, feed intake is suppressed, sometimes for long periods of time. Farms try to ensure salmon recommence feeding rapidly to reduce loss of condition which may lead to pinheading. The current research aimed to better define the term pinheading and examine factors effecting feed intake, aggression, growth performance, condition and nutritional status of fish around seawater transfer.

Pinheads and non-pinheads from a commercial farm were compared. All pinheads had condition factors ≤ 0.865. Pinheads had significantly ($p<0.05$) lower body weight, fork length, condition factor, visceral fat ratios, dry matter, total lipid and gross energy and significantly higher ash than non-pinheads, all indicating decreased condition. There was no significant difference in crude protein, osmolality, or digestive ability (based on trypsin activity).

Feeding frequency following seawater transfer was examined in fish fed eight meals per day before transfer. One meal per day following transfer resulted in a greater initial decrease in feed intake than four or eight and significantly increased feeding hierarchy strength (not significant for four or eight meals) before it decreased again to pre-transfer strength within three weeks of transfer. No differences between meal frequency treatments were observed in growth, condition or chemical composition. In a second experiment changing feeding frequency, whether increasing or decreasing, concurrently with seawater transfer had little effect on post-transfer feed intake. Following seawater transfer one meal per day fish had lower initial feed intake than eight meal fish regardless of pre-transfer frequency.

Feeding and dominance hierarchies were examined immediately before and following seawater transfer. Feeding ranks were stable (Kendalls coefficient of concordance) in both freshwater and seawater but no correlation existed between mean freshwater and seawater hierarchies. Dominance ranks were also stable in freshwater but not seawater

and mean dominance hierarchies in freshwater and seawater did not correlate. Findings suggest seawater hierarchies can not be predicted from freshwater hierarchies.

A period of feed-deprivation for the first fourteen days following seawater transfer resulted in feed-deprived fish being significantly out-competed by non-deprived fish in terms of feed intake, growth and final condition. Although not significant, aggression also tended to be lower for feed-deprived fish. Further findings suggest that at higher densities the advantage to non-deprived fish may be diminished.

Experiments show advantages of high feeding regimes immediately before and following seawater transfer and the difficulties in predicting performance of individuals in seawater based on their freshwater performance. They also suggests that fish that wait too long to recommence feeding may be at some disadvantage to those recommence sooner.

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This thesis is dedicated to my brother Tim… Now the adventure begins!

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List of abbreviations

ABARE	Australian Bureau of Agriculture and Resource Economics
AC	Absolute daily consumption
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BAPNA	a-N-benzoyl-DL-arginine-p-nitroanilide
BW	Body weight
°C	Degrees Celsius
CV	Coefficient of variation
d	day(s)
DI	Dominance index
DPIW	Department of Primary Industries and Water
FAA	Feed Anticipatory Activity
FAO	Food and Agriculture Organization of the United Nations
FCR	Feed conversion ratio
FW	Freshwater
g	gram(s)
GSI	Gonadal somatic index
h	hour(s)
HAC	Huon Aquaculture Company
HOGG	Head On Gilled and Gutted
HSI	Hepatosomatic index
K	Condition factor
Kg	Kilogram(s)
L	Litre(s)
max	maximum
MDI	Mean dominance index
mg	milligram(s)
min	minimum
min	minute(s)
ml	millilitre(s)
MSM	Mean share of the meal

nm	nanometres
ns	not significant
p	probability
PCSI	Pyloric somatic index
pNA	p-nitroaniline
SM	Share of the meal
SD	Standard Deviation
SEM	Standard error of the mean
SGR	Specific growth rate
Sp.	Species
SW	Seawater
µl	Microlitre(s)
µmol	Micromole(s)
U.V.	Ultraviolet
W	Weight
WW	Wet weight
1F	One meal per day
4F	Four meals per day
8F	Eight meals per day

Chapter 1

General Introduction

Matthew Flood

This research focuses on factors affecting feed intake, aggression, growth and condition of Atlantic salmon *Salmo salar* leading up to and following transfer from freshwater to seawater. In aquaculture seawater transfer of salmon occurs at the completion of smoltification when the fish are physiologically adapted for increased salinity (Blackburn and Clarke, 1987, McCormick, 1993). Despite physiological readiness seawater transfer can have a number of effects including alteration of feed intake (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996) and behaviour of the fish (Halls, 1994). It is well documented that correct management practices in the initial time following seawater transfer are critical for ensuring the survival and growth of these fish (Stead et al., 1996; Hjertenes, 1999), with poor animal husbandry believed to contribute to poor growth performance and losses in body condition (Johnstone, 1991; King, 1992a). The current thesis presents a comprehensive description of a wasting condition known as pinheading which occurs most commonly soon after seawater transfer in the Tasmanian Atlantic salmon aquaculture industry (King, 1992b,c). Feeding frequencies following seawater transfer and changes in feeding frequency simultaneously with seawater transfer were examined to determine their effects on feed intake, feeding hierarchies, growth and condition to try and determine whether specific regimes and changes in regimes affect competition which may lead to pinheading. The structure of dominance and feeding hierarchies were also examined to determine if and how seawater transfer affects these and whether there were any characteristics of individual salmon in freshwater that may indicate a high likelihood of these individuals becoming pinheads once transferred. Finally the effects of feed-deprivation in the weeks immediately following seawater transfer were examined to try and determine whether individual salmon delaying their recommencement of feeding in seawater increase their likelihood of being out-competed by individuals that commenced feeding sooner, and whether such delays may contribute to pinheading.

The following sections aim to put the current study into a wider context, firstly in terms of world and Australian aquaculture. As the thesis focused on Atlantic salmon undergoing seawater transfer the process of smoltification is briefly reviewed along with the documented effects of seawater transfer on feed intake and behaviour. A brief description of pinheading is presented along with an indication of how this condition has impacted Tasmanian aquaculture over recent years to highlight why this issue

warrants investigation. The effects of feeding regimes and how feeding frequency may effect the growth and condition of salmon in the early stages of seawater residence is then presented. Finally the effects of changing environmental conditions on feeding and aggression hierarchies are briefly investigated.

1.1 Aquaculture production of Atlantic salmon

Global production of all aquatic species from both capture fisheries and aquaculture in 2002 amounted to 133 million tonnes, aquaculture accounting for 39.8 million of this (FAO, 2004) with approximately 45000 tonnes from Australia (ABARE, 2003). World aquaculture production more than doubled in the 15 years up till 2000 largely in response to a decline in wild fisheries (Naylor et al., 2000). In 2002 the world aquaculture production of finfish specifically was 26 million tonnes with diadromous fish accounting for 2.59 million tonnes at a value of $6465 million US (FAO, 2004). Atlantic salmon are a native fish to the north Atlantic (McDowall and Tilzey, 1980) with the majority of the world's aquaculture production occurring in Norway, Chile and the United Kingdom (FAO, 2002). While total production in Australia is much lower than these countries it is still one of the five main species farmed nationally (ABARE, 2003) with a gross value of production of $113 million AUS in the year 2003/2004 (O'Sullivan et al., 2006). All Atlantic salmon currently in Australia are decedents of the River Philip population in Nova Scotia, Canada introduced to New South Wales from 1963-65. Since the initial introduction of fertilised eggs from Gaden Hatchery in New Sough Wales into Tasmania from 1984-86 (Ward and Grewe, 1993) the production of Atlantic salmon has increased rapidly from below 100 tonnes in 1986/1987 (Kailola et al., 1993) to 14940 tonnes in 2003/2004 (O'Sullivan et al., 2006). During 2001 the world wide production of Atlantic salmon was 880000 tonnes (FAO, 2002). The Tasmanian salmon farming industry is now comprised of nine companies (DPIW, 2006). Approximately 90% of the salmon produced in Australia is sold domestically with the remaining 10% exported to South East Asia, and much of this going to Japan (2001/2002 figures) (DPIW, 2006).

1.2 Smoltification of salmon

Smoltification, or the parr-smolt transformation, is the process by which anadromous salmon become competent for the migration from freshwater to seawater and involves several physiological, morphological and behavioural changes most of which occur in freshwater (Folmar and Dickhoff, 1980; McCormick and Saunders, 1987; Hoar, 1988). Physiologically smolts increase their hypo-osmoregulatory capacity (McCormick and Saunders, 1987; Björnsson et al., 1989; Sigholt et al., 1995) by changing the levels of circulating hormones which in turn influence osmoregulatory mechanisms (Willoughby, 1999a). Na^+-K^+ ATPase activity increases during this process (Saunders and Henderson, 1978; Boeuf et al., 1985) allowing fish to regulate their water and salt balance following introduction into seawater (Boeuf and Harache, 1982, 1984). Morphologically the darkly pigmented melanin bars (parr marks) on the flanks of the parr are visually obstructed by the deposition of purine crystals, specifically guanine and hypoxanthine, in the skin and scales giving the silvery body colouration of transformed smolt (Johnstone and Eales, 1967, 1968, 1970). At the same time condition factor is temporarily reduced due to utilisation of energy reserves (Pinder and Eales, 1969; Sheridan, 1989; Nordgarden, 2002) resulting in a more slender streamlined appearance (Hoar, 1988; Stefansson and Hansen, 1996). Behaviourally, prior to smoltification parr are aggressive in order to establish and maintain feeding territories (Keenleyside and Yamamoto, 1962). At completion of smoltification salmon rise in the water column and commence down stream migration, swimming with the current (Eriksson, 1984; Lundqvist and Eriksson, 1985). At this time aggression is replaced by schooling behaviour (Skilbrei et al., 1994).

Under culture conditions Atlantic salmon possess a freshwater hatchery phase and a marine grow-out phase (Willoughby, 1999b). In Tasmania Atlantic salmon are generally transferred directly from hatcheries into full strength seawater sea cages (H. King personal communication) where they are grown until harvest some nine to fifteen months later, at around 3.5 kg HOGG (Head On Gilled and Gutted) (D. Mitchell personal communication). Two environmental factors control the smoltification process; these are temperature and photoperiod (Saunders et al., 1990; Gaignon and Quemener, 1992; Stefansson et al., 1992). Temperature has been manipulated in hatcheries to

optimise growth while photoperiod has been manipulated to produce out-of-season smolt and marine pre-smolt (Duston and Saunders, 1992; Thrush et al., 1994; Duston and Saunders, 1995; Duncan and Bromage, 1998). Out-of-season smolts extend smolt production to eight or nine months of the year and effectively extend harvests at sea farms throughout the year (King, 1994b). Whilst in the wild the decision to go to sea has a physiological basis (Folmar and Dickhoff, 1980; Hoar, 1988) in aquaculture salmon are transferred as a group. Since not all individuals in salmon cohorts go through the parr-smolt transformation at exactly the same time it is likely that individuals will acclimate to increased salinity at slightly different rates (Stead et al., 1996).

1.3 Feed intake following transfer to sea

Following seawater transfer salmon have to deal with physiological changes associated with increased salinity (Usher et al., 1991), the stress imposed by transport from hatchery to sea cages (Willoughby, 1999b) and the environmental differences between the controlled freshwater hatcheries and the more variable sea cages (Bergout Anras et al., 2001) including differences in weather, light intensities, water depth, shape and structure of holding facilities and water velocity (Kestemont and Baras, 2001). As a result of seawater transfer feed intake of Atlantic salmon can be severely reduced for several weeks before returning to levels comparable with those in freshwater (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996). The re-establishment of feeding as soon as possible following transfer is critical for survival and growth of these fish (Hjertenes, 1999) with farms aiming to avoid situations where some fish end up losing condition, in extreme cases becoming pinheads (H. King personal communication). One of the documented problems with feeding strictly according to the guidelines of growth charts (Austreng et al., 1987; Cho, 1992) is that doing so has been found to result in suboptimal growth (Juell et al., 1993, 1994) with day to day variations resulting in overfeeding on some days and underfeeding on others (Jobling et al., 1995; Juell, 1995). Given that commercial salmon feeds can account for more than 60% of the operating costs of salmon production (Heen, 1993) efficient use of feed is economically very important to industry (Knights, 1985; Goddard, 1996). Despite this and given the day to day variations mentioned above, over-feeding by about 15 to 30% during the time following transfer to sea has been found to increase growth and health status of

fish with the economic benefits of successfully getting smolts feeding again outweighing any financial loss due to excess feeding (Hjertenes, 1999). The benefits gained by over-feeding most likely result from the fact that unrestricted feeding has been demonstrated to decrease competition for feed leading to increased homogeneity in feed acquisition and growth (McCarthy et al., 1992; Grant, 1993; Jobling, 1995).

1.4 Behaviour following transfer to sea

Immediately following seawater transfer the behaviour of salmon can differ from that generally witnessed after fish have settled into the seawater environment. Once settled in sea cages salmon usually display schooling behaviour continuously swimming in circles (Phillips, 1985; Juell and Westberg, 1993) with increasing densities shown to increase schooling behaviour (Juell and Westberg, 1993). The vertical distribution of caged salmonids is influenced by daily and seasonal variations in natural light intensity and the temperature stratification of the water column with fish generally swimming deeper in the cage during the day than at night (Sutterlin and Stevens, 1992; Huse and Holm, 1993; Fernö et al., 1995; Oppedal et al., 2001) and preferring deeper water in summer than winter (Huse and Holm, 1993; Fernö et al., 1995). During winter individuals also tend to be more evenly spread out vertically while more localized to specific depths in summer (Fernö et al., 1995). Feeding also affects vertical distribution with salmon swimming rapidly to the surface and gradually descending again as they reach satiation (Juell et al., 1994; Fernö et al., 1995). Despite these general trends, when Atlantic salmon are first transferred to sea they tend to dive deep into the sea cages, burrowing into the cage walls (Halls, 1994). This response is believed to be a result of the increase in light intensity for fish previously accustomed to lower light levels in hatchery tanks (Halls, 1994; Thrush et al., 1994). It is also believed to be one of the contributing factors to the decrease in feed intake following seawater transfer (Halls, 1994) and possibly contributes to some individuals losing condition following transfer (King, 1994a).

Rearing conditions such as high density and high feed availability in sea cages generally reduce aggression and hierarchy formation (Kjaftanson et al., 1988), and promote uniform growth (Huntingford and Thorpe, 1992). Reduced aggression is most likely due

to the high fish numbers and densities increasing the costs associated with dominance making it harder for dominant individuals to assert themselves (Huntingford and Thorpe, 1992; Grant, 1993; Sloman and Armstrong, 2002). As mentioned above the seawater migration of wild salmon is also generally associated with a decrease in aggression and increased schooling behaviour (Skilbrei et al., 1994) which would also reduce hierarchy formation. Despite the general lack of hierarchy formation dominance/submissive interactions have still been witnessed with individuals not performing well in seawater being crowded out of feeding areas and pushed into other parts of sea cages (Johnstone, 1991). It is possible that such behaviours may be indicative of restrictions in the environment given that fish in cage environments cannot move away from competitors. Doves for instance, while normally a non-aggressive species have been observed to fight to the death when spatially confined with other doves (Lorenz, 1952, 1966). In the wild it may be that not all salmon school however in sea cages non-schooling individuals will still have to compete with other individuals even though this competition may be outside actual hierarchical structures. The fact that competitive interactions are witnessed in sea cages demonstrates that even in rearing conditions known to reduce hierarchies aggression can still be an issue. It also highlights the need to feed in ways that reduce competition which may result in individuals being out-competed for feed resources by others.

1.5 Pinheading as an end point of unsuccessful seawater transfer

As previously mentioned there is considerable effort made by farms to re-establish feeding quickly following seawater transfer (Hjertenes, 1999) and it is possible individuals that delay recommencement of feeding may be out-competed when they do try and eat causing a loss of condition; in extreme cases this may contribute to the production of pinheads (King, 1992a). In Tasmania the term "pinheading" is used to describe Atlantic salmon that fail to grow in length while a considerable reduction in body weight occurs (King, 1992a) (See Plate 1.1). The problem occurs in both fresh and seawater but is a larger issue for industry in the seawater phase (H. King personal communication). The percentage of fish that pinhead in sea cages varies from year to year, averaging approximately 1.5% from 2001 to 2006 (D. Mitchell personal communication; N. Murfet personal communication) with individual sea cages as low as

0.001% and up to 12% (D. Mitchell personal communication). Presently freshwater hatcheries charge sea farm operations around $2 per fish for Atlantic salmon smolt at the time of transfer from hatcheries to sea cages. Approximately 6.25 million smolt are transferred from hatcheries to sea cages each year. At a rate of 1% pinheading the direct loss to industry is around $125,000 a year. By harvest time (~3.5 kg HOGG) at around $10 per kilogram this equates to $21.9 million potential loss of turnover which assuming a $1 per kilogram profit would equate to $2.2 million potential profit loss (D. Mitchell personal communication). A detailed description of the biology of pinheading is presented in Chapter 2.
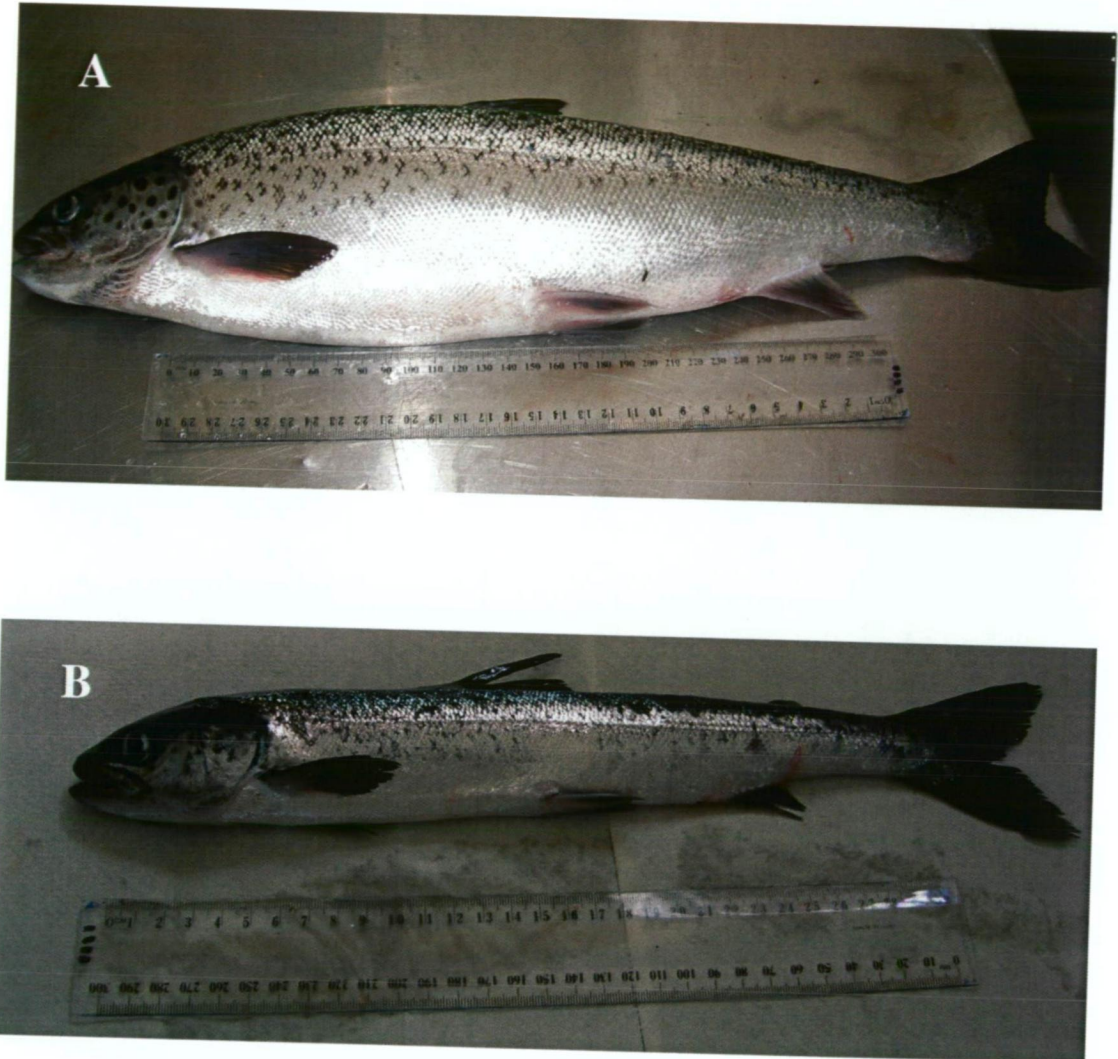
Plate 1.1 (A) Non-pinhead (B) Pinhead, with 30 cm rulers to provide scale.

1.6 The effects of feeding regimes

There are a number of factors that have been demonstrated to affect feed intake and feeding hierarchy formation. For example scattering of feed over a wide area rather than localised delivery reduces aggression by making feed less defensible (Huntingford and Thorpe, 1992; Grand and Grant, 1994; Ryer and Olla, 1995, 1996; McCarthy et al., 1999); increasing rations increases feed intake and reduces hierarchy formation by reducing competition for a limited resource (McCarthy et al., 1992; Moutou et al., 1998); while increasing the rate of feed delivery reduces aggression again making feed less defensible (Grant and Cramer, 1992; Hakoyama and Iguchi, 1997). Feeding frequency has also been documented to affect feed intake, growth, feed conversion efficiencies and the formation of hierarchies (Grayton and Beamish, 1977; Chua and Teng, 1978; Greenland and Gill, 1979; Jobling, 1983; Dwyer et al., 2002), with high feeding frequencies fed at high rations shown to reduce hierarchy formation (Jobling, 1983). In hatcheries feeding frequencies are generally quite high with fish fed as often as every 20 minutes (H. King personal communication). It is generally recommended that following seawater transfer Atlantic salmon be fed 5 to 6 times a day to help get them back onto feed as fast as possible (Hjertenes, 1999). Feeding only one or a few meals per day in the time following seawater transfer may contribute to the pinheading problem (H. King personal communication). It is also possible that changing the feeding frequency at seawater transfer may compound the already reduced initial feed intake of Atlantic salmon. Some changes in diet have been found to reduce feed intake (Toften and Jobling, 1997; Wybourne, 1997) and it is possible that changing feeding regime simultaneously with seawater transfer may have a similar effect especially given that fish often entrain to specific feeding times (Sanchez-Vazquez and Madrid, 2001).

1.7 Feeding and social dominance hierarchies

Changes in the environmental conditions in which fish are reared can affect both dominance and feeding hierarchies. Environmental changes can alter aggression levels and the strength and stability of dominance hierarchies, with stability generally decreased following change (McNicol and Noakes, 1984; Sloman et al., 2001; Snedden et al., 2006). The final structure of dominance hierarchies following environmental changes can also differ from the pre-change structure (Sloman et al., 2001; Sneddon et

al., 2006). In a similar way to that documented above regarding dominance hierarchies feeding hierarchies have also been shown to vary in strength and stability with dietary alterations (Toften and Jobling, 1997; Wybourne, 1997). It is possible that seawater transfer may also affect both feeding and dominance hierarchies. Pinheads tend to be recruited equally across the entire size range of groups transferred to sea (King, 1993) and given that larger fish are often dominant (Huntingford et al., 1990) they may also be recruited equally from all sections of the dominance and feeding hierarchies. If this is the case then predictions of which fish will perform poorly in seawater based on freshwater performance may not be possible. Previous research has already demonstrated that growth rates of individual Atlantic salmon held in groups in freshwater do not correlate with their growth rates following seawater transfer (Stead et al., 1996).

1.8 Aims of this study

The current study focused on the transfer of Atlantic salmon from freshwater to seawater and its effects on feed intake, aggression, growth, condition and nutritional status. The main objectives were to clearly define the pinheading condition, gain a better understanding of how feeding regimes can affect performance around seawater transfer and increase understanding of social processes that may contribute to the problem of pinheading. All experiments were carried out in small experimental systems allowing a large degree of environmental control while offering conditions known to intensify the effects of competition (Huntingford and Thorpe, 1992; Sloman and Armstrong, 2002). While the results from experiments using these systems may not be directly transferable to commercial settings, they allowed more intricate examination of hierarchies and the effects of hierarchies than could be measured in large commercial sea cages. The aims of the current study were as follows:

- To better define and characterise the pinheading condition in terms of morphological characteristics, osmoregulatory capability, whole body chemical composition and digestive capacity and to determine whether pinheads ingest feed in the seawater environment (Chapter 2, 3, 5 and 6).

- To determine the effects of feeding frequency immediately following seawater transfer on feed intake, feeding hierarchy formation, growth, condition and chemical composition. The hypothesis being that low feeding frequencies in seawater following previously high feeding frequencies in freshwater will result in lower performance than if feeding frequencies were kept higher. It was also hypothesised that low feeding frequency following seawater transfer contributes to the production of pinheads (Chapter 3).

- To determine the effect of changing feeding frequency at the time of seawater transfer on feed intake, growth, condition and chemical composition. The hypothesis being that changing feeding frequency, regardless of whether it is increasing or decreasing, will cause a decrease in feed intake and performance (Chapter 4).

- To determine the effect of seawater transfer on the stability and structure of dominance and feeding hierarchies, specifically examining whether hierarchies are stable in freshwater and seawater, whether hierarchies are broken down as a result of seawater transfer and whether the structure of post-transfer hierarchies differ from those of pre-transfer hierarchies. The hypothesis being that hierarchies change as a result of seawater transfer and thus performance in seawater will not be predictable based on performance in freshwater (Chapter 5).

- To determine the effect of a period of 14 days feed-deprivation immediately following seawater transfer on the competitive ability of feed-deprived individuals in competition with non-deprived individuals. Competitive ability being assessed in terms of feed intake, aggression, growth, condition and chemical composition. All measurements to be made following the first 14 days in seawater after fed and feed-deprived individuals are combined. The hypothesis being that delaying the recommencement of feeding following seawater transfer will decrease the competitive ability of feed-deprived fish and cause pinheading (Chapter 6).

1.9 Notes on this study

Each experimental chapter (Chapters 2-6) in this thesis has been prepared in the format of a manuscript for publication in a peer reviewed journal. For this reason there is some repetition of text especially in the materials and methods sections and reference lists. Experiments for chapters 5 and 6 were run simultaneously in separate sections of the same experimental system. There are a number of places throughout the thesis with information and data from personal communication with researchers and members of the aquaculture industry. Details for each individual at the time of communication are as follows:

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Chapter 2

Defining the Atlantic salmon pinhead

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Matthew Flood

2.1 Abstract

Pinheading is a term generally used to describe a loss of condition experienced by some Atlantic salmon (*Salmo salar*) after smoltification following transfer from freshwater hatcheries into seawater pens. Pinheads and non-pinheads were compared in order to better define this condition. Emphasis was placed on differences in morphology, osmoregulatory ability, whole body chemical composition, and digestive capacity. Pinheads were allocated by industry personnel to ensure relevant classification. Pinheads and non-pinheads were taken from one single cage population sampled at a single time point and a *t*-test used to compare the two groups. Pinheads had significantly ($p < 0.05$) lower body weight, fork length and condition factor than non-pinheads. The mean condition factor of pinheads was 0.74 with a maximum of 0.865. The low condition of pinheads was also evident in significantly lower visceral fat ratios. Pinheads had significantly lower proportions of dry matter, crude lipid and gross energy and significantly higher proportions of ash than non-pinheads. No significant difference was found between proportions of protein. Pinheads were found to have osmolalities comparable with non-pinheads. Pinheads had significantly higher hepatosomatic index (HSI) and gonadal somatic index (GSI) values than non-pinheads despite pinheads being emaciated. Furthermore, when fork length was used to represent fish size rather than body weight the ratios of fork length to liver weight and gonad weight showed the reverse of trends from HSI and GSI and these trends were still significant. No significant difference was found between pylorus weights as a percentage of total weight for pinheads and non-pinheads and there was also no significant difference found in activity of the protease enzyme trypsin between the two groups. It is proposed that pinheads are a fish with much poorer condition than other individuals within their cohort exemplified by low condition factor and reduced energy reserves. It is further proposed that these individuals do not have impaired osmoregulatory function and also may be capable of digesting feed if eaten.

Keywords: Atlantic salmon, Chemical composition, Condition, Digestive capacity, Failed smolt, Morphology, Osmoregulatory ability, Pinheading, Seawater, Smoltification, Transfer, Trypsin

2.2 Introduction

The term "pinheading" is in common use within the Tasmanian salmon aquaculture industry. It is generally used to describe Atlantic salmon (*Salmo salar*) that once transferred from freshwater hatcheries to seawater pens fail to grow in length while a considerable reduction in body weight occurs (King, 1992c). The term is also used to describe salmon in freshwater that stop eating and become emaciated (H. King personal communication) but appears to be used most often to refer to fish in seawater, probably due to their higher value in production and the greater effort put into reducing pinheading at this stage. Whilst pinheading is a common industry term, to our knowledge only Crane et al. (2000) mention it but do not describe the condition in any great detail. A similar condition in the Northern Hemisphere was described as "failed smolt syndrome" (Stradmeyer, 1994; Johnstone et al., 1999). This syndrome has been defined as "fish that had not fed since transfer to seawater... characterised by their thin appearance and general poor condition" (Stradmeyer, 1994). A number of other terms have been used to describe failed smolts including: non-starters (Stradmeyer, 1991), stunts (Björnsson et al., 1988; Varnavsky, 1992; Allen, 1998; Sheridan et al., 1998;) and culls (Allen, 1998). High levels of plasma growth hormone have been reported in coho (Folmar et al., 1982; Varnavsky et al., 1992) and Atlantic salmon stunts (Björnsson et al., 1988), suggesting the problem may be partly physiological (Johnstone et al., 1999).

Stradmeyer (1994) found that mortalities attributable to failed smolt syndrome occurred 7 to 9 weeks post-transfer to sea, while Johnstone et al. (1999) found still living failed smolt only began to appear at the surface of cages, as emaciated fish, 8 to 12 weeks after transfer (Johnstone et al., 1999). These fish did not die acutely but slowly as a result of emaciation over time (Johnstone et al., 1999). In Australia pinhead mortalities tend to start 5 to 8 weeks post-transfer and rise to a peak 12 weeks after transfer (King, 1992b). Triploid stocks have a higher incidence of pinheading than diploid stocks (King, 1993a) and in Scotland out-of-season smolts have produced higher numbers of failed smolt than spring smolts have (Johnstone et al., 1999). King (1993b) found that pinheads that died in the first two months post-transfer did not appear to have undergone any growth in length while those dying after two months were longer than fish at time of transfer. The main difference between pinheads and failed smolts is that

pinheading can be used to describe fish that have grown for a period of time after transfer before losing condition (resulting in a much longer fish), as well as the more common case where smolts do not grow once transferred (P. Lee personal communication). This difference in definition is supported by King (1993b) who stated that in contrast with conclusions made by Johnstone (1991) and Stradmeyer (1994) suggesting that failed smolt don't feed after transfer, a proportion of pinheads do in fact grow in sea pens indicating that they must commence feeding. In fact even the notion that failed smolts do not eat has been disputed as some failed smolt have been found with food in their guts (M. Porter personal communication), and in some cases they have been seen eating (Johnstone,1991; D. Mitchell personal communication).

To date there have been no papers published specifically defining pinheading or investigating possible causes. Despite this, on-farm trials and reports from both Australia and Scotland have ruled out some factors as contributors to either pinheading or failed smolt syndrome. In Scotland, Johnstone (1991) found no evidence of any disease resulting in failed smolt, no evidence of an inability to osmoregulate and no increase in failed smolt numbers with transfer to sea outside the smolt window (Johnstone et al., 1999). Also, the condition does not seem to be related to size of the fish at time of transfer (Johnstone et al., 1999). The findings were the same for Tasmanian pinheads (King, 1992a, 1993b). While it seems that an inability to osmoregulate is not the cause of pinheading, osmoregulatory failure may be the final cause of death (Johnstone, 1991; King, 1992a). Transfer to brackish water has been found to reduce post-transfer mortality but the proportion of mortalities attributable to pinheading remains constant (King, 1992b).

Other possible factors have not yet been fully examined. The effect of social interactions and formation of hierarchies is a possible causative factor since Johnstone (1991) observed that failed smolts would take food when offered but were soon out-competed by other fish and moved away from the feeding area. This observation has also been made independently by other members of the Scottish salmon aquaculture industry (D. Mitchell personal communication). Another factor still to be fully examined is that of husbandry practices such as handling and feeding after transfer. The possibility that husbandry plays a role in pinheading is supported by findings that

pinheading seems to be site specific rather than stock specific (King, 1992a). It has been speculated that fish may be traumatised during the process of transportation and transfer to seawater leading to decreases in feeding which may then be compounded by aggressive performance of successful fish (King, 1992a). There is also the possibility that fish may have been overfed prior to transfer (King, 1992a). It is known that during smoltification lipid levels are normally depleted (Pinder and Eales, 1969; Sheridan, 1989; Nordgarden, 2002), and fish exhibit a temporary decrease in condition factor as part of the parr-smolt transformation (Hoar, 1988; Stefansson and Hansen, 1996). It is possible that overfeeding may inhibit this depletion thus stopping the fish from fine-tuning the smoltification process (Johnstone, 1991; King, 1992a; Sheridan, 1989), especially given that increased adiposity had been found to reduce growth of salmon following seawater transfer (Jobling et al., 2002a,b)

Data collected through direct sampling in the present study has focused on the seawater phase for Tasmanian Atlantic salmon that developed into pinheads 9.5 months after transfer to sea cages. It is possible that pinheads occurring soon after seawater transfer may differ somewhat in characteristics from those sampled in the present study. The main aim of the current study was to better define the pinheading condition in Tasmanian grown Atlantic salmon. A number of factors were compared to determine how pinheads and non-pinheads differ including morphometric characteristics, most importantly condition factor but also other measures commonly used to estimate condition such as visceral fat ratio (Jarboe and Grant, 1996; Cho and Jo, 2002) and hepatosomatic index (HSI) (Storebakken et al., 1991; Liu and Liao, 1999). Differences in whole body chemical composition were also determined as another measure of condition. Despite previous research showing no differences in osmoregulatory ability, osmolalities were compared to determine osmocompetence. It is possible that part of the problem for pinheads may be reduced intestinal nutrient transport (Collie, 1985; King, 1992c). However, whilst attempts have been made to determine digestive capability of pinheads, insufficient faeces was collected from these fish (King, 1992c). The present study aimed to measure the activity of trypsin as an indicator of digestive function in Atlantic salmon (Preiser et al., 1975; Pringle et al., 1992).

2.3 Materials and methods

2.3.1 Atlantic salmon

Atlantic salmon were sampled from Huon Aquaculture Company located on the Huon Estuary in Tasmania Australia. Sampling occurred on 13 February 2004 from Pen 0308B holding all-female out-of-season smolts transferred to sea on 1 May 2003. The average weight at the time of transfer was 121 g with fish growing to an average weight of 1.37 kg by the last weight check prior to sampling (farm estimate taken when freshwater bathing). From seawater introduction until the salmon reached around 300 g, feeding was carried out using an adaptive Aquasmart program allowing feeding to occur through all daylight hours according to fish demand (Blyth et al., 1999). Once fish reached 300 g they were put onto a feeding regime of three meals a day, one at dawn, one around midday and one at dusk. Salmon were fed commercial extruded feed (3 mm Nutra Transfer 50/20, Skretting, Cambridge, Tasmania, Australia) immediately after transfer and followed in order by: 4 mm AH HT 50/20; then 4mm Apollo 45/17 (Skretting, Cambridge, Tasmania, Australia); 4, 6 then 8 mm 45/17 diet (Ridley Aqua Feeds, Brisbane, Queensland, Australia); 8 then 10 mm 45/25 diet (Ridley Aqua Feeds, Brisbane, Queensland, Australia).

2.3.2 On Farm sampling

Atlantic salmon were starved for approximately 24 hours prior to sampling. Fish were dip-netted out of the holding pen and placed into a large oxygenated transport bin containing seawater. Selection of pinheads and representative non-pinheads was made by farm technicians to ensure that fish were chosen according to industry practice. A photograph of each fish was taken and examined by farm staff at a later date to confirm the original classifications. After removal of fish from the analysis due to discrepancies in classification (3 fish), deformities (1 fish), and lost samples (2 fish), 8 pinheads and 8 non-pinheads were used for all further analyses.

Selected fish were transported to shore euthanased (benzocaine, 300 mg L^{-1}), weighed (nearest 0.1 g) and fork length measured (mm). Blood samples of approximately 1 ml were taken from the caudal blood vessels using heparin-treated needles and 1 ml

Tuberculin™ syringes. After needle removal samples were syringed into Eppendorf safe lock centrifuge tubes, placed on ice, and subsequently centrifuged for 3 min at 4000 g. Plasma was removed by pipette and stored in a second Eppendorf at -80°C for later analysis.

Fish were then dissected and the whole pylorus removed by cutting the digestive tract immediately posterior to the stomach and after the last caecum of the organ (allowing the inclusion of all the caeca). The pylorus was then rinsed with saline solution (0.2 μm filtered seawater at 34 ‰) to remove any remaining food or faeces. Fatty tissue not associated with the caeca was removed and the pylorus weighed. Fat was then scraped off by holding down the pylorus along the main tract with the back of a scalpel and removing the fat with another scalpel (blunt side) by pressing down (orienting second scalpel parallel to the first) and pulling away taking the fat with it. The pylorus, now free of fat was again weighed and the removed fat was returned to the carcass so that it would not be lost from later whole body chemical composition analysis. A section of the pylorus from the posterior end was then dissected to contain at least several caeca and in some cases the whole pylorus. Samples to be used for a trypsin assay, were wrapped in foil, frozen in a liquid nitrogen dry shipper (McLeese and Stevens, 1982), and transferred to -80°C for later analysis. The liver and gonads were then removed, weighed and replaced into the peritoneal cavity of whole fish. The whole fish was then transported on ice and subsequently frozen at -20°C.

2.3.3 Morphology

Morphology was compared for pinheads and non-pinheads using wet weight; fork length; condition factor (K = (weight g / length cm^3) × 100); pyloric fat ratio (weight of fat removed from the pylorus g / wet weight of the fish g × 100); pyloric somatic index (PCSI = (wet weight of the entire pylorus g with no visceral fat attached / wet weight of fish g) × 100); hepatosomatic index (HSI = (liver weight g / wet weight of fish g) × 100); gonadal somatic index (GSI = (gonad weight g / wet weight of fish g) × 100); liver weight to fork length ratio ((liver weight g / fork length mm) × 100); gonad weight to fork length ratio ((gonad weight g / fork length mm) × 100).

2.3.4 Whole body chemical analysis of fish

Sub-samples of minced fish were used to determine the chemical composition of fish whole bodies. Each complete fish was put through a mincer (Hobart, Model A200.11) three times. Hard material which had accumulated around the blades of the mincer was removed, softened by autoclaving, ground up using mortar and pestle and recombined with the rest of the mince. This was then put through the mincer two more times in order to ensure a homogeneous mixture. Two sub-samples of approximately 100 g of mince were taken for each fish and freeze dried.

Standard methods were used to measure the whole body chemical composition of each fish: dry matter (DM) was determined by freeze drying 100 g sub-samples to a constant weight followed by oven drying at 110°C overnight (~17 h) with corrections made for the small amounts of water previously lost during the autoclaving of hard matter; samples were ashed in a furnace at 550°C for 16 h (AOAC, 1995); crude protein (Kjeldahl using copper catalyst [N x 6.25]); crude lipid (Bligh and Dyer, 1959). Energy content was estimated using gross energy values for standard protein and lipid of 23.6 and 36.2 kJ g^{-1} respectively (Brafield, 1985); carbohydrate was not included (Shearer, 1994).

2.3.5 Osmolality measurements

To determine osmocompetence of fish osmolality of blood plasma was measured using a Vapro® Vapor Pressure Osmometer (Model 5520) giving measurements in Standard International (SI) units: mmol kg^{-1} (Webster, 1985). For each sample, 10 μl of plasma was used (Wescor Inc, 2000).

2.3.6 Trypsin activity

Trypsin activity was measured as previously described (Preiser et al., 1975; Pringle et al., 1992). Enzyme was extracted by homogenising 500 mg samples (where possible) of complete caecae in Tris HCl buffer using a Ystral homogeniser (D-79282 Ballrechten – Dottingen). The sample was then centrifuged (10000 g, 4°C, 20 min) to yield the enzyme extract supernatant. This method uses *a*-N-benzoyl-DL-arginine-p-nitroanilide

(BAPNA), a synthetic chromogenic trypsin-specific substrate to liberate p-nitroaniline (pNA) upon hydrolysis by trypsin (Preiser et al., 1975). Accordingly, enzyme extracted using Tris HCl buffer was combined with BAPNA reagent. This was incubated at 15°C for 10 min before the reaction was rapidly stopped using HCl. Sensitivity of this assay is increased using the Bratton-Marshall (BM) reaction (Bratton and Marshall, 1939) in which pNA turns purple in the presence of N-1-naphthylethylenediamine. Following the 10 minute incubation the Bratton Marshal reaction was then employed by adding, sodium nitrate solution, ammonium sulphamate, and N-1-naphthylethylenediamine at 3 minute intervals. The resulting purple coloured samples were read by spectrophotometer at 550 nm. The measurements of trypsin activity were expressed as total activity per g of caecal tissue (μmol pNA min^{-1}g^{-1}), total activity per pylorus (μmol pNA min^{-1} entire pylorus^{-1}) and total activity per gram of fish ((μmol pNA min^{-1} entire pylorus^{-1})/g weight fish).

The choice of incubation temperature for the enzyme and BAPNA was made for a number of reasons. Generally this decision is based on the temperature of the water the fish have been taken from and in past experiments dealing with salmon this has been 15°C (Pringle et al., 1992; Carter et al., 1994). In farm situations however the water temperature is variable. During 2003 the monthly mean water temperature at Huon Aquaculture at 5 meters depth ranged from 11.9°C to 17.3°C giving a mean for the year of 14.6°C. On our sampling day the water temperature at 5 meters was 17.4°C. The decision was made to use 15°C as this was both close to the 14.6°C (2003 mean), and allowed direct comparison with previous studies.

2.3.7 Statistical analysis

Values are reported as mean ± standard error of the mean (S.E.M.). Normality was assumed as sample sizes were too small to test for this. Homogeneity of variances were tested using an *F*-test (Underwood, 1997) and where variances were heterogeneous an adjusted degrees of freedom was used by SPSS to determine p values (Sokal and Rohlf, 1995). Data were analysed with unpaired *t*-tests using SPSS, version 11.5 (SPSS, 2002). One tailed tests were used for condition factor, length and weight as the definition of a pinhead is a fish that is lower in all of these factors. One tailed tests were also used for

trypsin data analysis as the hypothesis was that pinheads would have less trypsin activity than non-pinheads suggesting decreased digestive function. Osmalality data was also analysed with a one tailed test with the hypothesis that pinheads would have higher osmolalities if they were struggling to osmoregulate. All other analyses were made using two tailed tests. Differences were considered significant at $p < 0.05$.

2.4 Results

2.4.1 Morphology

Pinheads had significantly lower weight, fork length and K compared with non-pinheads (Table 2.1). The mean K of pinheads was 0.74 with a maximum of 0.865. PCSI (%) was not significantly different but the proportion of fat surrounding the pylorus was significantly lower in pinheads indicating a lower visceral fat ratio than non-pinheads. HSI was significantly different with liver sizes of pinheads being proportionally larger than those of non-pinheads. Similarly GSI was also found to be significantly higher in pinheads than non-pinheads. When liver and gonad weights were examined as a proportion of the fork length rather than weight the trends seen in HSI and GSI were reversed but remained significant.

2.4.2 Chemical composition

Pinheads had significantly higher proportions of ash (% wet weight) and significantly lower proportions of dry matter (% wet weight), crude lipid (% wet weight) and gross energy (kJ g^{-1} wet weight) than non-pinheads (Table 2.2). There was no significant difference in the proportion of crude protein (% wet weight) between pinheads and non-pinheads (Table 2.2).

2.4.3 Osmoregulation

There was no significant difference in osmolality between pinheads and non-pinheads (Table 2.3).

2.4.4 Trypsin activity

There was no significant difference in trypsin activity per gram of pyloric tissue (μmol pNA $min^{-1}g^{-1}$), per pylorus (μmol pNA min^{-1} pylorus $^{-1}$), or per gram of fish (μmol pNA $min^{-1}g$ of fish^{-1}) (Table 2.3). Whilst there was no significant difference in any of these cases the mean trypsin activity per pylorus was 0.325 μmol pNA min^{-1} pylorus $^{-1}$ for pinheads while it was 7 times higher for non-pinheads (2.311 μmol pNA min^{-1} pylorus $^{-}$

[1]). This finding can be explained by the size difference between pinheads and non-pinheads given that the mean size of non-pinheads was also 4 times higher than pinheads.

Table 2.1 Morphometric measurements of Atlantic salmon classified as pinheads or non-pinheads (mean ± S.E.M, n = 8 per treatment. For each parameter the mean is written above the S.E.M). Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Parameter	Unit	Category					
		Pinhead	Non-pinhead	t values	df	tails	p
Wet weight	(g)	327.9 47.4	1373.5 101.2	-9.36	14	1	< 0.001***
Length	(mm)	345.6 19.5	482.8 7.8	-6.52	14	1	< 0.001***
K	-	0.74 0.0336	1.22 0.0662	-6.43	14	1	< 0.001***
PCSI	(%)	1.82 0.15	1.78 0.13	0.19	14	2	0.852
% pyloric fat ●	(%)	0.15 0.062	2.13 0.155	-11.82	9.19	2	< 0.001***
HSI ●	(%)	1.07 0.089	0.80 0.037	2.75	9.34	2	0.022*
Liver weight x 100(%) Fork length		0.977 0.141	2.276 0.155	-6.20	14	2	< 0.001***
GSI ●	(%)	0.298 0.041	0.159 0.008	3.32	7.60	2	0.011*
Gonad weight x 100 Fork length	(%)	0.248 0.023	0.447 0.029	-5.40	14	2	< 0.001***

● Data found not to be homogeneous using an F test were tested with reduced degrees of freedom using SPSS's equal variances not assumed option.
K: condition factor; PCSI: pyloric somatic index; HSI: hepatosomatic index; GSI: gonadal somatic index (see section 2.3.3 for equations)

Table 2.2 Chemical composition (% wet weight) of Atlantic salmon classified as pinheads or non-pinheads (mean ± S.E.M, n = 8 per treatment. For each parameter the mean is written above the S.E.M). Levels of significance * p < 0.05; ** p < 0.01; *** p < 0.001.

Parameter	Category					
	Pinhead	Non-pinhead	t values	df	tails	p
Dry matter	23.04 0.83	34.73 0.58	-11.57	14	2	< 0.001***
Crude protein	17.20 0.37	18.11 0.20	-2.13	14	2	0.051
Crude lipid	2.93 0.67	14.76 0.66	-12.54	14	2	< 0.001***
Ash ●	2.99 0.20	2.11 0.06	4.21	8.11	2	0.003 **
Gross energy kJg^{-1} (wet weight)	5.12 0.30	9.62 0.23	-11.78	14	2	< 0.001***

● Data found not to be homogeneous using an F test were tested with reduced degrees of freedom using SPSS's equal variances not assumed option.

Table 2.3 Osmolalities and trypsin activity in Atlantic salmon classified as pinheads or non-pinheads (mean ± S.E.M, n = 8 per treatment. For each parameter the mean is written above the S.E.M). Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Parameter	Category					
	Pinhead	Non-pinhead	*t* values	*df*	tails	p
Osmolality						
Osmolality (mmol kg^{-1})	456.00 6.93	453.19 8.04	0.26	14	1	0.397
Trypsin activity						
μmol pNA min^{-1}g pylorus^{-1}	0.0834 0.0373	0.0783 0.0275	0.11	14	1	0.46
μmol pNA min^{-1} pylorus^{-1} ●	0.325 0.060	2.311 1.070	-1.85	7.04	1	0.053
μmol pNA min^{-1}g of fish^{-1}	0.00159 0.00078	0.00162 0.00075	-0.024	14	1	0.49

● Data found not to be homogeneous using an F test were tested with reduced degrees of freedom using SPSS's equal variances not assumed option.

2.5 Discussion

This study has clearly demonstrated many key differences between pinheads and non-pinheads and allows a comprehensive definition of pinheading. Using data collected through direct sampling and the incorporation of unpublished industry information, from internal reports and direct communication with industry personnel, the study presents an overview of the main characteristics of the condition as well as outlining its possible contributing factors. In definition our data supports the description that pinheads are fish that have stopped growing in length while a considerable reduction in body weight has occurred (King, 1992c). The present study has extended the definition by identifying specific morphometric and body composition characteristics which set pinheads and non-pinheads apart. Also of interest are the osmoregulatory and digestive capacity characteristics in which pinheads and non-pinheads do not differ. Morphometric characteristics that exemplify the low condition of pinheads include lower condition factor, less visceral fat, and smaller liver and gonad weight to length ratios than non-pinheads. In terms of chemical composition pinheads have less crude lipid, and less gross energy again characterising their low condition. In the present study there was no evidence to suggest that pinheads were more osmotically challenged than non-pinheads which supports previous findings (Johnstone, 1991; King, 1992a). Digestive capacity, estimated from trypsin activity, was not suppressed suggesting that pinheads are likely to be capable of digesting feed (protein) if consumed.

The terms pinheading (Tasmania) and failed smolt (Northern Hemisphere) are used in some but not all cases to describe the same condition. Pinheading is a blanket term for Atlantic salmon suffering an extreme loss of condition in freshwater (H. King personal communication), soon after seawater transfer (King, 1992b), or after a considerable time in seawater (P. Lee personal communication), as was also defined by samples in the present study. It is used most commonly (and in the present study) to describe this loss of condition post-seawater transfer whereas the term failed smolt is only used in the Northern Hemisphere and describes smolts that stop growing and begin to lose condition shortly following seawater transfer (Stradmeyer 1994; Johnstone, 1999). In a hierarchy of terms, failed smolts could be considered pinheads, but only pinheads apparent soon after seawater transfer could be considered failed smolt. Data collected

through direct sampling in the present study has focused on the seawater phase for
Tasmanian Atlantic salmon that developed into pinheads 9.5 months after transfer to sea
cages. It is likely that individuals from this cohort that pinheaded soon after transfer
would have died well before this time and that the pinheads examined in the present
study probably did eat for a time following transfer. The probability that these
individuals ate at some stage is supported by the fact that the mean weight of pinheads
at the time of sampling (328 g) was more than 2.5 times greater than the mean weight of
individuals from within the cohort at the time of seawater transfer (121 g). It is possible
that pinheads occurring soon after seawater transfer may differ somewhat in
characteristics from those sampled in the present study.

2.5.1 Morphology

Fish can be viewed crudely as being composed of two basic compartments, lean body
mass and lipid storage depots. During periods of starvation or feed shortage the
chemical components of these two compartments are mobilised at different rates to meet
energy demands (Jobling, 2001b). Generally there is a tendency to utilise lipid and
glycogen while conserving body protein. Even when protein is utilised mineral ash and
skeletal tissue tend to be conserved (Stirling, 1976; Boëtius and Boëtius, 1985). As a
result of the mobilisation and depletion of energy reserves fish generally suffer weight
loss becoming light for their length. The relationship between length and weight can
therefore be used to assess an individual's "well being" (Jobling, 2001b). Condition
factor (K) is an index that describes the relationship between length and weight with a
low value indicating low body weight for a given length (Jobling, 2001a). Fish with low
condition factors are considered to be in "poor condition" (Jobling et al., 1991, 1994). In
the present study condition factor was lower in pinheads than non-pinheads, the mean
value was 0.74 and very similar to an anecdotal industry value of 0.7 and below (I. Weir
personal communication). The maximum condition factor of any of the pinheads was
0.865. Mean weight and mean length were also found to be significantly lower in
pinheads supporting King's (1992d) definition that pinheads are fish that fail to grow in
length while a considerable reduction in body weight occurs. Visceral fat ratio (%) can
be used as an indication of condition, with a greater proportion of fat equalling higher
condition (Jarboe and Grant, 1996; Liu and Liao, 1999; Cho and Jo, 2002). Atlantic

salmon have been shown to mobilise reserves in the visceral cavity more rapidly than those elsewhere in the body (Einen et al., 1998). The current study found that pinheads had significantly less pyloric fat and therefore presumably less visceral fat than non-pinheads again demonstrating their low condition. No significant difference in PCSI showed that the ratio of pyloric matter to total mass was not affected by pinheading. An unexpected finding was that HSI was significantly higher in pinheads than in non-pinheads. Generally a high HSI would indicate higher condition (Storebakken et al., 1991; Moccia et al., 1998; Nordgarden et al., 2002) but pinheads are so thin that in proportion to their emaciated carcass even a normal sized liver was disproportionately large and lead to this numerical result. As these fish become emaciated they may be selectively using up resources from somatic tissue in preference to their organs. It is likely that each organ has a minimum functional size. As a proportion of fork length, rather than total body weight (as in the HSI) liver weights were significantly smaller in pinheads than non-pinheads. GSI was also found to be significantly larger in pinheads than non-pinheads but again as a proportion of fork length rather the total body weight the weight of gonads was significantly higher in non-pinheads. Both of these findings also suggest lower condition in pinheads.

2.5.2 Chemical composition of fish

The finding that pinheads had significantly less crude lipid than non-pinheads is partly explained by pinheads having a lower proportion of visceral fat. As a consequence of low lipid levels gross energy was significantly lower in pinheads. In fish there is a well recognised inverse relationship between carcass lipid and moisture, so that fish with higher lipid contents generally exhibit lower moisture content (Shearer, 1994; Liu and Liao, 1999). It is believed that this inverse relationship provides a mechanism for a homeostasis of tissue volume (Groves, 1970) keeping the combined percentage of moisture and lipid at approximately 80% of the wet weight of the fish (Jobling, 2001b). This general trend was found to hold true in the present study with significantly lower moisture content (higher % dry weight) observed in the high conditioned (high lipid) fish. Since osmoregulatory failure in seawater is linked with dehydration in salmonids (Franklin, 1992; Handeland et al., 2000) it was thought that if pinheads were not competent osmoregulators their dry weight measurements may indicate dehydration.

However, given the inverse relationship between lipid and moisture, fish in each group would have to have similar lipid levels in order to conclude that differences in moisture suggested dehydration. Finally, the loss of condition presumably increased the ratio of bone matter to other body tissues and explains the significantly higher % ash of pinheads. This is consistent with previous findings that mineral ash and skeletal tissue are conserved during the mobilisation of energy reserves (Stirling, 1976; Boëtius and Boëtius, 1985).

