Scale Growth Analysis of Atlantic salmon (*Salmo salar* Linnaeus) Unlocking Environmental Histories

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PhD

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Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of PhD is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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Abstract

Atlantic salmon (*Salmo salar* L.) populations have declined rapidly in recent years across all geographical ranges with populations becoming extinct within certain areas. Direct observation of the salmon's life is difficult and costly; therefore, scales remain the most widely used material to indirectly assess and monitor the recent changes in growth. Growth marks (circuli) in scales of Atlantic salmon are used to estimate age and to reconstruct growth histories. This thesis investigated mechanisms of circuli formation and the causes of variation in scale growth measurements. Comparison of scales from multiple body locations (Chapter 2) showed that growth, size and shape measurements varied significantly between body locations. Scale measurements taken from the sampling location recommended by ICES were sufficiently correlated with measurements from two adjacent locations in the posterior body region to facilitate conversion; calibration equations are presented for this purpose. Scale measurements from the anterior body region were highly variable and their use is not recommended. Scale size measurements from the recommended sampling location and from the two adjacent locations in the posterior body region were sufficiently correlated with fish fork length. Differences in scale size could potentially be used to determine the body location from which a scale was most likely sampled if this information has not been recorded (e.g. in archived scale collections); regression equations are presented for this purpose. Analysis of scales from experimentally reared Atlantic salmon post-smolts (Chapters 3 and 4), showed that scale growth and circuli number was proportional to fish growth under a range of different water temperatures and feeding conditions, justifying the use of these measurements as a proxy for growth. The rate of circuli deposition varied between temperature and feeding treatments and circuli number was proportional to cumulative degree day. Narrow inter-circuli spacings were observed during periods of slow growth at low temperatures and during periods of fast growth at high temperatures; therefore, circuli spacing should not be used to infer growth rates. In Chapter 5, scales from Atlantic salmon collected from three Irish rivers (Burrishoole, Moy and the Shannon) between 1954 and 2008 were analysed to determine if marine growth has changed during that period and to establish if trends are consistent across populations. Scale growth measurements and their temporal trends varied between populations. Post-smolt scale growth and circuli number were negatively correlated with SST (Burrishoole and Moy), NAO (Burrishoole) and AMO Burrishoole and Shannon). The results indicate that trends observed in one national index river may not be representative of change across all populations. The new knowledge generated in this thesis supports more accurate interpretation of scale growth measurements, furthers our understanding of this important species and ultimately benefits the future management of this species.

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Chapter 1.

General Introduction

1.1 Distribution

Atlantic salmon (*Salmo salar* L.) are an anadromous species, native to the temperate and sub-Arctic regions of the North Atlantic Ocean (Klemetsen *et al*., 2003), utilising rivers for both reproductive and juvenile stages and the marine environment for adult development and rapid growth (Mills, 1989). The species occur naturally along both the west and east coasts of the North Atlantic Ocean. In the northwest Atlantic, North American populations occur from approximately the Connecticut River in the south to Ungava Bay in the north, while in the Northeast Atlantic, the distribution ranges from the countries of northern Portugal to higher latitudes of Scandinavia (MacCrimmon and Gots 1979; Jensen *et al*., 2012; Figure 1.1). Due to this expansive range, the European stocks have been divided into two sub groups: a southern group ($< 62^{o}$ N) consisting of populations originating from Portugal, Spain, France, Ireland and the UK, and a northern group ($> 62^{o}$ N) comprising of stocks from Iceland, Norway, Russia and Sweden (Dadswell *et al*., 2010).

1.2 Ecology

The Atlantic salmon has a complex life cycle. The adult salmon return from the ocean to the natal river to lay their eggs (ova) within gravel depressions (redds) on the river bed during late autumn and winter. Once hatching of the ova occur, the alevin develops within the redd, feeding endogenously on a yolk sac. Once the yolk is depleted the newly developed fry emerge from the redd and begin to feed exogenously. The next stage of development is called the parr stage, depending on the origin or latitude of the river, this parr stage varies in length, lasting between one and seven years (Jensen and

Johnsen, 1982; Metcalfe and Thorpe, 1990). The parr undergo a process termed smoltification which involves morphological, physiological and behavioural changes, coinciding with increases in photoperiod and water temperature (McCormick *et al*., 1998) which prepare them for a marine existence (Hoar, 1988; Thorpe *et al*., 1998; Finstad and Jonsson, 2001). At this point the smolts begin the downward migration, predominantly at night to avoid predation (Hansen and Jonsson, 1985; Hvidsten *et al*., 1995) from their natal river to the sea. The seaward migration occurs from March to July; the timing of its onset depends on latitude (Jensen *et al*., 2012). Once a smolt enters the marine waters it is termed a post-smolt (Mills, 1989; Crozier and Kennedy, 1999). The entire North Atlantic Ocean (Figure 1.2) is utilised by Atlantic salmon during the marine phase of the life cycle until the point of sexual maturity, from one (one-sea-winter) two (two-sea winter) and even three to four years (multi-sea-winter). The stock structure varies with latitude; in southern latitudes one-sea-winter fish are most prevalent while two and multi-sea-winter fish are present at lower abundance. In contrast in more northerly regions two and multi-sea-winter fish are more abundant than the one-sea-winter fish. Salmon migrate from natal coasts to the pre/post adult feeding grounds in the Vøring Plateau region of the Norwegian Sea and to the multi-sea-winter adult feeding grounds off the east coast of Greenland.

Atlantic salmon are believed to be opportunistic feeders and are mainly found in the surface layers of the water column, occasionally diving to greater depths (Reddin and Shearer, 1987; Hislop and Shelton, 1993; Sturlaugsson, 1994; Jacobsen and Hansen, 2000; Holm *et al*., 2004; Reddin *et al*., 2006) and foraging on a diet of zooplankton

and nekton (Jacobsen and Hansen, 2000; Lacroix and Knox, 2005; Haugland *et al*., 2006). The main foraging grounds of the North American population are situated off the Greenland coast. The European stock complex has been observed to feed in the Norwegian Sea, an area characterised by a front that separates warmer Atlantic water to the south from the colder and less saline Arctic water to the north (Hansen and Jonsson, 1985; Jacobsen and Hansen, 2000). Atlantic salmon are assumed to inhabit areas with a narrow temperature range of between 8 and 12 °C (Friedland *et al*., 1993, 1998, 2000; Jonsson and Jonsson, 2004).

Ocean areas inhabited by Atlantic salmon are changing due to increasing sea surface temperatures and melting of sea ice (Lindsay *et al*., 2009). Furthermore, Todd *et al*. (2008) reports that sea surface temperature (SST) in the North East Atlantic have increased at a rate of between 0.5 and 1.5 °C per decade since the 1990's, this accelerated ocean surface warming may potentially have detrimental implications for a species with such a sensitive thermal preference. Richardson and Schoeman (2004) suggest that ocean warming leads to changes in the distribution of primary producers and negatively impacting fisheries. Friedland *et al.* (2012) reports that changes in food web composition have been associated with warming conditions in the Norwegian Sea resulting in poor growth and survival of salmon. Ultimately the oceanic environment is fluctuating and may attribute to changes in the oceanic environment will lead to changes in the distribution, abundance, growth and survival of many different organisms.

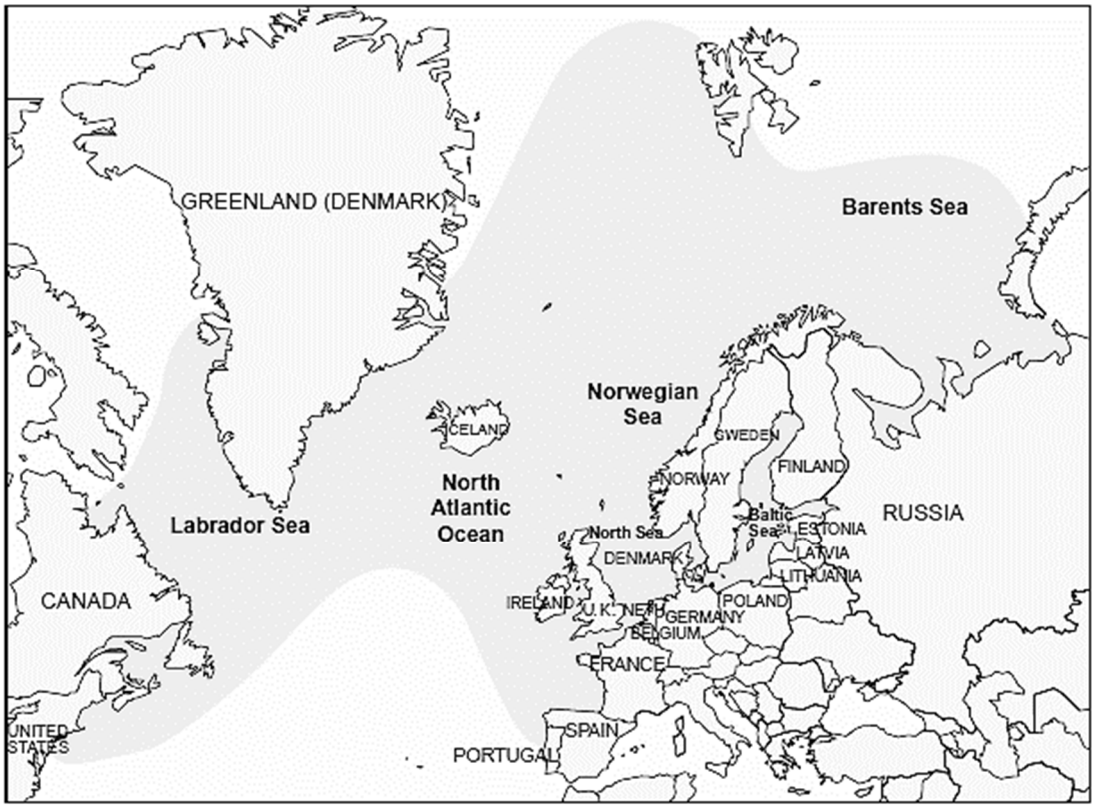

Figure 1.1. Assumed geographical distribution of Atlantic salmon in the North Atlantic Ocean and the associated countries that hold natural spawning populations of Atlantic salmon (Figure designed by Kari Sivertsen).

1.3 Understanding causes of decline of salmon populations

Historically Atlantic salmon were a highly abundant species, present in more than 2600 watersheds across the North Atlantic (WWF, 2001). Atlantic salmon populations have declined rapidly in recent years across all geographical ranges (Jonsson and Jonsson, 2009), with populations becoming extinct within certain areas (Russell *et al.*, 2012). Studies have linked survival during the marine phase to post-smolt growth rates, with a critical period occurring 4 to 5 months after ocean entry (Friedland *et al.*, 2000; McCarthy *et al.*, 2008). It is proposed that marine mortality is the main factor

underlying the demise of salmon stocks (Hansen and Quinn, 1998; Potter and Crozier, 2000; Friedland *et al*., 2005). The environmental conditions within the north Atlantic are changing and a substantial body of evidence links climate change to post-smolt growth and survival (Reddin and Shearer, 1987; Friedland *et al*., 1993, 1998; 2003; Jonsson and Jonsson, 2004; Todd *et al*., 2008). Increasing mortality is also thought to be driven by the synergistic effects of growth, pollution, disease, environmental factors (temperature and salinity influences, food availability), predators, freshwater influences and genetics (Figure 1.2.) (MacLean *et al*., 2003; Peyronnet *et al*., 2007). Evidence from retrospective growth studies suggest that growth rates have declined in recent decades in some European Atlantic salmon populations from both the southern and northern stock complexes. In relation to Irish populations, Peyronnet *et al.* (2007) reports that temporal growth changes and declines were evident over recent decades for salmon origination in the Burrishoole catchment in Co. Mayo. Most notably, a drastic growth decline was evident between the decades of the 1970's and 1980's corresponding with a rapid decline in return rates during this period. Friedland *et al.* (2000, 2009) also reports similar temporal changes evident in other European populations. It is not known if these growth and population declines seen in the Burrishoole and other European population which have been studied are indicative of other Irish rivers and investigation is needed to assess if the decline in growth is consistent across all populations.

The marine environment is so vast that the direct observation of each stage of the salmon's marine life poses huge difficulty (Hislop and Shelton, 1993). Research

surveys have helped to broaden our understanding of the ecology and population dynamics of Atlantic salmon in the marine environment (Holm *et al*., 1998, Holst *et al*., 2000, Anonymous, 2011). From these surveys, the initial marine juvenile growth (Jensen *et al*., 2012), migratory routes and swimming speeds (Mork *et al*., 2012), feeding and dietary patterns (Haugland *et al*., 2006) and the influences of environmental factors have been described. As direct observation is challenging and costly, scales are widely used to indirectly assess and monitor the recent changes in growth. Scales are the most easily obtained calcified structure, and can be obtained without need to sacrifice fish. Scale analysis is a very valuable tool that can be used to understand the Atlantic salmon's life in more depth.

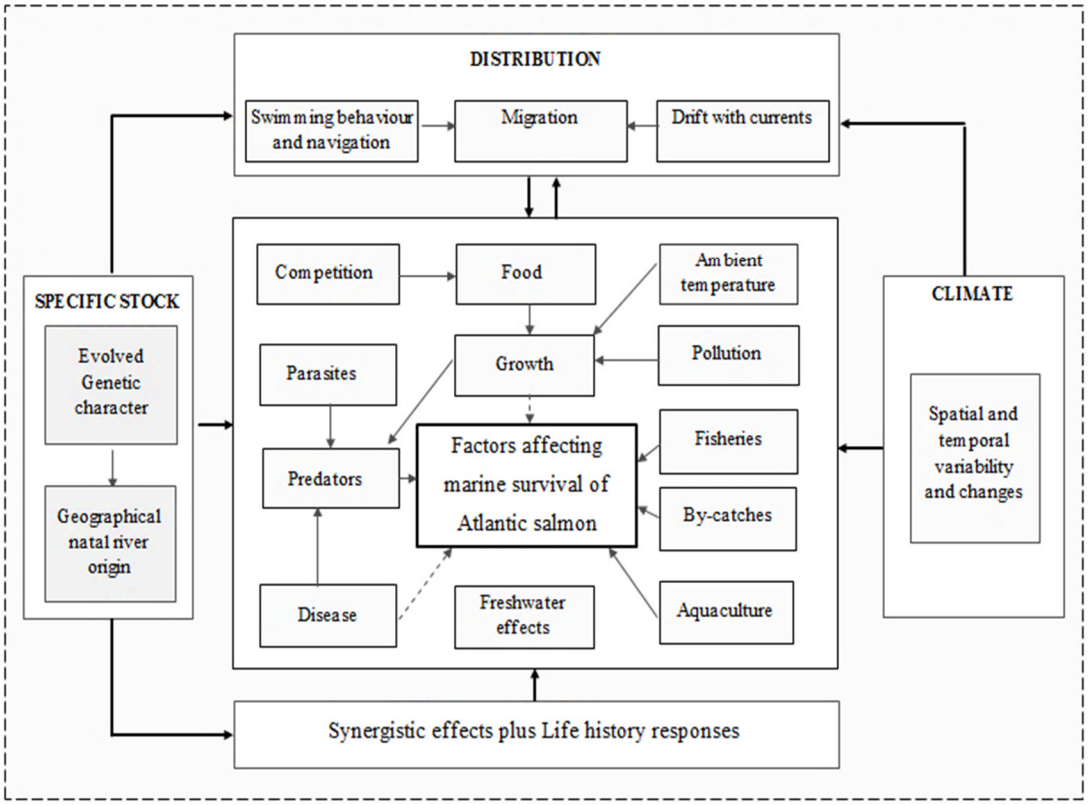

Figure 1.2. Associated factors affecting marine survival of Atlantic salmon (NASCO, 2012).

1.4 Management

Atlantic salmon populations are assessed annually by expert groups within each member state. In most North Atlantic salmon producing countries the assessment of Atlantic salmon stocks is conducted with reference to a conservation limits (CL) defined as the stock (number of spawners) that will achieve long-term average maximum sustainable yield (MSY) (ICES, 2016) identified from stock and recruitment curves. As Atlantic salmon are defined as short lived stocks, the overall abundance is sensitive to annual recruitment due to minimal age groups in the adult spawning stock. Therefore, the MSY approach is aimed at achieving a target escapement [MSY

$B_{escapement}$ (the biomass in numbers available to spawn)]. Each country is responsible for assessing stock levels on an annual basis. Similarly, different harvest rules may be applied in different countries for home water management e.g. in Ireland catches of Atlantic salmon are only permitted once this escapement target is achieved per river (ICES, 2016). The total return of salmon for each river is compared to the predetermined CL on an annual basis, and those rivers not meeting the CL are either open for angling by catch and release if attaining more than 65% of the CL but less than 100% or ultimately closed to angling if CL is below 65%. In relation to Irish Atlantic salmon populations, presently 143 Irish salmon rivers are monitored and assessed by means of CLs, with only 38% achieving the CL during 2015 (ICES, 2016). Once the annual assessments are completed, each member country provides the assessment results to the expert group within International Council for the Exploration of the Sea (ICES Working Group on North Atlantic Salmon). ICES provides updated fisheries statistics, stock assessment and advice to the North Atlantic Salmon Conservation Organization (NASCO), the regional fisheries management organisation responsible for managing salmon fisheries in international waters and high sea fisheries. For the purpose of national assessments used by ICES, each river CL may be totalled to provide a national estimate. When a summed river specific CL is not possible, ICES use a pseudo-stock–recruitment model to estimate adult returns based on catches raised by exploitation rates and unreported catch. Adult to adult stock recruitment curves for entire countries stocks can be generated in this manner to calculate a national CL [(Annex 6); ICES, 2016]. Specific advice is provided for fisheries in West Greenland and the Faroes relating to the status of stocks in stock

complexes for North American stock complex (NAC stock complex; Canada and the USA), northern Northeast Atlantic stocks (Northern NEAC stock complexes – Scandinavia, Russia and Northern Iceland) and southern Northeast Atlantic stock complexes (Southern NEAC; Ireland, UK, Spain, France, Southern Iceland).

Atlantic salmon numbers have had a marked decline in all of the countries reporting to ICES. In the case of Ireland, the estimated return rate in 1971 of one-sea-winter fish was 1,051,256 compared to 183,350 returns in 2015. Although two-sea-winter and multi-sea-winter fish are less abundant in Ireland compared to one-sea-winter populations, a similar decline in estimated return rates has also been reported ranging from 157,884 in 1971 to only 17,413 in 2015 (ICES, 2016).

1.5 Information from scales

Atlantic salmon scales are defined as elasmoid, being dermal in nature (Zylberberg *et al.*, 1992; Panfili *et al.*, 2002) and further termed as cycloid (derived from the Greek word *cyclo,* meaning circle) (Goodrich, 1907; Bertin, 1944; Panfili *et al.*, 2002). The scales are composed of a rigid organic surface layer primarily composed of calcium-based salts and a fibrous inner layer that is mainly collagen based, the anterior portion of the scale is embedded in the dermis and housed in the scale pocket, the posterior scale region further covered by an epithelial layer (Sire, 1988; Panfili *et al.*, 2002). The anterior region of each scale is overlapped by the posterior portion of the scale in front. This arrangement is termed imbricate (overlapping) (Bertin, 1944; Panfili *et al.*, 2002).

During scale growth, concentric rings referred to as circuli form on the superficial layer of each scale. This provides a record of growth during the entire life history of an individual fish (Dahl, 1911; Anonymous, 1984). Circuli are formed incrementally at a rate proportional to somatic growth (Panfili *et al*., 2002) and arranged sequentially as bands corresponding to specific periods of seasonal and annual growth – winter and summer. During the winter months, reductions in water temperatures, photoperiod and food supply result in a narrowing of circuli distances producing a darker winter band or annulus, with discontinuities in the circuli visible along its outside edge (Dahl, 1911; Anonymous, 1984). Once environmental conditions change after the winter months, growth rates again increase, producing wider circuli distances and the formation of a summer band on the scale (Anonymous, 1984). The deposition and arrangement of these circuli and the distances between them depict the age structure and somatic growth rate within both the freshwater and marine environments.

Recent developments in digital analysis have allowed substantial advances in the field of scale analysis. High resolution images may now be acquired and analysed by digital technology allowing for accurate fine scale temporal estimates of growth rates. Circuli spacings, counts and aggregate scale growth measurements may now be obtained from calibrated images using image analysis software, producing estimates of individual, population and stock growth histories. These retrospective growth studies provide a unique insight into the species use of the ecosystem, and indicate whether periodic changes in growth are apparent in monthly and overall growth rates and between populations (Peyronnet *et al*., 2007; Jensen *et al*., 2012).

When accurately calibrated, modern image analysis systems allow an experienced reader to make reliable measurements from scale images with a high degree of precision and accuracy. However, the identification of growth marks on a scale is prone to a certain degree of subjectivity, scale growth patterns in the freshwater and marine stages vary between populations and stocks. For example, in scales from southern populations, difficulties for a reader may arise at the point of marine migration on the scale as winter / spring water temperatures are higher in southern latitudes which is reflected on the scale as a gradual widening of circuli between the last freshwater winter annulus and initial marine circulus; therefore, distinguishing the point of marine migration on a scale may be problematic, leading to measurement error. Regarding northern stocks, difficulties may arise when scales are obtained from freshwater rivers with low winter / spring water temperatures, the fish originating from such rivers grow at a slower rate and as the first scales form at ~ 30 mm fork length (Warner and Havey, 1961), the first winter annulus may not be evident on the scale, leading to an ageing error. Measurements of growth marks on a scale are subject to various sources of error, both human and mechanical, that can affect the accuracy and precision of the measurements obtained, repeated readings can vary both within and between readers. Subsequent to training, intra and inter laboratory calibration exercises are a means of limiting reader error. Intra laboratory calibration exercises are a form of quality control - an individual reader is required to blindly read and measure one scale multiple times, a second reader then repeats the same process, and consistency of measurements within and between readers is examined (ICES, 2011,

2013). Similarly, inter-laboratory calibration exercises can be used to ensure that readings are consistent across laboratories, both nationally and internationally (Anonymous, 2010; NASCO, 2012).

Friedland *et al*. (1993) suggested marine circuli deposition rates of one circulus per week during summer months and bi weekly during winter months. A more recent study suggests that one circulus will be deposited every 6.3 days during initial post-smolt growth (Jensen *et al*., 2012). These proxy values are useful as an assessment of incremental scale growth over time and in the interpretation of the overall scale growth pattern; however, investigation is needed to validate circuli deposition rates.

From as early as the 1900's Atlantic salmon scale characteristics have been used both for ageing and growth purposes, providing estimations of population age and size, (Dahl, 1911; Gilbert, 1913; Rich, 1920; Warney and Havey, 1961; Bilton, 1975; Jensen and Johnsen, 1982). In more recent times fundamental questions about the nature and determinants of scale growth have been less studied, as rapid technological advancement has facilitated the collection of growth information from more and more populations and years. However, it is not yet clear what the main factors influencing scale growth and circuli deposition rates are or the effect that temperature and feeding may have on scale development.

Growth patterns on scales are used to reconstruct growth histories and indirectly assess and monitor temporal changes in growth (Peyronnet *et al*., 2007; McCarthy *et al*., 2008; Friedland *et al*., 2009; Jensen *et al*., 2012); if the rate of circuli deposition is

known, growth rates can be estimated over specific time periods (Friedland *et al*., 1993; Jensen *et al*., 2012). However, the periodicity of circuli deposition is not known and the main factors influencing scale growth and circuli deposition rates are not fully understood; little is known of the effect that temperature and feeding may have on scale development. Elucidating these mechanisms, would further understanding of scale growth patterns (growth, circuli number and circuli spacings) and allow for their more accurate interpretation. If growth rates can be accurately estimated for specific periods in the life history, these could then be related to environmental conditions, allowing us to examine the effect and magnitude of past environmental conditions and to more accurately predict the impacts of future change.

Investigations of long-term trends in scale growth integrate information from both archived and contemporary scale collections. Inconsistencies in sampling methods could introduce bias to these datasets. Scale sampling from a recommended standard body location (three to five rows above the lateral line, diagonally from the posterior edge of the dorsal fin to the anterior edge of the pelvic fin on the left side of the body) has been adopted since the mid 1980's (Anonymous, 1984). Historical scale collections obtained prior to 1984 may have contain scales obtained from other body locations and often the body location is not recorded. It is not known if scale growth measurements from different body locations will produce consistent results. Investigation of this source of variability in scale growth measurements would help to standardise scale analysis. If relationships between measurements taken from different body locations can be established, this would provide a means to convert

measurements and to integrate scale growth measurements taken from different body locations (when the body location is known). If scale size and shape measurements can be used to determine the body location from which a scale was sampled, this would facilitate the use of archived scales when body location has not been recorded. Ultimately this would help to standardise results, providing more accurate and hence reliable data from scales leading to more confidence in the outputs from scale studies. New knowledge would facilitate better, more informed, management to protect the species.

1.6 Objectives and thesis structure

Atlantic salmon scales are widely used to provide estimates of age and growth rates and to reconstruct population growth histories. Despite this, relatively little work has been conducted to validate the timing and rate of circuli formation and the effects of varying environmental factors on scale growth or to investigate the differences between scale measurements across the body. This thesis addresses these knowledge gaps by rearing salmon under controlled environmental conditions and examining scale circuli deposition rates and growth during the early post-smolt stages of the life cycle. These results are then compared to scale growth formation in wild samples with marine growth and patterns of growth inferred from the experimental information.

The present work is structured as six chapters with the first being an introduction followed by four chapters formatted as research papers. A synthesis of the results is presented in a final discussion chapter.

1.6.1 Chapter overview and objectives

Chapter 2; *Comparison of shape, growth and circuli counts of scales taken from various body locations of wild Atlantic salmon (Salmo salar* L.*) post-smolts and adults.* This study compared scale growth measurements obtained from various locations on the fish body. The objectives were to investigate if scale growth measurements obtained from the standard body location are significantly different than those obtained from other areas of the body, and if so, are the measurements sufficiently correlated to apply a conversion equation to measurements from non-standard locations. Scale size and shape measurements were also compared between body locations to determine if these features could be used to distinguish between scales from different body locations when the origin of the scale had not been recorded.

Chapter 3; *Experimental investigation of the effects of temperature and feeding regime on post-smolt scale growth, circuli deposition rates and circuli distances in Atlantic salmon (Salmo salar* L.*).*

The objective of this study was to investigate the effect of water temperature and feeding rate on the formation of circuli in the scales of Atlantic salmon post-smolts reared under controlled experimental conditions. By validating the periodicity of circuli formation and relating scale growth rates to rearing conditions this study seeks to inform interpretations of growth marks in scales of wild Atlantic salmon in relation to changes in the marine environment.

Chapter 4; *Experimental investigation of the effects of feeding regime on post-smolt growth scale in Atlantic salmon (Salmo salar* L.*).*

The objective of this study was to investigate the effect of feeding rate on the patterns of circuli in the scales of Atlantic salmon post-smolts reared under controlled experimental conditions. Relating scale growth rates to rearing conditions, this study seeks to inform interpretations of growth marks in scales of wild Atlantic salmon in relation to changes in the marine environment.

Chapter 5; *Decadal changes in post-smolt growth in three Irish populations of Atlantic salmon (Salmo salar* L.*).*

The objective of this study was to investigate if decadal trends in post-smolt growth were consistent across three Irish populations of Atlantic salmon and to establish whether marine environmental conditions affected marine growth in populations from geographically similar areas over a long time series.

Chapter 2.

Comparison of shape, growth and circuli counts of scales taken from various body locations of wild Atlantic salmon (*Salmo salar* L.) post-smolts and adults.

Submitted as:

Thomas, K., Brophy, D., Ó Maoiléidigh, N., Jensen, A.J., Jacobsen, J.A. and Fiske, P. Comparison of shape, growth and circuli counts of scales taken from various body locations of wild Atlantic salmon (*Salmo salar* L.) post-smolts and adults.

2.1 Abstract

Measurements obtained from Atlantic salmon (*Salmo salar* L.) scales are used to infer growth rates and to reconstruct growth histories. A standard body location recommended by ICES has been established for many years, however it is not always feasible to obtain samples from this location due to scale loss. Furthermore, archival scale sets may not indicate the body location at which the scale was sampled. It is unknown if growth measurements obtained from scales of body locations other than the recommended sampling location are comparable.

Growth, size and shape measurements were compared between scales obtained from the standard sampling location and scales obtained from other body locations of post-smolt and adult fish. Measurements varied significantly between body locations. Scale growth measurements from the recommended sampling location were sufficiently correlated with measurements from two adjacent locations in the posterior body region; these two areas would therefore suffice as an alternative sampling area if scales from the standard body location are unavailable; the calibration equation established in this study may be applied to facilitate a conversion of growth measurements comparable to the standard sampling location. Scale measurements from the anterior body region were highly variable and their use is not recommended for inclusion in growth studies. Scale size measurements (area and perimeter) from the recommended sampling location and from the two suggested alternative sampling locations were sufficiently correlated with fish fork length. Regression equations were established which could be used to determine if a scale originated from a body area other than the standard sampling location or from the two adjacent locations in the posterior body

(e.g. in archived scale collections). Therefore, if scale size measurement is lower than the expected value computed by the regression, the scale should be rejected as calibration of measurements would not be feasible.

2.2 Introduction

Atlantic salmon (*Salmo salar* L.) scales have been used for age determination from as early as the 1900's (Johnston, 1907; Dahl, 1911). Traditionally salmon scales were used only to estimate age and annual growth rate (Jensen *et al*., 2012). However, in recent times advances in image analysis have facilitated the extraction of higher resolution growth information. Growth rates can be accurately estimated over specific time periods (i.e. weekly, monthly and seasonally) based on circuli deposition and spacing. These measurements have been used to determine continent of origin (Lear and Sandeman, 1980), quantify spatial and temporal trends in growth and determine the environmental factors that may affect growth, survival and abundance of the species (Peyronnet *et al*., 2007; McCarthy *et al*., 2008; Friedland *et al*., 2009; Jensen *et al*., 2012).

The life history of the individual fish is recorded as concentric ridges on the outward facing surface of the scales. These ridges are commonly referred to as "circuli" on a scale. Originating from the centre of the scale or the scale focus, these circuli are arranged consecutively as bands coinciding with specific periods of seasonal growth. During the winter months, reductions in water temperatures, photoperiod and food supply result in a narrowing of circuli spacing producing a darker winter band or annulus, with discontinuities in the circuli visible along its outside edge (Dahl, 1911; Anonymous, 1984). Once environmental conditions change after the winter months, growth rates increase, producing wider circuli spacing and the formation of a summer band on the scale (Anonymous, 1984).

Scales of Atlantic salmon contain distinct zones corresponding to freshwater and marine residency, reflecting its anadromous life cycle. Winter and summer bands are evident in both the freshwater and marine zones. Growth patterns vary considerably between freshwater and marine environments and this is reflected in the circuli patterns within each zone. The end of the freshwater phase of the life cycle and the commencement of the seaward migration is identifiable on the scale as a change in the pattern and distance of the circuli spacings: either a sudden increase in circuli spacing coupled with more pronounced circuli, indicating faster marine growth, or a gradual increase in circuli spacing and in some instances a growth check (approx. three broken densely packed circuli) (Anonymous, 1984; Mc Carthy *et al*., 2008; Jensen *et al*., 2012).

Scales of Atlantic salmon are known to develop at different periods along the body. The scales first form when fry are ~ 30 mm fork length (L_F), along the lateral line directly posterior to the dorsal fin. Scale formation proceeds equally along the anterior and posterior locations along the lateral line and also toward the dorsal and ventral regions away from the lateral line. Body scalation has completed by the time the fry are ~ 50 mm fork length (Warner and Havey, 1961). Due to the progressive nature of scale formation, circuli counts and measurements can vary between scales from different body locations; this can be particularly pronounced in slower growing fish e.g. at higher latitudes where water temperatures and photoperiod are considerably less than that of more southerly latitudes (Bilton, 1975; Jensen and Johnsen, 1982).

Martynov (1983) states that total scale radius and circuli number will decrease with increasing distance from the lateral line. In 1984, an ICES expert group was established with a view to standardising sampling practices. Arising from this, a standard body location was assigned (three to five rows above the lateral line, diagonally from the posterior edge of the dorsal fin to the anterior edge of the pelvic fin on the left side of the body) (Anonymous, 1984).

Scales can be easily damaged or lost usually in the regions where the body is at its broadest (Johnston, 1907). Juveniles inhabiting fast flowing areas of rivers, mature adults spawning in redd's or fish exposed to mechanical objects may all incur significant scale loss. Scale regeneration is usually evident in the freshwater zone of the scale; complete loss of the inner matrix of circuli can render both the age and growth history indeterminable (Anonymous, 1984). Displaced scales may also be present, which will be evident as a portion of scale that has shifted or appears to have broken away from the original axis or direction of growth (Dahl, 1911). Although it may be possible to assign an age to the scale, it may not be possible to perform growth measurements as potential growth information may have been lost.

Generally, scales are easily obtained without need to sacrifice the specimen; however, to do the least harm, a limited numbers of scales are usually retrieved. Subsequent interpretation may be problematic if the scales are those that have regenerated (Anonymous, 1984). Furthermore, if the scales have not been sampled from the standard body location, inference from those scales might be biased. These problems

may be particularly acute for archival catalogues of scales sampled prior to 1984 as these may have been sampled outside the standard body locations.

The main objectives of this study was to establish if growth measurements obtained from the standard body location are significantly different from those obtained from other areas of the fish body, and if so, whether the measurements are sufficiently correlated to apply a conversion equation to measurements from non-standard locations. Scale size and shape measurements were also compared to determine if these features could be used to distinguish between scales from different body locations when the origin of the scale had not been recorded (as is the case for some archived scale collections).

This research is highly relevant to future scale studies as it would allow the use of scales collected from areas other than the standard body location when these scales are unavailable and would also facilitate the use of archival scales of unknown body location; ultimately providing a means to convert measurement and to standardise results, thus improving the accuracy and reliability of scale growth studies.

2.3 Methods

2.3.1 Sample collection

Atlantic salmon post-smolts were collected at sea by the Faroese vessel R/V Magnus Heinason during the international EU funded FP7 project, SALSEA-Merge survey in the Vøring Plateau Region of the Norwegian Sea in July of 2009. Fish were collected

from the upper 10 meters of the water column using surface trawls of a modified pelagic net. The tows were of three hours duration. Eighty-two salmon post-smolts of wild origin were collected for this study from a total sample size of 310 post-smolts, over eighteen surface trawls. Scales were sampled immediately after capture (NASCO, 2009). Returning adult salmon were sourced from ESB/Marine Institute salmon fixed trapping facility on the River Liffey, Co. Dublin Ireland. A sample of ten adult salmon of wild origin were obtained and frozen for later removal of scales.

2.3.2 Scale removal and processing

Scales were sampled from two body locations (location A and location E; Figure 2.1) for the post-smolt fish and from five body locations (location A to location E; Figure 2.1) for the adult fish. The scale sampling locations were positioned as follows: Location A (Loc_A); the standard body location sampling site, three to five rows above the lateral line, diagonally from the posterior edge of the dorsal fin to the anterior edge of the pelvic fin on the left side of the body (Anonymous, 1984). Location B (Loc_B); three to five rows below the lateral line, directly below the position of the first scale sample. Location C (Loc_C); the region between the adipose fin and the lateral line. Location D (Loc_D); the anterior section of the body, four to five rows above the lateral line and Location E (Loc_E); directly under the pectoral fin.

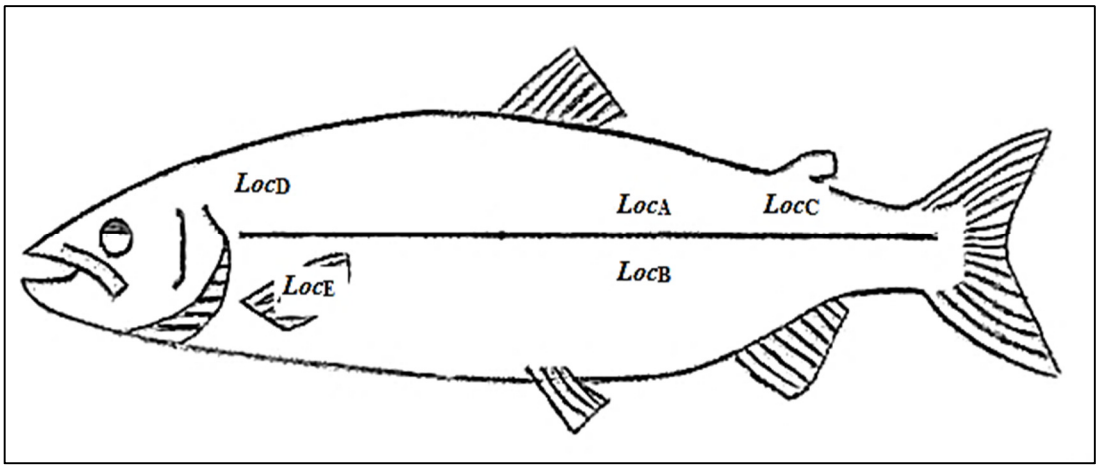

Figure 2.1. Body locations of scale samples obtained for this study.

All scales were placed in coded scale envelopes to dry and subsequently placed in a small petri dish. Between five and seven of the best scales (defined as showing an entire edge and clear focus) were selected using a stereo microscope and immersed in a 5% sodium hydroxide (NaOH) solution for a maximum duration of 30 seconds for post-smolt scales and a maximum duration of 1 minute for adult scales, to remove all traces of biological material that would impede light transmission under magnification without causing damage to the scale. The scales were then placed in distilled water for a few minutes to remove traces of the NaOH solution and subsequently mounted between a glass slide and cover slip and the age determined using transmitted light under a compound microscope.

2.3.3 Origin

As previously mentioned, post-smolt samples were part of the SALSEA Merge project; therefore, were originally included in genetic stock identification, the post-

smolt scale samples used in this study were genetically assigned to both Ireland and the UK (NASCO, 2012). The adult fish were of Irish origin as they were obtained from the River Liffey Co. Dublin.

2.3.4 Ageing

As salmon are anadromous, two distinct life stage components are identifiable on the scales, i.e. freshwater and marine zones. An annulus is defined as a region of a scale where successive bands of narrow circuli are followed by bands of widely spaced circuli. Three or more circuli may run together into one circulus in the region of densely packed circuli at the peripheral edge of the narrow band as the circuli run vertically down the scale margin (known as "cutting over"; Anonymous, 1984). Annuli were identified using these criteria and the circuli counted within the freshwater and marine zones [Figure 2.2 (a)].

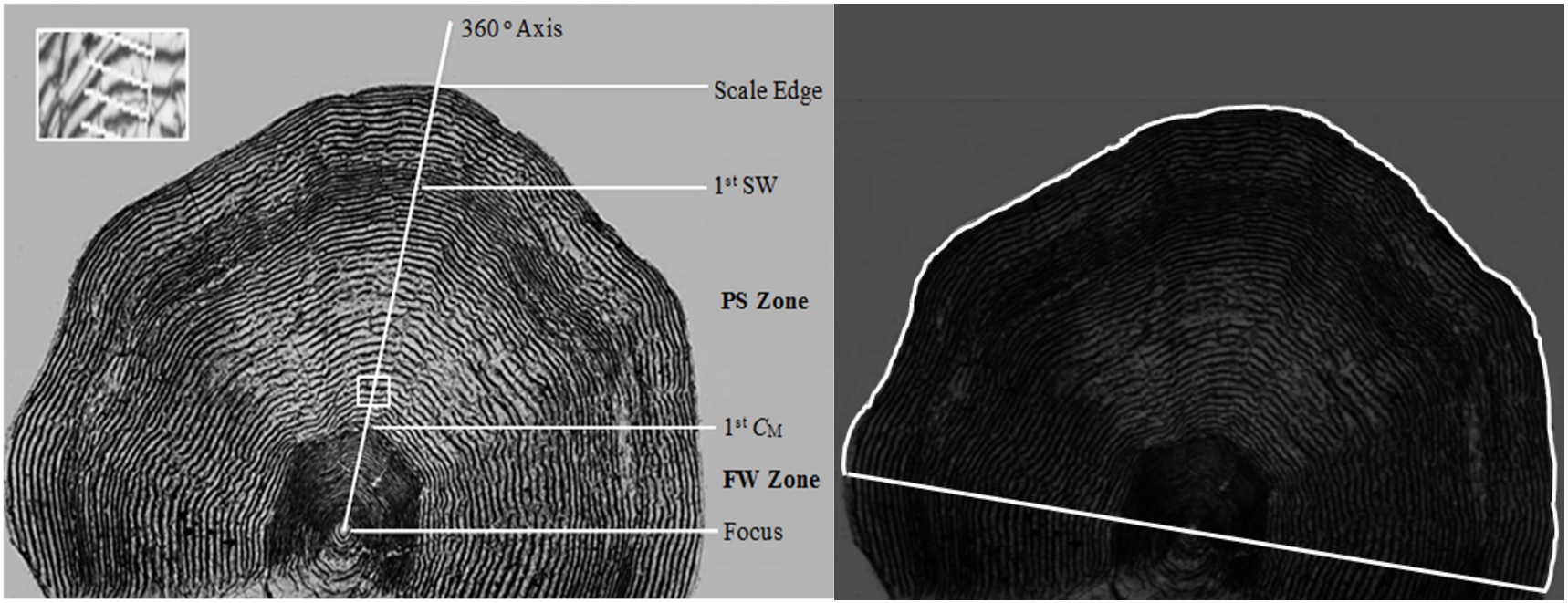

Figure 2.2 (a)

Figure 2.2 (b)

Figure 2.2 (a, b). (a) Image of an adult salmon scale displaying the 360° straight line axis used when obtaining measurements, both freshwater (FW), post-smolt (PS) and marine zones are illustrated. The circuli within the white rectangle on the main image are magnified in the inset on the upper left of the image (b) Image of an Adult scale displaying the region used for shape analysis (indicated by the white outline and transect).

Post-smolt scales were aged using a 40 X magnification. They were comprised of 40 one-year-old fish [one year residing in freshwater, followed by ~ four months of marine residency (1+0)], and 42 two-year-old fish [two consecutive years residing in freshwater, plus ~ four months of marine residency (2+0)]. The adult scales were aged under 12.5 X magnification and all fish were identified as being 2+2. fish (two consecutive years residing in freshwater, followed by a further two consecutive years residing in the marine environment before returning to fresh water where they were captured). Images of salmon scales were acquired and calibrated to the relevant objective using Image Pro Plus version 7.01 © software [Figure 2.2 (a)].

2.3.5 Scale shape analysis

Scales from different body regions showed differences in both size and shape (Figure 2.3). In order to quantify these differences and determine if they could be used to distinguish between scales from different locations, measurements of size [area (mm^2), perimeter (mm), height (mm) and width (mm)] and shape (circularity, aspect ratio, roundness and solidity) were obtained from calibrated scale images of ten post-smolts (Loc_A and Loc_E) and ten adult fish (Loc_A to Loc_E) using ImageJ software (Table 2.1). A straight line transect was traced horizontally through the scale focus and subsequently traced along the scale edge, concluding at the scale focus [Figure 2.2 (b)]. The size and shape measurements were automatically extracted from this outline.

2.3.6 Scale growth analysis

Measurements were extracted from calibrated scale images using Image Pro Plus version 7.01 © software. For the post-smolt scales, individual circuli were enumerated along a straight line transect along the 360° axis from the centre of the scale focus to the end of the last circulus of the freshwater zone to derive the freshwater circuli count. The aggregate length (mm) of the transect was used as a measurement of freshwater growth. The freshwater growth measurements were obtained in the same manner for the adult fish scales; however, freshwater circuli were not enumerated as they are more difficult to read in adult fish and there is a higher possibility of regeneration within the freshwater zone. The edge of the freshwater zone was identified by the increased circuli spacing representing sea entry (Jensen *et al*., 2012). In both the post-smolt and adult scales, measurements of the marine zone were taken along a straight line transect from the last freshwater circulus through to the scale edge. The marine zone in adult scales includes the post-smolt zone and the remaining distance from the 1st sea winter to the scale edge. The circuli were enumerated to obtain the marine circuli count, and the transect length was used as a measure of marine growth (mm) [Figure 2.2 (a)]. The freshwater growth (mm) and marine growth (mm) measurements were summed to give total scale radius measurement (mm).

