UNIVERSITETET
I OSLO

CEES

Spatio-temporal population structuring in complex environments: insights from the European grayling (*Thymallus thymallus*)

PhD thesis

University of Oslo
Faculty of Mathematics and Natural Sciences
Department of Biology

by

Claudia Junge

Series of dissertations submitted to the
Faculty of Mathematics and Natural Sciences, University of Oslo
No. 1099

ISSN 1501-7710

Cover: Inger Sandved Anfinsen.
Printed in Norway: AIT Oslo AS.

Produced in co-operation with Unipub.
The thesis is produced by Unipub merely in connection with the
thesis defence. Kindly direct all inquiries regarding the thesis to the copyright
holder or the unit which grants the doctorate.

Acknowledgements

I would like to thank many, many people. I am starting to realize, however, that I should have written this long time ago, so I will most likely forget to mention people now. So, if you read this and can remember a time you made me smile, told me about your exciting research, had a great idea, or just smiled in the hallway, I'd like to say Thank You!

I would like to thank my supervisors Asbjørn Vøllestad, Glenn-Peter Sætre and Craig Primmer, who were not only patient (which believe me, has probably not always been easy) but also shared their "science wisdom" and ideas during different times of my PhD. A special thanks, however, is going to Asbjørn who always had an open door and ear to listen, no matter how annoying the question or how short the time. Thank you very much! Famous quote: "shorter!" We still do need to battle out the question, however, which is the "better fish": salmonid, shark or eel…

Further, I would like to thank 'Team Grayling'. Thrond Haugen who has such a passion for grayling and who managed to lift our spirits on many frustrating field season or analysis occasions; famous quote: "hiihaa". During her PostDoc time here working on the Lesja grayling project, I have learned so much from Nicola Barson (Nicky), about science, what it means to be a good scientist and office mate (and medic). So thanks to both of you for sharing your science passion and seemingly never running out energy!

A big thank you also belongs to the 'Fish Group', aka the Vøllestad lab, and the good times during our 'fish coffee', which we are aware, for the record, sounds weird to everyone else! It was a great pleasure discussing science and "weird Norwegian habits" with you throughout the last years, so thanks to all of you.

I would also like to thank all the people from 'Team CEES' for all their help during the last years. From team 'CEES lab' I would like to especially thank Nanna Winger Steen and from the 'CEES administration dream team' my special thanks go to Tore Wallem, Gry Gundersen and Camilla Tømta for their time and effort during the last years. Thanks a lot!

I would like to very much thank Nils Christian Stenseth for creating such a stimulating research environment as the CEES, and making it such a great place to work at!

Thanks also to my various collaborators on the papers, you are about to read in my thesis, for their creative input, those papers would not be what they are now without them, and especially to Jaime Otero for his last minute R mission.

Kevin Feldheim introduced me to microsatellites in 2005, and what you can find out with them. That was a real WOW effect, so thanks a lot Kevin!

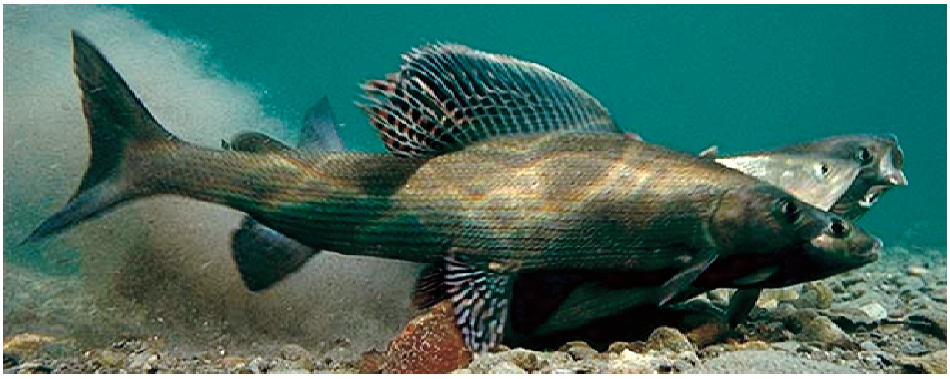
Further, great thanks belong to the many people who have helped with field work in Lesjaskogsvatnet, among them not only biologists but also "others" who helped for fun – Thomas Schütze (software engineer), Stefanie Netz (neurosurgeon), Øystein Langangen (astrophysicist), Mat Jackson (geologist) – it has been a great pleasure. Thank you, thank you, and thank you. I will never forget the famous "it jumped, it actually jumped!" thanks Mat!

On the personal side, I would like to thank all people in my life that have supported me, made me laugh or brought me coffee in critical times, thanks a lot! I am very grateful to my family who has always supported me and even learned to state "Yes, my daughter/sister/aunt/niece/ cousin works with fish!" without finding it weird themselves anymore. A special thanks goes to my mum, Waltraut Junge, who has always been there for me, trusting in me during my whole life, and my sister Mariana Schlicker for always being on my side. Dankeschön! I would also like to thank Irene Pahl who always made me smile whenever we talked. Special greetings also to my Paps who will hopefully keep smiling.

And last but not least all those great colleagues/friends who have made the last years not only work but also a lot of fun. Anna Nilson and Morten Helberg for being great friends, and the great time, soon times, in Mandal; Mali Skogen for always making me look at the bright and fun side, and for generally being a great friend; Thomas Schütze for many evenings of food and chat, and for always having supported me; Øystein Langangen and Annette Taugbøl for the many talking coffees and fun times; Sanne Boessenkool and Bastiaan Star for keeping me sane sometimes and for their energy aura (as promised, also thanks to Bastiaan for his creative input on the title of this thesis); Ryan Easterday for many head clearing coffee stops at his office; Thomas Marcussen for many evenings of social after-work science reflections; and the 'Carcassonne Geek Team': Eric de Muinck, Andrea Gerecht and Irene Parra for many great evenings.

I would very much like to thank my office mates Irene Parra and Art Bass, for their patience during the final months of my PhD, and interesting science and almost-science office conversations. Especially Irene, who managed to keep me alive during those months, which helped this thesis, by forcing me to have lunch, and to actually eat the food, thanks a lot!

Spatio-temporal population structuring in complex environments: insights from the European grayling (*Thymallus thymallus*)



Claudia Junge

Papers

Paper I:

Junge C, Primmer CR, Vøllestad LA, Leder EH (2010) Isolation and characterization of 19 new microsatellites for European grayling, *Thymallus thymallus* (Linnaeus, 1758), and their cross-amplification in four other salmonid species
Conservation Genetics Resources **2**: 219–223.

Paper II:

Junge C, Vøllestad LA, Barson NJ, Haugen TO, Otero J, Sætre G-P, Leder EH, Primmer CR (2011) Strong gene flow and lack of stable population structure in the face of rapid adaptation to local temperature in a spring spawning salmonid, the European grayling (*Thymallus thymallus*). *Heredity* **106**: 460–471.

Paper III:

Junge C, Kausrud KL, Haugen TO, Otero J, Barson NJ, Sætre G-P, Primmer CR, Vøllestad LA. Environmental fluctuations drive population genetic structure under a scenario of contemporary adaptation to divergent thermal habitats. *Manuscript*

Paper IV:

Barson NJ, Haugen TO, **Junge** C, Vøllestad LA. Rapid evolution mediated by plasticity but with strong constraints: contemporary adaptation to thermal shifts in European grayling (*Thymallus thymallus*).
.Manuscript

Paper V:

Junge C, Museth J, Hindar K, Kraabøl M, Vøllestad LA. Evaluating consequences of habitat fragmentation on two migratory salmonids: a snapshot before damming.
Manuscript

Table of Contents

1 Introduction

When colonizing a new habitat, organisms are often faced with novel and potentially fluctuating environmental conditions that exert strong selection pressures (Schluter 2000). The ability to adapt to these novel conditions may be critical for population persistence in these new environments (Chevin and Lande 2009). This is, however, easier said than done. Adaptive diversification and the resulting or foregoing population structuring can occur on different temporal and spatial scales, and can be aided or hindered by different evolutionary, ecological and anthropogenic factors. The aim of my thesis was therefore to investigate population structuring and divergence through time and space, and what influenced them.

Populations – the unit of change

The concept of a '*population'* is central to the fields of ecology, evolutionary biology, and conservation biology, yet, there is no consensus regarding a quantitative definition of a '*population'* (Waples and Gaggiotti 2006). In population genetics, however, the word '*population'* refers to a group of organisms of the same species living within a sufficiently restricted geographical area so that any member can potentially mate with any other member of the opposite sex (Hartl and Clark 2007), and will be used in that way throughout this thesis. Evolution is a process of change in the genetic makeup of populations, with the most basic component being change in allele frequencies with time. Most populations are grouped into smaller subpopulations within which mating usually takes place. When there is such population structure, there is almost inevitably some genetic differentiation, i.e. differing allele frequencies, among the subpopulations.

Several evolutionary forces like selection, genetic drift and gene flow affect genetic differentiation between populations. Genetic drift and selection can cause populations to diverge. Gene flow, on the other hand, tends to homogenize populations, although divergence between populations can occur despite ongoing gene flow if selection is strong enough (e.g. Hemmer-Hansen *et al*. 2007; Nadachowska and Babik 2009; Pavey *et al*. 2010; Richter-Boix *et al*. 2010). Conversely, if the level of gene flow exceeds the strength of selection, then local adaptation will be hindered by the continued introduction of alleles from other populations.

On the other hand, theoretical work suggests that gene flow might in some circumstances have a positive influence on adaptation (e.g. Holt and Gomulkiewicz 1997). It might mitigate negative effects of genetic drift in small populations by replenishing genetic variation and reducing the negative effects of inbreeding, and may thus facilitate adaptive evolution under certain circumstances (Alleaume-Benharira *et al*. 2006; Garant *et al*. 2007). The relative importance of positive and negative effects is, however, currently difficult to assess in nature (Lenormand 2002). To further complicate matters, genetic drift can aid divergence but might oppose adaptation due to its random nature. When either genetic drift or gene flow is able to overpower selection, local adaptation can be inhibited. Because of those complex interactions, population structuring can be very complex.

Salmonids have it all!

