Master Thesis

Phenotypic and ecological diversification in relation with habitat stability

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September 2014

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Abstract

Habitat stability is an important factor that strongly influences individuals and their interactions. Both the stability of a habitat and the most prevalent conditions in it can determine ecology and phenotype of individuals that inhabit it. This should be reflected by both the niche they occupy in the habitat and their phenotype. There is still a lack of studies showing causes and effects of individual specialization on individual and population niches. To study the influence of habitat stability on these factors, I analyzed the individual diets of and conducted phenotypic analyses on trout from environmentally stable groundwater fed and unstable surface water fed streams in the Lake Lucerne drainage. There were clear ecological differences between stream types. Individuals from the stable habitat showed a higher degree of specialization than those from unstable habitats. The population niche in the stable habitat, hence, consisted of many smaller, specialized individual niches, with most of the variation in the between-individual component. In the unstable habitat, it consisted of overlapping, generalist niches with a high within-individual component to variation. Overall, my study showed that habitat stability can be an important factor in determining the niche of individuals in the habitat. I also used microsatellites to show that each stream could be considered a separate replicate. I found statistically significant differences in phenotype, both color and shape.

Introduction

An overreaching factor that can shape whole communities and ecosystems is habitat stability. It influences various abiotic factors which can determine community composition. This effect has been described at lower trophic levels, i.e. in invertebrates (Death and Winterbourn, 1995), which can in turn structure predator communities through bottom up effects. However, effects of habitat stability have also been observed at higher trophic levels (Brydges *et al.*, 2008; Fitzpatrick *et al.*, 2009).

The selection pressures presented by an unstable habitat will differ greatly from those in a stable habitat. If the habitat is unstable, the populations that occupy it are forced to adapt to the adverse effects that are presented by the habitat. This could lead to selection on individuals that can adjust to the disturbances that occur (Clausen and Biggs, 1997). However, individuals should be adjusted to both average and extreme conditions. They will need to be adapted to the general conditions that are most prevalent in their habitat, but also able to cope with large disturbances when they occur. It can be hypothesized that this will lead to a mean phenotype that is shared by most individuals in a habitat, with little deviation from the optimum. This would suggest that population variation should be driven mostly by within-individual variation, rather than variation between individuals. The population would therefore be expected to consist of generalists, with all individuals occupying most or all of the total niche width of the population (Bolnick *et al.*, 2002).

In contrast, stable habitats offer different challenges. In stable ecosystems, the likelihood of extreme events is relatively low. Therefore, other factors can be decisive in shaping interactions both between individuals and between individuals and their environment. Competition is one of these factors, and can be the main selective pressure shaping communities. This can be interspecific competition if multiple species share habitat and niche. However, intraspecific competition and variation also strongly influence the outcome of ecological interactions (Bolnick *et al.*, 2011). Under the pressure of intraspecific competition, individual specialization is more likely to occur. This appears especially likely if a species is released from interspecific competition (Bolnick *et al.*, 2010). In such systems, it can be hypothesized that there exists a relatively high amount of between-individual variation, with individual niches representing only a small portion of the population niche. The specialization on specific prey types can also cause top down effects that then lead to feedback loops. This has been shown to be a large influence and could be especially true if the organism that shows high density is the top predator in the system (Svanbäck and Persson, 2004; Matich *et al.*, 2011).

To study the influence of stability, the pre-alpine region offers an ideal system. Streams in this region can have large changes in both the amount of water and speed of flow due to flooding. While some of the streams flood heavily and often, others do not. The reason for the greater stability of some of these streams can be found in the source of the water. Groundwater fed streams represent a stable habitat. These streams are fed by a relatively steady flow of groundwater, which changes little throughout the year. They also show high amounts of soft sediment accumulation, which is likely to be due to variation in flow. Flooding tends to reduce soft sediment accumulation (Whitledge and Rabeni, 1997; Lenzi and Marchi, 2000) in streams, so its presence suggests little flow variation. They also contain large amounts of vegetation. Flooding decreases plant biomass (Biggs, 1995), which is further

evidence that it is infrequent in these streams. Surface water fed streams receive most of their water from melt off and precipitation, which are inherently unstable. The amount of water and flow velocity can increase strongly when temperatures rise at the beginning of spring or during periods of heavy rainfall. This leads to frequent flooding and common occurrences of a flow velocity above the mean. Both stream types are often found at the same altitude in close proximity, feeding into each other or the same larger body of water. This allows pairwise comparisons to be made.

Both stream types are dominated by brown trout, *Salmo trutta* L., which are highly polymorphic and occupy a range of habitats (Pakkasmaa and Piironen, 2001; Klemetsen *et al.*, 2003). This makes them an ideal study organism to test for the influence of habitat stability. Trout feed on a variety of prey through different life stages, and often compete for the same resources (Alanärä *et al.*, 2001). They are also known to show both generalist and specialist feeding strategies (Bridcut and Giller, 1995). These traits allow the study of habitat factors that influence within-individual and between-individual components of the total niche width of populations.

In my study, I aimed to find out whether differences in habitat stability on a small scale would lead to population differentiation in *S. trutta*. For this, I studied streams with different stability in the Lake Lucerne drainage, i.e. groundwater and surface water fed. These streams differ greatly in several key habitat characteristics, both biotic and abiotic, in close proximity.

I hypothesize that there are consistent ecological differences between populations in the two different stream types I studied. Specifically, I expect a higher degree of individual specialization in the stable habitat. I further expected phenotype to differ significantly between stream types. To investigate this, I performed stomach content analyses on fish from both habitat types and calculated measures of population niche and individual specialization. To study phenotypic differences, I conducted geometric morphometrics and color analyses. Lastly, I conducted microsatellite analyses to determine whether the studied populations can indeed be considered independent sampling units.

Material and Methods

Study systems and populations

The study was conducted in groundwater and surface water fed streams. Ten streams (table 1) were chosen in a pairwise pattern, with two streams being tributaries to the same larger body of water. Surface water fed streams receive much of their water through precipitation, either directly or through snow melts. The water in groundwater fed streams is filtered through layers of soil before ending up in the stream. This leads to very different conditions in these streams. Groundwater fed streams have a more stable temperature and flow regime, are clearer and do not flood unless there is very heavy precipitation. Surface water fed streams are warmer in summer and colder in winter and flood quickly with precipitation. Floods also show higher flow than in groundwater fed streams.

All selected streams are part of the Lake Lucerne drainage, and the largest distance between two streams was 68.1 km, with the average distance being 32.57 km between any two streams and 4.47 km between stream pairs (Fig. 1).

Table 1: Streams for which trout were analyzed in the course of this study, with measures of habitat variables

Stream	Stream type	Coordinates (Lat/Long)	Altitude asl (m)
N2 Entwässerungskanal (N2)	Groundwater	46.967/8.349	443
Chli Schliere (CS)	Surface Water	46.951/8.282	438
Scheidgraben (SG)	Groundwater	46.978/8.407	405
Engelberger Aa (EA)	Surface Water	46.977/8.421	438
Schibenriedbach (SR)	Groundwater	46.837/8.175	420
Grosser Melchaa (GM)	Surface Water	46.890/8.260	484
Walenbrunnen (WB)	Groundwater	46.851/8.644	455
Gangbach (GB)	Surface Water	46.858/8.648	460
Schützenbrunnen (SB)	Groundwater	46.809/8.662	474
Kärstelenbach (KB)	Surface Water	46.769/8.670	513

For some analyses, namely habitat and community structure, data was drawn from a larger pool collected in the same sampling period by the same field team that collected the data from the streams that were picked for closer analysis (see tables 1 and 2, appendix).

