THE UNIVERSITY OF HULL

Applications of fish scale analysis to understand growth dynamics of fish populations

being a Thesis submitted for the Degree of

Doctor of Philosophy

In the University of Hull

by

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April 2012

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ACKNOWLEDGMENTS

I am extremely grateful to my supervisors Professor Ian Cowx and Dr Jon Harvey for their support and advice throughout this study. I am indebted to Dr Darren Rollins, whose work created the foundations for this thesis. Many thanks to Hull International Fisheries Institute’s students and staff for their assistance with project support, specifically for their assistance in collecting larval and juvenile fish for use in Chapter 3. Many thanks are also due to the various people from across the globe who politely replied to my emails regarding techniques and publication requests. I would also like thank Dr R. Britton, Gareth Davies and all staff from the Environment Agency for supplying the fish scales for this study. I am grateful to the Environment Agency and Centre for Ecology and Hydrology for allowing access to temperature and flow data. Finally I would like to extend my deepest gratitude to Emily Austen for her patience, tolerance, grammar, punctuation and overall editing skills, and most importantly, for her encouragement.

ABSTRACT

The use of hard structures to derive ecological information about fish populations is a fundamental tool in fisheries assessment, specifically the back-calculation of fish lengths. This study highlights the potential errors associated with correction factors (*c*) because of poor sampling and provides a validation of (*c*) values. In addition, classical fisheries assumptions about the relationship between scale radius and fish body length were tested. As a result, variability or error of correction factors can be reduced by having a minimum of 30 samples with at least 4 age classes represented. Alternatively the (*c*) provided can be used as a standard (*c*) factor for each species, eliminating the variance caused by poor sampling. Finally, the development of standard intercept values (based on observation of juvenile fish) should be promoted to replace or validate mathematically derived (*c*).

The ability to accurately determine the age and growth of fish is an important tool in fishery biology and therefore it is fundamental to this work that all steps should be taken to increase the accuracy of back-calculated length-at-age data and account for size when fish lay down scales. To account for potential error associated with a correction factor, larval fish were routinely sampled to identify patterns of squamation, providing preliminary reference data for correction factors used in back-calculation of fish length-at-age. Determination of the length at squamation for more specimens will allow for the derivation of standard correction factors for each species that can be used across the species' distribution.

Geometric morphometric (GM) analysis of fish scales has been shown to be a good discriminator of genera using a fixed landmark approach. However, freshwater fish scales are often irregular in shape; therefore it is not possible to identify identical locations on all individuals. This study provides evidence that scale morphology can be used to discriminate riverine fish species. The analysis of fish scale morphology is inexpensive, quick, non-destructive, and informative and could easily be added to existing monitoring programmes. This study highlights the potentially important and opportunistic information that can be gained from the GM analysis of fish scales. It is therefore anticipated that this study will be fundamental in shaping future fish population assessments.

It is recognised amongst scientists that fish growth rates vary across a catchment, with species typically achieving greater growth rates in their 'preferred' habitats. Similarly, previous authors have identified that growth variation exists for different species and populations. This study has found that the geographic location of a river/region influences the growth rates of freshwater fishes commonly found in England. The method of constructing regional growth curves and subsequent statistical analysis discussed in this study should be adopted by fisheries scientists, because current national growth rates may be unachievable in specific regions. Furthermore, current national curves are inappropriate for growth and population analysis because they may be biased by an individual river and/or region. This study is one of the few studies to examine the differences between regional recruitment success, and found similarities and differences at both the regional and national level. With recruitment success a key requirement of monitoring fish populations under the WFD, it is hoped the information provided here will aid fisheries scientists to understand the factors affecting regional and national recruitment success.

Studies on the impact of climate change on fish populations have typically focused on suggesting, rather than predicting, the effects on lentic species rather than lotic species. Furthermore, these studies often deal with American rather than European ecosystems. To address this, predicted changes in the climate of the UK were used to model likely influences on fish populations, expressed as the length of young of year (YOY) fish achieved by the end of the first growth period (May-September), juvenile and adult growth (annual growth increment, AGI) and recruitment success (year class strength, YCS), for three cyprinid fish. This study found that climate change is likely to increase the propensity for cyprinid fish to thrive, although the exact mechanism will depend on inter-annual variability in temperature rises and the timing of flow events. Notwithstanding the limitations of this study, it provides ecologists with a greater understanding of climate change and its potential impact on European, lotic fish populations.

1 GENERAL INTRODUCTION

Analysis of information obtained from fish scales is one of the most important tools available to biologists, because fish scales capture information on the biotic and abiotic factors influencing populations. Van Leeuwenhoek, a Dutch draper and pioneering microbiologist of the 17th century, is credited with being the first to recognize the importance of fish scales, identifying the relationship between marks on scales and the age of fish (Elliott & Chambers, 1996; Jackson, 2007). However, it was not until the start of the 20th century that scale reading became widely used as a technique for ageing fish; the initial species being cod (*Gadus morhua* L.) and salmon (*Salmo salar* L.), perhaps a reflection of their economic value. Subsequently, studies using fish scales as a narrative of ecological information gained momentum until the early 1990s, after which, their popularity stagnated under developments in other areas of science and fisheries ecology such as genetics. However, following recent expansion of research into climate change and obligations of European Member States under the Water Framework Directive (WFD; 2000/60/EEC) to assess age structure of fish populations, analysis of fish scales is regaining importance because of the array of ecological information documented by fish scales.

Fish scale analysis has been extensively used in scientific studies: age and growth (Schuck, 1949; Bagenal & Tesch, 1978; Mann, 1973); life history characteristics (Campana, 2001; Bolland *et al.*, 2007); population dynamics (Crisp *et al.*, 1975; Cowx, 1989); to interpret past biodiversity (Shackleton, 1987; McDowall & Lee, 2005); diet analysis of piscivorous species (Mauchline & Gordon, 1984; Britton & Shepherd, 2005; Miranda & Escala, 2007); ecological integrity of large rivers (Williams, 1967; Schiemer, 2000); stock identification (Jarvis *et al.*, 1978; Ibanez *et al*., 2007); trace-metal contamination (Mugiya *et al.*, 1991; Basu *et al.*, 2006; Lake *et al.*, 2006); comparative and phylogenetic studies (Lippitsch, 1992; Roberts, 1993); rapid isolation of DNA (Yue & Orban, 2001; Nielsen & Hansen, 2008) and taxonomic and evolutionary studies (Reif, 1980; Kuusipalo, 1998; Hutchinson *et al*., 2001; Sire & Huysseune, 2003; Sire & Akimenko, 2004). Thus, the array of information provided by fish scales is vast and their assessment provides fisheries scientists with vital ecological information.

Despite of the countless studies on age and growth of fish populations, derived from scales, the validity and accuracy of such information has always been questioned (Cragg-Hine & Jones, 1969; Klumb *et al.*, 1999a, 1999b; Panfili & Tomás, 2001; Musk *et al*., 2006). Equally, although ageing of fish scales is a well documented technique (Bagenal & Tesch, 1978), gaps in knowledge and vagaries in interpretation and analysis persist (Allen, 1976; Mann, 1973, 1974; Britton *et al*., 2010b; Pegg *et al*., 2011), because classic studies were based on single populations and often lacked scientific rigour (Mann & Steinmetz, 1985). Back-calculation is a fundamental tool in the analysis of the age and growth of fish (Campana, 2001). A key assumption of back-calculation is "the growth increment of the scale is on average a constant proportion of the growth increment of the fish" and instead of passing through the origin, the line must pass through the point where scale radius equals 0 and the length of the fish is a constant (*c*) (Francis 1990). The common practice of fisheries scientist is to set the value of the constant (*c*) mathematically rather than from biological data. Thus, potential errors exist surrounding (*c*) values, because a low or high (*c*) values will over or under estimate back-calculated length-at-age. Subsequently, measures to reduce error surrounding mathematically derived (*c*) should be derived. An alternative approach is to assess the potential use of a biologically derived value for (*c*). However, biological values of (*c*) are not readily available. Despite a few studies having questioned there use (Jones, 1959; Carlander, 1985; Gjøsæter, 1986), there is a need to analyse larval and juvenile fish to establish biological values for (*c*) to allow future assessment of their use and validity.

Although, recent studies have used DNA (Yue & Orban, 2001; Nielsen & Hansen, 2008) and stable isotope (Kennedy *et al.*, 2005; Grey *et al.*, 2009) analyses to assess fish populations, their application is often limited because of high cost per scale sample analysed; further, these techniques require degradation of the scale sample. Thus, a readily available technique capable of providing further ecological information at low cost and without destroying the scale sample would be preferred. Geometric morphometric (GM) approaches maybe one such solution (Ibanez *et al.*, 2007, 2009), because an individual's morphological characteristics are dictated by abiotic and biotic factors (Gomes & Monteiro, 2008; Kaliontzopoulou *et al.*, 2010), accordingly this approach may be a very powerful tool and the possibilities this approach could provide should be explored.

Fish populations are influenced by a number of environmental, physical and biological variables. As a result, scales narrate abiotic and biotic factors influencing an individual fish, which in turn can be aggregated with information from other individuals to understand population responses to such factors (Le Cren, 1958; Williams, 1967; Pitcher & MacDonald, 1973; Jezierska, 1974; Linfield, 1979; Cowx, 1988; Cowx & Welcomme, 1998). Environmental variables vary over a geographic range subsequently dictating species distribution and life history traits, Typically riverine fish populations are influenced by temperature (Summerfelt & Shirley, 1978; Sarvala & Helminen, 1996; Tolonen *et al.*, 2003), flow (Smith, 1991; Wootton, 1990; Penczak, 2007) and climate (Nunn *et al.,* 2007a). Although few studies have accessed the differences in fish age, growth and recruitment success over a geographic range, thus there is a need to address this dearth of information.

Furthermore, analysis of age and growth information increases the understanding of life history traits and population growth parameters such as growth rate, age at maturity, mortality rates and year class strengths (Cowx, 2001). It is these characteristics of fish scales, which makes them increasingly important in assessing potential impacts of changing climate. Importantly, there is increasing evidence that global climate change is already having measurable biological impacts (Daufresne *et al.*, 2003). Numerous literature reviews and ecological studies have been published suggesting possible effects of climate change. However, there is a definite need for more predictive studies, providing a greater scientific insight. Such studies have already published for various taxa; bats (Humphries *et al.*, 2002), butterflies (Fleishman *et al.*, 2001; Kerr, 2001; Oberhauser & Peterson, 2003; Crozier & Dwyer, 2006), birds (Sæther *et al.*, 2004; Gordo *et al.*, 2005; Lemoine *et al.*, 2007; Barbraud *et al.*, 2011), coral reef fish (Munday *et al.*, 2008), insects (Ungerer *et al.*, 1999), ladybirds (Samways *et al.*, 1999), lizards (Chamaille-Jammes *et al.*, 2006) and marine mammals (Bluhm & Gradinger, 2008). Consequently, fisheries scientist are concerned how climate change will influence fish populations and research regarding this matter should be promoted.

The intention of this thesis is to improve the tools and interpretation of ecological information derived from fish scales, through improving the understanding of techniques and critique of current practices, guiding biologists in relation to fisheries science and management. In particular, the European Commission has placed extensive pressure on countries to meet legislation i.e. Water Framework Directive. Improving tools available for fisheries management will help them achieve these obligations. This study also intends to initiate a review of current best practice guidelines for fish scale analysis. Overall this thesis aims to examine the factors influencing life history characteristics of fish species commonly found in English rivers, with a view to educate fisheries ecologists and managers. To this address this aim, the thesis was divided into key topics that are addressed in chapters 2 to 6. Specific objectives and hypotheses are provided at the start of each chapter.

Chapter 2 addresses the need to assess the potential error associated with correction factors, or the validation of such a value. This study tests the classic assumptions of back-calculation techniques and establishes minimum sample size and number of age groups required to calculate a biologically valid correction factor. Finally, this study aims to derive standard (*c*) values for inclusion in the Fraser-Lee (1920) formula as an alternative technique, for when sampling is inadequate.

Chapter 3 investigates the importance of biologically derived correction factors. The aim of this study will be met through the review of literature and analysis of larval fish specimens. This study will also provide reference data to establish intercept values for use in back-calculation of fish lengths, as an alternative method for obtaining correction factors.

Chapter 4 evaluates the possibility of adopting a GM approach to assess irregular shaped fish scales, typical of British freshwater riverine fish species, whilst still retaining the statistical power of the GM approach.

Chapter 5 investigates whether geographic variation influences the growth rates and recruitment success of freshwater riverine fishes commonly found within England. A further aim is to provide a clear and precise methodology for detailed analysis of growth curves.

Chapter 6 determines whether the predicted climate changes in the UK, are likely to influence fish populations, expressed as the length of young-of-year fish achieved by the end of their first growth period, juvenile and adult growth (Annual Growth Increment, AGI) and recruitment success (Year Class Strength, YCS) of three cyprinid fish species from temperature guilds (cold, cool and warm).

Chapter 7 summarises the information gained from the Chapters 2 to 6 in the context of management implications and provides recommendations for further research.

2 BACKCALCULATION: THE DEVIL'S ADVOCATE

2.1 INTRODUCTION

Fish scales capture information on an individual's life history that typically relate to variability in space and time. The derivation of this information often relies on the relationship between the size of scales and the body length of fish (Bagenal & Tesch, 1978; Horppila & Nyberg, 1999). This relationship is widely used in fisheries science to estimate body size at a younger age by "back-calculation" (Weisberg, 1986; Casselman, 1990); a technique often used to generate length-at-age data (Francis, 1990; Horppila & Nyberg, 1999). However, some back-calculation methods rely on an erroneous assumption, that fish and their scales begin to grow at the same time (Francis, 1990). Fraser (1916) and Lee (1920), commonly cited as Fraser-Lee (1920), were the first to propose a correction factor when back-calculating length-at-age, to account for body growth before the onset of scale formation (Klumb *et al.*, 1999a).

Fish length at scale formation has been the cause of much discussion since back-calculation was first proposed (Everhart, 1949). Despite Fraser-Lee (1920) being the most commonly used back-calculation formula (Carlander, 1981; Francis, 1990), the procedure does not appear to be well understood (Francis, 1990). The current practice for obtaining the correction factor, described as the length of the fish at the time of scale formation (Francis, 1990), is derived from the length intercept of the regression line between scale radius and fish length (Lee, 1920) or vice versa (Hile, 1941), rather than from observations of juvenile fish. Francis (1990) reviewed back-calculation practise and found 40% of the studies using Fraser-Lee (1920) calculated correction factors using the wrong regression. Francis (1990) suggested studies often use the wrong regression, but fails to provide any further information. Campana (1990) found regression and the Fraser-Lee (1920) back-calculation techniques are sensitive to variation in intercept values derived from the relationship between fish length and the size of hard structures. Subsequently, the effect of errors in estimating the age of fishes can have serious repercussions, especially because most fisheries dynamics models are based on ageing data thus they need to be accurate (Campana, 2001; Britton *et al.*, 2004b).

Few studies have discussed in detail the potential error associated with correction factors, or the validation of such a value. The aim of this study was to test classic assumptions that the relationship between fish length and scale radius is linear and that the correction factors derived from Hile (1941) are always lower than Fraser Lee (1920), as proposed by Ricker (1973). In addition, an assessment of the error surrounding correction factors derived from Fraser-Lee (1920) and Hile (1941) will be reviewed, to derive a minimum sample size and number of age groups required to calculate a biologically valid correction factor. Finally, this study aims to derive standard (*c*) values for inclusion in the Fraser-Lee (1920) formula as an alternative technique for when sampling is inadequate as suggested by Carlander (1982, 1985).

2.2 MATERIALS AND METHODS

2.2.1 *Sampling*

A diversity of fish species inhabit English rivers; including a number of freshwater families incorporating different reproductive guilds, e.g. eurytopic, limnophilic and rheophilic species (Schiemer & Waidbacher, 1992) (Table 2.1). Rheophilic fish species require flowing water to spawn and, as such, are usually found in fast-flowing, well-oxygenated stretches of river where the substratum is characterised by gravel or sand. Limnophilic species, on the other hand, prefer still water and prolific aquatic vegetation to deposit their eggs upon and, as such, are most abundant in isolated floodplain waterbodies or backwaters and oxbow lakes. Eurytopic species have less strict requisites for spawning, and are thus able to establish populations in a wider range of waterbodies than less adaptable species. Lithophilic species, for instance, deposit their eggs on gravel, while phytophils use aquatic vegetation as a substrate, phytolithophils use either vegetation or gravel, and psammophils use sand. Fish species can be further classified according to environmental and reproductive guilds (Davies et *al*., 2004; Welcomme *et al*., 2006). Fortunately, the diverse range of fish species found within the England the majority can be appropriately sampled by electric fishing. All fish data were acquired from Environment Agency (EA) fish monitoring surveys between 2003 and 2009. These surveys were conducted using electric fishing or netting, depending upon the most appropriate technique for the water being sampled. All fish captured during surveys were identified, measured (fork length, L_F, mm) and scale samples removed from the shoulder region for later laboratory analysis. All growth analysis was based on scales, read under a low powered microfiche, with a magnification of 20x or 30x (Bagenal & Tesch, 1978). To minimise errors in ageing of scales, a quality control procedure was followed, as described in Musk *et al.* (2006), where a secondary reader checked 10% of the aged scales. Where disagreement was found, the scale was reviewed to enable consensus to be reached. In addition to Musk *et al.* (2006), a second opinion was sought for any scales which proved difficult for the primary scale reader, outside of the quality control procedure.

Table 2.1 Riverine species commonly captured from English rivers, reproductive guilds according to Schiemer and Waidbacher (1992).

Family *Species*	**Vernacular name**	**Reproductive guild**
Anguillidae		
Anguilla anguilla (L.)	Eel	Eurytopic
Balitoridae		
Barbatula barbatula (L.)	Stone loach	Rheophilic A
Cobitidae		
Cobitis taenia L.	Spined loach	Rheophilic B
Cottidae		
Cottus gobio L.	Bullhead	Rheophilic A
Cyprinidae		
Abramis bjoerkna (L.)	Silver bream	Eurytopic
Abramis brama (L.)	Common bream	Eurytopic
Alburnus alburnus (L.)	Bleak	Eurytopic
Barbus barbus (L.)	Barbel	Rheophilic A
Cyprinus carpio (L.)	Carp	Eurytopic
Gobio gobio (L.)	Gudgeon	Rheophilic B
Leucaspius delineates (Heckel)	Sunbleak	Rheophilic A
Leuciscus cephalus (L.)	Chub	Rheophilic A
Leuciscus idus (L.)	Orfe (Ide)	Rheophilic A
Leuciscus leuciscus (L.)	Dace	Rheophilic A
Phoxinus phoxinus (L.)	Minnow	Rheophilic A
Rhodeus sericeus (Pallas)	Bitterling	Limnophilc
Rutilus rutilus (L.)	Roach	Eurytopic
Scardinius erythrophthalmus (L.)	Rudd	Limnophilic
Tinca tinca (L.)	Tench	Limnophilic

Table continued overleaf

Table continued

Esocidae		
Esox lucius L.	Pike	Eurytopic
Gaserosteidae		
Gasterosteus aculeatus L.	Three-spined stickleback	Eurytopic
Pungitius pungitius (L.)	Ten-spined stickleback	Limnophilic
Gobiidae		
Pomatoschistus microps (Kroyer)	Common goby	
Muglidae		
Liza ramada (Risso)	Thin-lipped grey mullet	
Percidae		
Gymnocephalus cernuus (L.)	Ruffe	Eurytopic
Perca fluviatilis L.	Perch	Eurytopic
Sander lucioperca (L.)	Zander	Eurytopic
Petromyzontidae		
Lampetra spp.	River/brook lamprey	Rheophilic A
Petromyzon marinus (Bloch)	Sea lamprey	Rheophilic A
Pleuronectidae		
Platichthys flesus (L.)	Flounder	Rheophilic C
Salmonidae		
Oncorhynchus mykiss (Walbaum)	Rainbow trout	Rheophilic A
Salmo trutta L.	Brown/sea trout	Rheophilic A
Salmo salar L.	Atlantic salmon	Rheophilic A
Salvelinus alpinus (L.)	Arctic charr	Rheophilic A
Thymallus thymallus (L.)	Grayling	Rheophilic A

2.2.2 *Relationship between fish length and scale radius*

The existence of a relationship between fish length and scale radius (or other hard structures) is a key assumption underlining back-calculation. This assumption was tested on data collected for each species for each river (population). Data collected from multiple sites within the same river were combined although it is recognised growth may vary between reaches. Linear least squares regression analysis was performed to establish whether a relationship exists between fish length and scale radius at time of capture. Individuals from all rivers were combined to provide an r^2 value and standard deviation for each species. In addition to linear least square analysis, other regression models were tested using curve estimation analysis in SPSS (V16), including logarithmic, quadratic, cubic (polynomial), power, compound, S (logistic curve (sigmoid)), logistic, growth and exponential.

2.2.3 *Back-calculation with correction factor*

The relationship between fish length and scale radius is calculated from two functions (Francis 1990). Francis (1990) stated these functions can be denoted as F and G, where $F(L)$ is the mean scales radius for fish of length (L), and $G(S)$ is the mean body length for fish with scale radius (S) is the mean body length for fish with scale radius. These functions, in their most common form (linear) are:

$$F(L) = a + bL \qquad \text{(equation 1)}$$

$$G(S) = c + dS \qquad \text{(equation 2)}$$

F is derived from the regression of S against L, and G from the regression of L against S.

The first readily acknowledged back-calculation formula is that described by Lea (1910), a formula developed by Knut Dahl and Einar Lea, subsequently known as Dahl-Lea (1910) who proposed scales grow in exact proportion to the total length of the individual (Bagenal & Tesch, 1978; Heidarsson *et al.*, 2006; Francis, 1990). Dahl-Leas' (1910) equation, describing the linear relationship between scale radius and fish length and assuming the relationship passes through the origin and can be written as:

$$L_t = (S_i/S_r)L_F \qquad \text{(equation 3)}$$

Lee (1920) investigated the hypothesis of Lea (1910) for a number of species and concluded the relationship between *S* against *L* did not pass through the origin. Instead growth increments of the scales are generally a constant proportion of the growth increment of the fish and Lee (1920) assumed the relationship passed through the intercept (S=0, L=c), where *c* as in equation 2. Lee (1920) converted the original approach described by Fraser (1916) and is subsequently known as the Fraser-Lee (1920) equation:

$$L_t = c + (L_F - c)(S_i/S_r) \qquad \text{(equation 4)}$$

Fraser-Lee (1920) described *c* as the length of the fish at the time of scale formation, with the assumption being that this length is the same for all scales from all fish in a given population. However, the current practice of most authors (and that followed by Lee (1920)) is to set *c* as the L-intercept of the regression line (equation 2), rather than direct observations of scale formation from juvenile fish.

Hile (1941) proposed a back-calculation technique that does not require the body-scale relationship to be linear or take any particular form. Hile's (1941) technique assumes the percentage or relative deviation of the radius of any single scale from the theoretical radius is constant at the time of formation of all annuli and at the time of capture. Hile's (1941) formula can be written as:

$$L_t = -\,(a/k) + (L_F + a/b)(S_i/S_r) \qquad \text{(equation 5)}$$

where *a* and *b* are as in equation 1. This is the same as the Fraser-Lee (1920) equation, (equation 4), except that *c*, the L-intercept in equation 2 is replaced by $-(a/b)$, the L-intercept of (equation 1). Ricker (1973) stated $-(a/b)$ is always less than *c*, so back-calculated lengths from (equation 5) will always be less than those from equation 4. The sole reason for the correction factor is to represent the fish length at scale formation. Consequently, the derived correction factor should be biologically valid, defined as a length greater than size at emergence (Pinder, 2001) and smaller than size at the expected length-at-age 1 (Britton, 2007), assuming that all fish lay down scales in the first calendar year of growth, which has been contested for later spawning species such as chub (Nunn, 2005).

All statistical analysis was performed using linear regression analysis in SPSS version 16.

2.2.4 *Correction factor error*

A Monte Carlo simulation was used to assess the potential error surrounding correction factors derived from Fraser-Lee (1920) and Hile (1941). The Monte Carlo method is based on the generation of multiple iterations to determine the expected value of a variable. The minimum and maximum (*c*) values for a given species and parameter (e.g. (*c*) factor derived from *L. cephalus* with a sample size of 15) were used to generate 1000 random iterations allowing the prediction of the estimated total error:

$$\varepsilon = \frac{3\sigma}{\sqrt{N}}$$

where 3 is a constant, σ is the standard deviation of the iterations, and N is the number of iterations. Total error was calculated for two parameters: 1, the number of individuals in a sample, classified into categories with increments of 15 individuals; 2, the number of age groups used in the analysis.

2.3 RESULTS

2.3.1 *Relationship between scale radius and body length*

Analysis of regression models (logarithmic, quadratic, cubic, power, compound, S (logistic curve (sigmoid)), logistic, growth and exponential) confirmed that linear least squares regressions is the most appropriate method to compare the relationship between fish length and scale radius, repeatedly providing one of the highest r^2 values and the most acceptable intercept values (Appendix 1), where acceptable values are those which are similar to chapter 3 (Table 2.2). There were strong linear relationships between fish length and scale radius for all species (Table 2.3). The lowest average r^2 values were for *G. cernuus* and *S. trutta* (sea trout), 0.23 and 0.48, respectively; although it is important to note the sample size for these two species was relatively low.

Ricker (1973) stated the back-calculation technique of Hile (1941) will always produce a correction factor less than Fraser-Lee (1920). To test this assumption correction factors were calculated for 19 species, totalling 931 separate populations (rivers) (Table 2.4). Correction factors derived from Hile (1941) were less than Fraser-Lee (1920) in 98.5% populations, Hile (1941) were greater than Fraser-Lee (1920) 2.2%

and equal to Fraser-Lee (1920) 3.3%. The relationships between Hile (1941) and Fraser-Lee (1920) for 9 species shows no clear relationship (Figures 2.4), possibly because of numerous outliers.

Table 2.2 Difference between correction factors (fish length at time scale radius is zero, mm) between linear regression, quadratic, S and cubic curves and the correction factor derived in Chapter 3 (50% median value), r^2 are in parentheses.

		Regression type			
Species	Chapter 3	Linear	Quadratic	S	Cubic
L. cephalus	31	37	-11	6	16
		(0.92)	(0.94)	(0.81)	(0.94)
L. leuciscus	30	32	12	6	25
		(0.86)	(0.86)	(0.77)	(0.86)
P. fluviatilis	33	55	53	6	54
		(0.77)	(0.77)	(0.63)	(0.77)
R. rutilus	32	30	18	4	18
		(0.91)	(0.91)	(0.80	(0.91)

Table 2.3 Linear relationship between scale radius and fork length and (*c*) values for inclusion in Fraser-Lee (1920) for 19 species. Intercept values for *C. carpio*, *O. mykiss* and *S. trutta* (sea trout), should be interpreted with caution because of their small sample size.

Species	Number of rivers	Number of individuals	r^2	Intercept (Fraser-Lee (*c*) mm)
A. bjoerkna	20	257	0.76**	30
A. alburnus	26	1885	0.63**	39
A. brama	101	4391	0.89**	19
B. barbus	50	1751	0.86**	50
C. *carpio*	3	5	0.94**	60
G. cernuus	15	68	0.23**	69
G. gobio	31	1552	0.62**	41
E. lucius	52	2575	0.85**	75
L. cephalus	134	23042	0.92**	37
L. leuciscus	132	17476	0.86**	32
O. mykiss	6	28	0.83**	6
P. fluviatilis	66	3812	0.78**	54
R. rutilus	168	39482	0.91**	30
S. erythrophthalmus	23	210	0.89**	21
S.lucioperca	5	58	0.76**	24
S. salar	13	401	0.89**	32
S. trutta (brown trout)	100	13315	0.76**	39
S. trutta (sea trout)	9	21	0.48**	142
T. tinca	8	53	0.86**	22
T. thymallus	31	1898	0.85**	58

*$P<0.05$; **$P<0.01$

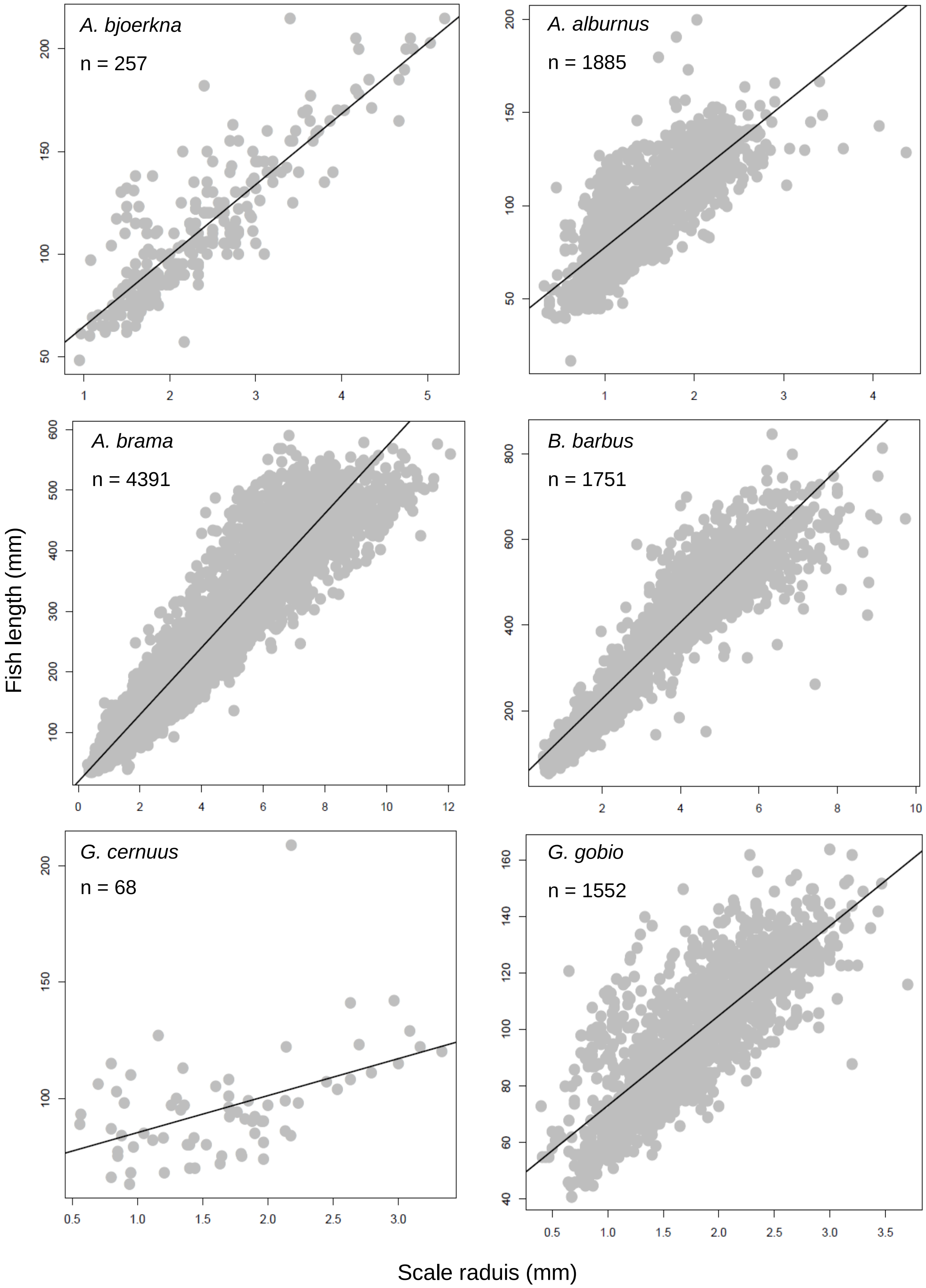

Figure 2.1 Relationship between scale radius and fish length for 18 species, where n is the number of individuals.

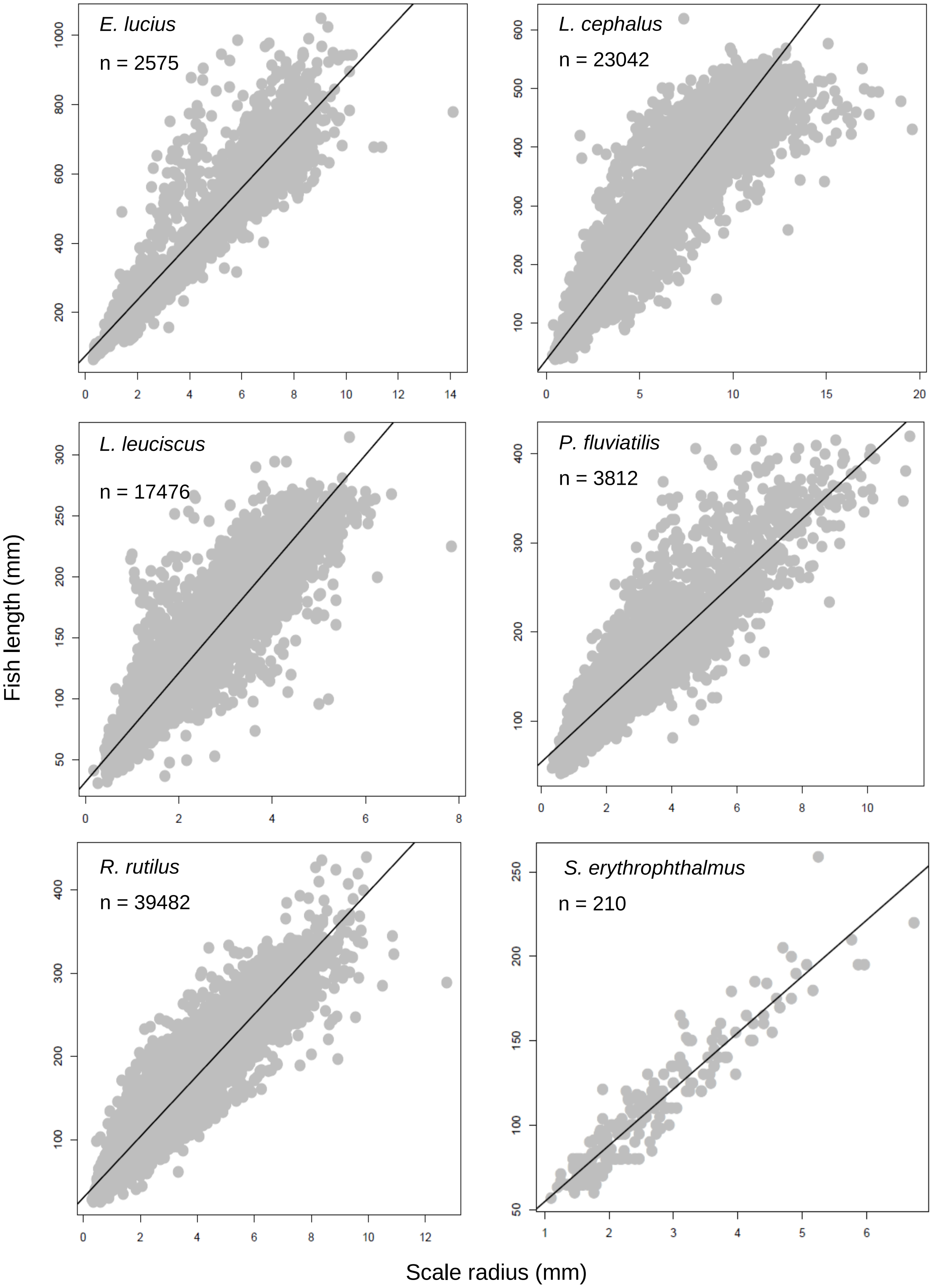

Figure 2.1 continued Relationship between scale radius and fish length for 18 species, where n is the number of individuals.

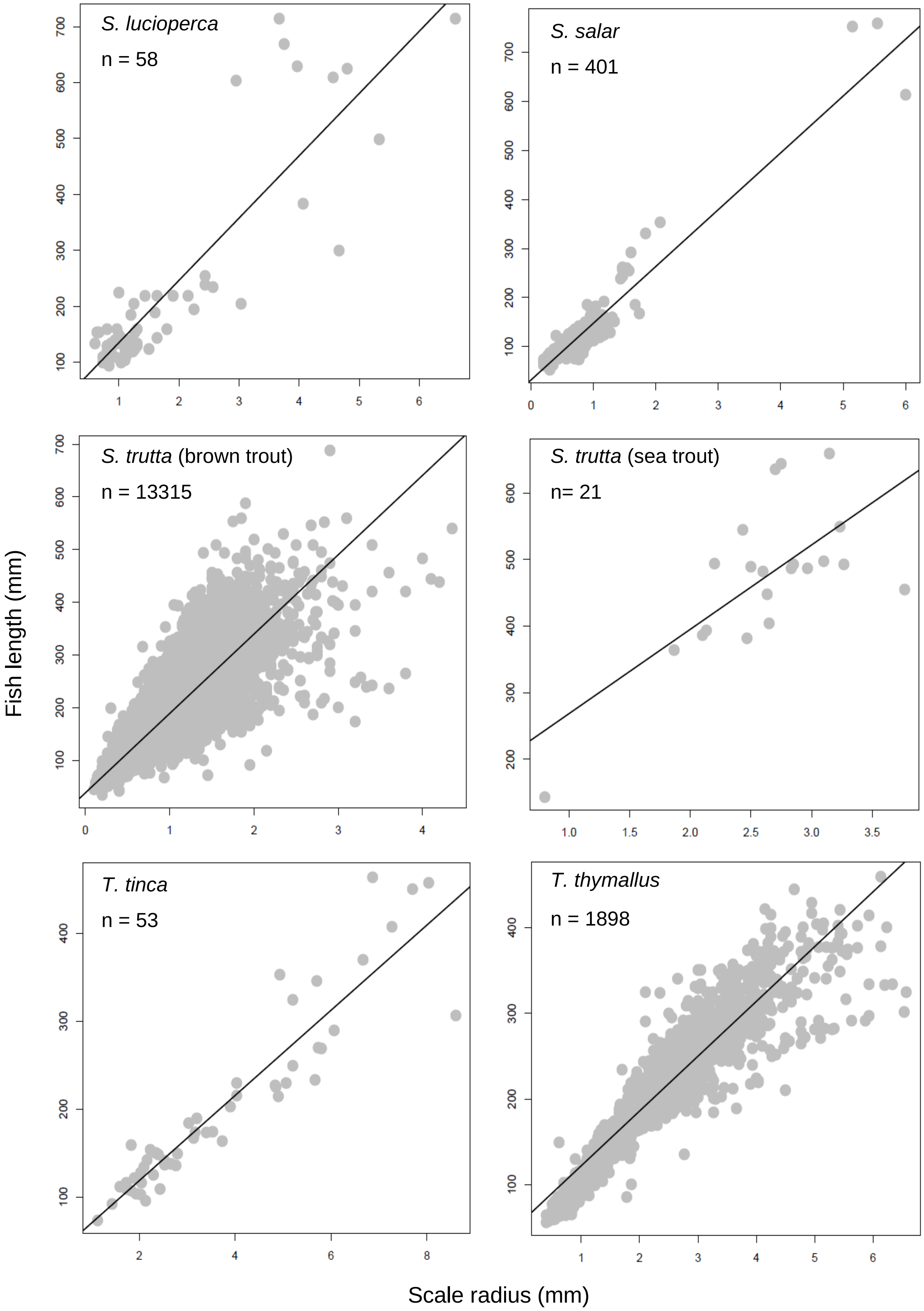

Figure 2.1 continued Relationship between scale radius and fish length for 18 species, where n is the number of individuals.

Table 2.4 Linear relationship between Hile (1941), H, and Fraser-Lee (1920), FL for 21 species, where n is the number of populations.

Species	n	H < FL	H > FL	H = FL
A. alburnus	26	26	0	0
A. bjoerkna	17	15	0	2
A. brama	94	85	4	5
B. barbus	50	48	1	1
C. carpio	1	0	0	0
E. Lucius	51	46	2	3
G. cernuus	10	8	1	1
G. gobio	29	26	0	3
L. cephalus	131	128	2	1
L. delineates	1	1	0	0
L. idus	1	0	0	1
L. leuciscus	125	121	0	4
O. mykiss	5	3	0	2
P. fluviatilis	61	59	2	0
R. rutilus	163	163	0	1
S. erythrophthalmus	15	15	0	0
S. lucioperca	5	5	0	0
S. salar	12	11	1	0
S. trutta (brown trout)	92	88	2	2
S. trutta (sea trout)	6	3	2	1
T. thymallus	29	25	2	2
T. tinca	7	4	1	2
Total count	931	880	20	31
Percentage		94.52%	2.15%	3.33%

The sole purpose of a correction factor is to represent the fish length when scales begin to form. For this reason, the derived correction factor should be biologically valid, defined as a length greater than size at emergence (Pinder, 2001) and smaller than size at the expected length-at-age 1 (Britton, 2007), assuming all fish fish lay down scales in their first year. For twelve species more correction factors derived from Fraser-Lee (1920) were biologically valid than values derived from Hile (1941); for two species (*G. cernuus* and *T. thymallus*) more correction factors were biologically acceptably using the Hile (1941) method, and for one species (*A. bjoerkna*) both formulae were equally acceptable (Table 2.5). The value of a correction factor can influence the growth data generated for a population (Figure 2.2). Furthermore, the influence of a correction factor is greatest in the 1st year of growth and declines as age increases. Two examples of the effect of low sample size and number of age groups on the correction factor derived from Fraser Lee (1920) for can be seen in Figure 2.3.

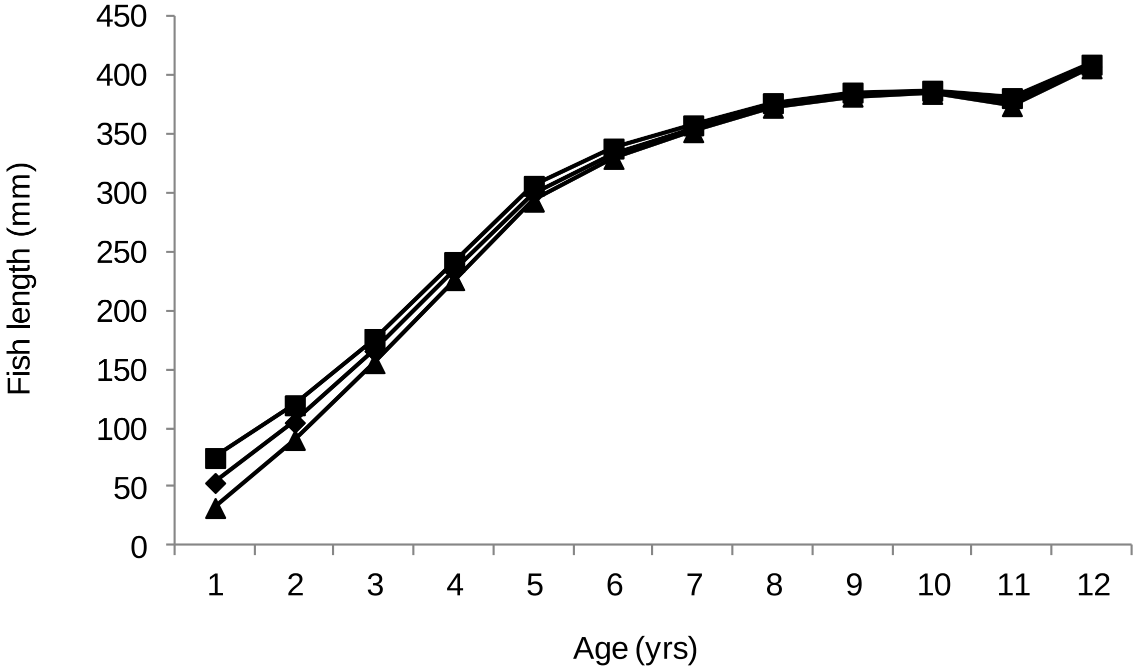

Figure 2.2 Influence of different correction factor in Fraser-Lee (1920) for *L. cephalus* captured from the river Rother 2007, where (▲) is (*c*) factor of 25, where (■) is (*c*) factor of 50 and (♦) is the Dahl-Lea (1910) BCF for comparison.

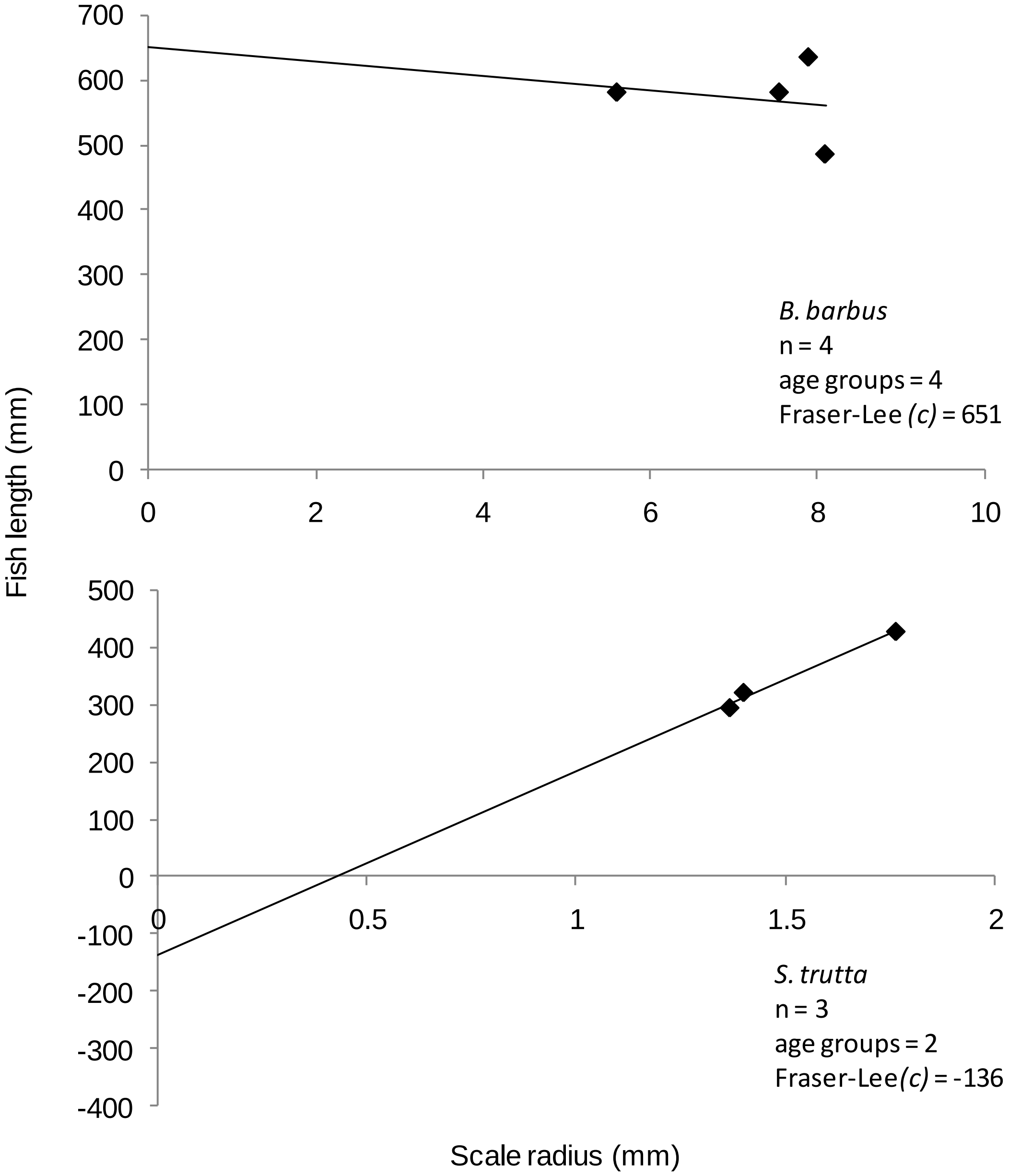

Figure 2.3 Examples of the effect of low sample size and number of age groups on the Fraser-Lee (1920) correction factor for two species (*B. barbus* and *S. trutta*).

Table 2.5 Biological validity of correction factor derived from Fraser-Lee (1920) and Hile (1941), where (n) is the number of rivers, Length at emergence is based on Pinder (2001), Ln is body length, expected length-at-age 1 is based on growth described by Britton (2007).

Species (n)	Ln at emergence (mm) Expected Ln at age 1 (mm)	Back-calculation formula	> Ln at t0	< Ln at age 1	Count of values not acceptable	Count of values acceptable	% acceptable
A. alburnus	5	Hile	12	0	12	3	20.00
(15)	43	Fraser-Lee	3	1	4	11	73.33
A. bjoerkna	5	Hile	4	0	4	3	42.86
(7)	41	Fraser-Lee	1	3	4	3	42.86
A. brama	5	Hile	19	4	23	10	30.30
(33)	48	Fraser-Lee	6	6	12	21	63.64
B. barbus	8	Hile	15	2	17	7	29.17
(24)	67	Fraser-Lee	6	7	13	11	45.83
G. cernuus	4	Hile	1	1	2	3	60.00
(5)	48	Fraser-Lee	0	3	3	2	40.00
G. gobio	5	Hile	10	1	11	4	26.67
(15)	48	Fraser-Lee	4	3	7	8	53.33
E. lucius	10	Hile	12	1	13	15	53.57
(8)	207	Fraser-Lee	5	2	7	21	75.00

Table continued overleaf

Table continued

L. cephalus	7	Hile	25	3	28	37	56.92
(65)	48	Fraser-Lee	4	12	16	49	75.38
L. leuciscus	8	Hile	20	1	21	39	65.00
(60)	53	Fraser-Lee	1	3	4	56	93.33
P. fluviatilis	5	Hile	5	0	5	19	79.17
(24)	75	Fraser-Lee	0	3	3	21	87.50
R. rutilus	6	Hile	8	2	10	63	86.30
(73)	39	Fraser-Lee	1	7	8	65	89.04
S. erythrophthalmus	5	Hile	6	0	6	2	25.00
(8)	41	Fraser-Lee	3	0	3	5	62.50
S. lucioperca	4	Hile	1	1	2	1	33.33
(3)	187	Fraser-Lee	0	1	1	2	66.67
S. trutta	-	Hile	30	3	33	8	19.51
(41)	89	Fraser-Lee	5	3	8	33	80.49
T. tinca	4	Hile	1	0	1	1	50.00
(2)	N/A	Fraser-Lee	0	0	0	2	100.00
T. thymallus	-	Hile	1	0	1	5	83.33
(6)	114	Fraser-Lee	3	0	3	3	50.00

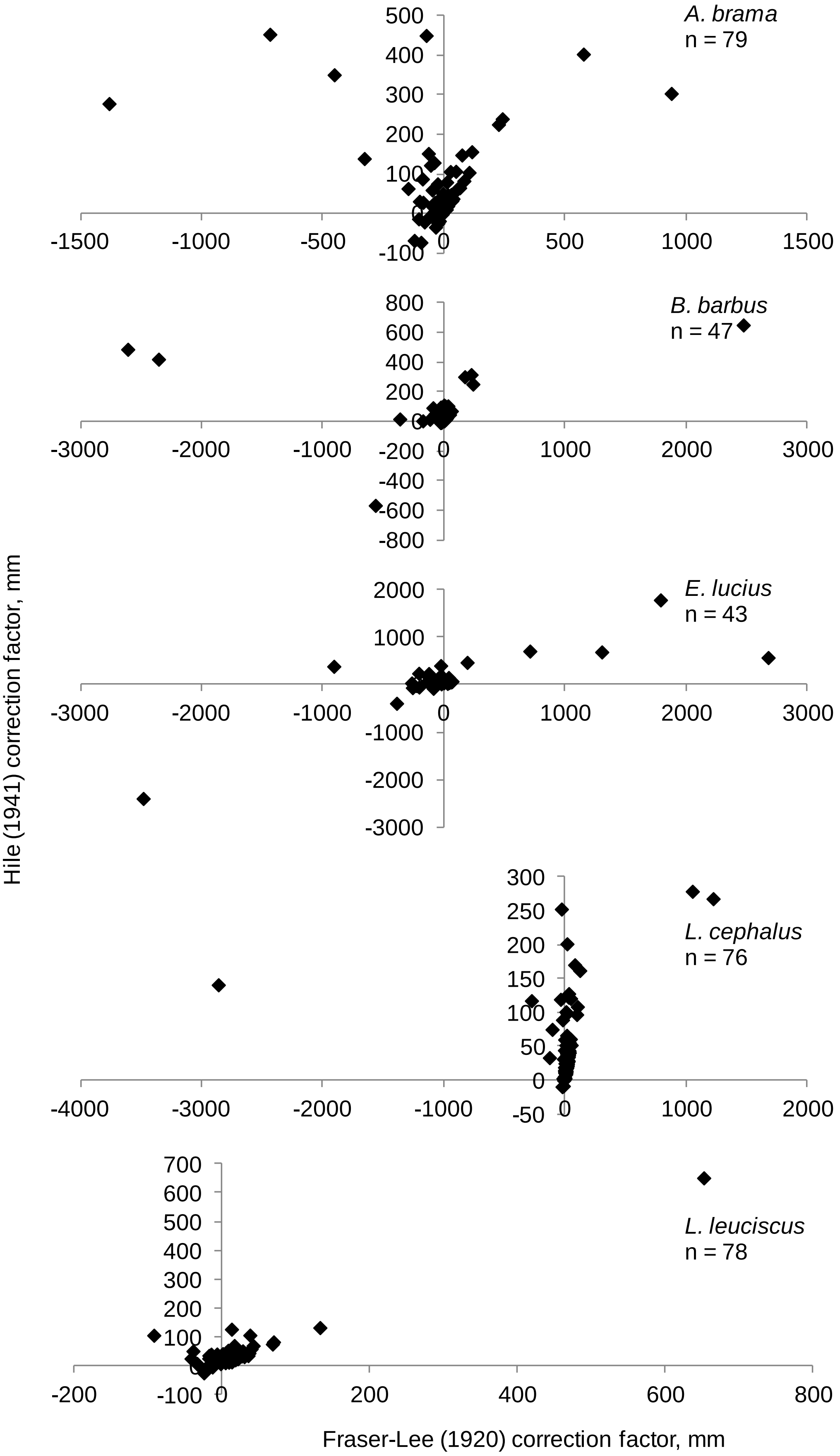

Figure 2.4 Relationships between correction factors derived from Fraser-Lee (1920) and Hile (1941), where n = number of populations.

