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Impact of in-stream habitat structures used in river restoration on fish populations: a feeding ecology approach

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Cover image:
Revitalized section of Scheidgraben stream in Ennetbürgen (canton Nidwalden, Switzerland)

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Abstract:

The aim of this project was to broaden our understanding of the increasingly popular revitalization projects on lowland streams. The main focus was on the effects caused by the installation of in-stream habitat structures (e.g. artificial fish shelters, tree branches/roots, stone banks) on different food web components and on the relationships that connect them. To obtain the necessary information, three streams that differed in the timing of restoration (one channelized, one restored about two years ago and one revitalized 6 years ago) were examined. The chemical and physical habitat characteristics of three separate reaches per stream were measured in addition to assessments of the fish populations and macroinvertebrate community compositions, and food web components for stable isotope analysis. Since fishes had to be sacrificed in order to obtain tissues for isotope analysis, gut content analysis was coupled to gain insight on fish feeding habits and to estimate the dietary overlap between the two main fish species: brown trout (*Salmo trutta*) and bullhead (*Cottus gobio*). The results showed no significant correlation between fish abundances of both species and in-stream habitat structure, while other habitat factors (e.g. proportion of macrophyte cover) resulted as more relevant variables in fish distributions. Also, the macroinvertebrate biodiversity was not statistically different among the different streams, while the composition of taxa and the most relevant functional feeding group differed for each stream according to the different habitat characteristics. Stable isotope analysis confirmed the observation that the three different streams presented singular characteristics, which limited the discrimination of the effects played by revitalization measures. Differences in the fish interaction between brown trout and bullhead were significant only in Scheidgraben, where the ratio bullhead/brown trout was dramatically low. Nevertheless, this factor also did not correlate significantly with in-stream habitat structures. The results suggest the installation of in-stream habitat structures are less effective in creating favourable habitat than other factors like in-stream macrophyte cover and substrate size distributions. The coupling of stable isotope analysis and fish gut content analysis was a powerful instrument for insights on food web dynamics, which might be applied before and after revitalization projects to assess their success.

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1. Introduction:

In the course of history, human beings were able to transform and adapt the surrounding environment to meet their needs. Among the ecosystems that have been impacted by human activity, there are the rivers. To control flooding, drain wetlands, improve river channels for navigation, control stream-bank erosion and improve river alignment, the watercourses have been engineered through the practice of channelization since the second half of the 19th century (Brookes et al. 1983). Research into the ecological and morphological effects of this practice started only in the second half of the 20th century, especially in the U.S.A, but also in England, West Germany and other European countries (Brookes et al. 1983). Many of such studies recognized that channelization caused the loss of in-stream cover (see figure 1), which results in lower fish densities, biomass and biodiversity (Brooker 1985). To counteract these deleterious effects, it has become widespread to install in-stream structures like deflectors, dams and shelters. According to Thompson (2006), in the 1930s in the U.S.A, the use of these structures was a nationwide practice and today the designs have remained for many of them largely unchanged. Many reports over the effects of these projects stated that in-stream structures were beneficial for fish populations, but it appears that deficiencies in the evaluation were present: e.g. many did not use modern statistical tests to examine their results, often there was a close relation between project designers and the evaluators, limited pre- and post-modification data, etc. (Thompson 2006).

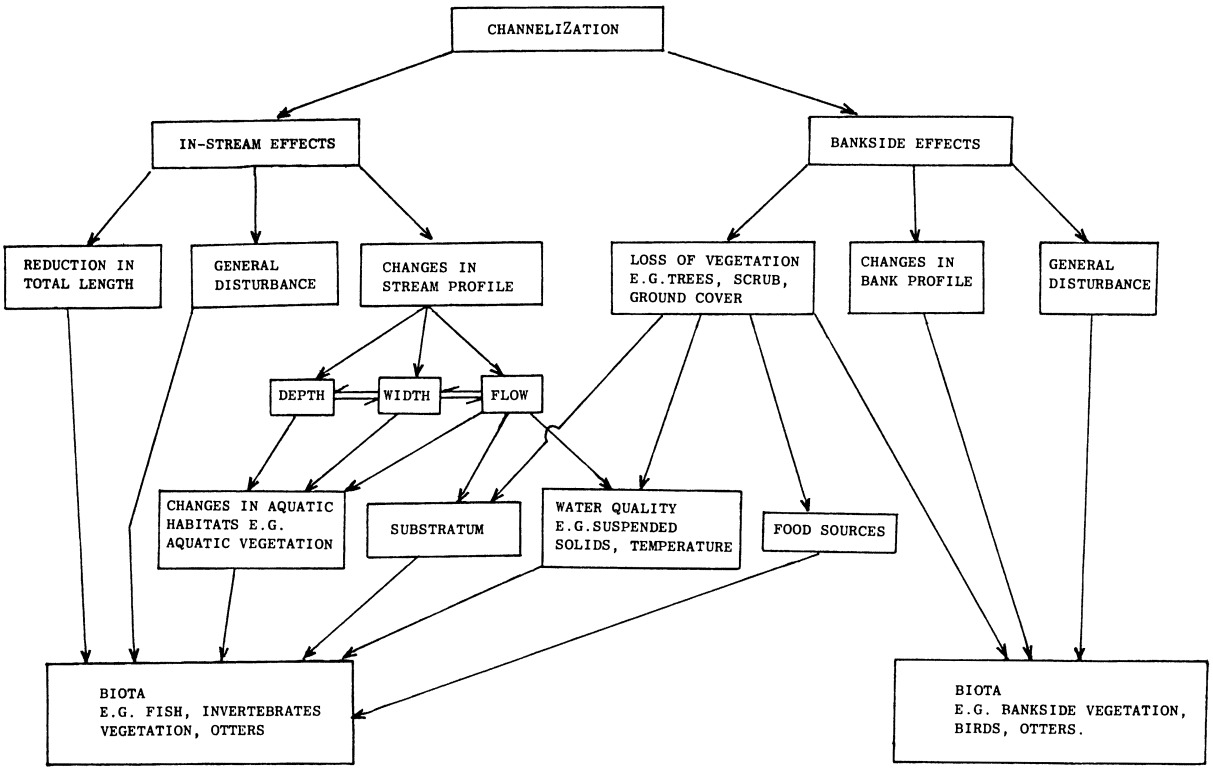

Figure 1: Potential effects of channelization on in-stream and bankside biota recognized by Brooker (1985).

Today, there is more extended knowledge on river degradation and revitalization than in the past. The number of studies on this topic has been increasing dramatically (see figure 2) and also the restoration measures applied to rivers have evolved and differentiated since the early 1990s. Depending on the degree of degradation and the aims of restoration projects, a particular set of restoration measures might be preferred to others. The complete reconfiguration of the stream channel is the most radical intervention. In this case, channel width, depth, slope, sinuosity and pattern are completely reshaped (Miller & Kochel 2009) in order to restore channel geomorphology, habitat complexity (Tullos et al. 2009) and river connectivity (Roni et al. 2013). In some cases, riverbed substrate is also modified to improve conditions for fish spawning by increasing the amount of gravel (Albertson et al. 2010; Utz et al. 2012). Another restoration measure, which is still in use, is the installation of in-stream structures: islands, deflectors (Boavida et al. 2010), weirs, cover structures, boulders, large woody debris (Whiteway et al. 2010). These structures create fish shelters and a greater variability in water flow and water depth, which are important requirements for riverine ecosystems (Jungwirth et al. 1995; Woolsey et al. 2005).

When a revitalization project is planned near urban or agricultural areas, it is likely to find high concentrations of nutrients and contaminants. The source of pollution must be eliminated or limited by a riparian buffer zone around the stream (Violin et al. 2011). It might also be necessary to re-establish complex processes that were part of the natural dynamics of a stream before degradation. Some authors claim that off-channel and marginal habitats are of particular importance in low gradient rivers (Pretty et al. 2003) because, according to the flood pulse concept, riverine food webs are significantly dependent on production derived from the floodplain (Bunn et al. 2003).

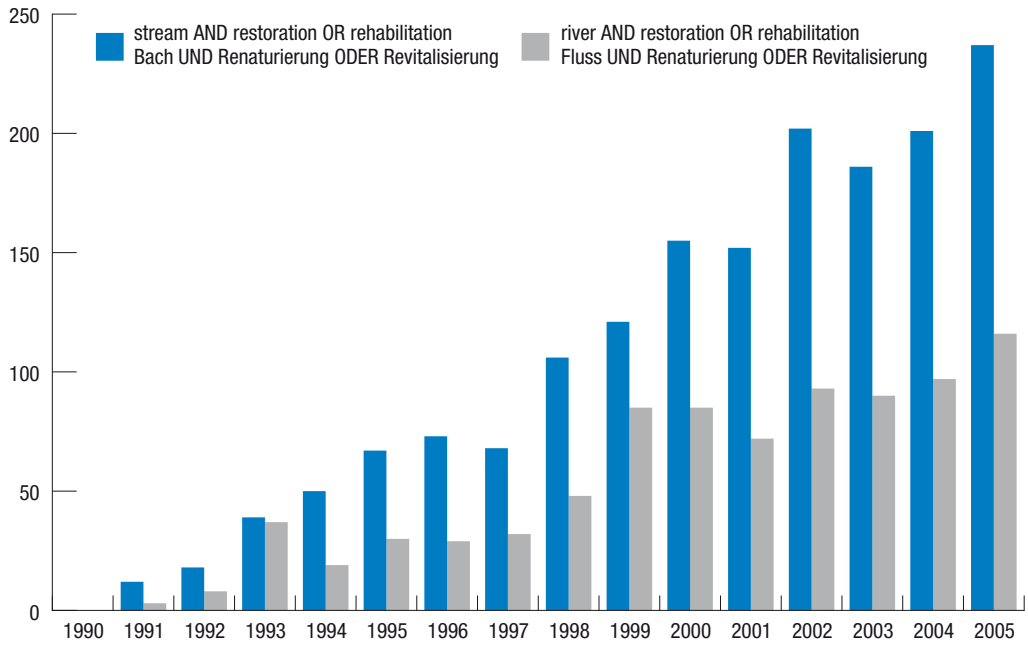

Figure 2: Number of international scientific publications between 1990 and 2005 containing the keywords river or stream and restoration or rehabilitation (Woolsey et al. 2005).

In Switzerland, rivers were not spared from degradation. As reported by the Federal Office for the Environment in 2009 (Zeh Weissman et al. 2009), some 22% of the ecomorphologically assessed rivers was evaluated as strongly degraded, artificial or culverted (see figure 3). Channelizations, which were built in the past to protect settlements from flooding, are not appropriate for this task today. Since the channels reduce stream width, the water is forced to flow more rapidly through these sections. This situation may reach dramatic consequences if a flooding event occurs and the channels are too narrow. In the last two decades, flooding events have been increasing, therefore new strategies for protection against flooding had to be developed. The authorities recognized that a wider riverbed was a necessary prerequisite for effective flood protection, which gave the possibility to integrate ecological improvement of rivers in flood prevention projects (Angelone et al. 2012). The increasing awareness among politicians and public on the importance of more natural rivers culminated in the revision of the water protection law, which was approved by the Swiss parliament at the end of 2009 and became effective 1 June 2011. This directive, among other things, obligates Swiss cantons to plan and implement revitalization projects. In the next 80 years, 4000 out of 15000 km of degraded watercourses are to be revitalized (Angelone et al. 2012). Given the new situation, it is not surprising that in Switzerland the number of revitalization projects has been increasing considerably in the last decade (e.g. Aare in canton Bern, Versoix in canton Geneva, Thur in canton Zurich, Rhone-Thur project in canton Valais and Thurgau).