2.5.3 Osmoregulation

No evidence was found that osmoregulatory ability was inhibited in pinheads which had almost identical osmolalities to non-pinheads. The mean values of 456 and 453 mmol kg^{-1} for pinheads and non-pinheads respectively were, however, higher than previously reported for Atlantic salmon (Parry, 1961; Prunet and Boeuf, 1985; Boeuf et al., 1989; Stagg et al., 1989; Warning and Moore, 2004). Osmolalities in freshwater typically range between 305 to 328 mmol kg^{-1} (Parry, 1961; Prunet and Boeuf, 1985; Boeuf et al., 1989; Stagg et al., 1989). Once transferred to seawater salmon must hypoosmoregulate, excreting salts in the hyperosmotic marine environment, to maintain the plasma electrolytes at about one third the seawater concentrations (Hoar, 1988). Osmolality values reported for Atlantic salmon transferred to seawater vary. Naturally migrating Atlantic salmon with osmolalities of approximately 328 mmol kg^{-1} in freshwater were found to increase their osmalalities to a stable value of 344 mmol kg^{-1} once they had migrated to sea (Parry, 1961). Osmolalities between 350 and 365 mmol kg^{-1} have been observed for smolting Atlantic salmon 24 h following transfer from freshwater to seawater ranging in salinity from 31 - 35‰ (Prunet and Boeuf, 1985; Boeuf, 1989; Stagg et al., 1989; Warning and Moore, 2004). Prunet and Bouef (1985) found that osmolality in fully smolted Atlantic salmon transferred to 35‰ seawater initially increased up to 24 hrs (approximately 355 mmol kg^{-1}) but then decreased to that of the freshwater controls and remained stable at 320 mmol kg^{-1} for the remaining 17 d of the experiment. Osmolalities above 400 mmol kg^{-1} are rare but have been observed 7 days following seawater transfer in non-smolting fish (Boeuf et al., 1989). In the present study it is possible that pinheads and non-pinheads alike had osmoregulatory problems associated with being out-of-season smolt, although after one experiment of three

months in seawater (Chapter 3) poorly performing out-of-season smolts still had a mean osmolality of approximately 343 mmol kg^{-1}. Also, it has been found in some cases that out-of-season smolts actually have better osmoregulatory ability than spring smolts (Lysfjord et al., 2004). One possible explanation for the high values in the present study is that samples were stored for too long at -80°C and evaporation occurred. Whilst the high values are of concern and absolute values would best be viewed cautiously, the finding of no significant difference between pinheads and non-pinheads does support previous findings for pinheads in Tasmania and failed smolt in the Northern Hemisphere (Johnstone,1991; King, 1992a). Osmolality in this study is presented in the standard international units of mmol kg^{-1} which is synonymous with the older units mOsm kg^{-1} (Webster, 1985). In some of the references above osmolality was reported using mOsm l^{-1}, however these units describe osmolarity not osmolality (Webster, 1985). Since osmometers measure osmolality and not osmolarity (Webster, 1985) it has been assumed that values given are accurate while units were not. In all cases the units have been changed to mmol kg^{-1} in the present study for both accuracy and consistency.

Whilst it has been found in both the present study and in previous studies (Johnstone,1991; King, 1992a) that pinheading is not caused by osmoregulatory failure it is interesting to note that survival of failed smolts in Scotland and pinheads in Australia has been found to be improved by returning these fish to freshwater (Johnstone, 1991; King 1992d). King (1992d) achieved 88% recovery of pinheads by removing them from cage populations and returning them to freshwater tanks, while only 5% recovered when removed but placed into seawater tanks. Sixty three % of fish which recovered in freshwater continued their recovery when placed back into seawater suggesting they were capable of osmoregulating. It is possible that life in sea cages may be more energetically costly than in freshwater tanks and this is supported by past findings that the incidence of failed smolt has been much lower in populations transferred from cages to cages (freshwater to seawater) than those transferred from tanks to cages (freshwater to seawater) (Johnstone et al., 1999), although it could be the change itself, whether it be from tanks to cages or cages to tanks, that causes problems. The combination of learning to feed and compete in a new environment and the additional stress of exposure to seawater may be contributing factors to pinheading and failed smolt syndrome despite the ability of these fish to osmoregulate.

2.5.4 Trypsin activity

Whilst it has been speculated that one of the characteristics of pinheads may be decreased digestive function (King, 1992c) there was no evidence to support this based on trypsin activity. Trypsin is a protease digestive enzyme synthesised by pancreatic tissue (Bishop and Odense, 1966) in the pyloric caeca (Blier et al., 2002). In the current study the mean activity of trypsin per gram of pyloric tissue was found to be 0.083 and 0.078 μmol pNA g^{-1}min^{-1} for pinheads and non-pinheads respectively. These values fall within the normal range for feeding animals of 0.075 to 0.135 μmol pNA g^{-1}min^{-1} determined using an incubation temperature of 15°C (Pringle et al., 1992; Carter et al., 1994), and while other studies using the same technique have found higher activities (around 0.2 to 0.4 μmol pNA g^{-1}min^{-1}) these higher values are presumably due to the higher incubation temperature (25°C) used (Einarsson et al., 1996, 1997). The results in the present study show that biochemically the pyloric tissue was capable of producing trypsin equally well in both pinheads and non-pinheads. To determine how this affected the fish as a whole trypsin activity was also examined in terms of the total activity per fish (μmol pNA min^{-1} pylorus^{-1}) and activity per gram wet weight of the entire animal (μmol pNA min^{-1}g of fish^{-1}). While neither of these were shown to be significantly different the trypsin activity of the whole fish was around 7 times greater in non-pinheads. This difference can be largely attributed to the fact that in absolute measures a larger fish will have a larger pylorus (even though they are not larger in proportion to weight). The pattern disappeared when the value was divided by the wet weight of the fish with activities per gram of fish being almost identical. This again suggests that pinheads are not inferior to non-pinheads in terms of trypsin production. However, whilst pinheads have similar capacity to produce trypsin and therefore digest protein it is important to note that their ability to absorb protein was not examined in the present study and this also could contribute to pinheading even if fish were eating.

Whilst in cage populations pinheads seem to avoid standard commercial feed pellets there have been observations made on Tasmanian farms that they will eat mussels mixed in with commercial feed that has been moistened with freshwater (I. Weir personal communication). Similar observations were made for failing smolt in Scottish

salmon farms. These individuals were seen to take interest in and subsequently eat chopped up mussels, chopped up sprat, crushed up crab, and other small crustaceans (D. Mitchell personal communication). Some farms have used moist pellets and hand feeding schedules to try and minimise failed smolt (Johnstone et al., 1999). In the present study the finding that trypsin activity is not reduced in pinheading Atlantic salmon suggests that these fish may well be capable of digesting feed and are simply not eating. Measurement of the amounts, or proportions in relation to the size of the fish of feed and faeces in the gastrointestinal tract of pinheads and non-pinheads may have helped shed light on whether pinheads had lower feed intake than non-pinheads. However the necessity of 24 hours starvation prior to sampling for trypsin activity meant that there was little in the intestines of any individuals. Interestingly pinheads isolated from cage populations and placed back in freshwater have been shown to start feeding again immediately (King, 1992c) as has been seen also for Scottish failed smolts (Johnstone et al., 1999). The fact that these pinheads seem capable of digestion and even capable of eating when removed from cage populations suggests that the problem of pinheading may be largely influenced by competition. It is possible that these fish are in some way compromised when first transferred to sea and become increasingly less able to compete with individuals who have started to feed successfully immediately upon transfer. Over time an association may be built up between taking pellets and being attacked by more competent individuals (D. Mitchell personal communication) and this concept is supported by the fact that these fish have been seen to avoid taking pellets but will eat the other forms of food mentioned above.

2.6 Conclusions

This study aimed to better define the term pinheading. Samples were taken at a single time point when pinheads were observed to be occurring and *t*-tests were used as a robust statistical approach to the analysis of parameters describing how pinheads and non-pinheads at exactly the same age and stage of development differ. It is worth noting that fish in this study had been in seawater for 9.5 months before our sampling was done and that patterns could be different from pinheads that emerged within the first 12 weeks following transfer. No evidence was found of pinheads having more difficulty osmoregulating than non-pinheads, nor was the activity of their protease enzyme trypsin lower, suggesting that these fish may be capable of digesting food if they ate. However, it is still possible that other aspects of digestion may be causing problems. From present findings a pinhead can be defined as a fish with significantly lower condition factor, length, weight, liver to fork length ratio, gonad to fork length ratio, visceral fat (%), crude lipid (% wet weight), and gross energy (kJ g^{-1} wet weight), and significantly greater dry matter (% wet weight) and ash (% wet weight) than other fish from the same population, cohort, and cage.

2.7 Acknowledgements

2.8 References

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Chapter 3

Manipulation of feeding frequency following transfer to sea in

Atlantic salmon

Matthew Flood

3.1 Abstract

The effects of feeding frequency immediately following seawater transfer of Atlantic salmon were investigated. Tanks of smolt were maintained in freshwater on a high feeding frequency (8 feeds per day – 8F) for a 27 d period prior to 2 d feed deprivation and transfer into near full strength seawater (30‰). Following transfer triplicate groups were allocated to low (1F), medium (4F) or high (8F) feeding frequencies while freshwater controls remained on the initial high feeding frequency (8F FW). Fish were kept on their new feeding regimes for 21 to 22 d. Individual feed intake was measured using X-radiography twice during each of the freshwater and seawater phases. The initial decrease in group feed intake following seawater transfer was significantly ($p < 0.05$) greater for the 1F treatment than 4F or 8F, however feed intake of 1F converged with the other treatments by the end of the experiment with no significant difference between treatments for mean feed intake during the seawater phase. The strength of the 1F feeding hierarchies increased significantly immediately following seawater transfer while not significantly increasing in the 4F or 8F treatments. This increase did not persist with the hierarchy strength in 1F returned to pre-transfer values within 3 weeks. There were no significant differences in growth performance between the treatments for seawater transferred fish. Freshwater controls had significantly higher weight gain and final condition factors (K), and significantly lower osmolalities than seawater transferred fish.

A second experiment was run aiming to examine the effects of the same feeding frequencies as experiment 1 on group feed intake, growth, and condition, over a longer period of time while removing the handling stress associated with anesthetising and X-radiography in the hope of detecting growth performance differences if they occurred. Smolts were however of poor quality and quickly presented symptoms of infection by a mixture of *Tenacibaculum* and *Vibrio spp.* Performance of these fish was poorer than seen in experiment 1 with lower feed intake, poorer FCRs and osmolalities on average 15 mmol kg^{-1} higher indicating decreased osmoregulatory capability. As a result of the poor performance of these fish the findings from this experiment were inconclusive.

3.2 Introduction

Feeding frequency has been shown to have a measurable effect on feed intake, growth and feed conversion ratio (FCR) in many species of fish, with increased frequency often increasing feed intake and/or growth performance (Grayton and Beamish, 1977; Chua and Teng, 1978; Greenland and Gill, 1979; Boujard and Leatherland, 1992; Dwyer et al., 2002), while decreasing the strength of feeding hierarchies when feeding to satiation (Jobling, 1983). The presence of hierarchies within groups of salmonids held in small experimental tanks is well documented (McCarthy et al.,1992; Moutou et al., 1998; Bailey et al., 2000; Suter and Huntingford, 2002), and normally most apparent with feed shortage (Slaney and Northcote, 1974). Competition for feed and the subsequent formation of hierarchies results in a wide disparity in individual feed intake (McCarthy et al., 1992; Grant, 1993; Jobling, 1995) which is thought to be a major contributor to increases in weight variation (growth depensation) between individuals within a groups (Davis and Olla, 1987; Weatherley and Gill, 1987; McCarthy et al., 1992; Jobling and Baardvik, 1994; Jobling, 1995; Shelverton and Carter, 1998; Petursdottir, 2002). This variation is not ideal commercially given that farms require a uniform size at harvest (Jobling et al., 1993a; Jobling et al., 1995). At its extreme, competition for feed and the formation of hierarchies may result in the emergence of pinheads, thin emaciated fish out-competed for feed by dominant individuals (Johnstone, 1991; King 1992a,b). It has been suggested that behaviour in the feeding arena may be an important factor in the expression of failed smolt syndrome, and that close attention to feeding regimes may help reduce the occurrence of the condition (Johnstone, 1999). Presumably the same could be suggested for pinheading.

Grayton and Beamish (1977) found that feed intake of rainbow trout *Oncorhynchus mykiss* will increase with the number of meals offered each day to some maximum point. Satiation time and the subsequent return of appetite seem to be the main factors limiting the number of meals a fish will take each day (Brett, 1971; Grove et al., 1978). While fish fed higher frequencies often consume larger quantities of feed, when the interval between meals is short the feed passes through the digestive tract more quickly, resulting in less effective digestion (Liu and Liao, 1999). It has also been suggested once the optimum feeding frequency is reached the increased cost of eating more small

meals will outweigh the benefits of increased energy intake (Ruohnen et al., 1998). The optimal number of daily meals also seems to be species-specific (Linnér and Brännäs, 2001). For more aggressive species such as rainbow trout higher feeding frequency can decrease growth performance while for less aggressive feeders such as Arctic charr *Salvelinus alpinus* higher frequency increases the chances of fish encountering pellets, thus increasing growth (Linnér and Brännäs, 2001).

Several studies failed to demonstrate an effect of feeding frequency on growth rate in Atlantic salmon in freshwater (Jørgensen and Jobling, 1992) or seawater (Thomassen and Fjæra, 1996; Johansen and Jobling, 1998). The seawater studies were, however, conducted in high density seawater cages (Thomassen and Fjæra, 1996; Johansen and Jobling, 1998) with rearing conditions in these cages known to reduce hierarchy formation (Kjartansson et al., 1988). It is possible that in small experimental tanks feeding regimes may affect growth and performance of Atlantic salmon in seawater.

Commercial feeding frequencies for Atlantic salmon in hatcheries in Tasmania tend to be high with feed being delivered as often as once every twenty minutes, while feeding frequency after seawater transfer may be reduced to only one or a few meals per day (H. King, personal communication). It is possible that reducing temporal availability of feed by reducing feeding frequency at the time of seawater transfer may promote increased competition leading to some fish being out-competed for feed causing a reduction in their condition and in extreme cases pinheading. In the case of failed smolt Johnstone et al. (1999) have suggested that disadvantaged fish that are predisposed to becoming failed smolts may adapt more successfully to the marine environment if more sympathetic feed management practices are employed. The present study was designed to examine whether the number of meals offered on a daily basis following direct transfer from freshwater to seawater had an affect on feed intake; growth; condition; hierarchy formation, strength and stability; and osmoregulatory capabilities of Atlantic salmon. In the most extreme case we were interested in whether treatments would lead to deterioration in condition to such an extent that some fish became pinheads. The effect of specifically 'changing' feeding frequency, i.e. going from low to high or high to low feeding frequencies simultaneously with seawater transfer will be examined in Chapter 4.

This study was comprised of three main parts:

1. A preliminary trial was set up to validate the accuracy of X-radiographic individual feed intake measurements (Talbot and Higgins, 1983) of Atlantic salmon fed one, five or ten meals over a one, nine or ten hour period respectively to ensure that ballotini glass beads incorporated into the feed would be retained in the gut of fish long enough for X-rays to be accurate. Comparisons between individual feed intake measured using X-radiography (summed for each tank to give a group total) and the known group intake of the tank measured by direct observation were used for this validation (3.3.3 and 3.3.4).

2. Experiment 1 aimed to examine the effects of low (1F), medium (4F) and high (8F) daily feeding frequencies during the first month following the seawater transfer of Atlantic salmon spring smolt. This followed a pre-transfer freshwater phase during which all groups of fish were fed a high meal frequency (8F) for one month to replicate industry practice. Following transfer a control remained in freshwater on the original feeding regime (8F FW). The aim of this experiment was to examine the effect of meal frequency on growth, growth depensation and final condition of fish and its effect on group and individual intake. Individual intake measurements were used to examine the formation of feeding hierarchies, their strength and stability in freshwater, seawater and between the two. Osmolality was examined to determine if any fish were osmo-compromised during the experiment, and to determine if feeding frequency had an effect on the osmoregulatory ability of salmon following introduction to seawater. By examination of the above parameters this experiment aimed to elucidate the contribution of feeding frequencies to the formation of pinheads and also to determine how fish that became pinheads (or were close to becoming pinheads) performed through time.

3. Experiment 2 aimed to advance findings from experiment 1 using identical treatments (without a freshwater control) but with fish held for 87 d in seawater rather than the 22 d of experiment 1. The aim was again to examine the effect feeding frequency on group feed intake, growth, and condition, over a longer period of time while removing the handling stress associated with anesthetising and X-radiography. It was believed that

reducing handling stress and extending the time of exposure to seawater may have made any differences due to treatment effects more pronounced, with increased time also allowing for the emergence of pinheads not seen in experiment 1.

3.3 Materials and methods

3.3.1 Experimental system and set up of experiments

Experiments were carried out at the School of Aquaculture, University of Tasmania (Launceston, Tasmania, Australia). During experiments fish were maintained in 300 L flat bottomed cylindrical tanks (Plate 3.1) in one of two separate systems. In both systems individual tanks were supplied with water from 3000 L temperature regulated reservoir tanks at a rate of approximately 13-15 L min^{-1}. The total volume of each system was approximately 7250 and 6500 L for the 12 tank and 9 tank systems respectively. Tanks had external stand pipes and were drained centrally displaying adequate centrifugal water flow to move uneaten feed and faeces to outlets. Solids were removed from the effluent water via dacron matting and a swirl separator, while nitrogenous wastes were treated by trickle biofiltration. Ambient light entered through overhead opaque skylights. During the freshwater (FW) phase water was continually replaced from the municipal water supply at a rate of approximately 10% d^{-1}, while during the seawater (SW) phase water was replaced by discrete water changes at least once per week equivalent to a rate of about 10% d^{-1}. There was adequate water flow into tanks to enforce exercise in the fish.

Each experimental tank was fitted with a belt feeder (Plate 3.1) with belts marked at hourly increments to align feed according to the specific feeding regimes (Plate 3.2). On each day the ration to be offered was divided into one (1F), four (4F) or eight (8F) equal meals (one, five or ten meals in the case of the preliminary experiment). In both experiment 1 and 2 the 1F commenced feeding at 0900 h; 4F had meals commencing at 0900, 1100, 1300, and 1500 h; and 8F were fed meals commencing at 0900, 1000, 1100, 1200, 1300, 1400, 1500, and 1600 h. The preliminary experiment had the same temporal spacing between meals as experiment 1 and 2 but in each treatment the first meal commenced at 0800 h. Meal duration among treatments varied and depended on the numbers of meals offered as an equal ration was offered to all treatments either at one meal or across multiple meals. 1F tanks fed for approximately 1.5 to 2 h; 4F tanks fed for approximately 30 to 45 min per meal and 8F tanks fed for approximately 15 to 30 min per meal. Belt feeders failed on several occasions, twice during experiment 1 and

ten times during experiment 2. In these cases the meals were pushed closer together and delivered over a shorter period of time within the normal hours of feeding. The term ration refers to the amount of feed supplied daily by belt feeders and not the amount ingested. The actual feed intake (amount ingested) was also calculated (see below). Rations and feed intake are expressed on a weight-specific basis of mg feed. g wet weight $^{-1}$. d^{-1} (mg g^{-1} d^{-1}). Tanks were fitted with mesh lids allowing pellets and light through but preventing escape by fish. Each water outlet was fitted with a mesh basket to collect uneaten feed pellets on days when feed intake was monitored (Plate 3.1) (Helland et al., 1996).

Water quality was monitored every 1 to 2 d throughout each experiment to ensure that the main parameters of chlorine, pH, ammonia, nitrite and nitrate remained within the recommended limits for salmon in FW (Wedemeyer, 1996) and SW (Tarazona and Muñoz, 1995). Water changes were used to reduce elevated levels.

3.3.2 Experimental diets

For the preliminary experiment and experiment 1 commercial feeds were hammer milled and re-pelleted with the inclusion of X-ray opaque Ballotini glass beads (no. 8.5, 400-450 µm, Jencons Scientific Ltd., Leighton Buzzard, UK) into feed to be used for X-ray measurements. Each milled diet was mixed with a Hobart mixer (Model A200.11) and re-pelleted to 4 mm pellet diameter with a laboratory pelletiser (California Laboratory Pellet Mill) to produce cold pressed feed. Pellets were dried at approximately 30°C for around 24 h and then refrigerated at 4°C until use. The preliminary experiment was formed by using a commercial diet (3 mm Salmon Grower 45/22, Skretting, Cambridge, Tasmania, Australia) combined with 1% binder (carboxymethyl cellulose CMC); 10% water and 3% ballotini in feed to be used for X-rays. Feed for experiment 1 was formed using kernels (extruded pellets taken before oil coating occurred) of another commercial feed (Nutra Transfer, Skretting, Cambridge, Tasmania, Australia) combined with 1% CMC; 15% fish oil; 8% water; and 1% ballotini in feed to be used for X-rays (part of Batch 2). Two batches of this diet were formed with near identical "as is" chemical composition (92.4 ± 0.09% dry matter, 39.1 ± 0.14% crude protein, 26.9 ± 0.41% crude lipid, 9.5 ± 0.07% ash (Batch 1); 91.0 ±

0.01% dry matter, 38.5 ± 0.01% crude protein, 26.4 ± 0.02% crude lipid, 9.3 ± 0.03% ash (Batch 2) (mean ± SD, n = 2)). For experiment 2 fish were fed using commercial extruded pellets (3 mm Atlantic LE, Skretting, Cambridge, Tasmania, Australia: 89.5 ± 0.04% dry matter, 38.8 ± 0.03% crude protein; 25.9 ± 0.29% crude lipid; and 7.7 ± 0.02% ash (mean ± SD, n = 2)). This was the same feed used in the commercial hatchery before fish were transported to the University of Tasmania.

3.3.3 Feed intake measurements

Measurements of group feed intake for each tank were made by counting the number of waste pellets recovered from the baskets placed under the water outlets of tanks (Helland et al., 1996). Pellets were removed from baskets and counted every 30 min when using re-pelleted feeds and every hour when using more stable extruded pellets. This was repeated throughout the hours of feeding each day, 8 h d^{-1} in experiments 1 and 2 and 10 h d^{-1} in the preliminary experiment. Feed intake was calculated as *feed ration – feed waste = feed intake*. The number of pellets collected was multiplied by the average pellet weight in order to determine the weight of uneaten pellets (*feed waste*). Feed intake is presented as absolute feed intake (mg d^{-1}) or intake relative to fish wet weight (mg g^{-1} d^{-1}) where 10 mg g^{-1} d^{-1} is equivalent to 1% BW d^{-1}.

X-Radiography was used to measure individual feed intake according to established techniques (Talbot and Higgins, 1983; Carter et al., 1996) using feed labelled with radio opaque glass marker beads (ballotini). Feeding on X-ray days was carried out using labelled feed (see 3.3.2) supplied in the same manner as normal pellets on non X-ray days. Fish were allowed 30 min after the completion of feeding before being removed from their tanks to prevent regurgitation of labelled feed (McCarthy et al., 1993). They were then removed one tank at a time, anaesthetized (benzocaine, 75 mg L^{-1}) over 2.5 min, and radiographs were taken using a Dong-A portable X-ray unit (Model: DA-70-P, Dong-A X-ray Co., Ltd, Chungnam, Korea). In experiment 1, where fish were PIT tagged, they were removed from the X-ray plate one at a time and identified. Their position on the X-ray plate was recorded and wet weight (to nearest 0.1 g) and fork length (mm) were measured. Fish were recovered in aerated water before being returned to their holding tanks. They were fasted for 1 d to aid in post X-ray recovery before

being returned to their normal feeding regimes. X-rays were processed in a dark room according to the manufacturer's instructions and the number of ballotini beads in the gut of each individual fish was counted by eye with the aid of a light box.

3.3.4 Preliminary experiment – Setting feeding frequency

This experiment aimed to validate the assumption that all ballotini beads ingested would be retained in the gut long enough for measurements to be made. Atlantic salmon were anesthetised (benzocaine, 75 mg L $^{-1}$) and wet weight (to nearest 0.1 g) and fork length (mm) measured. Twenty randomly selected fish were allocated to each of 6 tanks (51.8 ± 6.5 g, mean ± SD, n = 120). Tanks were allocated to a treatment of 1, 5 or 10 meals d^{-1} (1F, 5F or 10F) with 2 replicates of each and fish were fed using belt feeders between 0800 and 1800 h. The ration offered was in excess at a rate of 20 mg g^{-1} d^{-1} for 6 d beginning the day following allocation. On day 6 group feed intake was measured and used to determine the limiting ration which was used for the next 8 d. After a total of 13 d acclimation to the feeding regimes, group intake for each tank was measured and X-rays were taken. Using weight data from fish on the day of X-raying (64.6 ± 8.1 g, mean ± SD, n = 115) it was calculated that the ration offered and eaten was approximately 10 mg g^{-1} d^{-1} at this time. The temperature of the system was a constant 15°C over the course of the day. Group feed intake was compared with the cumulative individual feed intake measurements for each tank to ensure X-ray technique was accurate over these lengths of time. Tank 3 lost 5 of its 20 fish through escape from the tank prior to X-rays being taken but after feeding so data from these fish could not be analysed. The percentage of the total group feed intake accounted for in X-rays of individual fish was compared for each treatment.

3.3.5 Experiment 1 – Effect of feeding frequency immediately post-transfer to seawater on individual and group feed intake; growth and condition of Atlantic salmon.

For experiment 1 mixed sex diploid Atlantic salmon (*Salmo salar* L) parr were obtained from Wayatinah Salmon Hatchery (SALTAS, Tasmania, Australia) in April 2002, transported back to the School of Aquaculture, housed for approximately 6 months in a

4000 L FW recirculation system and maintained on 2 to 3 meals d^{-1} of a commercial extruded feed (3 mm Salmon Grower 45/22, Skretting, Cambridge, Tasmania, Australia).

At the beginning of the experiment fish were graded in order to obtain individuals of uniform size. Over 2 d on 7 and 8 October 2002 fish were anaesthetised (benzocaine, 75 mg L $^{-1}$), PIT (Passive Integrated Transponder) tagged, and wet weight (to nearest 0.1 g) and fork length (mm) were measured. Twenty-three fish were randomly allocated to each of 12 tanks (188.3 ± 10.4 g, mean ± SD, n = 276), nine tanks from one system and three from the second. PIT tags used were Destron 11 mm (small, model TX1400L) injectable transponders, which were inserted into the abdominal cavity using a syringe applicator through a small scalpel incision on the ventral side of the fish half way between the anus and pectoral fins. The tags were used to identify individual fish using a Trovan multireader Mark 3 (model MK3 multireader). After 1 d of recovery without feed, fish were hand fed 2 to 3 times d^{-1} on commercial extruded feed (3 mm Salmon Grower 45/22, Skretting, Cambridge, Tasmania, Australia) for 6 d before being started on re-pelleted experimental feed for 4 d (with the exception of one day when feeding was not carried out due to system pump failure). For 1 d fish were then hand-fed to satiation at each of 8 discrete meals, 1 h^{-1}, with the first at 0900 and the last at 1600 h (daylight savings time). The tank that ate the least was determined to have consumed approximately 13 mg g^{-1} d^{-1} and this was set as the daily ration in an attempt to ensure that feed intake in the FW phase would be close to uniform for all tanks. Tanks of fish were subsequently fed 8F d^{-1} using belt feeders for 26 d before being fasted for 2 d prior to direct transfer into near full strength SW (30‰), in accordance with present industry practice. Transfer to SW was achieved by emptying the reservoir completely and refilling it with SW. The water level in the experimental tanks was then dropped until only just enough remained to cover the fish. The pumps were then turned back on from the reservoir to the experimental tanks filling them with SW. To maintain standard protocols in the 3 FW control tanks in the second system the water level of the experimental tanks was also dropped and refilled in the same way with FW.

Following SW transfer triplicate tanks of fish were allocated to feeding regimes of 1F, 4F or 8F d^{-1} in SW tanks, while the 3 FW control tanks were maintained on the pre-

transfer regime of 8 feeds (8FFW). Fish were not fed on the day of SW transfer, feeding recommenced the following day. During the SW phase all groups continued to be fed on the fixed ration (13 mg g^{-1} d^{-1}) which had been determined during the FW phase. As a consequence of a severe reduction in feed intake following SW transfer this ration resulted in excess feeding on a daily basis during the SW phase. Despite this overall daily excess feeding was still unavoidably restricted at times during meals early in the day due to the restrictive nature of feed delivery using simple belt feeder technology. The ration offered was kept at 13 mg g^{-1} d^{-1} divided evenly into 1F, 4F or 8F depending on treatment (see 3.3.1). Rations were adjusted after each weighing and adjustments were also made on a daily basis to correct for a decline in total biomass of the tanks due to mortalities which were weighed on removal and not replaced. The systems were initially run at a mean temperature of approximately 15.5 ± 1.0°C but were decreased to approximately 13.5 ± 1.0°C two weeks prior to SW transfer to help postpone smoltification. Such decreased temperatures are known to help delay the smoltification process. This delay was important as it was nearing the end of the time period during which salmon are known to be viable smolt, i.e. the smolt window (Percival and Foster, 1988). Water temperature rose slightly on the day of transfer with SW entering experimental tanks at approximately 17.5°C. However, the system temperature had returned to 13°C when monitored the next day. This change in temperature did not appear to adversely affect the survival or behaviour of the fish. During the SW phase the mean salinity was approximately 30.6 ± 0.9 ‰. A heterotrophic bacterial bloom on 20 November 2002 was treated using a transportable U.V. lamp filter and a foam fractionator which was plumbed permanently into the system.

In order to assess feed intake on an individual basis salmon were X-rayed after eating ballotini labelled feed (see 3.3.3) twice in FW and twice in SW: 20 or 21 d pre-transfer; 6 or 7 d pre-transfer; 7 or 8 d post-transfer and 21 or 22 d post-transfer. The first X-ray day occurred after fish had been given 7 d to acclimate to the feeding regimes and 12 d to acclimate to the experimental re-pelleted feed. Each X-ray session was carried out over 2 d with half of the replicate tanks X-rayed on each day. At each X-ray tanks from each treatment and the sampling order of these tanks were chosen using random numbers. This process of randomly choosing tanks was repeated separately for each X-ray session. Waste pellets were monitored on X-ray days to determine group feed intake

and further verify that ballotini was retained within the fish long enough for X-rays to be taken. Group intakes were also monitored on the last feeding day before SW transfer and regularly after transfer. Belt feeder failure occurred only once on an X-ray day where it was found at 1300 h that one of the 4F feeders had stopped after delivering its first meal. To accommodate for this the feed was put on a different feeding belt with reduced time between meals. Fish were fed at 0900, 1300, 1415 and 1530 hr instead of the initial schedule of 0900, 1100, 1300, and 1500 h, and this was the last tank X-rayed on this day.

Once final X-rays were taken fish were returned to the system, deprived of feed for 1 d, before being euthanased (benzocaine, 300 mg L^{-1}). Tanks were removed in the same order as during final X-raying. Once euthanased individuals were identified by PIT tag and samples of approximately 1 ml of blood were taken from the caudal vein using heparin-treated needles and 1 ml Tuberculin™ syringes. Each sample was syringed into a Eppendorf safe lock centrifuge tube, placed on ice, and subsequently centrifuged for 3 min at 4000 g. Plasma was removed and stored in a second Eppendorf at -20°C for later analysis of osmolality. The PIT tags were then removed and the whole fish carcass was placed in labelled bags and stored in the freezer at -20°C for measurement of whole body chemical composition (see below). Four fish were selected from each tank for the assessment of whole body chemical composition with care being taken to select individuals representative of the full range of Ks within each tank, similar to Johansen and Jobling's (1998) approach used to select fish representative of the full range of weights. This was achieved by dividing the tanks into quartiles based on K and using random numbers to select one fish from each of these quartiles.

3.3.6 Experiment 2 – Effect of feeding frequency over three months post-transfer to seawater on group feed intake, growth and condition of Atlantic salmon.

This experiment was designed to complement experiment 1, aiming to examine the effect of feeding frequency on group feed intake, growth, and condition in SW, while removing the handling stress associated with anesthetising and X-raying. It was believed that by reducing these stresses and increasing the duration of the experiment greater growth would be achieved which would increase identifiable differences

between treatments. While experiment 1 was designed to examine the effect of feeding frequency on both and individual and group basis over a short period of time experiment 2 was designed to investigate only group dynamics over this longer period.

For experiment 2 mixed sex diploid out-of-season Atlantic salmon (*Salmo salar* L) smolt were obtained from the Florentine Salmon Hatchery (SALTAS, Tasmania, Australia) on 9 April 2003 and transported to the School of Aquaculture. They were held for 1 d in a 2000 L tank, one of 6 tanks in a large FW recirculation system. On 11 April 2003 fish were anaesthetised (benzocaine, 75 mg L^{-1}), and wet weight (to nearest 0.1 g) and fork length (mm) were measured. Twenty-five fish were randomly allocated to each of twelve tanks (127.1 ± 11.3 g, mean ± SD, n = 300) with 33‰ salinity. After 1 d of recovery without feed fish were hand fed to satiation once a day for 15 d after which time tanks of fish were allocated to the feeding regimes of 1F, 4F or 8F in triplicate and fed using belt feeders. Fish were kept in SW for a total of 87 d. Feed delivered using belt feeders was always to excess on a daily basis but was unavoidably restricted at times during meals early in the day due to the restrictive nature of feed delivery using simple belt feeder technology. At 3-weekly intervals fish were removed from tanks and weighed in a small bucket of water. Fish were not anesthetised in an effort to reduce anaesthetic related stress and to optimise growth. For this reason length data could not be obtained. Feed intake was monitored every 2 d throughout the experiment to determine group feed intake. Rations were adjusted after each weighing and on a daily basis to correct for a decline in total biomass of the tanks due to mortalities, which were weighed on removal and not replaced.

The experiment began at the end of an initial 15 d disease outbreak, tentatively identified as a mixture of *Tenacibaculum* (previously known as *Flexibacter*) and *Vibrio* spp. (Sajjadi, 2004). The delayed commencement was due to low feed intake during this outbreak period. The ration offered was set at 13 mg g^{-1} d^{-1} to provide consistency with experiment 1; however this amount of feed was found to considerably reduce water quality and was therefore lowered to approximately 6 mg g^{-1} d^{-1} for 7 d during which time feed intake slowly increased. This amount was still in excess of requirements on a daily basis. After 7 d the ration offered was again raised to 13 mg g^{-1} d^{-1}. The average temperature of the system throughout the experiment was 15.0 ± 0.7°C. The mean

salinity during the experiment was 31.8 ± 1.3‰. A heterotrophic bacterial bloom on 13 June 2003 was treated with a transportable U.V. lamp filter and a foam fractionator. Fish were not fed for 1 d following the bloom to allow water to clear.

The experiment continued for 87 d in SW, and after 1 d deprivation of feed fish were euthanased (benzocaine 300 mg L^{-1}) before wet weight (to nearest 0.1 g) and fork length (mm) were measured. Blood samples were taken as outlined in experiment 1.

3.3.7 Calculations

Feed conversion ratio (FCR) was calculated as:

$$FCR\ (g\ g^{-1}) = Weight\ of\ feed\ ingested\ (g)\ /\ Weight\ gain\ (g) \qquad [3.1]$$

In order to perform this calculation feed intake data from days on which group feed intake was measured was extrapolated to give values for days on which it was not (Carter and Hauler, 2000). Condition factor (K) was calculated as:

$$K = 100\ [Wet\ weight\ (g)\ /\ Fork\ length\ (cm)^3] \qquad [3.2]$$

Specific growth rate was calculated as:

$$SGR\ (\%\ d^{-1}) = 100\ ((\ln W_t - \ln W_i)/t) \qquad [3.3]$$

where W_i and W_t are the initial and final weights respectively and t is the time in days between the initial and final weighing. SGR describes exponential change in body mass with time (Jobling, 2001a) and was calculated and used to estimate the total weight of fish in each tank on each day of the experiment allowing a more accurate approximation of feed intake relative to fish weight (mg g^{-1} d^{-1}). SGR was therefore not shown as a direct measure of growth in the results.

Feeding hierarchies within each tank were determined by ranking all fish according to their individual feed intake. Share of the group meal (SM) was calculated for each fish

at each X-ray from individual intake as the percentage of the total feed consumed by all fish (that could be analysed) in a tank. SM (%) was calculated as follows:

$$SM\ (\%) = 100 \times AC\ /\ \textstyle\sum AC \qquad\qquad [3.4]$$

(Carter et al., 1994b) where AC is the absolute daily consumption rate (mg d^{-1}) of a fish and $\sum$ AC the sum of the absolute daily consumption rates for all fish in a group (tank) that could be analysed. Inter-individual variation in feed consumption was expressed as the mean share of meal (MSM) for each fish in both FW and SW. MSM was calculated as:

$$MSM\ (\%) = \textstyle\sum SM\ /\ n \qquad\qquad [3.5]$$

where n is the number of estimates of SM for each fish. MSM was used as an indication of the rank for each individual fish within the feeding hierarchy (McCarthy et al., 1992; Carter et al., 1994b).

The coefficient of variation was calculated as:

$$CV = 100\ (standard\ deviation\ /\ mean) \qquad\qquad [3.6]$$

CV is an expression of variability relating sample variability to the mean of the sample. CV was used in the present study to examine intra-individual (within-individual) variation in feed intake, known as CVc (McCarthy et al., 1992; Carter et al., 1994a), inter-individual (within-group) variation in individual feed intake (Jobling and Koskela 1996; Damsgård et al., 1997), and to give an indication of the variability in weight, length and K within groups (tanks) of fish. To examine growth depensation and depensation in length and K, the % change in coefficient of variation (ΔCV) for these parameters from the beginning (0) to the end (f) of the experiment was calculated as:

$$\Delta CV\ (\%) = 100\ ((CV_f/CV_i)\ -1) \qquad\qquad [3.7]$$

(Carter et al., 1996)

Intra-individual variation is the variability in daily feed intake (mg g^{-1} d^{-1}) of an individual fish over multiple days (McCarthy et al., 1992). In the present study CVc was calculated for consumption rate between the two X-rays in FW and for the consumption rate between the two X-rays in SW. CVc and MSM were used in conjunction to give an estimate of hierarchy strength (McCarthy et al., 1992). Inter-individual variation is the variability in individual feed intake (mg g^{-1} d^{-1}) within a group on a specific day. In the present study the magnitude and significance of inter-individual CV for feed intake was calculated as an indication of hierarchy strength for each tank on each X-ray day (Jobling and Koskela 1996; Damsgård et al., 1997).

3.3.8 Chemical analysis of diets and whole fish

Standard methods were used to measure the chemical composition of whole fish (exp 1) and feed: dry matter (DM) was determined by freeze drying fish to a constant weight followed by oven drying at 110°C overnight (~17 h); samples were ashed in a furnace at 550°C for 16 h (feed only) (AOAC, 1995); crude protein (Kjeldahl using copper catalyst [N x 6.25]); crude lipid (Bligh and Dyer, 1959). Energy content was estimated using gross energy values for standard protein and lipid of 23.6 and 36.2 kJ g^{-1} respectively (Brafield, 1985); carbohydrate was not included (Shearer, 1994).

3.3.9 Osmolality measurements

To determine osmocompetence of fish osmolality of blood plasma was measured using a Vapro© Vapor Pressure Osmometer (Model 5520) giving measurements in Standard International (SI) units: mmol kg^{-1} (Webster, 1985). For each sample, 10 µl of plasma was used (Wescor Inc, 2000) from frozen samples as described in 3.3.6.

3.3.10 Fish with low final condition (K) and low feed intake in SW tracked through time

In experiment 1 of all the fish transferred to SW the 9 or 10 with the lowest final condition or feed intake (in SW) were selected as possible candidates for pinheading. All fish transferred to SW with a K of less than 0.95 at the final weighing (a total of 9

fish) were examined in order to determine how their weight, length, K and individual

feed intake (mg g^{-1} d^{-1}) changed through time. Their positions in feeding hierarchies

before and after seawater transfer and their final osmolalities were also examined. Also,

all of the fish with an individual feed intake less than 2 mg g^{-1} d^{-1} at both SW X-rays (a

total of 10 fish) were examined in the same way. Whilst no statistical comparisons were

possible the data were still of interest in examination of the pinheading condition, and as

such a qualitative assessment of fish with poor final condition (K) and fish with low

feed intake in SW is presented in relation to mean data. In each case the trajectories of

the parameters listed above were graphed against the total mean of the entire SW

transferred experimental population.

3.3.11 Statistical analysis

Mean values are reported ± standard error of the mean (S.E.M.). All statistical analyses

were performed using SPSS version 11.5 (SPSS, 2002). Normality was assumed as

sample sizes were too small to test for this. Homogeneity of variance was tested

graphically by examination of residual plots in SPSS. Data were statistically analysed to

test for differences between treatments using one way ANOVA (Underwood, 1997).

Multiple comparisons were made using the Tukey test. Repeated measures ANOVA

were used to analyse the trajectories of weight and K through time (SPSS) with the

greenhouse geisser epsilon value used to reduce degrees of freedom of the F statistic to

correct for violation of the sphericity assumption. Multiple comparisons were made by

way of a series of pair wise repeated measures ANOVAs. Spearman's rank correlations

were used to examine correlations. The stability of each group's (tank's) feeding

hierarchy in FW and SW was shown by the magnitude and significance of the

correlation coefficient between feeding rank (SM) data from the pair of X-ray

measurements from FW and those from SW. Ranked MSM data from FW and SW were

also correlated to determine stability across the SW transfer. A significant negative

correlation between CVc and MSM was used to indicate the formation of a strong

hierarchy (McCarthy et al., 1992). Least squares regression analysis was used to

examine relationships between the final K and the final whole body chemical

composition parameters of (%) dry matter, crude protein, crude lipid and gross energy,

and ANCOVA was used to examine for treatment effects on chemical composition parameters. Differences were considered significant at $p < 0.05$.
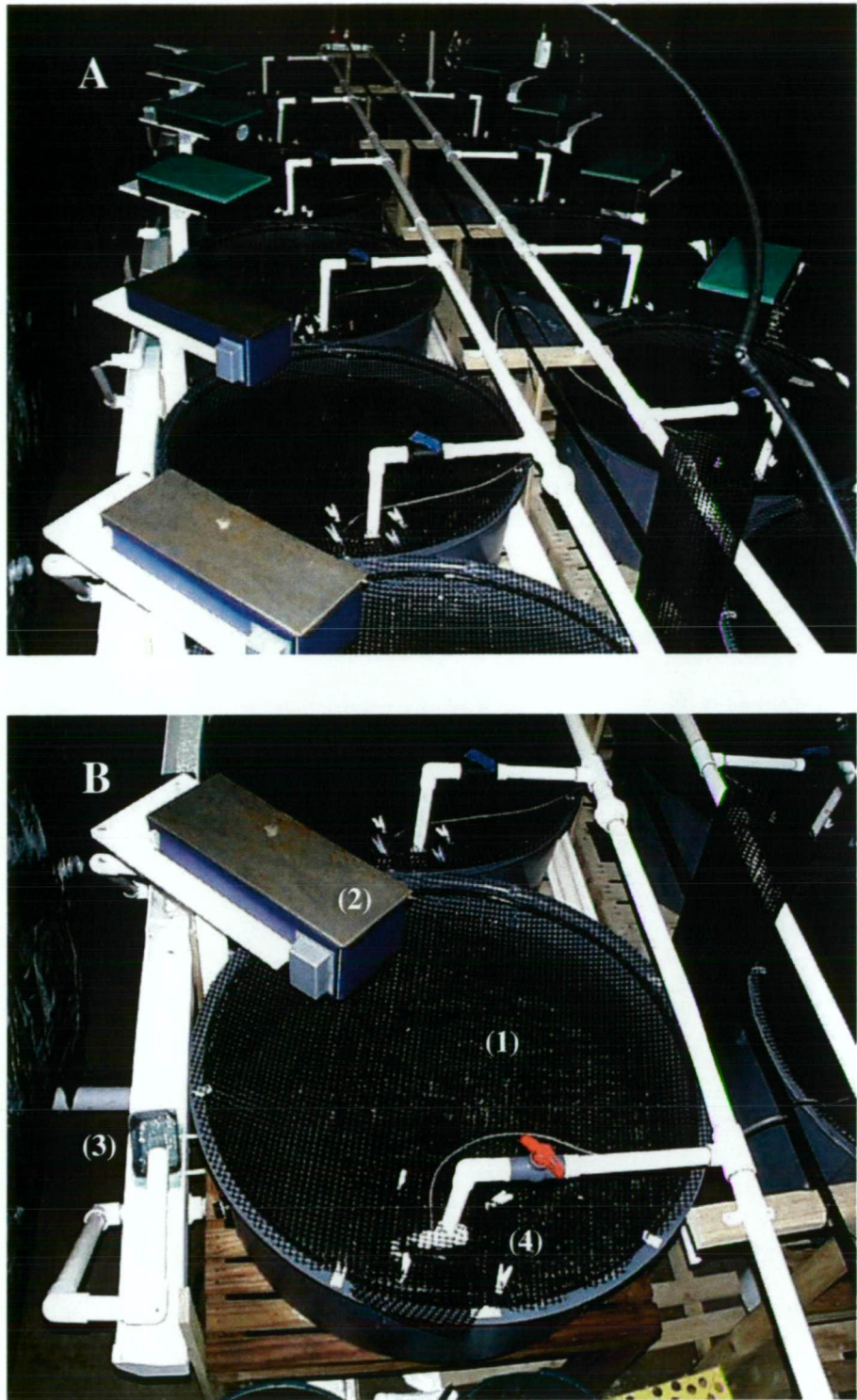
Plate 3.1 (A) Experimental system of 9 cylindrical 300 L tanks, each with a diameter of 110 cm. (B) Each tank (1) was fitted with a belt feeder (2) and excess feed collector (3). Inlets (4) faced in such a direction as to create centrifugal water flow.
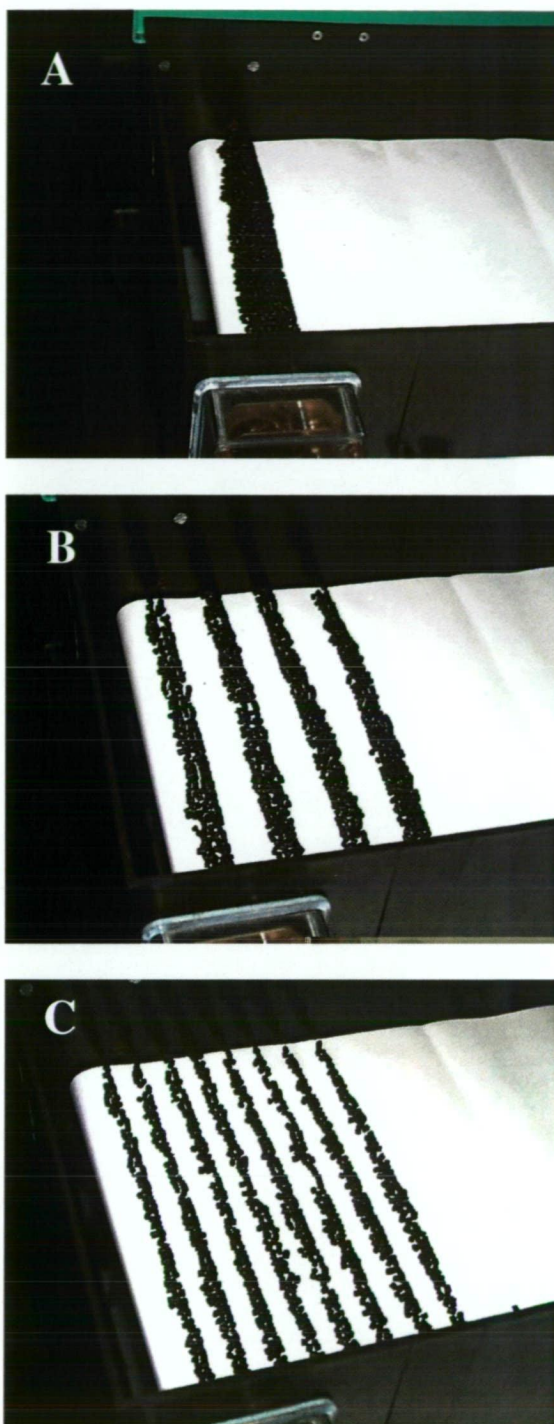
Plate 3.2 Belt feeders (30 cm across) with pellets lined up on each belt according to feeding frequency treatments. (A) One meal per day – 1F, (B) Four meals per day – 4F, (C) Eight meals per day – 8F.

3.4 Results

3.4.1 Preliminary experiment – Setting feeding frequency

There was no significant difference among the percentages of group feed intake accounted for by X-rays of all individual fish within tanks fed 1F, 5F or 10F (Figure 3.1). X-rays accounted for 89.5 to 98.0% of total group feed intake. Unaccounted ballotini beads were either lost in the system (stuck in stand pipes or the tank) or were eaten and defecated by fish before X-raying took place. The maximum number of meals for experiments 1 and 2 were set at 8 (over 8 h) to ensure all feeds occurred during daylight hours.

3.4.2 Growth performance

In experiment 1 mean survival was higher than 85% over the 9-week experiment and there was no significant difference in survival among treatments (Table 3.1). There were no significant differences in the final weight, final length, change in length, Δ CV weight or Δ CV length. The FW control fish had significantly higher weight gain than SW treatments but no difference was found between the SW treatments. There was also a significant difference in the trajectories of weight through time with fish transferred to SW showing reduced growth immediately following transfer which did not occur in fish kept in FW (Figure 3.2). The final K of FW fish was also found to be significantly greater than those transferred to SW but there were no significant differences in final K among any of the feeding frequency treatments used in SW (Table 3.1). A significant difference was found in the trajectories of K through time with a greater decrease in K for all groups of fish transferred to SW (Figure 3.3). The Δ CV K within tanks was also significant but in this case the difference was only between 4F fish and the 8F FW controls (Table 3.1). It is important to note that due to unresolved heterogeneity of variances there is a chance (due to type I error) that this difference in Δ CV K may not actually be significant and as such it must be viewed with some caution. The variation in K within tanks decreased in all cases but the percent decrease was only significantly less in 4F than 8F FW.

In experiment 2 mean survival was higher than 92% over the 9-week experiment. There was no significant difference in survival among feeding frequency treatments (Table 3.2). No significant differences were found for final weight, weight gain, final length, change in length, final K, change in K, change in CV weight, change in CV length, or change in CV K. There were also no significant differences in the growth trajectories for the three feeding frequencies (Figure 3.4).

3.4.3 Group feed intake

In experiment 1 group feed intake (mg g^{-1} d^{-1}) varied through time (Figure 3.5). After transfer to SW feed intake decreased in all treatment groups. Feed intake also decreased in the FW control (8F FW). All SW transferred groups had greater decreases than the control (8F FW), with 1F initially significantly lower than all other treatments. Over time feed intake in all treatments increased and within approximately 10 d all the SW treatments had converged to a similar feed intake. On the final day in which feed intake was measured, 21 d following SW transfer (day 42), there was no significant difference between any of the SW transferred treatments. Significant differences were only found on this day between 1F and 8F FW and between 8F and 8F FW (Figure 3.5). Mean feed intake (mg g^{-1} d^{-1}) across all 8 d in which feed intake was measured after transfer to SW was significantly lower for 1F than all other treatments, and significantly higher for 8F FW than all other treatments. There was no significant difference between 4F and 8F (Figure 3.6). Similarly the feed conversion during the SW phase was significantly better in the 8F FW treatment than 1F; no differences were found between any of the other treatments (Figure 3.7).

In experiment 2 group feed intake (mg g^{-1} d^{-1}) increased with time (Figure 3.8), but from the commencement of feeding regime treatments till the final weighing there were no clear differences in feed intake related to treatment. Mean feed intake (mg g^{-1} d^{-1}) was not significantly different among treatments, and nor was FCR (Figure 3.9). The standard error for FCR was quite large for the 8F treatment due to one tank in this treatment having a very poor growth but still eating feed (Figure 3.9). There was no obvious explanation for this excessively poor growth.

3.4.4 Individual feed intake and stability of feeding rank

During experiment 1 the percentage of group feed intake accounted for by X-rays of all individual fish within tanks was in most cases greater than 100% for 1F and less than 100% for 8F (Figure 3.10).

A significant positive correlation was found between the two sets of feeding ranks (SM) in FW (Table 3.3) in three of the groups transferred to SW (1F Tank 1; 4F: Tank 3; 8F: Tank 2). A significant positive correlation was also found between the two sets of feeding ranks (SM) in SW in seven of the groups transferred to SW (1F Tank 9; 4F Tank 3, 5 and 6; 8F Tank 2, 7 and 8). Only one of the SW transferred groups had a significant positive correlation between MSM in FW and SW (8F Tank 7). Two of the SW transferred groups that showed positive correlations in FW also showed positive correlations in SW but not between the two (4F Tank 3; 8F Tank 2). In the FW controls no significant correlations were found (Table 3.3).

CVs for inter-individual (within-group) variation in feed intake (mg g^{-1} d^{-1}) were not significantly different for any of the treatments during the FW phase. At the first individual feed intake measurement in SW the 1F treatment had a significantly larger CV than 8F FW treatment. 1F also had larger CVs than 4F and 8F however these differences were not statistically significant. By the second feed intake measurement in SW the CV of all of the SW treatments had decreased and no significant effect of the treatments was present. None of the final CV values were significantly different from the FW values (Figure 3.11).

During the FW phase only two groups had a significant negative correlation between CVc and MSM (8F Tank 2; 8F FW Tank 12) (Figure 3.12). During the SW phase the relationship between CVc and MSM was found to be significant and negatively correlated in two of the three tanks in both the 1F (Tank 1 and 4) and the 4F treatment (Tank 3 and 5), and none of the tanks from either the 8F or 8F FW treatment.

3.4.5 Chemical composition

The percentage of dry matter, crude lipid and gross energy all tended to increase with increasing K. For each of the three treatments in which fish were transferred to SW highly significant regressions were found for the relationships between dry matter and K, crude lipid and K, and gross energy and K. ANCOVA revealed no significant difference in the slopes of regressions for each treatment and no significant treatment effects. Common significant regressions are plotted with equations representing the relationships between condition factor (K) and tissue components

Figure 3.13). Crude protein was found to have a significant regression only for the 4F treatment and the relationship between condition factor and % crude protein is described by the equation: 4F % crude protein = -0.43 (± 0.39) + 0.10 (± 0.03) K (r^2 = 0.593; n = 12; p = 0.003).

3.4.6 Osmoregulation

In experiment 1 the mean tank osmolality at the end of the experiment was found to be significantly different but only between the SW treatments and the FW control (Figure 3.14). The CV within tanks was not found to be significantly different for any of the treatments (Figure 3.14). On examination of all the fish in the experiment there was no significant correlation between individual fish's values of K and osmolality (Figure 3.15).

In experiment 2 the mean tank osmolality at the end of the experiment was found to be significantly different with 1F fish having significantly higher osmolality values than 4F fish. This may not be biologically significant given that there was no significant difference between the 8F fish and other treatments (Figure 3.16). The CV within tanks was not found to be significantly different from any of the treatments (Figure 3.16). On examination of all fish in this experiment there was no significant correlation between individual fish values of K and osmolality (Figure 3.17). The mean osmolality readings for groups of fish transferred to SW in experiment 1 were lower (327.36 ± 3.49 mmol kg^{-1}, mean ± SD) than those for fish in experiment 2 (342.65 ± 8.59 mmol kg^{-1}, mean ± SD) (Figure 3.14 and Figure 3.16).