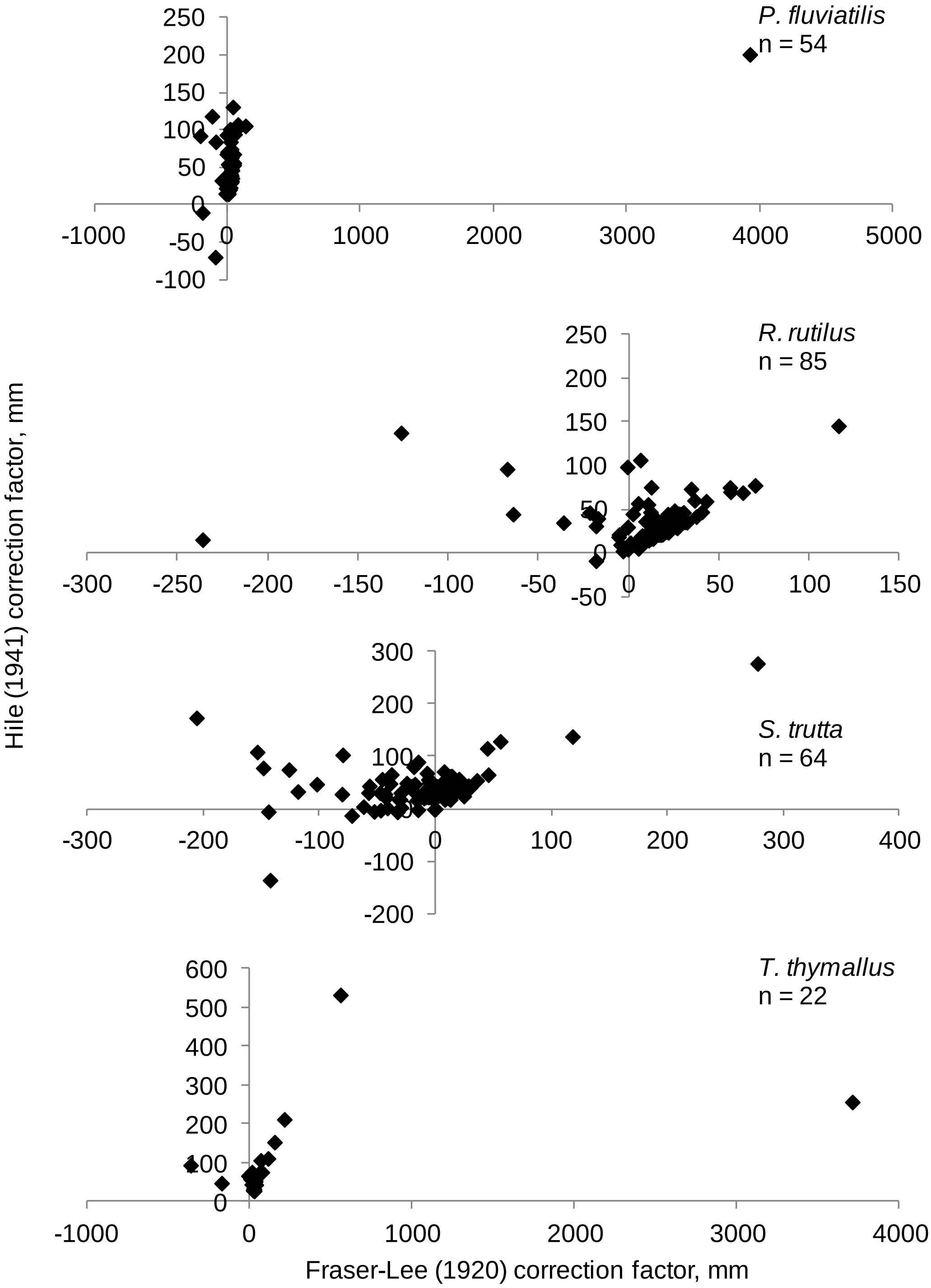

Figure 2.4 continued Relationships between correction factors derived from Fraser-Lee (1920) and Hile (1941), where n = number of populations.

2.3.2 *Correction factor error*

The associated total error of sample size and number of age groups are similar for correction factors derived from Fraser-Lee (1920) and Hile (1941), (Figures 2.5 and 2.6). The relationship between error surrounding correction factors derived from Fraser-Lee (1920) and Hile (1941) and sample size can be seen in Figures 2.5. The total error for *R. rutilus* and *S. trutta* is general low irrespective of sample size. Similarly, total error of correction factors derived for *L. leuciscus* is relatively low but decreased with increased sample size. Although, total error is very high for a number of species, this is reduced when sample size increases to over 30 for *E. lucius, L. cephalus*, *P. fluviatilis* and *T. thymallus*, and over 60 for *B. barbus*. A sample size of 30 reduces correction factors error for *A. brama* but increases again at 45, possible the level at 30 is exceptionally low or the value at 45 is exceptionally high. Perhaps, *A. bjoerkna* may have been wrongly as identified as *A. Brama,* which may have influenced sampling error.

The relationship between total error surrounding correction factors derived from Fraser-Lee (1920) and Hile (1941) and number of age groups can be seen in Figures 2.6. Similar to sample size the number of age classes from *R. rutilus* and *S. trutta* is low irrespective of number of age classes. Similar to sample size, *B. barbus* error is initially high and does not decrease until the number of age classes reaches 5. Likewise, *A. Brama* error at 4 does not decrease until 5 age groups. *Leuciscus cephalus* and *L. leuciscus* correction error and number of age classes, decreases by 3 age groups. *Thymallus thymallus* and *P. fluviatilis* error is initially high, but by the inclusion of 3 age groups this level of error has decreased. The peak for *E. lucius* error at 3 age groups is reduced by the inclusion of 4 age groups and is possibly related to samples often containing more fish aged 3+ then 2+. Similarly, there is no linear relationship between numbers of age groups; Figure 2.6 shows a decrease in variability in the derived correction factor and increasing number of age groups.

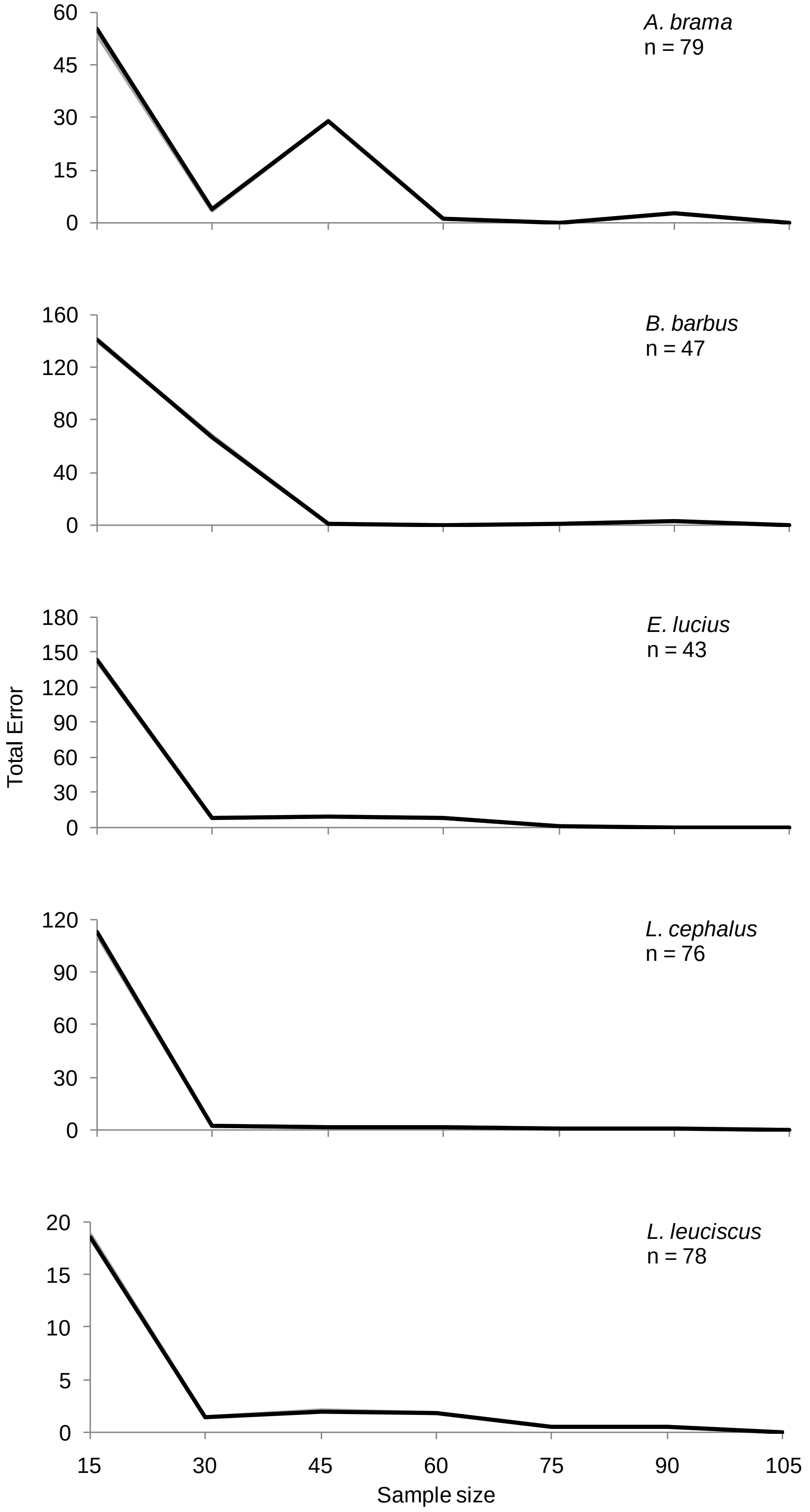

Figure 2.5 Relationship between sample size and total error of correction factors derived from Fraser-Lee (1920) (▬) and Hile (1941) (▬). Where n is the number of populations included in the analysis.

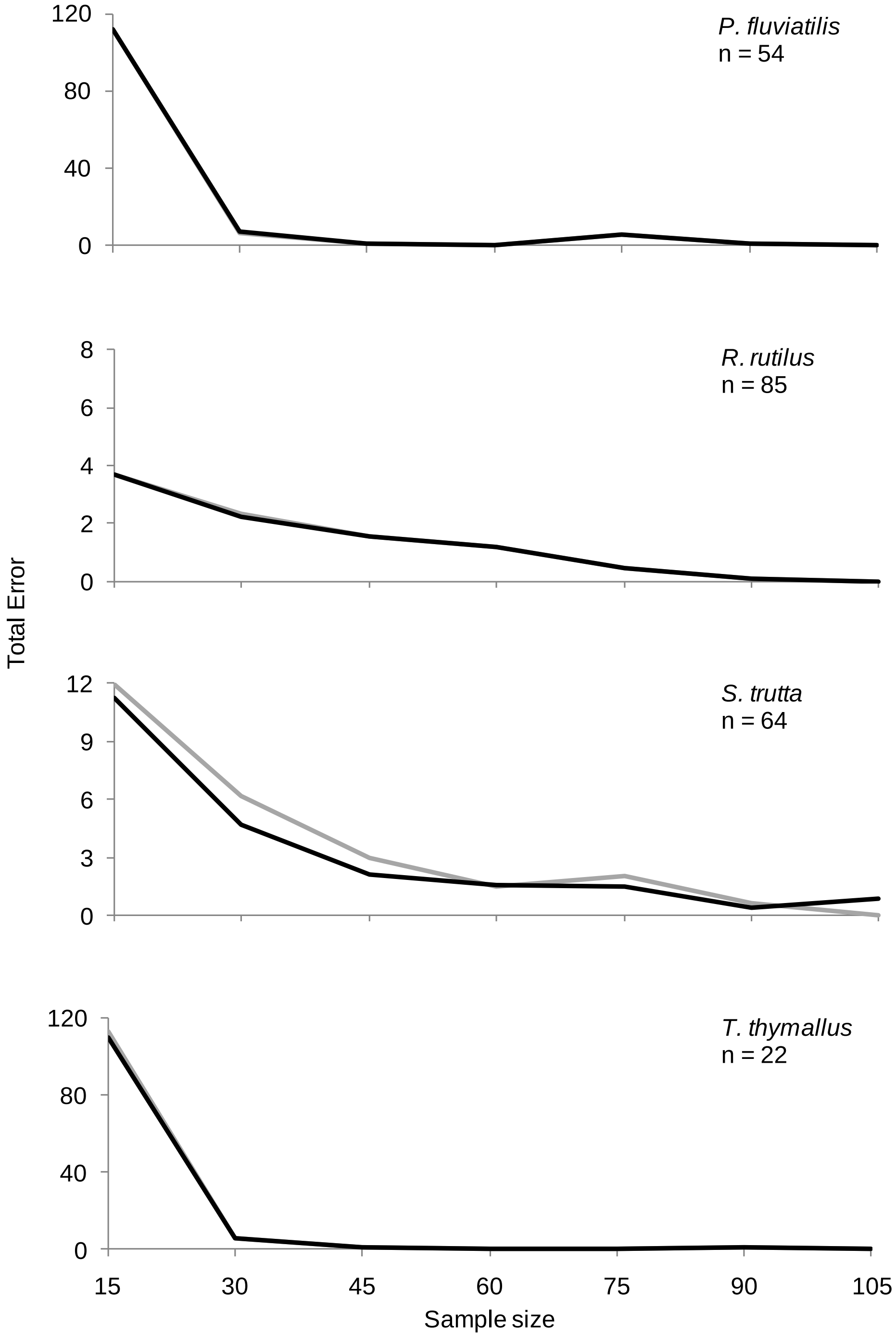

Figure 2.5 continued Relationship between sample size and total error of correction factors derived from Fraser-Lee (1920) (━) and Hile (1941) (━). Where n is the number of populations included in the analysis.

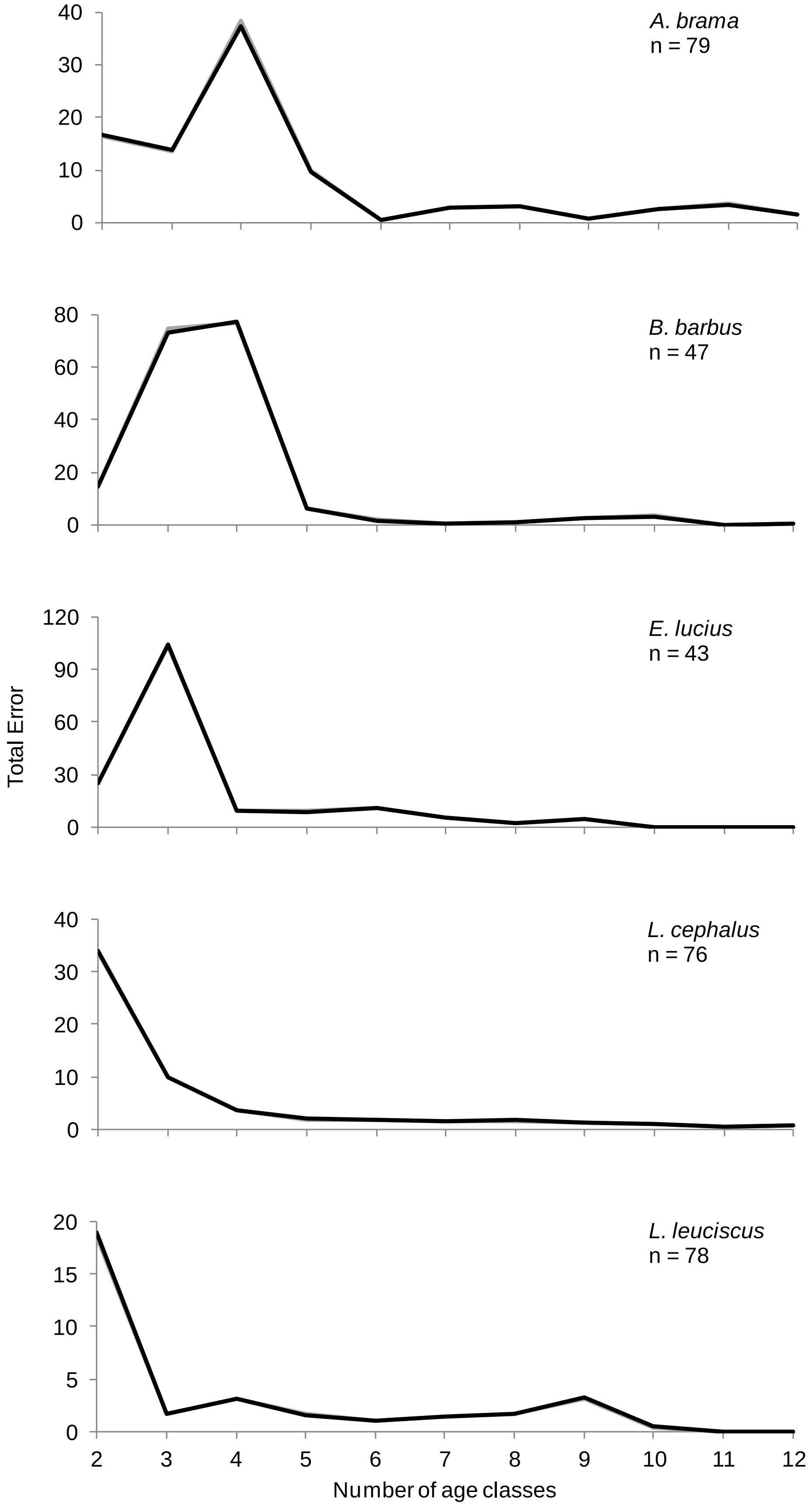

Figure 2.6 Relationship between number of age classes and total error of correction factors derived from Fraser-Lee (1920) (━) and Hile (1941) (━). Where n is the number of populations included in the analysis.

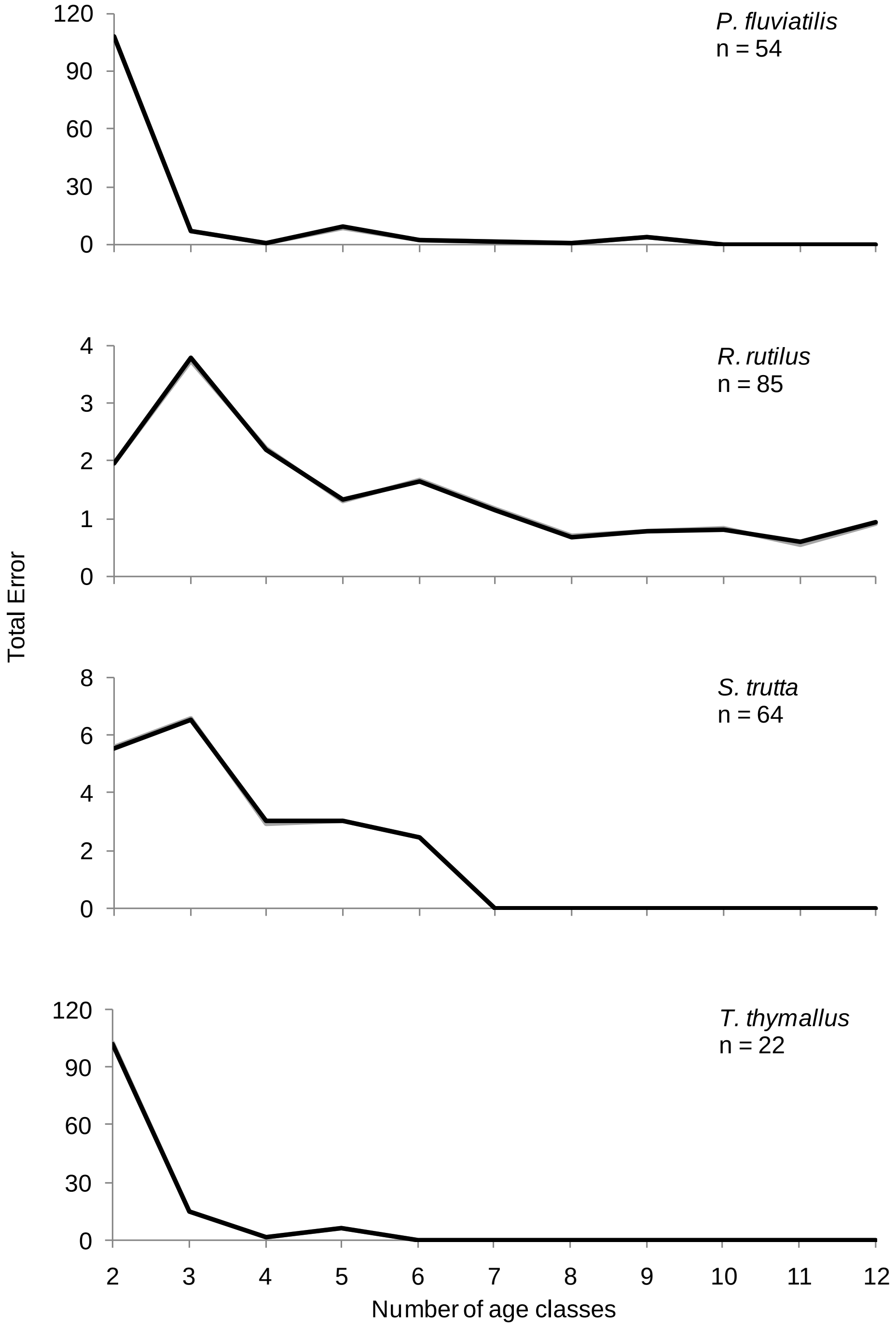

Figure 2.6 continued Relationship between number of age classes and total error of correction factors derived from Fraser-Lee (1920) (▬) and Hile (1941) (▬). Where n is the number of populations included in the analysis.

2.4 DISCUSSION

2.4.1 *Linear relationship between fish length and scale radius*

Most growth back-calculation procedures are based on the proportionality between fish length and some measure of a calcified hard structure (Carlander, 1981). The relationship is assumed to be of parametric form, with linear regression the most common (Weisberg, 1986). Whitney and Carlander (1956) questioned whether the body-scale relationship is always strictly linear and that caution should be exercised performing calculations that rely on this relationship. In agreement, linearity between body length and scale radius should always be tested as an assumption of back-calculation. The 19 species examined in this study exhibited linear relationship between fish length and scale radius, although not always a strictly linear relationship perhaps because scales samples were taken from different anatomical regions. Interestingly, derived (*c*) values represent the fish length when scales developed at that location (Carlander, 1982). Ibanez *et al.* (2008) suggested when the proportionality between the body length and scale radii is weak, back-calculation methods were poor in determining length at check formation.

Although a strong linear relationship (r^2 = 0.76) was found between *S. trutta* fish length and scale radius, the relationship is weaker for the migratory form – sea trout - returning from sea (r^2 = 0.48). Heidarsson *et al.* (2006) found a similar result and suggested this probably stems from the profound life history shift experienced when the fish migrate to the sea. In the sea, smolts encounter higher temperatures and environments that are rich with suitable prey, and the growth rate is greatly accelerated (Mills, 1989). Heidarsson *et al.* (2006) suggested this shift in general growth rate may affect the proportionality of growth of different body parts. However, it could be presumed any changes in the rate of the body growth would lead to a similar increase in scale size is questionable. Thus any great deviation from an r^2 of 1 is likely to be because scale samples were removed from different body regions, scales may have suffered erosion or be unsymmetrical, or error associated with inaccurately measuring a fish or its scales.

It is important to comment on other types of scale-body relationships. Several authors have used curvilinear body-scale relationships for back-calculation. However, a curvilinear relationship requires a better representation of a population than for linear regression (Carlander, 1985). When scales form, they are small platelets separated from each other. They quickly grow, proportionately faster than the fish length, until they overlap (in most species) (Carlander 1985; Chapter 3). Thus the body scale relationship is curvilinear early in life, but after the scales become overlapped, the relationship is linear. Often this curvilinear stage is completed before the first annulus and is thus of little significance in growth calculations (Carlander, 1985). As a result, Carlander (1982) recommended caution in the use of curvilinear relationships. An alternative scale-body relationship is the geometric mean regression. Carlander (1982) suggested the difference between a (*c*) factor derived from a linear regression would not vary significantly from one derived from a geometric mean regression. In addition this study identified that fish length-scale radius relationships other than linear, repeatedly provided (*c*) factors which were unacceptable, i.e. they differed too much from those patterns of squamation identified in Chapter 3. Subsequently, there is sufficient evidence that a linear regression is more than adequate at describing the scale-body relationship in freshwater fish species.

2.4.2 *Back-calculation with correction factor*

The correction factor derived by the Fraser-Lee (1920) and Hile (1941) methods can vary considerably. The Fraser-Lee (1941) correction factors were mostly smaller than those derived by the Fraser-Lee (1920), as suggested by Ricker (1973, 1984). This study has demonstrated how an increase in sample size and numbers of age groups significantly reduces the error of a correction factor and in turn back-calculated length-at-age. This study has suggested minimum samples size and number of age groups for 9 key, most commonly aged (personal observation), freshwater fish species and should be extended to more species when further data sets become available. An inaccurate correction factor will reduce the accuracy of data derived from back-calculation formulae. Importantly, the inclusion of a range of age classes may further reduce error surrounding correction factors.

There can be a false assumption among fisheries scientists, that their sample is a true representation of the population, when, often many samples do not include fish 1 and 2 year of age (Carlander, 1985). This study has confirmed the suggestion by Carlander (1982) that “most significant cause of the variation in (*c*) values probably is the difficulty in obtaining adequate samples”. Many factors can affect the derivation of a (*c*) value. For instance, the body region from which scales are taken and the radius at which scales are measured (Carlander, 1982). Whilst, Lager (1956) and Weese (1949) found that values for (*c*) increased successively with age. A high correction factor should be cause for concern, because it will result in a higher back-calculated length-at-age, and vice versa for a smaller correction factor. Similarly, Ricker (1973) and Carlander (1985) found that variance around (*c*) values increases with a decrease in length classes and with larger individuals. To compensate for this error, the use of standard (*c*) values in Fraser-Lee (1920) was recommended by Carlander (1982, 1985). Carlander (1985) stated where adequate data are available to describe the body scales regression, the regression for the particular population should be used, but if there are any questions about adequacy, the standard intercepts are probably better. These standards may need to be updated as further information is available, or research has been completed on the development of scales based on observations of juvenile fish species. Additionally, when research is complete on the observation of scale development in juvenile fish and how these values compare with (*c*) values. Nevertheless, the use of standard (*c*) values will improve the comparability of growth data (Carlander, 1982).

2.4.3 *Sampling error, sample size*

This study found Fraser-Lee (1920) derived more acceptable correction factors then Hile (1941); this may be partially because Hile (1941) produces values which are nearly always less than those calculated from Fraser-Lee (1941) and the length larval fish emerge. Although Fraser-Lee (1920) (*c*) values may be more acceptable, they are not necessarily more accurate, as the quality of the data and the many drivers of squamation and scale shape can impact on accuracy. Rollins (2009) obtained negative correction factor for dace and suggested this may be due to different growth rates between the juvenile and adult stages, where a slower growth rate during the adult stages produces a regression that underestimates the length intercept. Rollins (2009) concludes this is unlikely in a wild population, but the likelihood of this phenomenon increases for stocked fish because of the difference in growth rates pre and post-stocking. Unfortunately, this study found both formulae repeatedly failed to provide to a correction factor with a biological meaning, the sole purpose of their creation, and so the use of these Fraser-Lee (1920) and Hile (1941) back-calculation formulae becomes questionable.

Although a correction factor can provide a closer approximation of growth (Jones, 1959), not all authors are convinced of the validity of a correction factor, Regan (1911) and Van Oosten (1929) suggested once scales were formed they grew fast until they “caught up with” the growth of the body. A feasible hypothesis since a primary reason for the evolution of scales is to provide protection to an otherwise vulnerable body, therefore it would be reasonable to assume scales, at some point, must grow fast enough to catch up with body size, otherwise scales wouldn’t overlap one another, nor provide protection to the fish’s body. If scales did grow quickly until they caught up with the fish's body size, then a linear relationship between fish length and scale radius is important because Dahl-Lea (1910) would, potentially, be the only back-calculation formulae necessary.

2.4.4 *Which back-calculation technique?*

It would be naive to assume Fraser-Lee (1920) and Hile (1941) should be excluded from the choice of back-calculation techniques, other formulae incorporating a correction factor (e.g. Whitney & Carlanders' body proportion hypothesis (1956) and Ricker (1992)) may suffer similar problems and no literature is available on whether scale growth catches up with body length. Several authors have tried to validate the use of one formula over others, but they often find contrasting results (Campana, 1990; Francis, 1990; Horppila & Nyberg, 1999; Klumb *et al.*, 1999b; Campana, 2001; Johal *et al.*, 2001; Schirripa, 2002; Ibanez *et al.*, 2008). However, two formulae are repeatedly recommended, the Whitney and Carlander scale proportion hypothesis (1956) (Francis, 1990; Ibanez *et al*., 2008) and Hile (1941) (Francis, 1990; Ibanez *et al*., 2008; Rollins, 2009), it is important to note Whitney & Carlander's (1956) SPH formulae is the same as Hile (1941). Francis (1990) recommended, when the body-scale relationship is linear, the Fraser-Lee (1920) equation (which is based on a misunderstanding of the role of regression (Francis, 1990)) should be rejected in favour of (Hile, 1941) or (Whitney & Carlander, 1956). Similarly Rollins (2009) found Hile (1941) was the preferred method, providing the most consistent back-calculation values. However, the (*c*) values provided in this study should be used as a standard value for inclusion in the Fraser-Lee (1920) formula. These values are important because they eliminate the variance caused by poor sampling (Carlander, 1982).

It is not within the scope of this study to validate back-calculation formulas or the use of a correction factor. Instead it was to improve the understanding potential errors surrounding correction factors and provide criteria for sample size and number of age groups to reduce possible error in deriving correction factors and subsequent back calculated lengths. Indeed this study would be enhanced if it had validated back-calculation formulas, potentially demonstrating which correction factor, if any, is most suitable. Validation of back-calculation formulas is a topical debate (Francis, 1990). However, the only readily accepted validation technique is to recapture the same individual over several consecutive years, recording its length and acquiring scale samples. The number of individuals necessary to provide a statistically valid comparison would be relatively large, consisting of various age classes for over 16 species of various age classes; a very expensive data set to obtain especially since the sample size must be large enough to ensure a sufficient number of recaptures, subsequently; no such data set is currently available.

2.5 CONCLUSIONS

Accuracy in ageing procedures is paramount to provide robust estimates of individual and population growth variables (Campana, 2001). Thus further attention should be directed towards calculating a more biologically valid correction factor. Indeed, this study has met the suggestion of Campana (1990), that given an appropriate value for the regression intercept, the Fraser-Lee (1920) method will correctly predict mean back-calculated lengths, even in the presence of a growth effect. Campana (1990) concludes “the Fraser-Lee method is an appropriate starting point for the development of a new back calculation procedure, requiring only a better means of defining the intercept to be completed”. However, until a new technique is created and sufficiently validated, the sample size and number of age classes included in the back-calculation formulae should be maximised to reduce potential error around the correction factor. As a result, variability or error of correction factors can be reduced by having a minimum of 30 samples with at least 4 age classes represented. Alternatively the (*c*) derived in Table 2.2 can be used as a standard (*c*) factor for each species, eliminating the variance caused by poor sampling. Finally, the development of standard intercept values (based on observation of juvenile fish) should be promoted to replace or validate mathematically derived (*c*).

3 SQUAMATION OF FISH SCALES: STANDARD INTERCEPTS FOR BACK-CALCULATION OF FISH LENGTHS

3.1 INTRODUCTION

Skin is a complex epithelium and the most extensive interface between an animal and its external environment (Campinho *et al.*, 2007). The skin of teleost fish is covered with scales: flexible, calcified plates lying within shallow envelopes, or scale pockets, in the upper layers of the dermis (Bullock & Roberts, 1974). The posterior edge of the scale projects above the surface of the dermis overlapping the scales behind. Scales are covered by the epidermis, which consists of several layers of cells. The development of fish scales is known as squamation. For many species, squamation begins at a single locus on the lateral midline of the caudal peduncle: the wrist of the tail, (Andrews, 1970; White, 1977; Sire & Arnulf, 1990; Sire *et al.*, 1997).

Identifying the length of a fish when squamation is complete (*sc*) is a key aim for fisheries ecologists. Numerous authors have proposed mathematical formula to estimate the fish length at zero scale radius (*c*) (Fraser-Lee, 1920; Hile, 1941), although very few have derived a biological value, i.e. observations of scale formation in juvenile fish (Francis, 1990). Chapter 2 showed mathematically derived (*c*) factors can produce inaccurate back-calculated length data and that the error surrounding such values can be large. Consequently, there is a need to derive biologically determined (*c*) factor to replace mathematically derived values and provide more accurate back-calculated data.

The aim of this study was to derive and evaluate the importance of biologically derived (*c*) values and their influence on back-calculated data. The aim of this study will be met through three objectives: 1) to review the literature to identify size (fish length) and patterns of squamation; 2) to describe squamation processes in British freshwater riverine fishes, to improve understanding of fish physiology; and 3) to provide reference data to establish intercept values for use in back-calculation of fish lengths as an alternative method for obtaining correction factors (see Chapter 2 for issue).

3.2 MATERIALS AND METHODS

3.2.1 *Sampling*

Fish species inhabiting British rivers occupy a number of different habitats e.g. eurytopic, limnophilic and rheophilic. Fish species can be further classified according to environmental and reproductive guilds (Davies et *al*., 2004; Welcomme *et al*., 2006). Despite this, the post larval stages and juveniles of many fish can be readily captured using a micromesh seine net, set in shallow marginal water (Cowx *et al.*, 2001). For the purposes of this study, specimens were captured by Hull International Fisheries Institute (HIFI) from the rivers Trent, Ouse and Ancholme, approximately fortnightly between April and June and monthly from July and September in 2009 and 2010. The sampling technique was that described by Nunn *et al.* (2007a), where specimens were captured during daylight hours, using a micromesh seine (25-m long x 3-m deep, with 3-mm hexagonal mesh) set in a rectangle parallel to the bank either by wading or a small inflatable. The seine captured larvae as small as 5 mm standard length (L_S), although its efficiency was reduced for fishes <15 mm (Cowx *et al.*, 2001). In all cases, sampling was restricted to the margins in water <2 m deep, where water velocity was slowest and where 0+ year fishes tend to aggregate (Copp & Garner, 1995). The net was fished to the bank and captured fish were transferred to large water-filled containers prior to analysis. A sub-sample of fish was taken and preserved in formalin (4% formaldyhyde) and returned to the laboratory; the remaining samples were released. A hand net was also used to capture additional specimens from marginal areas, which preserved the condition of smaller specimens (<25 mm) better than seine netting. Care was taken during all collection, preservation and examinations to prevent damage to the integument or scale loss.

3.2.2 *Staining of specimens*

To increase the number of less frequently captured fish species, specimens from more than one river were combined. It was anticipated that combining rivers would not have a significant effect based on literature (Sire & Arnulf, 1990; Able *et al.,* 2009) and furthermore the sample sizes were too small to test for any significant difference between rivers. Individuals were measured to the nearest 0.1 mm fork length using callipers. It was possible specimens may have shrunk during preservation (Snyder, 1983; Treasurer, 1992; Sagnes, 1997; Paulet & Kaiser, 2004). Nevertheless, length measurements were not corrected for the effects of formalin since, for the sizes of fish in this study and the minimal times for which fish were kept in formalin, absolute shrinkage would have been small (~1 mm maximum) (Treasurer, 1992; Smith & Walker, 2003). Individuals to be stained were first washed in 95% ethyl alcohol (ETOH) for 1 - 5 minutes and then immersed in a solution containing Alizarin Red S to expose scales (adapted from Able & Lamonaca, 2006). The solution was made up by dissolving 1 g Alizarin Red S powder with 100 mL distilled water. Sodium hydroxide was then added until a pH of 4.1 - 4.3 was reached. Individuals were dipped in Alizarin solution and then washed in 95% ETOH. Individuals were analysed under a Leica stereomicroscope and re-stained in Alizarin solution until all possible scales were stained; see Figure 3.1 for example. Squamation patterns were illustrated on blank templates for individual species based on adult illustrations adapted from Maitland (2004). Thus, illustrations of representative stages of scale formation were standardised across all ontogenic stages. Shaded areas on the illustrations indicate the presence of scales, and the location of the lateral line is shown even before it has formed to provide a local landmark. The area behind the pectoral fin is not shaded to indicate better the location of the pectoral fins and thus provide another local landmark, similar to the method described by Able and Lamonaca (2006). To provide a final assessment, squamation pictures for each species were used to identify the smallest lengths scales began to appear at six specific locations (based on the author's personal observations), these were then plotted on blank templates.

Figure 3.1 Alizarin stained *S. lucioperca* of 27.8 mm L_F, developing scales can be seen as red circles along and below the later line between the caudal and anal fin.

3.2.3 *Deriving a correction factor*

Scale samples are typically taken from the shoulder region of a fish (above the lateral line but in front of the dorsal fin); as a result it is important to derive the body length when squamation at this location is complete. Binomial logistic regression was used to predict the stage of squamation per given fish length based on observations of juvenile fish of four species: *L. cephalus*, *L. leuciscus*, *P. fluviatilis* and *R. rutilus*. Additionally, logistic regression allows the prediction of a 50% median value for the length at squamation, i.e. the fish length at which squamation is achieved by 50% of fish. Ogive analysis (cumulative frequency distribution curve) provides graphical representation of the probability of complete squamation for a given fish length. Differences between back-calculated growth data generated with correction factors derived in chapter 2 by the Fraser-Lee (1920) and 50% median values (this chapter), were compared by two methods: 1) growth curves transformed into Ford Walford (1946) plots (ANCOVA); Chapter 5) first year growth (ANOVA). All statistical analysis was performed in R (Version 2.9.1).

3.3 RESULTS

3.3.1 *Squamation values from literature*

Analysis of available literature concluded that there was no single study which had attempted to record the size at squamation of freshwater fish found in the England. Instead, individual studies had noted the size at squamation of selected fish species, because the intention of these studies was not to investigate squamation and they do not follow a standardised technique, e.g. definition of squamation, type of length recorded or stage of squamation. Therefore, historical literature is open to interpretation and should be used cautiously (Table 3.1). One such example is Economou *et al.* (1991), who states *L. cephalus* (L.) squamation began at 15.9 mm and scalation began at 20.0 mm, but the authors fails to define squamation or scalation and provides no further explanation for two values. Analysis of literature also concluded that squamation has been found to begin at seven different locations for species commonly found within the British Isles (Figure 3.2, Table 3.2).

Table 3.1 Analysis of available literature on size at squamation of freshwater fish species found within the UK, were squamation is defined as the onset of scale development.

Species	**Length at squamation (mm)**	**Study**
B. barbus	21.0	(Sire & Arnulf, 1990)
L. cephalus	20.0 - 24.0	(Economou *et al.*, 1991)
L. leuciscus	19.0	(Kennedy, 1969)
R. rutilus	18.5	(Bagenal & Tesch, 1978)
	19.0 - 22.5	(Tong, 1986)
A. brama	17.0	(Kucharczyk *et al.*, 1998)
P. fluviatilis	18.0 - 28.0	(Spanovskaya & Grygorash, 1977)
S. lucioperca	34.0	(Priegel, 1964)
S. trutta	35.0	(Parrott, 1934)
	30.0	(Setna, 1934)
S. alpines	38.0	(Frost, 1978)
S. salar	30.0	(Jensen & Johnsen, 1981)

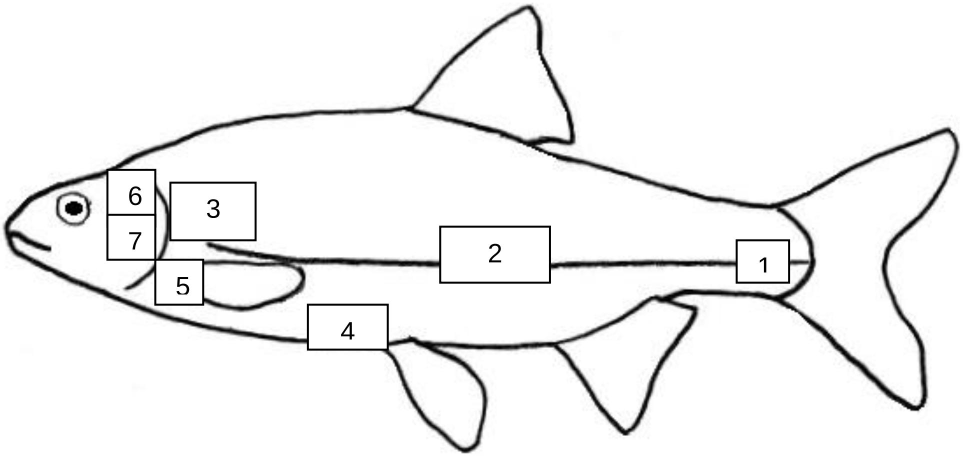

Figure 3.2 Localization of the different sites of the first scale appearance in a teleost fish. 1, medial region of the caudal peduncle; 2, middle region of the flank; 3, anterior region of the body near to the lateral line; 4, belly between pectoral and pelvic fins; 5, pectoral peduncle; 6 cranial vault; 7, opercular region. Redrawn from Sire and Arnulf (1990).

Table 3.2 Sites of scale appearance in teleost fishes. Data compiled from the literature. For one species, 2 or 3 sites indicate that scales are formed independently in 2 or 3 regions of the body. Generally, the first named is the site in which the scales appear first but sometimes can be formed simultaneously in 2 sites. Classification after Nelson (1984). For the localization of the sites on the fish body, see Figure 3.8. Adapted from Sire and Arnulf (1990).

Order	Family	Species	Site	References
Cypriniformes	Cyprinidae	*A. brama*	1	Segerstrale (1932)
		B. barbus	1	Sire & Arnulf (1990)
		B. bjoerkna	1	Frank (1956)
		C. carpio	3	Nozawa (1941); Balon (1958); McCrimmon & Swee (1966)
		Idus idus	1	Cala (1971)
		Pseudorasbora parva	3	Okada & Seishi (1936)
		R. rutilus	1	Balon (1955); Cala (1971)
Salmoniformes	Esocidae	*Esox americanus*	2-5	Jollie (1975)
		E. Lucius	2	Francklin & Smith (1960)
	Salmonidae	*S. salar*	2	Warner & Harvey (1961)
		S. trutta	2	Paget (1920); Parrott (1934); Setna (1934); Neave (1936)
		Salvelinus fontinalis	1	Elson (1939); Cooper (1971)
Cyprinodontiformes	Cyprinodontidae	*Rivulus marmoratus*	6-1-7	Park & Lee (1988)
Gasterosteiformes	Gasterosteidae	*Gasterosteus aculeatus*	3-1	Igarashi (1970)
Perciformes	Centrarchidae	*P. nigromaculatus*	1-4-3	Cooper (1971)
	Percidae	*P. fluviatilis*	1	Segerstrale (1933); Glenn & Mathias (1985)

3.3.2 *Patterns of scale formation*

The sampling technique sufficiently captured fish of various species and length suitable for staining with alizarin red solution (Figures 3.3). Although, the number of specimens captured for some species, this is perhaps a reflection of their low numbers within the study rivers e.g. *C. carpio*. Furthermore, the inclusion of species with local angling and economic importance (e.g. *B. barbus* and *T. thymallus*) was avoided where possible; similarly, species where a sufficient size range had been already been captured were released.

Staining of specimens, revealed scale formation began at caudal peduncle for all species. From here, scale formation for all species extended along the lateral line (or where it would be if formed) before extending to the anterior and posterior region of the body (Figures 3.4 and 3.5). The size at squamation varied more between families, i.e. Cyprinidae, Percidae and Salmonidae than within families.

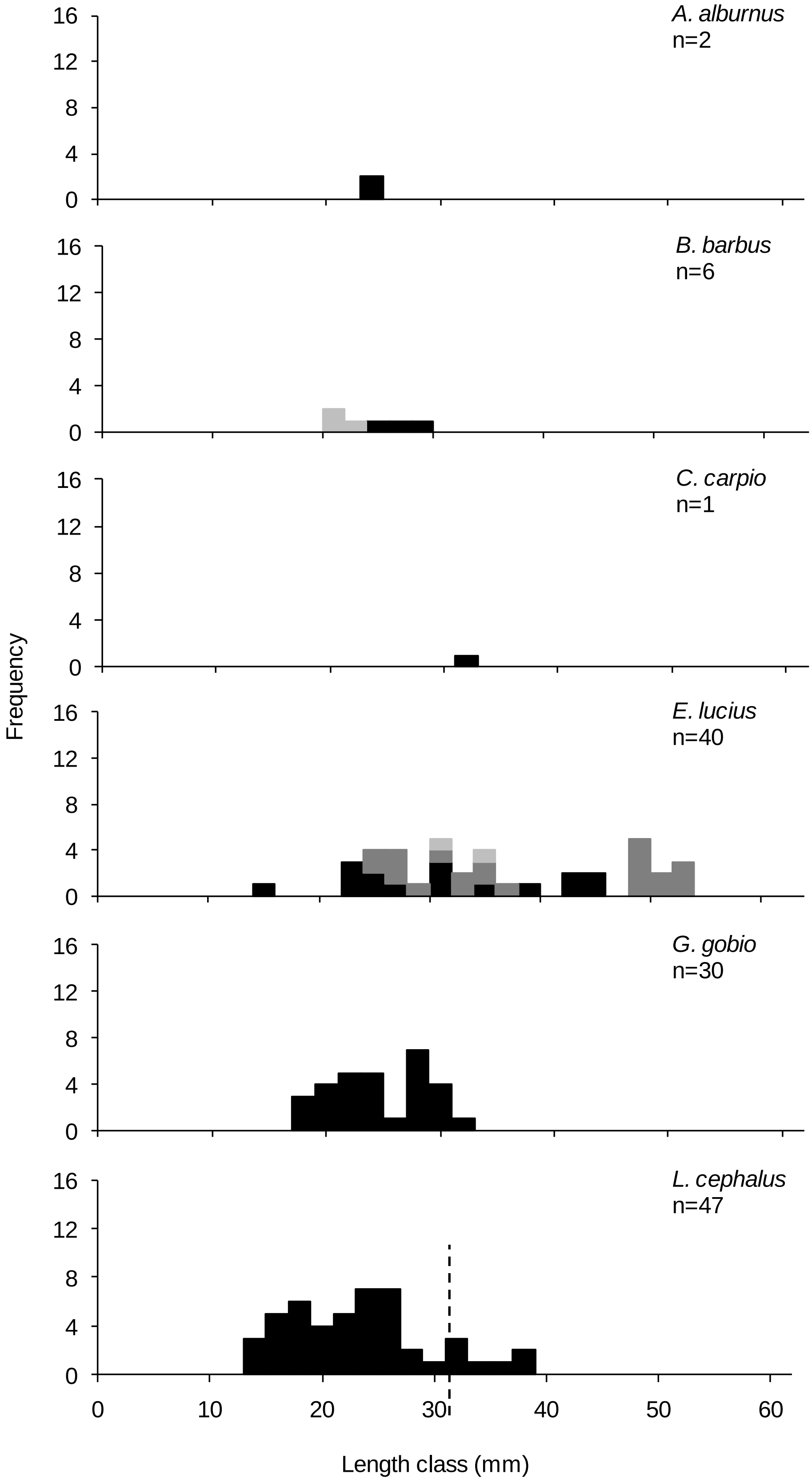

Figure 3.3 Length-frequency distribution of species analysed for squamation.Black represents River Trent fish, dark grey represents River Ancholme fish, light grey represents Yorkshire Ouse fish and dashed line is 50% median length at squamation, n is the sample size.

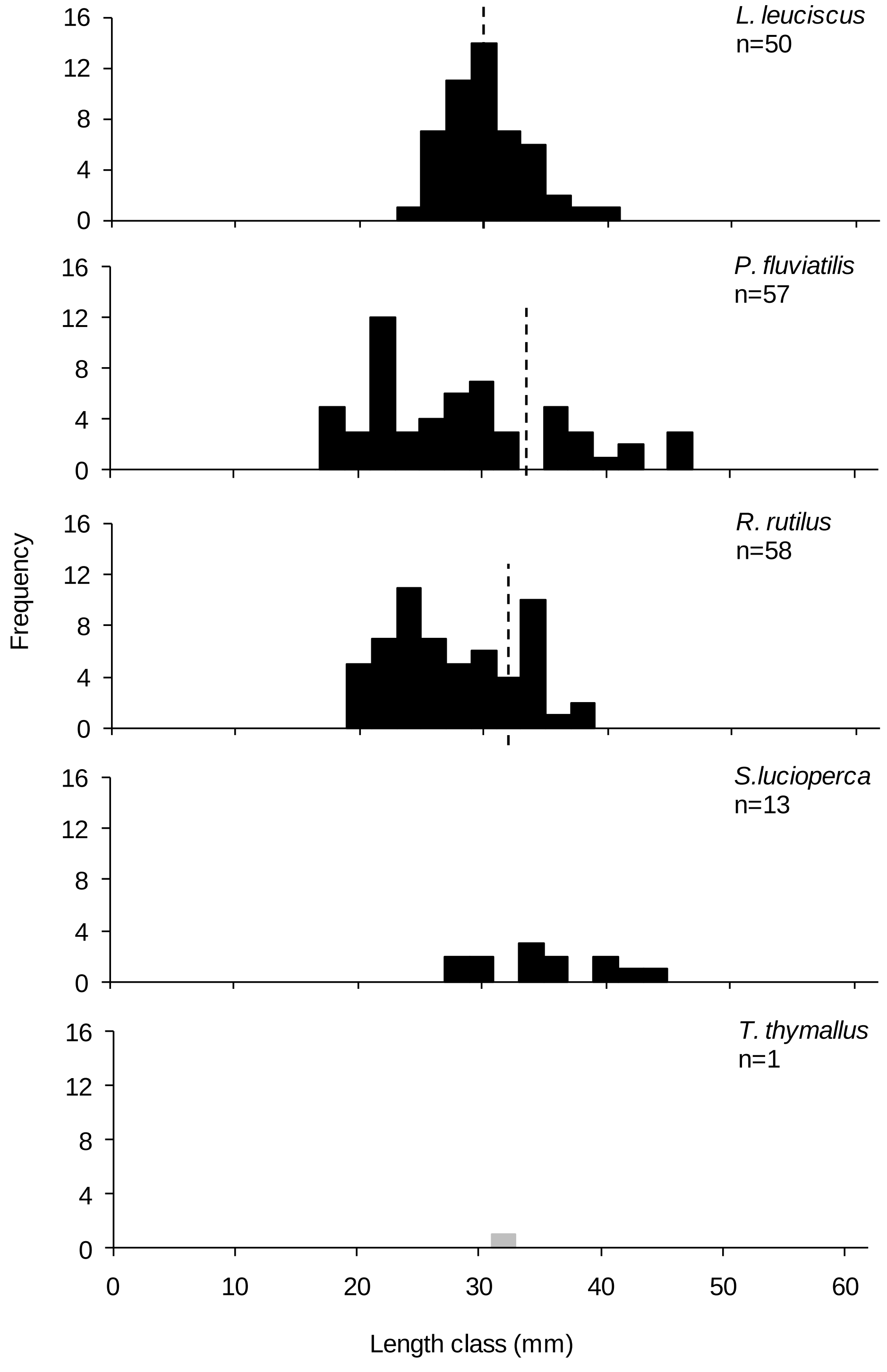

Figure 3.3 continued Length-frequency distribution of species analysed for squamation. Black represents River Trent fish, dark grey represents River Ancholme fish, light grey represents Yorkshire Ouse fish and dashed line is 50% median length at squamation, n is the sample size.