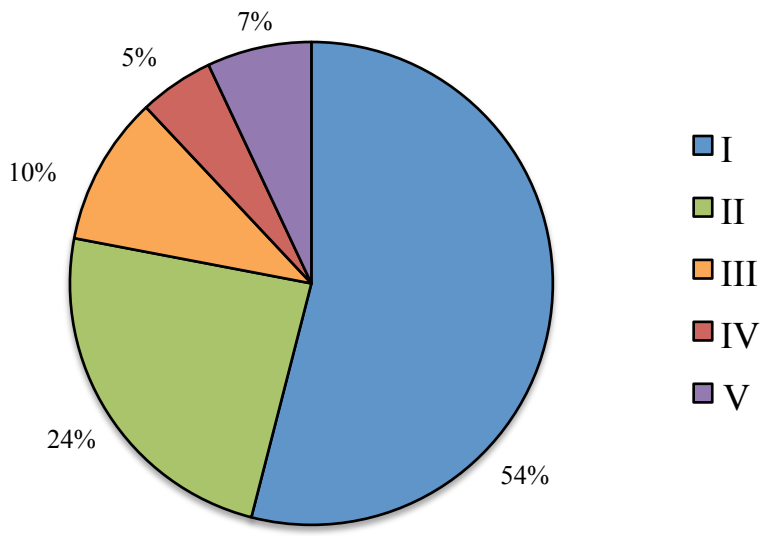

Figure 3: Subdivision of Swiss rivers into ecomophology classes. Class I is for natural state, class II for slightly modified state, class III for heavily modified state, class IV are artificial streams and class V for culverted streams. (Zeh Weissman et al. 2009).

Although knowledge on the effects of river degradation and revitalization practices has deepened greatly, various authors have reported that the number of revitalization failures is high (Albertson et al. 2010; Champoux et al. 2003; Haase et al. 2013). Pretty et al. (2003) claimed that the effects of restoration measures on river biota are poorly understood. Naiman et al. (2012) reported that past river restoration focused on recreating structural attributes based on the assumption that associated ecological functions will follow. Since restoration project expenses (which are usually considerably elevated, see table 1) are mostly covered with public money, it is important to legitimize these interventions to the public with significant positive results.

It has been suggested that a balance between physical habitat restoration and an understanding of trophic processes supporting biotic communities would improve restoration effectiveness by helping test restoration assumptions and leading to discovery of species interactions that influence management success (Naiman et al. 2012). Food web investigations, with the help of stable isotope analysis, have become a widespread technique in different research fields (e.g. feeding ecology, zoology, restoration ecology). Since $\partial^{13}C$ values of animal tissue are very close to those in their diet, the analysis of $\partial^{13}C$ ratios gives insight on the primary production source. On the other hand, $\partial^{15}N$ can be a useful parameter to analyze trophic position of food web components, given that $\partial^{15}N$ enrichment at each trophic level is rather constant (approximately 3.4 ± 1.1‰) (Wada et al. 1991).

Table 1: Median costs of restoration measures subdivided for goal categories in the U.S.A (Bernhardt et al. 2005).

Goal category	Median cost	Examples of common restoration activities
Aesthetics/recreation/education	$63,000	Cleaning (e.g., trash removal)
Bank stabilization	$42,000	Revegetation, bank grading
Channel reconfiguration	$120,000	Bank or channel reshaping
Dam removal/retrofit	$98,000	Revegetation
Fish passage	$30,000	Fish ladders installed
Floodplain reconnection	$207,000	Bank or channel reshaping
Flow modification	$198,000	Flow regime enhancement
In-stream habitat improvement	$20,000	Boulders/woody debris added
In-stream species management	$77,000	Native species reintroduction
Land acquisition	$812,000	
Riparian management	$15,000	Livestock exclusion
Stormwater management	$180,000	Wetland construction
Water quality management	$19,000	Riparian buffer creation/maintenance

The aim of this project was to broaden our understanding of the effects on lowland stream ecosystems from revitalization measures like the installation of in-stream structures, by investigating food web dynamics and the feeding ecology of fishes. The secondary aim of the project was to examine the effectiveness of stable isotope analysis in combination with gut content analysis as a technique for feeding ecology investigation.

The main research questions are the following:

1) Effects of revitalization on fish abundance

a) Is there a difference in fish abundance between channelized and revitalized stream reaches?

b) Do in-stream habitat structures have an influence on fish abundance?

2) Effects on the local food web

a) Is the macroinvertebrate community influenced by the revitalization measures?

b) Is there a difference in the food web structure between channelized and revitalized stream reaches?

3) Fish species interactions

a) Does the presence of in-stream habitat structure effect the interaction strength between trout and bullhead?

b) Is the dietary overlap between bullhead and brown trout influenced by revitalization measures?

Hypotheses to these research questions have been formulated as follows:

1a) Stream revitalization creates better conditions for fish populations, which is reflected by the higher abundance of fishes in revitalized reaches compared to those of channelized ones.

1b) In-stream structures increase the availability of fish shelters and the variability in flow velocity and water depth, which have a positive influence on fish abundance.

2a) Revitalization measures have a positive effect on the macroinvertebrate community. Revitalized streams show a higher macroinvertebrate biodiversity than unrestored streams.

2b) The higher availability of macroinvertebrate species in revitalized reaches supplies fish species with a more varied diet, which is reflected in the stable isotope contents of both bullhead and brown trout.

3a) The increased abundance of both brown trout and bullhead augment the competition between the species for available food items and the predatory pressure of brown trout on bullhead. The relative fitness of bullhead is reduced in the restored streams, while the $\partial^{15}N$ content of brown trout are higher due to an increased predation of bullhead.

3b) In channelized reaches, the food item choice is narrower, therefore the dietary overlap is higher between brown trout and bullhead.

2. Material and methods:

2.1 Study area and experimental design

Since the master thesis was constrained in time to 6 months, it was not possible to monitor the effects caused by river restoration on the ecosystem with a before-after control-impact (BACI) design. A complete factorial design was also rejected, because suitable sites were lacking. To overcome these limitations, we decided to examine three different streams with similar characteristics but different restoration levels. The experimental design comprised a gradient in in-stream habitat structures using 2 restored streams that differed in time after restoration and 1 channelized stream. The study area chosen for the project is located in the canton Nidwalden, in the proximity of the municipality of Stans (figure 4). The streams that best suited the project's purposes were the Mühlebach (located in Stans), the Lochrütibach (in Wolfenschiessen) and the Scheidgraben (in Ennetbürgen). For each stream, three different reaches of 30 meter length were selected (see table 2 and figures 5, 6 and 7).

Table 2: Coordinates and type of reaches for the three streams examined in the project.

Stream Reach	Coordinates	Reach type
Mühlebach 1	46°58`04.71"N 8°21`30.60"E	channelized
Mühlebach 2	46°58`05.20"N 8°21`38.26"E	channelized
Mühlebach 3	46°58`09.37"N 8°21`48.83"E	channelized
Lochrütibach 1	46°55`20.86"N 8°23`56.08"E	revitalized
Lochrütibach 2	46°55`34.39"N 8°23`53.83"E	revitalized
Lochrütibach 3	46°55`38.21"N 8°23`48.16"E	channelized
Scheidgraben 1	46°58`40.93"N 8°24`17.35"E	revitalized
Scheidgraben 2	46°58`43.90"N 8°24`22.98"E	revitalized
Scheidgraben 3	46°58`47.07"N 8°24`35.77"E	revitalized

Figure 4: Location of the three streams examined in the project on a 1:50000 map.

2.1.1 Mühlebach

The Mühlebach originates in Grossried and flows through the town of Stansstad, where it flows into Vierwaldstättersee (Lake Lucerne). With a width of about 3 meters and a gradient of 5‰, it belongs to the grayling fish-region (Schager & Peter, 2004). It has been partially restored in the last decade, but eastward from Oberstmühle the stream is still channelized. All three reaches chosen for this project are located in the channelized section (figure 5).

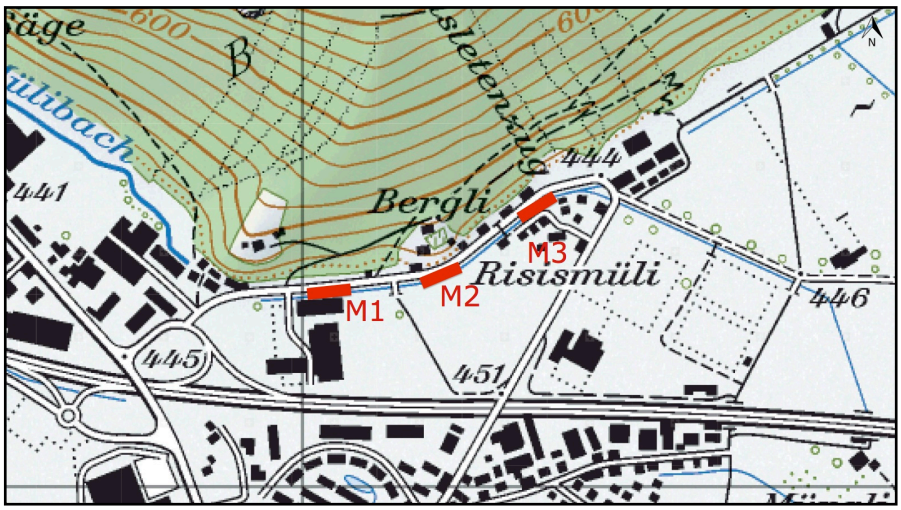

Figure 5: 1:5000 Map depicting the locations of the three stream reaches in Mühlebach.

2.1.2 Lochrütibach

The Lochrütibach is a groundwater fed stream that originates in Grunggis and flows through an area shared by industry and agricultural land until it reaches the river Engelberger Aa. This stream has been restored during 2011 as measure against flooding. The cross-section of the river has been broadened and more varied flowing conditions were created. The examined stream reaches were adapted to those of another project (conducted by Dr. Gregor Thomas) in order to collaborate during the fish sampling and minimize field disturbance. Two reaches were located in the revitalized area (L1 and L2, over the cantonal road), while one reach was in the channelized reach (L3 under the cantonal road)(figure 6).

Figure 6: 1:5000 Map depicting the locations of the three stream reaches in Lochrütibach.

2.1.3 Scheidgraben

The Scheidgraben originates near Ennetbürgen and runs along the military airport of Buochs until it flows into Vierwaldstättersee. This groundwater fed stream belongs to the grayling fish-region, with an approximate gradient of 5‰ and a width of 4 meters. It has been revitalized during 2006 with the installation of fish shelters, broadening and re-meandering of the riverbanks. The three examined reaches of Scheidgraben were all downstream of the merging point between Scheidgraben and Rotigraben in the restored part of the stream (figure 7).

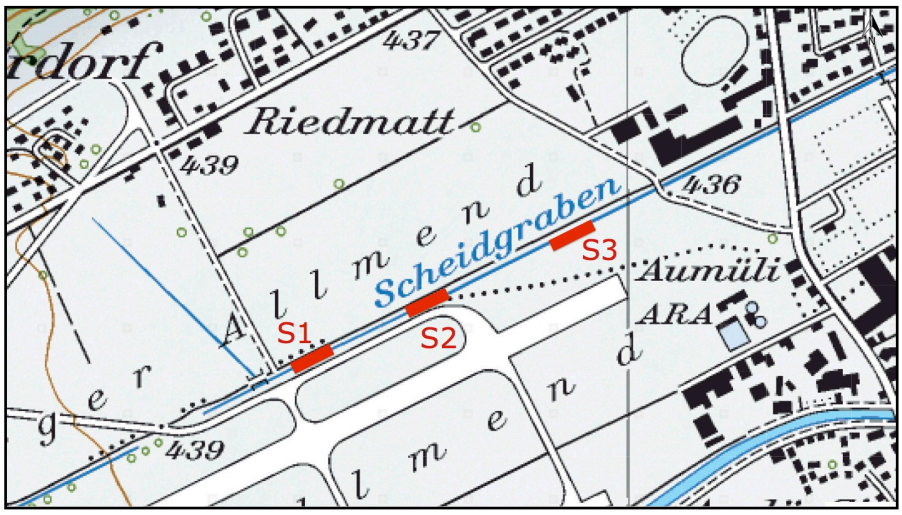

Figure 7: 1:5000 Map depicting the locations of the three stream reaches in Scheidgraben.

