

Selective and genetic constraints on the evolution of body size in a stream-dwelling salmonid fish

A. J. WILSON,* J. A. HUTCHINGS† & M. M. FERGUSON*

*Department of Zoology, University of Guelph, Guelph, ON, N1G 2W1, Canada

†Department of Biology, Dalhousie University, Halifax, NS, B3H 4J1, Canada

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Abstract

To examine constraints on evolution of larger body size in two stunted populations of brook charr (*Salvelinus fontinalis*) from a single river in Cape Race, Newfoundland, Canada, we measured viability selection acting on length-at-age traits, and estimated quantitative genetic parameters *in situ* (following reconstruction of pedigree information from microsatellite data). Furthermore we tested for phenotypic differentiation between the populations, and for association of high juvenile growth with early maturity that is predicted by life history theory. Within each population, selection differentials and estimates of heritabilities for length-at-age traits suggested that evolution of larger size is prevented by both selective and genetic constraints. Between the populations, phenotypic differentiation was found in length-at-age and age of maturation traits, whereas early maturation was associated with increased juvenile growth (relative to adult growth) both within and between populations. The results suggest an adaptive plastic response in age of maturation to juvenile growth rates that have a largely environmental basis of determination.

Introduction

Evolutionary constraints may be defined as restrictions or limitations on the course or outcome of evolution (Arnold, 1992). Although many types of constraints have been defined, these may be broadly separated into those that arise from genetic limitations and those that result from selection (Slichting & Pigliucci, 1998). In teleost fishes body size is generally considered to be a fitness-related trait, and as such may be subject to various forms of selection. For example, mortality processes may operate in a size-selective manner, with viability selection often acting according to a 'bigger is better' template (especially in juveniles; Sogard, 1997). This is a pattern of mortality that might be attributed to predation (particularly by piscivorous fish that are gape-limited predators; Mittelbach & Persson, 1998), or to differential overwinter mortality (Hunt, 1969; Henderson *et al.*, 1988; Post & Evans, 1989). Body size is also frequently associated with components of fitness other than survival. For example, there is typically a positive relationship between female

body size and fecundity (Kaeriyama *et al.*, 1995; Visman *et al.*, 1996; Morita & Takashima, 1998), and sexual selection (in the form of intra-male competition) may also favour large male size (Kitano, 1996).

However, the evolution of a trait under selection may be constrained by genetic factors such as the absence of a heritable component of phenotypic variance, or genetic correlations among traits that give rise to trade-offs among components of fitness (Roff, 2002). Thus, teleost body size is only expected to evolve if it has a significant genetic component of phenotypic variance. In salmonids, many laboratory-based studies have demonstrated the presence of additive genetic variance for size and growth traits (e.g. Nilsson, 1992; Gjedrem, 2000 and references therein). Despite this finding, and the expectation that selection is often likely to favour large size, the phenomenon of stunting (diminished growth relative to the potential of the species) is a relatively common occurrence in land-locked salmonids (e.g. see Leggett & Power, 1969; Reimers, 1979; Balon, 1980).

Stunting is usually considered to be a plastic response of growth rate to the environment, such that genetic variance may be relatively unimportant in the determination of size and growth (Heath & Roff, 1987; Hutchings,

Correspondence: A. J. Wilson, Department of Zoology, University of Guelph, Guelph, ON, N1G 2W1, Canada.
Fax: 519-767-1656; e-mail: awilso00@uoguelph.ca

1996). Resource limitation is the most likely cause of stunted growth (Ylikarjula *et al.*, 1999), and may result from density-dependent effects such as interference competition, or insufficient supply of one or more food components (Heath & Roff, 1996). Support for this perspective comes from several lines of evidence. For example, Heath & Roff (1987) failed to detect differences in growth rate when rearing fish from stunted and nonstunted populations under common laboratory conditions. Additionally, stunted growth has often been rapidly relieved by removal of fish biomass (e.g. Linlocken & Holt Seeland, 1996), or by increasing mortality rates so as to alleviate the resource limitation.

In addition to genetic factors, the evolution of larger body size in stunted populations might be constrained by local selection regimes. Although selection for large size may be common, it is not necessarily ubiquitous and in fact, small size may be adaptive in some cases. Instances of selection favouring small size are not well documented in teleost fishes. However, at a species level, adaptiveness of small size has been related to the efficiency of feeding on small prey, and to the potential for exploitation of spatially restricted habitats (Miller, 1979). It has also been suggested that, although selection on fecundity and mating success are more likely to favour large size in most organisms, body size may be limited by opposing selective forces operating primarily through viability (Blanckenhorn, 2000). Some instances of viability selection favouring small size have been reported in fishes, typically corresponding to selective predation on larger fish by nongape limited predators such as birds (Power *et al.*, 1989; Trexler *et al.*, 1994), bears (Konovalov & Shevlyakov, 1978) or human beings (Sinclair *et al.*, 2002).