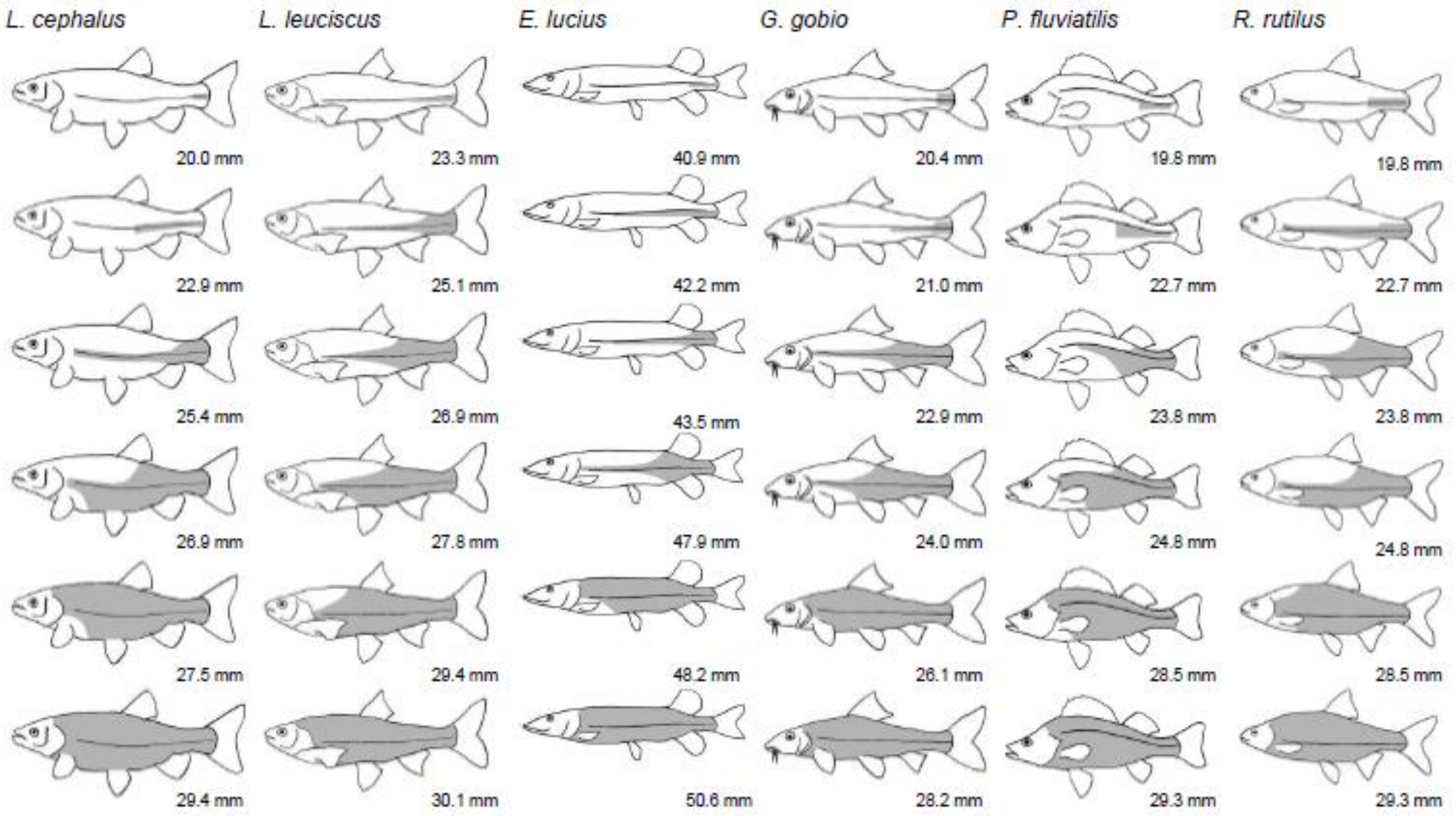

Figure 3.4 Squamation patterns of stained species, pictures and fish lengths dictate first appearance of scales at specific stages (i.e. appearance of scales at smallest length), shaded areas indicates the presence of scales.

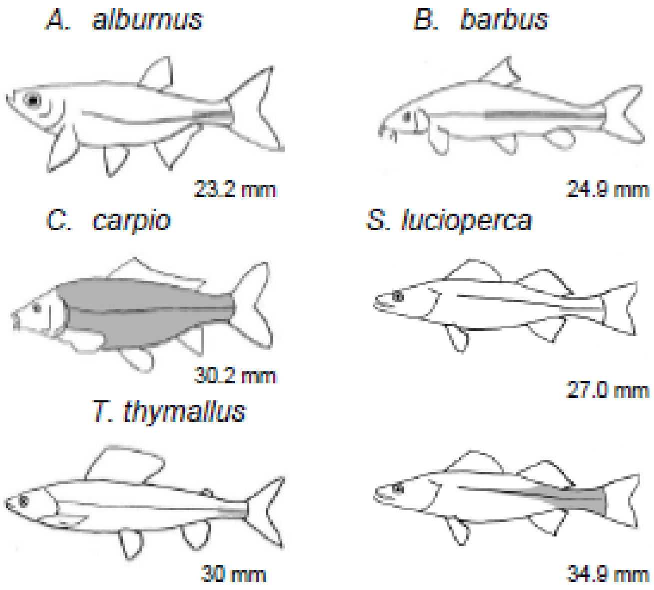

Figure 3.5 Squamation patterns of stained species of limited sample sizes, pictures and fish lengths dictate first appearance of scales at specific stages (i.e. appearance of scales at smallest length), shaded areas indicates the presence of scales.

3.3.3 *Predicting correction factors*

The ability to identify a generic length of complete squamation (*sc*) was investigated, for species where insufficient specimens were available. Firstly, a linear regression analysis was performed on the data provided in Figure 3.4 and 3.5 for species with complete squamation data (excluding *E. lucius*, *S. lucioperca* and *T. thymallus*), ($n = 30$, $r^2 = 0.839$, $P < 0.001$) and a length of 29.6 mm for complete squamation was predicted. A linear regression analysis was performed for all the data available for species with a complete pattern of squamation (excluding *E. lucius*), ($n = 33$, $r^2 = 0.828$, $P < 0.001$) and a correction factor 29.6 mm for complete scale formation was predicted. Subsequently, a value of 29.6 mm is proposed as a standard value for species with a similar growth rates. Although rather crude, a correction factor of 46.75 mm for *S. lucioperca* was predicted, however a greater sample size of specimens is necessary to validate this prediction.

To provide a more robust and scientifically valid (*c*) factor, binomial logistics allowed the prediction of a 50% median value for the length at squamation, i.e. the fish length at squamation achieved by 50% of fish: *L. cephalus* (31 mm), *L. leuciscus* (30 mm), *P. fluviatilis* (33 mm) and *R. rutilus* (32 mm). The 50% median values are similar to those from chapter 2 derived from Fraser-Lee (1920) for *L. cephalus* (37 mm), *L. leuciscus*

(32 mm) and *R. rutilus* (30 mm) but differed considerably for *P. fluviatilis* (54 mm) (Figure 3.7). Ogive analysis provides geographical representation of the probability of complete squamation for a given fish length (Figure 3.6). The 50% median and chapter 2 (Fraser-Lee, 1920) values were inserted into the Fraser-Lee (1920) back-calculation formulae and back-calculated data was compared. Generated growth curves were transformed into Ford Walford (1946) plots and an ANCOVA was performed. No significant differences was found between the growth curves of *L. cephalus* (d.f. = 1, F = 0.001, P = 0.975), *L. leuciscus* (d.f. = 1, F = 0.000, P = 0.990), *R. rutilus* (d.f. = 1, F = 0.000, P = 985) and *P. fluviatilis* (d.f. = 1, F = 0.036, P = 0.856). However, differences between back-calculated lengths decreased with age (Figure 3.8), *L. cephalus* (r^2 = 0.955, P < 0.001), *L. leuciscus* (r^2 = 0.998, P < 0.001), *P. fluviatilis* (r^2 = 0.998, P = 0.001) and *R. rutilus* (r^2 = 0.990, P < 0.001). ANOVA analysis revealed, there is a significant difference between first year back-calculated lengths of back-calculated length-at-age 1; *L. cephalus* (d.f. = 1, F = 16. 448, P = 0.000), and *P. fluviatilis* (d.f. = 1, F = 14.421, P = 0.001), but not for *L. leuciscus* (d.f. = 1, F = 0.772, P = 0.381) and *R. rutilus* (d.f. = 1, F = 1.897, P = 0.171).

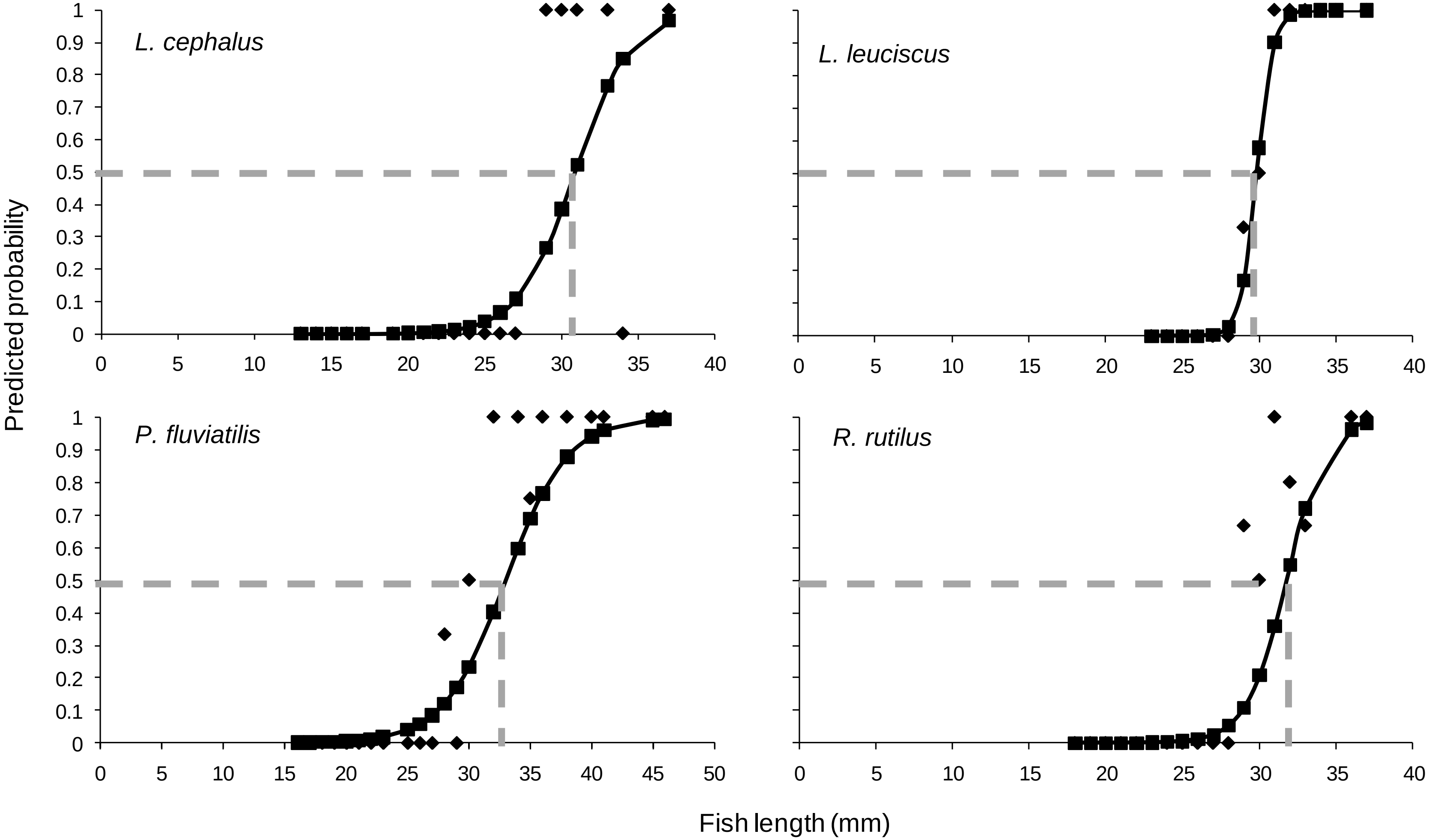

Figure 3.6 Ogive (fitted probability) of fish having scales developed at the shoulder region (■) and observed proportion in fish length (♦). Grey dashed line represents the 50% median value for fish lengths with complete squamation at the shoulder region.

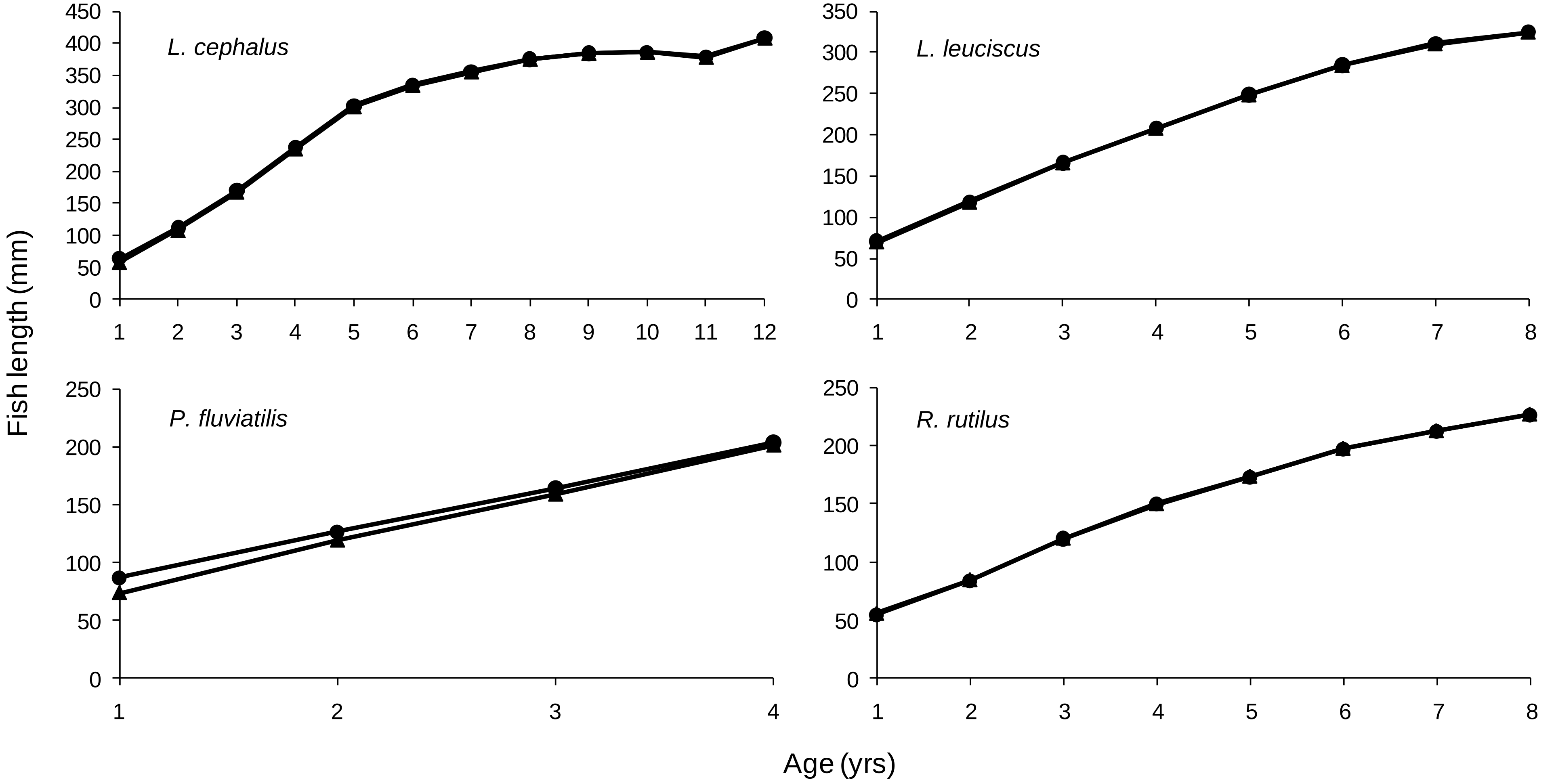

Figure 3.7 Fraser-Lee (1920) back-calculated fish length using c values from fish length and scale radius (Chapter 2) (▲) and 50% median of fish lengths with scales (●).

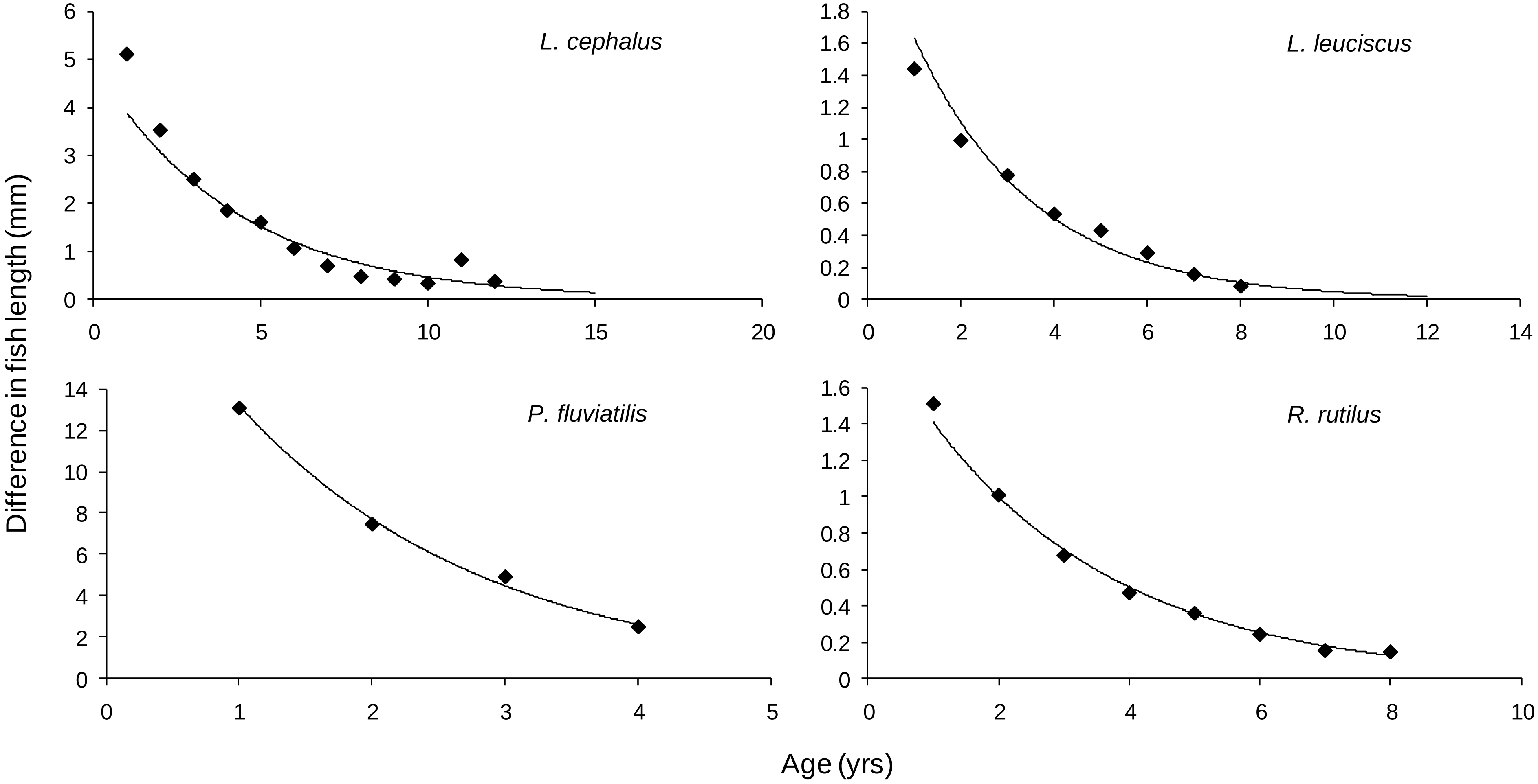

Figure 3.8 Difference in length between back-calculated length-at-age derived from (*c*) factors derived in Chapter 2 and 50% median of fish lengths with scales.

3.4 DISCUSSION

3.4.1 *Squamation initiation and patterns*

For all species, there was no single point of initial scale formation; instead several scales appeared together at a single area of the body and then appearance of further scales migrated from this location. Sire and Arnulf (1990) reviewing information from numerous authors reported a similar situation. Squamation appears to begin on the caudal peduncle for many families of freshwater fishes (Andrews, 1970; White, 1977; Sire & Arnulf, 1990; Penaz, 2001) and flatfishes (Able & Lamonaca, 2006), although other sites (up to seven) were discriminated for some species (Sire & Arnulf, 1990). Squamation has been found to begin at 3 different locations for species commonly found within the British Isles.

3.4.2 *Factors influencing initiation of squamation*

Sire and Arnulf (1991) suggested scale induction could be related to mechanical constraints imposed on a fish's skin during swimming and could be related to its morphology and behaviour. Squamation begins during the final transformation between larval and juvenile life stages (Copp, 1990; Penaz, 2001; Urho, 2002) and is considered an important morphological feature defining the end of the larval period (Gozlan *et al.*, 1999; Penaz, 2001; Urho, 2002; Able & Lamonaca, 2006; Able *et al.*, 2009). However, it is unlikely that development of a single character (e.g. fin ray formation, juvenile/adult body shape) could reflect all of the changes in morphology, ecology and behaviour that are typical of this transition phase and thus it cannot be used as a sole character to identify larval development stage (Urho, 2002; Able *et al.*, 2009).

Able *et al.* (2009) found the formation (onset, spatial development and completion) of scales generally appeared to be length-related and independent of whether the specimens were raised in the laboratory or wild-caught, providing evidence that squamation is independent of actual growth rate. This would make sense given ontogenic changes in body characteristics are related size not age (Sæle & Pittman, 2010). Consequently, combining specimens from different river catchments should not have influenced the results. Equally, several authors have found size at squamation is more related to length than to age (Armstrong, 1973; Sire, 1981; Sire & Arnulf, 1990), so combining specimens from different rivers and/or hatch dates, should not influence the results. Likewise, Sire and Arnulf (1990) suggested the length of the fry at scale formation is generally constant (within a range of 1 mm or less) within a species, but can vary between species from the same family. The squamation patterns found in the present study are similar to those observed for other species. Although no *B. barbus* were captured with complete squamation, Sire *and* Arnulf (1990) found *B. barbus* squamation had was still incomplete at 32.0 mm.

3.4.3 *Back-calculation with correction factor*

The possibility of predicting size at squamation for species with too few specimens was investigated and it can be concluded such a value is unsuitable for a number of reasons: Firstly, the origin of scale formation, is not always consistent within species (Sire & Arnulf, 1990), i.e. scale formation can begin at more than one location. Secondly, the size at squamation can vary between species within the same family. Thirdly, the present study did not divide specimens into classified stages of squamation (i.e. percentage of body to scale coverage), instead choosing to record stages of squamation through observed differences, principally because sample sizes were too small to accurately classify species into “defined” stages of squamation.

Identifying the length of a fish when squamation is complete at the location fish scales are typically taken is a key aim of fisheries ecologists. Currently, fisheries ecologists aim to find the body length at complete squamation, but this is not the correct practice, as scales samples are typically taken from the shoulder region. Despite the multiple functions and uses of fish scales, relatively little is known about their squamation or patterns of formation. Likewise, as previously mentioned, values of squamation obtained from the literature should be interpreted with caution, further highlighting the importance of further research into the squamation of fishes. Specifically, there is a definitive need to increase research into scale development and differences in the initiation of squamation between populations. This study has provided reference data for inclusion in back-calculation formula and has addressed the issue highlighted in chapter 2, accounting for potential erroneous calculation of stock assessment parameters.

This study has demonstrated that the (*c*) factor should be derived by the lengths at 50% median value rather than being mathematically derived from the relationship between fish length and scale radius (Chapter 2). Difference between back-calculated data derived with (*c*) factors from Fraser-Lee (1920) and 50% median were greatest in the first few years, with the greatest difference in the first year, a principal age used in the comparison of fish stock assessments (Nunn, 2005), thus the most important age.

Subsequently, the use of a standard correction factor (50% median value) provides a more accurate and biological valid back-calculated length data.

3.4.4 *Squamation and ageing error*

Species that adopt multiple or fractional spawning strategies, are more likely to have individuals across a wide length range (Bolland *et al.*, 2007; Nunn *et al.*, 2007b); later progeny having less time to grow in their first growth season, will have a mean length smaller than those of earlier progeny (Nunn *et al.*, 2007b). Subsequently smaller progeny spawned later in the year may not reach a size in their first year of life to lay down an annulus because they are of a size less than that when squamation commences. Furthermore the area of the body from which scales are removed for stock assessment is of importance, because scales are generally taken from the shoulder region, and this is the anatomical region where squamation begins last. Nunn *et al.* (2007b) captured fish that had survived the winter period at a length, or only just reaching a length, at which scale squamation commences at the caudal peduncle. Thus, there is the potential for the misinterpretation of these scales, because the first annuli may be very small around the focus of the scale or absent, further strengthening the importance of this study and future research into squamation for validating ageing of fish from scales and accounting for potential erroneous calculation of correction factors. Relating the size of squamation to size distribution of fish at the end of the first year of life to determine the proportion of fish which have reached the size of complete squamation is perhaps a fundamental step in the ageing procedure that has not been considered. This could be performed using a likelihood model to assess the probability of a proportion of the population having individuals with their first year check missing from their scales. It emphasises the importance of research into juvenile fish size distribution and growth to underpin back-calculation of growth studies. This is particularly crucial in species that exhibit batch spawning over an extended period such as *L. cephalus*.

3.5 CONCLUSIONS

Able and Lamonaca (2006) suggested the pattern of scale formation may be useful in improving the understanding of systematics, functional morphology and habitat use. However, more importantly, understanding the patterns of squamation of freshwater fish improves the ability to accurately determine the age and growth of fish.

Determination of squamation patterns for more specimens will allow for the derivation of standard correction factors for each species that can be used across the species' distribution. This study has also identified the possibility that *L. cephalus* maybe incorrectly aged because individuals can overwinter at a length at which squamation has not, or only just, occurred. Inaccurate ageing of fish (specifically *L. cephalus*) could have repercussions on the calculation of growth and recruitment success, in turn negatively affecting management decisions.

4 A GEOMETRIC MORPHOMETRIC APPROACH TO ANALYSE FISH SCALES

4.1 INTRODUCTION

Historically, fish scales have been widely used in scientific studies on: age and growth (Schuck, 1949; Bagenal & Tesch, 1978); life history characteristics (Campana, 2001; Bolland *et al.*, 2007); population dynamics (Crisp *et al.*, 1975; Cowx, 1989); diet analysis of piscivorous species (Britton & Shepherd, 2005; Miranda & Escala, 2007); ecological integrity of large rivers (Williams, 1967; Schiemer, 2000); stock identification (Jarvis *et al.*, 1978); trace-metal contamination (Mugiya *et al.*, 1991); rapid isolation of DNA (Yue & Orban, 2001; Nielsen & Hansen, 2008) and taxonomic and evolutionary studies (Kuusipalo, 1998; Sire & Huysseune, 2003).

Traditionally Fourier analysis has been used to analyse the morphology of fish scales (Jarvis *et al.*, 1978; Casselman *et al.*, 1981; Fraisse, 1990; Richards & Esteves, 1997; Poulet *et al.*, 2005). However, recent advances in computer science have made landmark-based Geometric Morphometrics (GM) more accessible. An increasingly popular technique, GM is more effective at capturing the shape of an individual and provides greater visualization of morphological differences. In addition, GM is statistically more powerful (Zelditch, 2004), identifying relationships between groups of individuals and morphologies versus other parameters (e.g. environmental or taxonomic parameters) (Rohlf & Marcus, 1993).

Ibanez *et al.* (2007) showed that GM analysis of fish scales was a good discriminator of genera and species within Mugilidae. However, this study also indicated that GM was less effective in discriminating populations from nearby areas. Similarly, Garduno-Paz *et al.* (2010) was able to detect differences in scale morphology between ecologically distinct phenotypes of *S. alpinus* coexisting in the same lake. In addition to discriminating stocks, variability in fish scale morphology between different anatomical regions of a fish’s body have been related to swimming mode (Ibanez *et al.*, 2009). These studies used a landmark-based GM approach; a technique reliant upon easily identifiable, fixed locations on all individuals. Unfortunately, freshwater fish scales are often irregular in shape, and it is not always possible to identify identical landmarks on all individual scales. Subsequently, the technique used in these studies cannot be readily applied to British freshwater riverine fish populations. As a result, there is a definite need for the development of a GM approach capable of assessing irregular shaped fish scales.

The aim of this study was to evaluate the possibility of adopting a GM approach to assess irregular shaped fish scales, typical of British freshwater riverine fish species, whilst still retaining the statistical power of a GM approach To test the possibility of developing a morphometric approach, four fish species were used (*B. barbus*, *L. cephalus*, *L. leuciscus* and *R. rutilus*) to assess the possibility of identifying different populations of the same species irrespective of any ontogenetic relationships between fish length and scale morphology. The development of a suitable GM approach would greatly improve the ecological information available from fish scale samples, specifically meta-population analysis within a river system. Importantly, the ability to identify fish populations from scales would improve the ability to identify stock fish from natural recruits or prey species of piscivores.

4.2 MATERIAL AND METHODS

4.2.1 *Sampling*

Fish populations were sampled by the Environment Agency during their routine monitoring programme, by electric fishing from a boat or wading, depending on the river and topographical conditions. All sampling was completed between 2003 and 2009. Fish were measured (fork length, L_F, mm) and a sample of scales taken from the same anatomical region on the fish’s body, below the dorsal fin but above the lateral line, before they were returned to the river. Scale samples were collected from 748 individuals, providing specimens from over a range of fish lengths, from four different species and six different river catchments (Table 4.1 and Figure 4.1).

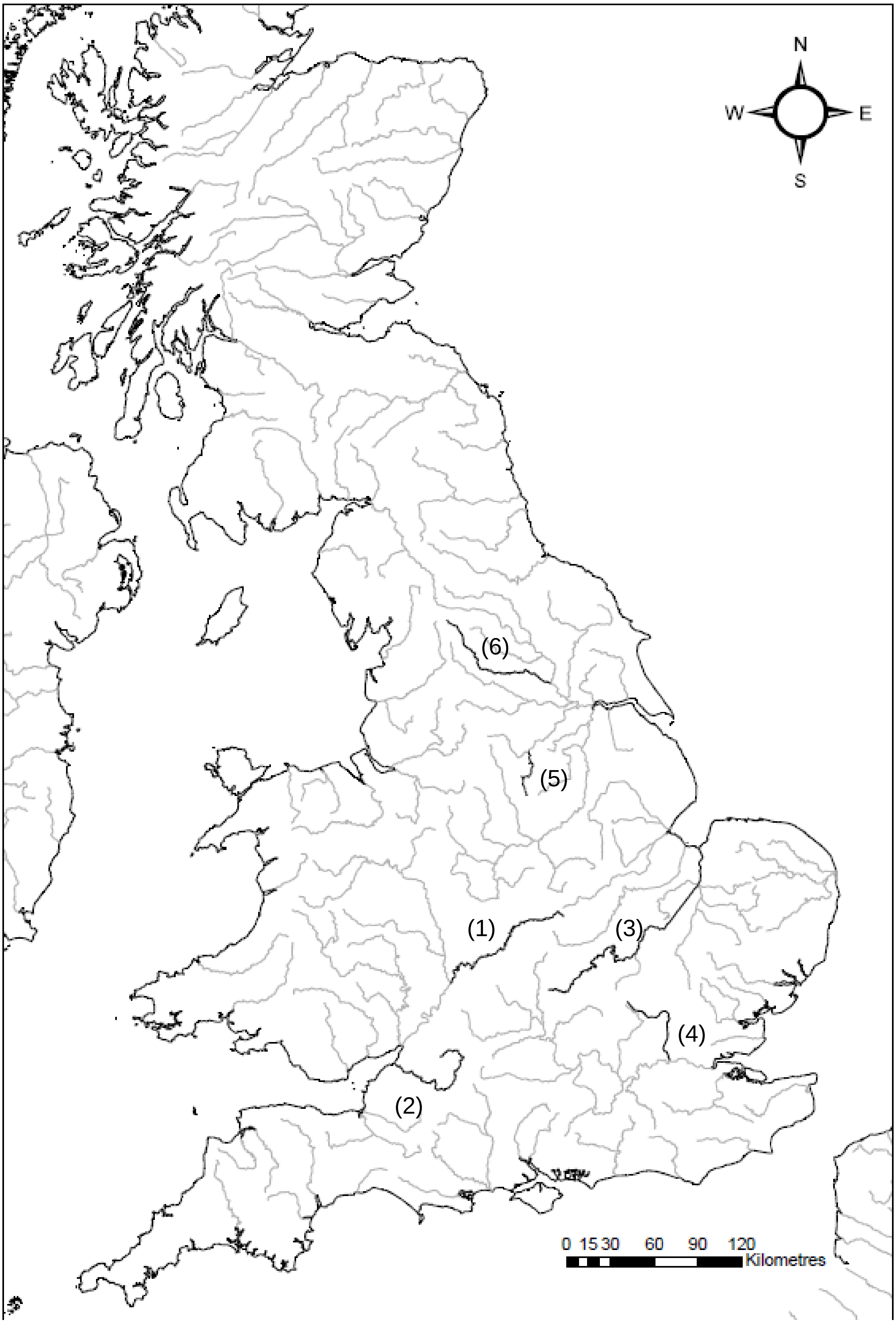

Figure 4.1 Map of study rivers, where (1) is Avon, (2) Bristol Avon, (3) Great Ouse, (4) Lee, (5) Rother and (6) Wharfe.

Table 4.1 Numbers and sizes of individuals used for analysis. n = number of individuals, number in parentheses are the mean ± SD fork lengths of individuals (mm).

River of origin	Species			
	B. barbus	*L. cephalus*	*L. leuciscus*	*R. rutilus*
Avon	n=74 (565 ± 171)	n=40 (192 ± 76)	n=45 (173 ± 51)	n=53 (189 ± 51)
Bristol Avon	n=0	n=27 (272 ± 65)	n=29 (135 ± 35)	n=37 (143 ± 45)
Gt Ouse	n=55 (618 ± 89)	n=22 (196 ± 97)	n=33 (134 ± 43)	n=50 (158 ± 57)
Lee	n=49 (385 ± 155)	n=0	n=0	n=0
Rother	n=38 (391 ± 111)	n=44 (230 ± 69)	n=30 (140 ± 43)	n=36 (127 ± 53)
Wharfe	n=47 (317 ± 162)	n=0	n=0	n=39 (106 ± 21)
Total	n=263 (473 ± 185)	n=133 (221 ± 81)	n=137 (148 ± 47)	n=215 (148 ± 56)

4.2.2 *Image analysis*

One scale per fish was photographed using a Leica stereo microscope and digital camera. Only one scale was photographed as per Ibanez *et al.,* 2009. Magnification was dependent on the size of the scale, adjusted to provide the largest possible picture to ensure precision. The photographed scales were selected according to two principles. Firstly, a scale could not be a replacement scale to ensure it held the entire historical record of the fish's life and the scale focus was an identifiable landmark location. Secondly, the scale had to be presumed truly representative of the species and individual, this was based on visual judgment of available scales for that individual. This method of pre-selecting scales based on these judgements requires the expertise of an individual on what constitutes a typical fish scale for each species.

Scale photographs were digitised using TPSDig2 (Rohlf, 2008a). Three landmarks were located along an axis running from the posterior to the anterior edge of the scales dividing the scale into two 'equal' halves. The contour of the scale was recorded using 16 equally distanced semi-landmarks (Figure 4.2). Semi-landmarks were slid according to Bookstein (1997), to account for the reduced accuracy of semi-landmarks compared with landmarks. The configurations of landmark coordinates for the sampled scales were superimposed (scaled, translated and rotated) using Generalised Procrustes Analysis (GPA) in TPSRelw (Rohlf, 2008b). This technique ensures only the shape of the scale is analysed. The centroid size (CS), defined as the square root of the summed square distance of all landmarks about their centroid, was calculated as a measure of overall scale size. Gomes and Monterio (2007) suggested this size variable provides a more complete measure of general size than traditional size estimates, such as standard length (L_S) and is a better estimator of isometric (not correlated with shape) size variation than the first principal component of multiple linear measurements.

Figure 4.2 Landmark and semi-landmark locations, 3 landmarks (numbered 1-3) and 16 semi-landmarks (numbered 4-19), displayed on a scale taken from a *R. rutilus,* 160 mm L_F.

4.2.3 *Morphology analysis*

Preliminary and exploratory analysis (Canonical Variants Analysis (CVA) and Principal Component Analysis (PCA)) was performed using MorphoJ (Klingenberg, 2008) which allowed a rapid assessment of individual scale shape morphology. This identified and corrected where necessary, potential outliers or errors within the data set. Furthermore, the ontogenic relationship between fish scale and fish length was identified and all other analysis was corrected for ontogeny.

Data sets were exported from MorphoJ for further analysis in various statistical packages with greater statistical and graphical options. Canonical Variants Analysis and regression analysis were performed in SPSS v17.0 (SPSS Inc. Chicago, Illinois), allowing discrimination between groups. Principal Component Analysis was performed in MORPHOLOGIKA^2 (O 'Higgins & Jones, 2006) allowing discrimination between individuals. Finally, principal component scores were imported into R (R-Core Development Team 2009, version 2.9.1), where Linear Discriminate Analysis (LDA) was performed to determine the validity of classify fish scales into their correct species and populations.

4.2.4 *Environmental variables*

Mean water temperature for all study rivers was obtained from the Environment Agency, mean discharge data were obtained from Centre for Ecology and Hydrology. These data sets allowed linear regression of scale shape and environmental variables (temperature or flow) to establish whether a relationship exists for all four species.

4.3 RESULTS

4.3.1 *Ontogeny*

A total of 261, 130, 137 and 212 *B. barbus, L. cephalus, L. leuciscus* and *R. rutilus* individuals were included in the analysis of ontogeny, of which ontogeny allometry (defined as the change in shape with size) accounted for 9.6%, 23.3%, 12.1% and 10.1% of the variation in scale morphology for each species, respectively. There is a positive relationship between fish length and scale morphology, regression plots displaying the ontogenetic relationship between fish scales and fish length for all four species are shown in Figure 4.3. There are no specific patterns or clusters, suggesting fish scales do not change over a short period (e.g. sexual maturity) but instead there is a general change in scale morphology over the life of an individual.

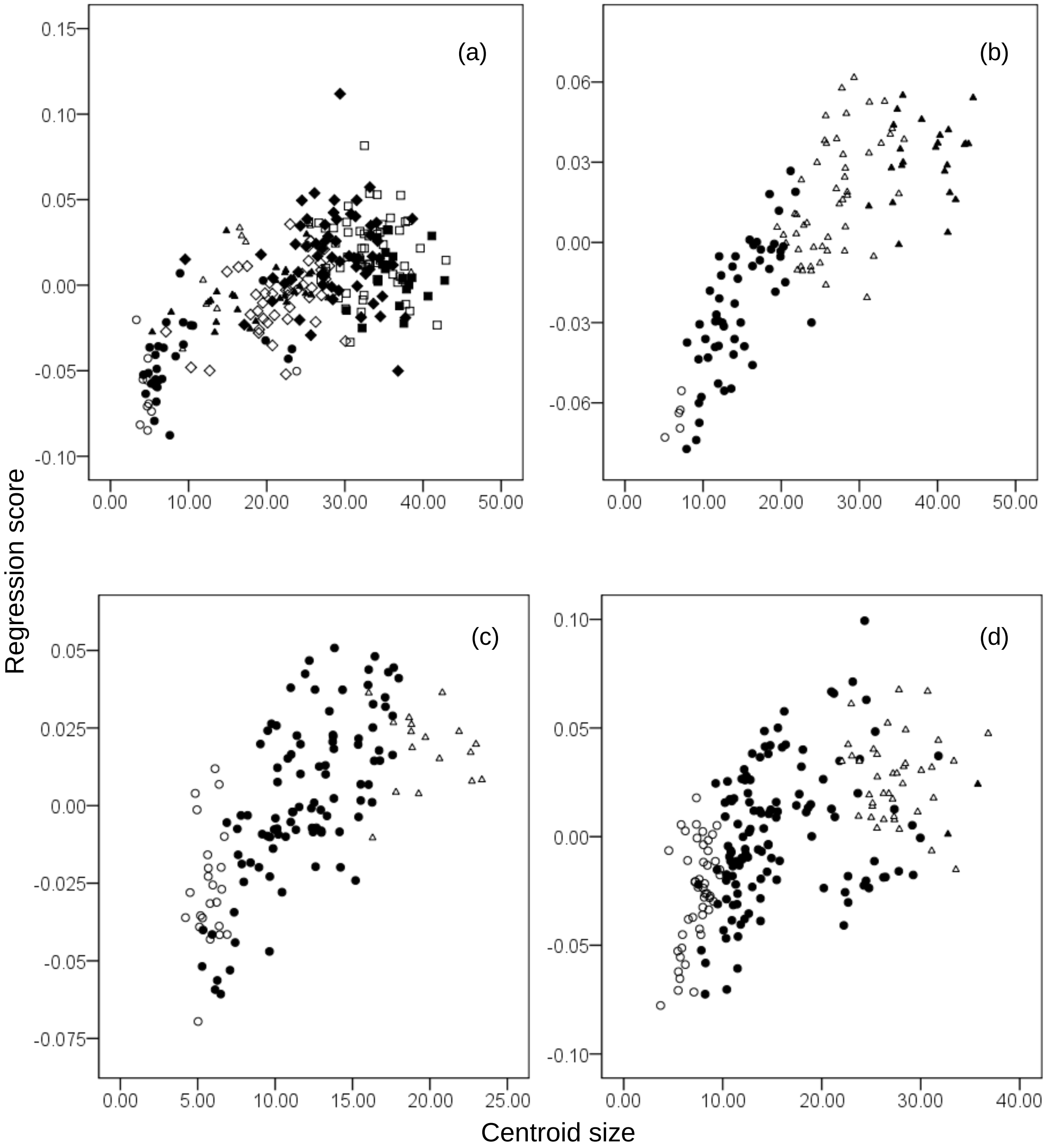

Figure 4.3 Regression plots of 100 mm length classes of *B. barbus* (a), *L. cephalus* (b), *L. leuciscus* (c) and *R. rutilus* (d). 100-mm class: (0-99 mm (○,0), 100 -199 mm (●,1), 200-299 mm (∆,2), 300-399 mm (▲,3), 400-499 mm (□,4), 500-599 mm (■,5), 600-699 mm (◊,6) and >700 mm (♦,7).

4.3.2 *Identifying species*

To assess whether scale morphology is related to species, PC plots were initially examined to see if there was any evidence of clustering by species, before employing discriminant analysis (see below). The general pattern of morphological differences described by the first two PCs was explored using transformation grids (Figure 4.4). The majority of individuals towards the left of the X axis in Figure 4.4 are *B. barbus*, while those towards the right are *L. cephalus*, *L. leuciscus* and *R. rutilus*. *Barbus barbus* scales are generally longer and narrower than the other three species studied and this is depicted in the transformation grid (Figure 4.4). The relative elongation of *B. barbus* scales reflects a larger area of insertion into the epidermis. Principal component analysis and canonical correlation analysis confirmed the different scale morphology of the four species (Figures 4.3 and 4.4). Species were separated principally on the first discriminant function; the first PC explained 90.2% of the total variance while the second accounted for 6.6%. A linear discriminant analysis (LDA) was also performed to identify differences between species and allow a cross-validation technique to be used, to determine the accuracy of correct classification. Using this function, *B. barbus* scales had the highest classification rate at 99.2%, whilst *L. leuciscus* had the lowest classification rate at 72.1% (Table 4.2).

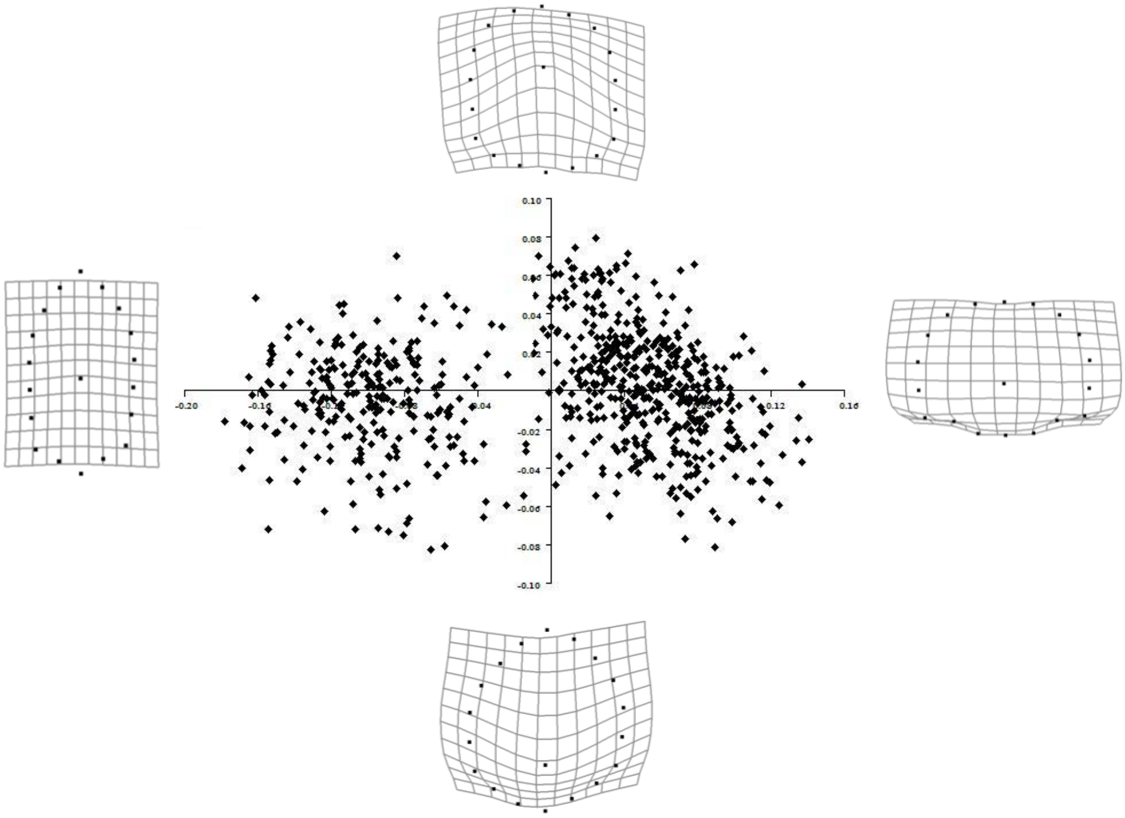

Figure 4.4 First two principal components (PCs) of shape of all individuals analysed. Thin plate spline transformation grids for the extreme points of each PC are shown. These are superimposed on the shapes, predicted when the average landmark configuration of all specimens is deformed into that of a hypothetical specimen positioned at the extreme of the PC of interest.

Table 4.2 Cross-validation between species.

			Predicted Group Membership				
		Species	*B. barbus*	*L. cephalus*	*L. leuciscus*	*R. rutilus*	Total
Original	Count	*B. barbus*	261.0	0.0	0.0	0.0	261
		L. cephalus	0.0	114.0	8.0	11.0	133
		L. leuciscus	0.0	12.0	104.0	20.0	136
		R. rutilus	0.0	12.0	24.0	178.0	214
	%	*B. barbus*	100.0	0.0	0.0	0.0	100
		L. cephalus	0.0	85.7	6.0	8.3	100
		L. leuciscus	0.0	8.8	76.5	14.7	100
		R. rutilus	0.0	5.6	11.2	83.2	100
Cross-validated	Count	*B. barbus*	259.0	0.0	1.0	1.0	261
		L. cephalus	0.0	109.0	9.0	15.0	133
		L. leuciscus	0.0	15.0	98.0	23.0	136
		R. rutilus	0.0	13.0	28.0	173.0	214
	%	*B. barbus*	99.2	0.0	0.4	0.4	100
		L. cephalus	0.0	82.0	6.8	11.3	100
		L. leuciscus	0.0	11.0	72.1	16.9	100
		R. rutilus	0.0	6.1	13.1	80.8	100

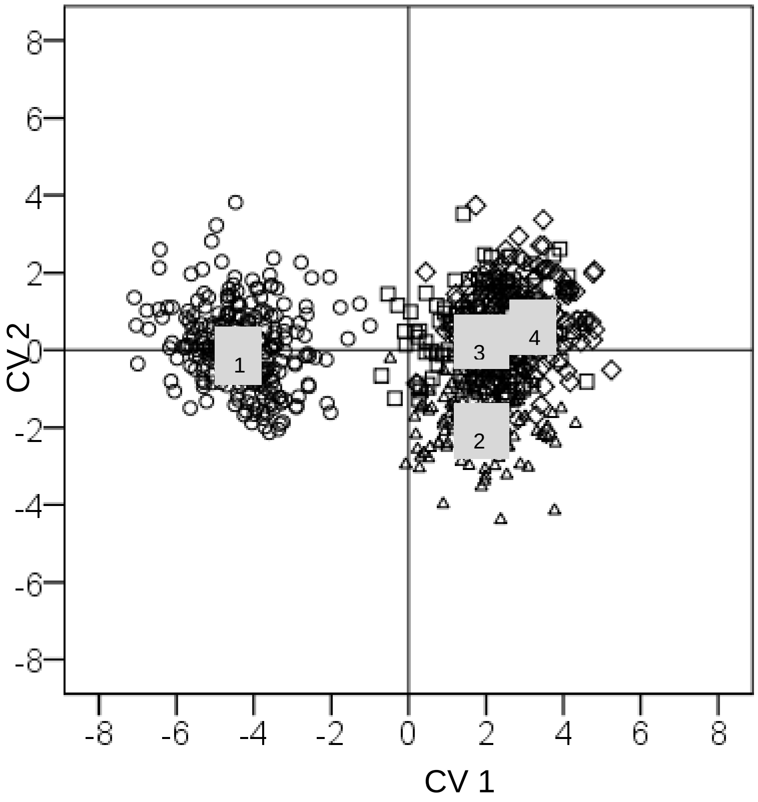

Figure 4.5 Canonical variates analysis of *B. barbus* (○, 1), *L. cephalus* (Δ, 2), *L. leuciscus* (□, 3) and *R. rutilus* (◊, 4). Numbers over the shaded squares are the number of centroids.

4.3.3 *Identifying populations*

Discriminant analysis revealed that it is possible to identify some individual populations. The first PC explained 47.2% of the total variance in *B. barbus* scales, while the second accounted for 24.3%. The River Wharfe had the highest classification of *B. barbus* at 71.7%, whilst the River Rother had the lowest at 43.2% (Table 4.3). The River Wharfe had more positive CV1 scores, while the River Great Ouse had more negative CV1 scores and positive CV2 scores (Figure 4.6). The River Lee had predominately negative CV1 and CV2 scores.

Table 4.3 Cross-validation between *B. barbus* populations.

