Local adaptation in brown trout early life-history traits: implications for climate change adaptability

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Knowledge of local adaptation and adaptive potential of natural populations is becoming increasingly relevant due to anthropogenic changes in the environment, such as climate change. The concern is that populations will be negatively affected by increasing temperatures without the capacity to adapt. Temperature-related adaptability in traits related to phenology and early life history are expected to be particularly important in salmonid fishes. We focused on the latter and investigated whether four populations of brown trout (*Salmo trutta*) are locally adapted in early life-history traits. These populations spawn in rivers that experience different temperature conditions during the time of incubation of eggs and embryos. They were reared in a common-garden experiment at three different temperatures. Quantitative genetic differentiation (Q_{ST}) exceeded neutral molecular differentiation (F_{ST}) for two traits, indicating local adaptation. A temperature effect was observed for three traits. However, this effect varied among populations due to locally adapted reaction norms, corresponding to the temperature regimes experienced by the populations in their native environments. Additive genetic variance and heritable variation in phenotypic plasticity suggest that although increasing temperatures are likely to affect some populations negatively, they may have the potential to adapt to changing temperature regimes.

Keywords: common-garden experiment; global warming; natural selection; phenotypic plasticity; Q_{ST} versus F_{ST} ; reaction norm

1. INTRODUCTION

Divergent natural selection due to spatially varying environments is expected to promote adaptive evolutionary responses in the absence of disruptive effects from random genetic drift and gene flow (Kawecki & Ebert 2004). However, when populations are small or gene flow is extensive, they are expected to be neutrally differentiated or genetically homogeneous, respectively. Hence, the evolutionary outcome is dictated by the relative strength of natural selection, migration and gene flow (Endler 1986). Despite its fundamental importance to the understanding of evolutionary trajectories, ecological speciation and its significance in relation to population management, knowledge about the extent of local adaptation among natural populations has been limited, albeit increasing during the past decade (Leinonen *et al.* 2008). Whereas some insight can be obtained by consideration of effective population size and migration rate based on molecular markers (Hansen *et al.* 2002; Dionne *et al.* 2008), direct demonstration of local adaptation involves either comparison of fitness among

populations in local and foreign environments, analysis of genes subject to selection or analysis of the between-population component of additive genetic variance in quantitative traits (Endler 1986; Kawecki & Ebert 2004).

One way of assessing the influence of natural selection on quantitative traits is by comparing population divergence at genetic markers affected solely by drift, gene flow and mutation (F_{ST}) to genetic divergence in quantitative traits potentially also affected by selection (Q_{ST}) (Merilä & Crnokrak 2001). When considering the relationship between Q_{ST} and F_{ST} , three scenarios can be envisaged. First, a higher divergence in quantitative traits than at neutral molecular markers ($Q_{ST} > F_{ST}$) is consistent with selection acting differently on those traits in separate populations. Second, the opposite scenario, i.e. $Q_{ST} < F_{ST}$, suggests that the same genotypes are favoured in different populations due to convergent selection. Third, if the two measures do not differ significantly, then genetic drift versus selection cannot be disentangled (Mckay & Latta 2002).

Genotypes often express a range of phenotypes in response to different environmental conditions, i.e. they exert phenotypic plasticity (Ward & Kelly 2004). The reaction norm describes this plasticity for a given genotype by relating inherent phenotypes to some environmental variable. As heritable differences in the norm of reaction have been demonstrated (Stinchcombe *et al.* 2004), natural selection could mould this plastic response if it has implications for fitness (Nussey *et al.* 2005;

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Richards *et al.* 2006). While genotype $\times$ environment interactions within populations suggest genetic variation for phenotypic plasticity, such interactions at the level of populations consistent with differences in selection regimes would indicate local adaptation in plasticity (Haugen & Vøllestad 2000; Hutchings *et al.* 2007).