"Salmonids are diverse, intriguing, beautiful – and very well studied" (Hendry and Stearns 2004). On top of that, they are ideally suited for the study of population structuring. Salmonid populations might be best described as 'population networks' or 'metapopulations' that occupy a variety of rearing and breeding habitats, and that are at least partially reproductively isolated owing to natal homing (for details on salmonids and their "features" see Hendry and Stearns (2004)). 'Metapopulations' are very dynamic systems, broadly defined as collections of local populations inhabiting discrete patches of suitable habitat, interacting through dispersal and persisting in a balance between stochastic extinctions and recolonizations (see Hanski and Gaggiotti 2004). Many salmonids are expected to exist as 'metapopulations', which is, however, often implied but rarely assessed in detail (but see Schtickzelle and Quinn 2007; for a synthesis see Rieman and Dunham 2000).

Salmonids are furthermore widely assumed to be adapted to their local environment which has recently been reviewed by Fraser *et al*. (2011). Therein, they point to the discrepancy between salmonids on the one hand being a paradigm for local adaptation but on the other hand the still poor knowledge of its extent, scale and molecular basis. Understanding local adaptation is, however, central to determining how quickly, and to what extent, particular salmonid populations will respond to e.g. habitat alterations, climate change and fisheries- or farming-induced evolution. Salmonids are economically and culturally important and have

therefore often been translocated and introduced into novel environments (Hendry and Stearns, 2004), resulting in a number of examples of rapid adaptation to novel environments (e.g. Haugen and Vøllestad 2001; Hendry 2001; Kinnison *et al*. 2001; Koskinen *et al*. 2002a).

In my thesis, I used the European grayling (*Thymallus thymallus*; see Fig. 1), a spring spawning salmonid, to study the spatio-temporal population structuring in complex environments. Grayling are distributed across a large part of Europe (Northcote 1995) and show very low levels of intra-population genetic diversity (Koskinen *et al*. 2002b) compared to other salmonids, but exhibit high levels of genetic divergence also at small geographical scales (Koskinen *et al*. 2001; for a review see Gum *et al*. (2009)) despite common long-range movements (Heggenes *et al*. 2006).

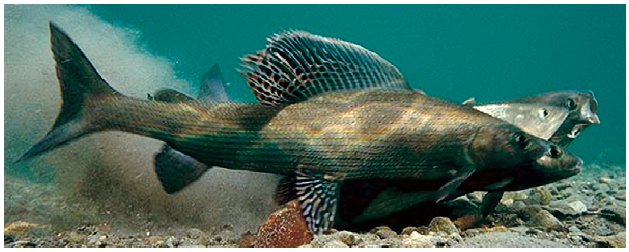


Figure 1: European grayling (*Thymallus thymallus*)

As a spring spawning salmonid, grayling spawning and early offspring survival are highly dependent on environmental conditions in spring, especially with respect to snow melt. In our main study system, Lake Lesjaskogsvatnet (Fig. 2 detail), variable topography along with large variability in the amount of accumulated snow leads to substantial variation in water flow and temperature among the different tributaries. These differences among tributaries lead to variation in both the spawning time of grayling, which may differ by three to four weeks, and the temperature experienced by developing offspring (Gregersen *et al*. 2008; Barson *et al*. 2009; Kavanagh *et al*. 2010), with strong evidence for local variation and adaptation in various life history traits (Gregersen *et al*. 2008; Kavanagh *et al*. 2010). This is especially interesting given that this is a very young grayling population system in its early phase of population divergence. The lake was colonized very recently by European grayling, about 20-25 grayling generations ago (Haugen and Vøllestad 2001). Subsequent dam construction

suppressed further migration into the lake therefore isolating it from the downstream founding river population. Recently, Pavey *et al*. (2010) studied a case of very recent ecological divergence despite gene flow in sockeye salmon that started around 100 generations before present, and reported that this is the most recent ecological divergence ever reported in a fish species following natural colonization. Here, we study a system that seems to have diverged even earlier following a semi-natural colonization, i.e. 20-25 generations ago, driven by differences in temperature between tributaries. Overall, very low neutral genetic diversity has been observed in the system (Koskinen *et al*. 2002), which is probably a result of serial bottlenecks caused by the founding of the original lake population as well as prior upstream translocations within the ancestral river system (see Barson *et al*. (2009) for details).

Lake Lesjaskogsvatnet is also the starting point of the River Gudbrandsdalslågen which terminates in Lake Mjøsa (Fig. 2). It is one of the largest rivers in Norway with 200 km of main river stem. Our 'Lågen' study area lies in a part of the river that comprises over 100 km without any anthropogenic migration barrier.

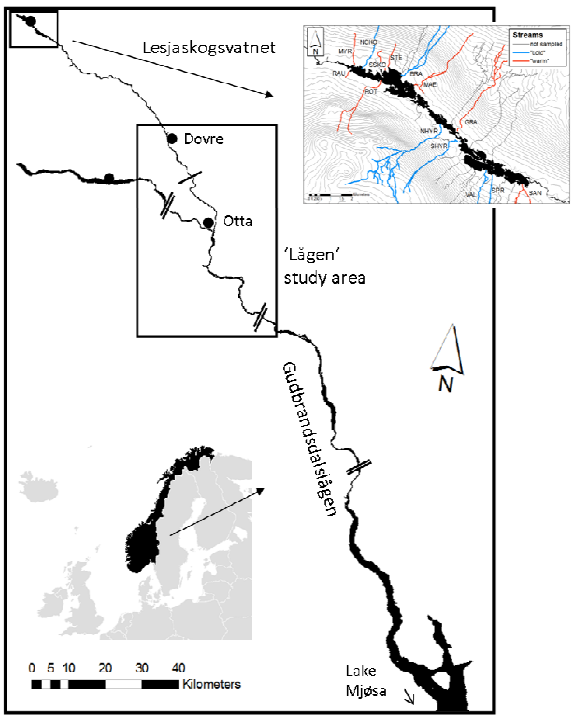


Figure 2: Map of the 'Lågen river system' with indicated migration barriers. Streams in Lesjaskogs-vatnet are labeled in blue for 'large-and-cold' and red for 'small-and-warm'.

The discovery of microsatellites, and other hypervariable genetic markers, has enabled the study of genetic differentiation and population subdivision at small scales, like this one, allowing for in-depth investigations of the different evolutionary forces and their relative

contributions (Koizumi *et al*. 2006; Räsänen and Hendry 2008; Gaggiotti *et al*. 2009) as well as their effects on contemporary adaptation (Garant *et al*. 2007).

Microsatellites

The PCR (=polymerase chain reaction) revolutionized not only molecular biology, but also the fields of organismal and population biology, by stimulating many powerful new approaches to genetic marker acquisition. The idea is surprisingly simple: amplify a single or a few copies of a piece of DNA in order to generate thousands to millions of copies of a particular target DNA sequence through cycles of repeated heating and cooling (see Fig. 3). This method enabled research on many species including those that are endangered because it allows for non-lethal sampling of very small quantities of e.g. tissue, blood, feathers, and faeces, for DNA extraction and subsequent PCR amplification of e.g. microsatellite repeats.

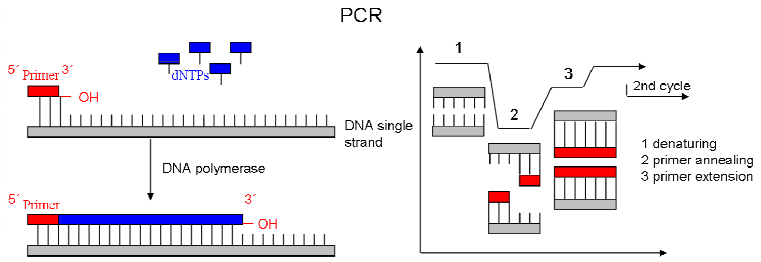


Figure 3: PCR. A sketch of the principle (left) and method (right).

In the late 1980s *Microsatellites* were discovered and soon found to be located throughout nuclear and chloroplast genomes and in the mitochondrial genomes of some species. *Microsatellites* are stretches of DNA that consist of short tandem repeats (1-6 bp). They mutate very rapid with mutation rates of around 10^{-4} events per locus per replication which often leads to multiple alleles at each locus. This high level of polymorphism makes them suitable for inferring relatively recent population genetic events and determining parentage. During replication slipped-strand mispairing might occur (the daughter strand temporarily becomes dissociated from the template strand and re-anneals to the "wrong" repeat) which results in an either longer or shorter strand because it contains a different number of repeats.

Another important feature of *Microsatellites* is based on the fact that they are codominant markers; which allows the identification all of the alleles that are present at a particular locus. This ability to distinguish between homozygotes (one same allele) and heterozygotes (two different alleles) means that we can calculate easily the allele frequencies for pooled samples (such as populations). Numerous analytical methods in population genetics are based at least partially on allele frequencies. (Avise 2004; Graur and Li 1999)

For all those reasons, microsatellites allow us to investigate the relative roles of the different evolutionary forces on population structuring, to understand the influence of the environment on populations especially with respect to changes over relatively short time (anthropogenic or climatic) and the way populations can adapt to that – given that they are developed for the species in question.

Although microsatellites have been earlier described for European grayling (e.g. Diggs and Ardren 2008; Koskinen and Primmer 1999; Sušnik *et al*. 1999; for a review of markers and studies see Gum *et al*. 2009), there is a growing realization that application of higher numbers of molecular markers can increase the accuracy of population genetic inferences (e.g. Koskinen *et al*. 2004). In addition, in cases where populations have low levels of genetic variation owing to e.g. habitat fragmentation or recent founder bottlenecks (see Barson *et al*. 2009; Koskinen *et al*. 2002) not all available markers may be polymorphic. We therefore developed new polymorphic *Thymallus thymallus* microsatellites and reported their cross-species amplification success in the closely related Arctic grayling (*Thymallus arcticus*) and three other salmonid species Atlantic salmon (*Salmo salar*), Arctic charr (*Salvelinus alpinus*) and brown trout (*Salmo trutta*; PAPER I). Those markers were then applied in an optimized panel to all further studies presented in this thesis.