			Predicted Group Membership					
		River	Avon	Gt. Ouse	Lee	Rother	Wharfe	Total
Original	Count	Avon	51.0	10.0	3.0	6.0	4.0	74
		Gt. Ouse	11.0	35.0	7.0	1.0	1.0	55
		Lee	9.0	4.0	33.0	1.0	2.0	49
		Rother	8.0	4.0	4.0	19.0	2.0	37
		Wharfe	4.0	2.0	1.0	2.0	37.0	46
	%	Avon	68.9	13.5	4.1	8.1	5.4	100
		Gt. Ouse	20.0	63.6	12.7	1.8	1.8	100
		Lee	18.4	8.2	67.3	2.0	4.1	100
		Rother	21.6	10.8	10.8	51.4	5.4	100
		Wharfe	8.7	4.3	2.2	4.3	80.4	100
Cross-validated	Count	Avon	41.0	12.0	4.0	11.0	6.0	74
		Gt. Ouse	13.0	26.0	10.0	4.0	2.0	55
		Lee	10.0	7.0	25.0	5.0	2.0	49
		Rother	8.0	5.0	6.0	16.0	2.0	37
		Wharfe	6.0	2.0	2.0	3.0	33.0	46
	%	Avon	55.4	16.2	5.4	14.9	8.1	100
		Gt Ouse	23.6	47.3	18.2	7.3	3.6	100
		Lee	20.4	14.3	51.0	10.2	4.1	100
		Rother	21.6	13.5	16.2	43.2	5.4	100
		Wharfe	13.0	4.3	4.3	6.5	71.7	100

Analysis of *L. cephalus* scales revealed the first PC explained 47.7% of the total variance while the second accounted for 35.6%. In the rivers Avon and Rother, 50% of *L. cephalus* were correctly classified, compared with 9.1% in the Great Ouse, which had the lowest classification rate (Table 4.4). Scales from the rivers Avon, Bristol Avon and Rother were generally separated in the CVA plot (Figure 4.6), but the CVA plot suggests the inclusion of the Great Ouse reduced the cross validation results.

Analysis of *L. leuciscus* scales revealed the first PC explained 60.7% of the total variance while the second accounted for 26.3%. In the Bristol Avon, 86.2% of *L.*

leuciscus were correctly classified, compared with 43.8% in the Rother, which had the lowest classification rate (Table 4.5). The CVA plot suggests there are differences in shape between the populations. The Bristol Avon has more positive CV1 scores, while the Great Ouse has more negative CV2 scores (Figure 4.6). The inclusion of the Rother scores may have reduced the cross validation scores.

Analysis of *R. rutilus* scales revealed the first PC explained 67.0% of the total variance while the second accounted for 16.9%. In the River Avon 80.8% of *R. rutilus* were correctly classified, compared with 29.7% in the Great Ouse, which had the lowest classification rate (Table 4.6). The rivers Rother and Wharfe fish have more positive CV1 scores in the CVA plot, while the River Avon has more negative CV1 scores (Figure 4.6). There were similarities between populations, suggesting environment conditions (e.g. temperature, flow and climate) between rivers may be similar and subsequently populations are displaying similar morphology.

Table 4.4 Cross-validation between *L. cephalus* populations.

			Predicted Group Membership				
		River	Avon	B. Avon	Gt. Ouse	Rother	Total
Original	Count	Avon	27.0	1.0	4.0	8.0	40
		B. Avon	2.0	21.0	1.0	3.0	27
		Gt. Ouse	1.0	3.0	13.0	5.0	22
		Rother	3.0	3.0	3.0	35.0	44
	%	Avon	67.5	2.5	10.0	20.0	100
		B. Avon	7.4	77.8	3.7	11.1	100
		Gt. Ouse	4.5	13.6	59.1	22.7	100
		Rother	6.8	6.8	6.8	79.5	100
Cross-validated	Count	Avon	20.0	1.0	7.0	12.0	40
		B. Avon	6.0	12.0	5.0	4.0	27
		Gt. Ouse	4.0	5.0	2.0	11.0	22
		Rother	8.0	6.0	8.0	22.0	44
	%	Avon	50.0	2.5	17.5	30.0	100
		B. Avon	22.2	44.4	18.5	14.8	100
		Gt. Ouse	18.2	22.7	9.1	50.0	100
		Rother	18.2	13.6	18.2	50.0	100

Table 4.5 Cross-validation between *L. leuciscus* populations.

			Predicted Group Membership				
		River	Avon	B. Avon	Gt. Ouse	Rother	Total
Original	Count	Avon	37.0	3.0	2.0	2.0	44
		B. Avon	0.0	28.0	1.0	0.0	29
		Gt. Ouse	2.0	2.0	26.0	2.0	32
		Rother	7.0	0.0	1.0	20.0	28
	%	Avon	84.1	6.8	4.5	4.5	100
		B. Avon	0.0	96.6	3.4	0.0	100
		Gt. Ouse	6.3	6.3	81.3	6.3	100
		Rother	25.0	0.0	3.6	71.4	100
Cross-validated	Count	Avon	27.0	4.0	7.0	6.0	44
		B. Avon	2.0	25.0	2.0	0.0	29
		Gt. Ouse	7.0	6.0	14.0	5.0	32
		Rother	9.0	1.0	5.0	13.0	28
	%	Avon	61.4	9.1	15.9	13.6	100
		B. Avon	6.9	86.2	6.9	0.0	100
		Gt. Ouse	21.9	18.8	43.3	15.6	100
		Rother	32.1	3.6	17.9	46.6	100

4.3.4 *Environmental variables*

There was no significant relationship between scale morphology and environmental variables (temperature and flow) using linear regression analysis (Figure 4.7). *B. barbus* scale shape and mean temperature (r^2 = 0.408, P = 0.246) and mean discharge (r^2 = 0.461, P = 0.208), *L. cephalus* and mean temperature (r^2 = 0.467, P = 0.317) and mean discharge (r^2 = 0.610, P = 0.219), *L. leuciscus* and mean temperature (r^2 = 0.307, P = 0.446) and mean discharge (r^2 = 0.463, P = 0.320) and *R. rutilus* and mean temperature (r^2 = 0.524, P = 0.167) and mean discharge (r^2 = 0.279, P = 0.360). The low significance values are a reflection of the small sample sizes.

Table 4.6 Cross-validation between *R. rutilus* populations.

			Predicted Group Membership					
		River	Avon	B. Avon	Gt. Ouse	Rother	Wharfe	Total
Original	Count	Avon	47.0	0.0	3.0	1.0	1.0	52
		B. Avon	0.0	25.0	6.0	0.0	6.0	37
		Gt. Ouse	3.0	6.0	33.0	3.0	3.0	48
		Rother	2.0	2.0	4.0	24.0	3.0	35
		Wharfe	1.0	5.0	7.0	4.0	22.0	39
	%	Avon	90.4	0.0	5.8	1.9	1.9	100
		B. Avon	0.0	67.6	16.2	0.0	16.2	100
		Gt. Ouse	6.3	12.5	68.8	6.3	6.3	100
		Rother	5.7	5.7	11.4	68.6	8.6	100
		Wharfe	2.6	12.8	17.9	10.3	56.4	100
Cross-validated	Count	Avon	42.0	0.0	6.0	3.0	1.0	52
		B. Avon	0.0	11.0	13.0	1.0	12.0	37
		Gt. Ouse	9.0	11.0	15.0	4.0	9.0	48
		Rother	7.0	3.0	9.0	11.0	5.0	35
		Wharfe	1.0	10.0	8.0	4.0	16.0	39
	%	Avon	80.8	0.0	11.5	5.8	1.9	100
		B. Avon	0.0	29.7	35.1	2.7	32.4	100
		Gt. Ouse	18.8	22.9	31.3	8.3	18.8	100
		Rother	20.0	8.6	25.7	31.4	14.3	100
		Wharfe	2.6	25.6	20.5	10.3	41.0	100

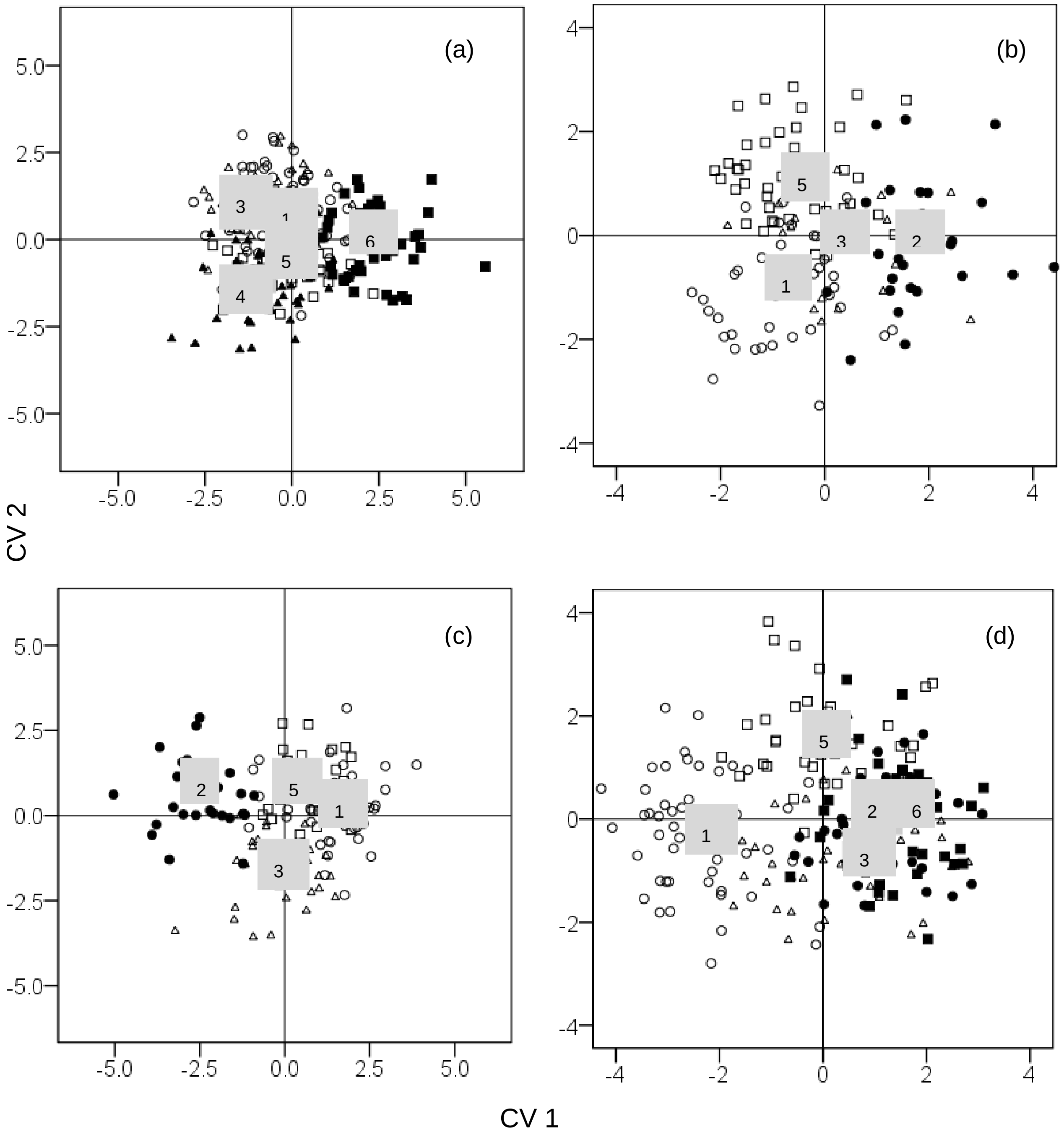

Figure 4.6 Canonical variates analysis of *B. barbus* (a), *L. cephalus* (b), *L. leuciscus* (c) and *R. rutilus* (d), from Avon (○, 1), Bristol Avon (●, 2), Great Ouse (∆, 3), Lee (▲, 4), Rother (□, 5) and Wharfe (■, 6). Numbers over the shaded squares are the centroid groups.

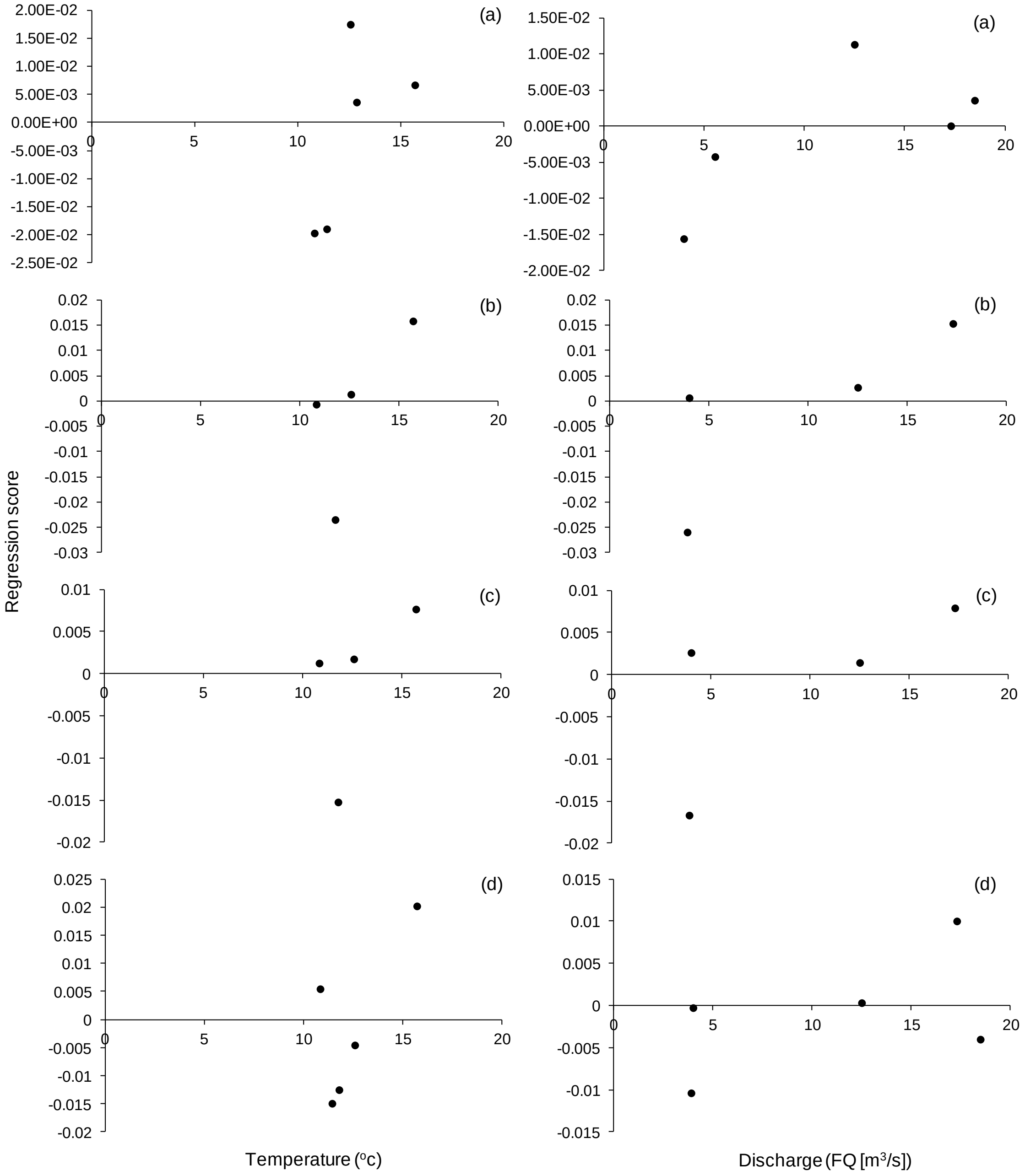

Figure 4.7 Relationship between scale morphology of four species ((a) *B. barbus*, (b) *L. cephalus*, (c) *L. leuciscus,* (d) *R. rutilus*) and mean temperature and discharge for rivers Avon, Bristol Avon, Great Ouse, Lee, Rother and Wharfe.

4.4 DISCUSSION

4.4.1 *Ontogeny*

This study provides evidence to confirm that fish species can be discriminated using a semi-landmark GM approach to analyse fish scale morphology. Furthermore this study has identified an allometric ontogenic relationship between scale morphology and fish length. It is possible heterochrony, defined as a developmental change in the timing of events, can lead to changes in size and shape (Klingenberg, 1998), although there is no clear “timing of change” between the ontogenetic development of fish scale morphology. Allometric ontogeny presents a confounding source of shape variation. Similar ontogenetic relationships are seen in otoliths that undergo morphology changes with age and environmental conditions (Monteiro *et al*., 2005). Camapana and Casselman (1993) found otolith morphology varied between ages, sexes and year classes and Simoneau *et al*. (2000) suggested part of this otolith-growth effect could be due to an intrinsic change in shape associated with allometric nature of the length/height relationship of an individual. It is possible much of the change in shape occurs when the various species shift diet from the juvenile to adult life stages, although this study provides no evidence of a specific shift. However, ontogenetic change in morphology will probably depend on a number of factors, including productivity of the river system, maturation of the individuals, competition with other species or within the population (intra-specific) and rate of growth. Since major shifts in growth rate can occur at these life events it is possible they lead to body morphology changes that are manifest through scale shape variability.

4.4.2 *Identifying species*

Scale morphology was successful at discriminating between species, despite not all individuals of the species being from the same catchment. Likewise, Campana & Casselman (1993) were able to discriminate between species using otolith morphology. Ibanez *et al.* (2009) suggested that differences in scale morphology between species might be linked to functional species-specific habits as well as to phylogenetic relationships. The body morphology of bats is capable of predicting microhabitat use (Crome & Richards, 1988; Barlow *et al*., 1997; Brigham *et al*., 1997) and diet (Sztencel-Jablonka *et al*., 2009). Similarly, the morphological features of a fish; size, shape and subsequent hydrodynamics influence swimming ability of fishes (Videler & Wardle, 1991; Videler 1993), which determines the habitat suitability for a species (McLaughlin & Grant, 1994). Differences in species morphology has been related to swimming ability through streamlining (Aleev, 1969; Hawkins & Quinn, 1996) and fin shape (Felley, 1984; Sambilay, 1990; Gerstner, 1999) and morphological characters that affect drag and thrust forces (Boily & Magnan, 2002). This study has demonstrated that scale morphology is a good discriminator between species, principally because of the evolutionary and morphological differences between species.

4.4.3 ***Morphological variation and phenotypic plasticity* (identifying populations)**

Fish populations display phenotypic plasticity in their scale morphology (Swain & Foote, 1999), however the accuracy of a semi landmark GM approach to identify populations based on scale morphology is questionable. The association between morphology and habitat is a central theme in evolutionary biology because it reflects the way organisms adapt to their surrounding environment (Kaliontzopoulou *et al.*, 2010), termed Ecomorphology (Motta *et al*., 1995). The relationship between morphology and environmental conditions has been related to habitat use of various taxa, including fishes (Aleev, 1969; Gatz, 1979a, 1979b; Webb, 1984), bats (Saunders & Barclay, 1992; Brigham *et al.*, 1997), birds (Ricklefs & Travis, 1980; Leisler & Winkler, 1985), lizards (Moermond, 1979, 1986; Kaliontzopoulou *et al.*, 2010) and insects (Harder, 1985; Moran, 1986). Although the discrimination of populations was unsuccessful for some species/populations others could be accurately identified based on scales morphology. Two such examples are the Wharfe *B. barbus* and Avon *R. rutilus*; *the former* tended to be compressed between the focus and lateral edge and laterally “pinched” before the posterior edge perhaps a reflection of the location scales samples were taken from on the fish or the distinct genetic structure of the population, the later scales tended to be compressed on posterior edge and push out on the lateral edges, perhaps a reflection of the deep body of the fish. In addition this study tested for potential relationships between scale morphology and environmental variables, specifically temperature and flow, but did not find any significant relationships, possibly because of their small sample size.

Subsequently, fish scale morphology could be a suitable discriminator of fish populations, similar to other phenotypic features - body morphology (Cadrin, 2000), meristic counts (Turan *et al.*, 2006), otolith morphology (Camapana & Casselman, 1993; Watkinson & Gillis, 2005), scale patterns (Watarai & Igarashi, 1992), scale annuli (Ibanez & Gallardo-Cabello, 2005) and scale morphology (De Pontual & Prouzet, 1987; Ibanez *et al*., 2009). Geographical isolation can result in the development of different morphological features between fish populations because the interactive effects of environment, selection and genetics on individual ontogenies produce morphometric differences within a species (Cadrin, 2000).

It is possible some of the study rivers were characteristically similar (e.g. gradient, substrate, depth profiles, temperature and flow), which could have resulted in inability to correctly classify the population from scales. Many environmental variables could have an influence on scale morphology, because fish scales are dynamic and capable of displaying changes in the environment through re-absorption of the material during times of stress and/or when growth ceases. Likewise, environmental factors have been shown to influence phenotypes directly, as well as indirectly via trait correlations and interactions with other environmental variables (Langerhans *et al.*, 2007). Scale morphology may represent an important phenotypic characteristic for fishes as they interact with the surrounding environment through their scales (Garduno-Paz *et al.*, 2010). Consequently, it is reasonable to assume that seasonal, location or habitat variability in growth characteristics of populations can influence scale morphology.

4.4.4 *Limitations*

In England and Wales, large numbers of farm-reared fishes are released annually to enhance fisheries (North, 2002). Britton *et al.* (2004b) stated that approximately 500,000 cyprinids, principally *A. brama*, *B. barbus, L. cephalus*, *L. leuciscus* and *R. rutilus,* are reared at the Environment Agency fish farm in Calverton and are stocked during 500 operations each year. Consequently, these stocked fish are homogenising the populations in England and Wales, and potentially affecting both the genetic diversity of the natural populations but also the phenotypic characteristics, including scale shape (Glover *et al.*, 2009; Hansen *et al.*, 2009). This could account for some of the morphological similarities between populations. However, Campana and Casselman (1993) re-evaluated published studies on various fish species and suggested that growth rates contributed more variation to regional differences in otolith shape than stock origin. Consequently, it is possible that variability in scale shape and thus discriminatory power in cyprinids in this study are more an outcome of environmental drivers than origin overriding any likely disruption from stocking. This will not be the case for genetic studies, in which origin is fixed to source not location.

Although all scales samples should have been taken from the same anatomical region, some scales may have been taken from other regions, or from near the extreme of the anatomical region. This is important because there is variation in scale shape within and between anatomical regions (Ibanez *et al.*, 2009). Future studies should, therefore, be more specific, dictating which scales to remove from an individual, e.g. the exact scale row and line number. Scales taken from specific anatomical regions may display more characteristic traits of that species, therefore further research into the impact of scale location on species classification may be needed.

4.5 CONCLUSIONS

This study highlights that scale morphology can be used to discriminate riverine fish species. There is sufficient evidence that with further development scale morphology may be capable of identify populations, although this may rely on populations being subject to different environmental conditions. The analysis of fish scale morphology is inexpensive, quick, non-destructive, and informative and could easily be added to existing monitoring programmes. Importantly, this study identified that scale morphology can be used to identify different species, subsequently GM analysis may be a suitable tool to identify prey species of piscivores. In conclusion, this and previous studies have highlighted the potentially important and opportunistic information that can be gained from the GM analysis of fish scales. It is therefore anticipated that this study will be fundamental in shaping future fish population assessments.

5 COMPARISON OF REGIONAL RECRUITMENT SUCCESS AND GROWTH

5.1 INTRODUCTION

Growth standards for UK fish species were historically developed by Hickley and Dexter (1979), who constructed expected lengths-at-age for A. *brama*, *L. cephalus*, *L. leuciscus* and *R. rutilus*. Later additions included *E. lucius* by Hickley and Sutton (1984) and *B. barbus* and *P. fluviatilis* by Cowx (2001). These original growth standards have been revised and further species added by Britton (2007). A similar concept to standard growth curves is the Relative Growth Index (RGI), an approach adopted in the United States by several authors (Hubert, 1999; Quist *et al.*, 2003; Jackson & Hurley, 2005; Jackson *et al.*, 2008).

It is recognised amongst fisheries scientists that the growth rates of fish vary within different ecological zones or tributaries within a catchment, with species typically achieving greater growth rates in their ‘preferred’ habitats (Cowx, 1988). Similarities and differences in growth rate have also been found between catchments for various species (e.g. Mann, 1973; Mann, 1974; Cowx, 2001; Copp *et al.*, 2007). The geographic location of a river can influence the growth rate of a species, and so current standard national growth rates may not be a true representation of regional growth and as a consequence could lead to mis-interpretation of fish population characteristics.

Rivers within the UK are dynamic, characterised by differences in length, width, gradient and rising at different elevations, whilst fish communities exhibit classical zonation between reaches (Huet, 1959; Cowx, 1988; Noble *et al*., 2007). Rivers are subject to different levels of precipitation and temperatures regimes that can potentially influence recruitment dynamics (Mooij *et al.*, 1995; Grenouillet *et al.*, 2001; Tolonen *et al.*, 2003; Nunn *et al.*, 2007a). However, few studies have assessed whether differences in recruitment dynamics exist between different regions of the UK. There is growing need for a greater understanding of fish populations, including recruitment success, as a result of the Water Framework Directive requirements and the limited information on temporal and spatial trends of cyprinid recruitment success.

The primary aim of this study was to determine whether geographic variation influences the growth rates of freshwater riverine fishes commonly found within England. To meet this aim growth curves were constructed and statistically compared for four key species [a broad generalist eurytopic species (*R. rutilus*), rheophilic gravel spawning species (*L. cephalus* and *L. leuciscus*) and a phytophilous spawner (*A. brama*)] in different Environment Agency (EA) regions; these regions typically encompass the major river catchments of England and Wales. A further aim was to provide a clear and precise methodology for detailed analysis of growth curves. The final aim of this study was to establish whether recruitment success varies between Environment Agency regions, providing further information on the temporal and spatial trends of cyprinid recruitment success at a regional and national level.

5.2 MATERIALS AND METHODS

Mean back calculated lengths-at-age for four species were assembled from age and growth data acquired from EA fish monitoring surveys between 2003 and 2009. All fish captured during surveys were identified, measured (fork length, L_F, mm) and scale samples removed for later laboratory analysis. All growth analysis was based on scales, read under a low powered microfiche, with a magnification of 20x or 30x. To minimise errors in ageing of scales, a quality control procedure was followed as described in Musk *et al.* (2006), where a secondary reader checked 10% of the aged scales. Where disagreement was found, the scale was reviewed to enable consensus to be reached. In addition to Musk *et al.* (2006), a second opinion was sought for any scales which proved difficult for the primary scale reader, outside of the quality control procedure.

Growth data were calculated for rivers where data was available for at least 15 individuals. Only rivers with multiple sampling sites were included in the analysis to gain a true representation of the river. When multiple surveys on a river (population) were available, the study with the largest sample size and/or age classes was selected. However, when necessary, multiple surveys were combined to increase the number of individuals available for analysis. Data sets were limited to 10 study rivers for each EA region (Table 5.1), rivers with the largest sample size and/or age classes were selected, reducing the influence of a specific region on the calculation of national YCS. The Welsh EA region was not included in the analysis because there were no cyprinid fish surveys available. Because of the possible difficultly in identification of *A. bjoerkna* and *A. brama* in the field, rivers which have had both species aged during 2003 and 2009, and/or individuals which displayed unusually growth from other individuals, suggesting they could be *A. bjoerkna* rather than *A. brama*, were excluded from the analysis.

Table 5.1 Study rivers used for construction and comparison of *A. brama*, *L. cephalus*, *L. leuciscus* and *R. rutilus* growth parameters. Geographic locations are displayed in Figures 5.1 - 5.4.

Region	River	River Code	Species			
			A. brama	*L. cephalus*	*L. leuciscus*	*R. rutilus*
Anglian	20ft Drain	1	✓			
	Ancholme	2	✓			
	Blackwater	3		✓	✓	
	Boston Drains	4				✓
	Bure	5			✓	✓
	Chelmer and blackwater	6			✓	✓
	Colne	7	✓	✓	✓	✓
	Ely Ouse	8	✓			
	Gipping	9		✓		
	Granta	10				✓
	Great Ouse	11	✓	✓		✓
	Lark	12		✓		
	Little Ouse	13			✓	
	Ouzel	14		✓		✓
	Pant	15		✓	✓	
	Saltes Lode	16	✓			
	Steeping	17	✓			✓
	Stone Bridge Drain	18	✓			
	Stour	19	✓	✓		✓
	Thurm	20	✓			
	Waveney	21		✓	✓	
	Wensum	22		✓	✓	✓
Midlands	Alne	23			✓	
	Anker	24		✓	✓	✓
	Aron	25		✓	✓	✓
	Arrow	26			✓	
	Blythe	27		✓		✓
	Coley brook	28	✓			
	Derwemt	29		✓	✓	✓
	Dove	30			✓	
	Idle	31				✓
	Rea	32			✓	
	Salwarpe	33		✓	✓	✓
	Soar	34		✓		✓
	Stour	35		✓		
	Tern	36		✓		✓
	Trent	37	✓	✓	✓	✓
	Wreake	38	✓	✓	✓	✓

Table continued overleaf

Table 5.1 continued

North East	Aire	39		✓		
	Calder	40		✓	✓	✓
	Dearne	41	✓	✓	✓	✓
	Derwent	42		✓	✓	✓
	Don	43		✓	✓	✓
	Hull	44	✓	✓	✓	✓
	Nidd	45		✓	✓	✓
	Ouse	46				✓
	Rother	47		✓	✓	✓
	Swale	48			✓	
	Ure	49		✓	✓	✓
	Wharfe	50		✓	✓	✓
North West	Abbey Fields	51				✓
	Dane	52		✓	✓	✓
	Douglas	53		✓		✓
	Etherow	54		✓		
	Glaze	55				✓
	Poever Eye	56				✓
	Tame	57		✓		
	Yarrow	58		✓	✓	✓
Southern	Adur	59		✓		
	Arun	60		✓	✓	✓
	Beult	61				✓
	Cuckmere	62		✓		
	Darent and Cray	63		✓	✓	✓
	Eden	64	✓	✓		✓
	Gt Stour	65		✓	✓	✓
	Medway	66		✓	✓	✓
	Ouse	67		✓	✓	✓
	Rother	68	✓	✓		✓
	Rother and RMC	69				✓
	Somerset Frome	70		✓		
	Wallington	71			✓	
	Wantsum	72	✓			✓

Table continued overleaf

Table 5.1 continued

South West	Avon	73		✓		
	Axe	74				✓
	Bristol frome	75		✓	✓	✓
	Brue	76		✓	✓	✓
	Chew	77			✓	✓
	Hampshire Avon	78		✓	✓	
	Huntspill	79	✓			✓
	Isle	80		✓	✓	✓
	Somerset Frome	81			✓	✓
	Stour	82		✓	✓	✓
	Tone	83		✓	✓	✓
	Yeo	84	✓	✓		✓
Thames	Cherwell	85		✓		✓
	Colne	86		✓	✓	✓
	Evenlode	87		✓	✓	
	Kennet	88	✓	✓	✓	✓
	Lee	89		✓	✓	✓
	Loddon	90		✓	✓	✓
	Mole	91		✓	✓	✓
	Thame	92	✓	✓		✓
	Thames	93	✓	✓	✓	✓
	Twarp	94	✓			✓
	Wey	95	✓	✓	✓	✓

Recruitment success (YCS) was calculated for each region/species. Data from sampling sites were pooled together per river. Rivers were weighted against the river with the largest number of individuals, to remove the influence of a specific river. These weighted values were summed, creating new weighted frequencies which represented that region. These new values were imported into the method of YCS according to Cowx and Frear (2004):

(1) The weighted number of fish in each cohort was used to determine the instantaneous mortality rate (Z) of the region using the linear relationship between the natural logarithm of the number of fish in each age group ($\ln N_t$) against age (t) according to $Z = \ln N_0 - \ln N_t$;

(2) The number of fish at time zero (N_0) was calculated independently for each age group using $N_0 = N_t / \exp(Z_t)$, where N_t is the number of fish at age t;

(3) The mean number of fish in each age group (N) was determined as $\Sigma^{t\max} N / t_{\max}$, where $t_{\max}$ is the total number of age groups represented;

(4) The YCS for each age group was determined by YCS at age $t = (N_0 / \bar{N}) \times 100$, where N_0 is the number of fish determined at time t_0 for age group t, i.e. YCS, which is then related to the year of birth, is calculated as an index standardised against a value of 100. A value greater than 100 is a strong year class and a lower value a weak year class.

National recruitment success was calculated similar to regional YCS. Except all rivers were weighted against the river with the largest number of individuals, to remove the influence of a specific river. These weighted values were summed, creating new weighted frequencies which represented all rivers. These new values were imported into the method of YCS according to Cowx and Frear (2004) and steps 1 to 4 repeated.

All fish lengths were back-calculated using the Hile (1941) method as described by (Francis, 1990), where:

$$L_t = -(a/k) + (L_F + a/k)(S_i/S_r) \qquad \text{(equation 6)}$$

S_i is scale increment and S_r is scale radius. This back calculation formula is the same as the Fraser-Lee equation (Francis, 1990), except that c, the L-intercept, is replaced by $-(a/k)$.

Data from sampling sites were pooled together per river. Using back calculated data, Walford (1946) plots for individual rivers were constructed, with mean length-at-age t (L_t) plotted against (L_t+1), to produce a straight line. From this, the intercept on the y axis (a) and slope of the line (k) were calculated and used to provide the growth parameters asymptotic length (L_∞) and the growth coefficient (K) for each river where:

$$L_\infty = a/(1-k) \qquad \text{(equation 7)}$$

$$K = -\ln(k) \qquad \text{(equation 8)}$$

ln is natural logarithm

To transform data into growth curves, the method used was that described by Hickley and Dexter (1979). To produce mean length-at-age for each study river, growth parameters derived from Walford (1946) plots were substituted into the equation:

$$L_t = L_\infty (1-k^t) \qquad \text{(equation 9)}$$

To transform individual river growth curves into regional growth curves. Growth curves for rivers within each region were capped at the maximum age in 75% of rivers (*A. brama*, age 13; *L. cephalus*, age 12; *L. leuciscus*, age 6; *R. rutilus*, age 7), these lengths at age where then plotted as Walford (1946) plots which enabled the calculation of L_∞ and k. These values are then substituted into equation 9, producing the regional growth curve. A similar method was used to construct national growth curves, regional growth curves capped at the maximum age in 75% of rivers were plotted as Walford (1946) plots enabling the calculation of L_∞ and k. These values are then substituted into equation 9, producing the national growth curve.

Transforming the growth data (individual river Hickey and Dexter (1979) growth curves) used to create the regional and national growth curves into straight lines (Walford (1946) plots), allowed regional and national growth differences to be compared using Analysis of Covariance (ANCOVA). All statistical analyses were completed in SPSS v16.0 (SPSS Inc. Chicago, Illinois).

The suitability of defining growth standards by EA regions was investigated, using a Bray-Curtis similarity matrix (Bray & Curtis, 1957) calculated in PRIMER (Plymouth Routines In Multivariate Ecological Research) statistical package, using the growth parameters length-at-age 1, length at maximum age in 75% of populations, L_∞ and K, and presented as a non-metric MultiDimensional Scaling (MDS) plot to test for an effect of geographic location with grouped average cluster analysis overlaid, at 80% and 95% similarities.

5.3 RESULTS

5.3.1 *Comparison of mean growth curves*

Inter-regional variation of the growth parameters asymptotic length and growth co-efficient were found (Table 5.2). Mean lengths-at-age were compared at two intervals; length-at-age 1 and the maximum age achieved in more than 75% of the study rivers. To show the goodness of fit of regional growth curves, individual river and regional growth standard curves are provided in Appendix 2 - 28.

Abramis brama mean length-at-age 1 varied between 48 mm (South West) and 65 mm (North East). The national mean length-at-age 1 was 55 mm (Figure 5.1). At age 13, the lowest mean length was 378 mm (South West) and the greatest mean length was 469 mm (Anglian), and the national mean length was 429 mm. The lowest asymptotic length was 531 mm (South West), the greatest asymptotic length was 733 mm (Anglian), while the national average asymptotic length was 609 mm (Table 5.2).

Leuciscus cephalus mean length-at-age 1 varied between 50 mm (Southern) and 58 mm (North East). The national mean length-at-age 1 was 55 mm (Figure 5.2). At age 12, the lowest mean length was 351 mm (North West), the greatest mean length was 427 mm (North East), and the national mean length was 416 mm. The lowest and greatest asymptotic lengths were 434 mm (North West) and 692 mm (South West), while the national average asymptotic length was 613 mm (Table 5.2).

Leuciscus leuciscus mean length-at-age 1 varied between 53 (Southern) and 60 mm (Midlands). The national mean length-at-age 1 was 55 mm (Figure 5.3). At age 6, the lowest mean length was 195 mm (Southern), the greatest mean length was 224 mm (North East), and the national mean length was 209 mm. The lowest and greatest asymptotic lengths were 262 mm (Thames) and 331 mm (North East), while the national average asymptotic length was 289 mm (Table 5.2).

Rutilus rutilus mean lengths of roach at age 1 varied between 40 (North East and North West) and 43 mm (Anglian). The national mean length-at-age 1 was 41 mm (Figure 5.4). At age 7, the lowest mean length was 188 mm (North West), the greatest mean length was 215 mm (Anglian), and the national mean length was 205 mm. The lowest and greatest asymptotic lengths were 297 mm (North West) and 382 mm (Midlands), while the national average asymptotic length was 350 mm (Table 5.2).

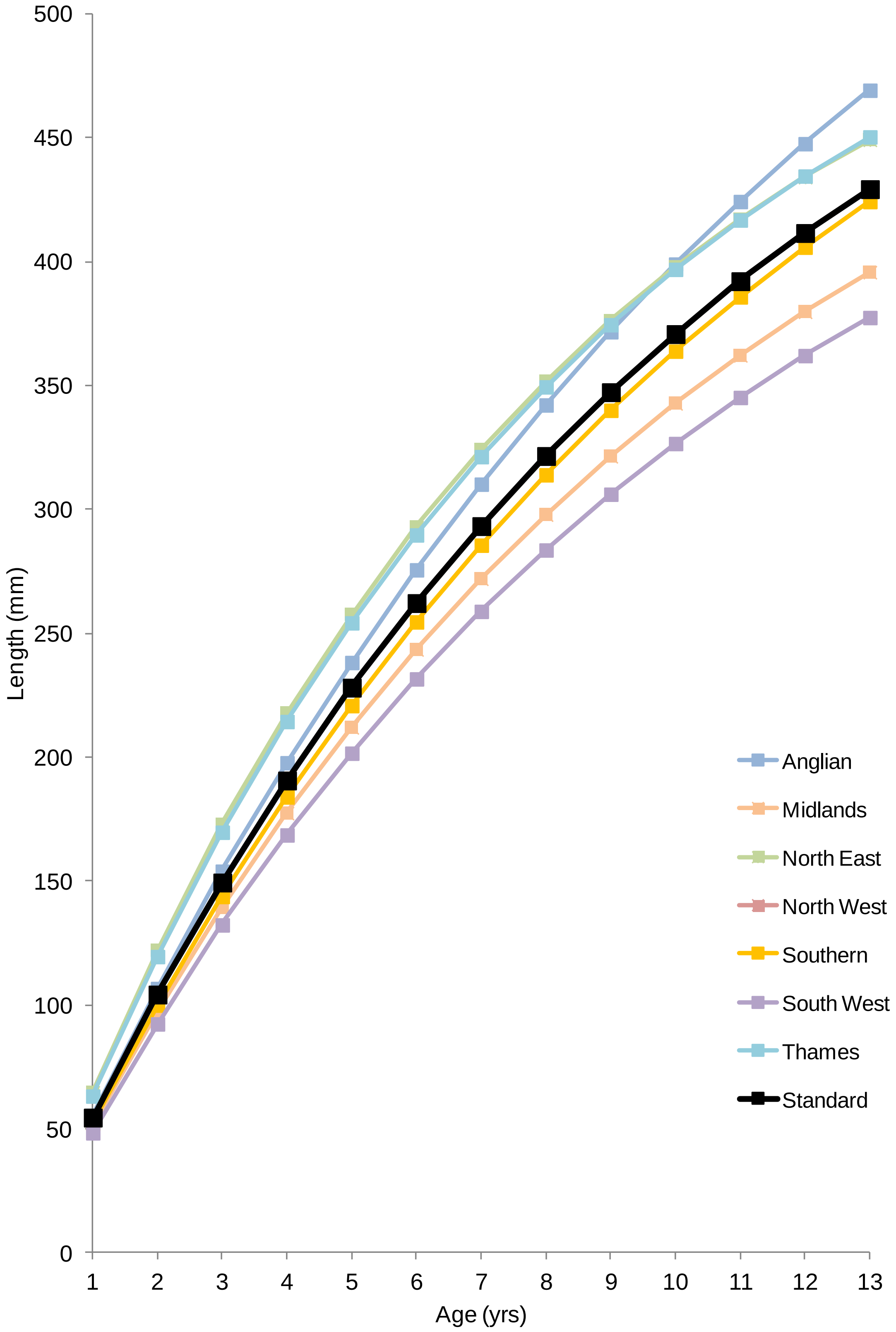

Figure 5.1 Environment Agency regional standard growth curves of *A. Brama,* based on data for rivers in Table 5.1.

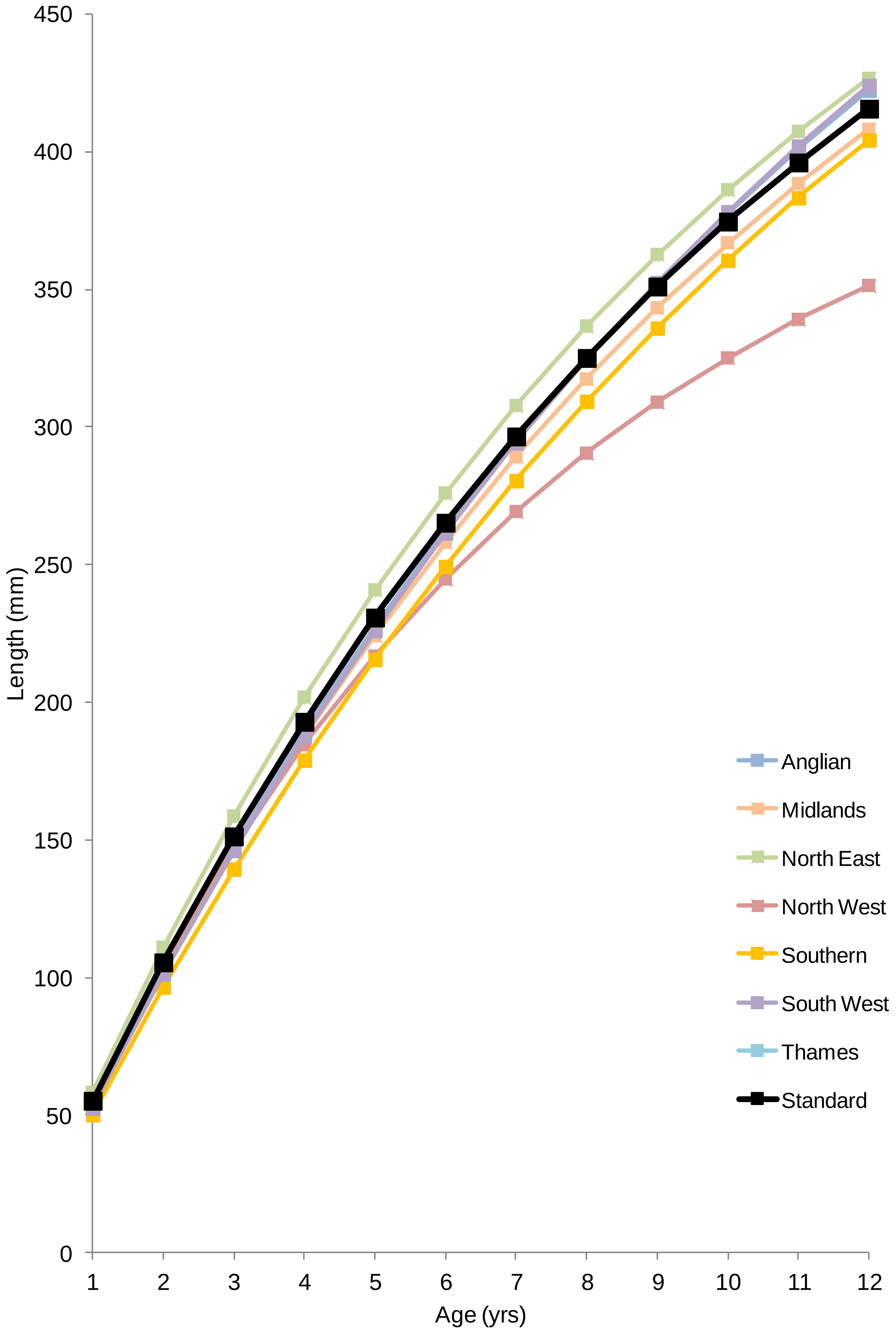

Figure 5.2 Environment Agency regional standard growth curves of *L. cephalus,* based on data for rivers in Table 5.1.

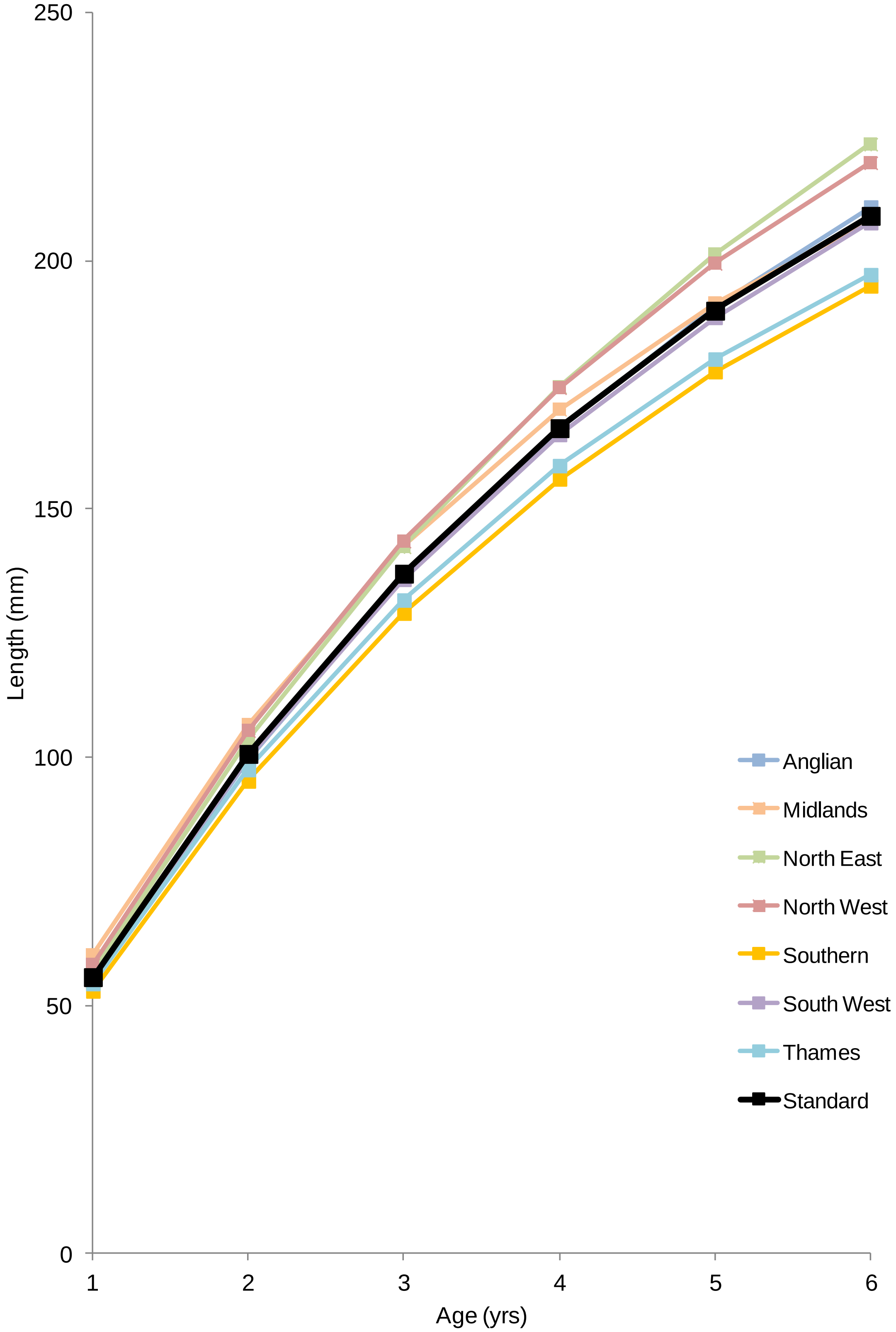

Figure 5.3 Environment Agency regional standard growth curves of *L. leuciscus,* based on data for rivers in Table 5.1.

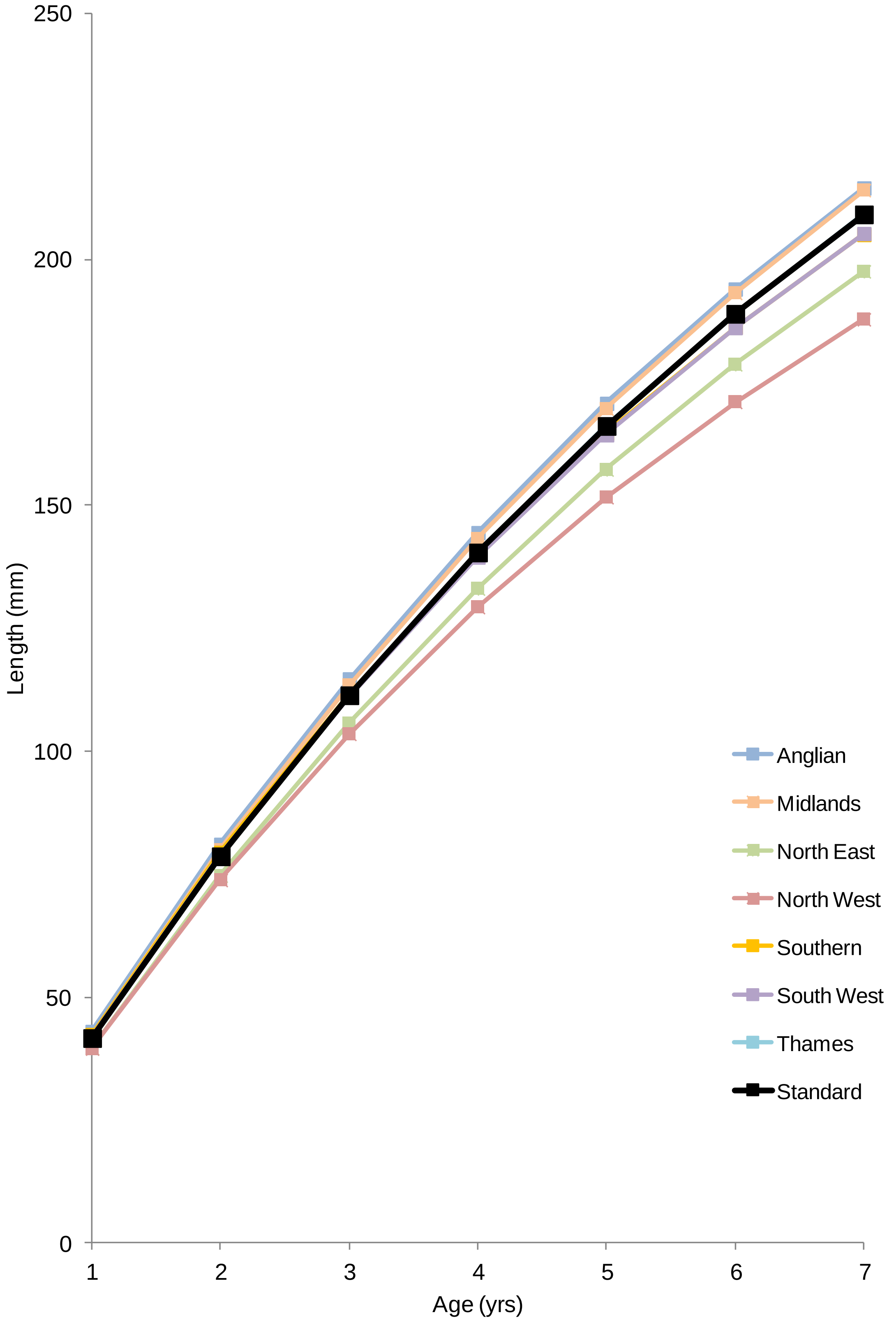

Figure 5.4 Environment Agency regional standard growth curves of *R. rutilus,* based on data for rivers in Table 5.1.

Table 5.2 Parameters of L_∞ (mm) and *k* for constructing length-at-age for each growth curve.