3.4.7 Fish with low final condition (K) and low feed intake in SW tracked through time

In experiment 1 fish with a condition factor less than 0.95 (a total of 9 fish) at the final experimental weighing were tracked retrospectively through time to determine how they differed from the whole experimental population (represented by the population mean). At the beginning of the experiment the weight and length of these fish were dispersed evenly above and below the mean. By the final weighing they were all below both the mean weight and mean length (Figure 3.18). Before SW transfer both weight and length of all of these individuals were increasing. Following transfer only 3 of the fish increased in weight while all increased very slightly in length. Their K values were less than the mean in every case at the initial and final weighing and only two fish showed any increase in condition during their time in SW. Feed intake was distributed evenly above and below the mean at both of the FW X-rays, however the feed intake of 7 of the individuals decreased to almost 0 mg g^{-1} d^{-1} after one week in SW, a much greater decrease than the mean experienced by the rest of the SW transferred population. Of these 7 fish 3 had commenced eating again (9.6 ± 3.6 mg g^{-1} d^{-1}, mean ± SD) by the time of the final X-ray while 4 fish remained very close to zero (0.6 ± 0.9 mg g^{-1} d^{-1}, mean ± SD).

Fish that had a weight specific feed intake less than 2 mg g^{-1} d^{-1} at both of the X-raying sessions after transfer to SW (a total of 10 fish) were also tracked through time as above. Four also had a final K less than 0.95 and as such were also in the previous analysis (fish below K = 0.95). As was the case above at the beginning of the experiment the weight and length of these fish was dispersed evenly above and below the mean and again by the final weighing they were all below the mean weight and length (Figure 3.19). Before transfer both weight and length of each of these individuals was increasing. However, all of these fish lost weight after transfer and continued to lose weight until the end of the experiment. All fish showed a very marginal increase in body length after transfer to SW. These fish were also dispersed evenly above and below the mean for K at the initial weighing but again by the final weighing the K of each of these fish was below the mean. Not one fish gained in K during their time in SW. Feed intake was distributed evenly above and below the mean at both of the FW X-rays before decreasing to and staying below 2 mg g^{-1} d^{-1} after transfer.

The positions in feeding hierarchies (based on MSM rank) for each of the fish that had a final K of less than 0.95 and those that ate less than 2 mg g^{-1} d^{-1} at both SW X-rays varied from 2nd most dominant to least dominant in the FW phase. Following seawater transfer all but 2 individuals held low (subordinate) positions in feeding hierarchies (Table 3.4). The final osmolality values for these fish were examined individually and compared with the mean osmolality value for the entire population of fish that were transferred to SW (Table 3.4). There was no evidence that any of these fish with poor condition or low feed intake were undergoing osmoregulatory failure. The poor conditioned fish had representatives from each treatment (1F, 3 fish; 4F, 2 fish; 8F, 4 fish) as did those with low feed intake (1F, 3 fish; 4F, 2 fish; 8F, 6 fish).
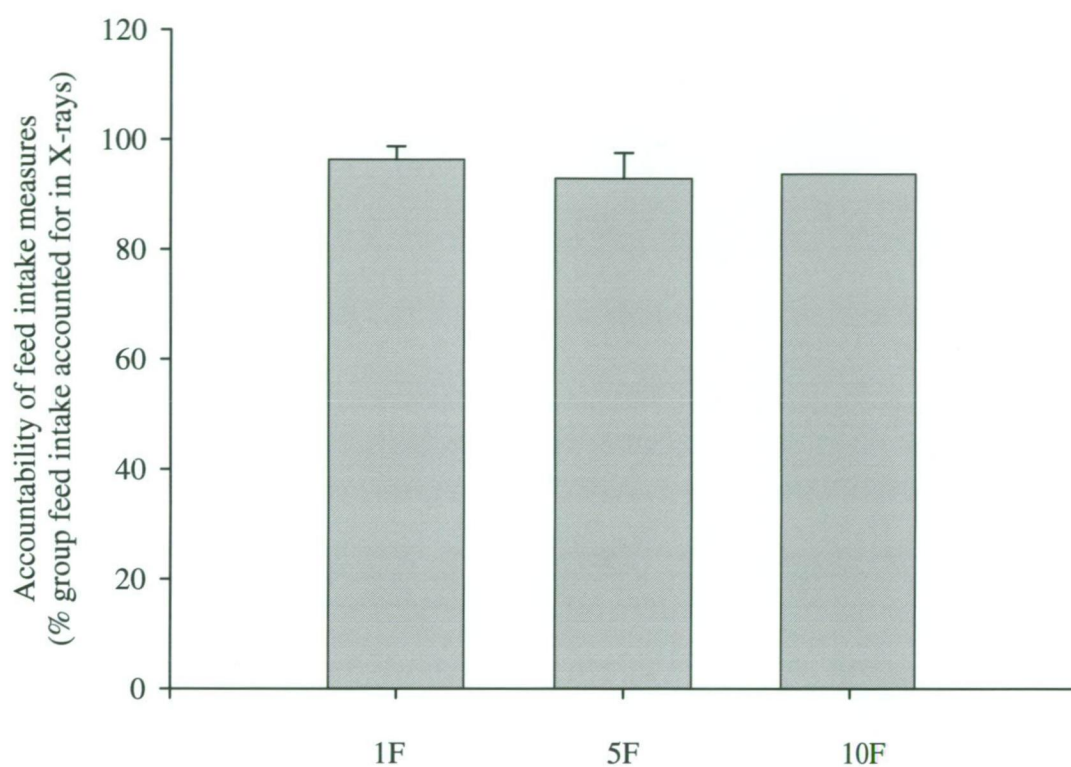
Figure 3.1 Accountability of feed intake measures (% group feed intake accounted for in X-rays) in preliminary experiment - The percentage (mean ± SD) feed intake accounted for in each treatment by X-radiography. Only one replicate for 10F. ANOVA showed no significant difference between means (F = 0.459, *df* 2,2, p = 0.685).

Table 3.1 Experiment 1 - performance of Atlantic salmon fed on feeding regimes of 1F, 4F, 8F or 8F FW after transfer to SW (mean ± S.E.M, n = 3 replicate tanks. For each parameter the mean is written above the S.E.M). Means with the same letter are not significantly different (Tukey's multiple comparison test $p < 0.05$). Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. FW = freshwater; SW = seawater.

Parameter	Unit	Treatment				F	df	p
		1F	4F	8F	8F FW			
WEIGHT								
Initial weight	(g)	70.6	71.8	71.9	69.5	0.614	3, 8	0.625
		1.5	1.5	1.5	1.0			
Final weight	(g)	120.9	128.5	126.4	138.3	3.779	3, 8	0.059
		4.6	2.8	3.9	3.5			
Weight gain	(g)	50.4[a]	56.8[a]	54.5[a]	68.8[b]	8.895	3, 8	0.006**
		3.5	1.3	2.6	2.7			
Δ CV weight	(%)	5.6	-6.9	10.8	-19.0	1.202	3, 8	0.369
		4.5	5.7	21.8	8.0			
LENGTH								
Initial length	(mm)	187.9	189.5	188.4	187.2	0.402	3, 8	0.755
		1.8	1.0	1.4	1.7			
Final length	(mm)	225.1	228.4	226.7	229.5	0.661	3, 8	0.599
		2.9	1.4	1.8	3.1			
Change in length	(mm)	37.1	38.9	38.3	42.3	3.266	3, 8	0.080
		1.3	0.6	1.4	1.4			
Δ CV length	(%)	-3.2	-15.9	0.3	-18.4	0.929	3, 8	0.470
		13.8	3.6	12.3	3.6			
CONDITION FACTOR								
Initial K		1.06	1.04	1.06	1.05	0.603	3, 8	0.631
		0.013	0.006	0.003	0.016			
Final K		1.05[a]	1.07[a]	1.07[a]	1.14[b]	12.029	3, 8	0.002**
		0.004	0.011	0.010	0.017			
Change in K		-0.0058[a]	0.0252[a]	0.0055[a]	0.0864[b]	23.575	3, 8	< 0.001***
		0.0104	0.0048	0.0108	0.0061			
Δ CV K ●	(%)	-12.6[ab]	-6.9[b]	-11.7[ab]	-28.8[a]	6.253	3, 8	0.017*
		4.7	5.9	1.0	0.8			
OVERALL SURVIVAL ● (%)		88.4	85.5	100	85.5	1.374	3, 8	0.319
		9.5	2.9		6.3			

● Data found not to be homogeneous but for which no transformation could remove heterogeneity.
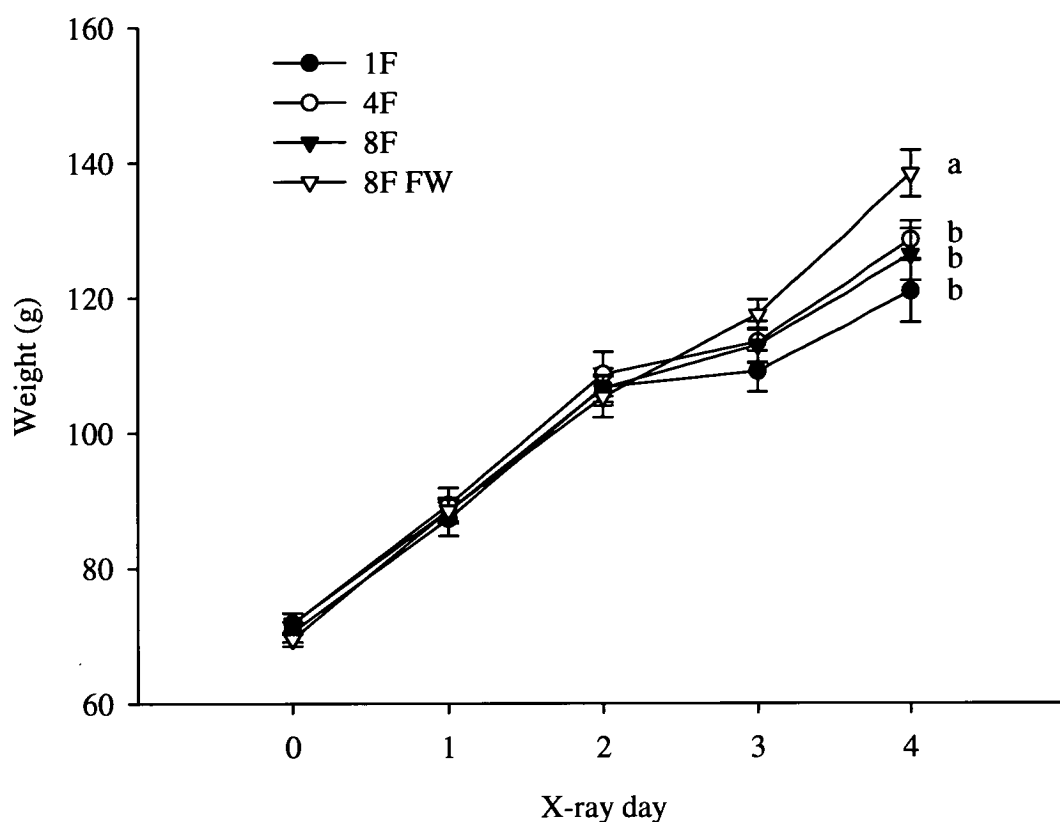
Figure 3.2 Experiment 1 - weight of each treatment (mean ± S.E.M) at initial weighing (shown as day 0) and at each X-ray. Repeated measures analysis using the greenhouse geisser epsilon value indicated that treatment trajectories behave significantly differently through time (F = 9.314, df = 4.614, 12.305, p < 0.001***). A series of pair wise repeated measures was then used to determine which specific treatments differed. Treatments with the same letter did not have significantly different growth trajectories through time.
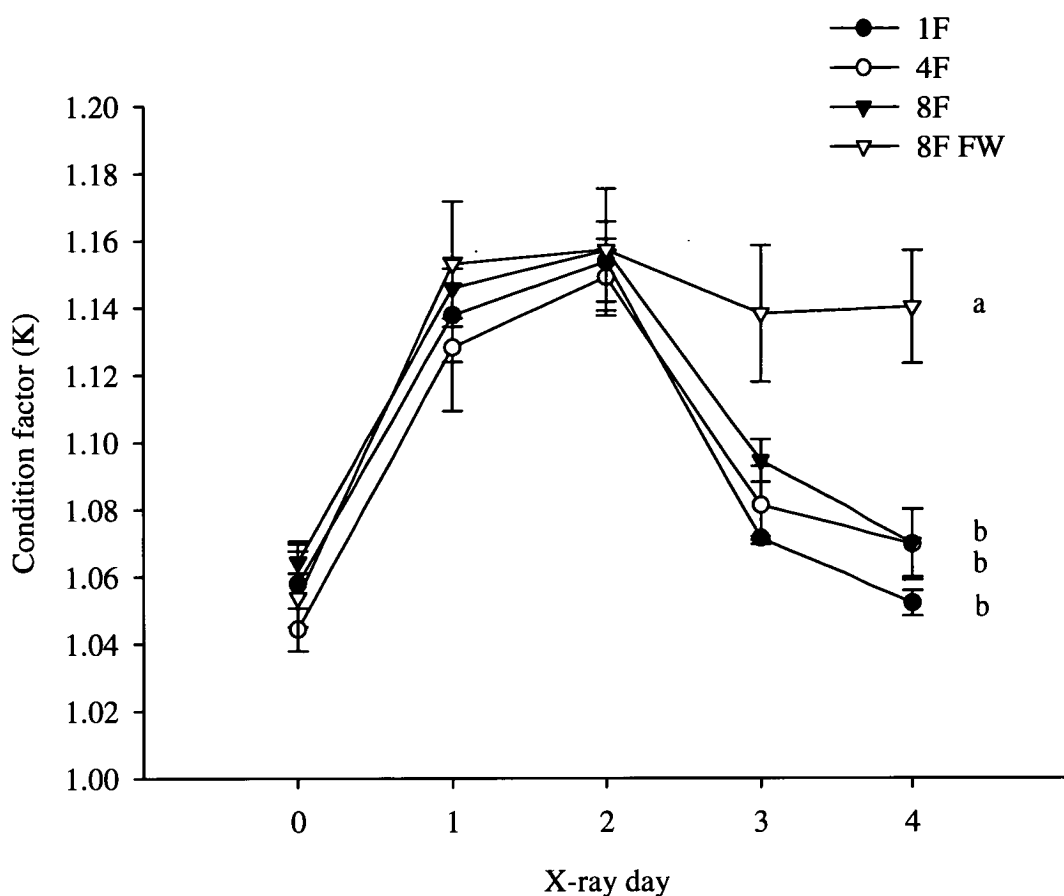
Figure 3.3 Experiment 1 – condition factor (K) of each treatment (mean ± S.E.M) at initial weighing (shown as day 0) and at each X-ray. Repeated measures analysis using the greenhouse geisser epsilon value indicated that treatment trajectories behave significantly differently through time (F = 9.045, df = 12, 32, p < 0.001***). A series of pair wise repeated measures was then used to determine which specific treatments differed. Treatments with the same letter did not have significantly different trajectories through time.

Table 3.2 Experiment 2 - performance of Atlantic salmon fed on feeding regimes of 1F, 4F, 8F after transfer to SW (mean ± S.E.M, n = 3 replicate tanks. For each parameter the mean is written above the S.E.M). Means with the same letter are not significantly different (Tukey's multiple comparison test $p < 0.05$). SW = seawater.

Parameter	Unit	Treatment			F	df	p
		1F	4F	8F			
WEIGHT							
Initial weight	(g)	127.5	126.9	126.9	1.521	2, 9	0.270
		0.3	0.1	0.3			
Final weight	(g)	153.5	162.3	143.0	1.530	2, 9	0.268
		7.5	9.5	6.0			
Weight gain	(g)	26.0	35.3	16.1	1.547	2, 9	0.265
		7.4	9.5	5.9			
Δ CV weight	(%)	309.2	381.7	343.8	1.072	2, 9	0.382
		45.7	32.0	23.9			
LENGTH							
Initial length	(mm)	222.2	221.9	222.1	0.074	2, 9	0.929
		0.5	0.7	0.6			
Final length	(mm)	238.4	240.5	236.6	0.958	2, 9	0.420
		1.0	2.8	1.8			
Change in length		16.2	18.6	14.5	1.067	2, 9	0.384
		1.4	2.8	1.6			
Δ CV length	(%)	109.7	145.7	120.6	1.060	2, 9	0.386
		17.8	17.2	18.8			
CONDITION FACTOR							
Initial K		1.16	1.16	1.16	0.050	2, 9	0.951
		0.009	0.013	0.008			
Final K		1.08	1.10	1.03	1.464	2, 9	0.281
		0.041	0.025	0.021			
Change in K		-0.079	-0.066	-0.131	1.677	2, 9	0.240
		0.032	0.023	0.024			
Δ CV K	(%)	223.9	271.7	217.7	0.622	2, 9	0.559
		37.6	31.5	42.6			
OVERALL SURVIVAL	(%)	93.0	93.0	92.0	0.100	2, 9	0.906
		2.5	1.9	0.0			
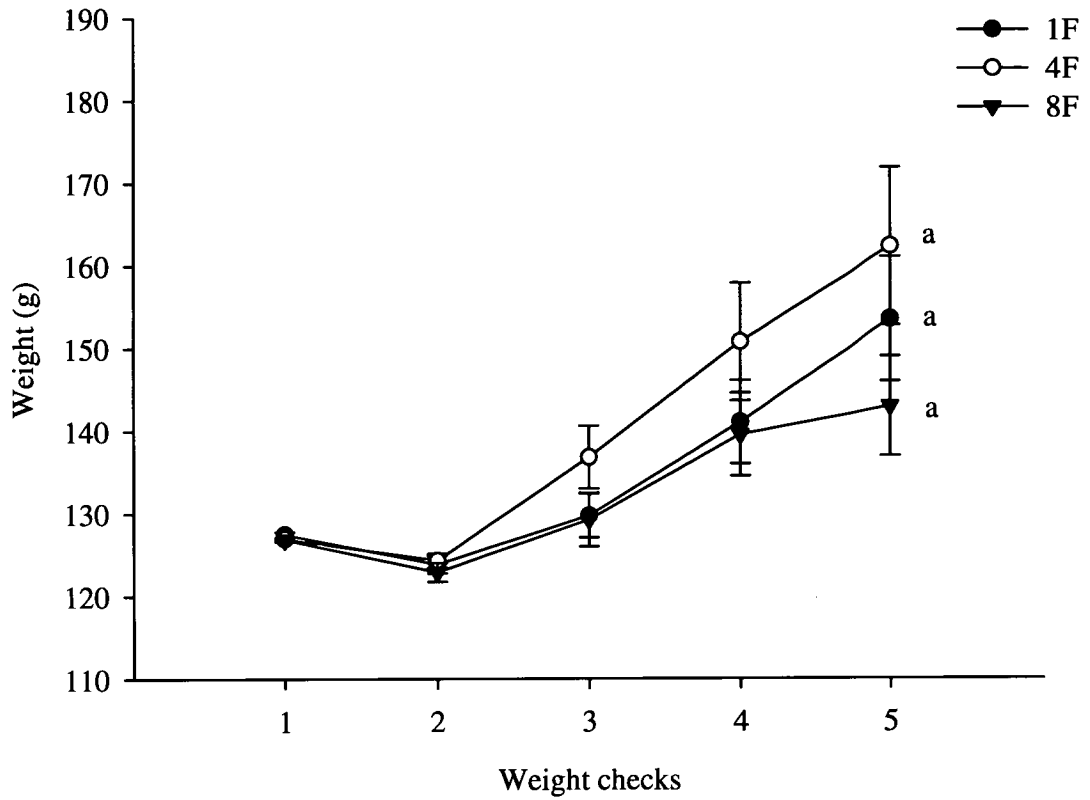
Figure 3.4 Experiment 2 - weight of each treatment (mean ± S.E.M) at three weekly weight checks. Repeated measures analysis using the greenhouse geisser epsilon value indicated no significant difference between the behaviour of treatment trajectories through time (F = 1.486; *df* = 2.211, 9.948; p = 0.274).
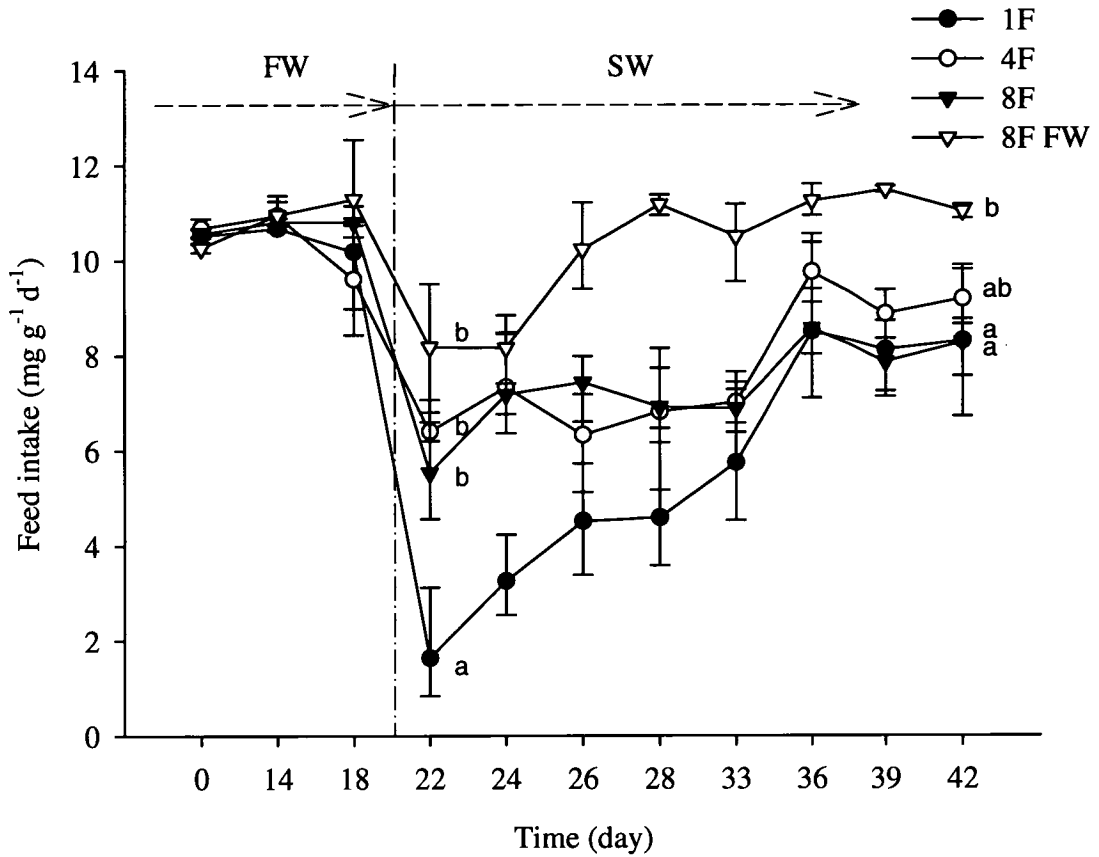
Figure 3.5 Experiment 1 – daily feed intake (mean ± max and min) for each treatment. Transfer to SW (̄ ̄ ̄) occurred 21 d after X-ray day 1. On the day following SW transfer (day 22) ANOVA found significant difference between treatments (F = 17.185, df 3, 8, p < 0.001***). Also on the final day on which feed intake was measured in SW (day 42) ANOVA found significant difference between treatments (F = 6.287, df 3, 8, p < 0.05*). Means with the same letter are not significantly different for each day (Tukey's multiple comparison test p < 0.05). No statistical comparisons were made between day 22 and day 42. FW = freshwater; SW = seawater.
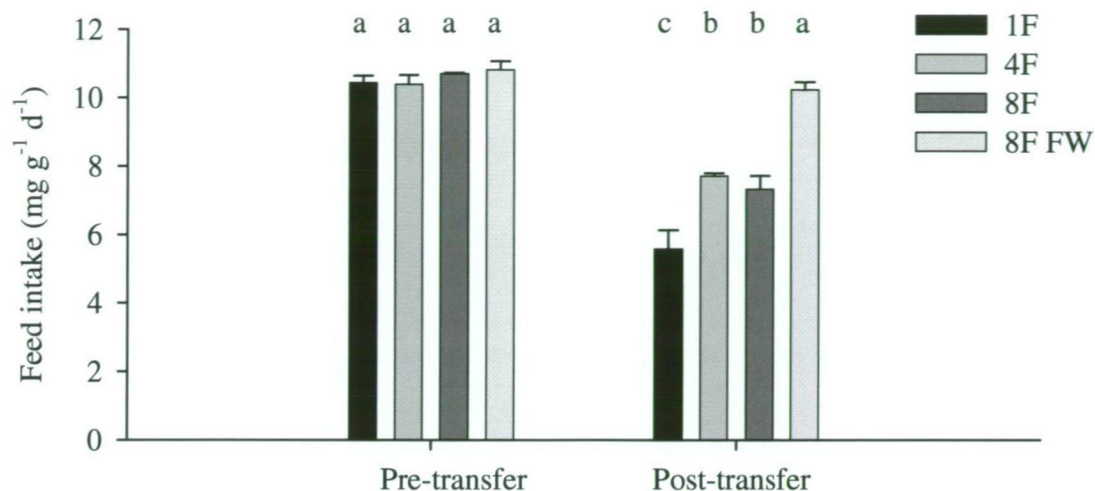
Figure 3.6 Experiment 1 – mean feed intake, mg g^{-1} d^{-1}, (mean ± S.E.M) for all 3 d during the FW phase combined and mean feed intake for all 8 d during the SW phase combined. ANOVA showed significant difference (F = 45.07, *df* 7, 16, p < 0.001***). Means with the same letter are not significantly different (Tukey's multiple comparison test p < 0.05). FW = freshwater; SW = seawater.
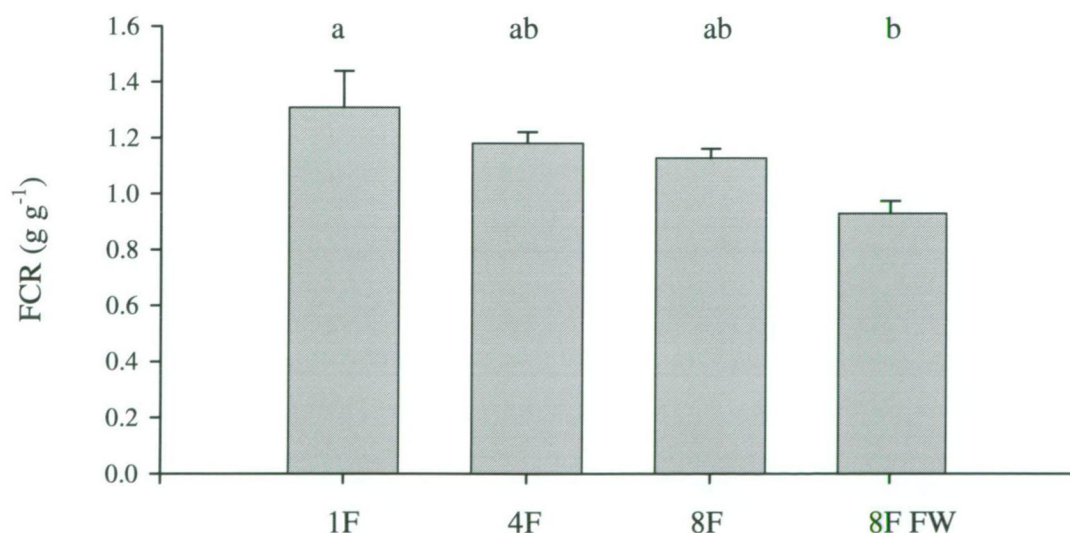
Figure 3.7 Experiment 1 - feed conversion ratio (FCR) for each treatment (mean ± S.E.M) calculated from the weighing 7 d prior to transfer till final weighing 21 d post transfer to SW (end of SW phase). ANOVA showed significant difference (F = 4.538, *df* 3, 8, p = 0.039*). Columns with the same letter are not significantly different (Tukey's multiple comparison test p < 0.05). FW = freshwater; SW = seawater.
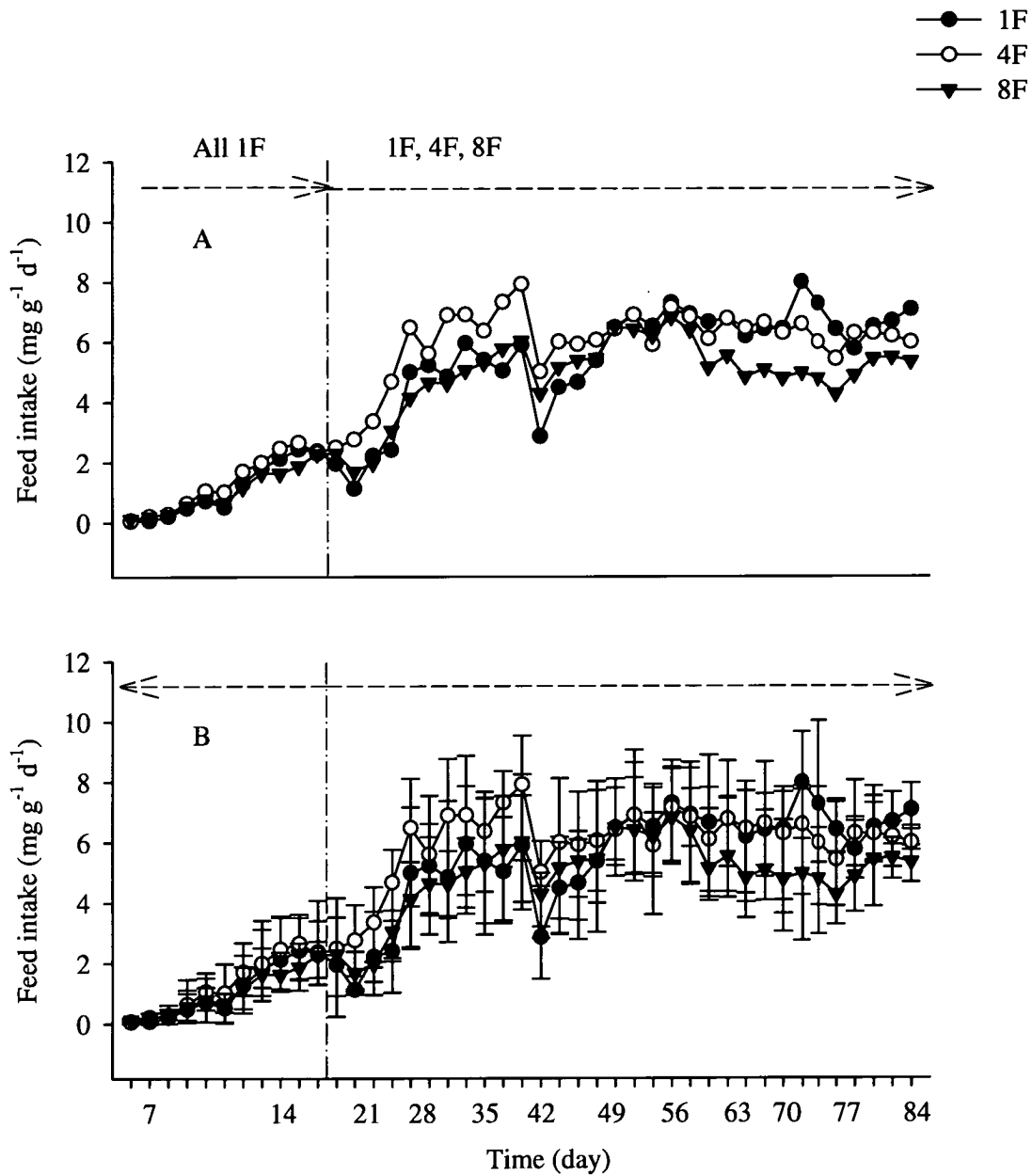
Figure 3.8 Experiment 2 – (A) daily feed intake (mean) for each treatment with day 0 being the first d any pellets were taken, (B) daily feed intake (mean ± max and min). Data was collected every d during the first 2 weeks (prior to commencement of treatments) and then every second d after treatments began. Commencement of treatments (1F, 4F, 8F) indicated with broken line (-----).
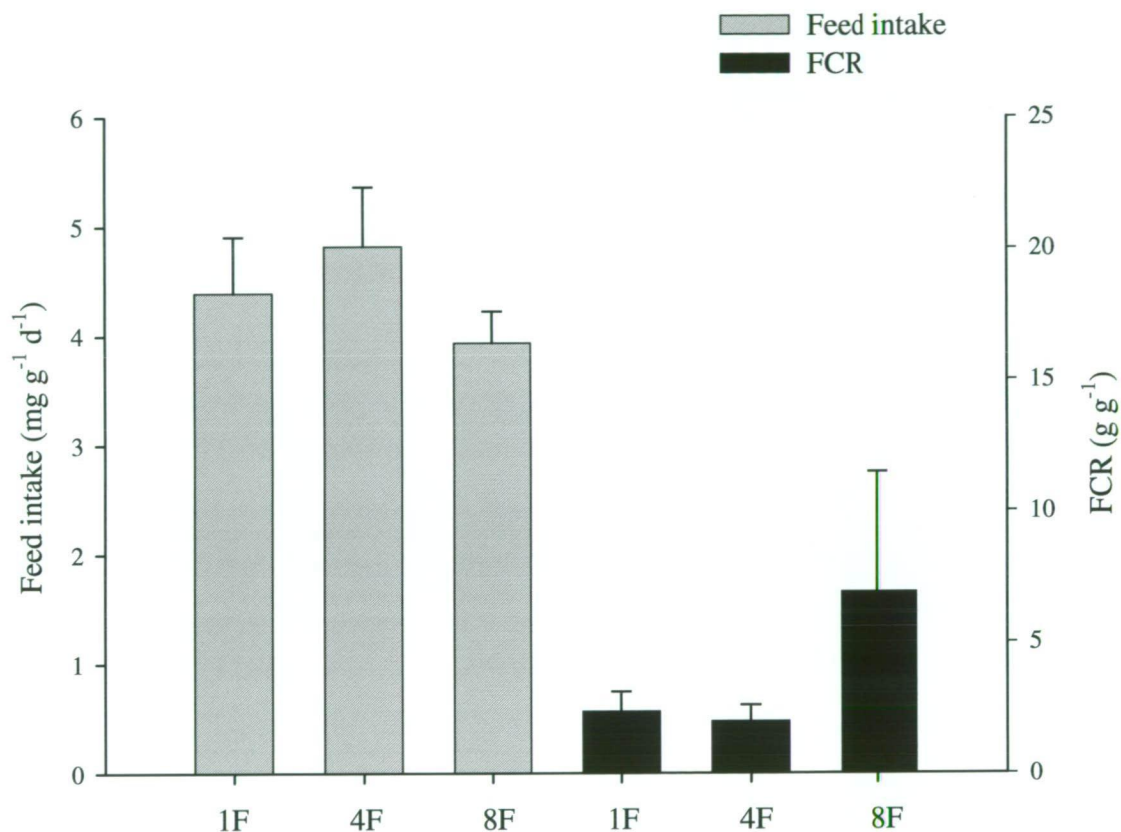
Figure 3.9 Experiment 2 – feed intake, mg g^{-1} d^{-1}, (mean ± S.E.M), and FCR for entire experiment (mean ± S.E.M). ANOVA showed no significant difference in either the group intake (F = 0.908, *df* 2, 9, p = 0.437), or feed conversion ratio (FCR) (F = 1.027, *df* 2, 9, p = 0.396).
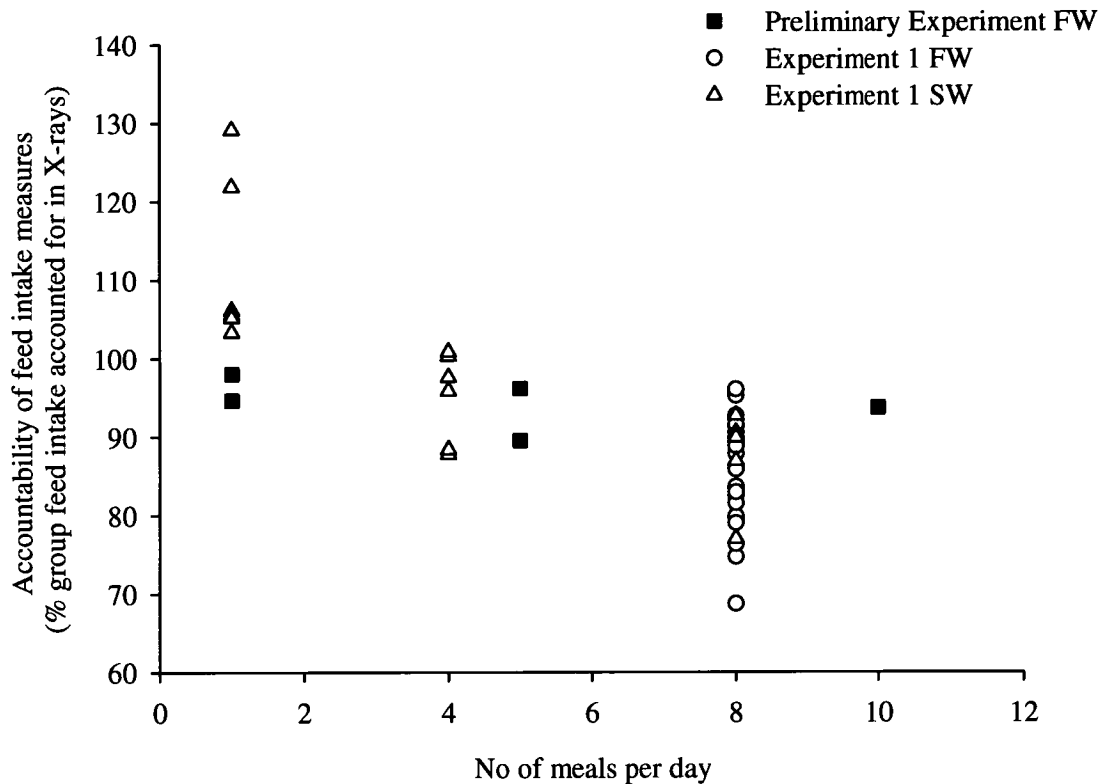
Figure 3.10 Accountability of feed intake measures (% group feed intake accounted for in X-rays) for preliminary experiment and experiment 1 - Feed intake accounted for in each treatment by X-radiography. Each symbol represents the percent of group feed intake accounted for in X-rays from each tank at single X-ray times. Different symbols are used to distinguish preliminary experiment fish X-rayed in FW, and experiment 1 fish X-rayed in FW and SW. FW = freshwater; SW = seawater.

Table 3.3 Spearman's correlation coefficients for feed intake rank (SM) correlations between each pair of X-rays in FW and SW and between the MSM ranks in FW and SW. Data are presented for each tank from each treatment. Levels of significance * $p < 0.05$; ** $p < 0.01$. FW = freshwater; SW = seawater.