2.2 Data sampling and elaboration

2.2.1 Habitat characterization

In each stream reach, in-stream habitat was characterized by measurements of physical and chemical parameters. Water depth, water flow velocity, riverbed substrate composition and in-stream vegetation cover were measured along transects, starting from the downstream end of the stream reach directed upstream and proceeding from the right riverbank to the left one. Each measurement started at the border between water and stream bank and was taken every 30 centimetres along the transect. The distances between each single transect depended on the stream type. Since channelized sections were more homogeneous than the revitalized ones, transects were measured every six meters. In Scheidgraben and the first two reaches of Lochrütibach, this distance was reduced to three meters to obtain a better resolution. In the case of the third reach of Lochrütibach, the first four transects were measured every six meters, while the remaining ones every three meters. This solution was adopted because the reach starts in a strictly channelized section and afterward opens to a more heterogeneous channel. To precisely locate the measurement position in the stream, two measuring tapes of 30 meters maximum length were used: the first one was placed along the stream to determine distances between transects, while the second one was used to measure the distance between the measurements along a single transect. To measure water depth, a folding carpenter's ruler was used. Water flow velocity was measured with a flow meter (Schiltknecht MiniAir 2), which has a programmed function that calculates a mean value from six sequential measurements. With the mean values of water flow, water depth and channel width, the water discharge was calculated for each transect as follows:

$$Q = A\bar{u} \tag{1}$$

with A as the cross section (calculated as product from width and depth) and u as the mean flow velocity.

The riverbed substrate composition and vegetation cover were visually determined similarly to the method described by Platts et al. (1983). Each substrate observation was determined by the predominant sediment class and categorized according to particle diameter size (table 3). The predominant type of vegetation (e.g. macrophytes, bryophytes or biofilm) defined the vegetation cover observed in a particular transect. From these two measurements, two additional factors were extrapolated for each transect: the proportion of substrate equal or larger than gravel not covered by macrophytes and the proportion of macrophyte cover. The first factor should give an estimation of the amount of substrate that is available to fishes as spawning habitat (Belica 2007; Tomlinson & Perrow 2003), while the second factor is an additional measure of fish shelters in the stream reach (Reyjol 2012).

Table 3: Sediment classification according to particle size diameter (Platts et al. 1983).

Particle diameter size [mm]	Sediment classification
610.1 or more	Large boulder
304.1 to 610.0	Small boulder
76.1 to 304.0	Cobble
4.81 to 76.0	Gravel
0.83 to 4.80	Sand
0.82 or less	Fine sediment

The in-stream habitat structures implemented during river restoration were quantified by measuring their approximate area with a folding carpenter's ruler and relating it to the area of the whole stream reach (similarly to the method proposed by Armin Peter in Woolsey et al. 2006). The reach area was calculated using the stream width data obtained with the transect measurements. The structures considered for this measurement were artificial fish shelters, tree branch bundles, tree roots, large in-stream rocks, riffles, stone banks and islands. These structures were selected because they are reported in the literature as beneficial for fish, enhancing the availability of fish shelters or creating a larger variability in water depth and flow velocity (Woolsey et al. 2006; Boavida et al. 2010; Champoux et al. 2006).

The chemical parameters of the streams were partially measured in the field and partially analysed in the laboratory. To measure conductivity, an electrical conductivity meter (WTW cond 3110) was used. Dissolved oxygen was measured with a Hach HQ40d Multi-Parameter meter and pH with a WTW ph 340i. Water samples were collected with 1L volume plastic bottle and delivered to the AuA laboratory at Eawag to analyse the concentrations of dissolved organic carbon (DOC), total organic carbon (TOC), total inorganic carbon (TIC), total nitrogen (TN), nitrate, ortho-

phosphate and total phosphor. The plastic bottles were rinsed three times with water from the streams before the water samples were taken.

2.2.2 Fish sampling

The project focused principally on two fish species: brown trout (*Salmo trutta*) and bullhead (*Cottus gobio*). These two species are very common in lowland streams, but are considered as near threatened in the Swiss red list of fishes. According to Kirchhofer et al. 2007 the populations of brown trout are threatened by fish stocking, which lowers the genetic diversity among trout populations. On the other hand the stocks of bullhead, although widespread in Switzerland, were estimated for over the half of them as small. To obtain information over the fish abundance and community composition, the fish assemblage in each stream reach was sampled by electrofishing. First of all, the measuring station was prepared before the beginning of sampling. This consisted of a table with a fish measuring board and a scale, two basins filled with water and supplied with oxygen from a tank and a third basin filled with water mixed with ethanol and eugenol (1mL of eugenol in 20 mL of ethanol for 30 liters of water) as anaesthetic. The upper and lower limits were closed with nets to prevent fishes from escaping into neighbouring areas. To maximize the efficiency of electrofishing, four people were employed: one person ran the electrofishing equipment (Grassl ELT 60 II 1.3 KW), a second person caught the stunned fishes with a net and passed them to a third person with a water filled bucket, who brought the fishes to the measuring station. The fourth person had to take the fishes from the collection basin and put them in the anaesthetic bath (not more than 5 exemplars each time to avoid over-exposure to anaesthetic). As soon as the fishes were anaesthetized, they were measured and weighed. Afterwards, they were kept for recovery in a separate water basin until the sampling ended.

A subsample of both brown trout and bullhead was taken for further investigation of feeding habits, while the rest of the catch was released. The sampled stream reach length of Lochrütibach was different to those of the other two streams because the sampling sites were adapted to those of the project of Dr. Gregor Thomas. Instead of three sections of only 30 meters length, three reaches of 100 meters length were sampled. Similarly to Brandner et al. (2013), Fulton's condition factor was calculated for each fish as follows:

$$CF = 100\frac{M}{L_T^3} \tag{2}$$

with M as the fish body mass (g) and L_T as the total length (cm). In Lochrütibach, the exemplars of bullhead (especially small ones) were not always weighed. Since for the Project of Dr. Thomas, every stream reach was sampled with three runs (only the first run was considered in this project), the amount of collected fishes was considerably higher compared to the other streams. Therefore, to

spare time, some weight measurements were overlooked. Nevertheless, length measurements were taken for all the individuals.

Two different measurements of fish community composition were taken: the first one was distributed over 22, 24 and 26 of April, while the second measurement was taken over 10 and 18 of July. During the planning of the experimental design, the idea was to mark a subsample of the caught bullheads after the first electrofishing session and to release them in the same stream reach. The recapture of the marked individuals in the second time would have given information over the growth rate of bullheads among the different stream reaches. Since no suitable marking technique was found, we decided to measure as an alternative the changes in size-class distribution. This should work as a proxy for growth rate, which might give insight on on-going dynamics in species interaction. Data on fish population previous to revitalisation in Scheidgraben were obtained from a report on the success of the revitalisation dated 7 May 2008 (Marrer, 2013), while data from an electrofishing sampling made in Lochrütibach on 30 November 2010 were provided by Dr. Gregor Thomas (table 4).

Table 4: Mean fish abundances for the revitalized streams before revitalization. The abundance values were normalized dividing them by reach length and a single mean value was calculated for the whole stream.

Stream (Date of sampling)	Mean brown trout abundance [individuals/m]	Mean bullhead abundance [individuals/m]
Lochrütibach (30.11.2010)	0.516	0.309
Scheidgraben (02.03.2005)	1.140	0.000

2.2.3 Food web analysis and feeding ecology

The combination of stable isotope analysis and stomach content analysis gives an interesting insight in fish feeding ecology. Stomach content analysis provides only short-term information about feeding habits, but provide data on taxonomic and size composition of diets. Stable isotope analysis provides middle-to-long-term information (weeks to month) on dietary habits, reflecting food that is actually assimilated by the consumer (Davis et al. 2012; Brandner et al. 2012).

The brown trout and bullhead subsamples taken for investigation of feeding habits were stored frozen until further preparation for analysis. First of all, they were weighed and measured. Afterwards, the stomach of all fish samples was excised from the fish and the content was extracted and stored in eppendorf tubes filled with 70% ethanol. If the digestion of the food items was not too advanced, they were counted and determined to the family level. Furthermore, the percentage contribution of all the food items to the whole gut content was estimated visually as suggested by Brandner et al. (2013) using a Leica M10 stereo-microscope with a volpi intralux 4000-1

lightsource and a glass fibre ringlight. From the gut content data, the index of food importance (I_{FI}) of prey item *i* among all items *j* for both brown trout and bullhead was calculated as follows:

$$I_{FI}(i) = 100O(i)V(i)\left(\sum_{n=1}^{j} O(i)V(i)\right)^{-1} \quad (3)$$

where O is the percentage of prey *i* among all prey items and V is the visually estimated proportion of volume of prey *i* (Brandter et al., 2013). Dietary overlap (O_D) between brown trout and bullhead was calculated with the Schöner Index:

$$O_D = 1 - \left(0.5\sum|p_a - p_b|\right)100^{-1} \quad (4)$$

with p_a as the percentage of a food item in species a and p_b as the percentage of the same food item in species b (Brandter et al., 2013).

Muscle tissue samples were collected from the fish specimen for $\partial^{13}C$ and $\partial^{15}N$ stable isotope content analysis. These samples were ideally composed only of white muscle, but in the case of very small individuals (< 50 mm) the entire body excluding head, tail and viscera was used for the analysis (Davis et al. 2012). In addition to fishes, other components of the food web (macroinvertebrates, macrophythes, bryophytes, filamentous algae and fine organic sediments) were collected for stable isotope analysis on 15 and 27 May and 6 June. The macroinvertebrates were collected using a 250-µm mesh kick-net from all major habitat types and placed in a sorting plate. The most common exemplars were then separated from the rest of the sample and placed into distinct 50mL tubes filled with water. The rest of the kick-net sample was afterward released in the stream. In order to empty the gut of the individuals, the tubes were left overnight in a refrigerated room at 4°C. If necessary, macroinvertebrates were separated from shells or cases, which might have an impact on the results of the analyses. The macrophyte, bryophyte and filamentous algae samples were carefully washed with water to remove other organisms.

All food web samples were dried for 48 hours at 60°C and then ground to a fine homogenous powder using a bead mill (Qiagen TissueLyser II). A small amount (approximately 0.5 mg for animal tissue, 1.5 mg for plant material and 3 mg for particulate organic matter) of the samples was then measured with a high precision Mettler Toledo UMX2 scale and packed in tin capsules (5x9 mm Säntis analytical). The capsules were then sent to the Surface Water Research and Management Group at Eawag Kastanienbaum for $\partial^{13}C$ and $\partial^{15}N$ content analysis, which was done with a stable isotope mass spectrometer (Elemental Analyzer Thermo Quest NC 2500 coupled with an Isotope Ratio Mass Spectrometer IsoPrime). According to the technician who analysed the isotopic contents of the samples, part of the results showed peaks with an excessive height. This means that the obtained values are not reliable and should be considered with caution. Unfortunately, the analysis of the first sample set took much more time than expected, therefore a repetition of the analysis for the problematic samples could not be done. The time limitation also

prevented a supplementary analysis of the food web, which was considered in order to get information over the changes in the isotopic contents during summer. Fish samples were taken on 18 (Mühlebach and Scheidgraben) and 31 July (Lochrütibach, only bullheads), while the rest of the food web was sampled on 29 August for Lochrütibach, 30 August for Mühlebach and 3 September for Scheidgraben. Nevertheless, the gut contents of fishes from the second sampling were analysed.