In addition to direct effects on fitness, low growth rates and small size may have consequences for other phenotypic traits. In teleost fishes, this is particularly true of life history traits that are intimately linked to growth (Roff, 1984). Recently some attention has been paid to the possibility of adaptive life history variation in stunted populations (Ylikarjula *et al.*, 1999). In salmonids, age at maturity frequently shows an association with growth rate, such that higher juvenile growth rate (relative to adult growth) is associated with earlier maturation (e.g. Thorpe, 1986). As fecundity increases as a function of body size, this association is predicted to increase female fitness, under the assumption (made on the basis of energetic limitation) that there is a trade-off between future adult growth and present fecundity (Hutchings, 1993).

The current work examines constraints on the evolution of body size in nonanadromous brook charr, *Salvelinus fontinalis* (Mitchill), by examining local selection regimes and by estimation of heritabilities for age-specific size traits. We focus on the system of Freshwater River, Cape Race, Newfoundland, Canada. This fluvial system contains two putative populations, both characterized by very small size of adult fish, compared with the range

reported for this species (Power, 1980). These populations, hereafter designated Lower and Upper (according to their relative positions in the river), show no evidence of genetic differentiation at microsatellite loci, although they do appear to be demographically isolated, with mark – recapture study having detected very little movement of individuals between populations (Wilson, 2002).

In the current study we describe the phenotypic distributions of age-specific size traits within each population and in the river as a whole. To test the hypothesis that selection regimes impose a constraint on the evolution of larger body size, we use a comparison of trait distributions between age classes to calculate differentials of viability selection (Endler, 1986). In order to examine the possibility that lack of heritable variation poses a genetic constraint on evolution, we estimate heritabilities for length-at-age traits. Estimates of trait heritabilities are made *in situ*. Several approaches for the estimation of quantitative genetic parameters in the absence of a known pedigree have been recently proposed that rely on the use of molecular markers to infer either relatedness or explicit relationships between individuals (e.g. Ritland, 2000; Thomas & Hill, 2000). The utility of these methods has been demonstrated by comparison to more conventional parameter estimation in a range of organisms including captive populations of salmonid fishes (Mousseau *et al.*, 1998; Wilson *et al.*, 2003). It is expected that extension of this methodology to natural populations of fishes will best be achieved in small, closed populations (such as Freshwater River) in which it will be possible to sample high proportions of candidate relatives (Wilson & Ferguson, 2002). In this case, we reconstruct sibships that can then be used in conjunction with phenotypic data to estimate genetic parameters using a restricted maximum likelihood (REML) methodology (Thomas & Hill, 2000). Microsatellite genotypes previously obtained for the fish in Freshwater River (Wilson, 2002) are used to reconstruct sibships. In order to further examine the basis of phenotypic determination in this system we test for phenotypic differentiation between the two populations and consider the results in the context of the local selection regimes and quantitative genetic architecture. In addition to length-at-age traits, we examine the age composition of mature fish, and test for the predicted adaptive association between high juvenile growth (relative to adult growth) and early age at maturity.

Methods

Sampling

Sampling was carried out from 1–3 October 1999. Fish of all ages were sampled by electrofishing from the environs of spawning sites used by the two putative populations (Lower, $n = 398$; Upper, $n = 201$). All fish were measured (fork length to the nearest millimetre), and scale

samples were taken from the region between the anterior edge of the dorsal fin and the lateral line. Maturity status and sex (of mature individuals) was recorded, this being easy to determine from external morphology and coloration as sampling was conducted just prior to the onset of the spawning season. Prior to maturity external sexing of this species is not possible, so no sex information was determined for juveniles. Caudal fin clips were used as an identification mark to prevent possible repeat sampling of any individuals.

Scale-reading methodology

Length-at-age data were generated using hard part analysis performed on scale samples from each fish sampled. Scales were wet-mounted on glass slides and examined by microscope. Digital images were captured and calibrated using *Northern Eclipse version 5.0*. Fish were subsequently aged by the identification of annuli (checks on the scale that are formed as the growth of the individual slows in the winter; Carlander, 1987). Additionally, scale radii (defined as the distance from the focus to the margin along the longest axis of the scale) were measured from the digital image, using the software package SIGMA SCAN PRO 5. Scale radius at each annulus was also measured. All scales used were symmetrical, of typical size and shape, and lacked any indication of damage or regeneration.

The Fraser–Lee equation was used to back-calculate a history of length-at-age data for each individual (Hile, 1970; Carlander, 1987). A natural log transformation of both the fork length and scale radius data was performed, as it improved the linearity of the relationship between these variables ($R^2 = 0.7734$). The equation used was thus:

$$\ln(l_x) = c + [(\ln(L) - c) \ln(s_x) / \ln(S)],$$

where l_x is the back-calculated estimate of fork length-at-age x , L is the actual fork length at capture, s is the scale radius at age x and S is the scale radius at capture. The constant c was calculated as 0.4512, the intercept of a linear regression of $\ln(L)$ on $\ln(S)$.

Validation of the ageing and back-calculation methodology was made possible by a second period of sampling from 7 to 9 June 2000. At this time individuals showing evidence of a caudal fin clip were resampled and their ages determined as previously described. Microsatellite genotypes previously obtained (Wilson, 2002) were then used to identify these recaptured individuals from the set of all fish initially sampled. Sixteen loci were used allowing individuals to be individually identified by unique genotypes. Sample size was limited as only 24 of 53 suspected recaptures had genotypes that matched individuals among the 599 initially sampled. The low number of recaptures was probably due to high mortality in the system and potentially some natural tag loss. We re-examined the genotypes of all fish that might have

corresponded to the unidentified putative recaptures if genotyping error had occurred (up to six alleles different between the two samples). However, no additional fish were identified and no errors in genotyping were detected. Of the 24 recaptures, two individuals were aged inconsistently, being assigned to the same age group, as they had been the previous year. One of these fish showed zero growth as measured by change in fork length, which if translated directly to zero scale growth would account for this error in ageing.