Over the past century, the average global temperature has increased by 0.7°C, and the International Panel of Climate Change (IPCC) predicts that even further increases are to be expected in the future (IPCC; <http://www.ipcc.ch>). Hence, *global warming* now seems to be reality (Kerr 2007). The fast rate of global change raises questions about the consequences for biodiversity in terms of species distributions and diversity, phenology and community structure (Walther *et al.* 2002). Moreover, there is limited knowledge about the role of genetic variation and the ability of natural populations to respond adaptively to current and future environmental change (van Heerwaarden & Hoffmann 2007; Gienapp *et al.* 2008). The expected evolutionary responses to global warming have recently been discussed by Bradshaw & Holzapfel (2008). They argued that phenology, e.g. reproducing at the optimal time of year, is the most critical factor in adaptation to climate change. Hence, evolutionary change is more likely to take place at traits and genes related to phenology when compared with thermotolerance. Nevertheless, the pronounced effects that temperature exerts on physiological processes, especially in poikilotherms, suggest that adaptation to local temperature regimes *per se*, involving physiology or behaviour, could also be critical. This particularly concerns phases of the life cycle where individuals are unable to 'escape' suboptimal temperature conditions by migration, e.g. during immobile juvenile life stages.

In salmonid fishes, adaptation to thermal conditions is probably of high importance during incubation of eggs and the critical phase that follows fry emergence. Timing of emergence from the nests to exogenous food availability and the remaining endogenous yolk reserves is essential for survival and is affected by temperature conditions within the river. During this critical phase, density-dependent competition for food and territory is fierce and predation is heavy (Elliott 1994). Together, these factors lead to mortality rates approaching 90 per cent in the early life stages in salmonids (Elliott 1994). Mortality during this period is generally considered to be size selective (Einum & Fleming 2000) as large fry are more resistant to starvation (Einum & Fleming 1999), less prone to size-limited predators (Werner & Gilliam 1984) and probably able to exploit larger food items (Wankowski 1979). Hence, early development and growth are critical for survival and highly affected by temperature.

Here, we investigate whether populations of brown trout (*Salmo trutta* L.) show evidence of adaptations in early life-history traits to their local environments, especially temperature regimes. Furthermore, we assess the consequences that increasing temperature will have in these populations and investigate whether they will be able to respond adaptively in early life-history traits to such environmental changes. We do this by rearing families from four different populations in a common environmental setting and testing for adaptive divergence by comparing genetic differentiation in five early life-history traits (Q_{ST}) with differentiation at 10 neutral molecular markers (F_{ST}).

We replicate the experiment using three different temperatures, 2, 5 and 8°C, and test for temperature effects as well as genotype $\times$ environment interactions.

2. MATERIAL AND METHODS

(a) Study populations

Adult brown trout were collected by electrofishing repeatedly during autumn/winter 2004/2005 from four watersheds, all located in the Jutland Peninsula, Denmark: Norring Møllebæk River (NOR); Lilleaa River (LIL); Karup River (KAR); and Lake Hald (HAL) (figure 1). The habitats of these populations differ in terms of physical conditions and temperature regimes. NOR is a small river (approx. 6 km long, 1.7 m wide, on average, and 0.1–0.5 m deep) that flows into LIL, but gene flow is restricted due to an impassable dam established more than 200 years ago. As a consequence, the population consists exclusively of resident trout. LIL is a larger river (approx. 34 km long, 5.1 m wide, on average, and 0.2 to more than 1 m deep) that flows into the Gudena River, which has its outlet into the brackish Randers Fjord and subsequently into the Kattegat Sea. LIL is inhabited by a large population of mainly anadromous brown trout. The water temperature in NOR and LIL is highly affected by the prevailing air temperature and hence winter and spring temperatures are on average low, approximately 3–5°C (figure 1 in the electronic supplementary material). HAL is a 342 hectare lake and is part of the Gudena River system. It has an average depth of 13 m and receives influent water from six small tributaries (0.3–4.0 km long, 0.6–2.2 m wide and 0.02–0.6 m deep), which serve as spawning areas of the local brown trout population. The mean winter/spring temperature within the tributaries is approximately 6–7°C and stable due to extensive upwelling and feeding by groundwater (figure 1 in the electronic supplementary material). HAL is isolated from allochthonous immigrant brown trout due to an impassable dam in the outlet river. The trout from the experiments were sampled from two tributaries, the Dollerup and Mostgaard Rivers. A study by Jensen *et al.* (2005) showed that extensive gene flow occurs among tributaries and that trout in the lake can be considered a single population. KAR is mainly inhabited by anadromous brown trout. It is a large river (approx. 75 km long, 17 m wide, on average, and more than 2 m deep at several places) that flows into the Limfjord. The temperature regimes vary along the watershed, with some tributaries fed by groundwater whereas other parts of the system are more susceptible to ambient winter air temperatures.

