Variation in early life history traits among populations of grayling (*Thymallus thymallus*); different temperature reaction norms

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Master of Science thesis

2013

CEES

Centre for Ecological and Evolutionary Synthesis

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University of Oslo, Norway

2013

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https://www.duo.uio.no/

Trykk: Reprosentralen, Universitetet i Oslo

Acknowledgements

Denne oppgava har blitt skrevet ved CEES på Universitet i Oslo i perioden februar 2012 – august 2013.

Først,tusen takk til veilederen min Asbjørn Vøllestad for hjelp til å gjennomføre eksperimentet, og for hjelp og veiledning med skriveprosessen.

Takk til alle som har vært med på felt- og labarbeid. Thrond Haugen og Erica Leder, takk for at dere fanget fisk til eksperimentet, og takk til Anders Herland og Jørgen Haddeland for hjelp til å passe på forsøksdyra mine.

Tusen takk til vennene mine My Hanh, Kjersti, Helena, Sunniva og Gunn Kristin for motivasjon og oppmuntring. Takk for at du hjalp meg med R, Helena og gjorde at jeg klarte å få til statistikken til slutt. My Hanh, Kjersti og Gunn Kristin, takk for at dere har lest igjennom, kommet med gode kommentarer på innhold og rettet skrivefeil.

Til slutt, tusen takk til familien min. Takk for all støtte og motivasjon gjennom studietida.

Anette

Blindern, Oslo

August 2013

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Abstract

The differences in early life history traits among populations of grayling in Lake Lesjaskogvatn and surrounding lakes have been extensively studied the last decades. These populations are the offspring of a small group of grayling that migrated from the river Gudbrandsdalslågen over a century ago. However, the founder populations have never before been compared to the original population in Gudbrandsdalslågen. This study was a common garden experiment with different temperatures comparing the original population of Gudbrandsdalslågen with two newly-founded populations. The experiment had three different temperature treatments and followed the grayling from fertilization to after hatching. The traits studied were timing of eye development, timing of hatching, larvae growth rates and yolk sac consumption rates. Comparison of reaction norms for timing of eye development and hatching showed that there are genetic differences among the populations. The results for growth rate and yolk sac consumption rate were inconclusive due to lack of sample size in this part of the experiment.

Introduction

Climate change can be a challenge for all living organisms; when the global temperature rises local environments may change and a population adapted to a habitat may become less fit (Parmesan and Yohe 2003). An increase in temperature may alter food webs, change life history traits and have a negative effect on physiology, especially in fish that are very sensitive to temperature changes in the water (Ficke, Myrick et al. 2007, Rijnsdorp, Peck et al. 2009). Populations faces risks of extinction and environments may lose biodiversity when the environment changes (Thomas, Cameron et al. 2004). To survive climate changes, such as those predicted following the global climate change, populations can migrated to a more suitable habitat or adapt to the new environment (Malcolm, Markham et al. 2002, Walther, Post et al. 2002). For organisms living in freshwater migration is limited to lakes and rivers, making it more challenging to migrate. When migration is not possible, the solution for freshwater organisms may be adjusting to the changed environment and that can happen in two ways; either through phenotypic plasticity or by rapid evolution (Stearns 1989, Stearns 1992).

Phenotypic plasticity is the capacity of a single genotype to exhibit a range of phenotypes in response to variation in a heterogeneous environment (Fordyce 2006). The level of plasticity in each individual may be important for a population to survive following a climate change; therefore plasticity may be favoured by natural selection. Evolution by natural selection is defined as a change in gene frequencies, and phenotypic plasticity does not change the genotype, therefore the phenotypic response of each individual is not necessarily adaptive. However, the individuals that display phenotypic plasticity may have a higher fitness in a changing environment than those that have a fixed phenotype, and the ability to alter phenotypes by plasticity as thus favoured by natural selection (Westeberhard 1989). To adapt to environmental changes an organism's life history will change. Life history theory is the study of important traits in an organism's life associated with reproduction and survival (Stearns 1992). Timing of reproduction, number of offspring and size of offspring are some life history traits that are being studied in evolutionary biology (Beacham and Murray 1990, Hutchings 1993, Haugen and Rygg 1996, Hutchings and Jones 1998, Schiemer, Keckeis et al. 2002, Papakostas, Vollestad et al. 2010). A norm of reaction is a way to visualize phenotypic plasticity; it illustrates the range of possible phenotypes that a single genotype can develop if exposed to different environmental conditions (Stearns 1989)(figure 1). Reaction norms show how a genotype is express in different environments but does not explain the underlying

mechanisms and evolution of phenotypic plasticity; they only describe their variable and heritable natures. A change in a population's reaction norm requires a genetic change facilitated by evolution.

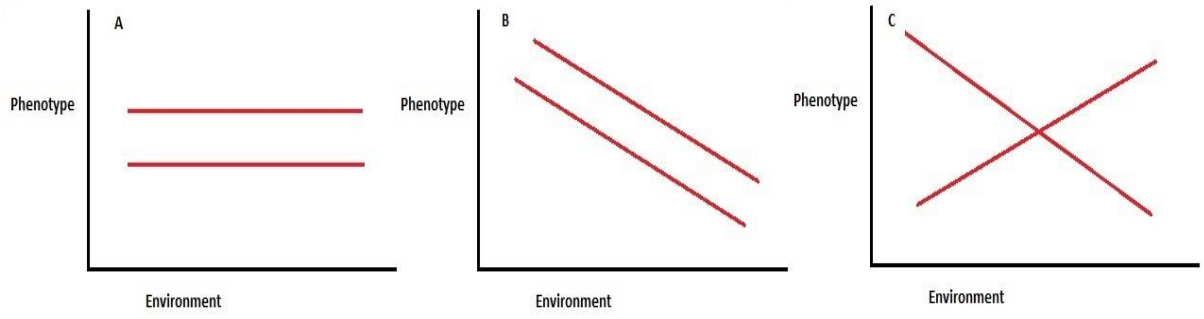

Figure 1 Examples of reaction norms illustrating how phenotypes of two different genotypes (red lines) respond to changes in the environment. In panel A the genotypes produce parallel reaction norms with zero slopes, indicating that environment has no effect on the phenotype (no plasticity) but there may be a genetic difference. Panel B shows an effect of the environment and the genotype have the same plasticity (same slope), but there is no genotype x environment (GxE) interaction. In panel C the two genotypes respond differently to a range of environments. When the slopes cross there is a GxE interaction. GxE interactions show that each genotype is more fit in a certain environment and may outperform the other genotype.