Fork length in the fall of 1999 was estimated for the recaptured fish using the back-calculation procedure. Estimated and actual fall 1999 fork lengths were then compared; the mean difference (estimated fork length minus actual fork length) was found to be -1.9 mm. A paired-sample t -test showed no significant difference between estimated and actual fork lengths ($P = 0.70$). On this basis, the Fraser–Lee back-calculation procedure was deemed to be appropriate.

Data treatment

Length-at-age traits

Summary statistics were calculated for length-at-age traits (l_x) in each population and in the river overall. In order to test for differentiation in length-at-age traits between populations, t -tests were used to compare mean values after first verifying the normality of each length-at-age data set (by visual inspection of histograms and use of the Shapiro–Wilkes test). Significant deviation from normality was actually found in one case (age 0 + fish from the Lower population), but given the robustness of the t -test with large enough sample sizes (Brown & Rothery, 1993), no data transformation was applied. Comparisons were also made for each sex (after exclusion of all juveniles).

Selection on length-at-age

Initial attempts to examine selection on length-at-age traits by cohort tracking were prevented by insufficient numbers of recaptures being made for statistical analysis. As a result, selection on length-at-age traits was measured by comparing pre- and post-selection distributions for each age-specific length. Actual fork length at age x data were used as the pre-selection distribution. The post-selection distribution was obtained by estimating length-at-age x for all individual fish of age $x + 1$ or older, using the back-calculation procedure. This method necessarily makes the assumption that fish of age $x + 1$ are representative of fish aged x after a year of viability selection, with the back-calculation procedure correcting the post-selection sample for growth. Thus, the effect of fork length on survival over a year-long period of selection is examined. Standardized selection differentials i and j were calculated as measures of linear and nonlinear selection, respectively (Brodie *et al.*, 1995). These are measures of the proportional change in mean

(*i*) and variance (*j*) of a trait, and their significance and confidence limits can be readily estimated (see Endler, 1986). The analysis was performed for the set of all fish (for each population and for the river as a whole), and also for each sex separately (mature fish only).

Association between age of maturity and juvenile growth rate

Maturity as age 1+ was defined as early maturity (EM) and was tested for phenotypic association with high juvenile growth rate by comparing mean lengths of mature and immature fish of age 1+. The null hypothesis that mature 1+ fish were no larger than immature 1+ fish was tested in the overall river and in each population separately using one-tailed *t*-tests. In order to test for phenotypic association of age of maturity and juvenile growth rates across populations, the age composition of mature fish was described for each population by calculating the percentage of all mature fish in each age class. This was carried out for both sexes combined and for males and females separately. Heterogeneity χ^2 test was used to test the null hypothesis that population-specific age distributions were the same. Whilst length-at-age 0+ can be used as a measure of absolute juvenile growth rate (see below), a metric of juvenile growth rate relative to adult growth rate was defined as relative juvenile growth (RJG). Where:

$$RJG = (l_{1+} - l_{0+}) / (l_{3+} - l_{2+}).$$

This metric was calculated for all fish aged 3+ or older, and the mean values found for each population overall and for each sex. Population mean values were compared to test the hypothesis that increased juvenile growth rate relative to adult growth rate is associated with earlier age of maturity (Hutchings, 1993). It should be noted that this metric RJG is not perfect as it is based on growth rates of mature fish that were also expending energy into reproduction. An ideal comparison would be between the growth rate of juveniles, and that of adults expending all surplus energy into growth.

Estimation of quantitative genetic parameters

Within each age class individuals were partitioned into groups of putative full siblings using microsatellite data

previously obtained (Wilson, 2002). These genetic data comprised microsatellite genotypes for 16 loci with two to 16 alleles per locus (Table 1). There were no significant deviations from Hardy-Weinberg proportions observed either at individual loci, or over all loci, and no significant linkage disequilibrium was detected between pairs of loci in either population (Wilson, 2002). On this basis it is assumed that the loci used were selectively neutral and independent.

Sibling groups were partitioned using the Markov chain Monte Carlo (MCMC) approach described by Smith *et al.* (2001) which maximizes an overall likelihood score on the basis of pairwise likelihood ratios of being full siblings or unrelated. The algorithm is constrained such that within a group of putative siblings the genotypes at each locus must be able to be derived from a single parental pair. Multiple runs were performed to assess solution convergence, and reconstructed sibships were based on chain lengths of between 2 million (4+ age class) and 5 million (0+ age class) iterations. Sibship groups from each age class were then combined to give the complete reconstructed pedigree under the assumption that no full-sibling relationships exist between fish of different age classes. Reconstructed pedigrees were used in conjunction with phenotypic data for length-at-age traits and EM to estimate quantitative genetic parameters. In order to maximize sample sizes both actual and estimated length-at-age values were used, and separate sex-specific analyses were not performed. Genetic parameters were estimated for fish in the river as a whole using an REML procedure (implemented in VCE4; Groeneveld, 1994) under the sire model:

$$y_i = \mu + a_{si} + e_i,$$

where y_i is a phenotypic observation for individual *i*, μ is the population mean, a_{si} is the random sire effect and e_i is a residual error term. Estimates were made of trait heritabilities for length-at-age traits (l_{0+} , l_{1+} , l_{2+} and l_{3+}) and EM. As EM was scored as a binary trait, heritability estimates were transformed to the liability scale (Falconer & Mackay, 1996). Genetic correlations between EM and absolute juvenile growth rates were also estimated from the same model, using l_{0+} as a metric of juvenile growth rate. Population-specific estimates were obtained by