2.2.4 Biodiversity assessment

The macroinvertebrate community was examined by taking biodiversity samples on 15 May for Mühlebach, 27 May for Scheidgraben and 6 June for Lochrütibach. As for the stable isotope analysis, the samples were taken with a 250-µm mesh kick-net and collected in plastic bags with some water. To take a representative sample of the biodiversity, the most abundant of the available habitats were examined. Afterwards, all specimens were fixed in 70% ethanol and all macroinvertebrates were sorted and counted. The identification level of the macroinvertebrates was different among individuals: Diptera larvae were determined to the family or tribe rank, while Trichoptera larvae, Plecoptera larvae, Coleoptera, Ephemeroptera larvae, Mollusca, Crustacea, Megaloptera larvae, Hirudinea and Heteroptera were determined to genus. Diptera larvae, Coleoptera and Ephemeroptera larvae were determined with the determination keys from Tachet et al. (2000). Plecoptera larvae were identified with Lubini, Knispel and Vinçon (2000). Mollusca exemplars were determined with Hausser (2005). Trichoptera larvae were identified with Waringer and Graf (2011).

The different macroinvertebrate taxa were also subdivided into functional feeding groups with the help of Bouchard (2004). The resulting data matrix of taxa presence among stream reaches was used to calculate three diversity indices. Taxa Richness value is the simple count of the different taxa present in each location. Shannon's diversity index (H) was calculated with the formula:

$$H = -\sum_{i=1}^{R} p_i \ln p_i \tag{5}$$

where R is the number of taxa and p_i is the proportion of the individuals of the *i*th taxa in the total number of individuals of the site. Simpson's index (D) was calculated as follows:

$$D = \frac{\sum_{i=1}^{R} n_i(n_i - 1)}{N(N-1)} \tag{6}$$

where n_i is the number of individuals belonging to the *i*th taxa and N is the total number of individuals of the site. The last two indices differ in their sensitivity to taxas: Shannon index is more sensitive to the rare ones, while Simpson's D is on the other hand more responsive to the dominant ones (Nagendra 2002).

A second kick-net sampling took place on 3 September for Scheidgraben and 11 September for both Mühlebach and Lochrütibach. Since the sorting, counting and identification of these samples would have been as time consuming as for the first sampling, only the number and type of taxa was verified. For this reason, there is no information available about the number of individuals per taxa and consequently no Shannon's or Simpsons's diversity index. The data obtained from this sampling are nevertheless still informative on the seasonal changes in macroinvertebrates community composition.

2.3 Statistical analysis and results visualisation

Statistical analysis of the collected data was performed with the help of the opensource software Rstudio version 0.97.551 developed by R Development Core Team (2011). Part of the data elaboration, indices calculation and results visualization were carried out with the help of Microsoft Excel 2008 for Mac version 12.1.0.

2.3.1 Effects of revitalization on fish abundance

To test if the differences among streams in fish abundance were significant, an analysis of variance (ANOVA) was used. First of all, to verify the normal distribution of the data, the Shapiro-Wilk test was implemented. If the test resulted with p-values less than 0.05, then the hypothesis of normal distribution of the data was rejected. In this case, logarithmic transformation was considered as a possible intervention to improve the normal distribution of the data. For both brown trout and bullhead abundance data, the logarithmic transformation was applied. Since fish abundances were measured on two different sampling session, the best solution to control the effects that sampling date might have on the response variable was to apply a two-way ANOVA with sampling date as block factor. To test for differences among revitalized and channelized stream reaches, a two-way ANOVA with sampling date as block factor was used. If the ANOVA was significant, then Tukey's honest significance difference test (Tukey's HSD) was applied to the model in order to calculate the significance of each comparison.

Multiple linear regression was used to investigate the influence that different habitat factors might have on fish abundance. In order to obtain the best model possible, different techniques were applied: diagnostic plots, partial residual plots, variable transformation and variable selection. The four standard diagnostic plots in Rstudio are Tukey-Anscombe plot (residuals vs. fitted values), Normal plot, Scale-Location plot and leverage plot. The model diagnostic plots were used to control that the model assumptions were met and to identify unusual observations. If the model presented leverage points (observations that forced the regression fit to adapt to it), observations were controlled for possible errors (e.g. typing errors) and variable transformations were considered. Partial residual plots depict each predictor of the model versus the response variable without the

estimated effect of all the other predictors. If this relation was not linear, the predictor in the plot was transformed (e.g. logarithmic, square-root or arcsine transformation) and re-plotted to see if variable transformation was effective. Variable selection was made with stepwise regression based on Akaike's information criterion (AIC). With the help of this technique, the number of predictors present in the model was reduced, so that only the most influential predictors were part of the model.

2.3.2 Effects on the local food web

Nonmetric Multidimensional Scaling (NMDS) was applied to the matrix of taxa observed during the macroinvertebrate biodiversity assessment sampled among the different stream reaches to visualize how similar the macroinvertebrate communities were. These taxa were then pooled into functional feeding groups and the same technique applied to these values to obtain information over the most relevant functional feeding groups across the streams.

To investigate differences across streams in the biodiversity indices of macroinvertebrate communities, one-way ANOVA was used. In the case of taxa richness, which has been obtained from both biodiversity samples taken during the project, a two-way ANOVA with sampling date as a block factor was calculated. With this method, the possibility of a change in taxa richness caused by seasonality was tested. To test for correlation between biodiversity indices and the presence of in-stream habitat structures, tests based on Pearson's product-moment correlation coefficient were calculated. For the analysis of stable isotope contents across the examined streams, one-way ANOVAs and Tukey's HSD tests were used.

2.3.3 Fish species interactions

One-way ANOVAs and Tukey's HSD tests were used for determining differences across the streams for the bullhead to brown trout ratio, the peak distances of the normal distributions fitted for the fish length distributions, the fish condition factor and the dietary overlap.

Correlation tests based on Pearson's product-moment correlation coefficient were used in this section for testing the following relationships: peak distances of the normal distributions fitted for the bullhead length distributions against bullhead to brown trout ratio and in-stream structure area, dietary overlap against bullhead to brown trout ratio, in-stream structure area and taxa richness.

3. Results:

3.1 Data collection

3.1.1 Habitat characterization

The data obtained during the transect measurements are summarized for each stream reach in table 5. Part of them was used as predictors in the multiple linear regression with brown trout and bullhead abundance as response factors, i.e. mean discharge, proportion of substrate (not covered by macrophytes) equal or larger than gravel (% substr.), proportion of substrate covered with macrophytes (% macroph.) and ratio of in-stream structure area to total reach area.

Table 5: Summary of data obtained from transects measurements for each stream reach. % substr. represents the proportion of substrate (not covered by macrophytes) equal or larger than gravel, while % macroph. is for the proportion of substrate covered with macrophytes.

Stream reach	Mean width [m]	Total Reach Area [m^2]	Mean Discharge [m^3/s]	% substr.	% macroph.	Mean Depth Variance	Mean Flow Variance	In-stream structure/ total area
M1	2.76	66.6	0.32	10.0	53.3	243.5	0.012	0.00
M2	3.00	72.0	0.28	25.0	31.9	305.0	0.008	0.00
M3	2.46	58.5	0.26	17.3	34.7	91.6	0.016	0.00
S1	4.66	143.3	0.57	82.5	17.5	163.0	0.070	0.24
S2	3.44	103.1	0.43	86.6	13.4	281.3	0.074	0.20
S3	4.31	129.1	0.47	64.9	29.9	259.0	0.072	0.09
L1	4.48	134.4	0.26	74.2	17.9	492.7	0.041	0.06
L2	3.84	115.8	0.31	84.7	10.4	358.1	0.078	0.11
L3	2.29	61.2	0.41	5.4	10.0	222.2	0.110	0.04

The chemical/physical parameters of water samples are reported in table 6. The values of DOC, TOC, TN, nitrate, ortho-phosphate (o-P) and total phosphor (G-P) were evaluated according to five condition categories suggested by the Federal Office for the Environment (Liechti 2010). Since these values were all smaller than the half of the target values required by the Water Protection Ordinance (WPO Annex 2 Number 12), they were evaluated as very good.

Table 6: Summary of the water chemical and physical parameters measured on the field (conductivity, temperature, pH and oxygen) or analyzed in the laboratory (DOC, TOC, TN, Nitrate, o-P and G-P). The blue columns represent the classification of the values according to Liechti (2010) as very good.

Stream Reach	Conduct. [µS/cm]	T [°C]	pH	O_2 [mg/L]	DOC [mg/L]	TOC [mg/L]	TN [mg/L]	Nitrate [mg/L]	o-P [µg/L]	G-P [µg/L]
M1	375	9.8	7.9-8.1	8.84	0.8	1.2	1.1	1.0	1.0	5.8
M2	370	10.8	7.9-8.1	9.10	0.9	0.9	1.0	1.0	3.6	4.7
M3	375	11.4	8.0-8.2	9.28	0.8	1.1	1.0	1.0	2.4	7.2
S1	401	10.7	7.8	8.80	1.0	1.3	1.2	1.2	2.2	6.4
S2	401	11.5	7.9	9.00	1.0	1.2	1.2	1.2	3.4	7.9
S3	401	12.7	8.0	9.40	0.9	1.3	1.2	1.1	1.8	8.1
L1	323	8.9	7.7	8.40	0.6	0.7	1.0	1.0	1.2	2.5
L2	319	9.8	7.7	9.05	0.6	0.7	1.0	1.0	0.9	2.1
L3	317	9.8	7.8	8.95	0.7	0.7	1.0	1.0	1.2	2.4

3.1.2 Fish sampling

The electrofishing data obtained from the two distinct sampling sessions are found in table 7 and figure 8. The values of fish abundance were normalized for the reach length in order to obtain comparable data among streams (abundances were divided by reach length). The fish species other than brown trout and bullhead that were caught are the following: pike (*Esox lucius)* found in Mühlebach (M1 both fishing sessions, M2 only 24 April), burbot (*Lota lota*) found in Scheidgraben (1 individual in S1 and 3 individuals in S3 on 26 April, only 1 individual in S3 on 18 July), European brook lamprey (*Lampetra planeri*) found in Scheidgraben (4 individuals in S3 on 26 April) and European perch (*Perca fluviatilis*) found in Scheidgraben (1 individual in S3 on 26 April). The condition factor of the bullhead population in L3 is missing for 10 July because none of the sampled individuals was weighed.

Table 7: Summary of the fish population data sampled on the two separated fishing sessions (end April and mid July). The data reported are normalized fish abundances (divided by reach length), bullhead to brown trout ratio and mean condition factor of fishes.