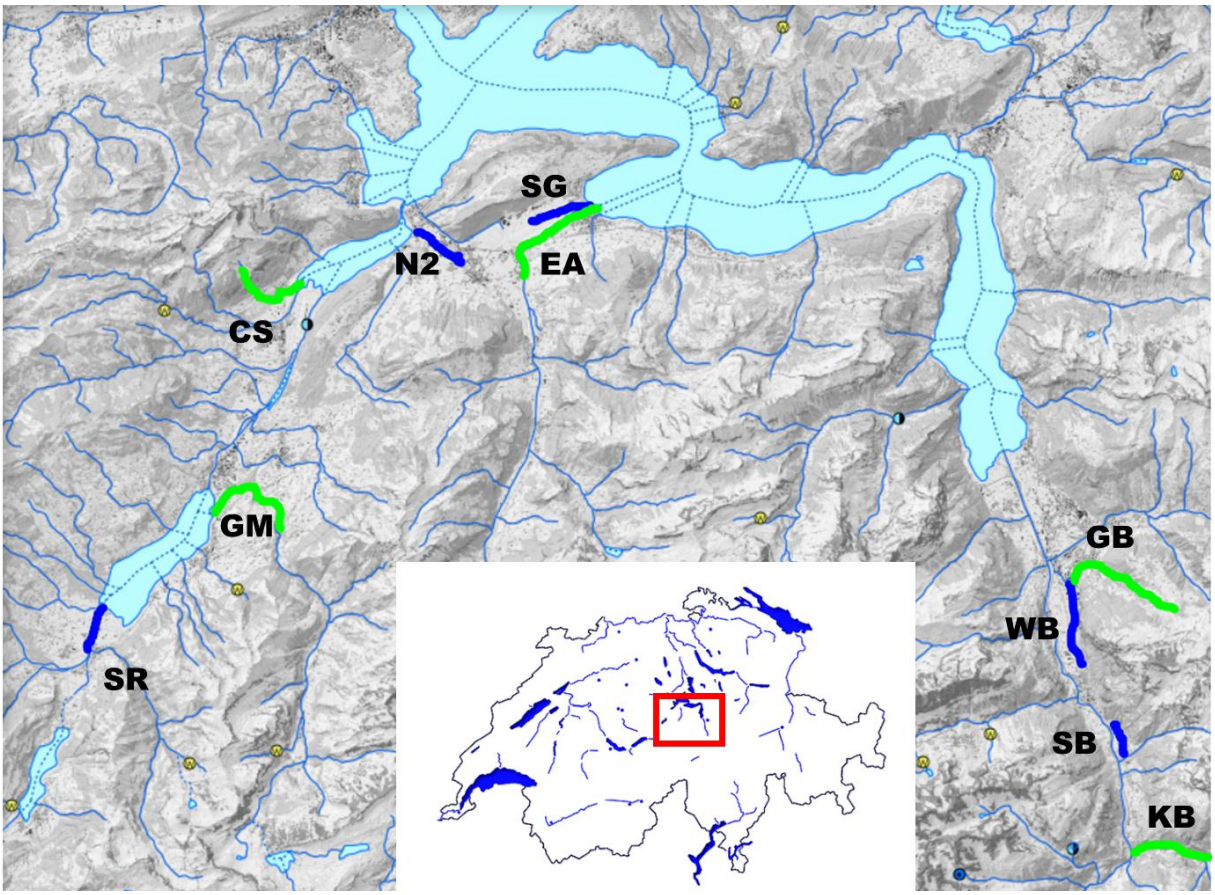

Figure 1: Map of all streams from which phenotypic and ecological analyses of trout were conducted. Groundwater fed streams are in blue, surface water fed streams in green. SR = Schibenriedbach, GM = Grosser Melchaa, CS = Chli Schliere, N2 = N2 Entwässerungskanal, SG = Scheidgraben, EA = Engelberger Aa, GB = Gangbach, WB = Walenbrunnen, SB = Schützenbrunnen, KB = Kärstelenbach. Red box shows location of sampling site in Switzerland

Environmental variables

Prey communities were sampled by kicking 10 times at 5 locations within the stream to include invertebrate species inhabiting the different substrates present at a sampling site. A fine meshed (250 µm) kick net was used and samples were stored in ethanol.

Temperature and conductivity were taken at each sampling site. Additionally, the habitat was visually assessed and percentages of substrate and flow types were recorded. The categories for substrate were overhead cover, vegetation (submerged and emergent), mud, sand, gravel, cobbles and large stones. An estimated percentage of the fished stretch was assigned to each substrate.

Flow regimes were classified as riffles, runs, fast runs, waterfalls and shallow water, analogous to substrate type.

In the quantitative fishing stretches, 10 transects were designated and the depth was taken at 5 points on the transect. The width of the transect was also measured. Flow velocity was measured at each of the points in the first transect. The deepest point for each stretch was also recorded.

The Shannon information formula (Shannon, 1963) was used to calculate habitat diversity measures.

Fish collection

Fish were caught by electrofishing in rivers around Lake Lucerne in Switzerland in September and October 2013. Six of the surveys were carried out quantitatively by blocking off the survey stretch with a block net up and downstream. Depending on the number of fish present, either 2 or 3 passes were made, which allowed the calculation of densities for the stretch. The other four surveys were done semi-quantitatively, as the physical characteristics did not allow quantitative fishing. This was done by fishing a transect in the upstream direction.

Total sample size for this study was 195 fish, with 20 from 9 rivers and 15 from one river, Scheidgraben. For the genetic analyses, five fish caught earlier in the year in Scheidgraben were added to achieve even sample sizes for all streams. All fish were photographed, weighed, and measured, then preserved in Formalin. Fin clips were taken and stored in 100% analytical ethanol.

Two sorts of pictures were taken during fieldwork. Cuvette pictures were taken in good lighting in a Plexiglas photo cuvette, with the fish anaesthetized beforehand with MS-222. A color bar was included for later calibration of the image. The fish were then euthanized with MS-222 and standard pictures were taken from above against a gray background with the fish stretched naturally and the fins spread. The camera was fixed in position to ensure a 90° angle to the fish.

Microsatellite analyses

DNA extraction from fin clips was done using a BioSprint 96 DNA Blood Kit and BioSprint 96 workstation (QIAGEN GmbH, Hilden, Germany), following the instructions of the manufacturer.

14 markers in two multiplexes were used (see table 2). These markers were previously published and described in various sources (Slettan *et al.*, 1995; O'Reilly *et al.*, 1996; Estoup *et al.*, 1998; Estoup *et al.*, 2000; Keller *et al.*, 2011).

PCR was performed on a witec ag TC-412 with 5µl of master mix, 0.5µl of primer mix and 3.5µl of water per sample. To this, 1.5µl of DNA were added. The PCR was initialized at 94°C for 15min. The DNA was then denatured for 30s at 94°C, allowed to anneal at 54°C for 90s, followed by an elongation of 90s at 72°C. This cycle was repeated 35 times. The final elongation was done for 30 minutes at 60°C.

The finished PCR product was diluted at a ratio of 1:7 for sequencing. It was then sequenced on an ABI 3130XL (Applied Biosystems International) according to the manufacturer's instructions and the markers were scored using GeneMapper 4.0 (Applied Biosystems International).