Table 1 Variability of microsatellite markers used, indicating number of alleles (*n*) and observed heterozygosity (H_o) at each locus.

Locus	<i>n</i>	H_o	Locus	<i>n</i>	H_o
SFO 12 LAV	2	0.516	BHMS 328	2	0.344
SFO 18 LAV	4	0.396	BHMS 356	4	0.531
SFO C28	4	0.626	BHMS 462	2	0.276
SFO D91	16	0.806	BHMS 7-001	2	0.504
SFO D100	6	0.658	BHMS 7-033	4	0.516
SCO 19	14	0.773	STR 85 INRA	12	0.698
SSA 20.19 NUIG	2	0.480	OMY 38 DU	3	0.518
SSA 85 DU	2	0.450	OMY RGT46 TUF	2	0.114
Mean	5.1	0.513			

exclusion of all individuals sampled in the alternate population from the analysis. Standard errors were also estimated using VCE4 and used to determine significance of estimated parameters.

Results

Length-at-age traits

Summary data confirm the system is characterized by small size of fish, a finding that is also common to both populations considered separately (Table 2). Nevertheless, there are clear differences in length-at-age between the two populations. Considering all fish, mean fork length is significantly higher for all age classes in the

Upper population (two-tailed *t*-tests, all $P \leq 0.01$). For mature fish, further comparisons reveal that the Upper population has consistently greater mean length-at-age in all age classes of females and males. For the females, differences were significant in all age classes but 1+ (where sample size was limited). The same qualitative pattern is repeated for male fish, although differences in mean values are not significant.

Selection on length-at-age

Significant differentials of selection acting on length-at-age traits were detected within the river overall and also within each population (Table 3). Overall, there is evidence of linear selection favouring increased juvenile

	Age class	Mean length, mm (SD, <i>n</i>)			<i>P</i>
		Overall	Lower	Upper	
All fish	0+	56.7 (5.3, 253)	56.3 (5.4, 214)	58.7 (4.4, 39)	0.011
	1+	82.9 (9.4, 92)	81.1 (8.4, 75)	90.7 (9.9, 17)	<0.001
	2+	109.6 (11.4, 111)	106.4 (9.5, 55)	112.8 (12.2, 56)	0.002
	3+	126.1 (13.8, 93)	120.3 (10.6, 33)	129.3 (14.4, 60)	0.002
	4+	133.1 (15.4, 34)	122.2 (8.4, 12)	139.1 (15.2, 22)	<0.001
	5+	162 (–, 1)	–	162.0 (–, 1)	–
Mature females	1+	93 (6.5, 12)	91.6 (5.2, 9)	97.3 (9.1, 3)	0.192
	2+	110.7 (11.6, 79)	106.8 (10.1, 41)	114.9 (11.7, 38)	0.001
	3+	125.6 (13.1, 73)	120.0 (10.0, 25)	128.6 (13.6, 48)	0.006
	4+	132.2 (14.1, 30)	122.2 (8.4, 12)	138.9 (13.1, 18)	<0.001
	5+	162 (–, 1)	–	162 (–, 1)	–
Mature males	1+	86.6 (10.2, 35)	84.8 (10.2, 21)	89.3 (9.8, 14)	0.201
	2+	107 (10.6, 32)	105.2 (7.6, 14)	108.4 (12.5, 18)	0.401
	3+	126.8 (16.8, 21)	121.4 (12.8, 8)	132.2 (17.7, 12)	0.157
	4+	139.8 (25, 4)	–	139.8 (25.0, 4)	–

Table 2 Summary of mean length-at-age data for all age classes of *Salvelinus fontinalis* in Freshwater River overall and in each population; *P* values are those obtained from two-tailed *t*-tests to compare mean between populations.