2.3.7 Statistical analysis

Four scale size measurements (area, perimeter, height and width), four scale shape indices (circularity, aspect ratio, roundness and solidity) and five scale growth measurements (freshwater circuli number, marine growth, marine circuli number and

scale radius) were compared between body locations using a series of repeated measure ANOVAs. For the comparison of scale growth measurements, smolt age and body location were included as a fixed factors and fish ID as a random factor. Smolt age was a between-subject factor (nested within fish ID) and body location was a within subject factor. For the comparison of scale size and shape measurements, 1+0 post-smolt, 2+0 post-smolt and adult scale measurements were analysed separately and the models contained just two factors: body location (fixed) and fish ID (random). In all cases, measurements were compared between body locations using Tukey's post-hoc procedure.

Pearson's correlations were used to establish the relationship between scale growth measurements from the different body locations. The relationship between fish L_F and scale size/shape measurements per body location were also established. Age groups were analysed separately. Regression equations between L_F and size measurements (area and perimeter) were established to predict scale size of standard location (Loc_A) and non-standard locations (Loc_B to Loc_E for adult fish). Regression equations were derived to predict growth measurements for the standard location (Loc_A) based on measurements taken at non-standard locations (Loc_E for post-smolts, Loc_B to Loc_E for adult fish). All statistical analysis was conducted using the MINITAB statistical package. An alpha level of 0.05 was used for all significance tests.

2.4 Results

The summary statistics for each of the size, shape and growth variables are shown in Tables 2.2 (a, b) and Table 2.3, while the results of the statistical comparisons are summarised below and in Table 2.4.

2.4.1 Scale size and shape

Visual assessment of scale appearance and statistical comparison of scale size and shape confirmed that these features were characteristic of body location and could potentially be used to distinguish between scales from different body locations.

2.4.1.1 Post-smolt scales; variation in appearance, size and shape

Post-smolt scales from location A and location E showed clear differences in appearance [Figure 2.3 (a, b)]. In scales from Loc_E , the growth patterns in the freshwater zone were less well defined compared to Loc_A i.e. fewer circuli were visible and there was little circuli deposition between annuli [Figure 2.3 (b)]. This made freshwater age estimation more difficult. The marine zone of the scales from Loc_E was also smaller relative to scales from Loc_A. However, scales from both locations were similar in terms of growth pattern and the point of seaward migration was well-defined in both. The beginning of the marine zone could also be unambiguously identified.

The repeated measures ANOVAs confirmed that scales from the two body locations differed in size and shape. All measured scale size parameters (area, perimeter, height

and width) were significantly smaller in scales obtained from body Loc_E compared to Loc_A for both age groups [ANOVA, $p<0.001$; Table 2.4]. Two of the four measured shape indices (aspect ratio and roundness) showed significant differences between body locations of both age groups [ANOVA, $p\leq0.001$; Table 2.2 (b); Table 2.4].

2.4.1.2 Adult scales; variation in appearance size and shape

In adult fish, scales from locations Loc_A, Loc_B and Loc_C were visually similar in both size and appearance and the freshwater and marine ages were clearly distinguishable. The freshwater zone of Loc_D and Loc_E of the adult scales were less similar in shape and size compared to scales from the other three locations sampled; however, the freshwater and marine zones were clearly discernible.

The repeated measures ANOVAs revealed significant variation in scale size and shape between body locations. There were no significant differences for area measurements between Loc_A and Loc_B (ANOVA, $p=0.882$). All other pairwise comparisons for area measurements differed significantly [ANOVA, $p<0.001$; Table 2.4]. Height was not significantly different between Loc_A and Loc_B (ANOVA, $p=0.364$). For both perimeter and width measurements there were no significant differences between Loc_A and Loc_B (ANOVA, $p=0.548$; $p=0.865$), respectively. All other pairwise comparisons were significantly different [$p\leq0.028$; Table 2.4].

2.4.1.3 Correlations between fish length and scale size/shape measurements

The scale size parameters (area, perimeter and width and height) were mostly significantly positively correlated with L_F [$p \leq 0.044$; Table 2.2 (a)] except width in scales from Loc_E for 1+0 post-smolts ($p=0.078$) and height in scales from Loc_A for 1+0 post-smolts and adult fish ($p=0.092$; $p=0.238$), respectively. The strength of the correlations varied between locations, but were generally strong [Figure 2.4; Table 2.2 (a)]. The scale shape parameters were not significantly correlated with L_F [$p>0.05$; Table 2.2 (b)], except for circularity at Loc_A in the adult fish ($p=0.019$) and circularity ($p=0.030$), aspect ratio ($p=0.024$) and roundness ($p=0.021$) at Loc_B in the adult fish [Figure 2.4; Table 2.2 (b)]. The results suggest that the size parameters area and perimeter are the best indicators of fish size. These fish size/scale size relationships could be used to screen for scales from non-standard body locations among archive scale collections by applying the generated regression equations shown in Table 2.5.

Figure 2.3 (a)

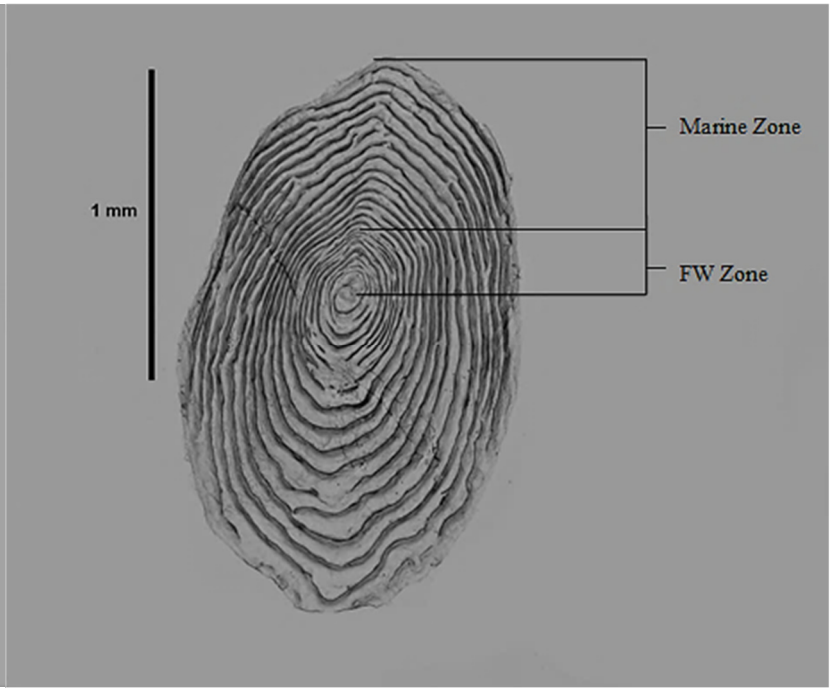

Figure 2.3 (b)

Figure 2.3 (a, b). Images of scales taken from the same 2-year-old (2+0) Atlantic salmon post-smolt viewed under 40X magnification (scale bar =1mm). Freshwater (FW) and marine zones are clearly indicated (a) Scale from location A (Loc_A) (b) Scale from location E (Loc_E).

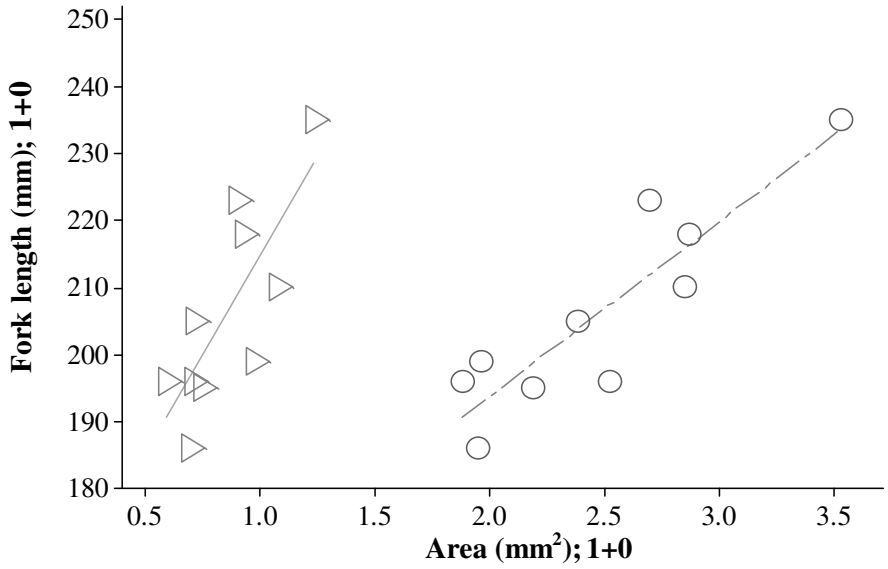

Figure 2.4 (a)

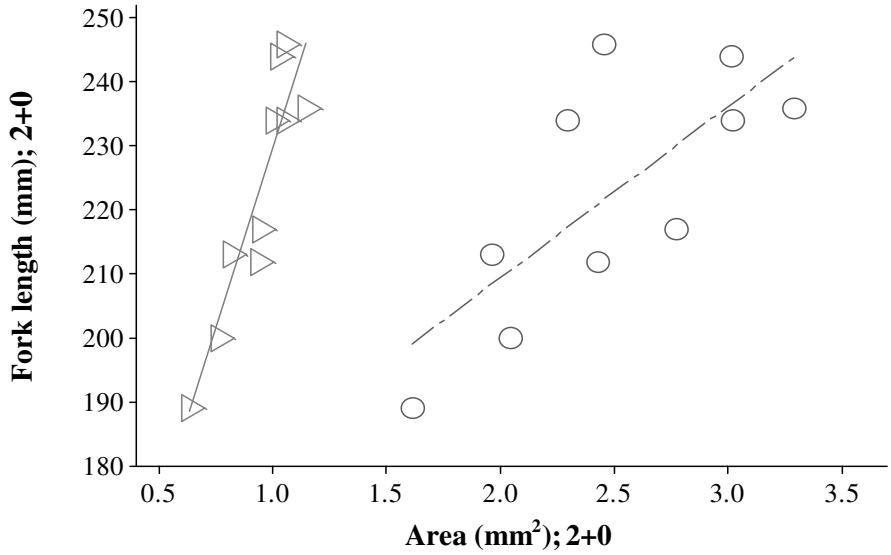

Figure 2.4 (b)

Figure 2.4 (a, b). Linear relationships between fish fork length (L_F; mm) and size parameters for scales from the sampled body locations (a) 1-year-old (1+0) post-smolts (b) 2-year-old (2+0) post-smolts (○ — - , Loc_A; ▷ —— , Loc_E).

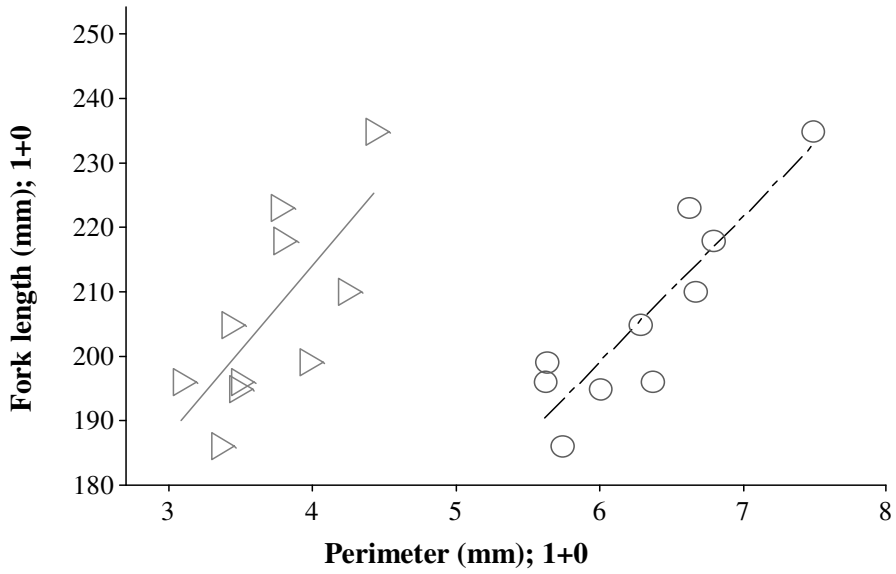

Figure 2.4 (c)

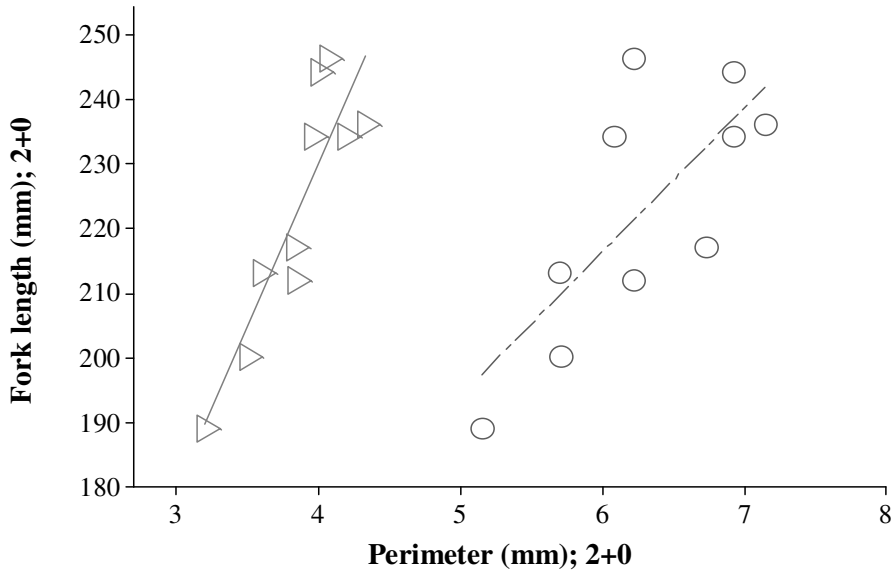

Figure 2.4 (d)

Figure 2.4 (c, d). Linear relationships between fish fork length (L_F; mm) and size parameters for scales from the sampled body locations (c) 1-year-old (1+0) post-smolts (d) 2-year-old (2+0) post-smolts (○ — - , Loc_A; ▷ —— , Loc_E).

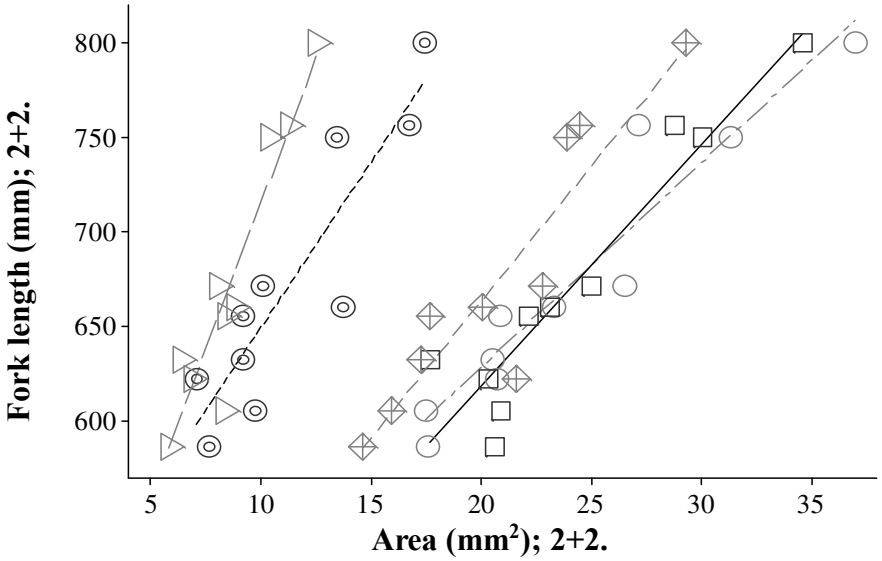

Figure 2.4 (e)

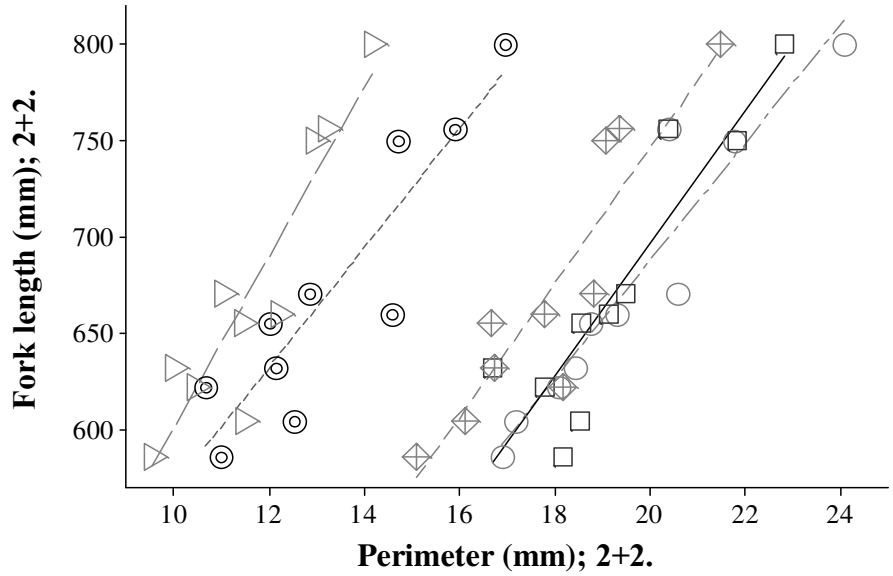

Figure 2.4 (f)

Figure 2.4 (e, f). Linear relationships between fish fork length (L_F; mm) and size parameters for scales from the sampled body locations of adult fish (○ — –, Loc_A; □ ——, Loc_B; ◈ – – –, Loc_C; ⊙ - - - -, Loc_D; ▷ — —, Loc_E).

2.4.2 Scale growth

2.4.2.1 Post-smolt scales: variation in growth measurements

All of the growth measurements examined showed significant variation between age groups and between body locations (ANOVA, $p<0.001$). For freshwater growth, freshwater circuli number, marine growth and marine circuli number, the interactions between smolt age and body location were also significant (ANOVA, $p<0.001$; Table 2.4) indicating that the magnitude of the difference between body locations varied between the two age groups. All scale growth measurements were greater in scales from Loc_A compared to scales from Loc_E (ANOVA, $p<0.001$; Figure 2.5; Table 2.3).

The percentage differences for 1+0 and 2+0 age fish, respectively were: overall scale radius 47.9% and 45.6%; freshwater growth 24.5% and 30.4%; freshwater circuli number 10.9% and 12.8%; marine growth 23.5% and 15.3% and marine circuli number 29.5% and 20.2%. The 1+0 post-smolts had considerably fewer freshwater circuli and smaller freshwater growth than that of the 2+0 fish [ANOVA, $p<0.001$; Figure 2.5 (a, b)]. The mean marine growth and marine circuli count of the 1+0 fish was greater than that of the 2+0 post-smolts for both locations [ANOVA, $p<0.001$; Figure 2.5(c, d)].

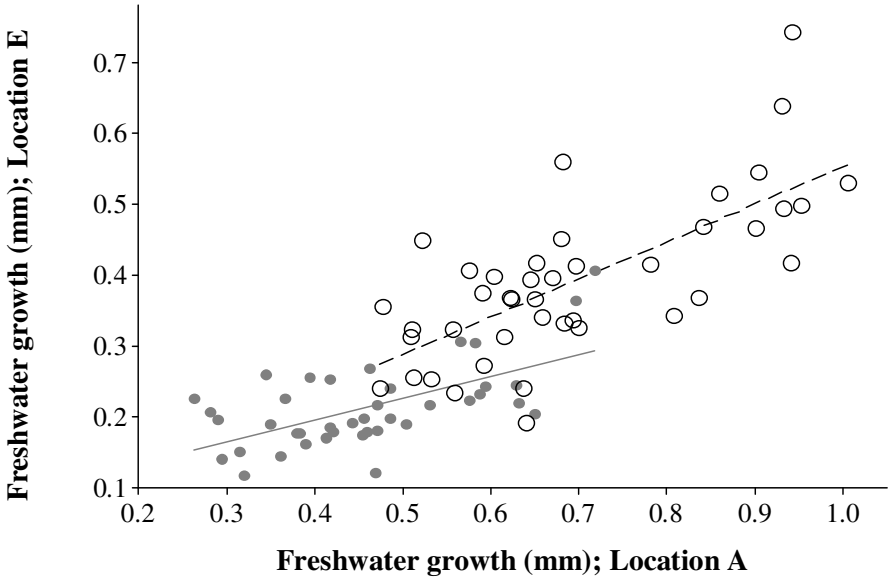

Figure 2.5 (a)

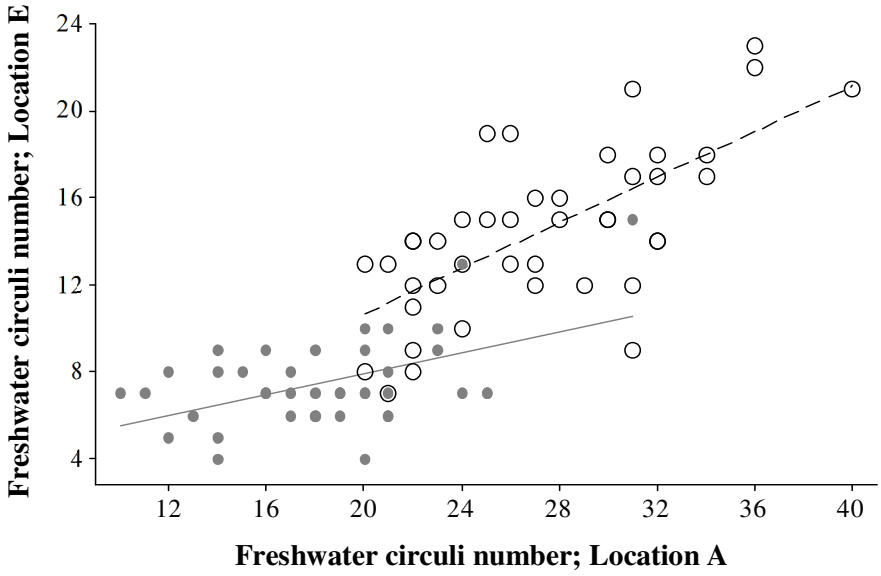

Figure 2.5 (b)

Figure 2.5 (a, b). Linear relationships between measured growth parameters for both age groups between scales from location A (Loc_A) and location E (Loc_E) (a) Freshwater growth (G_{FW}; mm) (b) Freshwater circuli number (C_{FW}) [1-year-old (● ——— , 1+0) and 2-year-old (○ - - - - , 2+0) post-smolts].

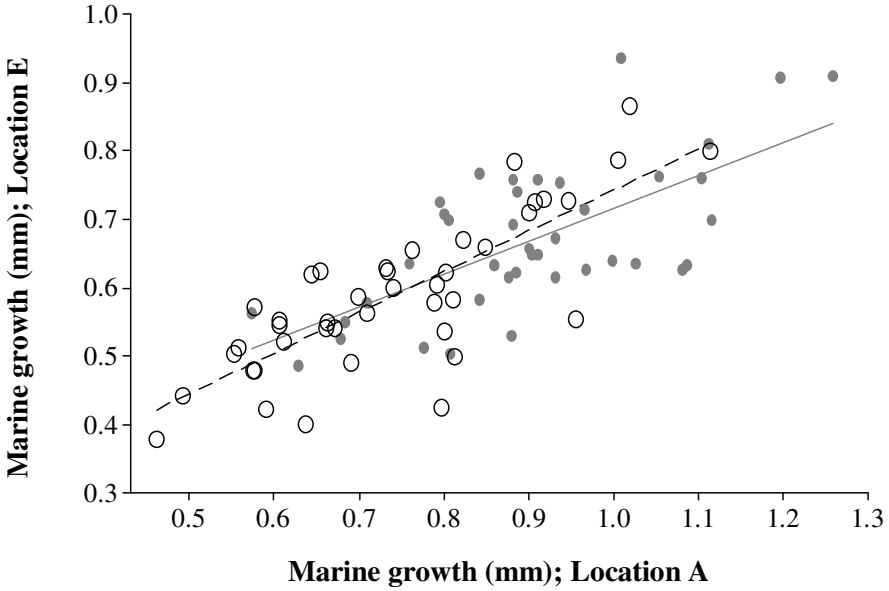

Figure 2.5 (c)

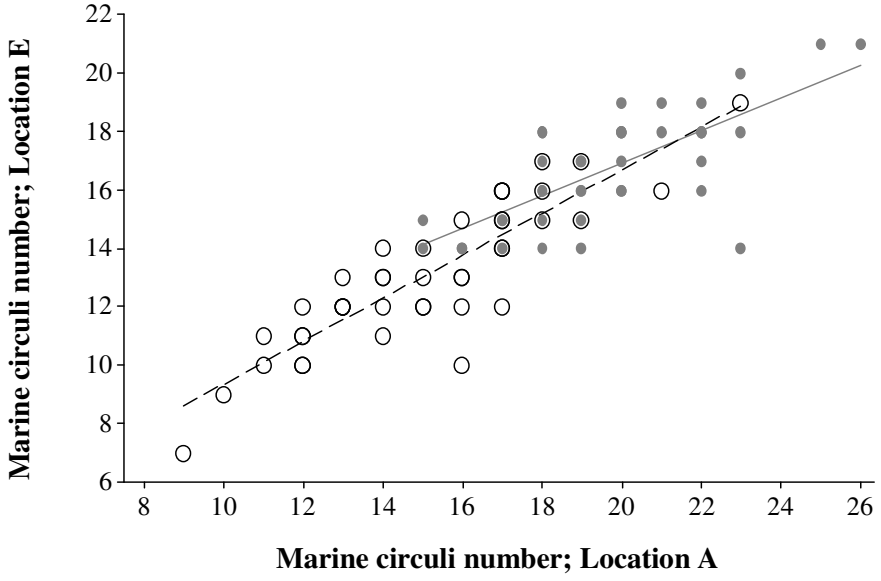

Figure 2.5 (d)

Figure 2.5 (c, d). Linear relationships between measured growth parameters for both age groups between scales from location A (Loc_A) and location E (Loc_E) (c) Marine growth (G_M; mm) (d) Marine circuli number (C_M) [1-year-old (● ——, 1+0) and 2-year-old (○ - - - - , 2+0) post-smolts].

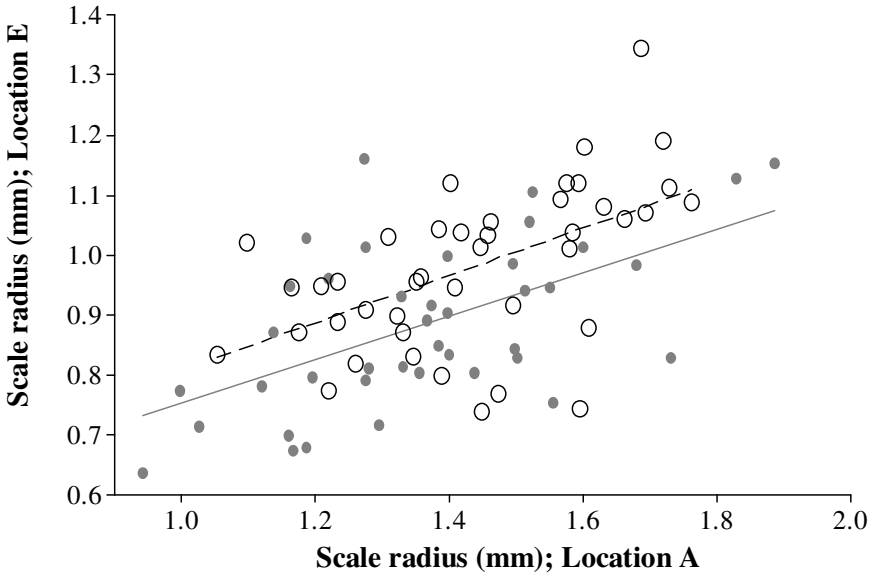

Figure 2.5 (e)

Figure 2.5 (e). Linear relationships between scale radius (R_S; mm) measurements for both age groups between scales from location A (Loc_A) and location E (Loc_E) [1-year-old (● ——, 1+0) and 2-year-old (○ - - - - , 2+0) post-smolts].

2.4.2.2 Adult scales: variation in growth measurements

Loc_C showed the largest mean freshwater growth measurements followed by locations Loc_B, Loc_A, Loc_D and Loc_E, respectively. Loc_B had the highest mean marine growth. Loc_B also had the highest mean marine circuli count, followed by Loc_A, Loc_C, Loc_D and Loc_E, respectively. Loc_B had the largest scale radius [Table 2.2 (a, b)]. All four scale growth measurements (freshwater growth, marine growth, marine circuli number and scale radius) showed significant variation between body locations in the adult fish (ANOVA, $p<0.001$; Table 2.4). There was no significant difference in marine growth between Loc_A and Loc_C (ANOVA, $p=0.081$; Table 2.4). Marine circuli count showed

no significant variation between Loc_A and Loc_B (ANOVA, p=0.231; Table 2.4) or between Loc_A and Loc_C (ANOVA, p=0.313; Table 2.4). Scale radius was not significantly different between Loc_A and Loc_C (ANOVA, p=0.645; Table 2.4). All other pairwise comparisons were significant ($p \leq 0.014$; Table 2.4).

2.4.2.3 Correlations between fish length and scale growth measurements

For both the post-smolt and adult scales, growth measurements for Loc_A were significantly positively correlated with the equivalent measurements from all other body locations (Figure 2.5; Figure 2.6; Table 2.6). The strength of the correlations varied between body locations. Measurements from Loc_A tended to be most strongly correlated with those from Loc_B and Loc_C (R^2=0.70-0.95). Correlations with measurements from Loc_D and Loc_E were weaker, particularly for the post-smolt scales (R^2=0.24-0.76).

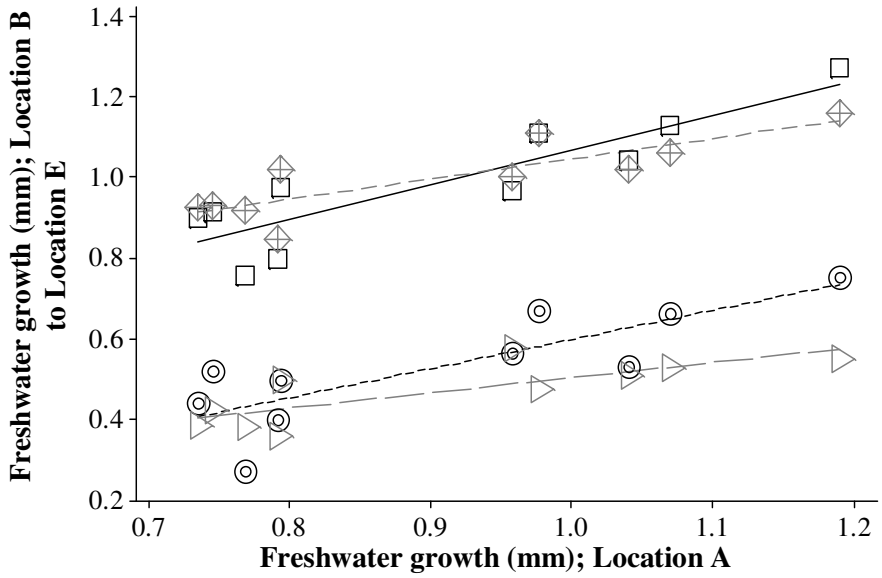

Figure 2.6 (a)

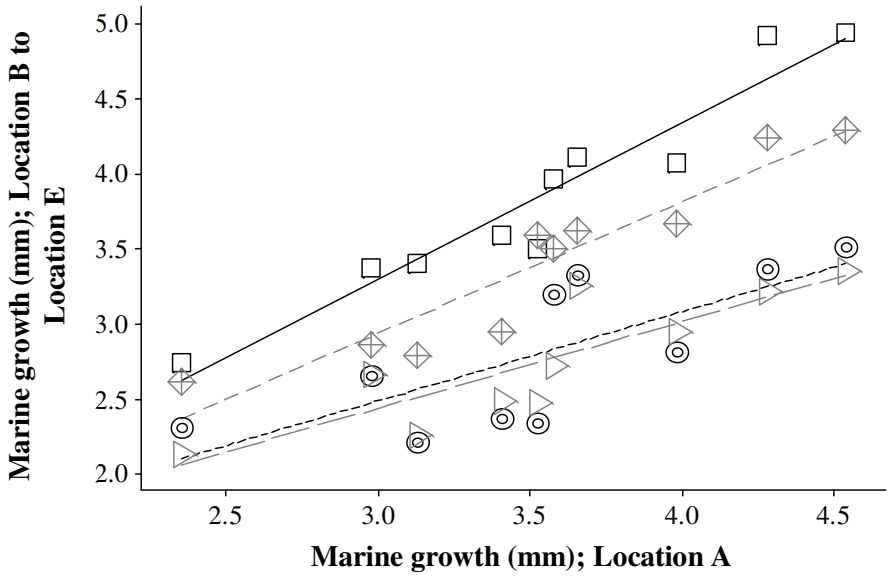

Figure 2.6 (b)

Figure 2.6 (a, b). Linear relationships between measured growth parameters of adult fish, between scales from location A (Loc_A) to location E (Loc_E) (a) Freshwater growth (G_{FW}; mm) (b) Marine growth (G_M; mm) (□ ——, Loc_B; ◈ — — —, Loc_C; ◎ - - - -, Loc_D; ▷ — —, Loc_E).

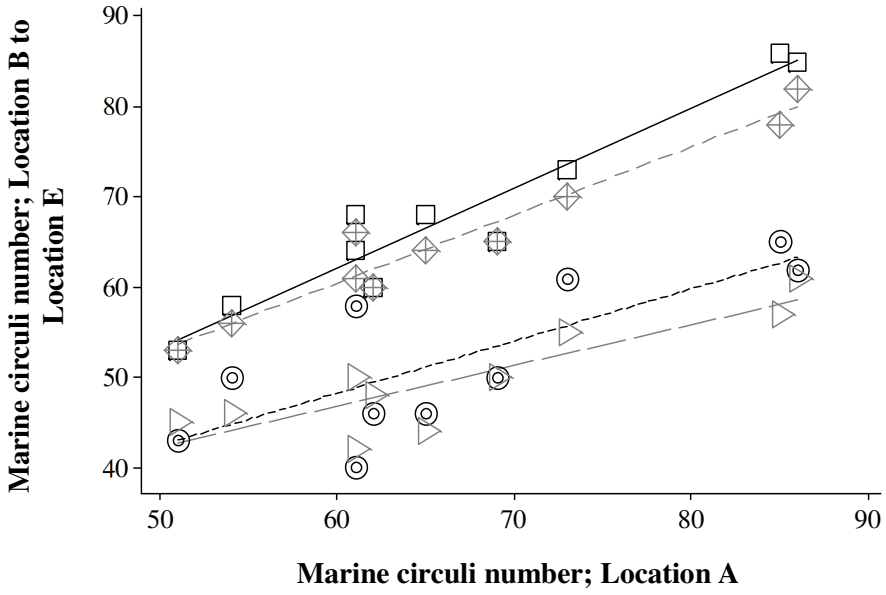

Figure 2.6 (c)

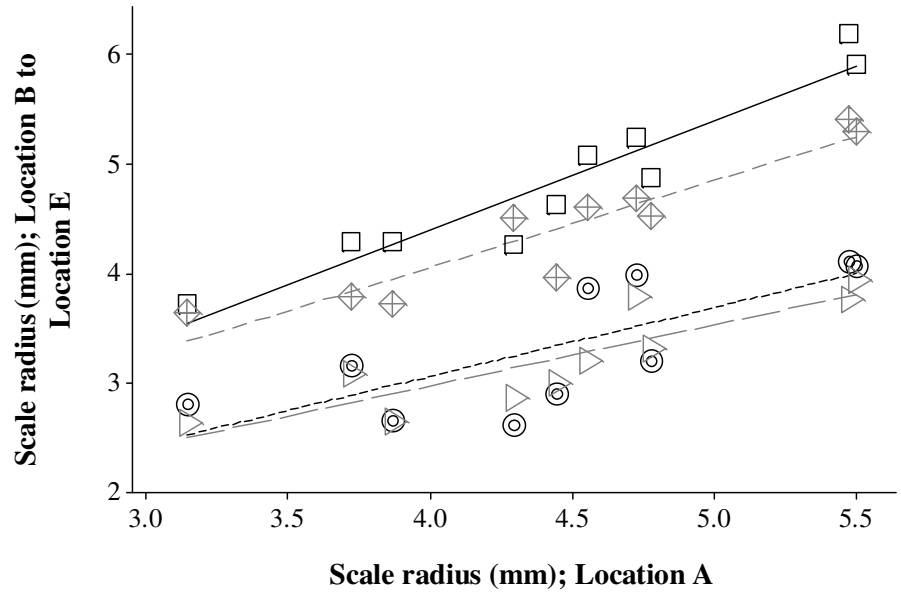

Figure 2.6 (d)

Figure 2.6 (c, d). Linear relationships between measured growth parameters of adult fish, between scales from location A (Loc_A) to location E (Loc_E) (c) Marine circuli number (C_M) (d) Scale radius (R_S; mm) (□ ——, Loc_B; ◈ — — —, Loc_C; ◎ - - - -, Loc_D; ▷ — —, Loc_E).

2.5 Discussion

The results of this study show that significant differences in growth patterns occur between scales obtained from specific body locations for both post-smolt and adult fish. Therefore, measurements derived from non-standardised body locations will produce inconsistent estimates of growth. The differences were particularly pronounced when scales taken from the anterior region of the body (Loc_D and Loc_E) were compared to scales taken from the posterior region (Loc_A, Loc_B and Loc_C). Scales from the anterior locations were smaller and had consistently fewer circuli than scales from the posterior. This is consistent with the timing of scale development; body scalation begins in the posterior region of the body and then progresses to the anterior regions (Warner and Havey, 1961; Bilton, 1975). Consequently, measurements of scales from Loc_D and Loc_E would lead to overall underestimation of growth.

When sampled at the standard scale sampling site (Loc_A) or within close proximity (Loc_B and Loc_C), scale growth measurements were strongly and positively correlated with each other (R^2>0.70), particularly for the marine portion of the scales. This suggests that measurements from one location could be converted to the equivalent measurements for the other location using linear regression with a reasonable degree of accuracy. Scale measurements from Loc_D and Loc_E were less strongly correlated with scale measurements from the standard location and the use of conversion equations for these locations would be subject to a larger degree of error.

Consistencies in the measurements of marine growth obtained from different posterior body locations show that reliable growth information can be obtained from locations

other than the standard sampling site. Although marine growth measurements from Loc_B varied significantly from the other two posterior locations *(*Loc_A and Loc_C) the measurements obtained from Loc_B were strongly correlated with those from Loc_A (R^2=0.92); therefore, a correction could be applied for the marine growth measurements between location Loc_B and Loc_A, if necessary. These findings are reassuring, as post-smolt growth has been linked to survival (Peyronnet *et al*., 2007) and measurements from the marine portion of the scale are widely used in studies of marine survival over broad temporal and geographical range (Friedland *et al*., 2000; Friedland *et al*., 2003; Friedland *et al*., 2005; Hubley *et al*., 2008).

The results of this study have important implications for the application of scale growth information to ecological and fishery related questions. With developments in digital analysis techniques, scale analysis has advanced rapidly in recent times. Precise measurements of circuli spacings, counts and aggregate scale growth measurements can be obtained and growth rate can be calculated over short periods of time (Friedland *et al*., 2005; Peyronnet *et al*., 2007; Jensen *et al*., 2012). Researchers are using both historical and contemporary scale material to examine spatial and temporal variation in growth and to increase understanding of the factors contributing to trends in growth and survival (Peyronnet *et al*., 2007; McCarthy *et al*., 2008; Friedland *et al*., 2009; Hogan and Friedland, 2010).

Where circuli counts are used to estimate the duration of marine residency, scale measurements obtained from the anterior end of the body could lead to substantial underestimation. In the marine environment, it is estimated that circuli of Atlantic

salmon in the early post-smolt phase are deposited every 6.3-days (Jensen *et al*., 2012). We observed a mean difference of three (1+0 post-smolts) and two (2+0 post-smolts) marine circuli number between scales from Loc_A and Loc_E. Therefore, the duration of marine residency would be underestimated by 18.9 and 12.6-days for 1+0 and 2+0 fish, respectively when using scales from Loc_E instead of Loc_A. The duration of the marine residency is one of the indicators used to determine the region of origin (Lear and Sandeman, 1980; Reddin, 1986; Reddin and Friedland, 1999; Jensen *et al*., 2012); therefore, underestimation of this parameter could lead to inaccurate assignment of origin, particularly when other indicators of origin such as freshwater age or genetics are not available.

The extent to which scale growth patterns vary between body locations is likely to depend on the stock and the temperatures at which the fish develop. The timing and progression of body scalation (Warner and Havey, 1961) as well as the rate of freshwater circuli formation (Jensen and Johnsen, 1982) are known to be temperature dependent. The differences in scale growth patterns between body locations that are reported here relate to Atlantic salmon from southern stocks. The influence of body location on scale growth patterns in salmon from other stocks would warrant further investigation.

Based on the findings of this study, we recommend that where possible scales are obtained from the standard body location (Loc_A) and that only an adequate number of scales (<10) are removed to ensure that other locations are not unintentionally sampled. If scales from the Loc_A are not available due to scale loss, scales can be

derived from Loc_C, followed by Loc_B. Where necessary, measurements should be converted using the appropriate linear regression obtained from a sub-sample of scales from multiple body locations for the corresponding cohorts and stock. For contemporary collections of scales, it is important to ensure that the body location from which the scales have been obtained is clearly recorded and that methods of scale sampling are standardised between operators. With regard to historical scale archives, especially those collected before 1984, the possibility that scales may have been derived from locations other than the standard sampling site must be considered. For example, when large numbers of scales are contained in an envelope this can indicate that scales originate from more than one body location. Substantial variation in scale shape and size from fish of the same body length may also reflect inconsistencies in the sampling location (Anas, 1963; LaLanne, 1963; Pearson, 1966; Major *et al.*, 1972; Scarnecchia, 1979; Jensen *et al.*, 2012). Such inconsistencies, if not accounted for, could lead to underestimation of age, freshwater and marine growth and back calculated body lengths as well as a misinterpretation of temporal trends in growth. The results of this study confirm that scale size and shape indices differ significantly between certain body locations. In addition scale size is significantly correlated with fish length and the nature of the fish size/scale size relationship is specific to each body location. The established regression equations between fish size and scale size (area and perimeter) generated in this study could identify if a scale originated from a location other than Loc_A, Loc_B and Loc_C (Loc_B and Loc_C have been proposed as alternative sampling locations within this study; regressions have also been described for these locations). The regression equations would inform the reader of the expected size measurement with a degree of accuracy (R^2=0.74-0.95), comparing the value

computed by the equation to the measurement from the scale of unknown origin would inform the reader if the scale originated from Loc_A or Loc_B and Loc_C; if the measurement falls below the expected value/s, the scale should be rejected as a conversion factor cannot be applied.

Proper calibration is vital to ensure that growth measurements are consistent and comparable across studies (Bilton and Jenkinson, 1969; Fukuwaka, 1998; Copeland *et al*., 2007; Wilson *et al*., 2009). Inter-reader scale reading calibration exercises have been conducted between international laboratories in recent times, notably as part of the SALSEA Merge project (NASCO, 2008) and the Celtic Sea Trout project (Anonymous, 2010). These exchanges have helped to standarise the interpretation of scale growth measurements amongst readers working from images of the same scales. Numerous studies have been conducted on the differences found between scales of Pacific salmon (*Oncorhynchus sp*) (Anas, 1963; LaLanne, 1963; Pearson, 1966; Major *et al*., 1972; Scarnecchia, 1979). Similar studies do not appear to have been conducted for Atlantic salmon. However, the implications arising from the analyses of scales from different body locations and the integrity of results have previously been addressed (ICES, 2011, 2013). Progress and improvements to current scale analyses for Atlantic salmon will require further studies and collaborations across geographic areas and stocks to ensure accuracy of information and appropriate application of results.