A common way of studying reaction norms is to perform common garden experiments, and test for differences in the slope and elevation of the plastic response (Hutchings 2011). During a common garden experiment, individuals from populations originally living in different habitats are exposed to the same environment. If the populations have similar reaction norms (similar slopes) but differ in elevation the difference between them are most likely due to phenotypic plasticity(figure 1, panel B). Further, if the slopes of the reaction norms are different, this is clearly due to genetic effects (figure 1, panel C).

In relation to freshwater fish in Norway, there is a well-documented example of how newly founded populations of grayling (*Thymallus thymallus*) in the Lesja area in Norway have adapted to different environmental conditions (Gregersen, Haugen et al. 2008, Junge, Vollestad et al. 2011). The grayling population of Lesjaskogvatn was founded by a small group of grayling from the river Gudbrandsdalslågen that dispersed into Lesjaskogvatn through a man-made connection in the late 1880s. There are no natural migration routes between the river and the lake (due to waterfalls), therefore the gene flow between

Gudbrandsdalslågen and Lesjaskogvatn have been non-existent or limited for the last 130 years. In the early 1900s grayling from Lesjaskogvatn was further transported into other nearby lakes in the mountains – and has since dispersed into Aursjøen and several other lakes (Haugen and Vollestad 2001). The grayling populations of Lesjaskogvatn and nearby lakes (figure 2) have been studied over the two last decades (Haugen and Rygg 1996, Barson, Haugen et al. 2009, Thomassen, Barson et al. 2011). The studies have focused on life history evolution and how life history traits have changed over the last century, mainly focusing on early development (timing to eye development and hatching, early growth and energy use)(Haugen and Vollestad 2001). The founder populations experienced a genetic bottleneck when they were split up from the main population and have had short time (20-25 generations) to adapt to the new environment. The bottleneck effect is a type of genetic drift where a small group of individuals are separated from the rest of the population and become the origin of a new population (Nei, Maruyama et al. 1975). Since it is only a small group of founder-individuals the genetic variance of the new population is expected to be lower than the original (Junge, Vollestad et al. 2011). Hence, the founder populations are expected to have reduced ability to adapt to new conditions. In cases when a bottleneck has occurred, the founder population makes for a good study in how they have adapted to the new environment and the results may tell something about how populations are able to survive if the environment changes (i.e. climate change). Earlier studies have showed genetically-based differences in larval growth and yolk-to-body-size conversion efficiency between populations from different tributaries in Lesjaskogvatn (Kavanagh, Haugen et al. 2010, Thomassen, Barson et al. 2011). The same has been found when comparing populations from Lesjaskogvatn and populations from some other alpine lakes that received grayling during later years (Haugen and Vollestad 2000, Koskinen, Haugen et al. 2002). However, no studies have compared the newly-founded populations with the original population living in Gudbrandsdalslågen.

This study compares the early life history of grayling from the original population in Gudbrandsdalslågen with two newly-founded populations. The two main questions that I ask are:

1. Are there any differences in timing of early life history traits (time of eye development, time of hatching) between the original and the founder populations?
2. Do developmental and growth rates differ during early development?

To answer these questions I performed a common garden experiment with three populations and three treatment temperatures.

Materials and Methods

The study organism

European grayling (*Thymallus thymallus)* is a freshwater species in the family Salmonidae that is commonly found in northern Europe. In southern Norway, its distribution is limited to the river Glomma and its associated rivers and lakes. Unlike most Salmonidae species grayling spawn in the spring after ice break when water temperature has risen to minimum 4 °C, in this area it is usually late May to June (Northcote 1995). Both male and female mature at the same time when they are 3 or 4 years old and they spawn every year after. Before the spawning period the grayling most likely comes back to their natal tributary where the eggs are deposited under a small layer of gravel and left unattended until the larvae has matured and swim up.

The study area

The fish in this study were captured in three different locations in northern Oppland, Norway (Gudbrandsdalslågen, Lesjaskogvatn and Aursjøen). The first group of grayling was caught at Otta (OT) in the river Gudbrandsdalslågen; here the grayling spawn in the middle of the river. The other two groups, one from Steinbekken (ST) in Lake Lesjaskogvatn and one from Kvita (KV) in Lake Aursjøen, spawn in small tributaries. Lesjaskogvatn is a fairly large lake located 611 meters above sea level with two main river outlets; Rauma on the west side and Gudbrandsdalslågen on the east side. Both lakes are located in mountainous area. Lesjaskogvatn is closest to the Gudbrandsdalslågen and Aursjøen at a higher altitude (figure 2).