	A. brama			*L. cephalus*			*L. leuciscus*			*R. rutilus*		
	L_∞ (S.D.)	*k*	*K*	L_∞ (S.D.)	*k*	*K*	L_∞ (S.D.)	*k*	*K*	L_∞ (S.D.)	*k*	*K*
Anglian	733 (342)	0.92	0.08	669 (112)	0.92	0.08	302 (66)	0.82	0.20	371 (324)	0.88	0.12
Midlands	553 (161)	0.91	0.10	617 (122)	0.91	0.09	264 (36)	0.77	0.26	382 (156)	0.89	0.12
North West	-	-	-	434 (69)	0.87	0.14	306 (73)	0.81	0.19	297 (53)	0.87	0.14
North East	566 (21)	0.89	0.12	608 (93)	0.90	0.10	331 (73)	0.83	0.22	341 (175)	0.88	0.12
Southern	627 (59)	0.92	0.09	659 (269)	0.92	0.08	264 (66)	0.80	0.21	335 (149)	0.87	0.14
South West	531 (83)	0.91	0.10	692 (101)	0.92	0.08	288 (35)	0.81	0.23	342 (79)	0.88	0.13
Thames	580 (57)	0.89	0.12	613 (66)	0.91	0.09	262 (91)	0.79	0.21	367 (206)	0.89	0.12
National	609 (74)	0.91	0.09	613 (85)	0.91	0.09	289 (55)	0.81	0.21	350 (252)	0.88	0.13

5.3.2 *Comparison of growth curves*

No significant differences (ANCOVA test) were found between the Southern and Midlands growth curves for *A. brama, L. cephalus* and *R. rutilus* (Table 5.3). There was no significant difference in growth curves for *L. leuciscus,* between the North East and North West regions or for *L. cephalus* and *R. rutilus*. There was also no significant between the Thames and Midlands regions for *L. cephalus* and *R. rutilus*.

There were no significant differences of *A. brama* growth curves between Midlands and South West or Southern, or between Thames and North East n regions (Table 5.3). Similarly the Southern region *A. brama, L.cephalus or R. rutilus* growth curves was not significantly different from the national growth curve. There were no significant differences between the growth curves of *L. leuciscus* in the Anglian and North West regions, in the North East and North West regions and in the Southern and Thames regions. There was also no significant difference between the Anglian, Midlands or South West regions and the national growth curve (Table 5.3).

There were no significant differences between the growth curves of *R. rutilus* in the Anglian and Midlands or Midlands and Thames regions, in the Southern and South West regions, Southern and Thames or between the South West and Thames regions. There was also no significant difference between the Midlands, North East, North West, Southern, South West or Thames regions and the national growth curve (Table 5.3).

There were no significant differences between the growth curves of *L. cephalus* from the Anglian region and the North East,South West or Thames, between the North East and South West, between the Midlands region and Southern and Thames regions and the national growth curve (Table 5.3). Comparison of length-at-age 1 from rivers in North East region highlighted that *L. cephalus* in the River Don catchment had a greater length-at-age 1 than other North East rivers; Dearne (F = 17.17, *d.f.* 1,585, $P <$ 0.001), Rother (F = 10.13, *d.f.* 1,576, P = 0.002) and Don (F = 20.95, *d.f.* 1,527, $P <$ 0.001), and Don fish were longer than expected (personal observation of young of year (YOY)).

5.3.3 *Growth parameters and mortality rate*

All four species exhibited a significant and inverse relationship between asymptotic length (L_∞) and growth co-efficient K (r^2 = 0.57-0.76, P < 0.001; Figure 5.5). The negative relationship between instantaneous mortality (Z) rate and growth coefficient was significant for all four species, (r^2 = 0.31-0.56, P < 0.001; Figure 5.6). There were no regional relationships or trends between asymptotic length, growth co-efficient and mortality rate (Figures 5.5 and 5.6).

Table 5.3 ANCOVA of regional growth rates, F values and their significant values, the degree of freedom for all tests was 1.

		Midlands	North East	North West	Southern	South West	Thames	National
A. brama	Anglian	32.222**	0.412	-	24.983**	63.391**	0.863	25.418**
	Midlands		10.589**	-	3.190	1.044	20.250**	7.603**
	North East			-	11.284**	78.697**	0.006	10.493**
	North West				-	-	-	-
	Southern					26.112**	15.960**	0.991
	South West						65.945**	47.233**
	Thames							14.889**
L. cephalus	Anglian	4.383*	3.811	86.038**	12.538**	0.026	0.733	5.833*
	Midlands		16.440**	41.620**	1.134	5.085*	2.700	0.002
	North East			211.995**	35.196**	3.588	12.671**	29.380**
	North West				37.185*	98.118**	134.128**	84.493**
	Southern					14.777**	10.793**	1.711
	South West						1.271	8.096**
	Thames							4.834*
L. leuciscus	Anglian	0.076	6.534*	2.339	10.056**	3.70	6.972*	0.063
	Midlands		5.661*	1.622	12.342**	0.778	9.319**	0.304
	North East			0.074	26.303**	9.923**	23.879**	8.672**
	North West				11.091**	4.231*	8.621**	6.913*
	Southern					7.719**	0.356	12.129**
	South West						4.813*	0.225
	Thames							7.509**
R. rutilus	Anglian	0.480	26.220**	17.566**	6.927**	7.654**	5.138*	16.591**
	Midlands		8.721**	5.803*	1.871	1.995	0.787	2.459
	North East			0.072	3.007	3.115	7.557**	3.927
	North West				1.523	1.611	4.371*	2.276
	Southern					0.000	0.468	0.029
	South West						0.533	0.025
	Thames							1.233

*=P<0.05; **=P<0.01

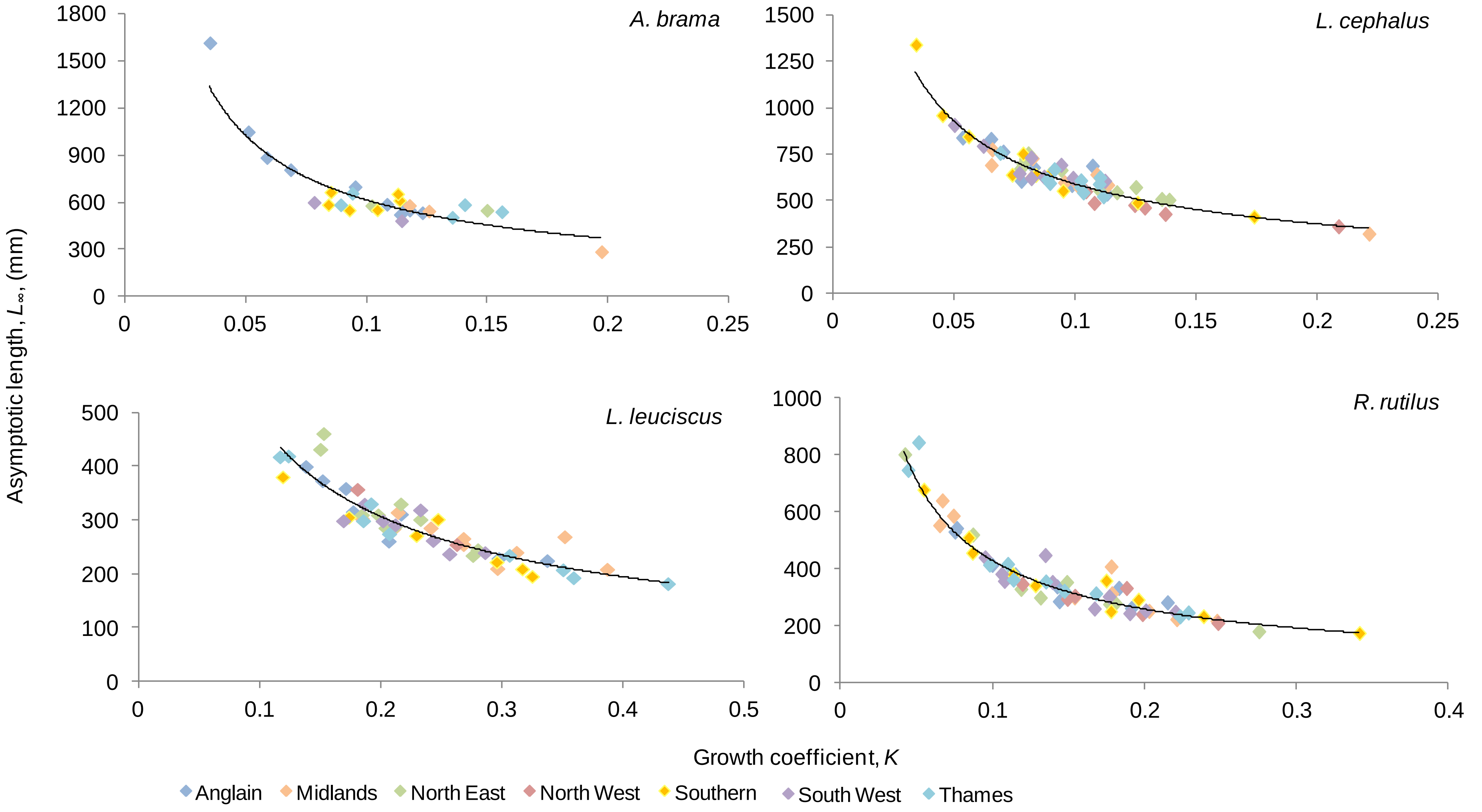

Figure 5.5 Relationship of von Bertalanffy growth model parameters growth co-efficient (*K*) and asymptotic length **(***L*∞) for four species, from different Environment Agency regions.

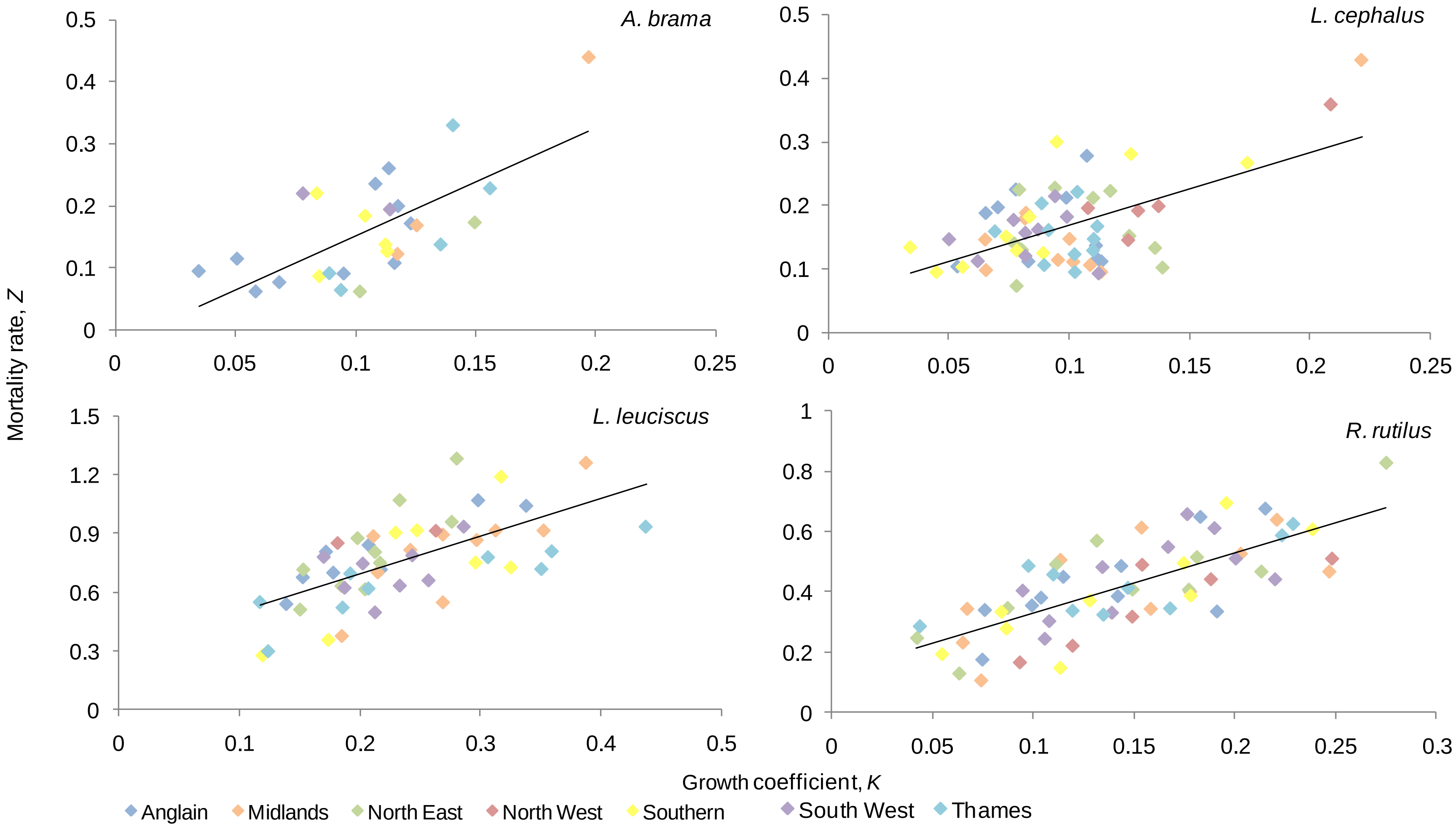

Figure 5.6 Relationship of von Bertalanffy growth model parameter growth co-efficient (*K*) and instantaneous mortality rate (*Z*) for four species, from different Environment Agency regions.

The relative patterns in YCS (Figures 5.7 - 5.10) suggests there are considerable inter-annual and regional variations for *R. rutilus*, *L. leuciscus*, *L. cephalus* and *A. brama*. However, similarities were present between regions for specific years. There appears to be a cyclical trend in recruitment success of *A. brama* and *L. cephalus* (Figures 5.7 and 5.8). The period 1993 - 1997 and 2000 - 2005 stand out as good years for the national recruitment of *A. brama* and *L. cephalus*. This trend may be applicable to *L. leuciscus* and *R. rutilus* (Figures 5.9 and 5.10), although insufficient data are available to confirm the trend. The analysis indicated that the strong year classes of 2002 - 2003 have not been repeated in recent years with recruitment success being weak. Despite recruitment success varying between and within regions for all species, there is strong evidence that some years are exceptional for recruitment irrespective of species or region, one such example is 2003.

Spearman rank correlation was used to test for correlations in *A. brama* YCS between regions and against the national average (Table 5.4). There was generally little correlation in the YCS pattern for *A. brama* between regions other than for Anglian, Southern & Thames. Only recruitment success in the Thames and Anglian regions correlated with the national average, perhaps because these regions contributed most rivers to the National average.

Spearman rank correlation was used to test for correlations in *L. cephalus* YCS between regions and against the national average (Table 5.4). Regional recruitment patterns are unclear for *L. cephalus*, although there are strong correlations between Midlands, Thames, Southern and South West regions. Recruitment success in the North East was only correlated with Thames. All regions, except North West, correlate strongly with the National average.

Spearman rank correlation was used to test for correlations in *L. leuciscus* YCS between regions and against the national average (Table 5.4). The correlation between all regions and National average were strong or very strong for *L. leuciscus*.

Spearman rank correlation was used to test for correlations in *R. rutilus* YCS between regions and against the national average (Table 5.4). For *R. rutilus* most regions have good correlations except for North West. Recruitment success for the *R. rultius* in North West was only correlated to the National average. Similar to *L.* cephalus and *L. leusicus*, there were strong correlations between YCS of *R. rutilus* between Southern, South West and Thames regions. However, similar to *L. cephalus* there were no correlations in recruitment success of *R. rutilus* between North East and North West regions.

Table 5.4 Spearman rank correlation of regional and national YCS for four species, r_s values and their significant values.

		Midlands	North East	North West	Southern	South West	Thames	National
A. brama	Anglian	0.097	-0.005	-	0.551*	-0.030	0.544*	0.850**
	Midlands		0.592*	-	-0.025	-0.080	-0.195	0.177
	North East			-	-0.179	0.211	-0.096	0.081
	North West				-	-	-	-
	Southern					-0.251	0.591**	0.563*
	South West						-0.124	0.092
	Thames							0.838**
L. cephalus	Anglian	0.458	0.046	0.490*	0.445	0.740**	0.811**	0.814**
	Midlands		0.495*	0.529*	0.877**	0.641**	0.625**	0.826**
	North East			0.207	0.381	0.279	0.619**	0.710**
	North West				0.623**	0.323	0.065	0.253
	Southern					0.672**	0.618**	0.777**
	South West						0.723**	0.949**
	Thames							0.860**
L. leuciscus	Anglian	0.595**	0.912**	0.718**	0.629**	0.793**	0.862**	0.773**
	Midlands		0.698**	0.793**	0.878**	0.833**	0.802**	0.902**
	North East			0.791**	0.727**	0.875**	0.865**	0.908**
	North West				0.698**	0.903**	0.943**	0.835**
	Southern					0.838**	0.824**	0.886**
	South West						0.984**	0.894**
	Thames							0.904**
R. rutilus	Anglian	0.736**	0.889**	0.318	0.877**	0.921**	0.921**	0.961**
	Midlands		0.780**	-0.126	0.940**	0.819**	0.643*	0.780**
	North East			0.196	0.899**	0.899**	0.903**	0.914**
	North West				-0.042	0.191	0.292	0.651**
	Southern					0.890**	0.776**	0.890**
	South West						0.895**	0.943**
	Thames							0.952**

* = P<0.05; ** = P<0.01

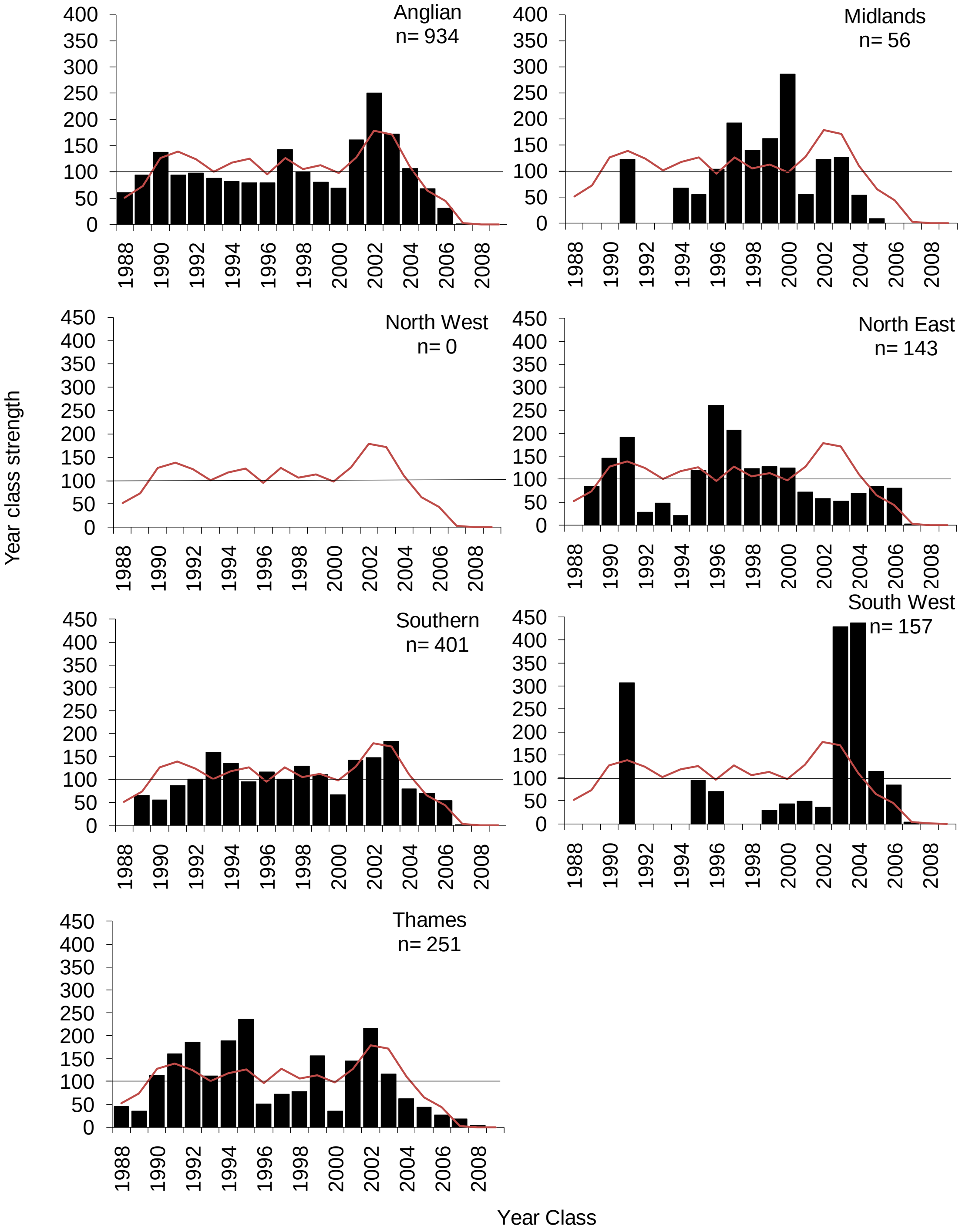

Figure 5.7 Regional *A. brama* recruitment success (YCS), line at 100 indicates standard, other line indicates the national average YCS. Note that data were not necessary available from 1988 for each region, however the first year YCS has been drawn indicates the earliest year data were available.

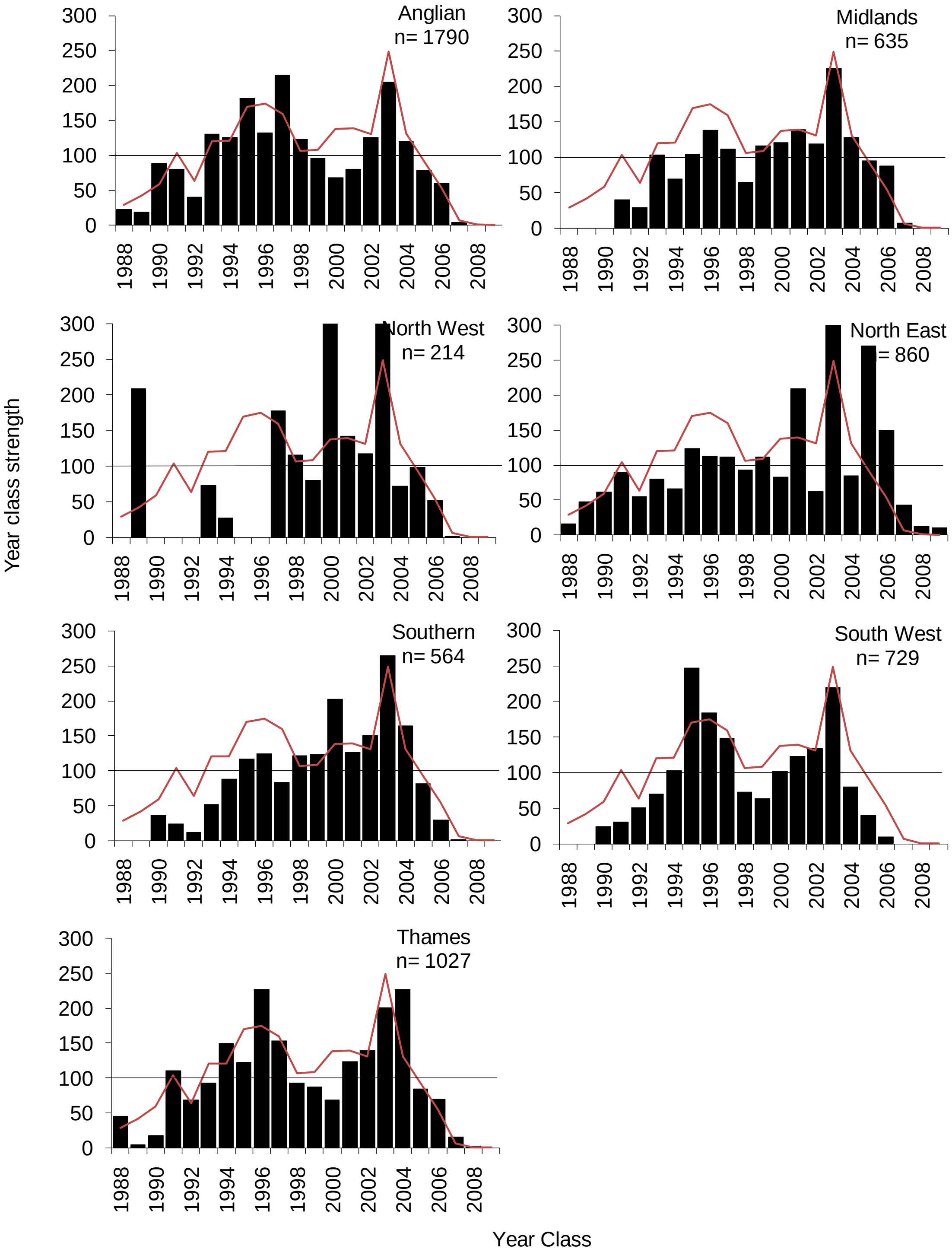

Figure 5.8 Regional *L. cephalus* recruitment success (YCS), line at 100 indicates standard, other line indicates the national average YCS. Note that data were not necessary available from 1988 for each region, however the first year YCS has been drawn indicates the earliest year data were available.

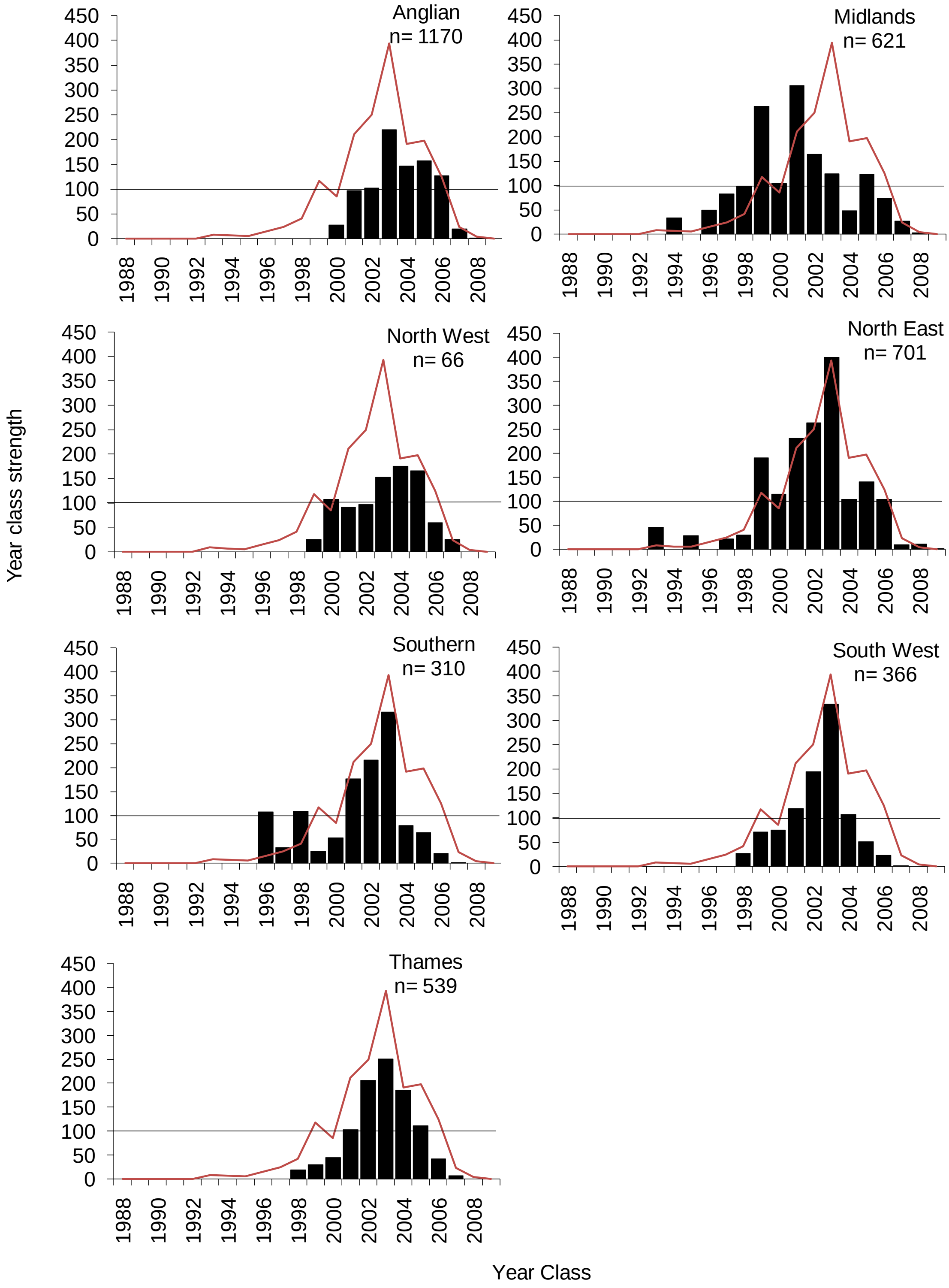

Figure 5.9 Regional *L. leuciscus* recruitment success (YCS), line at 100 indicates standard, other line indicates the national average YCS. Note that data were not necessary available from 1988 for each region, however the first year YCS has been drawn indicates the earliest year data were available.

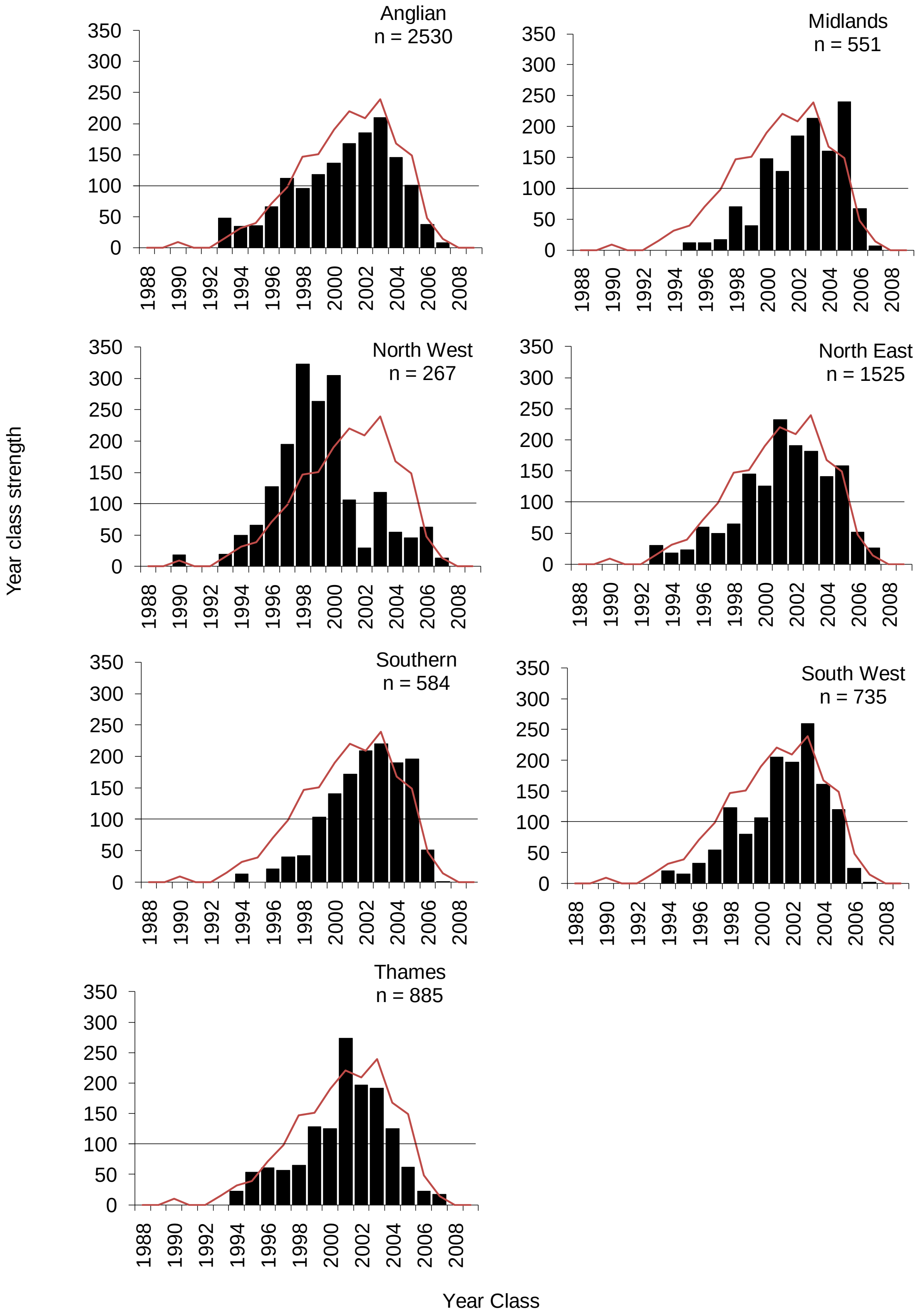

Figure 5.10 Regional *R. rutilus* recruitment success (YCS), line at 100 indicates standard, other line indicates the national average YCS. Note that data were not necessary available from 1988 for each region, however the first year YCS has been drawn indicates the earliest year data were available.

5.3.4 *Suitability of defining growth standards by EA regions*

The suitability of defining growth standards by EA regions was investigated, using a Bray-Curtis similarity matrix (Bray & Curtis, 1957) calculated in PRIMER (Plymouth Routines In Multivariate Ecological Research) statistical package, using the growth parameters length-at-age 1, length at maximum age in 75% of populations, L_∞ and K, and presented as a non-metric MultiDimensional Scaling (MDS) plot to test for an effect of geographic location with grouped average cluster analysis overlaid, at 80% and 95% similarities. There was no clear pattern of regional growth variations (Figure 5.11), specifically a north – south divide.

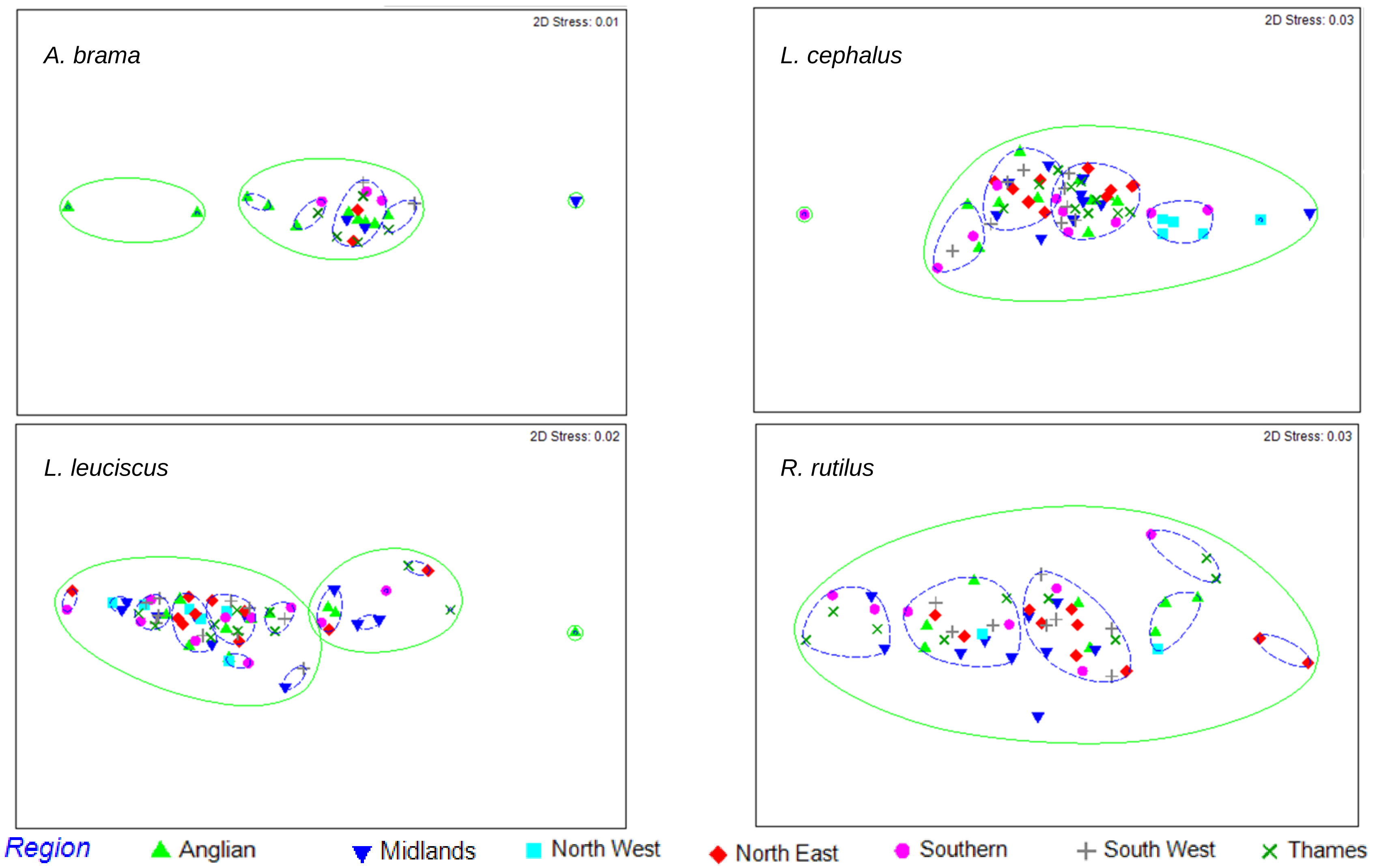

Figure 5.11 Non-metric Multi-Dimensional Scaling (MDS) of Regional growth variables (Length at age 1 and at 75% maximum age, L_∞ and K) with similarity grouped average cluster overlaid, at 80% (green) and 95% (blue dashed) similarities.

5.4 DISCUSSION

5.4.1 *Statistical analysis of growth curves*

The use of graphs to display growth rates is a common practice (Hickley & Dexter, 1979), but there isn't a widely accepted method to compare growth rates statistically. Although the method proposed by Allen (1976) has been used by several authors (Craig, 1980; Maximovich & Guerassimova, 2003), this study has found it to be inappropriate and over complicated; removing too much variance from the data sets, resulting in significance values (P) that are either unrealistically low or high. This study describes a method of statistical analysis, a simple one-way ANCOVA of Walford (1946) plots that can be easily and quickly repeated for multiple data sets. This method can be adapted to test statistically inter and intra specific differences in populations, by comparing multiple individuals or average population Walford (1946) plots of back calculated length data.

5.4.2 *Growth parameters and mortality*

Growth coefficient (K), asymptotic length (L_∞), mortality (Z) are collectively referred to as Beverton-Holt variants (Charnov, 1993), with each parameter being correlated to the other. An inverse relationship exists between asymptotic length (L_∞) and the growth coefficient (K), such that a high rate of growth leads to smaller asymptotic length (see for example Iglesias *et al.*, 1997; MacMillan *et al.*, 2002; Siegfried & Sanso, 2006; Britton, 2007). Mortality is positively related to the growth coefficient, and so in turn asymptotic length. The fecundity of an individual is related to its size; hence a population with a greater asymptotic length will have a greater fecundity, reach sexual maturity at a lower age and have an increased reproductive effort (Morita & Morita, 2002; Vollestad & L'abbe-Lund, 1990). Therefore, fish populations from rivers and/or regions with a greater asymptotic length will have lower mortality and greater reproductive effort than populations with a lower asymptotic length.

5.4.3 *Factors influencing growth*

The factors influencing the growth of fishes are often complex and inter-related, and include temperature (Kempe, 1962; Williams, 1967; Cragg-Hine & Jones, 1969; Broughton & Jones, 1978), density dependence (Burrough & Kennedy, 1979; Tolonen *et al.*, 2003), food composition/availability (Mann *et al.*, 1997), zonation (Cowx, 1988; Cowx, 1989), latitudinal gradient (Braaten & Guy, 2002; Heibo *et al.*, 2005) and sex

(Williams, 1967; Cragg-Hine & Jones, 1969; Mann, 1973). The velocity of a river is another factor that can influence growth (Nunn *et al.*, 2007a), as fish maintain their position in the water column by swimming against a current reducing the energy available for growth (East & Magnan, 1987; Wootton, 1990). Despite the multiple and often inter-linked factors influencing the growth of riverine fishes, temperature, discharge and climate are generally accepted as being the most important (Nunn *et al.,* 2007a; Rollins 2009) and can vary considerably between Environment Agency regions (Orr *et al*., 2010).

5.4.4 *Variations in regional growth*

This study found intra-regional differences in the growth coefficient and asymptotic length of all four species. This could be related to differences in the physical characteristics or topography of the rivers. It is unlikely that the variations were a result of differences in sampling efficiency between years and/or rivers, because, when available, data from several years were combined and a standard sampling protocol was adopted. Therefore, it is unlikely that intra-regional variation adversely affected the regional or national growth curves, but suggests considerable plasticity between fish populations. The reason is thus likely related to the different species occupying habitats that match their optimal environmental requirements and thus achieving the faster growth. This is discussed in relation to fish species environmental requirements and regional variation in these requirements.

Growth rates for a species may alter within a catchment, for example Cowx (1988) found species achieved their fastest growth in zones where the river topography portrayed their 'preferred' habitat characteristics. As conditions depart from the 'preferred' habitat characteristics their numerical dominance and/or growth rate declines (Cowx, 1988). Catchment variations in growth have been shown for various species by numerous authors (Mann, 1973, 1974; Vollestad & L'abbe-Lund, 1990; Oliveira *et al.*, 2002; Abdoli *et al.*, 2007; Copp *et al.*, 2007).

Blanck *et al.* (2007) published data on species traits and habitat preferences, grouping fish species according to their hydraulic, temperature and oxygen level preferences. Using these groups, it is possible to suggest an explanation for the differences in catchment or regional growth rates. *Abramis brama*, an eurythermic species, tolerates low oxygen levels (able to live in habitat where oxygen decreases periodically to 1.5 - 3.0 mg L^{-1}) and prefers deep, fast flowing microhabitats and warm waters (optimal growth temperature between 20 and 28 °C) (Blanck *et al.*, 2007), with a temperature between 12 - 20 °C for reproduction (Cowx, 2001). The asymptotic length of *A. brama* was greatest in the Anglian region. This suggests the environmental requirements and preferred habitats for this species are found in this region, more so than in other regions. This suggestion is further strengthened because Cowx (2001) states *A, brama* prefer lowland reaches; slow flow, deep backwaters, vegetated areas, mud/silt substrate, a description typical of the topography of many of the rivers found within the Anglian region.

The Anglian region had a high asymptotic length for all species. This suggests it may have more optimal conditions that meet the environmental requirements and preferences for all species studied. The absence of sufficient numbers of *A. brama* in the North West region may be due unsuitability of the rivers and natural geographical distribution, as it is predominately a salmonid region.

Similarly, Blanck *et al.* (2007) stated *L. cephalus*, a eurythermic species, requires a high oxygen level (able to live in habitat where oxygen decreases periodically to 2.5 - 3.0 mg L^{-1}) and prefer deep, fast flowing microhabitats and cool waters (optimal growth temperature between 14 and 23 °C). Cowx (2001) states chub require a minimum temperature of 18 - 20 °C for reproduction. The greatest asymptotic lengths of *L. cephalus* were found in the Southern and South West regions. This suggests these regions provide the environmental requirements and preferred habitats for *L. cephalus*, more so than other regions. The Met Office (1971 - 2008) annual average regional temperatures suggest these two regions are warmer than other regions, approximately 2.5 °C warmer than the Northern regions of England.

According to Blanck *et al.* (2007), *L. leuciscus* is a stenothermic species requiring a high oxygen level (able to live in habitat where oxygen decreases periodically to 2.5 - 3.0 mg L^{-1}) and prefer deep, fast flowing microhabitats and cool waters (optimal growth temperature between 14 and 23 °C). The greatest asymptotic lengths of *L. leuciscus* were found in the North East and North West. This suggests the environmental requirements and preferred habitats for this species are found in these regions, more so than in other regions. The Met Office (1971-2008) annual average regional temperatures suggest these two regions are cooler than other regions, approximately 2.5 °C cooler than the southern regions of England. Cowx (2001) also classifies *L. cephalus* and *L. leuciscus* preferring the same habitat but *L. leuciscus* requiring a minimum of temperature of 6 - 9 °C for reproduction, which is considerable less than for *L. cephalus*. Although *L. leuciscus* and *L. cephalus* have similar environmental requirements and habitat preferences, *L. leuciscus* prefer cooler water than *L.*

cephalus, resulting in greater asymptotic lengths for *L. leuciscus* in the most northern regions.

Blanck *et al.* (2007) described *R. rutilus* as an eurythermic species requiring a low oxygen level (able to live in habitat where oxygen decreases periodically to 1.5 - 3.0 mg L^{-1}), similar to *A. brama*, and preferring deep, slow flowing microhabitats and cool waters (optimal growth temperature between 14 and 23 °C). Cowx (2001) states *R. rutilus* require a minimum of temperature of 7 - 21 °C (14 - 18 °C optimal) for reproduction. The greatest asymptotic lengths were found from the Midlands and Thames region, suggesting these two regions provide the environmental requirements and preferred habitats for *R. rutilus*, more so than other regions.

5.4.5 *Ageing error and quality control*

A rigorous quality control method using at least three experienced workers was incorporated into the scale ageing process to minimise ageing errors. However, the length of *L. cephalus* at age 1 in the North East region was higher than expected. Comparison of length-at-age 1 from rivers in this region highlighted that *L. cephalus* in the River Don catchment (rivers Dearne and Rother) had a greater length-at-age 1 than *L. cephalus* in other rivers. There are two possible explanations for this result. Economou *et al.* (1991) noted, scales do not form on *L. cephalus* until fork length is > 15 mm. Therefore it is possible that the first check on *L. cephalus* scales from the rivers Don, Dearne and Rother was either too small to be noticed or missing. Alternatively, Amisah and Cowx (2000) found that the growth of fish in the river Don appears to be better than standard growth rates for fish of all ages, with the exception of *T. thymallus*, because the river is highly productive and fish densities relatively low. This suggests there may not be an ageing error and the growth curves are a true reflection of the rivers at the time of sampling.

5.4.6 *Variations in recruitment success*

A wide range of biotic (e.g. competition, predation, disease) and abiotic (e.g. physicochemical, physical habitat and water chemistry) factors influence recruitment success. Likewise, water temperature, discharge and the position of the NWGS have been found to strongly influence the recruitment success and growth parameters of cyprinid fish populations within English rivers (Nunn *et al.*, 2007a). In some rivers, river discharge (rather than water temperature) may be the key factor that determines YCS (Nunn *et al.*, 2007a). Although these factors account for a great deal of the variance, other factors such as parasite loading (Longshaw *et al.*, 2010) and available food resources (Mills & Mann, 1985) will play an important role. Cowx (2001) suggested years in which a strong year class is prevalent in one species do not necessarily result in strong year classes in other coexisting species. Occasionally, specific years appear to be good years for recruitment irrespective of species or region, for example 2003. Likewise, YCS and growth differed from the National standards for *L.* cephalus from the North West region and for *A. brama* from the North West, Midlands, North East and Southern regions. Suggesting species from regions which have different recruitment patterns from the national standard may also have different growth from the national standard. However, it is possible that recruitment success derived for *L. leuciscus* and *R. rutilus* may not be a true representation, because the recruitment patterns appear to have been influenced by an abundance of surveys between 2003 and 2007. Although, the method described by Cowx and Frear (2004) has been used in successfully in several studies (Bolland *et al.*, 2007; Nunn *et al*., 2007a; Rollins 2009), therefore it is anticipated that the results in this study are correct.

5.4.7 *Suitability of defining growth standards by EA regions*

There was no clear pattern of regional growth variations, specifically a north – south divide for all four species, suggesting predicting expected growth of riverine fish may be more complex than geographic region. It is anticipated that the addition of variables such as altitude, latitude, longitude, river length and width would provide a better separation of growth characteristics and allow the development of model capable of predicting more accurate growth standards. However, until such information is ready available for each sampling location and/or river, the use of EA regions to define growth standard does provide an additional assessment which maybe more suitable than a national standard.

5.5 CONCLUSIONS

This study has found that the geographic location of a river/region influences the growth rates of freshwater fishes commonly found in England, highlighting the plasticity of cyprinid fish species between neighbouring Environment Agency regions. Numerous authors have shown geographical location and environmental conditions of a river can influence the growth of fish species. This study has shown environmental variables influence the growth of fish populations and provides evidence for the importance of regional growth curves. The method of constructing regional growth curves and subsequent statistical analysis, discussed in this study, should be adopted by fisheries scientists because current national growth rates may be unachievable in specific regions. Furthermore, current national curves are inappropriate for growth and population analysis because they may be biased by an individual river and/or region. This study has addressed this issue and provided more accurate national growth curves. This study is one of few to examine the differences between regional recruitment success, and found similarities and differences at both the regional and national level. With recruitment success a key requirement of monitoring fish populations under the WFD, it is hoped the information provided here will help fisheries scientists to understand the factors affecting regional and national recruitment success.

6 WILL CLIMATE CHANGE INFLUENCE CYPRINID FISH POPULATION DYNAMICS IN RIVERS?

6.1 INTRODUCTION

Climate change is a global phenomenon. The average air temperature of the Earth has increased by 0.06 °C per decade over the last century (Intergovernmental Panel on Climate Change, IPCC, 2009; scientifically referenced as Jenkins *et al.*, 2009) and Europe has been referred to as the cauldron of climate change. Predicted changes in temperature and precipitation (IPCC, 2009) are expected to result in all areas of the UK becoming warmer, more so in summer than in winter, and precipitation decreasing during summer and increasing during winter. These predicted changes in climate will undoubtedly have a major influence on the terrestrial and aquatic ecosystems.

There is increasing evidence that global climate change is already having measurable biological impacts (Daufresne *et al.*, 2003). Numerous literature reviews and ecological studies have been published suggesting possible effects of climate change. However, there is a definite need for more predictive studies, providing a greater scientific insight. Such studies have already been published for various taxa, including; bats (Humphries *et al.*, 2002), insects (Samways *et al.*, 1999; Ungerer *et al.*, 1999; Fleishman *et al.*, 2001; Kerr, 2001; Oberhauser & Peterson, 2003; Crozier & Dwyer, 2006), birds (Sæther *et al.*, 2004; Gordo *et al.*, 2005; Lemoine *et al.*, 2007; Barbraud *et al.*, 2011), coral reef fish (Munday *et al.*, 2008), lizards (Chamaille-Jammes *et al.*, 2006), marine mammals (Bluhm & Gradinger, 2008) and freshwater fishes (Graham & Harrod, 2009).

Changes in precipitation and temperature regimes or patterns have significant effects on the distribution and abundance of plants and animals (Hauer *et al.*, 1997). Ectothermic animals, such as riverine fish (specifically poikilothermic fish), are an ideal study organism for climate studies, because temperature and flow govern their biological processes and behaviour. Combined with the inability to relocate outside of their drainage basin, riverine fish species are highly vulnerable to broad-scale environmental changes (Grant *et al.*, 2007; Buisson *et al.*, 2008). Furthermore, fish are key predators in the majority of aquatic ecosystems and, as such, fluctuations in their population dynamics can have implications for ecosystem functioning as a whole (Nunn *et al.*, 2007a).

Studies on the impact of climate change on fish populations have typically focused on predicting the effects on lentic species (Casselman, 2002; Casselman *et al.*, 2002; Andersen *et al.*, 2006; Sharma *et al.*, 2007; Suski & Ridgway, 2007; Ellis *et al.*, 2008; Venturelli *et al.*, 2010) rather than lotic species (Daufresne *et al.*, 2003; Daufresne & Boët, 2007; Buisson *et al.*, 2008). Furthermore, these studies often deal with American rather than European ecosystems. Studies that consider the effects on lotic species have addressed the issue of temperature changes, but generally neglected the influence of flow. Consequently, there is a need to predict the impact of changes in temperature, flow and climate on lotic fish populations.

The aim of this study was to determine whether the predicted climate changes in the UK (IPCC, 2009) are likely to influence fish populations, expressed as the length young-of-year (YOY) fish achieved by the end of the first growth period (May-September), juvenile and adult growth (annual growth increment, AGI) and recruitment success (year class strength, YCS), for three cyprinid fish species from three different temperature guilds (cold, cool and warm), i.e. *L. leuciscus*, *L. cephalus* and *R. rutilus* respectively.