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Table 2.1. Size and shape parameters.

Size parameters	**Shape indices**
Area (S_A) mm^2	Circularity (S_{Cir}) = (4π*area/perimeter^2)
Perimeter (S_{Per}) mm	Aspect ratio (S_{Ar}) = (major_axis/minor_axis)
Height (S_H) mm	Roundness (S_{Rn}) = (4*area/(π*major_axis^2)
Width (S_W) mm	Solidity (S_{Sol}) = (area/convex area)

Table 2.2 (a). Scale size measurements for post-smolt and adult Atlantic salmon.

Variable†	Stage‡	Age	Loc§	Mean ± SD	Regression with L_F†		
					r	*p*	S.level^
	PS	1+0	Loc_A	2.5 ± 0.52	0.90	**<0.001**	*
	PS	1+0	Loc_E	0.86 ± 0.20	0.78	**=0.008**	*
	PS	2+0	Loc_A	2.5 ± 0.54	0.74	**=0.014**	*
	PS	2+0	Loc_E	0.94 ± 0.16	0.91	**<0.001**	*
S_A	AD	2+2.	Loc_A	24.3 ± 6.3	0.95	**=0.001**	*
	AD	2+2.	Loc_B	24.4 ± 5.3	0.94	**=0.001**	*
	AD	2+2.	Loc_C	20.8 ± 4.5	0.91	**<0.001**	*
	AD	2+2.	Loc_D	11.4 ± 3.7	0.90	**<0.001**	*
	AD	2+2.	Loc_E	8.7 ± 2.2	0.94	**<0.001**	*
	PS	1+0	Loc_A	6.3 ± 0.6	0.90	**<0.001**	*
	PS	1+0	Loc_E	3.7 ± 0.42	0.73	**=0.017**	*
	PS	2+0	Loc_A	6.3 ± 0.65	0.75	**=0.013**	*
	PS	2+0	Loc_E	3.9 ± 0.34	0.89	**=0.001**	*
S_{Per}	AD	2+2.	Loc_A	19.5 ± 2.2	0.95	**<0.001**	*
	AD	2+2.	Loc_B	19.3 ± 1.9	0.90	**<0.001**	*
	AD	2+2.	Loc_C	17.9 ± 1.9	0.91	**<0.001**	*
	AD	2+2.	Loc_D	13.3 ± 2.1	0.91	**<0.001**	*
	AD	2+2.	Loc_E	11.6 ± 1.5	0.91	**<0.001**	*
	PS	1+0	Loc_A	2.2 ± 0.21	0.84	**=0.002**	*
	PS	1+0	Loc_E	1.2 ± 0.12	0.58	=0.078	ns
	PS	2+0	Loc_A	2.1 ± 0.22	0.79	**=0.006**	*
	PS	2+0	Loc_E	1.3 ± 0.12	0.77	**=0.009**	*
S_W	AD	2+2.	Loc_A	6.6 ± 0.84	0.95	**<0.001**	*
	AD	2+2.	Loc_B	6.5 ± 0.58	0.76	**=0.011**	*
	AD	2+2.	Loc_C	5.9 ± 0.59	0.83	**=0.003**	*
	AD	2+2.	Loc_D	4.4 ± 0.70	0.91	**<0.001**	*
	AD	2+2.	Loc_E	3.6 ± 0.49	0.84	**=0.002**	*
	PS	1+0	Loc_A	1.7 ± 0.20	0.56	=0.092	ns
	PS	1+0	Loc_E	1.0 ± 0.17	0.74	**=0.014**	*
	PS	2+0	Loc_A	1.7 ± 0.17	0.64	**=0.044**	*
	PS	2+0	Loc_E	1.0 ± 0.12	0.80	**=0.006**	*
S_H	AD	2+2.	Loc_A	5.3 ± 0.56	0.41	=0.238	ns
	AD	2+2.	Loc_B	5.1 ± 0.63	0.81	**=0.005**	*
	AD	2+2.	Loc_C	4.8 ± 0.63	0.85	**=0.002**	*
	AD	2+2.	Loc_D	3.6 ± 0.57	0.71	**=0.022**	*
	AD	2+2.	Loc_E	3.5 ± 0.39	0.78	**=0.008**	*

Variable†; L_F (fork length), S_A (area), S_{Per} (perimeter), S_W (width), S_H (height). Refer to Table 2.1. **Stage**‡; post-smolt (PS), adult (AD). **Loc**§; (locations). Refer to Figure 2.1. **S. level**^; (significance level) ;<0.05; *, ns; no significance.

Table 2.2 (b). Scale shape measurements for post-smolt and adult Atlantic salmon.

Variable†	Stage‡	Age	Loc§	Mean ± SD	Regression with L_F†		
					r	*p*	S.level^
	PS	1+0	Loc_A	0.77 ± 0.019	0.61	=0.064	ns
	PS	1+0	Loc_E	0.78 ± 0.024	0.45	=0.187	ns
	PS	2+0	Loc_A	0.78 ± 0.016	0.63	=0.053	ns
	PS	2+0	Loc_E	0.79 ± 0.018	0.013	=0.971	ns
S_{Cir}	AD	2+2.	Loc_A	0.79 ± 0.029	0.72	**=0.019**	*
	AD	2+2.	Loc_B	0.81 ± 0.031	0.68	**=0.030**	*
	AD	2+2.	Loc_C	0.80 ± 0.019	0.42	=0.231	ns
	AD	2+2.	Loc_D	0.79 ± 0.021	0.004	=0.991	ns
	AD	2+2.	Loc_E	0.79 ± 0.024	-0.077	=0.833	ns
	PS	1+0	Loc_A	1.4 ± 0.092	-0.21	=0.554	ns
	PS	1+0	Loc_E	1.3 ± 0.055	-0.58	=0.078	ns
	PS	2+0	Loc_A	1.4 ± 0.063	-0.11	=0.772	ns
	PS	2+0	Loc_E	1.2 ± 0.064	-0.28	=0.437	ns
S_{Ar}	AD	2+2.	Loc_A	1.5 ± 0.057	-0.19	=0.593	ns
	AD	2+2.	Loc_B	1.4 ± 0.067	-0.70	**=0.024**	*
	AD	2+2.	Loc_C	1.4 ± 0.059	-0.096	=0.792	ns
	AD	2+2.	Loc_D	1.3 ± 0.060	-0.076	=0.834	ns
	AD	2+2.	Loc_E	1.1 ± 0.044	-0.38	=0.278	ns
	PS	1+0	Loc_A	0.71 ± 0.047	0.23	=0.525	ns
	PS	1+0	Loc_E	0.78 ± 0.034	0.59	=0.074	ns
	PS	2+0	Loc_A	0.72 ± 0.031	0.10	=0.789	ns
	PS	2+0	Loc_E	0.84 ± 0.044	0.29	=0.415	ns
S_{Rn}	AD	2+2.	Loc_A	0.68 ± 0.025	0.16	=0.651	ns
	AD	2+2.	Loc_B	0.74 ± 0.038	0.71	**=0.021**	*
	AD	2+2.	Loc_C	0.73 ± 0.031	0.064	=0.860	ns
	AD	2+2.	Loc_D	0.77 ± 0.035	0.065	=0.859	ns
	AD	2+2.	Loc_E	0.90 ± 0.037	0.39	=0.257	ns
	PS	1+0	Loc_A	0.98 ± 0.010	0.48	=0.159	ns
	PS	1+0	Loc_E	0.98 ± 0.0094	-0.027	=0.940	ns
	PS	2+0	Loc_A	0.98 ± 0.0079	0.44	=0.204	ns
	PS	2+0	Loc_E	0.98 ± 0.004	-0.011	=0.976	ns
S_{Sol}	AD	2+2.	Loc_A	0.98 ± 0.014	0.53	=0.113	ns
	AD	2+2.	Loc_B	0.98 ± 0.008	0.38	=0.284	ns
	AD	2+2.	Loc_C	0.98 ± 0.010	0.086	=0.810	ns
	AD	2+2.	Loc_D	0.98 ± 0.008	-0.18	=0.613	ns
	AD	2+2.	Loc_E	0.97 ± 0.013	-0.11	=0.772	ns

Variable†; L_F (fork length), S_{Cir} (circularity), S_{Ar} (aspect ratio), S_{Rn} (roundness), S_{Sol} (solidity). Refer to Table 2.1. **Stage**‡; PS (post-smolt), AD (adult). **Loc**§; (locations). Refer to Fig. 2.1. **S. level**^; (significance level); <0.05; *, ns; no significance.

Table 2.3. Scale growth measurements for post-smolt and adult Atlantic salmon.

Variable*	Stage†	Age	Loc‡	Mean	±	SD
G_{FW}	PS	1+0	Loc_A	0.46	±	0.12
	PS	1+0	Loc_E	0.21	±	0.059
	PS	2+0	Loc_A	0.69	±	0.15
	PS	2+0	Loc_E	0.39	±	0.11
	AD	2+2.	Loc_A	0.91	±	0.16
	AD	2+2.	Loc_B	0.99	±	0.16
	AD	2+2.	Loc_C	1.0	±	0.10
	AD	2+2.	Loc_D	0.53	±	0.14
	AD	2+2.	Loc_E	0.47	±	0.080
C_{FW}	PS	1+0	Loc_A	18.4	±	4.4
	PS	1+0	Loc_E	7.5	±	2.1
	PS	2+0	Loc_A	27.3	±	4.9
	PS	2+0	Loc_E	14.5	±	3.8
G_M	PS	1+0	Loc_A	0.91	±	0.15
	PS	1+0	Loc_E	0.67	±	0.11
	PS	2+0	Loc_A	0.74	±	0.15
	PS	2+0	Loc_E	0.59	±	0.11
	AD	2+2.	Loc_A	3.5	±	0.64
	AD	2+2.	Loc_B	3.9	±	0.69
	AD	2+2.	Loc_C	3.4	±	0.59
	AD	2+2.	Loc_D	2.8	±	0.50
	AD	2+2.	Loc_E	2.7	±	0.43
C_M	PS	1+0	Loc_A	19.7	±	2.6
	PS	1+0	Loc_E	16.7	±	2.0
	PS	2+0	Loc_A	15.1	±	2.9
	PS	2+0	Loc_E	13.1	±	2.5
	AD	2+2.	Loc_A	66.7	±	11.8
	AD	2+2.	Loc_B	68.0	±	10.8
	AD	2+2.	Loc_C	65.5	±	9.1
	AD	2+2.	Loc_D	52.1	±	8.8
	AD	2+2.	Loc_E	49.8	±	6.1
R_S	PS	1+0	Loc_A	1.4	±	0.22
	PS	1+0	Loc_E	0.88	±	0.14
	PS	2+0	Loc_A	1.4	±	0.18
	PS	2+0	Loc_E	0.98	±	0.13
	AD	2+2.	Loc_A	4.5	±	0.74
	AD	2+2.	Loc_B	4.8	±	0.78
	AD	2+2.	Loc_C	4.4	±	0.63
	AD	2+2.	Loc_D	3.3	±	0.61
	AD	2+2.	Loc_E	3.2	±	0.47

Variable*; *GFW* (freshwater growth), C_{FW} (freshwater circuli number), G_M (marine growth), C_M (marine circuli number), R_S (scale radius). **Stage**†; PS (post-smolt), AD (adult). **Loc**‡; (locations). Refer to Fig. 2.1.

Table 2.4. Comparisons of measurements for post-smolt and adult Atlantic salmon.

Parameter			Size‡ *p*			
Stage*	**Age**	**Loc†**	S_A	S_{Per}	S_W	S_H
PS	1+0	Loc_A, Loc_E	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PS	2+0	Loc_A, Loc_E	**<0.001**	**<0.001**	**<0.001**	**<0.001**
AD	2+2.	Loc_A, Loc_B	=0.882	=0.548	=0.865	=0.364
AD	2+2.	Loc_A, Loc_C	**=0.002**	**<0.001**	**=0.003**	**=0.027**
AD	2+2.	Loc_A, Loc_D	**<0.001**	**<0.001**	**<0.001**	**<0.001**
AD	2+2.	Loc_A, Loc_E	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Parameter			Shape‡ *p*			
Stage*	**Age**	**Loc†**	S_{Cir}	S_{Ar}	S_{Rn}	S_{Sol}
PS	1+0	Loc_A, Loc_E	=0.738	**0.001**	**<0.001**	=0.873
PS	2+0	Loc_A, Loc_E	=0.638	**<0.001**	**<0.001**	=0.728
AD	2+2.	Loc_A, Loc_B	**=0.028**	**<0.001**	**=0.001**	=0.095
AD	2+2.	Loc_A, Loc_C	=0.110	**<0.001**	**=0.003**	=0.120
AD	2+2.	Loc_A, Loc_D	=0.758	**<0.001**	**<0.001**	=0.573
AD	2+2.	Loc_A, Loc_E	=0.431	**<0.001**	**<0.001**	=0.828

Parameter			Growth‡ *p*				
Stage*	**Age**	**Loc†**	G_{FW}	C_{FW}	G_M	C_M	R_S
PS	1+0	Loc_A, Loc_E	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
PS	2+0	Loc_A, Loc_E	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
AD	2+2.	Loc_A, Loc_B	**=0.010**	**-**	**=0.001**	=0.231	**=0.001**
AD	2+2.	Loc_A, Loc_C	**=0.014**	-	=0.081	=0.313	=0.645
AD	2+2.	Loc_A, Loc_D	**<0.001**	-	**<0.001**	**<0.001**	**<0.001**
AD	2+2.	Loc_A, Loc_E	**<0.001**	-	**<0.001**	**<0.001**	**<0.001**

*Stage; PS (post-smolt), AD (adult). †Loc (locations). Refer to Fig. 1. ‡Variable; G_{FW} (freshwater growth), C_{FW} (freshwater circuli number), G_M (marine growth), C_M (marine circuli number), R_S (scale radius). Size; S_A (area), S_{Per} (perimeter), S_W (width), S_H (height). Refer to Table I. Shape; S_{Cir} (circularity), S_{Ar} (aspect ratio), S_{Rn} (roundness), S_{Sol} (solidity). Refer to Table 2.1.

Table 2.5. Regression between fork length (L_F; mm) and size measurements S_A (area; mm^2) and S_{Per} (perimeter; mm) at Loc_A for post-smolt and Loc_A to Loc_C for adult Atlantic salmon.

Variable*	Stage	Age	Regression Equation	R^2	p
S_A	PS	1+0	Loc_A = - 3.867 + (0.03077(L_F))	0.80	**<0.001**
	PS	2+0	Loc_A = - 2.113 + (0.02068(L_F))	0.74	**=0.014**
	AD	2+2.	Loc_A = - 32.25 + (0.08387(L_F))	0.95	**=0.001**
	AD	2+2.	Loc_B = - 22.74 + (0.06991(L_F))	0.94	**=0.001**
	AD	2+2.	Loc_C = - 18.23 + (0.05792(L_F))	0.90	**<0.001**
S_{Per}	PS	1+0	Loc_A = - 0.966 + (0.03533(L_F))	0.80	**<0.001**
	PS	2+0	Loc_A = 0.672 + (0.02521(L_F))	0.75	**=0.013**
	AD	2+2.	Loc_A = - 0.194 + (0.02928(L_F))	0.95	**<0.001**
	AD	2+2.	Loc_B = 3.493 + (0.02352(L_F))	0.90	**<0.001**
	AD	2+2.	Loc_C = 4.706 + (0.02678(L_F))	0.91	**<0.001**

*Stage; PS (post-smolt), AD (adult). Loc_A, Loc_B, Loc_C (location A) Refer to Fig. 1.

Table 2.6. Regression of growth measurements from Loc_A compared with the equivalent measurements from the other body locations for post-smolt and adult Atlantic salmon.

Variable†	Stage‡	Age	Regression Equation	R^2	p
G_{FW}	PS	1+0	$Loc_A = 0.196 + (1.23(Loc_E))$	0.38	**<0.001**
	PS	2+0	$Loc_A = 0.320 + (0.959(Loc_E))$	0.51	**<0.001**
	AD	2+2.	$Loc_A = 0.011 + (0.908(Loc_B))$	0.78	**=0.001**
	AD	2+2.	$Loc_A = - 0.502 + (1.41(Loc_C))$	0.70	**=0.002**
	AD	2+2.	$Loc_A = 0.414 + (0.930(Loc_D))$	0.67	**=0.004**
	AD	2+2.	$Loc_A = 0.138 + (1.64(Loc_E))$	0.62	**=0.007**
C_{FW}	PS	1+0	$Loc_A = 10.8 + (1.02(Loc_E))$	0.24	**=0.001**
	PS	2+0	$Loc_A = 14.4 + (0.895(Loc_E))$	0.47	**<0.001**
G_M	PS	1+0	$Loc_A = 0.279 + (0.934(Loc_E))$	0.44	**<0.001**
	PS	2+0	$Loc_A = 0.109 + (1.07(Loc_E))$	0.64	**<0.001**
	AD	2+2.	$Loc_A = 0.120 + (0.887(Loc_B))$	0.92	**<0.001**
	AD	2+2.	$Loc_A = 0.081 + (1.01(Loc_C))$	0.89	**<0.001**
	AD	2+2.	$Loc_A = 0.840 + (0.963(Loc_D))$	0.57	**=0.011**
	AD	2+2.	$Loc_A = 0.024 + (1.28(Loc_E))$	0.74	**=0.001**
C_M	PS	1+0	$Loc_A = 3.90 + (0.943(Loc_E))$	0.53	**<0.001**
	PS	2+0	$Loc_A = 1.50 + (1.04(Loc_E))$	0.76	**<0.001**
	AD	2+2.	$Loc_A = - 4.73 + (1.05(Loc_B))$	0.93	**<0.001**
	AD	2+2.	$Loc_A = - 15.8 + (1.26(Loc_C))$	0.95	**<0.001**
	AD	2+2.	$Loc_A = 12.2 + (1.05(Loc_D))$	0.60	**=0.008**
	AD	2+2.	$Loc_A = - 16.4 + (1.67(Loc_E))$	0.76	**=0.001**
R_S	PS	1+0	$Loc_A = 0.570 + (0.898(Loc_E))$	0.33	**<0.001**
	PS	2+0	$Loc_A = 0.705 + (0.746(Loc_E))$	0.29	**<0.001**
	AD	2+2.	$Loc_A = 0.054 + (0.907(Loc_B))$	0.91	**<0.001**
	AD	2+2.	$Loc_A = - 0.404 + (1.10(Loc_C))$	0.88	**<0.001**
	AD	2+2.	$Loc_A = 1.39 + (0.917(Loc_D))$	0.58	**=0.011**
	AD	2+2.	$Loc_A = 0.056 + (1.37(Loc_E))$	0.76	**=0.001**

Variable†; G_{FW} (freshwater growth), C_{FW} (freshwater circuli number), G_M (marine growth), C_M (marine circuli number), R_S (scale radius). **Stage**‡; PS (post-smolt), AD (adult).

Chapter 3.

Experimental investigation on the effects of temperature and feeding regime on post-smolt scale growth, circuli deposition rates and circulus spacing in Atlantic salmon (*Salmo salar* L.).

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Thomas, K., Hansen, T., Brophy, D., Ó Maoiléidigh, N. and Fjelldal, P.G. Experimental investigation on the effects of temperature and feeding regime on post-smolt scale growth, circuli deposition rates and circulus spacing in Atlantic salmon (*Salmo salar* L.).

3.1 Abstract

Proxy values of scale circuli deposition rates are used to estimate growth of Atlantic salmon (*Salmo salar* L.) over time; however, the periodicity of circuli deposition rates have never been experimentally validated. Atlantic salmon post-smolts were reared in seawater in a controlled laboratory experiment for 12 weeks following fluorescent marking. Fish were exposed to one of three constant temperature treatments (15 °C, 10.5 °C and 6 °C) and one of two feeding treatments [constant feeding or interrupted feeding (starvation period over a 14-day block)]. Across all treatments, scale growth rates were proportional to somatic growth rates which justifies the use of scale growth measurements as a proxy of growth. Circuli deposition rate was mostly proportional to somatic growth and was dependant on temperature and feeding regime; at 15 °C circuli deposition rates surpassed the growth rate causing a decoupling effect between the circuli deposition rate and somatic growth. Circuli deposition rates contrasted from 4.8 d circulus $^{-1}$ at 15 °C (constant feeding) to 15.1 d circulus $^{-1}$ at 6 °C (interrupted feeding). When time was expressed relative to cumulative degree day, no differences were detected between the 15 °C and 10.5 °C temperature treatments, this suggested that cumulative degree day was a better predictor of circuli deposition rate than time expressed as day. Circuli spacing did not reflect growth rate; narrow spaced circuli occurred during periods of starvation at 6 °C but also during periods of high growth associated with 15 °C.

3.2 Introduction

Over the last three decades, Atlantic salmon (*Salmo salar* L.) has declined over most of its range, despite reductions in fishing pressure and measures to protect critical habitats (Friedland *et al*., 2009). In the European stock complex, the decline was more pronounced in southern populations compared to northern populations (Parrish *et al*., 1998; Potter *et al*., 2004; Chaput, 2012; Jensen *et al*., 2012; Mills *et al*., 2013). Various changes in oceanic conditions in the Northern Atlantic are thought to contribute to declines in survival including ocean warming and sea surface temperature (SST) fluctuations as well as reduced food availability and the northerly shift of prey species (Reddin and Friedland, 1993; Friedland *et al.*, 1998; Beaugrand and Reid, 2003; Rikardsen *et al*., 2004; Reddin *et al*., 2011; Jensen *et al*., 2012).

In Atlantic salmon the seaward migration from natal rivers occurs during spring, and is initiated progressively later at increasing latitudes (Jensen and Johnsen, 1982; Otero *et al*., 2014). In the productive marine environment, salmon undergo rapid and excessive growth (Gross, 1987; Økland *et al*., 1993; Dietrich and Cunjak, 2007). However, mortality rates are high during the period of initial sea migration and the subsequent few months of marine habitation (Thorpe, 1994; Jacobsen and Hansen, 2000; MacLean *et al*., 2000; Sturlaugsson, 2000; Rikardsen *et al*., 2004; Davidsen *et al*., 2009; Strand *et al*., 2011). Several field investigations have focused on marine growth, ecology and feeding of Atlantic salmon during this critical period (Jacobsen and Hansen, 2000; Haugland *et al*., 2006; Jensen *et al*., 2012 and Mork *et al*., 2012 and Anonymous, 2012). These studies provide evidence that survival and recruitment

of European salmon is linked to ocean climate, feeding and post-smolt growth (Peyronnet *et al*., 2007; McCarthy *et al*., 2008; Todd *et al*., 2008; and Friedland *et al*., 2000,2009). It has been hypothesised that faster growth during the post-smolt period leads to lower overall mortality which in turn results in a higher adult return rate (Friedland *et al*., 2009).

Analyses of growth marks in scales are widely used to indirectly assess and monitor temporal changes in growth. Scales form and grow incrementally at a rate proportional to somatic growth (Panfili *et al*., 2002). The entire life history of an individual fish is recorded as concentric rings referred to as circuli. The time a fish spends in both freshwater and marine environments and how both environments are utilised, is engraved in the growth patterns and spacing between these circuli, making it feasible to reconstruct individual growth histories (Dahl, 1911; Anonymous, 1984). In Atlantic salmon, many retrospective growth studies have linked post-smolt growth rates to survival, recruitment and ocean climate (Reddin and Shearer, 1987; Friedland *et al*., 1993, 1998; Jonsson and Jonsson, 2004; Todd *et al*., 2008).

Field observations suggest that in Atlantic salmon, circuli are deposited at a rate of 1 every 6.3 days (Jensen *et al*., 2012). Therefore, measurements of scale circuli can potentially be used to reconstruct past growth histories with high temporal resolution. Linking these estimations with environmental data, can help to identify drivers of change in growth and detect when marked changes in growth rate have occurred. However, the periodicity of circuli formation has never been experimentally validated.

The rate and nature of circuli deposition may vary with temperature and feeding conditions making it difficult to compare results across populations and to interpret temporal change.

The objective of this study was to investigate the effect of water temperature and feeding regime on the formation of circuli in the scales of Atlantic salmon post-smolts marked by the fluorochrome dye – Calcein, upon experiment commencement and reared under controlled experimental conditions. By validating the periodicity of circuli formation and relating scale growth rates to rearing conditions this study seeks to inform interpretations of growth signatures in scales of wild Atlantic salmon in relation to changes in the marine environment.

3.3 Methods

All experimental work using Atlantic salmon was conducted ethically and in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway, following the Norwegian Regulation on Animal Experimentation 1996. This experiment was conducted at the Institute of Marine Research (IMR) Matre research station in Matredal Norway (60^{o} N) and ran for a duration of twelve weeks from the 22^{nd} of May 2013 to the 14^{th} of August 2013.

One-year-old Atlantic salmon smolts of the same Norwegian hatchery strain (Aqua Gen AS, Trondheim, Norway) reared at an ambient freshwater temperature of 6 °C were used for this experiment.

3.3.1 Smolt marking

Prior to the commencement of the experiment, 756 fish [Fork length = 185 ± 12.0 mm (mean ± standard deviation (SD)) and weight = 60.8 ± 11.02 g (mean ± standard deviation (SD))] were starved for 24 hours before being marked by calcein, a fluorochrome dye (wavelength: excitation/emission 495/515 nm) by means of osmotic induction using the Mohler method (Mohler, 2003). A 5% salt solution was prepared by adding non-iodized NaCl to 3.5% saline tank water. A 1% calcein solution was made up by adding calcein powder to freshwater. Sodium bicarbonate was added to this solution until the calcein powder was fully dissolved. The fish were removed from the holding tank using a hand net and contained within the net until the procedure was complete. Subsequently the net was immersed in the saline bath for 3.5 minutes to begin the osmotic process, and then dipped in a bath of freshwater and gently shaken to remove excess salt. Finally, the net was immersed in the calcein bath for a further 3.5 minutes. At this point, 36 smolts were sacrificed, in order to verify that the marking method was effective. The remaining 720 fish (hereafter referred to as post-smolt) were transferred to the experimental unit and randomly divided between experimental seawater tanks.

3.3.2 Experimental design

Experimental fish were held in 1 X 1 m closed marine tanks at three temperatures: 15 °C, 10.5 °C and 6 °C. To reduce potential thermal stress/shock and mortality, the water temperatures in the 10.5 °C and 15 °C treatments were gradually increased over a period of 48 and 96 hours, respectively. After thermal acclimation, temperatures were

held constant throughout the experiment and were automatically controlled throughout. Thermal sensors alerted within one minute if a fluctuation of ± 1 °C occurred. The experimental temperatures (15 °C, 10.5 °C and 6 °C) were chosen with reference to sea surface temperature (SST) profiles from the SALSEA Merge research surveys (NASCO, 2012). The highest catches of post-smolts occurred within a temperature range of 9 °C to 12 °C. Therefore, 10.5 °C was chosen to represent the mid-range of the temperatures that post-smolts are exposed to during migration and initial habitation within nursery grounds in the wild marine environment. The other two temperatures 15 °C and 6 °C were chosen to investigate the effect of exposure to temperatures above and below the normal range, on scale growth. Four tanks were held at each experimental temperature treatment.

The photoperiod used in the experiment [(L.D; 24:0) twenty-four-hours daylight] corresponded to the light conditions in the Norwegian Sea during the month of May. Two 18W fluorescent daylight tubes (OSRAM L 18 W/840 LUMILUX, OSRAM GmbH, Augsburg, Germany) mounted under water in the tank center, were used to produce 960 LUX of constant light. The fish were fed to excess on a commercial dry salmon feed (Nutra Olympic, Skretting AS, Averøy, Norway) using automated revolving feeders (ARVO-TEC T Drum 2000, Arvotec, Huutokoski, Finland) attached to the lid. Feeders were set to dispense food for one second followed by a brief pause, the length of the pause depending on the increasing food requirement of the growing fish; i.e. at week 12 over a 24-hour period, the feeders dispersed food, 369 times with a pause of 233 seconds, between each feeding revolution. The fish in two of the four

tanks were exposed to a constant feeding regime over the duration of the experiment, in the other two tanks an interrupted feeding regime was used i.e. fish were starved for 14 -days from the start of week 7 to the end of week 8. The photoperiod and feeders were controlled automatically by electronic software (Normatic AS, Norfjordeid, Norway).

3.3.3 Post-smolt sampling

Sampling was conducted at the same time (09:00) each week. Three fish were randomly selected and removed from each tank using a hand net and placed in individual containers containing a lethal dose of the anaesthetic 2-Phenoxyethanol solution (0.6 ml / l). Individual fork lengths (mm) and weights (g) were recorded and fish fins, eyes and the operculum were physically inspected and checked for signs of erosion and cannibalism. Scales were then removed from the recommended standard location (i.e. three to five rows above the lateral line, diagonally from the posterior edge of the dorsal fin to the anterior edge of the pelvic fin on the left side of the body) (Anonymous, 1984) and stored in pre labeled envelopes.

3.3.4 Scale analysis

Post-smolt scales were wet mounted on glass slides, between a cover glass and viewed using a Leica DMRE fluorescent compound microscope. An I3 filter was used to excite the calcein mark at 495/515 nm. A mercury light box transmitted blue light through the scale to produce a brilliant green mark in the location of the calcein (Figure 3.1). Images were captured using Image Pro Plus version 7.01 © software. Scale

measurements were taken along a 360° axis in a straight line transect from the centre of the scale focus to the edge. The distances from the focus to the calcein mark (freshwater growth, mm) and from the end of the calcein mark to the scale edge (marine growth, mm) were measured. The circuli within the marine portion of the scale were counted (marine circuli number) and the spacing between each circuli enumerated (circulus spacing, mm) (Figure 3.1).

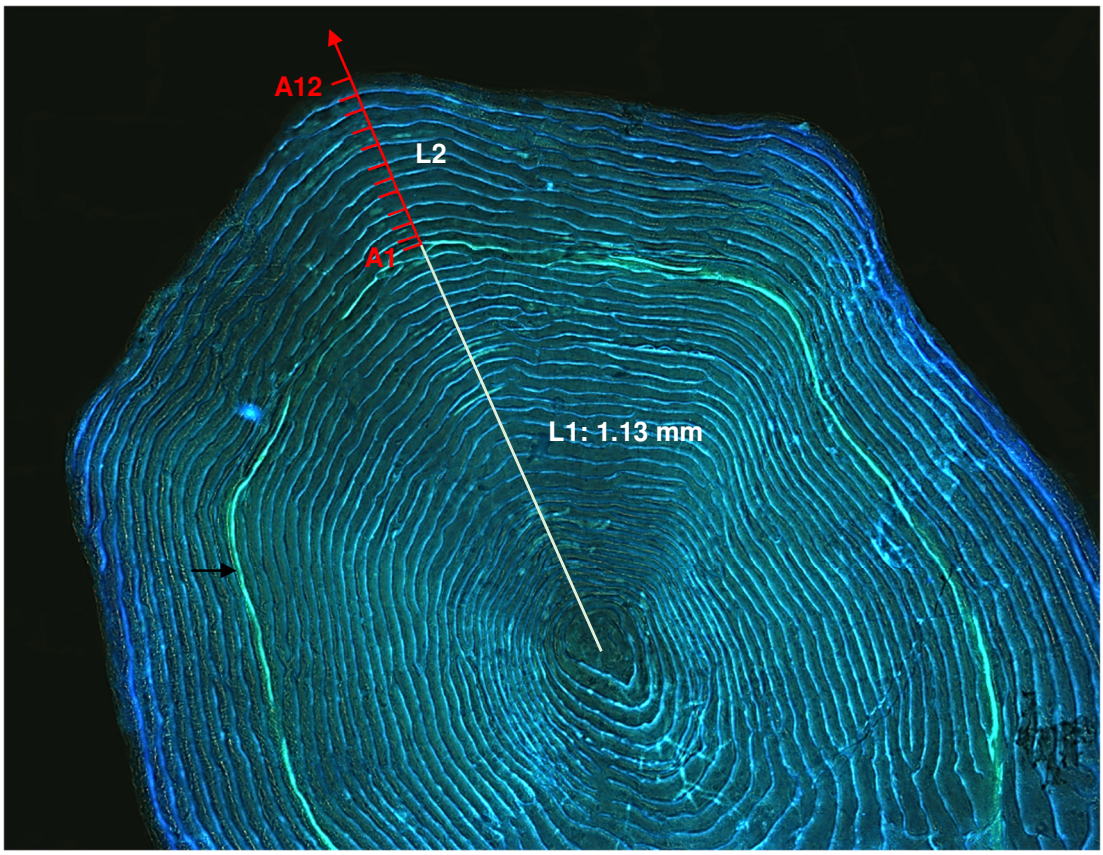

Figure 3.1. Image of a post-smolt scale acquired using fluorescent microscopy, clearly showing the calcein mark (arrow). The 360º straight line axis used when obtaining measurements, coupled with the freshwater transect (L1; length, mm) and marine transect (L2; A1-A12); circuli number and circuli spacing) are illustrated.

3.3.5 Statistical analysis

The analysis was conducted in two stages. Firstly, the effect of temperature on fish growth and scale growth was investigated by comparing fork length and scale measurements between the three temperature treatments (15 °C, 10.5 °C and 6 °C) that received constant feeding. In the second stage, the effect of a short period of starvation on scale growth was investigated by comparing fork length and scale measurements between the constant and interrupted feeding treatments at the each of the three temperatures. Fork length, freshwater growth, marine growth, circulus spacing and scale radius were compared between treatments using a series of nested ANCOVAs. Freshwater scale growth measurements were compared between treatments to confirm that there were no pre-existing differences in growth that could bias the subsequent marine growth analyses. Treatment was included as the fixed factor and time as the co-variate. For the comparison of fish and scale growth between temperature treatments, time was expressed in two ways: firstly, as week number and secondly as cumulative degree days (CDD). CDD was calculated as follows:

Equation 3.1

$$\sum_{i=1}^{n} \frac{MaxT_i + MinT_i}{2}$$

Where $MaxT_i$ and $MinT_i$ are the maximum and minimum temperatures recorded on day *i,* respectively and n is the duration of the experiment at the time of scale sampling.

For the comparison of feeding treatments only the variable week number was used as the covariate.

Tanks were nested within treatments. If there was no significant difference in growth between tanks within a treatment, data for replicate tanks were pooled and the analysis was re-run. Marine circulus deposition rate (CDR_{Day}) was calculated by dividing the day number at time of sampling by the number of circuli after the calcein mark on the scale. For the comparison of temperature treatments, marine circulus deposition rate was also expressed relative to degree day by dividing CDD at the time of sampling by the number of circuli after the calcein mark on the scale. This variable is referred to as marine circulus degree day deposition rate (CDR_{CDD}). Circuli deposition rates were compared between treatments using Kruskal-Wallis tests were applied when variables were either non-normally distributed and/or displayed unequal variances) Mann-Whitney post-hoc tests were then conducted. The relationship between circulus spacing and circuli number was compared between treatments using a series of repeated measure ANOVAs. Treatment was included as a fixed factors and fish ID as a random factor and circuli number as the co-variate.

All statistical analysis was conducted using the MINITAB statistical package. An alpha level of 0.05 was used for all significance tests.

3.4 Results

The mortality rate was monitored throughout the experiment. A mortality rate of 2.9% was recorded within the initial 24-hours of the experiment. After the initial day, the

mortality rate was negligible throughout the remainder of the experiment (Table 3.1). Scale growth measurements for each treatment are summarised in Table 3.2. ANCOVA confirmed that there were no differences in freshwater growth between any of the temperature or feeding treatments (p=0.734), therefore, there were no pre-existing differences in growth that could bias comparisons of marine growth and circuli deposition rates.

3.4.1 Effect of temperature on scale growth

3.4.1.1 Marine growth

Marine growth measurements [mean ± standard deviation (SD) mm] recorded in the scales at week 12 were highest in the 15 °C temperature treatment (0.59 ± 0.074) followed by 10.5 °C (0.42 ± 0.065) and 6 °C (0.22 ± 0.036). The rate at which scale size increased during the course of the experiment varied between the three temperature treatments [Figure 3.2 (a)]. The ANCOVA confirmed that the slope of the relationship between marine growth and week number was significantly different between treatments [ANCOVA, $p<0.001$; Table 3.3 (a)]. Linear regressions were derived to describe the relationship between marine growth (y) and week (x) at each temperature treatment (Table 3.4). This showed that scale growth rates increased with temperature with average growth rates of 0.0071 mm d^{-1}, 0.0058 mm d^{-1} and 0.0025 mm d^{-1} at temperatures 15 °C, 10.5 °C and 6 °C, respectively. When marine growth was plotted against CDD the difference between temperature treatments was much less marked [Figure 3.2 (b)]. However, a significant difference in the slope of the relationship between marine growth and CDD was detected [ANCOVA, $p<0.001$;

Table 3.3 (b)]. Post-hoc pairwise comparisons confirmed that no significant was found between the 15 °C and 6 °C treatments (p=0.123) or between the 15 °C and 10.5 °C treatments (p=0.052). The 10.5 °C treatment significantly differed to 6 °C temperature treatment (p=0.006). Linear regressions were derived to describe the relationship between marine growth (y) and CDD (x) and at each temperature treatment (Table 3.4). The rate at which the size of the scale increased with degree day was greatest at 10.5 °C, followed by 15 °C and 6 °C with growth rates of 0.00055 mm cdd^{-1} ,0.00048 mm cdd^{-1} and 0.00041 mm cdd^{-1}, respectively.

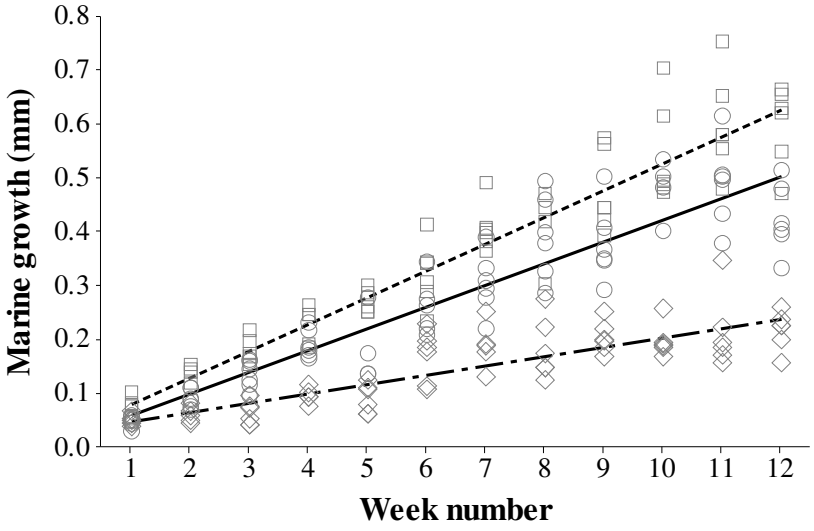

Figure 3.2 (a)

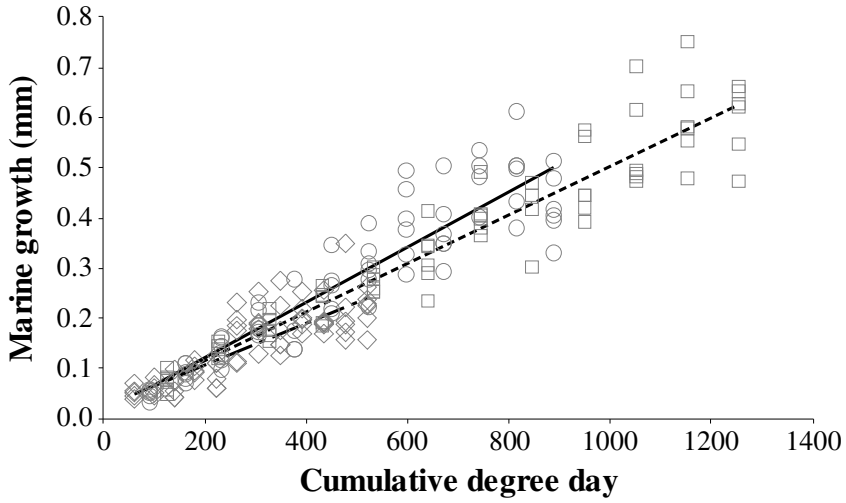

Figure 3.2 (b)

Figure 3.2 (a, b). (a) Marine growth (mm) per temperature treatment by time; weeks (b) Marine growth (mm) per temperature treatment by time; cumulative degree day (CDD); [F_C (constant feeding); □ - - - - ,15 °C (F_C); ○ —— ,10.5 °C (F_C); ◇ — - ,6 °C (F_C)].

3.4.1.2 Marine circuli number

The rate of circuli deposition increased with temperature; the numbers [mean ± standard deviation (SD)] of circuli recorded in the scales at week 12 were 16.8 ± 1.7, 10.8 ± 0.98 and 6.2 ± 0.75 at 15 °C, 10.5 °C and 6 °C, respectively [Figure 3.3 (a)]. CDR_{Day} was significantly different between the three temperature treatments (Kruskal-Wallis, $p<0.001$) [Figure 3.3 (c); Table 3.2]. CDR_{CDD} showed less variation between the three temperature treatments [Figure 3.3 (d)]. However, a significant difference was detected between the three temperature treatments (Kruskal-Wallis, $p<0.05$). Mann-Whitney post-hoc tests confirmed that CDR_{CDD} at 6 °C was significantly higher than the 10.5 °C ($p=0.024$; Table 3.2) and 15 °C treatments ($p=0.008$; Table 3.2). There was no difference in CDR_{CDD} between the 10.5 °C and 15 °C treatments ($p=0.553$; Table 3.2).

The relationship between week/day (x) and circuli number (y) was described by linear regression [Figure 3.3 (a); Table 3.4]. Circuli were deposited at a rate of 0.20 circulus d^{-1}, or 5.1 d circulus $^{-1}$ at 15 °C; 0.13 circulus d^{-1}, or 7.8 d circulus $^{-1}$ at 10.5 °C and 0.06 circulus d^{-1}, or 16.2 d circulus $^{-1}$ at 6 °C. The relationship between degree day (x) and circuli number (y) was also described by linear regression [Figure 3.3 (b); Table 3.4]. The rate of circuli deposition was established as 75.2 cdd circulus $^{-1}$ at 15 °C; 80.6 cdd circulus $^{-1}$ at 10.5 °C; and 97.0 cdd circulus $^{-1}$ at 6 °C.

3.4.1.3 Marine circulus spacing

Circulus spacing [mean ± standard deviation (SD) mm] over the 12-week period was widest at 10.5 °C (0.040 ± 0.0.0074) followed by 6 °C (0.039 ± 0.0075) and 15 °C (0.037 ± 0.0050), respectively [Figure 3.3 (e); Table 3.2].

In all three temperature treatments, circulus spacing increased slightly at the start of the experiment. At 10.5 °C and 15 °C circulus spacing remained steady during the middle of the experiment and narrowed towards the end. At 6 °C the circulus spacing measurements fell steadily from circulus three onwards. During the middle of the experiment the circuli in scales from the 10.5 °C treatment appeared wider than the corresponding circuli from the other treatments [Figure 3.3 (e)]. The ANCOVA confirmed that the slope of the circulus spacing/circulus number relationship was significantly different between temperatures (ANCOVA, temperature*circulus number, p=0.003). The main temperature effect was not significant [p=0.450; Table 3.3 (a)]. Post-hoc pairwise comparisons confirmed that no significant difference was found between circulus spacing in the 10.5 °C and 6 °C treatment (p=0.084) or between the circulus spacing at 15 °C and 6 °C (p=0.365). A significant difference was detected between the 15 °C and 10.5 °C circulus spacings measurements (p=0.004).