Understanding early population structuring

As discussed earlier, genetic differentiation between populations and therefore population structure is affected by several evolutionary forces like selection, genetic drift and gene flow whose interactions are not always clear or easily to decipher. One way of investigating the importance of the different processes influencing population divergence and adaptation is to study the early phases when a species invades a set of new environments. By doing this, it

may be possible to better understand the relative roles of genetic drift and selection together with the opposing effect of gene flow for divergence. One important question is whether population structuring is required before adaptive divergence can proceed or whether adaptive divergence can occur simultaneously with or even precede the development of isolation (see Dieckmann *et al*. 2004). The Lesjaskogsvatnet grayling system is a very young system in its early phase of population divergence with only about 20-25 generations (Haugen and Vøllestad 2001) since its colonization. On top of that, the combination of environmentally dependent adaptive differences and ongoing gene flow makes this system well-suited for investigating whether a scenario of 'isolation by adaptation' or 'adaptation by isolation' (see Dieckmann *et al*. 2004) can better explain the development of local adaptation in the very early stages of adaptive divergence. To investigate this question we analyzed neutral genetic structure and its stability over time, using microsatellite markers and samples from almost a decade of sampling. Furthermore, we used the analysis of temporal stability to assess the strength of temporal stochasticity in comparison to fluctuations in gene flow using a decomposed pairwise regression (DPR) analysis (Koizumi *et al*. 2006) in order to investigate whether the system is more influenced by drift or gene flow. (PAPER II)

What influences population structure and divergence?

Population structure can be aided or hindered by a variety of factors. Among those are environmental conditions experienced at different life history stages. Many organisms reproduce seasonally and must respond to a variety of cues indicating proper conditions for reproduction. Shifts in phenology, i.e. changes in the timing of events, therefore, seem to be common results of changing environmental conditions (Bradshaw and Holzapfel 2006, 2008). Environmental variability may then lead to an isolation-by-time (IBT) structure, where timing of environmental cues is the main driver of divergence (Hendry and Day 2005). Isolation-by-time could lead to ‘adaptation-by-time’ when differences in reproductive timing that lead to reduced gene flow coincide with differences in selective environments (Hendry and Day 2005). If differentiation is maintained primarily by timing of important life history events, the opportunity for gene flow and thus the strength of the IBT signal may vary as environmental conditions vary among years. Temperature, for example, is an important reproductive cue in many organisms. For salmonids that spawn in rivers and streams with environmental

conditions that differ strongly the response to these cues must differ among populations. Together with the well-documented propensity of salmonids to be highly philopatric (Hendry and Stearns 2004), this may lead to strong population structuring and also local adaptation (see Fraser *et al*. 2011). However, gene flow may still be common among populations, and differences in environmental conditions among years may potentially facilitate or constrain gene flow. In the Lesjaskogsvatnet lake system, previous studies showed a weak but significant signal of isolation-by-distance (IBD; Barson *et al*. 2009; PAPER II). However, the strength of this signal seemed to be subject to temporal fluctuations (PAPER II), possibly related to environmental variation among years that could, through its influence on spawning time, affect the level of among-stream migration. We therefore investigated in detail how among-year variation in local environmental conditions may influence reproductive isolation and impact on the isolation-by-distance signal, and show that climate interacts with geography to either facilitate or constrain gene flow (PAPER III). We used a set of environmental data to estimate spawning times for the various grayling populations during different years where observations were lacking, and the differences between them. Those spawning time differences together with geographic distances were then used to test for an influence on genetic distances (PAPER III).

Global climate change is predicted to result in rapid environmental shifts (Meehl *et al*. 2007). Adaptive responses therefore need to be rapid to avoid maladaptation leading to population extinction (McLaughlin *et al*. 2002; Chevin *et al*. 2010; Chevin and Lande 2010). The relative contribution of genetic, plastic and ecological change to responses to climate change is still under debate (Gienapp *et al*. 2008; Chevin and Lande 2010). The picture is complicated as phenotypic plasticity can also evolve (Via and Lande 1985; Schlichting and Pigliucci 1998), and it is possible that evolving plasticity can accelerate evolutionary responses making this interaction between plastic and genetic change non-trivial (Lande 2009). In the Lesjskogsvatnet grayling system, the delayed warming of the ‘cold’ streams resulted not only in a delay in the spawning date of up to four weeks but also in a cumulative lower developmental temperature experience for ‘cold’ deme offspring (Gregersen *et al*. 2008; Barson *et al*. 2009; Kavanagh *et al*. 2010). This difference in growth period and temperature experience during development has lead to differences in growth rate and muscle

development despite the short time since the colonization (Kavanagh *et al*. 2010). We therefore investigated the potential that evolution of plasticity could have facilitated this rapid adaptation to an environmental perturbation following the colonization of a new habitat (PAPER IV). A common garden experiment was conducted to test for adaptive differentiation among cold and warm spawning demes. By rearing grayling individually at four developmental temperatures, we tested for the signature of adaptation by evolution of phenotypic plasticity in early life-history traits among grayling occupying divergent habitat types within Lesjaskogsvatnet. Additionally, we compared Q_{ST}, a standardized measure of genetic differentiation of a quantitative trait among populations (Spitze 1993) to the expected distribution of Q_{ST} for neutral traits and F_{ST} (Whitlock and Guillaume 2009), to assess whether the trait changes we recorded could be explained purely by stochasticity in these small semi-isolated populations. The average Q_{ST} of a neutral quantitative trait is expected to be equal to the mean F_{ST} of neutral loci (Spitze 1993). If Q_{ST} is lower than F_{ST} this is interpreted as evidence of stabilizing selection and if Q_{ST} is higher of divergent selection.

Anthropogenic barriers to migration like dams have a huge impact on population structure as they fragment or even lead to the loss of previously continuous habitats, therefore leading to one of the greatest threats to biodiversity (Hanski and Gaggiotti 2004). In rivers, habitat fragmentation is usually caused by construction of dams for hydropower production or irrigation. Permanent barriers like dams not only directly degrade or alter aquatic habitats and alter nutrient flows and dynamics, but they also prevent migrations between vital habitats (Jungwirth 1998; Lucas and Baras 2001). Barriers to migration compromise the metapopulation dynamics of habitat specialists by impeding re-colonization, shifting (Williams *et al*. 2008) or even preventing life history migrations (e.g. access to spawning or nursery grounds, e.g. Dauble *et al.* 2003) and reducing gene flow (e.g. Neraas and Spruell 2001; Meldgaard *et al*. 2003). The background for this study (PAPER V) was the hydroelectric development plans in the River Gudbrandsdalslågen (see Fig. 2), and the need for a scientific assessment of consequences for migratory salmonids, i.e. brown trout (*Salmo trutta*) and European grayling (*Thymallus thymallus*). The respective part of the river comprises over 100 km without any anthropogenic migration barrier, an excellent opportunity for a 'baseline' study in an un-fragmented river section as a 'snapshot before damming'. The

new hydropower stations intent to use two existing potential migration barriers, i.e. a dam and natural waterfalls, which are just upstream that area. We used an integrative approach combining population genetics with telemetry to (i) assess population connectivity (movement/level of gene flow), (ii) identify vital habitats (for spawning, feeding and wintering) and (iii) predict genetic consequences of hydropower development by assessing the pre-regulation genetic structure of trout and grayling populations (PAPER V).

2 Material and Methods

Isolation of microsatellites

European grayling DNA library enriched for microsatellite sequences was obtained from a non-commercial microsatellite enrichment service which utilises the hybridisation capture method outlined in Glenn and Schable (2005). Sequences containing microsatellites were screened and selected as described in Leder *et al*. (2008). Amplification success and levels of polymorphism were initially assessed using an M13-tailing procedure (Oetting *et al*. 1995) to screen eight individuals from two different populations (details can be found in PAPER I). The level of polymorphism was assessed by genotyping 24 individuals from a population in southeastern Finland (Puruvesi) and 22 from our study population Lesjaskogsvatnet, shown to have very low microsatellite diversity (Koskinen *et al*. 2002). The final optimization was conducted with end-labelled primers.

Sampling and genotyping

In Lake Lesjaskogsvatnet grayling were caught from 15 spawning populations during spawning runs May/June between 2001 and 2009 (see Fig. 2; PAPER II, III and V) in total 1485 individuals, some streams were sampled only once and others for up to seven years. At capture, all fish were anesthetized, measured (fork length), sexed, and fin clips were excised from the adipose fin and stored in 96% ethanol.

In the mid-section of River Gudbrandsdalslågen system, 80 km in the main river stem and 30 km in the main tributary River Otta, grayling and trout were sampled in 2008 and 2009 at five locations (see Fig. 3; paper IV). 172 trout and 199 grayling were captured above and below the Eidefoss dam and the Rosten rapids and waterfalls as well as further downstream in the main river stem. All fish were captured by rod fishing during early spring (end of March – April), measured (fork length, mm) and a small tissue sample from one of the pelvic fins was taken for later genotyping. 194 of them were also tagged with radio tags (see below). Further grayling samples from the Gudbrandsdalslågen river system below Hunderfossen, below Harpefoss and Lesjaskogsvatnet were obtained and used for comparison.

DNA was extracted using either the DNeasy® Blood & Tissue Kit (Qiagen) or the E.Z.N.A. Tissue DNA Kit (Omega). All samples were genotyped for a set of microsatellite loci: 19 (paper II and III), 18 (paper IV) and 12 (paper V) comprising some previously used ones, plus some of the newly developed microsatellite markers (paper I). For details, please see the respective papers. Briefly, multiplex PCRs (using 1x Qiagen Multiplex PCR Master Mix) with annealing temperatures between 58 and 60°C, were run and subsequently combined for electrophoresis on an ABI3730xl Genetic Analyzer (also ABI3130xl in paper I and V). Trout samples were genotyped for one study, and details can be found in PAPER V. All genotypes were scored using GeneMapper 4.0 software (ABI) and genotype data were converted for further analysis using GenAlEx 6.2 (Peakall and Smouse 2006).

Population genetics analyses

For all genotype datasets, basic population genetics statistics were conducted (details can be found in the papers). In short: Descriptive statistics of microsatellite diversity, i.e. unbiased expected and observed heterozygosity, allele frequencies and mean number of alleles per locus were calculated in GenAlEx 6.2 (Peakall and Smouse 2006). Allelic richness was estimated in FSTAT 2.9.3.2 (Goudet 2001). GENEPOP version 4.0.7 (Rousset 2007) was used to test for significant deviations from Hardy-Weinberg and linkage equilibrium. We corrected for multiple tests by applying sequential Bonferroni corrections (Rice 1989), and also the Bernoulli method (Moran 2003). We tested for population differentiation by performing exact G tests, implemented in GENEPOP, to estimate the *p*-values for genic differentiation between each population pair at every locus and over all loci. In order to assess the statistical power when testing for genetic differentiation, we conducted several simulations using the computer program POWSIM (Ryman and Palm 2006). To estimate the degree of differentiation, pairwise F_{ST} values and global F_{ST} were calculated (Weir and Cockerham 1984; GENEPOP, FSTAT).

Subsequently, several methods and programs have been used to further investigate the population structure in the two study systems. For details please see the PAPERS II and V.