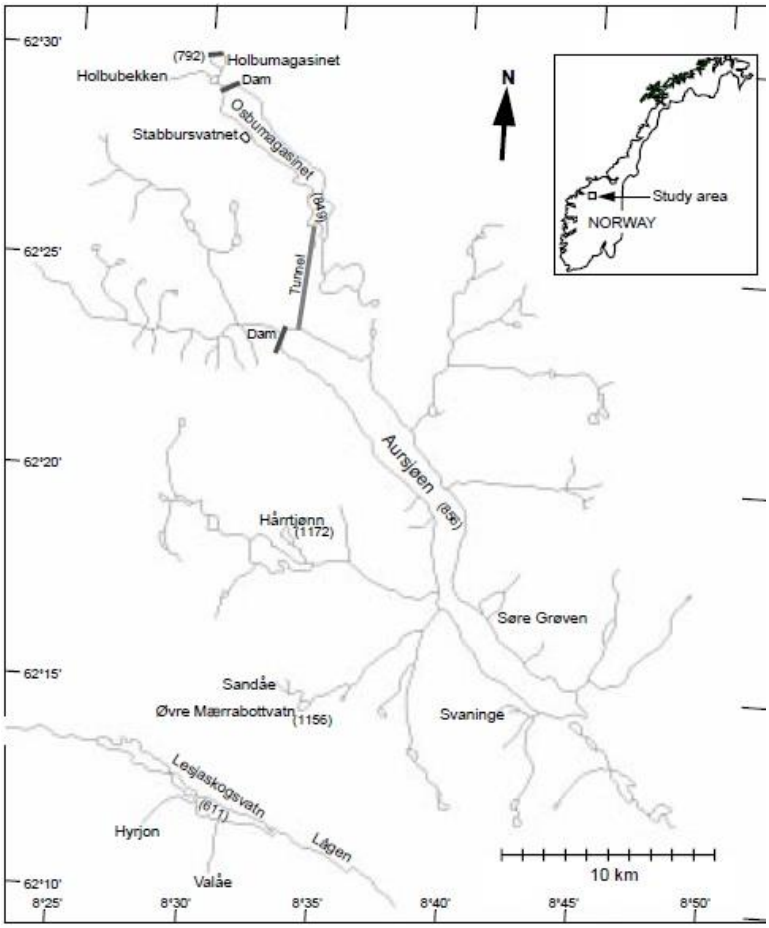

Figure 2 Map of the study area. Steinbekken is located in the north-western part of Lesjaskogvatn, Kvita is located in Aursjøen and Otta further south (not on the map) in Gudbrandsdalslågen (named Lågen on this map).

Field sampling and experiment design

The fish were caught in June 2012 during the spawning period. In Otta, the fish was caught on the spawning location with rod and line. At Kvita and Steinbekken the fish were caught during migration to the spawning sites with fyke nets. Gametes were stripped from the mature fish in the field, stored in plastic bags with air and kept cold on ice during transportation to the University of Oslo. The first population to spawn was Otta, they were caught on the 4th of June and the eggs were fertilized on the 5th of June. The fish in Steinbekken were the second population to spawn and were caught on the 7th of June and fertilized the day after. Finally, fish from Kvita was caught on the 28th of June and the eggs were fertilized on the 29th of June. Due to cold weather during the spawning season, the migration and spawning in Kvita was delayed and very few mature fish were captured. Other populations in the area were even

more delayed, and some probably were not able to spawn at all (T. Haugen and E. Leder, personal communication).

In Oslo the eggs from each female were divided in five batches and sperm from one male were added to each batch of eggs for fertilization. This was all done in a climate room (at the University of Oslo) at 8 degrees. A total of five males and five females were used, and this full factorial design created 25 full-sib families for the Steinbekken and Otta populations. The limited number of females captured in Kvita lead to only 15 families created for that population (five males and three females). After fertilization and cleaning, the fertilized eggs from each family were distributed randomly onto three standard 24 wells culture plates with one egg in each well (figure 3). The wells were filled with water with the same temperature as the climate room (8 °C). Three culture plates were made for each family and placed in three different rooms with temperatures of 6, 8 and 10 °C to simulate different environments. Each plate was marked with population and family number. Other studies (Kavanagh, Haugen et al. 2010) have shown that grayling cannot develop successfully at temperatures above 12 degrees, therefore the temperatures chosen for this experiment was 6, 8 and 10 °C (later referred to as cold, medium and warm). The temperatures in the three climate rooms were monitored using HOBO temperature loggers to get the actual temperature in the rooms. During the whole period the temperature did not fluctuate much so the mean temperatures were used when calculating degree days. The temperature in the warm room was the one closest to the targeted temperature of 10 °C with a mean (±SD) temperature during the whole experiment of 10.1±0.1 °C. The temperature in the medium room was a little bit lower than was aimed for with a mean temperature of 7.3±0.1 °C and the cold room had a mean temperature of 6.2 ±0.1°C. During the experiment the eggs/larvae were monitored once every day, water was refilled when needed and time of eye development and hatching were noted for each individual.