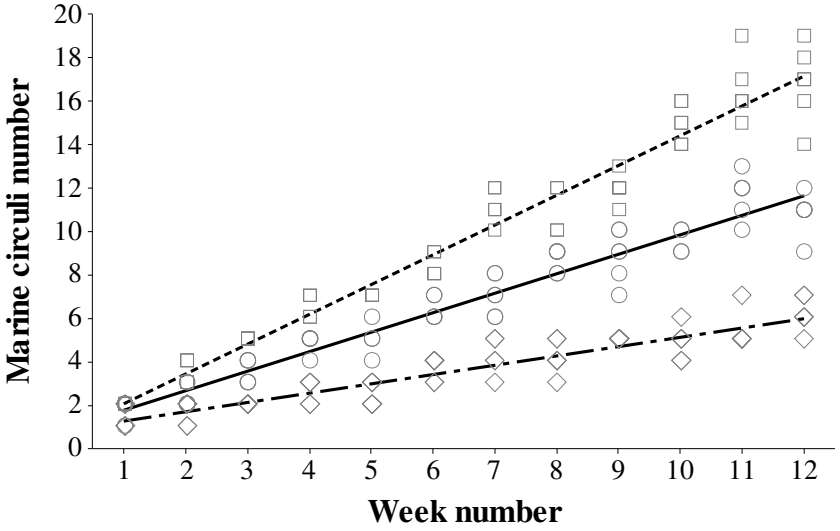

Figure 3.3 (a)

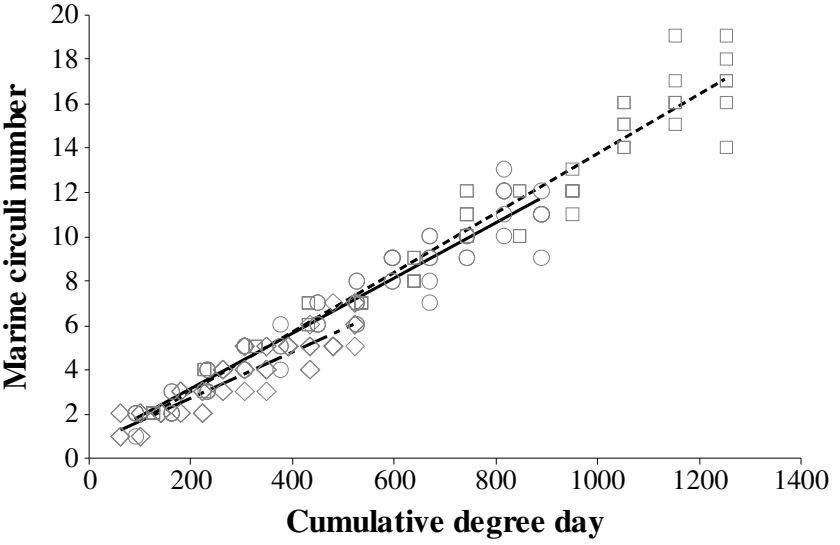

Figure 3.3 (b)

Figure 3.3 (a, b). (a) Marine circuli number per temperature treatment by time; weeks (b) Marine circuli number per temperature treatment by time; cumulative degree day; [F_C (constant feeding); □ - - - - ,15 °C (F_C); ○ —— ,10.5 °C (F_C); ◇ — - ,6 °C (F_C)].

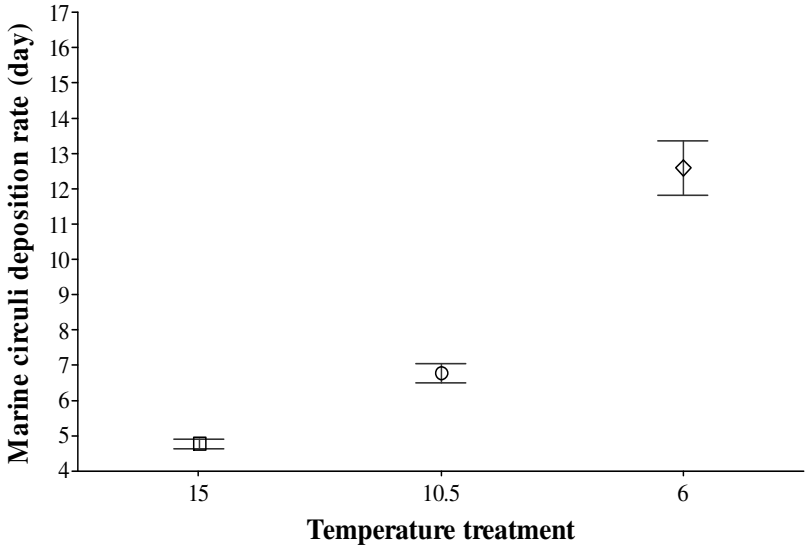

Figure 3.3 (c)

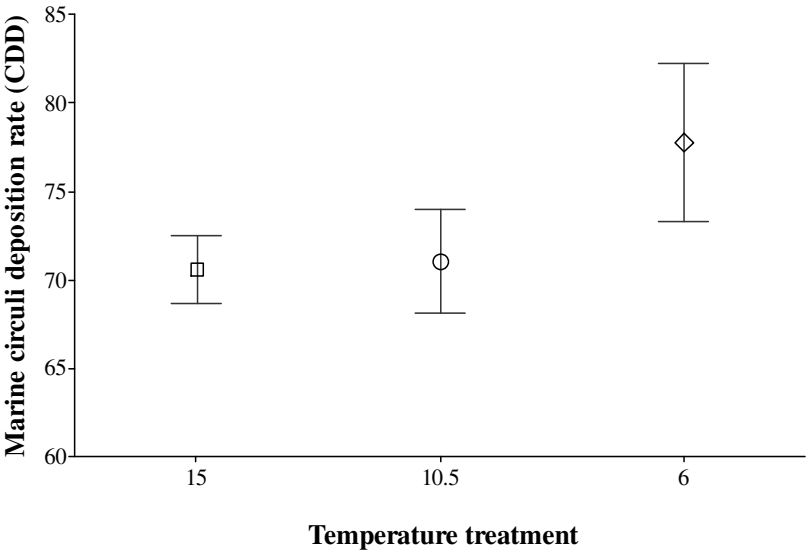

Figure 3.3 (d)

Figure 3.3 (c, d). (c) Marine circuli deposition rate per day (d) Marine circuli deposition rate per cumulative degree day (CDD); [F_C (constant feeding); □ ,15 °C (F_C); ○ ,10.5 °C (F_C); ◇ ,6 °C (F_C)]; Error bars are 95% confidence intervals.

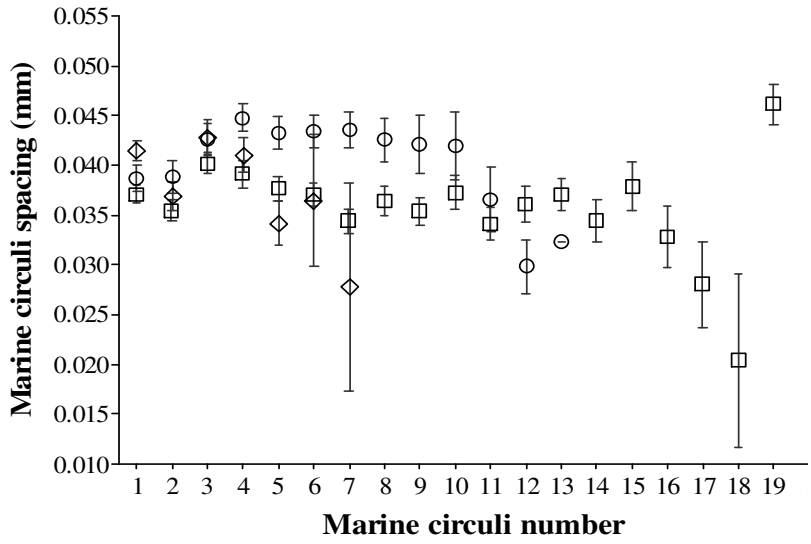

Figure 3.3 (e)

Figure 3.3 (e). Marine circulus spacing (mm) per circuli number; [F_C (constant feeding); □ ,15 °C (F_C); ○ ,10.5 °C (F_C); ◇ ,6 °C (F_C)]; Error bars are 95% confidence intervals.

3.4.1.4 Fish fork length

Average fish fork length measurements [mean ± standard deviation (SD) mm] were highest in the 15 °C temperature treatment (226.3 ± 22.9) followed by 10.5 °C (222.5 ± 22.1) and 6 °C (203.5 ± 15.4) treatments, respectively [Table 3.2]. The rate at which fish length increased during the course of the experiment varied between the three temperature treatments [Figure 3.4 (a)]. The ANCOVA confirmed that the slope of the relationship between fish fork length and week number was significantly different between temperature treatments [$p<0.001$; Table 3.3 (a)]. The main effect of temperature treatment was not significantly different between treatments [ANCOVA, $p=0.797$; Table 3.3 (a)]. Post-hoc pairwise comparisons showed no significant

difference between 15 °C and 10.5 °C ($p=0.322$); however, the fork length at 6 °C differed to 15 °C temperature treatment ($p<0.001$) and the 10.5 °C temperature treatment ($p<0.001$).

A linear regression was derived to describe the relationship between fork length (y) and day/week (x) at each temperature treatment (Table 3.4). This showed that fish length increased with temperature with average growth rates of 0.83 mm d^{-1}, 0.75 mm d^{-1} and 0.39 mm d^{-1} at temperatures 15 °C, 10.5 °C and 6 °C, respectively.

The rate at which fish length increased with degree day varied between the three temperature treatments [Figure 3.4 (b)]. ANCOVA confirmed that the slope of the relationship between fish fork length and CDD differed significantly between the three temperature treatments at 15 °C, 10.5 °C and 6 °C [$p<0.001$; Table 3.3 (b)]. Post- hoc pairwise comparisons found no significant difference for fish fork length and CDD between the 15 °C and 6 °C treatments ($p=0.451$), the 10.5 °C and 6 °C treatments ($p=0.504$); however, the 15 °C and 10.5 °C temperature treatment differed ($p=0.024$).

Linear regressions were derived to describe the relationship between fork length (y) and CDD (x) for each temperature treatment (Table 3.4). The rate at which fish length increased with degree day was greatest at 10.5 °C (0.072 mm cdd^{-1}) followed by 6 °C (0.064 mm cdd^{-1}) and 15 °C (0.0563 mm cdd^{-1}), respectively. ANCOVA confirmed that the slope of the relationship between fish length and scale radius did not differ significantly between the three temperature treatments [$p=0.712$; Table 3.3 (a); Figure 3.4 (c)].

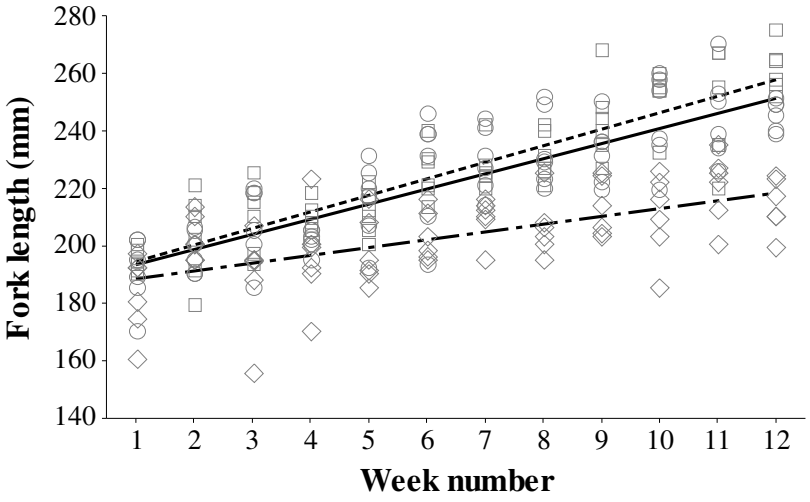

Figure 3.4 (a)

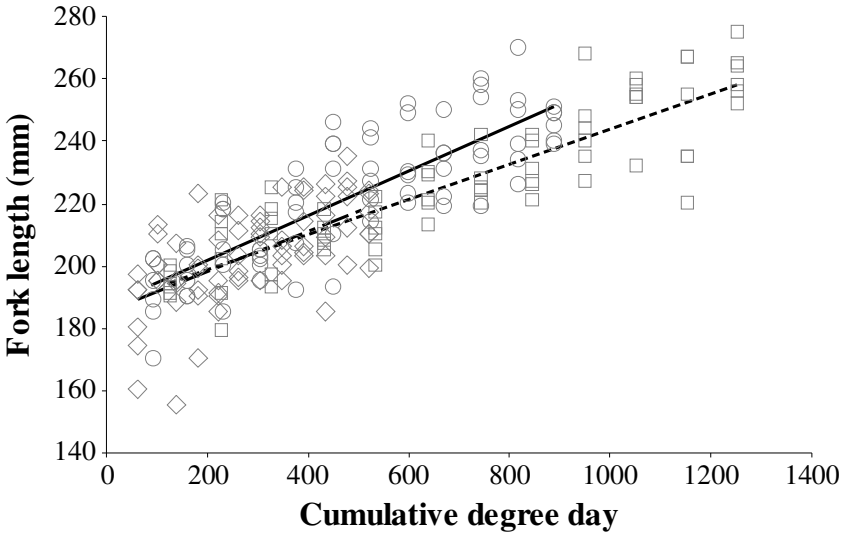

Figure 3.4 (b)

Figure 3.4 (a, b). (a) Fork length (mm) per temperature treatment by time; weeks (b) Fork length (mm) per temperature treatment by time; cumulative degree day; [F_C (constant feeding); □ - - - - ,15 °C (F_C); ○ —— ,10.5 °C (F_C); ◇ — - ,6 °C (F_C)].

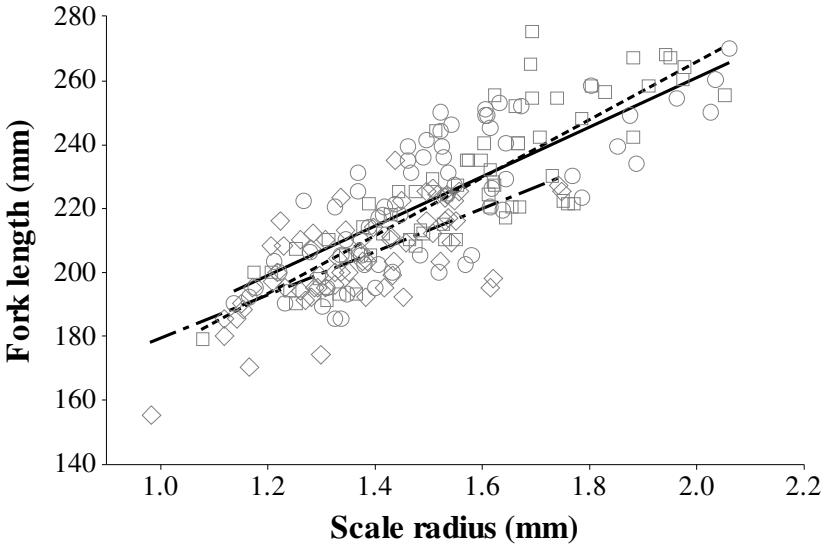

Figure 3.4 (c)

Figure 3.4 (c). Fork length (mm) /scale radius (mm) per temperature treatment [F_C (constant feeding); □ - - - - ,15 °C (F_C); ○ —— ,10.5 °C (F_C); ◇ — – ,6 °C (F_C)].

3.4.2 Effect of feeding on scale growth

3.4.2.1 Marine growth

From weeks 1 to 7, there were no significant differences in growth between the two feeding treatments at each of the three temperature treatments (ANCOVA, p=0.214). This confirmed that fish in the continuous feeding and the interrupted feeding treatments had grown at the same rate prior to the starvation period. The effects of starvation on scale growth became evident when the feeding treatments were compared at weeks 8 to 12 [Table 3.2; Table 3.3 (d)].

The rate at which scale size increased between weeks 8 and 12 showed variation between the two feeding treatments [Figure 3.5 (a-c)]. ANCOVA confirmed that the slope of the relationship between marine growth and time (week number) differed

significantly between the two feeding treatments at 15 °C [p<0.001; Table 3.3 (d)] and 10.5 °C [p=0.031; Table 3.3 (d)]. No significant difference was detected between the feeding treatments at 6 °C [p=0.064; Table 3.3 (d)]. The main effect of feeding treatment was significant at 15 °C [p=0.009; Table 3.3 (d)] and 10.5 °C [p=0.003; Table 3.3 (d)], with the continuous feeding treatments showing significantly higher marine growth than the interrupted treatments of 0.060 ± 0.022 mm [mean difference ± standard deviation (SD)] and 0.070 ± 0.020 mm [mean difference ± standard deviation (SD)] at 15 °C and 10.5 °C, respectively [Figure 3.5 (a, b)]. Feeding treatment did not negatively affect growth at 6 °C [p=0.243; Figure 3.5 (c); Table 3.3 (d)].

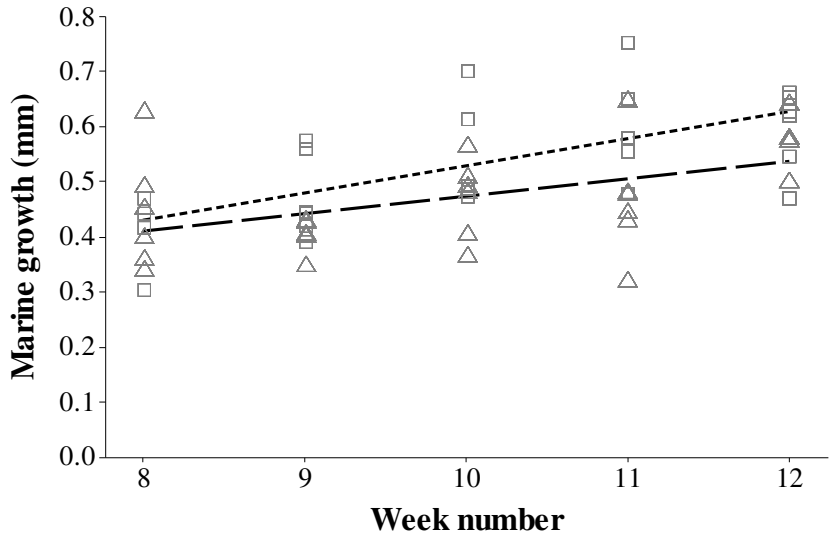

Figure 3.5 (a)

Figure 3.5 (a). Marine growth (mm) per time; feeding treatment at 15 °C; [F_C (constant feeding), F_I (interrupted feeding); □ - - - - ,15 °C (F_C); △ ——,15 °C (F_I)].

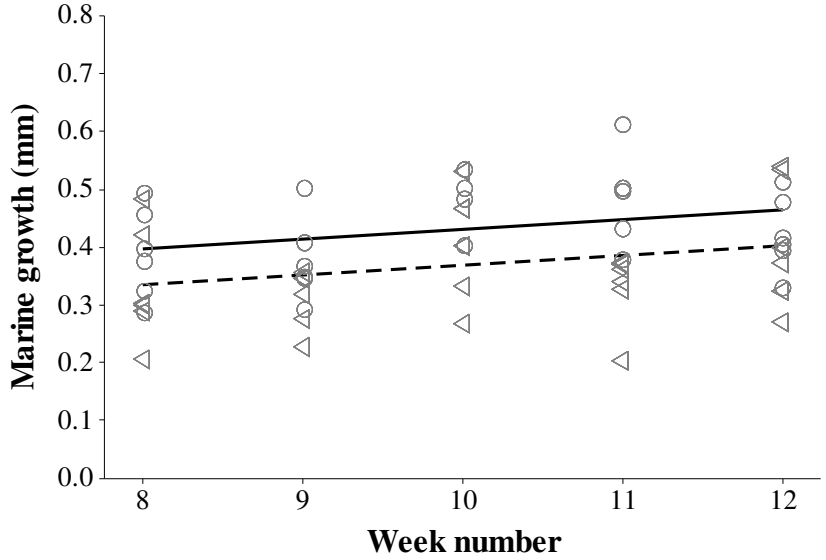

Figure 3.5 (b)

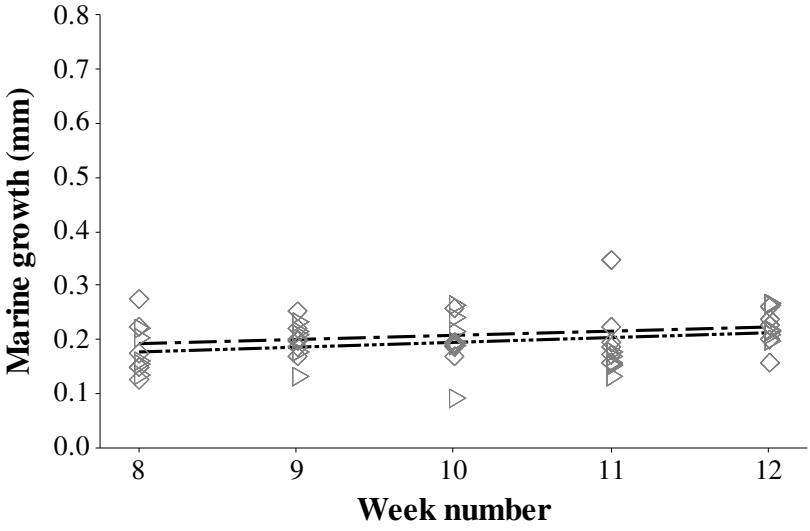

Figure 3.5 (c)

Figure 3.5 (b, c). (b) Marine growth (mm) per time; feeding treatment at 10.5 °C (c) Marine growth per time; feeding treatment at 6 °C; [F_C (constant feeding), F_I (interrupted feeding); ○ —— ,10.5 °C (F_C); ◁ – – – ,10.5 °C (F_I); ◇ — - ,6 °C (F_C); ▷ — - - - ,6 °C (F_I)].

3.4.2.2 Marine circuli number

There was no significant difference in marine circuli number between the continuous feeding and interrupted feeding treatments across the temperature treatment (p=0.966) from weeks 1 to 7. From weeks 8 to 12, fewer circuli were deposited in fish from the interrupted feeding treatment compared to the continuous feeding treatment at both 15 °C and 10.5 °C with a difference [mean difference ± standard deviation (SD)] of 1.5 ± 0.54 and 1.5 ± 0.31, respectively [Figure 3.6 (a - c)]. CDR_{Day} was significantly different between the feeding treatments at 15 °C (ANCOVA, p=0.003) and 10.5 °C (ANCOVA, p<0.001) but no difference of CDR_{Day} was found between the feeding treatments at 6 °C (ANCOVA, p=0.201). Circuli deposition rate was much slower in fish from the interrupted feeding treatment compared to the continuous feeding treatment. No difference in deposition rate was evident between the feeding treatment at 6 °C [Figure 3.6 (d); Table 3.2].

3.4.2.3 Marine circulus spacing

When the relationship between circulus spacing and circuli number was compared between the continuous feeding and interrupted feeding treatments from weeks 1 to 7 and again at weeks 8 to 12, across the three temperature treatments; 15 °C, 10.5 °C and 6 °C, respectively, no significant differences in the slopes (feeding treatment*circulus number) (ANCOVA, p=0.269) or intercepts (feeding treatment) (ANCOVA, p=0.070) were found, showing that the short starvation event did not affect the width between the circuli. [Figure 3.6 (e-g); Table 3.2; Table 3.3 (e)].

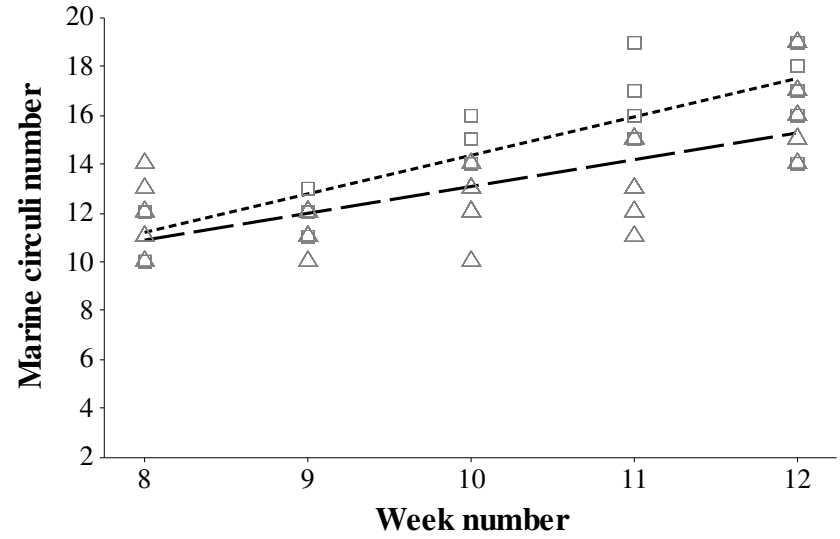

Figure 3.6 (a)

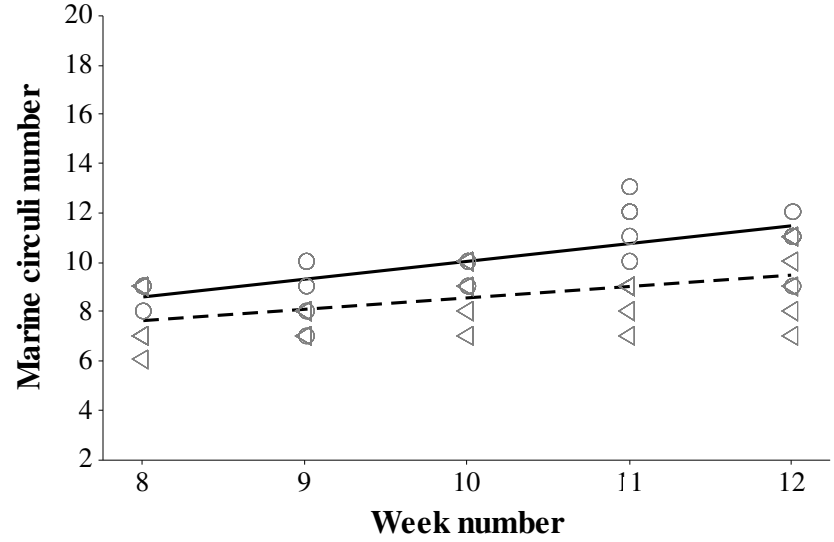

Figure 3.6 (b)

Figure 3.6 (a, b). (a) Marine circuli number per time; feeding treatment at 15 ℃ (b) Marine circuli number per time; feeding treatment at 10.5 ℃ [F_C (constant feeding), F_I (interrupted feeding); □ - - - - ,15 ℃ (F_C); △ ——,15 ℃ (F_I); ○ —— ,10.5 ℃ (F_C); ◁ ———,10.5 ℃ (F_I)].

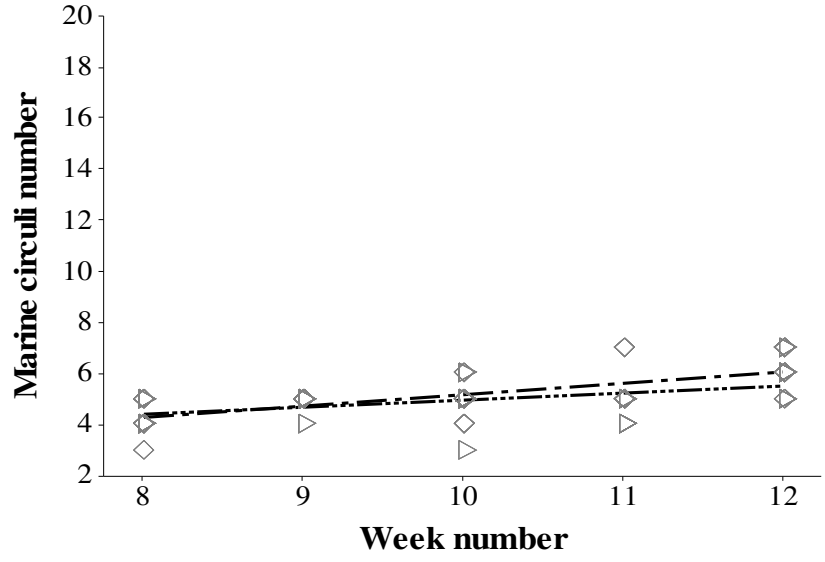

Figure 3.6 (c)

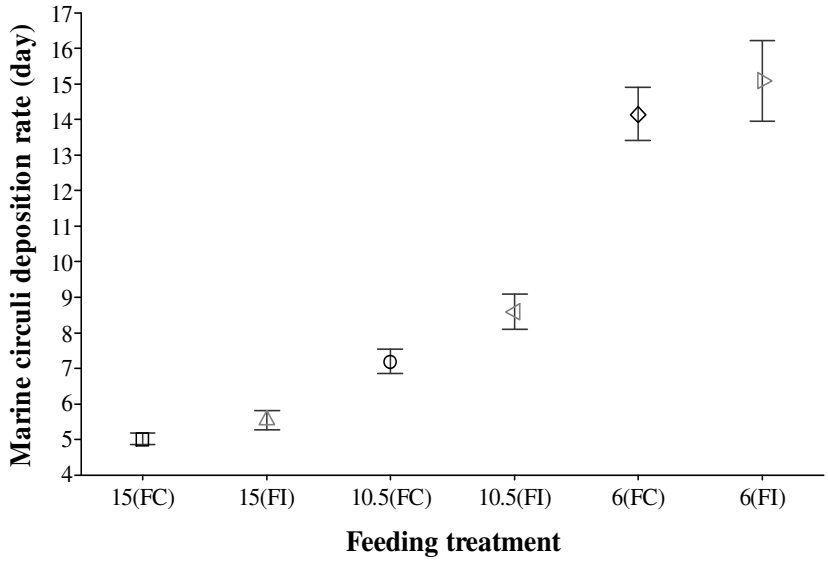

Figure 3.6 (d)

Figure 3.6 (c, d). (c) Marine circuli number per time; feeding treatment at 6 °C (d) Marine circulus deposition rate / day per feeding treatment [F_C (constant feeding), F_I (interrupted feeding); □ ,15 °C (F_C); △,15 °C (F_I); ○ ,10.5 °C (F_C); ◁ ,10.5 °C (F_I); ◇ — - ,6 °C (F_C); ▷— - - - ,6 °C (F_C)]; Error bars are 95% confidence intervals.

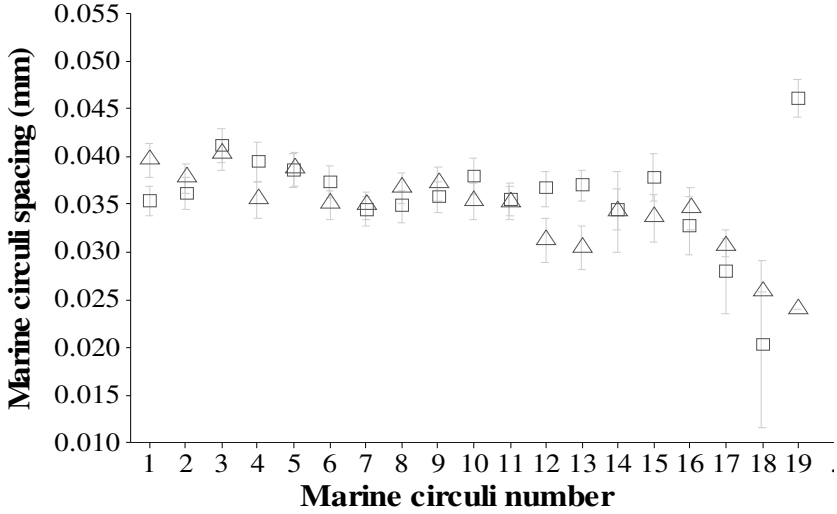

Figure 3.6 (e)

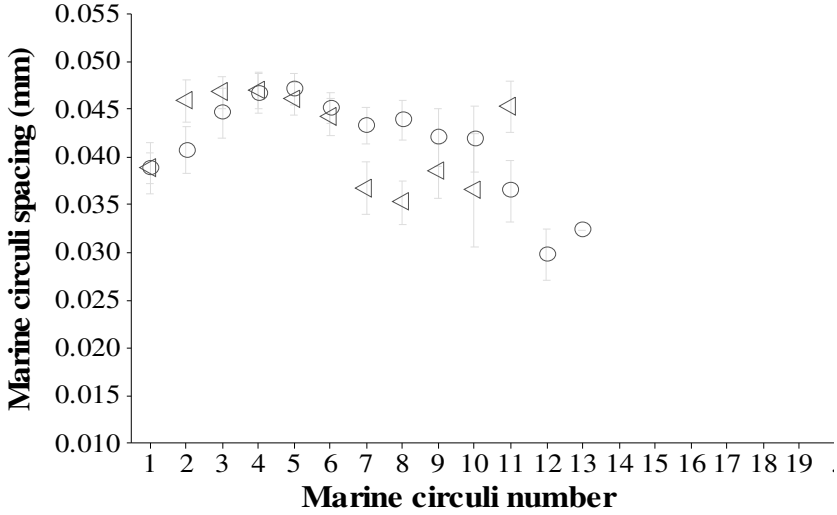

Figure 3.6 (f)

Figure 3.6 (e, f). (e) Marine circulus spacing (mm) per circuli number; feeding treatment at 15 °C (f) Marine circulus spacing (mm) per circuli number; feeding treatment at 10.5 °C [F_C (constant feeding), F_I (interrupted feeding); □ ,15 °C (F_C); △ ,15 °C (F_I); ○ ,10.5 °C (F_C); ◁ ,10.5 °C (F_I)]; Error bars are 95% confidence intervals.

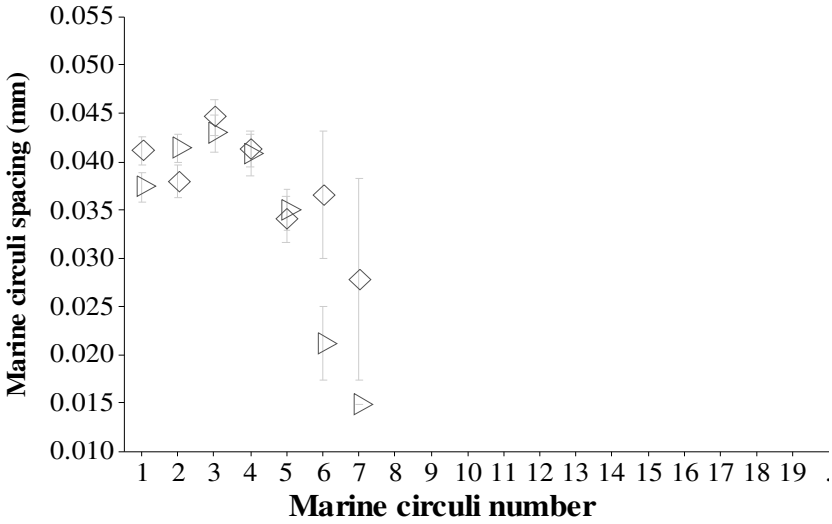

Figure 3.6 (g)

Figure 3.6 (g). Marine circulus spacing (mm) per circuli number; feeding treatment at 6 °C [F_C (constant feeding), F_I (interrupted feeding); ◇ ,6 °C (F_C); ▷ ,6 °C (F_C)]; Error bars are 95% confidence intervals.

3.4.2.4 Fish fork length

From weeks 1 to 7, there were no significant differences in growth between the two feeding treatments at each of the three temperature treatments (ANCOVA, p=0.181). The rate at which scale size increased between weeks 8 and 12 showed variation between the two feeding treatments at each temperature [Table 3.2; Table 3.3 (c)]. ANCOVA confirmed that the slope of the relationship between fish fork length and time differed significantly between the two feeding treatments at 15 °C [p<0.001; Table 3.3 (c)] and 10.5 °C [p=0.001; Table 3.3 (c)]. No significant difference was found between fork lengths and time at 6 °C [p=0.253; Table 3.3 (c)]. The main effect

of feeding treatment was also significant at 15 °C [p=0.008; Table 3.3 (c)] and 10.5 °C [p=0.004; Table 3.3 (c)], the continuous feeding treatments had significantly larger fork lengths [mean difference ± standard deviation (SD) mm] than the interrupted treatment of 9.4 ± 3.4 mm and 9.4 ± 3.0 mm at 15 °C and 10.5 °C, respectively [Figure 3.7 (a, b)]. No significant difference was found between the feeding treatments at 6 °C [p=0.284; Figure 3.7 (c); Table 3.3 (c)].

ANCOVA confirmed that the slope of the relationship between fish fork length and scale radius did not differ significantly between the two feeding treatments at 15 °C, 10.5 °C or 6 °C [p=0.379; Figure 3.7 (d); Table 3.3 (c)].

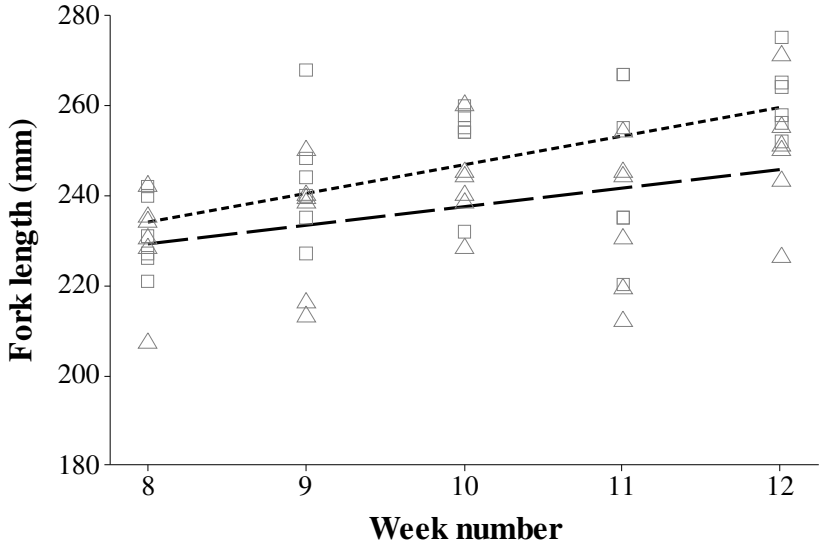

Figure 3.7 (a)

Figure 3.7 (a). Fork length (mm) per time; feeding treatment at 15 °C [F_C (constant feeding), F_I (interrupted feeding); □ - - - - ,15 °C (F_C); △ ——— ,15 °C (F_I)].

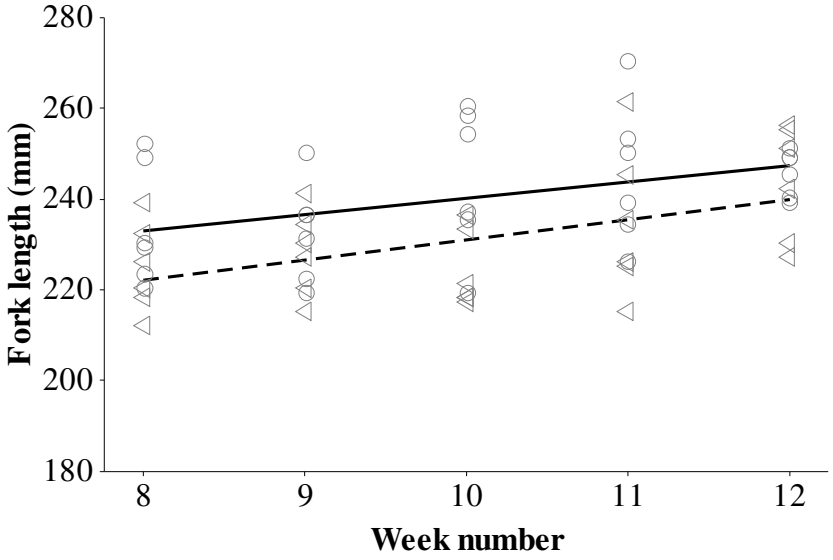

Figure 3.7 (b)

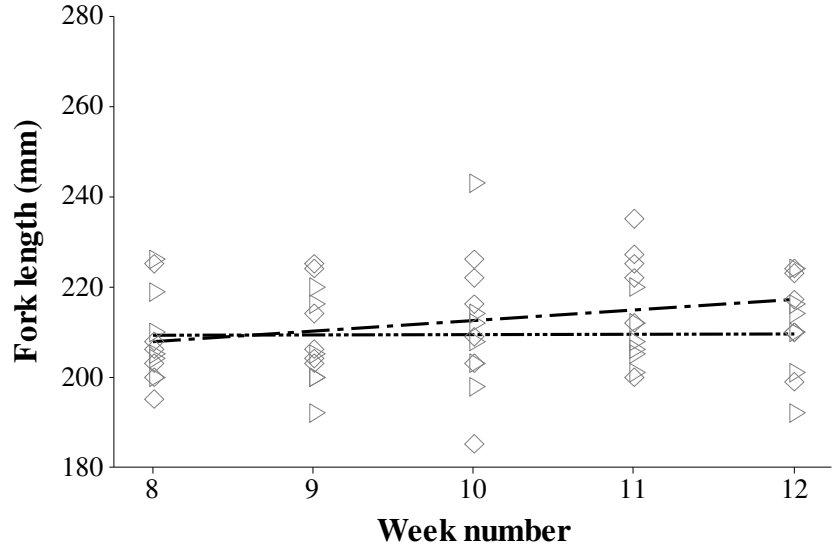

Figure 3.7 (c)

Figure 3.7 (b, c). (b) Fork length (mm) per time; feeding treatment at 10.5 °C (c) Fork length (mm) per time; feeding treatment at 6 °C [F_C (constant feeding), F_I (interrupted feeding); ○ ——,10.5 °C (F_C); ◁ – – –,10.5 °C (F_I); ◇ — - ,6 °C (F_C); ▷ — - - -,6 °C (F_C)].

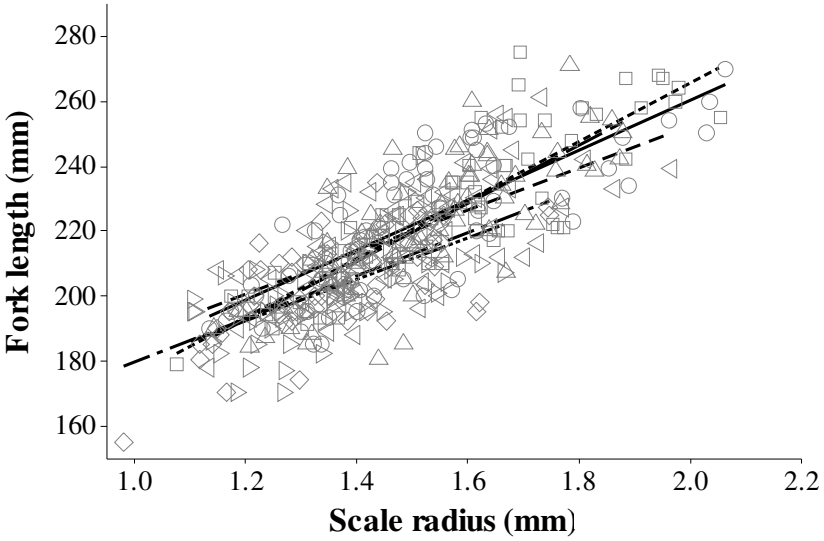

Figure 3.7 (d)

Figure 3.7 (d). Scale radius (mm) per fork length (mm); feeding treatment at 15 °C, 10.5 °C and 6 °C [F_C (constant feeding), F_I (interrupted feeding); □ - - - - ,15 °C (F_C); △ ——,15 °C (F_I); ○ ——,10.5 °C (F_C); ◁ – – –,10.5 °C (F_I); ◇ — – ,6 °C (F_C); ▷— - - - ,6 °C (F_C)].

3.5 Discussion

This experiment investigated the effect of both water temperature and food availability on somatic growth and scale growth of Atlantic salmon post-smolts during the first three months of marine habitation. The results show that growth and scale characteristics were influenced by both the temperature and feeding conditions during rearing, agreeing with previous experimental studies conducted on somatic growth of Atlantic salmon (Handeland *et al.*, 2000, 2003, 2008; Beakes *et al.*, 2014). Scale radius and circuli number increased with water temperature and decreased due to starvation. The differences in scale growth rates between treatments generally reflected the

differences found in body growth rates, supporting the use of scale measurements to infer growth rates. However, fish length and scale radius appeared to respond differently to cumulative degree day, indicating a mechanistic difference in these responses. In addition, narrow inter-circuli spacings were observed during periods of slow growth at low temperatures and during periods of fast growth at high temperatures. These findings highlight the importance of considering temperature histories when using scale measurements to reconstruct fish growth.