	Trait	Overall		Lower		Upper	
		<i>L</i>	<i>j</i>	<i>i</i>	<i>j</i>	<i>i</i>	<i>j</i>
All fish	l_{0+}	+1.65***	+1.79***	+1.38***	+1.72***	+2.02***	+2.81***
	l_{1+}	+1.12***	+0.16	+1.20***	+0.37	+0.44	+0.04
	l_{2+}	+0.06	+0.05	–0.01	–0.03	–0.03	–0.07
	l_{3+}	–0.3	–0.06	–0.72*	–0.018	–0.18	–0.21
Females	l_{1+}	+0.08	+1.41	–0.05	+2.69	–0.23	+0.14
	l_{2+}	–0.07	–0.03	–0.10	+0.08	–0.24	–0.07
	l_{3+}	–0.3	–0.08	–0.73*	–0.15	–0.09	–0.10
Males	l_{1+}	+0.63**	+0.11	+0.61*	–0.15	+0.51	+0.36
	l_{2+}	+0.46	+0.33	+0.49	+0.80	+0.41	+0.14
	l_{3+}	–0.13	+1.17	–	–	–0.41	+1.08

Differentials were calculated for all age classes with sufficient sample size (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Table 3 Differentials of linear (*i*) and non-linear (*j*) selection acting on length-at-age traits of *Salvelinus fontinalis* in Freshwater River overall and in each population.

growth, as measured by length-at-age 0+ ($i = +1.65$, $P < 0.001$). The same pattern of size-selective mortality is found in the 1+ fish ($i = +1.12$, $P < 0.001$). Sex-specific differentials show that this selection is not only acting on juvenile 1+ fish, but also on the males of this age ($i = +0.63$, $P < 0.01$). There is no evidence for nonlinear selection acting on the overall, or sex-specific, length-at-age traits (Table 3), except for the case of l_{0+} for which an increase in variance was detected in the post-selection sample ($j = +1.79$, $P < 0.001$).

In the Lower population, linear differentials indicate selection for increased l_{0+} ($i = +1.38$, $P < 0.001$) and l_{1+} ($i = +1.20$, $P < 0.001$). Interestingly there is evidence for a reversal of this directional selection in older fish, with selection favouring smaller l_{3+} . In fact as there were no males older than 3+ at the lower site from which to estimate the post-selection sample, this result can be attributed entirely to effects on female 3+ fish. This is reflected by the differential i for this age group of females in this population ($i = -0.73$, $P < 0.05$). Nonlinear selection acting on l_{0+} was also detected ($j = +1.72$, $P < 0.001$).

In the Upper population, selection favouring increased length-at-age 0+ ($i = +2.02$, $P < 0.001$) is also seen. However, there is no evidence for linear selection acting on any other age class. Sex-specific differentials confirm this pattern with no significant directional selection acting on any mature fish. Again nonlinear selection acting on l_{0+} was detected ($j = +2.81$, $P < 0.001$).

Association between age of maturity and juvenile growth rate

Scale analysis revealed that all individuals aged 2+ or older were mature and all 0+ individuals were immature. The 1+ age class thus contained a mixture of juvenile and mature fish. Overall mature fish of age 1+ were found to be significantly larger than immature individuals of the same age (one-tailed t -test, $P < 0.001$). The same result was found within the Lower population (one-tailed t -test, $P < 0.001$). In the Upper population this relationship could not be tested as the sample size of immature fish was small ($n = 39$) and comprised entirely of individuals from the 0+ age class.

The two populations differed in the percentage composition of mature fish by age class (Fig. 1). For both sexes combined, the age composition of mature fish in the Lower population is shifted to the left (i.e. to younger ages) relative to the Upper population (Fig. 1a). In particular 1+ fish constitute 23.1% of the mature fish in the Lower population, but only 10.9% in the Upper. Thus, there is a greater incidence of maturity at age 1+ in the Lower population. This pattern is further demonstrated by the fact that the modal age class of mature fish in the Lower population is 2+ compared with 3+ in the Upper, and the Upper shows a greater contribution to the spawning stock from age classes 4+ and 5+. The difference between the

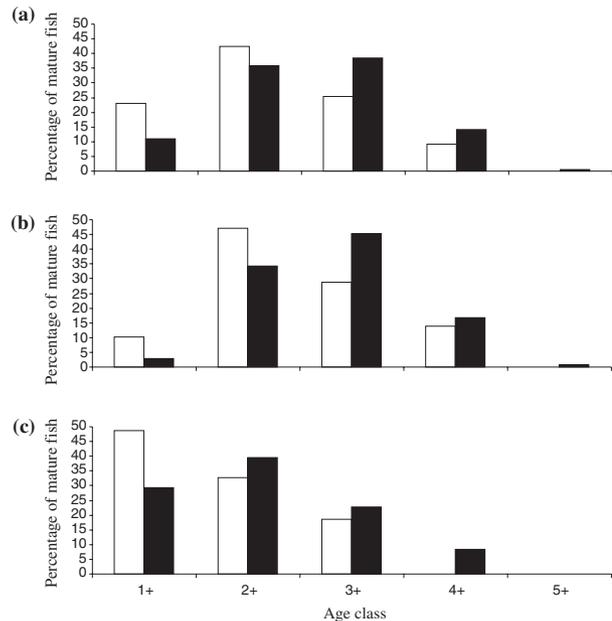


Fig. 1 Age composition of mature fish in the Lower (white) and Upper (black) populations. Panel (a) shows all fish, panel (b) females only and panel (c) males only.

two distributions is statistically significant ($\chi^2 = 12.46$, d.f. = 3, $P = 0.005$).

The same qualitative pattern is seen in each sex separately with the distributions in the Lower population shifted left relative to the Upper (Fig. 1b,c). Again there are differences in modal classes. For females, the largest group is the 2+ in the Lower and 3+ in the Upper; for males, it is 1+ in the Lower and 2+ in the Upper. The difference in distributions is significant for females ($\chi^2 = 10.43$, d.f. = 3, $P = 0.015$) but not for males ($\chi^2 = 4.03$, d.f. = 2, $P = 0.133$). Sample sizes were considerably smaller for males (Table 2).