Table 2: Microsatellite markers used including which multiplex they were used in, size in base pairs, dye, and source

Marker Name	Multiplex	Size (bp)	Dye	Source
Ssa100	1	90 - 130	Yellow	unpublished, Giger et al. 2006, supplementary material
Ssa197	1	105 - 200	Red	OReilly et al. 1996
Ssa85	1	100 - 125	Green	OReilly et al. 1996
SsoSL417	1	160 - 217	Green	Slettan et al. 1995
Str15	1	190 - 245	Blue	Keller et al. 2011
Str2	1	295 - 421	Blue	Estoup et al. 1998
Str73	1	135 - 165	Blue	Keller et al. 2011
T3_13	1	175 - 264	Yellow	Estoup et al. 1998
SsoSL438	2	100 - 122	Red	Keller et al. 2011
Str543	2	120 - 176	Blue	Keller et al. 2011
Str591	2	145 - 200	Red	Estoup et al. 2000
Str60	2	85 - 115	Green	Keller et al. 2011
Str85	2	140 - 199	Yellow	Keller et al. 2011
Strutta12	2	115 - 255	Green	Poteaux et al. 1999

Diet

Stomach contents were analyzed for all studied fish. The stomachs were cut out, weighed, then opened and the contents extracted. The empty stomach was weighed again to determine weight of the contents and discarded. The taxa were identified and grouped. Results were analyzed on the level of order.

Measures of individual level specialization and niche width were calculated using the R-Package RInSp (Zaccarelli *et al.*, 2013).

Color analysis

Color was analyzed on an RGB scale. For this, cuvette pictures were used. In a first step, all pictures were subjected to a white and black balance using Adobe Photoshop, with the color bar on the cuvette used as a reference. The fish were then marked with circles along the sideline. All larger fish (n=172, mean TL=157 mm) were marked with ten circles, while few smaller ones had 7 (n= 1, TL 107mm), 8 (n = 8, mean TL=101 mm) or 9 (n = 14, mean TL= 134 mm). Photoshop's color selection tool was used to determine the RGB values in a 5x5 pixel square at a point inside the circle. The red, blue and green values were all recorded separately.

Morphometric analyses

Geometric morphometrics were conducted based on the standard pictures taken during fieldwork. 19 landmarks were placed on the head and body of the fish (Fig. 2).

tpsDig (Rolf, 2005) was used to place the landmarks, and tpsUtil (Rolf, 2008) was used to collate the files. A covariance matrix and wireframe were then generated in MorphoJ (Klingenberg, 2011) and a principal component analysis was performed. The fish was analyzed as a whole, and also split up as only the head and only the body.

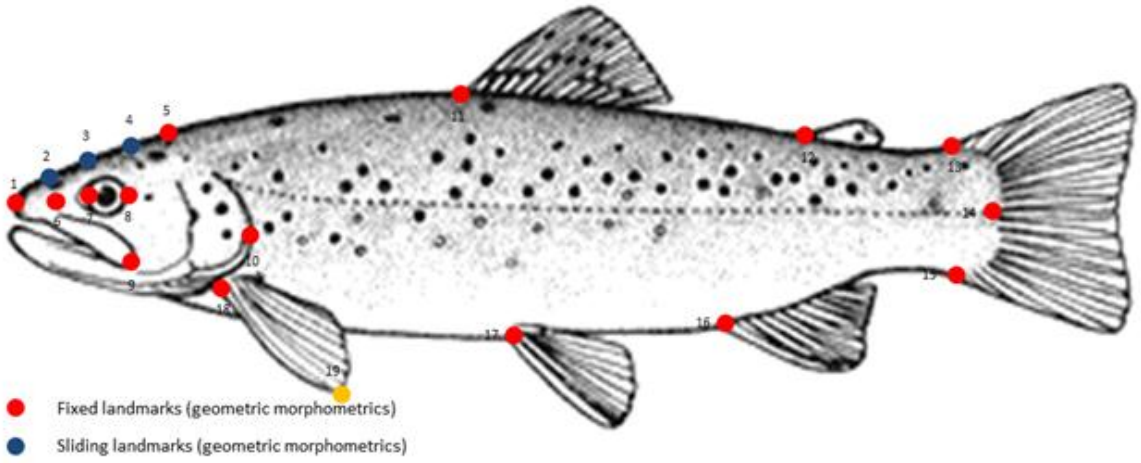

Figure 2: Landmarks used for morphometric analyses, these being 1. Tip of the snout; 2-4, sliding landmarks that follow the curve of the head; 5. End point of the skull; 6. Middle of the nostril; 7. Most anterior part of the eye; 8. Most posterior part of the eye; 9. Most posterior-ventral part of the maxilla; 10. Most posterior part of the operculum; 11. Where the first ray of the dorsal fin inserts; 12. Where the most anterior part of the adipose fin inserts; 13. Dorsal transition from body to caudal fin; 14. End of the lateral organ and transition to caudal fin; 15. Same as 13 but ventral; 16. Where the most anterior ray of the anal fin inserts; 17. Where the most anterior ray of the pelvic fin inserts; 18. Where the most dorsal ray of the pectoral fin inserts in the fleshy part of the pectoral fin; 19. Most posterior part of the most ventral ray of the pectoral fin (not used in analysis).

Statistical analysis

Statistical analysis was done using SPSS 2.6.2.2 Differences between stream types were tested with independent sample t-tests. ANCOVAs were used to control for total length and condition influencing PC scores. Levene's test was used to assess homogeneity of variance.

Structure 2.3.4 (Pritchard *et al.*, 2000; Falush *et al.*, 2003; Hubisz *et al.*, 2009) was used to look for neutral population differentiation. The Locprior model, which uses sampling locations as prior information for clustering, was used to look for clusters within the genetic data. The range of K was set to include the possibility of each sampling site being distinct (K=1-10). The analysis was then performed with 10'000 burn-in steps and 100'000 MCMC (Markov chain Monte Carlo) steps and 10 independent runs per K. Support for different Ks was assessed using STRUCTURE HARVESTER (Earl and vonHoldt, 2012).

Arlequin (Excoffier and Lischer, 2010) was used to calculate F_{st}-values between populations and their significance.

Results

Habitat

The average depth of the channel of the rivers was 16.5 cm for surface water and 47.7 cm for groundwater fed streams, which was a significant difference (independent samples t-test, $F = 27.32$, $p < 0.001$). The deepest point for each stream did not differ significantly between stream types (independent samples t-test, $F = 2.13$, $p = 0.17$). The mean width was also not significantly different between stream types (independent samples t-test, $F = 0.67$, $p = 0.42$), but there was a strong trend towards higher variance in the surface water fed streams (Levene's test for median shows p-value of 0.061, while the p-value for the mean is significant, $p < 0.01$, with an F of 8.42).

Some substrate types differed significantly by stream type as well (Fig. 3). Surface fed streams showed an average of 0.46% submerged vegetation, while groundwater fed streams had an average of 29.11% (independent samples t-test, $F = 7.34$, $p < 0.05$). Cobbles were more likely to be present in surface fed streams (independent samples t-test, 43.64% versus 10.11%, $F = 24.82$, $p < 0.001$). The substrate diversity (Fig. 4) did not differ significantly between stream types (independent samples t-test, $F = 1.55$, $p = 0.23$).

