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Parallelism in thermal growth response in otoliths and scales of brown trout (*Salmo trutta* L.) from alpine lakes independent of genetic background

Jens Thaulow[1,2] Thrond O. Haugen[1] and Reidar Borgstrøm[1]

[1]Department of Ecology and Natural Resource Management, Norwegian University of Life Sciences, 1432 Ås, Norway

[2]Norwegian Institute for Water Research, Gaustadallèen 21, N-0349 Oslo, Norway

Jens Thaulow, present address, Norwegian Institute for Water Research, Gaustadallèen 21, N-0349 Oslo, Norway

Correspondence: J. Thaulow, Norwegian Institute for Water Research, Gaustadallèen 21, N-0349 Oslo, Norway. E-mail: jens.thaulow@gmail.com

Running headline: Thermal otolith- and scale growth response

Abstract

Low density in natural populations of salmonids has predominantly been managed by stocking of non-native conspecifics. Due partly to domestication, introduced non-native fish may be maladapted under natural conditions. Interbreeding between introduced and wild individuals may therefore impair local adaptation and potentially population viability. Brown trout (*Salmo trutta* L.) from three headwaters (with stocked fish) and three inter-connected lakes (with native fish) on the Hardangervidda mountain plateau, Southern Norway, were tested for differences in thermal effects on scale- and otolith growth. Otolith- and scale annuli widths from immature brown trout showed positive correlation with mean annual summer temperature for all six sampled populations. In mature individuals, a similar positive thermal correlation was evident for the otoliths only. Inter-annuli width measurements from scales indicate a halt in somatic growth for brown trout in this alpine environment when reaching ages between 7 and 9 winters, coinciding with age at maturity. Our study indicates that otolith growth follows summer temperature even when individuals do not respond with somatic growth in these populations, and that introduced brown trout and introgressed populations have similar thermal growth responses. Due to the continued otolith growth after stagnation in somatic growth and the impact of fluctuations in summer temperature, the utilization of otolith annuli widths for back-calculation of length-at-age should be treated with caution.

Keywords: temperature; somatic growth; otolith; scales; AFLP

Introduction

Growth in fish is highly dependent on temperature (Elliott 1976a, b), and a large number of studies have demonstrated adaptations to local temperature conditions both in marine and freshwater environments (Haugen and Vøllestad 2000; Jensen et al. 2008; Sanford and Kelly 2011). Brown trout occur from lowland to high mountain areas, experiencing a wide range of habitat conditions and temperature regimes. Local adaptations to the prevailing thermal conditions are therefore expected, and genetic variation in growth-related traits among populations is likely to arise as a result from these potential temperature adaptation processes (e.g., Haugen and Vøllestad 2000; Jensen et al. 2008). With maximum rations, brown trout (*Salmo trutta* L.) may have an optimum growth at temperature around 13 $^{\circ}$C (Elliott 1975). In alpine areas in Norway, however, long winters with ice-covered lakes and much snow prevail, and the short open water season, combined with low temperatures, may highly affect annual growth of brown trout in such areas (Borgstrøm 2001; Borgstrøm and Museth 2005). Stocking of non-native individuals, with the aim of establishing new populations or improving fish recruitment for harvesting, has been a common practice in these mountain areas. Inter-breeding between introduced and native individuals is well documented (Hansen and Mensberg 2009), but the long-term outcome from such introgression into native populations is not easily predicted, and represents a situation where gene flow and natural selection act in concert (Hansen 2002). The result from these interacting processes may impact the native population's local adaptation (e.g., Gilk et al. 2004; McGinnity et al. 2009; Skaala et al. 2012).

Estimation of age structure and individual growth in fish populations has been an essential component in the study of population dynamics in both marine and freshwater species (Francis 1990; Morita and Matsuishi 2001; Francis and Campana 2004). Accuracy and precision of scale-based ageing have been shown to decline after sexual maturity, and are therefore most applicable for immature individuals (Alvord 1954; Jonsson 1976). In contrast, otoliths continue to grow throughout the entire lifetime of an individual (Campana and Neilson 1985) irrespective of somatic growth (Nordeng 1961; Aass 1972 Wright et al. 1990), but with decreasing annual increments as age increases (e.g., Black et al. 2005; Weisberg et al. 2010; Stocks et al. 2011). Otoliths are therefore considered the most accurate structure for age determination (Jonsson 1976; Burnham-Curtis and Bronte 1996; Buckmeier et al. 2012). However, most studies addressing the decoupling of somatic growth and otolith growth have been restricted to larval and juvenile fishes under artificial conditions and on relatively short time scales, most often less than one year in duration (Mosegaard et al. 1988; Secor and Dean 1989; Wright et al. 2001; Fey 2006).

In this study, we take advantage of an alpine brown trout system where three headwater lakes comprise populations established by stocking of individuals of unknown, but non-local origin. Downstream lakes hold original populations that have experienced gene flow from the headwater populations to varying degrees (Sønstebø et al. 2008). This system therefore offers conditions for comparing otolith- and scale growth in populations with different genetic backgrounds and the effect of a possible gene flow on thermal effects on these ageing structures. Brown trout in this alpine area reach ages between 30 and 40 years (Svalastog 1991), but as seen elsewhere, may stagnate in growth after maturation, especially in dense populations (Jensen 1977; Borgstrøm 1994; Wootton 1999).

The objective of the study was to explore the thermal responses in two growth-increment traits (otoliths and scales) of brown trout from introduced and native populations. The two growth increment traits are expected to show differentiated thermal responses. In general, scale growth stagnates after maturation in dense populations, while otoliths continue to grow and form annuli irrespective of somatic growth stagnation of the fish (e.g., Nordeng 1961). Furthermore, otolith growth has been shown to correlate with summer temperature, and possibly other environmental factors, irrespective of age (Black et al. 2005, 2013). However, since all the studied brown trout populations live in sub-optimum temperature conditions with short growth seasons, we expect natural selection to be strong and, hence, prevail over genetic drift and gene flow so as to favour converging thermal growth-increment responses among populations.

Materials and methods

Study area and population characteristics

All six lakes included in the study are located in the upper reaches of the River Kvenna watercourse on the Hardangervidda mountain plateau, at altitudes from 1167 to 1282 m above sea level (ASL) (Table 1; Fig. 1a). The lakes are ice covered from October-November to June-July, with large variations in ice break-up time among years, but always starting first in the lowermost lake. April snow depth in the area varies annually between less than one meter to more than three meters, and the ice break-up is highly dependent on the spring snow depth, as shown for the lake Litlosvatn (Borgstrøm 2001). All lakes are relatively shallow, with Litlosvatn being the deepest (about 25 m maximum depth; Table 1).