Mean RJGs were higher at the lower site in accordance with the predicted association between EM and high juvenile growth rate relative to adult growth (Table 4). The differences were marginally significant using a one-tailed t -test for the sexes combined ($P = 0.075$), and for females alone ($P = 0.087$), but not for males.

Genetic parameters

Significant trait heritabilities were found for all traits examined in Freshwater River overall (Table 5), with values being low–moderate (range from 0.148 to 0.346). In addition a significant positive genetic correlation was detected between EM and l_{0+} . Within the Lower population alone heritabilities for l_{1+} , l_{2+} and l_{3+} were significant but estimates for l_{0+} and EM were not. However, the estimate of genetic correlation between

Table 4 Mean (SD) values of relative juvenile growth of *Salvelinus fontinalis* in Freshwater River, *P* values are from one-tailed *t*-tests comparing mean between populations with the alternate hypothesis that mean RJG is greater in the Lower population than in the Upper.

	Lower	Upper	<i>P</i>
All fish	2.69 (3.31)	1.96 (0.86)	0.075
Females only	2.79 (3.62)	1.96 (0.86)	0.087
Males only	2.22 (1.49)	1.95 (1.40)	0.522

Table 5 Estimates of trait heritabilities and genetic correlations (SE) in Freshwater River overall and for each population.

Trait(s)	Heritability/genetic correlation		
	Overall	Lower	Upper
l_{0+}	0.346 (0.036)*	0.014 (0.040)	0.038 (0.045)
l_{1+}	0.304 (0.045)*	0.261 (0.124)*	0.001 (0.040)
l_{2+}	0.248 (0.057)*	0.276 (0.093)*	0.000 (0.000)
l_{3+}	0.148 (0.068)*	0.229 (0.139)*	0.094 (0.111)
EM	0.330 (0.090)*	0.020 (0.035)	–
l_{0+} –EM	1.000 (0.002)*	0.873 (0.120)*	–

*Denotes significantly greater than zero at $P < 0.05$.

these latter two traits was still found to be significantly greater than zero. Within the Upper population alone there was no evidence for significant heritabilities for any traits. Note that given a lack of phenotypic variance for EM in the sample for this population no parameters for this trait could be estimated.

Discussion

Brook charr in Freshwater River as a whole exhibit stunted growth, with mean lengths-at-age being low in comparison with the potential for this species (Power, 1980). Nevertheless, there is also convincing evidence for phenotypic differentiation, with fish from the Upper population being larger, on average, in each age class, reflecting higher rates of absolute growth. Given this observed differentiation, selective and genetic constraints on evolution are most appropriately considered in a population-specific context.

In the Lower population, evolution of larger body size in adult fish is constrained by the selection regime. Although heritable components of variance and significant linear selection differentials are found for the traits l_{1+} and l_{3+} , the direction of selection varied with the age of mature fish. Selection favoured increased body size in the 1+ fish but there is a reversal of this size-selective mortality in the 3+ age class. Thus, viability effects of large size in mature fish are age-specific, and phenotypic evolution will likely be constrained by a now-later trade-off of age-specific fitness components. Linear selection for increased l_{1+} may result in part from

selection on juveniles (discussed below), but this size-selective mortality is also operating on the 1+ males.

Although the current study does not directly examine the underlying causes of selection, it is known that male salmonids can incur size-specific costs of reproduction that impact post-reproductive survival (Kitano, 1996). The cause of the selection favouring decreased l_{3+} in females is also unknown and there is no human exploitation (or other predation-induced mortality) that might be expected *a priori* to differentially target larger mature fish. This result also differs from an earlier study of Cape Race populations of brook charr (that included fish from the Lower population in which overwinter survival of post-reproductive females increased significantly with body size; Hutchings, 1994). This contrast may reflect fluctuating selection, (particularly because recent winters have been milder than those of the late 1980s when data previously reported were obtained). Alternatively, given that the time period of selection examined here is a year (as opposed to a winter), it is possible that previously detected associations between body size and survival (Hutchings, 1994) might be masked by mortality processes operating over the longer timescale.

Evolution of larger juvenile size in the Lower population is constrained by a lack of heritable variation for l_{0+} . This might be due to high components of environmental or nonadditive genetic variance, or to loss of additive genetic variance that is expected in a trait under sustained selection. The presence of linear selection for increased l_{0+} is consistent with the generally observed 'bigger is better' template of size-selective mortality in juvenile teleosts (see Sogard, 1997 for review). Differential overwinter mortality is a likely selective agent, with smaller fish having lower energy reserves and thus being more susceptible to starvation (e.g. Henderson *et al.*, 1988; Hutchings, 1994), and intraspecific predation may also be a factor. Cannibalism is known in this species (Power, 1980) and in Freshwater River (personal observation). There is also strong evidence of nonlinear selection acting on mean length-at-age 0+ with an increase in variance in the post-selection samples suggesting the possibility of disruptive selection. Disruptive selection on body size in fish has been linked to the maintenance of male size dimorphism (with large and small size morphs employing alternate strategies to maximize reproductive success; Fleming & Gross, 1994), but has not been previously reported in juveniles.