Treatment	Group (Tank no.)	FW X-ray 1 vs 2	SW X-ray 3 vs 4	MSM FW vs MSM SW
1F	1	0.417 * (n = 23)	0.309 (n = 23)	0.334 (n = 23)
	4	0.331 (n = 22)	0.279 (n = 22)	-0.291 (n = 22)
	9	-0.238 (n = 17)	0.681 ** (n = 16)	0.256 (n = 16)
4F	3	0.770 ** (n = 16)	0.489 * (n = 19)	-0.168 (n = 15)
	5	0.152 (n = 19)	0.710 ** (n = 19)	-0.047 (n = 19)
	6	0.420 (n = 21)	0.666 ** (n = 21)	0.164 (n = 21)
8F	2	0.429 * (n = 23)	0.528 ** (n = 23)	0.285 (n = 23)
	7	-0.534 * (n = 15)	0.569 ** (n = 23)	0.796 ** (n = 15)
	8	0.205 (n = 23)	0.617 ** (n = 23)	0.300 (n = 23)
8F FW	10	0.223 (n = 20)	0.245 (n = 20)	0.110 (n = 20)
	11	-0.113 (n = 19)	-0.049 (n = 18)	-0.038 (n = 18)
	12	0.338 (n = 23)	0.292 (n = 22)	0.015 (n = 22)
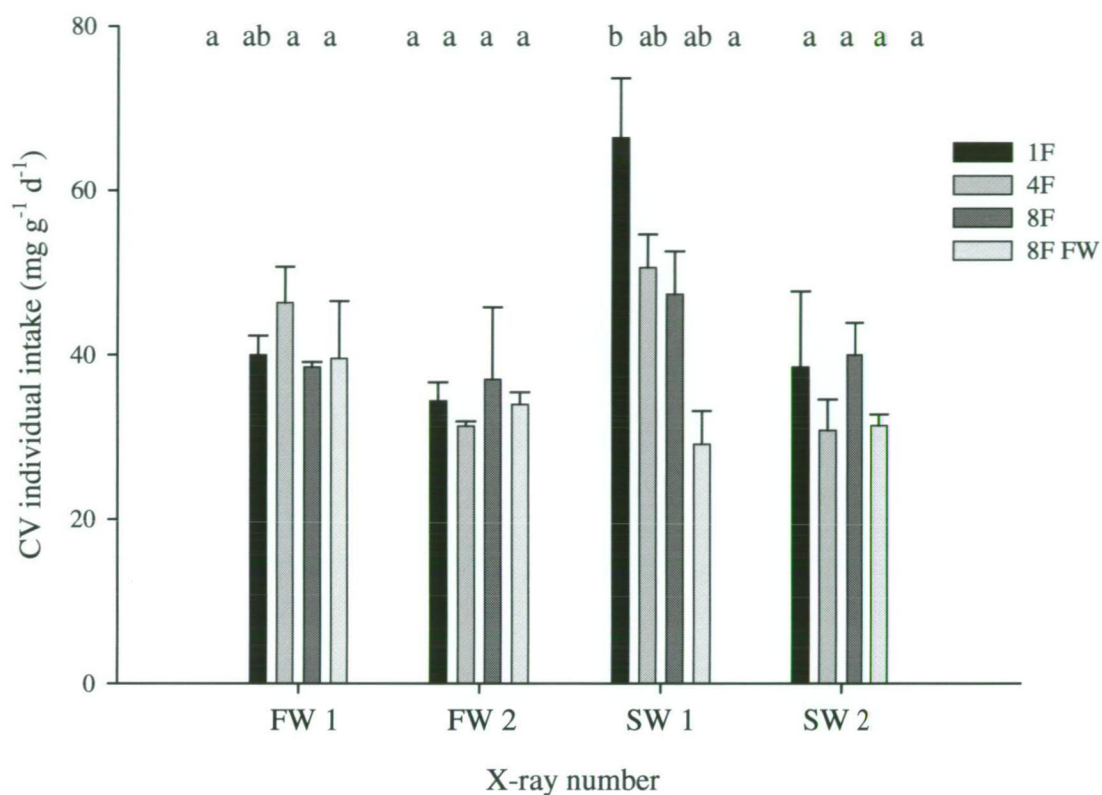
Figure 3.11 Experiment 1 - CV for each treatment (mean ± S.E.M) for feed intake by individual fish at each X-ray. ANOVA showed a significant difference (F = 3.690, *df* 15, 32, p = 0.001***). Means with different letters are significantly different (Tukey's multiple comparison test p < 0.05). FW = freshwater; SW = seawater.
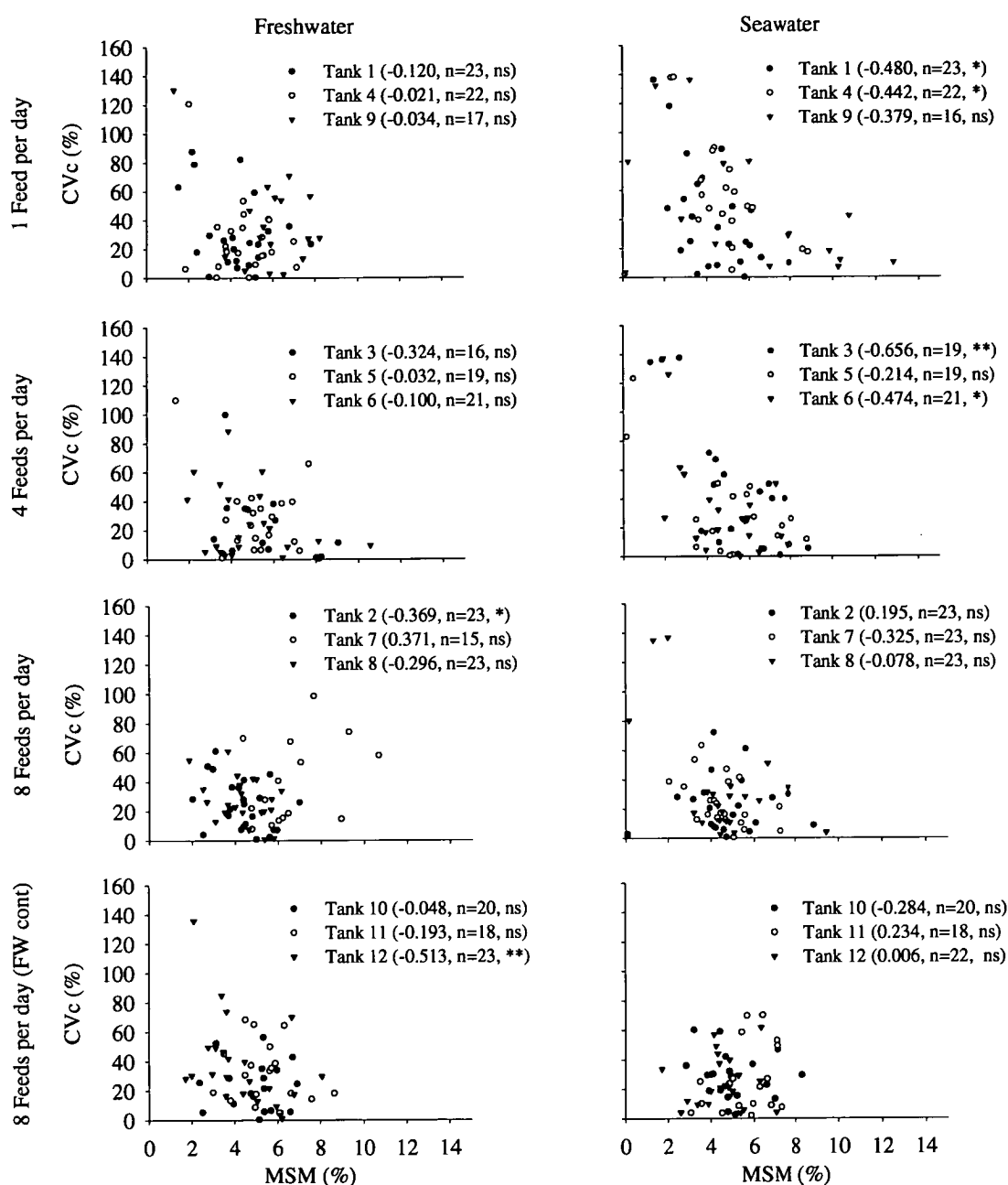
Figure 3.12 Experiment 1 – relationship between CV in weight specific consumption (mg g^{-1} d^{-1}) and MSM (mean percentage share of meal) for individual salmon based on absolute daily consumption rate (mg d^{-1}). Spearman correlation coefficient, n and significance are shown for each tank in during FW and SW phase of the experiment. FW = freshwater; SW = seawater.
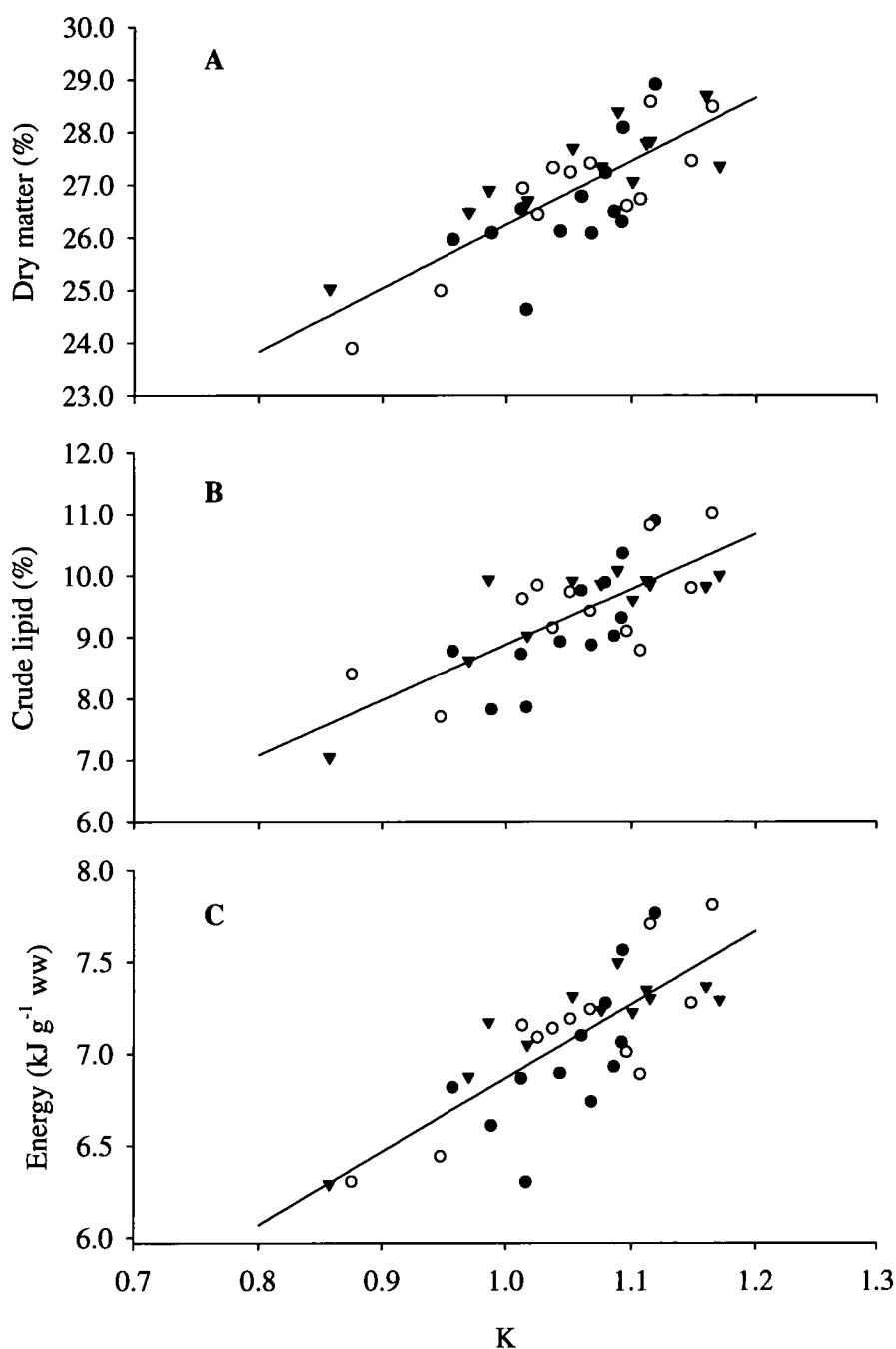
Figure 3.13 Experiment 1 – scatterplots showing the effects of condition factor on the chemical composition (% wet weight) of whole bodies. Significant common regressions lines (pooled for treatments) are plotted (n = 36). (A) dry matter = 14.18 (± 1.76) + 12.07 (± 1.66) K (r^2 = 0.608; n = 12; p < 0.001). (B) crude lipid = -0.117 (± 1.48) + 9.00 (± 1.40) K (r^2 = 0.549; n = 12; p < 0.001). (C) gross energy (kJ.g^{-1} ww) = 2.87 (± 0.60) + 4.00 (± 0.57) K (r^2 = 0.596; n = 12; p < 0.001). Fish from each of the SW treatments are indicated by different symbols: ● 1F; ○ 4F; ▼ 8F. SW = seawater.
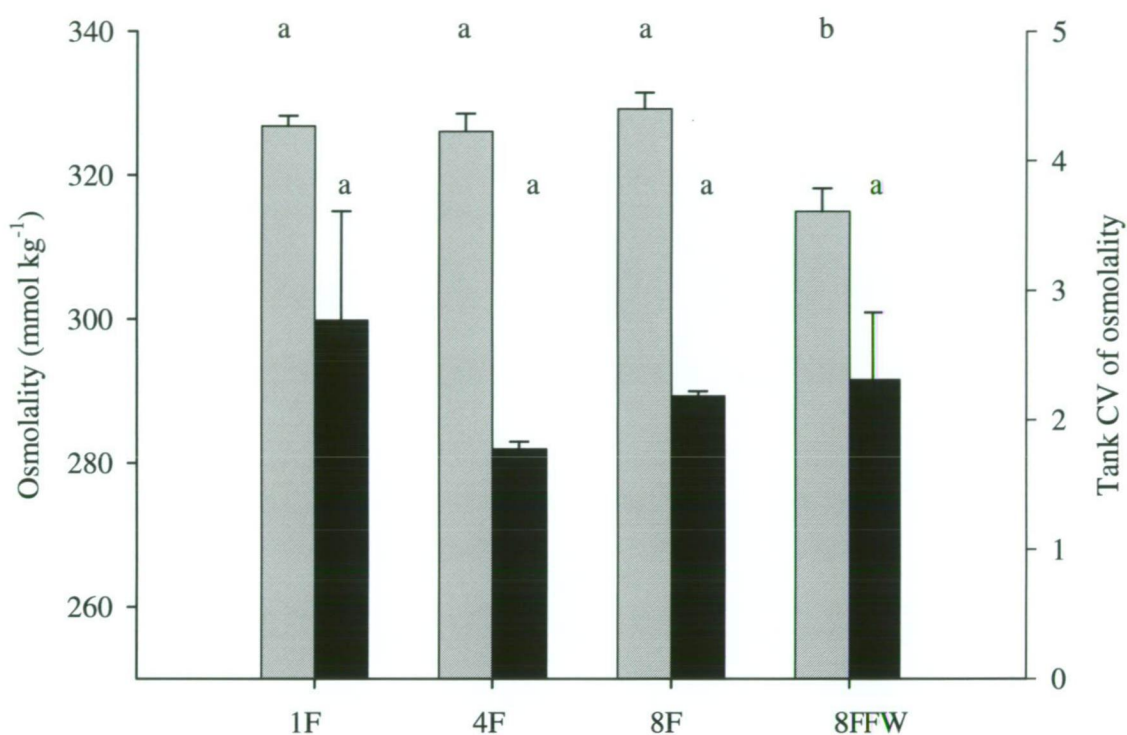
Figure 3.14 Experiment 1 - osmolality of each treatment (mean ± S.E.M) and CV for osmolality within tanks of each treatment (mean ± S.E.M). ANOVA showed significant difference (F = 6.779, *df* 3, 8, p = 0.014*) for mean tank osmolality. Osmolality means with different letters are significantly different (Tukey's multiple comparison test p < 0.05). No significant differences were found for CV of osmolality within tanks (F = 0.679, *df* 3, 8, p = 0.589).
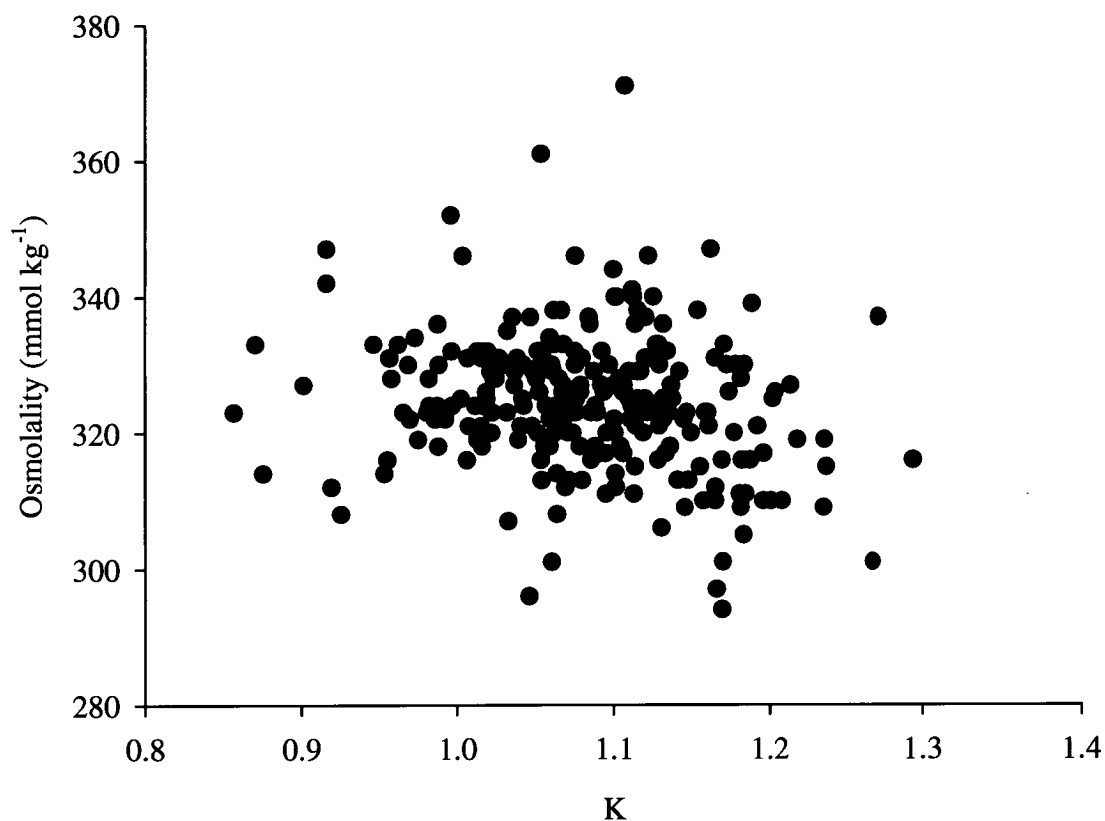
Figure 3.15 Experiment 1 – correlation between osmolality (mmol kg^{-1}) and K at the final sampling, after 23 d in SW. All individuals from all treatment levels transferred to sea have been pooled for this analysis. No significant correlation was found using Spearman's correlation (correlation coefficient = 0.101, n = 189, p = 0.083). SW = seawater.
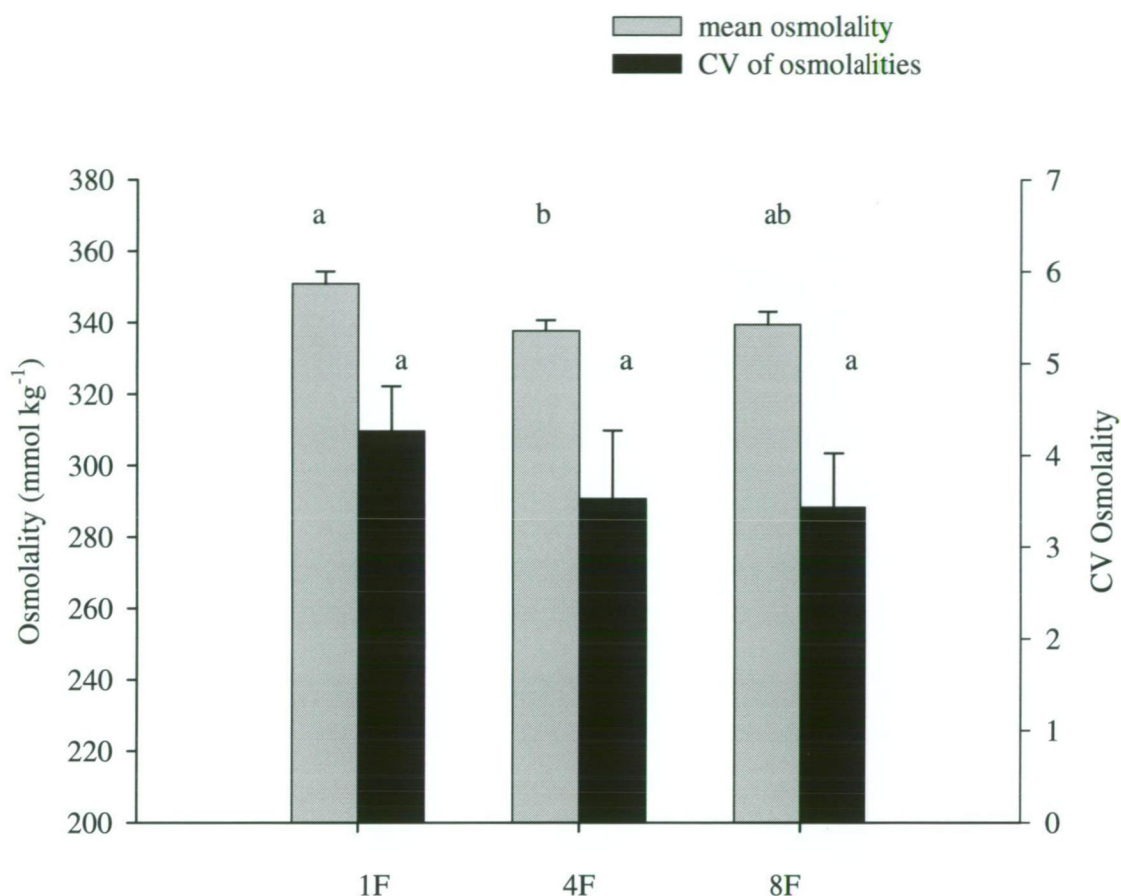
Figure 3.16 Experiment 2 - osmolality of each treatment (mean ± S.E.M) and CV for osmolality within tanks of each treatment (mean ± S.E.M). ANOVA showed significant difference (F = 4.511, *df* 2, 9, p = 0.044*) for mean tank osmolality. Tukey's multiple comparison test (p < 0.05) did not find a significant difference and it was therefore assumed that the difference was between the largest and smallest mean osmolality. Osmolality means with different letters are significantly different. No significant differences were found for CV of osmolality within tanks (F = 0.546, *df* 2, 9, p = 0.597).
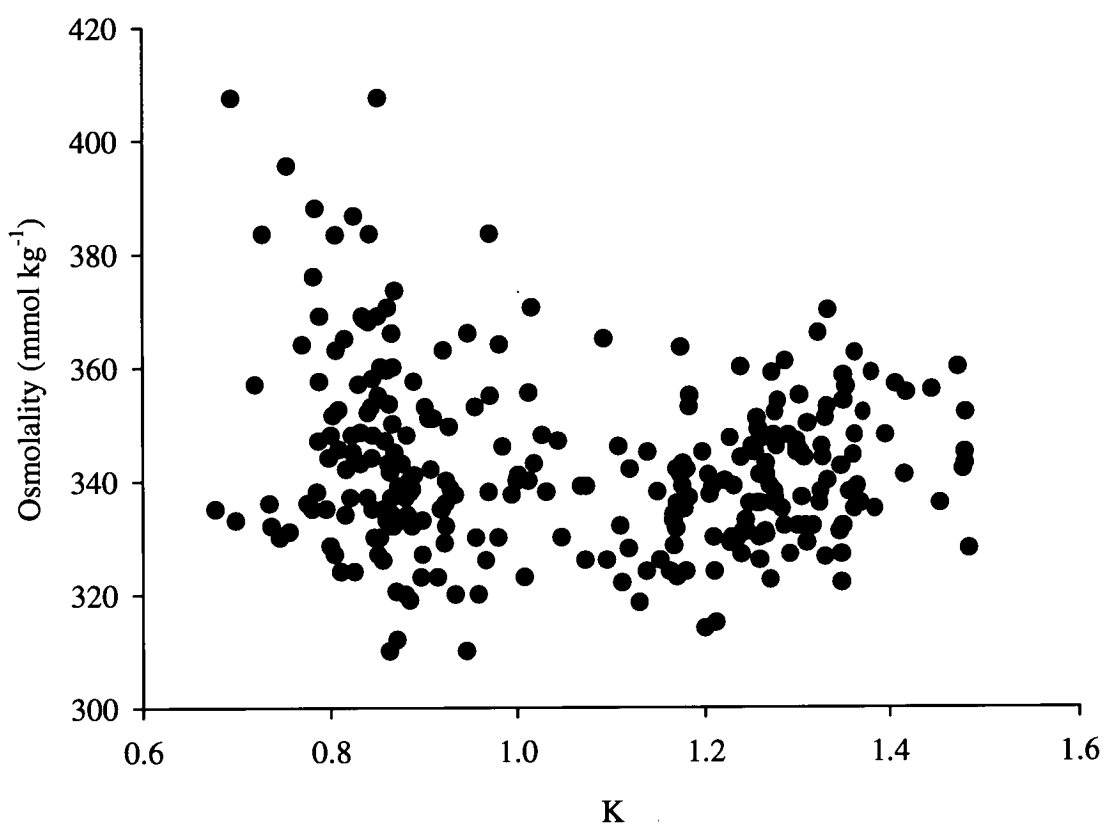
Figure 3.17 Experiment 2 – correlation between osmolality (mmol kg^{-1}) and K at the final sampling, after 87 or 88 d in SW. All individuals from all treatments levels have been pooled for this analysis. No significant correlation was found using Spearman's correlation (correlation coefficient = -0.085, n = 278, p = 0.079). SW = seawater.
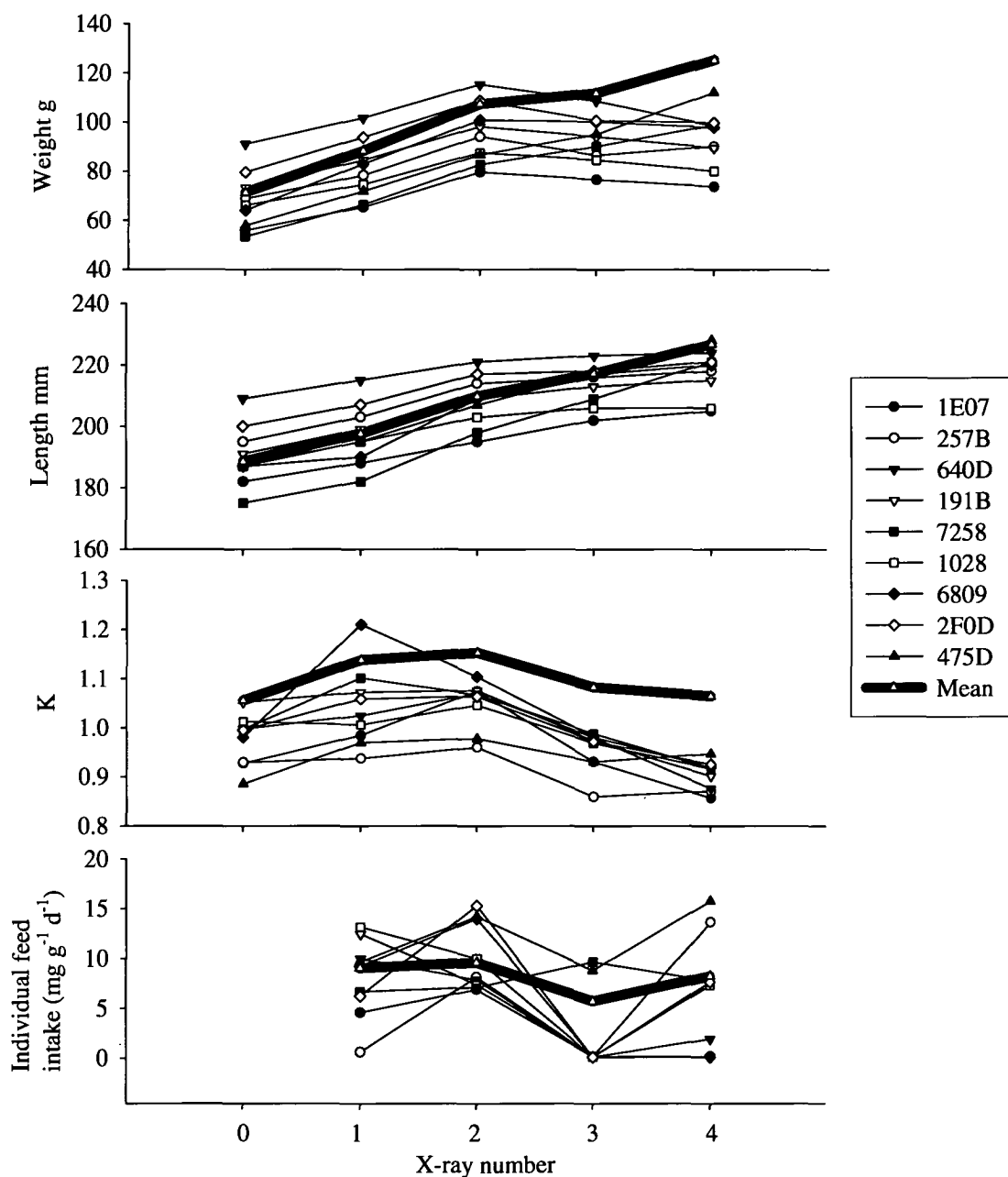
Figure 3.18 Experiment 1 – line graphs track all individual fish with a final K less than 0.95 through time from initial weighing till final X-ray. Fish were transferred to SW between X-ray 2 and 3. Graphs show: (A) weight, (B) length (C) K and (D) individual feed intake mg g^{-1} d^{-1}. Each thin line indicates an individual fish while the thick line shows the mean trend of all fish in the experiment which were transferred to SW. SW = seawater.
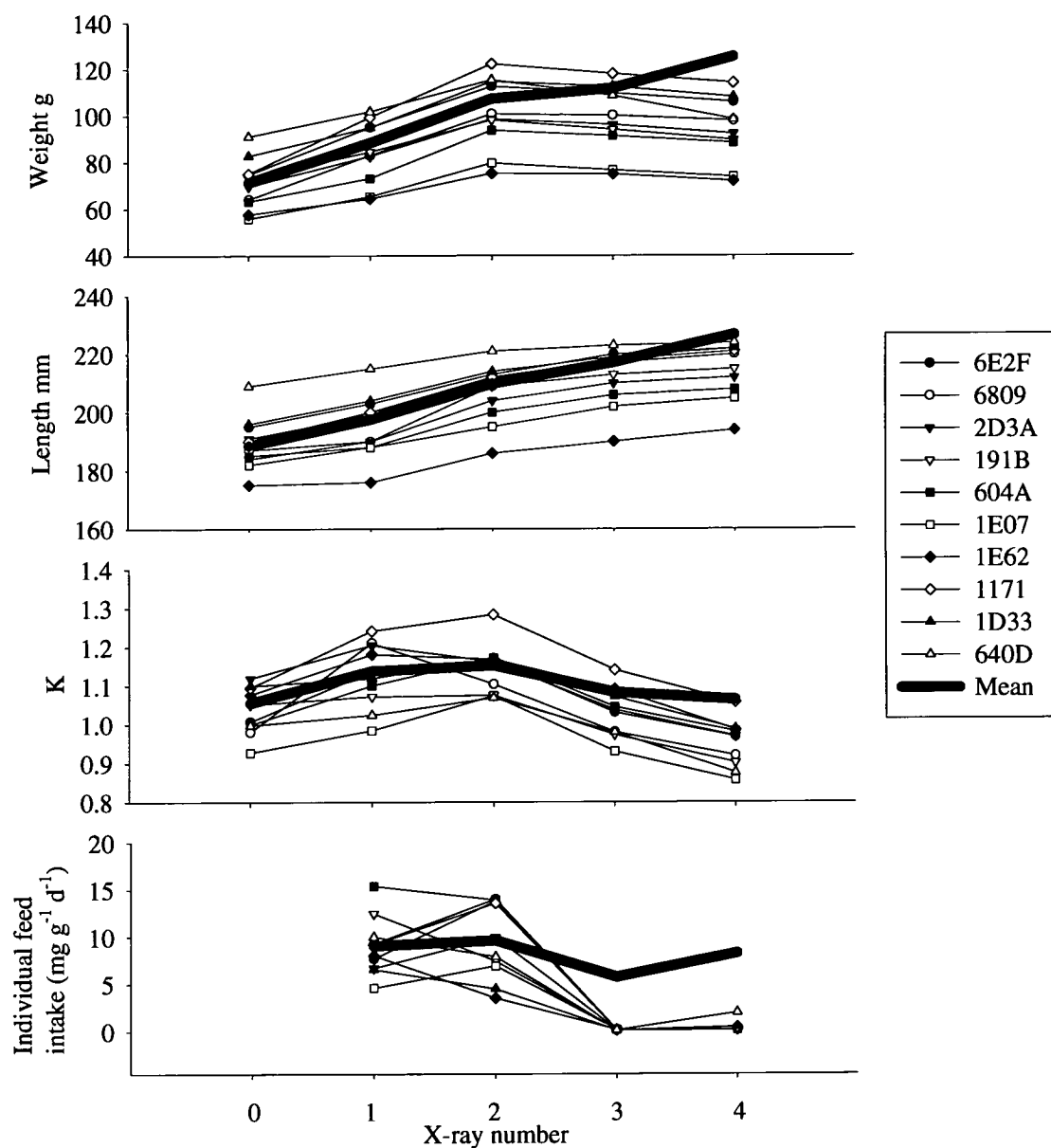
Figure 3.19 Experiment 1 – line graphs track all individual fish with intake of less than 2 mg g^{-1} d^{-1} at X-ray 3 and 4 through time from initial weighing till final X-ray. Fish were transferred to SW between X-ray 2 and 3. Graphs show: (A) weight, (B) length (C) K and (D) individual feed intake mg g^{-1} d^{-1}. Each thin line indicates an individual fish while the thick line shows the mean trend of all fish in the experiment which were transferred to SW. SW = seawater.

Table 3.4 Experiment 1 – final osmolalities and MSM rank positions in FW and SW of Atlantic salmon that had a final K of less than 0.95 and those that ate less than 2 mg g^{-1} d^{-1} on each of the X-ray feed intake measurement days in SW. Mean of all fish transferred to SW is also presented for comparison. FW = freshwater; SW = seawater.

Parameter PIT tag number	Osmolality (mmol kg^{-1})	MSM rank FW	MSM rank SW	MSM of n fish	Comments
1E07	323	23	22	23	These four fish had both a K less than 0.95 and also
640D	314	7	18	19	ate less than 2 mg g^{-1} d^{-1} during both of the days on
191B	327	11	16	16	which X-raying was carried out in SW.
6809	312	6	22	23	
257B	333	21	19	22	These five fish had a final K less than 0.95.
7258	347	22	9	23	
1028	342	12	21	23	
2F0D	308	8	23	23	
475D	333	5	2	15	
6E2F	330	4	22	22	These six fish ate less than 2 mg g^{-1} d^{-1} during both
2D3A	322	12	15	15	days on which X-raying was carried out in SW.
604A	324	2	22	23	
1E62	322	23	23	23	
1171	319	3	15	16	
1D33	318	21	22	23	
Mean	328				This is the mean osmolality value for all fish in the experiment that were transferred to SW.

3.5 Discussion

The two experiments in the present study used three different feeding frequency regimes on Atlantic salmon smolt following seawater transfer to investigate their effect on feed intake, growth and condition. Experiment 1 focused on feed intake by individuals and the assessment of hierarchy strength and stability in both fresh and seawater. Experiment 2 focused on group feed intake once fish were transferred to seawater, aiming to give a better overall picture of the post-transfer affects of feeding frequency on feed intake and growth by increasing growing time in seawater and reducing handling stress associated with X-rays in experiment 1. In experiment 1 feeding frequency did not result in significant differences in overall survival, final weight, final length or final condition; nor did they result in significant differences in growth depensation or depensation in length or K. However for some of the parameters listed above differences were detected between seawater treatments and the freshwater controls. Feed intake was significantly affected by feeding frequency as was hierarchy strength and the stability of hierarchies through time. The chemical composition of fish was not affected by feeding frequency however it was affected by the condition factor of individual fish within tanks. Neither feeding frequency nor K influenced osmoregulatory ability.

In experiment 2 out-of-season smolt were of poor quality and quickly presented symptoms of infection by a mixture of *Tenacibaculum* and *Vibrio spp.* (Sajjadi, 2004). These fish grew poorly, ate little and consequently had very large FCRs. Variation within treatments was high for all parameters investigated and only one of these showed any significant difference between treatments. This difference was in osmolality measurements but given the poor health of these fish it is unclear whether it was due to treatments or associated with health status. The osmolality values were on average 15 mmol kg^{-1} higher in experiment 2 than those in experiment 1 indicating decreased osmoregulatory capability. Whilst the majority of the results from experiment 2 show no significant difference it is not clear whether this would be the case if healthy fish had been used. In experiment 2 feed intake was lower and FCR poorer than those from experiment 1 both indicating the lower quality of these smolt. From this point on the discussion will mainly explore the findings of experiment 1.

3.5.1 Ballotini validation of X-radiographic feed intake measurements

When using X-radiography adequate preliminary studies should assess possible limitations of the technique for the species of choice and under the given experimental conditions (Jobling et al., 1993b, McCarthy et al., 1993). It is important to validate that ballotini are retained within the fish long enough for X-rays to detect all feed eaten (Jobling et al., 1993b; McCarthy et al., 1993; Jobling et al., 2001). In the present study the validity of using X-radiography was assessed for fish fed for up to 10 hours and on multiple meals. During the preliminary study there was no indication that higher feeding frequencies or longer feeding times resulted in reduced accountability of feed intake in comparison with lower meal numbers and shorter periods of feeding. However, the decision was made to reduce the feeding time from 10 to 8 hours to ensure all feeding occurred during daylight hours. Despite the lack of difference in percentages of group feed intake accounted for by X-rays in the preliminary study in many cases in experiment 1 the group feed intake was overestimated in X-rays for 1F tanks and underestimated in 8F tanks. The greater than 100% accounted for in the 1F fish may have been due to over counting of the large number of waste pellets resulting from delivery of the entire ration at one feed. The large numbers of uneaten pellets could have broken into smaller fragments each of which would have been recorded as an entire pellet. This is likely as the pellets were cold pressed and not extruded. For the less than 100% in 4F and 8F tanks it is possible that fish were defecating out ballotini before X-raying despite findings to the contrary in the preliminary study.

3.5.2 Growth and feed intake

Results from past experiments designed to evaluate the effect of feeding frequency on growth are conflicting. Some found that larger numbers of meals resulted in greater growth in salmonids and other fish (Holm et al., 1990; Hung et al., 2001; Linnér and Brännäs, 2001) and others found no differences (Grayton and Beamish, 1977; Jarobe and Grant, 1996; Hung et al., 2001; Petursdottir, 2002). Differences may be attributable to factors such as levels of aggression. In one study increasing the meal frequency to 32 meals per day resulted in lower growth for rainbow trout than 8 meals per day, which was explained by increased activity and energy expenditure (Linnér and Brännäs, 2001). Ration is also an important factor influencing the overall effect of feeding frequency.

Catfish fed 1, 2 or 3 meals per day had significantly better growth on 2 and 3 meals than on 1 when fish were fed to satiation at each meal, whereas no difference was found using restricted rations (Hung et al., 2001).

In previous research differing feeding regimes offered to Atlantic salmon in freshwater have been found to have no significant effect on growth (Jørgensen and Jobling, 1992). Similar results were also found in seawater cages of Atlantic salmon fed a fixed but not limiting daily ration using automatic feeders delivering 3, 9, 27, or 81 meals per day (Thomassen and Fjæra, 1996). In the present study there was also no evidence for any effect of feeding frequency on the growth of Atlantic salmon smolt following seawater transfer despite differences in feed intake.

Feed consumption in Atlantic salmon can be severely reduced for several weeks following seawater transfer before returning to levels comparable with those in freshwater (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996). This was clearly the case in the present study however feed intake for 1F was initially significantly lower than the higher feeding frequencies and may have been attributable to a decrease in the number of feeds from 8 to 1. While the group feed intake of all seawater transferred treatments converged on similar and non-significantly different values after 21 days in seawater the mean feed intake measured across all 8 days in which feed intake was measured in seawater was still lower for the 1F treatment than the 4F or 8F treatments. The final convergence of the 1F treatment to similar feed intake as 4F and 8F treatments may be evidence of the 1F fish increasing their stomach capacity over time as they adapted to their new feeding regime (Grayton and Beamish, 1977; Jobling 1982, 1983; Ruohnen and Grove, 1996; Wu et al., 2002). The final convergence of 1F to 4F and 8F may help to explain the lack of difference in growth between the groups.

Since dominant fish tend to eat more than subordinate fish (McCarthy et al., 1992; Brännäs and Alanärä, 1993) it is expected that variation in weight (growth depensation) within a group of fish should mirror feeding hierarchy strength (Petursdottir, 2002). Growth depensation can be determined by examining the coefficient of variation for weight at multiple time points during the growth period investigated (Petursdottir, 2002)

or by examining the change in coefficient of variation for weight over the period of time (Carter et al., 1996). Increasing variation in weight of individuals within tanks occurs with increased hierarchy strength (Carter et al., 1996; Petursdottir, 2002). It has been preposed that since pinheads are likely to be out-competed by non-pinheading individuals (King, 1992a) stronger hierarchies will increase pinheading. In the current study growth depensation offered no evidence of hierarchy strengths differing among feeding regimes over the seawater phase as a whole. Accordingly the numbers of pinheads from each feeding regime treatment should not differ. While experiment 1 was not run long enough to result in true pinheads ($K \leq 0.865$) fish that had low final condition factors and/or low feed intake following transfer were recruited approximately equally from each treatment.

The fixed ration of 13 mg g^{-1} d^{-1} offered to all groups throughout the experiment resulted in unrestricted feeding on an entire day basis following seawater transfer as a result of the severe reduction in feed intake caused by the transfer and it is known that the strength of a feeding hierarchy can be reduced by unrestricted feeding (Jobling, 1983; McCarthy et al., 1992; Johansen and Jobling, 1998). Groups on high frequencies may develop strong feeding hierarchies when rations are low due to competition for restricted feed at each meal (McCarthy et al., 1992; Hakoyama and Iguchi, 1997; Johansen and Jobling, 1998; Moutou et al., 1998), while unrestricted feeding at multiple meals can result in lower hierarchy formation than unrestricted feeding over fewer meals (Jobling, 1983). In the present study the hierarchy strength of 1F fish increased significantly immediately following transfer to seawater. However, within 3 weeks in seawater the hierarchy strength of 1F groups had returned to their pre-transfer value. Evidence has been found in fish for the synchronising effect of scheduled feeding (Spieler, 1992) with the timing and number of meals per day known to influence feeding entrainment. In general synchronisation is stronger when fish are offered a single meal than when offered multiple meals during the day (Sanchez-Vazquez and Madrid, 2001) and when it occurs feeding entrainment can only be detected after several feeding cycles (Sanchez – Vazquez et al., 1997). It is possible that the initially larger CVs for individual intake measurements in the 1F treatment may have been due to some fish within the 1F groups more quickly adapting to a single meal than others. Despite the initial increase in hierarchy strength of the 1F treatment not affecting growth or

growth depensation the increased strength alone represents a disadvantage to some fish within these groups. Given the importance of getting fish back onto feed as soon as possible following seawater transfer (Hjertenes, 1999) avoiding both the initial increase in hierarchy strength and the exaggerated decrease in feed intake associated with one feed per day would help maximise feed intake of all individuals within the groups.

The rank stability of both dominance and feeding hierarchies have been examined in previous studies (McCarthy et al., 1999; Carter and Davies, 2004). In the present study stability of feeding hierarchy was examined over three periods within freshwater, between freshwater and seawater, and within seawater. There were a number of significant positive correlations detected between pairs of rank measurements in freshwater and pairs of rank measurements in seawater. It has been found in previous studies that dominance hierarchies can vary between groups within the same treatment as specific individuals will influence the effect of social interactions within that group (Symons, 1968; Winberg et al., 1991). The possible resulting lack of apparent pattern based on treatments may therefore often be attributable to an individual group effect rather than a treatment effect (Carter et al., 1994b, 1996). In the present study patterns of stability were not completely clear but there was generally more stability within the seawater phase for fish in the 8F treatment than the 4F and in the 4F than the 1F. Interestingly this was the reverse of the pattern seen for hierarchy strength using both correlations of MSM vs CVc and also CV of individual intakes following transfer. The MSM vs CVc analysis showed no evidence of strong hierarchies within any of the 8F tanks once transferred to seawater suggesting that feed intake by these fish may be determined by exploitation (scramble) competition and not interference competition related to position in social hierarchy (Branch, 1984; Carter et al., 1994b). Disregarding specific treatment effects it is interesting to note that overall there is a trend (not consistent across all tanks) for hierarchies to be stable in freshwater, lose stability across the transfer from freshwater to seawater and again become stable once in seawater. Given that stability was generally not present across the seawater transfer it is possible that feeding hierarchies established in seawater differed from those in freshwater. In a study on jackdaws (*Corvus monedeulla* L) it was found that dominant individuals were never the first to try a novel feed (Katzir, 1983) and other studies investigating novel diets have suggested that lower ranked fish would risk a higher intake of novel feed if

the potential benefit of eating was greater for lower ranked fish than for individuals more dominant in the hierarchy (Wybourne, 1997; Carter and Davies, 2004). It is possible that dominant fish in the present study initially had lower appetite in the new seawater environment which provided opportunity for subordinate fish to feed more. Changes in feed intake between individuals may have contributed to a change in feeding hierarchy once transferred to seawater. This will be examined in more detail in Chapter 5.

3.5.3 Chemical composition of fish

The chemical composition of fish is mainly a reflection of nutritional (energy) status due to changes in the water and fat content which are inversely related to each other (Storebakken et al., 1991; Shearer, 1994). Feed intake or ration will determine nutritional status to some extent but depending on many factors ration may drive protein and skeletal growth rather than fat deposition (Shearer, 1994). While feeding frequency can affect feed intake, several studies have failed to demonstrate any significant effect of feeding frequency on the chemical composition of fish (Grayton and Beamish, 1977; Jarboe and Grant, 1996; Johansen and Jobling, 1998). In the present study despite differences in feed intake there were no differences in chemical composition among feeding regime treatments.

As fish lose condition (K) their energy reserves are mobilised and depleted (Jobling, 2001b). Generally lipids and glycogen decrease while proteins are conserved (Brett et al., 1969; Black and Love, 1986; Weatherley and Gill; 1986) and moisture levels increase (Stirling, 1976; Shearer, 1994). In the present study while there was also no difference attributable to feeding frequency treatments there were linear relationships between condition factor and lipid content and condition factor and moisture content. The findings support those from Chapter 2 that low conditioned fish have lower lipid levels and more water than fish with higher condition factors. Also there was a linear trend between condition factor and gross energy, with energy increasing in fish of higher condition factor again reflecting the finding from Chapter 2.

3.5.4 Osmoregulation

Past research has found no evidence of a link between pinheading and osmoregulatory failure (King 1992a; Johnstone et al., 1999). These findings are consistent with the present study in which there was no evidence of any correlation between condition factor and osmoregulatory ability. There was also no difference in osmoregulatory ability or CV of osmoregulatory ability among feeding frequency treatments in experiment 1. In experiment 2 the mean osmolalties were in all cases higher than those seen in experiment 1. The mean osmolality of 327 mmol kg^{-1} in experiment 1 were similar to those reported for fully smolted Atlantic salmon (320 mmol kg^{-1}) following 17 d in full strength seawater (Prunet and Bouef, 1985) while 343 mmol kg^{-1} from experiment 2 suggests osmotic incompetence. This may have been caused by the history of disease as disease has previously been linked to increased osmolalities in salmonids (Moles, 1997). Lysfjord et al. (2004) found that out-of-season smolts can in some cases have better osmoregulatory ability than spring smolts suggesting that the higher values in the present study are more likely the result of the poor quality of these specific smolts than the use of out-of-season smolts per se. Whilst significant differences were found in mean osmolality for experiment 2 they only existed between the 1F and 4F treatment and given the history of disease and lower performance of these fish it is unclear whether this finding is meaningful. Both low condition and low feed intake are characteristics of pinheads (King, 1992b, 1993). Whilst experiment 1 did not result in pinheads, fish with low condition (K < 0.95) at the end of the experiment or not eating (intake less than 2 mg g^{-1}d^{-1}) during the seawater phase were examined for osmolality. Osmolality values for these fish varied little from the mean value for all fish transferred to seawater, suggesting that pinheading may not be the result of osmoregulatory failure.

3.5.5 Fish with low final condition (K) and low feed intake in SW tracked through time

Whilst no fish considered true pinheads (as defined in Chapter 2, K ≤ 0.865) were seen at the end of experiment 1 the fish with the lowest final condition factors and lowest feed intake during the seawater phase were examined to determine how they changed through time. In all cases where feed intake in seawater was low at both feed intake measurements fish deteriorated in condition factor following seawater transfer. Given

more time in seawater these fish may have become pinheads (King, 1992b, 1993). Interestingly the converse situation in which condition factor was low at both measurements taken following seawater transfer did not in all cases correlate with low feed intake at both measurements in seawater. It is possible that given time the individuals with higher feed intake may have improved in condition rather than continuing on to become pinheads. It follows that low feed intake soon after transfer may be a better indicator of an individual's likelihood of becoming a pinhead than an initially poor condition factor. Pre-transfer data indicates that the fish with low feed intake at both measurements in seawater were recruited from both above and below the mean weight which is consistent with previous observations that pinheads tend to be recruited equally across the entire size range of groups transferred to sea (King, 1993). Data from the present study also indicate recruitment from above and below the population mean for length, condition and individual feed intake measured in freshwater before transfer.

3.6 Conclusion

Reducing feeding frequency had no short term effects on growth or condition of Atlantic salmon smolts following seawater transfer. However the reduction to one meal per day did cause a significantly greater decrease in feed intake immediately following transfer than occurred with higher feeding frequencies. It is unclear whether this was the result the lower feeding frequency per se or whether it was due to the change in feeding frequency from 8F to 1F. Further research examining the effect of a change in feeding frequency, be it low to high or high to low, is needed to answer this question. This is addressed further in chapter 4.

Feeding a single meal also significantly increased the initial strength of feeding hierarchy in seawater, however feeding hierarchy strengths returned to pre-transfer levels within three weeks or transfer. The results from the present study suggest that in order to help reduce competition for feed (which may lead to pinheading), feeding frequency should be kept high immediately following seawater transfer of Atlantic salmon. Whilst no actual pinheading $(K \leq 0.865)$ occurred in experiment 1 fish deemed most likely to become pinheads (low feed intake and K) were recruited from individuals above and below the mean weight, length, condition factor and feed intake in freshwater. These fish showed no signs of osmoregulatory failure. The current study indicated that feeding hierarchies in seawater may differ from those that existed in freshwater. Further research is required to determine the extent to which hierarchies are affected by seawater transfer. This is addressed further in chapter 5.

3.7 Acknowledgements

3.8 References

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Chapter 4

The effects of changing feeding frequency simultaneously with

transfer to sea in Atlantic salmon

Matthew Flood

4.1 Abstract

The effects of changing feeding frequency simultaneously with transfer from freshwater to seawater were investigated. Tanks of Atlantic salmon *Salmo salar* smolt were kept in freshwater and fed either a low (1F) or a high (8F) feeding frequency for 26 d prior to 2 d feed deprivation and transfer to near full strength seawater (30‰). Following transfer half the groups on each feeding frequency remained on their pre-transfer regime while the other half were swapped. Fish were kept in seawater for 40 d on these feeding regimes. Changing feeding frequency did not cause a greater decrease in feed intake than keeping the pre-transfer feeding regime, however regardless of the pre-transfer feeding regime groups offered 1F had a significantly ($p < 0.05$) greater decrease in feed intake immediately following transfer than those offered 8F. Over time the daily feed intake values for 1F converged on those of 8F and overall there was no significant difference in mean feed intake for any of the treatments during the seawater phase. Prior to transfer, daily feed intake of 1F was also initially lower than 8F but converged on that of 8F. During the freshwater phase overall mean feed intake of 8F was significantly greater than 1F, possibly causing the significantly greater overall increase in weight and length in both treatments fed 8F in freshwater compared with the 1F treatments. Feed conversion ratios (FCRs) were significantly higher for the 1F-8F treatment than other treatments showing poorer feed conversion; however, despite significance the FCR value was only slightly higher. There were no significant differences in chemical composition of fish among treatments. No significant differences in ΔCV of weight, length or condition factor (K) suggested no overall differences in hierarchy formation among treatments. The main finding of this experiment was that a single meal per day (1F) immediately following seawater transfer causes an initially greater decrease in feed intake than a higher feeding frequency (8F) regardless of pre-transfer feeding regime.

Keywords: Atlantic salmon, Change, Chemical composition, Feeding frequency, Feed intake, Freshwater, Growth, Hierarchy, Seawater, Smoltification, Transfer

4.2 Introduction

Changes in diet can positively or negatively alter feed intake in groups of fish (Carter and Davies, 2004). For example both the addition of oxytetracycline (Toften and Jobling, 1997) and the replacement of fish meal with pea protein (Wybourne, 1997) have been demonstrated to cause initial decreases in feed intake before subsequent steady increases. Feed intake in Atlantic salmon, *Salmo salar*, is also affected by changes such as seawater transfer, which can severely reduce feed intake for several weeks before returning to levels comparable with those in freshwater (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996). In the case of diet and possibly seawater transfer decreases may be due to unfamiliarity or neophobia (Forbes, 1999) associated with change to a new diet or environment. It is also possible that in a similar way changes such as alterations to feeding frequency may result in decreased feed intake. The affect of feeding frequency on feed intake has been examined with varying results in both salmonid (Grayton and Beamish, 1979; Jobling, 1983; Thomassen and Fjæra, 1996; Ruohonen et al., 1998; Linner and Brännäs, 2001) and non-salmonid species (Andrews and Page, 1975; Chua and Teng, 1978; Jobling, 1982; Jarobe and Grant, 1996; Hung et al., 2001; Dwyer et al., 2002). Increasing feeding frequency in some but not all cases increased growth by improving feed intake (Ishiwata, 1969; Grayton and Beamish, 1977; Linner and Brännäs, 2001).

A change in feeding frequency may also cause feed intake to decrease due to neophobia, entrainment to the old feeding regime or physiological and morphological adaptations associated with the previous feeding regime. In terms of entrainment scheduled feeding is documented to have a synchronising effect on fish (Spieler, 1992) with some species demonstrating entrainment to feeding at specific meal times (Sanchez-Vazquez and Madrid, 2001). Entrainment is evident by feeding anticipatory activity exhibited in the hours prior to feeding time (Spieler, 1992), with entrainment to feeding time and feed anticipation possibly helping animals prepare physiologically for a meal and aid in feed acquisition (Comperatore and Stephan, 1987). When feeding is limited to certain specific times digestive and metabolic rhythms are set accordingly (Sanchez-Vazquez and Madrid, 2001). In terms of physiological and morphological adaptations fish may,

for example, adapt to infrequent meals eating more per meal, and develop larger gut capacities over time (Jobling, 1983, 1983; Ruohonen and Grove, 1996).

In hatcheries juvenile Atlantic salmon are fed as frequently as every twenty minutes, while after transfer to sea pens frequency may be reduced to only one or two meals per day (H. King personal communication). Decreasing feeding frequency simultaneously with transfer to seawater causes a greater decrease in feed intake than keeping feeding frequency high before and after transfer (Chapter 3). It is not clear whether this decrease is the result of a decrease or a change in feeding frequency. Such changes in feeding frequency may also affect the formation of feeding hierarchies which have been shown to form in groups of salmonids fed low feeding frequencies (1 meal every 2nd day) (Jobling, 1983) and low rations (McCarthy et al., 1992; Jobling et al., 1993; Moutou et al., 1998). The present study examined the effect of changing feeding frequency simultaneously with transfer from freshwater to seawater on feed intake, growth, feed conversion efficiency (FCR) and whole body composition of Atlantic salmon. Fish were fed on a high (8 feeds per day) or low (1 feed per day) daily feeding frequency regime in freshwater and then either swapped or kept on the same frequency once transferred to seawater. One feed per day was chosen as the lowest feeding frequency farms are likely to employ following seawater transfer (H. King personal communication) while 8 feeds was the maximum number of feeds possible using belt feeders (Chapter 3). The main aim was to determine whether a decrease or a change in feeding frequency had the greater effect on post-transfer feed intake and performance of Atlantic salmon.

4.3 Materials and methods

4.3.1 Experimental system and experimental organisation

The experiment was carried out at the School of Aquaculture, University of Tasmania (Launceston, Tasmania, Australia). During the experiment fish were maintained in 300 L flat bottomed cylindrical tanks (see Chapter 3) each supplied with water from a 3000 L temperature regulated reservoir tank at a rate of approximately 15-16.5 L min^{-1}. The total volume of the system was approximately 7250 L. Tanks had external stand pipes and were drained centrally displaying adequate centrifugal water flow to move uneaten feed and faeces to outlets. Solids were removed from the effluent water via dacron matting and a swirl separator, while nitrogenous wastes were treated by trickle biofiltration. A U.V. lamp filter was used to minimise bacterial levels in the system and a foam fractionator was used to remove dissolved proteins during the seawater (SW) phase. Ambient light entered through overhead opaque skylights. The average water temperature throughout the experiment was $15.5 \pm 0.6°C$. The average salinity during the SW phase was 31.8 ± 1.3 ‰. During the freshwater (FW) phase water was continually replaced from the municipal water supply at a rate of approximately 10% d^{-1}, while during the SW phase water was replaced by discrete water changes at least once per week equivalent to a rate of about 10% d^{-1}.

Each experimental tank was fitted with a belt feeder in which belts were marked at hourly increments to align feed according to the specific feeding regimes (see Chapter 3). On each day the ration to be offered was divided into one (1F) or eight (8F) equal meals. 1F commenced feeding at 0900 h; while 8F were fed meals commencing at 0900, 1000, 1100, 1200, 1300, 1400, 1500, and 1600 h. Meal duration between treatments varied and depended on the number of meals offered because an equal ration was offered to all treatments either at one meal or over eight meals. 1F tanks fed for approximately 1.5 to 2 h and 8F tanks fed for approximately 15 to 30 min per meal. The term ration refers to the amount of feed supplied daily by belt feeders and not the amount ingested. The actual feed intake (amount ingested) was also calculated (see below). Tanks were fitted with mesh lids allowing pellets and light through but

preventing escape by fish. Each water outlet was fitted with a mesh basket to collect uneaten feed pellets on days when feed intake was monitored (Helland et al., 1996).

Water quality was monitored every 1 to 2 d throughout the experiment to ensure that the main parameters of chlorine, pH, ammonia, nitrite and nitrate remained within the recommended limits for salmon in FW (Wedemeyer, 1996) and SW (Tarazona and Muñoz, 1995). Water changes were used to reduce elevated levels.

4.3.2 Feed intake measurements

Measurements of total feed intake for each tank were made by counting the number of waste pellets recovered from the baskets placed under the water outlets of tanks (Helland et al., 1996). Pellets were removed from baskets and counted every hour for each of the 8 h of feeding. Feed intake was calculated as *feed ration – feed waste = feed intake*. The number of pellets collected was multiplied by the average pellet weight in order to determine the weight of uneaten pellets (*feed waste*). Feed intake is presented relative to fish wet weight (mg g^{-1} d^{-1}) where 10 mg g^{-1} d^{-1} is equivalent to 1% BW d^{-1}.

4.3.3 Feeding and growth experiment

Mixed sex diploid Atlantic salmon (*Salmo salar*) spring smolt were obtained from the Florentine Salmon Hatchery (SALTAS, Tasmania, Australia) on the 2 October 2003 and transported to the School of Aquaculture. They were held for 3 d in a 4000 L FW recirculation system during which time they were acclimated from Florentine's water temperature of 7.5°C to the experimental temperature of 15°C. On 6 October 2003 fish were anaesthetised (benzocaine, 75 mg L^{-1}), and wet weight (to nearest 0.1 g) and fork length (mm) were measured. Twenty-five fish were randomly allocated to each of 12 tanks (116.0 ± 15.9 g, mean ± SD, n = 300). After 1 d recovery without feed 6 tanks of fish were allocated to a feeding regime of 1F and 6 to 8F. All tanks of fish were fed commercial extruded pellets (3 mm Nutra Transfer 45/26, Skretting, Cambridge, Tasmania, Australia: 91.8 ± 0.03% dry matter; 39.7 ± 0.09% crude protein; 30.3 ± 0.27% crude lipid; 10.0 ± 0.01% ash (mean ± SD, n = 2)). Feed was delivered by belt feeders for 26 d prior to 2 d feed-deprivation before direct transfer into near full strength

SW (30‰), in accordance with present industry practice. Transfer to SW was achieved by emptying the reservoir completely and refilling it with SW. The water level in the experimental tanks was then lowered until only just enough remained to cover the fish. The pumps were then turned back on from the reservoir to experimental tanks filling them with SW. Following SW transfer groups of fish were allocated to new feeding regimes, half of the 1F tanks from FW remained 1F in SW while the other half were swapped to 8F. Similarly half of the 8F tanks from FW remained 8F in SW while the other half were swapped to 1F. This resulted in 4 treatments, 1F-1F; 1F-8F; 8F-1F; 8F-8F each in triplicate and each chosen by random numbers to be 1F or 8F in FW and 1F or 8F in SW. Fish received their first feed in SW on the day following SW transfer. Individual weights were measured twice during this experiment, at initial allocation and termination. Initial weights were used to determine all daily ration values presented. During the entire experiment feeding was always to excess on a daily basis but was unavoidably restricted at times during meals early in the day due to the restrictive nature of feed delivery using simple belt feeder technology. To ensure feeding was in excess on a daily basis, and in most cases in excess on a per meal basis, the ration was increased periodically throughout the experiment as required. Initially in FW the ration offered was 15 mg g^{-1} d^{-1} divided evenly into 1F or 8F depending on the treatment (see 4.3.1). The ration was then raised to 20 mg g^{-1} d^{-1} during the FW phase and 20, 22.5, 25.0 and then 30 mg g^{-1} d^{-1} during the SW phase. Rations were corrected on a daily basis for any decline in total biomass of the tanks due to mortalities which were weighed on removal and not replaced. Group feed intake was measured every second day throughout the experiment.

The experiment finished on the 17 December 2003 after 40 d in SW and 1 d deprivation of feed. Tanks were selected in random order (chosen using random numbers), and fish euthanased (benzocaine, 300 mg L^{-1}) before wet weight (to nearest 0.1g) and fork length (mm) were measured. The liver and gonads were then removed, weighed and replaced into the peritoneal cavity of whole fish. The whole fish carcass was placed in a labelled bag and stored in the freezer at -20°C.

4.3.4 Calculations

Feed conversion ratio (FCR) was calculated as:

$$\text{FCR (g g}^{-1}\text{)} = \text{Weight of feed ingested (g) / Weight gain (g)} \qquad [4.1]$$

In order to perform this calculation feed intake data from days on which group feed intake was measured was extrapolated to give values for days on which it was not (Carter and Hauler, 2000). Condition factor (K) was calculated as:

$$K = 100 \left[\text{Wet weight (g) / Fork length (cm)}^3 \right] \qquad [4.2]$$

Specific growth rate was calculated as:

$$\text{SGR (\% d}^{-1}\text{)} = 100 \left((\ln W_t - \ln W_i)/t \right) \qquad [4.3]$$

where W_i and W_t are the initial and final weights respectively and t is the time in days between the initial and final weighing. SGR describes exponential change in body mass with time (Jobling, 2001a) and was calculated and used to estimate the total weight of fish in each tank on each day of the experiment allowing a more accurate approximation of feed intake relative to fish weight ($\text{mg g}^{-1} \text{d}^{-1}$). SGR was therefore not shown as a direct measure of growth in the results. The coefficient of variation was calculated as:

$$CV = 100 \text{ (standard deviation / mean)} \qquad [4.4]$$

CV is an expression of variability relating sample variability to the mean of the sample. It was used in this study to give an indication of the variability within groups (tanks) of fish. CV was calculated for weight, length and K data. To examine growth depensation, and depensation in length and K the % change in coefficient of variation (ΔCV) from the beginning (i) to the end (f) of the experiment was calculated as:

$$\Delta CV \text{ (\%)} = 100 \left((CV_f/CV_i) - 1 \right) \qquad [4.5]$$

(Carter et al., 1996).

4.3.5 Chemical analysis of diets and whole fish

Standard methods were used to measure the whole body chemical composition of whole fish and feed: dry matter (DM) was determined by freeze drying fish or feed to a constant weight followed by oven drying at 110°C overnight (~17 h); samples were ashed in a furnace at 550°C for 16 h (feed only) (AOAC, 1995); crude protein (Kjeldahl using copper catalyst [N x 6.25]); crude lipid (Bligh and Dyer, 1959). Energy content of fish was estimated using gross energy values for standard protein and lipid of 23.6 and 36.2 kJ g^{-1} respectively (Brafield, 1985); carbohydrate was not included (Shearer, 1994). Five fish from each tank were selected using random numbers and 10 g samples of homogeneous dry matter from each fish were pooled for measurement of whole body chemical composition.

4.3.6 Statistical analysis

Mean values are reported ± standard error of the mean (S.E.M.). All statistical analyses were performed using SPSS version 11.5 (SPSS, 2002). Normality was assumed as sample sizes were too small to test for this. Homogeneity of variances was tested graphically by examination of residual plots in SPSS. Data were statistically analysed to test for differences between treatments using one way ANOVA (Underwood, 1997). Multiple comparisons were made using the Tukey test. Differences were considered significant at $p < 0.05$.

4.4 Results

4.4.1 Growth performance

Mean survival was higher than 92% for all treatments with no significant differences between treatments (Table 4.1). There were no significant differences between treatments in initial weight, initial length or initial K. Final weight, weight gain, final length and change in length all showed the same general trend with values increasing for treatments as follows: 1F-8F < 1F-1F < 8F-1F < 8F-8F. The final weight for 1F-8F was significantly lower than both treatments fed 8F in FW (8F-1F and 8F-8F), while 1F-1F was only significantly lower than 8F-8F. Weight gain followed the same general pattern with only 1F-8F and 8F-8F differing significantly (Table 4.1). Final length of 1F-8F was significantly lower than both treatments fed 8F in FW while 1F-1F was intermediate being not significantly different from any other treatment. Change in length had similar results with 1F-8F significantly lower than both treatments fed 8F in FW (8F-1F and 8F-8F), while 1F-1F was only significantly lower than 8F-8F. The 2 treatments fed 1F in FW did not differ significantly in final weight, weight gain, final length or change in length, nor did the 2 treatments fed 8F in FW (Table 4.1). There were no significant differences in final K or change in K, nor were there any significant differences in Δ CV weight, length or K over the period of the experiment (Table 4.1).

4.4.2 Feed intake

Group feed intake (mg g^{-1} d^{-1}) varied through time (Figure 4.1). During the FW phase feed intake increased rapidly following allocation to the experimental system and plateaued after around 12 d. Throughout the FW phase fish fed 1F had lower feed intake than those fed 8F on both a daily basis (Figure 4.1) and as an overall mean for the entire FW phase (Figure 4.2). After day 12 feed intake of 1F continued to increase slightly and converged on 8F by the time of SW transfer (Figure 4.1). The mean feed intake immediately prior to SW transfer was approximately 13.5 mg g^{-1} d^{-1} for 8F and 12 mg g^{-1} d^{-1} for 1F. After transfer feed intake decreased for all treatments and was lower for 1F. Immediately following transfer both treatments fed 1F decreased to a feed intake around 3.5 mg g^{-1} d^{-1} while both treatments fed 8F only decreased to around 6.5 mg g^{-1} d^{-1}. Intake for the 1F treatments were significantly lower than 8F treatments

on the day following SW transfer (Figure 4.1). After 7 d in SW, feed intake was similar for all treatments. Feed intake in all groups decreased after 8 d in SW coinciding with a large water change and subsequently intake continued to increase in general until the termination of the experiment after 42 d in SW (Figure 4.1). The overall mean intake during the SW phase was not significantly different for any of the treatment groups (Figure 4.2). FCR during the experiment as a whole was found to be significantly greater for fish on the 1F-8F feeding regime treatment than any of the other feeding regime treatments; however the mean FCR values were all quite similar so it is questionable whether this is biologically significant (Figure 4.3).

4.4.3 Chemical composition

There was no significant difference in dry matter, crude protein, crude lipid or gross energy between treatments despite values for parameters being slightly higher in treatments fed 8F in FW than those fed 1F during this time (Table 4.2). Heterogeneity of variances was found for dry matter, crude lipid and gross energy data which could not be corrected for using transformations. Results were, however, viewed as valid since heterogeneity of variances leads to increased probability of type I error, which is the erroneous rejection of actual non-significance (Underwood, 1997).

Table 4.1 Performance of Atlantic salmon fed on feeding regimes in FW and SW of 1F-1F, 1F-8F, 8F-1F or 8F-8F (mean ± S.E.M, n = 3 replicate tanks. For each parameter the mean is written above the S.E.M). Means with the same letter are not significantly different (Tukey's multiple comparison test $p < 0.05$). FW = freshwater; SW = seawater.