Spatial population structure

Spatial population structure was investigated mainly in two ways: (i) through genetic cluster analysis using Markov chain Monte Carlo (MCMC) simulations (paper IV) and (ii) in a linear fashion by assessing isolation-by-distance aiming furthermore to understand the relative contributions of genetic drift and gene flow during the early phase of adaptive differentiation (paper II).

The program STRUCTURE 2.3 (Pritchard *et al*. 2000) was used to infer spatial population structuring among the five sampling locations in the Lågen study system for grayling and trout (PAPER IV). It uses a Markov chain Monte Carlo (MCMC) simulation to assign individuals to genetic clusters (K) on the basis of their multilocus genotypes. The analysis detects clusters under the assumption of Hardy-Weinberg and linkage equilibrium within each cluster, including new models that make explicit use of sampling location information which can potentially help to detect weak structuring (see Hubisz *et al*. 2009). (PAPER IV)

Under migration-drift equilibrium populations are expected to exhibit a significant correlation between their genetic and geographic distance, termed 'isolation by distance' (IBD; Wright 1943). IBD was tested by correlating genetic distances ($F_{ST}/(1-F_{ST})$; Rousset 1997) with geographic distances (km), measured as the shortest water distance between tributary mouths. However, by using standard regression analysis on all pairwise plots information on local specialties is lost, i.e. sub-population specific characters which are in turn responsible for the relative strengths of genetic drift and gene flow. Since we expected differences between the different Lesjaskogsvatnet spawning sub-populations due to different stream characteristics, we applied the decomposed pairwise regression (DPR) analysis introduced by Koizumi *et al*. (2006) in PAPER II. Briefly, after regressing genetic against geographic distance for all pairwise comparisons, putative outlier populations were detected (and removed) based on systematic bias of the regression residuals. The true outlier populations were then identified by choosing the best model based on the corrected Akaike Information Criteria (AIC_c). For each of the true outlier populations, pairwise genetic and geographic distances were regressed separately against all non-outlier populations, and each non-outlier population was further regressed against all other non-outlying populations to investigate the relative patterns of gene flow and drift (Koizumi *et al*. 2006). (PAPER II)

Material and Methods

Spatio-temporal population structure

The temporal stability of the Lesjaskogsvatnet grayling population structure was tested by (i) assessing how much of the total genetic variation is explained by either spatial or temporal variation through performing a hierarchical analysis of molecular variance (AMOVA) in Arlequin 3.11 (Excoffier *et al*. 2005), and (ii) assessing the signal of 'isolation by distance' through time by partitioning the dataset into years and performing Mantel tests, as described previously (PAPER III).

Detection of migrants

We used two different methods to evaluate dispersal between trout and grayling populations in the Lågen study system. (i) We used GeneClass2 (Paetkau *et al*. 2004; Piry *et al*. 2004) with the following settings: likelihood computation L_home / L_max (Paetkau *et al*. 2004), Rannala and Mountain's (1997) Bayesian criterion for likelihood estimation and Paetkau *et al*.'s (2004) re-sampling method. (ii) The assignment test implemented in STRUCTURE 2.3 (Pritchard *et al*. 2000) was used to detect putative migrants along with any individuals with recent immigrant ancestry. The assignment test implemented in STRUCTURE is a fully Bayesian method that uses geographical sampling location as prior population information, and assumes with a user-specified prior probability (v) that an individual is an immigrant (Pritchard *et al*. 2000).

Effective population sizes and bottlenecks

Short-term effective population sizes (N_e) for the different spawning populations were estimated based on (i) linkage disequilibrium as a one-time estimation (LDNe 1.31; Waples and Do 2008; PAPER V) and (ii) short-term allelic frequency changes between sampling periods using a method that allows for migration (MNe 1.0; Wang and Whitlock 2003; see Fraser *et al*. (2007) for a detailed method comparison; PAPER II).

We used the program BOTTLENECK 1.2.02 (Cornuet and Luikart 1996; Piry *et al*. 1999) to detect population bottlenecks. Since, in a recently bottlenecked population, the level of heterozygosity expected under Hardy–Weinberg equilibrium (observed H_E) exceeds the level

expected in a population at mutation-drift equilibrium (H_{EQ}; Piry *et al*. 1999), this signature is detected by the program.

Telemetry

A total of 127 brown trout and 67 European grayling were radio-tagged at different sections of River Gudbrandsdalslågen and River Otta, and positioned during ≥ 8 weeks (PAPER V). The fish were positioned once a week from March/April to December in 2008 and 2009. Home ranges for each radio-tagged fish positioned more than 7 weeks are presented as the total length of the river section employed, including the extreme points of the positions. Median values across all tagged fish of each location were then used to describe the distribution of individual home ranges.

Common garden experiments

Mature grayling were captured on their spawning run into four streams (two 'cold', two 'warm') during June 2007 and 2008, anesthetized, and their eggs and sperm stripped and a fin clip was taken for genetic analysis. Eggs were fertilized at ~8°C laboratories. Grayling were then reared individually in a common garden at four developmental temperatures. Generally, each male was crossed with two to three females. In 2007 all females were unique but in 2008 maternal in addition to paternal half-sib families were produced. Following fertilisation, eggs were placed into individual wells of a 48-well culture plate and eggs from each family were split between three temperatures for incubation (5.2± 0.2, 6.3± 0.2 and 10.5±0.3°C) in 2007 and reared at 8.2±0.3°C in 2008. Measurements were performed using photographs and subsequent image analysis. (PAPER IV)

We tested for elevated plasticity in 'cold' deme offspring relative to the 'warm' demes, and for a correlation between the slope of the reaction norm and the elevation in the cold thermal environment. Early and late embryonic survival rates were used to test for shifts in the lower thermal tolerance limit, i.e. in the direction of the local adaptive shift, previous work suggested no difference in the upper thermal limit (Kavanagh *et al*. 2010). Analysis of the lower limit allowed us to test if the thermal window has evolved or just the plasticity within this window. For each trait we used an animal model, a form of mixed effects model (Kruuk

2004; Wilson *et al*. 2009), to estimate the additive genetic variance, heritability and evolvability (Houle 1992). By decomposing the variance components the animal model allowed us to check for conformity to the model assumption of additive genetic control and to examine the potential for genetic constraints.

In order to assess whether the trait changes that we observed could be explained by random genetic drift in the small semi isolated spawning populations, we estimated Q_{ST} over the population set. Q_{ST} is the quantitative genetic analogue of F_{ST}. Nineteen microsatellite loci were genotyped for 42-44 mature individuals caught in the four streams in 2008 (see PAPER II for loci and methods). We tested for neutrality using LOSITAN (Beaumont and Nichols 1996; Antao *et al*. 2008), and one locus showing signals of balancing selection was removed from the analysis as a precaution. Weir and Cockerham's (1984) F_{ST} and its 95% confidence intervals were calculated in FSTAT v2.9.3.2 (Goudet 1995, 2001). Since bias in the comparison between Q_{ST} and F_{ST} can arise from sampling error and within population stochasticity (Whitlock and Guillaume 2009), we minimized this effect by comparing the estimated Q_{ST} and its HPD intervals to both F_{ST} and the predicted neutral Q_{ST} following the method proposed by Whitlock and Guillaume (2009). We estimated the neutral distribution of the between population variance. A distribution of Q_{ST} was estimated using the results from the simulation of between population variance and the point estimate of the within population variance. To take account of sampling error in the estimation of F_{ST}, the neutral expectation of F_{ST} - Q_{ST} was calculated. Variance components for each locus were calculated in NEMO (Guillaume and Rougemont 2006) and these were used to create bootstrap estimates of F_{ST} as described in (Whitock and Guillaume 2009). Each iteration of neutral Q_{ST} was subtracted from a bootstrap estimation of F_{ST} to create the neutral expectation for F_{ST} - Q_{ST}.

Modeling

We utilized existing microsatellite data from 12 spawning populations in Lesjaskogsvatnet (Fig. 1) that were collected during spawning runs in May/June 2001-2008, and analyzed previously in PAPER II). In addition, we have, for PAPER III, added samples collected in 2009 when six of the spawning populations were re-sampled. Based on the criteria stated in PAPER III, the final genetics dataset included data from the years 2001 and 2004-2009 and a total of 1261 individuals collected from the 10 spawning populations, in total 33 population-

year samples, with sample sizes between 19 and 69 individuals. Pairwise F_{ST} values were calculated between local populations within the same year (Weir and Cockerham 1984) using GENEPOP version 4.0.7 (Rousset 2007). F_{ST} is a fixation index (Wright 1951), the portion of the total genetic variance that is attributable to differences between populations, and is a commonly used measure of genetic distance which when regressed against geographic distance provides a measure of isolation-by-distance (IBD). When using measures of 'time' one should be able to apply the same logic to test for isolation-by-time (IBT; see Hendry and Day 2005). All estimated F_{ST} values were subsequently transformed as suggested by Rousset (1997) so that the response variable used in all analyses below is $F_{ST}/(1-F_{ST})$, annotated as FST from now on for simplicity.

A set of environmental data was used to estimate the predicted spawning time for the various grayling populations during the different years. It was necessary to estimate some spawning dates as we did not have direct observations and measurements for all years and populations. We used a combination of local environmental data, i.e. geography, stream characteristics, water temperature and spawning observations, and regional and global weather data to make these estimates. Our modeling approach went from meteorological data to stream water temperatures to spawning time to population genetic structure.

In short: We reconstructed stream water temperatures from metrological data (T). We assumed the actual daily mean stream temperature (W) of each stream i would be a function of the month (m) where measures where taken, the mean air temperature the current day, and the average temperature over the previous week. A linear model was fitted to the water temperature data, allowing interactions between stream identity and the other covariates. The onset of spawning was predicted based on the estimated stream temperatures using the approach of Kavanagh et al. (2010). Briefly, a generalized additive model (GAM) was fitted to data on spawning data and stream water temperature. Daily values of either 0 or 1 were assigned to each stream depending on whether spawners were observed (value=1) or not (value=0). As temperature predictor, we used accumulated temperature sums over 4 degrees C (W_4), starting on May 20. The probability of spawning (Pr(S)) was modeled using the binary arrays of spawning/no spawning as response and various predictors including temperature sum, day number of year (t), winter (December-March) NAO index and stream type (G,

classified as cold or warm). From 1996 to 2009 the onset of spawning (*S*) was recorded in 52 occasions for certain combinations of streams (*i*, *j*) and years (*y*). For the rest of the streams and years the onset of spawning was predicted from the model. These spawning observations (and predictions) were used to calculate pairwise differences in the onset of spawning between streams within each year. These comparisons lead to 64 points to be used on further FST modeling. Therefore, the FST values were modeled as a function of the geographic distances (*WD* or *SD*) and the spawning distance using linear models allowing for plausible interactions between candidate covariates.