NOR has never been stocked to our knowledge. KAR and HAL have previously been stocked with exogenous trout, but two studies have shown that this has had virtually no effect on the indigenous gene pools (Hansen *et al.* 1993; Hansen 2002). LIL has been subject to some stocking in the 1980s and the early 1990s with trout of same origin as those stocked in KAR, but as natural reproduction in the river has always been high, we assume that this has had limited effect on the indigenous gene pool. Nevertheless, if stocking had affected KAR and LIL significantly, we would expect these two populations to show more similarity for the measured traits.

(b) Rearing experiment

Following collection, fishes were transported to the hatchery facilities at the Danish Centre for Wild Salmon and kept until stripping for eggs or milt. For all individuals, fork length was measured to the nearest millimetre and an adipose fin clip was

Figure 1. Map showing the locations of the four populations from Jutland, Denmark: Norring Møllebæk River (NOR); Lilleaa River (LIL); Karup River (KAR); and Lake Hald (HAL).

taken for later molecular analyses. Using a paternal half-sib mating design (North Carolina Design 1; Comstock & Robinson 1952), experimental families were established for each of the four populations by crossing each male with two or three females, using dry fertilization (eggs and sperm are mixed and subsequently water is added). For NOR 13 full-sib families nested within 5 half-sib families were established (i.e. 5 males and 13 females were used); for KAR 17 full-sib families nested within 6 half-sib families (6 males and 17 females); for LIL 21 full-sib families nested within 8 half-sib families (8 males and 21 females) and for HAL 17 full-sib families nested within 6 half-sib families (6 males and 17 females). Owing to pronounced differences in spawning time among the populations, fertilization took place over a total period of 45 days. Individuals from NOR were the first to ripen (all families were

established on 1 December 2004) followed by LIL (families established between 5 and 20 December 2004). Ripening of individuals from KAR and HAL occurred later and spanned a longer time period. Thus, for KAR, the first families were established on 20 December 2004 and the last crosses were completed on 3 February 2005. For HAL, families were established between 10 January and 25 February 2005. Each lot of eggs was divided into three batches, which were incubated in separate compartments in conventional hatchery troughs at 2, 5 and 8°C. Coolers and heaters controlled by thermostats ensured that the temperature within each trough was kept constant (not deviating more than $\pm 0.5^\circ\text{C}$) at the appropriate temperature during the whole period of incubation. From each female, a sample of unfertilized eggs was collected, and individual eggs were weighed after drying at 70°C for 14 h.

(c) Trait measurements

We focused on five early life-history traits: incubation time (time to hatching); alevin length (length at hatching); yolk sac volume; swim-up length (length at first feeding); and growth rate. Incubation time (measured in number of degree-days) was recorded by counting hatched alevins each day until all eggs were hatched. A sample of 20 newly hatched alevins was collected and total length as well as length and width of yolk sac was measured using an ocular with a measuring reticle on a stereo magnifier at the lowest magnification ($1.67\times$). Yolk sac volume was estimated by $V = (\pi/6) \times L \times H^2$ (Blaxter & Hempel 1963), where L is length and H is the width of the yolk sac. At the time when the majority of the fry had become positively phototactic and neutrally buoyant, i.e. the time of swim-up, 20 fry were collected and digitally photographed lying next to a micrometre scale, using a Canon IXUS 500 digital camera. Lengths were subsequently measured using the software TPSDIG2 v. 2.04 (Rohlf 2005). Finally, growth rate for each family was estimated from the relationship $G = (L_{SU} - L_H) / \Delta D$, where L_{SU} and L_H are lengths at swim-up and hatching, respectively, and ΔD is the number of degree-days passed between these stages.