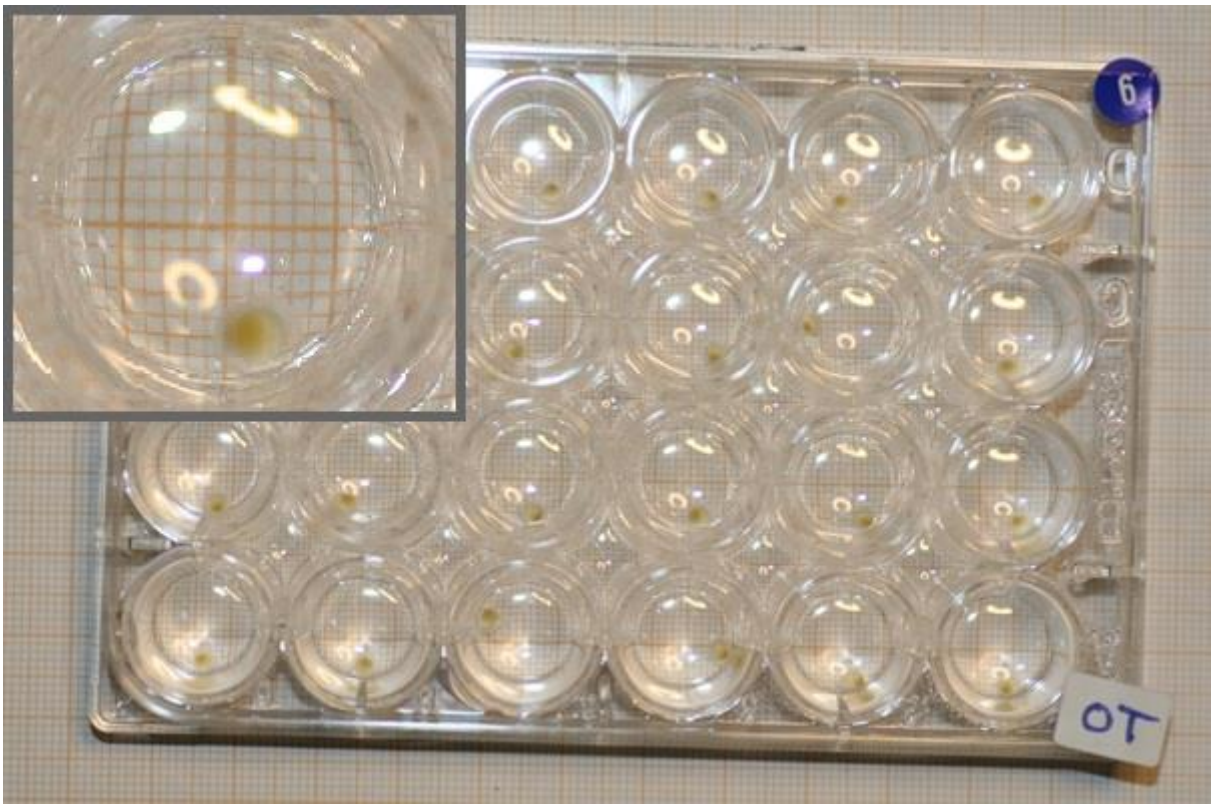

Figure 3 Standard culture plate with 24 eggs of grayling (*Thymallus thymallus).*This image show eggs from family number 6 of the Otta population in the medium treatment temperature. The 12 eggs on the left side were used in another study and the 12 egg on the right side were studied in this experiment. Magnification of one egg inside the grey frame.

Two days after fertilization all of the plates with the eggs were photographed with a Canon EOS 350D camera with a fitted 90 mm Tamron macro lens and a measuring scale placed underneath the plate. The eggs/larvae were photographed three times during their development; two days after fertilization, right after hatching and 70 degree days (estimated from temperatures of 6, 8 and 10 °C, not the actual temperatures of the climate rooms) after hatching when the experiment ended. After finishing the experiment the images were used to measure egg size, yolk sac size and larvae length at hatching and at the end of the experiment.

Image analysis

For measuring egg size, yolk sac size and larvae length I used ImageJ (http://rsbweb.nih.gov/ij/), an image processing program that measures pixels in the images and after calibrated to a known distance converts the pixels to millimetre. The first

measurements were done two days after fertilization to measure initial egg size. All the egg at this time had the same shape of a sphere and a straight line across the eggs was used to measure the diameter. The last two sets of images (from hatching and end of experiment) both larvae length and yolk sac area was measured. Larvae length was measured from the tip of the head to the visible end of the notochord (figure 4). The shapes of the yolk sacs differed but most of them were ellipses and were measured using the ellipse tool in ImageJ to give the area (mm^2) of the yolk sacs. A freehand drawing tool were used to draw the outline of odd shaped yolk sacs where the ellipse form did not fit, and the number of pixels in the area were converted to square millimetre (figure 4).

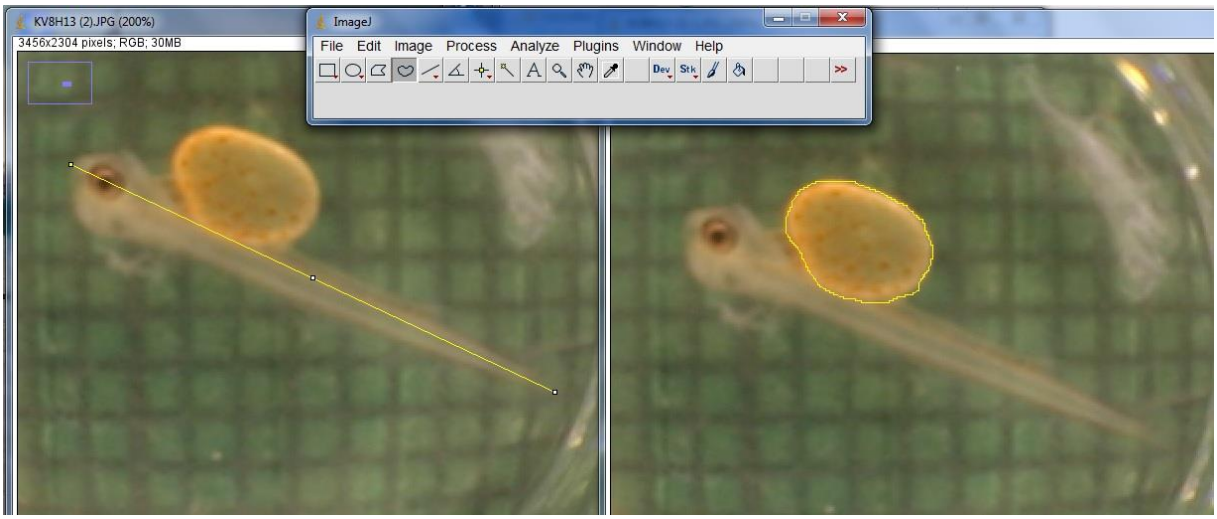

Figure 4 Magnification of an image of a grayling larva from the Kvita population in medium treatment temperature taken right after hatching. The surface underneath the larvae is a measuring scale where one square is 1mm x 1mm. Here, the image processing program ImageJ was used to measuring larvae length, measured from the tip of the head to the end of notochord and yolk sac area was in this case measured from a freehand drawing of the yolk sac because of the non-ellipse shape.