Sampling Date	Stream Reach	Abund. Trout [ind./m]	Abund. Bullhead [ind./m]	Bullhead / trout	Abund. other fishes	Condition factor trout	Condition factor bullhead
22.4.'13	L1	0.29	1.37	4.72	0	1.007	1.143
22.4.'13	L2	0.10	0.63	6.30	0	0.945	1.080
22.4.'13	L3	0.28	0.14	0.50	0	0.992	1.224
24.4.'13	M1	0.60	0.67	1.11	1	1.070	1.042
24.4.'13	M2	0.43	0.73	1.69	1	0.987	1.045
24.4.'13	M3	1.17	1.87	1.60	0	1.043	1.086
26.4.'13	S1	1.90	0.73	0.39	1	0.938	0.964
26.4.'13	S2	1.90	0.07	0.04	0	0.958	1.074
26.4.'13	S3	1.50	0.10	0.07	8	1.267	1.099
10.7.'13	L1	0.53	1.05	1.98	0	1.041	1.106
10.7.'13	L2	0.12	1.21	10.08	0	0.994	1.246
10.7.'13	L3	0.28	0.29	1.04	0	1.149	NA
18.7.'13	M1	0.43	0.57	1.31	1	1.090	1.144
18.7.'13	M2	0.60	1.00	1.67	0	1.066	1.114
18.7.'13	M3	1.80	1.57	0.87	0	1.088	1.097
18.7.'13	S1	5.40	0.30	0.06	0	1.111	1.210
18.7.'13	S2	2.73	0.07	0.02	0	1.100	1.251
18.7.'13	S3	4.03	0.10	0.02	1	1.159	1.120

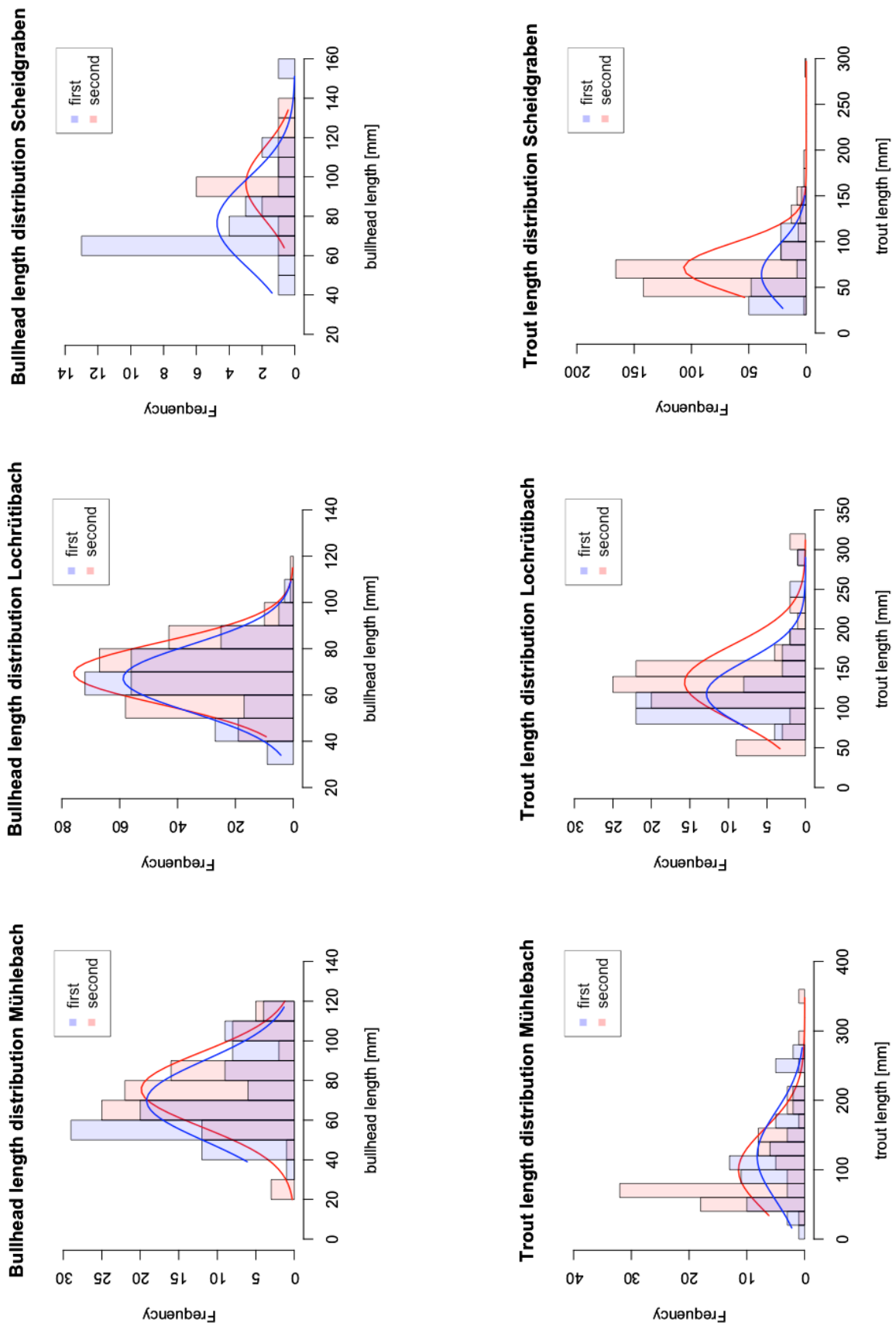

Figure 8: Graphic representations of the fish length distributions for bullhead and brown trout in the three different streams. In blue are represented the data from the first fishing session, while in red are the values from the second session. Normal distribution curves have been fitted and plotted for both fishing sessions.

3.1.3 Food web analysis and feeding ecology

The data concerning the food importance index and the dietary overlap among bullhead and brown trout from the first sampling are summarized in figure 9. The data from the different reaches were pooled in the same stream and the different macroinvertebrate families were summarized according to order so that the results are easier to read. For the complete data of every stream reach see appendix 1. The dietary overlap between populations of the same species from different streams was calculated and is reported in table 8. The food importance values were pooled according to the functional feeding group to give an overview that might be helpful in the explanation of stable isotope contents of fishes (table 9).

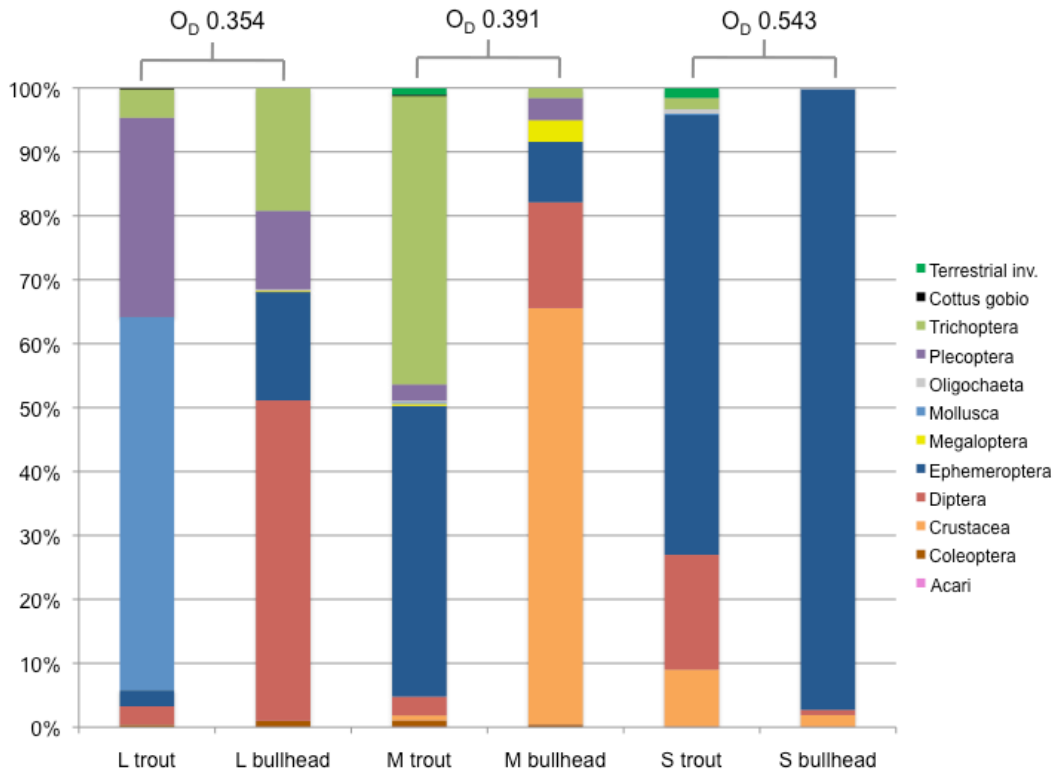

Figure 9: Bar plot representing the food importance indices I_{FI} for prey items found in the fish gut contents from different streams (L stands for Lochrütibach, M for Mühlebach and S for Scheidgraben). The dietary overlaps O_D values calculated between brown trout and bullhead individuals from the same stream are also reported.

Table 8: Dietary overlaps O_D values calculated between individuals of the same fish species coming from different streams (L stands for Lochrütibach, M for Mühlebach and S for Scheidgraben).

	L/M	L/S	M/S
O_D bullhead across streams	0.474	0.215	0.344
O_D brown trout across streams	0.303	0.162	0.537

Table 9: Indices of food importance I_{FI} for prey items found in the fish gut contents from different stream pooled according to functional feeding group.

	Lochrütibach		Mühlebach		Scheidgraben	
Functional feeding group	Brown trout	Bullhead	Brown trout	Bullhead	Brown trout	Bullhead
Collectors	4.61	82.17	49.07	90.55	96.35	100.00
1st Predators	2.84	3.09	1.17	4.23	0.18	0.00
2nd Predators	0.16	0.00	0.15	0.00	0.00	0.00
Scrapers	60.85	3.92	0.26	0.00	0.25	0.00
Shredders	31.15	9.83	48.37	4.79	3.19	0.00

Data on $\partial^{13}C$ and $\partial^{15}N$ stable isotope contents were pooled among streams into categories. The primary producers were subdivided into algae, macrophytes and bryophytes. Macroinvertebrate samples were categorized into functional feeding groups (collectors, scrapers, shredders and predators). Brown trout, bullhead and sediment data remained independent categories. The resulting data are depicted in figures 10, 11 and 12, while the mean values for each food web component of every stream are reported in appendix 2.

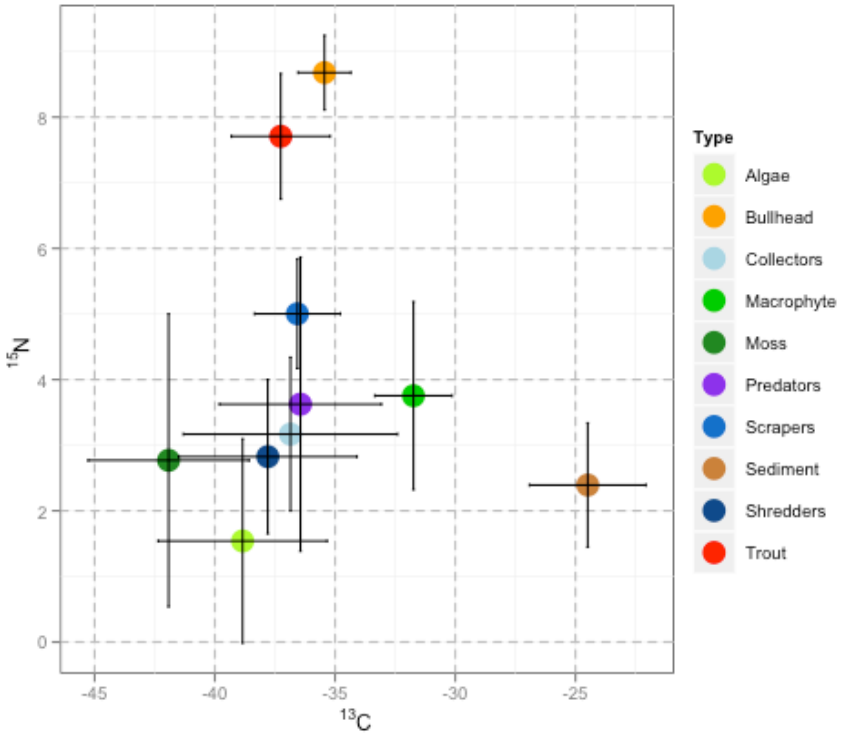

Figure 10: Graphic representation summarizing the stable isotope contents of the food web components sampled in Mühlebach.