(d) Data analysis

An experimental design based on the F1 progeny of field-caught individuals potentially produces biased estimates of genetic variance components through confounding maternal effects (Lynch & Walsh 1998). Therefore, we analysed only paternally derived genetic variance components. Variance components of the observed phenotypic variability were estimated by applying a linear animal dam model (Mrode 2005)

$$Y_{ijk} = \mu + S_i + D_j + T_k + e_{ijk},$$

where μ is the population mean; S is the random effect due to sires; D is the random effect due to dams nested within sires; T is the fixed effect due to incubation temperature; and e is the error term. The variance components were estimated by fitting linear animal models using a restricted maximum-likelihood (REML) method in the DMUAI software with an implemented parameter update method (AI-REML with EM crash recovery; Jensen & Madsen 1994). In the cases of deviations from normality and unbalanced experimental design, REML is still efficient and provides estimates with little bias, despite losing some of its optimality properties (Hoeschele *et al.* 1987).

In order to evaluate population divergence in the quantitative traits, we estimated Q_{ST} values for each trait: $Q_{ST} = \sigma_{GB}^2 / (\sigma_{GB}^2 + 2\sigma_{GW}^2)$, where σ_{GB}^2 and σ_{GW}^2 represent the between- and within-population paternal additive genetic variance components, respectively (Merilä & Crnokrak 2001). We estimated 95% CIs for Q_{ST} using the direct simulation data method (O'Hara & Merilä 2005).

A nested mixed-model ANOVA was applied to analyse trait variance using the following model:

$$Y_{ijklm} = \mu + T_i + P_j + S_{ijk} + D_{ijkl} + P \times T + S \times T + e_{ijklm},$$

where T and P are the fixed effects from temperature and population, respectively; S is the random effect due to sire; and D is the random effect due to dam nested within sire. $P \times T$ and $S \times T$ are the interactions between population and temperature and between sire and temperature, respectively. As egg size is a key factor of maternal effects (Kamler 2005),

linear regressions of egg weight on individual trait measurements in each full-sib family were fitted and the residuals extracted before conducting the mixed-model nested ANOVA. In this way, most maternal effects are expected to be eliminated. Pairwise differences between the mean of the traits (within population, between temperature and within temperature, between populations) were tested by means of two-tailed t -tests and the level of significance was adjusted by the false discovery rate procedure (Benjamini & Yekutieli 2001). The statistical software JMP (SAS Institute Inc., Cary, NC, USA) was used for these analyses.

(e) Molecular analyses

DNA was extracted using the DNeasy Tissue Kit 250 (Qiagen, Düsseldorf, Germany). We analysed 10 microsatellite loci: *Str15*; *Str60*; *Str73* (Estoup *et al.* 1993); *SsOSL417*; *SsOSL311* (Slettan *et al.* 1995); *SsOSL438* (Slettan *et al.* 1996); *Ssa85*; *Ssa197* (O'Reilly *et al.* 1996); *SsHaeIII4.20* (GenBank accession no. U10050; Goodier 1994, unpublished data); and *T3-13* (Estoup *et al.* 1998). Annealing temperatures were 52°C (*SsOSL417*, *SsHaeIII4.20*), 54°C (*Str73* and *Ssa197*) and 58°C (*Str15*, *Str60*, *T3-13*, *SsOSL311*). A stepwise procedure of 6×6 cycles using annealing temperatures of 54, 53, 52, 51, 50 and 49°C was used for the loci *SsOSL438* and *Ssa85*. The amplified microsatellite loci were analysed on an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA), according to the recommendations of the manufacturer.

Exact tests for deviation from the Hardy–Weinberg proportions were conducted using a Markov Chain Monte Carlo method implemented in the GENEPOP v. 3.1 software package (Raymond & Rousset 1995). Tests for gametic-phase disequilibria between pairs of loci, as well as estimation of allelic richness, a measure of the number of alleles independent of sample size and observed and expected heterozygosity, were conducted using the program FSTAT v. 2.9.3 (Goudet 1995). Genetic differentiation was quantified by estimating F_{ST} following Weir & Cockerham (1984), and the associated 95% CI was estimated by bootstrapping over loci using FSTAT.