3 Results and Discussion

Markers for population structure

We found 19 new polymorphic *Thymallus thymallus* microsatellites. We reported genetic diversity indices and equilibrium test results based on two populations, as well as their cross-species amplification success in four other salmonids (for details see Table 1 and 2 in PAPER I). Compared to earlier reported *T. thymallus* markers, these newly developed microsatellites were highly polymorphic with the mean number of alleles per locus for both populations together totaling 9.64; 8.32 (Puruvesi) and 4.5 (Lesjakogsvatnet), the latter one being notably higher than the numbers previously reported in the same lake system (1.8 in Koskinen *et al*. (2002) and 2.6 in Barson *et al*. (2009)) which was the motivation for the isolation of those new markers. Furthermore, one third of the loci showed heterozygosities >0.7 making them not only useful markers for parentage studies but also a valuable resource for conservation genetic studies of European grayling. Most grayling populations appear to show signals of historical bottlenecks (Swatdipong *et al*. 2010) which means, having a wide range of markers to choose from is extremely useful.

Early population structuring

We here used microsatellite data from almost a decade of sampling to investigate early population structuring and its temporal stability (PAPER II), as well as the roles of gene flow (PAPER II) and plasticity (PAPER IV) in a scenario of contemporary adaptation to divergent thermal habitats in the Lesjaskogsvatnet grayling system.

Gene flow and plasticity in adaptive divergence

Both plasticity and gene flow can constrain or promote adaptive divergence (Crispo 2008). Plasticity can allow populations to reach new optima without genetic change (Price *et al*. 2003; Ghalambor *et al*. 2007), whereas high gene flow allows recombination and can result in genetic swamping (Lenormand 2002). However, gene flow also contributes genetic diversity to small isolated populations, therefore increasing the variation that natural selection can act upon and increasing the strength of selection (Garant *et al*. 2007). Gene flow can also rescue

small populations from extinction during the early stages post colonization by buffering against negative population growth until the population is sufficiently well adapted (Lenormand 2002).

In Lesjaskogsvatnet, we found an overall weak but significant correlation between genetic and geographic distance suggesting a regional equilibrium allowing for divergence despite ongoing gene flow (PAPER II). This trend however, does not seem to be associated with the temperature-dependent divergence previously observed in the system (see Kavanagh *et al*. 2010). Thus, is seems that habitat specific adaptation in this system has preceded the development of consistent population sub-structuring and in the face of high levels of gene flow from divergent environments. More detailed assessment of specific local populations, through decomposed pairwise regression (DPR) analysis (PAPER II), indicated that they may in fact be affected differently by gene flow and drift (see Fig. 4 – higher contributions from drift (B) to gene flow (D)) and possibly also extinction-recolonization dynamics, but for the majority of populations and years gene flow appears to be dominant to drift; with variation among years (see below: *spatio-temporal population structure*).

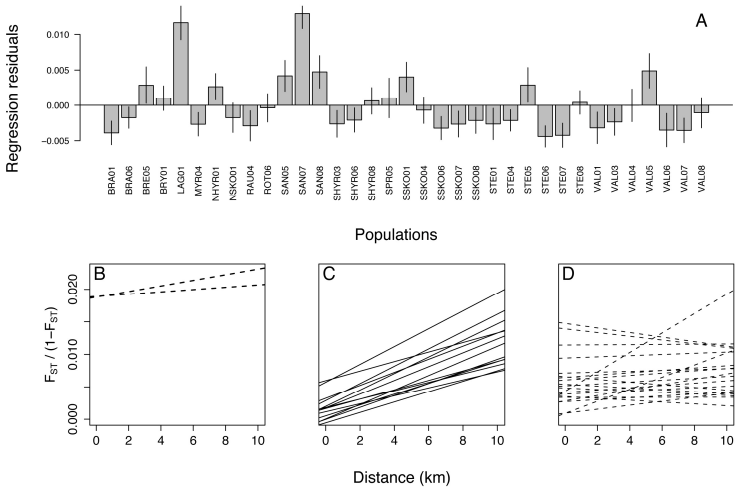


Figure 4: Decomposed pairwise regression (DPR) analyses. A: Average residuals and 95% CIs from the IBD regression. B-D: DPR of (F_{ST}/(1-F_{ST}) vs. km for each population-year combinations; each of the two 'true' outlier populations was regressed with 33 non-outlier populations (B; drift), each of the 33 non-outlier populations was regressed with the other 32 populations showing statistically significant (C; IBD) and non-significant (D; gene flow) regressions.

Several factors may favor divergence despite gene flow in the Lesjaskogsvatnet system. Firstly, given the short period since colonization and the estimated evolvability for the diverging traits (PAPER IV), it is likely that selection is acting on standing genetic variation in the reaction norm, as opposed to requiring the invasion of new mutations. In Hendry *et al*'s (2001) model of divergence with gene flow in quantitative traits, adaptation is predicted even with high levels of gene flow if the heritability of the trait is moderate to high and can be achieved over short time periods (50 generations to reach equilibrium). Secondly, the plastic responses in the timing traits measured appear to be in the same direction as the adaptive response (PAPER IV) leading to weaker directional selection against immigrants than if there were counter-gradient selection (Crispo 2008). Although we did not estimate fitness, the plastic response of the warm population is in the same direction as the elevation of plasticity in the cold adapted population and so we assume this represents adaptive plasticity (PAPER IV). Additionally, the parallelism in responses between offspring from streams with similar spring temperatures suggests that the reaction norms are adaptive. Nevertheless direct tests of fitness of the ecotypic variation we have recorded are required to confirm the adaptive nature of these changes. Cogradient variation is expected to reduce the cost of dispersal and thus allow higher gene flow among selective environments (Crispo 2008). De Jong (2005) modeled adaptation of phenotypic plasticity and found that ecotypes could develop with moderate migration, as is suggested by estimates of neutral genetic structuring in Lesjaskogsvatnet (Barson *et al*. 2009; PAPER II).

Spatio-temporal population structure

Since the evolution of specializations can be very vulnerable to demographic perturbations, it is important to study the early phase where a system might not be in equilibrium yet to understand the development of the type of local adaptation for which salmonids are famous (see e.g. Ronce and Kirkpatrick 2001). One of the main questions we aimed to address in PAPER II was therefore whether or not this system is in equilibrium, which would assume a stable population structure. We tested for both migration-drift equilibrium, i.e. 'isolation by distance', and mutation-drift equilibrium, as evidenced by an absence of bottleneck signatures. None of the performed tests to detect these equilibria convincingly revealed a stable system. Signals of population bottlenecks were observed approximately 2/3 of the 35

population-year samples and isolation-by-distance was observable in only three of six years. A possible explanation for such a temporal pattern is that IBD may be unstable during the initial phase of its establishment (for details see Castric and Bernatchez 2003). Here, a combination of sampling issues, fluctuating environmental conditions and possibly fluctuating population dynamics could result in the observed pattern in this very young and thus yet unstable system. In our study, a lack of temporal stability was, furthermore, suggested by (i) the non-grouping of temporal samples in a principal component analysis, and (ii) the analysis of molecular variance (AMOVA) that showed that a significant amount of the overall variance was accounted for by temporal variance in addition to the underlying spatial variation. Thus, both temporal and spatial genetic variation is evident in this initial phase following colonization of Lesjaskogsvatnet.

The weak overall signal of isolation-by-distance seemed to be subject to temporal fluctuation, possibly related to environmental variation among years that through its influence on spawning time could affect the level of among-stream migration. There was, however, no correlation in any of the years between genetic distances, i.e. $F_{ST}/(1- F_{ST})$ and spawning time distances measured as the difference in days between the spawning onsets. To further investigate this relationship between environmental factors, geographic distance, spawning time and genetic structure we conducted a further study (see below, PAPER III).

Environmental fluctuations drive phenology and population structure

Spawning tributary specific characteristics like exposition and width, determine how fast the stream will warm up in spring and will be able to keep a steady temperature that is suitable for spawning. Since temperature is the cue for the onset of spawning, the environmental conditions therefore determine the reproductive timing. This determines hereupon the amount of gene flow between the different spawning populations.

The opportunity for gene flow among subdivided populations is clearly dependent on the geographic configuration of the landscape. But, as we show in PAPER III, gene flow is, moreover, also facilitated or constrained by local-scale temporal variation in environmental conditions. Our results clearly show a shift in the relative contribution of geography (geographic distance) and ecology (spawning time difference) that is driven by environmental

variation. In the case of large differences between spawning times, ecology seems to constrain gene flow, therefore maintaining population structure based on spawning time difference (isolation-by-time; see Fig. 5, 18 days). In a scenario of simultaneous spawning, however, geographic distance leads to an isolation-by-distance pattern (see Fig. 5, 0 days). This shows that within very small geographic scales the dominant isolating mechanism can vary depending on the climatic conditions. Thus, the strength of the reproductive isolation among populations depends on the local environmental conditions. Weakened strength of this isolation may reduce the opportunity for development of local adaptation, or lead to the breakdown of established adaptations through swamping of local genepools by migrants.

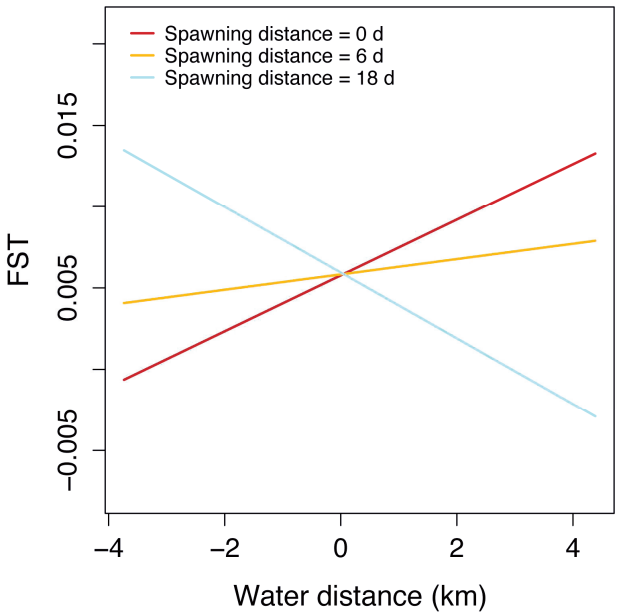


Figure 5. Effects plot from the linear regression model showing the relationship between FST and water distance for three different values of spawning distance: 0, 6, and 18 days of separation. Note that predictors were centred.