To estimate the measurement errors 30 newly hatched individuals were measured twice and the mean (±SD) difference between the two were 0.25± 0.16 mm^2(5%) for yolk sac area and 0.19±0.13 mm (2%) for larvae length. Larvae length is a more accurate measurement because of the straight line and that the start and end is easy to determine, whereas the errors in yolk sac area is estimated to be higher. The reason for this may be that some of the yolk sacs have an unusual shape and the positioning of the larvae can make it difficult to determine where the outline of the yolk sac is.

Statistical analysis

All statistical analysis was done using the open source program R 2.13.1 (http://www.r-project.org/) for Windows.

For different reasons (see Discussion), there was a varying number of individuals in each family. Therefore, family means was used in the statistical analysis. The statistics for timing of eye development and hatching are based on data from the eggs that survived to hatching.

The data was analysed by fitting a Generalized Linear Model (GLM) with a Gaussian distribution. I used a general model for all the analysis:

$$Y\sim P + T + P * T + \text{egg size}$$

Here, P is population, T is treatment, T*P is the interaction between population and treatment, and egg size is the mean family egg size measured two days after fertilization. Mean family egg size is used as a covariate as development and size often depend on egg size (Gregersen, Haugen et al. 2008). The response variable Y is degree days from fertilization to development of eye pigmentation and to hatching.

An ANOVA F-test was used to test the effect of egg size, treatment, population and the interaction between treatment and population on the response variables.

The statistics for larvae growth rate and yolk sac consumption rate are based on data from the larvae that survived to the end of the experiment. Due to low number of larvae in the cold treatment, the statistical analysis for growth rate and yolk sac consumption rate only consider the warm and medium treatment (see Discussion). Only 50% of the larvae that hatched survived to the end (table 1 and 2). Larvae growth rate and yolk sac consumption rate are calculated as:

(Larvae length at end of experiment – larvae length at hatching) / (day degrees at end – day degrees at hatching)

(Yolk sac area at end of experiment – yolk sac area at hatching) / (day degrees at end – day degrees at hatching)

The data was analysed by fitting a Generalized Linear Model (GLM) with a Gaussian distribution. I used a general model for all the analysis:

$$Y \sim P + T + P * T$$

Here, P is population, T is treatment, and T*P is the interaction between population and treatment. The response variable Y is larvae growth rate and yolk sac consumption rate.

Results

When this experiment was planned the intention was to have five males and five females paired (25 families) from five populations of grayling. From Otta and Steinbekken there were lots of eggs from all the females but very small amounts of sperm from some of the males. All families from Steinbekken had eggs that developed except one family but from Otta there were only 14 families with fertilized eggs or eggs that developed properly. Of the nine unsuccessful Otta families five of them were by the same male and the two others had different father but the same mother. In Kvita there was trouble getting enough females, in the end five males and three females were captured and all of the eggs of one mother did not get fertilized, with the result of only ten successful families from the Kvita population. No fish showed up at the other two spawning streams, therefore I only had eggs from three populations and not five as planned.

In total, 48 families were produced (OT: 14, ST: 24, KV: 10) and distributed at three temperatures. This gives 144 families and with 12 eggs on each plate sums up to a total of 1728 eggs that were followed in the experiment. 742 eggs from 111 families developed to hatching (table 1) and 368 larvae from 71 families survived to the end of the experiment (table 2).

Table 1 Number of grayling eggs that hatched. The experiment had three treatment groups of warm, medium and cold temperatures, and the eggs were from three different populations; Otta (OT), Steinbekken (ST) and Kvita (KV). This sample was used in the analysis of timing of eyeing and hatching.

Population	Total	Warm	Medium	Cold
OT	228	111	106	11
ST	316	188	73	55
KV	194	56	67	71
Total	738	355	246	137

Table 2 Number of grayling eggs surviving to the end of the experiment. The experiment had three treatment groups of warm, medium and cold temperatures, and the eggs were from three different populations; Otta (OT), Steinbekken (ST) and Kvita (KV). None of the eggs from Otta survived to the end in the cold treatment (see Discussion). This sample was used in the analysis of larvae growth rate and yolk sac consumption rate.

Population	Total	Warm	Medium	Cold
OT	72	67	5	0
ST	174	152	15	7
KV	122	29	38	55
Total	368	248	58	62

Timing of eye development

Timing of eye development (eyeing) was estimated as the mean number of degree days when eye pigment was visible for each family. Eye developed within a few days for each population, hence small variance within the populations (see details in Appendix, table 7). The reaction norms showed only a small difference between the three populations (figure 5). In all populations the eggs in medium temperature developed eyes first and the cold treatment was the slowest. In cold treatment all the populations used almost the same number of degree days to develop eyes. While in the medium ST and KV were similar, using more time than OT. In the warm treatment OT and ST developed eyes at similar time, while KV used more time.

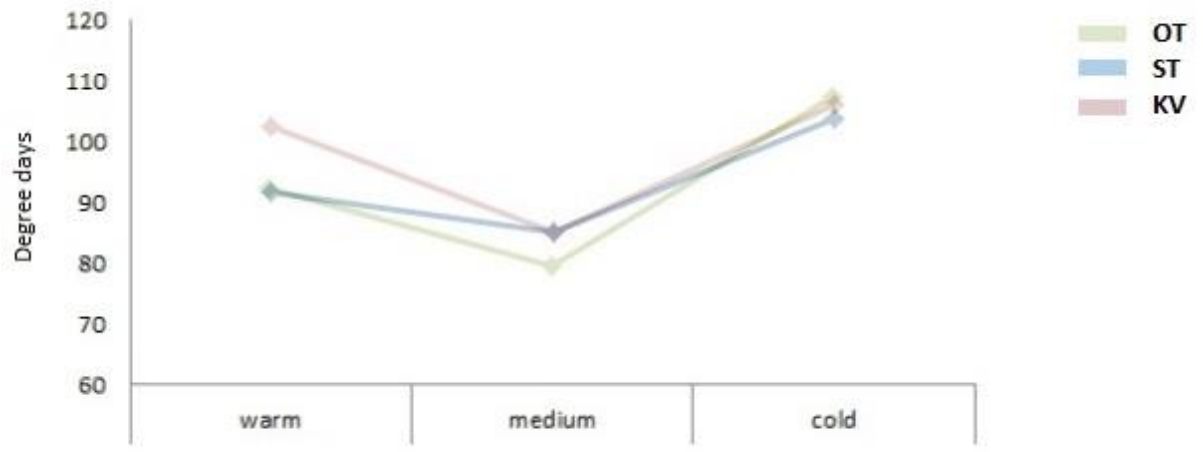

Figure 5 Reaction norms showing degree days from fertilization to eye development in population Otta (OT), Steinbekken (ST) and Kvita (KV) at warm, medium and cold treatment.