3. RESULTS**(a) Molecular analyses**

Summary statistics for the 10 microsatellite loci are presented in table S1 of the electronic supplementary material. No linkage disequilibrium between pairs of loci or deviations from the Hardy–Weinberg equilibrium were observed after correction for multiple tests (data not shown). The overall F_{ST} estimate was 0.065 (95% CI: 0.043–0.091).

(b) Rearing experiment

Mortality from the eyed stage to the swim-up stage was low and did not differ among populations. Therefore, mortality is not considered further in the study. The estimated total additive genetic variance (V_A), maternal component (V_M), environmental component (V_E), phenotypic variance (V_P) and narrow-sense heritability (h^2) are presented in table 1. For alevin length, yolk sac volume, swim-up length and growth rate, significant additive genetic variance, V_A , was observed and point estimates of narrow-sense heritability, h^2 , ranged from 0.57 to 0.77. For incubation time, no significant V_A was observed. The estimates of Q_{ST} were high for alevin

Figure 2. Overall estimate of Q_{ST} in the populations of brown trout across all three rearing temperatures for five early life-history traits; incubation time; alevin length; yolk sac volume; swim-up length and growth rate, along with overall estimate of F_{ST} for the 10 microsatellite loci. Horizontal bars represent 95% CIs, obtained by direct simulation in the case of Q_{ST} and bootstrapping over loci in the case of F_{ST} . The vertical dotted line indicates the upper confidence limit of the F_{ST} estimate. Q_{ST} is significantly higher than F_{ST} for alevin and swim-up lengths.

Table 1. The additive genetic variance, V_A , the maternal component, V_M , and the environmental variability, V_E , followed by their standard errors (s.e.), estimated using the animal dam model (AD): phenotype = population mean + V_A + V_M + V_E . $V_M = V_G + V_{ME}$, where V_G is the genetic component of the dam; V_{ME} is the variance due to the maternal environment of the dam; and V_E is the environmental variability. The narrow-sense heritability, h^2 , is obtained by dividing V_A by V_P , where $V_P = V_A + V_E + V_M$. (These parameters were estimated for the following traits: incubation time; alevin length; yolk sac volume; swim-up length and growth rate. Asterisk indicates that the additive genetic variance is significant at the 5% level.)

trait	V_A	V_M	V_E	V_P	h^2	Q_{ST}	95% CI
incubation time	8×10^{-7} (298)	18.36 (39.56)	3625 (331)	3643	2.2×10^{-10}	0.004	0.00–1.00
alevin length	0.014 (0.0013)*	0.011 (0.0037)	1×10^{-7} (0.001)	0.025	0.57	0.49	0.14–0.86
yolk sac volume	0.0005 (0.00005)*	1.7×10^{-4} (5.8×10^{-5})	1.4×10^{-7} (3.8×10^{-5})	0.0007	0.71	0.53	0.00–0.93
swim-up length	3.144 (0.3238)*	1.365 (0.482)	7.7×10^{-6} (0.28)	4.5	0.70	0.68	0.21–0.98
growth rate	8×10^{-7} (2.6×10^{-7})*	1×10^{-7} (1.00)	4×10^{-7} (2.2×10^{-7})	1.3×10^{-6}	0.62	0.62	0.00–1.00

Table 2. Summary statistics for nested mixed-model ANOVA with dams nested within sires. (Dam and sire were set as random effects, and population and temperature were treated as fixed effects. For each of five early life-history traits; incubation time; alevin length; yolk sac volume; swim-up length and growth rate, F values with degrees of freedom in parentheses are presented. Asterisks indicate the level of significance. n.s., not significant, * $p < 0.001$.)