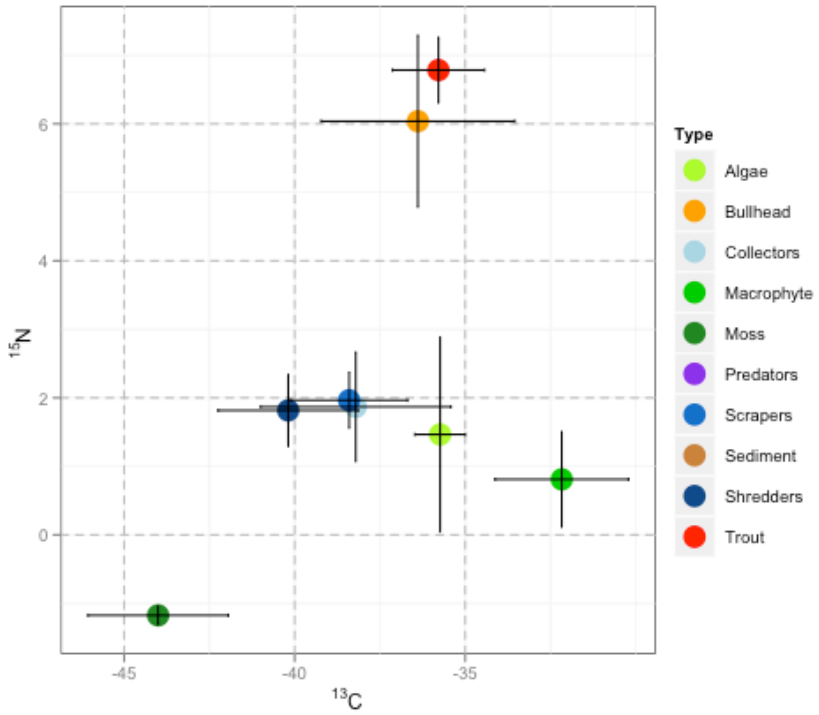

Figure 11: Graphic representation summarizing the stable isotope contents of the food web components sampled in Lochrütibach.

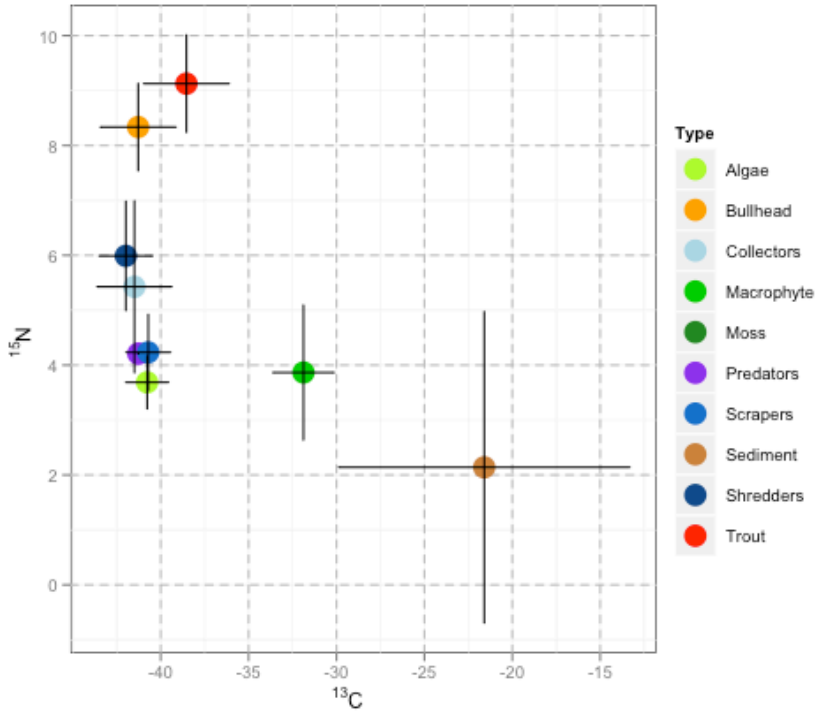

Figure 12: Graphic representation summarizing the stable isotope contents of the food web components sampled in Scheidgraben.

3.1.4 Biodiversity assessment

The values obtained for Taxa Richness, Simpson's D and Shannon's index during the first biodiversity assessment are presented for each stream reach in table 10. The table with the complete data on the observed taxa is in appendix 3 and 4. The second assessment of macroinvertebrates diversity showed some differences in the detected taxa compared to the first time. Some genera were not encountered anymore (13 taxa), while others were found for the first time (4 taxa). Among the missing taxa there were: *Annitella* sp., *Sericostoma* sp., *Chloroperla* sp., *Hydraena* sp., *Bythinella* sp., *Gyraulus* sp., *Planorbarius* sp., Hemerodromiinae, Stratiomyidae, Tipulidae, *Sialis* sp., *Helobdella stagnalis* and *Microvelia* sp.. The newly encountered taxa were the following: *Agabus* sp., *Haliplus* sp., *Platambus* sp. and *Theodoxus* sp.. The value of Taxa Richness obtained from the second assessment are summarised in table 10.

Table 10: Summary of the different biodiversity indices obtained for each stream reach. The two different series of values for taxa richness refer to the two separated samples of macroinvertebrates community, which took place on different dates. The first sampling took place end of May-begin of June, while the second sampling took place at the beginning of September.

Diversity index	M1	M2	M3	S1	S2	S3	L1	L2	L3
Taxa Richness 1st	24	26	28	22	16	22	21	29	25
Taxa Richness 2nd	20	23	23	23	19	18	21	22	24
Shannon index	1.90	3.57	2.07	1.46	1.98	1.97	2.05	2.25	2.09
Simpson D	0.80	0.76	0.76	0.64	0.82	0.79	0.83	0.84	0.81

3.2 Data analysis

3.2.1 Effects of revitalization on fish abundance

ANOVA on fish abundance among streams showed significant differences for both brown trout ($p=9.243*10^{-6}$) and bullhead populations (p=0.004)(see figure 13). Tukey's HSD test on ANOVA models showed that for brown trout, each stream population was significantly different from the others (Mühlebach-Lochrütibach p=0.008, Scheidgraben-Lochrütibach $p=5.9*10^{-6}$, Mühlebach-Scheidgraben p=0.004). In the case of bullhead, Scheidgraben was significantly different from Mühlebach (p=0.003) and Lochrütibach (p=0.024).

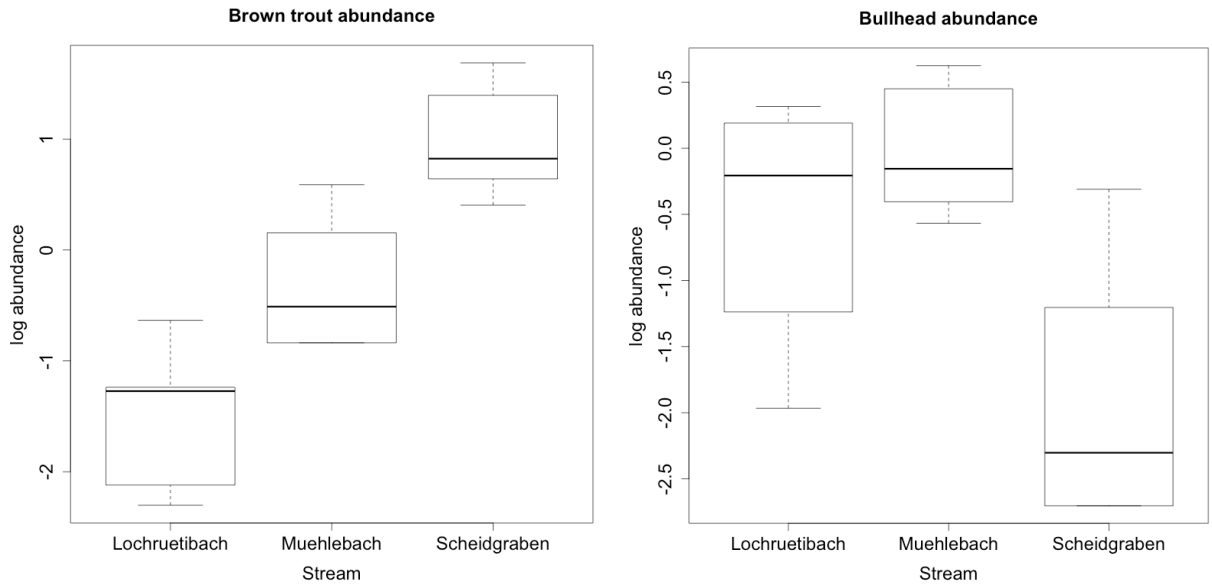

Figure 13: Differences of brown trout and bullhead abundances among the three examined streams.

The fish abundance between channelized and revitalized stream reaches was not significantly different (see figure 14) for both brown trout (p=0.375) and bullhead (p=0.201).

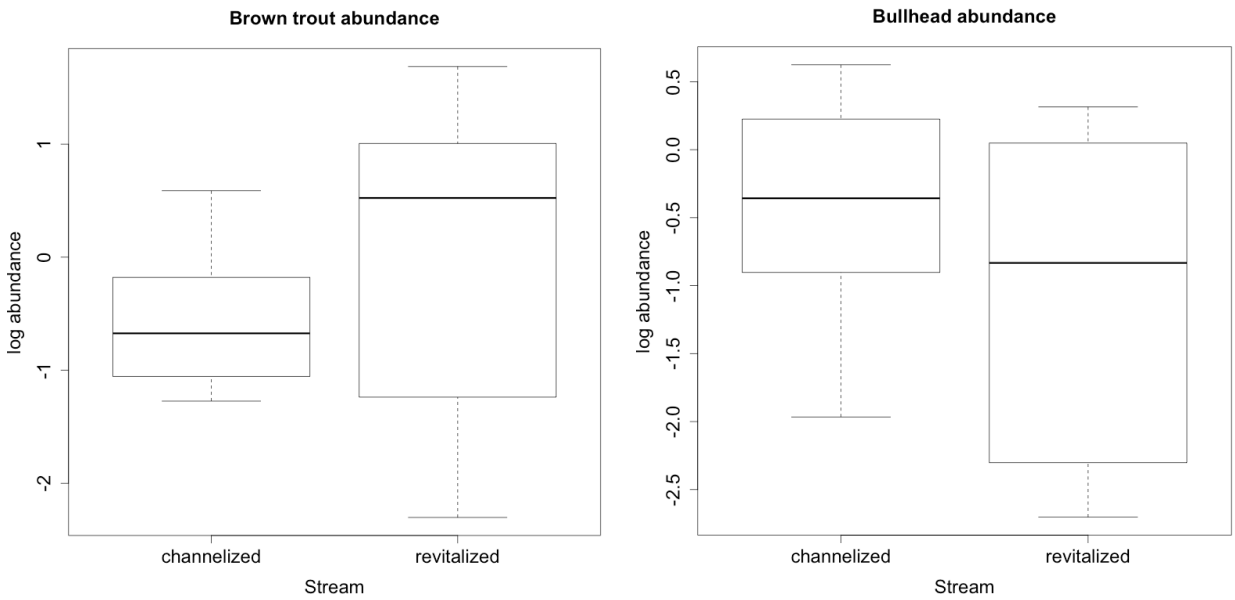

Figure 14: Differences between channelized and revitalized stream reaches in abundance of brown trout and bullhead.