Brown trout is the only fish species present in the studied lakes, and has been present on the Hardangervidda mountain plateau for as much as 6500 year (Indrelid 1985). The brown trout populations in the three downstream-connected lakes, Kollsvatn (KOL), Litlosvatn (LIT), and Kvennsjøen (KVE), most likely represent populations of these original fish, that have not been stocked in modern times, and are only influenced by downstream migration from the headwater lakes (Sønstebø et al. 2008). As part of a population assessment study, KOL brown trout individuals were tagged during 1988 and 1989. Individuals from this KOL tagging programme were recaptured in LIT during July-August 1993, clearly demonstrating down-stream migration to occur between these lakes. Based on original tagging numbers and the number of recaptures, more than 700 individuals from the 1988 – 1989 population in KOL may still have been present in LIT in 1993. The populations in the three headwater lakes Krokavatn (KRO), Skavatn (SKA), and Nedre Vassdalsvatn (NVV) are, on the other hand, isolated by impassable upstream waterfalls (Fig. 1a). Brown trout populations were therefore established in these headwater lakes by two stocking episodes in KRO and SKA during the 1930s (Tomas Sekse, previous leader of the local Mountain board, pers. comm.), and in mid 1940s in NVV (Jakob J. Eitrheim, participated in the stocking, pers. comm.), with fish of non-local, but unknown origin. However, according to Jakob J. Eitrheim (pers. comm.), it is very likely that the NVV population was founded by broodstock from a lowland population. SKA was re-stocked in 1974 and 1982, also using fish of non-local and unknown origin.

According to local fishermen, individuals larger than two kg were common in all six populations during the 1950 – 1960s (Jakob H. Eitrheim and the late Ingvald N. Øvregård, pers. comm.). Natural recruitment to the populations varies, with several weak or missing year-classes during the last decades, while others have been exceptionally strong (Borgstrøm and Museth 2005). This variation in annual recruitment is most likely governed by variations in summer temperatures and snow conditions (Borgstrøm and Museth 2005). According to gillnet catches in July 2003 – 2004, the strong year-classes 1997 and 1999 doubled the population density in most of the lakes compared to densities in July 1989 – 1993 (unpublished, own data). Due to the strong year-classes, all populations have been dense during the last years, as indicated by high catch per unit effort by gillnets, and relatively small individual size of captured individuals (unpublished, own data).

Water temperature

Annual mean summer temperatures (1 July – 15 September) for the years 2004 – 2008 were recorded by temperature loggers (TinyTag^{12}, Gemini Data Loggers, UK) at 2 m depth in open water of LIT and in the outlet stream of KRO (Fig. 1a,b).

Sampling

All brown trout were sampled by gill net fleets consisting of the mesh sizes 16.5, 19.5, 22.5, 26, 29, 35, 39, and 45 mm (knot to knot measure), except for KVE where only rod fishing was used, allowing both fast and slow growers within each age-class to be sampled. A small number was captured by rod fishing. From 2009 – 2011, 240 brown trout were collected from the six lakes, and subjected to amplified fragment length polymorphism (AFLP) fingerprinting (Table 1). A subset of these fish, and additional collections from 2008 to 2012 were selected for analyses of the otolith and scale inter-annular widths, yielding a total of 247 individuals for biological analyses (Table 1). In addition, 110 females sampled from KOL in 2009 – 2012 were used as a standard for age at maturation (Fig. 1c). Length of all sampled brown trout were measured to the nearest mm (L_T), and sex and maturity stage were determined according to (Dahl 1917). Sagitta otoliths were removed and cleaned, stored in individual paper envelopes together with scale samples, and left to dry before age determination and back-calculation of length at age. Tissue samples (fin clips) for AFLP analysis were stored in 96 % ethanol before DNA extraction with the DNeasy® Tissue kit (Qiagen) according to manufacturer's guidelines.

Molecular analysis

Amplified fragment length polymorphism fingerprinting of samples was performed according to Vos et al. (1995) with some minor modifications: 300 ng genomic DNA was digested in 50 µl volumes containing 1x RL-buffer (10mM Tris-aceate pH 7.5, 10 mM Mg-aceate, 50 mM potassium-aceate, and 5 mM DTT), 5 u *Eco*RI and *Mse*I restriction enzymes (Fermentas) for 2 h at 37 °C. Adaptors were ligated to cutting sites for 3 h at 37 °C by adding 10 µl of 1 x RL-buffer containing 5 mM *Eco*RI and 50 mM *Mse*I adaptors to the 50 µl digestion volume. Selective preamplification was performed in a 50 µl reaction volume containing 5 µl ligated product, 1x PCR-buffer, 1 u *Taq*, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 75 ng *Eco*RI (5´-GACTGCGTACCAATTCA-3´) and 75 ng *Mse*I (5´-GATGAGTCCTGAGTAAC-3´) primers complementary to ligated adaptors. Thermo-cycling conditions included 30 cycles of: denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. The quality and quantity of the preamplified products were determined on a 1.0 % agarose gel and diluted (1:99) with ddH_2O. Selective amplification with six primer combinations, with a two base extension to the *Eco*RI and *Mse*I preamplification primers, respectively, (i.e., A+AG/C+CT, AG/GT, CT/GT, CT/TA, GC/CG, and GT/CG) was performed in a 12 µl reaction volume using 2 µl pre-diluted product, 1 x PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTP, 1 u *Taq* polymerase, 50 µg γ-^{33}P-ATP labeled *Eco*RI-primer, and 30 µg *Mse*I-primer. Thermo-cycling conditions were: denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 1 min, followed by 11 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s (decreasing 0.7 °C for every cycle), and extension at 72 °C for 1 min, followed by 24 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 1 min, followed by a final extension step at 72 °C for 10 min. PCR products were size separated by 5 % polyacrylamide gel electrophoresis (PAGE) run at 80 W for 1.5 hours. AFLPs were visualized by placing dried gels onto KODAK BioMax MR films for approximately 48 hours. AFLP fragments of equal fragment lengths were scored by naked eye as present (1) and absent (0) and arranged in a binary matrix. A subset of the samples (12.5 %) was independently genotyped twice for error rate estimation by direct comparison of scoring mismatches.

Genetic data analyses

The genetic structure of five of the six populations included in this study has already been investigated (Sønstebø et al. 2007, 2008). However, with the inclusion of NVV, new and updated genetic analyses of all sampled populations were performed. The number of scored polymorphic loci and percentage of polymorphic loci (%P) in each of the sampled populations were calculated using GENALEX v6.5 (Peakall and Smouse 2006, 2012). This program was also used to carry out a principal coordinate analysis (PCoA) and pairwise population PhiPT (analog to F_{ST} for dominant markers) with tests for statistical significance based on 9 999 permutations. All analyses were performed using polymorphic loci only.

Otolith and scale analyses

Otoliths from 247 of the brown trout sampled between 2008 and 2012 were sectioned through the center, the fragment surfaces gently polished with no 1200 polish paper, and the surface subsequently burnt over an ethanol flame until winter zones appeared as dark-brown bands (Fig. 2) (Christensen 1964; Power 1978). The sectioned and burnt otoliths were placed in plasticine immersed in propandiol, and photographed at 40 x magnification by a Leica DFC

320 digital camera, mounted on a dissecting microscope. Most of our sampled fish belong to the year-classes 1997 – 2002, and since juveniles seem to use the streams during the first years after hatching, we have avoided analysing the annual growth during the stream residence of the sampled individuals. Accordingly, we have studied the growth pattern in both immature fish (age ≤ 7 years), and mature fish (≥ 8 years) restricted to the annual growth during the years 2004 – 2008. The differentiation between immature and mature fish was obtained from maturity stages of female fish from KOL indicating a shift from being immature until age 7 to maturity in older fish (Fig. 1c). Annuli formed in these years were measured along the otolith and scale radii (Fig. 2). Since the otoliths are not cut at exactly the same transect, the relative widths of the 2004 – 2008 annuli have been used for statistical analyses, with the width of the 2004 annulus set to 100. Imprints of three to five scales from each fish were made in a celluloid strip, magnified in a microfilm reader, and annuli widths measured from scale centre to the edge (Fig. 2). Scales were used for back-calculation of length-at-age in the same years (i.e., 2004 – 2008), according to the Lea-Dahl method (Dahl 1910; Lea 1910), and individual annual growth increments were based on these back-calculated lengths. For direct comparison of annual growth increments in otoliths and scales, scale annuli widths were also converted to relative size.