Disentangling isolating mechanisms is vital when studying population divergence. Recent studies have shown that geography and ecology together contribute to divergence (Dionne *et al*. 2008; Schwartz *et al*. 2010; Gomez-Uchida *et al*. 2011). Their relative contributions,

however, seem to vary with spatial scale, with an increasing role of ecology at smaller scales (Dionne *et al*. 2008; Gomez-Uchida *et al*. 2011). We could here show those relative contributions of ecological, here spawning time, differences, and geographical distance, which seem to be dependent on environmental conditions (PAPER III), within a very small spatial scale where spawning streams are well within grayling cruising distance. The variation in strength of the spawning time isolation among years, and the interaction between this strength and the strength of the isolation-by-distance signal, could explain why we previously found temporally fluctuating signals of isolation-by-distance (IBD; PAPER II). Variation in local climate here leads to among population variation in annual timing of reproduction/spawning. If some of this variation has a genetic basis, then the presence of different genotypes, with associated particular behaviors and life history traits, may increase the biocomplexity of the system.

These climate induced fluctuations in gene flow are likely to coincide with fluctuations in selection intensity against immigrants. Environmental conditions that determine spawning time, and therefore spawning time differences, are also linked to the thermal conditions experienced by developing offspring. These different thermal conditions, experienced by the different populations, are thought to result in adaptive divergence among populations (Kavanagh *et al*. 2010; Barson *et al*., manuscript). Thus, environmental coupling of both gene flow and divergent selection could be expected to reduce the efficiency of divergent selection among tributaries in this system. Years of increased gene flow would also be years of decreased selection against immigrants. These fluctuations in the strength of both selection and isolation will have consequences for the persistence of locally adapted phenotypes.

The reproductive isolation among the various grayling populations is then maintained by a combination of geography and innate tendencies to search for the natal stream (homing; see Hendry & Stearns 2004), and differential responses to environmental cues. This differential response, whether plastic or adaptive, leads to grayling from different spawning streams experiencing different environmental conditions during early development (Kavanagh *et al*. 2010). Evidence does show that this has lead to genetic differences in a number of early life-history traits, most probably due to rapid local adaptation to the temperature driven differences in local conditions (Kavanagh *et al*. 2010; PAPER IV).

The role of plasticity

By rearing grayling individually at four developmental temperatures, we tested for the signature of adaptation by evolution of phenotypic plasticity in early life-history traits among grayling occupying divergent habitat types within Lesjaskogsvatnet, and found significant Q_{ST} differences of quantitative traits between 'cold' and 'warm' spawning populations in developmental traits (time of eyeing and time of hatching) and survival (PAPER IV).

We found contemporary shifts in the slope and elevation of the reaction norm for the timing of major developmental events. In addition, a signature of adaptation by phenotypic plasticity in the correlation between elevation of the reaction norm in the new environmental conditions and its slope was detected. This rapid response (i.e. within 20-25 generations) suggests potential resilience to temperature shifts resulting from climate change. However, no shift in the thermal tolerance window was evident, despite rapid adaptation within it. As a result this initial rapid response may not translate into resilience against further perturbations of the environment in the same direction. In Lesjaskogsvatnet, adaptation to a shift outside of the thermal tolerance window seems to have required a shift in spawning time (phenology) coupled with the elevation in plasticity of early life-history traits (Barson *et al*. 2009; Kavanagh *et al*. 2010). Thus, responses to anthropogenic disturbance or colonization of novel habitats may require multifarious responses to accommodate the resulting environmental perturbations. It is unlikely that these shifts have resulted from genetic drift in small populations as Q_{ST} exceeded both F_{ST} and the neutral expectation of Q_{ST} for time of eyeing (E_T) and time of hatching (H_T) and the shifts were concordant within stream temperature types with striking parallelism evident in the reaction norms (Figure 3).

Adaptation to climate change is likely to depend on standing genetic variation. This study suggests that this can lead to adaptation of developmental rates to novel conditions within contemporary time frames that can partially compensate for a change in temperature of approximately two degrees centigrade (see below). However, plasticity is likely to be limited by the costs of plasticity (Lind and Johansson 2009) suggesting that the range of perturbations that can be accommodated through plasticity must likewise be limited. Here we see evidence of these constraints through the stasis of the thermal tolerance window despite rapid adaptation of slope and elevation within it.

Anthropogenic impacts on connectivity: movement and gene flow

We have here assessed the population connectivity in River Gudbrandsdalslågen for trout and grayling by investigating both the short-term movement of the two species using telemetry and the more long-term level of gene flow using a set of population genetic tools (PAPER V). Much more extensive movement was observed in grayling (>60 km), which has also been documented in earlier studies in a nearby river where some individuals moved over 150 km (Heggenes *et al*. 2006). Individuals of both species ranged freely within the study area with regular movement between spawning, feeding and wintering areas. The movement was, however, constrained by the dams and waterfalls. The population genetic analysis on the other hand revealed possible upstream migration for trout, but not grayling (probably due to species differences in swimming ability), and downstream gene flow for both species. The population structuring detected was very different for trout and grayling. Most of the five trout sampling populations are significantly differentiated from each other and result in separate genetic clusters (Fig. 6A), except for the two furthest downstream populations which constitute one 'downstream' population. In grayling, however, only the population above the natural waterfalls is differentiated from the other four sampling populations, (Fig. 6B), which is most likely explained by the recent immigration history.

One important potential consequence of fragmenting this river landscape and manipulating water flow is that highly migratory genotypes may become less fit since migratory opportunity will decrease and costs will potentially increase. This may lead to reduced population growth rate and potentially alter the demographic structure. Especially the reduction in water flow over large stretches of the river will select for less migratory genotypes in both species. The loss of particular genotypes, with associated particular behaviors and life history traits, may reduce the biocomplexity of the system and reduce overall population resilience. Recent studies on sockeye salmon (*Oncorhynchus nerka*) and cod (*Gadus morhua*) do show that (meta)populations with complex structures are more resilient towards environmental change than less complex ones – named the portfolio effect (Hilborn *et al*. 2003; Olsen *et al*. 2008; Schindler *et al*. 2010).

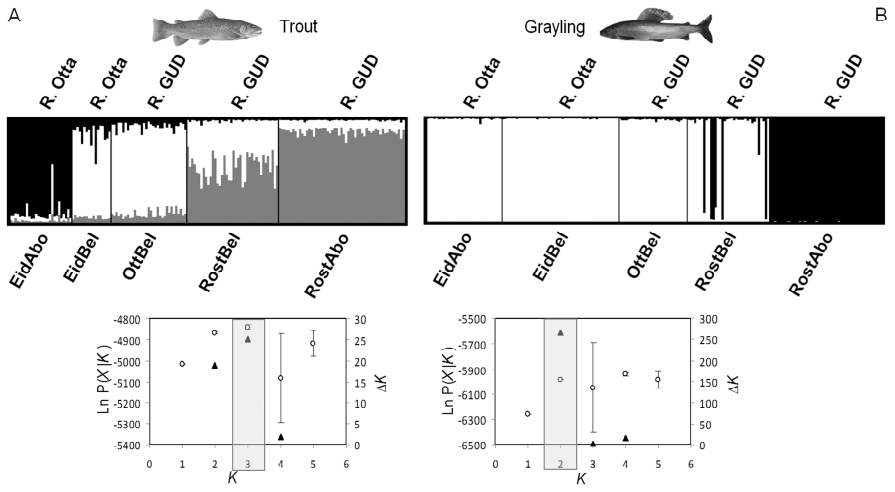


Figure 6: STRUCTURE results for inference of the number of genetic clusters for trout (A) and grayling (B). Top: Proportional membership (Q) of individuals to genetic clusters (K) for $K = 3$ (trout) and $K = 2$ (grayling). Each vertical bar represents a single individual and individuals are ordered by geographical sampling location. Shades (black, grey, white) correspond to genetic clusters. Bottom: Both ln $P(X|K)$ (the likelihood of the data given K; open circles) and ΔK (the standardized second order rate of change of ln $P(X|K)$; filled triangles) are plotted as a function of K. Error bars (where discernible) of ln $P(X|K)$ indicate standard deviations.

The two methods applied here, telemetry and population genetics, did not always lead to the same conclusions on the population and species level, which illustrates the necessity for not only 'multi-method' but also 'multi-species' approaches in order to address such complex questions as 'population connectivity'. One immediate outcome from this study was the preparation of revised construction proposals due to an interference with identified spawning areas for grayling and/or trout.

Furthermore, the results from this study now offer a unique opportunity to follow a "controlled" fragmentation and its ecological and genetic consequences in a well studied population system including two species with contrasting life histories. Evolutionary changes and species-specific responses can so be tracked over time, potentially allowing the identification of the main factors and processes disrupting population dynamics.

4 Summary

I have hopefully shown in my thesis that it takes a variety of approaches to answer such complex questions as the relative roles of evolutionary forces especially on early population structuring together with the effects of space and time.

We investigated early population structuring in the lake system Lesjaskogsvatnet that has recently been colonized by European grayling showing evidence for local variation and adaptation in various life history traits (Gregersen *et al*. 2008; Kavanagh *et al*. 2010). We found that this 'metapopulation' is not yet in equilibrium and detected a weak but significant signal of genetic structuring based on geographic distance allowing for divergence despite ongoing gene flow (PAPER II). Interestingly, this trend did not seem to be associated with the temperature-dependent divergence in the system (see Kavanagh *et al*. 2010). Several factors may favor divergence despite gene flow in the Lesjaskogsvatnet system: (i) given the short period since colonization and the estimated evolvability for the diverging traits (PAPER IV), it is likely that selection is acting on standing genetic variation in the reaction norm, as opposed to requiring the invasion of new mutations; and (ii) the plastic responses in the timing traits measured appear to be in the same direction as the adaptive response (PAPER IV) leading to weaker directional selection against immigrants than if there were counter-gradient selection (Crispo 2008).