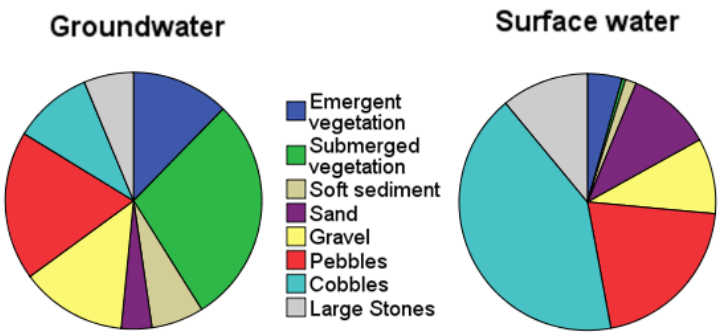

Figure 3: Mean proportion of substrate for each stream type, groundwater fed on left and surface water fed on right

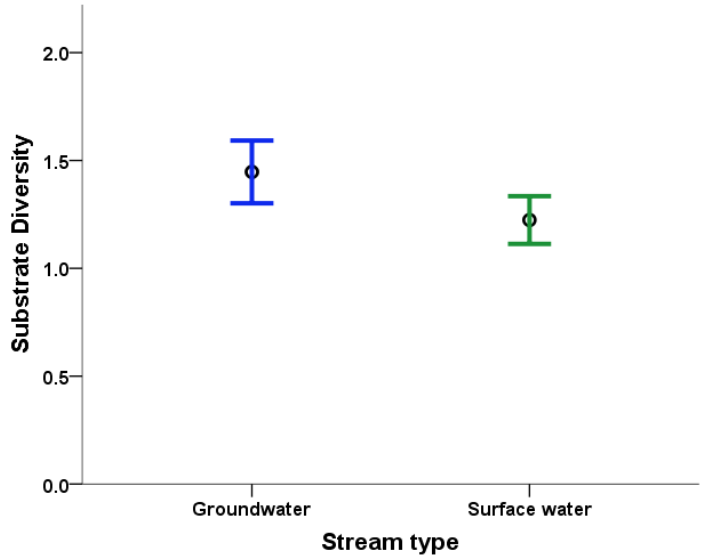

Figure 4: Substrate diversity as measured by the Shannon information formula. Mean value for groundwater fed streams is on the left in blue, for surface water fed on the right in green. The error bars show +-SE

Flow regime was very different between stream types (Fig. 5). Groundwater fed streams were dominated by runs, which comprised a mean 81% of the whole stretch, while surface water showed a mean of 22.9% (independent samples t-test, $F= 29.64$, $p < 0.001$). Alpine streams were much more likely to contain riffles, showing a mean of 45.1% versus 2.8% for groundwater (independent samples t-test, $F = 17.97$, $p < 0.001$). The mean flow diversity (Fig. 6) was 0.95 for alpine fed streams and 0.47 for groundwater fed streams (independent samples t-test, $F = 4.02$, $p = 0.059$).

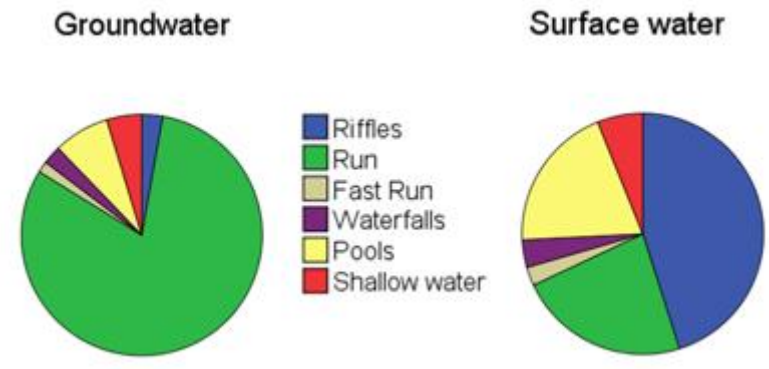

Figure 5: Mean flow regime by stream type, groundwater fed on left and surface water fed on right

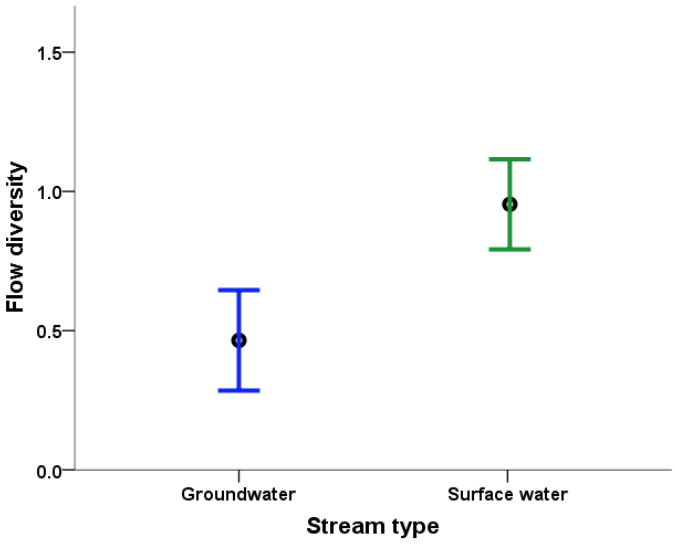

Figure 6: Flow diversity as measured by the Shannon information formula. Mean value for groundwater fed streams is on the left in blue, for surface water fed on the right in green. The error bars show +-SE

There was a clear trend towards different invertebrate densities between stream types, with an average of 255 individuals per sample in surface water and 854 in groundwater fed streams (independent samples t-test, F=4.51, p = 0.053). Community composition differed strongly as well (see table 3, figure 7).

Table 3: Mean percentage of taxa in both stream types, with F and p values for independent samples t-test. GW = groundwater fed streams, SW = surface water fed streams

Taxa	SW (%)	GW (%)	F-value	p - value
Coleoptera	0.98	30.77	13.51	0.003
Diptera	24.29	4.36	2.97	0.108
Trichoptera	9.79	2.06	7.72	0.016
Ephemeroptera	54.23	10.49	23.76	<0.001
Plecoptera	9.79	0.66	9.03	0.01
Gammarids	0.47	35.91	18.71	0.001
Gastropoda	0.03	14.79	7.37	0.018

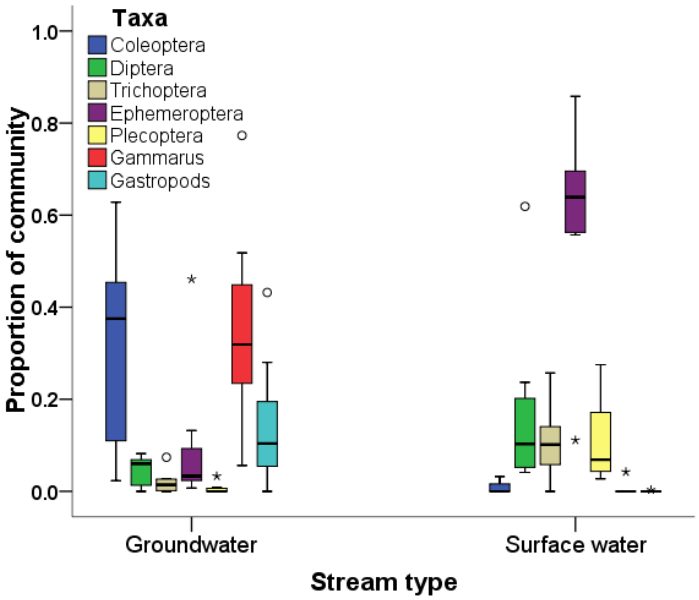

Figure 7: Mean proportional composition of invertebrate community by stream type. Boxes represent the interquartile range, lines across boxes indicate median values and whiskers extend to maximum and minimum values. Circles mark outliers and asterisks mark extreme values.

Trout density was not significantly higher in either stream type (independent samples t-test, F = 2.99, p = 0.101) (see table 1, appendix, for densities).