Statistical analyses

In order to estimate within- and among-population variance components that were as much as possible freed from non-genetic environmental effects a linear mixed effects model (LME, e.g., Pinheiro and Bates 2000) approach was applied for both otolith- and scale-based growth increments, to identify possible differences between immature (≤ 7 years) and mature individuals (≥ 8 years) (Fig. 1c). In particular, we included the extrinsic variables water temperature (i.e., mean relative annual summer temperature calculated in the same manner as the relative width of scale and otolith) and population density (see below) to both account for and quantify the effect of density-independent and density-dependent processes on individual growth, respectively. By including principal component values from the PCoA analysis as a candidate predictor variable, we aimed at retrieving eventual gene-flow effects on individual growth. Fish ID and Population were included as random factors (random intercepts).

Differences in suitable habitat area and depth, and time of ice-off may all influence annual individual growth in the studied lakes. However, in accordance with literature (Jensen 1977; Jenkins et al. 1999; Vøllestad et al. 2002) we assume that individual growth rates and population density is negatively correlated in brown trout. Since direct or indirect estimates of population densities (e.g., CPUE-data) only was available from the catch year, and not from years at which the back-calculated size increments actually were realised, we fitted scale-based back-calculated length-at-age to the Gompertz growth equation (e.g., Ricker 1979) and used the L_∞ -parameter as an indirect index of population density. This parameter, the asymptotic length, averages individual growth potential across many years and may therefore provide a reliable proxy for the average population density across years relevant for our samples. In addition to the L_∞ -parameter, the Gompertz growth function has two parameters, G and g. The G parameter is the instantaneous growth rate at age 0 (i.e., t=0), and the g parameter is the instantaneous rate at which G decreases as age increases:

$$L_t = L_\infty e^{-e^{(G-gt)}}$$

As expected, the L_∞ parameter was negatively associated with $\text{CPUE}_{20\text{-}40\text{cm}}$ derived from 2004 – 2007 gillnet surveys conducted in five out of the six study lakes ($r_p = -0.77$, n=5, p=0.07). Unfortunately, no such CPUE data exists for the last lake (KVE), leaving us with the L_∞ parameter as the most complete density-related metric available for our analyses.

Model selection was conducted using AICc following routines described in Zuur et al (2009). Model averaging was undertaken for models yielding ΔAICc ≤ 4 (Richards 2008). All statistical analyses were conducted using R version 3.2.0 (R Core Team 2015), with the lme4 library. The influence.ME and AICcmodavg packages were used for LME model validation/diagnostics and model selection, and the nls2 library used for fitting the Gompertz model. Type III test statistics for the fitted LME models were performed using the Wald F test with Kenward-Roger degrees of freedom (Kenward & Roger 1997), as implemented in the car package in R.

Results

Water temperature

Mean annual summer temperature in LIT during 2004 – 2008 was 9.97 °C: but varied substantially among years: 12.0, 8.6, 13.1, 6.7, and 9.0 °C for each of the years 2004 – 2008, respectively. Mean daily summer temperature never fell below 1.2 °C or exceeded 17.2 °C. Additional temperature measurements from the KRO outlet stream, showed the same yearly variation as LIT (Fig. 1b), and were not used in further analyses.

Growth potential

The Gompertz length-at-age parameters, obtained from back-calculated length-at-age data from scale samples, indicate variation in growth potential among lakes. Highest and lowest growth potentials occurred in KVE and SKA, with asymptotic lengths at 38.4 and 27.8 cm, respectively (Supplementary Table 1).

Population genetics

A total of 139 polymorphic AFLP loci were scored from the six primer combinations, with an estimated error rate of 1.2 %. Mean percent polymorphism among all sampled populations was 75.9 % (± 5.7 % (SE)), ranging from 49.6 % (NVV) to 86.3 % (KOL) (Supplementary Table 2). All pairwise F_{ST} estimates were significant at the 5 % level (Supplementary Table 2). The three stocked populations generally showed the strongest differentiation; whereas among the three introgressed populations genetic differentiation was low, but increased downstream (i.e. KVE was more differentiated from KOL than LIT). Genetic differentiation between SKA and KOL was low, compared to SKA *vs.* all other sampled populations. Principal coordinate analysis separated sampling location KRO, with an intermediate positioning of SKA and KOL from NVV, LIT and KVE along principal component (PC) 1, explaining 62.3 % of the genetic variation (Fig. 3). Sampling location NVV was separated from LIT and KVE along PC2, explaining 20.2 % of the variation. Principal component 3, explaining 13.9 %, separated SKA from KOL.

Mean annual growth of scales and otoliths

Mean relative annuli widths from otoliths and scales across all study populations were positively associated with mean annual summer temperature for all five years (Fig. 4). Only mean relative annuli widths of scales from mature individuals showed no association with temperature. Individual representation of this correlation was consistent from each of the study populations, showing no strong deviation between populations, except KVE where no difference in increment width of otoliths and scales was recorded in mature individuals (Fig. 4).

Intrinsic and extrinsic effects on otolith growth

There was little support in the data for differentiated temperature effect among ages in otolith increment, as age*temperature interaction effect models attained high AICc values compared to their additive counterpart models (Supplementary Table 3). Little support was found for density-regime effects (i.e., L_∞) on otolith increment, as ΔAIC>9 for candidate models including this variable. Models including year as a fixed effect (not shown) generally had little support compared to those including temperature as a substitute for year effect. There was more support for models including random population effect rather than using genetic structure predictors (principal components, PC1 – PC3).

The temperature effect on otolith increment was highly significant and positive (Fig. 5a). As can be interpreted from the most supported model parameter estimates displayed in Supplementary Table 4, otolith increment was highest at age 2 and generally decreased as age increased.

The highest ranked model involved differential random intercepts and slopes of temperature among populations (i.e., (1+st.T|Population)). However, this model attained an AICc value that was just 1.5 units lower than the simpler second-ranked model which included no random slope effect for temperature. A likelihood ratio test between the two top models revealed that they were not significantly different ($\chi^2 = 4.55$, df = 2, p = 0.103). There is therefore little support for among-population differentiation in the thermal otolith-increment responses. Since the two top-ranked models both received substantial AICc weights (0.67 and 0.32, respectively) model averaging was undertaken for the fixed effects parameters (Supplementary Table 3).

Intrinsic and extrinsic effects on scale growth

There was strong support in the data for differentiated temperature effects among ages, as the three best supported models, entailing more than 0.99 of the AICc weight, included an age*temperature interaction effect on back-calculated growth increment (Supplementary Table 5). In contrast to the otolith increment analysis, there was high support (AIC weight = 0.99) for density-regime effects (i.e., L_∞) on length increment, where the most supported model structure including this variable as an additive effect. As expected, the density-regime effect coefficient was positive (0.092 ± 0.017 (SE)), indicating that individual growth rates are higher in lakes with large L_∞. There was more support for models including random population effects than those using genetic structure predictors (principal components, PC1 – PC3). Models including age*year effects (not among the 15 top-ranked models) received less support than those including age*temperature effects.