Parameter	Unit	Treatment				F	df	p
		1F-1F	1F-8F	8F-1F	8F-8F			
WEIGHT								
Initial weight	(g)	116.1	115.9	116.1	116.0	0.181	3, 8	0.906
		0.2	0.2	0.2	0.1			
Final weight	(g)	232.3^{ab}	225.6^{a}	251.7^{bc}	258.8^{c}	7.500	3, 8	0.010*
		3.7	8.2	6.2	3.5			
Weight gain	(g)	116.2^{ab}	109.7^{a}	135.6^{ab}	142.8^{b}	7.093	3, 8	0.012*
		3.9	8.4	6.3	3.6			
Δ CV weight	(%)	83.8	78.4	59.4	48.2	0.343	3, 8	0.795
		31.1	36.1	23.8	18.5			
LENGTH								
Initial length	(mm)	214.1	213.9	213.8	213.4	0.317	3, 8	0.813
		0.6	0.2	0.1	0.7			
Final length	(mm)	270.0^{ab}	266.4^{a}	276.2^{b}	277.7^{b}	9.708	3, 8	0.005**
		1.5	2.0	1.5	1.8			
Change in length	(mm)	56.0^{ab}	52.5^{a}	62.3^{bc}	64.3^{c}	11.015	3, 8	0.003**
		1.0	1.9	1.4	2.1			
Δ CV length	(%)	40.7	35.3	39.3	21.9	0.151	3, 8	0.926
		22.6	32.0	18.1	9.5			
CONDITION FACTOR								
Initial K		1.18	1.18	1.18	1.19	0.189	3, 8	0.901
		0.013	0.004	0.003	0.011			
Final K		1.16	1.17	1.18	1.19	0.467	3, 8	0.713
		0.017	0.026	0.016	0.021			
Change in K		-0.0227	-0.0149	-0.0080	0.0016	0.212	3, 8	0.886
		0.0239	0.0285	0.0181	0.0174			
Δ CV K	(%)	60.3	73.1	34.7	56.1	0.201	3, 8	0.893
		15.3	59.6	15.1	32.4			
OVERALL SURVIVAL ● (%)		98.7	92.0	100.0	100.0	3.300	3, 8	0.079
		1.3	4.0					

● Data found not to be homogeneous but for which no transformation could remove heterogeneity.
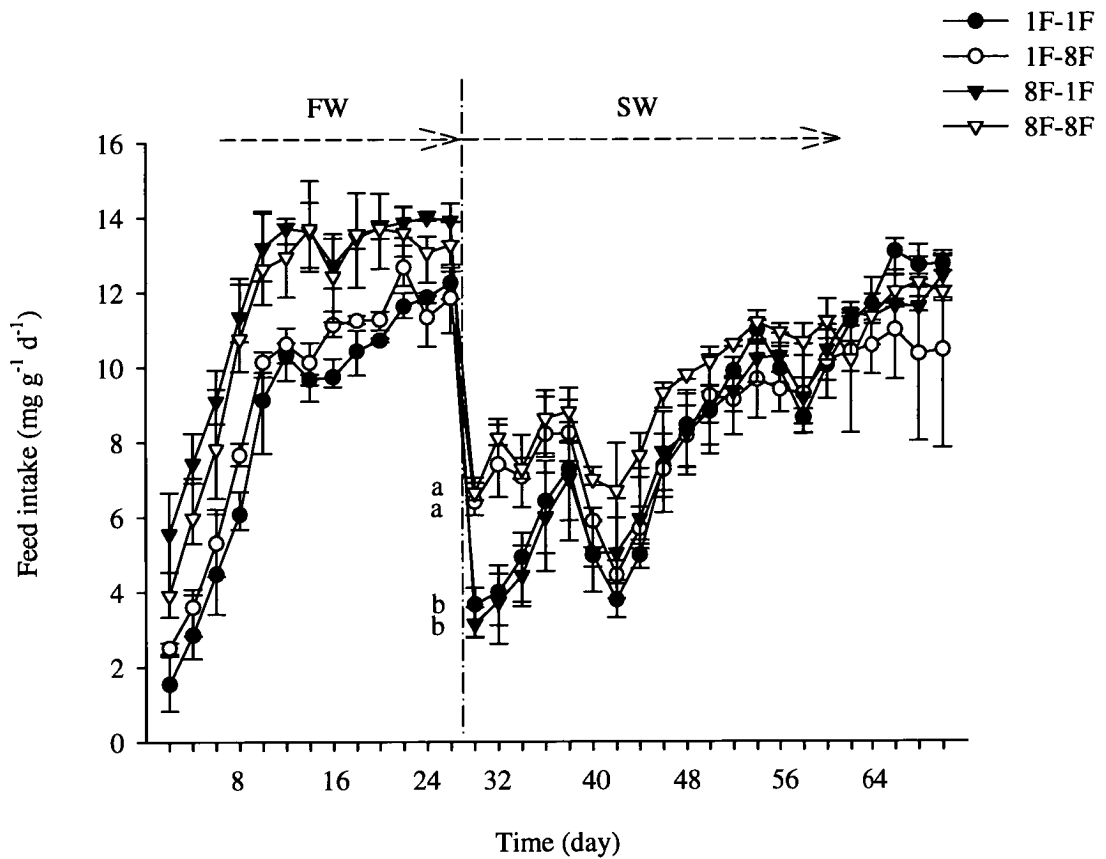
Figure 4.1 Daily feed intake (mean ± max and min) for each treatment. Transfer to SW (_ _ _ _) occurred 30 d after the initial weighing and allocation of fish to tanks. On the d following SW transfer (day 31) ANOVA found significant difference between treatments (F = 35.551, *df* 3, 8, p < 0.001***). Means with the same letter are not significantly different for this day (Tukey's multiple comparison test p < 0.05). FW = freshwater; SW = seawater.
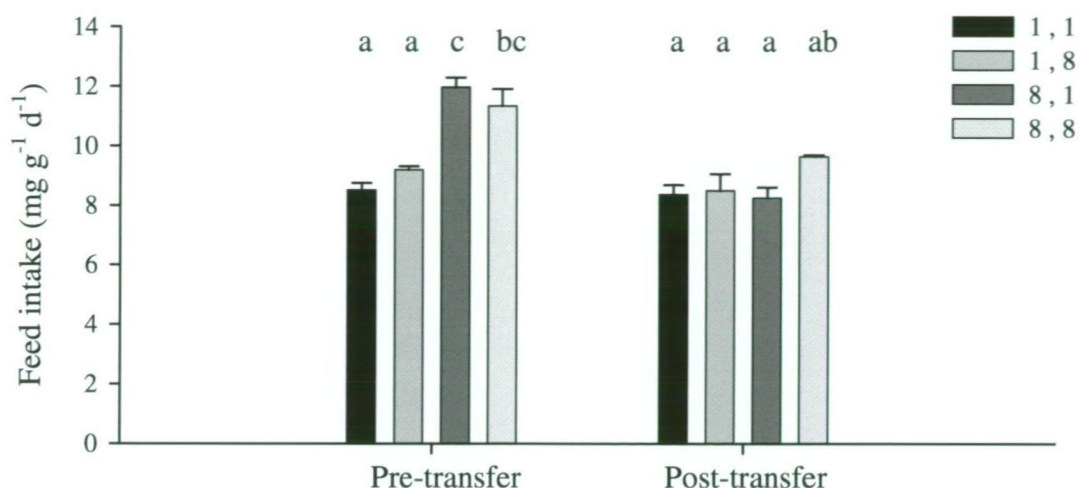
Figure 4.2 Feed intake, mg g^{-1} d^{-1}, (mean ± S.E.M) for 13 d during FW phase and 21 d during SW phase. ANOVA showed significant difference (F = 15.80, *df* 7, 16, p < 0.001***). Means with different letters are significantly different (Tukey's multiple comparison test p < 0.05). FW = freshwater; SW = seawater.
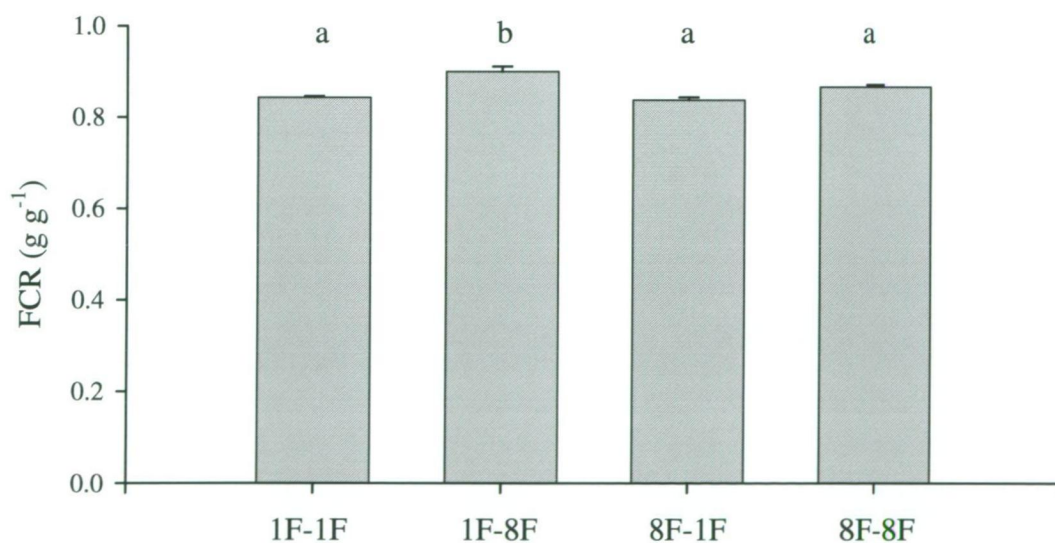
Figure 4.3 Feed conversion ratio (FCR) for each treatment (mean ± S.E.M) calculated from the initial weighing until final weighing (30 d FW and 42 d SW). ANOVA showed significant difference (F = 16.43, *df* 3, 8, p < 0.001***). Columns with the same letter are not significantly different (Tukey's multiple comparison test p < 0.05). FW = freshwater; SW = seawater.

Table 4.2 Chemical composition (% wet weight) of Atlantic salmon fed on feeding regimes in FW and SW (FW-SW) of 1F-1F, 1F-8F, 8F-1F or 8F-8F (mean ± S.E.M, n = 3 replicate tanks. For each parameter the mean is written above the S.E.M). FW = freshwater; SW = seawater.

Parameter	Treatment				F	*df*	p
	1F-1F	1F-8F	8F-1F	8F-8F			
Dry matter ●	31.02	31.12	31.34	32.13	2.379	3, 8	0.146
	0.08	0.52	0.34	0.19			
Crude protein	16.42	16.59	16.67	16.79	1.262	3, 8	0.351
	0.04	0.17	0.19	0.11			
Crude lipid ●	12.78	12.74	13.04	13.74	2.499	3, 8	0.134
	0.01	0.51	0.27	0.11			
Gross energy kJg^{-1} ● (wet weight)	8.50	8.53	8.66	8.94	2.567	3, 8	0.127
	0.01	0.20	0.13	0.07			

● Data found not to be homogeneous but for which no transformation could remove heterogeneity.

4.5 Discussion

In the present study changing feeding frequency simultaneously with transfer from freshwater to seawater was investigated in order to determine if the exaggerated decrease in feed intake seen to occur when shifting from a high feeding frequency in freshwater (8F) to a low (1F) feeding frequency in seawater (see chapter 3) was the result of the change in feeding frequency itself or a decrease in feeding frequency. In the present study changing feeding frequency was not found to affect daily feed intake following seawater transfer however post-transfer feeding frequency in its own right did have an effect with fish fed once per day (1F) initially having significantly lower feed intake than fish fed eight times per day (8F). Overall there was no significant difference in mean feed intake during the seawater phase between any of the treatments and treatments had no affect on the whole body chemical composition of fish. Pre-transfer feeding regime did have a significant effect; overall feed intake was higher for 8F than for 1F. Due to the lack of differences in mean feed intake following seawater transfer the differences in growth during the experiment would appear to be related to the greater pre-transfer intake of 8F than 1F rather than the feeding frequency post-transfer or the change in feeding frequency over transfer. However, this could not be tested as weights were only taken at the beginning and end of the experiment to minimise stress at seawater transfer.

4.5.1 Feed intake and growth

In the present study it was hypothesised that a change in feeding frequency imposed simultaneously with transfer from freshwater to seawater may decrease feed intake more than seawater transfer alone due to neophobia (Forbes, 1999) associated with the new feeding regime, entrainment to the old feeding regime or physiological and morphological adaptations associated with previous feeding regime. However, changing feeding frequency, irrespective of whether increased or decreased, did not result in a greater decrease in feed intake than was seen in the unaltered treatments. This does not support the concept that the fish had entrained to their initial feeding regimes despite the fact that periodic feeding can act as a potential zeitgeber or synchroniser of an activity (Edmonds, 1977; Boulos and Terman, 1980) being one of the most potent

environmental synchronisers of endogenous rhythms (Sanchez-Vazquez and Madrid, 2001), and despite 27 d acclimation to feeding regimes in freshwater. If entrainment had occurred presumably fish kept on the same feeding frequency across transfer would perform better than those for which feeding frequency was changed. Besides feeding frequency the time of feeding is an important factor affecting increased feed intake (Hung et al., 2001). Atlantic salmon have been found to feed predominantly during daylight hours (Hoar, 1942; Higgins and Talbot, 1985; Thorpe et al., 1988; Jørgensen and Jobling, 1992). It has been found that preferences for particular feeding times during the day in fish relate to circadian rhythms (Boujard and Leatherland, 1992; Kadri et al., 1997) and studies of Atlantic salmon have demonstrated that greatest feed intake usually occurs during the first hours of daylight, intake then decreases during the middle of the day and increases again around dusk (Kadri et al., 1991; Blyth et al., 1993, 1999; Juell et al., 1994a). The 1F treatment fish in the current study were fed at 0900 h and it is possible that feeding outside of the natural peak feeding times may have resulted in greater differences between 1F and 8F treatments.

The lower feed intake observed in low feeding frequency fish (1F) immediately following seawater transfer, which occurred regardless of pre-transfer feeding regime, is consistent with previous findings that, when fed to satiation, low feeding frequencies tend to lead to lower overall feed intake for a number of fish species (Ishiwata, 1969; Andrews and Page, 1975; Grayton and Beamish, 1977; Hung et al., 2001; Dwyer et al., 2002). The present study indicates that feed intake immediately following transfer is dictated by the number of feedings offered and does not reflect a response to change. One of the ways that fish adapt to restricted feeding regimes is to increase feed intake (Jobling, 1982). Sockeye salmon *Oncorhynchus nerka* increase feed consumption per meal with increasing time between meals up to 25 hours (Brett, 1971). In the present study there was a convergence of daily feed intake for 1F to similar values as the 8F during both the fresh and seawater phase of the experiment. This is consistent with the finings of Grayton and Beamish (1977) that rainbow trout fed to satiation at one meal per day initially ate significantly less than fish fed 3 or 6 meals per day but after the first ten days there were no significant differences between these treatments. The convergence in feed intake values for groups of fish fed 1F in freshwater to similar values as those fed 8F just prior to seawater transfer is evidence that the 1F fish may be

becoming hyperphagic (ingesting abnormally large amounts of feed) and increasing their stomach capacity as they adapted to their feeding regime (Grayton and Beamish, 1977; Jobling, 1982, 1983; Ruohnen and Grove, 1996; Wu et al., 2002). Brown trout *Salmo trutta* fed on a low feed frequency have been observed to increase their intake so much at each meal that they "bulged markedly" (Brown, 1951). This additional intake is believed to help fish to compensate for not being able to eat later in the day (Ruohnen and Grove, 1996). In the present study if hyperphagia did occur in freshwater this ability was not carried through transfer as fish kept on 1F across transfer decreased to a similar value as those transferred from 8F to 1F. Again there is evidence of hyperphagia occurring during the seawater phase with 1F fish converging on similar feed intake values with 8F groups. Fish displaying hyperphagia would be expected to have larger stomachs and or intestines (Jobling 1982, 1983) and whilst hyperphagia may have occurred it cannot be confirmed by stomach size in the current study as this was not measured.

Growth in fish is affected by both the amount of feed consumed and the efficiency of assimilation of this feed (Buurma and Diana, 1994). Optimal feeding strategies have been found to improve growth, and feed conversion ratios (FCR) (Goddard, 1996; Kubitza and Lovshin, 1999; Dwyer et al., 2002) and while fish on restricted feeding regimes have been shown to improve their feed utilisation by increasing conversion efficiency (Brown, 1957) there was no evidence of this in the present study. The 1F-8F treatment had significantly higher overall FCRs than the other treatments but the actual difference in the mean was very small and whilst statistically significant presumably not biologically significant. Higher feeding frequencies have been shown in many species to result in greater growth in fish (Jobling, 1982; Holm et al., 1990; Hung et al., 2001; Linner and Brännäs, 2001; Dwyer et al., 2002) presumably as a result of feed intake increasing as feeding frequency increases (Ishiwata, 1969; Grayton and Beamish, 1977). In the present study fish fed 8F during the freshwater phase had significantly larger final weights and lengths than those fed 1F. These significant differences mirror the results for intake in the freshwater phase during which fish fed 1F had significantly lower mean intake than fish fed 8F. While changing feeding regime did not affect feed intake post-transfer the 1F and 8F regimes did have significantly different mean feed intakes during the freshwater phase.

Since dominant fish tend to eat more than subordinate fish (McCarthy et al., 1992; Brännäs and Alanärä, 1993; Damsgård et al., 1997) it would be expected that variation in weight, or change in variation in weight over a period of time (growth depensation) within a group should mirror hierarchy status (Carter et al., 1996; Petursdottir, 2002). In the current study there was no evidence of any difference in the strength of hierarchies within each treatment based on growth depensation, or depensation in length or condition.

4.5.2 Chemical composition of fish

Feed intake or ration can affect chemical composition of fish with greater feed intake generally increasing deposition of lipid stores (Storebakken et al., 1991) and reducing body moisture (i.e. increase the percent dry matter) as a result of the inverse relationship between water and lipid (Shearer, 1994; Liu and Liao, 1999). Generally the percentage of protein remains reasonably stable unless lipid and glycogen stores become depleted (Shearer, 1994; Jobling, 2001b). As previously mentioned in the present study there was significantly greater mean feed intake in the two treatments fed 8F in the freshwater than the 1F treatments and a lack of significance between any of the treatments in seawater. Despite the final chemical composition of the freshwater 8F treatments having slightly higher dry matter, crude protein, crude lipid, and gross energy than 1F treatments the differences were not significant. The convergence of feed intake values of 1F fish to those of 8F fish in both fresh and seawater and the similar FCR values for each treatment may help to explain the lack of significance. The present findings are consistent with many cases where overall feeding frequency has been shown not to affect the chemical composition of fish (Grayton and Beamish, 1977; Jarobe and Grant, 1996; Johansen and Jobling, 1998).

4.6 Conclusion

Changing feeding frequency simultaneously with transfer from freshwater to seawater did not result in a greater decrease in feed intake immediately following transfer than keeping the same feeding frequency pre and post-transfer. The decrease in feed intake was initially significantly greater for 1F fish than 8F fish, regardless of pre-transfer feeding regime, but overtime the intake of 1F fish converged on the intake values of the 8F fish, and a similar convergence was seen during the freshwater phase. Despite initial differences following transfer mean feed intake for the entire time in seawater did not differ significantly for any treatment, however mean feed intake during the freshwater phase was significantly less for the two 1F treatments. Final weights and lengths, and change in weight and length over the period of the experiment mirrored the results for pre-transfer feed intake with less growth in fish fed 1F during the FW phase. FCR was significantly greater for 1F-8F fish suggesting poorer feed conversion in this group however given the similarity of the actual value with other groups it is questionable whether this finding is of biological significance. No differences were found in body composition of fish from any of the treatments.

4.7 Acknowledgements

4.8 References

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Chapter 5

Changes in dominance and feeding hierarchies in Atlantic salmon as a result of transfer from a freshwater to a seawater environment

Matthew Flood

5.1 Abstract

The effects of transfer from freshwater to seawater on the dominance and feeding hierarchies of Atlantic salmon *Salmo salar* were investigated. Following a period of 32 days in freshwater, four tanks of individually colour tagged Atlantic salmon (n = 8) underwent direct transfer into near full strength seawater (30‰) where they remained at a mean salinity of 33.6‰ for 43 days. Throughout the experiment tanks were fed one meal per day. During the 25 days immediately prior to seawater transfer and the 25 days immediately following seawater transfer aggression and feed intake were measured. Transfer to seawater caused group feed intake in each tank to decrease greatly and on the day immediately following transfer no aggressive interactions were observed. Both aggression and feed intake increased rapidly over the following 7 day period. Feeding rank was significantly stable in freshwater during the 25 days immediately prior to seawater transfer and was also significantly stable in the 25 days immediately following seawater transfer but there was no significant correlation between mean feeding ranks in freshwater and seawater suggesting the feeding hierarchies may have been broken down and reformed as a result of transfer. Dominance hierarchy stability was significant in three of four tanks during the freshwater phase however stability was not significant for any group during the seawater phase, and there was also no significant correlation between mean dominance ranks in freshwater and seawater. These findings suggest that dominance hierarchies may be broken down before being slowly reformed to result in a different hierarchy structure in seawater than that which existed in frehswater. There were no correlations between dominance and feeding ranks in either freshwater or seawater. There was little evidence that final condition of fish following transfer can be predicted using either feeding or dominance hierarchies, pre- or post-seawater transfer, and no evidence that fish becoming pinheads could be identified by examining pre-transfer characteristics.

Keywords: Aggression, Atlantic salmon, Dominance, Feeding, Feed intake, Freshwater, Hierarchies, Pinheads, Seawater, Smoltification, Social, Stability, Transfer

5.2 Introduction

Social hierarchies have been demonstrated to have an effect on feed intake with dominant fish in general gaining greater access to feed than subordinates (Fausch, 1984; Basquill and Grant, 1998; McCarthy et al., 1999; Gilmour et al., 2005). Since dominance hierarchies based on aggression are believed to be a manifestation of social dominance hierarchies (Francis, 1988) aggressive interactions are often used to calculate a dominance index and thus assign relative social status (Barlow and Ballin, 1976; Winberg et al., 1991; McCarthy et al., 1999; Carter and Davies, 2004). Altering the environment of fishes is known to have marked affects on aggression levels and the strength and stability of dominance hierarchies, with stability generally decreased as a result of such changes (McNicol and Noakes, 1984; Sloman et al., 2001; Snedden et al., 2006). Along with aggression and dominance hierarchies, feed intake and feeding hierarchies are similarly known to be affected by change which can alter overall feed intake along with strength and stability of feeding hierarchies (Carter and Davies, 2004). Examples of environmental changes shown to effect aggression and dominance hierarchies include changing water flow rates and changing level of turbulence to which fish are exposed (McNicol and Noakes, 1984; Sneeden et al., 2006), while changes demonstrated to effect feed intake and feeding hierarchies include the introduction of novel or altered diets (e.g. addition of oxytetracycline) (Toften and Jobling, 1997; Wybourne, 1997; Carter and Davies, 2004). While it is known that feed intake in Atlantic salmon can be reduced following seawater transfer for several weeks before returning to levels comparable with those in freshwater (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996), the effects of seawater transfer on dominance and feeding hierarchies are not well documented.

While dominant individuals are often capable of monopolising feed sources (McCarthy et al., 1999) there is evidence that the decisions by an individual to feed or not feed may incorporate the relative importance of risks and benefits, with decisions based on factors including the energetic status of the individual (Bateson, 2002; Sloman and Armstrong, 2002; Carter and Davies, 2004). Top ranking individuals are often more conservative than mid- to low-ranking individuals when exploring novel conditions as they have relatively more to lose and less to gain by taking risks (Katzir, 1982, 1983). Mikheev et

al. (1994) found that in a novel environment small fish with relatively low energy reserves emerged from shelters to compete for feed earlier than large fish, and in coho salmon *Oncorhynchus kisutch* predation risk was also found to lower overall levels of aggression and increase access to feed for smaller fish (Reinhardt, 1999). It is possible that socially dominant fish with relatively high energy reserves may ignore feed once placed into a novel environment, possibly due to perceived risk of predation, while subordinates may need to feed sooner due to lower energy levels (Sloman and Armstrong, 2002).

While socially dominant individuals may simply not be the first to eat in a new environment, it is also possible that dominance status itself may be state- or context-specific (Sloman and Armstrong, 2002). It has been shown that dominant sticklebacks exposed to a change in environment either became more aggressive or were deposed, and it was speculated that changing the environment may place different energetic demands on individual fish either increasing or decreasing the competitive success and as such increasing or decreasing their rank position in social hierarchies (Sneddon et al., 2006). In the wild the decision to go to sea has a physiological basis (Folmar and Dickhoff, 1980; Hoar, 1988) whereas in aquaculture they are transferred as a group and some individuals may not be physiologically or behaviourally prepared. Within a group there may be graded levels of preparedness and ability to adapt to seawater (King, 1992a) which may result in restructuring of the social hierarchy. This is supported by findings that growth performance in Atlantic salmon in freshwater does not correlate with performance in seawater (Stead et al., 1996).

Previous findings suggest feeding hierarchies in freshwater and seawater may differ (Chapter 3). The reasons for such differences are not clear. It may be that socially dominant fish in freshwater continue to be socially dominant in seawater but due to perceived risks of feeding in a new environment these individuals do not eat initially, allowing subordinate fish to take the risk of eating first. Alternatively it is possible that if dominance hierarchies are "state- or context-specific" they may be completely broken down and reformed as a result of seawater transfer. In the present study groups of Atlantic salmon were transferred from freshwater to seawater, with fish monitored before and after transfer in order to determine the effect of the transfer on both

dominance and feeding hierarchies. The main aims of the study were to determine whether dominance and feeding hierarchies change as a result of transfer to seawater, if so how they change, and how feeding and dominance hierarchies relate to each other. Also of interest was the effect of feeding and dominance hierarchies, both before and after transfer, on the final condition and performance of the salmon, and whether position in dominance and feeding hierarchies before or after transfer could help to predict which fish would become pinheads.

5.3 Materials and methods

5.3.1 Experimental system and set up of experiment

The experiment was carried out at the School of Aquaculture, University of Tasmania (Launceston, Tasmania, Australia). During the experiment fish were maintained in four 300 L flat bottomed cylindrical tanks (Plate 5.1) each supplied with water from a 3000 L temperature regulated reservoir tank at a rate of approximately 17.5-18 L min^{-1}. The total volume of the system was approximately 7250 L. Tanks had external stand pipes and were drained centrally displaying adequate centrifugal water flow to move uneaten feed and faeces to outlets. Solids were removed from the effluent water via dacron matting and a swirl separator, while nitrogenous wastes were treated by trickle biofiltration. A U.V. lamp filter was used to minimise bacterial levels in the system and a foam fractionator was used to remove dissolved proteins during the seawater (SW) phase. Ambient light entered through overhead opaque skylights. The average water temperature throughout the experiment was 14.3 ± 1.0°C. The average salinity during the SW phase was 33.6 ± 1.2‰. During the freshwater (FW) phase water was continually replaced from the municipal water supply at a rate of approximately 10% d^{-1}, while during the SW phase water was replaced by discrete water changes at least once a week equivalent to a rate of about 10% d^{-1}. There was adequate water flow into tanks to enforce exercise in the fish with inlets under the water level (Plate 5.2B) to minimise disruption of surface water allowing clear video footage of fish behaviour to be taken. Each experimental tank was fitted with mesh fencing to prevent escape by fish while not obstructing the view from above (Plate 5.1). The tanks were surrounded by black plastic, to avoid disturbance of fish, with a small rectangular viewing window, which could be closed when not in use, in the front for observation of feed intake (Plate 5.2).

Water quality was monitored every 1 to 2 d throughout the experiment to ensure that the main parameters of chlorine, pH, ammonia, nitrite and nitrate remained within the recommended limits for salmon in FW (Wedemeyer, 1996) and SW (Tarazona and Muñoz, 1995). Water changes were used to reduce elevated levels.

5.3.2 Fish

All-female diploid Atlantic salmon (*Salmo salar*) spring smolt were obtained from Wayatinah Salmon Hatchery (SALTAS, Tasmania, Australia) on the 5 August 2004 and transported to the School of Aquaculture. They were placed in a 4000 L FW recirculation system at 8°C (2°C higher than Wayatinah's water temperature) where they were held for 21 days. Fish were graded to obtain individuals of uniform size and over a 2 d period were anaesthetised (benzocaine, 75 mg L^{-1}), PIT (Passive Integrated Transponder) tagged, and wet weight (nearest 0.1 g) and fork length (mm) were measured before 8 fish were randomly allocated to each of 4 tanks (97.1 ± 1.7 g, mean ± SD, n = 32). PIT tags used were Destron 11 mm (small, model TX1400L) injectable transponders, which were inserted into the abdominal cavity using a syringe applicator through a small scalpel incision on the ventral side of the fish half way between the anus and pectoral fins. Following tag insertion incisions were sealed with a 3:1 mixture of orahesive protective powder (E. R. Squibb and Sons Ltd, Hounslow, Middlesex, England) and cicatrin antibiotic (The Wellcome Foundation, London, England) to help wounds heal and prevent infection (Porter et al., 1998). PIT tags were used to identify individual fish using a Trovan multireader Mark 3 (model MK3 multireader). Fish were held for a further 25 d before each individual was tagged with two coloured buttons (C. Noble personal communication) for individual identification under video cameras and for individual feed intake (University of Tasmania ethics approval number A0007035). The technique used was similar to that described in Bailey et al. (2000) using coloured beads, Hakoyama and Iguchi (1997) using coloured ribbon, and Johnsson et al. (1996) using coloured wooden beads.

Fish were removed one tank at a time. Each individual was then anaesthetised (benzocaine, 75 mg L^{-1}), PIT tag number was recorded, and weight (nearest 0.1 g) and fork length (mm) were measured. Two coloured buttons were then secured, one to each side of the fish just posterior to the dorsal fin. A sterilised size 4 sewing needle with a grade 2.0 braided silk suture (Sherwood Davis and Geck, St Louis, USA) was passed through the first button, the flesh on the dorsal side of the spine and then the second button. The needle was then passed back through the buttons and fish in the opposite direction. Orahesive protective powder containing cicatrin antibiotic (see above) (Porter et al., 1998) was applied under buttons to help wounds heal and prevent infection. The

buttons were then pulled tightly up against the flanks of the fish, two reef knots were tied and a drop of superglue was used to secure the knots (C. Noble personal communication). The fish were revived by holding them under their tank outlet before being returned to the tank to fully recover. Periodically button tags would be dislodged from fish necessitating replacement; each fish had its buttons replaced due to dislodgement between one and five times (1 time, 1 fish; 2 times, 7 fish; 3 times, 11 fish; 4 times, 11 fish; 5 times, 2 fish) during the actual experimental period. On replacement the buttons were placed in a new location ranging from just anterior to just posterior to the dorsal fin in order to allow healing of flesh at the previous buttoning site. Button replacement only occurred on days immediately following those on which digital footage was taken. This allowed at least one full day for fish to recover from buttoning and disturbance before footage was taken again.

After initial button tagging the salmon faced into but did not swim against the current, instead they positioned themselves on their pectoral and caudal fins on the tank floor reacting little to feed presented. The fish were held for a further 38 d following buttoning during which time they slowly commenced swimming against the current and feeding. Button tags which had been pulled out during this time were replaced and the day following tag replacement all individual fish were anaesthetised (benzocaine, 75 mg L^{-1}) and wet weight (nearest 0.1 g) and fork length (mm) were measured (114.8 ± 9.0 g, mean ± SD, n = 32). No fish were lost during the pre-experimental period. The experiment then commenced with fish fed one meal per day and video footage taken (see below). Fish were kept in FW for 32 d before being fasted for 2 d prior to direct transfer into near full strength SW (30‰), in accordance with present industry practice, on day 35 of the experiment.

Transfer to SW was achieved by emptying the reservoir completely and refilling it with SW. The water level in the experimental tanks was then lowered until only just enough remained to cover the fish. The pumps were then turned back on from the reservoir to experimental tanks filling them with SW. A pen of 14 spare button tagged fish kept in the reservoir tank were randomly (using random numbers) divided into two groups to be used in a 24 h salinity challenge. Blood samples of approximately 1 ml were taken from half of these fish immediately prior to SW transfer and for the second half 24 h

following transfer from the caudal vein using heparin-treated needles and 1 ml Tuberculin™ syringes. Samples were then syringed into Eppendorf safe lock tubes, placed in ice, and subsequently centrifuged for 3 min at 4000 g. Plasma was removed and stored in a second Eppendorf at -80°C for later analysis. Intermediate weights were not measured to avoid disturbance of fish while undertaking behavioural observations. Only initial and final weights were taken. Fish received their first feed in SW on the day following transfer and were kept in SW for a further 43 d before the experiment finished.

Final sampling occurred over one day with tanks selected in random order (chosen using random numbers) and fish euthanased (benzocaine, 300 mg L^{-1}) before wet weight (to nearest 0.1 g) and fork length (mm) were measured. The liver was then removed, weighed, returned and the whole fish carcass placed in a labelled bag and stored at -20°C for measurement of whole body chemical composition (see below).

5.3.3 Feeding and feed intake

Fish were fed a commercial extruded salmon feed (3 mm Skretting Nutra Transfer 47/26, Skretting Cambridge, Tasmania, Australia: 90.0 ± 0.02% dry matter, 40.1 ± 0.11% crude protein, 28.4 ± 0.11% crude lipid, 8.6 ± 0.05% ash (mean ± SD, n = 2)). From the commencement of the experiment feeding occurred once a day through a clear feeding tube (permanently positioned on the side of the tank) connected to a funnel outside the black plastic for feed delivery without disturbance to fish (Plate 5.2). Feed was delivered at a rate of approximately 0.5 pellet s^{-1} (slightly slower immediately following SW transfer) until satiation was achieved. Satiation was defined as the time when 4 pellets lay simultaneously on the base of the tank. Feeding lasted approximately 3 to 5 min and meals commenced, at 1000, 1005, 1010 and 1015 h for tanks 1, 2, 3 and 4 respectively. Feeding in this way ensured feed was delivered in a spatially and temporally defensible way. Feed intake for individual fish was measured every 3 d throughout the experiment (number of pellets eaten multiplied by the average pellet weight). Fish were observed through the viewing window and the button colour code was recorded on audio cassette each time a pellet was taken by an individual. Number of pellets consumed by each fish within the feeding session was scored by playing back

the cassette at a later date. Feed intake is presented as absolute feed intake (mg d^{-1}) or intake relative to fish wet weight (mg g^{-1} d^{-1}) where 10 mg g^{-1} d^{-1} is equivalent to 1% BW d^{-1}.

Share of the meal eaten by each individual was calculated as a percentage of the total amount consumed by the group:

$$SM\ (\%) = 100 \times AC\ /\ \textstyle\sum AC \hspace{3cm} [5.1]$$

(Carter et al., 1994) where AC is the absolute daily consumption rate (mg d^{-1}) of a fish and $\sum$ AC the sum of the absolute daily consumption rates for all fish in a group (tank) that could be analysed. Ranked share of meal data was used to determine the stability of the feeding hierarchies in the 25 d period immediately prior to SW transfer and the 25 d period immediately following SW transfer. The period of time following transfer that stability of hierarchies could be assessed was restricted by the occurrence of mortalities. For fish to be included in the assessment of stability they needed to be alive on each day included in the analysis. During the first 25 d in SW only two tanks lost one fish each. These fish were removed from all stability analyses. After the first 25 d mortalities in all tanks increased hence stability was not measured after this point. The mean share of meal (MSM) was used to calculate the overall feeding hierarchy during the FW phase and SW phase. The MSM was calculated as:

$$MSM\ (\%) = \textstyle\sum SM\ /\ n \hspace{3cm} [5.2]$$

(McCarthy et al., 1992) where n is the number of estimates of SM for each fish. MSM was used to assign a rank for each individual fish within the feeding hierarchy (McCarthy et al., 1992; Carter et al., 1994).

5.3.4 Analysis of fish behaviour

Agonistic behaviour was measured in each tank for 15 min twice a day: 15 min immediately before and 15 min immediately after the daily meal, but not during the meal as feeding behaviour was intense and disruptive to the surface water making video

footage unclear. Behavioural measurements were taken around the meal as comparisons

between aggression and feeding hierarchies were to be made, similar to those in

McCarthy et al. (1999). Behavioural observations for fish tend to range from around 5

(e.g. Oliveira and Almada, 1996) to 30 minutes per day (Olsen et al., 1996) in previous

studies and the decision was made to use the upper end of this range in the present study

to increase accuracy of estimates. Observations were made on 8 d prior to SW transfer

(days 11, 14, 17, 20, 23, 26, 29, 32) and 8 d following SW transfer (days 36, 39, 42, 45,

48, 51, 54, 57). On the first day following transfer (day 36) no aggressive interactions

occurred during monitoring times and hence no dominance index or dominance rank

could be determined for any fish. Consequently day 36 was removed from the stability

analysis. Day 11 in FW was also removed so that stability was assessed on 7 d prior to

and 7 d following transfer. These days were also used to assess the stability of feeding

hierarchies (see above). Footage was captured by colour digital video cameras (Model:

C 500R CCD, Swann Communications Pty. Ltd., Victoria, Australia) mounted above

each tank and recorded for each tank simultaneously using a Chateau XP digital

surveillance network system (Chateau Technical Corp, Taipei, Taiwan) on a PC (Plate

5.3). The agonistic behaviours of Atlantic salmon identified by Keenleyside and

Yamamoto (1962) were adapted for the present study: charging (swimming directly and

quickly towards another fish), nipping (biting another fish without prior approach),

chasing (a succession of repeated charges possibly with attempts to nip the retreating

fish). Frontal and lateral displays (Keenleyside and Yamamoto, 1962) were not

witnessed in the present study, presumably due to the necessity to swim against the

current in this particular experiment, and as such were not included. In each interaction

the losing fish would either flee or be displaced from its original position. The winner

and loser were recorded for each distinct encounter, with an encounter defined as a

group of one or more behaviours separated by no activity (Olsen and Jarvi, 1997; Carter

and Davies, 2004). To assign each fish a dominance rank a dominance index (DI) was

calculated as:

$$DI = A^+ / (A^+ + A^-)$$ [5.3]

(Barlow and Ballin, 1976; Carter and Davies, 2004), where A^+ and A^- are the number of

aggressive acts performed (winner) and received (loser), respectively, for a given

individual. Individual values for DI can range between 0 (lost all encounters) and 1 (won all encounters). In the few cases where there were no recorded behaviours for an individual, and DI could not be calculated, the fish were ranked below fish for which $A^+ > A^-$ and above fish where $A^+ < A^-$, i.e. they were given a DI value of 0.5 (Carter and Davies, 2004). Dominance index (DI) rank was used to determine the stability of the social hierarchy in the 25 d prior to SW transfer and the 25 d following SW transfer. The mean dominance index (MDI) was used to calculate the overall social hierarchy prior to SW transfer and again following SW transfer. The MDI was calculated as:

$$MDI = \Sigma \, DI \, / \, n \qquad\qquad [5.4]$$

(McCarthy et al., 1999), where n is the number of estimates of DI for each fish. MDI was used to assign a rank for each individual fish within the dominance hierarchy.

5.3.5 Further calculations

Condition factor (K) was calculated as:

$$K = 100 \, [\text{Wet weight (g)} / \text{Fork length (cm)}^3] \qquad\qquad [5.5]$$

Specific growth rate was calculated as:

$$SGR \, (\% \, d^{-1}) = 100 \, ((\ln W_t - \ln W_i)/t) \qquad\qquad [5.6]$$

where W_i and W_t are the initial and final weights respectively and t is the time in days between the initial and final weighing. SGR describes exponential change in body mass with time (Jobling, 2001) and was calculated and used to estimate the total weight of fish in each tank on each day of the experiment allowing a more accurate approximation of feed intake relative to fish weight ($mg \, g^{-1} \, d^{-1}$). SGR was therefore not shown as a direct measure of growth in the results.

5.3.6 Chemical analysis of diets and whole fish

Standard methods were used to measure the chemical composition of whole fish and feed: dry matter (DM) was determined by freeze drying fish or feed to a constant weight followed by oven drying at 110°C overnight (~17 h); samples were ashed in a furnace at 550°C for 16 h (feed only) (AOAC, 1995); crude protein (Kjeldahl using copper catalyst [N x 6.25]); crude lipid (Bligh and Dyer, 1959). Energy content of fish was estimated using gross energy values for standard protein and lipid of 23.6 and 36.2 kJ g^{-1} respectively (Brafield, 1985); carbohydrate was not included (Shearer, 1994).

5.3.7 Osmolality measurements

To determine whether fish were physiologically ready to be transferred to SW osmolalities of blood plasma samples taken in the 24 h salinity challenge (see 5.3.2) were measured using a Vapro$^{©}$ Vapor Pressure Osmometer (Model 5520) giving measurements in Standard International (SI) units: mmol kg^{-1} (Webster, 1985). For each sample, 10 μl of plasma was used (Wescor Inc, 2000).

5.3.8 Pinheads tracked through time

All of the fish with K ≤ 0.865 at the termination of the experiment or at the time of their death (a total of 5 fish) were deemed to be pinheads (Chapter 2). These fish were examined in order to determine how their individual feed intake (mg g^{-1} d^{-1}) changed through time and how they compared with non-pinheading fish in terms of individual feed intake through time; initial and final weight, length, K; mean individual feed intake (mg g^{-1} d^{-1}) during both the FW and SW phase; final chemical composition; and HSI. Whilst no statistical comparisons were possible the data were still of interest in examination of the pinheading condition, and as such a qualitative assessment of pinhead performance is presented in relation to mean data. In all cases the individual values for each pinhead are compared to the mean values calculated for non-pinheading fish. The positions of pinheads in the overall feeding and dominance hierarchies in FW and SW were also examined.

5.3.9 Statistical analysis

Mean values are reported ± standard error of the mean (S.E.M.). Normality was assumed as sample sizes were too small to test for this. Homogeneity of variances was tested graphically by examination of residual plots in SPSS. Data were statistically analysed to test for differences between means using one way ANOVA (Underwood, 1997) or independent samples *t*-tests. Kendall's coefficient of concordance was used to examine the stability of individual feeding and dominance ranks of fish in groups between days on which measurements were made for both the period in FW and that in SW (Sokal and Rholf, 1995; McCarthy et al., 1999; Carter and Davies, 2004). Spearman's correlations were used for all correlation analysis. With the exception of Kendall's coefficient of concordance all statistical analyses were performed using SPSS version 11.5 (SPSS, 2002). Differences were considered significant at $p < 0.05$.
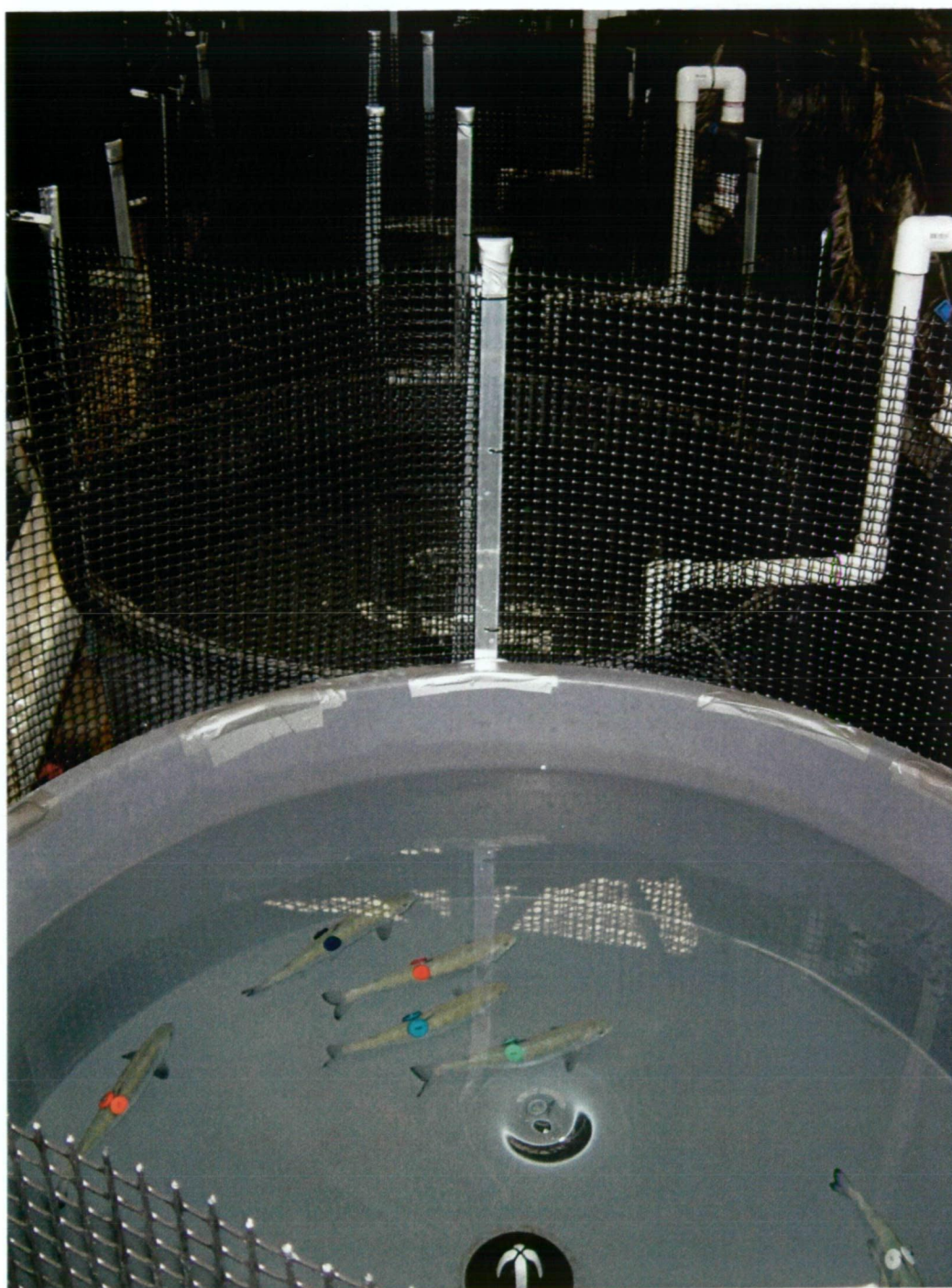
Plate 5.1 Experimental system of 4 cylindrical 300 L tanks, each with a diameter of 110 cm (salmon approximately 22 cm fork length).
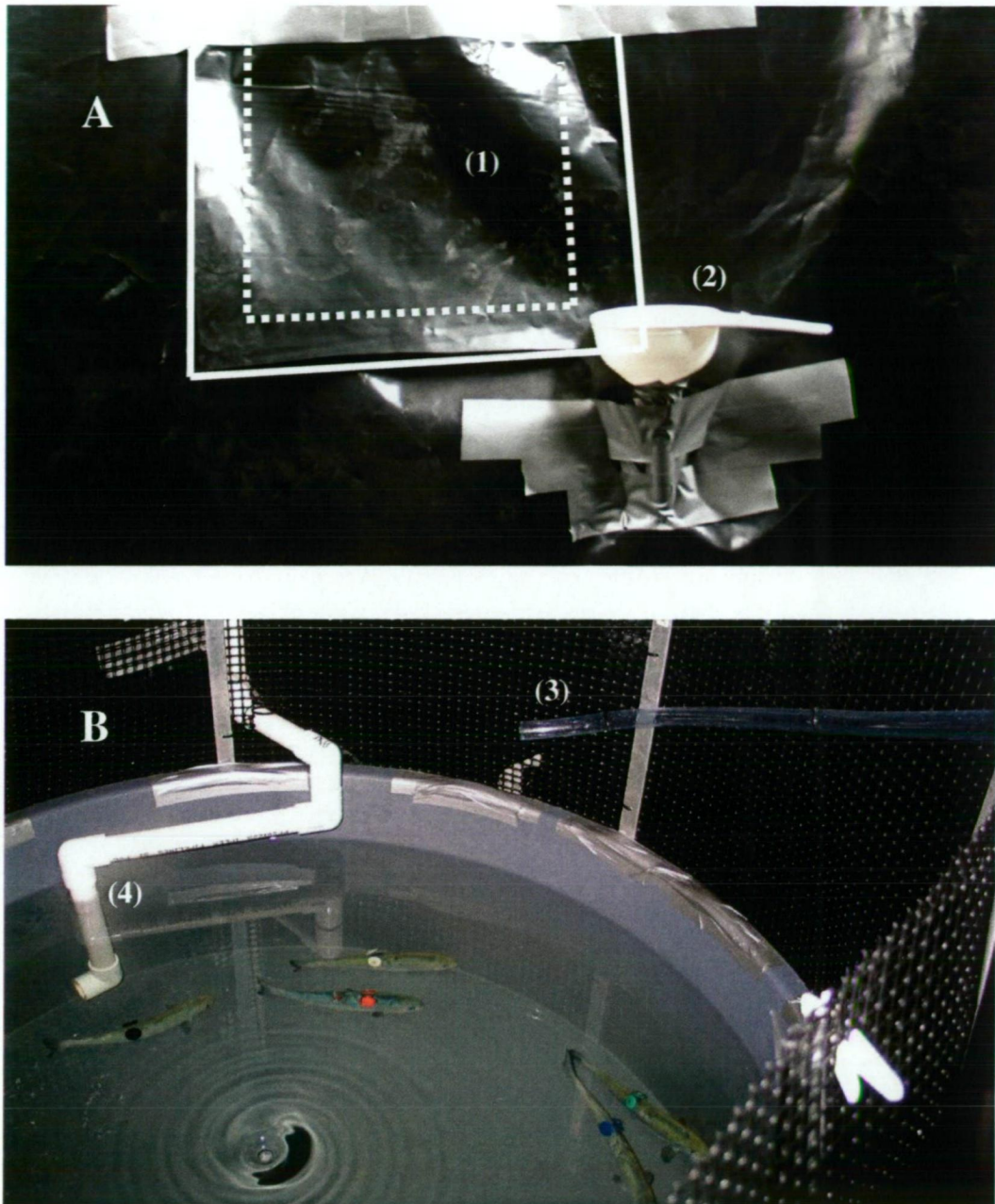
Plate 5.2 (A) View from outside black plastic showing positioning of small rectangular viewing window (1) (25 cm across - indicated by broken white line) with black plastic cover (35 cm across - indicated by unbroken white line) and a feeding funnel on the right (2) connected to the feeding tube. (B) The view through the viewing window with black plastic cover lifted showing clear feeding tube (3) and white PVC water inlet (4) (salmon approximately 22 cm fork length).
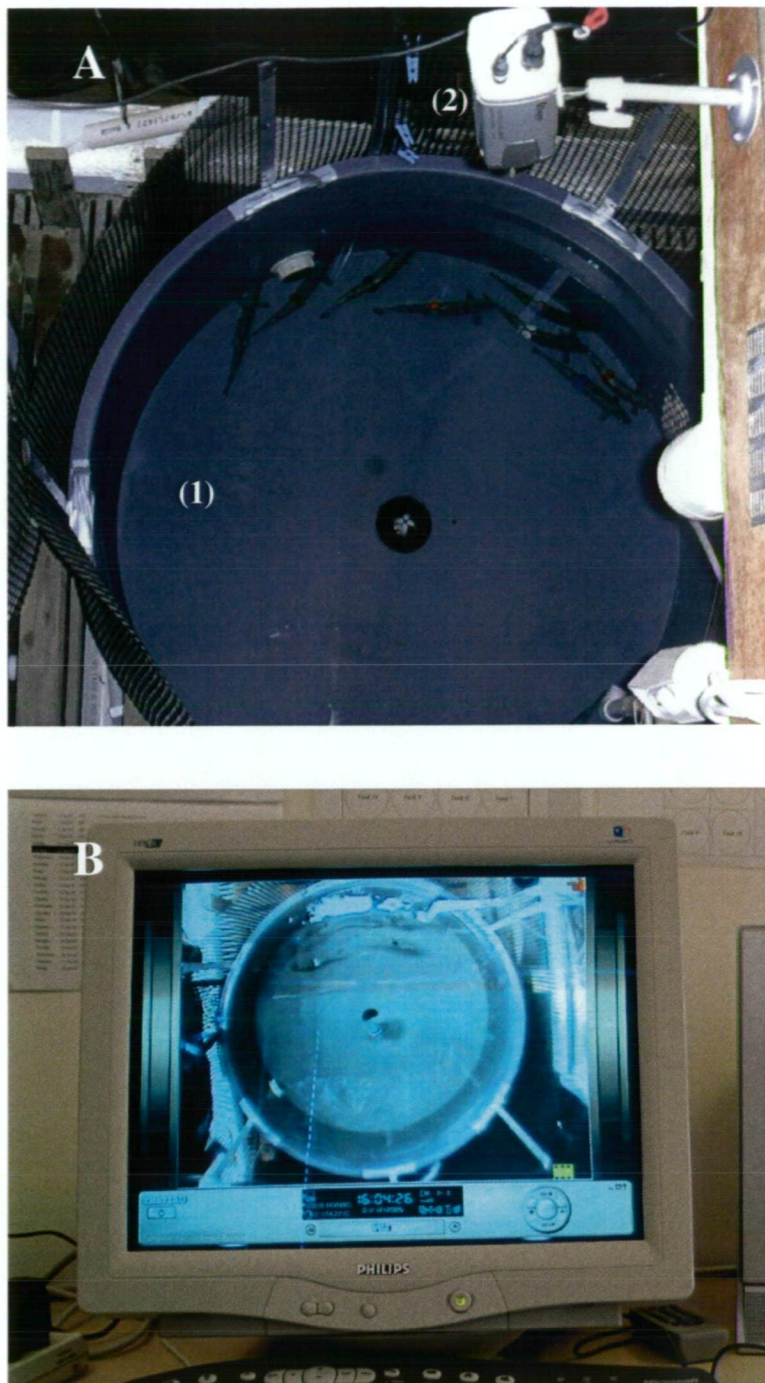
Plate 5.3 (A) View from above experimental tank (1) with a diameter of 110 cm (salmon approximately 22 cm fork length), showing positioning of video camera (2) (5.5 cm wide across base). (B) View of computer screen (36.5 cm across) showing image captured using video camera surveillance system.

5.4 Results

5.4.1 Growth

At the time of initial allocation to experimental tanks the weight and length of fish were 97.1 ± 1.7 g and 204.5 ± 4.9 mm (mean ± SD, n = 32). At the commencement of the experiment, 66 d after initial allocation the weight and length were 114.8 ± 9.0 g and 220.1 ± 5.9 mm (mean ± SD, n = 32) and by the completion of the experiment, 79 d following commencement, fish had grown to a weight and length of 140.7 ± 32.3g and 239.2 ± 13.4 mm (mean ± SD, n = 21).

5.4.2 Twenty-four hour salinity challenge

There was a significant difference between osmolality in FW and after 24 h exposure to SW (t = -3.847, df 12, p = 0.002**); it increased from 328.14 ± 2.6 to 344.6 ± 3.4 mmol kg^{-1} (mean ± S.E.M).

5.4.3 Group feed intake and aggression

Group feed intake (mg g^{-1} d^{-1}) varied through time (Figure 5.1). During the FW phase feed intake remained reasonably constant for each tank with a mean value of 5.5 mg g^{-1} d^{-1}. Immediately following SW transfer feed intake decreased to a mean of 1.1 mg g^{-1} d^{-1} before rapidly increasing over the following 7 d period. Feed intake continued in general to increase at a slower and variable rate up until the termination of the experiment. Aggression on a group basis also varied through time (Figure 5.2). During the 8 d monitored in FW the mean daily number of attacks (all agonistic behaviours combined) per group (tank) was 51.4 ± 20.7 (during 30 min observation). On the day immediately following transfer there were no aggressive encounters in any of the tanks during observation. Aggression also increased fairly rapidly over the 7 d following SW transfer. During the remaining 7 d of monitoring in SW the mean daily number of attacks was 30.8 ± 17.4 (during 30 min observation). Both before and after SW transfer the percentage of attacks immediately prior to the meal was higher than immediately following. Over the 7 d in which stability was assessed in FW an average of 79.4 and 20.6% of attacks occurred during the 15 min prior to and following the meal

respectively. Over the 7 d in which stability was assessed in SW an average of 67.1 and 32.9% of attacks occurred during the 15 min prior to and following the meal, respectively.