Phenotype – habitat correlation

Morphometrics

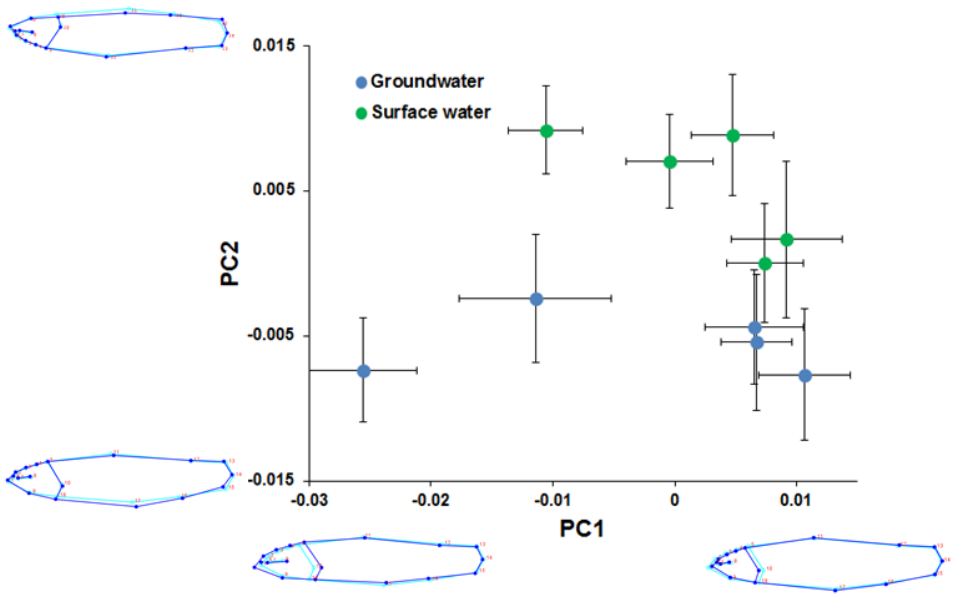

Figure 8: Mean PC scores for groundwater fed streams in blue and surface water fed streams in green, error bars for PCs show 95% CI. The wireframes show the lowest and highest score recorded for PC1 and PC2 in dark blue, with the average individual for the whole dataset in light blue, to indicate morphometric changes with high (top for PC1, right for PC2) and low (bottom for PC1, left for PC2) PC scores.

PC1 and PC2 cumulatively explained 46.93% of the variation in shape, with PC1 accounting for 28.87% and PC2 for 18.06%. PC1 and PC2 of the principal component analysis separated individuals from groundwater and surface water fed streams (Fig. 8).The PCA showed that the differences were mostly in head length and body shape, with fish from groundwater fed streams showing a deeper body and a shorter head. Conversely, fish from surface water fed streams showed shallower bodies. The relationship for PC1 was significant when controlling for length and condition (ANCOVA, $F = 8.44$ $p < 0.01$), as was that for PC2 (ANCOVA, $F = 53.1$, $p < 0.001$). Influence of length on PC1 (ANCOVA, $F = 79.05$, $p < 0.001$) and PC2 (ANCOVA, $F = 6.27$, $p < 0.05$) was significant. The influence of condition on PC1 (ANCOVA, $F = 9.77$, $p < 0.01$) and PC2 (ANCOVA, $F = 13.99$, $p < 0.001$) was also significant.

Levene's test showed that variance for PC1 was significantly higher in groundwater fed streams ($F = 19.61$, $p < 0.001$), suggesting higher variation in shape in groundwater fed streams. There was no difference in the variance for PC2 (Levene's test, $F = 0.06$, $p = 0.81$).

Color

Color analysis showed higher sums of RGB values for individual fish in surface water fed streams than groundwater fed streams (Fig. 9). High sums indicate that the color approaches white, while low sums indicate the color approaches black. This matched the qualitative observation that fish from groundwater fed streams tended to be brown, while fish from surface water fed streams tended to be silvery.

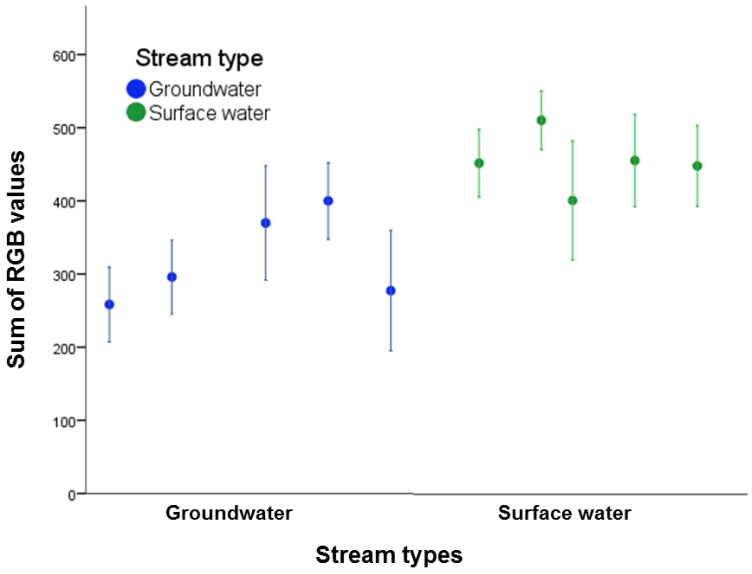

Figure 9: Mean sum of red, green and blue values obtained through color analysis for each stream. The means are grouped by stream type, with groundwater fed streams on the left in blue and surface water fed streams on the right in green. Error bars show +-SD

The difference in coloration of fish from different stream types was significant (independent samples t-test, $F = 147.82$, $p < 0.001$).

Microsatellite analyses

Out of 45 pairwise comparisons among the 10 streams, 37 were significant. 8 out of 10 stream pairs showed significant F_{st}-values (table 3, appendix). This suggest that the populations surveyed are for the most part discrete replicates, with relatively little gene flow between populations.

Diet

Morphology and diet

The morphology of the head, without considering landmarks on the body, was significantly related to diet (R^2 = 0.048, p < 0.01). This relationship was present in both groundwater and surface water fed streams (Fig. 10). High PC1 values indicated a downturned mouth.

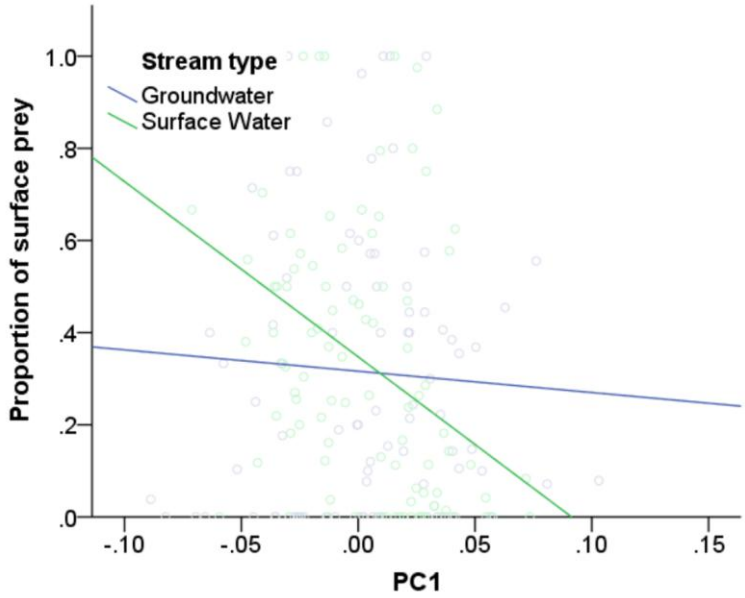

Figure 10: Correlation between PC1 and proportion of surface prey eaten. Lines indicate the slope of the regression. Individual data points are shown for all individuals.

Diet composition

Trout consumed a relatively high number of surface insects. Percentages of surface insects in the diet were similar for both stream types, at 31.65% for groundwater and 34.21% for surface water fed streams.