The relationship between scale radius and fish length indicated that scale length was proportional to fish length and this relationship was consistent across both the temperature and feeding treatments. A similar result was reported by Beakes *et al.* (2014) for juvenile steelhead (*Oncorhynchus mykiss*) reared at different temperatures and feeding regimes. Scale radius measurements from Atlantic salmon are generally used to infer growth rates, particularly during the post-smolt period to the first sea winter (Friedland *et al.*, 2000, 2009). The results of this study validate the use of scale radius measurements as a proxy for fish size as this relationship appears to be independent of environmental factors.

The number of circuli present in the post-smolt portion of a scale are presumed to be proportional to the time spent in the marine environment, although the likely effects of temperature are acknowledged. Circuli deposition rates estimated from field studies vary; according to Hubley *et al.* (2008) and Friedland *et al.* (2009) circuli are formed at a rate of 7 d circulus $^{-1}$ in summer and 14 d circulus $^{-1}$ during winter months while

Jensen *et al*. (2012) estimate a formation rate of 6.3 d circulus $^{-1}$ during summer. These estimates are commonly used to reconstruct growth histories in retrospective growth studies. In this study, circuli deposition rates were comparable with previous field estimates, varying from 4.8 d circulus $^{-1}$ at 15 °C (constant feeding) to 15.1 d circulus $^{-1}$ at 6 °C (interrupted feeding). The results confirm that marine circuli are deposited at irregular intervals and circuli deposition is dependent on temperature and feeding. Therefore, using general deposition rates as a means of evaluating and reconstructing growth histories of Atlantic salmon of unknown or different origin and varying thermal histories, may produce erroneous results.

When circuli deposition rate was expressed relative to cumulative degree day, the observed rates of deposition were 0.0103, 0.0125 and 0.0133 circulus cdd^{-1} at 6 °C, 10.5 °C and 15 °C, respectively. No difference was evident between the 15 °C and 10.5 °C treatments, showing that at these two temperatures circuli deposition is a reliable indicator of cumulative temperature history. While marine circuli deposition rate (CDR_{CDD}) was significantly higher at 6 °C compared to the other two temperature treatments, this difference was much smaller than that observed when circuli deposition rate was expressed in days (CDR_{Day}). Therefore, if a fish's cumulative temperature history can be estimated from recorded SST values [e.g. Meteorological Office Hadley Centre (HadISST)] records along its migration route, the time of formation of each circulus could be estimated using a deposition rate of ~0.01 circulus cdd^{-1}. This should allow for a more accurate reconstruction of chronological growth histories than can be achieved when a constant daily deposition rate is assumed,

although the effect of variations in food supply on circuli deposition rate must also be considered as a potential source of error.

While feeding cessation caused fewer circuli to be deposited in the scale at 15 °C and 10.5 °C, it had no apparent effect on circuli deposition at 6 °C. Previous studies suggest that osmotic stress may be more severe for post-smolts at temperatures less than 7 °C (Sigholdt and Finstad, 1990; Handeland *et al*., 2000). The fish reared at 6 °C may have suffered from some form of osmotic stress leading to lower growth rates. Growth may be so impaired at this temperature that the additional stress of reduced food supply does not reduce it further.

It has been proposed that the spacing between circuli reflect fish growth rates; it is thought that during periods of fast growth widely spaced circuli are deposited in the scale (Friedland *et al*., 1993). The results of this study are not consistent with this assumption. The circulus spacings in the 10.5 °C treatment were on average, 11% wider compared to the other two temperature treatments. In the 15 °C treatment scale and body growth rates were higher and more circuli were deposited on the scale. However, these circuli were narrower than those observed in scales from the 10.5 °C treatment and were more similar to those from slower growing fish from the 6 °C treatment. While scale radius was 29% higher at 15 °C compared to 10.5 °C, circuli number was 46% higher and thus the circuli were more tightly packed. In an experimental study of juvenile *Oncorhynchus mykiss,* Beakes *et al.* (2014) observed that while scale growth and circuli deposition rates were lower at 8 °C relative to

higher temperatures, circuli were more widely spaced at 8 °C. This was attributed to suppressed circuli formation at decreased temperatures. Therefore, the experimental evidence shows that circulus spacing is not reflective of growth rate. This corroborates field observations reported by Peyronnet *et al.* (2007) who found that in one-sea-winter Atlantic salmon returns, average inter-circuli distances were lower but average fish lengths were higher in the 1980's compared to the 1990's. Based on these results, they suggested that marine circuli spacing may not accurately describe growth.

Jensen *et al.* (2012) observed that circuli deposited during the early stage of the marine migration were narrower in one-year-old Atlantic salmon post-smolts of southern origin than in post-smolts from Northerly populations. They suggested that this was indicative of poor growth and consequently higher mortality of Atlantic salmon from southern populations. However, based on the results of this study, the narrow circuli spacing in the southern fish could be attributed to higher sea surface temperatures (SST) at lower latitudes, resulting in rapid deposition of narrowly spaced circuli.

In this study, temperatures in each treatment were held constant at 15 °C, 10.5 °C and 6 °C. Apart from the 14-day starvation period in the interrupted feeding treatments, food supply was high and continuous and all other conditions were stable throughout the experiment. The marine environment is much more variable; water temperatures, salinities, photoperiod and productivity continually fluctuate with latitude and according to daily, seasonal and annual cycles. The experimental conditions may not be directly comparable with conditions experienced by wild Atlantic salmon in the

natural environment. Salmon post-smolts preferentially inhabit areas with a narrow temperature range of between 8 °C and 12 °C (Friedland *et al*., 1993, 1998, 2000; Jonsson and Jonsson, 2004). In addition, fish in the wild may be exposed to more severe food shortages than in this experiment. The results demonstrate how somatic and scale growth respond to experimentally manipulated temperature and feeding conditions. Further investigative studies in more variable mesocosm environments are needed to more fully understand the extent to which scale growth marks in Atlantic salmon reflect natural environmental fluctuations.

The results of this study confirm that temperature strongly influences somatic growth, scale growth and circuli patterns. Circuli number is reflective of cumulative temperature history rather than time spent at sea and circuli spacing is not a reliable indicator of growth rate. The study highlights the importance of considering temperature history when interpreting scale measurements. The 14-day starvation period decreased growth and circuli deposition rates but did not affect the circuli spacing. Further investigation is required to assess the impact of prolonged or repeated starvation on scale and body growth.

Acknowledgements

We thank the scientific and technical personnel of Matre research station, IMR Norway, involved in this experiment. This study was funded by the Marine Institute, Ireland, the Institute of Marine Research, Norway and the Loughs Agency, N. Ireland

Table 3.1. Overview of time and mortality rate per temperature treatment; week and cumulative degree days (CDD).

Treatment	15 °C			10.5 °C			6 °C		
Week	**CDD**	**M Rate***	**M Rate – 24H‡**	**CDD**	**M Rate***	**M Rate – 24H‡**	**CDD**	**M Rate***	**M Rate – 24H‡**
1	122.8	9	0	88.7	5	0	58.7	11	4
2	224.5	1	1	159.1	0	0	97.8	0	0
3	325.9	1	1	229.9	0	0	136.7	0	0
4	429.0	0	0	302.5	0	0	178.0	0	0
5	531.8	0	0	374.4	0	0	219.6	0	0
6	637.2	0	0	448.5	0	0	261.0	0	0
7	742.1	0	0	522.6	0	0	303.4	0	0
8	844.8	0	0	595.6	0	0	345.8	0	0
9	948.3	0	0	668.4	0	0	389.1	0	0
10	1050.3	0	0	741.4	1	1	432.7	0	0
11	1151.5	1	1	814.6	1	1	476.7	1	1
12	1252.1	0	0	888.0	0	0	519.9	2	2

*; M Rate (mortality rate), ǂ; M Rate – 24H (mortality rate excluding the initial 24 hours of experiment).

Table 3.2. Results of scale growth measurements (mean ± SD) per treatment; marine growth (G_M; mm), marine circuli number (C_M), circulus spacing (S_{CM}; mm), circuli deposition rate per day (CDR_{Day}) and fork length (L_F; mm).

Variable	Treatment*		Weeks 1 to 12 Mean ± SD	Weeks 8 to 12 Mean ± SD
G_M	15 °C	F_C	0.36 ± 0.18	0.53 ± 0.10
		F_I	0.33 ± 0.15	0.47 ± 0.092
	10.5 °C	F_C	0.28 ± 0.15	0.43 ± 0.080
		F_I	0.26 ± 0.13	0.36 ± 0.094
	6 °C	F_C	0.15 ± 0.072	0.20 ± 0.046
		F_I	0.13 ± 0.066	0.19 ± 0.044
C_M	15 °C	F_C	9.8 ± 4.9	14.5 ± 2.6
		F_I	9.1 ± 4.2	13.0 ± 2.2
	10.5 °C	F_C	6.7 ± 3.3	9.9 ± 1.5
		F_I	6.2 ± 2.6	8.4 ± 1.3
	6 °C	F_C	3.7 ± 1.6	5.1 ± 0.92
		F_I	3.5 ± 1.5	4.9 ± 0.85
S_{CM}	15 °C	F_C	0.037 ± 0.0050	0.037 ± 0.0045
		F_I	0.037 ± 0.0046	0.036 ± 0.0041
	10.5 °C	F_C	0.040 ± 0.0074	0.043 ± 0.0064
		F_I	0.041 ± 0.0075	0.042 ± 0.0058
	6 °C	F_C	0.039 ± 0.0075	0.040 ± 0.0056
		F_I	0.038 ± 0.0068	0.039 ± 0.0044
CDR_{Day}	15 °C	F_C	4.8 ± 0.54	5.0 ± 0.46
		F_I	5.0 ± 0.82	5.5 ± 0.70
	10.5 °C	F_C	6.8 ± 1.2	7.2 ± 0.84
		F_I	7.3 ± 1.7	8.6 ± 1.3
	6 °C	F_C	12.6 ± 3.2	14.1 ± 2.0
		F_I	13.0 ± 3.7	15.1 ± 3.0
L_F	15 °C	F_C	226.3 ± 22.9	247.0 ± 15.6
		F_I	221.7 ± 20.9	237.6 ± 14.9
	10.5 °C	F_C	222.5 ± 22.1	240.2 ± 13.4
		F_I	218.9 ± 17.6	230.8 ± 13.2
	6 °C	F_C	203.5 ± 15.4	212.6 ± 11.7
		F_I	201.4 ± 14.9	209.5 ± 10.8

*; F_C (constant feeding), F_I (interrupted feeding).

Table 3.3 (a). Results of general linear models comparing scale and fish measurements between temperature treatments per week; scale radius (S_R; mm), fork length (L_F; mm), marine growth (G_M; mm), marine circuli number (C_M), circulus spacing (S_{CM}; mm) and circuli deposition rate per day (CDR_{Day}).

Response	Model terms*	DF	F	p	R^2
S_R	Fork length	1	308.04	**<0.001**	0.68
	Temperature	2	1.35	0.261	-------
	(Fork length*Temperature)*	2	0.34	0.712	-------
	Error	196	-------	-------	-------
L_F	Week number	1	376.3	**<0.001**	0.73
	Temperature	2	0.23	0.797	-------
	Week *Temperature	2	16.4	**<0.001**	-------
	Error	210	-------	-------	-------
G_M	Week number	1	1036.7	**<0.001**	0.9
	Temperature	2	0.24	0.786	-------
	Week *Temperature	2	74.6	**<0.001**	-------
	Error	194	-------	-------	-------
C_M	Week number	1	2789.4	**<0.001**	0.96
	Temperature	2	0.15	0.857	-------
	Week *Temperature	2	251.8	**<0.001**	-------
	Error	194	-------	-------	-------
S_{CM}	Week number	1	8.8	**0.003**	0.12
	Temperature	2	0.80	0.450	-------
	Week*Temperature	2	3.9	**0.022**	-------
	Error	194	-------	-------	-------

* Interaction term removed if p>0.15 and analysis re-run (Fork length*Temperature).

Table 3.3 (b). Results of general linear models comparing scale and fish measurements between temperature treatments per cumulative degree day (CDD); scale radius (S_R; mm), fork length (L_F; mm), marine growth (G_M; mm), marine circuli number (C_M), circulus spacing (S_{CM}; mm) and circuli deposition rate per day (CDR_{Day}).

Response	Model terms	DF	F	p	R^2
L_F	CDD	1	260.4	**<0.001**	0.73
	Temperature	2	0.17	0.841	-------
	CDD*Temperature	2	2.6	0.078	-------
	Error	210	-------	-------	-------
G_M	CDD	1	667.6	**<0.001**	0.90
	Temperature	2	0.27	0.767	-------
	CDD*Temperature	2	4.3	**0.015**	-------
	Error	194	-------	-------	-------
C_M	CDD	1	1746.1	**<0.001**	0.96
	Temperature	2	0.42	0.656	-------
	CDD*Temperature	2	8.6	**<0.001**	-------
	Error	194	-------	-------	-------
S_{CM}	CDD	1	7.9	**0.005**	0.12
	Temperature	2	0.83	0.436	-------
	CDD*Temperature	2	5	**0.008**	-------
	Error	194	-------	-------	-------

Table 3.3 (c). Results of general linear models comparing scale and fish measurements between feeding treatments; scale radius (S_R; mm) and fork length (L_F; mm).

Response	Treatment*	Model terms‡	DF	F	p	R^2
S_R	All	Fork length	1	723.8	**<0.001**	0.61
		Feeding	1	1.7	0.109	-------
		(Fork length*Feeding)‡	1	1.1	0.379	-------
		Error	597	-------	-------	-------
L_F	15 °C	Week	1	18.6	**<0.001**	0.32
	F_C, F_I W2	Feeding	1	7.6	**0.008**	-------
		(Week*Feeding)‡	1	0.85	0.36	-------
		Error	57	-------	-------	-------
L_F	10.5 °C	Week	1	13.5	**0.001**	0.28
	F_C, F_I W2	Feeding	1	9	**0.004**	-------
		(Week*Feeding)‡	1	0.14	0.709	-------
		Error	57	-------	-------	-------
L_F	6 °C	Week	1	1.3	0.253	0.042
	F_C, F_I W2	Feeding	1	1.2	0.284	-------
		(Week*Feeding)‡	1	1.2	0.287	-------
		Error	57	-------	-------	-------

*F_C (constant feeding), F_I W2 (2 week interupted feeding). ‡Interaction term removed if p > 0.15 and analysis re-run (Fork length*Feeding; Week*Feeding).

Table 3.3 (d). Results of general linear models comparing scale and fish measurements between feeding treatments; marine growth (G_M; mm) and marine circuli number (C_M).

Response	Treatment*	Model terms‡	DF	F	p	R^2
G_M	15 °C	Week	1	26.4	**<0.001**	0.39
	F_C, F_I W2	Feeding	1	7.3	**0.009**	-------
		(Week*Feeding)‡	1	1.3	0.264	-------
		Error	55	-------	-------	-------
G_M	10.5 °C	Week	1	4.9	**0.031**	0.21
	F_C, F_I W2	Feeding	1	9.7	**0.003**	-------
		(Week*Feeding)‡	1	0.01	0.940	-------
		Error	54	-------	-------	-------
G_M	6 °C	Week	1	3.6	0.064	0.08
	F_C, F_I W2	Feeding	1	1.4	0.243	-------
		(Week*Feeding)‡	1	0.01	0.941	-------
		Error	55	-------	-------	-------
C_M	15 °C	Week	1	90.0	**<0.001**	0.67
	F_C, F_I W2	Feeding	1	2.3	0.135	-------
		Week*Feeding	1	4.0	0.050	-------
		Error	54	-------	-------	-------
C_M	10.5 °C	Week	1	32.2	**<0.001**	0.52
	F_C, F_I W2	Feeding	1	26.6	**<0.001**	-------
		(Week*Feeding)‡	1	1.7	0.200	-------
		Error	54	-------	-------	-------
C_M	6 °C	Week	1	24.4	**<0.001**	0.32
	F_C, F_I W2	Feeding	1	2.0	0.159	-------
		(Week*Feeding)‡	1	1.8	0.180	-------
		Error	54	-------	-------	-------

*F_C (constant feeding), F_I W2 (2 week interupted feeding). ‡Interaction term removed if p > 0.15 and analysis re-run (Week*Feeding).

Table 3.3 (e). Results of general linear models comparing scale and fish measurements between feeding treatments; circulus spacing (S_{CM}; mm) and circuli deposition rate per day (CDR_{Day}).

Response	Treatment†	Model terms‡	DF	F	p	R^2
S_{CM}	15 °C	Week	1	1.72	0.196	0.036
	F_C, F_I W2	Feeding	1	0.45	0.505	-------
		(Week*Feeding)‡	1	0.20	0.656	-------
		Error	54	-------	-------	-------
S_{CM}	10.5 °C	Week	1	1.75	0.192	0.039
	F_C, F_I W2	Feeding	1	0.45	0.507	-------
		(Week *Feeding)‡	1	0.73	0.396	-------
		Error	54	-------	-------	-------
S_{CM}	6 °C	Week	1	5.3	**0.026**	0.14
	F_C, F_I W2	Feeding	1	2.6	0.116	-------
		Week*Feeding	1	2.3	0.136	-------
		Error	54	-------	-------	-------
CDR_{Day}	15 °C	Week	1	0.1	0.756	0.21
	F_C, F_I W2	Feeding	1	1.8	0.189	-------
		Week*Feeding	1	3.3	0.077	-------
		Error	54	-------	-------	-------
CDR_{Day}	10.5 °C	Week	1	10	**0.003**	0.39
	F_C, F_I W2	Feeding	1	24.9	**<0.001**	-------
		(Week*Feeding)‡	1	1.2	0.285	-------
		Error	54	-------	-------	-------
CDR_{Day}	6 °C	Week	1	4.2	**0.044**	0.11
	F_C, F_I W2	Feeding	1	2.0	0.160	-------
		(Week*Feeding)‡	1	1.8	0.188	-------
		Error	55	-------	-------	-------

†F_C (constant feeding), F_I W2 (2 week interupted feeding). ‡Interaction term removed if p>0.15 and analysis re-run (Week*Feeding).

Table 3.4. Linear regression equations for marine growth (G_M; mm), marine circuli number (C_M), circulus spacing (S_{CM}; mm), circuli deposition rate per day (CDR_{Day}) and fork length (L_F; mm).

Treatment*		Time‡	Regression Equation	R^2	p
15 °C	F_C	CDD	G_M = 0.00048*CDD + 0.020	0.90	**<0.001**
	F_I W2	CDD	G_M = 0.00039*CDD +0.064	0.84	**<0.001**
	F_C	Week	G_M = 0.050*Week + 0.029	0.90	**<0.001**
	F_I W2	Week	G_M = 0.040*Week + 0.071	0.84	**<0.001**
10.5 °C	F_C	CDD	G_M = 0.00055*CDD + 0.011	0.84	**<0.001**
	F_I W2	CDD	G_M = 0.00042*CDD +0.058	0.65	**<0.001**
	F_C	Week	G_M = 0.040*Week + 0.017	0.84	**<0.001**
	F_I W2	Week	G_M = 0.030*Week + 0.063	0.65	**<0.001**
6 °C	F_C	CDD	G_M = 0.00041*CDD + 0.025	0.69	**<0.001**
	F_I W2	CDD	G_M =0.00037*CDD + 0.025	0.72	**<0.001**
	F_C	Week	G_M= 0.017*Week + 0.030	0.69	**<0.001**
	F_I W2	Week	G_M = 0.016*Week + 0.029	0.72	**<0.001**
15 °C	F_C	CDD	C_M = 0.013*CDD + 0.45	0.96	**<0.001**
	F_I W2	CDD	C_M = 0.011*CDD +1.27	0.92	**<0.001**
	F_C	Week	C_M = 1.4*Week + 0.69	0.96	**<0.001**
	F_I W2	Week	C_M = 1.2*Week + 1.5	0.92	**<0.001**
10.5 °C	F_C	CDD	C_M = 0.012*CDD + 0.71	0.94	**<0.001**
	F_I W2	CDD	C_M = 0.0093*CDD +1.6	0.85	**<0.001**
	F_C	Week	C_M = 0.90*Week + 0.86	0.94	**<0.001**
	F_I W2	Week	C_M =0.68*Week + 1.7	0.85	**<0.001**
6 °C	F_C	CDD	C_M = 0.010*CDD + 0.69	0.86	**<0.001**
	F_I W2	CDD	C_M = 0.0091*CDD +0.83	0.83	**<0.001**
	F_C	Week	C_M = 0.43*Week + 0.81	0.86	**<0.001**
	F_I W2	Week	C_M =0.39*Week + 0.93	0.83	**<0.001**
15 °C	F_C	CDD	L_F = 0.056*CDD + 187.5	0.78	**<0.001**
	F_I W2	CDD	L_F = 0.044*CDD +191.4	0.57	**<0.001**
	F_C	Week	L_F = 5.8*Week + 188.5	0.78	**<0.001**
	F_I W2	Week	L_F = 4.5*Week + 192.2	0.57	**<0.001**
10.5 °C	F_C	CDD	L_F = 0.072*CDD + 187.5	0.68	**<0.001**
	F_I W2	CDD	L_F = 0.052*CDD +193.6	0.57	**<0.001**
	F_C	Week	L_F = 5.2*Week + 188.4	0.68	**<0.001**
	F_I W2	Week	L_F = 3.8*Week + 194.2	0.57	**<0.001**
6 °C	F_C	CDD	L_F = 0.064*CDD + 185.3	0.37	**<0.001**
	F_I W2	CDD	L_F = 0.060*CDD +184.3	0.35	**<0.001**
	F_C	Week	L_F = 2.7*Week + 186.0	0.37	**<0.001**
	F_I W2	Week	L_F = 2.6*Week + 184.9	0.35	**<0.001**

* F_C (constant feeding), F_I (interrupted feeding). ǂ CDD (Cumulative degree day).

Chapter 4.

Experimental investigation of the effects of feeding regime on post-smolt scale growth in Atlantic salmon (*Salmo salar* L.).

To be submitted as:

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Experimental investigation of the feeding regime on post-smolt scale in Atlantic salmon (*Salmo salar* L.).

4.1 Abstract

Atlantic salmon (*Salmo salar* L.) post-smolts were reared in a controlled laboratory experiment for 12 weeks following fluorescent marking and transfer to seawater. Fish were exposed to one of four feeding treatments: constant feeding, starved for 7-days (W1 interrupted feeding), starved for 14-days (W2 interrupted feeding) and starved intermittently for four periods of 7-days (28-days total) (W4 interrupted feeding). Significant differences in somatic growth, scale growth and circuli deposition rates were observed between the constant feeding treatment and the latter two interrupted feeding treatments. Across all treatments, scale growth rates and circuli deposition rates were proportional to fish growth rates. However, circuli spacing did not reflect growth rate. The highest somatic, scale growth and circuli deposition rates were observed in the constant feeding treatment, followed by the W1 interrupted feeding, W2 interrupted feeding and W4 interrupted feeding treatments, respectively. Daily scale growth and circuli deposition rates were described using linear regression, the regressions from chapter three were incorporated into this chapter also. Thus, this study highlights the importance of incorporating feeding history when investigating scale growth.

4.2 Introduction

The immediate period after sea entry is a critical stage in the life history of Atlantic salmon (*Salmo salar* L.). Following the demanding physiological smoltification process and migration period, post-smolts have variable and even depleted energy reserves (McCormick *et al*., 1998; Steffansson *et al*., 2003); therefore, successful foraging is of the utmost importance for growth, condition and survival during this initial stage (Levings *et al*., 1994; Thorpe, 1994; Haugland *et al*., 2006).

Atlantic salmon populations have been in decline over recent decades across their entire range (Parrish *et al*., 1998; Klemetsen *et al*., 2003; Jonsson and Jonsson 2004). Declines have been more pronounced in southern populations compared to their northern equivalents (Potter *et al*., 2004; Chaput, 2012; Jensen *et al*., 2012; Mills *et al*., 2013). Key factors associated with this demise are linked to warming sea surface temperatures (Todd *et al*., 2008) coupled with reduced prey availability and the changing spatial and temporal distribution of prey species (Rikardsen *et al*., 2004; Haugland *et al*., 2006). Numerous investigative studies suggest that poor growth during the post-smolt stage is directly linked to high rates of marine mortality and diminished recruitment (Peyronnet *et al*., 2007; Friedland *et al*., 2009).

Studies have also indicated that post-smolt growth and survival are intrinsically linked to ocean climate (Reddin and Shearer, 1987; Friedland *et al*., 1993, 1998; 2003; Jonsson and Jonsson, 2004; Todd *et al*., 2008), and between spawning stock biomass (SSB) of pelagic fish, plankton abundance and adult return rates have also been

detected. Jensen *et al*. (2012) suggest that annual variation in the post-smolt growth rate in the initial few months at sea, is directly influenced by food availability rather than sea surface temperature (SST). They showed negative correlations between pelagic fish abundance SSB and post-smolt growth over a four-year period in the feeding areas at the Vøring Plateau in the Norwegian Sea, whereas no link between SST and post-smolt growth was found during this same period. Beaugrand and Reid (2003) correlated changes in the plankton abundance with the European salmon recruitment rates, while Hvidsten *et al.* (2009) found a significant correlation between the proportion of fish larva in post-smolt stomachs and the abundance estimate of returning adult fish to the River Orkla in a Norwegian Fjord system. There is substantial evidence; therefore, that variability in feeding conditions during the marine phase can shape the dynamics of salmon populations and could contribute to observed declines.

Scale analysis has been extensively used to reconstruct growth history in Atlantic salmon (Friedland *et al*., 1993; Peyronnet *et al.*, 2007; McCarthy *et al*., 2008; Hubley *et al*., 2008; Friedland *et al*., 2009; Jensen *et al*., 2012; Todd *et al*., 2014). A positive correlation between the rates of scale growth and fish growth appears to be a common feature among fish (Fisher and Pearcy, 1990; Nicieza and Brãna, 1993; Fukuwaka, 1998; Heidarsson *et al*., 2006; Beakes *et al*., 2014; Walker and Sutton, 2016). Therefore, scales provide an invaluable chronological record that can be used to interpret the salmon's exploitation of the environment. Recent developments in image analysis allow for the investigation of growth rate at fine temporal scales. The resulting

estimates may then be compared with environmental and biological indicators to identify drivers of change in Atlantic salmon growth and recruitment (McCarthy *et al*., 2008; Friedland *et al*., 2009; Jensen *et al*., 2012).

Many previous studies have focused on the importance of temperature in shaping Atlantic salmon population characteristics (Friedland *et al*., 1993, 1998, 2003), and in assessing the predominant prey groups foraged by Atlantic salmon post-smolts (Holst *et al*., 1996; Shelton *et al*., 1997; Jacobsen and Hansen, 2000; Haugland *et al*., 2006). However, there are few studies linking feeding and food availability with scale growth rates of Atlantic salmon.

Experimental evidence confirms that the influence of temperature on fish growth during the marine phase is reflected in scale growth and circuli number (chapter three, of this thesis). The relationship between scale growth and fish growth is not affected by a 2-week period of food deprivation. It is not known if more prolonged or repeated periods of starvation could disrupt the relationship or lead to an obscuring of scale circuli. Therefore, the objective of this study was to investigate the effects of different feeding regimes on somatic growth, scale growth and circuli formation on scales of Atlantic salmon post-smolts reared under controlled experimental conditions. The results will inform interpretations of growth characteristics in scales of wild Atlantic salmon in relation to changes in fish growth and relationships with environmental variables.

4.3 Methods

All experimental work using Atlantic salmon was conducted ethically and in accordance with the laws and regulations controlling experiments and procedures on live animals in Norway, following the Norwegian Regulation on Animal Experimentation 1996.

This experiment was conducted at the Institute of Marine Research (IMR) Matre research station in Matredal Norway (60^{o} N) and ran for a duration of twelve weeks from the 22nd of May 2013 to the 14th of August 2013. One-year-old Atlantic salmon smolts from a Norwegian hatchery strain (Aqua Gen AS, Trondheim, Norway) reared at 6 °C ambient freshwater were used for this experiment.

4.3.1 Smolt marking

Prior to the commencement of the experiment, 504 fish [Fork length = 187 ± 12.0 mm (mean ± standard deviation (SD)) and weight = 63.9 ± 11.8 g (mean ± standard deviation (SD))] were starved for 24 hours before being marked by calcein, a fluorochrome dye (wavelength: excitation/emission 495/515 nm) by means of osmotic induction using the Mohler method (Mohler, 2003). A 5% salt solution was prepared by adding non-iodized NaCl to 3.5% saline tank water. A 1% calcein solution was made up by adding calcein powder to freshwater. Sodium bicarbonate was added to this solution until the calcein powder was fully dissolved.

The fish were removed from the holding tank using a hand net and contained within the net until the procedure was complete. Initially the net was immersed in the saline

bath for 3.5 minutes to begin the osmotic process, and then dipped in a bath of freshwater and gently shaken to remove excess salt. Finally, the net was immersed in the calcein bath for a further 3.5 minutes. At this point, 36 smolts were sacrificed, in order to verify that the scales had been sufficiently marked. The remaining 480 fish (hereafter referred to as post-smolt) were transferred to the experimental unit and randomly divided between the experimental marine tanks.

4.3.2 Experimental design

Fish were reared in 1 X 1 m closed marine tanks with a water temperature of 10.5 °C, salinity of 35‰ and a dissolved oxygen level of >90%. To reduce potential thermal stress/shock and mortality, the water temperatures treatments were gradually increased over a period of 48 hours. Once thermal acclimation was reached, temperature was held constant throughout the experiment and automatically controlled throughout. If a fluctuation of ± 1 °C occurred, a sensor sounded within one minute. The experimental temperature was chosen with reference to sea surface temperature (SST) profiles from the SALSEA Merge research surveys (NASCO, 2012). The highest catches of post-smolts occurred within a temperature range of 9 °C to 12 °C. Therefore, 10.5 °C was chosen to represent the mid-range of the temperatures that post-smolts are exposed to during migration and initial habitation within nursery grounds in the wild marine environment.

Eight tanks were held at the experimental temperature and allocated to four feeding treatments. The fish in the first feeding treatment were exposed to a constant feeding

regime throughout the experiment. Fish in the second treatment (W1 interrupted feeding) were starved for 7-days throughout week 8; fish in the third treatment (W2 interrupted feeding) were starved for 14-days from week 7 to the end of week 8 and fish in the final treatment (W4 interrupted feeding) were starved for a total of 28-days; 7-days at weeks 4, 6, 8 and 10. The fish were fed to excess on a commercial dry salmon feed (Nutra Olympic, Skretting AS, Averøy, Norway) using automated revolving feeders (ARVO-TEC T Drum 2000, Arvotec, Huutokoski, Finland) attached to the lid. The photoperiod used in the experiment [(L.D; 24:0) twenty-four hours daylight] reflected the light conditions in the Norwegian Sea during the month of May. Two 18W fluorescent daylight tubes (OSRAM L 18 W/840 LUMILUX, OSRAM GmbH, Augsburg, Germany) mounted under water in the tank center, were used to produce 960 LUX of constant light. The photoperiod and feeders were controlled automatically by electronic software (Normatic AS, Norfjordeid, Norway).

4.3.3 Post-smolt sampling

Sampling was conducted at the beginning of each experimental week. In the interrupted feeding treatments, starvation commenced at the beginning of the experimental week. Therefore, the effect on scale characteristics would become evident on samples obtained during subsequent weeks. Three fish were randomly selected and removed from each tank using a hand net and placed in individual containers containing a lethal dose of the anaesthetic 2-Phenoxyethanol solution (0.6 ml / l). Individual fork lengths (mm) and weights (g) were recorded and fish fins, eyes and the operculum were physically inspected and checked for signs of erosion. Scales

were then removed from the recommended standard location [i.e. three to five rows above the lateral line, diagonally from the posterior edge of the dorsal fin to the anterior edge of the pelvic fin on the left side of the body (Anonymous, 1984)] and stored in pre labeled envelopes.

4.3.4 Scale analysis

Post-smolt scales were wet mounted on glass slides, between a cover glass and viewed using a Leica DMRE fluorescent compound microscope. An I3 filter was used to excite the calcein mark at 495/515 nm. A mercury light box transmitted blue light through the scale to produce a brilliant green mark in the location of the calcein. Images were captured using Image Pro Plus version 7.01 © software. Scale measurements were taken along a straight line transect from the centre of the scale focus to the edge. The distances from the focus to the end of the calcein mark (freshwater growth mm) and from the end of the calcein mark to the scale edge (marine growth mm) were measured. The circuli within the marine portion of the scale were counted (marine circuli number) (Figure 4.1).

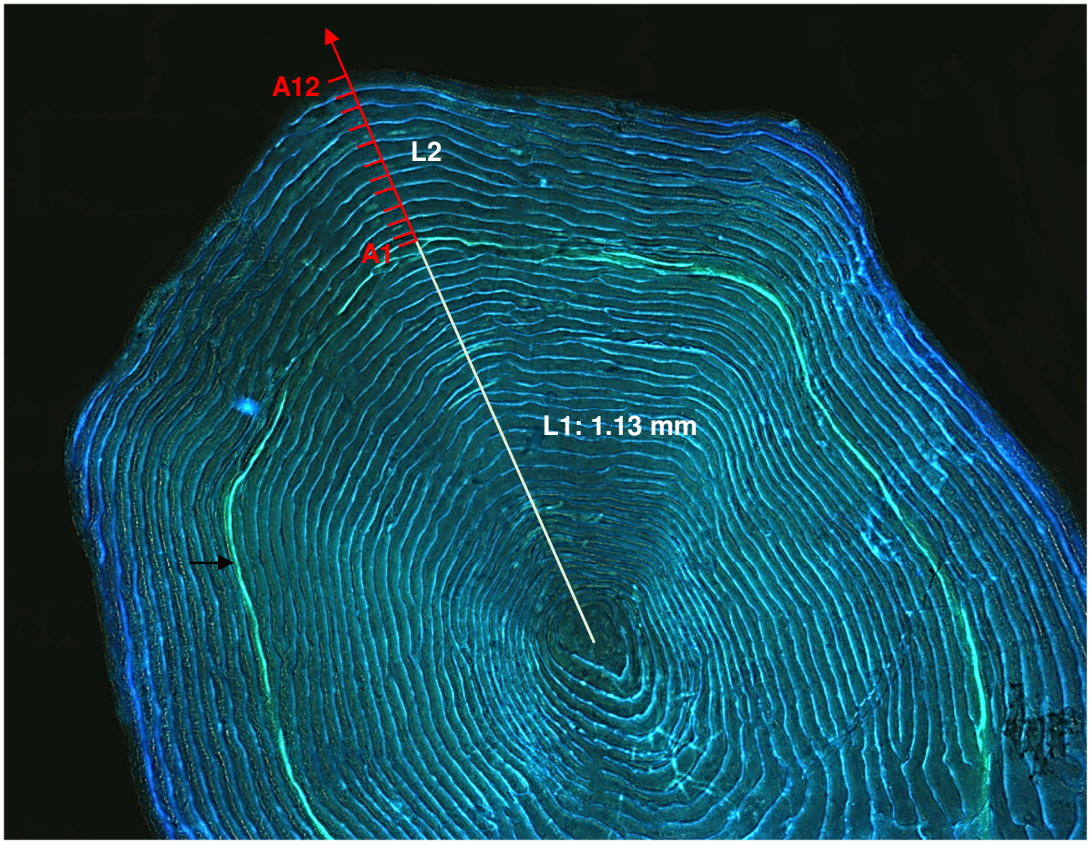

Figure 4.1. Image of a post-smolt scale acquired using fluorescent microscopy, clearly showing the calcein mark (arrow). The 360° straight line axis used when obtaining measurements, coupled with the freshwater transect (L1; length, mm) and marine transect (L2; A1-A12); circuli number and circuli spacing) are illustrated.

4.3.5 Statistical analysis

The analysis was conducted in two stages. Firstly, the effect of varying feeding regimes on fish growth and scale growth was investigated by comparing fork length and scale measurements over the experimental duration (weeks 1 to 12) between the four feeding treatments (constant feeding, W1 interrupted feeding, W2 interrupted feeding and W4 interrupted feeding treatments). In the second stage, the growth measurements derived from the constant feeding treatment were compared against

each of the interrupted feeding treatments separately for the periods after starvation was initiated (weeks 9 to 12 for the W1 interrupted feeding treatment, weeks 8 to 12 for the W2 interrupted feeding treatment and weeks 5 to 12 for the W4 interrupted feeding treatment).

Fork length, freshwater growth, marine growth, circulus spacing and scale radius were compared between treatments using a series of nested ANCOVAs. Freshwater scale growth measurements were compared between treatments to confirm that there were no pre-existing differences in growth that could bias the subsequent marine growth analyses. Treatment was included as the fixed factor and time as the co-variate. Tanks were nested within treatments. If there was no significant difference in growth between tanks within a treatment, data for replicate tanks were pooled and the analysis was re-run.

Marine circulus deposition rate (CDR_{Day}) was calculated by dividing the day number at time of sampling by the number of circuli post calcein mark produced on the scale. Circuli deposition rates were compared between feeding treatments using one-way ANOVAs. Kruskal-Wallis tests were performed when variables were either non-normally distributed and/or displayed unequal variances.

The relationship between circulus spacing and circuli number was compared between feeding treatments using a series of repeated measure ANCOVAs. Treatment was included as a fixed factor and fish ID as a random factor and circuli number as the co-variate.

All statistical analysis was conducted using the MINITAB statistical package. An alpha level of 0.05 was used for all significance tests.

4.4 Results

The mortality rate was monitored throughout the experiment. A mortality rate of 1.9% occurred in the initial 24 hours Consequent to this, the mortality rate was negligible throughout the remainder of the experiment (Table 4.1). Scale growth measurements for each feeding treatment are summarised in Table 4.2. ANCOVA confirmed that there were no differences in freshwater growth between any of the feeding treatments ($p=0.119$), therefore, there were no pre-existing differences in growth that could bias comparisons of marine growth and circuli deposition rates. There were no significant differences in growth between the constant feeding treatment and each of the interrupted feeding treatments prior to the individual starvation regimes (ANCOVA, $p\geq0.162$). This confirmed that fish across all feeding treatments had grown at the equal rates prior to the starvation period.

4.4.1 Fork length

Fish from the constant feeding treatment had the largest fork length [mean ± standard deviation (SD) mm] (222.5 ± 22.1) followed by the W1 interrupted feeding treatment (219.4 ± 19.9) the W2 interrupted feeding treatment (218.9 ± 17.6) and W4 interrupted feeding treatment (213.4 ± 18.5) (Table 4.2).

When the whole experimental period was examined, some differences in fish growth rates was observed between feeding treatments. The ANCOVA confirmed that the slope of the relationship between fork length and week number did not differ significantly between the constant feeding and W1 interrupted feeding treatment (ANCOVA, p=0.383) or between the constant feeding and W2 interrupted feeding treatment (Kruskal-Wallis, p=0.275). A significant difference was evident between the constant feeding and W4 interrupted feeding treatment [ANCOVA, p=0.009; Figure 4.2 (a-c), Table 4.2].

The rate at which fish length increased from weeks 9 to 12 between the constant feeding and W1 interrupted feeding treatment showed little variation [Figure 4.2 (a); Table 4.2]. There was no significant difference in the slope of the relationship between fish fork length and time (week 9 to 12) (ANCOVA, p=0.104) or the main effect of feeding between the two feeding treatments (ANCOVA, p=0.391).

The effect of starvation on fork length was evident when the constant feeding treatment and the W2 interrupted feeding treatments were compared at weeks 8 to 12. Although ANCOVA confirmed that the slope of the relationship between fork length and time (weeks 8 to 12) did not differ significantly [p=0.709; Figure 4.2 (b); Table 4.2], the main effect of feeding treatment was significant [p=0.004; Figure 4.2 (b); Table 4.2]. Growth was higher in the continuous feeding treatment by 9.4 ± 3.0 [mean difference ± standard deviation (SD) mm] compared to the W2 interrupted feeding treatment.

A starvation effect was also observed when the constant feeding and W4 interrupted feeding treatment at weeks 5 to 12 were compared. The slope of the relationship between fork length and time (weeks 5 to 12) differed significantly between the two feeding treatments (ANCOVA, p=0.001), the main effect of feeding treatment was also significant (ANCOVA, p=0.004). Growth was significantly higher in the continuous feeding treatment compared to the W4 interrupted feeding treatment with a mean difference of 12.6 ± 3.6 [mean difference ± standard deviation (SD) mm] found [Figure 4.2 (c)].

ANCOVA confirmed that the slope of the relationship between fish length and scale radius did not differ significantly between the constant feeding treatment and W1, W2 and W4 interrupted feeding treatments over the entire experimental duration and pre / post starvation periods [p=0.379; Figure 4.2 (d)] indicating that the proportionality of scale growth and fish growth were not influenced by feeding regime.

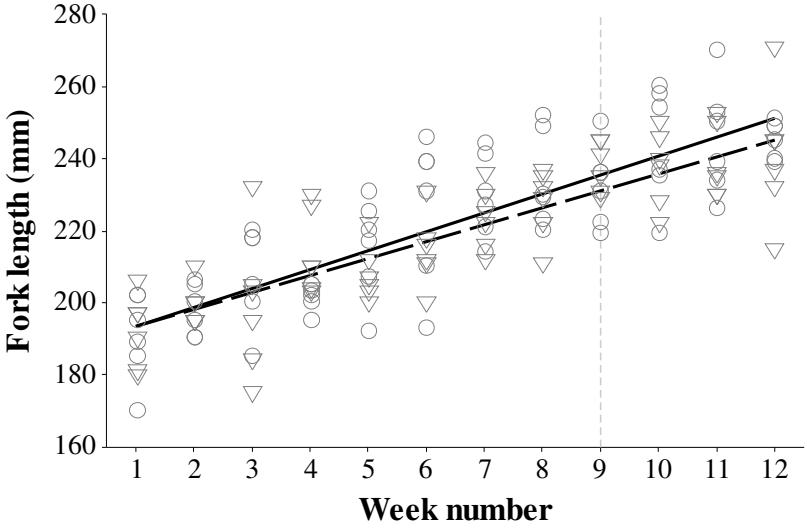

Figure 4.2 (a)

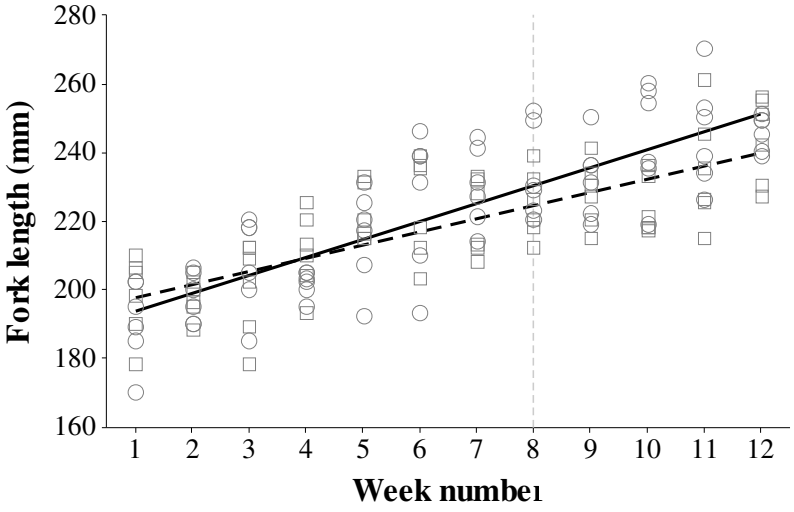

Figure 4.2 (b)

Figure 4.2 (a, b). Fork length (mm) against time (a) treatment; F_C and W1 (b) treatment; F_C and W2 [○—— ,($F_{C;}$ constant feeding); ▽ — — ,(W1; 1 week interupted feeding); □ – – – ,(W2; 2 week interupted feeding); Reference lines indicate the point at which the effect of starvation was observed on the scale; - - - -].