5.4.4 Individual feeding ranks

Transfer to seawater appears to affect the structure of feeding hierarchies, while significant stability in hierarchies was seen in both freshwater and seawater. The stability of a group's feeding hierarchy was shown by the magnitude and significance of Kendall's coefficient of concordance. This showed the level of concordance between feeding ranks of all individuals in a group over the 7 d monitored in FW and the 7 d monitored in SW (Table 5.1). Feeding ranks showed stability in every group in both FW and SW however there was no significant correlation between the overall feeding rank in FW and the overall feeding rank in SW based on MSM during the two periods. These results suggest that while feeding hierarchies in FW and SW were stable the hierarchy structure in each environment was different. The fish with the highest feeding rank in SW originally had the highest feeding rank in FW in two of the four groups (tank 2 & 3) while in the remaining two groups (tank 1 & 4) these fish were originally ranked 2 (out of 7 fish) and 4 (out of 8 fish) respectively. The fish with lowest feeding rank in SW originally had the lowest feeding rank in FW in only one group (tank 4) while initially being ranked 1 (out of 7 fish), 7 (out of 8 fish), and 2 (out of 7 fish) in the other three groups (tanks 1, 2 & 3, respectively).

No significant correlation was found between overall feeding rank in FW and final weight, final K, dry matter, crude protein, crude lipid, gross energy or HSI in three groups (tanks 1, 2 & 4) (Table 5.2). In one group (tank 3) a significant negative correlation was found between feeding rank and chemical composition measures (dry matter, crude lipid and gross energy). Similarly there were no correlations in SW in 3 groups. However in tank 4 there was a significant correlation between feeding rank and HSI (Table 5.3). Most of the directly measured chemical composition parameters (i.e. dry matter, crude protein and crude lipid) correlated with each other. Only crude lipid and crude protein did not correlate (Table 5.4).

5.4.5 Behavioural interactions

The stability of a group's dominance hierarchy was also shown by the magnitude and significance of Kendall's coefficient of concordance, again showing the level of concordance between dominance ranks of all individuals in a group over the 7 d monitored in FW and 7 d monitored in SW (Table 5.5). Dominance ranks showed stability in all but one group (tank 3) during the 7 d of observations in FW but no stability was seen for any group during the SW phase and there was no significant correlation between the overall dominance rank in FW and the overall dominance rank in SW based on MDI during the two periods. The fish with the highest dominance rank in SW originally had the highest dominance rank in FW in only one of the four groups (tank 4) while initially being ranked 2 (out of 7 fish), 4 (out of 8 fish), 6 (out of 7 fish) for the other three groups (tanks 1, 2, & 3, respectively). The fish with the lowest dominance rank in SW originally had the lowest dominance rank in FW in only one group (tank 3) while initially being ranked 4 (out of 7 fish), 7 (out of 8 fish), 3 (out of 8 fish) for the other three groups (tanks 1, 2 & 4, respectively).

There was little evidence of dominance rank relating to feeding rank. During the FW phase there was a significant positive correlation between the overall dominance rank and the overall feeding rank in only one group (tank 2) and no significant correlation was found between overall dominance and feeding rank for any group in SW (Table 5.6). During the FW phase the fish with the highest dominance rank also had the highest feeding rank in only one of the four groups (tank 2) while having a feeding rank of 4 (out of 7 fish), 2 (out of 7 fish), and 7 (out of 8 fish) for the other three groups (tanks 1, 3 & 4, respectively). The fish with the lowest dominance rank also had the lowest feeding rank in only one of the four groups (tank 2) while having a feeding rank of 1 (out of 7 fish), 4 (out of 7 fish), and 3 (out of 8 fish) for the other three groups (tanks 1, 3 & 4, respectively). During the SW phase the fish with the highest dominance rank had the highest feeding rank in only one of the four groups (tank 1) while having a feeding rank of 4 (out of 8 fish), 3 (out of 7 fish), and 6 (out of 8 fish) for the other three groups (tanks 2, 3 & 4, respectively). The fish with the lowest dominance rank did not have the lowest feeding rank in any case, but rather were ranked 5 (out of 7 fish), 2 (out of 8 fish), 6 (out of 7 fish), and 3 (out of 8 fish) (tanks 1, 2, 3 & 4, respectively).

There was no significant correlation between mean dominance rank in FW and final weight, final K, dry matter, crude protein, crude lipid, gross energy or HSI in two groups (tank 2 & 3)

Table **5.7**). In one group (tank 1) a significant negative correlation was found between dominance rank and all chemical composition parameters i.e. dry matter, crude protein, crude lipid and gross energy; and in the final group (tank 4) a significant negative correlation was only found between dominance rank and gross energy. The same correlations using overall dominance hierarchy in SW rather than FW were not significant for any parameter in any of the four groups (Table 5.8).

5.4.6 Chemical composition of fish

Significant positive correlations were found for the relationship between K (fish condition) and dry matter, crude protein, crude lipid, and gross energy (Figure 5.3).

5.4.7 Pinheads tracked through time

Fish identified as pinheads ($K \leq 0.865$) had initial weight, length and K both above and below the mean initial values of these parameters for non-pinheads (Table 5.9), however in every case their final weight, length and K were below the mean final values for non-pinheads. Over the length of the experiment all pinheads lost weight while non-pinheads gained weight. In every case the increase in length for pinheads was lower than the non-pinhead mean, and the loss of condition of pinheads was at least 3 times greater than the mean loss of condition experienced by non-pinheads (only 4 non-pinheads gained condition throughout the experiment). During the FW phase the mean overall daily feed intake for each of the individual pinheads was larger than the mean of non-pinheads for all but one individual (030C – see Table 5.9), however following SW transfer mean daily feed intake of each individual pinhead, calculated across the entire SW phase, was less than the mean for non-pinheads. Despite this it was found that on some days during which feed intake was monitored in SW the amount eaten ($mg\ g^{-1}\ d^{-1}$) by pinheads did exceed the mean value for non-pinheads, however this occurred less frequently as the

time post-transfer increased (Figure 5.4). Whilst every pinhead ingested less overall than non-pinheads following SW transfer in no case did an individual pinhead fail to ingest feed during this time (Table 5.9). Pinheads were distributed across all ranks of the feeding and dominance hierarchies during the 25 d leading up to SW transfer. However, for all but one individual fish, pinheads were ranked close to the bottom of the feeding hierarchies and all pinheads fell below the first two ranks in the dominance hierarchies during the first 25 d in SW (Table 5.9).

Due to mortalities before the end of the experiment only two pinheads were still alive at the final sampling, hence there are only two values for pinheads for each of the chemical composition parameters and HSI (Table 5.9). Dry matter, crude lipid, and gross energy of pinheads were lower in both pinheads than the non-pinhead mean, while crude protein was in one pinhead lower and in the other almost identical to the non-pinhead mean. HSI was lower than the non-pinhead mean for one pinhead and higher for the other. During the SW phase three of the five pinheads died (60 %) while only eight of the twenty-seven non-pinheads died (29.6%).

5.4.8 Button tagging

Button tag colour combination had no significant effect on the mean number of attacks received by each fish calculated across all the d in which aggression was observed (F = 1.555, *df* 7, 24, p = 0.197), and no significant effect on the mean feed intake (mg g^{-1} d^{-1}) calculated across the entire experimental period (F = 1.376, *df* 7, 24, p = 0.260). Hence there was a high degree of confidence in using colour button tags as a method of identification of fish to determine hierarchies.
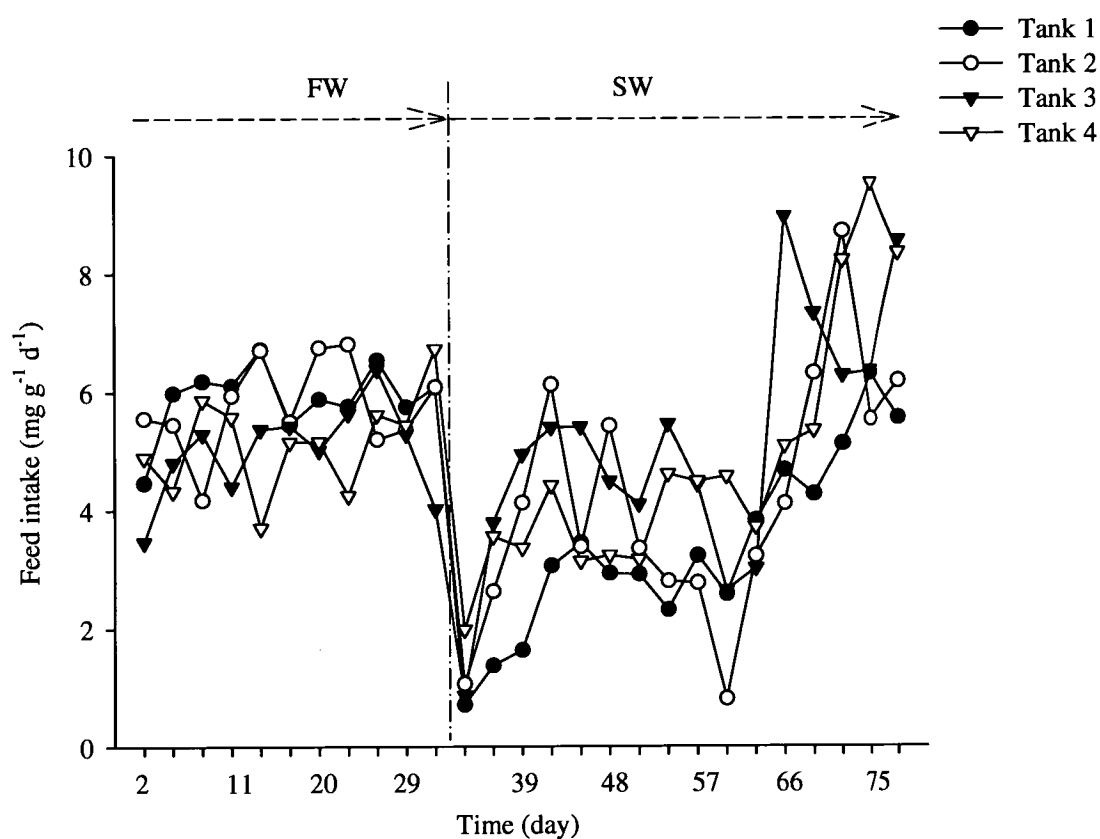
Figure 5.1 Daily feed intake for each tank. Transfer to SW (·-··) occurred 34 d after the initial weighing. FW = freshwater; SW = seawater.
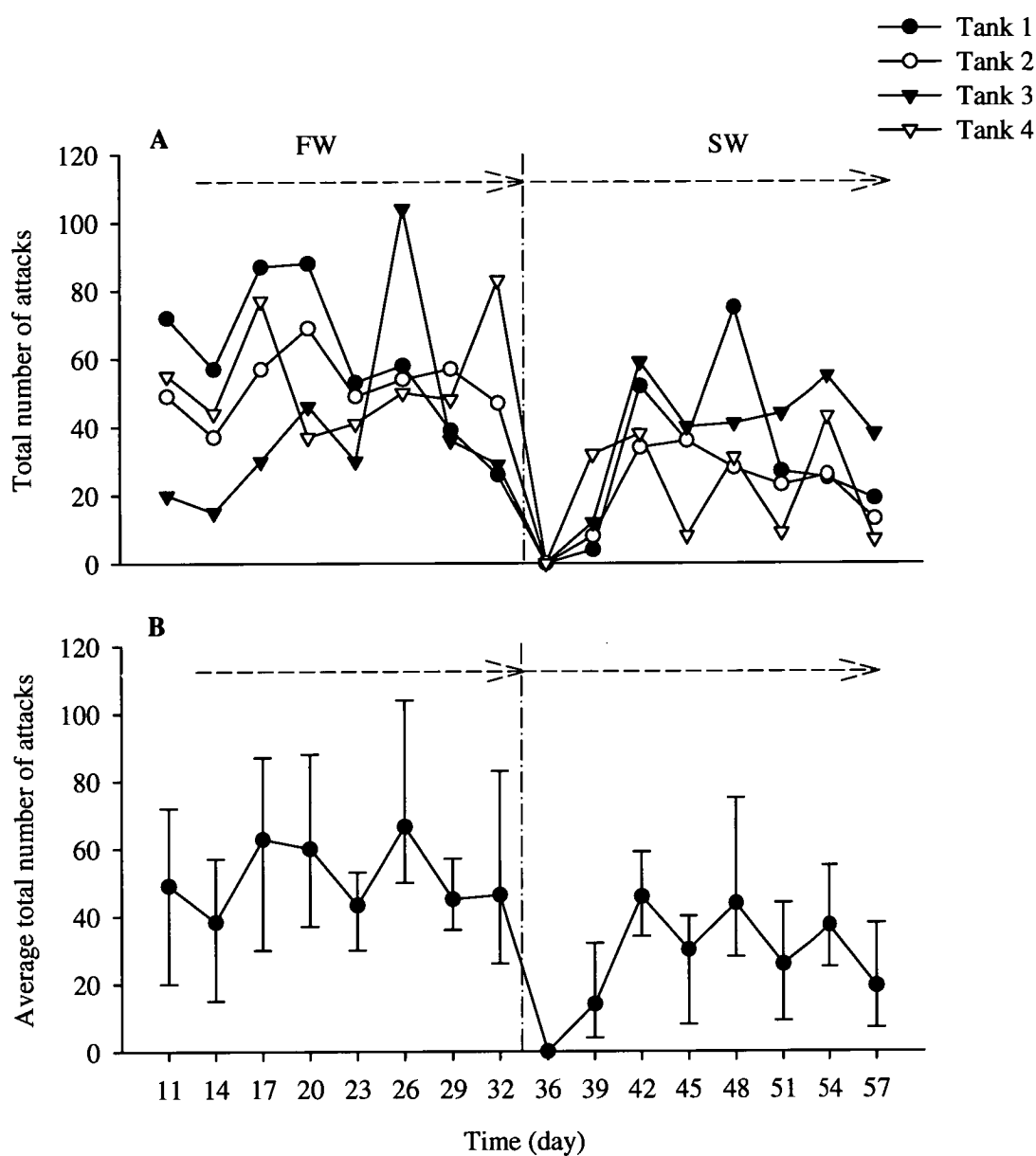
Figure 5.2 (A) Total number of aggressive interactions in each tank on each d of video footage. (B) Mean number of aggressive interactions in all tanks combined ± max and min. FW = freshwater; SW = seawater.

Table 5.1 Analysis of stability in individual feeding ranks, based on share of meal (SM), in two feeding periods (FW and SW) and correlation between the mean feeding rank (MSM) for the two periods (FW vs SW) for Atlantic salmon in four groups. Data are presented as Kendall's coefficient of concordance (W) (corrected for ties) using χ^2 statistic and Spearman's rank correlation. Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. FW = freshwater; SW = seawater.

| Parameter | | Feeding period | | Spearman's rank correlation |
| | | Kendall's coefficient of concordance | | |
Group (Tank no.)		FW	SW	MSM FW vs MSM SW
1	(n = 7)	0.524 **	0.720 ***	0.000
2	(n = 8)	0.570 ***	0.323 *	0.548
3	(n = 7)	0.392 *	0.442 **	-0.179
4	(n = 8)	0.675 ***	0.553 ***	0.262

Table 5.2 Spearman's correlation between ranked feeding hierarchies *before transfer to SW* based on mean share of meal (MSM) against final weight (g), final condition factor (K), chemical composition (% wet weight) and HSI (%) of individual fish in two feeding periods (FW and SW) for Atlantic salmon in four groups. Levels of significance * p < 0.05; ** p < 0.01; *** p < 0.001. FW = freshwater; SW = seawater.

Group	Tank 1	Tank 2	Tank 3	Tank 4
Pre-seawater transfer feeding hierarchy vs:				
Final weight	0.179 (n = 7)	-0.381 (n = 8)	-0.607 (n = 7)	-0.667 (n = 8)
Final K	0.214 (n = 7)	-0.333 (n = 8)	-0.500 (n = 7)	-0.476 (n = 8)
Dry matter	-0.300 (n = 5)	-0.700 (n = 5)	-1.000 ** (n = 5)	-0.200 (n = 6)
Crude protein	-0.100 (n = 5)	-0.400 (n = 5)	-0.500 (n = 5)	-0.771 (n = 6)
Crude lipid	-0.300 (n = 5)	-0.700 (n = 5)	-0.900 * (n = 5)	0.257 (n = 6)
Gross energy kJ g^{-1}	-0.300 (n = 5)	-0.700 (n = 5)	-1.000 ** (n = 5)	0.086 (n = 6)
HSI	-0.300 (n = 5)	-0.100 (n = 5)	0.000 (n = 5)	0.314 (n = 6)

Table 5.3 Spearman's correlation between ranked feeding hierarchies *following transfer to SW* based on mean share of meal (MSM) against final weight (g), final condition factor (K), chemical composition (% wet weight) and HSI (%) of individual fish in two feeding periods (FW and SW) for Atlantic salmon in four groups. Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. FW = freshwater; SW = seawater.

Group	Tank 1	Tank 2	Tank 3	Tank 4
Post-seawater transfer feeding hierarchy vs:				
Final weight	-0.429 (n = 7)	-0.619 (n = 8)	0.071 (n = 7)	-0.690 (n = 8)
Final K	-0.536 (n = 7)	-0.524 (n = 8)	-0.036 (n = 7)	-0.690 (n = 8)
Dry matter	-0.400 (n = 5)	-0.300 (n = 5)	0.000 (n = 5)	-0.029 (n = 6)
Crude protein	-0.300 (n = 5)	-0.100 (n = 5)	-0.500 (n = 5)	0.486 (n = 6)
Crude lipid	-0.400 (n = 5)	-0.300 (n = 5)	0.400 (n = 5)	-0.029 (n = 6)
Gross energy	-0.400 (n = 5)	-0.300 (n = 5)	0.000 (n = 5)	0.086 (n = 6)
HSI	-0.400 (n = 5)	-0.500 (n = 5)	0.500 (n = 5)	-0.829 * (n = 6)

Table 5.4 Spearman's correlation between chemical composition parameters (% wet weight) of whole bodies for Atlantic salmon (n = 21). Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	Dry matter	Crude protein	Crude lipid
Dry matter	1.000		
Crude protein	0.604 **	1.000	
Crude lipid	0.957 **	0.409	1.000

Table 5.5 Analysis of stability in individual dominance ranks, based on dominance index (DI), in two feeding periods (FW and SW) and correlation between the mean dominance index (MDI) for the two periods (FW vs SW) for Atlantic salmon in four groups. Data are presented as Kendall's coefficient of concordance (W) (corrected for ties) using χ^2 statistic, and Spearman's rank correlation. Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. FW = freshwater; SW = seawater.

Parameter		Feeding period Kendall's coefficient of concordance		Spearman's rank correlation
Group (Tank no.)		FW	SW	MDI FW vs MDI SW
1	(n = 7)	0.464 **	0.266	0.643
2	(n = 8)	0.347 *	0.179	0.048
3	(n = 7)	0.161	0.180	-0.071
4	(n = 8)	0.569 ***	0.109	0.262

Table 5.6 Spearman's rank correlation between the mean share of meal (MSM) and mean dominance index (MDI) in two feeding periods (FW and SW) for Atlantic salmon in four groups. Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. FW = freshwater; SW = seawater.

Group (Tank no.)		FW	SW
1	(n = 7)	-0.286	0.643
2	(n = 8)	0.738 *	-0.262
3	(n = 7)	0.357	0.571
4	(n = 8)	-0.143	0.071

Table 5.7 Spearman's correlation between ranked dominance hierarchies *before transfer to SW* based on mean dominance index (MDI) against final weight (g), final condition factor (K), chemical composition (% wet weight) and HSI (%) of individual fish in two feeding periods (FW and SW) for Atlantic salmon in four groups. Levels of significance * p < 0.05; ** p < 0.01; *** p < 0.001. FW = freshwater; SW = seawater.

Group	Tank 1	Tank 2	Tank 3	Tank 4
Pre-seawater transfer dominance hierarchy vs:				
Final weight	-0.357 (n = 7)	-0.071 (n = 8)	-0.571 (n = 7)	-0.214 (n = 8)
Final K	-0.214 (n = 7)	0.024 (n = 8)	-0.393 (n = 7)	-0.095 (n = 8)
Dry matter	-0.900 * (n = 5)	-0.700 (n = 5)	-0.500 (n = 5)	-0.771 (n = 6)
Crude protein	-1.000 ** (n = 5)	-0.400 (n = 5)	-0.500 (n = 5)	-0.371 (n = 6)
Crude lipid	-0.900 * (n = 5)	-0.700 (n = 5)	-0.600 (n = 5)	-0.714 (n = 6)
Gross energy	-0.900 * (n = 5)	-0.700 (n = 5)	-0.500 (n = 5)	-0.886 * (n = 6)
HSI	0.500 (n = 5)	-0.100 (n = 5)	-0.500 (n = 5)	0.257 (n = 6)

Table 5.8 Spearman's correlation between ranked dominance hierarchies *following transfer to SW* based on mean dominance index (MDI) against final weight (g), final condition factor (K), chemical composition (% wet weight) and HSI (%) of individual fish in two feeding periods (FW and SW) for Atlantic salmon in four groups. Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. FW = freshwater; SW = seawater.

Group	Tank 1	Tank 2	Tank 3	Tank 4
Post-seawater transfer dominance hierarchy vs:				
Final weight	-0.429 (n = 7)	0.095 (n = 8)	0.429 (n = 7)	-0.357 (n = 8)
Final K	-0.321 (n = 7)	0.071 (n = 8)	0.571 (n = 7)	-0.286 (n = 8)
Dry matter	-0.700 (n = 5)	-0.100 (n = 5)	0.100 (n = 5)	-0.143 (n = 6)
Crude protein	-0.600 (n = 5)	-0.300 (n = 5)	-0.800 (n = 5)	0.143 (n = 6)
Crude lipid	-0.700 (n = 5)	-0.100 (n = 5)	0.300 (n = 5)	-0.371 (n = 6)
Gross energy	-0.700 (n = 5)	-0.100 (n = 5)	0.100 (n = 5)	-0.429 (n = 6)
HSI	-0.300 (n = 5)	0.300 (n = 5)	0.700 (n = 5)	0.257 (n = 6)
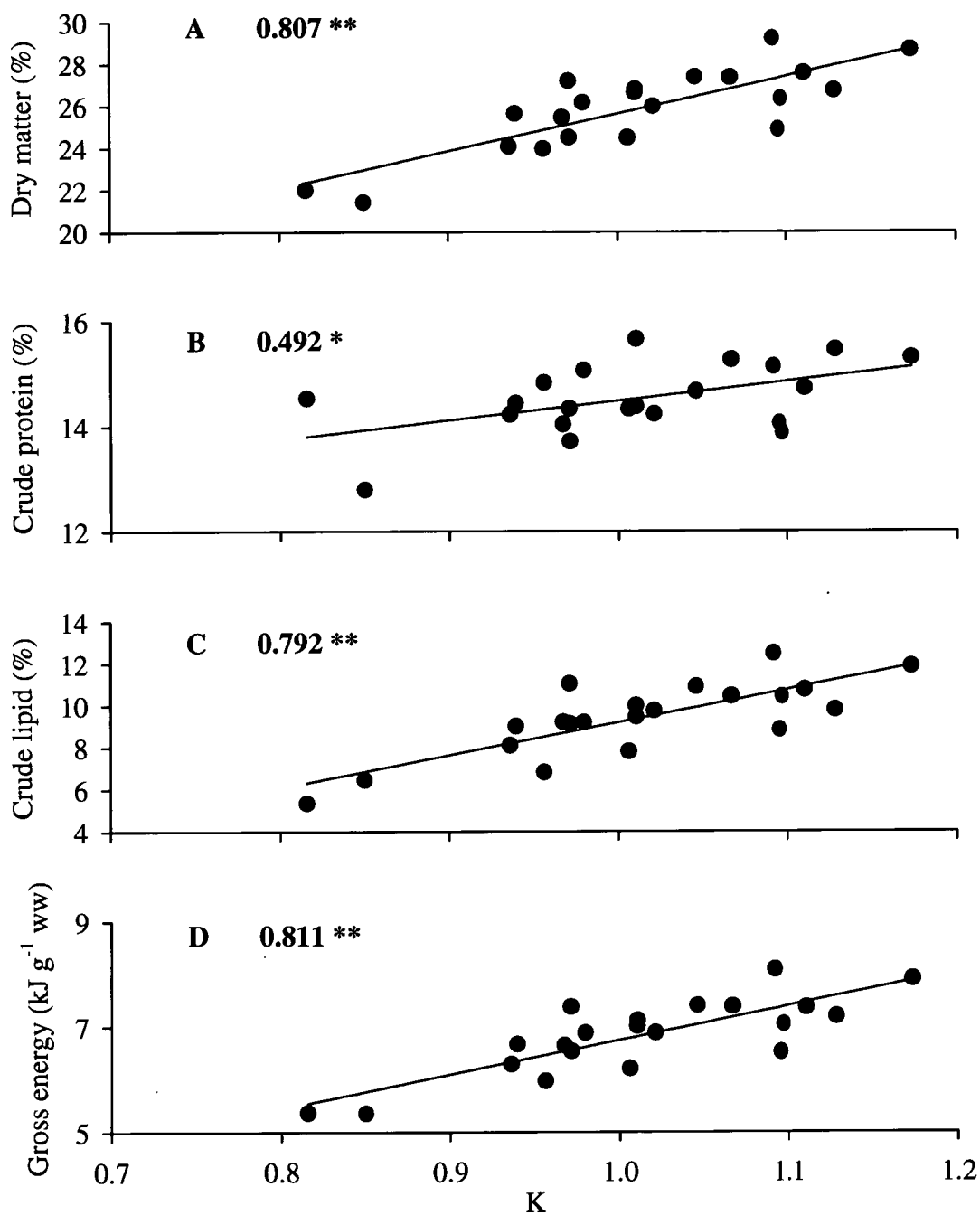
Figure 5.3 Correlations between condition factor and the chemical composition (% wet weight) of whole bodies for Atlantic salmon (n = 21). (A) dry matter, (B) crude protein, (C) crude lipid, (D) gross energy (kJ.g^{-1} ww). Spearman's correlation coefficients are shown with levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 5.9 Characteristics of Atlantic salmon determined to be pinheads (i.e. K ≤ 0.865) at the termination of the experiment or at the time of their death. For fish that died during the experiment the number of days spent in SW is given. FW = freshwater; SW = seawater.

PIT tag number	Unit	1D74	2A32	2D32	030C	736F	Non-pinhead mean
Initial weight	(g)	105.2	116.4	120.1	92.0	103.4	**116.2**
Final weight	(g)	96.8	97.9	106.1	70.1	95.4	**145.4**
Weight gain	(g)	-8.4	-18.5	-14.0	-21.9	-8.0	**29.2**
Initial length	(mm)	216	228	220	206	220	**220**
Final length	(mm)	225	229	231	203	227	**241**
Change in length	(mm)	9	1	11	-3	7	**20.2**
Initial K		1.044	0.982	1.128	1.053	0.971	**1.084**
Final K		0.850	0.815	0.861	0.838	0.816	**1.030**
Change in K		-0.194	-0.167	-0.268	-0.215	-0.155	**-0.053**
Mean FW intake	(mg g^{-1} d^{-1})	6.8	7.0	7.7	3.8	6.2	**5.4**
Mean SW intake	(mg g^{-1} d^{-1})	3.9	0.2	0.1	2.3	0.8	**4.4**
FW MSM rank		3	4	1	7	3	
FW MDI rank		6	1	7	3	8	
SW MSM rank		2	6	7	8	7	
SW MDI rank		4	3	6	3	5	
Dry matter	(% wet weight)	21.4				22.0	**26.3**
Crude protein	(% wet weight)	12.8				14.5	**14.6**
Crude lipid	(% wet weight)	6.5				5.3	**9.7**
Gross energy	(% wet weight)	5.4				5.4	**7.0**
HSI	(%)	3.9				1.5	**2.6**
Days post transfer until mortality		N/A	38	36	27	N/A	
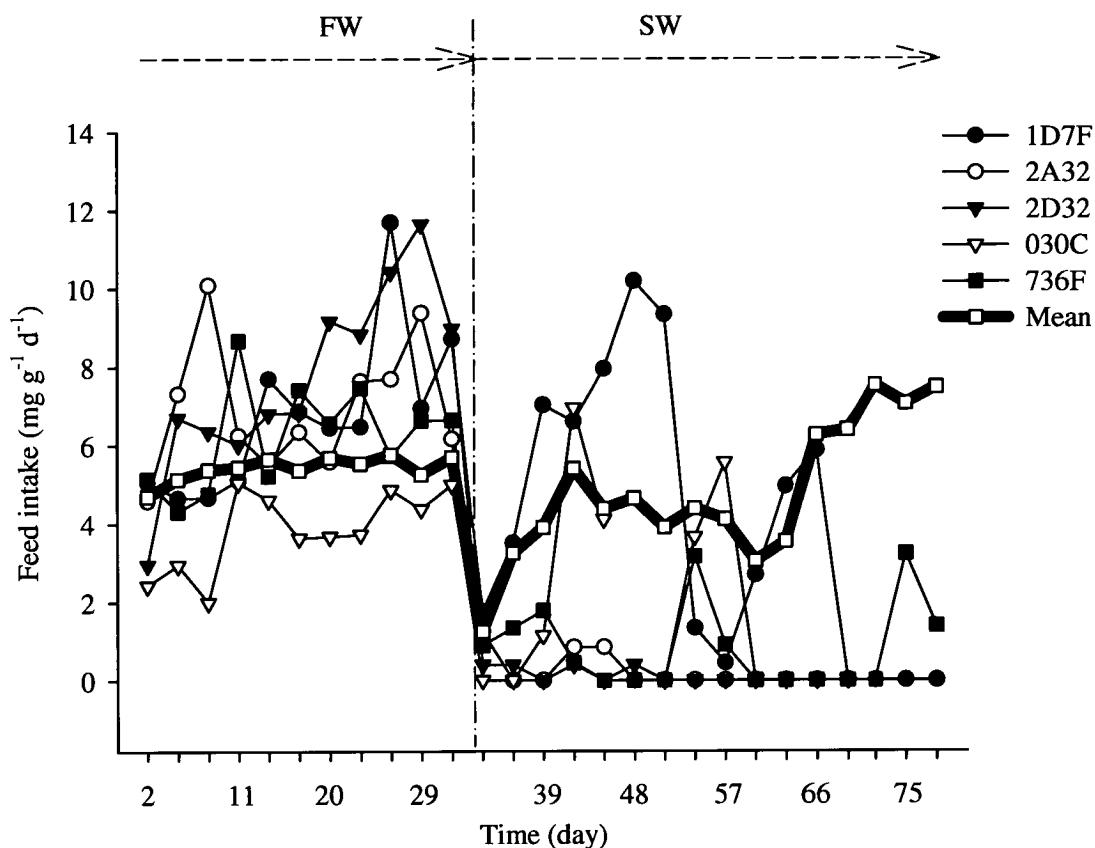
Figure 5.4 Daily feed intake of five (all) individual fish classified as pinheads at time of death or termination of experiment (i.e. fish with K ≤ 0.865) through the entire period of the experiment. Each thin line indicates an individual pinhead while the thick line shows the mean trend of all non-pinheading fish (K > 0.865). Transfer to SW (_.._.) occurred 34 d after the initial weighing. FW = freshwater; SW = seawater.

5.5 Discussion

5.5.1 Group feed intake and aggression

In the present study the results of a 24 h salinity challenge suggest that fish were fully smolted at the time of seawater transfer showing that the choice of time for seawater transfer in this experiment was appropriate to the physiological state of the salmon (Blackburn and Clarke, 1987). While freshwater and 24 h post-transfer osmolalities were significantly different the freshwater value of 328 mmol kg^{-1} was typical of freshwater osmolalities for Atlantic salmon (Prunet and Boeuf, 1985) and the 24 h post-transfer reading of 345 mmol kg^{-1} fell within the range of values previously reported for fully smolted Atlantic salmon (Prunet and Boeuf, 1985).

The reduction in feed intake immediately following seawater transfer and a subsequent increase were consistent with previous findings (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996). It has been suggested that this decrease may be the result of physiological challenge associated with adaptation to the hyperosmotic seawater environment (Stead et al., 1996). Despite the suggestion that the greatest levels of aggression in territorial animals occur following introduction into new habitats when territory boundaries and social hierarchies are first being established (Cutts et al., 2002), in the present study there were no aggressive encounters during the first day in seawater. Aggression increased over the following 7 days concurrently with the rapid increase in feed intake. These features suggested that transfer may initially cause dominance hierarchies to break down. Changes in environmental conditions are known to have varying affects on levels of aggression within groups of fish. For example, increasing water flow has been shown to decrease aggression (McNicol and Noakes, 1984) but increased turbulence to have no affect (Snedden et al., 2006). The changes in levels of aggression are presumably specific to specific environmental changes. For example decreased aggression due to increased water flow is probably explained by increased schooling behaviour that decreases aggression (Christiansen and Jobling, 1990). In the present study it is possible that the initial absence of aggressive behaviours was the result of prioritising energy expenditure to acclimation and/or a result of stress.

In the present study both before and after transfer to seawater a greater proportion of attacks occurred before the meal than after. This pattern of aggression may be an example of feeding anticipatory activity (FAA). While most studies on FAA have focused on swimming and demand-feeding rhythms (Sanchez-Vazquez and Madrid, 2001), Weber and Spieler (1987) found that agonistic behaviour would also entrain to meal times. Scheduled feeding is known to be a zietgeber, a signal that can entrain fish (Purser and Chen, 2001), with this entrainment typically manifesting itself as a pronounced increase in activity in the hours leading up to feeding (Spieler, 1992; Mistlberger, 1994). Given that the feeding regime in the present study was only one meal per day it is likely that this difference was an example of FAA as synchronisation is generally found to be stronger when fish are offered a single daily meal than multiple meals (Sanchez-Vazquez and Madrid, 2001). While it seems FAA may be occurring in the present study for confirmation this would have to be tested under constant conditions, for example 24 hour light and feed deprivation using a full day profile (Purser and Chen, 2001; Sanchez-Vazquez and Madrid, 2001).

Observations were made during the current study that suggest that the decrease in feed intake in Atlantic salmon often associated with seawater transfer (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996) may be due to difficulty in swallowing pellets following transfer. While no quantitative data were collected many fish were observed immediately following seawater transfer to take a pellet into their mouth but after initial capture to reject it. A number of actions occurred that seemed to be associated with trying, in many cases unsuccessfully, to swallow the pellet. Actions included varying combinations, and in some cases all of the following: flaring of the gills, what appeared to be chewing, coughing and choking, opening and shutting of the mouth and shaking of the entire body. In many cases after struggling for a time the pellet was then spat out and the process would start again with another pellet. Once fish did manage to seemingly forcefully swallow the first pellet they seemed quite able to easily swallow following pellets in most cases. Despite these observations in most cases even fish that were unable to eat did try to swallow pellets. Similar observations have been documented for startfeeding of alevins with fish taking pellets and spitting them out many times before they "learn" to swallow (Willoughby, 1999).

5.5.2 Feeding hierarchy stability

Spatial localisation of feed has been shown to give dominant fish the advantage of defending positions close to the feed point source leading to monopolisation of feed resources and the establishment of stable feeding hierarchies (Thorpe et al., 1990; Huntingford and Thorpe, 1992). In the present study point source feeding was used (Davis and Olla, 1987; McCarthy et al., 1999) and feeding rank was found to be stable during both the freshwater phase leading up to seawater transfer and the seawater phase immediately following transfer, though there was no correlation between mean feeding rank in freshwater and mean feeding rank in seawater. This lack of correlation suggests that the feeding hierarchy may be broken down and reformed as a result of seawater transfer, with the reformed hierarchy not resembling that which existed in freshwater. Such changes in the structure of feeding hierarchies have been reported for changes in diet however they tend to be temporary reverting to their original structure over time (Katzir, 1983; Wybourne, 1997). Whilst there was evidence from some groups that the fish at the top of the feeding hierarchy in seawater was also the top of the feeding hierarchy in freshwater this was not the case in all groups. These findings suggest that the lack of correlation between freshwater and seawater feeding hierarchies was not simply the result of hierarchies being non-linear, i.e. one dominant fish and all others having variable ranks (Carter and Davies, 2004). The fact that there were few cases where feeding rank in either freshwater or seawater correlated with final parameters of weight, condition factor, chemical composition or HSI, all of which give an indication of the final condition, demonstrates that in the present study feeding hierarchies, both in fresh and seawater, were poor predictors of final condition.

There is evidence that the relative importance of risks and benefits of foraging to each individual in a group influences the choices an animal makes (Batesone, 2002; Sloman and Armstrong, 2002). For example, jackdaws with high social ranks were less exploratory and never the first to try novel feeds (Katzir, 1983) or to explore new environments (Katzir, 1982) while animals restricted by dominant individuals developed patterns of behaviour such as quickly using new resources when they were made available to compensate for their restrictions (Katzir, 1982, 1983). Few studies have addressed these questions in fish. Carter and Davies (2004) investigated the effects of dietary changes on feed intake and stability of feeding hierarchies finding that

changing diets tended to reduce hierarchy stability possibly related to the balance between potential risks and benefits associated with consuming a novel diet. Wybourne (1997) also found that when feeding a novel diet lower ranked fish increased their relative share of the meal initially while meal share of higher ranked individuals decreased. Whether an animal is willing to take risks appears to depend on factors including its energetic status (Bateson, 2002), and it has been found that when dominant and subordinate Atlantic salmon, both of which had been exposed to a predator (model), differ in their hunger level the hungrier fish will return to feeding first regardless of social status (Gotceitas and Godin, 1991). It would therefore be possible that the fish that were eating following seawater transfer were in fact not dominant fish but rather those that had a greater appetite. Appetite reflects a complex integration of internal factors, including nutritional status and stress level, and external factors including access to feed (Vahl, 1979; Goddard, 1996). Presumably if this was the case there would have been instability in feeding hierarchy (during the 25 days during which stability was analysed following seawater transfer) as the more socially dominant fish slowly began to eat and out-compete less socially dominant fish. The fact that stability was present suggests that this was not occurring and it was not simply a case of poorer fish needing to eat and therefore risking eating in the new environment. Rather some aspect of the new seawater environment actually changed the feeding hierarchies of these fish suggesting that specific adaptations leading to competitive advantage may differ between fresh and seawater environments. The lack of correlation is consistent with findings of Stead et al. (1996) who found that growth performance in freshwater was not significantly correlated with growth performance in seawater. They suggested this was due to not all salmon undergoing the parr-smolt transformation at exactly the same time, and consequently each individual taking different amounts of time to adapt to the increased salinity when transferred as a group. In the current study the stability in feeding hierarchy also suggests that feeding is not likely to be the result of exploitation (scramble) competition, which is defined as indirect competition with other individuals through use of a common resource (Branch, 1984; Carter et al., 1994; McCarthy et al., 1999).

5.5.3 Dominance hierarchy stability

In this study dominance rank was stable in most groups during the freshwater phase leading up to seawater transfer but was not stable in any group during the seawater phase immediately following transfer. The pattern of stability before transfer and lack of stability immediately following transfer is consistent with past findings that while after their initial formation dominance hierarchies tend to become and remain stable in constant conditions (Forkman and Haskell, 2004); environmental disturbance or changes in environment tend to reduce stability (Sloman et al., 2001; Sneddon et al., 2006). Dominance in the present study was monitored in the seawater phase immediately following the change of environment from freshwater to seawater, presumably during which time initial hierarchy formation was occurring for the seawater phase. It is likely that hierarchies would have become more stable following this initial formation period however in the present experiment monitoring of dominance hierarchies after 25 d post-seawater transfer was not possible due to the occurrence of mortalities.

There was no correlation between the mean dominance ranks in freshwater or seawater, or even a tendency for the top and bottom ranked fish to retain their rank across transfer. This may in part be due to the lack of stability during the seawater phase but it is likely, given that mean feeding ranks changed after seawater transfer, and given the lack of any aggression immediately following seawater transfer, that the dominance hierarchies also broke down and were in the process of reforming to be different from those in freshwater. Environmental perturbations or changes such as increased densities and increased turbulence have been demonstrated to cause a breakdown in dominance hierarchies of fish with reformed hierarchies different from those seen before the change (Sloman et al., 2001; Snedden et al., 2006). Dominant sticklebacks, for example, responded to environmental variation by either becoming more aggressive or being deposed (Snedden et al., 2006). The fact that there were very few cases where mean dominance hierarchy rank in either freshwater or seawater showed any correlation with final nutritional status (weight, condition factor, chemical composition or HSI) suggested that long term performance in seawater is not predicted by position in the dominance hierarchy during the freshwater or seawater phase.

5.5.4 Feeding rank vs dominance rank

Dominant individuals generally gain preferential access to feed over subordinates (Basquill and Grant, 1998; Vervaecke et al., 1999), and this is certainly the case in stream living salmonids (Fausch, 1984; Hughes, 1992; Gilmour et al., 2005). Despite this general trend, in experimental systems the way feed is offered can have profound effects on feed acquisition. Feed delivered in a spatially localised way has been shown to allow monopolisation by dominant individuals; while feed delivered in a spatially dispersed manner (scatter feeding) is more difficult to defend and allows more equal access to all individuals (Grand and Grant, 1994; Ryer and Olla, 1995, 1996; McCarthy et al., 1999). As a result spatially localised feeding can lead to positive correlation between overall dominance and feeding ranks, while scatter feeding is likely to result in little correlation between the two (McCarthy et al., 1999). In the current study despite being point source fed there was only one group that had a significant positive correlation between dominance and feeding hierarchy in freshwater and no groups showed correlation between these two in seawater. Where dominance hierarchies and feeding hierarchies did not correlate it has been suggested that instead of a linear dominance hierarchy one individual was dominant (Carter and Davis 2004). However in this study in freshwater and seawater there was only one group where the highest dominance ranked individual had the highest feeding rank. Whilst in seawater the lack of correlation between the two might be partially explained by the lack of stability in the dominance hierarchy during the freshwater phase in most cases dominance hierarchy was stable. It is also worth noting that the button tagging technique used in the present study to identify each individual fish, while being visually quite successful did lead to more handling of fish than would be desired possibly affecting the present findings. While such handling could possibly result in individual fish falling in and out of the feeding hierarchy, as they are less likely to feed post-handling, the stability of the feeding hierarchies suggests that this was not occurring.

Apart from spatial localisation of feed there are a number of other factors that will decrease the ability of dominant fish to successfully monopolise feed, including decreasing the time between arrival of feed items (temporal clumping) (Grant and Cramer, 1992; Hakoyama and Iguchi, 1997), increasing group size (McCarthy et al., 1999), and increasing the ration level (McCarthy et al., 1992; Moutou et al., 1998). The

group size in the present study was small (n = 8) and the feed items delivered in a localised manner and slowly enough (0.5 pellet s^{-1}) for monopolisation by dominant individuals at such group sizes (Hakoyama and Iguchi, 1997). It is however possible that feeding to satiation and not using a restricted ration may have allowed behaviourally subordinate fish to access feed after dominants were satiated. Interestingly it has been found in one study (Bailey et al., 2000) on Arctic charr that even where dominance and feeding hierarchies correlated there was still a large deviation between the actual rank scores with the most aggressive fish in only four out of twelve groups also having the greatest feed intake. In that study the group size was small (n = 4), and feed pellets were delivered in very small numbers (5 pellets between 4 fish) at a single point source.

5.5.5 Pinheads tracked through time

The present study produced five fish that were considered to be pinheads. Pre-transfer data from each of these individuals compared with mean values for non-pinheads show they were recruited from both above and below the mean weight, length and condition supporting previous findings that pinheads tend to be recruited equally across the entire size range of groups transferred to sea (King, 1993). In this experiment there were positive correlations between condition factor and all chemical composition parameters measured, i.e. fish with a poor condition factor also had low dry matter (i.e. a high moisture content), low levels of crude lipid and gross energy, and reduced protein content. Given their poor condition factors it was not surprising that the pinheads that survived till the end of the experiment were found to have values below the mean for each of these chemical composition parameters also.

Present data also indicates that feed intake for pinheads during the freshwater phase was above average, and pinheads were also recruited from all ranks in the freshwater dominance and feeding hierarchies. After transfer to seawater all but one of the pinheads had low mean feeding ranks and none held positions at the top of the dominance hierarchy. However, whilst they were not the highest ranked neither were they the lowest. It has been suggested that failed smolts may be individuals that are physically out-competed by more dominant fish once in seawater (Johnstone, 1991),

however given that individuals with lower dominance rankings did not become pinheads it seems unlikely that social status is the cause but may rather be a symptom. Also, while it has been suggested in the northern hemisphere that failed smolt may not eat following transfer (Johnstone, 1991) in the present study some of the pinheads actually exceeded the mean feed intake of non-pinheads for a few days after transfer to seawater. All pinheading fish ate some feed after transfer.

5.6 Conclusion

This study supports previous findings that changing environmental conditions causes a lack of stability in dominance hierarchies. Feeding hierarchies were found to be stable in both the freshwater and seawater phase of this experiment but there was no correlation between the two suggesting feeding hierarchy in seawater is distinctly different from that in freshwater and one can not be predicted from the other. Dominance hierarchies were found in most groups to be stable in freshwater but not in seawater and again the overall dominance hierarchies in the freshwater and seawater phase did not correlate. There was also a lack of correlation between overall dominance rank and feeding rank in both freshwater and seawater. Overall it seems that seawater transfer breaks down and restructures both dominance and feeding hierarchies. Results of the present study indicate that there is little the structure of dominance or feeding hierarchies in freshwater are likely to be able to tell us about dominance and feeding hierarchies in seawater, and that there is no evidence suggesting that fish that are lower in either hierarchy in freshwater should be at a disadvantage to more dominant fish from freshwater, at least during the first weeks following seawater transfer. In farming situations any advantage that dominant fish may have had over subordinates would presumably be even further reduced, given that densities are larger and fish numbers are much greater in farms than those used in the present study. In general as density and fish number increase the formation of social hierarchies are reduced due to reducing agnostic behaviours and decreasing dominance stability (Li & Brocksen, 1977; Fleming and Johansen, 1984; Kjartansson et al., 1988) as the costs of dominance presumably become too high (Huntingford and Thorpe, 1992; Sloman and Armstrong, 2002).

5.7 Acknowledgements

5.8 References

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Chapter 6

Effect of feed-deprivation immediately following seawater

transfer on feeding, dominance and growth of Atlantic salmon

Matthew Flood

6.1 Abstract

The effects of feed-deprivation immediately following transfer from freshwater to seawater in Atlantic salmon *Salmo salar* were investigated with respect to differences in aggression, feed intake, growth and body composition between fed and feed-deprived fish following the combination of the two. Following a period of 32 days in freshwater, four tanks of individually marked (colour tagged and pit tagged) Atlantic salmon (n = 8) underwent direct transfer into near full strength seawater (30‰) where they remained at a mean salinity of 33.6‰ for 43 days. Throughout the experiment groups were fed one meal per day. In the 14 days immediately following seawater transfer two groups were deprived of feed while the remaining two were fed. Four fish from each feed-deprived group were then combined with 4 fish from each fed group to create four new groups containing half fed and half feed-deprived individuals. Comparisons between fed and feed-deprived fish were made between the time of combining these two treatment groups (14 days post seawater transfer) and the end of the experiment. Following the recombination feed-deprived fish were found to have significantly (p < 0.05) lower mean feed intake and although differences were not significant there were trends for feed-deprived fish to perform lower numbers of attacks and sit lower in the dominance hierarchies. Following recombination feed-deprived fish lost weight while fed fish continued to achieve positive growth. By the end of the experiment feed-deprived fish had significantly lower weight, length and condition factor and their chemical composition indicated lower nutritional status with dry matter, crude lipid, crude protein and gross energy all significantly lower in feed-deprived individuals. Three of four pinheads came from the feed-deprived treatment. A second experiment was run with a larger number of fish (n = 24) specifically to examine growth. The experiment was set up identically to that described above but individuals were not button tagged and no individual assessments were made for feed intake or aggression. Within these groups there was no significant difference in final weight, length or condition factor; change in weight, length or condition factor; or any of the chemical composition parameters. These results are possibly the result of higher fish numbers and densities in this experiment than in the initial behavioural experiment. The main finding from the current experiment was that feed-deprivation immediately following seawater can result

in decreased competitive ability for individuals in direct competition with non feed-deprived fish.

Keywords: Aggression, Atlantic salmon, Compensatory growth, Competition, Dominance, Feed-deprivation, Feeding, Feed intake, Freshwater, Hierarchies, Pinheads, Seawater, Smoltification, Social, Stability, Transfer

6.2 Introduction

Transfer from freshwater to seawater is known to reduce feed intake of Atlantic salmon for several weeks before returning to levels comparable with those in freshwater (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996). It is likely that variation exists between individuals in the amount of time spent in seawater before they recommence feeding given that not all fish undergo the parr-smolt transformation at exactly the same time and hence all individuals will acclimate to increased salinity at slightly different rates (Stead et al., 1996). Periods of feed restriction are often followed by compensatory or catch up growth in many species of mammals, birds (Wilson and Osbourne, 1960) and fish (Jobling et al., 1994; Wang et al., 2000; Tian and Quin, 2003), including salmonids (Dobson and Holmes, 1984; Maclean and Metcalfe, 2001; Nikki et al., 2004). Compensatory growth usually occurs concurrently with increased feed intake (hyperphagia) and improved feed conversion efficiency (Miglavs and Jobling, 1989; Russel and Wootton, 1992; Broekhuizen et al., 1994; Boujard et al., 2000). Whilst it may be predicted that salmon that have waited days or weeks to recommence feeding following seawater transfer would go through a period of compensatory growth, it is also apparent that re-entering the feeding population exposes an individual to increased competition. While aggressive behaviour increases in salmonids with increasing hunger (Symons, 1968; Dill et al., 1981), over time physical condition will deteriorate due to depletion of energy reserves (Jürss et al., 1986; Black and Love, 1986; Weatherley and Gill, 1987; Einen et al., 1998) which can decrease competitive ability. Johnsson et al. (1996) found that while fasting initially caused an increase in the dominance status of rainbow trout, the positive effect of increasing feeding motivation was soon offset by declining energy reserves presumably reducing competitive ability.

It is possible that a loss of competitive ability due to prolonged postponement of feeding in seawater may contribute to the formation of pinheads and failed smolts. Pinheads and failed smolts may be individuals that are traumatised during seawater transfer, or may not be physiologically or behaviourally ready for transfer (King, 1992a; D. Mitchell personal communication). Disrupted feeding behaviour following seawater transfer may cause initial feed-deprivation which is then compounded by social stresses resulting from activity of the more aggressive fish in the population (Johnstone, 1991). It has

been suggested that the tendency to be out-competed may lead to a negative conditioning, with subordinate fish learning that attempting to take feed pellets equates to being attacked by more dominant fish, leading them to avoid pellets (D. Mitchell personal communication). There are a number of observations that support this explanation. Johnstone (1991) observed that failed smolts would take feed when offered but were soon crowded out of the feeding area by "healthy feeding" fish, and it has also been found that pinheads and failed smolts isolated from cage populations and placed back in freshwater tanks will immediately start feeding on commercial pellets (King, 1992b; Johnstone et al., 1999). Failed smolts have also been shown to recover in seawater when placed into separate sea cages from the rest of the population (D. Mitchell personal communication). There is evidence that when failed smolts and pinheads do eat they do so in a way that does not put them into direct competition with more aggressive fish. In cage populations in Tasmania pinheads have been witnessed to avoid standard commercial feed pellets but eat mussels mixed in commercial feed that has been moistened with freshwater (I. Weir personal communication). Similar observations on Scottish farms have been made for failed smolt which again avoid commercial pellets but will eat chopped mussels, chopped sprat, crushed crab, and other small crustaceans (D. Mitchell personal communication).

The aim of the current experiment was to determine whether a period of 14 days feed-deprivation immediately following seawater transfer would result in the deprived fish being out-competed by feeding fish. The main focus was to determine whether a delay in the recommencement of feeding following seawater transfer caused fish to be locked out of feeding resulting in loss of condition, and in extreme cases pinheading. The effect feed-deprivation had on the performance of deprived fish in comparison to fed fish was also examined in terms of growth, condition factor and nutritional status.

6.3 Materials and methods

The following sections outline two experiments designed to determine the effect of 14 days feed-deprivation immediately following seawater transfer on Atlantic salmon in direct competition with individuals not having undergone feed-deprivation. The first experiment (behavioural experiment) focused on small numbers of fish and examined competitive abilities in terms of aggression and feed intake. The second experiment (growth experiment) focused on growth performance of larger numbers of fish than the behavioural experiment without aggression or individual feed intake measurements.

6.3.1 Experimental system and set up of experiments (behavioural and growth experiments)

Experiments were carried out at the School of Aquaculture, University of Tasmania (Launceston, Tasmania, Australia). During the experiments fish were maintained in two blocks of four 300 L flat bottomed cylindrical tanks each supplied with water from a single 3000 L temperature regulated reservoir tank at a rate of approximately 17.5-18 L min^{-1}. The total volume of the system was approximately 7250 L. Tanks had external stand pipes and were drained centrally displaying adequate centrifugal water flow to move uneaten feed and faeces to outlets. Solids were removed from the effluent water via dacron matting and a swirl separator, while nitrogenous wastes were treated by trickle biofiltration. A U.V. lamp filter was used to minimise bacterial levels in the system and a foam fractionator was used to remove dissolved proteins during the seawater (SW) phase. Ambient light entered through overhead opaque skylights. The average water temperature throughout the experiment was 14.3 ± 1.0°C. The average salinity during the SW phase was 33.6 ± 1.2‰. During the freshwater (FW) phase water was continually replaced from the municipal water supply at a rate of approximately 10% d^{-1}, while during the SW phase water was replaced by discrete water changes at least once a week equivalent to a rate of about 10% d^{-1}. There was adequate water flow into tanks to enforce exercise in the fish with inlets under the water level in the behavioural experiment tanks to minimise disruption of surface water allowing clear video footage of fish behaviour to be taken. In the behavioural experiment each experimental tank was fitted with mesh fencing to prevent escape by fish while not

obstructing the view from above. The tanks were surrounded by black plastic, to avoid disturbance of fish, with a small rectangular viewing window, which could be closed when not in use, in the front for observation of feed intake (see chapter 5). In the growth experiment water inlets were just above the water surface and tanks were fitted with mesh lids allowing pellets and light through but preventing escape by fish (see chapter 5).