As can be interpreted from the most supported model parameter estimates displayed in Supplementary table 6, back-calculated length increment was highest for age 2 and generally decreased as age increased. The temperature effect on back-calculated length increment decreased with increasing age, resulting in very low increment responses for ages above seven years (Fig. 5b).

Discussion

This study documents parallel thermal otolith- and scale-increment responses among interconnected alpine brown trout populations. Although the two growth-related traits differ in their age-specific temperature responses, virtually all populations displayed parallel thermal responses for both traits. These findings should be interpreted in light of significant neutral among-population genetic structuring and recent introgression of non-native genotypes into the studied populations.

The annual increment in scales and otoliths did not match in mature fish (> 7 years) from the study area, since scale growth seems to be linked to somatic growth until around age seven, corresponding to age at maturation, while otolith increment width was positively correlated with summer temperature, not only in juveniles, but also in mature individuals which exhibit very little or no somatic growth. Similar findings related to scale growth versus otolith growth are known from previous studies of brown trout (Jonsson 1976) and other salmonid species e.g. lake trout (Burnham-Curtis and Bronte 1996) (*Salvelinus namaycush* Walbaum 1792), Arctic charr (*Salvelinus alpinus* (L.)) (Nordeng 1961), whitefish (*Coregonus lavaretus* L.) (Skurdal et al. 1985), and vendace (*Coregonus albula* L.) (Aass 1972). Temperature responses have also been observed in lake trout where otolith annuli widths were related to fluctuations in August air temperature over a 22-year interval in an Alaskan lake (Black et al. 2013). Likewise, brown trout showed high correlation between somatic growth and the number of days with temperatures above 7 °C in a sub-alpine Norwegian lake (Hesthagen et al. 2004).

Somatic growth rate is often found to have a maximum response at an intermediate temperature within the species' range of tolerance (Webb 1978). For brown trout, the range over which feeding and growth occurs seems to be 2.0 – 19 °C, with an optimum around 13 °C when fed maximum rations (Elliott 1975; Elliott 1981; Forseth et al. 2009), indicating that optimum growth temperature may not be reached every year or only in limited periods during the ice-free season in the study area. In the juvenile stage, somatic and otolith growth of fish seems to match (Fey and Hare 2012; Baumann et al. 2013), however, beyond age at maturity, somatic growth ceases due to allocation of energy into reproduction (Hile 1970). Despite this expected decrease or halt in somatic growth, otoliths have been shown to continue growing in adult fish, suggesting that otolith growth is linked to metabolism rather than somatic growth *per se* (Mosegaard and Titus 1987; Mosegaard et al. 1988), a hypothesis that has received support from findings of positive correlation between oxygen consumption and otolith growth (Wright et al. 2001). The identified significant positive correlation between annual otolith increments and temperature may accordingly represent a metabolic response rather than the effects of local adaptation or plasticity (Gauldie and Nelson 1990; Yamamoto et al. 1998; Wright et al. 2001). However, uncoupled otolith- and somatic growth has been observed in unfavourable growth conditions, e.g., limited food supply (Campana 1983; Volk et al. 1984) or hyperoptimal temperatures (Mosegaard et al. 1988), indicating that a direct linkage between otolith increment and somatic growth is unlikely. Positive correlation between otolith growth and temperature may therefore potentially result from, and thus be used as an indicator of, years with environmental conditions beneficial for high growth and high metabolic rate. Under favourable conditions, the somatic growth potential may be realized even in older individuals, as was observed in mature individuals from KVE. This lake holds the largest potential for individual growth as evident from the Gompertz estimates of asymptotic length. The five remaining locations most likely do not provide the same growth potential even under favourable growth temperatures, probably due to higher population densities, as indicated by the estimated asymptotic lengths. Several studies have shown that individual growth in salmonids may increase when population density is decreased (Jensen 1977; Donald and Alger 1989; Klemetsen et al. 2002; Janhunen et al. 2010), or translocated to more favourable habitats (Rosseland 1956), as also shown for e.g. European perch (*Perca fluviatilis* L.; Persson 1986).

The among-population parallelism in the positive association between mean summer temperatures and width of otolith increments in mature, growth-stagnated fish indicates that the same thermal growth response has been favoured in all populations, irrespective of genetic background. The origin of the stocked fish in the three lakes KRO, SKA, and NVV remains enigmatic, however the AFLP analysis indicates high genetic diversity among these populations. The genetic differentiation among the sampled populations was generally in accordance with previous studies in five of the lakes (Sønstebø et al. 2007, 2008), as well as the stocking history of NVV. Although elevation above sea level, snow conditions, and ice-off varies among the lakes, the temperature profiles show relatively small differences, as seen in LIT and KRO during the years 2004 – 2008, and the differences seem to be proportional and comparable. The study system therefore provides a relevant situation for studying scale- and otolith growth responses in multiple populations under natural conditions, whereas a more controlled experiment under a common-garden design would be problematic due to the large time scope (age range: 6 – 15 years) that would be required. Despite genetic differentiation, especially among the three headwater lakes, stabilizing selection may have resulted in convergent adaptation in the thermal growth responses. This should be interpreted in light of the relatively short time since population establishment or most recent restocking, indicating that substantial selection enforced by the region-specific temperature regime must have occurred during 3 – 10 generations (assuming age at maturity = 7 years). Adaptation of developmental and life-history traits to local thermal conditions has been found to occur over relatively short time in introduced salmonids (for examples see: Koskinen et al. 2002; Kavanagh et al. 2010; Crozier and Hutchings 2014; 6 – 30 generations, reviewed in Fraser et al. 2011). In fitness-related traits like individual growth, adaptation may take place despite the commonly documented low heritability for such traits (Mousseau and Roff 1987) due to either high selection intensities (Kingsolver et al. 2001) or high residual genetic variance that allows evolution via complex genetic architecture pathways (Merilä and Sheldon 2000). In fact, a study of multiple Nordic populations of brown trout raised under common-garden conditions found no evidence for temperature adaptation in somatic growth performance (Forseth et al. 2009), which strongly suggests canalization of thermal reaction norms of growth performance in this species.

In conclusion, despite the presence of profound genetic differentiation among the studied populations, especially for the three introduced headwater populations, we found no indication of differentiation in thermal response patterns for either of the two growth-related traits (i.e., annual otolith and scale increments) among populations. This parallelism in thermal growth-response pattern indicates that the study populations have experienced stabilizing selection with respect to these traits. The comparison of somatic growth with annuli widths in scales and otoliths, showed that reliable scale annuli were only deposited in the juvenile stage. After maturation, the failure to produce distinct annuli in scales indicates somatic growth stagnation. However, this halt in somatic growth did not seem to affect the continuous formation of otolith annuli, and, most importantly, their positive growth correlation to temperature. The positive correlation between otolith increment width and temperature, irrespective of age and somatic growth, suggests that otoliths, besides being valuable for age determination, may have a value as indicators of difference in mean annual summer temperature in past years, as well. Due to continued otolith growth, dependence on fluctuations in summer temperature, the utilization of otolith annuli widths for back-calculation of growth/length-at-age should be treated with caution.