6.2 MATERIALS AND METHODS

6.2.1 *Study rivers and sampling*

This study used fish scales collected during routine monitoring of fish populations conducted by the Environment Agency (EA) to calculate population dynamics of *L. cephalus, L. leuciscus* and *R. rutilus* from the rivers Don, Great Ouse, Lee and Warwickshire Avon. These rivers were selected to model the influence of climate change because they are either major tributaries or the parent river of the four longest rivers within England (Figure 6.1; Table 6.1).

Sampling was carried out by electric fishing from a boat or wading, depending upon the most appropriate method for the river and conditions. All sampling was completed between May and September. The rivers Don, Great Ouse and Lee were sampled in 2006, 2007 and 2008, while the River Avon was sampled in 2003, 2004 and 2006. All samples were combined for each river. Fish were measured (fork length, L_F, mm) and a sample of scales taken from the shoulder region, below the anterior insertion of the dorsal fin, before they were returned to the river.

Table 6.1 Characteristics of study rivers.

River	Catchment	Parameters			
		Elevation (m)	Length (km)	Catchment area (km^2)	Gradient (m/km)
Don	Yorkshire Ouse	485	114	1849	4.25
Great Ouse	Great Ouse	115	230	3400	0.5
Lee	Thames	115	68	1420	1.69
Warwickshire Avon	Severn	150	137	2670	1.09

6.2.2 *Back-calculation and length-at-age 1*

Fish scales were examined under a projection microscope with a 20x magnification. To minimise errors in ageing of scales, a quality control procedure was followed as described in Musk *et al.* (2006), where a secondary reader checked 10% of the aged scales. Where disagreement was found, the scale was reviewed to enable consensus to be reached. In addition to Musk *et al.* (2006), a second opinion was sought for any scales which proved difficult for the primary scale reader, outside of the quality control procedure. Scale sample data sets for each river catchment were combined, and fish lengths (L_t) for age t were back-calculated using the Hile (1941) method, as described by Francis (1990):

$$L_t = -(a/k) + (L_F + a/k)(S_i/S_r) \quad \text{(equation 10)}$$

where L_F is fish length (mm), S_i scale increment, S_r scale radius and a/k where a and k are the intercept and slope of the regression between S_r and L_F, respectively.

6.2.3 *Annual growth increment*

Annual growth increment (AGI) was calculated according to Kempe (1962), using back-calculated length-at-age data for each river. The average length increment at each age, I, II, III . . . X, was calculated and used as a standard. The growth of each year class in each year was then calculated as a percentage of this standard. The mean growth rate in each year was calculated as a mean of these percentages for each age (Mann, 1973).

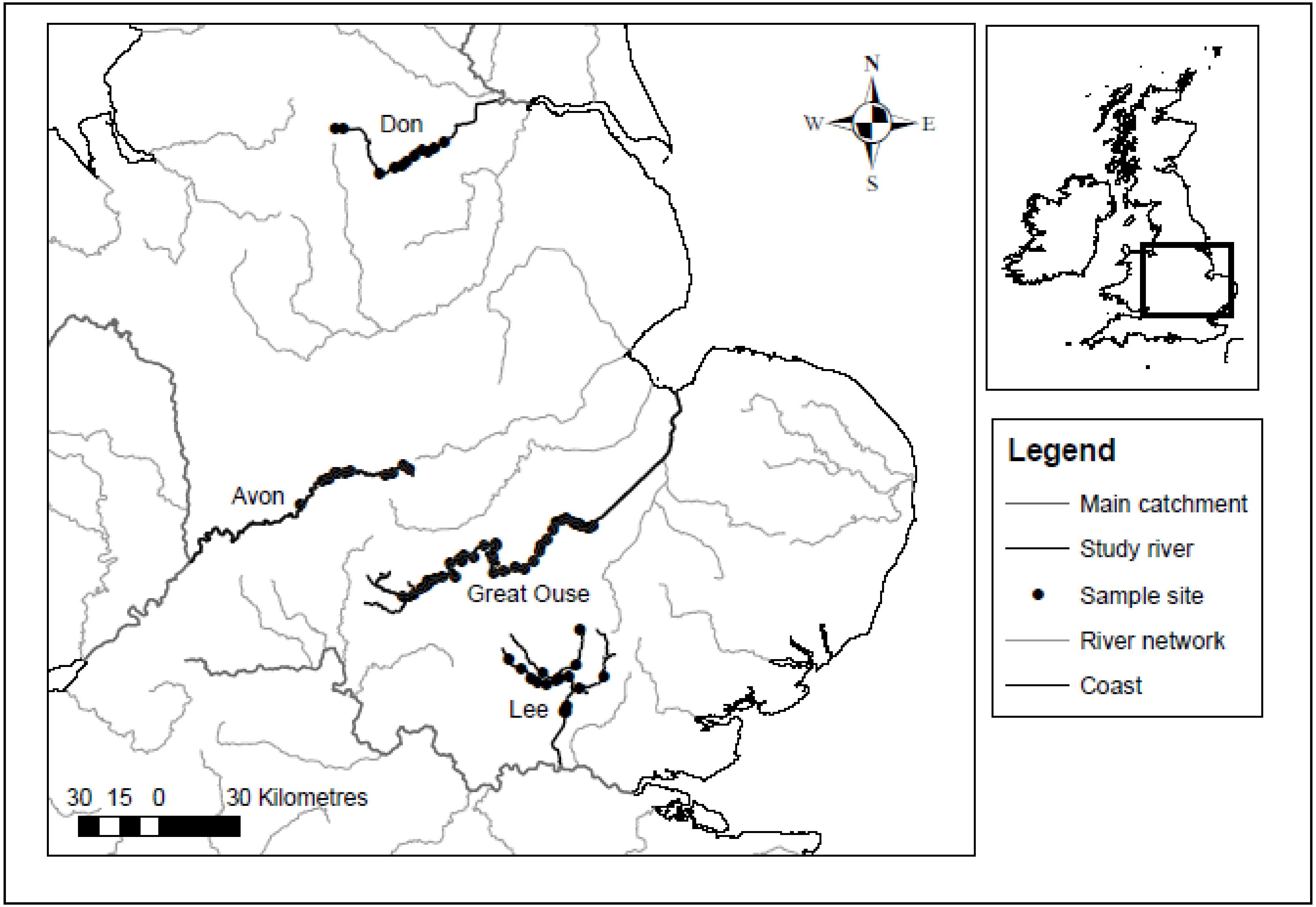

Figure 6.1 Geographic location of the sample sites and study rivers.

6.2.4 *Recruitment success*

To assess the possible influence of climate change on recruitment success (YCS). Year class strength was calculated for each river according to Cowx and Frear (2004):

(1) The number of fish in each cohort was used to determine the instantaneous mortality rate (Z) of the target population using the linear relationship between the natural logarithm of the number of fish in each age group ($\ln N_t$) against age (t) according to;

$Z = \ln N_0 - \ln N_t$ (equation 11)

(2) The number of fish at time zero (N_0) was calculated independently for each age group using

$N_0 = N_t / \exp(Z_t)$, (equation 12)

where N_t is the number of fish at age t;

(3) The mean number of fish in each age group (N) was determined as

$\Sigma^{tmax} N / t_{max}$ (equation 13)

where t_{max} is the total number of age groups represented;

(4) The YCS for each age group was determined by YCS at age

$t = (N_0 / \bar{N}) \times 100$ (equation 14)

where N_0 is the number of fish determined at time t_0 for age group t, i.e. YCS, which is then related to the year of birth, is calculated as an index standardised against a value of 100. A value greater than 100 is a strong year class and a lower value a weak year class.

6.2.5 *Water temperature*

Mean daily water temperature data for the rivers Avon, Don and Lee (1989-2007 inclusive) were obtained from the Environment Agency and for the Great Ouse from the Centre for

Ecology and Hydrology (1989 to 1993 inclusive). Where water temperature data was missing for specific periods on specific rivers, it was estimated from a direct relationship with the River Avon (Table 5.2), which had the most complete data set of daily mean average temperature for all years.

Table 6.2 Linear regression relationships between water temperature (cumulative degree-days >12 °C, April-September inclusive) in the rivers W. Avon, Don, Great Ouse and Lee, where n equals the total number of individuals from each of the four rivers.

Linear regression	***P***	***r^2***	**N**
Lee = W. Avon (0.86) + 4.244	<0.001	0.810	94
Don = W. Avon (0.788) + 2.652	<0.001	0.804	226
Great Ouse = W. Avon (0.889) + 5.02	<0.001	0.890	214

Growth was presumed to occur at temperatures greater than 12 °C (April-September inclusive) (Mann, 1973; Mann, 1976; Mills & Mann, 1985; Britton *et al.*, 2004a; Nunn *et al.*, 2007a). Temperature was expressed as cumulative sum of degree days for each month, over a 19-year period (1989-2007 inclusive). Cumulative degree days were calculated as:

$$D_d = \sum_{i=1}^{d} (T_t - T_{th}), T_t \geq T_{th} \qquad \text{(equation 15)}$$

where *i* the first day that temperature (*T*) exceeded the analysed threshold T_{th} (12 °C), *d* was the last day that temperature (*T*) exceeded T_{th}.

A further temperature parameter tested in the models was the difference in cumulative degree days (°C) greater than 12 °C between months, i.e. the degree days (°C) difference from April to May, May to June, and so on. This parameter will be referred to as cumulative degree day increment (TI).

6.2.6 *River discharge*

Mean daily river discharge data (1989-2008 inclusive) were obtained from the EA gauging stations at Evesham (W. Avon), North Bridge (Don), Feildes Weir (Lee) and Lees Brook (Great Ouse). These data were used to calculate 50, 70 and 90 percentile flow values (Q_{50},

Q_{70} and Q_{90}) and the cumulative sum of discharge days above the basal discharge rate (i.e. the long-term mean daily discharge calculated for each month using the 19-year data set) from April to September. Cumulative discharge days were calculated as:

$$D_d = \sum_{i=1}^{d} (D_t - D_{th}), D_t \geq D$$

(equation 16)

Where *i* the first day that discharge (*D*) exceeded the monthly basal rate (D_{th}), *d* was the last day that discharge (*D*) exceeded D_{th}. Cumulative discharge days will be referred to as (F), a further parameter was the logarithm of flow, which will be referred to as (LF).

6.2.7 *North Wall Gulf Stream*

The monthly position of the NWGS (1989-2008 inclusive) was obtained from the Plymouth Marine Laboratory, UK (http://www.pml.ac.uk/gulfstream/data.htm). A positive value indicates a displacement of the NWGS to the north of the long-term mean location, while a negative value indicates a movement south.

6.2.8 *Modelling*

The primary aim was to relate fish population variables with river temperatures, discharges and position. The analysis was established at two levels: annual and monthly.

At the annual level, multiple regression models were created for each fish population parameter (Length-at-age 1, AGI, YCS) using three explanatory variables: cumulative degree days (T), cumulative discharge days (F) and annual position of North Wall of the Gulf Stream (NWGS).

At the monthly level, explanatory variables were further divided; cumulative degree days (T), cumulative degree days increment (TI), cumulative discharge days (C), cumulative discharge days logarithm (LF) (Naperian log transformed) and NWGS position were used as monthly variables for April, May, June, July, August and September (i.e. for cumulative degree days T^{April}, T^{May}, T^{June}, T^{July}, T^{August}, $T^{September}$).

All models were created using the same technique in Statgraphics v5.0. Statistical descriptive analysis was performed, with special attention paid to testing the normality (Skewness and Kurtosis analysis) and testing for significant correlation between variables.

Regression tools were used to identify the best General Linear Model (GLM); r^2, r^2-ajusted and Cp Mallows' coefficient. These coefficients were created for all possible models and helped to follow how the addition of new significant variables influenced their goodness-of-fit scores (Mallows' Cp coefficient), which is calculated as follows:

If p regressors are selected from a set of q, then:

$$Cp = \frac{SSE_p}{S^2} - N + 2P \qquad \text{(equation 17)}$$

where $SEEp$ is the error sum of squares for the model with p regressors, S^2 the residual mean square after regression on the complete set of q and N the sample size.

Cp Mallow's coefficient is essentially a special case of Akaike (AIC) Information Criterion and is commonly used in GLM as the criterion for choosing the best subset of predictor variables when a best subset regression analysis is being performed, i.e. best model is the one with the minimum or an acceptably small value of Cp Mallows' coefficient. This measure of the quality of fit for a model tends to be less dependent (than r^2) on the number of effects in the model, and hence, it tends to find the best subset that includes only the important predictors of the respective dependent variable.

The best models, those finally selected, were not influenced by the order variables were entered, because a combinational procedure makes all possible models from a set of independent variables. The final step was to confirm the robustness of the best models, by obtaining the same best models through back-stepwise regression. A back-stepwise regression selects variables based on the significance level of the regression coefficient using Mallows' coefficient and r^2-ajusted. Using back-stepwise regression all parameters of models were calculated (regression coefficients and their standard error, residual analysis, Durbin-Watson autocorrelation test) and the autocorrelation among residuals and between them and variables was estimated.

6.2.9 *Climate change predictions and Monte Carlo simulation*

Summer temperature and precipitation predictions for Central England were used as a representative value for the whole of the UK to predict the impact of climate change. The IPCC (2009) predicted a 10% probability of a 2 °C rise in summer temperature and 50% decrease in summer precipitation, a 4 °C rise in summer temperature and 30% decrease in summer precipitation at a 50% probability, and a 6 °C rise in summer temperature and a 10% increase in summer precipitation at a 90% probability. The IPCC (2009) report stated that the central estimates at the 50% probability level are likely to be exceeded, while those at the 10 and 90% are unlikely to be exceeded. These predicted changes in UK climate were used to manipulate the original data sets, e.g. a 2 °C rise, resulted in the addition of 2 °C to original temperature data sets and the number of degree days recalculated. These new variables were then substituted into the appropriate models.

A Monte Carlo simulation was used to calculate the total error of the final predictive models, based on the generation of multiple random numbers to mimic a statistical population. The first step of the Monte Carlo simulation was to generate random values for each of the explanatory variables. Assuming uniform distribution, it was possible to generate random numbers and then multiple these by the range of each variable. The range is the difference between the minimum and maximum value. Median and standard deviations were calculated for 1000 iterations, subsequently allowing the total error for each model to be calculated.

6.3 RESULTS

6.3.1 *Model Validity*

At the annual level, 8 models were tested for each fish population parameter (YCS, Length-at-age 1, AGI). However, in monthly level, the number of general linear models (GLM) tested was 270,464 for each fish population parameter (length-at-age 1, AGI and YCS). Although the sample data sets are quite small, potentially reducing the accuracy of predictions, the information obtained through the multiple regression models is presumed to be ecologically relevant because the final explanatory variables chosen by the models had biological as well as statistical meaning.

Homogeneity among rivers was tested by ANOVA; the only significant difference was between length-at-age 1 and AGI of *L. cephalus*. Any potential influence of pseudo-replication was presumed to be theoretical, because this is a retrospective study made by linear regression analysis without an experimental design plot, in which the variable river was not included as a factor. In addition, sampling was during different days, temperatures, discharges and distances to NWGS position. According to basic hypothesis of linear regression models, there cannot be a correlation between variables (autocorrelation); in one case out of nine, a significant correlation was found between explanatory variables and residuals (AGI, LF4 , r = -0.293). The residual independency of data was tested using Durbin-Watson (DW) for the best models, and only the length-at-age 1 for *L. leuciscus* model had a significant residual autocorrelation (DW = 2.61, $P < 0.01$).

Hydrological variables are highly correlated because of temporal patterns of seasons. A common way to avoid this is through time-series theory (eliminate the seasonal effects, differentiation of data, smoothing). This was investigated, but further problems were encountered when attempting to create new coefficients (ARMA, ARIMA coefficients); because only one value per year was available for some parameters, while data sets were incomplete or over a short time period for other parameters. Consequently, a significant dimensional reduction was not provided by Principal Component Analysis and no advantages were found by applying the method.

Multi-colinearity appeared in many models, increasing with the number of variables considered in the model and usually between variables from the same month. Conversely, the less monthly variables considered, the lower multi-colinearity, but also the lower variability explained. Multi-colinearity was accepted to identify which monthly variables influence annual dependent variables. Colinearity can affect models by increasing standard error of regression coefficients and hence the stability of them; as a way to show the stability of models standard error of regression coefficient is included in Tables 6.3 to 6.5.

Some attempts were made to mitigate multi-colinearity by selecting the most efficient variables. From the time series analysis reasonable differentiation was found between cumulative degree days increments (TI) and cumulative degree days (T). Auto-correlation was investigated among monthly variables. Cumulative degree days increment was better than cumulative degree days because TI presented more significant independent months. Nevertheless, the nine models finally selected had Variance Inflation Factors (V.I.F.) less than 10 (Tables 6.3 to 6.5), which is a standard threshold for allowing multi-colinearity (Belsley *et al.*, 1980; Myers, 1990).

Homogeneity was tested using ANOVA; none of the three variables were significantly different for *R. rutilus* (YCS: *F* = 0.96, d.f. = 3,33, *P* = 0.424), (Length-at-age 1: *F* = 2.29, d.f. = 3,33, *P* = 0.09), (AGI: *F* = 0.32, d.f. = 3,33, *P* = 0.81) or *L. leuciscus* (YCS: *F* = 1.43, d.f.= 3,33, *P*= 0.251), (Length-at-age 1: *F* = 2.62, d.f. = 3,33, *P* = 0.067), (AGI: *F* = 0.14, d.f. = 3,33, *P* = 0.938), but there were significant differences in *L. cephalus* fish length-at-age 1 (*F* = 18.40, d.f. = 3,43, *P* = 0.001) and AGI (*F* = 6.71, d.f. = 3,43, *P* = 0.001) but not for YCS (*F* = 0.03, d.f.= 3,43, *P* = 0.992).

All multiple regression models were statistically significant with r^2 ranging between 0.231 for *R. rutilus* YCS and 0.815 for *L. leuciscus* length-at-age 1 (Tables 6.3 to 6.5). The most commonly included explanatory variables, included in the best models, were derived from the position of the NWGS (number of occurrences, 18), cumulative degree-day increment (number of occurrences, 17), cumulative discharge days (number of occurrences, 16) and cumulative temperature degree days (number of occurrences, 1).

6.3.2 *Environment variables and climate change predictions*

The NWGS had the greatest influence on length-at-age 1, AGI and YCS of the three species, highlighting the important role overall climate drivers, for which NWGS is a surrogate, have on fish population dynamics. Unexpectedly, flow was not an important monthly variable for YCS of *L. leuciscus* and *L. cephalus* despite these being rheophilic. Cumulative degree-day increment played an important role in determining length-at-age 1 of all three species and YCS and AGI of *L. cephalus* and *R. Rutilus.* No consistent relationships were found between species and monthly flow variables, although high flow in the early summer tended to affect AGI of *L. cephalus* negatively, while flow negatively influenced AGI of *L. leuciscus* in late summer (August and September).

The Intergovernmental Panel on Climate Change (2009) predictions were first applied to temperature and flow variables independently then cumulatively (Table 6.6). Changes in flow negatively influenced the length-at-age 1 of all three species at all three probabilities. However, negative effects were reduced by the predicted increase in temperature. This is apparent for all *L. leuciscus* models, but not *L. cephalus* or *R. rutilus* at 10% or 50% probability levels. Overall there were more negative or negligible effects as a result of a change in flow than positive. By contrast, there were more positive effects of temperature than negative or negligible effects. Interestingly *R. rutilus* (warm temperature guild) were more negatively influenced by increases in temperature than *L. leuciscus* and *L. cephalus*, cold and cool guilds respectively. Monte Carlo simulations showed the total error for each predicted model was less than 5%, except *R. rutilus* YCS (less than 30%), which further confirms the robustness and accuracy of the final models.

Table 6.3 Length-at-age 1 models for *L. cephalus*, *L. leuciscus* and *R. rutilus*. Cumulative degree-day increment (TI), cumulative discharge (F), Naperian logarithm of cumulative discharge (LF), standard error (S.E.), variance inflation factor (V.I.F.), squared correlation coefficient (r^2).

Species	Parameter	Estimate	S.E.	V.I.F.	*P* value
L. cephalus	CONSTANT	40.622	1.432		<0.001
(r^2 = 0.539)	TI$^{\text{May}}$*F$^{\text{May}}$	0.0002	$6.61*10^{-5}$	1.263	<0.001
	TI$^{\text{June}}$*F$^{\text{June}}$	0.0001	$5.81*10^{-5}$	1.052	<0.051
	TI$^{\text{September}}$*F$^{\text{September}}$	-0.0003	$8.50*10^{-5}$	1.245	<0.004
	NWGS$^{\text{April}}$	-2.264	0.754	1.421	<0.005
	NWGS$^{\text{July}}$	2.209	0.745	2.075	<0.005
	NWGS$^{\text{August}}$	-2.919	1.142	1.912	<0.015
L. leuciscus	CONSTANT	29.080	2.220		<0.001
(r^2 = 0.815)	TI$^{\text{May}}$	-2.072	0.485	5.958	<0.001
	TI$^{\text{July}}$	4.862	0.655	3.124	<0.001
	TI$^{\text{September}}$	-4.862	0.871	9.558	<0.001
	LF$^{\text{July}}$	1.755	0.659	1.298	<0.017
	NWGS$^{\text{May}}$	-2.472	0.621	2.255	<0.002
	NWGS$^{\text{July}}$	-2.800	0.541	7.441	<0.001
	NWGS$^{\text{September}}$	4.695	1.011	9.760	<0.001
R. rutilus	CONSTANT	51.078	1.950		<0.001
(r^2 = 0.585)	TI$^{\text{September}}$	0.089	0.017	1.484	<0.001
	LF$^{\text{April}}$	-0.369	0.125	1.281	<0.007
	LF$^{\text{May}}$	0.516	0.170	1.286	<0.006
	NWGS$^{\text{April}}$	1.672	0.448	1.509	<0.005

Table 6.4 Annual Growth Increment models for *L. cephalus*, *L. leuciscus* and *R. rutilus*. Cumulative degree-day increment (TI), cumulative discharge (F), Naperian logarithm of cumulative discharge (LF), standard error (S.E.), variance inflation factor (V.I.F.), model squared correlation coefficient (r^2).

Species	Parameter	Estimate	S.E.	V.I.F.	*P* value
L. cephalus	CONSTANT	66.181	13.377		<0.001
(r^2 = 0.504)	TI^{May}	0.092	0.045	2.415	<0.049
	TI^{June}	0.107	0.045	8.813	<0.024
	TI^{July}	0.123	0.060	9.696	<0.047
	$\text{TI}^{\text{September}}$	-0.174	0.052	3.262	<0.002
	LF^{April}	-1.462	0.399	1.270	<0.001
	LF^{May}	-1.091	0.386	1.518	<0.008
	NWGS^{May}	-6.440	1.968	4.457	<0.002
	$\text{NWGS}^{\text{June}}$	5.059	2.117	3.215	<0.022
	$\text{NWGS}^{\text{July}}$	-3.108	1.175	3.395	<0.012
	$\text{NWGS}^{\text{August}}$	8.158	2.514	6.091	<0.003
L. leuciscus	CONSTANT	102.724	1.523		<0.001
(r^2 = 0.626)	$\text{Flow}^{\text{April}}$	0.350	0.128	1.414	<0.013
	$\text{Flow}^{\text{August}}$	-0.495	0.214	1.214	<0.032
	$\text{Flow}^{\text{September}}$	-0.629	0.142	1.496	<0.001
R. rutilus	CONSTANT	100.210	1.008		<0.001
(r^2 = 0.438)	$\text{TI}^{\text{August}}$	0.066	0.020	1.010	<0.002
	$\text{LF4}^{\text{April}}$	0.434	0.172	1.254	<0.017
	LF5^{May}	-0.618	`0.237	1.287	<0.014
	NWGS^{May}	1.968	0.707	1.287	<0.009

Table 6.5 Year Class Strength models for *L. cephalus*, *L. leuciscus* and *R. rutilus*. Cumulative degree-day increment (TI), cumulative discharge (F), Naperian logarithm of cumulative discharge (LF), Standard error (S.E.), variance inflation factor (V.I.F.), model squared correlation coefficient (r^2).

Species	Parameter	Estimate	S.E.	*V.I.F.*	*P* value
L. cephalus	CONSTANT	46.389	29.273		<0.121
(r^2 = 0.527)	TI^{June}	0.518	0.200	3.697	<0.014
	TI^{July}	0.864	0.263	4.022	<0.002
	$NWGS^{April}$	50.790	9.402	3.125	<0.001
	$NWGS^{May}$	-76.599	13.271	4.364	<0.001
	$NWGS^{August}$	32.300	13.410	3.734	<0.021
	$NWGS^{September}$	-23.520	10.254	2.635	<0.027
L. leuciscus	CONSTANT	-58.369	63.841		<0.037
(r^2 = 0.422)	T^{June}	11.091	2.632	1.294	<0.016
	$NWGS^{June}$	21.504	10.055	2.559	<0.045
	$NWGS^{September}$	-16.847	6.713	2.323	<0.021
R. rutilus	CONSTANT	75.466	8.837		<0.001
(r^2 = 0.231)	$TI^{July}*F^{July}$	0.007	$2.7*10^{-3}$	5.668	<0.013
	$TI^{August}*F^{August}$	0.016	$5.6*10^{-3}$	4.063	<0.008
	$TI^{September}*F^{September}$	0.002	$8.8*10^{-4}$	1.866	<0.033

Table 6.6 IPCC predictions applied to fish population models, T and F represent temperature and flow respectively, dictating which parameters have been manipulated, where length is length-at-age 1 and the values in the table are the predicted change (%).

Species	Model	Parameter and% Probability								
		T	T	T	F	F	F	T and F	T and F	T and F
		10%	50%	90%	10%	50%	90%	10%	50%	90%
L. cephalus	Length	0.1	0.2	0.3	-2.4	-1.2	5.2	-2.3	-0.8	6.6
L. leuciscus	Length	30.9	8.1	9.8	-0.9	-0.8	-0.3	30.0	7.3	9.4
R. rutilus	Length	-0.5	-0.9	-1.4	-1.2	-1.0	-0.8	-1.7	-2.0	-2.2
L. cephalus	AGI	2.3	3.8	4.9	4.5	2.8	0.4	6.8	6.6	5.4
L. leuciscus	AGI	-	-	-	-4.8	4.2	6.1	-4.8	4.2	6.1
R. rutilus	AGI	-	-	-	0.6	0.5	0.4	0.7	0.6	0.5
L. cephalus	YCS	2.6	4.5	6.0	-	-	-	2.6	4.5	6.0
L. leuciscus	YCS	19.0	45.0	71.2	-	-	-	19.0	45.0	71.2
R. rutilus	YCS	1.8	2.2	2.6	-4.2	-3.9	-7.1	-4.1	-3.6	-5.5

6.4 DISCUSSION

6.4.1 *Growth parameters*

This study predicted how the growth parameters of riverine fish species will be affected by climate change. *Leuciscus leuciscus* (cold water preference) was predicted to benefit from climate change, more so than other species, especially in terms of annual growth and recruitment success. Future predictions of flow regimes were predicted to negatively influence the length-at-age 1 of all three species; however these effects were offset by predicted increases in temperature for *L. leuciscus* and *L. cephalus* (i.e. cold and cool water preference) but not for roach (warm water preference). Flow was not an important monthly variable for YCS of *L. leuciscus* and *L. cephalus* despite these being rheophilic. Subsequently, *L. leuciscus* and *L. cephalus* YCS were predicted to increase with an increase in temperature. Likewise, Mills and Mann (1985) stated recruitment success (YCS) of cyprinid fishes in most cases appears to be determined by growth in the first year of life, with recruitment frequently highest in years when water temperatures are higher than average. However, although flow was not an important monthly variable, it may play an important role in future climate models.

6.4.2 *Water temperature*

Cumulative degree-days increment played an important role in determining length-at-age 1 of all three species and YCS and AGI of *L. cephalus* and *R. rutilus*. Despite several authors investigating the role of temperature on population characteristics, few have investigated cumulative degree-days increment**.** Cumulative degree-days increment in June and July contributed more to AGI, and thus larger size at the end of the first year of growth, than cumulative degree-days increment in August and September. These relationships were generally consistent between species, although *R. rutilus* AGI appeared to be more positively influenced by cumulative degree-days increment in August. The early summer period is critical during larval and juvenile life stages as high water temperature has positive effects on growth and survival; but is reduced through predation (Mills, 1982), winter starvation (Karas, 1990) and washout during high flow events (Mann, 1973; Heggenes & Traaen, 1988). Faster growth of juvenile fishes also increases their swimming performance and habitat accessibility (Mills & Mann, 1985), providing a greater opportunity to utilise larger and more energetically profitable prey earlier in life (Keast & Eadie, 1984).

6.4.3 *River discharge*

No consistent relationships were found between species and monthly flow variables, although high flow in the early summer tended to affect AGI of *L. cephalus* negatively, while flow negatively influenced AGI of *L. leuciscus* in late summer (August and September). Jensen and Johnsen (1999) and Nunn *et al.* (2007a) suggested that moderate flows at sensitive life stages, typically the larval period post-hatch, may cause higher mortality than considerably higher flows at less sensitive stages. This could be cause for concern, because all models predicted a reduced length-at-age 1 for all three species, but the reduced precipitation rates predicted by IPPC (2009) scenarios would result in fewer high flood events during critical periods.

6.4.4 *North Wall Gulf Stream*

The NWGS is not predicted to shift outside of its current range within the present century (IPPC 2009). The NWGS had the greatest influence on current status models of the three species, highlighting the important role overall climate drivers, for which NWGS is a surrogate, have on fish population dynamics. This role can only be assumed to continue with a changing climate.

6.4.5 *Model predictions*

There is considerable agreement between the results of this study and previous research (Daufresne *et al.*, 2009; Graham & Harrod, 2009), strengthening the validity of the predictive models. This study improves the understanding of interactions between key abiotic variables in a changing climate, with each environmental driver having a predominant effect at different times (Cattanéo, 2005). The predictive models are in agreement with studies that suggest high temperatures, particularly associated with low discharge in the first growth year, generally produce strong year classes (Cowx, 2001). Flow variables were a more important factor for *L. cephalus* and *L. leuciscus* (rheophilic) than *R. rutilus* (eurytopic).

Longshaw *et al.* (2010) suggested that additional biotic factors need to be considered when determining factors affecting recruitment success. They, and Feist and Longshaw (2008), found disease can be an important factor driving recruitment success, and ultimately year class strength. Furthermore, Johnson *et al.* (2009) concluded that some of the changes predicted to occur in UK rivers over the next 50-100 years will lead to shifts in endemic fish disease dynamics and may facilitate the emergence of most pathogens. These changes are expected to be detrimental in most cases to the fish host.

Based on the findings of the current study, predicted changes in temperature and river discharge across the UK as a result of climate change (Jenkins *et al.*, 2009) are likely to have either a negligible or positive influence on fish populations. This study predicts these changes will result in improved annual growth rates after the first year, greater overwinter survival and stronger year classes, possibly linked to increased food availability (Grenouillet *et al.*, 2001; Nunn *et al.*, 2003; Johnson *et al.*, 2009). Even small increases in water temperature are likely to result in improved population status, overriding the reduced flow. However, Daufresne *et al.* (2009) argued that climate warming in aquatic systems would result in reduced body size. Therefore, predicting the impact on fish communities in temperate European rivers remains speculative, although it is important to note that this study was conducted in a single large river catchment. Tributaries and head waters compose over 2/3 of total stream lengths in typical river drainage systems (Buisson *et al.*, 2008). It can be presumed tributaries will see the first signs of climate change, because they are less stable, being more dependent on temperature and precipitation than large lowland rivers. Interestingly, Elliot and Elliott (2010) found small increases (<2·5 °C) in winter and spring would be beneficial for growth with 1 year-old salmon smolts. However, water temperatures would have to increase by about 4 °C in winter and spring, and 3 °C in summer and autumn before they had a marked negative effect on trout (cold preference) growth.

The impacts of climate change are further complicated because spawning of many riverine coarse fish species is triggered by temperature, although in some cases photoperiod can play an equally important role (Baras & Philippart, 1999; Norberg *et al.*, 2004). An increase in spring temperatures caused by climate change may result in spawning events occurring earlier in the year (Gillet & Quetin, 2006; Daufresne *et al.*, 2009). Whilst this could extend the growing season and counteract the reduced lengths predicted in this study, there would need to be synchronicity of food availability (Li & Mathias, 1987). It is possible that protracted or multiple spawning species such as *L. cephalus* and *G. gobio* (Nunn *et al.*, 2007b) would have a distinct advantage over earlier spawners, as the risk of failure in larval development and exogenous food mismatch, leading to larval mortality, would be reduced.

6.4.6 *Limitations*

Competitive interactions between riverine fishes, in contrast to lake fishes, remain somewhat superficial (Resetarits, 1997). There are numerous studies showing the presence of competition among stream fishes (Resetarits, 1997). However, other studies have suggested environmental variability, behavioural, morphological and physiological adaptations play a greater role in river systems than competition amongst individuals, similarly average population densities are often far below the maximum (Gilliam *et al.*, 1993). Furthermore, river systems are generally unstable, fish populations being influenced by low and high flow events, as is well documented (Jensen & Johnsen, 1999; Nunn *et al*., 2007a). Hence, interactions between riverine fishes are complex and involve various interacting variables. Including these interactions in the predictive models would have been difficult and is beyond the scope of a single study.

Caution must be paid to the overall effect on fish populations (and communities) because several successive strong year classes may result in intra-specific competition for food resources and inter-specific competition between species. This may lead to a change in the dominance of species with the communities, which may be exacerbated by a displacement of more northerly species (e.g. *E. lucius, G. gobio, L. leuciscus, P. fluviatilis* and *S. trutta*) by southern species and warm-water species (e.g. *A. alburnus*, *L. cephalus, B. barbus* and *R. rutilus*) (Daufresne *et al.*, 2004; Daufresne *et al.*, 2009). Invasive species are also likely to increase as a result of climate change (Britton *et al.,* 2010a). Wilson (1990) suggested that under gradual climate change no species would have time to eliminate (by the way of competitive exclusion) others before being itself constrained by the environment. As a consequence, the communities would always include a mixture of species favoured by the current vs. previous climate.

This study did not investigate inter-seasonal variation as it used IPCC (2009) seasonal predictions rather than monthly predictions. Similarly, IPCC (2009) central England predictions were used for modelling. Therefore, this study has not considered regional and inter-regional variation between the interactions of climate and a river catchment. This study concentrated on modelling climate change impacts on the summer growth period of fish populations, but did not take into account possible implications of overwintering survival (Griffiths & Kirkwood, 1995). Climate change could cause rivers to become unsuitable for specific populations, increasing temperatures greater than species' tolerance limits (Buisson *et al.*, 2008). Combined with water abstraction increased siltation and low precipitation rates, river temperatures could increase even further with the extreme event of rivers running dry (Mann, 1996; Malmqvist & Rundle, 2002). The general effects of climate change on freshwater systems will likely be increased water temperatures, decreased dissolved oxygen levels, and the increased toxicity of pollutants (Ficke *et al.*, 2007), which could have further detrimental effect on fish populations.

6.5 CONCLUSIONS

Overall the impact of climate change on ecosystems relies on the interactions between invertebrates, mammals, birds and fish species. Effects on ecosystems can only be understood with continued research into predicting the impact climate change on theses taxonomic groups. Climate change is likely to increase the propensity for cyprinid fishes to thrive, although the exact mechanism will depend on inter-annual variability in temperature rises and the timing of flow events. Notwithstanding the limitations of this study, it provides ecologists with a greater understanding of climate change and its potential impact on European, lotic fish populations.

7 GENERAL DISCUSSION

7.1 Introduction

The focus of this thesis was to illustrate the array of ecological information available from fish scales collected during routine monitoring programmes. Squamation (the development of fish scales) is integral to understanding fish physiology and ecology. All fish species studied displayed similar patterns of squamation; beginning at the caudal fin peduncle and spreading laterally (Chapter 3). Because fish scales are not present at the time of fish hatch, it is common practice for back-calculation formulae to incorporate a correction factor to account for the difference between fish length and size at squamation. However, large errors can occur as correction factors vary with sample size and the number of age groups used in their calculation. Fortunately, correction factor error can be minimised by adopting species specific criteria (Chapter 2). The back-calculation of fish lengths has been used by several authors to identify differences in growth between locations and populations. Fish growth plasticity is evident across different regions of the UK, with longitudinal regions having significantly similar growth in contrast to latitudinal. Equally, geographic variation also existed for recruitment success (Chapter 5). Growth characteristics and population dynamics are governed by environment variables and climate events. Consequently, changes in global climate would have a direct effect on fish populations. Current predictions (IPCC, 2009) suggest it is likely the propensity for cyprinid fishes to thrive will increase, although the exact mechanism will depend on inter-annual variability in temperature rises and the timing of flow events (Chapter 6). Similarly, abiotic and biotic factors dictate the morphological characteristics of an individual. As a result, geometric morphometric analysis of fish scales is a good discriminator of species, fish length and river of origin, irrespective of allometric ontogeny (Chapter 3).

7.2 Conclusions and recommendations

7.2.1 *Squamation of freshwater fishes*

The error surrounding correction factors can be very large. Correction factors derived from larval fish could provide standard values for use in back-calculation formulae, minimising error. **It is recommended further individuals and species are analysed using the technique described in Chapter 3, to provide (*c*) values for species with insufficient sample sizes. Additionally, samples should be collected from various**

rivers to assess whether growth rate influences squamation patterns. These investigations would improve the understanding the factors influencing squamation e.g. day length, temperature or growth rate. Reducing correction factor error is of high importance to fisheries ecologist reliant on back-calculation formulae. Therefore, all possible steps should be taken to improve the accuracy of fish lengths derived from fish scales.

Nunn (2005) captured overwintering *L. cephalus* less than 20 mm, a length less than the size squamation is completed (Chapter 3). **It is recommended that 0+ *L. cephalus* are analysed to assess the impact of over-wintering at or below the length of scale squamation is complete and the impact this has on the reliability of ageing fish from scales**. In addition, this study would provide information on whether squamation is age or length dependent. Improving the accuracy of scale reading would ultimately reduce any miscalculation of age and avoid incorrect assessment of such techniques as year class strength estimations and mortality assessments on which management decisions are made. Additionally, this study would allow an assessment of whether compensatory growth exists in *L. cephalus*, i.e. where smaller progeny catch up in size with the faster growing individuals.

7.2.2 *Review of back-calculation procedures*

Correction factors are often assumed to be a compulsory inclusion in back-calculation formulae, to account for the difference between body length and scale development. The error surrounding a correction factor can vary over a large range, depending on sample size and number of age classes used in the calculation. **It is recommended that further research is conducted on correction factors derived from other back-calculation formulae, extending the results of this study.** Developing more detailed criteria of appropriate sample sizes and age classes would improve the accuracy of back-calculation formulae. Increasing data available on a population through back-calculation is a widely used technique in fisheries ecology. Subsequently it is anticipated correction factor criteria would be widely adopted amongst fisheries ecologists.

The greatest apprehension regarding back-calculation is validation. **It is recommended that a large scale investigation is conducted to assess which back-calculation formulae should be used.** This investigation will need to be completed for all species commonly aged during routine monitoring programmes, because it is possible the accuracy of back-calculation formulae may vary between species. The length of farmed fish, of various ages, should be measured (fork length, mm), scaled and individually Passive Integrated Transponder (PIT) tagged, then released into a river. These individuals will need to be recaptured each year for several consecutive years, each year recording its length and acquiring scale samples. The number of individuals necessary to provide a statistically valid comparison would be need to be relatively large, consisting of various age classes, to ensure an acceptable recapture rate. Identifying the most accurate back-calculation formulae should be of upmost importance for fisheries managers as each year thousands of fish are aged during routine monitoring programmes and currently the interpretation of such data is open to dispute; this study would address these concerns.

7.2.3 *Regional plasticity in growth and recruitment success*

National standard growth rates of fish have been updated by several authors, but very few have investigated regional variation in growth. Site specific information is readily available for a number of rivers, but fish are known to migrate large distances, subsequently the site where fish are captured may not be a true representation of their home range, e.g. below a weir in a heavily canalised river. **It is recommended, factors influencing site specific and regional variation in growth are further investigated. Reach specific environmental data combined with regional climate data would be a substantial data set capable of explaining a high proportion of variation in growth.** Currently, published national growth standards do not contain details regarding samples sizes and/or spread of rivers used to create them. **It is recommended, the information used to build standard growth rates are made available or future growth standards should publish this information to facilitate the understanding and minimise regional bias. Furthermore, it is highly recommended updated growth standards are adopted by the appropriate governing bodies.** The ability to understand further factors influencing growth would assist fisheries managers in meeting the requirements of the Water Framework Directive. In addition, nationally accepted standard growth rates will allow generic comparisons of growth rates, reducing possible misinterpretation because of outdated or biased growth standards.

Regional and national growth standards calculated in this study represent just a small portion of the species found in rivers throughout England, and only included limited information regarding life history traits. **It is recommended that the regional and national growth standards studied are extended to encompass a larger number of species. Also, further information on life history traits would allow impacts of**

climate change to be assessed. Indeed further information is needed on the growth of fish species not commonly caught and those species where little is known of their growth, e.g. *T. thymallus and G. cernuus.* Detailed information of the growth and life history traits of further species would improve understanding of fish species and provide reference data for the assessment of fish populations.

The EU Water Framework Directive (WFD) is a major driver influencing the management of water in European Member States. It requires that all inland and coastal waters within defined river basin districts must reach at least good ecological status by 2015 and defines how this should be achieved through the establishment of environmental objectives and ecological targets for surface waters. The WFD has been responsible for reducing sources of pollution into rivers, and in turn, is associated with improved water quality of many rivers. As a result, improvements in water quality would, in theory, manifest itself in fish population dynamics, especially species preferring good water quality. **It is recommended that a study is completed to access whether improvements in water quality are correlated to improvements in fish populations. Fish growth is plastic. As a result, any changes in water quality should be manifested in growth rates. Comparisons of historical growth rates with present day growth may be a useful tool, especially if other factors are removed, i.e. changes in water temperature or abstraction.** An evaluation of the responses of the fish growth to water quality improvements could be used as a reference when legislation is reviewed. However, the implications of a non-significant correlation between water quality and fish growth would be a cause for concern, but, may highlight water quality is not a key factor influencing fish populations.

The Water Framework Directive indirectly influences biodiversity, through changes in habitat and water quality improvements. Changes in biodiversity may alter competition from sympatric species and influence their growth characteristics. **It is recommended that growth characteristics of riverine fish populations are continued to be monitored to identify changes related to the Water Framework Directive. Furthermore, there is a definite need to model species interactions, because changes in life history characteristics may have serious implications, especially species protected under the EU Habitats Directive (92/43/EEC).** Similarly, changes in fish diversity may also influence other non-fish species i.e. white clawed crayfish (*Austropotamobius pallipes*) or otters (*Lutra lutra*). Hence, networking of species interactions will require a detailed study of the entire aquatic system to provide a robust and biologically valid model suitable for modelling changes to the ecosystems.

7.2.4 *Predicting the influence of climate change on fish populations*

Global climate change is unequivocal, it is evident these changes will have an influence on aquatic and terrestrial animals. Climate model projections indicate the global surface temperature is likely to rise a further 1.1 to 6.4°C during the 21st Century, with concurrent rises in the temperature regimes of freshwater bodies. **It is recommended that fisheries monitoring is continued, in conjunction with assessing key environmental variables (temperature, precipitation and North Wall Gulf Stream) to assess the impacts of any future climate change on growth rate, life history traits, recruitment success and species composition.** Through the monitoring of prevailing climate in conjunction with the results of historic assessments of climate, future trends can be identified. These trends can be used to assess any changes that have occurred in the same time frame, where possible, through the assessment of growth rate and YCS and, with the results of future fisheries monitoring, an assessment of any changes in population characteristics. The regional plasticity of growth and recruitment success identified in Chapter 5, suggests national modelling of environmental influences on fish populations may be inadequate for some species, because of the high geographic variation between regions. **Therefore, it is recommended that there is regional and nation modelling of the impact of climate change on fish populations. Specifically, regional modelling should be promoted for species that display high variability around key biological process, e.g. time of spawning and emergence.** Predicting the impacts of climate change on fish populations must be an important task for fisheries ecologists, as it will enable mitigating measures that mitigate changes in climate and support continued existence of fish species, especially those threatened.

Temperature is a key variable governing the biological process of fishes, specifically freshwater fish species. However there is a definite lack of information regarding species specific temperature tolerances. **Thus, it is recommended that a detailed analysis of species temperature preferences and tolerances is made. It is important this study incorporates different life stages, because a species temperature preference will change with age.** Through the identification of specific temperature preferences, statistical modelling of fish growth parameters and environmental variables will be more accurate and will improve the advice given to fisheries managers.

Very few studies have used data collected during routine fish monitoring programmes, similar to those conducted by the EA, to model climate change. However, the potential error surrounding back-calculated lengths-at-age data (Chapter 2) could have serious repercussion. Specifically the error from the incorrect calculation of the correction factor (to account for the length of the fish when the scale radius is zero) could be integrated into the modelling, making the results erroneous. This possible source of error is even more important considering the small (< 10%) predicted changes in fish populations (Chapter 6). **It is recommended that an investigation is conducted to assess the influence of correction factor error on the predictions from climate change models using back-calculated length-at-age data.** Furthermore, this study should investigate how the use of a biologically derived (50% median, Chapter 3) or a mathematically derived (individual river or standard, Chapter 2) correction factors could influence the climate change predictions. **In addition, it is recommended that the impact of incorrectly ageing *L. cephalus,* which have over-wintered at or below the length squamation is complete, on climate change models which rely on accurate ageing is investigated**.

7.2.5 *Future applications of geometric morphometrics*

To determine age, growth and other statistics of riverine fish populations the Environment Agency routinely sample populations to meet the requirements of the Fisheries Monitoring Programme. The Environment Agency’s ‘Best Practice Guidelines’ suggest fish scales are removed from the same anatomical region to ensure consistently of scale morphology. Because it is essential, a consistent methodology in scale collection is implemented across the Fisheries Function and scales samples collected are adequate for ageing and subsequent data analysis. However, it is unclear from what basis the ‘Best Practice Guidelines’ have chosen the anatomical region to remove a scale sample. **It is recommended an investigation is conducted to assess, statistically, which anatomical regions exhibit the least variation in scale morphology. Furthermore, this study would provide sufficient data to assess variation in back-calculated length-at-age derived from scale samples from different anatomical regions.** These two compatible studies will provide scientific guidelines to accompany the Best Practice Guidelines set out by the Environment Agency. It is further anticipated that this study would attract attention from other governing authorities, nationally and globally.

Scale morphology is a good discriminator of an individual’s characteristics, being dictated by abiotic and biotic variables. As a result, it is anticipated scale morphology will be correlated to body morphology of an individual. **It is recommended a pilot study is conducted to assess whether scale and body morphology are**

correlated. If a correlation exists, then scale morphology may be an appropriate tool to study body morphology of populations. Furthermore, body morphology may be related to the sex of an individual, allowing a cost effective tool to assess the sex ratio of a population.

Nationally, the Environment Agency annually conducts hundreds of fish surveys, resulting in the ageing of approximately 50,000 fish scales each year. Globally, the number of fish aged annually is estimated at over 1 million (Campana & Thorrold, 2001). However, information derived from these surveys can be contentious because fish scale analysis requires further research and statistical validation. Despite this, ageing of fish scales is still the most important tool available for fisheries ecologist, biologists and managers, because fish scales narrate the fish’s environment variables; physical, geographic and climatic. Consequently, the ability to derive further ecological information, other than age and growth, from scale samples is advantageous. Accordingly it is foreseen that information in this thesis will be adopted as a guide for fisheries ecologists, influencing best practice guidelines of fish monitoring programmes.

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APPENDIX 1 Comparison of different methods of regression analysis between scale radius and fork length and (*c*) values for inclusion in Fraser-Lee (1920) for 19 species, where the number of rivers and individuals used in the analysis are the same as in Table 2.2.