Water quality was monitored every 1 to 2 d throughout the experiment to ensure that the main parameters of chlorine, pH, ammonia, nitrite and nitrate remained within the recommended limits for salmon in FW (Wedemeyer, 1996) and SW (Tarazona and Muñoz, 1995). Water changes were used to reduce elevated levels.

6.3.2 Fish – Behavioural experiment

All-female diploid Atlantic salmon (*Salmo salar*) spring smolt were obtained from Wayatinah Salmon Hatchery (SALTAS, Tasmania, Australia) on the 5 August 2004 and transported to the School of Aquaculture. They were placed in a 4000 L FW recirculation system at 8°C (2°C higher than Wayatinah's water temperature) where they were held for 22 days. Fish were graded to obtain individuals of uniform size and over a 2 d period were anaesthetised (benzocaine, 75 mg L^{-1}), PIT (Passive Integrated Transponder) tagged, and wet weight (nearest 0.1 g) and fork length (mm) were measured before 8 fish were randomly allocated to each of 4 tanks (95.7 ± 1.7 g, mean ± SD, n = 32). PIT tags used were Destron 11 mm (small, model TX1400L) injectable transponders, which were inserted into the abdominal cavity using a syringe applicator through a small scalpel incision on the ventral side of the fish half way between the anus and pectoral fins. Following tag insertion incisions were sealed with a 3:1 mixture of orahesive protective powder (E. R. Squibb and Sons Ltd, Hounslow, Middlesex, England) and cicatrin antibiotic (The Wellcome Foundation, London, England) to help wounds heal and prevent infection (Porter et al., 1998). PIT tags were used to identify individual fish using a Trovan multireader Mark 3 (model MK3 multireader). Fish were held for a further 23 d before each individual was tagged with two coloured buttons (C. Noble personal communication) for individual identification under video cameras and for individual feed intake (University of Tasmania ethics

approval number A0007035). The technique used was similar to that described in Bailey et al. (2000) using coloured beads, Hakoyama and Iguchi (1997) using coloured ribbon, and Johnsson et al. (1996) using coloured wooden beads.

Fish were removed one tank at a time. Each individual was then anaesthetised (benzocaine, 75 mg L^{-1}), PIT tag number was recorded, and weight (nearest 0.1 g) and fork length (mm) were measured. Two coloured buttons were then secured, one to each side of the fish just posterior to the dorsal fin. A sterilised size 4 sewing needle with a grade 2.0 braided silk suture (Sherwood Davis and Geck, St Louis, USA) was passed through the first button, the flesh on the dorsal side of the spine and then the second button. The needle was then passed back through the buttons and fish in the opposite direction. Orahesive protective powder containing cicatrin antibiotic (see above) (Porter et al., 1998) was applied under buttons to help wounds heal and prevent infection. The buttons were then pulled tightly up against the flanks of the fish, two reef knots were tied and a drop of superglue was used to secure the knots (C. Noble personal communication). The fish were revived by holding them under their tank outlet before being returned to the tank to fully recover. Periodically button tags would be dislodged from fish necessitating replacement; each fish had its buttons replaced due to dislodgement between one and five times (1 time, 1 fish; 2 times, 2 fish; 3 times, 15 fish; 4 times, 8 fish; 5 times, 6 fish) during the actual experimental period. On replacement the buttons were placed in a new location ranging from just anterior to just posterior to the dorsal fin in order to allow healing of flesh at the previous buttoning site. Button replacement only occurred on days immediately following those on which digital footage was taken. This allowed at least one full day for fish to recover from buttoning and disturbance before footage was taken again.

After initial button tagging the salmon faced into but did not swim against the current, instead they positioned themselves on their pectoral and caudal fins on the tank floor reacting little to feed presented. The fish were held for a further 38 d following buttoning during which time they slowly commenced swimming against the current and feeding. Button tags which had been pulled out during this time were replaced and the day following tag replacements all individual fish were anaesthetised (benzocaine, 75 mg L^{-1}) and wet weight (nearest 0.1 g) and fork length (mm) were measured

(113.7 ± 8.3 g, mean ± SD, n = 32). No fish were lost during the pre-experimental period. The experiment then commenced with fish fed one meal per day and video footage taken in behavioural tanks (see below). Fish were kept in FW for a further 32 d before being fasted for 2 d prior to direct transfer into near full strength SW (30‰), in accordance with industry practice, on day 35 of the behavioural experiment.

Transfer to SW was achieved by emptying the reservoir completely and refilling it with SW. The water level in the experimental tanks was then lowered until only just enough remained to cover the fish. The pumps were then turned back on from the reservoir to experimental tanks filling them with SW. A pen of 14 spare button tagged fish kept in the reservoir tank were randomly (using random numbers) divided into two groups to be used in a 24 h salinity challenge. Blood samples of approximately 1 ml were taken from half of these fish immediately prior to SW transfer and for the second half 24 h following transfer from the caudal vein using heparin-treated needles and 1 ml Tuberculin™ syringes. Samples were then syringed into Eppendorf safe lock tubes, placed in ice, and subsequently centrifuged for 3 min at 4000 g. Plasma was removed and stored in a second Eppendorf at -80°C for later analysis. Intermediate weights were not measured to avoid disturbance of fish while undertaking behavioural observations. Fish were weighed only at the beginning of the experiment, at reallocation (see below) and at the end of the experiment. Fish in tanks 2 and 4 (chosen using random numbers) received their first feed in SW on the day following transfer while 1 and 3 were not fed for 14 d. On the 15th d following SW transfer (day 50) all fish within the experiment were reallocated. Half of the fish from each tank were combined with half of the fish from another tank so that all tanks contained half fish that had been fed and half fish that had been feed-deprived during the first 14 d in SW; this period of 14 d will be referred to from this point on as the treatment period. Button code combinations were sorted using random numbers and divided accordingly into two groups, identical in each of the four tanks to ensure that no colour was in a tank twice following reallocation (Figure 6.1). In no case were fish put back into their original tank and in all cases the combination of the two origin tanks was unique. Tank 1 fish were originally from tank 3 and 4; tank 2 originally tank 1 and 4; tank 3 originally tank 1 and 2; and tank 4 originally tank 2 and 3 (Figure 6.2). Reallocation was achieved by placing fish into four 100 L containers corresponding to their original tank number. Fish from each tank were

identified by their button colour combination as well as their PIT tag numbers, and placed into a 100 L container on the opposite side of the room corresponding to their new tank number. Once all fish had been reallocated each individual had its PIT tag number checked for a second time before being weighed by placing a bucket of water on a scale, taring the scales and placing the un-anesthetised fish in the water. The newly allocated groups were then placed back into their new tanks in the experimental system and kept in SW for a further 29 d with the experiment finishing 79 d after its commencement.

Final sampling occurred over one d with tanks selected in random order (chosen using random numbers) and fish euthanased (benzocaine, 300 mg L^{-1}) before wet weight (to nearest 0.1 g) and fork length (mm) were measured. The liver was then removed, weighed, returned and the whole fish carcass was placed in a labelled bag and stored in the freezer at -20°C for measurement of whole body chemical composition (see below).

6.3.3 Fish – Growth experiment

A second experiment was run to determine the effect of a period of feed-deprivation on growth in fish in larger groups not affected by the stress of button tagging. At the time of PIT tagging for the behavioural experiment a further 96 fish weighing between 100 and 108 g were PIT tagged and distributed equally between four tanks. The day following the commencement of the behavioural experiment all growth experiment fish were combined in a large holding container before being anaesthetised (benzocaine, 75 mg L^{-1}), identified using their PIT tag code, and wet weight (nearest 0.1 g) and fork length (mm) were measured before 24 fish were randomly allocated to each of 4 tanks (134.2 ± 12.1 g, mean ± SD, n = 96). Fish were not individually tagged with coloured buttons. The fish were transferred to SW simultaneously with the behavioural experiment fish. Fish in tanks 2 and 3 (chosen using random numbers) received their first feed in SW on the day following transfer while 1 and 4 were not fed for 14 d (the treatment period). On the 15th d following SW transfer (day 49) all fish within this experiment were also reallocated as was described for behavioural fish with the exception that the original tanks were randomly (using random numbers) divided into two groups as there was no need to prevent doubling up of button code combinations.

The same procedure was used as for the behavioural experiment; however when it came time to scan the fish for the second time and weigh them the scanner malfunctioned. The newly allocated groups were then placed back into the experimental system while a replacement PIT tag scanner was sourced. Two days later (day 51) these fish were again removed from their tanks identified by their PIT tag numbers and individually weighed in a bucket of water before being replaced into the system. In this experiment fish were individually weighed at allocation (day 0), again on the day prior to SW transfer (day 33), immediately following reallocation of feed-deprived and fed fish (day 51), between reallocation and final sampling (day 70) and at final sampling (day 78). On days between allocation and final sampling weights were taken by placing a bucket of water on a scale, taring the scales and placing the un-anaesthetised fish in the water. The final sampling occurred on the 19 Jan 2004 as described for the behavioural experiment above.

6.3.4 Feeding and feed intake

Fish were fed a commercial extruded salmon feed (3 mm Skretting Nutra Transfer 47/26, Skretting Cambridge, Tasmania, Australia: $90.0 \pm 0.02\%$ dry matter, $40.1 \pm 0.11\%$ crude protein, $28.4 \pm 0.11\%$ crude lipid, $8.6 \pm 0.05\%$ ash (mean $\pm$ SD, n = 2)). From the commencement of the behavioural experiment feeding occurred once a day through a clear feeding tube (permanently positioned on the side of the tank) connected to a funnel outside the black plastic for delivery without disturbance to fish (see chapter 5). Feed was delivered at a rate of approximately 0.5 pellets s^{-1} (slightly slower immediately following SW transfer and immediately following reallocation) until satiation was achieved. Satiation was defined as the time when 4 pellets lay simultaneously on the base of the tank. Feeding lasted approximately 3 to 5 min and meals commenced, at 1030, 1035, 1040 and 1045 h for tanks 1, 2, 3 and 4 respectively. Feeding in this way ensured feed was delivered in a spatially and temporally defensible way. Feed intake for individual fish was measured every 3 d throughout the experiment (number of pellets eaten multiplied by the average pellet weight). Fish were observed through the viewing window and the button colour code was recorded on audio cassette each time a pellet was taken by an individual. Number of pellets consumed by each fish within the feeding session was scored by playing back the cassette at a later date. Feed

intake is presented as absolute feed intake (mg d^{-1}) or intake relative to fish wet weight (mg g^{-1} d^{-1}) where 10 mg g^{-1} d^{-1} is equivalent to 1% BW d^{-1}.

Share of the meal eaten by each individual was calculated as a percentage of the total amount consumed by the group:

$$SM\ (\%) = 100 \times AC\ /\sum AC \qquad\qquad [6.1]$$

(Carter et al., 1994a) where AC is the absolute daily consumption rate (mg d^{-1}) of a fish and $\sum$ AC the sum of the absolute daily consumption rates for all fish in a group (tank) that could be analysed. Ranked share of meal data was used to determine the stability of the feeding hierarchies in the 22 d immediately following the reallocation to combine fed and feed-deprived fish following the treatment period. The period of time following the reallocation during which stability of hierarchies could be assessed was restricted by the occurrence of mortalities. For fish to be included in the assessment of stability they needed to be alive on each day included in the analysis. One tank had lost one fish during the 14 d starvation period following SW transfer while another lost one fish within 5 days of the reallocation. These fish were removed from all stability analyses. After the first 22 d following reallocation more mortalities occurred and hence stability was not measured after this point. The mean share of meal (MSM) was used to calculate the overall feeding hierarchy during this 22 d period following reallocation. The MSM was calculated as:

$$MSM\ (\%) = \sum SM\ /\ n \qquad\qquad [6.2]$$

(McCarthy et al., 1992) where n is the number of estimates of SM for each fish. MSM was used to assign a rank for each individual fish within the feeding hierarchy (McCarthy et al., 1992; Carter et al., 1994a).

Fish in the growth trial were fed the same feed used in the behavioural experiment once a day by hand from the commencement of the experiment. The feed was always delivered to the same position in the tank though no feeding tubes were used in this experiment. Feed was delivered at a rate of approximately 1.5 pellets s^{-1} (slightly slower

immediately following SW transfer and immediately following reallocation), approximately 3 times the rate of the behavioural experiment in which each tank contained 1/3 the number of fish. Feed was delivered continuously until satiation was achieved. Satiation was defined as the time when 8 pellets lay simultaneously on the base of the tank. Feeding lasted approximately 3 to 5 min with meals being delivered between 0930 and 1000 h for each tank with the order changed daily using random numbers.

6.3.5 Analysis of fish behaviour

Agonistic behaviour was measured in each tank for 15 min twice a day: 15 min immediately before and 15 min immediately after the daily meal, but not during the meal as feeding behaviour was intense and disruptive to the surface water making video footage unclear. Behavioural measurements were taken around the meal as comparisons between aggression and feeding hierarchies were to be made, similar to those in McCarthy et al. (1999). Behavioural observations for fish tend to range from around 5 (e.g. Oliveira and Almada, 1996) to 30 minutes per day (Olsen et al., 1996) in previous studies and the decision was made to use the upper end of this range in the present study to increase accuracy of estimates. Observations were made on 8 d following the reallocation to combine fed and feed-deprived fish after the treatment period (days 51, 54, 57, 60, 63, 66, 69, 72). On the first day following reallocation (day 51) no aggressive interactions occurred during monitoring times for 1 of the 4 tanks and hence no dominance index or dominance rank could be determined for this tank of fish. The decision was made to remove this day from the stability analysis for all 4 tanks. The remaining 7 d were also used to assess the stability of feeding hierarchies (see above). Footage was captured by colour digital video cameras (Model: C 500R CCD, Swann Communications Pty. Ltd., Victoria, Australia) mounted above each tank and recorded for each tank simultaneously using a Chateau XP digital surveillance network system (Chateau Technical Corp, Taipei, Taiwan) on a PC (see chapter 5). The agonistic behaviours of Atlantic salmon identified by Keenleyside and Yamamoto (1962) were adapted for the present study: charging (swimming directly and quickly towards another fish), nipping (biting another fish without prior approach), chasing (a succession of repeated charges possibly with attempts to nip the retreating fish). Frontal and lateral

displays (Keenleyside and Yamamoto, 1962) were not witnessed in the present study, presumably due to the necessity to swim against the current in this particular experiment, and as such were not included. In each interaction the losing fish would either flee or be displaced from its original position. The winner and loser were recorded for each distinct encounter, with an encounter defined as a group of one or more behaviours separated by no activity (Olsen and Jarvi, 1997; Carter and Davies, 2004). To assign each fish a dominance rank a dominance index (DI) was calculated as:

$$DI = A^+ / (A^+ + A^-)$$
[6.3]

(Wineberg et al., 1991; Carter and Davies, 2004), where A^+ and A^- are the number of aggressive acts performed (winner) and received (loser), respectively, for a given an individual. Individual values for DI can range between 0 (lost all encounters) and 1 (won all encounters). In the few cases where there were no recorded behaviours for an individual, and DI could not be calculated, the fish were ranked below fish for which $A^+ > A^-$ and above fish where $A^+ < A^-$, i.e. they were given a DI value of 0.5 (Carter and Davies, 2004). Dominance index (DI) rank was used to determine the stability of the social hierarchy in the 22 d immediately following the reallocation (i.e. combining fed and feed-deprived fish). The mean dominance index (MDI) was used to calculate the overall social hierarchy during this time. The MDI was calculated as:

$$MDI = \Sigma DI / n$$
[6.4]

(McCarthy et al., 1999) where n is the number of estimates of DI for each fish. MDI was used to assign a rank for each individual fish within the dominance hierarchy.

6.3.6 Further calculations

Feed conversion ratio (FCR) was calculated as:

$$FCR \ (g \ g^{-1}) = \text{Weight of feed ingested (g)} / \text{Weight gain (g)}$$
[6.5]

In order to perform this calculation feed intake data from d on which group feed intake was measured was extrapolated to give values for d on which it was not (Carter and Hauler, 2000). Condition factor (K) was calculated as:

$$K = 100 \text{ [Wet weight (g) / Fork length (cm)}^3] \quad\quad [6.6]$$

Specific growth rate was calculated as:

$$SGR \text{ (\% d}^{-1}) = 100 \text{ ((ln } W_t - \ln W_i)/t) \quad\quad [6.7]$$

where W_i and W_t are the initial and final weights respectively and t is the time in days between the initial and final weighing. SGR describes exponential change in body mass with time (Jobling, 2001a) and was calculated and used to estimate the total weight of fish in each tank on each day of the experiment allowing a more accurate approximation of feed intake relative to fish weight (mg g^{-1} d^{-1}). SGR was therefore not shown as a direct measure of growth in the results.

6.3.7 Chemical analysis of diets and whole fish

Standard methods were used to measure the chemical composition of whole fish and feed: dry matter (DM) was determined by freeze drying fish or feed to a constant weight followed by oven drying at 110°C overnight (~17 h); samples were ashed in a furnace at 550°C for 16 h (feed only) (AOAC, 1995); crude protein (Kjeldahl using copper catalyst [N x 6.25]); crude lipid (Bligh and Dyer, 1959). Energy content of fish was estimated using gross energy values for standard protein and lipid of 23.6 and 36.2 kJ g^{-1} respectively (Brafield, 1985); carbohydrate was not included (Shearer, 1994).

6.3.8 Osmolality measurements

To determine whether fish were physiologically ready to be transferred to SW osmolalities of blood plasma samples taken in the 24 h salinity challenge (see 5.3.2) were measured using a Vapro© Vapor Pressure Osmometer (Model 5520) giving

measurements in Standard International (SI) units: mmol kg^{-1} (Webster, 1985). For each sample, 10 μl of plasma was used (Wescor Inc, 2000).

6.3.9 Pinheads tracked through time

All of the fish with K ≤ 0.865 at the termination of the experiment or at the time of their death (a total of 4 fish) were deemed to be pinheads (Chapter 2). These fish were examined in order to determine how their individual feed intake (mg g^{-1} d^{-1}) changed through time and how they compared with non-pinheading fish in terms of individual feed intake through time; initial and final weight, length, K; mean individual feed intake (mg g^{-1} d^{-1}) during both the FW and SW phase; final chemical composition; and HSI. Whilst no statistical comparisons were possible the data were still of interest in examination of the pinheading condition, and as such a qualitative assessment of pinhead performance is presented in relation to mean data. In all cases the individual values for each pinhead are compared to the mean values calculated for non-pinheading fish. The positions of pinheads in the overall feeding and dominance hierarchies in FW and SW were also examined as was the number of pinheads resulting from fish fed immediately following SW transfer and those feed-deprived for 14 d.

6.3.10 Statistical analysis

Mean values are reported ± standard error of the mean (S.E.M.). Normality was assumed as sample sizes were too small to test for this. Homogeneity of variances was tested graphically by examination of residual plots in SPSS. Data comparing feed intake and attacks received by each button colour code were statistically analysed to test for differences between means using one way ANOVA (Underwood, 1997). All other means were analysed using independent samples t-tests. Repeated measures ANOVAs were used to analyse the trajectories of weight through time (SPSS) with the greenhouse geisser epsilon value used to reduce degrees of freedom of the F statistic to correct for violation of the sphericity assumption when needed. Kendall's coefficient of concordance was used to examine the stability of individual feeding and dominance ranks of fish in groups between days on which measurements were made following reallocation, combining fed and feed-deprived fish (Sokal and Rholf, 1995; McCarthy et

al., 1999; Carter and Davies, 2004). Spearman's correlations were used for all correlation analysis. With the exception of Kendall's coefficient of concordance all statistical analyses were performed using SPSS version 11.5 (SPSS, 2002). Differences were considered significant at $p < 0.05$.
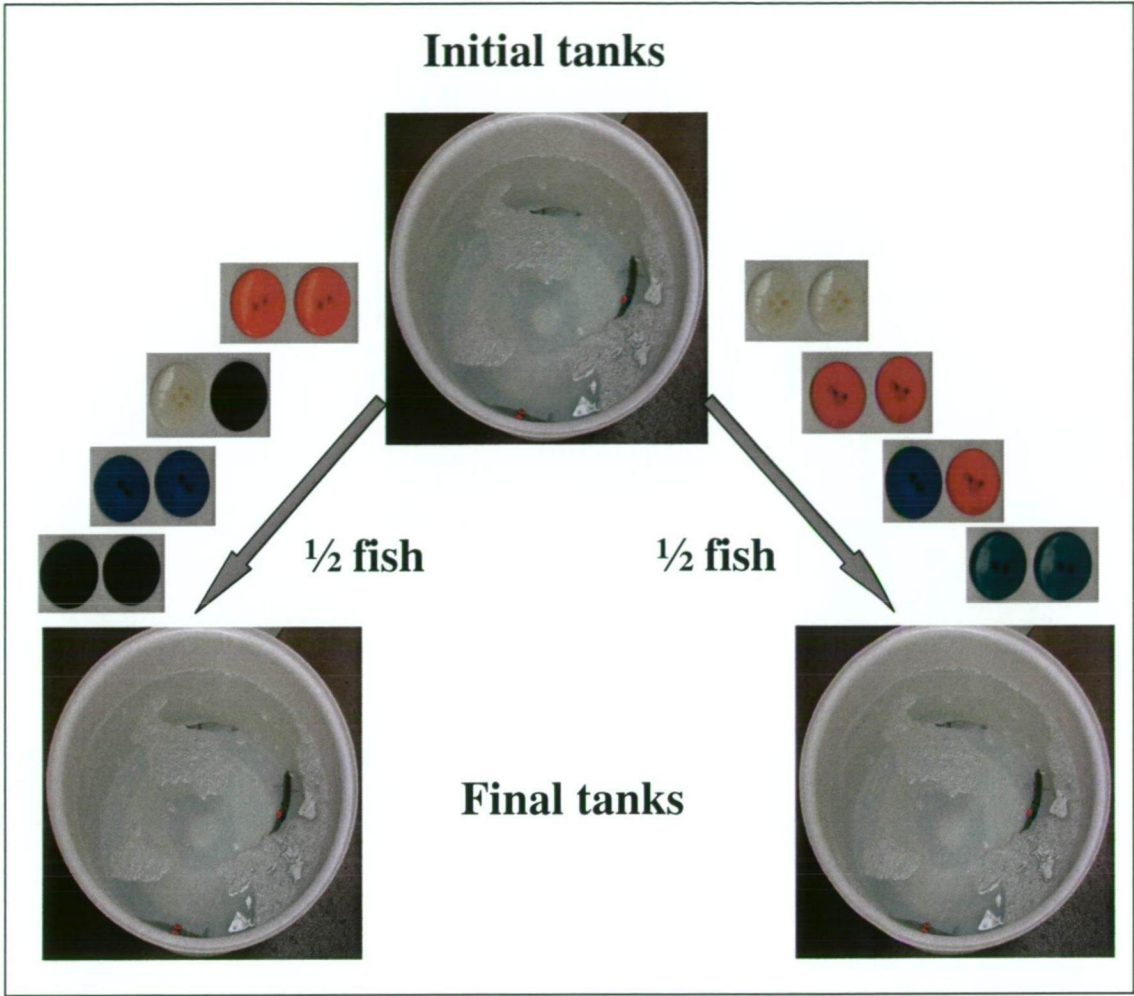
Figure 6.1 Each tank from the fed or feed-deprived treatment in the behavioural experiment was halved and reallocated following the treatment period as shown here.
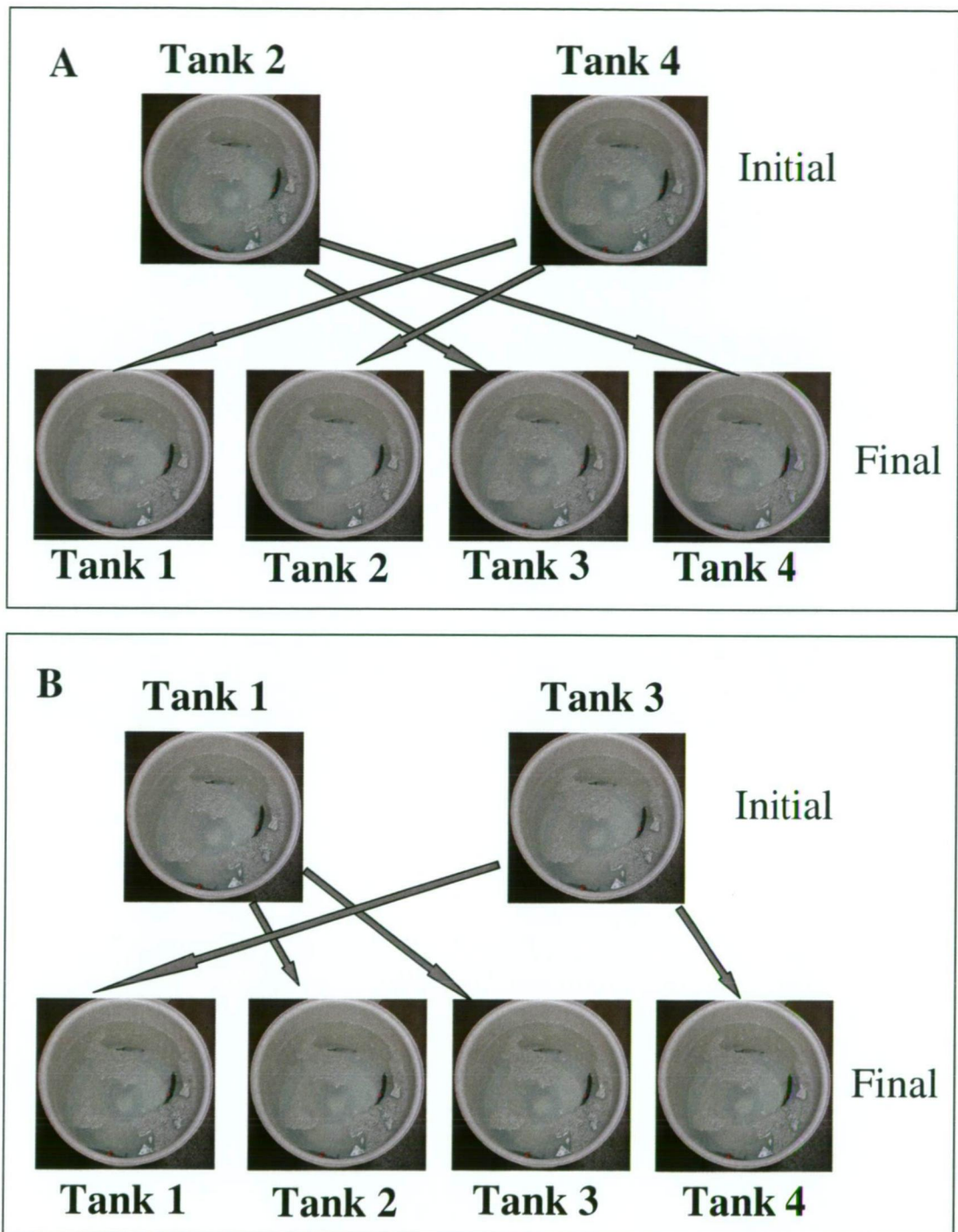
Figure 6.2 Diagrams show the reallocation of fish in order to gain half fed and half feed-deprived individuals. (A) Two tanks labelled "initial" were the fed treatment tanks. Each was halved and allocated to two of the tanks labelled "final". (B) Two tanks labelled "initial" were feed-deprived treatment tanks. Again each was halved and allocated to two of the tanks labelled "final". This resulted in each "final" tank containing 4 fed and 4 feed-deprived individuals.

6.4 Results

Results from both experiments will be included together under each of the following sections.

6.4.1 Growth and performance

In the behavioural experiment mean survival was higher than 81% over the 11-week experiment and there was no significant difference in survival between fed and feed-deprived fish (Table 6.1). Random selection of tanks for each treatment (fed or feed-deprived) resulted in the chance occurrence that fed fish displayed a significantly larger initial weight than feed-deprived fish, with fed fish 6.2% larger. There was no significant difference between initial length or K for the two treatments. At reallocation the fed fish were significantly larger by weight than feed-deprived fish, with fed fish 14.5% larger. The final weight, weight gain (reallocation to final), final length, change in length (initial to final), final K and change in K (initial to final) were all significantly greater for fed than feed-deprived fish, with fed fish 28.1% larger by weight at final sampling. There was no significant difference between the HSI of fed and feed-deprived fish (Table 6.1). There was a significant difference between the trajectories of weight through time with fed fish having greater growth than feed-deprived fish between the commencement of the experiment and the reallocation following the treatment period (Figure 6.3). Growth slowed slightly between the reallocation and final weighing for fed fish while fish that had been feed-deprived continued to lose weight and the overall gap in weight between fed and feed-deprived fish became greater. On an individual basis it was found that only 5 of the 16 feed-deprived fish had positive growth between reallocation (following the treatment period) and the end of the experiment. Values for these 5 fish were comparable with fed treatment fish.

In the growth experiment mean survival was higher than 79% over the 11-week experiment and there was no significant difference in survival between fed and feed-deprived fish (Table 6.2). There was no significant difference between the initial weight, length or K for the two treatments. At reallocation the weight of fed fish was significantly higher than feed-deprived fish, however, there was no significant difference between final weight, weight gain (reallocation to final), final length, change

in length (initial to final), final K, change in K (initial to final) or HSI (Table 6.2). There was a significant difference between the trajectories of weight through time (Figure 6.4). Between initial weighing and SW transfer (day 33) the trajectories were indistinguishable from each other. During the period between transfer and reallocation (day 51), i.e. the treatment period, the weight of feed-deprived fish decreased while fed fish continued to gain weight at a much reduced rate. While the trajectories were split after the reallocation the distance between the lines and gradients of the lines suggests similar growth performance of both groups following the treatment period, with lack of significance in final weights suggesting some level of convergence by the end of the experiment.

6.4.2 Twenty-four hour salinity challenge

There was a significant difference between osmolality in FW and after 24 h exposure to SW ($t = -3.847$, df 12, p = 0.002**); it increased from 328.14 ± 2.6 to 344.6 ± 3.4 mmol kg^{-1} (mean ± S.E.M).

6.4.3 Group feed intake and aggression

Group feed intake (mg g^{-1} d^{-1}) varied through time (Figure 6.5). During the FW phase intake for fed and feed-deprived fish was almost identical rising from a mean of 4.0 mg g^{-1} d^{-1} up to a maximum of 6.5 mg g^{-1} d^{-1} just prior to SW transfer. Immediately following SW transfer feed intake decreased to a mean of 1.8 mg g^{-1} d^{-1}, for fish fed during the treatment period only, before increasing rapidly over the following 7 d period (till day 45). There was a decrease in feed intake on day 48 presumably due to 6 of the 16 fish in the fed treatment requiring button replacement 2 d prior to this (day 46). During the treatment period feed intake of feed-deprived fish was obviously 0 mg g^{-1} d^{-1}. The day following reallocation to combine fed and feed-deprived fish mean daily feed intake for both treatment groups was 1.5 mg g^{-1} d^{-1}. Mean daily feed intake climbed steadily until the termination of the experiment with the fed treatment always having higher intake than the feed-deprived treatment. While the maximum and minimum error bars did overlap for the two groups on most days overall mean intake for the period from reallocation till termination of the experiment was significantly

higher for fed treatment fish than those feed-deprived (Figure 6.6). FCR calculated for the period between reallocation and termination of the experiment was significantly higher (positive value) for fed than feed-deprived treatment fish (negative value) (Figure 6.7).

Aggression on a group basis varied through time during the 22 d following reallocation after the treatment period (Figure 6.8). During the 7 d monitoring to assess hierarchy stability the mean daily number of attacks per group (tank) was 56.6 ± 23.4 (during 30 min observation). During this time an average of 57.3 and 42.7% of the attacks observed occurred during the 15 min monitoring immediately prior to the meal and immediately following the meal, respectively. On most days the mean number of aggressive interactions performed by fish from the fed treatment were greater than those performed by the feed-deprived treatment (Figure 6.8B), however, there was no significant difference between the average number of attacks (calculated across the 8 d monitoring) performed by each treatment ($t = 1.997$, df 6, p = 0.093). And whilst there was also a trend showing that the average position held by individuals in the dominance hierarchy was higher for fed fish than feed-deprived fish this difference was also not significant (Figure 6.9).

6.4.4 Individual feeding ranks

The stability of a group's feeding hierarchy was shown by the magnitude and significance of Kendall's coefficient of concordance. This showed the level of concordance between feeding ranks of all individuals in a group over the 7 d monitored during the 22 d immediately following reallocation after the treatment period (Table 6.3). Feeding ranks showed stability in every group. The fish with the highest feeding rank came from the fed treatment in three of the four groups (tanks 1, 3 & 4), and the fish with the lowest feeding rank came from the feed-deprived treatment in three of the four groups (tanks 3, 4 & 5).

In 3 of the 4 tanks there were significant negative correlations between overall feeding rank and final weight, final K, dry matter, crude protein, crude lipid, gross energy, and HSI (Table 6.4). In one group (tank 1) correlation existed between feeding rank and

final weight, and feeding rank and crude protein; in another (tank 3) between feeding rank and final weight, feeding rank and dry matter, feeding rank and crude lipid and feeding rank and gross energy; and in the third (tank 4) between feeding rank and final K, feeding rank and dry matter, feeding rank and crude protein, feeding rank and crude lipid, and feeding rank and gross energy. The directly measured chemical composition parameters (i.e. dry matter, crude protein and crude lipid) all correlated with each other (Table 6.5).

6.4.5 Behavioural interactions

The stability of a group's dominance hierarchy was also shown by the magnitude and significance of Kendall's coefficient of concordance, again showing the level of concordance between ranks of all individuals in a group over the 7 d monitored during the 22 d immediately following reallocation after the treatment period (Table 6.3). Dominance ranks showed stability in all groups during the 7 d of observations, however in all cases the magnitude of significance was less than that observed for feeding rank stability. The fish with the highest dominance rank came from the fed treatment in three of the four groups (tanks 1, 3 & 4), and the fish with the lowest dominance rank came from the feed-deprived treatment in all four groups.

There was little evidence of dominance rank relating to feeding rank. There was no significant correlation between the mean dominance rank and the mean feeding rank in any group for the 7 d monitored. Despite this lack of statistical correlation there was evidence of non-linear hierarchy formation as the fish with the highest dominance rank also had the highest feeding rank in three of the four groups (tanks 2, 3 & 4) while having a feeding rank of 2 (out of 7 fish) in the final group. The fish with the lowest dominance rank also had the lowest feeding rank in only one of the four groups (tank 1) while having a feeding rank of 5 (out of 7 fish), 7 (out of 8 fish), and 7 (out of 8 fish) in the other three groups (tanks 2, 3, & 4, respectively).

There was no significant correlation between mean dominance rank and final weight, final K, dry matter, crude protein, crude lipid, gross energy or HSI in three of the groups (tanks 1, 2 & 3) (Table 6.6). In the final group (tank 4) there was a significant

correlation found between dominance rank and final weight, and dominance rank and all chemical composition parameters.

6.4.6 Chemical composition of fish, behavioural and growth experiment

At final sampling the feed-deprived treatment fish had significantly less dry matter, crude protein, crude lipid and gross energy than the fed treatment fish in the behavioural experiment (Table 6.7). At final sampling there was no significant difference in dry matter, crude protein, crude lipid, or gross energy between feed-deprived and fed treatment fish in the growth experiment (Table 6.8).

6.4.7 Pinheads tracked through time

Four fish were identified as pinheads (K ≤ 0.865) in the behavioural experiment, 3 of which came from the feed-deprived treatment. The pinheads had initial weight, length and K both above and below the mean initial values of these parameters for non-pinheads (Table 6.9), however in every case their final weight, length and K were below the mean final values for non-pinheads. Over the length of the experiment all pinheads lost weight while non-pinheads gained weight. Any increase in length for pinheads was lower than the non-pinhead mean increase, and the loss of condition of pinheads was at least 2 times greater than the mean loss of condition experienced by non-pinheads. During the FW phase the mean overall daily feed intake for pinheads fell above and below the mean for non-pinheads, however following SW transfer mean daily feed intake of each individual pinhead, calculated across the entire SW phase, was less than the mean for non-pinheads. Despite this it was found that on some days during which feed intake was monitored in SW the amount of feed eaten (mg g^{-1} d^{-1}) by pinheads did exceed the mean value for non-pinheads (Figure 6.10). While every pinhead ate less over the course of the entire SW period than non-pinheads in no case did an individual pinhead fail to ingest feed during this time (Figure 6.10). Pinheads were ranked from the middle to the bottom of both feeding and dominance hierarchies (Table 6.9). Two of the 4 pinheads held the lowest positions in the dominance hierarchy and both also held the second lowest positions in their feeding hierarchies. Both of these fish came from the feed-deprived treatment.

Due to mortalities before the end of the experiment only two pinheads were still alive at the final sampling, hence there are only two values for pinheads for each of the chemical composition parameters and HSI (Table 6.9). Dry matter, crude protein, crude lipid, and gross energy were lower in both pinheads than the non-pinhead mean. HSI was lower than the non-pinhead mean in one pinhead and higher for the other. In the growth trial 6 fish were considered to be pinheads at either final sampling or at time of their death and three of these came from each of the fed and feed-deprived treatments.

6.4.8 Button tagging

Button tag colour combination had no significant effect on the mean number of attacks received by each fish calculated across the 7 d during which stability of dominance rank was monitored (F = 0.428, *df* 7, 23, p = 0.874), and no significant effect on the mean feed intake (mg g^{-1} d^{-1}) calculated across the entire experimental period (F = 0.590, *df* 7, 24, p = 0.758). Hence there was a high degree of confidence in using colour button tags as a method of identification of fish to determine hierarchies.

Table 6.1 Performance of Atlantic salmon in behavioural study, fed or feed-deprived for 14 d following transfer to SW (mean ± S.E.M, n = 4 replicate groups. For each parameter the mean is written above the S.E.M). SW = seawater.

Parameter	Unit	Treatment					
		Fed	Feed-deprived	t values	df	tails	p
WEIGHT							
Initial weight	(g)	117.1	110.3	3.858	6	2	0.008**
		1.2	1.3				
Weight at mixing	(g)	129.1	112.8	4.350	6	2	0.005**
		0.9	3.6				
Final weight	(g)	136.5	106.6	6.405	6	2	< 0.001***
		2.4	4.0				
Weight gain (mix to final)	(g)	7.4	-6.3	4.165	6	2	0.006**
		2.9	1.5				
LENGTH							
Initial length	(mm)	220.3	216.7	2.280	6	2	0.063
		1.3	0.9				
Final length	(mm)	235.9	224.6	3.787	6	2	0.009**
		1.4	2.7				
Change in length (initial to final)	(mm)	15.6	7.9	2.612	6	2	0.040*
		0.75	2.9				
CONDITION FACTOR							
Initial K		1.10	1.08	0.624	6	2	0.555
		0.017	0.008				
Final K		1.03	0.93	6.770	6	2	< 0.001***
		0.008	0.013				
Change in K (initial to final)		-0.060	-0.151	3.801	6	2	0.009**
		0.019	0.015				
HSI	(%)	2.70	2.60	0.366	6	2	0.727
		0.081	0.251				
OVERALL SURVIVAL	(%)	93.8	81.3	1.414	6	2	0.207
		6.3	6.3				

Figure 6.3 Behavioural experiment - weight of each treatment (mean ± S.E.M) at the commencement of the experiment (day 0), at mixing of fed and feed-deprived fish (day 50) and at final sampling (day 79). Repeated measures analysis indicated that treatment trajectories behave significantly differently through time from day 0 to 79 (F = 20.220, df = 2, 12, p < 0.001***). FEED-DEPRIVATION = 14 d period following SW transfer (⁻⁻⁻⁻); FW = freshwater; SW = seawater.

Table 6.2 Performance of Atlantic salmon in growth study, fed or feed-deprived for 14 d following transfer to SW (mean ± S.E.M, n = 4 replicate groups. For each parameter the mean is written above the S.E.M). SW = seawater.

Parameter	Unit	Treatment					
		Fed	Feed-deprived	*t* values	*df*	tails	p
WEIGHT							
Initial weight	(g)	134.2	134.3	-0.103	6	2	0.922
		0.4	0.3				
Weight at mixing	(g)	166.1	155.4	3.769	6	2	0.009**
		2.2	1.7				
Final weight	(g)	188.6	171.2	2.260	6	2	0.065
		4.2	6.4				
Weight gain (mix to final)	(g)	22.5	15.7	1.089	6	2	0.318
		2.3	5.8				
LENGTH							
Initial length	(mm)	230.6	230.3	0.260	6	2	0.803
		1.1	0.2				
Final length	(mm)	263.9	257.6	2.124	6	2	0.078
		1.0	2.8				
Change in length (initial to final)	(mm)	33.3	27.4	1.813	6	2	0.120
		1.8	2.8				
CONDITION FACTOR							
Initial K		1.09	1.10	-0.282	6	2	0.787
		0.012	0.006				
Final K		1.01	0.99	0.925	6	2	0.390
		0.017	0.013				
Change in K (initial to final)		-0.081	-0.105	1.161	6	2	0.290
		0.010	0.018				
HSI	(%)	1.18	1.31	-1.573	6	2	0.167
		0.034	0.075				
OVERALL SURVIVAL	(%)	91.7	79.2	1.342	6	2	0.228
		5.9	7.2				

Figure 6.4 Growth trial - mean weight of each treatment (mean ± S.E.M) at the commencement of the experiment (day 0), at transfer (day 33), at mixing of fed and feed-deprived fish (day 51), intermediate weight check (day 70) and at final sampling (day 78). Repeated measures analysis using the greenhouse geisser epsilon value indicated that treatment trajectories behave significantly differently through time from day 0 to 78 (F = 7.306, df = 1.189, 7.134, p = 0.027*). FEED-DEPRIVATION = 14 d period following SW transfer (⁻·⁻·); FW = freshwater; SW = seawater.
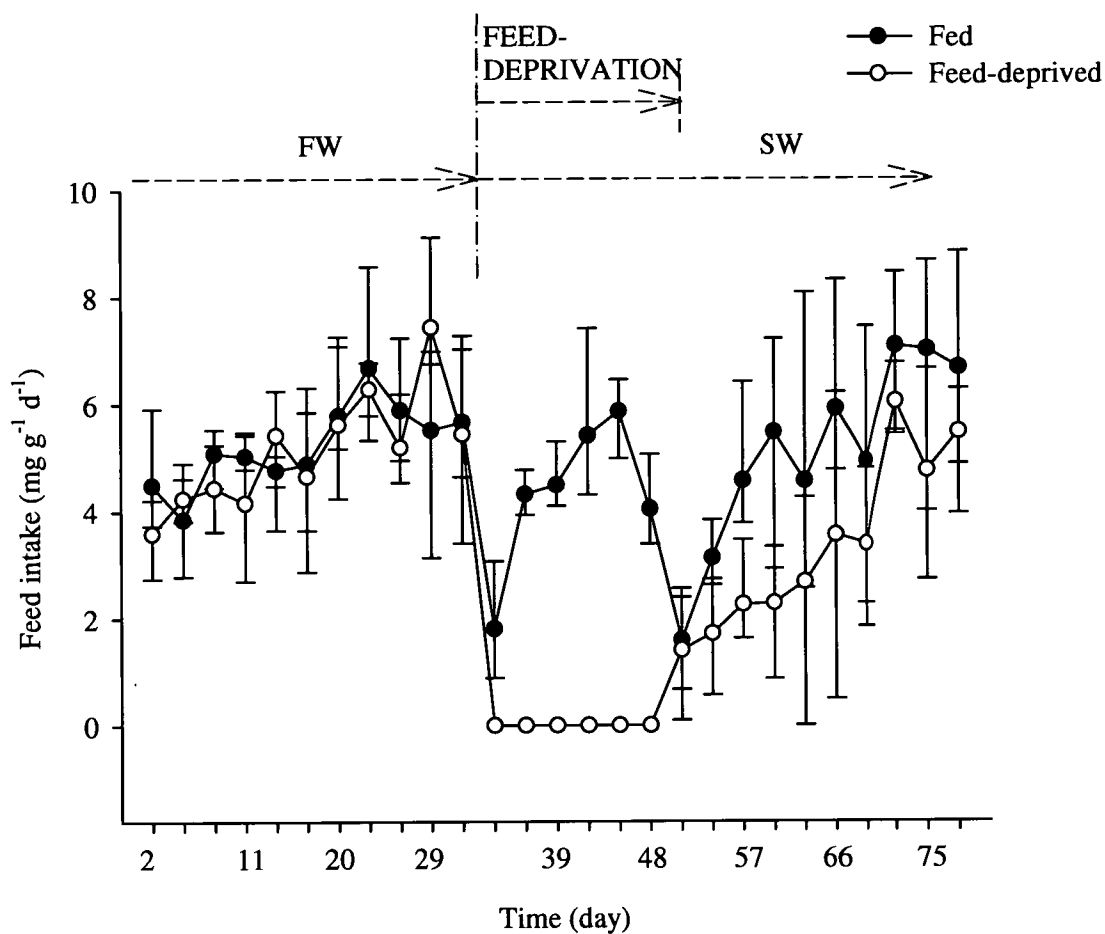
Figure 6.5 Mean daily feed intake (± max and min) for fish fed or not fed for 14 d immediately following SW transfer. Transfer to SW occurred 34 d after the initial weighing. FEED-DEPRIVATION = 14 d period following SW transfer (⁻ ⁻ ⁻ ⁻); FW = freshwater; SW = seawater.
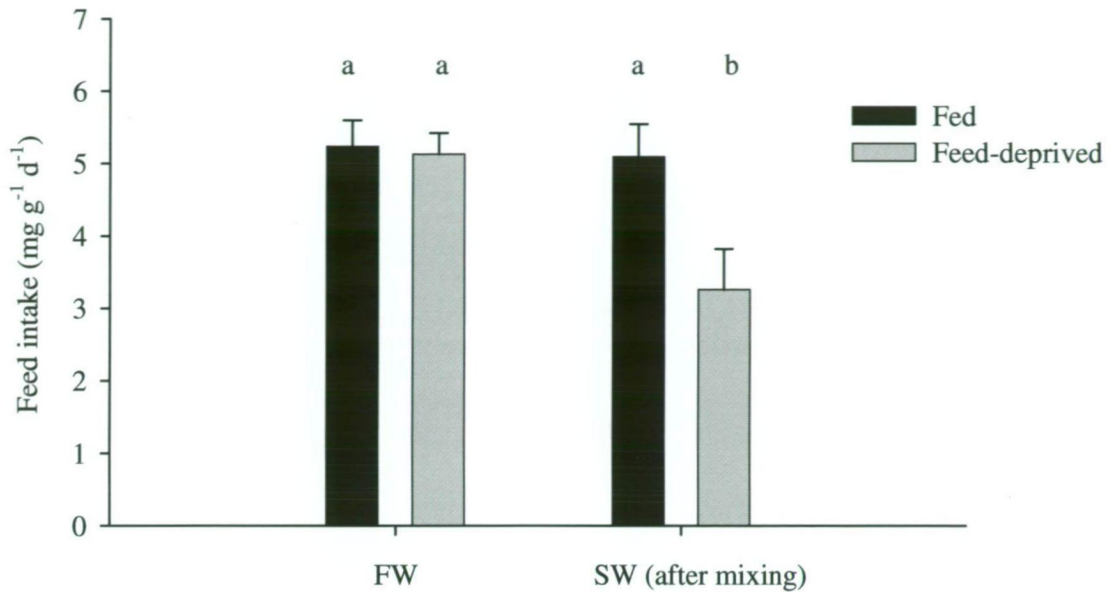
Figure 6.6 Group feed intake, mg g^{-1} d^{-1}, (mean ± S.E.M) for 11 d during FW phase and 10 d during SW phase. ANOVA showed significant difference (F = 4.918, *df* 3, 12, p = 0.019*). Means with different letters are significantly different (Tukey's multiple comparison test p < 0.05). FW = freshwater; SW = seawater.
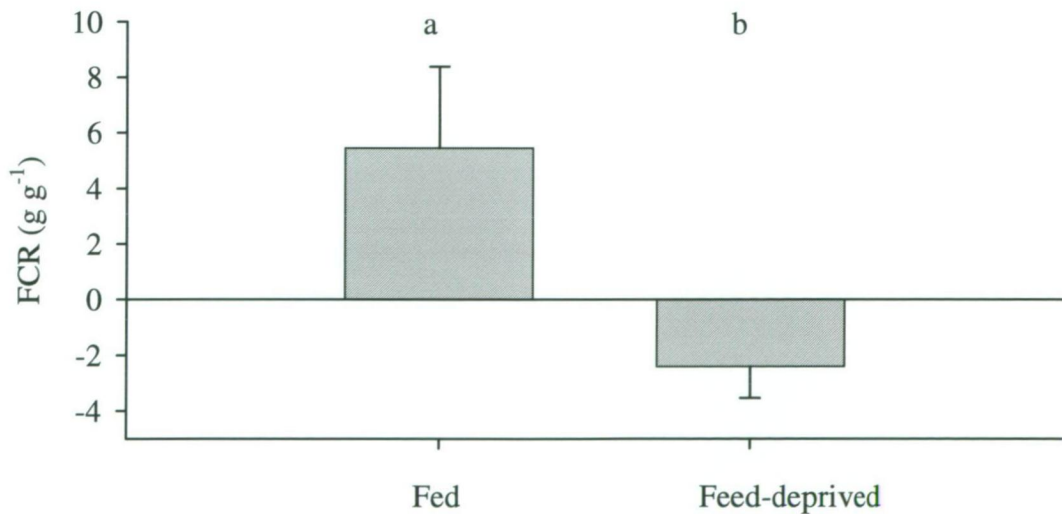
Figure 6.7 Feed conversion ratio (FCR) for each treatment (mean ± S.E.M) calculated from the date of mixing fed and unfed fish (15 d post SW transfer) until final weighing (29 d). *t*-test showed significant difference (*t* = 2.505, *df* 6, p = 0.046*).
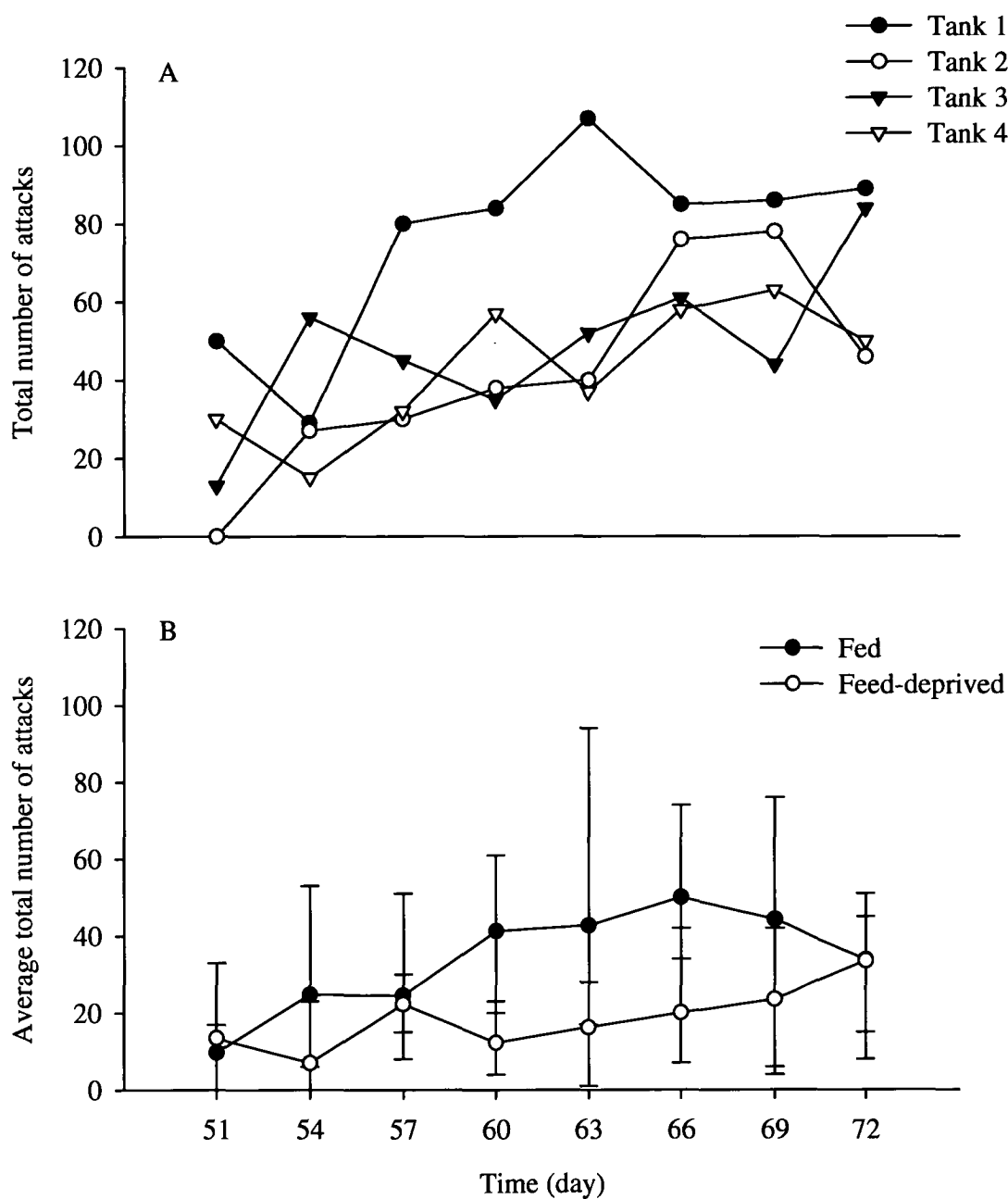
Figure 6.8 (A) Total number of aggressive interactions initiated in each tank during 30 min of observations on each d of video footage (fed and feed-deprived fish were combined on day 50). (B) The number of aggressive interactions in tanks attributable to each treatment (mean ± max and min).
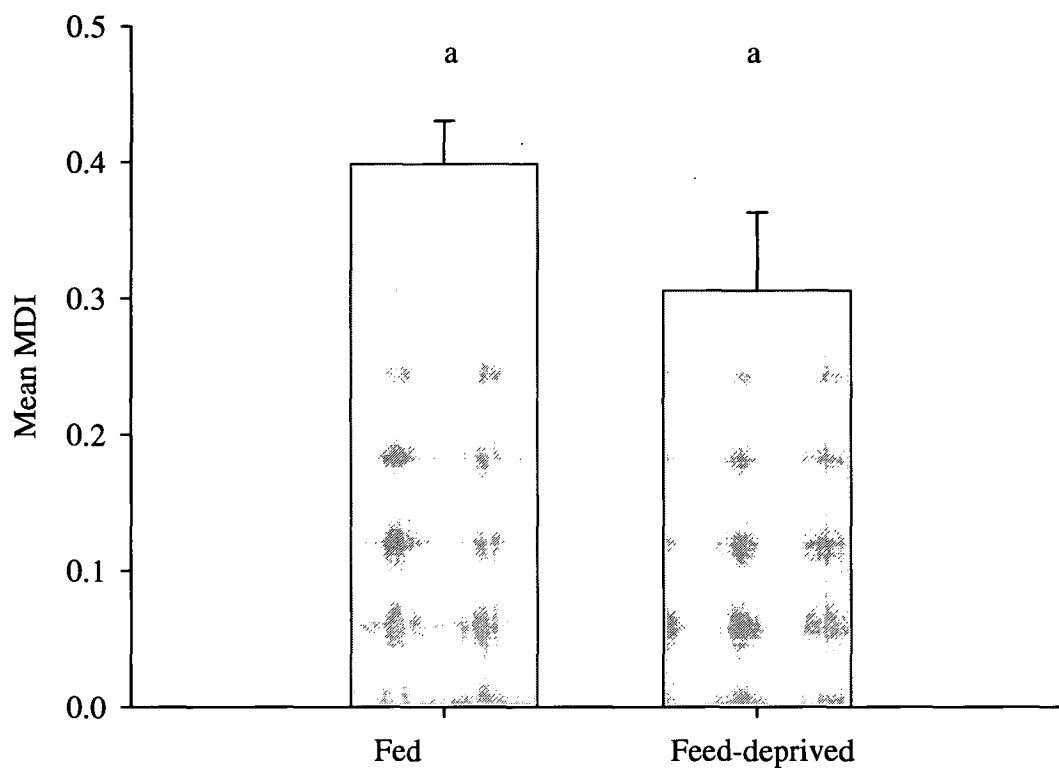
Figure 6.9 Treatment mean of mean dominance index (MDI) (± S.E.M) over 22 d following mixing of feed-deprived and fed fish (mean of 7 d data). t-test showed no significant difference ($t = 1.412$, df 6, p = 0.208).