Timing of eye development was significantly influenced by temperature (table 3). Also population and the interaction between population and treatment had a significant effect on the response. The size of the eggs at fertilization had no effect on the timing of eye development.

Table 3 Summary of test statistics for the effects on timing of eye development. Pop is population, Treat is treatment, T*P is the interaction between population and treatment, and egg size is the size of the eggs measured two days after fertilization. Df indicates degrees of freedom, for the relevant model term, P taken to be significant (P<0,05) are in bold. R^2 for this model is 0,95.

Variable	Df	F-value	P
Pop	2	111.1	**<0.001**
Treat	2	778.1	**<0.001**
P*T	4	36.5	**<0.001**
Egg size	1	0.6	0.436

Timing of hatching

Timing of hatching was estimated as the mean number of degree days at hatching for each family.

The reaction norms for OT and ST were similar in all the treatments; the embryos developed faster in the medium temperature treatment and slowest in the cold (figure 6). KV used the same amount of degree days to hatch in warm and medium treatment, and significantly more time in cold treatment (see detail in Appendix, table 8). In warm treatment all the populations hatched at almost the same point, in medium and cold there is a difference between KV and the others but the difference is the same among medium and cold.

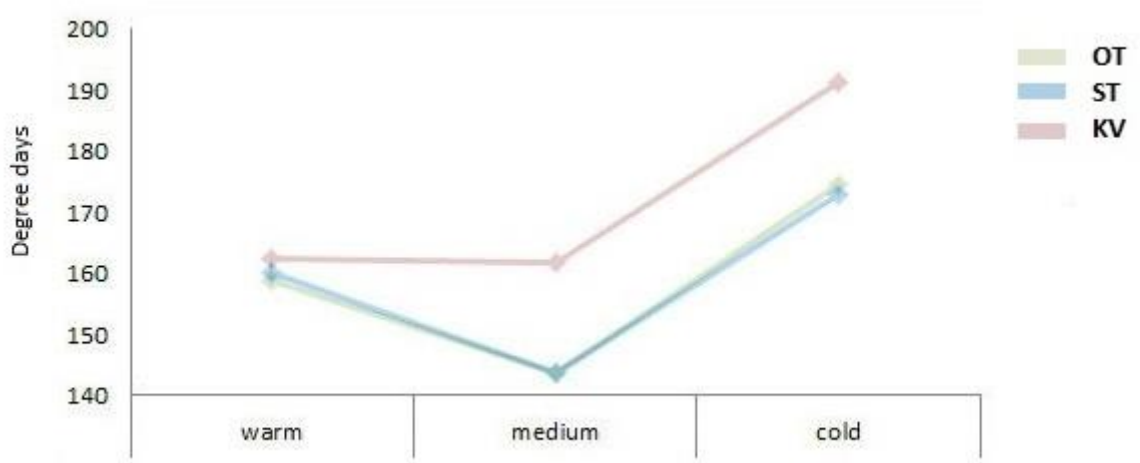

Figure 6 Reaction norms showing degree days from fertilization to hatching in population Otta (OT), Steinbekken (ST) and Kvita (KV) at warm, medium and cold treatment.

Timing of hatching was significantly influenced by temperature and population (table 4). The interaction between population and treatment also had a significant effect on the response. The size of the eggs at fertilization had no effect on the timing of hatching.

Table 4 Summary of test statistics for the effects on timing of hatching. Pop is population, Treat is treatment, T*P is the interaction between population and treatment, and egg size is the size of the eggs measured two days after fertilization. Df indicates degrees of freedom, for the relevant model term, P taken to be significant (P<0,05) are in bold. R^2 for this model is 0,94.

Variable	Df	F-value	P
Pop	2	197.8	**<0.001**
Treat	2	587.0	**<0.001**
P*T	4	24.5	**<0.001**
Egg size	1	0.5	0.474

Larvae growth rate and yolk sac consumption rate

Results from the cold treatment have been excluded from the analysis due to lack of larvae surviving to the end of the experiment in the cold temperature treatment (see Discussion). The reaction norms for larvae growth rates differ among the populations (figure 7). ST and KV have higher growth rate in the warm treatment than medium (see details in Appendix, table 9). OT has lower growth rates than the other populations in both treatments and OT have higher rate in medium than warm.