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Figure legends

Fig. 1 a) Southern Norway with enlarged map of the study area. Solid bars in rivers denote upstream impassable waterfalls and arrow out of Kvennsjøen indicates water flow direction. b) Summer water temperature measured from 1 July – 15 September in the years 2004 – 2008 at 2 meters depth in Lake Litlosvatn (black circle) and outlet stream from Lake Krokavatn (grey circle). c) Frequency of 110 immature (grey bars) and mature (black bars) female brown trout from Lake Kollsvatn with ages 3 – 15 winters (age-class 9 is only represented by two individuals). The individuals were sampled in the years 2009 – 2012

Fig. 2 Paired scale and otolith from individual brown trout. Scale (a) and section of broken and burnt otolith (b) from a seven winters old brown trout (year-class 2002) captured in the Lake Krokavatn, in July 2009, and scale (c) and otolith (d) from a 15 winter old brown trout captured in Lake Kollsvatn, in July 2012. End of winter zones are marked by horizontal lines. Grouped lines at the scale margin (c) indicate stagnation since annuli numbers did not concur with that found in the corresponding otolith (d). The 2006 annulus is marked

Fig 3 Principal coordinate analysis of the six analysed brown trout populations from the Hardangervidda mountain plateau

Fig. 4 Mean relative annuli widths of otoliths and scales from brown trout in the six studied populations (blue = scales, red = otoliths) divided into two groups representing mature (solid lines) and immature fish (broken lines) together with mean relative summer temperature for the years 2004 – 2008. Mean relative values are calculated with the width of the 2004 annulus, or temperature, set to 100, respectively. Mature individuals are represented by the 1997 year-class (except KVE represented by year-classes 1998 and 1999) and immature by year-class 2002 (except NVV represented by year-class 2003). Population abbreviations are given in Table 1

Fig. 5 Prediction plot for the average effect of water temperature on a) relative otolith increment, here exemplified using age 2, and b) on age-specific back-calculated annual growth increment from scales. Predictions were retrieved from fixed-effects parameter estimates of the most supported LME-model, provided in Table 5 and Table 7 for otolith (a) and scales (b), respectively. Year-specific temperature values are indicated along the x-axis.

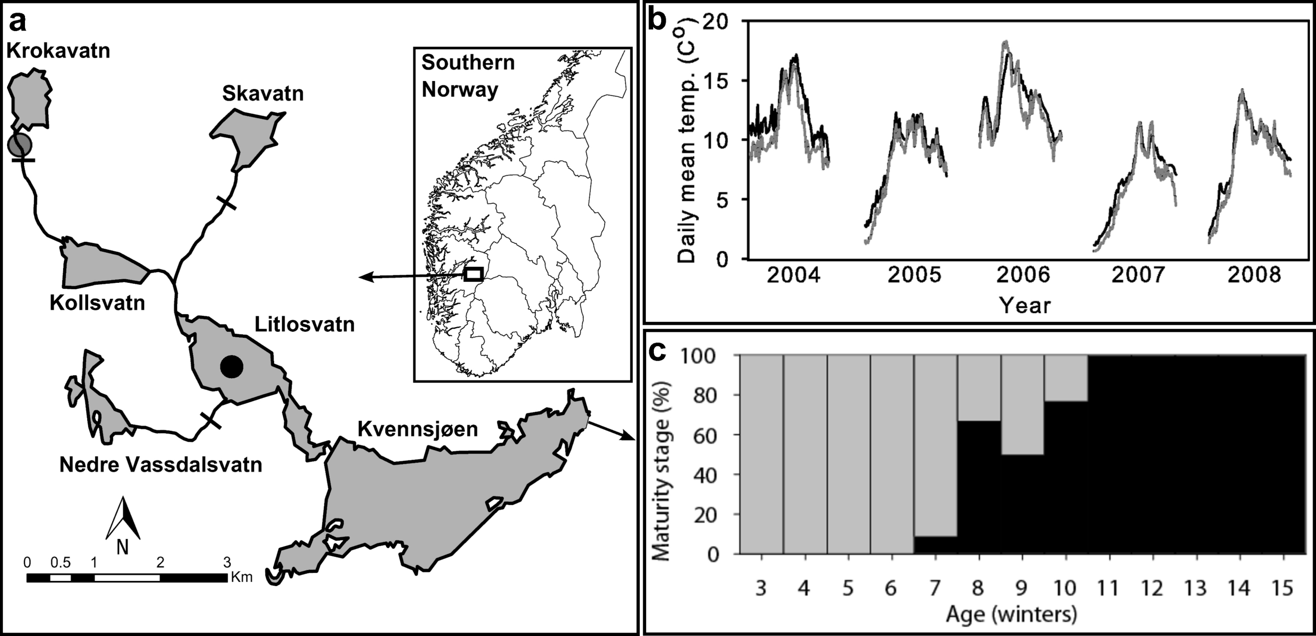
A) The relative increment is predicted in relation to the year 2004 increment. Shaded area represents the 95 % confidence bound. The temperature effect (i.e., the slope) is the same for all ages, but differs slightly in their respective intercepts. b) Between age 7 and 9 back-calculated annual growth is close to 0