effect	incubation time	alevin length	yolk sac volume	swim-up length	growth rate
temperature	14.37 (1)*	21.38 (1)*	0.07 (1) n.s.	0.11 (1) n.s.	610.62 (1)*
population	0.31 (3) n.s.	200.43 (3)*	333.57 (3)*	238.54 (3)*	118.44 (3)*
sire (population)	1.08 (5) n.s.	96.90 (5)*	0.506 (5) n.s.	75.3 (5)*	59.45 (5)*
dam (population, sire)	0.32 (33) n.s.	86.40 (33)*	30.39 (33)*	93.21 (33)*	50.91 (33)*
population $\times$ temperature	0.60 (6) n.s.	20.09 (6)*	5.21 (6)*	36.63 (6)*	57.17 (6)*
temperature $\times$ sire	0.28 (12) n.s.	21.91 (12)*	13.30 (12)*	31.70 (12)*	29.03 (12)*

length, yolk sac volume, swim-up length and growth rate, and significantly exceeded the estimate of F_{ST} for alevin and swim-up lengths (figure 2).

The results from the nested mixed-model ANOVA are presented in table 2. Significant population differences were observed for alevin length, yolk sac volume, swim-up length and growth rate, but not for incubation time. A significant temperature effect was observed for alevin length, growth rate and incubation time. However, for alevin length and growth rate, populations differed in their response to temperature, and a significant

population $\times$ temperature interaction was evident (table 2; figure 3). This indicates genetic differentiation in phenotypic plasticity among populations. Also, for alevin length and growth rate, a significant sire $\times$ temperature interaction was observed, indicating the presence of genetic variation for phenotypic plasticity within populations (table 2). The two-tailed t -tests between pairs of populations within temperatures revealed that most populations were significantly different from each other for most traits (table S2 in the electronic supplementary material). Likewise, the pairwise tests

Figure 3. Reaction norm curves to varying temperatures (2, 5 and 8°C) for five early life-history traits, (a) incubation time, (b) alevin length, (c) yolk sac volume, (d) swim-up length and (e) growth rate, in four populations of brown trout. Grey dashed line, Norring Møllebæk River (NOR); black solid line, Lilleaa River (LIL); grey solid line, Karup River (KAR); black dashed line, Lake Hald (HAL). The y-axis represents the residuals from linear regression of egg weight on individual trait values. This procedure is expected to remove most maternal effects.

among temperatures within populations revealed several pairwise differences (table S3 in the electronic supplementary material).

4. DISCUSSION

(a) Local adaptation

Two independent lines of evidence suggest adaptation of populations to their local environments. First, the comparison of Q_{ST} and F_{ST} showed that the populations had diverged to a significantly higher extent in two early life-history traits, alevin and swim-up lengths, than expected from the sole effects of genetic drift and migration. Second, we found heritable differences in the way that populations responded to differing temperatures, i.e. heritable differences in their phenotypic plasticity. By conducting our experiment in a common-garden setting, we sought to remove confounding environmental effects that could have introduced a bias into both types of analyses. Nevertheless, possible sources of error remain, which need to be discussed.

Maternal effects may cause serious problems for both analyses of Q_{ST} and reaction norms. The estimates of Q_{ST} were obtained using only the sire additive genetic variance, which is expected to decrease the influence of maternal

effects on the estimates. However, the influence of maternal effects on Q_{ST} estimates still cannot be ruled out. For instance, correlation between genotype and environment may increase phenotypic variance between females from different populations, which could result in maternal effects inflating Q_{ST} . In the analysis of population differences in reaction norms, we sought to remove most maternal effects by standardizing with egg weight and using the residuals in the subsequent analyses. We thereby expect that most non-genetic maternal influence has been eliminated and that the significant dam effect identified by the nested mixed-model ANOVA (table 2) represents mainly genetic maternal influence. This can be further substantiated by considering that NOR and HAL exhibited the lowest mean egg weight (NOR: 0.017 g, s.d. 0.004; HAL: 0.025 g, s.d. 0.011; LIL: 0.037 g, s.d. 0.019; KAR: 0.031 g, s.d. 0.006) and yet exhibited divergent reaction norm curves (figure 3).