We detected spatial and temporal genetic variation, with inter-annually fluctuating signals of isolation-by-distance, possibly related to environmental variation among years that through its influence on spawning time could affect the level of among-stream migration (PAPER II). Variation among streams in how temperature develops during spring determines the spawning onset and therefore the opportunity for gene flow between the different spawning populations. This is a critical relationship given the strong evidence for local adaptation in various early life history traits in this system (Kavanagh *et al*. 2010; also see paper IV). To further investigate this relationship between environmental factors, geographic distance, spawning time and genetic structure, we estimated spawning times based on local, regional, and global environmental data and modeled the effects of spawning time differences between populations on the genetic distance between them. Our results clearly show a shift in the

relative contribution of geographic distance (hence IBD) and spawning time difference (hence IBT) that is driven by environmental variation. In the case of large differences between spawning times, ecology seems to constrain gene flow, therefore maintaining population structure based on spawning time difference (IBT). In a scenario of simultaneous spawning, however, geographic distance leads to an IBD pattern. If conditions are becoming more similar, i.e. due to changing climatic conditions the streams warm up at more similar times therefore leading to similar spawning times and also more similar temperature profiles in the streams, the Lesjaskogsvatnet 'metapopulation' could lose its biocomplexity. This can cause the 'metapopulation' to become less resilient to new disturbances, an effect proposed by Schindler *et al*. (2010). Especially in the face of changing climatic conditions this might have dramatic consequences for population persistence (Chevin and Lande 2009).

This is also highly relevant with respect to anthropogenic "perturbations" that may fragment complex population systems. This might cause the loss of particular genotypes, with associated particular behaviors and life history traits, therefore reducing the biocomplexity of the system and the overall population resilience (see Hilborn *et al*. 2003; Olsen *et al*. 2008; Schindler *et al*. 2010). In PAPER IV, we aimed to assess the potential ecological and evolutionary impact of imposing new migration barriers on trout and grayling, and showed extensive within-river movement of both species with regular movement between spawning, feeding and wintering areas. When fragmenting this population system through hydropower dams, highly migratory genotypes may become less fit since migratory opportunity will decrease and costs will potentially increase. This may lead to reduced population growth rate, potentially altering the demographic structure. Especially the reduction in water flow over large stretches of the river will select for less migratory genotypes in both species. The results from this study now offer a unique opportunity to follow a "controlled" fragmentation and its ecological and genetic consequences in a well studied population system including two species with contrasting life histories. Evolutionary changes and species-specific responses can so be tracked over time, potentially allowing the identification of the main factors and processes disrupting population dynamics. The application of our findings furthermore led to revised construction proposals due to an interference with important spawning areas – a win for science and nature.

Summary

An obvious next step to further understand the adaptive differences in Lesjaskogsvatnet, is an investigation of loci potentially affecting traits under divergent selection. Such candidate loci have previously revealed genetic differentiation between temporally divergent migratory runs in Chinook salmon, suggesting an influence on migration and spawning timing (see O'Malley *et al*. 2007). This promises to be very interesting when applied to the Lesjskogsvatnet grayling in a scenario of contemporary adaptation to divergent thermal habitats.

And it goes on and on and on, and it goes on it and on and on…

(Taio Cruz)

5 References

Alleaume-Benharira M, Pen IR and Ronce O. 2006. Geographical patterns of adaptation within a species' range: interactions between drift and gene flow. *Journal of Evolutionary Biology* **19**: 203-215.

Antao T., Lopes A, Lopes RJ, Beja-Pereira A and Luikart G. 2008. LOSITAN: A workbench to detect molecular adaptation based on a F-st-outlier method. Bmc Bioinformatics 9.

Avise JC. 2004. *Molecular Markers, Natural History, and Evolution*. Sinauer Associates; 2nd edition.

Barson NJ, Haugen TO, Vøllestad LA and Primmer CR. 2009. Contemporary isolation-by-distance, but not isolation-by-time, among demes of European grayling (*Thymallus thymallus*, Linnaeus) with recent common ancestors. *Evolution* **63**: 549-556.

Beaumont MA and Nichols RA. 1996. Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London Series B-Biological Sciences* **263**: 1619-1626.

Bradshaw WE and Holzapfel CM. 2006. Evolutionary response to rapid climate change. *Science* **312**: 1477-1478.

Bradshaw WE and Holzapfel CM. 2008. Genetic response to rapid change: it's seasonal timing that matters. *Molecular Ecology* **17**: 157-166.

Castric V and Bernatchez L. 2003. The rise and fall of isolation by distance in the anadromous brook charr. *Genetics* **163**: 983-996.

Chevin L-M and Lande R. 2009. When do adaptive plasticity and genetic evolution prevent extinction of a density-regulated population? *Evolution* **64**: 1143-1150.

Chevin L.-M, Lande R and Mace GM. 2010. Adaptation, Plasticity, and Extinction in a Changing Environment: Towards a Predictive Theory. *PLoS Biology* **8**: e1000357.

References

Cornuet JM and Luikart G. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* **144**: 2001-2014.

Crispo E. 2008. Modifying effects of phenotypic plasticity on interactions among natural selection, adaptation and gene flow. *Journal of Evolutionary Biology* **21**: 1460-1469.

Dauble DD, Hanrahan TP, Geist DR and Parsley MJ. 2003. Impacts of the Columbia River Hydroelectric System on main-stem habitats of fall chinook salmon. *North American Journal of Fisheries Management* **23**: 641–659.

De Jong G. 2005. Evolution of phenotypic plasticity: patterns of plasticity and the emergence of ecotypes. *New Phytologist* **166**: 101-117.

Dieckmann U, Doebeli M, Metz JAJ and Tautz D. 2004. *Adaptive speciation*. Cambridge University Press: Cambridge, UK.

Diggs MD and Ardren WR. 2008. Characterization of 12 highly variable tetranucleotide microsatellite loci for Arctic grayling (*Thymallus arcticus*) and cross amplification in other *Thymallus* species. *Molecular Ecology Resources* **8**: 828–830.

Excoffier L, Laval G and Schneider S. 2005. Arlequin version 3.0: an intergrated software package for population genetics analysis. *Evolutionary Bioinformatics Online* **1**: 47-50.

Franks SJ and Weis AE. 2009. Climate change alters reproductive isolation and potential gene flow in an annual plant. *Evolutionary Applications* **2**: 481-488.

Fraser D and Hansen MM, Østergard S, Tessier N, Legault M, Bernatchez L. 2007. Comparative estimation of effective population sizes and temporal gene flow in two contrasting population systems. *Molecular Ecology* **16**: 3866–3889.

Fraser D, Taylor E, Hansen M and Bernatchez L. 2011. Genetics of local adaptation in salmonids: what we have learned in 20 years and where to go from now? *Heredity* **106**: 404-420.

Gaggiotti OE, Bekkevold D, Jorgensen HBH, Foll M, Carvalho GR, Andre C and Ruzzante DE. 2009. Disentangling the effects of evolutionary, demographic, and environmental factors

influencing genetic structure of natural populations: Atlantic herring as a case study. *Evolution* **63**: 2939-2951.

Garant D, Forde SE and Hendry AP. 2007. The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology* **21**: 434-443.

Ghalambor CK, McKay JK, Carroll SP and Reznick DN. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Functional Ecology* **21**: 394-407.

Gienapp P, Teplitsky C, Alho JS, Mills JA and Merilä J. 2008. Climate change and evolution: disentangling environmental and genetic responses. *Molecular Ecology* **17**: 167-178.

Glenn TC and Schable NA. 2005. Isolating microsatellite DNA loci. *Methods in Enzymology* **395**: 202-222.

Goudet J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet J (1995). FSTAT (version 1.2) a computer program to calculate F-statistics. *Journal of Heredity* **86**: 485-486.

Graur D and Li W-H. 1999. *Fundamentals of Molecular Evolution*. Sinauer Associates; 2 Sub edition.

Gregersen F, Haugen TO and Vøllestad LA. 2008. Contemporary egg size divergence among sympatric grayling demes with common ancestors. *Ecology of Freshwater Fish* **17**: 110–118.

Guillaume F and Rougemont J. 2006. Nemo: an evolutionary and population genetics programming framework. *Bioinformatics* **22**:2556-2557.

Gum B, Gross R and Geist J. 2009. Conservation genetics and management implications for European grayling, *Thymallus thymallus*: synthesis of phylogeography and population genetics. *Fisheries Management and Ecology* **16**: 37-51.

Hanski I and Gaggiotti OE, eds. 2004. *Ecology, Genetics, and Evolution of Metapopulations*. Elesvier Academic Press, San Diego.

References

Haugen TO and Vøllestad LA. 2001. A century of life-history evolution in grayling. *Genetica* **112–113**: 475-491.

Hartl DL and Clark AG. 2007. *Principles of Population Genetics*. Sinauer Associates, Inc.; 4th edition.

Heggenes J, Qvenild T, Stamford MD and Taylor E. 2006. Genetic structure in relation to movements in wild European grayling (*Thymallus thymallus*) in three Norwegian rivers. *Canadian Journal of Fisheries and Aquatic Sciences* **63**: 1309-1319.

Heggenes J and Roed KH. 2006. Do dams increase genetic diversity in brown trout (*Salmo trutta*)? Microgeographic differentiation in a fragmented river. *Ecology of Freshwater Fish* **15**: 366-375.

Hemmer-Hansen J, Nielsen EE, Frydenberg J and Loeschcke V. 2007. Adaptive divergence in a high gene flow environment: Hsc70 variation in the European flounder (*Platichthys flesus* L.). *Heredity* **99**: 592-600.

Hendry AP. 2001. Adaptive divergence and the evolution of reproductive isolation in the wild: an empirical demonstration using introduced sockeye salmon. *Genetica* **112-113**: 515-34.

Hendry AP and Stearns SC, eds. 2004. *Evolution illuminated: Salmon and their relatives*. Oxford University Press: New York.

Hendry AP, Day T and Taylor EB. 2001. Population mixing and the adaptive divergence of quantitative traits in discrete populations: A theoretical framework for empirical tests. *Evolution* **55**: 459-466.

Hilborn R, Quinn TP, Schindler DE and Rogers DE. 2003. Biocomplexity and fisheries sustainability. *Proceedings of the National Academy of Sciences of the United States of America* **100**: 6564-6568.

Holt RD and Gomulkiewicz R. 1997. How does immigration influence local adaptation? A reexamination of a familiar paradigm. *American Naturalist* **149**:563-572.

Houle D. 1992. Comparing evolvability and variability of quantitative traits. *Genetics* **130**: 195-204.