Species	**Regression type**									
	Linear	Logarithmic	Quadratic	Cubic	Compound	Power	S	Growth	Exponential	Logistic
A. alburnus	0.63**	0.63**	0.65**	0.65**	0.61**	0.65**	0.58**	0.61**	0.61**	0.61**
	(39)	(78)	(19)	(33)	(50)	(75)	(5)	(4)	(50)	(0)
A. bjoerkna	0.76**	0.71**	0.76**	0.76**	0.72**	0.72**	0.66**	0.72**	0.72**	0.72**
	(30)	(46)	(37)	(54)	(54)	(60)	(5)	(4)	(54)	(0)
A. brama	0.89**	0.83**	0.90**	0.91**	0.84**	0.92**	0.71**	0.84**	0.84**	0.84**
	(19)	(17)	(-20)	(36)	(74)	(67)	(6)	(4)	(74)	(0)
B. barbus	0.86**	0.86**	0.88**	0.90**	0.80**	0.92**	0.84**	0.80**	0.80**	0.80**
	(50)	(83)	(-43)	(-8)	(109)	(114)	(7)	(5)	(109)	(0)
C. carpio	0.94**	0.84*	0.97*	0.99	0.96**	0.91*	0.79*	0.96**	0.96**	0.96**
	(-83)	(-440)	(106)	(603)	(84)	(26)	(7)	(4)	(83)	(0)
E. lucius	0.85**	0.79**	0.86**	0.86**	0.85**	0.91**	0.69**	0.85**	0.85**	0.85**
	(75)	(81)	(39)	(59)	(146)	(138)	(7)	(5)	(146)	(0)

Table continued overleaf

Table continued

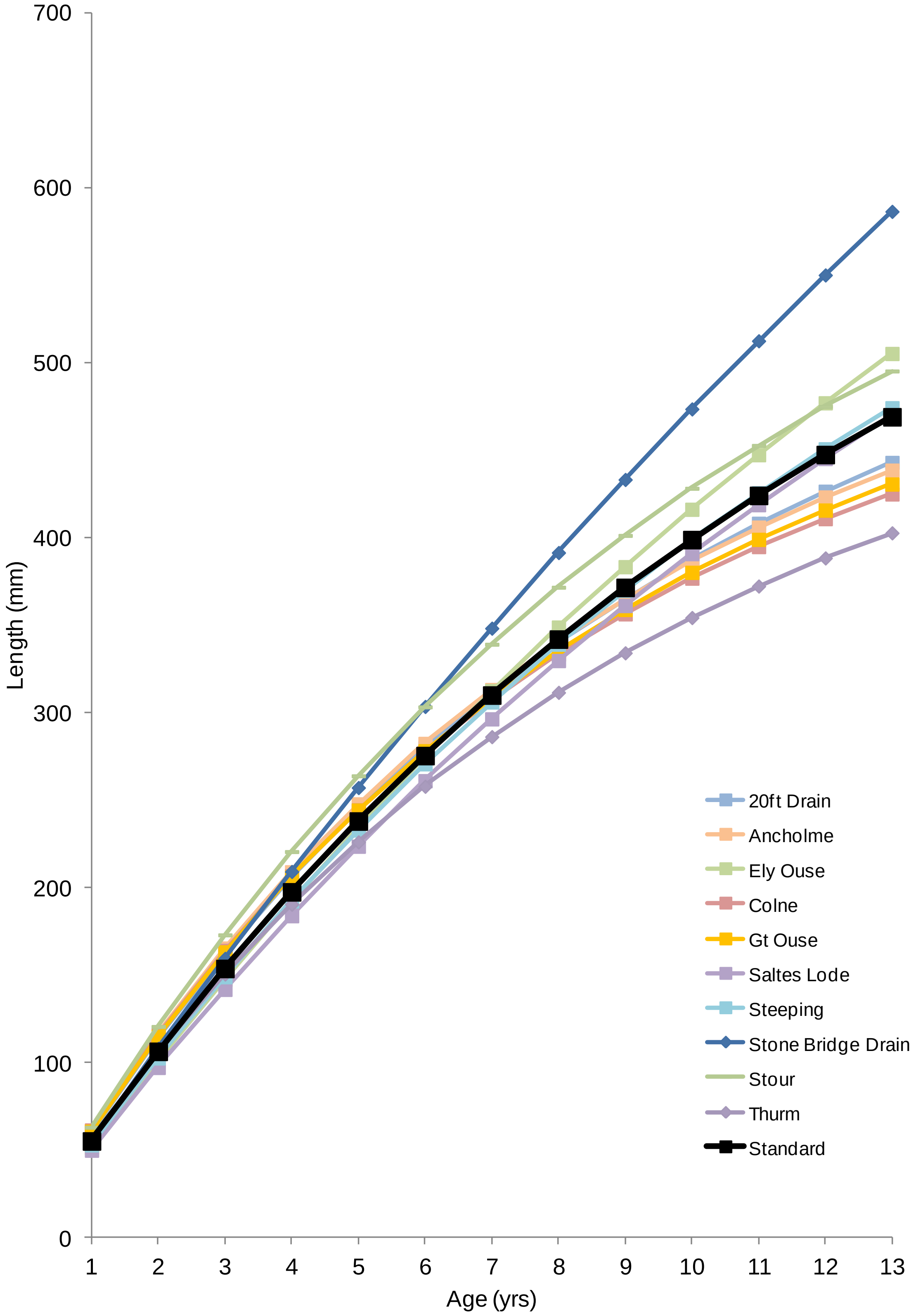
G. cernuus	0.23**	0.17*	0.28**	0.30**	0.25**	0.18**	0.11*	0.25**	0.25**	0.25**
	(69)	(87)	(96)	(141)	(72)	(86)	(5)	(4)	(72)	(0)
G. gobio	0.62**	0.60**	0.63**	0.63**	0.61**	0.61**	0.53**	0.61**	0.61**	0.61**
	(41)	(72)	(36)	(64)	(52)	(71)	(5)	(4)	(52)	(0.)
L. cephalus	0.92**	0.89**	0.94**	0.94**	0.85**	0.95**	0.81**	0.85**	0.85**	0.85**
	(37)	(-31)	(-11)	(16)	(89)	(61)	(6)	(5)	(89)	(0)
L. leuciscus	0.86**	0.83**	0.86**	0.87**	0.82**	0.88**	0.77**	0.82**	0.82**	0.82**
	(32)	(63)	(12)	(25)	(58)	(72)	(6)	(4)	(58)	(0)
O. mykiss	0.83**	0.85**	0.86**	0.87**	0.86**	0.89**	0.86**	0.86**	0.86**	0.86**
	(6	(233)	(-92)	(41)	(74)	(207)	(6)	(4)	(74)	(0)
P. fluviatilis	0.77**	0.70**	0.77**	0.77**	0.73**	0.77**	0.63**	0.73**	0.73**	0.73**
	(55)	(61)	(53)	(54)	(80)	(79)	(6)	(4)	(80)	(0)
R. rutilus	0.91**	0.86**	0.91**	0.91**	0.84**	0.92**	0.80**	0.84**	0.84**	0.84**
	(30)	(41)	(18)	(18)	(59)	(60)	(6)	(4)	(59)	(0)

Table continued overleaf

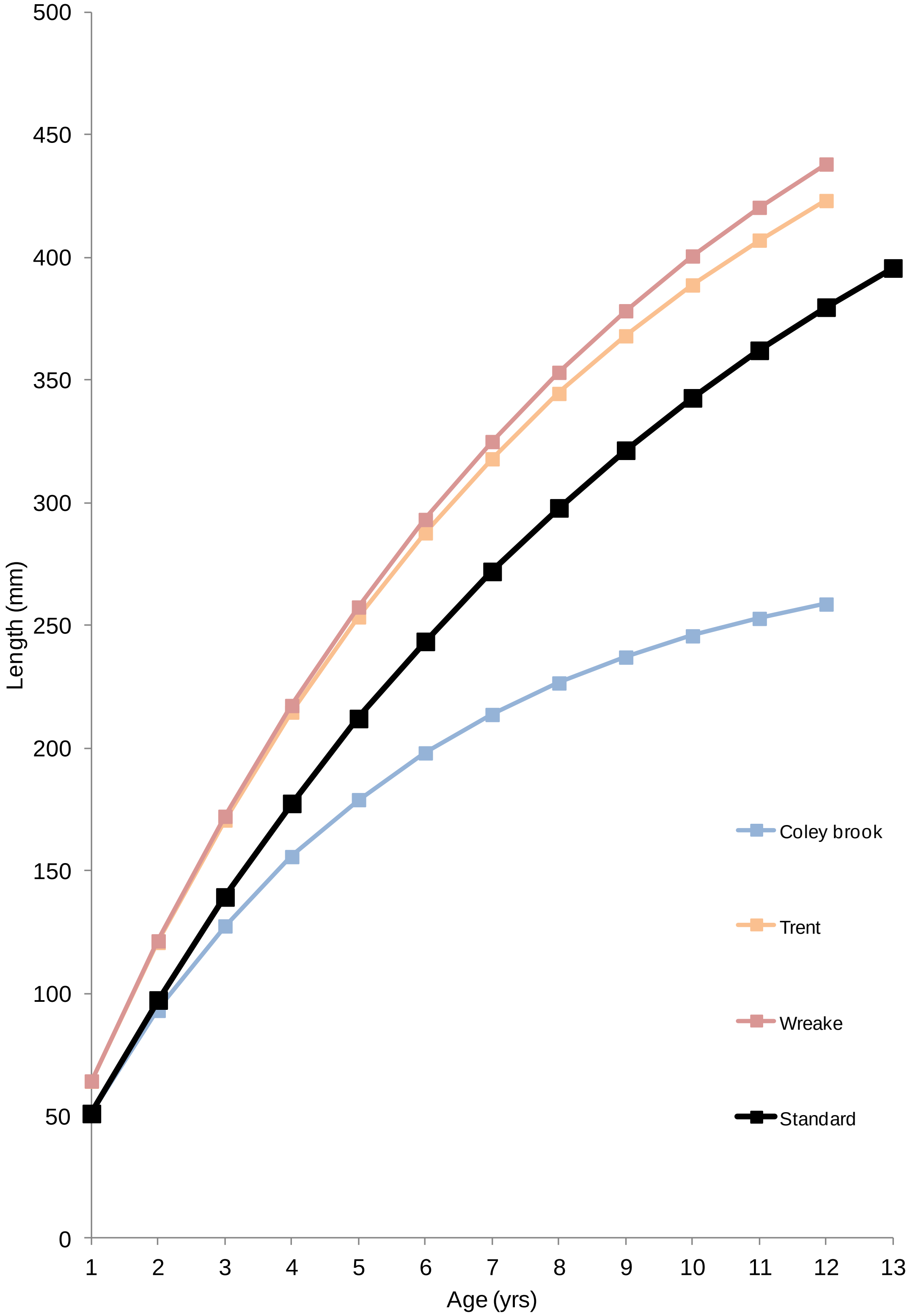
Table continued

S. erythrophthalmus	0.89**	0.86**	0.89**	0.89**	0.85**	0.89**	0.84**	0.85**	0.85**	0.85**
	(21)	(26)	(10)	(29)	(49)	(50)	(6)	(4)	(49)	(0)
S. lucioperca	0.76**	0.69**	0.76**	0.78**	0.78**	0.76**	0.59**	0.78**	0.78**	0.78**
	(24)	(135)	(4)	(108)	(95)	(136)	(6)	(5)	(95)	(0)
S. salar	0.89**	0.52**	0.90**	0.93**	0.73**	0.79**	0.56**	0.73**	0.73**	0.73**
	(32)	(157)	(41)	(78)	(76)	(142)	(5)	(4)	(76)	(0)
S. trutta	0.76**	0.71**	0.77**	0.77**	0.75**	0.82**	0.69**	0.75**	0.75**	0.75**
(brown trout)	(39)	(203)	(13)	(28)	(74)	(189)	(6)	(4)	(74)	(0)
S. trutta	0.48*	0.57**	0.62**	0.65**	0.58**	0.74**	0.80**	0.58**	0.58**	0.58**
(sea trout)	(142)	(222)	(-184)	(140)	(166)	(206)	(7)	(5)	(166)	(0)
T. thymallus	0.85**	0.85**	0.88**	0.88**	0.79**	0.91**	0.85**	0.79**	0.79**	0.79**
	(58)	(106)	(9)	(17)	(90)	(110)	(6)	(5)	(90)	(0)
T. tinca	0.86**	0.81**	0.86**	0.88**	0.88**	0.90**	0.81**	0.88**	0.88**	0.88**
	(22)	(-8)	(25)	(141)	(79)	(66)	(6)	(4)	(79)	(0)

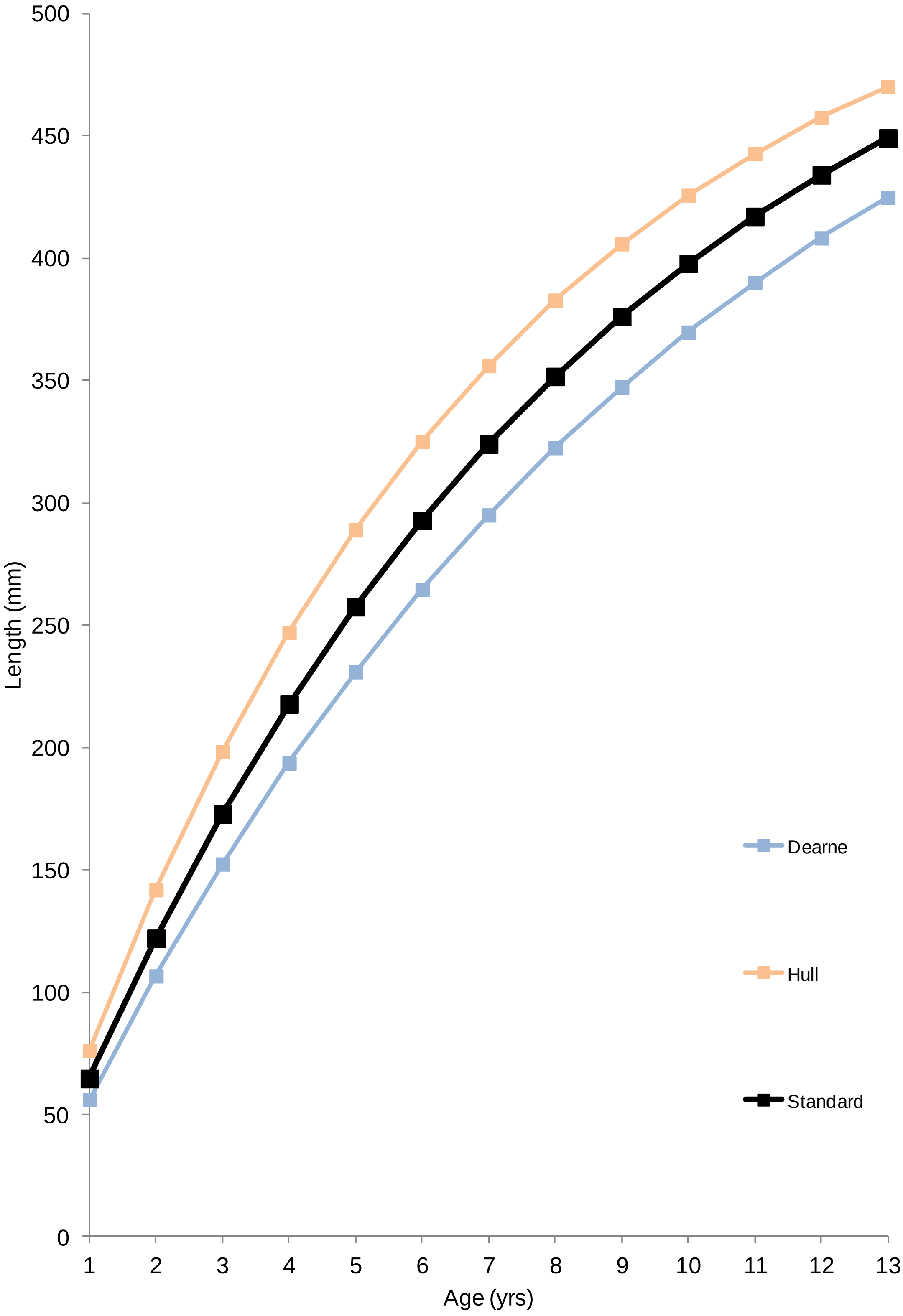
*=P<0.05; **=P<0.01



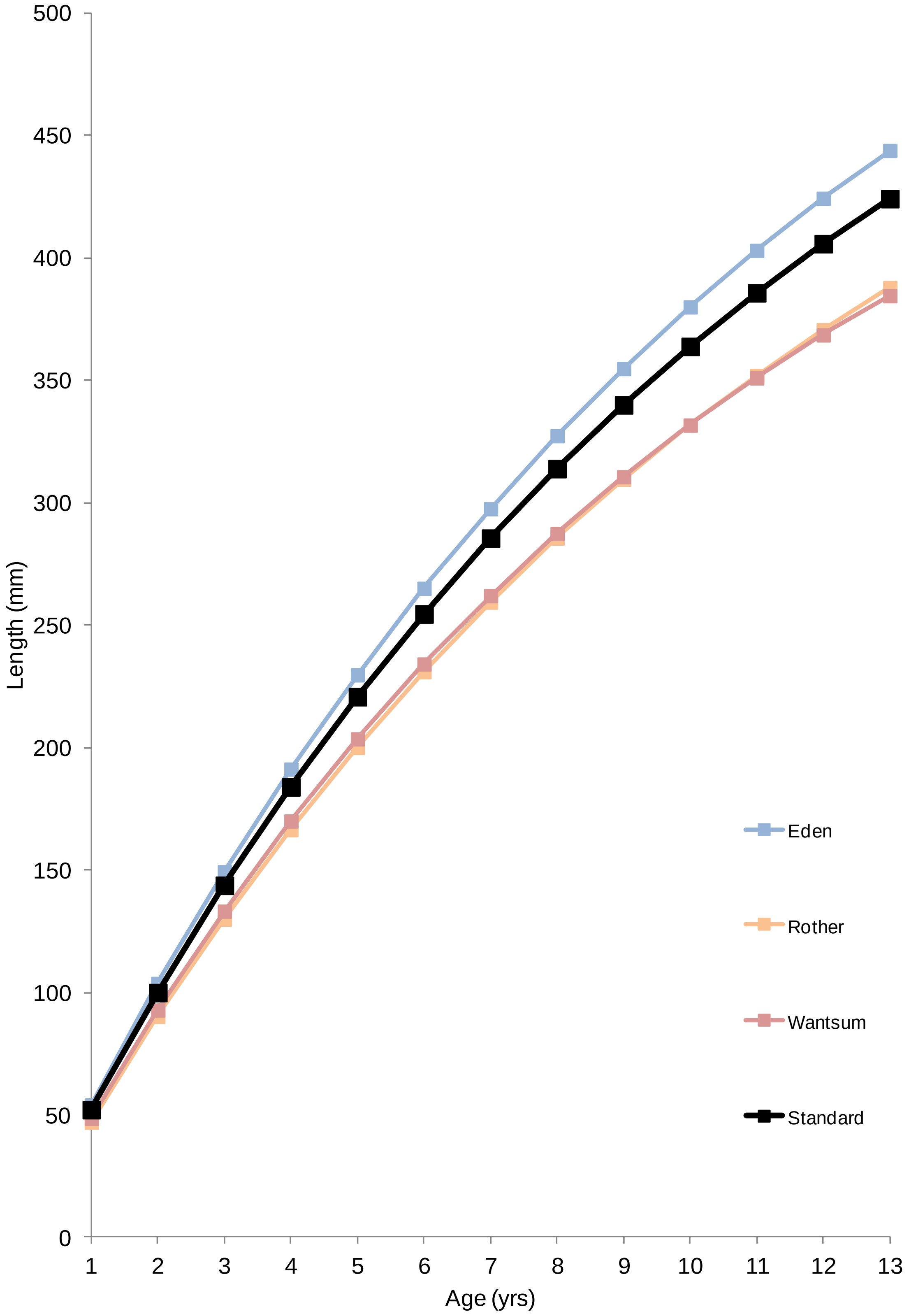
APPENDIX 2 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *A. brama* used to create the standard growth curve (black) for the Environment Agency Anglian region.



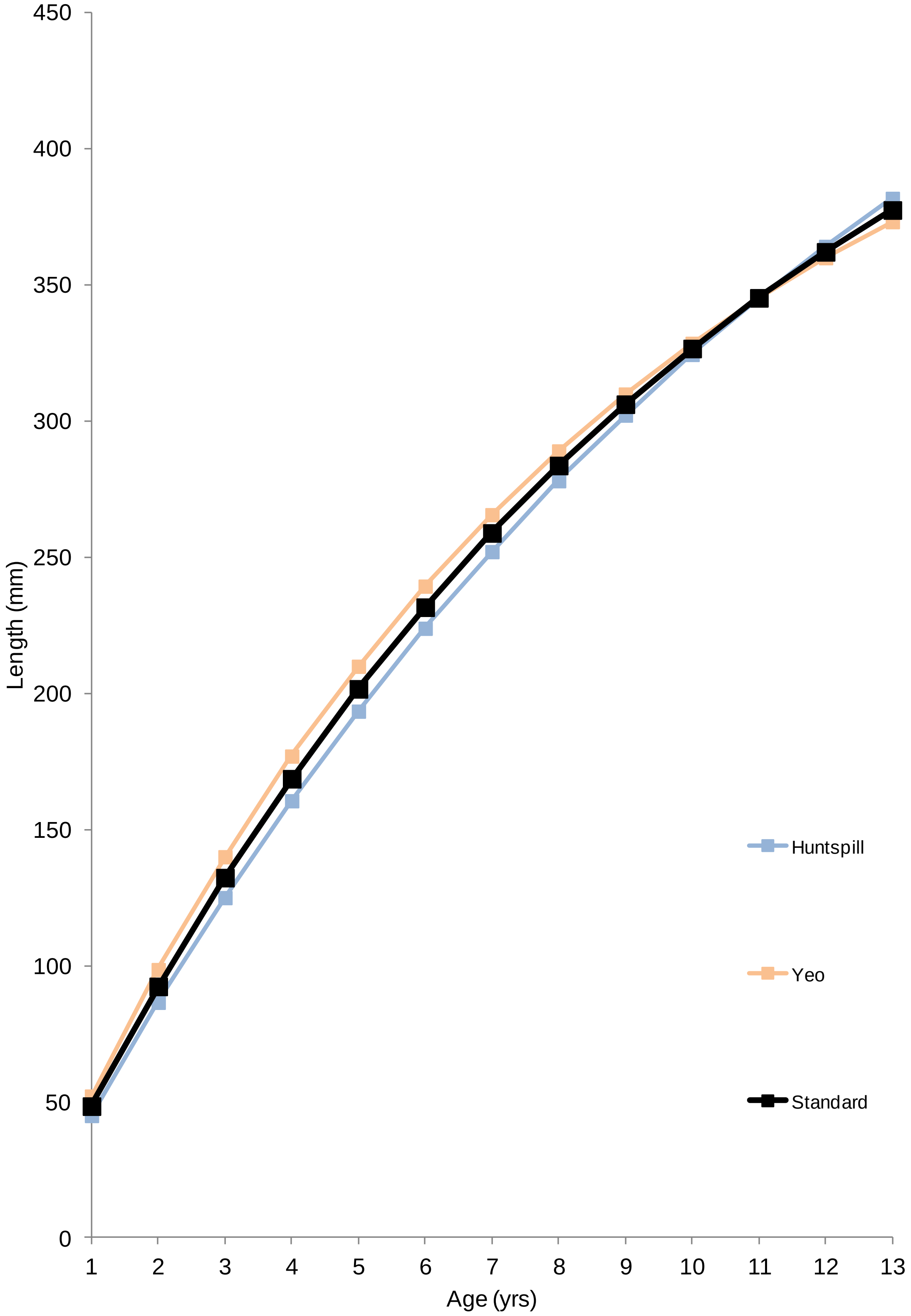
APPENDIX 3 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *A. brama* used to create the standard growth curve (black) for the Environment Agency Midlands region.



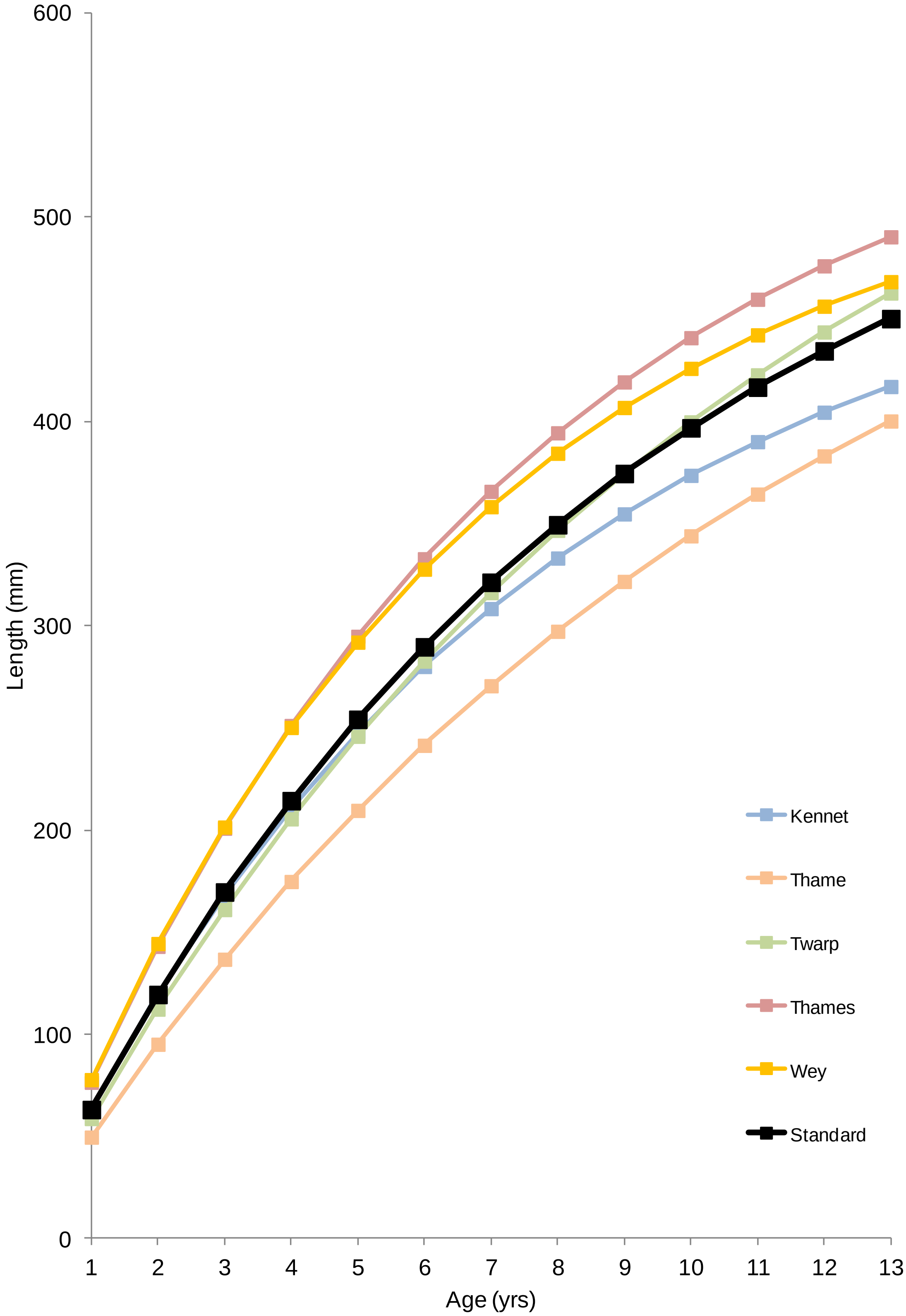
APPENDIX 4 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *A. brama* used to create the standard growth curve (black) for the Environment Agency North East region.



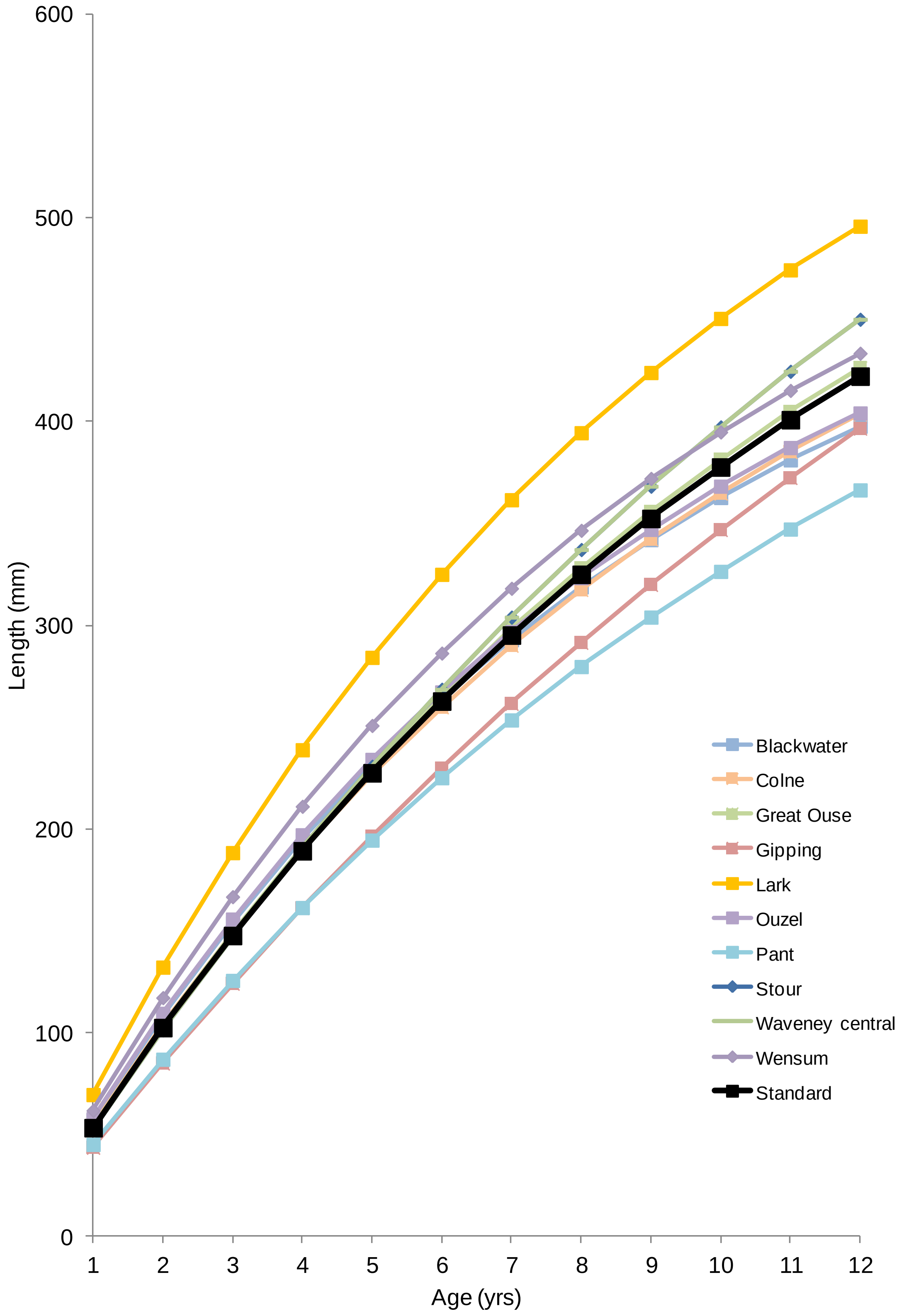
APPENDIX 5 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *A. brama* used to create the standard growth curve (black) for the Environment Agency Southern region.



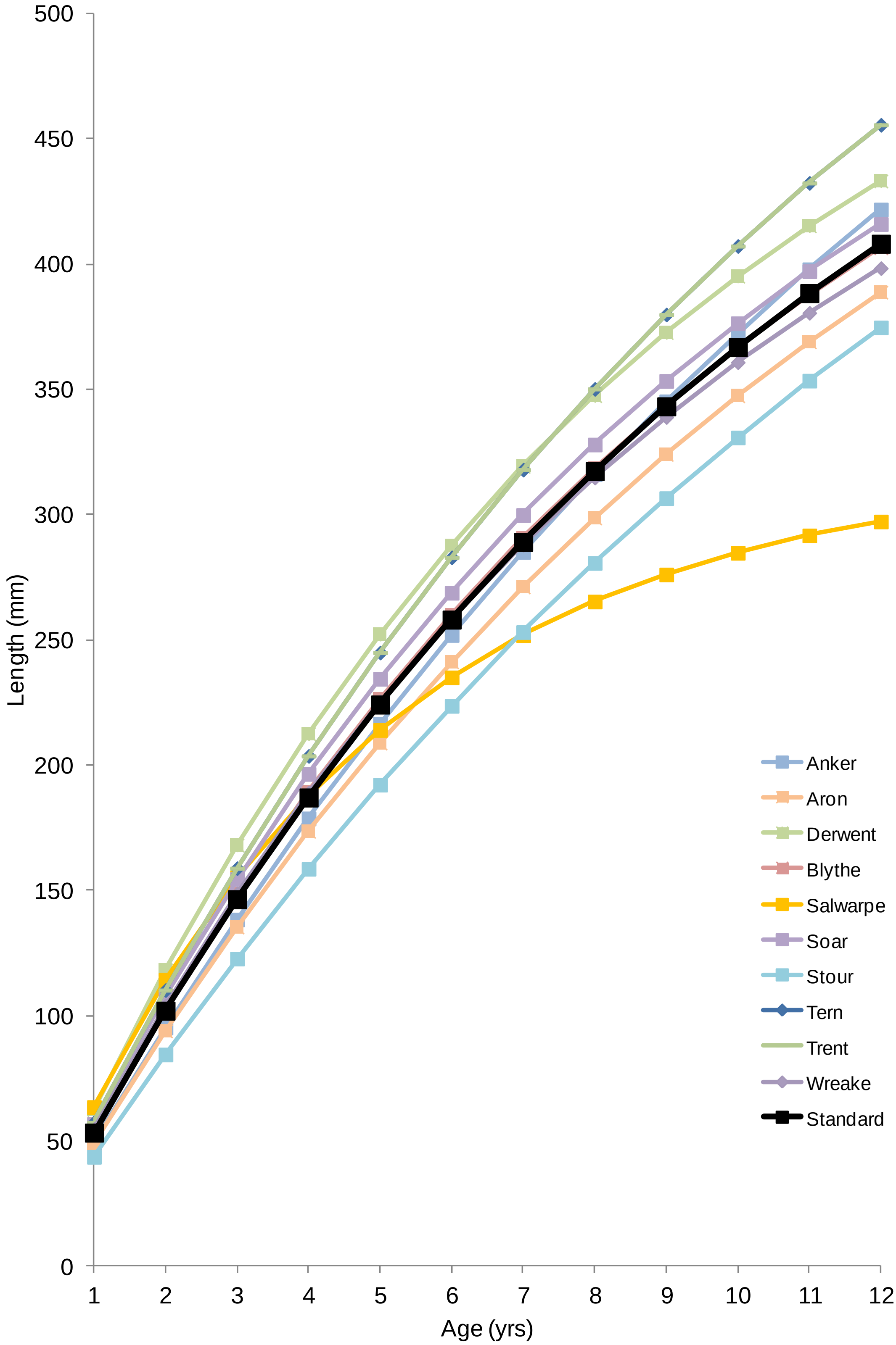
APPENDIX 6 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *A. brama* used to create the standard growth curve (black) for the Environment Agency South West region.



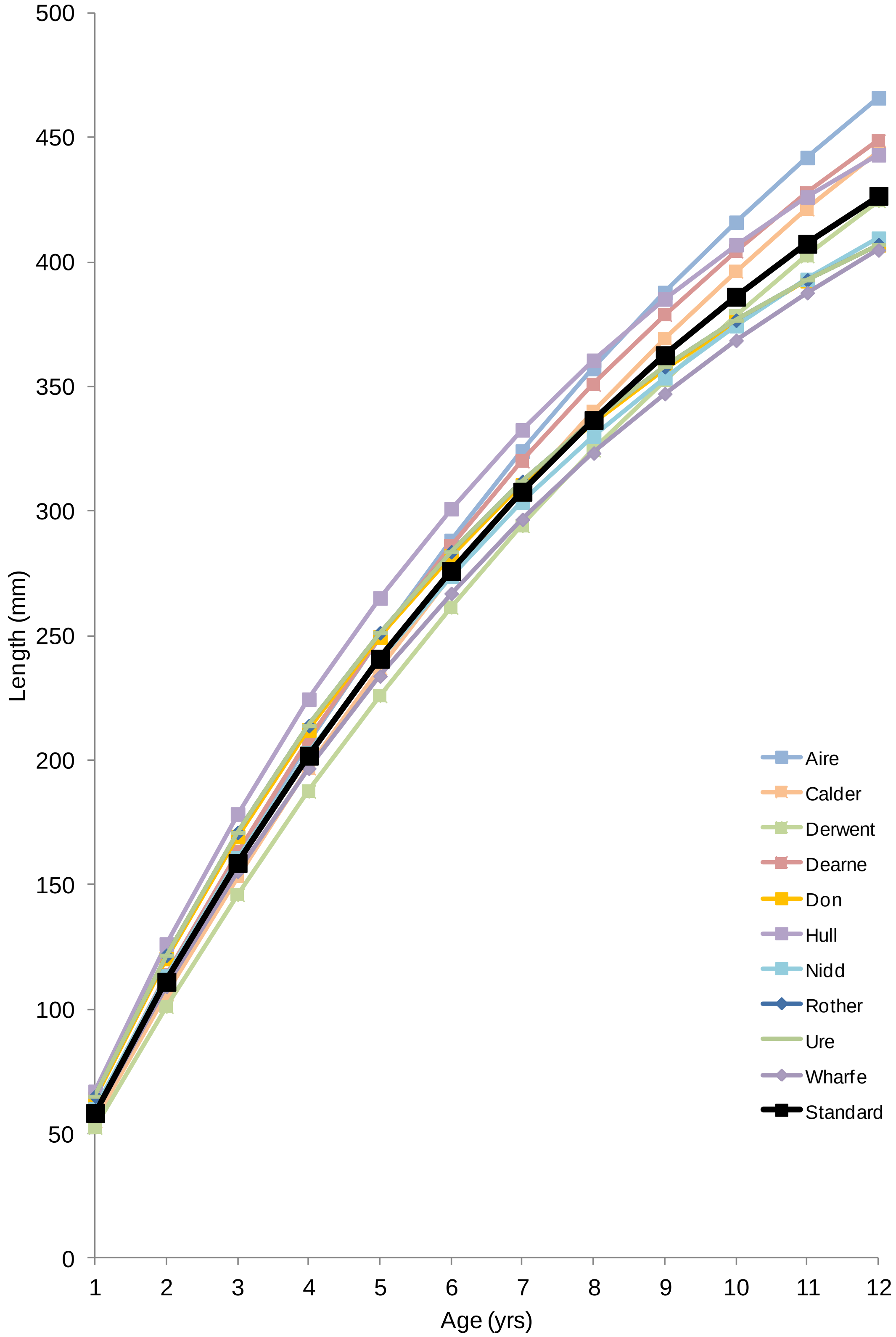
APPENDIX 7 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *A. brama* used to create the standard growth curve (black) for the Environment Agency Thames region.



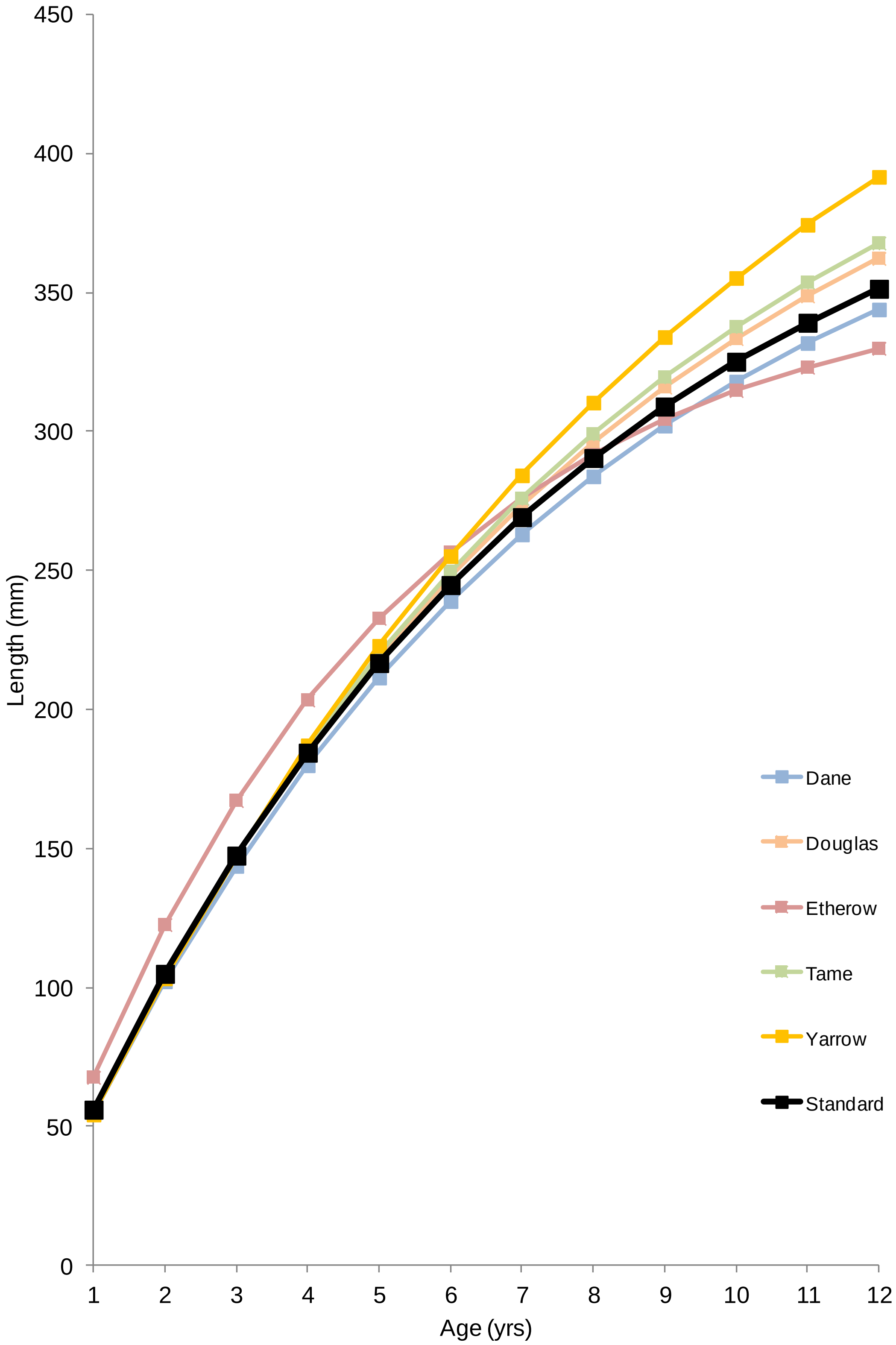
APPENDIX 8 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. cephalus* used to create the standard growth curve (black) for the Environment Agency Anglian region.



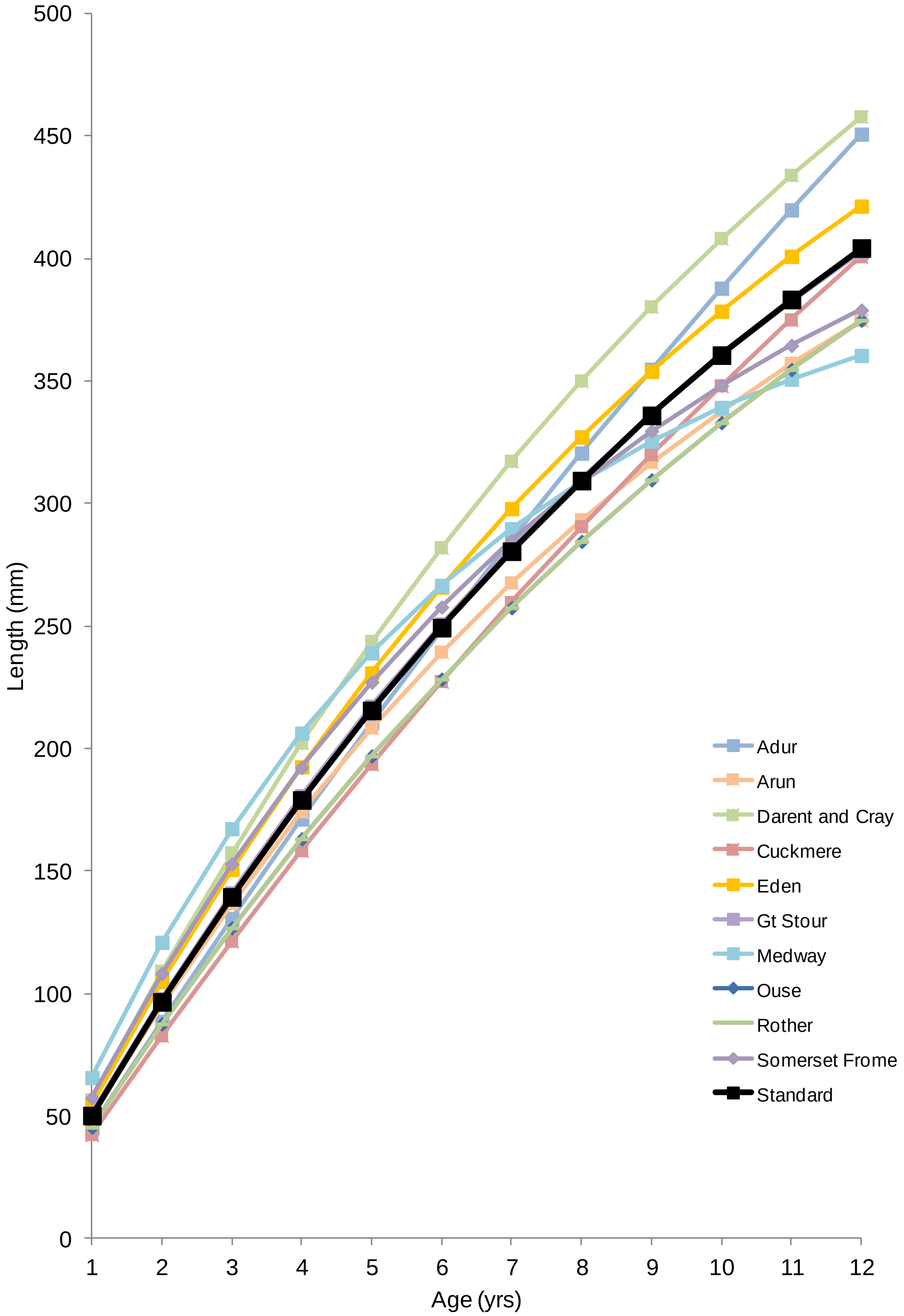
APPENDIX 9 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. cephalus* used to create the standard growth curve (black) for the Environment Agency Midlands region.



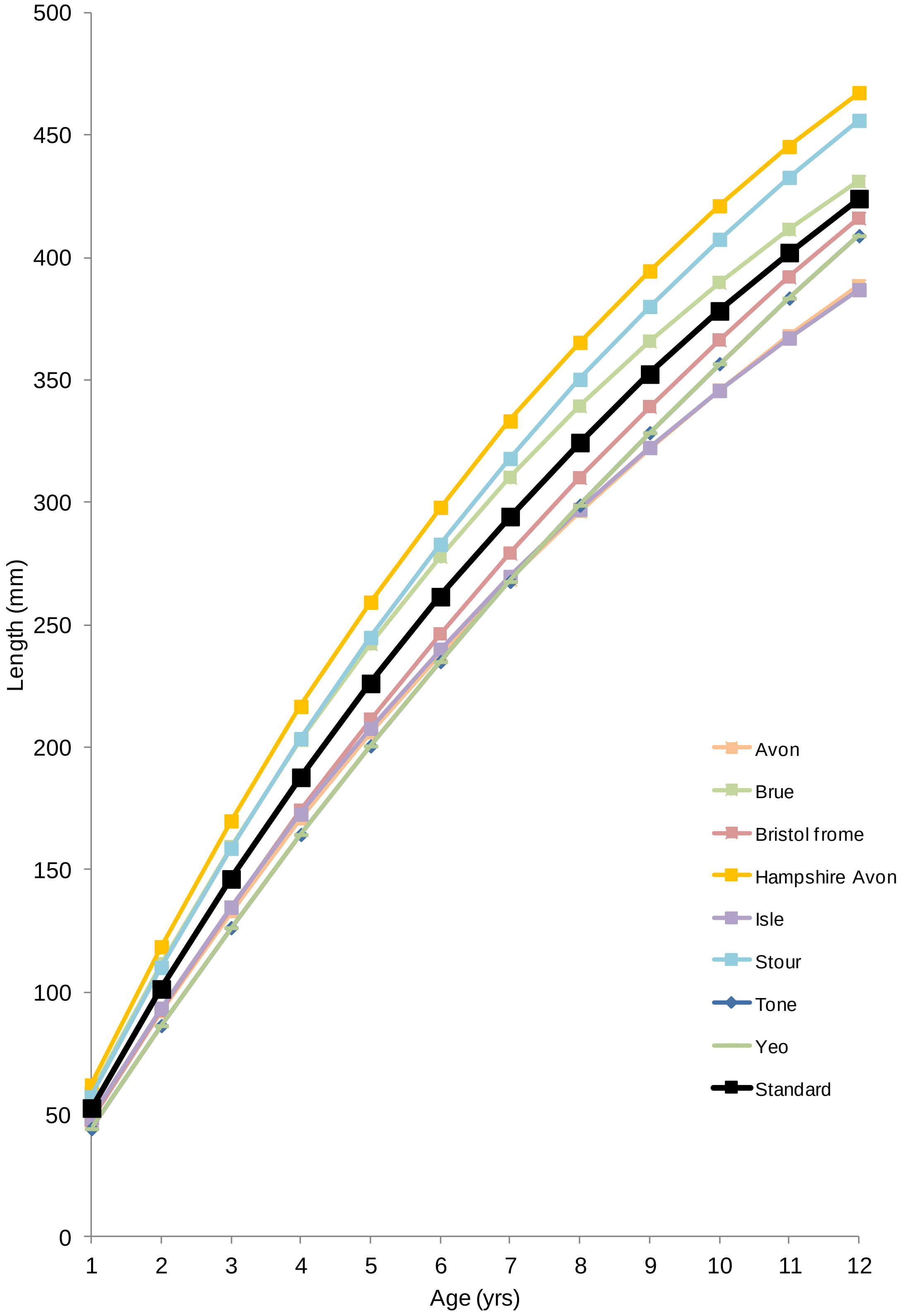
APPENDIX 10 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. cephalus* used to create the standard growth curve (black) for the Environment Agency North East region.



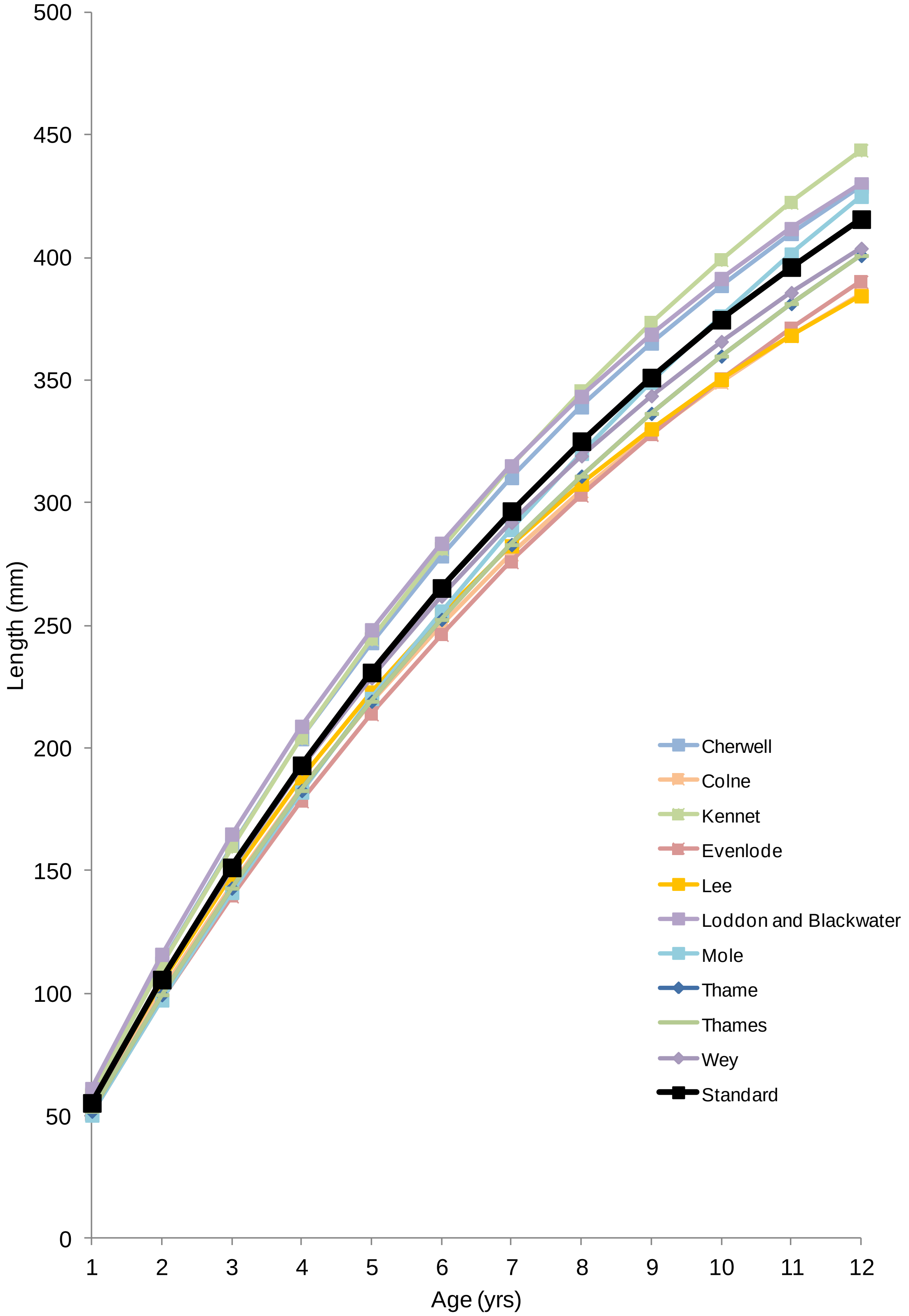
APPENDIX 11 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. cephalus* used to create the standard growth curve (black) for the Environment Agency North West region.



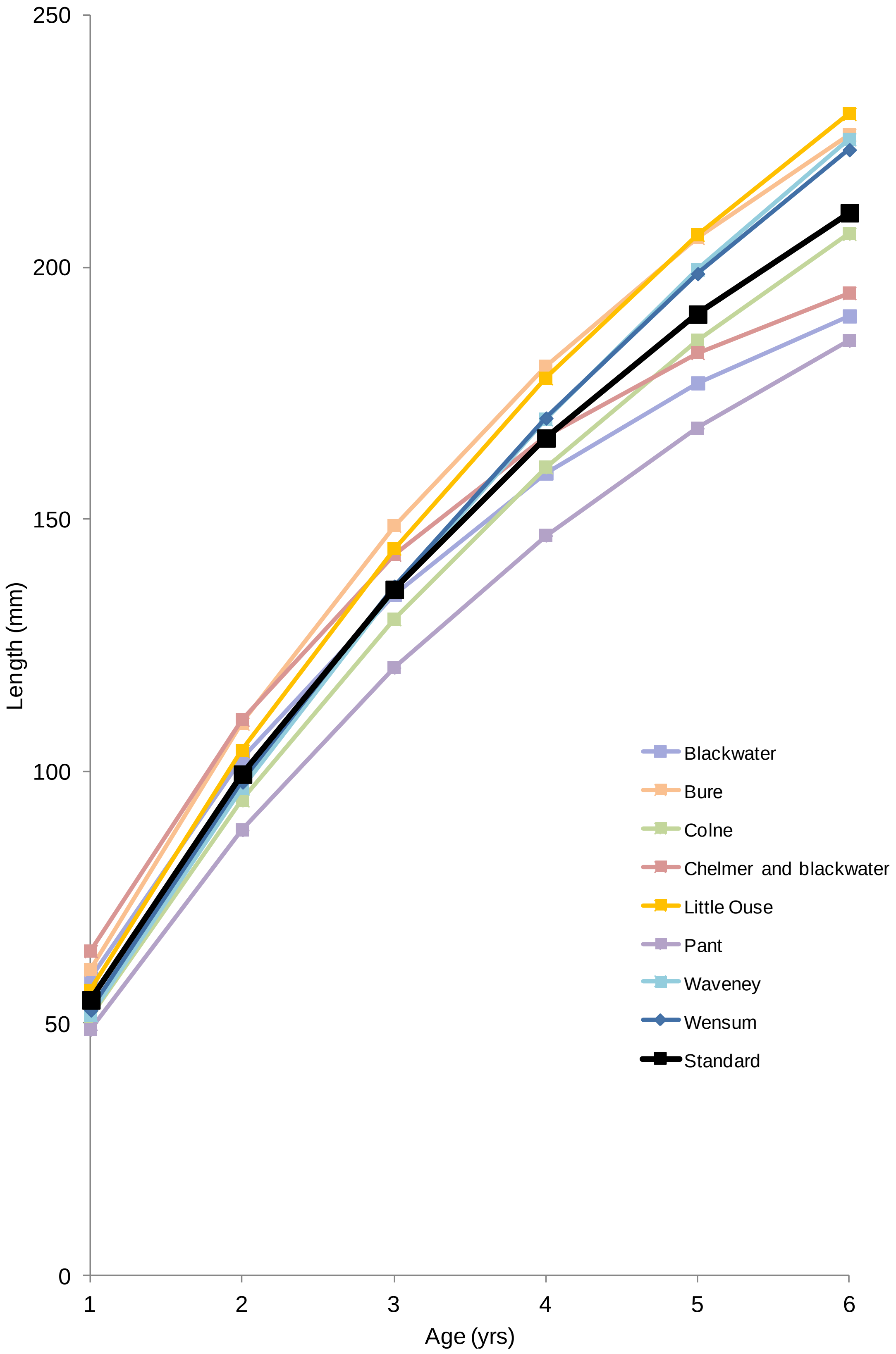
APPENDIX 12 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. cephalus* used to create the standard growth curve (black) for the Environment Agency Southern region.