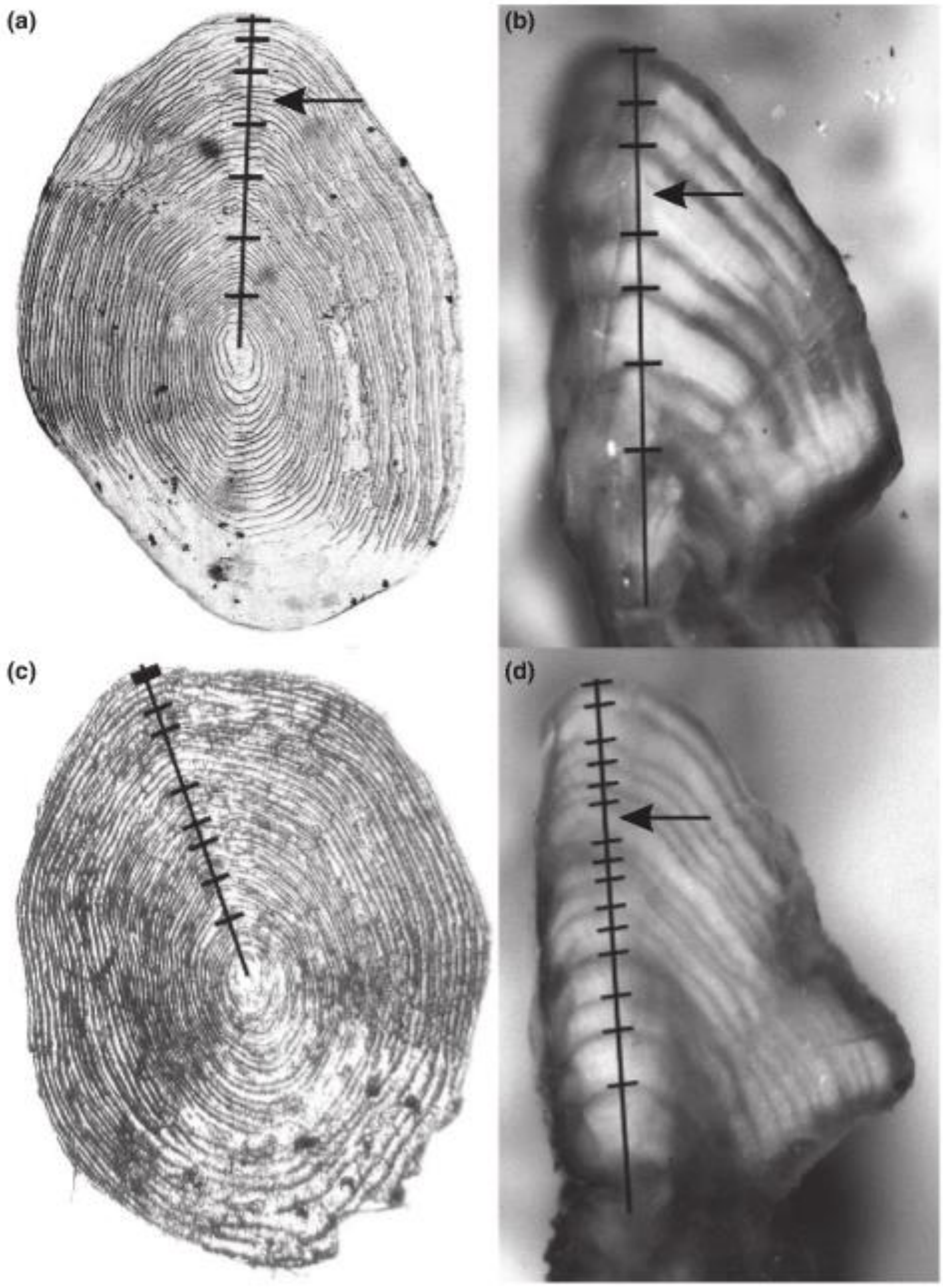
Tables

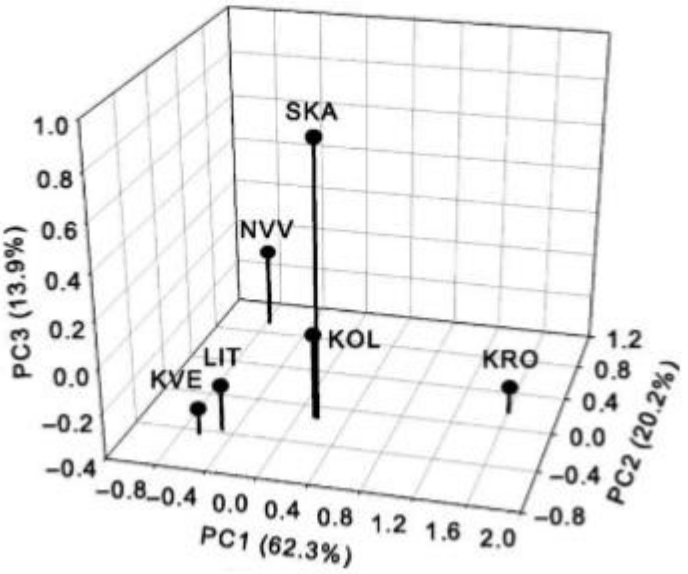
Table 1 Number (*N*) of brown trout used in the biological analyses/AFLP analysis, with population abbreviation, sex (male/female/not available), altitude, surface area, maximum depth, and stocking history of the sampled lakes

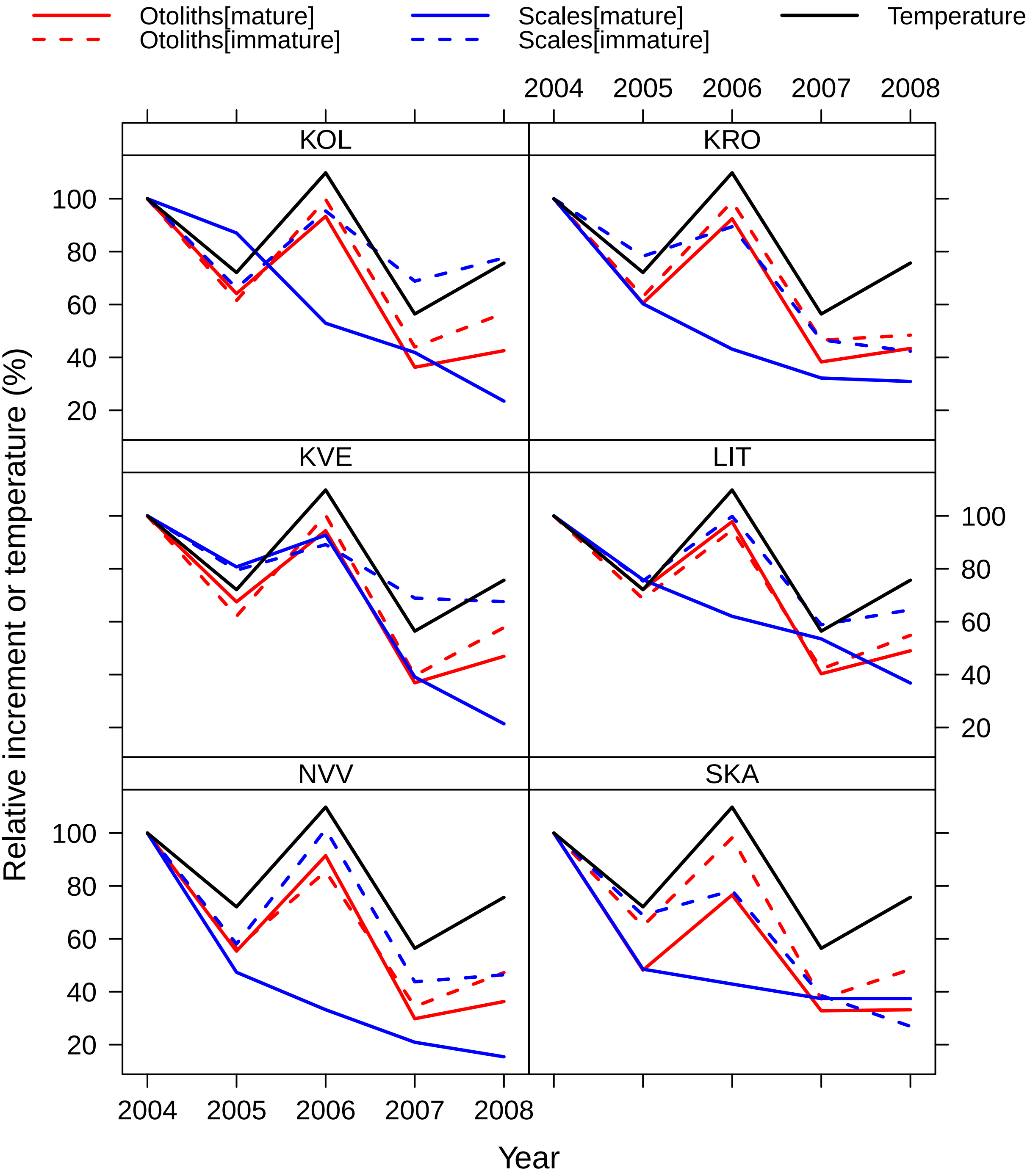
Lake	Abbreviation	*N*	Sex M/F/NA	Altitude (m)	Lake size (km^2)	Maximum depth (m)	Stocking
Krokavatn Nedre	KRO	30/40	13/17/0	1236	0.42	16	1935*
Vassdalsvatn	NVV	37/40	27/8/2	1282	0.47	shallow	*c.* 1945*
Skavatn	SKA	28/40	12/16/0	1249	0.47	15	1935*, 1974 and 1982
Kollsvatn	KOL	64/40	22/25/17	1182	0.62	13	Not stocked
Litlosvatn	LIT	58/40	11/22/25	1172	1.52	25	Not stocked
Kvennsjøen	KVE	30/40	17/13/0	1167	5.09	shallow	Not stocked