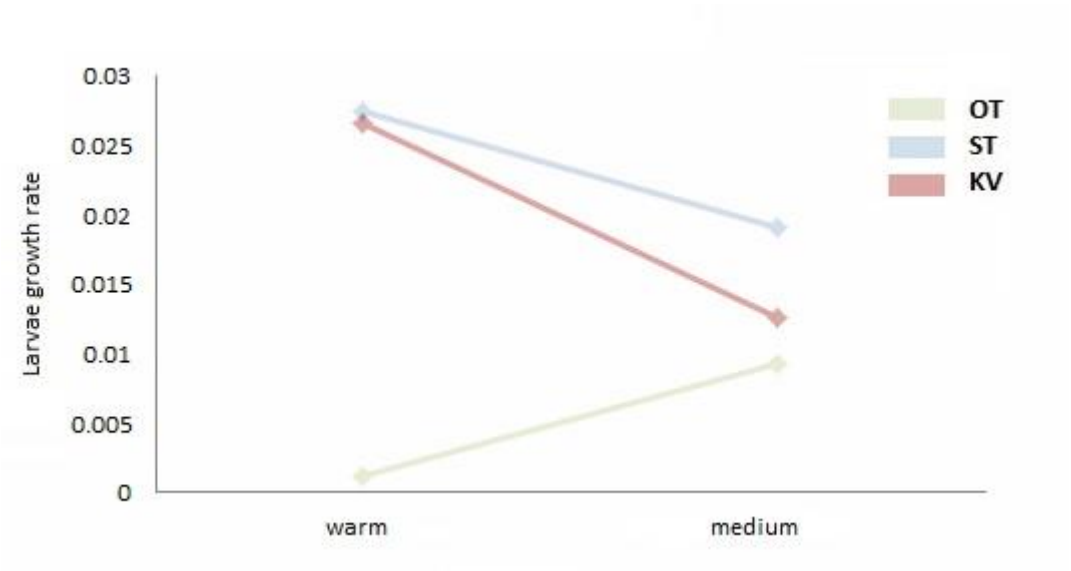

Figure 7 Reaction norms for larvae growth rates in population Otta (OT), Steinbekken (ST) and Kvita (KV) at warm, medium and cold treatment. Cold treatment is left out due to lack of data in ST and OT (appendix).

Population and treatment both had a significantly effect on larvae growth rate (table 5). However, the interaction between population and treatment was not significant.

Table 5 Summary of test statistics for the effects on larvae growth rate. Pop is population, Treat is treatment, T*P is the interaction between population and treatment. Df indicates degrees of freedom, for the relevant model term, P taken to be significant (P<0,05) are in bold. R^2 for this model is 0,48.

Variable	Df	F-value	P
Pop	2	16.9	**<0.001**
Treat	1	13.8	**<0.001**
P*T	2	1.9	0.153

KV has the highest yolk sac consumption rate among the populations (figure 8). Both KV and ST had higher rates in medium treatment than in warm. OT had lowest yolk sac consumption rate in warm treatment, while in medium treatment the rate was higher.

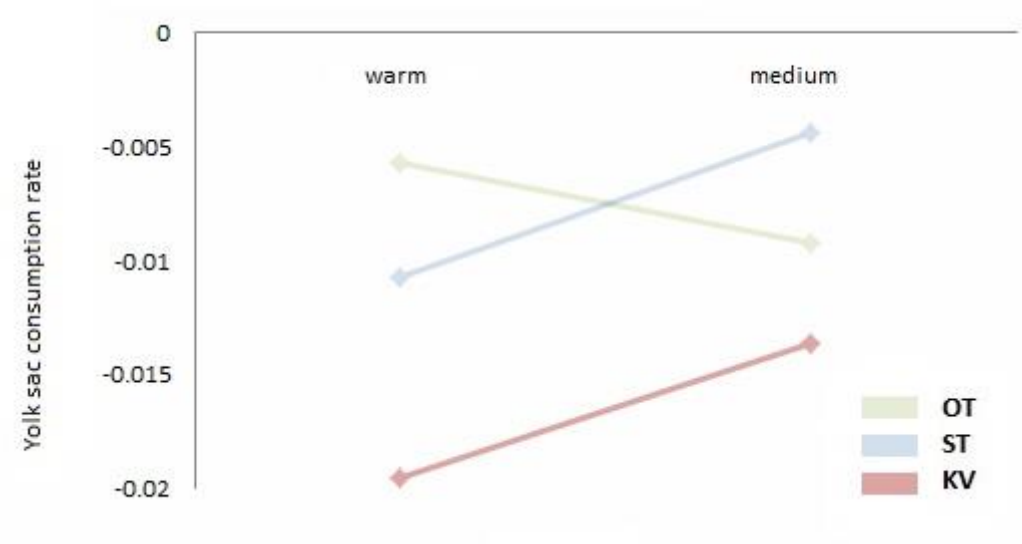

Figure 8 Reaction norms for yolk sac consumption rates in population Otta (OT), Steinbekken (ST) and Kvita (KV) at warm, medium and cold treatment. Cold treatment is left out due to lack of data in ST and OT.

When testing the effects on yolk sac consumption rate the statistics show that all three variables, population, treatment and interaction between the two, had an effect on the yolk sac consumption rate (table 6).

Table 6 Summary of test statistics for the effects on yolk sac consumption rate. Pop is population, Treat is treatment, T*P is the interaction between population and treatment. Df indicates degrees of freedom, for the relevant model term, P taken to be significant (P<0,05) are in bold. R^2 for this model is 0,51.

Variable	Df	F-value	P
Pop	2	17.5	**<0.001**
Treat	1	7.6	**0.008**
P*T	2	3.8	**0.030**

Discussion

The results from this study showed that there are genetic differences in early development among population of grayling in Otta, Steinbekken and Kvita. The common garden experiment with different temperatures produced different reaction norms among the populations indicating genetic differences (figure 5, 6, 7 and 8). Temperature had a significant effect on all the traits studied (table 3, 4, 5 and 6).