Further bias may be present in the comparisons of Q_{ST} and F_{ST} , the latter of which could be biased if natural selection (or hitch-hiking selection) acts on some of the microsatellite loci. However, conducting the neutrality test by Beaumont & Nichols (1996) did not provide evidence

for selection (data not shown). Another potential problem relates to the dependence of F_{ST} on within-population heterozygosity (Hedrick 1999), which causes the maximal obtainable F_{ST} at highly variable loci to be determined by the level of homozygosity. However, the maximal F_{ST} for the 10 microsatellite loci calculated according to Meirmans (2006) was 0.279. This vastly exceeds the observed F_{ST} estimate of 0.065 and, consequently, F_{ST} is unlikely to be bounded. There are no similar procedures available to assess whether Q_{ST} is bounded (Leinonen *et al.* 2008), but if Q_{ST} was underestimated this would have a conservative effect on the results.

In total, we find it likely that the high Q_{ST} values for alevin and swim-up lengths and the differences in phenotypic plasticity between populations reflect genetically based local adaptation. Residual maternal effects remain the most serious concern, and we cannot rule out that this has affected the results. If this was to be completely eliminated, second or later generations of common-garden-reared individuals should be analysed; though for a species such as brown trout with a generation time of 3.5 years, this becomes highly demanding in terms of facilities and resources.

Two related studies comparing F_{ST} and Q_{ST} for early life-history traits in populations of grayling, *Thymallus thymallus* (Koskinen *et al.* 2002), and brook charr, *Salvelinus fontinalis* (Perry *et al.* 2005), reached similar conclusions regarding local adaptation. These authors found evidence of divergent natural selection acting on alevin length and yolk sac volume. Koskinen *et al.* (2002) and Perry *et al.* (2005) additionally reported high differentiation for growth rate due to natural selection. In the present study, we also observed high estimates of Q_{ST} for growth rate, but due to a very wide CI for this trait it did not deviate significantly from F_{ST} .

The populations in the present study also showed adaptive divergence in their response to varying temperatures for two traits: alevin length and growth rate. These differences coincided with the temperature conditions within their natal rivers. Thus, individuals originating from LIL, with average winter temperatures of approximately 3–5°C (figure 1 in the electronic supplementary material), showed the largest alevin length at 2 and 5°C and were significantly smaller at 8°C (figure 3). By contrast, alevins from HAL, with groundwater-fed tributaries and winter temperatures of approximately 6–7°C (figure 1 in the electronic supplementary material), were larger at 8°C than at 5°C (figure 3). KAR alevins were also larger at the higher temperatures. It is more difficult to unequivocally ascribe this to environmental conditions. Temperature regimes vary throughout the river system, and the individuals used for the common-garden experiment were caught in the lower part of the river and could in principle be derived from different parts of the river system. Since size in the early life stages in salmonid fishes generally correlate with survival (Einum & Fleming 2000), the observed size difference could have important consequences for fitness.

For growth rate, the observed patterns were less clear. KAR and HAL showed similar reaction norms for growth rate, but as stated above, this cannot be ascribed unequivocally to similarities in temperature regimes. The lack of correspondence between the highest growth rate and the prevailing temperature within the respective rivers

could be due to trade-offs between growth and other traits important to fitness. Growth rate has been suggested to be correlated with risk of starvation (Gotthard *et al.* 1994), locomotor performance (Cano & Nieceza 2006), lifespan (Metcalfe & Monaghan 2001) and adult migratory life history (Fraser *et al.* 2007). The highest growth rate is therefore not necessarily expected to be found at the optimal temperature. In a related study on grayling, Haugen & Vøllestad (2000) found a similar pattern for growth rate. In this study, the population experiencing the highest temperature in the natural environment also grew better at high temperatures, but for two other populations there was no obvious correspondence. By contrast, a population experiencing temperatures of approximately 9°C in the natural environment actually grew the slowest at this temperature during the experiment. Hence, predicting fitness from growth rate *per se* seems not possible, and trade-offs with regard to this trait might be common in natural environments (Fraser *et al.* 2007).

In all populations, the highest growth rate (per degree-day) was observed at the lowest temperature, 2°C. This could be due to generally low activity levels at this temperature, allocating most of the predetermined energy reserves into growth. Alternatively, growth rates could be upregulated by some sort of compensatory growth in response to the low growth per day, which is experienced at this low temperature. The results for incubation time mirrored the findings for growth rate, as eggs hatched at a lower number of degree-days in all populations (see Hendry *et al.* (1998) for a similar finding). Thus, early development (incubation and growth) seems to take place at a higher rate when temperatures are low, possibly as a response directed towards limiting the prolonged development time under these conditions.