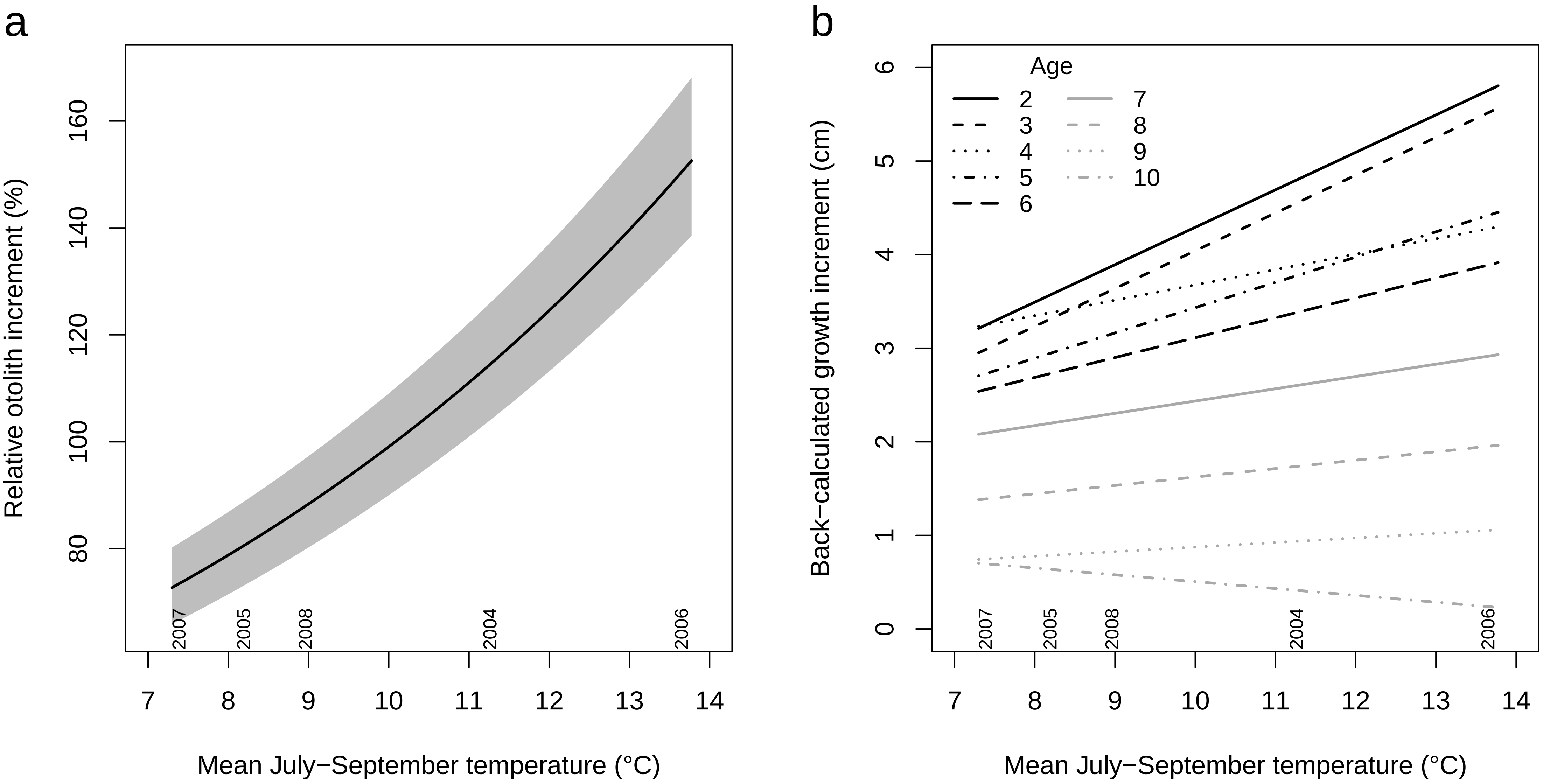
* Assumed fishless before stocking


a
Krokavatn
Skavatn
Kollsvatn
Litlosvatn
Nedre Vassdalsvatn
Kvennsjøen
Southern Norway
N
0
0.5
1
2
3
Km
b
Daily mean temp. (C°)
0
5
10
15
20
2004
2005
2006
2007
2008
Year
c
Maturity stage (%)
0
20
40
60
80
100
3
4
5
6
7
8
9
10
11
12
13
14
15
Age (winters)


(a)
(b)
(c)
(d)


SKA
NVV
KOL
LIT
KVE
KRO
PC3 (13.9%)
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
−0.4
PC1 (62.3%)
−0.8
−0.4
0.0
0.4
0.8
1.2
1.6
2.0
PC2 (20.2%)
1.2
0.8
0.4
0.0
−0.4
−0.8


Otoliths[mature]
Otoliths[immature]
Scales[mature]
Scales[immature]
Temperature
KOL
KRO
KVE
LIT
NVV
SKA
2004
2005
2006
2007
2008
20
40
60
80
100
Relative increment or temperature (%)
Year


a
Relative otolith increment (%)
80
100
120
140
160
2007
2005
2008
2004
2006
7
8
9
10
11
12
13
14
Mean July−September temperature (°C)
b
Back−calculated growth increment (cm)
0
1
2
3
4
5
6
Age
2
3
4
5
6
7
8
9
10
2007
2005
2008
2004
2006
7
8
9
10
11
12
13
14
Mean July−September temperature (°C)

Supplementary tables

Supplementary Table 1 Gompertz parameter estimates for the six populations. L_∞ = asymptotic length, G = instantaneous growth rate at age = 0 and g is the instantaneous rate at which G decreases as age increases, LCL and UCL = lower and upper 95 % confidence limits, respectively

	L_∞			G			g		
Pop	Est	LCL	UCL	Est	LCL	UCL	Est	LCL	UCL
KOL	35.94	34.45	37.68	1.175	1.069	1.292	0.310	0.277	0.345
KRO	36.38	34.83	38.24	1.286	1.148	1.441	0.356	0.312	0.403
KVE	38.40	35.95	41.65	1.235	1.094	1.399	0.326	0.276	0.381
LIT	33.37	31.82	35.24	1.140	1.032	1.259	0.326	0.288	0.367
NVV	35.29	32.75	38.65	1.161	0.991	1.360	0.342	0.280	0.411
SKA	27.80	26.67	29.12	1.205	1.078	1.345	0.380	0.336	0.427

Supplementary Table 2 Pairwise genetic differentiation between sampled populations and percent polymorphic loci scored in each population (%P). All pairwise comparisons are significant at the < 5 % level. Population abbreviations are given in table 1

Pop	%P	KRO	NVV	KOL	LIT	KVE
KRO	71.2 %					
NVV	49.6 %	0.339				
KOL	86.3 %	0.137	0.130			
LIT	84.9 %	0.247	0.127	0.031		
KVE	80.6 %	0.271	0.136	0.065	0.016	
SKA	82.7 %	0.189	0.181	0.051	0.096	0.118

Supplementary Table 3 Model selection parameters, ranked according to AICc, for the top 15 LME models estimating the relative otolith increment on ln scale. Predictor variables that are given in parentheses correspond to random effects, and the remainder are fixed effects. df = degrees of freedom, AICc = Akaike's Information Criterion corrected, ΔAICc = AICc difference towards most supported model, AICcWt = AICc weight among all fitted models, Resid. LL = residual log likelihood, st.T = standardized temperature ((x-mean)/SD), L_∞ = asymptotic length, PC1-PC3 = principal component 1 – 3 from a neutral genetic PCA (used as proxy for relative genetic distance)