Table 6.3 Analysis of stability in individual feeding ranks, based on share of meal (SM), individual dominance ranks, based on dominance index (DI), and correlation between the mean share of meal (MSM) and mean dominance index (MDI) for Atlantic salmon in four groups with half of fish in each group fed and the other feed-deprived for 14 d following transfer to SW. Data are presented as Kendall's coefficient of concordance (W) (corrected for ties) using χ^2 statistic and Spearman's rank correlation. Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. SW = seawater.

Group (Tank no.)		Kendall's coefficient of concordance		Spearman's rank correlation
		SM	DI	MSM vs MDI
1	(n = 7)	0.584 ***	0.503 **	0.321
2	(n = 7)	0.581 ***	0.368 *	0.250
3	(n = 8)	0.545 ***	0.299 *	0.429
4	(n = 8)	0.671 ***	0.459 **	0.619

Table 6.4 Spearman's correlation between ranked feeding hierarchies based on mean share of meal (MSM) after mixing of fed and feed-deprived fish in SW against final weight (g), final condition factor (K), chemical composition (% wet weight) and HSI (%) of individual fish for Atlantic salmon in four groups with half of fish in each group fed and the other feed-deprived for 14 d following transfer to SW. Levels of significance * p < 0.05; ** p < 0.01; *** p < 0.001. SW = seawater.

Group	Tank 1	Tank 2	Tank 3	Tank 4
Feeding hierarchy vs:				
Final weight	-0.857 * (n = 7)	-0.750 (n = 7)	-0.881 ** (n = 8)	-0.690 (n = 8)
Final K	-0.750 (n = 7)	-0.679 (n = 7)	-0.690 (n = 8)	-0.833 * (n = 8)
Dry matter	-0.607 (n = 7)	0.321 (n = 7)	-0.821 * (n = 7)	-0.857 * (n = 7)
Crude protein	-0.857 * (n = 7)	0.036 (n = 7)	-0.571 (n = 7)	-0.857 * (n = 7)
Crude lipid	-0.500 (n = 7)	0.321 (n = 7)	-0.821 * (n = 7)	-0.821 * (n = 7)
Gross energy kJ g^{-1}	-0.607 (n = 7)	0.214 (n = 7)	-0.821 * (n = 7)	-0.857 * (n = 7)
HSI	-0.429 (n = 7)	-0.714 (n = 7)	0.143 (n = 7)	-0.714 (n = 7)

Table 6.5 Spearman's correlation between chemical composition parameters (% wet weight) of whole bodies for Atlantic salmon (n = 28). Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

	Dry matter	Crude protein	Crude lipid
Dry matter	1.000		
Crude protein	0.742 **	1.000	
Crude lipid	0.951 **	0.556 **	1.000

Table 6.6 Spearman's correlation between ranked dominance hierarchies based on mean dominance index (MDI) after mixing of fed and feed-deprived fish in SW against final weight (g), final condition factor (K), chemical composition (% wet weight) and HSI (%) of individual fish for Atlantic salmon in four groups with half of fish in each group fed and the other feed-deprived for 14 d following transfer to SW. Levels of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. SW = seawater.

Group	Tank 1	Tank 2	Tank 3	Tank 4
Dominance hierarchy vs:				
Final weight	-0.500 (n = 7)	0.250 (n = 7)	-0.357 (n = 8)	-0.810 * (n = 8)
Final K	-0.571 (n = 7)	-0.143 (n = 7)	-0.333 (n = 8)	-0.667 (n = 8)
Dry matter	-0.393 (n = 7)	0.536 (n = 7)	-0.571 (n = 7)	-0.929 ** (n = 7)
Crude protein	0.071 (n = 7)	0.036 (n = 7)	-0.571 (n = 7)	-0.929 ** (n = 7)
Crude lipid	-0.500 (n = 7)	0.429 (n = 7)	-0.357 (n = 7)	-0.821 * (n = 7)
Gross energy	-0.393 (n = 7)	0.321 (n = 7)	-0.571 (n = 7)	-0.929 ** (n = 7)
HSI	-0.393 (n = 7)	-0.750 (n = 7)	0.143 (n = 7)	-0.357 (n = 7)

Table 6.7 Chemical composition (% wet weight) of Atlantic salmon in behavioural study, fed or feed-deprived for 14 d following transfer to SW (mean ± S.E.M, n = 4 replicate groups. For each parameter the mean is written above the S.E.M). SW = seawater.

Parameter	Treatment					
	Fed	Feed-deprived	t values	df	tails	p
Dry matter	26.48	24.14	4.383	6	2	0.005**
	0.26	0.46				
Crude protein	14.77	13.90	4.794	6	2	0.003**
	0.11	0.14				
Crude lipid	9.99	8.21	4.139	6	2	0.006**
	0.27	0.34				
Gross energy kJg^{-1}	7.10	6.25	4.677	6	2	0.003**
(wet weight)	0.10	0.15				

Table 6.8 Chemical composition (% wet weight) of Atlantic salmon in growth study, fed or feed-deprived for 14 d following transfer to SW (mean ± S.E.M, n = 4 replicate groups. For each parameter the mean is written above the S.E.M). SW = seawater.

Parameter	Treatment					
	Fed	Feed-deprived	t values	df	tails	p
Dry matter	28.51	27.26	2.092	6	2	0.081
	0.45	0.39				
Crude protein	16.48	16.11	1.418	6	2	0.206
	0.23	0.13				
Crude lipid	10.16	9.51	1.550	6	2	0.172
	0.21	0.36				
Gross energy kJg^{-1} (wet weight)	7.57 0.12	7.24 0.16	1.599	6	2	0.161

Table 6.9 Characteristics of Atlantic salmon determined to be pinheads (i.e. K ≤ 0.865) at the termination of the experiment or at the time of their death. For fish that died during the experiment the number of days spent in SW is given. The mean feed intake in SW is presented only during the time following mixing of fed and feed-deprived fish when all fish were being offered feed. SW = seawater.

PIT tag number	Unit	4820	3A40	1B09	5921	Non-pinhead mean
Initial weight	(g)	104.6	114.8	128.7	111.7	113.5
Final weight	(g)	73.1	93.0	100.4	96.6	125.5
Weight gain	(g)	-31.5	-21.8	-28.3	-15.1	12.0
Initial length	(mm)	206	225	228	223	218
Final length	(mm)	204	225	233	229	232
Change in length	(mm)	-2	0	5	6	14
Initial K		1.197	1.008	1.086	1.008	1.092
Final K		0.861	0.816	0.794	0.804	1.000
Change in K		-0.335	-0.191	-0.292	-0.203	-0.093
Mean FW intake	(mg g^{-1} d^{-1})	2.020	5.778	4.391	5.937	5.259
Mean SW intake (post feed-deprivation)	(mg g^{-1} d^{-1})	2.249	4.494	0.000	0.236	4.168
MSM rank following mixing		7	4	8	7	
MDI rank following mixing		8	4	4	8	
Dry matter	(% wet weight)	22.7			20.4	25.7
Crude protein	(% wet weight)	12.8			13.8	14.4
Crude lipid	(% wet weight)	7.5			4.5	9.4
Gross energy	(% wet weight)	5.7			4.9	6.8
HSI	(%)	3.7			2.1	2.6
Days post transfer until mortality		N/A	40	39	N/A	
Treatment		Feed-deprived	Feed-deprived	Fed	Feed-deprived	
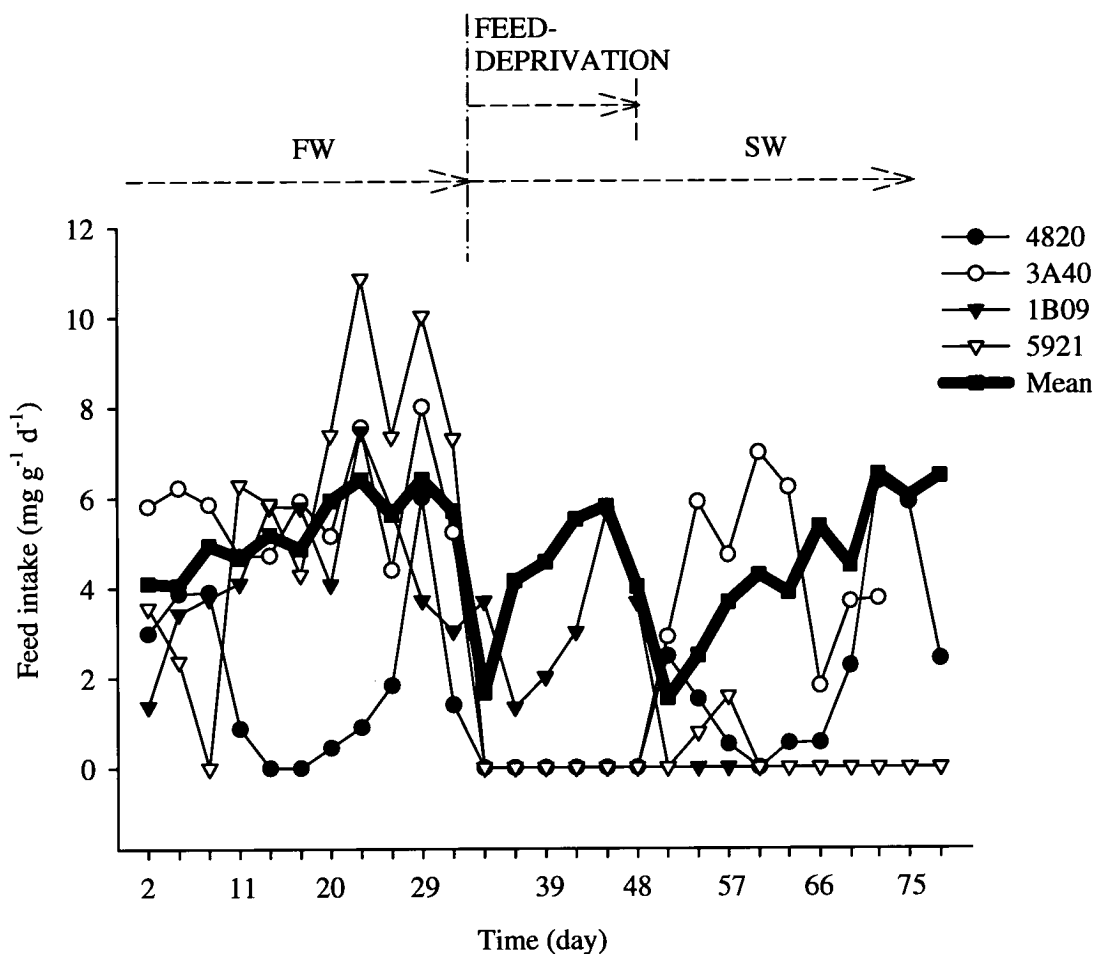
Figure 6.10 Daily feed intake of four (all) individual fish classified as pinheads at time of death or termination of experiment (i.e. fish with K ≤ 0.865) through the entire period of the experiment. Each thin line indicates an individual pinhead while the thick line shows the mean trend of all non-pinheading fish (K > 0.865). Transfer to SW (-·--·-) occurred 34 d after the initial weighing. FEED-DEPRIVATION = 14 d period following SW transfer; FW = freshwater; SW = seawater. Fish 1B09 was from the treatment fed during the first 14 d in seawater while fish 4820, 3A40, and 5921 were from the feed-deprived treatment.

6.5 Discussion

The behavioural experiment in this study has demonstrated that a period of feed-deprivation immediately following seawater transfer (14 days) has a significant negative effect on the feed intake when feed-deprived fish were combined with fish that were fed immediately following transfer. There was also a tendency for the feed-deprived fish to perform fewer attacks and to sit lower in the dominance hierarchy although the differences were not statistically significant. In the behavioural experiment feed-deprived fish had lower growth and lower final condition, as described by condition factor and chemical composition parameters. This was not the case in the growth experiment, using higher numbers and densities of fish, in which the growth of feed-deprived fish following combination with fed fish parallelled the fed fish. As stated in Chapter 5 the results of a 24 hour salinity challenge indicated that salmon were fully smolted at the time of seawater transfer showing that the choice of time for seawater transfer in this experiment was appropriate to the physiological state of the salmon (Blackburn and Clarke, 1987).

6.5.1 Feed intake and aggression

Fish that were fed during the first 14 days in seawater showed a reduction in feed intake immediately following transfer consistent with previous findings that consumption is normally reduced with several weeks between transfer and the time when most fish are feeding again (Usher et al., 1991; Jørgensen and Jobling 1994; Stead et al., 1996). In aquaculture the time between arrival in seawater and recommencement of feeding may vary considerably from individual to individual. This is believed to be a result of salmon undergoing the parr-smolt transformation at different times and therefore each individual adapting to the increased salinity over slightly different periods (Stead et al., 1996). It has been speculated that prolonged periods of non-feeding may contribute to the creation of pinheads and failed smolt with individuals that take longer to recommence feeding possibly being out-competed once they do try to feed by individuals already established in the feeding hierarchy (Johnstone, 1991; King, 1992a). Such suggestions are consistent with the model put forward and tested by Johnsson et al. (1996) that predictions of competitive ability are time-dependent, i.e. that

competitive alibility changes with the length of time an animal is deprived of feed (Johnsson et al., 1996). The model suggested that motivation based on hunger would initially increase and then plateau while competitive ability would increase rapidly, reach a peak and then slowly diminish. It was found that rainbow trout deprived of feed for 3 days out-competed non-deprived fish in terms of feed intake and aggression, that there was no difference between the two groups after 6 and 9 days deprivation, and that by 12 days there was a tendency for the non-deprived fish to out-compete deprived individuals (Johnsson et al., 1996). In general aggression levels in fish appear to increase with increasing hunger (Symons, 1968; Dill et al., 1981), however the subsequent depletion of energy reserves as fish physically deteriorate from not feeding (Jürss et al., 1986; Black and Love, 1986; Weatherly and Gill, 1987; Einen et al., 1998), will presumably deplete competitive ability by providing progressively less energy to perform costly aggressive behaviours (Johnsson et al., 1996).

Based on the above findings by Johnsson et al. (1996) the feed-deprivation period of 14 days in the current study was quite possibly long enough for a reduction in competitive ability. Fish were point-source-fed as spatial localised feeding has been shown to promote competition giving dominant fish the advantage of defending positions close to the feed source and thereby monopolising the feed (McCarthy et al., 1999). Dominance ranks in this study were found to be stable through the 22 days following the combining of fed and feed-deprived individuals, suggesting little change occurred in the positions individuals held within the dominance hierarchies throughout this monitoring period. Overall there was a tendency for previously feed-deprived fish to have lower levels of aggression and sit lower in the dominance hierarchies than previously fed fish, which suggest that the competitive ability of feed-deprived fish may be reduced as a result of starvation; it is important to note however the differences were not significant. The lack of significance may be due to natural variability in the ability of individuals to cope with transfer to seawater (Stead et al., 1996), unrelated to the treatments in the present study. Despite this lack of significance the fact that in three of the four groups the most dominant fish came from the fed treatment, while in all groups the least dominant fish was from the feed-deprived treatment, suggests that feed-deprivation does cause some level of competitive disadvantage. Feeding ranks were also found to be stable throughout the 22 days following the combining of fed and feed-deprived individuals,

suggesting little change occurred in positions held by individuals in the feeding hierarchy throughout the monitoring period. Feed-deprived individuals had significantly lower feed intake than fed fish. This again suggests a general reduction in competitive ability of these fish which would limit compensatory growth following feed-deprivation given that this is usually accompanied by an increase in feed intake (hyperphagia) (Miglaves and Jobling, 1989; Hayward et al., 1997; Wang et al., 2000). The overall reduction in competitive ability was further supported by the fact that in three of the four groups the fish with the highest position in the feeding hierarchy were from the fed treatment, and in three of the four groups the fish with the lowest position in the feeding hierarchy came from the feed-deprived treatment.

Whilst in terms of aggression and feed consumption there was suggestion that fed individuals generally out-competed feed-deprived individuals no groups showed correlation between mean feeding and dominance ranks. Where dominance hierarchies and feeding hierarchies have been found not to correlate it has been suggested that instead of linear dominance hierarchies there may be one individual which is dominant to all other fish while other fish don't have fixed ranks (Carter and Davis, 2004). There was evidence that this was the case in three of the four groups in this study where the individual holding the highest position in the dominance hierarchy also held the highest position in the feeding hierarchy, and in fact in the fourth tank the second most dominant fish held the highest position in the feeding hierarchy. It was also found that in three of the four tanks the fish with the lowest position in the dominance hierarchy held either the lowest or second lowest position in the feeding hierarchy.

As was found in the previous chapter (Chapter 5) a greater proportion of attacks occurred before the meals than after meals. It is possible that this pattern of aggression is an example of feeding anticipatory activity (FAA) (see Chapter 5).

6.5.2 Growth and condition

In the behavioural experiment of the present study, in a number of the groups significant negative correlations existed between mean feeding ranks and parameters such as final weight, final condition, and the chemical composition parameters measured. However

such correlations were only found for one group when mean dominance rank was considered rather than mean feeding rank. Where differences did exist they indicated that in general fish at the top of the feeding hierarchies and, to a lesser extent, dominance hierarchies tended to perform better than individuals lower in the hierarchies.

Given the differences in feed intake and dominance between fed and feed-deprived fish it is not surprising that in the behavioural experiment feed-deprivation was found to have significant negative effects on the final weight and weight gain; final length and change in length; final condition factor and change in condition factor; and also to significantly affect trajectories of weight through time with negative growth in feed-deprived individuals, and positive growth in fed individuals following the combination of the two. While periods of feed restriction are often followed by a period of compensatory growth (Dobson and Holmes, 1984; Tian and Quin, 2003; Nikki et al., 2004), the present study suggests that competition with non feed-deprived individuals can limit feed intake thereby limiting growth of previously feed-deprived fish. Only 5 of the 16 feed-deprived fish had positive growth and this growth was comparable but not above that of fed-treatment fish. While animals undergoing compensatory growth often have increased feed conversion efficiency (Miglavs and Jobling, 1989; Russell and Wootton, 1992; Boujard et al., 2000) here feed-deprived fish had negative feed conversion ratios. Feed intake was below maintenance and consequently FCR was negative. It is worth noting that the high FCR values in the fed treatment fish also indicate poor feed conversion which may be attributable to handling stress associated with button tagging and continual replacement of these tags, or may be related to high levels of aggression within the groups.

It is important to note that while the random selection of tanks for each treatment resulted in feed-deprived fish having significantly lower initial weight than fed fish the difference was small (fed fish were 6.2% larger) in comparison with the difference following feed-deprivation (fed fish 14.5% larger). It is also important to realise that differences in weight gain between the two treatments were of more importance than final weights. In a study using rainbow trout 12 days feed-deprivation resulted in fed fish being 37.4% larger than feed-deprived fish, despite no significant difference

initially (Johnsson et al., 1996). It seems, therefore, that the small initial difference in weight in this study would have had a negligible effect in comparison to the actual period of feed-deprivation on feed intake, aggression and growth following recombination of fed and feed-deprived fish. Given the increase in magnitude of difference between mean weights of fed and feed-deprived fish the differences seen are likely to be reliable and not simply artefacts of the initial difference.

Interestingly the effects on weight, length and condition seen in the behavioural experiment were not parallelled in the growth experiment. Only during the period of feed-deprivation itself did feed-deprived fish lose weight, and even in this period there was no significant effect on length or condition factor. Following the period of feed deprivation both fed and feed-deprived fish grew at a similar rate, and while the growth trajectories did not visually seem to merge there was no significant difference between fed and feed-deprived fish after 27 days following the feed-deprivation period. The significant difference in weight following feed-deprivation and lack of significance by the end of the experiment indicates some level of compensatory growth and indicates that fish were not out-competed in terms of growth or condition. Weight lost as a result of feed-deprivation was recovered following the recommencement of feeding. Given that increasing fish numbers and densities are thought to increase the costs associated with dominance (Huntingford and Thorpe, 1992; Sloman and Armstrong, 2002) and that such increases have been shown to reduced the formation of social hierarchies (Li and Broksen, 1977; Fleming and Johansen, 1984; Kjartansson et al., 1988) it is possible that the lack of difference in growth and condition were due to higher fish numbers and densities reducing the formation of feeding and dominance hierarchies. Within the current study increased numbers of individuals could not be separated from increased densities to determine which of these was the key factor given that all tanks were the same size and increasing the number of fish automatically increased fish density. Since this experiment was designed to look at group response it may be the case that a small proportion of fish were negatively affected by feed-deprivation and that differences may have been masked by examining the group as a whole. This seems unlikely however, as both the fed and feed-deprived treatments resulted in the same number of pinheads. Unfortunately due to the experimental design the group feed intake of fed and feed-

deprived fish could not be separated from each other so no indication of overall ability to compete for feed could be attained.

6.5.3 Chemical composition of fish

Fish that are feed-deprived will suffer weight loss as their energy reserves are mobilised and depleted (Jobling, 2001b). Starvation generally causes initial decreases in lipid and glycogen while proteins are conserved (Brett et al., 1969; Black and Love, 1986; Weatherly and Gill, 1986) and moisture levels generally increase (Stirling, 1976; Shearer, 1994). The decrease in condition of feed-deprived fish by the end of the behavioural experiment was evident in examination of their chemical composition. Dry matter, crude protein, crude lipid, and gross energy were all significantly lower in feed-deprived fish than in fed fish. The low crude lipid and protein with the consequently low gross energy all indicate a reduction in nutritional state. The low percentage of dry matter indicates an increase in moisture content which is inversely proportional to lipid levels in fish (Shearer, 1994; Liu and Liao, 1999) again indicating loss of lipid stores. It seems that in the behavioural experiment competition and the reduction in feed intake by feed-deprived fish may have prevented them from recovering their lipid and protein reserves in the 27 days between the combining of fed and feed-deprived fish and the end of the experiment.

In the growth experiment no significant differences were found between any of the chemical composition parameters. This is consistent with findings of no differences in growth or condition following the period of feed-deprivation. It again suggests that at higher fish numbers and densities the formation of dominance and feeding hierarchies may be restricted overriding the negative effects normally associated with long periods of feed-deprivation followed by competition with non-feed-deprived fish (Johnsson et al., 1996).

6.5.4 Pinheads tracked through time

The behavioural experiment in the current study produced four fish that were considered to be pinheads. Three of these fish came from the feed-deprived treatment supporting

the concept that postponing the recommencement of feeding in seawater may play a role in the production of pinheads (King 1992a). Data taken at the commencement of the experiment, 34 days before transfer to seawater, suggest that the pinheads were recruited from above and below the mean initial weight, initial length, and initial condition factor of non-pinheading fish, supporting previous findings that pinheads tend to be recruited equally across the entire size range of groups transferred to sea (King 1993). Data also suggests that pinheads were individuals with feed intake above and below the non-pinheading mean measured across the freshwater phase. All of the pinheads had lower final weights than the non-pinhead average, all but one was shorter in length than the non-pinhead average and all of these fish had lost weight and condition while non-pinheads had on average increased in weight and condition. After transfer the one pinhead from the fed treatment did ingest feed during the treatment period but this intake was below the mean intake of non-pinheads and there was a lag before intake began to increase. Following the combination of fed and feed-deprived fish three of the four pinheads had average feed intakes below the mean. These three fish also held the lowest or second lowest positions in feeding rank and two of these three also held the lowest positions in the dominance rank. These two fish were also the only two that lived till the end of the experiment at which time they had lower dry matter, crude protein, crude lipid and gross energy than the mean values measured for non-pinheads, all indication their low nutritional status. The fact that both of these fish were from the feed deprived treatment, that both held positions at the bottom of both the dominance and feeding hierarchies and that both became pinheads adds further support to the concept that a period of not eating immediately following seawater transfer may contribute to the production of pinheads by reducing the competitive ability of these fish. This is consistent with work from previous studies showing that fish that recommenced feeding sooner after seawater transfer were at an advantage over those that started feeding later in terms of opportunity for growth (Stead et al., 1996). However, the fact that not all the feed-deprived fish became pinheads suggests that while feed-deprivation may exacerbate the problem it is probably not the sole cause. It is also interesting to note that at the higher fish numbers and densities used in the growth trial both the fed and feed-deprived treatments resulted in the same number of pinheads.

6.6 Conclusion

This study supports the concept that individuals that wait prolonged periods of time to recommence feeding once transferred from freshwater to seawater increase their risk of being out-competed for feed once they do try and eat by individuals that began eating earlier. Fish that were feed-deprived in the behavioural experiment were found on average to have significantly lower feed intake in the period following combination with fed fish. Whilst not significantly different there was also a trend for the average number of attacks performed and the average position held in the dominance hierarchies to be higher in fed fish. It was also found that in general the individual fish at the top of both the feeding and dominance hierarchies were from the fed treatment, while in general individual fish at the bottom of these hierarchies were from the feed-deprived treatment. In the behavioural experiment fish from the feed-deprived treatment lost weight following their combination with fed fish while the fed fish gained weight. By the end of the experiment feed-deprived fish had lower weight, length and condition factors than fed fish. They also had significantly lower dry matter, crude protein, crude lipid and gross energy indicating lower nutritional status. Of the fish from this experiment that were considered to be actual pinheads four came from the feed-deprived treatment and two of these were at the bottom of both feeding and dominance hierarchies.

Interestingly it was found in the growth trial, using higher fish numbers and densities than the behavioural study, that whilst feed-deprived fish lost weight during the period of feed-deprivation their growth following the recommencement of feeding after being combined with fed treatment fish was not suppressed. Feed-deprived fish grew at a similar rate to the fed fish actually showing some indication of compensatory growth as by the end of the experiment whilst the weight of fed fish was still greater than feed-deprived fish the difference was not significant. Nutritionally these fish were also found not to differ significantly from fed fish at the end of the experiment in terms of dry matter, crude protein, crude lipid and gross energy; and both treatments produced the same number of pinheads. Overall it seems that a period of feed-deprivation may decrease competitive ability and increase the chances of a fish experiencing problems associated with such a decrease. In farming situations with much higher numbers of fish than used in the present study it is possible that the associated decreases in aggression

may result in these problems being less pronounced. However it is possible that trauma or physiological lack of readiness for transfer may cause problems to continue for a prolonged period and may result in a greater reduction in competitiveness than would have been achieved by only 14 days feed-deprivation.

6.7 Acknowledgements

6.8 References

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Chapter 7

General Discussion

Matthew Flood

7.1 Overview of study

The current study focused on the period of time around seawater transfer of Atlantic salmon, examining factors affecting feed intake, aggression, growth and condition. Once transferred to sea, salmon have to contend with a new set of environmental variables which include increased salinity, and changes in temperature, photoperiod, currents and predator exposure (Brett, 1979; Usher et al., 1991; Stead et al., 1996; Bégout Anras et al., 2001). In the wild the decision to go to sea has a physiological basis (Folmar and Dickhoff, 1980; Hoar, 1988) and coincides with a loss of condition (Pinder and Eales, 1969; Sheridan, 1989; Nordgarden, 2002) and decreased aggression (Skilbrei et al., 1994), previously associated with defence of territories in streams (Keenleyside and Yamamoto, 1962). In aquaculture seawater transfer generally occurs when fish are known to be physiologically adapted for increased salinity, i.e. when they are smolts (Blackburn and Clarke, 1987; McCormick, 1993). Regardless of physiological readiness once the salmon are introduced into seawater their feed intake can be severely reduced for several weeks before returning to levels comparable with those in freshwater (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996). It is likely that variation exists between individuals in the amount of time spent in seawater before the recommencement of feeding given that not all fish undergo the parr-smolt transformation at exactly the same time and hence all individuals will acclimate to increased salinity at slightly different rates (Stead et al., 1996). Fish that recommence feeding quickly following seawater transfer are believed to be at an advantage to those that commence feeding later (Johnstone, 1991; Stead et al., 1996). The current study aimed to increase understanding of how seawater transfer effects feeding, behaviour, growth performance, condition and nutritional status during the period leading up to and immediately following seawater transfer and provide a better understanding of what is required to ensure the survival and growth of these fish. Farms have a vested interest in trying to ensure that salmon recommence feeding again as soon as possible following seawater transfer (Hjertenes, 1999) and such information may help them to achieve such goals. One of the main objectives following seawater transfer is to avoid promoting circumstances whereby some fish lose condition, in extreme cases becoming emaciated and eventually dying, a condition known as pinheading in Tasmania (King, 1992b) (see chapter 2).

Pinheads and non-pinheads were examined in the present study in order to better define and characterise the pinheading condition. Manipulation of feeding frequency was used to determine how feeding frequency following seawater transfer and changes in feeding frequency from freshwater to seawater affect salmon on both a group and individual basis. Dominance and feeding hierarchies were examined both before and after seawater transfer to determine what effect transfer would have on their stability and structure; and feed-deprivation was used to simulate a situation in which competition between individuals choosing to feed early following seawater transfer and those delaying the recommencement of feeding following seawater transfer could be studied.

Pinheads were defined as fish with lower condition than non-pinheads, exemplified by their condition factors (K) at or below 0.865. While these fish did have lower feed intake than non-pinheads during the seawater phase they did eat. Findings from feeding frequency experiments demonstrated the benefits of feeding higher numbers of meals both before and after seawater transfer while no evidence was found that meal frequency affected pinheading rates. With respect to hierarchies both feeding and dominance hierarchy stability and structure were affected by seawater transfer demonstrating that prediction of fish performance in seawater can not be made based on freshwater performance. Evidence was also found that feed-deprivation of 14 days following seawater transfer can reduce the competitive ability of feed-deprived individuals in comparison to non-deprived individuals suggesting that prolonged delay of recommencement of feeding in seawater may contribute to pinheading. In a broad sense the current study contributed to the examination of various aspects of hierarchy dynamics currently being discussed in animal behaviour literature. These include the effects of 'change' on feeding and dominance hierarchies (Katzir, 1982, 1983; Carter and Davies, 2004; Sneddon et al., 2006), the importance of risks and benefits in decision making (Bateson, 2002; Sloman and Armstrong, 2002; Carter and Davies, 2004), the state or context-specific nature of hierarchy structures (Vervaecke et al., 1999; Sloman and Armstrong, 2002; Sneddon et al., 2006), and the formation and maintenance of hierarchies through time (Forkman and Haskell, 2004; Sneddon et al., 2006). Previous work in the areas listed above has been carried out on a range of animals including primates, birds and fish. Using Atlantic salmon as a model the current project adds to a

limited but growing body of literature on the effects of 'change' on group dynamics of fish (Wybourne, 1997; Carter and Davies, 2004), by examining the effects of seawater transfer and feeding regimes on feeding and dominance hierarchy structure, stability and strength.

All experiments run throughout this study (Chapters 3-6) were carried out in small scale experimental systems (see section 7.5) allowing a large degree of environmental control (Bégout Anras et al., 2001) while offering conditions known to intensify the effects of competition (Huntingford and Thorpe, 1992; Sloman and Armstrong, 2002). The parameters on which most focus was placed throughout this study were feed intake and aggression, both of which were measured using documented methods. Both X-radiography (Talbot and Higgins, 1983; McCarthy et al., 1993; Jobling et al., 1993) and direct observation of individuals while feeding (Carter and Davies, 2004) were used to measure individual feed intake. X-radiography required no external markings to identify fish and allowed for measurement of feed intake from large numbers of fish within in each group (tank), however the number of measurements was limited by the requirements for recovery of fish between X-raying days (McCarthy et al., 1993; Jobling et al., 2001) and costs associated with X-ray development. Direct observations on the other hand limited the number of fish that could be studied due to the requirement for unique visual marking of each fish. Direct observations did however allow more frequent measurements of feed intake than X-radiography with minimal disturbance to the fish (Jobling et al., 2001). Group feed intake was measured throughout the study by quantifying waste feed from tanks (Helland et al., 1996). A digital surveillance network system was used to capture footage to assess aggression levels and also assign dominance ranks to fish using a dominance index (Barlow and Ballin, 1976; Carter and Davies, 2004). Whilst this technique offers accurate measurement of aggression and dominance hierarchies it is very time consuming limiting the amount of footage that can be assessed.

7.2 Pinheading

The term pinheading is in common use within the Tasmanian salmon industry describing fish that fail to grow in length while a considerable reduction occurs in body

weight (King, 1992b). The current study has helped to better define the condition in terms of morphological characteristics, osmoregulatory capability, whole body chemical composition and digestive capacity (Chapter 2); and examined some of the possible contributing factors in the area of feeding and social behaviour (Chapters 3, 4, 5 and 6). Pinheads tend to be recruited equally across the entire size range of groups transferred to sea (King, 1993) and findings from the current study support this. Salmon in poor condition following 23 days in seawater (Chapter 3) and actual pinheads (Chapter 5 and 6) were recruited from a range of initial (freshwater) weights, lengths and conditions both above and below the mean values for non-pinheading fish. Overall it would seem performance in freshwater is not a good indicator of how fish will perform once they are transferred to sea. This is in agreement with findings by Stead et al. (1996) that specific growth rates of individuals in seawater did not correlate with those previously seen in freshwater. That the structure of both feeding and dominance hierarchies in seawater differ from those in freshwater is also consistent with previous findings that other environmental changes can cause hierarchies to change (McNicol and Noakes, 1984; Snedden et al., 2006) and highlights the difficulties in trying to determine which fish will perform poorly following seawater transfer (Chapter 5). It has been speculated that disrupted feeding behaviour following seawater transfer may cause an initial feed-deprivation which could then be compounded by social stresses resulting from the more aggressive fish in the population leading to the production of failed smolt (Johnstone, 1991) and presumably pinheads. Findings in the current study suggest that while feed-deprivation of 14 days immediately following seawater transfer may contribute to pinheading the effect was reduced when the fish number and density within the tanks were increased (Chapter 6).

One aspect of pinheading not clear in the past is whether pinheads emerging soon after seawater transfer feed in the seawater environment. It has been suggested that fish suffering a similar condition (failed smolts) in the northern hemisphere may not feed (Johnstone, 1991; Stradmeyer, 1994). While some individuals with poor condition (Chapter 3) did not ingest feed on the two days feed intake was measured using X-rays it is possible that these individuals did eat on other days. In Chapter 5 and 6 all pinheads did ingest feed during the seawater phase, although at reduced levels, and data collected from the analysis of trypsin enzyme, the activity of which gives an indication of

digestive function (Preiser et al., 1975; Pringle et al., 1992), also suggest that these fish may be able to digest feed (Chapter 2). Whilst there is no doubt that some fish may not eat in seawater it seems likely that not eating at all following transfer is not a prerequisite for individuals to become pinheads. It is more likely that the amount ingested causes the problem. In the current study (Chapter 5 and 6) feed intake of pinheads was reduced in seawater, declining in most cases with time, presumably as the individuals approached death.

The work carried out on pinheading in the current study (Chapter 2) provides a clear definition of the condition, in terms of morphology, osmoregulatory ability, whole body chemical composition and digestive capacity and may help to formalise the use of the term "pinheading" for both industry and research purposes. A clarification of how this condition relates to a similar condition (failed smolt) described in the northern hemisphere has also been presented.

Two areas thought to possibly contribute to the problem of pinheading that were not examined in this study were light shock and over-conditioning in freshwater. Large increases in light intensity when going from indoor tanks in hatcheries to exposed sea cages could potentially cause light shock which may affect the performance of some individuals possibly contributing to pinheading (King 1994). It has also been suggested that fish that have very high condition factors before transfer may be disadvantage following transfer since in the wild smolts tend to be long slender fish, not the highly conditioned fish that freshwater hatcheries produce (King, 1992a). Work done on lipostatic regulation of feed intake has demonstrated that fish with high adiposity tend to consume less feed, grow more slowly and deposit less fat than leaner fish (Johansen et al., 2002, 2003). Adiposity has also been found to affect growth immediately following seawater transfer with greater adiposity at the time of transfer resulting in decreased growth, believed to result from lower feed intake (Jobling et al., 2002a,b). Decreases in growth and feed intake of fish with higher adiposity may contribute to pinheading. Further work is needed to determine the importance of both light intensity at transfer and pre-transfer adiposity on pinheading.

7.3 Feeding frequencies (Chapters 3 and 4)

As stated previously one of the main issues for industry in the time immediately following seawater transfer is ensuring that salmon commence feeding again as soon as possible to try and avoid disadvantaging any individuals. Improvement in feeding strategies is believed to be one of the key ways to reduce problems such as pinheading (D. Mitchell personal communication; H. King personal communication). Feeding frequency is a well documented factor affecting feed intake, growth, feed conversion efficiencies and the formation of hierarchies in many fish species (Grayton and Beamish, 1977; Chua and Teng, 1978; Greenland and Gill, 1979; Jobling, 1983; Dwyer et al., 2002). The presence of hierarchies is normally most apparent when there is feed shortage (Slaney and Northcote, 1974) and hierarchy formation is stronger when low rations are used (McCarthy et al., 1992; Moutou et a., 1998). High rations fed at high frequency have been shown to reduce hierarchy formation (Jobling, 1983). While feeding frequencies are normally quite high in hatcheries (H. King personal communications) and recommendations suggest the smolt should be fed 5 to 6 times a days following transfer (Hjertenes, 1999) it has been suggested that in some cases they may be fed as infrequently as one meal per day during this time (H. King personal communication). The present study has shown that feeding a high feeding frequency (eight meals per day) prior to seawater transfer reduced to one meal per day immediately following transfer caused a significantly greater decrease in feed intake initially than offering four or eight meals per day following transfer. This is consistent with previous findings that higher meal numbers increase feed intake, presumably by offering more opportunities for fish to feed (Grayton and Beamish, 1977). In the current study one meal per day following transfer also caused a significant increase in the strength of feeding hierarchies immediately following transfer which did not occur with four or eight meal per day. This indicates that feed was not shared equally among individuals and hence some fish may have been disadvantaged. While these findings are consistent with those from Jobling (1983) showing that unrestricted feeding at multiple meals results in less hierarchy formation than unrestricted feeding over fewer meals, the pattern did not persist with hierarchy strength in one meal per day groups returning to their pre-transfer value within 3 weeks. Given the importance of getting fish back onto

feed as soon as possible (Hjertenes, 1999) high meal numbers should be utilised to avoid the initial increase in hierarchy strength and maximise feed intake.

One question unanswered above is whether the greater initial decrease in feed intake in the one meal per day groups was the result of the low meal number alone or whether a change in meal frequency, be it increasing or decreasing, concurrently with seawater transfer could contribute. Changes in diet have been found to reduce feed intake (Toften and Jobling, 1997; Wybourne, 1997) and it was thought that changing feeding frequency may have had a similar effect, especially given that fish often entrain to specific feeding times (Sanchez-Vazquez and Madrid, 2001). However findings from the current study (Chapter 4) indicate that changing feeding frequency in its own right has little effect on post-transfer feed intake and that fish fed only one meal will ingest less during initial time in seawater than those on eight meals per day regardless of any pre-conditioning.

Based on the findings of the current study Atlantic salmon should be fed in excess more often than once per day both pre- and post-seawater transfer as larger numbers of meals increased feed intake both before and immediately following seawater transfer while initially decreasing the strength of post-transfer feeding hierarchies and thus reducing the variation in feed intake among individuals in a group. Although higher fish numbers of sea cages would probably decrease the formation of hierarchies in their own right (Kjartansson et al., 1988), following these recommendations would simply be to err on the side of caution. Also presumably even in these large cages increased numbers of feeding opportunities may help to get fish back onto their feed as quickly as possible following transfer.

One of the main questions generated by the current study is how applicable the findings are to larger commercial scale cage populations. To determine this further work could to be done repeating some trials from the current study in commercial sea cages using commercial feeding systems. Within the salmon industry in Tasmania the feeding technologies between farms do differ. While some farms use adaptive Aquasmart systems delivering feed to newly transferred seawater smolts according to their demand throughout daylight hours, others utilise less frequent un-automated feeding (D.

Mitchell personal communication; H King personal communication). By re-running the feeding frequency experiments it could be determined how much of the benefit gained by using higher feeding frequencies in small systems is transferable to commercial scale operations.

On a more general note regarding the running of experiments examining feeding frequencies it is worth noting that the experiments in the present study were difficult to manage due to the need to constantly remove and quantify uneaten feed pellets associated with feeding to excess. In studies where a restricted ration is fed this problem is presumably avoided as fish would ingest all feed offered (Holm et al., 1990; Jarobe and Grant, 1996; Linnér and Brännäs, 2001). Other studies avoid the issue by hand feeding until satiation is reached (Hung et al., 2001) however large numbers of tanks and high feeding frequencies make this even more difficult than the quantifying of uneaten pellets. In cases where fish are fed in excess by hand or using automatic feeders, measurements of uneaten feed are required to accurately assess actual feed intake (Chua and Teng, 1978; Helland et al., 1996; Bendiksen et al., 2002). When such measurements are required over long periods throughout the day they would be more streamlined and manageable with the implementation of systems designed to automate the counting of waste pellets such as the Aquasmart AQ1 waste pellet sensor previously described by Chen (2000). Further to this Helland et al. (1996) has also outlined a manual and inexpensive method of quantifying feed intake of large numbers of tanks of fish fed extruded pellets using wire baskets similar to those used in the current study. Using this technique pellets need only be collected and weighed once per day and taking into account the recovery of dry matter (i.e. correcting for loss due to leaching in the waste feed) an estimate can be made of the amount of feed consumed by the fish. It is important to note that this technique requires very stable pellets (extruded only) and has the limitation of not providing information on whether individual meals during the day are to satiation or restricted. The technique also requires the oven or freeze drying of all uneaten feed samples taken so can still be quite time consuming.

7.4 Feeding and social dominance hierarchies (Chapters 3, 5 and 6)

Altering environmental conditions has been demonstrated to affect group feed intake and aggression, and the stability and structure of dominance and feeding hierarchies in fish (McNicol and Noakes, 1984; Sloman and Armstrong, 2002; Sneddon et al., 2006). The change in environment from freshwater to seawater was found to have similar effects. In the current study the structure of feeding hierarchies in seawater differed from those in freshwater (Chapter 3 and 5) as did dominance hierarchies (Chapter 5). This is consistent with work on sticklebacks showing changes in environments alter hierarchical structure (Sneddon et al., 2006). The current findings suggest there is little the relative performance of an individual in freshwater can tell us about its likely performance once transferred to a seawater environment which is consistent with previous findings of a lack of correlation between individual growth rates in fresh and seawater (Stead et al., 1996). In the present study there was also a complete lack of aggression immediately following seawater transfer (Chapter 5), which points to a break down in dominance hierarchies seemingly nullifying any pre-existing structure and allowing the hierarchies to form anew in seawater. This is consistent with previous findings showing that changes in the environment can reduce dominance hierarchy stability (Sloman et al., 2001; Sneddon et al., 2006). Information from work on hierarchies suggests that knowledge of relative performance of individual fish in freshwater offers little information to farms in terms of predicting their relative performance following seawater transfer. On a broader scale this study has added to information on the importance of risks and benefits in decision making for animals (Batesone, 2002; Sloman and Armstrong, 2002). Through examination of feeding and dominance hierarchies little evidence was found that the decision to eat following seawater transfer was dependent on risks and benefits. Instead it seems that state- or context-specific physiological capacity in the new seawater environment may have played a far greater role in deciding which individuals would eat.

Another aspect of dominance and feeding hierarchies examined in the present study was the effect of feed-deprivation following seawater transfer on subsequent competitive ability. One reason fish may perform poorly in seawater is that they "wait" too long to recommence feeding once transferred and are subsequently out-competed by individuals

that started feeding earlier (Johnstone, 1991; Stead et al., 1996) (Chapter 6). In small groups in the current study a period of 14 days of feed-deprivation immediately following seawater transfer adversely affected some individuals when placed with fish that were fed during the same 14 days. Feed-deprived fish had significantly lower feed intake and while the difference was not significant they also tended to be less aggressive than fed fish. Compensatory growth which is well documented in fed-deprived fish following recommencement of feeding (Dobson and Holmes, 1984; Maclean and Metcalfe, 2001; Nikki et al., 2004) was not seen. These findings are consistent with previous findings that while in the short term feed-deprivation can increase competitive capabilities, prolonged fed-deprivation can reduced these capabilities (Johnsson et al., 1996). It is important to note that not all feed-deprived fish competed poorly in these small groups and it would seem that at higher fish numbers and densities fed fish have less success in out-competing feed-deprived individuals (Chapter 6).

Although the tanks of Atlantic salmon in all experiments of the present study were set up as replicates differences in the dynamics of feeding and dominance hierarchies were observed among replicate groups of the same treatments in many of the experiments. Similar observations have been made in greenback flounder *Rhombosolea tapirina* (Carter et al., 1996). It has been suggested that groups of fish represent unique combinations of individuals and hence these groups do not all exhibit the same trends when treated in the same way (Carter et al., 1994, 1996). This explanation helps to explain the differences observed in groups of fish in the current study.

There are a few noteworthy points regarding the running of the hierarchy experiments. Firstly, one of the requirements of studying interactions of individuals is the ability to clearly identify each and this generally requires unique markings. While the coloured button technique used in the present study (C. Noble personal communication) was visually very useful the constant dislodgement and subsequent replacement of buttons required unavoidable repeated handling. Similar problems have been described using the same technique with pieces of coloured ribbon rather than buttons (Hakoyama and Iguchi, 1997). The increased handling required to replace dislodged tags may have affected behaviour and feed intake in Chapters 5 and 6 and as such the results may need to be viewed with some caution. It is important to note, however, that this was the best

technique available at the time given the experimental set up used. Also, the stability found in feeding hierarchies suggests that the tagging did not significantly affect hierarchy structure. Further work is needed to develop a system for visually tagging fish that does not damage the fish, lasts for long periods of time, does not require handling of fish during the experiments and makes fish easy to identify using overhead video cameras.

A second issue relating to the study of feeding hierarchies immediately following seawater transfer (Chapters 3 and 5) is that the reduction in feed intake and slow increase over time causes difficulties in the setting of rations. Hierarchies are known to be induced by restricted rations (McCarthy et al., 1992; Moutou et a., 1998) and it is known that feeding in a spatially localised manner can allow dominant fish to monopolise feed resources (Thorpe et al., 1990; Huntingford and Thorpe, 1992; McCarthy et al., 1999). As such it would have been ideal to use restricted rations when studying the effects of a change such as seawater transfer on feeding and dominance hierarchies. The main problem with feeding a restricted ration during a period of changing voluntary feed intake, such as is generally seen immediately following seawater transfer (Usher et al., 1991; Jørgensen and Jobling, 1994; Stead et al., 1996), is that one can not accurately predict how much the fish will eat on any given day. As a result in the current study all experiments relied on feeding to satiation. One interesting finding from studying dominance and feeding hierarchies was that there was little correlation between the two (Chapter 5). This may have been due to satiation feeding allowing subordinate fish to eat pellets later in the meal. It is possible that similar problems in allocating specific rations would be faced in other studies dealing with change, whether a change of environments or parameters such as diets which have also been shown to initially decrease feed intake in some situations (Toften and Jobling, 1997; Wybourne, 1997). Unfamiliarity or neophobia associated with a change to a new environment or diet may cause such decreases (Forbes, 1999).

7.5 Scale and environmental control

Findings in the current study, with the exception of those from Chapter 2, were based on experiments run in small tank systems and therefore the scale of experiments and the

amount of control attained over environmental variables needs to be taken into account in the interpretation of results and their application to a commercial scale. In experimental tank systems the numbers of individuals reared in each tank are far fewer than those in sea cages. In sea cages fish are exposed to variation in parameters such as temperature, light intensity, currents, salinity, exposure to predators, etc, while in indoor tank environments these parameters can be controlled or minimised (Bégout Anras et al., 2001). As a result the complexity of rearing environments in indoor tanks is lower than sea cages and indoor tanks allow much greater control over environmental parameters for experimenters and farmers (Willoughby, 1999; Bégout Anras et al., 2001). One possible limitation in using small indoor experimental systems for studies examining the formation of pinheads following seawater transfer is that the proportion of fish that pinhead tends to be low, averaging around 1.5% in industry in recent years (D. Mitchell personal communication; N. Murfet personal communication). Despite the resulting concern that pinheading may not occur in experimental systems pinheads were produced. While no statistical comparisons were possible qualitative assessments of pinhead performance could still be presented in relation to mean data collected for non-pinheading fish.

A major difference between small experimental systems and large commercial sea cage set ups relates to competition. In sea cages numbers of fish and densities are much higher than those seen in small experimental systems. Increases in these parameters have been shown to result in reduced aggression (Li and Brocksen, 1977; Fleming and Johansen, 1984; Kjartansson et al., 1988), most likely related to the increases in the cost of dominance with increasing group size decreasing the ability of dominant fish to assert their dominance (Huntingford and Thorpe, 1992; Grant, 1993; Sloman and Armstrong, 2002). In the present study seawater transfer alone was enough for dominance hierarchies to be reduced in stability, consistent with findings from other alterations to environments (Sloman et al., 2001; Snedden et al., 2006). Given that previous findings suggest that hierarchies tend to become stable in constant conditions (Forkman and Haskell, 2004) it is quite possible they would have stabilised again in seawater given adequate time since environmental conditions in tank systems are quite consistent. It is also possible however that in sea pens the constant perturbations in

environmental parameters may preclude the formation of stable hierarchies all together. Given the complexity of cage environments even if hierarchies were able to form they may not be stable, instead remaining in a constant state of structural change. This too would reduce the chances of any of the individuals being truly dominant and may add another dimension to the explanation of why aggression and hierarchy formation is reduced in sea cages (Kjartansson et al., 1988).

Further work is needed to determine whether the environmental instabilities associated with sea cage environments cause instabilities in dominance hierarchies which may contribute to observations that in sea cages hierarchy formation tends to be suppressed (Kjartansson et al., 1988). Experiments using small sea cages exposed to the normal environmental fluctuations of large sea cages, with small enough numbers of individuals to allow the expression of dominance but may help elucidate this.

Running experiments in indoor tanks systems in the current study may have affected the results obtained. However it is important to note that tank systems allow a more intricate examination of patterns that would be difficult to measure in sea cages. Findings such as the change in hierarchies from freshwater to seawater and the resulting suggestion that this would make it difficult to determine which fish would perform well in seawater are still meaningful when looking at a larger scale. If anything the probable decreases in hierarchy stability in sea cages would only accentuate this lack of predictive power. In terms of feeding frequencies while absolute feed intake following seawater transfer may differ slightly and while the strength of feeding hierarchies may have been artificially inflated due to the formation of stronger hierarchies in smaller tank groups, the results are most likely still meaningful with the resulting guidelines at worst being conservative.

7.6 Overall Summary

The most important outcomes, findings and recommendations from this study were:

- Pinheads have been defined as fish that have stopped growing in length while a considerable reduction in body weight has occurred (Chapter 2).

- Pinheads have lower condition factors (K ≤ 0.865), visceral fat, total body lipid, and gross energy than non-pinheading fish (Chapter 2).

- There is no evidence of pinheads being more osmotically compromised than non-pinheading fish in seawater (Chapter 2).

- The digestive capacity of pinheads, estimated from trypsin activity was not suppressed suggesting that pinheads are likely to be capable of digesting feed (protein) if consumed (Chapter 2).

- The term 'pinheading' in Tasmania is synonymous with 'failed smolt' in the northern hemisphere when used to describe pinheads appearing soon after transfer. However unlike 'failed smolt' 'pinheading' can also describe the same condition occurring after considerable time in seawater or less commonly before seawater transfer while still in freshwater (Chapter 2).

- Evidence from throughout this study suggests that pinheads do eat in the seawater environment though they consume less than non-pinheads and the amounts ingested may be reduced through time (Chapter 5 and 6).

- Feeding frequencies should be kept high immediately following seawater transfer of Atlantic salmon as feeding only one meal per day resulted in initially lower group feed intake than was seen using higher feeding frequencies (Chapter 3).

- Feeding one meal per day immediately following seawater transfer also significantly increased the initial strength of feeding hierarchies which may have increased the level of competition between individuals and could contribute to problems such as pinheading. Despite this possibility there was not evidence of a link between feeding frequencies and low condition factors (Chapter 3).

- Feeding frequency did not affect the number of fish in poor condition following seawater transfer suggesting that given enough time for the formation of pinheads feeding frequency may not have affected the percentage (Chapter 3).

- Feeding one meal per day during the time leading up to seawater transfer caused lower group feed intakes than eight meals per day (Chapter 4).

- The number of meals offered per day pre-transfer had no significant effect on feed intake following transfer. Tanks fed one meal per day after transfer had a greater decrease in feed intake immediately following seawater transfer than those fed eight meals per day regardless of pre-transfer regime (Chapter 4).

- The structure of feeding and dominance hierarchies in freshwater are poor indicators of the structure of feeding and dominance hierarchies in seawater. Hierarchies seem to be broken down and reformed as a result of seawater transfer (Chapter 5).

- While pinheads were distributed across all ranks of the feeding and dominance hierarchies in freshwater they were ranked close to the bottom of the feeding hierarchies in the time immediately following seawater transfer. While not being ranked at the bottom of dominance hierarchies in seawater all pinheads were below the first two ranks during this time (Chapter 5).

- A period of 14 days feed-deprivation following seawater transfer caused fish held at low numbers and densities to have lower feed intake than non feed-deprived fish following combination of fed and feed-deprived individuals. While there was also a trend for decreased aggression of feed-deprived fish, differences were not significant. Findings suggest that fish that fail to eat soon after transfer may have a higher risk of becoming pinheads than those that commence feeding sooner (Chapter 6).

7.7 References

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