In the Upper population selection regime was found to be generally similar to that in the Lower population, although several differences were apparent. For example, there was no evidence of selection against large females in the 3+ age class, and selection for increased l_{1+} was not detected. This latter result is probably because fish of this age from the Upper population are already large enough to escape the selection pressure that is operating. Hence, it would appear that the reduced absolute growth rate in

the Lower population is associated with an increased time over which size-selective mortality is operating. Although both linear and nonlinear selection were found to operate on the juvenile fish, there was no evidence for significant heritability of l_{0+} (or for any length-at-age traits). Thus, in the Upper population evolution of large body size is also prevented by both genetic and selective constraints. It is notable that significant values of h^2 were detected for length-at-age traits in the Lower population but not the Upper. This might reflect differing levels of genetic variance between the populations (a possibility discussed below), or the presence of a greater component of environmental variance in the Upper population.

In the river as a whole, heritable variation for l_{0+} was detected, a result that contrasts with the nonsignificant values determined for each population alone. Given that there is limited movement of individuals between populations on an ecological timescale (Wilson, 2002), this probably reflects upward bias in the overall estimates of trait heritabilities due to sharing of environment by siblings. Alternatively, this finding could be explained by local selection regimes acting to reduce additive genetic variance within each population (especially the Upper), while preserving variance in the river as a whole. Under this scenario, the observed phenotypic differentiation of length-at-age traits between the Lower and Upper populations might reflect local adaptation. However, based on an extensive microsatellite study (Wilson, 2002), there is no evidence for genetic differentiation between the populations. Although the correlation between molecular and quantitative measures of genetic variability cannot be assumed (Reed & Frankham, 2001), the microsatellite study indicated a freedom of gene flow that would pose a challenge to the origin and maintenance of phenotypic differentiation through local adaptation (or genetic drift). Furthermore, the similarities between the population-specific selection regimes are generally more striking than the differences, providing no evidence for selection favouring divergent phenotypes. Thus, differentiation in length-at-age traits might be most parsimoniously explained as phenotypic plasticity, although common-garden experiments would be useful in testing this conclusion.

As predicted, a positive phenotypic association between EM and high juvenile growth rate (as indicated by l_{0+}) was determined in the river overall and in the Lower population. Furthermore, between the populations, a tendency towards earlier maturity in the Lower population was associated with greater juvenile growth rates (as indicated by mean RJG). This association has previously been demonstrated in females across rivers in the Cape Race area (Hutchings, 1993). Here we also find it across populations within Freshwater River, and in both sexes. Delaying reproduction might generally allow surplus energy to be used for growth, providing fitness benefits in the form of increased survival, mating success or fecundity (Roff, 2002). Although selection differentials

indicate that survival of larger adult fish is generally no greater, an increase of fecundity with body size has been previously reported in this system (Hutchings, 1993, 1996). Hence, for females with higher juvenile growth, earlier reproduction becomes favoured as the inherent benefit of a shorter generation time is coupled with an increased age-specific fecundity. Hutchings (1996) predicted that females in Freshwater River (Lower population) should all mature at age 2+ regardless of growth rate, whereas the optimal age of maturity could be 3+ or 4+ in other Cape Race rivers depending on an individual's growth rate. The predicted lack of variance in female age of maturity in Freshwater River arose from the fact that a combination of slow growth and high mortality results in a low possibility of realizing any benefits from delaying reproduction (Hutchings, 1996). Our results suggest that there is in fact variation in female age of maturity because some were even able to reach maturity as early as age 1+. In males, the association between earlier reproduction and higher RJG was qualitatively similar to that seen in females (although less convincing quantitatively, perhaps due to limited sample sizes). This might imply that to some extent male mating success also increases with size, an implication consistent with previous work on salmonids (e.g. Kitano, 1996).

The abundance of mature 1+ fish in Freshwater River as a whole, and the tendency for earlier maturation in the Lower population, are consistent with the idea that age of maturity may typically be lower in stunted populations. However, the nature of the link between stunting and EM is difficult to interpret. In the river overall, and in the Lower population alone, significant genetic correlations between l_{0+} and EM were detected, consistent with the hypothesis of genetic coupling between age of maturation with juvenile growth rate (Thorpe *et al.*, 1983). However, accurate estimation of genetic correlation usually depends on large sample size (Falconer & MacKay, 1996), and in this case sample sizes are relatively small. Furthermore, the overall estimate is subject to bias (as discussed above), whereas in the Lower population h^2 values for these traits were not significantly greater than zero. Although there is some evidence for a genetic basis to this covariation of traits this result should be treated with caution.

Alternatively, age of maturation might be expressed as an adaptive plastic response to environmentally determined juvenile growth rates, a mechanism consistent with the lack of heritable variation for juvenile growth found in the current study. A recent model describing the effects of growth conditions on size structure of fish populations suggests that adaptive plastic responses in age of maturity can either alleviate, or exacerbate, stunting that primarily results from resource limitation (Ylikarjula *et al.*, 1999). In Freshwater River, the extent to which interpopulation differences might be determined by differential survival (affecting optimal age of maturity and thus having indirect effects on growth

trajectories) as opposed to differential resource abundance (affecting growth directly) is an open question.