Model structure	df	AICc	ΔAICc	AICcWt	ResID. LL
age + st.T + (1+st.T | Population) + (1 | ID)	15	109.71	0	0.67	-39.64
age + st.T + (1 | Population) + (1 | ID)	13	111.21	1.5	0.32	-42.44
age + st.T + L_∞ + (st.T | Population) + (1 | ID)	16	119.08	9.36	0.01	-43.29
age + st.T + PC1 + PC2 * PC3 + (1 | ID)	16	119.86	10.15	0	-43.69
age + st.T + L_∞ + (1 | Population) + (1 | ID)	14	119.93	10.21	0	-45.78
age + st.T + L_∞ + PC1 + PC2 * PC3 + (1 | ID)	17	123.21	13.49	0	-44.33
age + st.T + L_∞ + PC1 * PC2 + (1 | ID)	16	126.91	17.2	0	-47.21
age + st.T + L_∞ + PC1 * PC2 + PC3 + (1 | ID)	17	127.92	18.21	0	-46.69
age + st.T + L_∞ + PC1 + (1 | ID)	14	130.7	20.99	0	-51.16
age + st.T * L_∞ + (1 | Population) + (1 | ID)	15	131.45	21.74	0	-50.51
age + st.T + L_∞ + PC1 + PC2 + PC3 + (1 | ID)	16	133.34	23.62	0	-50.43
age + st.T + L_∞ + PC2 + (1 | ID)	14	136.55	26.84	0	-54.09
age + st.T + L_∞ + (1 | ID)	13	138.71	29	0	-56.19
age * st.T + (1 | Population) + (1 | ID)	21	144.78	35.07	0	-50.97
age*st.T + L_∞ + (1 | Population) + (1 | ID)	22	153.53	43.82	0	-54.31

Supplementary table 4 Parameter estimates (fixed effects) and corresponding test statistics and variance components (random effects) for the most supported LME model structure analysing annual otolith increment. The fixed effects parameters have been model averaged with the second-ranked model in Supplementary table 3. The model accounts for 69 % of the total variation. The intercept represents the age 2 coefficient. The test statistics are based on Type III F tests with Kenward-Roger df. st.T = standardized water temperature ((x-mean)/SD), where mean = 9.89 °C and SD = 2.59

Parameter estimates			Test statistics					Variance components		
Term	Estimate	SE	Variable	Df	Res.Df	F	p	Groups	Variance	SD
Intercept	4.5859	0.0507	age	8	810.74	102.08	<0.0001	ID	0.0509	0.2256
age 3	-0.1336	0.0296	st.T	1	6.04	736.41	<0.0001	Population	0.0097	0.0984
age 4	-0.2724	0.0291						st.T	0.0003	0.0183
age 5	-0.3447	0.0286						Residual	0.0390	0.1998
age 6	-0.3766	0.0299								
age 7	-0.4337	0.0323								
age 8	-0.6006	0.0355								
age 9	-0.7549	0.0353								
age 10	-0.9848	0.0405								
st.T	0.2962	0.0076								

Supplementary table 5 Model selection parameters, ranked according to AICc, for the top 15 LME models estimating the scale-based back-calculated growth increment in brown trout from six brown trout populations on the Hardangervidda mountain plateau. Predictor variables provided in parentheses correspond to random effects, and the remainders are fixed effects. df = degrees of freedom, AICc = Akaike's Information Criterion corrected, ΔAICc = AICc difference towards most supported model, AICcWt = AICc weight among all fitted models (the relative support), Resid. LL = residual log likelihood, st.T = standardized water temperature ((x-mean)/SD), L_∞ = asymptotic length

Modnames	df	AICc	ΔAICc	AICcWt	ResID. LL
age*st.T + L_∞ + (1 | Population) + (1 | ID)	22	3473.4552	0.00	1.00	-1714.27
age*st.T * L_∞ + (1 | Population) + (1 | ID)	39	3478.1133	4.66	0.10	-1698.62
age*st.T + (1 | Population) + (1 | ID)	21	3483.4449	9.99	0.01	-1720.30
age*st.T + (st.T | Population) + (1 | ID)	23	3487.6076	14.15	0.00	-1720.30
age + year + L_∞ + (1 | ID)	16	3495.4107	21.96	0.00	-1731.46
age + year + L_∞ + (1 | Population) + (1 | ID)	17	3497.2806	23.83	0.00	-1731.36
age*st.T + L_∞ + PC1 * PC2 + (1 | ID)	16	3503.4951	30.04	0.00	-1735.50
age*st.T + L_∞ + (1 | ID)	13	3504.4403	30.99	0.00	-1739.06
age*st.T + L_∞ + PC1 * PC2 + PC3 + (1 | ID)	17	3505.5235	32.07	0.00	-1735.49
age*st.T + L_∞ + PC1 + PC2 * PC3 + (1 | ID)	17	3505.5235	32.07	0.00	-1735.49
age*st.T + L_∞ + PC2 + (1 | ID)	14	3505.9286	32.47	0.00	-1738.78
age*st.T + L_∞ + (1 | Population) + (1 | ID)	14	3506.1001	32.64	0.00	-1738.86
age*st.T + L_∞ + PC1 + (1 | ID)	14	3506.4347	32.98	0.00	-1739.03
age*st.T + L_∞ + PC1 + PC2 + PC3 + (1 | ID)	16	3507.0052	33.55	0.00	-1737.26
age*st.T + L_∞ + (st.T | Population) + (1 | ID)	16	3507.7118	34.26	0.00	-1737.61

Supplementary table 6 Parameter estimates (fixed effects) and corresponding test statistics and variance components (random effects) for the most supported LME model structure analysing annual back-calculated growth increment. The fixed effects parameters have been model averaged with the second-ranked model in Table6. The model accounts for 62 % of the total variation. The intercept represents the growth-age 2 coefficient. L_∞ = asymptotic length; st.T = standardized water temperature ((x-mean)/SD), where mean = 9.89 °C and SD = 2.59. The test statistics are based on Type III F tests with Kenward-Roger df

Parameter estimates		
Term	Estimate	SE
Intercept	1.0670	0.5903
age 3	-0.2526	0.1618
age 4	-0.5915	0.1621
age 5	-0.8458	0.1542
age 6	-1.1623	0.1637
age 7	-1.8278	0.1635
age 8	-2.6352	0.1809
age 9	-3.3776	0.1890
age 10	-3.7480	0.5521
st.T	1.0343	0.2397
age 3*st.T	0.0128	0.2858
age 4*st.T	-0.6095	0.2466
age 5*st.T	-0.3343	0.2595
age 6*st.T	-0.4874	0.2721
age 7*st.T	-0.6897	0.2661
age 8*st.T	-0.8069	0.2792
age 9*st.T	-0.9046	0.2636
age 10*st.T	-1.2431	0.6335
L_∞	0.0918	0.0166

Test statistics				
Variable	Df	Res.Df	F	p
age	8	996.58	71.597	<0.0001
st.T	1	647.15	17.261	<0.0000
age*st.T	8	946.08	6.231	<0.0001
L_∞	1	5.22	22.738	0.0044

Variance components		
Groups	Variance	SD
ID	0.1895	0.4354
Population	0.0043	0.0655
Residual	1.0829	1.0406