Timing of eye development and hatching

It is clear from both the statistical analysis and the slope of the reaction norms that temperature had an effect on early development in all the populations (figure 5 and 6, table 3 and 4). The statistical analysis showed that there was an interaction between population and treatment, hence that there is a genetic difference among the populations. So far, the results were in line with previous studies of the same topic (Jungwirth and Winkler 1984, Haugen and Vollestad 2000, Kavanagh, Haugen et al. 2010, Thomassen, Barson et al. 2011). These studies also showed that early development was faster in warmer temperatures. However, in this study early development (eyeing and hatching) went fastest in medium temperature and slowest in coldest. An explanation for this unexpected result may be that in the warm treatment the eggs suffered temperature stress and thus, reducing the speed of development. In a study by Kavanagh et al. grayling could not develop at temperatures above 12 °C, indicating an upper thermal tolerance level (Kavanagh, Haugen et al. 2010). The mean temperature in

the warm treatment was 10.1±0.1 °C but during the experiment there were several temperature peeks around 11 °C. In Kavanagh et al.'s study the treatment temperatures were 8 and 12 °C, in 8°C the grayling developed normally, while at 12°C showed high mortality and high rate of malformations, indicating that the upper thermal tolerance limit is close to 12 °C. In my experiment the mortality was not higher in the warm treatment but with temperature close to thermal tolerance limit may be the cause of slow development.

There is a similarity among the populations' reaction norms with the exception of KV (figure 6), especially at hatching were OT and ST have similar reaction norms but KV develops slower in medium and cold treatment. In nature, OT and ST are the most similar with relatively warm temperatures, while KV is colder due to higher altitude. Both ST and KV originate from the OT population but KV has gone through two bottlenecks (first dispersing into Lesjaskogvatn then into Aursjøen). After two bottleneck events, genotype frequencies will most likely be different from the original populations. Hence, there being a genetic difference between KV and two others. The differences between the KV population and the original population (OT) have originated in within the last 20-25 generations and are a result of rapid evolution. Thus, indicating that local adaptation can happen in a short period of time.

Larvae growth rate and yolk sac consumption rates

The reaction norms for larvae growth rate and yolk sac consumption rate showed that there are differences among the populations. In warm treatment OT had very slow larvae growth, while ST and KV had significantly faster growth. In medium temperature, OT grew faster than in warm but is still the slowest population. ST and KV grew faster than OT in medium temperature but their growth was fastest in warm temperature. According to the statistical analysis, the interaction between population and treatment have an effect on yolk sac consumption but not on larvae growth. However, the slopes of the reaction norms indicated that there is a genetically based difference among the population in both larvae growth rate and yolk sac consumption rate.

The growth rate and yolk sac consumption rate are expected to increase with temperature (Kamler 2008). The results for ST and KV follow these expectations with a higher larvae growth rate and yolk sac consumption rate in the warm treatment than in medium treatment (figure 7 and 8). Moreover, the results for OT are opposite with a decreased rates in warmer temperatures.

The results from this part of the study are difficult to analyse and inconclusive due to low sample number in some of the groups. No larvae survived to the end in OT cold treatment and very few survived in ST cold treatment. Therefore, the analysis was done without the cold treatment. However, the number of surviving larvae in medium treatment is also very low for OT and ST, and this may be the reason for the unexpected results in this part of the study.

The amount of eggs and larvae in this experiment decreased throughout the experiment. Only 43% of the eggs hatched. One of the reasons for this low percentage is that a lot of the eggs in the cold treatment from OT and ST dried out early in the experiment. Some of the eggs did not develop at all and may have been unfertilized. In OT cold many eggs dried out but some made it to hatching. However, before the end of the experiment all the larvae were dead, leaving no data for larvae growth rate and yolk sac consumption rate in this group.

There was a high mortality rate in the period between hatching and the end of the experiment, indicating that the experiment should have ended earlier (table 1 and 2). Of all the hatched larvae (742) only 50% survived to the end of the experiment who ended 70 degree days after hatching (calculated from theoretical temperatures in the climate rooms). The reason for so many larvae dying before the end of the experiment may be lack of energy or the space in the wells became too small for the larvae to continue development. For future studies, the growth rate should be measured within a shorter period of time after hatching, to reduce the mortality of hatched larvae.

Conclusion

There are differences in timing of eyeing and hatching among populations of grayling, and reaction norms from a common garden experiment with different temperatures demonstrate that the differences are genetic.

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Appendix

Table 7 An overview of mean degree days with standard deviation from fertilization to eye development in each population (Otta =OT, Steinbekken =ST and Kvita =KV) and treatment group. The sample size for eyeing was the same as for hatching. Most of the embryos that developed eyes continued to hatch, and the data used in both the analysis for eyeing and hatching are from all the larvae that hatched.

Population	Treatment	Degree days	Standard deviation	Sample size
OT	warm	92	0	111
ST	warm	92	3.3	188
KV	warm	102	0	56
OT	medium	79	1.2	106
ST	medium	85	0.9	73
KV	medium	85	0.7	67
OT	cold	107	1.6	11
ST	cold	104	0.7	55
KV	cold	106	2.2	71

Table 8 An overview of mean degree days with standard deviation from fertilization to hatching in each population (Otta =OT, Steinbekken =ST and Kvita =KV) and treatment group.

Population	Treatment	Degree days	Standard deviation	Sample size
OT	warm	159	4.2	111
ST	warm	160	4.1	188
KV	warm	163	2.8	56
OT	medium	144	2.4	106
ST	medium	144	0.7	73
KV	medium	162	3.6	67
OT	cold	175	3.2	11
ST	cold	173	2.3	55
KV	cold	191	3.7	71

Table 9 An overview of mean larvae growth rates and mean yolk sac consumption with standard deviation in each population (Otta =OT, Steinbekken =ST and Kvita =KV) and treatment group. No larvae in cold treatment from OT survived to the end of the experiment.

Population	**Treatment**	**Larvae growth rate (mm)**	**Standard deviation**	**Yolk sac consumption rate (mm^2)**	**Standard deviation**
OT	warm	0.777	0.006	-0.377	0.003
ST	warm	2.020	0.009	-0.817	0.005
KV	warm	2.067	0.006	-1.468	0.006
OT	medium	0.752	0.006	-0.728	0.007
ST	medium	1.536	0.010	-0.361	0.004
KV	medium	1.062	0.002	-1.155	0.005
OT	cold				
ST	cold	1.953	0.007	-1.236	0.014
KV	cold	1.968	0.006	-0.719	0.002