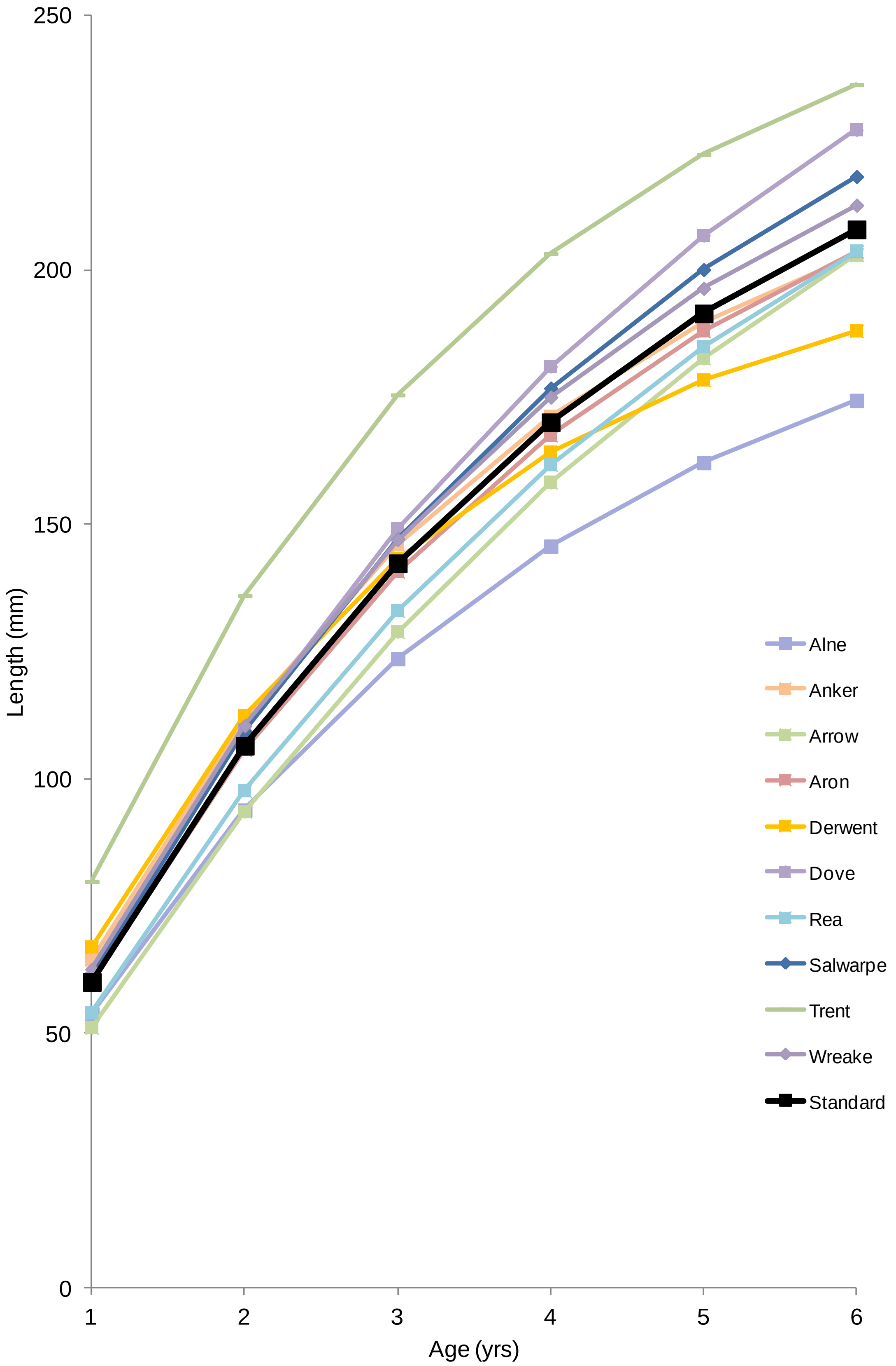
APPENDIX 13 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. cephalus* used to create the standard growth curve (black) for the Environment Agency South West region.



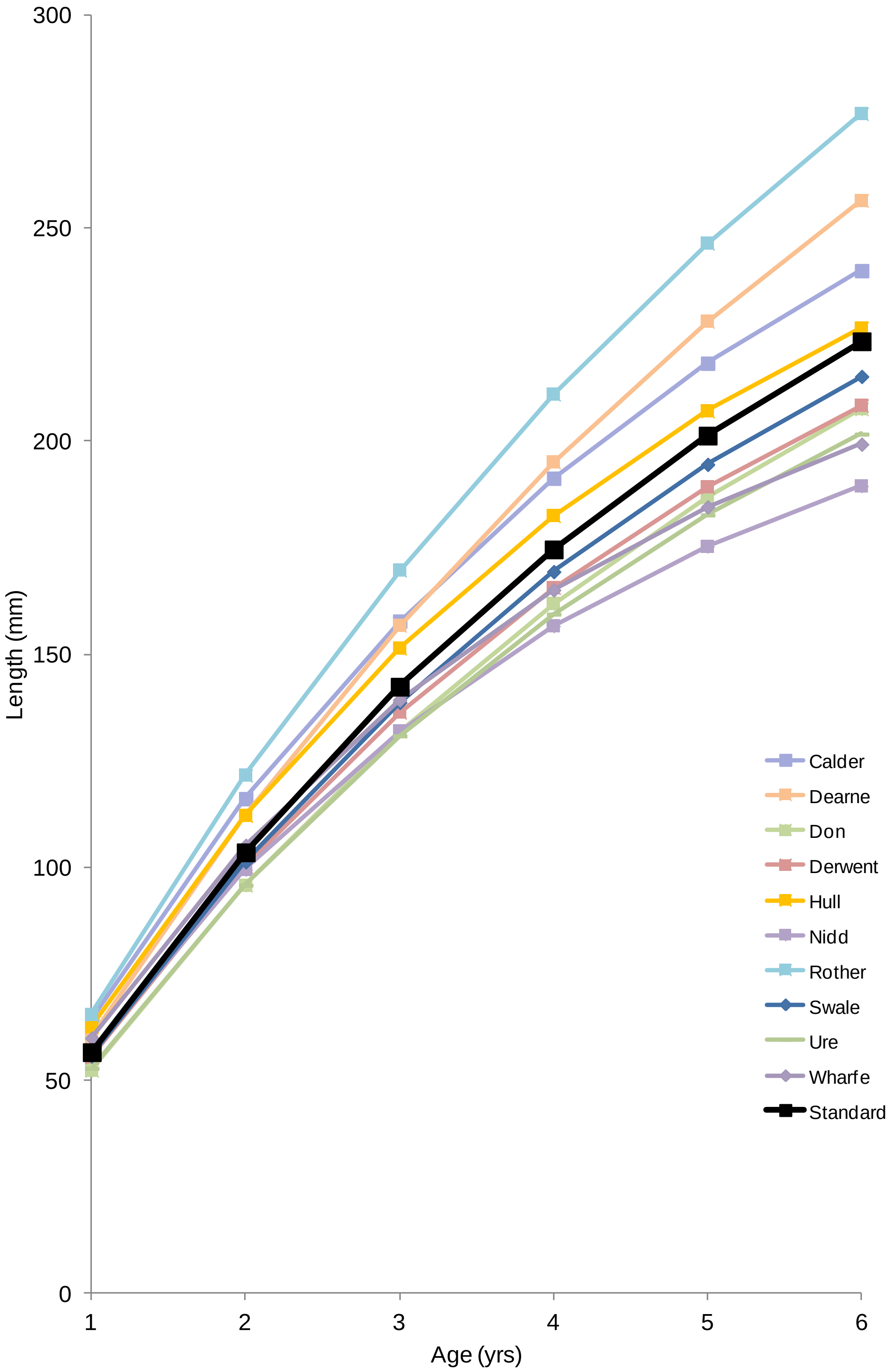
APPENDIX 14 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. cephalus* used to create the standard growth curve (black) for the Environment Agency Thames region.



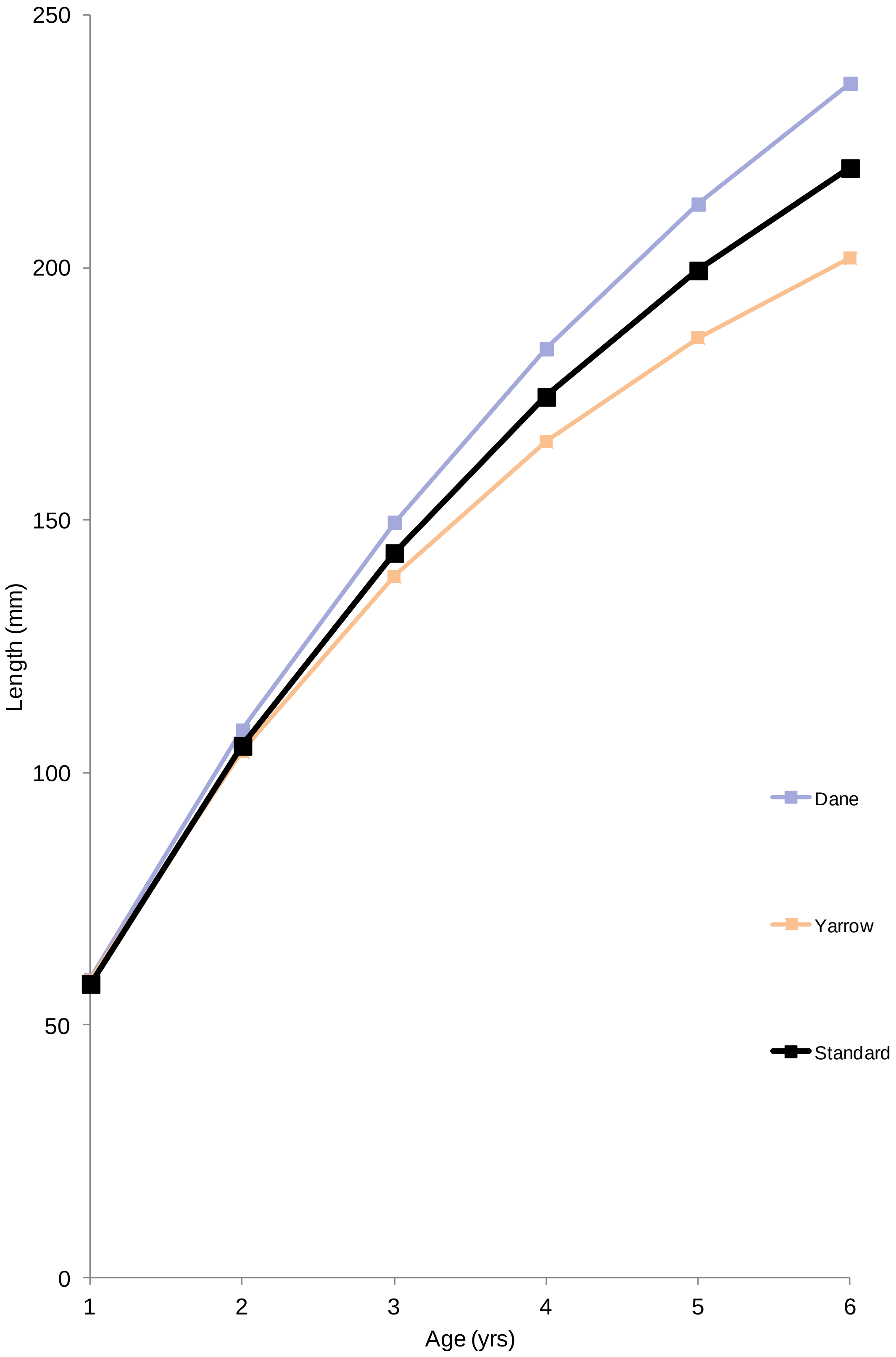
APPENDIX 15 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. leuciscus* used to create the standard growth curve (black) for the Environment Agency Anglian region.



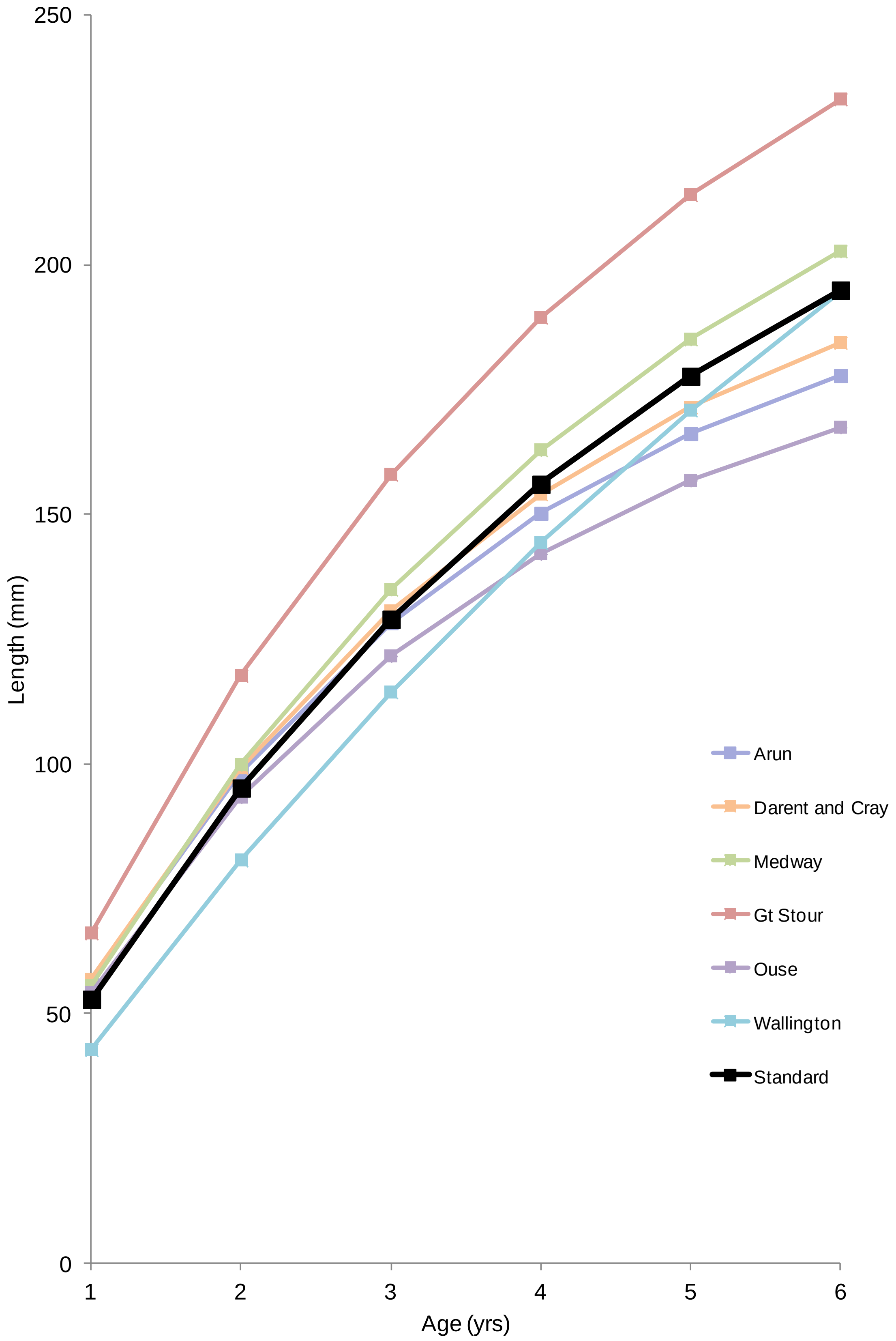
APPENDIX 16 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. leuciscus* used to create the standard growth curve (black) for the Environment Agency Midlands region.



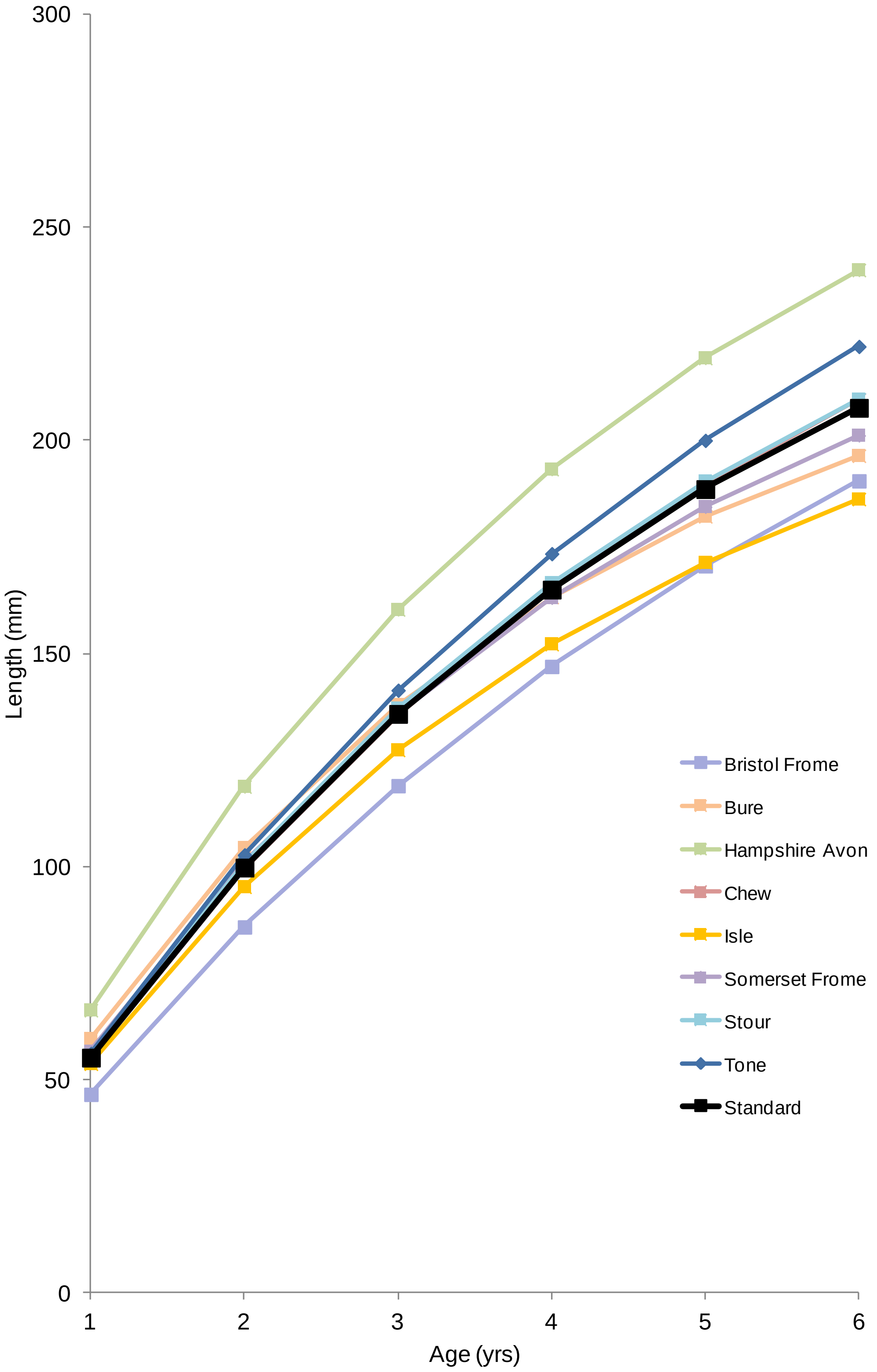
APPENDIX 17 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. leuciscus* used to create the standard growth curve (black) for the Environment Agency North East region.



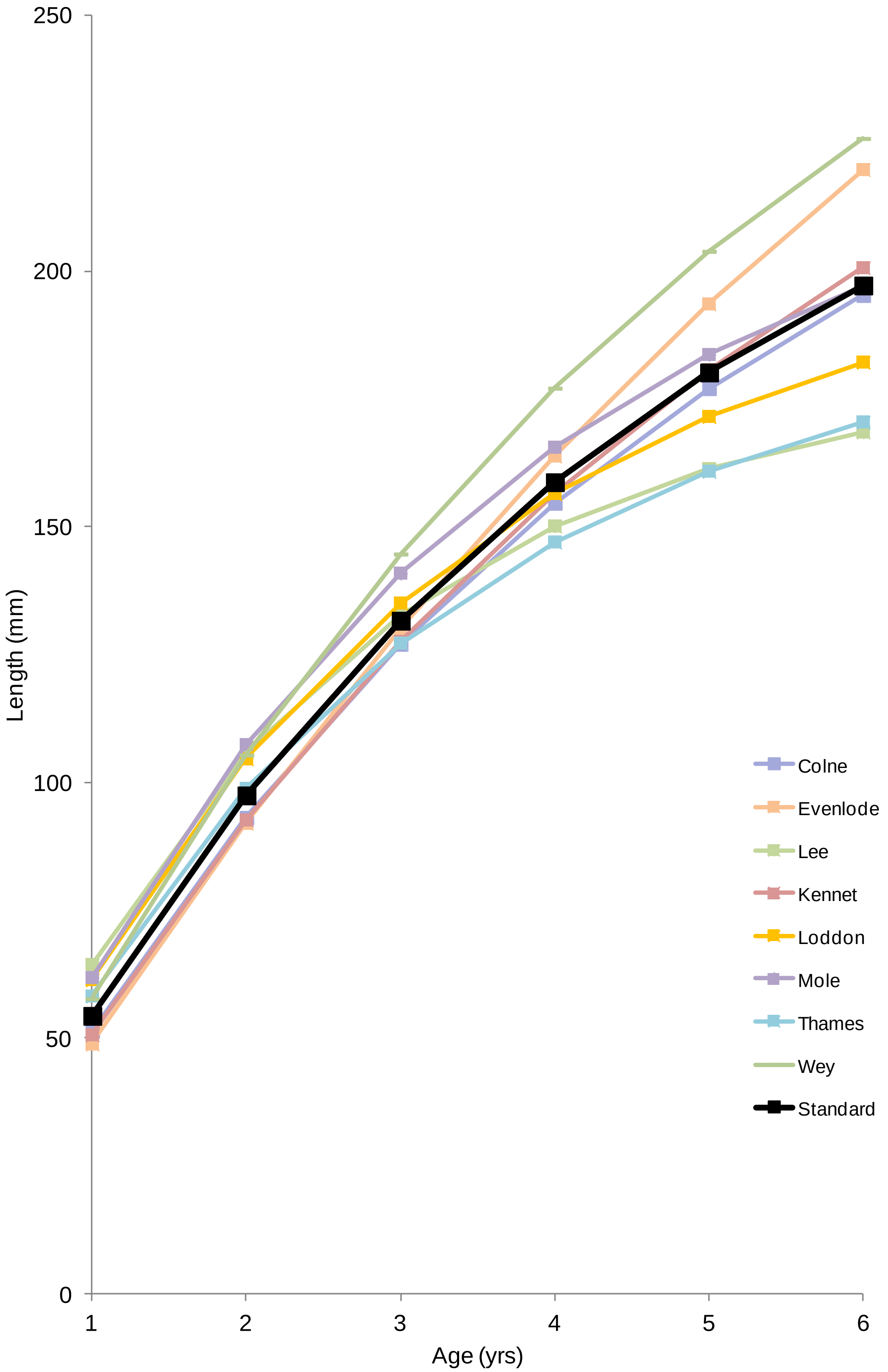
APPENDIX 18 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. leuciscus* used to create the standard growth curve (black) for the Environment Agency North West region.



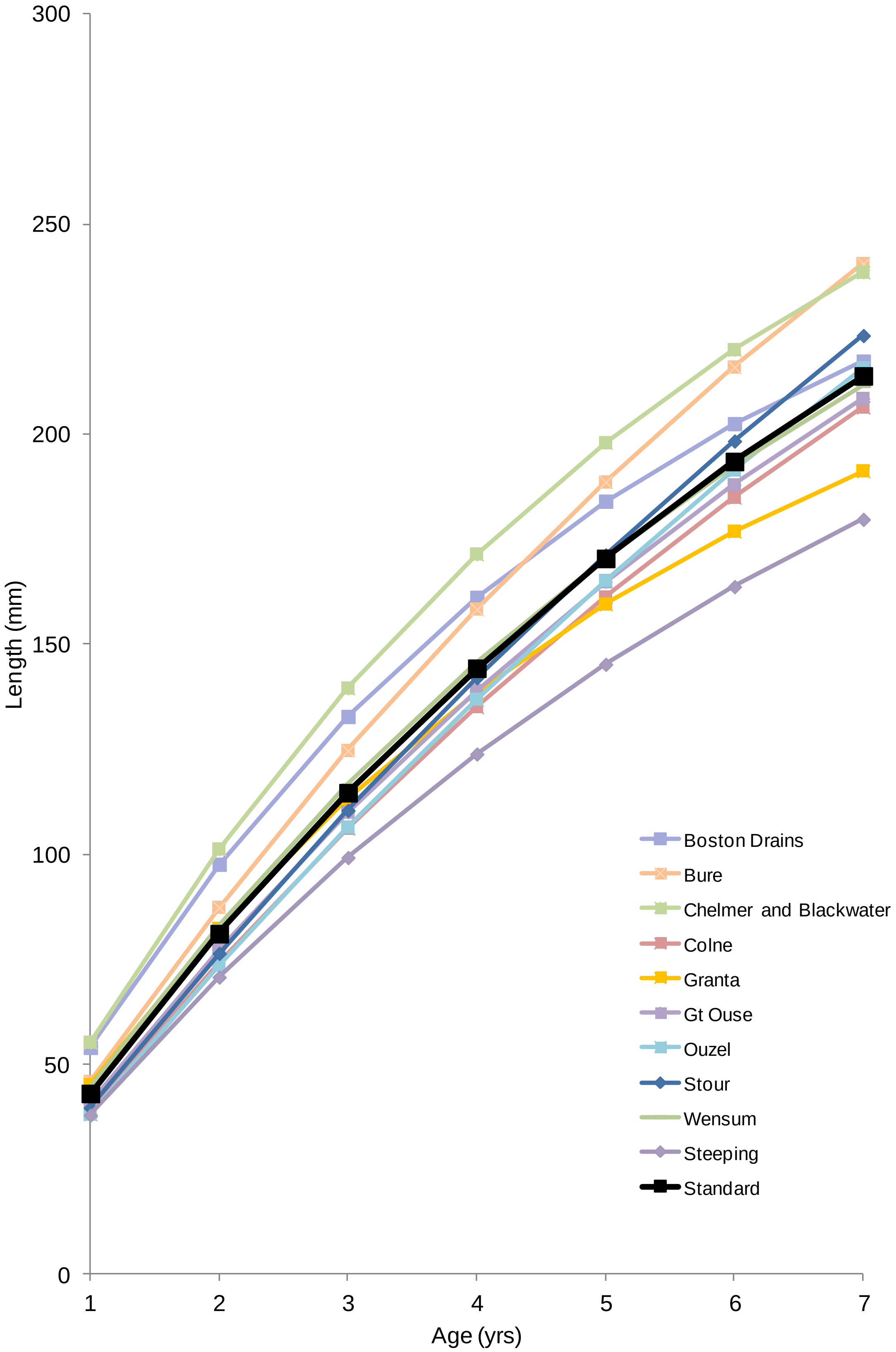
APPENDIX 19 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. leuciscus* used to create the standard growth curve (black) for the Environment Agency Southern region.



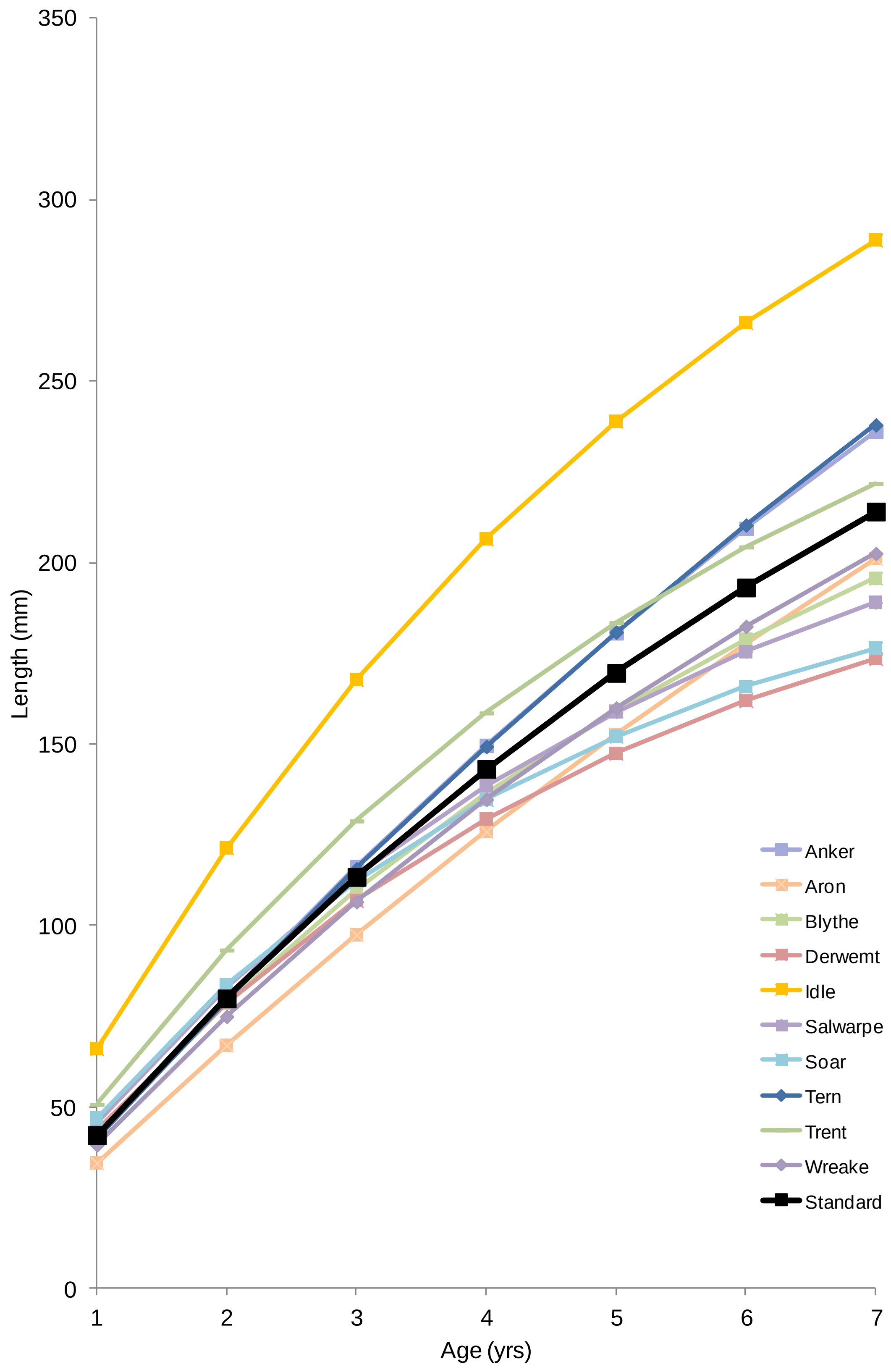
APPENDIX 20 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. leuciscus* used to create the standard growth curve (black) for the Environment Agency South West region.



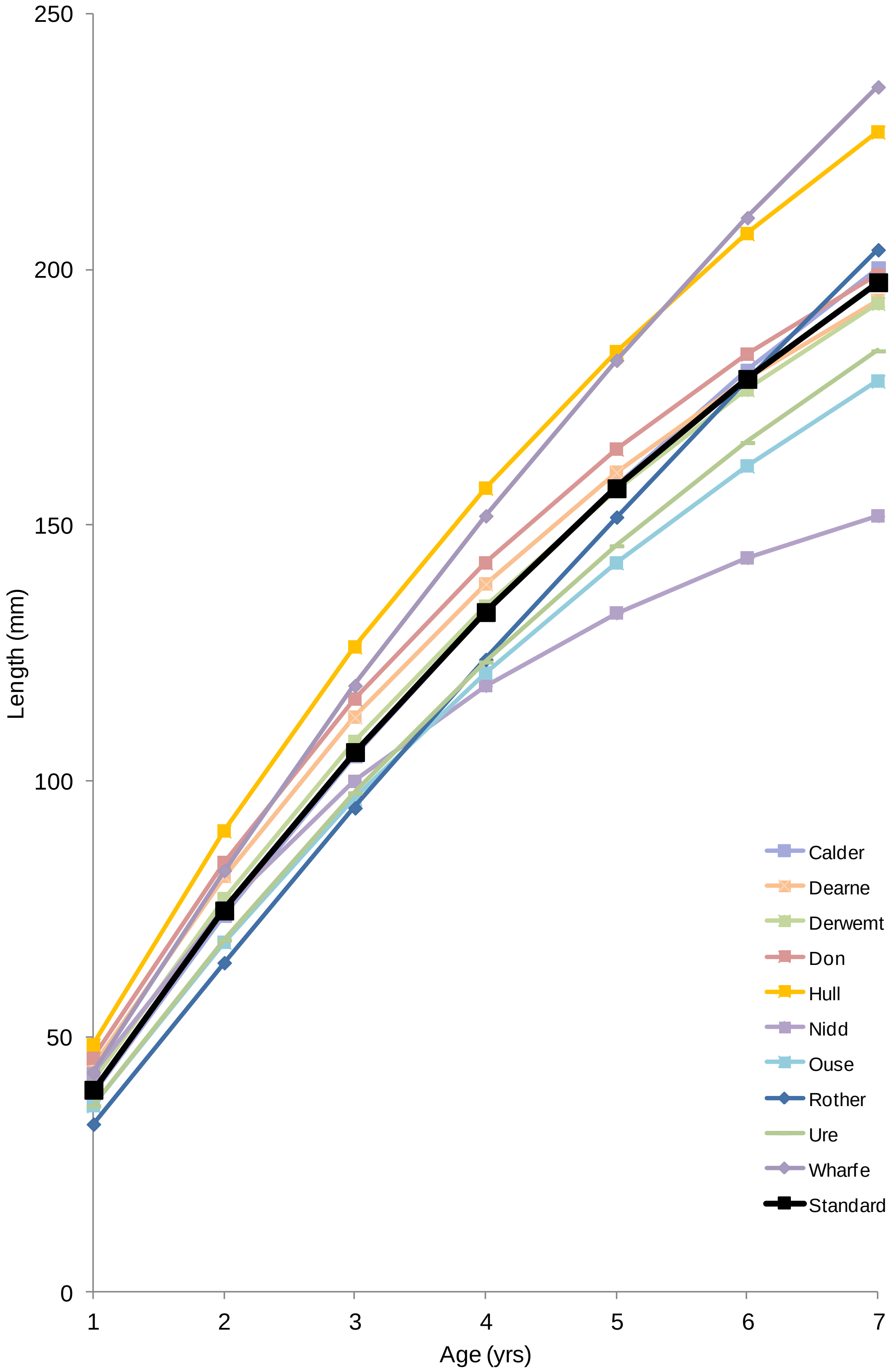
APPENDIX 21 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *L. leuciscus* used to create the standard growth curve (black) for the Environment Agency Thames region.



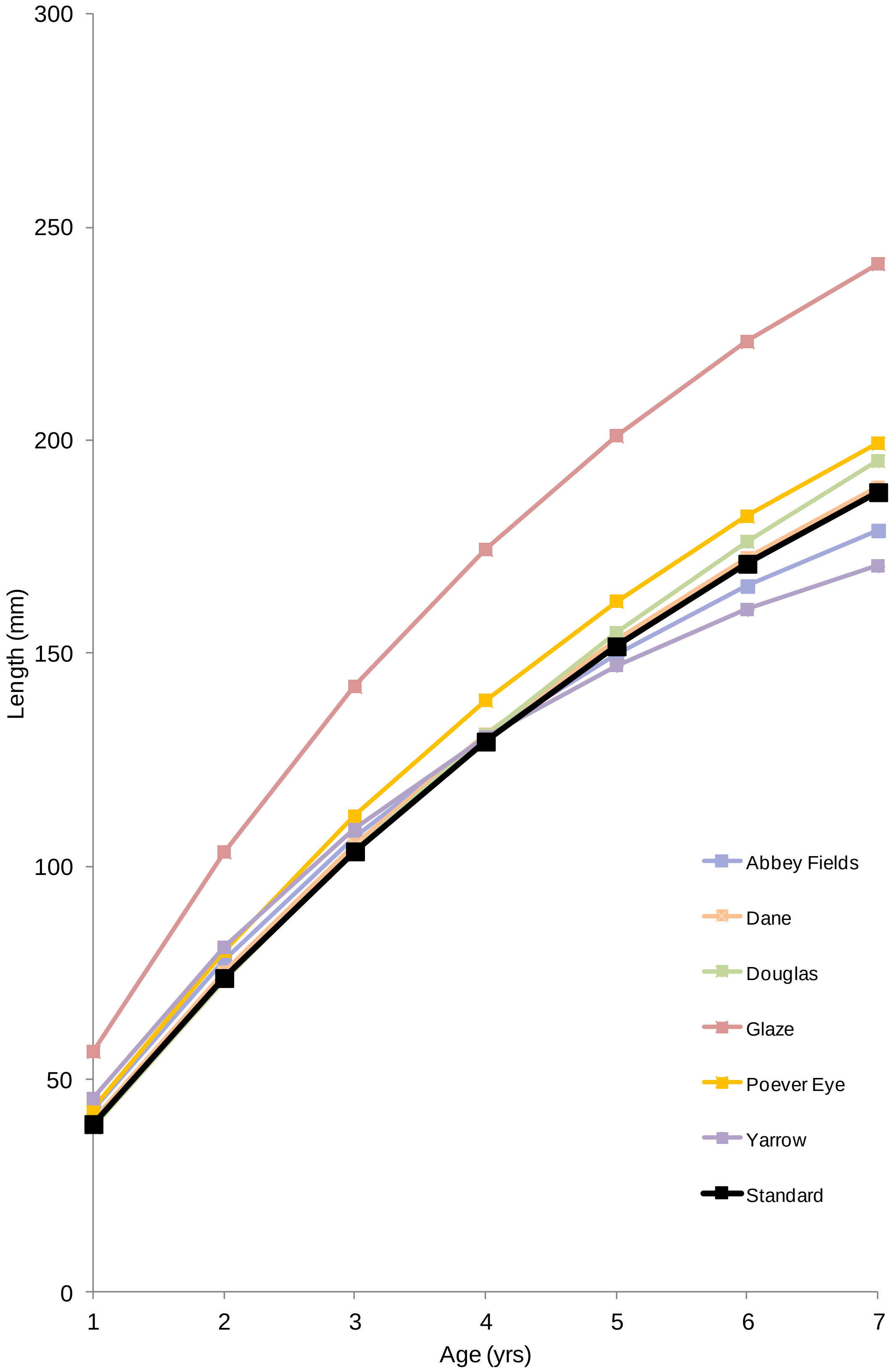
APPENDIX 22 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of R. rutilus used to create the standard growth curve (black) for the Environment Agency Anglian region.



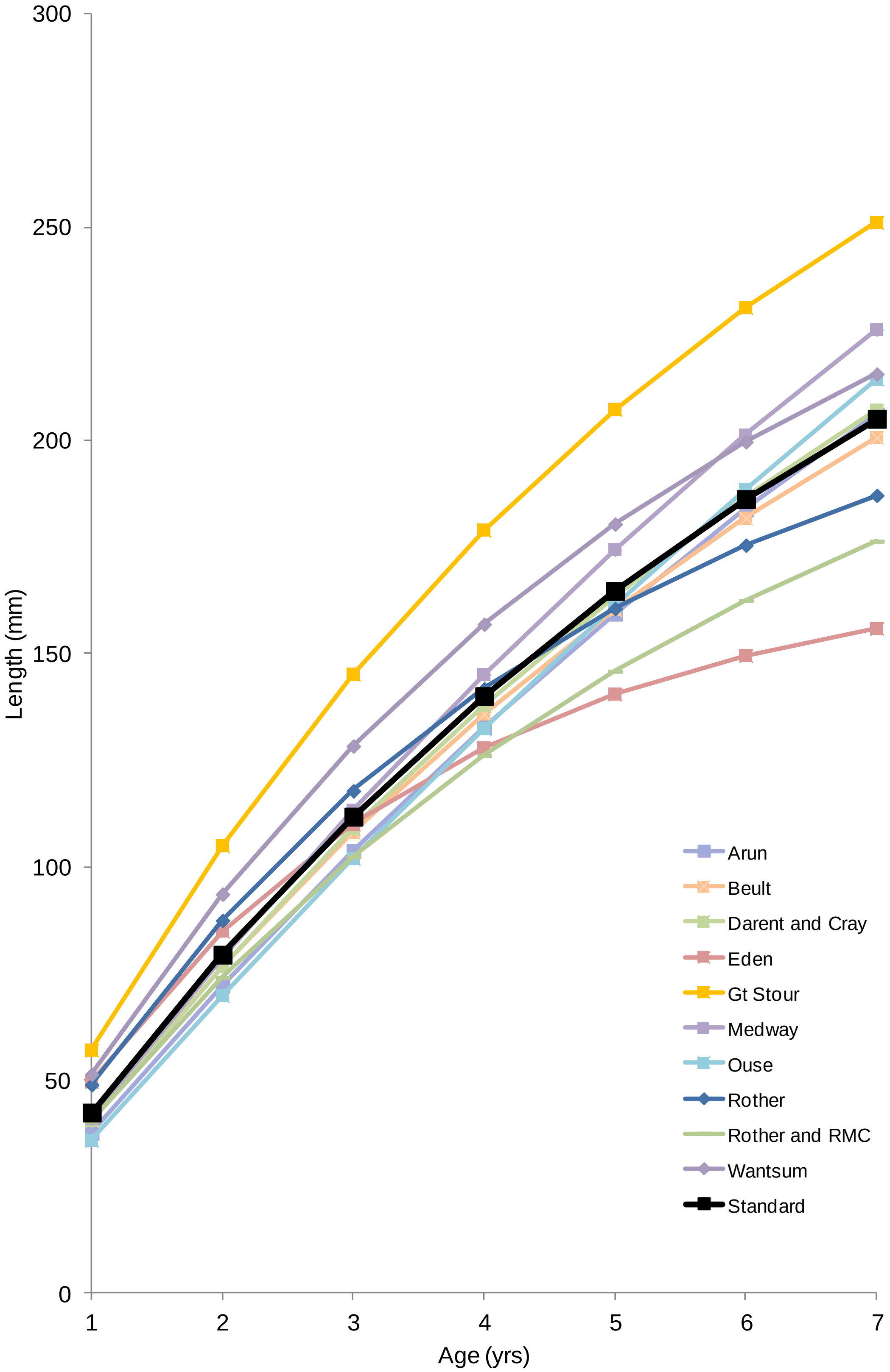
APPENDIX 23 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *R. rutilus* used to create the standard growth curve (black) for the Environment Agency Midlands region.



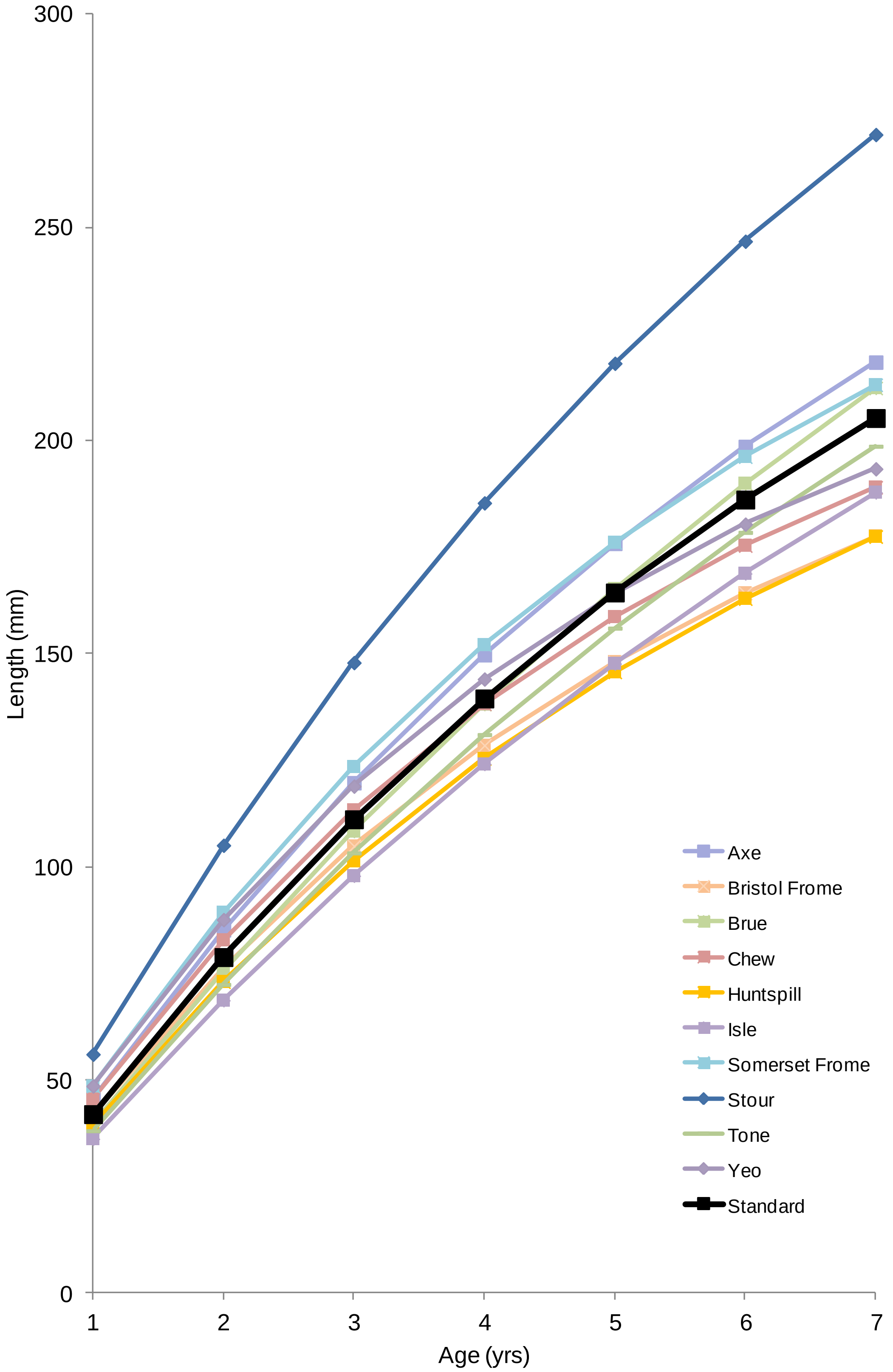
APPENDIX 24 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *R. rutilus* used to create the standard growth curve (black) for the Environment Agency North East region.



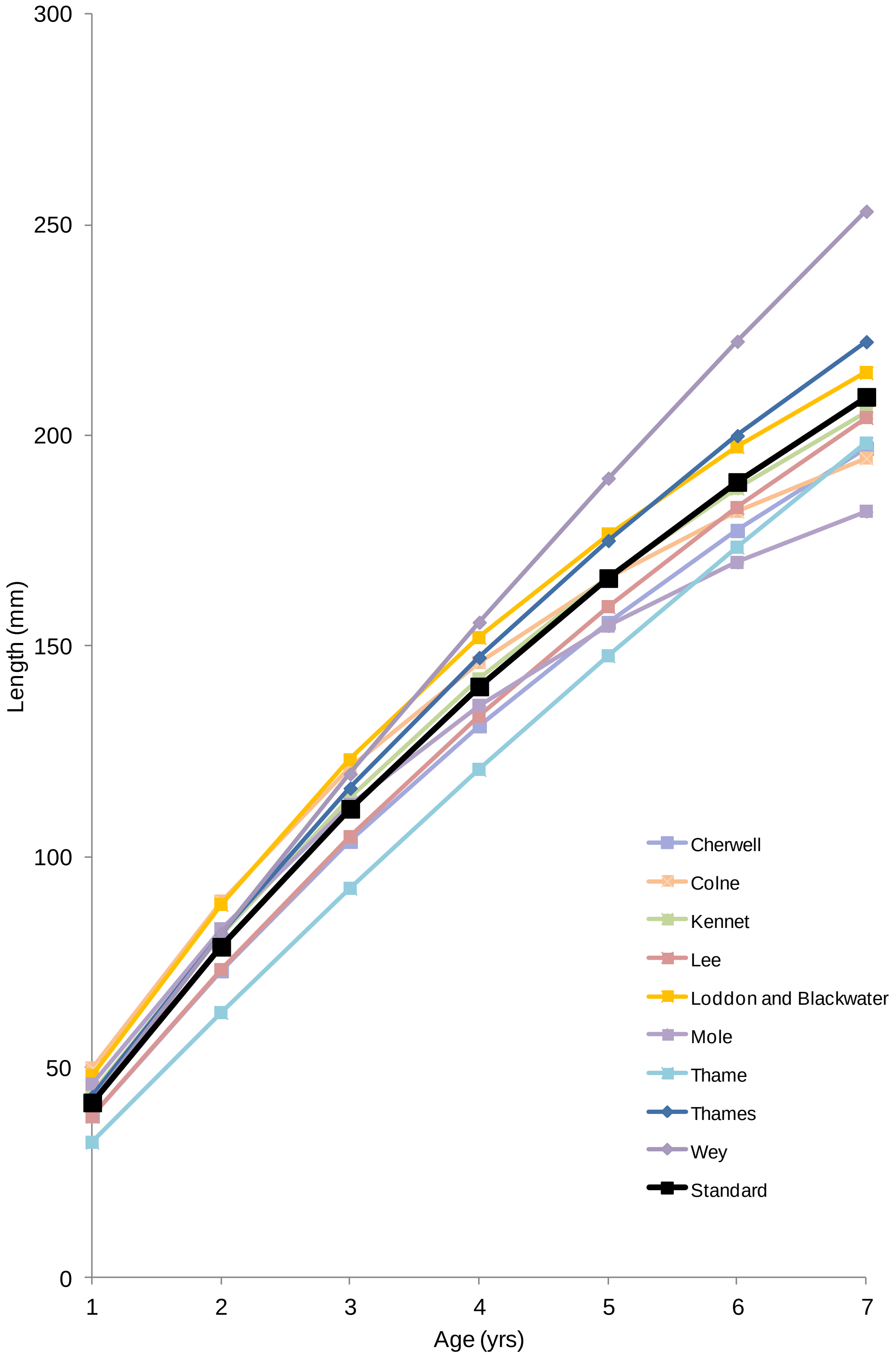
APPENDIX 25 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *R. rutilus* used to create the standard growth curve (black) for the Environment Agency North West region.



APPENDIX 26 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *R. rutilus* used to create the standard growth curve (black) for the Environment Agency Southern region.



APPENDIX 27 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *R. rutilus* used to create the standard growth curve (black) for the Environment Agency South West region.



APPENDIX 28 Growth curves (calculated according to Hickley and Dexter (1979)) for the individual populations of *R. rutilus* used to create the standard growth curve (black) for the Environment Agency Thames region.