The major prey in surface water fed streams were ephemeroptera, comprising 35.57% of prey items eaten. In groundwater fed stream, they only accounted for an average of 9.34% (independent samples t-test, F = 42.73, p < 0.001). Gammarids were a major food source in groundwater fed streams, making up a mean of 16.03% of prey. In surface water fed streams, they represented less than 1% of prey items (independent samples t-test, F= 26.28, p < 0.001). Fish in groundwater fed streams were also more likely to consume both gastropods and coleoptera larvae, with mean contribution to total prey being 6.02% (independent samples t-test, F = 9.43, p < 0.01) and 4.94% (independent samples t-test, F = 9.43 and 7.27, p < 0.01). Neither of these comprised major food sources in alpine streams, accounting for 0.08% and 1.8%.

Total number of prey items per stomach was significantly higher in surface water fed streams than groundwater fed streams, at 25.68 per fish versus 44.43 per fish (independent samples t-test, F =

8.28, p < 0.01). The total weight of prey items as measured by weight of stomach content did not differ (independent samples t-test, F = 2.76, p = 0.1).

Niche characteristics

Within-individual component (WIC), between-individual component (BIC) and total niche width (TNW) for all populations showed clear differences. Namely, BIC was significantly higher in groundwater (0.5927) fed streams than in surface water (0.2713) fed streams (independent samples t-test, F = 24.04, p < 0.01, see Fig. 11). The ratio between WIC and TNW showed a clear trend towards being lower in Groundwater fed streams, at 0.59 versus 0.77 for surface water fed (independent samples t-test, F = 4.81, p-value 0.06).

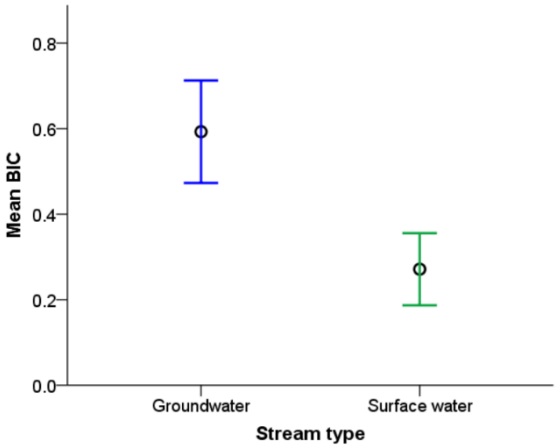

Figure 11: Mean between-individual component of dietary variation both stream types. Error bars show +- SD

WIC was nearly identical between groundwater (0.9112) and surface water (1.0056), showing no significant difference between stream types (independent samples t-test, F = 0.18, p = 0.68). TNW was higher in groundwater (1.5039) than surface water (1.2769) fed streams, but also not significantly so (independent samples t-test, F = 1.48, p = 0.26).

Discussion

My results show clear ecological and phenotypic differences between populations from groundwater fed and those from surface water fed streams. The analysis of habitat variables shows that groundwater fed streams are stable, while surface water fed streams are not. My results show the importance of stability, or the lack thereof, in shaping both individual and population niches.

The clearest support for my hypotheses is found in the ecology of individuals that inhabit a certain stream type. There are large differences in diet, and these can be linked to the environment. This was apparent in the stomach content analyses that I conducted. In part, these results were to be expected, as trout in different stream types had different available resources. However, my results show more than individuals feeding on the prey available to them. The ratio of the between-individual component (BIC) and within-individual component (WIC) of the total niche width (TNW), as well as comparing each of these measures of niche width and individual specialization (Araújo *et al.*, 2011), shows strong indications that individuals from the stable habitat are more specialized. The ratio of BIC and WIC presents a measure of individual specialization by comparing what part of the available resources are used by single individuals, compared to the population as a whole. If this ratio is high, it indicates that individuals are specialists, not generalists, utilizing only a portion of the prey the whole population is feeding on. The strong trend toward a higher ratio in groundwater fed streams was the first factor supporting stronger individual specialization in the stable habitat. The individual measures themselves further support this hypothesis. TNW does not differ significantly between stream types, which suggests that the populations as a whole are utilizing the same breadth of resources. BIC on the other hand is significantly higher in groundwater fed streams than in surface water fed streams, showing that the TNW in these habitats is composed of many small, specialized individual niches.

From the totality of my results, it is likely that the stability in groundwater fed streams allows individuals to specialize on differing food items, thereby decreasing the competitive pressure that comes from feeding on the same resource. Additionally, prey items are generally larger in groundwater fed than in surface water fed streams, suggesting individuals focus on optimal prey items that allow them to maximize their energy balance. Conversely, fish in surface water fed streams occupy an unstable environment. The low BIC and ratio of BIC to WIC show that TNW in these systems is defined by broad, overlapping individual niches, which are indicative of generalists. This may allow them to switch between available prey items, which differ with the changing conditions in the stream. It has been shown that individual specialization is often stronger in stable habitats (Svanbäck *et al.*, 2009) than in unstable habitats, which is further supported by my results.

In addition to the ecological differences, the mean phenotype also differed strongly between stream types. Condition and total length are factors that often strongly influence PC values for morphology. This is apparent in my results as well, with both factors showing highly significant contributions to PC1 and PC2. However, controlling for them actually increases the influence of stream type on PC1 and does not change it for PC2. This suggests that the effect of stream type may be even stronger than I observed, and could be partially obscured by length and condition.

The main cause for these differences may be in environmental factors that are different between the stream types, rather than habitat stability. The shape of individuals from either stream type appears to be well adjusted to the average flow regime of the streams they inhabit. It has been shown in the blacktail shiner (*Cyprinella venusta*) that lack of flow produces individuals with, among other characteristics, deeper bodies and shorter heads (Haas *et al.*, 2010). I found both of these characteristics in my study. Deeper body forms are often seen in environments where maneuverability is important over swimming speed, such as in habitats with high amounts of vegetation (Svanbäck and Eklöv, 2004; Andersson *et al.*, 2006). This allows, e.g., for more efficient and faster changes of direction and braking, which is beneficial for searching for and foraging on prey in a structured environment. In total, these characteristics suggest that individuals inhabiting groundwater fed streams show a phenotype well adapted to a structured habitat with low flow. In surface water fed streams, I found that the trout were more shallow-bodied and streamlined. In fast current, streamlined fish can be selected for (Alexandre *et al.*, 2014), as they present less resistance to the flow. This may allow individuals to expend less energy and avoid being washed away during flood events. The pattern of trout in fast flowing streams showing shallower, more fusiform body shape than those in slow flowing water has been observed before in Swiss brown trout (Stelkens *et al.*, 2012).

Overall, the first measure of phenotype I studied showed significant differences between stream types. This is likely to be caused by the different flow regimes, these being slow, stable flow in groundwater and fast flow caused by flooding in the surface water fed streams.

The second measure of phenotype I studied was coloration. Camouflage can be a potentially important factor influencing the color of individuals in an environment. Bird predation is present in the streams I surveyed. Juvenile trout may additionally be preyed on by mature individuals or other fish predators present in the stream. Experiments have shown that both brown trout and related brook trout *Salvelinus fontinalis* can and do match their color to their background to avoid predation (Donnelly and Whoriskey, 1991; Westley *et al.*, 2013). To be effectively camouflaged, individuals need to be adjusted to the color and type of substrate they encounter most often in their habitat. The substrate in groundwater fed streams consisted mostly of soft sediment and vegetation. The habitats in surface water fed streams consisted mostly of rocky, lighter colored substrates. Floods also greatly increase suspended sediments in the water (Lenzi and Marchi, 2000). These sediments give the water a milky coloration. Individuals from ground water fed streams were darker, more brownish in color, while those from surface water fed streams were brighter and more silvery. Background matching presents a reasonable explanation for these color differences.