The investigation made with the help of multiple linear regression on the possible influential factors of fish abundance resulted in two different models for brown trout and bullhead. The best model obtained for bullhead is summarized in the following R output:

```
Call:
lm(formula = log(bull.abund) ~ trout.abund + prop.substr +
log(prop.macroph) +
    log(taxa.rich) + other.fish, data = abund.mlr)

Residuals:
     Min       1Q   Median       3Q      Max
-0.97524 -0.48059  0.08537  0.44775  0.94601

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)       -11.54015    4.24593  -2.718  0.01868 *
trout.abund        -0.34381    0.13655  -2.518  0.02701 *
prop.substr         1.65619    0.71600   2.313  0.03925 *
log(prop.macroph)   1.35945    0.39217   3.466  0.00466 **
log(taxa.rich)      4.08692    1.33563   3.060  0.00990 **
other.fish         -0.30524    0.09887  -3.087  0.00941 **
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.7056 on 12 degrees of freedom
Multiple R-squared: 0.7214,   Adjusted R-squared: 0.6053
F-statistic: 6.213 on 5 and 12 DF,  p-value: 0.004561
```

The model shows that trout abundance and the presence of other fish species (e.g. pike, burbot) have a negative correlation with bullhead abundance, while proportion of substrate that is equal or larger than gravel, proportion of substrate covered with macrophytes and macroinvertebrate taxa richness have a positive correlation. Overall, the model has a significant p-value of 0.005 and the R^2 value is 0.605. The potential predictors that were eliminated during variable selection are in-stream structures area and water discharge. In the case of brown trout, the best model obtained had this output:

```
Call:
lm(formula = log(trout.abund) ~ log(bull.abund) +
log(discharge) + log(prop.substr) + log(prop.macroph) +
other.fish, data = abund.mlr)

Residuals:
     Min       1Q   Median       3Q      Max
-1.09603 -0.22461 -0.01377  0.39004  1.31522

Coefficients:
                    Estimate Std. Error t value Pr(>|t|)
(Intercept)           5.1466     1.4096   3.651  0.00332 **
log(bull.abund)      -0.2706     0.2384  -1.135  0.27846
log(discharge)        2.5741     0.9707   2.652  0.02110 *
log(prop.substr)      0.3923     0.1888   2.078  0.05984 .
log(prop.macroph)     1.5365     0.3914   3.925  0.00202 **
other.fish           -0.2138     0.1164  -1.837  0.09102 .
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' '
1

Residual standard error: 0.7435 on 12 degrees of freedom
Multiple R-squared: 0.7071,   Adjusted R-squared: 0.5851
F-statistic: 5.795 on 5 and 12 DF,  p-value: 0.006001
```

This time only water discharge and proportion of substrate covered with macrophytes had a significant influence in the model. Both predictors correlated positively with brown trout abundance. The factor "proportion of substrate equal or bigger than gravel" is very near significance with a p-value of 0.0598, while the presence of other fishes is much less near significance. Bullhead abundance, although not significant, was still kept in the model, which had an overall significance of p=0.006 and R^2 value of 0.585. In this model, two factors were completely eliminated by variable selection: macroinvertebrate taxa richness and in-stream structures area. The simple correlation of fish abundance with in-stream habitat structures was almost significantly negative for bullhead (p=0.062) and significantly positive for brown trout (p=0.008).

3.2.2 Effects on the local food web

With the help of NMDS, it was possible to visualize the similarities and differences of the macroinvertebrate communities from the first biodiversity assessment among the stream reaches (figure 15).

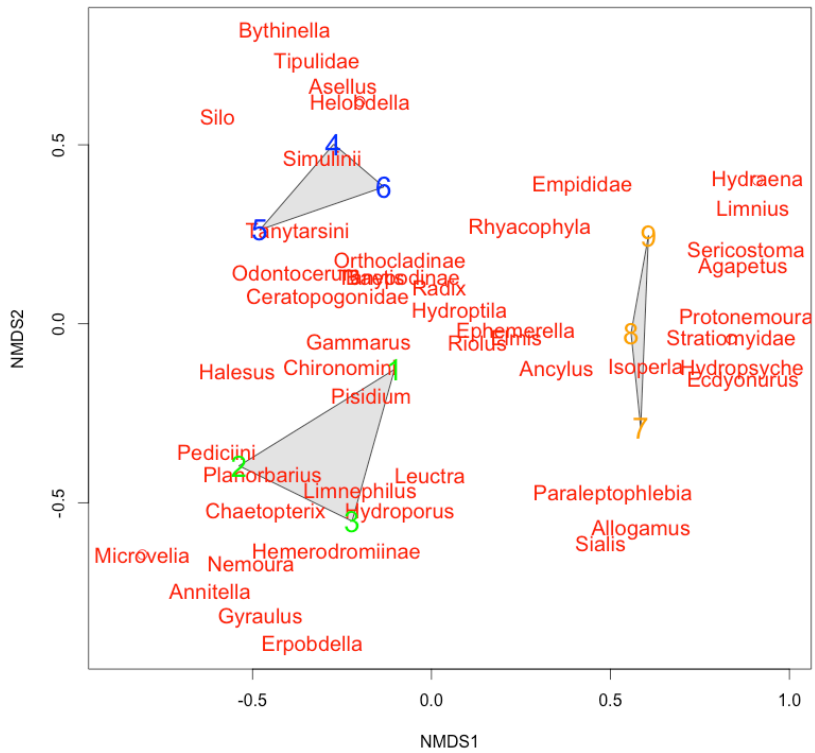

Figure 15: NMDS plot of the first biodiversity assessment. Points 1, 2 and 3 represent the reaches of Mühlebach, points 4, 5 and 6 those of Scheidgraben and points 7, 8 and 9 represent the points of Lochrütibach. The three taxa names that overlap in the middle of the figure are: *Odontocerum*, *Baetis* and Tanypodinae. *Ephemerella, Elmis* and *Riolus* might also be difficult to distinguish.

By pooling the macroinvertebrates into the different functional feeding groups and doing NMDS, the obtained graph (figure 16) shows which functional feeding group is dominant across the stream reaches.

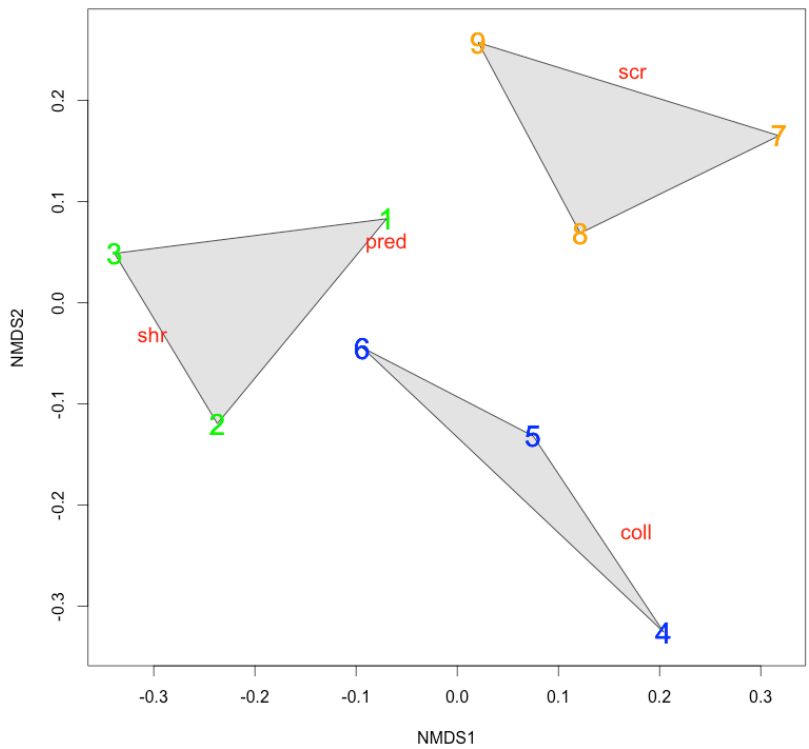

Figure 16: NMDS plot of the first biodiversity assessment with macroinvertebrate taxa pooled into functional feeding groups (collectors, scrapers, shredders and predators). Points 1, 2 and 3 represent the reaches of Mühlebach, points 4, 5 and 6 those of Scheidgraben and points 7, 8 and 9 represent the points of Lochrütibach.

Among streams, no significant differences in macroinvertebrate community biodiversity were found for all three indices (p=0.053 for taxa richness, p=0.361 for Shannon index and p=0.375 for Simpson' D). By taking out L3 from the analysis to control a potential influence of the channelized reach of Lochrütibach, no substantial change was observed. In the case of taxa richness, also the comparison of values from the first sampling with those of the second sampling to test for seasonality resulted in a non-significant difference (p=0.101). No significant correlation was found between biodiversity indices and in-stream structures (p=0.805 for taxa richness, p=0.321 for Shannon Index, p=0.752 for Simpson's D).

The stable isotope analysis showed that bullheads in Lochrütibach had a significantly lower amount of $\partial^{15}N$ stable isotope compared to Mühlebach ($p < 1*10^{-7}$) and Scheidgraben ($p < 1*10^{-7}$). In the case of brown trout, Scheidgraben had a significantly higher value compared to those of Lochrütibach ($p=1*10^{-7}$) and Mühlebach ($p=6.46*10^{-5}$). In Scheidgraben, the content of $\partial^{13}C$ stable isotope in bullheads was lower than the values obtained for the other two streams ($p=1*10^{-7}$ Lochrütibach and $p < 1*10^{-7}$ Mühlebach). The $\partial^{13}C$ values of brown trout only differed between

Lochütibach and Scheidgraben (p=0.009), with the latter one having the smallest value. Muhlebach had a mid-value that did not significantly differ from the other two extremes.

3.2.3 Fish species interactions

The bullhead to brown trout ratio was significantly different among streams ($p=9.51*10^{-6}$, see figure 17). The ratio in Scheidgraben was significantly lower than both Lochrütibach ($p=1.7*10^{-6}$) and Mühlebach ($p=2.09*10^{-5}$). By taking out stream reach L3 from the analysis, the results do not change in significance, although the ratio for Lochrütibach almost was significantly higher than that of Mühlebach (p=0.053).

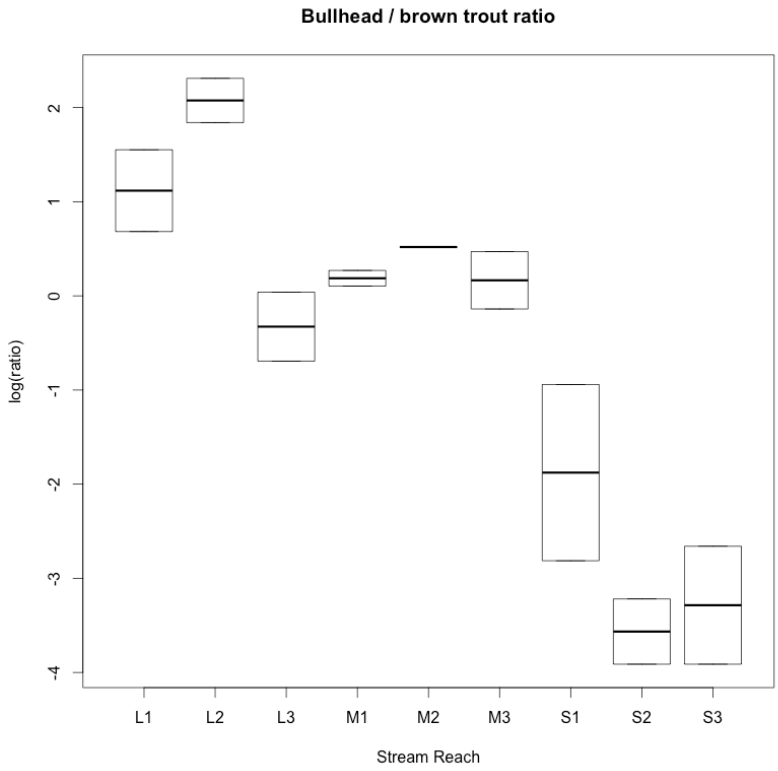

Figure 17: Bar plots of the bullhead to brown trout ratio among the different stream reaches.

The peak differences in the normal distributions fitted for the length distribution plotted in figure 8 are summarized in table 11. The values obtained for brown trout showed no significant differences among streams (p=0.441), while for bullhead a significant difference was found between Scheidgraben and Lochrütibach (p=0.037). The correlation between peak differences and bullhead to brown ratio was significant ($p=3.7*10^{-4}$), while the correlation between peak differences and in-stream structure area was not significant (p=0.235).

Table 11: Summary of the distances measured between the peaks of the normal distributions fitted to the fish length distribution plots of both fishing sessions for each stream reach (also depicted in figure 8).

Stream Reach	Trout distribution maxima distances [mm]	Bullhead distribution maxima distances [mm]
M1	20.21	8.49
M2	-8.14	3.69
M3	-32.22	5.15
S1	6.21	12.15
S2	19.77	23.74
S3	12.43	40.77
L1	19.83	3.74
L2	-4.58	3.36
L3	-2.20	-14.31

ANOVA testing for relevant differences among stream reaches in fish condition factor was not significant for both brown trout (p=0.104) and bullhead (p=0.436). The dietary overlap between brown trout and bullhead also was not significantly different among streams (p=0.461). No significant correlation was found between dietary overlap and bulhead / brown trout ratio (p=0.457), in-stream structure area (p=0.159) or taxa richness (p=0.796).