In this work, microsatellite markers provide pedigree information that cannot otherwise be determined in natural populations of fishes (Wilson & Ferguson, 2002) and this allows estimation of quantitative genetic parameters. Marker-assisted estimation of quantitative genetic parameters is a relatively new field and empirical studies have been limited to date (but see Ritland & Ritland, 1996; Mousseau *et al.*, 1998; Thomas *et al.*, 2002; Wilson *et al.*, 2003). Although this strategy has enormous advantages in allowing estimation of genetic parameters *in situ* without prior knowledge of pedigree structure, it is also important to acknowledge the limitations. For example, marker-assisted estimation of genetic parameters is dependent on the relationship structure of the population studied, such that samples must contain significant proportions of related individuals (Ritland, 2000; Thomas *et al.*, 2002). In this case the apparent site fidelity of brook charr (Wilson, 2002), coupled with the fact that Freshwater is a closed system (preventing migration out of the study area) probably increase the utility of the marker-assisted approach. Nevertheless, a limitation of the current work is that the accuracy of pedigree reconstruction cannot be evaluated. As there is currently no method for determining confidence levels in the pedigree as reconstructed by the sibship partitioning procedure (C. Herbinger, personal communication), it is important to note that standard errors for genetic parameters estimated under REML do not incorporate error from the pedigree reconstruction procedure. Any error in pedigree reconstruction (inclusion of unrelated individuals into sibships or partitioning of siblings across multiple families) is expected to cause a downward bias of heritability estimates (Thomas *et al.*, 2000). Furthermore, we assume each age class is comprised of unrelated full-sibling families, a model of the true pedigree that may be violated. For example, multiple matings leading to half-sibling relationships are known to occur in some salmonids (e.g. Taggart *et al.*, 2001). The need to assume a model of pedigree structure can be removed entirely by using estimates of pairwise relatedness to estimate heritability in a regression-based approach (Ritland, 1996). Unfortunately work to date has found that this method often yields less reliable estimates of genetic parameters (for methodological comparisons see Thomas & Hill, 2000; Wilson *et al.*, 2003). In the current study, use of the regression method yielded estimates of trait heritabilities that were generally outside the true parameter space ($0 \leq h^2 \leq 1$), with confidence intervals that spanned zero (results not shown).

However, violations of the assumed model of pedigree structure may have only a limited impact as most of the information used to estimate heritability will come from close relatives (i.e. full siblings; Thomas & Hill, 2000). This view is supported by an empirical study (Wilson *et al.*, 2003), although partitioning of half-siblings into

reconstructed full-sibships, and the sharing of parents across sibships will likely cause some downward bias of genetic parameters. It should therefore be noted that the absence of heritable variation in length-at-age traits in the Upper population could be due, at least in part, to a high incidence of multiple mating in this population and/or to poor performance of the sibship reconstruction algorithm. Further exploring the effects of error in pedigree reconstruction on the accuracy and bias of resultant heritability estimates represents an important avenue of further research, as does the extension of the MCMC methodology to permit multiple classes of relationship in the model of pedigree structure (Thomas & Hill, 2002).

Further limitations of the current study stem from the assumption of steady-state conditions that are necessary as cohorts could not be followed through time (Arnold & Wade, 1984; Endler, 1986). For example, we assume that differences in pre- and post-selection samples are due to differential mortality alone, and not the result of temporal differences in environment that may impose different patterns of mortality, or allow different potential for growth across years. Increased confidence in the results presented herein comes from the fact that differences between pre- and post-selection samples are qualitatively consistent if the post-selection sample is broken up by actual age class (results not shown). Hence, the findings show stability across year classes (as far as sample sizes will permit examination) and post-selection samples have not been biased by any unusual events in a single age class. However, it is important to note that this assumption may sometimes be violated. For example, Good *et al.* (2001) found different patterns of size-selective mortality in juvenile Atlantic salmon between consecutive summers and related this finding to hydro-climatic events. As sibship groups were reconstructed within each age class, any interannual differences in growth conditions will be analogous to sharing of environment by relatives and might cause upward bias of heritability estimates as well as affecting selection differentials. These ambiguities can best be resolved by use of a cohort tracking approach, which would also permit examination of multiple fitness components. In this context molecular markers might also be used for parentage analysis (e.g. Marshall *et al.*, 1998), allowing cross-generational study and hence estimation of total fitness. However, in practice (and as was found in the current study), application of such methods will be limited to systems where repeated sampling of individuals is possible.

In conclusion, constraints on body size in Freshwater River probably arise from a combination of selective and genetic factors. At least within the Lower population, there is evidence for heritable components of adult body size traits, although larger size does not generally confer a viability advantage on mature fish. Although there is evidence for linear selection favouring increased juvenile

size in both Lower and Upper populations, population-specific heritability estimates of juvenile size traits are not significant, suggesting that this selection is unlikely to cause an evolutionary response. Phenotypic differentiation between the two populations in length-at-age traits might best be explained as plasticity arising from environmental effects, although further study is required to fully resolve this. The mechanism underlying the observed adaptive association between juvenile growth and age of maturation remains ambiguous and also provides an avenue for further study.

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