(b) *Adaptability to future climate change*

There is growing evidence that the climate is currently changing at an unprecedented rate, at least partly as a result of anthropogenic activities (Kerr 2007). The projections from the climate models adopted by the Danish Meteorological Institute (www.dmi.dk) suggest marked future changes within northern Europe, including Denmark, with annual average temperature expected to increase by 0.7–4.6°C by the year 2100. This implies milder winters with increased precipitation as well as warmer and drier summers. Additionally, a higher frequency of extreme events is predicted, such as heavy rainfalls and storms.

In this study, we found a significant effect of temperature on three traits, incubation time, alevin length and growth rate, indicating that if the projected rise in temperature within northern Europe becomes reality, it might have detrimental impacts on some natural populations of brown trout. The susceptibility to climate change seems to vary among populations according to adaptation to current local environments. Thus, the suggested adaptation to relatively low temperatures within LIL and NOR would leave these populations more susceptible to increased temperatures compared with, for example, the HAL population, which shows adaptation to higher water temperatures during the early life stages. The ability of some of these populations to adapt to altered conditions could be essential for population viability. The finding of heritable variation

in reaction norms for alevin length and growth rate (sire $\times$ temperature interaction) suggests that there is a potential to respond adaptively to altered temperature regimes. However, this ability will depend on the rate of change in temperature as the populations are not expected to be able to adapt to the altered conditions if the selective pressure becomes very strong (e.g. Gienapp *et al.* 2008).

Our study focused exclusively on adaptability to climate change in juvenile life-history traits, but we note that traits related to phenology could be at least as important (Bradshaw & Holzapfel 2008). In salmonid fishes, spawning time has been suggested to control timing of fry emergence (Beacham & Murray 1987; Hebert *et al.* 1998) and may furthermore have a genetic basis (Gharrett & Smoker 1993; Quinn & Adams 1996; Hebert *et al.* 1998). The four brown trout populations of the present study showed pronounced differences in spawning time. It is possible that this has a genetic component, although testing this in a common-garden design would be demanding in terms of time and resources. If genetic divergence in spawning time exists between populations, then there may be a potential for adapting to altered seasonal timing despite the very low observed heritability for incubation time. However, this potential would then reside in parental traits related to phenology rather than juvenile life-history traits.

While climate model projections suggest increased temperatures in the future, they also predict future increase in climatic variability, e.g. increased variance in temperature around the mean and increased frequency of extreme events. Increased temporal variance in environmental variables is normally expected to favour phenotypic plasticity in labile traits, i.e. traits able to respond instantly to environmental change, such as physiological and behavioural traits (Scheiner 1993), although this depends on the degree of environmental unpredictability (Hard 1995). Moreover, local adaptation to specific thermal regimes may in fact turn out to be highly maladaptive if the regimes become more unpredictable (Pertoldi & Bach 2007; Kristensen *et al.* 2008). The results of this study emphasize these points. The observed temperature-related local adaptation may become maladaptive under future thermal regimes, whereas on the other hand the populations exhibited heritable variation for reaction norms in growth rate. Increased plasticity in this trait might be expected to evolve as a result of increased climatic variation.

(c) Conclusions

In conclusion, although we cannot completely rule out the influence of maternal effects, our results suggest the presence of local adaptation in the populations of brown trout in early life-history traits as well as in their plastic response to different temperatures. While these local adaptations are the product of past natural selection and are expected to increase fitness in the respective populations, they might prove to be maladaptive under future temperature conditions, as projected from climate change models. However, the finding of additive genetic variance and heritable variation in phenotypic plasticity suggests that there is potential for these populations to adapt to future selection regimes, given a gradual environmental change that will allow selection to occur. Finally, it should be noted that adaptive responses to climate change in traits related to phenology, such as spawning time differences, may be at

least as important as adaptability in early life-history traits, and a comprehensive assessment of the evolutionary potential of populations should ideally include both types of traits.

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