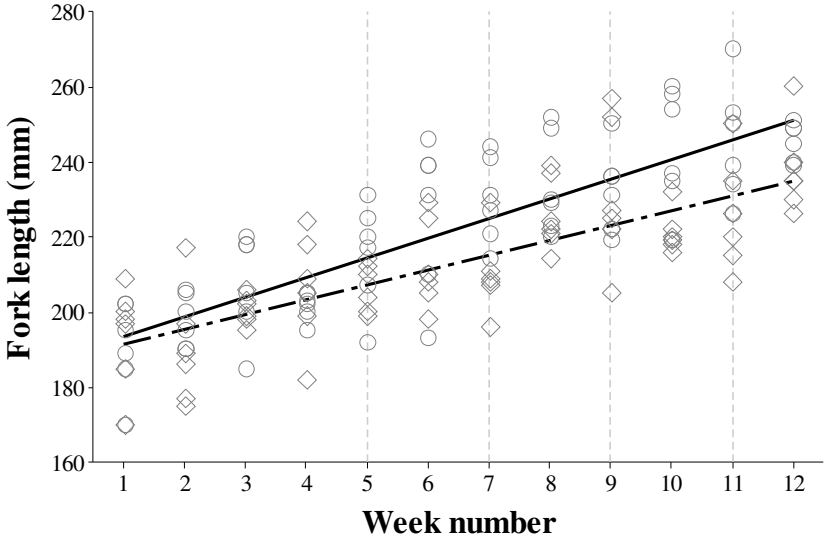

Figure 4.2 (c)

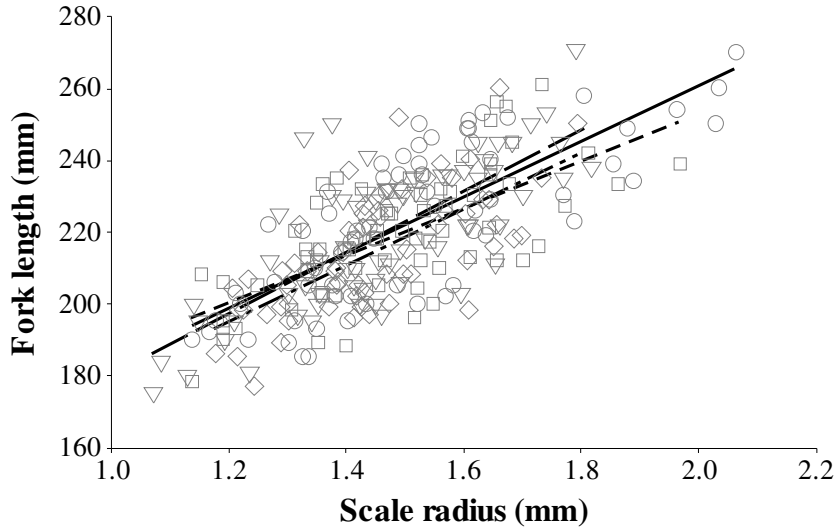

Figure 4.2 (d)

Figure 4.2 (c, d). Fork length (mm) against time (c) treatment; F_C and W4. (d) Fork length (mm) / scale radius (mm) per feeding treatment [○ ——— ,($F_{C;}$ constant feeding); ▽ ——— ,(W1; 1 week interupted feeding); □ – – – ,(W2; 2 week interupted feeding); ◇ — – ,(W4; 4 alternate week interupted feeding); Reference lines indicate the point at which the effect of starvation was observed on the scale; - - - -].

4.4.2. Marine growth

The highest scale growth [mean± standard deviation (SD) mm] was observed in both the constant feeding (0.28 ± 0.15) and the W1 interrupted feeding treatment (0.28 ± 0.15) followed by the W2 interrupted feeding treatment (0.26 ± 0.13) and W4 interrupted feeding treatment (0.23 ± 0.10) [Figure 4.3 (a-c); Table 4.2].

When the whole experimental period was examined, some variation in scale growth rates was observed between feeding treatments. ANCOVA confirmed that there was a significant difference in the slope of the relationship between marine growth and week number between the constant feeding and W2 interrupted feeding treatments ($p<0.001$), indicating that scale growth rate was reduced by the two-week starvation period [Figure 4.3 (b)]. The slope of the relationship between marine growth and week number was not significantly different between the constant feeding and the W4 interrupted feeding treatment (ANCOVA, $p=0.120$). However, when the constant feeding and W4 interrupted feeding treatments were compared, a significant difference in the intercept of the marine growth-week number relationship was detected ($p<0.001$), reflecting the fact that starvation was initiated earlier in the experiment (week 4). The marine growth measurements were significantly lower in scales from the W4 interrupted feeding treatment compared to the constant feeding treatment [Figure 4.3 (c); Table 4.2]. When the constant feeding and W1 interrupted feeding treatments were compared, neither the slope (ANCOVA, $p=0.628$) nor the intercept (ANCOVA, $p=0.544$) of the relationship between marine growth and week number was significantly different, indicating that one-week of starvation did not significantly impact scale growth rate.

ANCOVA showed that the slope of the relationship between marine growth and time (week 9-12) did not differ significantly between the constant feeding and the W1 interrupted feeding treatments (ANCOVA, $p=0.150$). The main effect of feeding treatment was also not significant ($p=0.653$). This confirmed that the one-week starvation period did not have a significant effect on scale growth. However, effects of a two-week period of starvation on scale growth were evident. When the constant feeding and W2 interrupted feeding treatments were compared at weeks 8 to 12, the slope of the relationship between marine growth and time (week 8 to 12) did not differ significantly [ANCOVA, $p=0.940$; Figure 4.3(b)] but the intercept was significantly different ($p=0.003$). Growth was significantly higher by 18% in the continuous feeding treatment compared to the W2 interrupted feeding treatment (Table 4.2).

The effects of starvation were also evident when the constant feeding treatment was compared to the W4 interrupted feeding treatment at weeks 5 to 12. ANCOVA confirmed that the slope of the relationship between marine growth and time significantly differed between the two feeding treatments [ANCOVA, $p=0.006$; Figure 4.3 (c)]. The main effect of feeding treatment was also significant ($p<0.001$). Growth was higher by 28% in the continuous feeding treatment compared to that of the interrupted feeding treatment (Table 4.2).

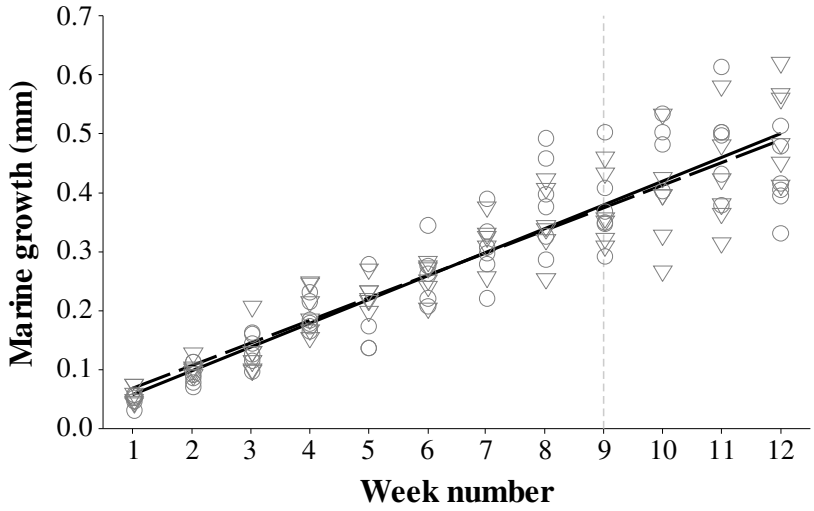

Figure 4.3 (a)

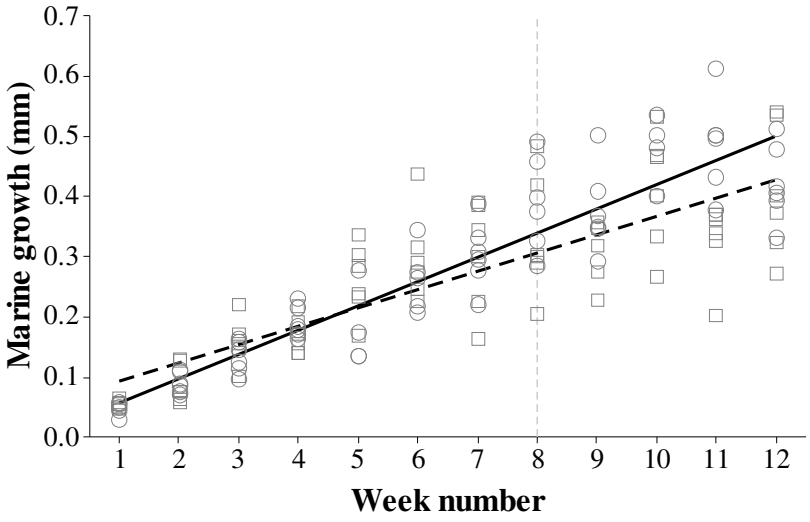

Figure 4.3 (b)

Figure 4.3 (a, b). Marine growth (mm) against time (a) treatment F_C and W1 (b) treatment F_C and W2 [○ ——— , (F_C; constant feeding); ▽ — — ,(W1; 1 week interupted feeding); □ ——— ,(W2; 2 week interupted feeding; Reference lines indicate the point at which the effect of starvation was observed on the scale; - - - -].

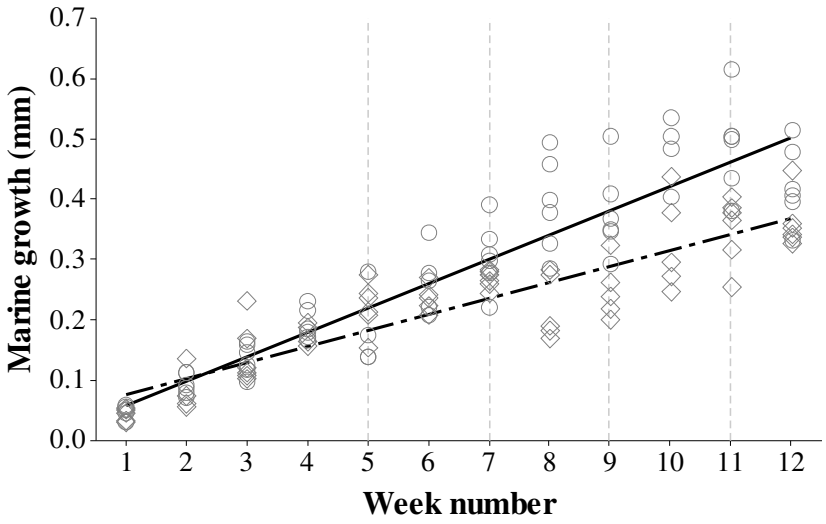

Figure 4.3 (c)

Figure 4.3 (c). Marine growth (mm) against treatment; F_C and W4 [○—— ,(F_C; constant feeding); ◇ — – ,(W4; 4 alternate week interupted feeding); Reference lines indicate the point at which the effect of starvation was observed on the scale; - - - -].

4.4.3 Marine circuli number

The rate of circuli deposition decreased due to starvation; the mean numbers of circuli [mean± standard deviation (SD)] recorded in the scales over the duration of the experiment was highest in the constant feeding treatment (6.7 ± 3.3) followed by the W1 interrupted feeding (6.6 ± 3.0), W2 interrupted feeding (6.2 ± 2.6) and W4 interrupted feeding treatments (6.0 ± 2.7) [Figure 4.4 (a-c); Table 4.2].

When all weeks were analysed; CDR_{Day} did not differ significantly between the constant feeding and W1 interrupted feeding treatments (ANOVA, p=0.665), or between the constant feeding and W2 interrupted feeding treatments (Kruskal-Wallis, p=0.075). A significant difference was detected between the constant feeding and W4

interrupted feeding treatments. CDR_{Day} was faster in the constant feeding treatment compared to the W4 interrupted feeding treatment by 15% (ANOVA, $p<0.001$).

CDR_{Day} was not significantly affected by feeding manipulation between the constant feeding and W1 interrupted feeding treatment from weeks 9 to 12 [ANOVA, $p=0.184$; Figure 4.4 (d)]. The effects of starvation on CDR_{Day} became evident when the constant feeding and W2 interrupted feeding treatments were compared at weeks 8 to 12. CDR_{Day} was significantly faster by 18% in the continuous feeding treatment compared to the W2 interrupted feeding treatment [ANOVA, $p<0.001$; Figure 4.4 (d)]. Also, when the continuous feeding and W4 interrupted feeding treatments were compared at weeks 5 to 12, CDR_{Day} was significantly faster in the continuous feeding treatment compared to the W4 interrupted feeding treatment by 15% [Kruskal-Wallis, $p<0.001$; Figure 4.4 (d)].

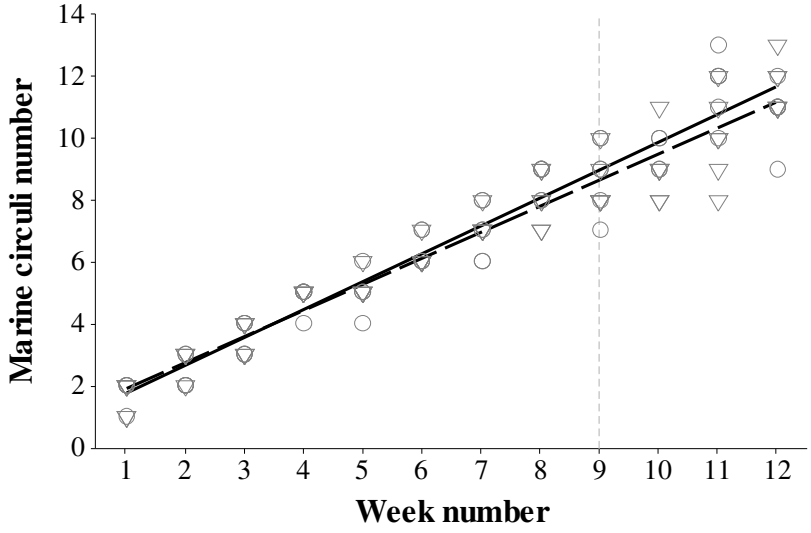

Figure 4.4 (a)

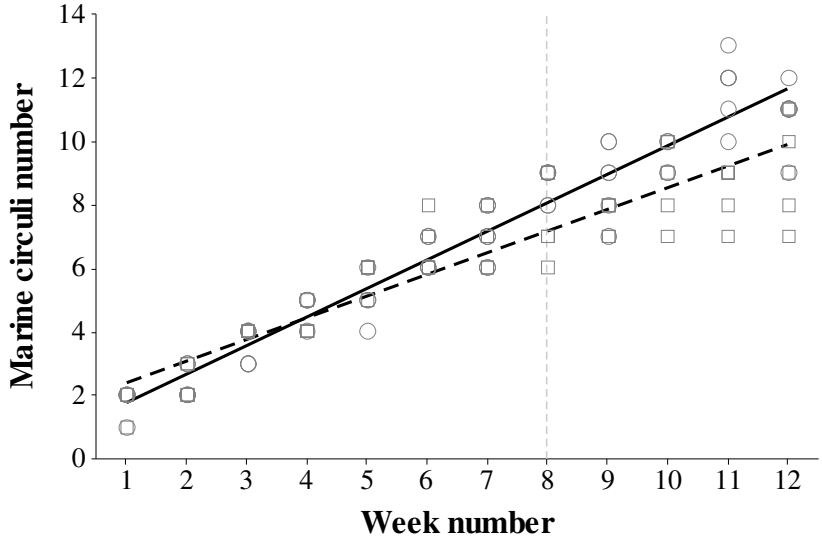

Figure 4.4 (b)

Figure 4.4 (a, b). Marine circuli number against time (a) treatments; F_C and W1 (b) treatments; F_C and W2 [○ —— ,(F_C; constant feeding); ▽ — — ,(W1; 1 week interupted feeding); □ – – – ,(W2; 2 week interupted feeding); Reference lines indicate the point at which the effect of starvation was observed on the scale; - - - -].

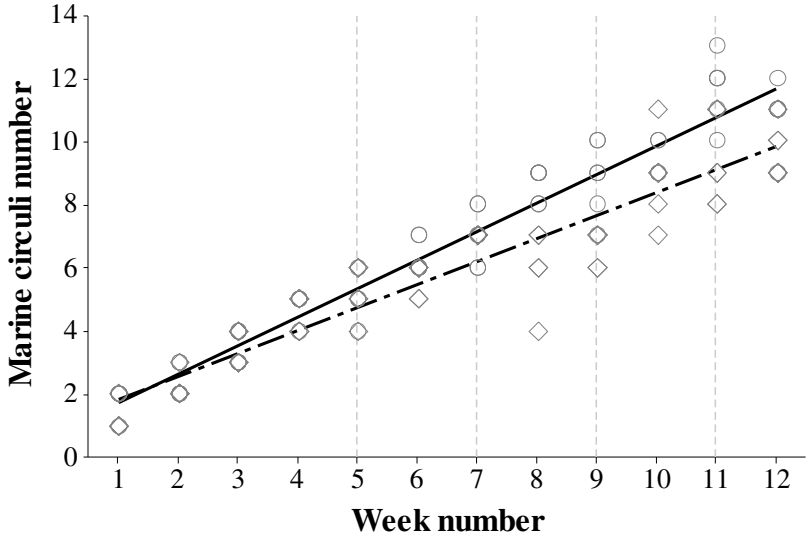

Figure 4.4 (c)

Figure 4.4 (c). Marine circuli number against time; treatments F_C and W4 [○——, (F_C, constant feeding); ◇ —— –,(W4; 4 alternate week interupted feeding); Reference lines indicate the point at which the effect of starvation was observed on the scale; - - - -].

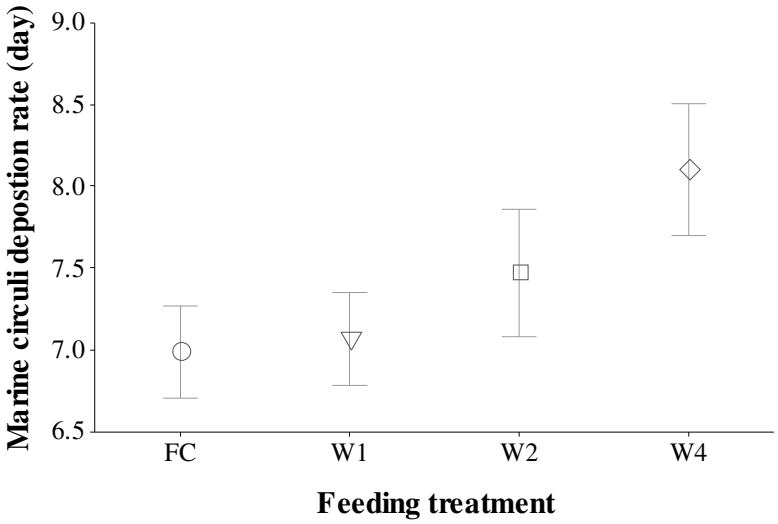

Figure 4.4 (d)

Figure 4.4 (d). Marine circulus deposition rate / day per feeding treatment [○,(F_C; constant feeding); ▽ ,(W1; 1 week interupted feeding); □ ,(W2; 2 week interupted feeding); ◇ ,(W4; 4 alternate week interupted feeding)]; Error bars are 95% confidence intervals.

4.4.4 Marine circuli spacing

In all four feeding treatments, circulus spacing increased slightly at the start of the experiment. In the constant feeding treatment, circulus spacings remained relatively constant during the middle of the experiment but narrowed towards the end of the experiment. In the W1 interrupted feeding treatment circulus spacing measurements narrowed from circulus seven, increasing in width again by circulus 10. The circulus spacing measurements in the W2 interrupted feeding treatment decreased in width from circulus 6, remaining at a similar width through circulus 7 to 10 with an increased width at the final circulus. In the W4 interrupted feeding treatment circulus spacing

steadily decreased from circuli 4 with a slight increase in width at circulus 10 before decreasing again for the final circulus.

When all weeks were assessed, the ANCOVA confirmed that the slope of the circulus spacing/circulus number relationship (feeding treatment*circulus number) was not significantly different between constant feeding and the W1 interrupted feeding (ANCOVA, p=0.601) or between the constant feeding and W2 interrupted feeding treatments (ANCOVA, p=0.457). The main feeding effect was also not significant between the constant feeding and W1 interrupted feeding treatments (ANCOVA, p=0.296) or the constant feeding and W2 interrupted feeding treatments (ANCOVA, p=0.206) [Figure 4.5 (a, b); Table 4.2]. The slope of the circulus spacing/circulus number relationship was significantly different however, between the constant feeding and the W4 interrupted feeding treatment (ANCOVA, p=0.003). A significant difference was further detected in the main feeding effect, the constant feeding treatment displayed 10% wider circuli spacings compared to the W4 interrupted feeding treatment [p=0.013; Figure 4.5 (c)].

No significant differences in the slopes (feeding treatment*circulus number) or intercepts (feeding treatment) were found between the constant feeding and the W1 interrupted week 9 to 12 (ANCOVA, p=0.204) or between the constant feeding and W2 interrupted feeding treatments from week 8 to 12 (ANCOVA, p=0.350). This suggests that the short starvation event did not affect the width between the circuli [Figure 4.5 (a, b); Table 4.2]. Starvation had a negative effect on circuli spacing

between the constant feeding and W4 interrupted feeding treatments from week 5 to 12. The ANCOVA confirmed that the slope of the circulus spacing/circulus number relationship was not significantly different between the two treatments (ANCOVA, feeding treatment*circulus number, p=0.50). However, the main feeding effect was significant; circulus spacings were 10% wider in scales from the constant feeding treatment than in the W4 interrupted feeding treatment [ANCOVA, p=0.002; Figure 4.5 (c); Table 4.2].

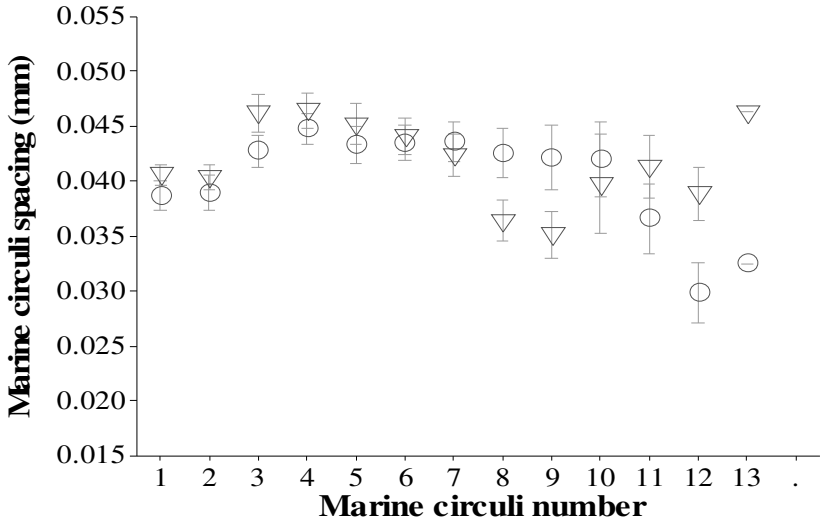

Figure 4.5 (a)

Figure 4.5 (a). Marine circulus spacing (mm) per circuli number; treatment; F_C and W1 [○,(F_C; constant feeding); ▽ ,(W1; 1 week interupted feeding)]; Error bars are 95% confidence intervals.

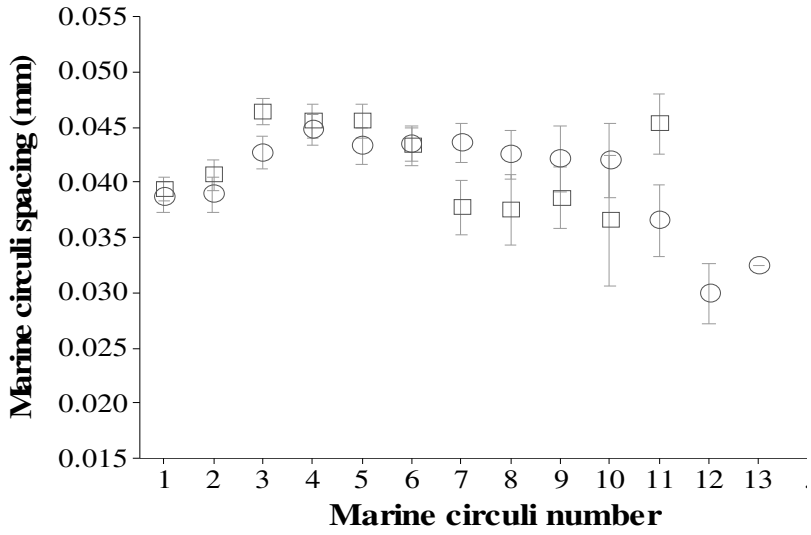

Figure 4.5 (b)

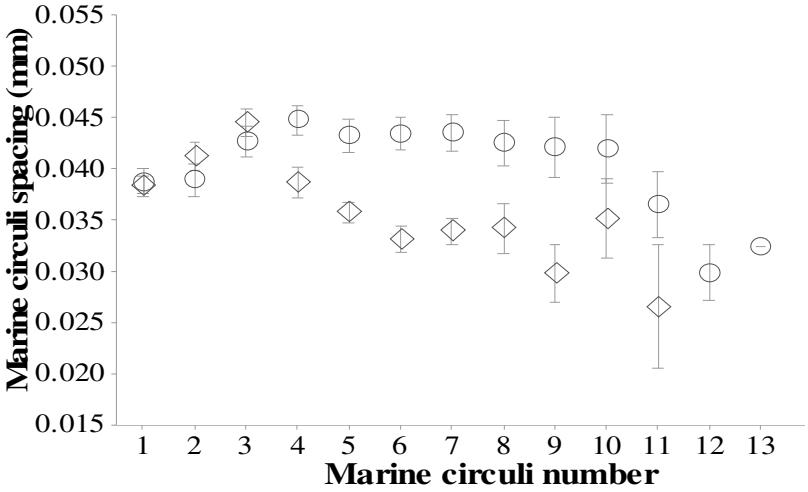

Figure 4.5 (c)

Figure 4.5 (b, c). Marine circulus spacing (mm) per circuli number (b) treatment; F_C and W2 (c) treatment; F_C and W4 [○,($F_{C;}$ constant feeding); □ ,(W2; 2 week interupted feeding); ◇ ,(W4; 4 alternate week interupted feeding)]; Error bars are 95% confidence intervals.

4.4.5 Daily growth rates

The relationships between day and each of the growth variables were described for each treatment using linear regression. These equations were combined with the relationships derived from the temperature experiments in chapter three (Table 4.3). The slopes of each regression were used to provide an estimate of daily fish and scale growth rates and circuli deposition rates for each combination of temperature and feeding conditions. Mean circulus spacing at each circulus number was also calculated for each treatment. Estimated scale growth rates, circuli deposition rates and circulus spacing values were regressed against fish growth rates to determine if the proportionality between scale measurements and fish growth was constant across treatments.

Estimated daily fish growth rates were strongly correlated with daily scale growth rates (R^2=0.96) confirming that the proportionality of fish growth and scale growth was constant across all the experimental treatments [Figure 4.6 (a)]. Daily fish growth rate was correlated with circuli deposition rate but the correlation was not as strong as that with scale growth rate (R^2=0.81). Circuli deposition rates in the 15 °C (constant and interrupted feeding treatments) were considerably higher than predicted while circuli deposition rates in the 6 °C and 10.5 °C treatments were lower than predicted [Figure 4.6 (b)]. When the higher temperature treatments were exlcuded, the regression fit improved considerably (R^2=0.99). The results suggest that at 15 °C there was a decoupling of scale growth and circuli deposition.

There was no correlation between daily fish growth rate and mean circulus spacing [R^2 =0.10; Figure 4.6 (c)].

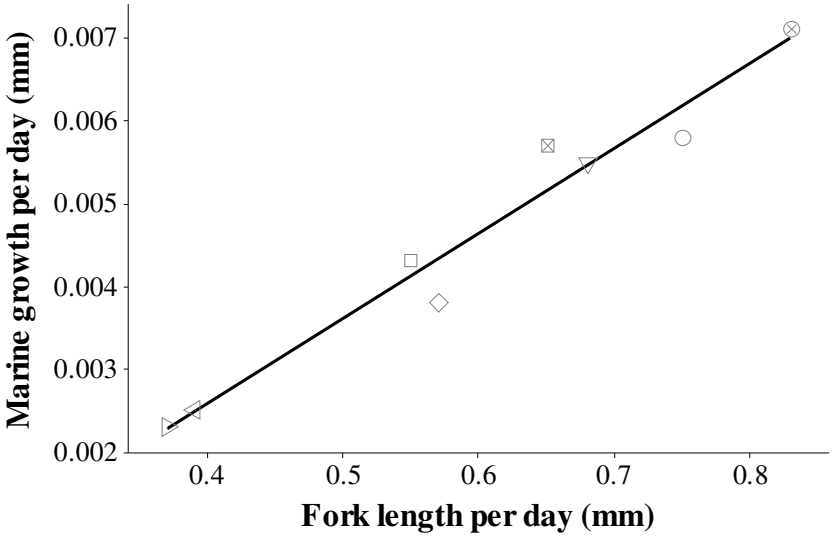

Figure 4.6 (a)

Figure 4.6 (a). Marine growth per day (mm) / fork length per day (mm) [○, (10.5 °C; $F_{C;}$ constant feeding); ▽ , (10.5 °C; W1; 1 week interupted feeding); □ , (10.5 °C; W2; 2 week interupted feeding); ◇,(10.5 °C; W4; 4 week alternate interupted feeding); ⊗ ,(15 °C*;* $F_{C;}$ constant feeding); ⊠ , (15 °C; W2; 2 week interupted feeding) ◁ ,(6 °C*;* $F_{C;}$ constant feeding); ▷ , (6 °C; W2; 2 week interupted feeding)].

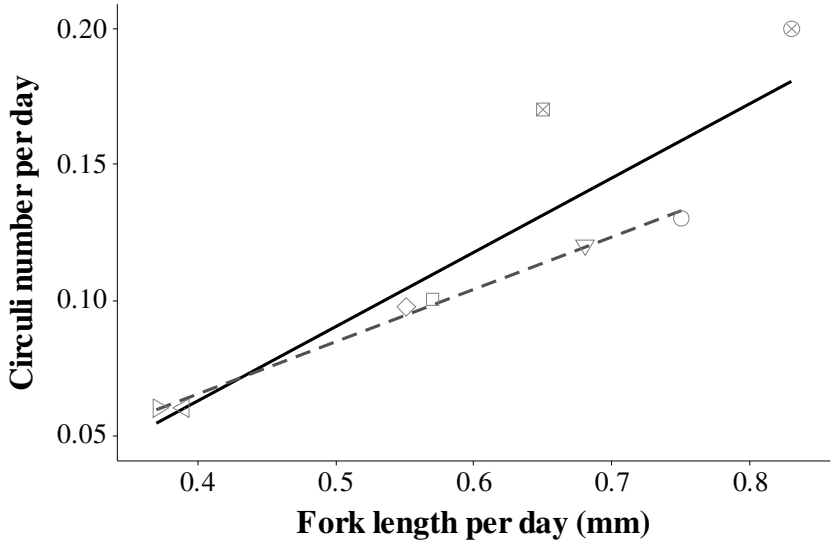

Figure 4.6 (b)

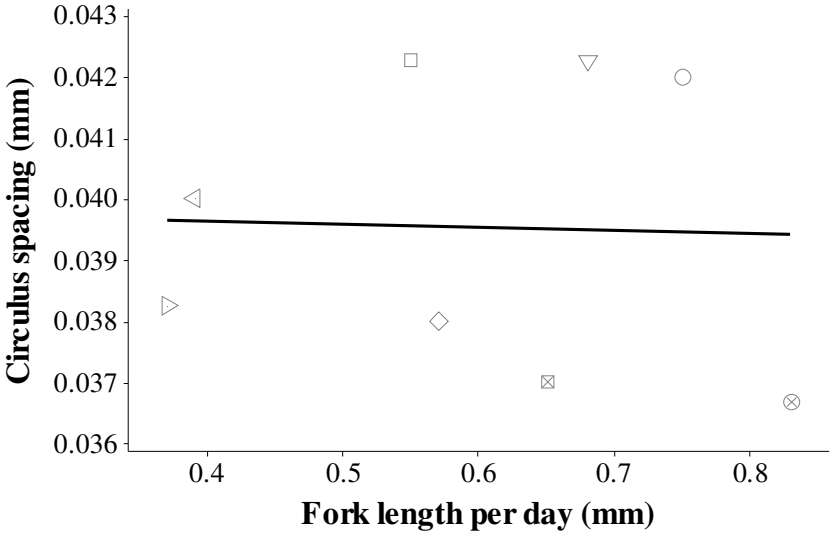

Figure 4.6 (c)

Figure 4.6 (b, c). (b) Marine circuli number per day / fork length per day (mm) (c) Marine circulus spacing (mm) / fork length per day (mm) [○, (10.5 °C; $F_{C;}$ constant feeding); ▽, (10.5 °C; W1; 1 week interupted feeding); □ ,(10.5 °C; W2; 2 week interupted feeding); ◇,(10.5 °C; W4; 4 week alternate interupted feeding); ⊗ ,(15 °C*;* $F_{C;}$ constant feeding); ⊠ **,**(15 °C; W2; 2 week interupted feeding) ◁ **,**(6 °C*;* $F_{C;}$ constant feeding); ▷ ,(6 °C; W2; 2 week interupted feeding)].

4.5 Discussion

This study investigated the effect of food availability on somatic growth and scale growth of Atlantic salmon post-smolts during early marine habitation. The results show that fish growth and scale characteristics were influenced by feeding conditions during rearing. Scale growth and circuli number were not negatively impacted by the seven-day starvation event; however, a decrease in both was evident when the duration of starvation was increased. The differences in scale growth rates between treatments corresponded to the differences found in body growth rates. To further investigate this result, the daily fish growth and marine growth rates established in chapter three where integrated with the daily growth rates established during this study. Figure 4.6 (a) clearly indicates that scale growth during the marine phase is proportional to fish growth, across the range of temperature and feeding conditions examined. This further supports the use of scale measurements to infer fish growth rates.

There was little correlation between circulus spacing and fish growth rate. In this study, narrow circuli spacings were observed during periods of slow growth corresponding to periods of intermittent feeding. In chapter three, narrow circulus spacings coincided with fast growth at high temperatures. These findings highlight the importance of considering environmental factors when employing scale measurements to reconstruct fish growth.

Scale radius measurements from Atlantic salmon are regularly used to reconstruct growth rates, particularly during the post-smolt period to the first sea winter (Friedland *et al*., 2000, 2009). The results of this and the previous chapter support the use of scale

radius measurements as a proxy for growth rates across a range of temperature and feeding conditions.

Circuli deposition rates are estimated to be 7 d circulus $^{-1}$ in summer and 14 d circulus $^{-1}$ during winter (Hubley *et al*., 2008; Friedland *et al*., 2009) while Jensen *et al.* (2012) estimated a formation rate of 6.3 d circulus $^{-1}$ during the initial few months of marine residency. In this study, circuli deposition rates were comparable with these given estimates, varying from 7.0 d circulus $^{-1}$ in the constant feeding treatment to 8.1 circulus $^{-1}$ in the W4 interrupted feeding treatment. The results confirm that circuli deposition rate is dependent on both temperature and feeding rate. To investigate this further, the daily growth rates between fork length and circuli deposition were compared using the results from this chapter plus four treatments from chapter three. Although the initial relationship was good at R^2=0.81, it was evident that the points relating to the 15 °C treatments deviated from the overall trajectory of growth rate, suggesting a decoupling of the circuli. Many studies have reported decoupling of otolith and fish growth where otolith growth somatic growth under particular temperature or feeding rates conditions and otolith growth is no longer proportional to somatic growth. Mosegaard *et al.* (1988) reported that Arctic charr, *Salvelinus alpinus* (L.) otolith growth rates became decoupled from somatic growth rates due to varying temperatures. Decoupling between otolith growth and somatic growth have been documented in larval and juvenile fish (Hare and Cowen, 1995; Takasuka *et al*., 2008; Stormer and Juanes, 2016).

In this instance the accelerated circuli deposition rates observed at 15 °C surpassed the growth rate; therefore, causing a decoupling effect between the circuli deposition rate and body growth. To clarify that this was the case, a second regression fit was included omitting these two stray points and the remaining treatments displayed an excellent correlation suggesting that circuli deposition rates at elevated temperatures are independent of scale and somatic growth rates. Therefore, applying general deposition rates as a means of assessing and reconstructing growth histories of Atlantic salmon of unknown temperature and feeding histories may produce erroneous results if fish have experienced elevated temperatures of this magnitude during their migration.

Once consistent feeding is achieved, the number of circuli present in the post-smolt portion of a scale reflects thermal history rather than the time in the marine environment (chapter three; Thomas *et al*., in prep). However, starvation exceeding one-week reduces the number of circuli deposited. Large spatial and temporal differences in feeding occur in the marine environment (Rikardsen *et al*., 2004; Haugland *et al*., 2006). This presents a challenge when trying to relate individual circuli to distinct periods of time.

Circuli spacing is also used to interpret growth history with the assumption being that periods of fast growth produce widely spaced circuli in the scale (Fisher and Pearcy, 1990; Friedland *et al*., 2000, 2009; Jensen *et al*., 2012). The results reported in chapter three suggested however, that circuli spacing is not an accurate indicator of growth. At higher temperatures, narrow circuli spacings indicated rapid growth as both scale

growth and circuli deposition rates were significantly higher than the other temperature treatments investigated. However, the circulus spacing amongst the highest temperature treatment produced significantly narrower circulus spacings, which if assessed alone would lead to the assumption of poor growth.

The results of this study further corroborate the assumptions from chapter three, that thermal history is required to fully investigate circulus spacing. In this study the circulus spacings measurements were similar across the continuous feeding and the W2 interrupted feeding treatment throughout the experiment despite the W2 interrupted feeding treatment having significantly lower scale growth and decreased circuli deposition rate. The only indication that narrow circuli spacing reflected decreased growth was in the W4 interrupted feeding treatment; therefore, a narrowing of scale circuli may indicate faster growth due to elevated temperatures or slower growth due to prolonged periods of low food availability.

Figure 4.6 (c) which incorporated the daily growth rates established during this study and that of chapter three further highlights that circuli spacing is not truly representative of growth rate as no correlation is evident between fish growth rates and circuli spacings. Hence, the experimental evidence shows that circulus spacing is not reflective of growth rate. This corroborates the results reported by Peyronnet *et al.* (2007) who found that in returning one-sea winter Atlantic salmon, mean circulus spacing was lower during a period of high growth (1980's) compared to a period of slow growth (1990's) and suggested that this measure may not be a reliable indicator of fish growth, particularly during poor growth conditions.

In this study, temperatures in each treatment were held constant at 10.5 °C. Apart from the stated starvation periods conducted in the interrupted feeding treatments, fish were fed in excess and all other conditions were stable throughout the experiment. The experimental conditions may not be directly comparable with conditions experienced by wild Atlantic salmon in the natural environment. Fish in the wild may be exposed to more severe food shortages than reflected in this experiment. Due to the low mortality rate throughout this experiment, the extent to which severe food shortages would affect somatic growth, scale characteristics and survival were not fully achieved and further investigative studies with extended starvation periods would be required to fully understand the extent to which starvation effects Atlantic salmon.

The results of this study confirm that feeding influences somatic growth, scale growth and circuli patterns. Circuli spacing is not a reliable indicator of growth rate. The fourteen-day starvation period decreased growth and circuli deposition rates but did not affect the circuli spacing. The study highlights the importance of considering prey abundance and feeding history when interpreting scale measurements and further investigation is required to assess the impact of prolonged or repeated starvation on scale and body growth.

Acknowledgements

We thank the scientific and technical personnel of Matre research station, IMR Norway, involved in this experiment.

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Table 4.1. Overview of mortality rate over time per feeding treatment.

	F_C *		W1†		W2‡		W4§	
Week	**M Rate⊥**	**M Rate - 24H‖**	**M Rate⊥**	**M Rate - 24H‖**	**M Rate⊥**	**M Rate - 24H‖**	**M Rate⊥**	**M Rate - 24H‖**
1	2	0	3	0	1	0	3	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	1	1
7	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	1	1
9	0	0	0	0	0	0	0	0
10	0	0	1	1	1	1	1	1
11	0	0	1	1	1	1	0	0
12	0	0	0	0	0	0	1	1

ǂ Treatment; *; F_C (constant feeding), †; W1 (1 week interupted feeding), ‡; W2 (2 week interupted feeding), §; W4 (4 alternate week interupted feeding), ⊥ ; M Rate (mortality rate), ‖; M Rate – 24H (mortality rate excluding the initial 24 hours of experiment).

Table 4.2. Results of scale and growth measurements (mean ± SD) per feeding treatment; marine growth; (G_M; mm) marine circuli number (C_M) circuli spacing (S_{CM}; mm), circuli deposition rate per day (CDR_{Day}) and fork length; mm (L_F; mm).

Variable	Treatment*	Mean ± SD			
		Weeks 1 to 12	Weeks 5-12†	Weeks 8-12‡	Weeks 9-12§
G_M	F_C	0.28 ± 0.15	0.37 ± 0.11	0.43 ± 0.080	0.44 ± 0.079
	W1	0.28 ± 0.15	----- -- ------	---- -- ------	0.43 ± 0.096
	W2	0.26 ± 0.13	----- -- ------	0.36 ± 0.094	----- -- ------
	W4	0.23 ± 0.10	0.28 ± 0.071	---- -- ------	----- -- ------
C_M	F_C	6.7 ± 3.3	8.7 ± 2.2	9.9 ± 1.5	10.3 ± 1.5
	W1	6.6 ± 3.02	---- -- -----	---- -- -----	9.8 ± 1.6
	W2	6.2 ± 2.6	---- -- -----	8.4 ± 1.3	---- -- ----
	W4	6.0 ± 2.7	7.3 ± 1.9	---- -- -----	---- -- ----
S_{CM}	F_C	0.040 ± 0.0074	0.042 ± 0.0065	0.043 ± 0.0064	0.043 ± 0.0063
	W1	0.041 ± 0.0063	------- -- ---------	------ -- ----------	0.043 ± 0.0043
	W2	0.041 ± 0.0075	------- -- ---------	0.042 ± 0.0058	------ -- ---------
	W4	0.038 ± 0.0059	0.038 ± 0.0039	------ -- ---------	------ -- ---------
CDR_{Day}	F_C	6.8 ± 1.2	7.2 ± 0.85	7.2 ± 0.84	7.4 ± 0.86
	W1	6.9 ± 1.2	---- -- ----	---- -- -----	7.7 ± 0.15
	W2	7.3 ± 1.7	---- -- ----	8.6 ± 1.3	---- -- ------
	W4	7.9 ± 1.6	8.3 ± 1.5	---- -- -----	---- -- ------
L_F	F_C	222.5 ± 22.1	234.0 ± 16.4	240.2 ± 13.4	241.8 ± 13.2
	W1	219.4 ± 19.9	------- -- ---	------- -- ----	238.7 ± 11.5
	W2	218.9 ± 17.6	------- -- ---	230.8 ± 13.2	------- -- ----
	W4	213.4 ± 18.5	221.4 ± 15.3	------- -- ----	------- -- ----

Treatment‡; F_C (constant feeding), W1 (1 week interupted feeding), W2 (2 week interupted feeding), W4 (4 alternate week interupted feeding). † Effects of starvation on scales in W4; ‡Effects of starvation on scales in W2; § Effects of starvation on scales in W1.

Table 4.3. Linear regression equations describing the relationships between day and marine growth (G_M; mm), marine circuli number (C_M) and fork length (L_F; mm).