The overall differences in phenotype again showed significant differences between stream types, though the mechanism behind this may be different. Environmental factors other than stability could be a stronger influence in the phenotypic diversification I observed in my study.

Lastly, the microsatellite analyses showed that there is relatively high reproductive isolation between individual sites, even the stream pairs that are in very close proximity. For this reason, they can be considered to be distinct replicates for this study.

In total, the results of my study suggest that stability can lead to one population showing much more individual specialization with the same total niche width, showing the importance of looking at variation not only on a population level, but also individual level. This seems especially important if looking at habitats with different stability. Furthermore, adaptation to two different types of habitat, even on a small scale, can produce very different phenotypes in the populations inhabiting them. Lastly, my microsatellite analyses show that reproductive isolation may arise and the potential for the fixation of these phenotypes exists at relatively small spatial scales.

Acknowledgements

Thanks go to EAWAG for hosting and funding this master's thesis. Thanks also go to Alba Stamm, Johannes Hellmann, Julian Junker, Michael Häberli and Fishec for their help during fieldwork, and Anna Humbel for the analysis of the invertebrate kicksamples. Further thanks go to Salome Mwaiko for her help in the lab. Finally, special thanks got to Jakob Brodersen for his supervision and help during this master's project.

Statement of Authorship

I declare that I have used no other sources and aids other than those indicated. All passages quoted from publications or paraphrased from these sources are indicated as such, i.e. cited and/or attributed. This thesis was not submitted in any form for another degree or diploma at any university or other institution of tertiary education.

Kastanienbaum, 15.09.2014

References

Alanärä, A., Burns, M. D. & Metcalfe, N. B. (2001). Intraspecific resource partitioning in brown trout: the temporal distribution of foraging is determined by social rank. *Journal of Animal Ecology* **70**, 980-986.

Alexandre, C. M., Quintella, B. R., Ferreira, A. F., Romão, F. A. & Almeida, P. R. (2014). Swimming performance and ecomorphology of the Iberian barbel Luciobarbus bocagei (Steindachner, 1864) on permanent and temporary rivers. *Ecology of Freshwater Fish* **23**, 244-258.

Andersson, J., Johansson, F. & Söderlund, T. (2006). Interactions between predator- and diet-induced phenotypic changes in body shape of crucian carp. *Proceedings of the Royal Society B: Biological Sciences* **273**, 431-437.

Araújo, M. S., Bolnick, D. I. & Layman, C. A. (2011). The ecological causes of individual specialisation. *Ecology Letters* **14**, 948-958.

Biggs, B. J. F. (1995). The contribution of flood disturbance, catchment geology and land use to the habitat template of periphyton in stream ecosystems. *Freshwater Biology* **33**, 419-438.

Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., Rudolf, V. H., Schreiber, S. J., Urban, M. C. & Vasseur, D. A. (2011). Why intraspecific trait variation matters in community ecology. *Trends in ecology & evolution* **26**, 183-192.

Bolnick, D. I., Ingram, T., Stutz, W. E., Snowberg, L. K., Lau, O. L. & Paull, J. S. (2010). Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B: Biological Sciences*.

Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M. & Svanbäck, R. (2002). MEASURING INDIVIDUAL-LEVEL RESOURCE SPECIALIZATION. *Ecology* **83**, 2936-2941.

Bridcut, E. E. & Giller, P. S. (1995). Diet variability and foraging strategies in brown trout (Salmo trutta): an analysis from subpopulations to individuals. *Canadian Journal of Fisheries and Aquatic Sciences* **52**, 2543-2552.

Brydges, N. M., Heathcote, R. J. & Braithwaite, V. A. (2008). Habitat stability and predation pressure influence learning and memory in populations of three-spined sticklebacks. *Animal Behaviour* **75**, 935-942.

Clausen, B. & Biggs, B. (1997). Relationships between benthic biota and hydrological indices in New Zealand streams. *Freshwater Biology* **38**, 327-342.

Death, R. G. & Winterbourn, M. J. (1995). Diversity Patterns in Stream Benthic Invertebrate Communities: The Influence of Habitat Stability. *Ecology* **76**, 1446-1460.

Donnelly, W. A. & Whoriskey, F. G. (1991). Background-Color Acclimation of Brook Trout for Crypsis Reduces Risk of Predation by Hooded Mergansers Lophodytes cucullatus. *North American Journal of Fisheries Management* **11**, 206-211.

Earl, D. & vonHoldt, B. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4**, 359-361.

Estoup, A., Gharbi, K., SanCristobal, M., Chevalet, C., Haffray, P. & Guyomard, R. (1998). Parentage assignment using microsatellites in turbot (Scophthalmus maximus) and rainbow trout (Oncorhynchus mykiss) hatchery populations. *Canadian Journal of Fisheries and Aquatic Sciences* **55**, 715-723.

Estoup, A., Largiadèr, C. R., Cornuet, J. M., Gharbi, K., Presa, P. & Guyomard, R. (2000). Juxtaposed microsatellite systems as diagnostic markers for admixture: an empirical evaluation with brown trout (Salmo trutta) as model organism. *Molecular Ecology* **9**, 1873-1886.

Excoffier, L. & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Mol Ecol Resour* **10**, 564-567.

Falush, D., Stephens, M. & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**, 1567-1587.

Fitzpatrick, S. W., Brasileiro, C. A., Haddad, C. F. & Zamudio, K. R. (2009). Geographical variation in genetic structure of an Atlantic Coastal Forest frog reveals regional differences in habitat stability. *Molecular Ecology* **18**, 2877-2896.

Haas, T. C., Blum, M. J. & Heins, D. C. (2010). Morphological responses of a stream fish to water impoundment. *Biology Letters* **6**, 803-806.

Hubisz, M. J., Falush, D., Stephens, M. & Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Mol Ecol Resour* **9**, 1322-1332.

Keller, I., Taverna, A. & Seehausen, O. (2011). Evidence of neutral and adaptive genetic divergence between European trout populations sampled along altitudinal gradients. *Molecular Ecology* **20**, 1888-1904.

Klemetsen, A., Amundsen, P. A., Dempson, J. B., Jonsson, B., Jonsson, N., O'Connell, M. F. & Mortensen, E. (2003). Atlantic salmon Salmo salar L., brown trout Salmo trutta L. and Arctic charr Salvelinus alpinus (L.): a review of aspects of their life histories. *Ecology of Freshwater Fish* **12**, 1-59.

Klingenberg, C. P. (2011). MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Resour* **11**, 353-357.

Lenzi, M. A. & Marchi, L. (2000). Suspended sediment load during floods in a small stream of the Dolomites (northeastern Italy). *CATENA* **39**, 267-282.

Matich, P., Heithaus, M. R. & Layman, C. A. (2011). Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. *Journal of Animal Ecology* **80**, 294-305.

O'Reilly, P. T., Hamilton, L. C., McConnell, S. K. & Wright, J. M. (1996). Rapid analysis of genetic variation in Atlantic salmon (Salmo salar) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 2292-2298.

Pakkasmaa, S. & Piironen, J. (2001). Morphological differentiation among local trout (Salmo trutta) populations. *Biological Journal of the Linnean Society* **72**, 231-239.

Pritchard, J. K., Stephens, M. & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* **155**, 945-959.

Rolf, F. (2005). tpsDig, digitize landmarks and outline, version 2.05. (Department of Ecology and Evolution, S. U. o. N. Y. a. S. B., ed.).

Rolf, F. (2008). tpsUtil, file utility program. (Department of Ecology and Evolution, S. U. o. N. Y. a. S. B., ed.).

Shannon, C. E. (1963). *Mathematical theory of communication*: University Illinois Press.