Hubisz MJ, Falush D, Stephens M and Pritchard JK. 2009. Inferring weak population structure with the assistance of sample group information. *Molecular Ecology Resources* **9**: 1322-1332.

Jungwirth M. 1998. River continuum and fish migration-going beyond the longitudinal river corridor in understanding ecological integrity. In Jungwirth M, Schmutz S, Weiss S, eds. *Fish Migrations and Bypasses*. Oxford Fishing News Books, Oxford. 438pp.

Kavanagh KD, Haugen TO, Gregersen F, Jernvall J and Vollestad LA. 2010. Contemporary temperature-driven divergence in a Nordic freshwater fish under conditions commonly thought to hinder adaptation. *BMC Evolutionary Biology* **10**: 350.

Kinnison MT, Unwin MJ, Hendry AP and Quinn TP. 2001. Migratory costs and the evolution of egg size and number in introduced and indigenous salmon populations. *Evolution* **55**: 1656-1667.

Koskinen MT and Primmer CR. 1999. Cross-species amplification of salmonid microsatellites which reveal polymorphism in European and Arctic grayling, Salmonidae: *Thymallus* spp. *Hereditas* **131**: 171–176.

Koskinen MT, Piironen J and Primmer CR. 2001. Interpopulation genetic divergence in European grayling (*Thymallus thymallus*, Salmonidae) at a microgeographic scale: implications for conservation. *Conservation Genetics* **2**: 133-143.

Koskinen MT, Haugen TO and Primmer CR. 2002a. Contemporary fisherian life-history evolution in small salmonid populations. *Nature* **419**: 826-830.

Koskinen MT, Nilsson J, Veselov AJ, Potutkin AG, Ranta E, Primmer CR and 2002b. Microsatellite data resolve phylogeographic patterns in European grayling, *Thymallus thymallus*, Salmonidae. *Heredity* **88**: 391-401.

References

Koskinen MT, Hirvonen H, Landry P-A and Primmer CR. 2004. The benefits of increasing the number of microsatellites utilized in genetic population studies: an empirical perspective from analyses of grayling (*Thymallus thymallus*) evolutionary relationships. *Hereditas* **141**: 61–67.

Koizumi I, Yamamoto S and Maekawa K. 2006. Decomposed pairwise regression analysis of genetic and geographic distances reveals a metapopulation structure of stream-dwelling Dolly Varden charr. *Molecular Ecology* **15**: 3175-3189.

Kruuk LEB. 2004. Estimating genetic parameters in natural populations using the 'animal model'. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**: 873-890.

Lande R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *Journal of Evolutionary Biology* **22**: 1435-1446.

Leder EH, Karaiskou N and Primmer CR. 2008. Seventy new microsatellites for the pied flycatcher *Ficedula hypoleuca* and amplification in other passerine birds. *Molecular Ecology Resources* **8**: 874-880.

Lenormand T. 2002. Gene flow and the limits to natural selection. *Trends in Ecology and Evolution* **17**: 183-189.

Lind MI, and Johansson F. 2009. Costs and limits of phenotypic plasticity in island populations of the common frog *Rana temporaria* under divergent selection pressures. *Evolution* **63**: 1508-1518.

Lucas MC and Baras E. 2001. *Migration of Freshwater Fishes*. Oxford: Blackwell Science, 420 pp.

McLaughlin JF, Hellmann JJ, Boggs CL and Ehrlich PR. 2002. Climate change hastens population extinctions. *Proceedings of the National Academy of Sciences of the United States of America* **99**: 6070-6074.

Meehl GA, Stocker TF, Collins WD, Friedlingstein P, Gaye AT, Gregory JM, Kitoh A, Knutti R, Murphy JM, Noda A, Raper SCB, Watterson IG, Weaver AJ, Zhao Z-C. 2007. Global Climate Projections in Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M and Miller HL, eds. *Climate Change 2007: The Physical Science Basis*. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Meldgaard T, Nielsen EE and Loeschcke V. 2003. Fragmentation by weirs in a riverine system: A study of genetic variation in time and space among populations of European grayling (*Thymallus thymallus*) in a Danish river system. *Conservation Genetics* **4**:735-747.

Moran MD. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* **100**: 403-405.

Nadachowska K and Babik W. 2009. Divergence in the face of gene flow: the case of two newts (Amphibia: Salamandridae). *Molecular Biology and Evolution* **26**: 829-841.

Neraas LP and Spruell P. 2001. Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. *Molecular Ecology* **10**: 1153-1164.

Northcote TG. 1995. Comparative biology and management of Arctic and European grayling (Salmonidae, *Thymallus*). *Reviews of Fish Biology and Fishereries* **5**:141-194.

Oetting WS, Lee HK, Flanders DJ, Wiesner GL, Sellers TA and King RA. 1995. Linkage analysis with multiplexed short tandem repeat polymorphisms using infrared fluorescence and M13 tailed primers. *Genomics* **30**: 450-458.

Olsen EM, Knutsen H, Gjøsæter J, Jorde PE, Knutsen JA and Stenseth NC. 2008. Small-scale biocompexity in coastal Atlantic cod supporting a Darwinian perspective on fisheries management. *Evolutionary Applications* **1**: 524-533.

O'Malley KG, Camara MD and Banks MA. 2007. Candidate loci reveal genetic differentiation between temporally divergent migratory runs of Chinook salmon (*Oncorhynchus tshawytscha*). *Molecular Ecology* **16**: 4930–4941.

References

Paetkau D, Slade R, Burden M, Estoup A. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* **13**: 55-65.

Pavey SA, Nielsen JL, Hamon TR. 2010. Recent ecological divergence despite migration in sockeye salmon (*Oncorhynchus nerka*). *Evolution* **64-6**: 1773-1783.

Peakall R, Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**: 288-295.

Piry S, Luikart G, Cornuet JM. 1999. BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity* **90**: 502-503.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A. 2004. GENECLASS2: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity* **95**: 536-539.

Price TD, Qvarnstrom A, Irwin DE. 2003. The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**: 1433-1440.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. *Genetics* **155**: 945-959. http://pritch.bsd.uchicago.edu/structure.html

Rannala B, Mountain JL. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America* **94**: 9197-9201.

Räsänen K, Hendry AP. 2008. Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecology Letters* **11**: 624-636.

Rice WR. 1989. Analysing tables of statistical tests. *Evolution* **43**: 223-225.

Richter-Boix A, Teplitsky C, Rogell B, Laurila A. 2010. Local selection modifies phenotypic divergence among *Rana temporaria* populations in the presence of gene flow. *Molecular Ecology* **19**: 716-731.

Rieman BE, Dunham JB. 2000. Metapopulations and salmonids: a synthesis of life history patterns and empirical observations. *Ecology of Freshwater Fish* **9**: 51-64.

Ronce O, Kirkpatrick M (2001). When sources become sinks: Migrational meltdown in heterogeneous habitats. *Evolution* **55**: 1520-1531.

Rousset F 1997. Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* **145**: 1219-1228.

Rousset F. 2007. Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux. *Molecular Ecology Notes* **8**: 103-106.

Ryman N, Palm S. 2006. POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. *Molecular Ecology Notes* **6**: 600-602.

Schlichting CD, Pigliucci M. 1998. *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer Associates Inc., Sunderland, MA.

Schindler DE, Hilborn R, Chasco B, Boatright CP, Quinn TP, Rogers LA, Webster SE. 2010. Population diversity and the portfolio effect in an exploited species. *Nature* **465**: 609-612.

Schluter D. 2000. *The Ecology of Adaptive Radiation.* Oxford University Press, Oxford, UK.

Schtickzelle N, Quinn TP. 2007. A metapopulation perspective for salmon and other anadromous fish. *Fish and Fisheries* **8**: 297-314.

Spitze K. 1993. Population structure in *Daphnia obtusa*: quantitative genetic and allozymic variation. *Genetics* **135**: 367-374.

Sušnik S, Snoj A, Dovč P (1999) A new set of microsatellite markersfor grayling: bfro014, bfro015, bfro016, bfro017 and bfro018. *Animal Genetics* **30**: 462–478.

References

Swatdipong A, Primmer CR and Vasemagi A. 2010. Historical and recent genetic bottlenecks in European grayling, *Thymallus thymallus*. *Conservation Genetics* **11**:279-292.

Via S, Lande R. 1985. Genotype-environment interaction and the evolution of phenotypic plasticity. *Evolution* **39**:505-522.

Wang J, Whitlock MC. 2003. Estimating effective population size and migration rates from genetic samples over space and time. *Genetics* **163**: 429-446.

Waples RS. 2004. Salmonid insights into effective population size. Pages 295-314 in Hendry, AP, Stearns SC, eds. *Evolution Illuminated. Salmon and their relatives*. Oxford University Press, New York.

Waples RS, Do C. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources* **8**: 753-756.

Waples RS, Gaggiotti O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* **15**: 1419-1439.

Weir BS, Cockerham CC. 1984. Estimating F-statistics for the analysis of population-structure. *Evolution* **38**: 1358-1370.

Whitlock MC, Guillaume F. 2009. Testing for Spatially Divergent Selection: Comparing Q(ST) to F-ST. *Genetics* **183**: 1055-1063.

Williams JG, Zabel RW, Waples RS, Hutchings JA, Connor WP. 2008. Potential for anthropogenic disturbances to influence evolutionary change in the life history of a threatened salmonid. *Evolutionary Applications* **1**: 271-285.

Wilson A.J, Réale D, Clements MN, Morrissey MM, Postma E, Walling CA, Kruuk LEB, Nussey DH. 2009. An ecologist's guide to the animal model. *Journal of Animal Ecology* **79**: 13-26.

Wright S. 1943. Isolation by distance. *Genetics* **28**: 114-138.

Paper I

Isolation and characterization of 19 new microsatellites for European grayling, *Thymallus thymallus* (Linnaeus, 1758), and their cross-amplification in four other salmonid species

Conservation Genetics Resources

Paper II

Strong gene flow and lack of stable population structure in the face of rapid adaptation to local temperature in a spring spawning salmonid, the European grayling (*Thymallus thymallus*)

Heredity

Paper III

Environmental fluctuations drive reproductive phenology and population genetic structure under a scenario of contemporary adaptation to divergent thermal habitats

Manuscript

Paper IV

Rapid evolution mediated by plasticity but with strong constraints: contemporary adaptation to thermal shifts in European grayling (*Thymallus thymallus*)

Manuscript

Paper V

Evaluating consequences of habitat fragmentation on two migratory salmonids: a snapshot before damming

Manuscript