Treatment*		Time	Regression Equation	R^2	p
10.5 °C	F_C	Day	G_M = 0.0058*Day + 0.012	0.84	**<0.001**
10.5 °C	W1	Day	G_M = 0.0055*Day + 0.022	0.86	**<0.001**
10.5 °C	W2	Day	G_M = 0.0043*Day + 0.059	0.65	**<0.001**
10.5 °C	W4	Day	G_M = 0.0038*Day + 0.047	0.78	**<0.001**
15 °C	*F*c	Day	G_M = 0.0071*Day + 0.055	0.90	**<0.001**
15 °C	W2	Day	G_M =0.0057*Day + 0.065	0.84	**<0.001**
6 °C	F_C	Day	G_M =0.0025*Day + 0.027	0.69	**<0.001**
6 °C	W2	Day	G_M =0.0023*Day + 0.027	0.72	**<0.001**
10.5 °C	F_C	Day	C_M = 0.13*Day + 0.73	0.94	**<0.001**
10.5 °C	W1	Day	C_M = 0.12*Day + 0.97	0.94	**<0.001**
10.5 °C	W2	Day	C_M = 0.097*Day + 1.6	0.85	**<0.001**
10.5 °C	W4	Day	C_M = 0.10*Day + 1.0	0.87	**<0.001**
15 °C	*F*c	Day	C_M = 0.20*Day + 0.50	0.96	**<0.001**
15 °C	W2	Day	C_M = 0.17*Day +1.3	0.92	**<0.001**
6 °C	F_C	Day	C_M = 0.062*Day + 0.75	0.86	**<0.001**
6 °C	W2	Day	C_M = 0.055*Day + 0.88	0.83	**<0.001**
10.5 °C	F_C	Day	L_F = 0.75*Day +187.7	0.68	**<0.001**
10.5 °C	W1	Day	L_F = 0.66*Day +188.0	0.68	**<0.001**
10.5 °C	W2	Day	L_F = 0.54*Day +193.7	0.57	**<0.001**
10.5 °C	W4	Day	L_F = 0.57*Day +187.0	0.56	**<0.001**
15 °C	*F*c	Day	L_F = 0.83*Day +187.7	0.78	**<0.001**
15 °C	W2	Day	L_F = 0.65*Day +191.5	0.57	**<0.001**
6 °C	F_C	Day	L_F = 0.39*Day +185.6	0.37	**<0.001**
6 °C	W2	Day	L_F = 0.36*Day +184.5	0.35	**<0.001**

* F_C (constant feeding), W1 (1 week interupted feeding), W2 (2 week interupted feeding), W4 (4 alternate week interupted feeding).

Chapter 5.

Decadal changes in post-smolt growth in three Irish populations of Atlantic salmon (*Salmo salar* L.).

To be submitted as:

Thomas, K., Brophy, D. and Ó Maoiléidigh, N. Decadal changes in post-smolt growth in three Irish populations of Atlantic salmon (*Salmo salar* L.).

5.1 Abstract

In this study, growth marks in scales from Atlantic salmon (*Salmo salar* L.) originating from three Irish rivers (Burrishoole, Moy and the Shannon) were analysed to investigate if growth changes occurred during key periods from 1950's to 2008. In particular, the post-smolt growth, post-smolt circuli number and first summer maximum measurement were measured and compared by decade between populations. Scale growth measurements and their temporal trends varied between populations, with a most notable decline evident in the Burrishoole river.

Correlations between scale growth measurements and oceanographic variables sea surface temperature (SST), North Atlantic oscillation (NAO) and Atlantic Multidecadal oscillation (AMO). Post-smolt scale growth and circuli number were negatively correlated with SST in the Burrishoole and Moy rivers, NAO in the Burrishoole river and AMO in the Burrishoole and Shannon rivers. Broad scale decadal decreases in growth rates which correspond to reported declines in return rates of Atlantic salmon were evident across populations and the results indicate that trends observed in one national index river may be representative of change across all populations

5.2 Introduction

Atlantic salmon (*Salmo salar* L.) populations have declined across their geographical range in recent decades (Parrish *et al*., 1998). This reduction is mainly attributed to poor survival in the marine environment and has not responded to reduced fishing effort in all Atlantic salmon fishing jurisdictions (Friedland *et al*., 2000, Jonsson and Jonsson, 2003, Peyronnet *et al*., 2007, Friedland *et al*., 2009, Reddin *et al*., 2011). Mortality is believed to be most severe during the first few months at sea for post-smolts and marine survival rates for some stocks have been correlated with post-smolt growth during the first year at sea (Fisher and Pearcy, 1990; Holtby *et al*., 1990; Eriksson, 1994; Salminen *et al*., 1995). Evidence suggests that the decline in growth is linked to a range of synergistic effects; freshwater influences, pollution, disease, environmental factors (temperature and salinity influences, food availability) abundance of predators, fish origin and climate change (Figure 1.1) (Ricker, 1962; Neilson and Geen, 1986; Friedland *et al*., 1996; Friedland *et al*., 2000; MacLean *et al*., 2003, Peyronnet *et al*., 2007).

Evidence from scale analysis suggests that temporal changes in growth occurred in recent decades in some European populations of Atlantic salmon (Friedland *et al*., 2000, 2009) including one Irish population (the Burrishoole) with a notable decline in growth occurring in the period post 1970 (Peyronnet *et al*., 2007). These changes in growth coincided with the persistent decline in marine survival and are intrinsically linked to climate change (Reddin and Shearer, 1987; Friedland *et al*., 1993, 1998; 2003; Jonsson and Jonsson, 2004; Todd *et al*., 2008). However, it is not yet known if

the changes in growth observed in the Burrishoole are mirrored in salmon populations from other Irish rivers.

Atlantic salmon scales have been commonly used as a means to age and infer growth rates since the early 1900's (Johnston, 1907; Dahl, 1911; Peyronnet *et al.*, 2007; McCarthy *et al.*, 2008; Friedland *et al.*, 2009; Jensen *et al.*, 2011; Jensen *et al.*, 2012). In Ireland, scale samples have been obtained from various rivers throughout the country over the last century. Scale samples were obtained from adult salmon in numerous settings; draft net fisheries, angling catches, fish returning to trapping facilities and weirs, biological sampling in rivers, drift net fisheries [prior to the fishery closer in 2007 (ICES, 2007)], and fish markets. The Marine Institute holds this national scale archive which consists of salmon scales stored in paper scale envelopes or permanently mounted on glass slides, covering a time series from the early 1920's to the present. This is a unique catalogue of historical importance; the information stored within this archive is valuable in aiding the understanding of changes among Atlantic salmon populations over time.

In this study archived scales obtained from three Irish rivers, were measured using digital imaging software to determine if scale growth during the marine phase has changed during the period 1952 to 2008 and to establish if trends are consistent across populations. Given the lack of consistency in the years for which scale samples were available from each river, the resulting growth parameters were compared within and between rivers by decade. Mean growth trajectories in each decade were examined to

identify specific periods within the post-smolt region of the scale when growth anomalies occurred. Correlations between scale growth, sea surface temperature (SST) and global climatic indices North Atlantic oscillation (NAO) and Atlantic multidecadal oscillation (AMO) were investigated. Information from this historical material provides a unique insight into periodic changes in the species' use of the marine ecosystem, and in the possible link between marine growth and survival between populations.

5.3 Methods

5.3.1 Scale collections

Temporal changes in growth of Atlantic salmon was examined using scales from three rivers; Burrishoole system, River Moy and River Shannon. A previous study (Peyronnet *et al*., 2007) used the Burrishoole scale sets which is comprised of scales collected from returning one-sea-winter adult salmon from the 1960's to 1990's. In this study, the time series was extended to included scales from the 2000's. The Burrishoole scales analysed prior to 1980 were of wild origin, from 1981 to 1999 the scale samples were a random mix of both wild and hatchery fish and finally from 2000 on, all scale samples were obtained from fish of hatchery origin. Initially, a full inventory was conducted of all historic scale material available for the Moy and the Shannon, which is held at the Marine Institute research station in Newport, Mayo (Table 5.1). All scales from the Moy and Shannon were from fish of wild origin. The River Shannon collection comprised of 53.7% and 46.3% one and two-sea-winter fish, respectively while the river Moy collection comprised of 71.1% one-sea-winter and

28.9% two-sea-winter fish. Where available, fifty scales per year from each river were randomly selected. This number was chosen to obtain an acceptable level of sample representativeness and to obtain a precise estimate of marine growth for each year and population. The majority of scale samples had previously been mounted on glass slides. The remaining scales were stored in envelopes; these were wet mounted between a glass slide and cover slip for this analysis.

5.3.2 Scale analysis

Scales were viewed using transmitted light under a compound microscope. The best scales (defined as showing an entire edge and clear focus) were selected and high resolution images were acquired and measurements taken using Image Pro Plus version 7.01 © software. A straight line transect was drawn along the 360° axis from the centre of the focus of the scale, to the last circulus of the freshwater zone of the scale to record the freshwater measurement (Figure 5.1). The point on the scale representing sea entry was identified by the increased circuli spacing at outer edge of the freshwater growth zone (Jensen *et al*., 2012). A caliper line was then drawn along the same axis, from the last freshwater circulus through to the scale edge. Circuli were enumerated manually and circuli spacings within the marine zone computed.

The first winter annulus was identified by computing a five-point running average of circuli spacings from the seawater entry mark to the edge and finding the first minimum following Mc Carthy *et al.* (2008). The averaging reduces the effect of measurement error and anomalies within the scale. The sum of the circuli spacings from the beginning of the marine zone to the winter annulus and the number of circuli

deposited was used as the post-smolt growth measurement and post-smolt circuli number, respectively. The highest circuli spacing within the post-smolt region was also identified and used as a measure of maximum growth (first summer maximum). (For continuity with previous studies, first summer maximum measurements were included in this study. However, as the results of chapters three and four of this thesis report that circulus spacing is highly variable and should not be used to infer growth rates; the first summer maximum measurements were mainly to display the growth trajectory.

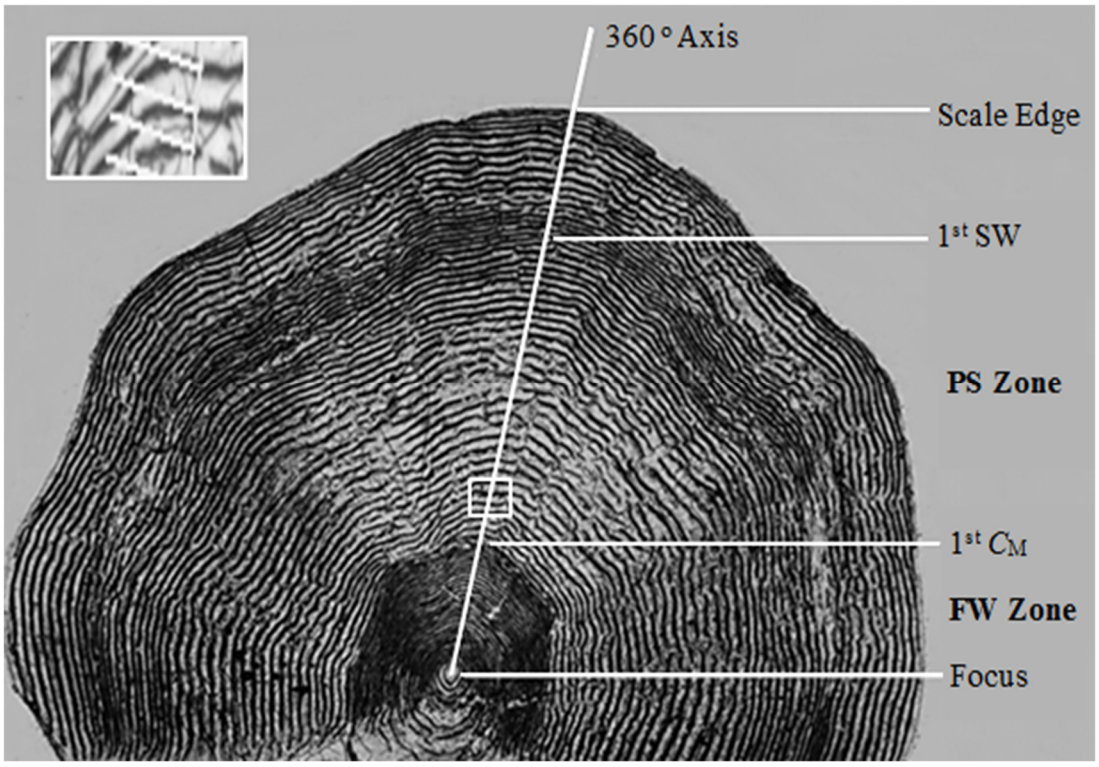

Figure 5.1. Image of an adult salmon scale displaying the 360° straight line axis used when obtaining measurements, both freshwater (FW), post-smolt (PS) and marine zones are illustrated; the first marine circuli (1st CM) and first sea winter annulus (1st SW) are clearly defined. The circuli within the white rectangle on the main image are magnified in the inset on the upper left of the image.

5.3.3 Environmental parameters

5.3.3.1 Sea Surface Temperatures

Mean annual and summer (July to September) sea surface temperature (SST) data was obtained for the period 1954 to 2008 from the Extended Reconstructed Sea Surface Temperature (NOAA ERSST.v3) (Smith *et al*., 2008) between 67°N to 75° N and 10° W to 15° E in the Norwegian Sea region; a known feeding ground of Atlantic salmon (Holm *et al*., 2000; Jensen *et al*., 2012). Local mean annual and spring (March to May; corresponding with average timing of smolt migration) SST measurements at each river mouth were also extracted using the nearest 2° latitude x 2° longitude ERSST grid.

5.3.3.2 Climatic parameters

The North Atlantic oscillation (NAO) is a pattern of atmospheric variability that has a significant impact on oceanic conditions. It affects precipitation, wind speed, evaporation plus the exchange of heat between the ocean and atmosphere, and its effects are most strongly experienced in winter. The NAO index is a measure of the strength of the sea-level air pressure gradient between the Azores and Iceland. During the positive phase of NAO index, there is a strengthening of the Icelandic low-pressure system and the Azores high-pressure system, which produces stronger mid-latitude westerly winds, with colder and drier conditions over the western North Atlantic and warmer and wetter conditions in the eastern North Atlantic. During the negative phase

of the NAO index, the pressure gradient is reduced, and the effects tend to be reversed (Hurrell *et al*., 2003; Jonsson and Jonsson, 2004; ICES, 2017).

The Atlantic Multidecadal oscillation (AMO) is a broad scale signal indicator of variations in North Atlantic Ocean climate (Friedland *et al*., 2014) and is determined from the de-trended annual mean of SST variability over the North Atlantic region including 0° to 70° N, 75°W to 7.5°W, utilizing a 5° grid (Enfield *et al*., 2001). Detrending is intended to remove recent global climate change effects induced by increasing greenhouse emissions. The AMO has an approximate periodicity of between 20 to 40 years with major oscillations between warm and cool conditions. Knight *et al*. (2005), Todd *et al*. (2011) and Friedland *et al*. (2014) report that since the turn of the century, the North Atlantic has been experiencing a strong warm period and in the period from 1960 through 1990 cold periods were reported. Mean annual and winter (January through March) data sets for the North Atlantic Oscillation (NAO) and the Atlantic Multidecadal Oscillation (AMO) were obtained for the 1950's through to the 2000's from NOAA Earth system research laboratory.

5.3.4 Statistical analyses

The analysis was conducted in two stages. Firstly, the three scale growth measurements (post-smolt growth, post-smolt circuli number and first summer maximum) were compared between decades for each river separately using a series of one way ANOVAs. In the second stage, the three scale growth measurements (post-smolt growth, post-smolt circuli number and first summer maximum) were compared

between rivers for each decade separately using a series of one way ANOVAs. Kruskal-Wallis tests were applied when variables were either non-normally distributed and/or displayed unequal variances.

Pearson's correlations were used to determine if the two scale measurements (post-smolt growth measurements and post-smolt circuli numbers) were related to the environmental variables, across the three rivers. An alpha level of 0.05 was used. In any time series, sequential observations are non-independent i.e. they are more similar to each other than observations from other parts of the time series. The temporal auto correlation violates the statistical assumptions and can lead to type I error. To account for temporal auto correlation, the effective degrees of freedom were calculated using the procedure suggested by Pyper and Peterman (1998) and Garrett and Petrie (1981). The effective degree of freedom (N^{eff}) was estimated by:

Equation 5.1:

$$\frac{1}{N^{eff}} \approx \frac{1}{N} + \frac{2}{N}\sum_{j=1}^{N}\frac{N-j}{N}\rho XX(j)\rho YY(j)$$

Where N^{eff} is the effective degrees of freedom, N is the sample size, and $\rho XX(j)$ and $\rho YY(j)$ are the autocorrelations of the X and Y time series at lag j, with a lag of 5.

Pearson's correlations were further used to determine if the two scale measurements (post-smolt growth measurements and post-smolt circuli numbers) were linearly

related between the three rivers. Statistical analysis was conducted using the MINITAB statistical package. Corrections for temporal autocorrelation were conducted using Rstudio.

5.4 Results

5.4.1. Temporal changes in post-smolt growth

5.4.1.1 Burrishoole river

The post-smolt growth declined from the 1970's to the 2000's, with the most pronounced decline occurring from the 1970's to the 1980's [Figure 5.2 (a, b)]. Post-smolt scale growth was significantly higher during the 1960's and the 1970's than in the 1980's (ANOVA, $p<0.001$; $p<0.001$), 1990's (Kruskal-Wallis, $p<0.001$; $p<0.001$) and 2000's (Kruskal-Wallis, $p<0.001$; $p<0.001$), respectively (Table 5.2). During the 2000's post-smolt growth was significantly lower than in all other decades (Kruskal-Wallis, $p<0.001$).

5.4.1.2 River Moy

Post-smolt scale growth increased from the 1960's to the 1980's, and then declined between the 1980's and 1990's [Figure 5.2 (a)]. Growth in the 1980's was significantly higher than in the 1950's, 1960's, 1990's and 2000's (ANOVA, $p<0.001$). Growth in the 1990's and 2000's was significantly lower than in all preceding decades [ANOVA, $p<0.001$; Figure 5.2 (b); Table 5.2]

5.4.1.3 River Shannon

Post-smolt scale growth increased steadily from the 1950's to the 1970's [Figure 5.2 (a)]. Growth in the 1960's was significantly higher than in the 1950's (ANOVA $p=0.027$). Growth in the 1970's was significantly higher than in the 1950's and 1960's (ANOVA, $p<0.001$).

5.4.2 Temporal changes in circuli number

5.4.2.1 Burrishoole river

Mean circuli numbers showed the same trends as the post-smolt growth measurements, declining from the 1970's to the 2000's, with the most pronounced decline occurring from the 1970's to the 1980's [Figure 5.3 (a)].

Circuli number was significantly higher in the 1960's and 1970's than in the 1980's (ANOVA, $p<0.001$; $p<0.001$), 1990's (Kruskal-Wallis, $p<0.001$; $p<0.001$) and 2000's (Kruskal-Wallis, $p<0.001$; $p<0.001$), respectively and was significantly higher in the 1980's compared to the 1990's (Kruskal-Wallis, $p=0.003$) and 2000's (Kruskal-Wallis, $p=0.001$) [Figure 5.3 (b); Table 5.2]

5.4.2.2 River Moy

Mean circuli number showed little variation between the 1950's and 1980's and then declined between the 1980's and 1990's [Figure 5.3 (a)]. Mean circuli number in the 1990's was significantly lower than in the 1950's, 1970's, 1980's and 2000's [ANOVA, $p<0.001$; Figure 5.3 (b); Table 5.2].

5.4.2.3 River Shannon

The average circuli numbers were high during the 1950's and steadily increased during the 1960's through to the 1970's [Figure 5.3 (a)]. Significant differences were detected between decades (Kruskal-Wallis, $p<0.001$). Circuli number in the 1960's was significantly higher than in the 1950's (ANOVA $p<0.001$). Circuli number in the 1970's was significantly higher than in the 1950's and in the 1960's [Kruskal-Wallis, $p<0.001$) [Figure 5.3 (a, b); Table 5.2].

5.4.3 Temporal changes in first summer maximum values

Figure 5.5 illustrates the generalised scale growth pattern in each decade for the three rivers studied. The shape of the trajectories has changed and their maximum height has reduced over time in both the Burrishoole and the Moy populations. The width of the first summer maximum decreased from the 1980's to the 2000's for the Burrishoole and from the 1990's and 2000's in the Moy.

5.4.3.1 Burrishoole river

The width of the first summer maximum decreased from the 1960's to the 1980's [Figure 5.4 (a, b)] and then remained relatively stable for the rest of the time-series. This measurement was significantly higher in the 1960's, compared to the other four decades [ANOVA, $p<0.001$; Figure 5.4 (a-c); Table 5.2].

5.4.3.2 River Moy

The width of the first summer maximum increased from the 1970's to the 1980's and then declined from the 1980's to the 2000's. The measurement was significantly lower in the 2000's compared to all other decades [ANOVA, $p<0.001$; Figure 5.4 (a-c); Table 5.2].

5.4.3.3 River Shannon

The width of the first summer maximum showed little variation from the 1950's to 1970's and no significant differences were detected [ANOVA, $p=0.131$; Figure 5.4 (a-c); Table 5.2].

5.4.4. Inter-river comparison of growth

5.4.4.1 Inter-river comparison of decadal post-smolt growth

The highest post-smolt growth was observed in the Shannon population across the three decades investigated. Post-smolt growth was significantly higher in the Shannon than Moy in the 1950s (ANOVA, $p<0.001$). During the 1960's, the Shannon displayed a significantly higher post-smolt growth than both the Moy (ANOVA, $p<0.001$) and the Burrishoole (Kruskal-Wallis, $p<0.001$) [Figure 5.2 (a, b); Table 5.2]. The decline in growth occurred later in the Moy (1980's to 1990's) than in the Burrishoole population (1970's to 1980's). Post-smolt growth was significantly higher in the Moy than in the Burrishoole in the 1980's (ANOVA, $p<0.001$), 1990's (Kruskal-Wallis, $p<0.001$) and 2000's (ANOVA, $p<0.001$) [Figure 5.2 (a); Table 5.2].

5.4.4.2 Inter-river comparison of circuli number

Circuli number was similar in the Shannon and Moy populations in the 1950's (Kruskal-Wallis, p=0.343) but higher in the Shannon in the 1960's (Kruskal-Wallis), p=0.007) and 1970's (Kruskal-Wallis, p=0.001). Circuli numbers were lowest in the Burrishoole population throughout the time-series. The decline in circuli numbers occurred earlier in the Burrishoole population (1970's to 1980's) than in the Moy population (1980's to 1990's) compared to post-smolt growth results. In the 1960's (Kruskal-Wallis, $p<0.001$) and 1970's (Kruskal-Wallis, $p<0.001$) circuli numbers were significantly higher in the Shannon population than the Burrishoole [Figure 5.3 (a, b); Table 5.2]. Circuli number was similar in the Moy and Burrishoole populations in the 1950's (ANOVA, p=0.104). Circuli number was significantly higher in the Moy than in the Burrishoole throughout the 1970's (Kruskal-Wallis, p=0.010), 1980's (Kruskal-Wallis, $p<0.001$), 1990's (Kruskal-Wallis, $p<0.001$) and 2000's (ANOVA, $p<0.001$) [Figure 5.3 (a, b); Table 5.2].

5.4.4.3 Inter-river comparison of first summer maximum values

The width of the first summer maximum (mm; ±95% confidence intervals) was highest in salmon from the Shannon collected during the 1950's to 1970's. In contrast to the post-smolt growth and circuli counts, first summer maximum values were higher in scales from the Burrishoole than from the River Moy in all decades except the 1980's. The width of the first summer maximum decreased from the 1960's to the 1980's in the Burrishoole and from the 1980's to the 2000's in the Moy [Figure 5.4 (a, b); Table 5.2].

Figure 5.5 illustrates the generalised scale growth pattern in each decade. The size of the first summer maximum reflects the decline previously shown. The position of the first summer maximum (i.e. the circuli pair between which this spacing occurs) has also varied over time but suggest that the first summer maximum measurement is located at a higher circuli pair number for the Moy in more recent years [Figure 5.4 (c)]. The widest circuli spacing measurement was observed in the Shannon which differed significantly to the Moy and Burrishoole [Figure 5.4 (a-c); Table 5.2].

5.4.4.4. Correlations with environmental variables

The winter NAO was negatively correlated with both the post-smolt growth measurement and post-smolt circuli numbers in the Burrishoole ($p=0.013$ and $p=0.009$), respectively. The relationship remained significant after correction for temporal autocorrelation. No significant relationship was found for the Moy or the Shannon. Likewise, the annual NAO showed no significant correlations with any variable (Table 5.3).

The annual AMO was negatively correlated with the post-smolt growth measurement ($p<0.001$) and post-smolt circuli number ($p<0.001$) in the Burrishoole and with the post-smolt growth measurement in the Shannon ($p=0.043$). Significant negative relationships were found between the winter AMO and the post-smolt growth measurement in the Burrishoole ($p=0.005$) and Shannon ($p=0.017$) and post-smolt circuli number in the Burrishoole ($p=0.009$) [Figure 5.7 (a, b)]. All correlations with

AMO remained significant after correction for temporal auto-correlation. No significant relationships between scale growth measurements and AMO were found for the Moy (Table 5.3).

The post-smolt growth measurement in fish from the Burrishoole ($p=0.001$) and Moy and post-smolt circuli number in fish from the Burrishoole were negatively correlated with both annual North Atlantic SST ($p=0.001$; $p=0.032$; $p=0.005$), respectively and summer North Atlantic SST ($p<0.001$; $p=0.037$; $p=0.003$), respectively. Relationships remained significantly correlated after correction for temporal autocorrelation. No significant relationships with SST were found for the Shannon (Table 5.3). Both the post-smolt growth measurement and the post-smolt circuli number in the Burrishoole displayed significant negative relationships with the local annual summer SST ($p=0.002$; $p=0.007$), respectively and the local summer SST ($p=0.001$; $p=0.003$), respectively. All relationships remained significant after correction for temporal autocorrelation [Table 5.3; Figure 5.8 (a-d)].

5.4.4.5. Cross correlations between rivers

Correlations between rivers in annual mean post-smolt growth and post-smolt circuli numbers were examined to determine if there was any consistency in the temporal trends. No significant correlations were evident between the river Moy and Shannon during the 1950's (post-smolt growth; $r = -0.041$, $p=0.651$; circuli number; $r = 0.013$, $p=0.886$), the 1960's (post-smolt growth $r = 0.200$, $p=0.110$; circuli number; $r = 0.196$, $p=0.118$) or the 1970's (post-smolt growth $r = 0.200$, $p=0.110$; circuli number; $r =$

0.196, p=0.118). No significant correlations were evident between the Burrishoole and Shannon during the 1960's (post-smolt growth r = -0.040, p=0.451; circuli number; r = -0.084, p=0.114) or during the 1970's (post-smolt growth r = -0.015, p=0.894; circuli number; r = 0.031, p=0.777). The Burrishoole and Moy were then assessed from the 1960's through to the 2000's with no significant correlations found between rivers in the 1960's (post-smolt growth r = -0.212, p=0.090; circuli number; r = -0.036, p=0.778), the 1970's (post-smolt growth r = 0.117, p=0.340; circuli number; r = 0.215, p=0.078), 1980's (post-smolt growth r = -0.091, p=0.390; circuli number; r = -0.146, p=0.162), 1990's (post-smolt growth r = -0.067, p=0.462; circuli number; r = 0.167, p=0.064) or the 2000's (post-smolt growth r = 0.119, p=0.155; circuli number; r = -0.002, p=0.985).

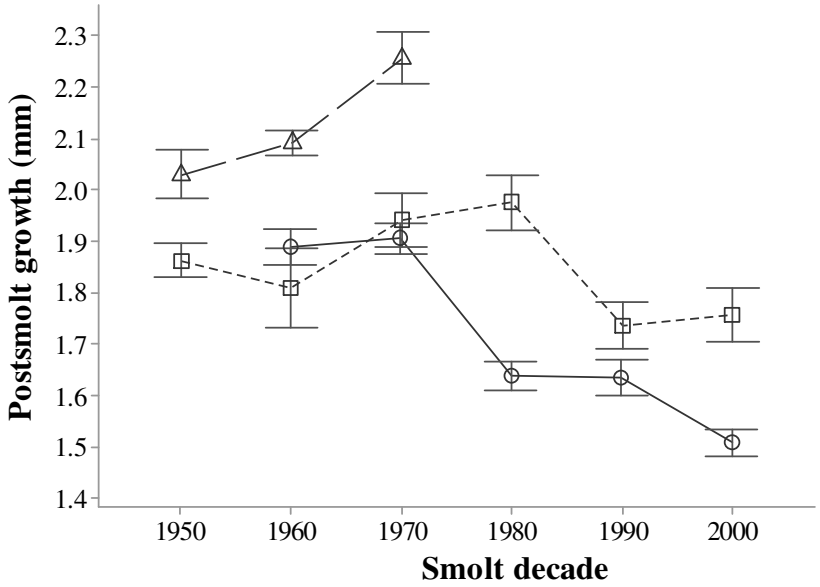

Figure 5.2 (a)

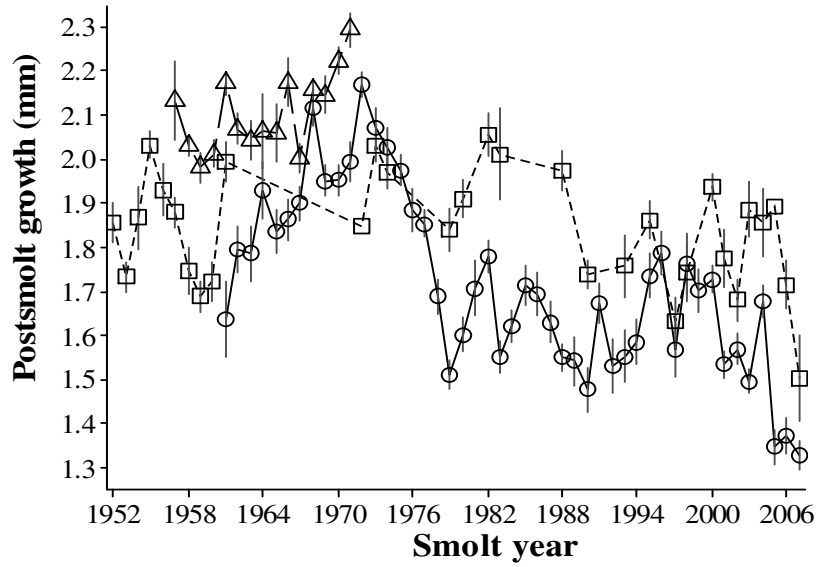

Figure 5.2 (b)

Figure 5.2 (a, b). (a) Post-smolt growth (mm) by decade (b) Post-smolt growth (mm) by year (○ ——— , Burrishoole; □ – – – , Moy; △ — — , Shannon); Error bars are 95% confidence intervals.

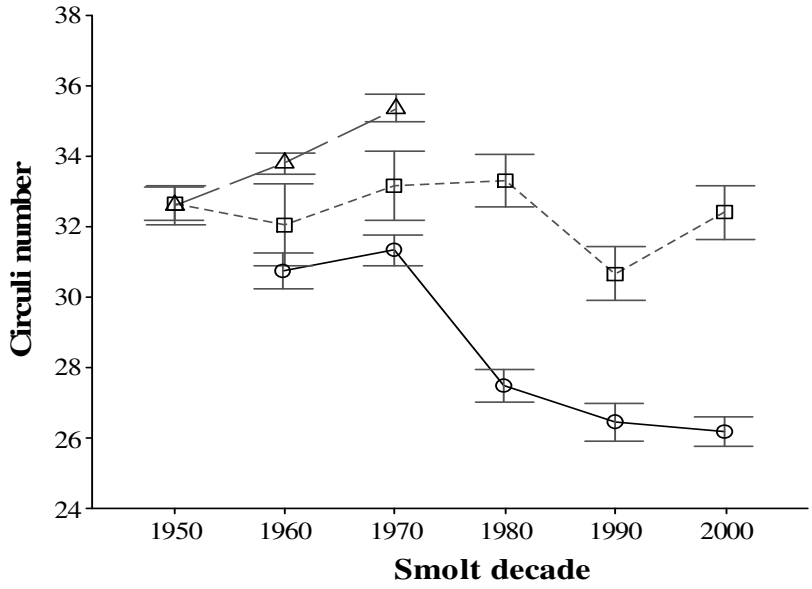

Figure 5.3 (a)

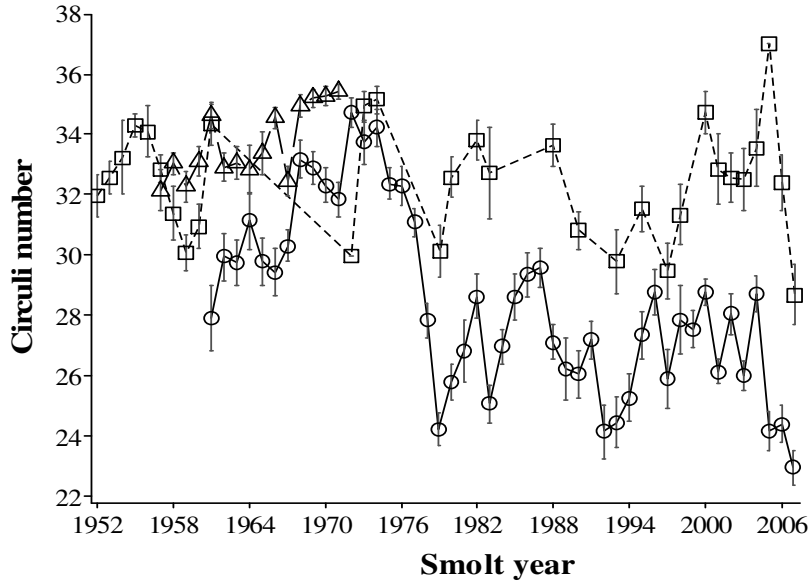

Figure 5.3 (b)

Figure 5.3 (a, b). (a) Post-smolt circuli number by decade (b) Post-smolt circuli number by year (○ —— , Burrishoole; □ – – – , Moy; △ —— , Shannon); Error bars are 95% confidence intervals.

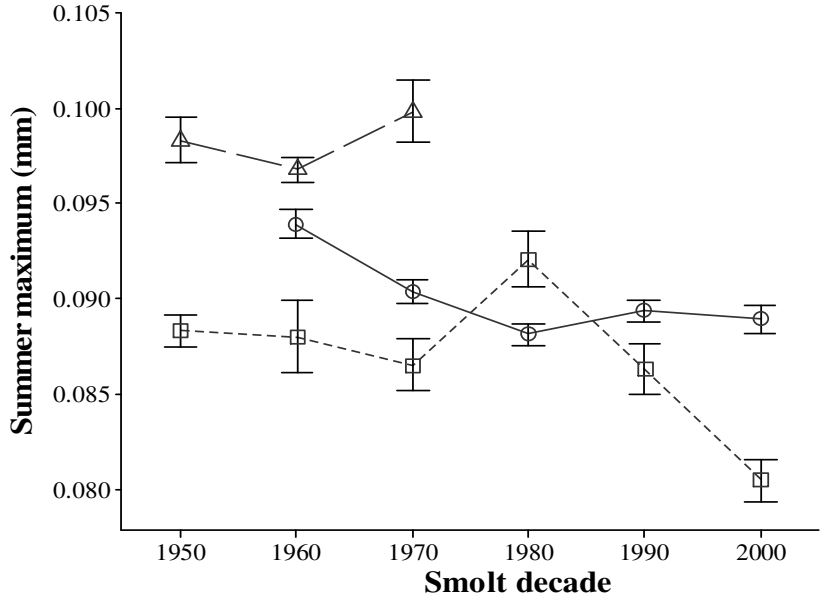

Figure 5.4 (a)

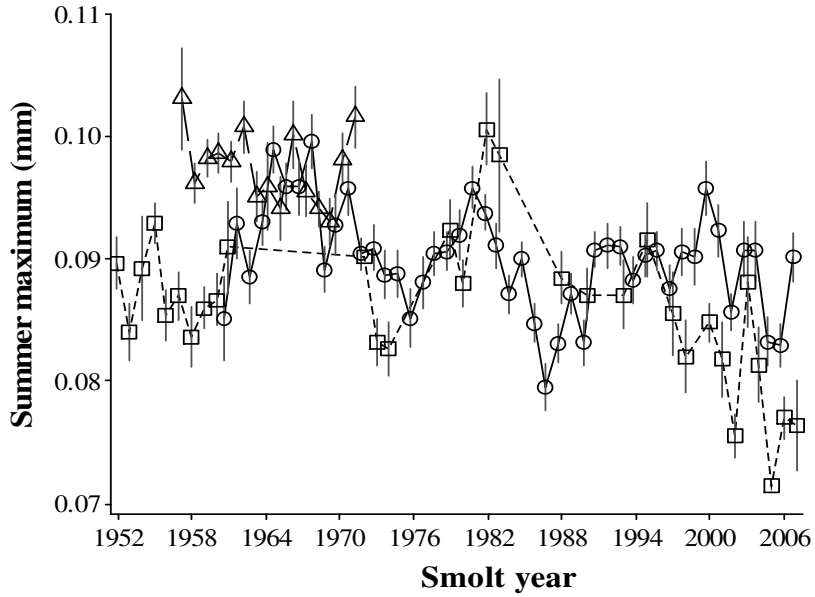

Figure 5.4 (b)

Figure 5.4 (a, b). (a) First summer maximum (mm) by decade (b) First summer maximum (mm) by year (○ —— , Burrishoole; □ – – –, Moy; △ —— , Shannon); Error bars are 95% confidence intervals.

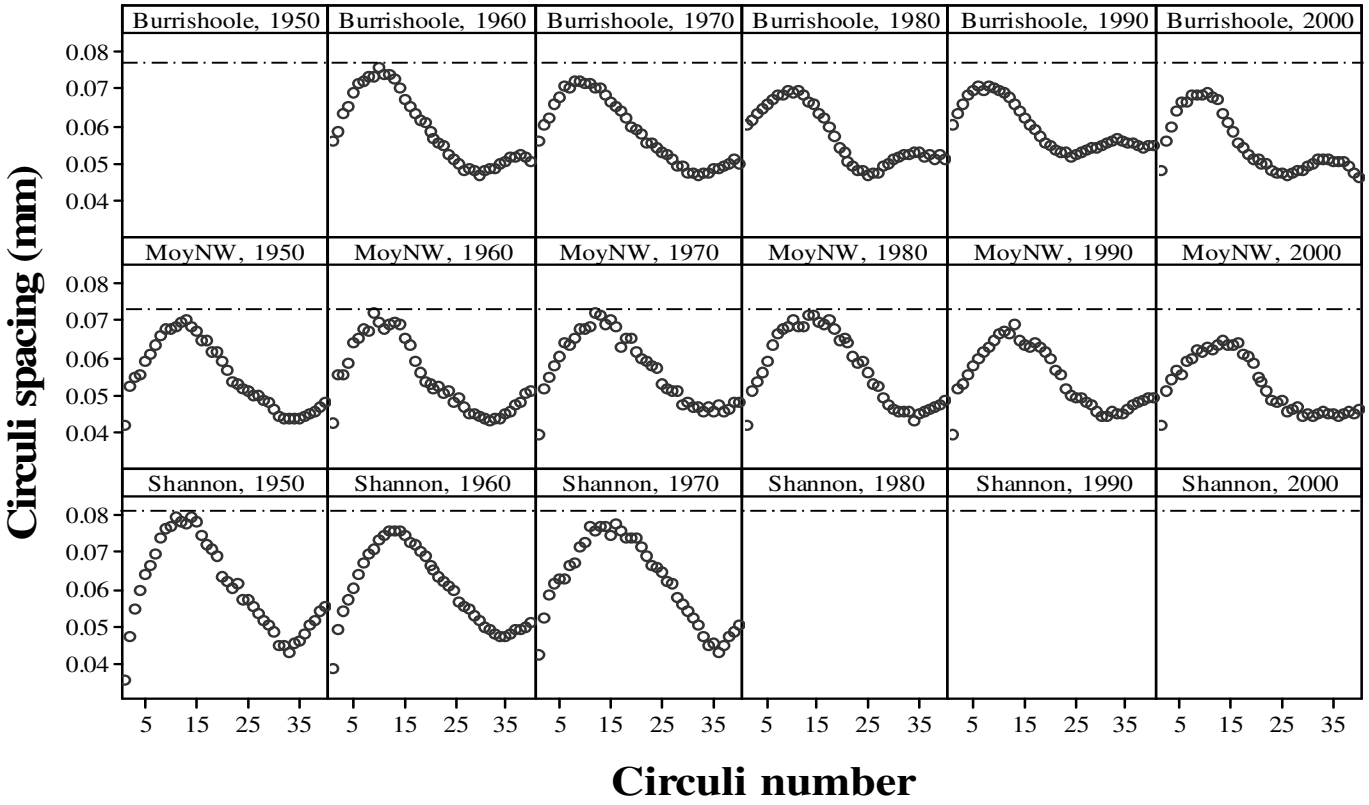

Figure 5.5. Mean circuli spacing (mm) per circuli number by river, peaks indicate the first summer maximum (mm) after smolt migration (-·-·- ; indicates the widest circulus spacing (mm) per river).

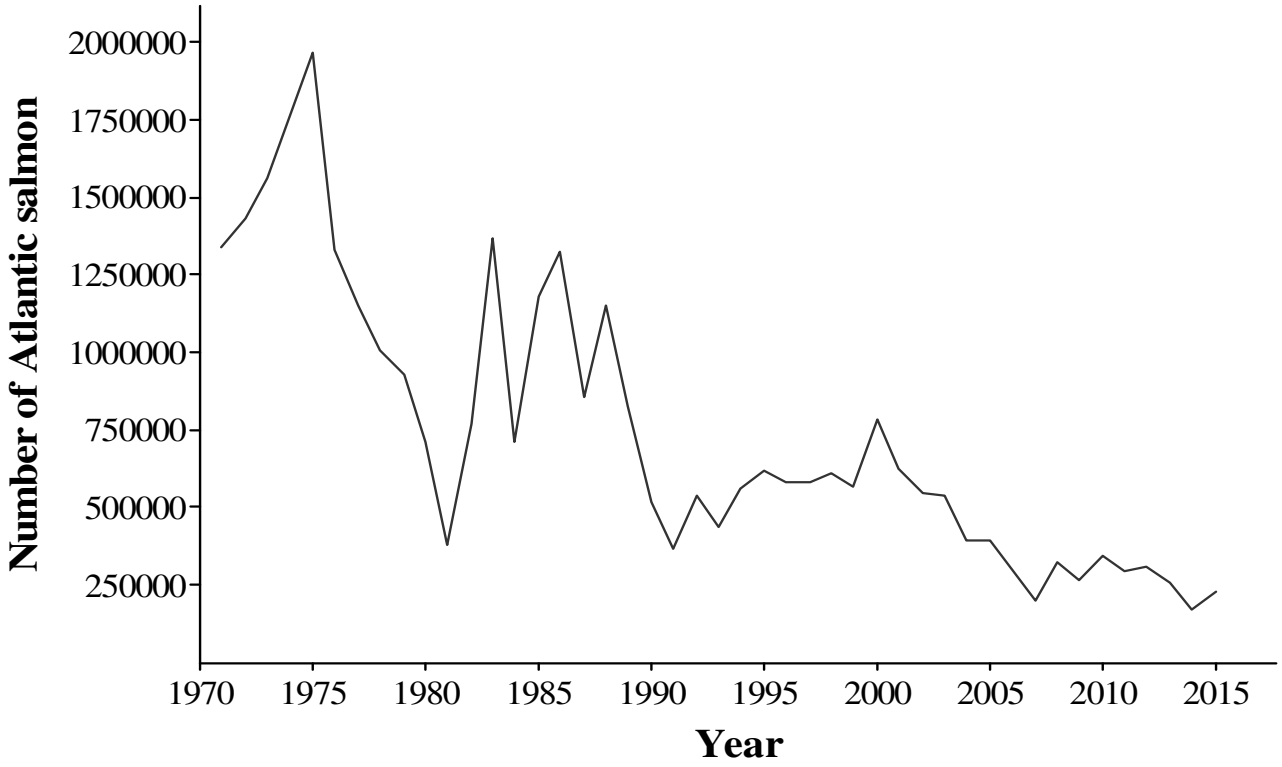

Figure 5.6. Time series of recruitment estimates for North Atlantic salmon estimated from the pre-fishery abundance by ICES of maturing one sea winter (1SW) salmon returns.