Slettan, A., Olsaker, I. & Lie, Ø. (1995). Atlantic salmon, Salmo salar, microsatellites at the SSOSL25, SSOSL85, SSOSL311, SSOSL417 loci. *Animal Genetics* **26**, 281-282.

Stelkens, R. B., Jaffuel, G., Escher, M. & Wedekind, C. (2012). Genetic and phenotypic population divergence on a microgeographic scale in brown trout. *Molecular Ecology* **21**, 2896-2915.

Svanbäck, R. & Eklöv, P. (2004). Morphology in perch affects habitat specific feeding efficiency. *Functional Ecology* **18**, 503-510.

Svanbäck, R. & Persson, L. (2004). Individual diet specialization, niche width and population dynamics: implications for trophic polymorphisms. *Journal of Animal Ecology* **73**, 973-982.

Svanbäck, R., Pineda-Krch, M. & Doebeli, M. (2009). Fluctuating Population Dynamics Promotes the Evolution of Phenotypic Plasticity. *The American Naturalist* **174**, 176-189.

Westley, P. A. H., Stanley, R. & Fleming, I. A. (2013). Experimental Tests for Heritable Morphological Color Plasticity in Non-Native Brown Trout (<italic>Salmo trutta</italic>) Populations. *Plos One* **8**, e80401.

Whitledge, G. W. & Rabeni, C. F. (1997). Energy sources and ecological role of crayfishes in an Ozark stream: insights from stable isotopes and gut analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 2555-2563.

Zaccarelli, N., Bolnick, D. I. & Mancinelli, G. (2013). RInSp: an r package for the analysis of individual specialization in resource use. *Methods in Ecology and Evolution* **4**, 1018-1023.

Map from maps.geo.admin.ch and http://www.infosport.ch/kanu/levels/navlevel.htm, accessed on 11.09.2014 at 11:00.

Appendix

Stream	Stream type	Coordinates	Altitude AMSL (m)	Max depth (cm)	Mean depth of channel (m)	Mean Width (m)	Length of transect (m)	Trout Density (ind/m^2)
Altibach	SW	46.830/ 8.159	539.9	45	0.14	3.63	23	19.16
Chli Schliere	SW	46.940/ 8.256	440.3	85	0.21	6.65	29	11.41
Chli Schliere	SW	46.951/ 8.282	438	80	0.26	8.74	58.6	6.25
Chli Schliere	SW	46.951/ 8.281	506.7	NA	0.12	7.81	87	1.32
Dorfbach	SW	46.898/ 8.623	394.5	84	0.66	4.47	32	4.89
Gangbach	SW	46.858/ 8.648	459.5	25	0.19	3.19	22	38.45
Giessen	GW	46.898/ 8.619	450.5	71	0.47	3.97	26	12.6
Klosterbach	GW	46.851/ 8.644	437	NA	0.69	2.58	21.9	21.24
Klosterbach	GW	46.886 / 8.610	423.4	51	0.4	4.19	23	87.14
Leewasser	GW	46.997/ 8.613	431.4	NA	0.7	5.41	NA	NA
Meisibach	SW	47.000/ 8.612	510.1	101	0.11	2.88	60	9.26
N2 Entwäss.kanal	GW	46.943/ 8.258	443.1	NA	0.35	2.73	57	152.42
Scheidbächli	SW	46.967/ 8.349	443.4	35	0.94	1.45	25.3	84.68
Schibenriedbach	GW	47.065/ 8.432	419.6	91	0.25	3.53	26	74.2
Schützenbrunnen	GW	46.809/ 8.662	474.3	95	0.53	3.18	25	31.45
Steinibach	GW	46.809/ 8.662	445.6	NA	0.15	2.51	49	116.5
Steinibach	SW	47.016/ 8.302	500.3	45	0.2	5.21	40.5	2.84
Walenbrunnen	GW	46.927/ 8.395	455	75	0.57	3.66	32.5	31.15
Würzenbach	SW	47.050/ 8.337	566.8	30	0.13	0.92	24	113.58
Würzenbach	SW	47.065/ 8.366	429.8	NA	0.14	3.24	29	0
Würzenbach	SW	47.082/ 8.400	495.1	85	0.23	3.71	28	64.5

Table 1: Sites, selected habitat variables and trout densities. GW = groundwater, SW = surface water

Site	Coordinates	Alt. asl	Col	Dip	Tri	Ephem	Plec	Gam	Gast	NA	Total
Alpbach	46.819166/ 8.645791	483	0	5	0	74	30	0	0	0	109
Altibach	46.830877/ 8.15915	540	0	8	23	82	21	0	0	3	137
Engelberger Aa	46.977368/ 8.41759	444	2	146	68	350	50	0	0	0	616
Gangbach	46.858017/ 8.648272	460	20	48	76	476	24	28	2	0	674
Isenthalbach	46.918939/ 8.597084	427	2	39	3	7	12	0	0	0	63
Kärstelenbach	46.76912/ 8.670678	513	0	9	5	37	3	0	0	0	54
Chli Schliere	46.951661/ 8.282083	438	0	6	10	127	4	0	0	1	148
Klosterbach	46.997182/ 8.613108	437	11	29	2	3	0	370	53	11	478
Klosterbach	46.88752/ 8.608678	424	751	10	17	65	10	315	24	3	1197
Leewasser	47.005831/ 8.622901	440	10	20	0	112	0	92	0	9	243
Lochrütibach	46.922461/ 8.398911	496	996	144	150	60	0	114	570	0	2034
N2 Entwäss.kanal	46.967683/ 8.349633	NA	605	0	0	29	0	835	144	0	1613
Schibenriedbach	NA	420	76	12	5	6	6	58	19	0	182
Urner Reuss	46.769185/ 8.670184	514	0	32	61	132	12	0	0	0	237
Walenbrunnen	46.851501/ 8.644853	455	42	4	6	31	1	48	101	1	234

Table 2: Sites and estimated invertebrate numbers from kick samples.

	N2	CS	SR	GM	EA	WB	GB	SB	KB	SG
N2		**<0.001**	0.009	<0.001	0.036	0.063	0.014	0.009	0.063	<0.001
CS	**0.0247*** **		0.018	<0.001	0.027	0.189	0.009	0.108	<0.001	0.045
SR	0.0157**	0*		**<0.001**	0.027	0.036	0.015	0.018	0.018	<0.001
GM	0.0120***	0.0219***	**0.0167*** **		<0.001	0.009	0.016	<0.001	0.027	<0.001
EA	0.0205*	0.0121*	0.0108*	0.0231***		0.135	0.007	0.018	0.009	**0.009**
WB	0.0168	0.0217	0.0154*	0.0136**	0.0072		**<0.001**	0.108	0.108	<0.001
GB	0.0350*	0.0155**	0.0331*	0.0219*	0.0158**	**0.0197*** **		0.009	<0.001	<0.001
SB	0.0320**	0.0173	0.0129*	0.0170***	0.0149*	0.0118	0.0229**		**0.603**	0.009
KB	0.0122	0.0250***	0.0126*	0.0292*	0.0090**	0.0146	0.0423***	**0.0192**		<0.001
SG	0.0262***	0.0113*	0.0064***	0.0198***	**0.0141** **	0.0168***	0.0226***	0.0232**	0.0202***	

1: F_{ST} values of all pairwise comparisons between sites, stream pairs are marked in bold. N2 = N2 Entwässerungskanal, CS = Chli Schliere, SR = Schibenriedbach, GM = Grosser Melchaa, EA = Engelberger Aa, WB = Walenbrunnen, GB = Gangbach, SB = Schützenbrunnen, KB = Kärstelenbach, SG i= Scheidgraben. * marks F_{ST}s below 0.05, ** below 0.01, and * below 0.001.**