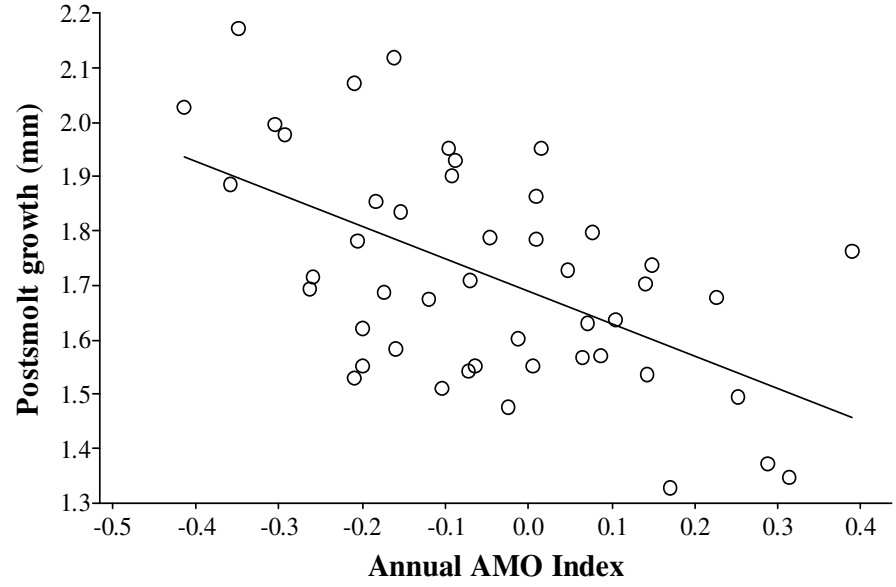

Figure 5.7 (a)

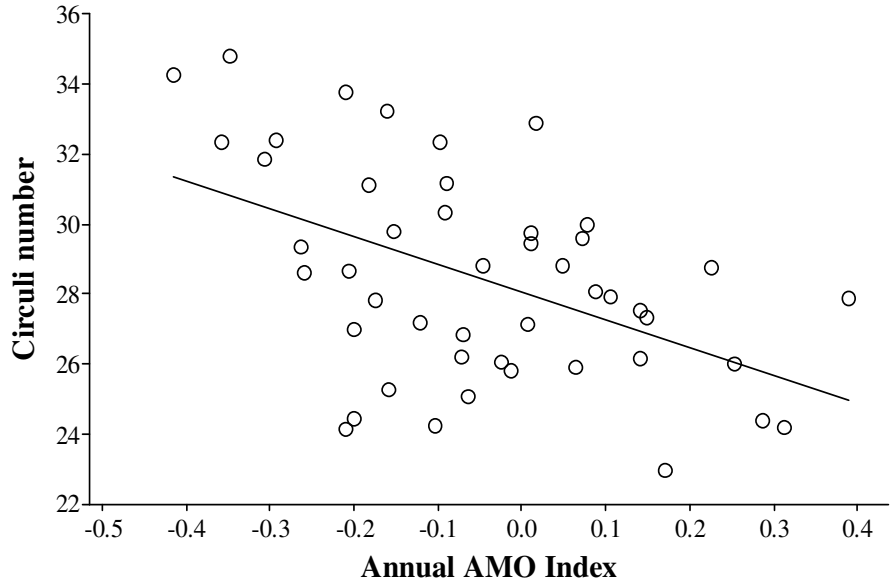

Figure 5.7 (b)

Figure 5.7 (a, b). Correlations between Annual AMO index and the Burrishoole river (a) post-smolt growth (mm) (b) post-smolt circuli number.

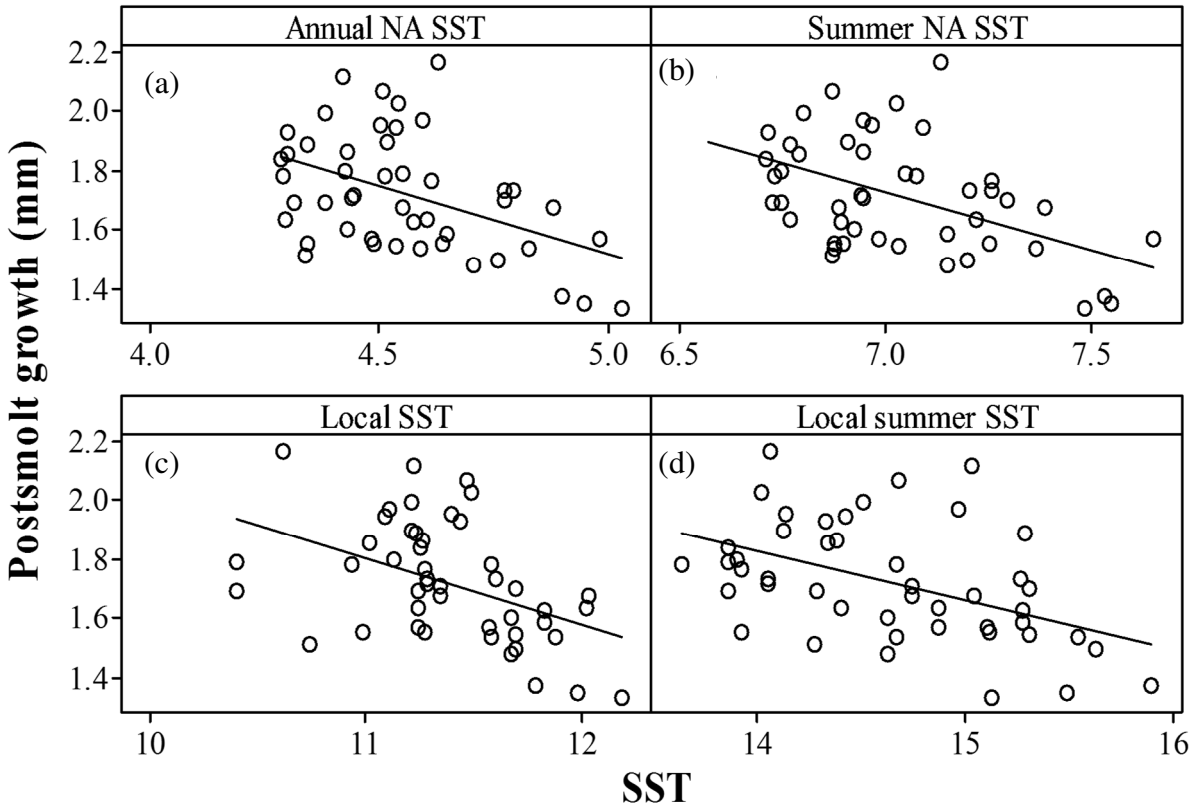

Figure 5.8 (a-d). Correlations between sea surface temperature (SST) and post-smolt growth (mm) in the Burrishoole river (a) Annual North Atlantic SST (b) Summer North Atlantic SST (c) Local SST (d) Local summer SST.

5.5 Discussion

The environment of the north-east Atlantic has changed in recent years and represents a less productive environment for Atlantic salmon post-smolts. Changes in food web composition related to warming conditions has resulted in poor growth and survival of Atlantic salmon (Friedland *et al.*, 2009). These environmental changes may be a factor contributing to the decreased return rates of adult salmon (Beaugrand *et al.*, 2002). The growth measurements inferred from scales during this study displayed patterns of decrease which coincide with the abrupt declines in salmon recruitment from the late 1970's, as reflected in the annual recruitment indices produced by ICES (Figure 5.6; ICES, 2016). Previous studies have similarly shown that growth of Atlantic salmon during the marine phase has decreased over the last thirty years impacting the recruitment indices (Crozier and Kennedy, 1999; Peyronnet *et al.*, 2008).

Within this study, comparisons of scale measurements revealed differences in post-smolt growth, circuli number and first summer maximum measurements between salmon from the three rivers. Although changes in scale growth were largely consistent across populations, differences between populations were also observed. In general, the Shannon population displayed the fastest rates of marine growth, the Burrishoole population had the slowest growth rates and salmon from the river Moy showed intermediate growth, with declining growth occurring later in the river Moy than the Burrishoole. Furthermore, temporal changes in the shape of the scale growth trajectories were also detected.

Atlantic salmon are distributed over large areas of the north Atlantic Ocean. Marine feeding grounds utilised by Atlantic salmon vary between stock complexes. Fish of North American origin appear to remain mainly in the north-west Atlantic (Reddin *et al*., 2012). However, a proportion may move into the north-east Atlantic during marine residency (Jacobsen *et al*., 2012) as evidenced by salmon tagged in North America but recovered in the Faroes fishery. European salmon are known to migrate to the same marine nursery grounds in the Norwegian Sea area in the north-east Atlantic (Holm *et al*., 2000, 2004), with a proportion of the southern European multi-sea-winter populations feeding in the North-west Atlantic. Hansen (1993) reports various sea age classes of Northern European origin salmon derived from the same population present within the same marine area simultaneously. It is therefore assumed that European populations originating from the same geographical region, migrating to sea at a similar time, would encounter comparable environmental factors during the initial post-smolt migration.

Salmon from the Shannon population displayed much higher marine growth rates than salmon from the Moy and the Burrishoole during the 1960's and 1970's. This may suggest that fish migrating from the River Shannon utilised different feeding grounds to that of the other two rivers at some point during the post-smolt marine residency. Within this study, scales were randomly selected from the scale archive. The fifteen years of growth data obtained from the River Shannon consisted of both one and two-sea-winter fish. The hypothesis that growth differs between sea age classes and the possibility that Shannon fish utilised other more productive nursery grounds is

plausible. Jonsson *et al*. (1991) states that Norwegian multi-sea-winter populations tend to grow faster than the one-sea-winter populations. Across seven north Atlantic rivers, post-smolt growth of one-sea-winter salmon was significantly lower compared to both two and three sea winter salmon (Jensen *et al*., 2011). Nicieza and Braña (1993) reported a similar finding in salmon from Spanish rivers; the growth increment during the first year at sea was greater amongst the two-sea-winter salmon than the one-sea-winter fish originating from the Narcea and Esva rivers. An opposite result was found for River Cares however, as no significant differences were detected between the sea age classes.

In respect to differences between one and two-sea-winter fish, a similar proportion of one and two-sea-winter scales were analysed from the River Shannon and Moy in early stage of the time series (1958 to 1970). The growth measurements relating to the River Shannon were much higher than those from the river Moy during these initial decades. Therefore, it is difficult to ascertain if the higher growth rates reported in the River Shannon are due to differences in sea age class itself or the assumption that the two-sea-winter fish inhabited different and more productive feeding areas. Jensen *et al*. (2011) suggests that differences in migration routes of one and two-sea-winter fish may occur at times during the first year at sea. Due to the geographical distances between rivers and marine feeding grounds, it may not be feasible for potential two-sea-winter fish to migrate to areas other than the feeding grounds shared by one-sea-winter fish during the earliest post-smolt period. However, a segregation may occur at some point with one and two-sea-winter fish residing in different areas when the

first winter annulus is formed. The distribution within the marine environment is dependent on various factors such as SST, ocean currents plus genetic factors controlling population specific migrations (Hansen and Quinn, 1998; Holm *et al*., 2004). A study conducted by Aykanat *et al*. (2015) on Atlantic salmon populations from the River Teno, divided populations of various freshwater and sea age classes into sub-populations within the river. Subtle genetic differences were detected between the overlapping sub-populations. It was suggested that this may explain local phenotypic divergence including differences in juvenile growth rate, age at maturity and sizes of sea age classes.

Furthermore, conditions during the freshwater phase may have preconditioned the Shannon fish toward enhanced marine growth. Compensatory growth is the term used to describe a period of fast growth that follows a period of reduced growth in Atlantic salmon (Morgan and Metcalfe, 2001). Periods of food shortages or decreased temperatures impede growth rates and this malnourishment may reduce fish size compared to fish with abundant food resources. Once food becomes more readily available, these smaller fish may compensate and replenishes lipid reserves in turn causing a catch up effect with well-nourished cohorts (O'Connor *et al*., 2014). However, it has been indicated that fish that have undergone compensatory growth show decreased performance and increased mortality over long time scales (Morgan and Metcalfe, 2001; Johnsson and Bohlin, 2006; Lee *et al*., 2013). If the freshwater conditions within the Shannon system were less productive than those within the River Moy and Burrishoole, the higher post-smolt growth reported in the Shannon fish may

be attributed to compensatory growth during the marine migration. Therefore, an alternative reasoning may relate to one-sea-winter fish maturing earlier than older sea age classes. Sea age at maturity is positively associated with growth rate during the first year at sea, there appears to be a positive association between poor first year growth at sea and early maturation. Once there is no advantage in remaining in the marine environment, maturation occurs earlier (Jonsson *et al*., 2003).

The scale growth measurements from the Burrishoole river displayed the lowest growth measurements of all rivers across all but one decade. The growth trajectories of salmon from the river Moy followed a different pattern to salmon from the Burrishoole despite the close geographical proximity of the two rivers. These growth differences may be due to the origin of the fish. Scales analysed from the Burrishoole river included both wild and hatchery reared fish. From 1962 to 1980 when the highest growth rates were recorded, all Burrishoole fish were of wild origin. The scales analysed from 1981 to 1999 were predominantly from hatchery-reared fish and in the 2000's when growth rates were lowest all scales were from hatchery reared fish. Evidence suggests that hatchery fish do not respond to changes in environmental conditions as well as those of wild origin. Peyronnet *et al*. (2007) suggests that hatchery fish may be subject additional mortality events compared to wild counterparts. Hatchery fish are reared in a protected enclosure in the absence of predators and with a constant food supply. However, on release into the marine environment, they must quickly adapt to hunting for food and evading predators (Sundström and Johnsson, 2001; Jonsson *et al*., 2003).

Various studies have concluded that differences occur between the survival rates of wild and hatchery fish. In the Burrishoole the survival of one-sea-winter wild salmon was higher than the ranched salmon (Piggins and Mills, 1985). In the Baltic Sea, wild salmon survival rates were over four times higher than cultured salmon (Saloniemi *et al*., 2004). A study on the Irma in Norway, reported differences between the survival rates of returning wild and hatchery Atlantic salmon, survival rate which was a proxy of recapture rate was significantly higher for wild fish compared to hatchery fish (Jonsson *et al*., 2003).

Differences between wild and hatchery fish may be due to genetic factors, or may be caused by differences in the juvenile rearing environment, or a combination of these effects. Alternatively, the differences in marine growth between salmon from the Burrishoole and the river Moy might occur due to differences in the timing of the marine migration. This seems unlikely however, as Atlantic salmon populations originating from similar latitudes are assumed to migrate at similar times (Kennedy and Crozier, 2010; Jensen *et al*., 2012). Salmon from the river Moy may have utilised a different migratory route, fed at different marine feeding grounds or fed more efficiently at the same feeding grounds compared to Burrishoole salmon (MacKenzie *et al*., 2012). Whatever the explanation, the observed differences in growth between rivers shows that temporal trends in Atlantic salmon populations show localised variation.

Reductions in circuli spacing may reflect periods of reduced food supply and reduced somatic growth (as shown in chapter four) or may also occur during periods of rapid growth at particularly high temperatures (chapter three). The plots of circuli-spacing against circuli number (Figure 5.5) show that inter-circuli distances increase steadily over the initial growth period at sea, peaking at a maximum that corresponds with the first summer at sea. This is followed by a gradual decline in circuli spacings until the narrowest inter circuli distance which is recorded as the first winter minimum. This general pattern of scale growth varied over time across the three rivers. The width of first summer maximum declined over the time series and the width of the winter minimum increased. These changes in patterns may reflect changes within the marine environment; perhaps growing conditions have become more homogenous throughout the year. However, it has been suggested that circuli spacing is not a reliable indicator of short term growth (Peyronnet *et al.*, 2007; Beakes *et al.*, 2014; Thomas *et al.*, in prep). The results from chapters three and four of this thesis reported that narrow circulus spacings coincided with increased growth at elevated temperatures and that narrow circuli spacings occurred during periods of slow growth corresponding to periods of intermittent feeding. In this study the first summer maximum decreased over time. If circuli spacing was assessed alone, this change may indicate periods of increased feeding at higher temperatures; however, the post-smolt growth measurement also decreased over time which would not occur if favourable conditions were present. Therefore, it is difficult to identify the specific cause of the changing trajectory over the time series.

The relationship between growth and the environmental variables; SST, NAO and AMO was also explored in this research. Atlantic salmon post-smolts are generally found in the upper layers of the water column (Holm *et al*., 2000) and are sensitive to thermal fluctuations. Therefore, SST is an important variable to assess coupled with the climatic drivers that further impact SST, the NAO and AMO. The distribution, mortality, and marine growth of Atlantic salmon have been linked to SST variability (Reddin and Shearer, 1987; Friedland *et al*., 2000, 2009). SST variability has been associated with mortality rates of European and North American salmon stock complexes.

Previous studies suggest that a positive NAO coupled with elevated SST resulted in lower abundance of *Calinus finmarchicus* in the north-east Atlantic Ocean (Planque and Reid, 1998; Beaugrand *et al*., 2002). Salmon abundance and marine growth are strongly influenced by SST (Niemela *et al*., 2004; Jensen *et al*., 2011).

Elevated temperatures accelerate the metabolism, respiration, and oxygen demands of fish. Therefore, increases in fish metabolic rate may reduce the availability of food supply due to increased feeding. As temperature is a known driver effecting all physiological processes most notably within ectotherms (Hoar, 1953; Fry, 1971), fluctuations in SST will affect Atlantic salmon and the way in which they utilise the environment. This was evident during this study, as it was found that SST was negatively correlated with post-smolt growth from both the Burrishoole and the river Moy. Decreasing growth measurements coincided with an increase in SST. Similarly, Friedland *et al*. (2009) reported a negative relationship between SST and summer post-

smolt growth in the Norwegian Sea while McCarthy *et al*. (2008) found a correlation between SST and the post-smolt growth of salmon from the Drammen river in Norway during the fourth and fifth sea months. However, Jensen *et al*. (2012) found no significant relationship between SST and post-smolt growth in the Norwegian Sea.

In relation to Atlantic salmon recruitment, the AMO appears to be a more closely linked climate related index than the NAO (Friedland *et al*., 2009). The results from this study suggests some synchrony between this environmental index and growth indices. The annual AMO was negatively correlated with growth measurements from the Burrishoole and the River Shannon. Fluctuations in the AMO have been related to broad scale ecosystem change (Nye *et al*., 2014). Within the north-east Atlantic, fluctuations in the AMO have been related to changes in productivity within areas supporting juvenile salmon, resulting in lower post-smolt growth during the positive phase of the AMO coupled with lower recruitment rates (Friedland *et al*., 2009). The positive phase of the AMO is believed to affect north-west Atlantic salmon in a different manner. AMO related warming is assumed to modify the predator field affecting the mortality rate of salmon at ocean entry and during the early marine phase (Friedland *et al*., 2003, 2009; Friedland and Todd, 2012; Nye *et al*., 2014).

When analysing any extended time series of biological measurements, possible methodological inconsistencies must be considered. Within this study, sources of potential errors were identified. Firstly, scales obtained prior to 1984 may not have originated from the standard body location (Anonymous, 1984). The results of chapter

two showed that scales obtained from body areas other than the standard sampling location contain fewer circuli and have smaller marine and scale radius growth measurements. Therefore, higher growth rates observed earlier in the time series are unlikely to be due to differences in scale sampling methods. Secondly, different readers from two laboratories analysed the scales used in this study. With regard to the Burrishoole dataset, scales from 1962 to 1999 were analysed at an American laboratory for a previous PhD thesis (Peyronnet, 2006). All other scales were analysed in the Marine Institute laboratory in Newport. Scale readers within both agencies were trained by the same expert reader and measurements were cross calibrated between different laboratories. Furthermore, the readers in the Marine institute laboratory were trained by an experienced reader within the agency and work was cross checked. Therefore, within this study scale reading was conducted in a consistent manner and differences between readers or laboratories are unlikely to bias the results.

Overall this study found that each of the Atlantic salmon populations examined showed differences in scale growth during the marine phase. The results indicate that each population responded differently to their environment. Growth reductions over time were detected most notably at the later stages of the 1970's which corresponds with the reported declines of Atlantic salmon. Environmental factors may also have had an effect on growth rates as negative relationships were established between growth indices and SST, AMO and NAO.

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Table 5.1 Details of river, time frames and samples analysed within this study; period relates to post-smolt year.

River	Period	No. of years / decade	No. of years	No. of samples
Burrishoole	1961-1969	1960 (9)	47	2153
	1970-1979	1970 (10)		
	1980-1989	1980 (10)		
	1990-1999	1990 (10)		
	2000-2007	2000 (8)		
Moy	1952-1959	1950 (8)	33	784
	1960-1961	1960 (2)		
	1972-1974, 1979	1970 (4)		
	1980, 1982-1983, 1987-1989	1980 (6)		
	1990, 1993, 1995, 1997-1998	1990 (5)		
	2000-2007	2000 (8)		
Shannon	1957-1959	1950 (3)	15	643
	1960-1969	1960 (10)		
	1970-1971	1970 (2)		

Table 5.2 Results of post-smolt growth (PSG; mm) marine circuli number (Circ No.) and first summer maximum (FSM; mm) measurements per river.

		River								
		Burrishoole			Moy			Shannon		
Variable	**Decade**	**Mean ± SD**								
PSG	1950	------	--	------	1.86	±	0.28	2.03	±	0.27
	1960	1.92	±	0.35	1.81	±	0.30	2.09	±	0.27
	1970	1.92	±	0.32	1.94	±	0.21	2.3	±	0.23
	1980	1.65	±	0.33	1.98	±	0.26	-----	--	------
	1990	1.65	±	0.41	1.74	±	0.26	-----	--	------
	2000	1.51	±	0.28	1.76	±	0.32	-----	--	------
Circ No.	1950	------	--	-----	32.7	±	4.0	32.6	±	3.1
	1960	31.1	±	5.0	32.2	±	4.6	33.8	±	3.3
	1970	31.6	±	5.0	33.2	±	4.0	35.4	±	1.9
	1980	27.7	±	5.5	33.3	±	3.6	------	--	------
	1990	26.7	±	6.2	30.7	±	4.3	------	--	------
	2000	26.2	±	4.4	32.4	±	4.7	------	--	------
FSM	1950	------	--	-------	0.088	±	0.014	0.098	±	0.013
	1960	0.094	±	0.014	0.088	±	0.015	0.097	±	0.014
	1970	0.090	±	0.013	0.087	±	0.01	0.10	±	0.015
	1980	0.088	±	0.013	0.092	±	0.014	------	--	-------
	1990	0.089	±	0.013	0.086	±	0.015	------	--	-------
	2000	0.089	±	0.015	0.081	±	0.013	------	--	-------

Table 5.3 Correlations between post-smolt growth (PSG; mm) and circuli number (Circ No.) against environmental variables for all three rivers. * Indicates P level associated with statistical significance following temporal autocorrelation.

	River											
Variable*	Burrishoole				Moy				Shannon			
	PSG		**Circ No.**		**PSG**		**Circ No.**		**PSG**		**Circ No.**	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
Annual NAO	-0.24	0.11	-0.22	0.13	-0.06	0.73	-0.21	0.24	-0.15	0.60	-0.24	0.39
Winter NAO	**-0.36***	**0.013**	**-0.38***	**0.009**	-0.16	0.38	-0.20	0.27	0.12	0.67	-0.087	0.76
Annual AMO	**-0.55***	**<0.001**	**-0.49***	**<0.001**	-0.31	0.080	0.020	0.91	**-0.53***	**0.043**	-0.42	0.12
Winter AMO	**-0.40***	**0.005**	**-0.38***	**0.009**	-0.31	0.079	0.017	0.92	**-0.61***	**0.017**	-0.43	0.11
Annual NA SST	**-0.46***	**0.001**	**-0.40***	**0.005**	**-0.37***	**0.032**	-0.008	0.97	0.23	0.41	0.14	0.62
Summer NA SST	**-0.50***	**<0.001**	**-0.42***	**0.003**	**-0.37***	**0.037**	-0.011	0.95	0.20	0.47	0.11	0.70
Local SST	**-0.45***	**0.002**	**-0.39***	**0.007**	-0.25	0.17	0.17	0.35	-0.18	0.53	-0.31	0.26
Local summer SST	**-0.47***	**0.001**	**-0.42***	**0.003**	-0.24	0.17	0.016	0.93	-0.063	0.86	-0.051	0.86

*Variable; NAO (North Atlantic Oscillation); AMO (Atlantic Multidecadal Oscillation); SST (Sea surface temperature ^{o}C).

Chapter 6.

General Discussion

6.1 Overview

Scales remain the most widely collected biological material in fish. The growth patterns and measurements available from scales provides an integrated snapshot of the entire lifecycle and a record of how the fish has responded to its environment. Developments in digital analysis techniques has advanced scale analysis of Atlantic salmon (*Salmo salar* L.) rapidly in recent times. Precise measurements of circuli spacings, counts and aggregate scale growth measurements can be obtained (Friedland *et al*., 2005; Peyronnet *et al*., 2007; Jensen *et al*., 2012) and proxy values of growth rate can be calculated over short periods of time (6-14 d circulus $^{-1}$; Friedland *et al*., 1993; Hubley *et al*., 2008; Jensen *et al*., 2012; Todd *et al*., 2014) which examine spatial and temporal variation in growth, increasing our understanding of the factors contributing to trends in growth and survival (Peyronnet *et al*., 2007; McCarthy *et al*., 2008; Friedland *et al*., 2009; Hogan and Friedland, 2010). Within these studies both historical and contemporary scale samples are included in the analysis. However, scales have been shown to form at different stages along the body (Warner and Havey, 1961), scale shape and size along with the produced scale measurements may vary between scales from different body locations. Knowledge gaps are present within the field of scale analysis; the implications of analysing scales of unknown body location have never been investigated therefore it unknown if growth measurements obtained from scales from various body locations are comparable with measurements from the standard sampling location. Furthermore, the estimates of growth rates commonly used in scale studies have never been experimentally validated so it is unclear at what rate circuli deposition occurs or if environmental factors have an effect on scale

growth. This thesis addressed these knowledge gaps by investigating if differences in growth measurements were evident between the standard sampling body locations and other body locations (chapter two); by rearing salmon under controlled environmental conditions and examining scale circuli deposition rates and growth during the early post-smolt stages of the life cycle (chapters three and four); by comparing scale growth patterns from three Atlantic salmon populations and to establish if environmental factors affected growth (chapter five) with marine growth and patterns of growth inferred from the experimental information of chapters three and four.

The aims of chapter two were achieved. Firstly; the results of this study showed that significant differences in growth, size and shape measurements occur between scales obtained from the standard sampling location and scales obtained from the body locations investigated. It was determined that two locations in the peripheral body region would suffice as an alternative sampling area if required as growth measurements were sufficiently correlated with measurements from the recommended sampling location; a calibration equation was established which allows for a conversion of measurements between these locations to achieve comparable measurements to those at the standard sampling location. Growth measurement differences were particularly pronounced when scales taken from the anterior region of the body were compared to scales taken from the standard sampling location and their use is not recommended for inclusion in growth studies as calibration is not possible. Secondly; it was determined the scale size measurements can be used to distinguish between scales from different body locations. The results revealed that

scale size is significantly correlated with fish length and the nature of the fish size/scale size relationship is specific to each body location. Therefore, the generated regression equations can be used to objectively identify scales that are likely to originate from a location other than the standard sampling location or two alternative sampling locations. The findings of this study are important to the scientific community as the results not only highlight the importance of scale selection, they also highlight the implications for the future collection of scales of Atlantic salmon. The findings should instil confidence in scale analyst and managers for the future integrity of scale studies. The results verify that measurements derived from non-standardised body locations will produce inconsistent estimates of growth if uncorrected. This study confirms that archival scale collections may be used within scale studies once a scale fits a certain size criterion as reported within this study. Furthermore; in instances when scales from the standard sampling location are unavailable due to scale loss, scales samples should be obtained from the peripheral body region. The generated calibration equation should then be used to facilitate directly comparable growth results which will lead to more confidence in the results generated in growth studies.

The aims of chapters three and four were achieved and shall be reported simultaneously for this general discussion due to the similarities in experimental design, the combination of results from chapter three and chapter four and the overall suggestions being discussed. The results of these studies showed that marine growth is the most reliable indicator of somatic growth as the relationship between scale growth and somatic growth was proportional across all treatments, justifying the use

of scale measurements as a proxy for growth as this relationship appears to be independent of environmental factors. The rate of circuli deposition was dependant on temperature and feeding regime and was generally proportional to fish growth but with some decoupling of the relationship at 15 °C. Deposition rates varied from 4.8 d circulus^{-1} at 15 °C (constant feeding) to 15.1 d circulus^{-1} at 6 °C (interrupted feeding), confirming that marine circuli are deposited at irregular intervals. Cumulative degree day was therefore a more reliable predictor of circuli deposition rate than day although the rate of circuli deposition per degree day was significantly lower at 6 °C compared to the 15 °C and 10.5 °C treatments. Deposition rates varied from 0.0133 circulus cdd^{-1} at 15 °C to 0.0103 circulus cdd^{-1} at 6 °C, and a proxy value of 0.01 circulus cdd^{-1} was established. Circuli spacings were highly variable and did not reflect growth rate; narrow spaced circuli occurred during periods of starvation at 6 °C when growth was depressed, but also during periods of rapid growth at 15 °C. The findings of this study are extremely relevant to the scientific community; circuli deposition rate has now been experimentally validated under different environmental factors which have shown that marine circuli are deposited at irregular intervals and using general deposition rates as a means of evaluating and reconstructing growth histories of Atlantic salmon may produce erroneous results. The impacts of temperature, growth rate and food supply on circulus spacing were complex and as circuli spacings did not accurately reflect growth, it is recommended that this measurement is not used to assess growth. These findings highlight the importance of considering temperature and feeding histories when using scale measurements to reconstruct fish growth and the results further our current understanding of scale growth properties and can inform

investigations of declining marine growth in Atlantic salmon based on interpretations of scale growth patterns with more accuracy. Alternating the commonly used proxy value of 7 d circulus^{-1} (Friedland *et al.*, 1993) to the value of 0.01 circulus cdd^{-1} reported in this study will not only allow for a more accurate reconstruction of growth histories, it will also provide more insight into the potential negative effects of climate induced increases in sea surface temperature. Using the thermal history along with interrogating the scale growth patterns from various populations and stocks would identify if growth is declining (southern populations) or ultimately increasing (northern populations) and would provide knowledge as to the effect that a changing environment is having on this species and help to identify which populations are most at risk from these changes.

The aims of chapter four were achieved. The results showed that scale growth measurements and their temporal trends varied between populations from the three Irish rivers (Burrishoole, Moy and the Shannon) investigated using archived scales collected from 1954 and 2008. Changes in scale growth measurements were largely consistent across the three rivers over time. The highest growth rates were observed in the River Shannon followed by the Moy and Burrishoole. Post-smolt scale growth and circuli number were negatively correlated with SST (Burrishoole and Moy), NAO (Burrishoole) and AMO Burrishoole and Shannon). Retrospective scale studies commonly include circuli spacing into studies mostly in the form of putative monthly growth rates and compare these estimated monthly rates with environmental variables to assess if there are any causative affects. However, as the results from both chapters

three and four of this study recommended that circuli spacing may not accurately describe growth and circuli deposition rate too variable, this study did not incorporate the use of circuli spacing, proxy circuli deposition rates or estimated putative monthly growth rates so the results are not comparable with previous studies. However, the main finding from this study is that that trends observed in one national index river may not be representative of change across all populations.

6.2 Building understanding of Atlantic salmon at sea

6.2.1 Migratory shifts due to climate change

In recent times pelagic fish have been found further north and are present in areas where they have not been present in significant numbers previously (Montero-Serra *et al*., 2014). In the northeast Atlantic, studies showed that southern fish moved north into the English Channel, Celtic Sea and North Sea and within the North Sea species moved poleward over the last few decades (Perry *et al*., 2005; Simpson *et al*., 2011). Also, there is evidence that distribution of Atlantic salmon in the north Atlantic have changed and they have been reported in areas where they were previously less common or absent (Jensen *et al*., 2014). These types of migratory changes are indicative of increasing temperatures, which cause changes in composition, abundance and distribution of the planktonic crustaceans (Jacobsen and Hansen, 2000; Beaugrand *et al*., 2002; Beaugrand and Reid, 2003).

The results obtained from scale analysis can be used an indicator of change and detect shifts in life history. The new knowledge generated in chapters three and four of this thesis will aid in the interpretation of scale growth patterns as the effects of both

temperature and feeding rate were explored. In chapter five of this study, post-smolt growth in the river Moy and Burrishoole was negatively correlated with the annual and summer North Atlantic SST. However, to fully examine this relationship and the growth patterns on a scale, it would be beneficial to have more accurate SST relating to an area and time as opposed to a large transect averaged over specific times. Therefore, to fully understand the impacts of a changing marine environment and to relate this to scale analysis with a higher resolution, additional sampling at sea surveys coupled with telemetry studies is needed to provide more accurate real time data of SST, migratory patterns and biological indices.

6.2.2 Scientific surveys

Atlantic salmon post-smolts and adults are an occasional bycatch within pelagic fish surveys in the Atlantic (ICES, 2017). During the 1990's and 2000's, the species was targeted by scientific surveys using a modified pelagic net within the Eastern Atlantic (Shelton *et al.*, 1997; Holst *et al.*, 2000) and within the North-western Atlantic (Lacroix and Knox, 2005; Sheehan *et al.*, 2011;). These surveys gave us valuable information on the salmons' presence within the North Atlantic; the predominant areas inhabited (Holm *et al.*, 2000), the diet and foraging rates (Haugland *et al.*, 2006; Sheehan *et al.*, 2012; Melle in prep), age profiles (Haugland *et al.*, 2006; Jensen *et al.*, 2011), origins (Verspoor *et al.*, 2012) and the species movements, swimming speeds and migrations in the ocean (Mork *et al.*, 2012; Sheehan *et al.*, 2012). However, dedicated salmon sampling programmes at sea are costly. A potential means of monitoring within the marine ecosystem would be to modify existing marine pelagic

surveys carried out annually in relevant areas. This incorporation has been suggested on both sides of the North Atlantic. A suggestion by Therriault *et al*. (1998) and further endorsed by Sheehan *et.al*. (2012) advises incorporating surface trawling into the Fisheries and Oceans Canada Atlantic Zone Monitoring Programme. This survey covers transects from southern Nova Scotia to southern Labrador; a region previously surveyed for post-smolts. Furthermore, ICES (2016) suggests incorporating survey trawling for post-smolts into pelagic surveys within the North-eastern Atlantic, most notably i.e. the International Ecosystem Survey of the Nordic Seas (IESSNS) which is implemented by research institutes from Iceland, the Faroes and Norway each summer since 2007. The survey covers areas of the North Atlantic which are known migratory and nursery areas favoured by Atlantic salmon.

Continued sampling over a longer time period is vital for gaining more insight into the environmental and ecological characteristics of the fish during specific periods of the marine lifecycle. These surveys would assist with further monitoring of the environment and would aid in identifying if and when changes are occurring within the environment. Surveying specified transects annually would also identify if changes in Atlantic salmon migratory patterns were occurring and also indicate whether changes occur in the amount and type of both competitive and predatory fish, directly impacting salmon survival due to pry competition plus predation. Changes in planktonic assemblages affecting productivity is another important assessment.

As with previous post-smolt surveys; the incorporation of a device for the collection of viable samples of salmonids is of importance. This device known as a fish lift (Holst and McDonald, 2000) or closed aquarium connected to the trawl cod-end, in turn this aquarium holds live fish providing viable samples such as bacterial and virology samples, blood and tissue samples, gonadal development samples and external parasite levels/samples. Furthermore, a haul could be sub-sampled providing the opportunity for non-lethal sampling such as scale and genetic samples with release back into the environment to reduce impacts of a species that is already in decline during a vulnerable part of its life cycle. Retrospective scale studies have incorporated environmental variables (SST, NAO and AMO), plankton indices and stock spawning biomass and assessed whether environmental factors have an effect on growth (Friedland *et al*., 2003; Peyronnet *et al*., 2007). Therefore, analysing scale samples obtained from these surveys for age and growth properties and relating the associated biological variables i.e. stomach content/feeding, plankton indices plus environmental variables, would provide more direct comparisons between marine growth and environmental (SST)/biological variables as opposed to using estimated values from large marine transects. In chapters three and four of this thesis the results showed that the impacts of temperature, growth rate and food supply on scales are complex, and although this thesis experimentally validated circuli deposition rates expressed as cumulative degree day, field studies in a natural mesocosm would build on the new knowledge generated in this thesis. If we know the life history of these fish, we can further explore and understand the growth patterns displayed on a scale.

6.2.3 Tagging studies

Telemetry is a very important modern method which complements both previous and current marine investigations within the marine environment. Advances in telemetry facilitate direct observation of individual fish and their environment (Drenner *et al*., 2012; Crossin *et al*., 2017). Acoustic, satellite and data storage tags (DST) relay vital information regarding temperature profiles, depths, swimming speeds and migratory routes. SALSEA Track is a collaborative international programme supported by NASCO with twelve main projects which aim to track salmon along their inshore and oceanic migration routes (NASCO, 2016). The first year at sea is critical for Atlantic salmon due to the high rate of marine mortality occurring within this period (Hansen and Quinn, 1998; Potter and Crozier, 2000; Friedland *et al*., 2009). Information collected from tagging projects produces real time data on the fish' environment, the results provide vital information on the areas inhabited plus the duration of residency within these areas coupled with a thermal profile. Furthermore, scale samples obtained from returning tagged fish gives an opportunity to fully interrogating measurements and deposition rates in scales and in further understanding how scale growth is influenced by the environment. Relating scale growth marks to the environmental data obtained from tagging data would aid in interpretation of scale patterns. This would help us fully interpret the information recorded on the scale coupled with the results found in chapters three, four and five and would further increase or understanding of scale pattern in turn leading to less subjectivity and confidence in results of previous and future studies.

6.3 Continuation of research

In recent years, scale analysis has progressed through the use of digital analysis tools however, the mechanisms driving scale growth are still poorly understood as is the implications of analysing scales of an origin other than the recommended sampling location. This thesis investigated these growth mechanisms for the first time.

In relation to chapter two, the results were inclusive of southern populations only and further work would be needed to assess if the results from this study would be applicable to more Northern counterparts most notably within the freshwater region of the scale. Due to the nature of declines of wild Atlantic populations and the closures of fisheries within countries, it would not be ethically justifiable to sample scales from numerous body locations from live wild fish; therefore, this work is limited to deceased fish obtained in traps or within designated fisheries. Samples could be obtained through collaboration with the international sampling programme in West Greenland as this sampling programme is conducted annually during the Greenlandic fishing season, scale samples could potentially be collected from numerous wild adult fish to incorporate fish from both southern populations along with more Northern and North American populations. Lastly, the origin of the scale sample should be included on the envelope, this is in practise in certain organisations but should be recommended as standard practise internationally; therefore, it would ensure standardisation and continuity of results.

In this thesis, it was established in chapters three and four how scale growth is influenced by temperature and feeding conditions early in the post-smolt phase under controlled laboratory conditions. Further experimental work could build on this new knowledge by investigating scale growth under more variable conditions and over a longer time period within a mesocosm setting. By altering the conditions within the experimental tanks and examining the impact on scale growth, future studies could build on from the present work and give us further insight into the mechanisms driving growth within a more natural environment. Within this, research, feeding was designated into weekly blocks and altering the feeding regime by quantity i.e. full feed, half feed, quarter feed over a longer time frame would complement the results presented in this thesis and give us further scope into assessing the effects of feeding on both scale and somatic growth. Within the last decade, anomalies in the form of a growth check have become apparent on scales of some wild fish (ICES, 2011). This growth anomaly occurs within the first few months at sea and has been suggested to represent a check caused by unstable conditions at sea i.e. a thermocline or lack of feeding. In this study a similar growth check was not apparent, but it was noted that the check would be difficult to identify due to the short duration of the experiment, as surplus scale growth would be a requirement to identify if a growth check would occur. Therefore, to progress this research in the future, it would be advisable to increase the experiment length by a number of weeks.

To further investigate the effects that temperature has on scale and somatic growth, a further study could incorporate the methods and results from this study and progress

further by altering the daily temperatures within the tanks, alternating the temperatures by a block of time per day i.e. a higher temperature during the initial twelve hours per day with a decreased temperature for the remaining twelve hours would aid in further assessing the implications of temperature changes on the Atlantic salmon. The scope of the research within this thesis was to investigate scale dynamics and somatic growth. To progress this research further, the effects of temperature and feeding regimes on the fish itself could be conducted, food consumption rates at varying temperatures, the physiological changes occurring due to elevated or decreased temperature and the effects that climate change may have on the species growth and maturation processes. This type of research would require a much longer duration, but would provide more information on these processes. Monitoring the stress levels on the fish over time would indicate how the fish cope with extreme temperature changes and dietary fluctuations. All of these suggestions would help us to probe further into the environmental issues that the species are now faced with.

To progress the research conducted in chapter five, it would be beneficial to extend the time series. As the status of the Atlantic salmon populations within Europe has not recovered since 2008 (end of the time series analysed within this research), it would therefore be helpful to investigate further and monitor scale growth to assess whether growth over the last decade has remained stable or declined further. Also, it would be beneficial to analyse more Irish rivers within a study to facilitate all regions within the country and to monitor whether differences in growth rates are more apparent within certain areas/populations. Furthermore, as Atlantic salmon scales are available both

regionally and internationally it would be beneficial to expand the work on the archived scales by incorporating other national collections. A collaborative programme that shared scale images and measurements between laboratories would help to ensure consistency across laboratories and stimulate more research. In Ireland, various organisation hold scale sets that could be combined into a national archive thus making them more accessible. Similarly, in other countries, multiple collections could be consolidated. Scale analysis is subjective. Intra and inter laboratory calibrations are key to ensuring comparability between readers and laboratories. As collaborative studies do occur between agencies, conducting a calibration study between the various laboratories at the onset of work is of prime importance to the integrity of the research, this type of exercise would ensure continuity for present and future studies that incorporate these data sets.

Stable isotope analysis has been used to examine the diet and migration of Atlantic salmon (MacKenzie *et al*., 2012; Dixon *et al*., 2012; Vuori *et al*., 2012) by portioning the scale into zones i.e. first winter, second winter. Incorporating stable isotope analysis into retrospective scale growth studies would aid in the interpretation of growth patterns. Studies could be segregated by sea age class, stock complex, nationally and also at a population level. As reported in chapter five, the River Shannon displayed the highest growth measurements of the three rivers analysed. As this river contained both one and two-sea-winter age classes, the possibility that two-sea-winter fish inhabited different feeding areas was suggested. Stable isotope analysis would assist in testing this hypothesis by comparing stable isotope signatures in the post-

smolt portion of the scale between one-sea-winter and two-sea-winter fish, to confirm if the groups were feeding in different areas or on different prey items. Furthermore, stable isotope analysis could be used to assess differences in growth between populations, as reported in chapter five. Comparing the scales between rivers would give further insight into dietary conditions encountered during specific marine stages and would assist in interpreting the differences in scale patterns and growth measurements. Finally, stable isotope analysis coupled with the growth measurements inferred from scales pre and post decline era warrants further work and would assist in identifying whether ecological conditions changed over time.

To conclude; this thesis has generated new information which will support more accurate interpretations of scale growth patterns, furthers our understanding of this important species and ultimately benefits the future management of Atlantic salmon.

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