4. Discussion:

4.1 Effects of revitalization on fish abundance

The research questions for this section were the following:

1a) Is there a difference in fish abundance among channelized and revitalized stream reaches?

1b) Do in-stream habitat structures have an influence on fish abundance?

The fish data collected in the field from Mühlebach, Lochrütibach and Scheidgraben showed that a simple comparison between channelized (M1, M2, M3 and L3) and revitalized (L1, L2, S1, S2 and S3) stream reaches was not an effective approach to the experimental design of this project. By pooling Scheidgraben and Lochrütibach together for revitalized streams, fish abundances were not significantly different from those of the channelized reaches (see figure 14). Although the streams were chosen because of their similarity to represent an ideal alternative to BACI approach, it is evident that the streams have characteristics that need to be considered by doing separate analysis of the streams. The expectation of higher fish abundance in revitalized streams compared to channelized ones was not met. The brown trout population of Lochrütibach was the least abundant of the three, while the same was true for bullhead in Scheidgraben.

Data on fish abundance in Scheidgraben before revitalization showed that fish abundances for both brown trout and bullhead were lower than those measured during this project (tables 4 and 7) (bullhead was not even present in 2005). In Lochrütibach, on the other hand, only the bullhead population increased after revitalization, while the brown trout population has become smaller (tables 4 and 7). Although the project in Scheidgraben, and partially also in Lochrütibach, were successful in increasing fish populations, it appears that overall the fish abundances have not improved in absolute terms compared to channelized reaches. These results show that revitalization may not be as effective as expected in creating better conditions for fishes than those of channelized streams (as suggested by Naiman et al. 2012 and Pretty et al. 2003).

The analysis with multiple linear regression of factors that might be critical in the determination of suitable habitat for fishes showed interesting results for both brown trout and bullhead abundance. It appears that the installation of in-stream habitat structures, like those considered in this project (e.g artificial fish shelters, stone banks, etc), did not significantly influence the abundance of both fish species. Although the simple correlation between in-stream structure area and fish abundance scored significantly for at least brown trout, it seems that other factors play a more relevant role. According to the multiple linear regression, the model is much more relevant for the presence of macrophytes in the stream. It might be that the shelter offered by

this kind of vegetation is more suitable by fishes than the artificial counterpart. Macrophytes also play other roles, e.g. as resting habitat from hydraulic conditions and as food reservoir (Reyjol 2012), which might be lost in streams with low in-stream vegetation cover.

Another factor that had a significant positive correlation with bullhead abundance and almost significant (p=0.060) to brown trout abundance is the proportion of substrate that is equal or larger than gravel. This is not surprising since higher values for this factor reflects a higher availability of suitable substrate to fish spawning (Cienciala & Hassan, 2013).

The direct predation exerted by brown trout on bullhead is a plausible explanation for the negative correlation between bullhead and brown trout abundances. This also is partially true for the presence of other fishes than bullhead and brown trout. In fact, pike, burbot and European perch are known to count bullhead among their suitable prey (Zbinden et al. 2004), while this doesn't hold for European brook lamprey. It is interesting that the model fitted for bullhead abundance found a significant positive correlation between taxa richness and fish abundance, while the same thing was not true for brown trout. It might be that bullhead are more affected by the competition for macroinvertebrate prey than brown trout and therefore they might prefer sites with higher biodiversity where the availability of macroinvertebrate taxa is greater. Since bullhead have a mainly crepuscular feeding habit and brown trout a broader time range of activity with peaks at dawn and dusk (Belica 2007; Tomlinson & Perrow 2003), there is an imbalance between the two species: brown trout may be active during the whole hunting time of bullhead, but not vice versa.

Brown trout also showed a positive significant correlation to discharge, which was not significant for bullhead. This might be explained by the fact that bullhead have benthic habits due to the lack of a swim bladder. Living near the ground, where the water current is slower, makes this species less susceptible to variation in this factor. Brown trout on the other hand are adapted to live in the stream current, therefore it is not surprising that discharge has an influence. Lastly, the water chemistry values obtained from the lab analyses (see table 6) showed that the water quality in all of the three streams was very good. Given these circumstances, no chemical parameter was used as possible predictor in the analysis on fish abundance.

In summary, hypothesis 1a was partially rejected since the abundance of brown trout in Lochrütibach and that of bullhead in Scheidgraben were significantly lower than those in the channelized Mühlebach. The hypothesis 1b was rejected for bullhead because no significant positive correlation was found between in-stream structures and fish abundance. For brown trout, the hypothesis was partially rejected since an indication of a positive correlation was found.

4.2 Effects on the local food web

The research questions on food web were the following:

2a) Is the macroinvertebrate community influenced by the revitalization measures?

2b) Is there a difference in the food web structure among channelized and revitalized stream reaches?

The ANOVA testing for differences in biodiversity indices showed that the three streams did not differ significantly from one to another. Nevertheless, in the case of taxa richness the p-value was nearly significant (p=0.053) and Tukey HSD resulted with Scheidgraben nearly having less taxa compared to Mühlebach (p=0.054) and Lochrütibach (p=0.079). In the case of the other two indices, the results obtained for the three reaches of Scheidgraben seem to be lower than those from the other streams (see table 10).

The values of taxa richness obtained from the second biodiversity assessment showed that no significant difference in biodiversity was found between the communities sampled at the end of May / beginning of June and those sampled at the beginning of September. Overall, the second assessment showed the absence of some taxa and the appearance of others that were not present during the first assessment, which might explain the low variation in the index. On the other hand, the second assessment was much less thorough than the first one, since not every individual of the sample was sorted out from the samples. Therefore, it might be that the second estimation is not optimal for detecting seasonal changes.

The plot obtained from the NMDS (see figure 15) shows that the macroinvertebrate communities sampled in the various reaches are very similar within the same stream, but are clearly distinct across the different streams. An example of this difference is the presence of taxa like *Agapetus* sp., *Protonemoura* sp., *Hydropsyche* sp. and *Ecdyonurus* sp. exclusively in Lochrütibach, where they are relatively common. Other taxa like *Baetis* sp., *Radix* sp., *Gammarus* sp. and Orthocladinae are, on the other hand, commonly found in each stream. A significant difference found during the macroinvertebrate biodiversity assessment is the almost completely absence of Plecoptera in Scheidgraben. Only during the second biodiversity assessment individuals of the genus *Nemoura* sp. were found (no individuals were found in the gut contents of fishes as well). This might be an important absence, since stoneflies (Plecoptera) are considered among the macroinvertebrates used as indicators for water quality due to their sensitivity to water pollution, i.e. Ephemeroptera, Plecoptera and Trichoptera (EPT). Particularly surprising was the absence of Odonata larvae in the samples collected from all of the examined streams. However, flying adults were observed during the fieldwork, therefore it might be that sampling was suboptimal in this

aspect. The data obtained for dietary overlap between same fish species from different streams (see table 8) also reflects the diversity of taxa among streams. In particular, this is evident for Lochrütibach and Scheidgraben, where bullheads have only the 21.5 % of the diet in common and brown trout had only 16.2 %.

The NMDS for the functional feeding groups gives the following information: in Lochrütibach the most relevant group is represented by scraper macroinvertebrates, in Mühlebach mainly by shredders and for Scheidgraben in part by collectors. This differentiation underlines the different characteristics of the streams. Lochrütibach had the lowest levels of in-stream vegetation and the riparian vegetation as well was weakly developed, which suggests a low amount of organic material to break down. This is also reflected by low values measured for DOC and TOC in the water samples. It is therefore reasonable to think that scrapers, which rely on algal production, might be more abundant in Lochrütibach than collectors or shredders. In the cases of Scheidgraben and Mühlebach, where vegetation is more abundant, collectors and shredders play a major role. According to the River Continuum model, shredders are expected to dominate the upper reaches of streams, while collectors increase in importance more downstream (Hawkins & Sedell 1981). This might explain the trends for Mühlebach and Scheidgraben because the first one was sampled farther from the mouth of the river compared to the latter one.

The $\partial^{13}C$ stable isotope contents of the samples collected from the various streams (see figures 10, 11 and 12) suggest that the primary source of carbon is represented by algae in Scheidgraben and Mühlebach. The $\partial^{13}C$ values of functional feeding groups of macroinvertebrates indeed reflect those measured for algal samples (although the variability in the samples from Scheidgraben is high). This is also true for the values of both fish species sampled, which reflect those of their diet (i.e. macroinvertebrates). In the case of Lochrütibach, the $\partial^{13}C$ values of macroinvertebrates were not similar to that of algae. It might be that in Lochrütibach the source of carbon is not represented by in-stream production but from riparian vegetation. Unfortunately no samples from terrestrial vegetation were analysed. However, literature reports riparian producers being $\partial^{13}C$ depleted compared to in-stream producers (Turner & Edwards 2012), which is consistent with the values obtained for macroinvertebrates. Another possibility is that the carbon source is partially represented by algae and partially by mosses, which would explain the position halfway for the macroinvertebrates samples. Nevertheless, this explanation might not be sufficient, because scrapers do not feed on fine or coarse particulate organic matter (FPOM and CPOM), which might also contain material from mosses. Therefore this might not explain why the $\partial^{13}C$ value of scrapers deviates so much from that of algae.

The $\partial^{15}N$ stable isotope contents of the food web components reflect the general expectations: values increase in concurrence with higher trophic position. It is surprising that

predatory macroinvertebrate taxa from Scheidgraben have similar $\partial^{15}N$ values of scrapers. Since predators were sampled only in one reach of Scheidgraben, this might not be a highly representative value for this functional feeding group. Mühlebach also presents a singularity: the isotopic content of bullhead is higher than that of brown trout. Since the trout individuals sampled from every stream are not greater than 165 mm, the direct predation rate on bullhead for these samples is expected to be low. This might explain why the isotope values of both fishes are so similar among streams. The exceptional case of Mühlebach might be explained by looking at the indices of food importance for functional feeding groups (see table 9). Although the differences are not extreme, predatory macroinvertebrates have a higher importance for bullhead than for trout, which might be reflected in the higher isotopic values. It has also been reported by Gaudin (1985), that bullhead (in laboratory conditions) prey on brown trout fry. Predation on other fish larvae might increase the isotopic content of *C. gobio*, but the value measured for Mühlebach is the same as that from Scheidgraben, where the brown trout $\partial^{15}N$ value was higher than that of bullhead.

Lochrütibach had the lowest value of $\partial^{15}N$ contents in bullhead samples. Again, a possible explanation is given by the indices of food importance (table 9). The gut contents of bullhead in Lochrütibach showed a higher importance played by scrapers, which have lower $\partial^{15}N$ values compared to the other groups due to their diet of algae.

The significantly higher content of $\partial^{15}N$ in brown trout from Scheidgraben was consistent with a general higher content of this stable isotope in the whole food web. Cabana & Rasmussen (1996) reported that the increase in $\partial^{15}N$ contents of primary consumers correlated with the increase in human population density and that this shift likely reflects the high $\partial^{15}N$ of human sewage. From the nitrate and total nitrogen values obtained from the water samples, it seems that Scheidgraben has higher values compared to the other streams. However, the water parameters for Scheidgraben were evaluated as very good. It is possible that the proximity of the stream to the airport of Buochs might play a role, which should be further investigated.

In summary, hypothesis 2a was rejected because the three biodiversity indices used for macroinvertebrate community were not significantly different among the three streams. Scheidgraben even showed a negative trend, having among the lowest values for the indices. Hypothesis 2b was partially rejected because some differences in isotopic contents of fishes were found significant, but this is inconsistent with the macroinvertebrate biodiversity.

4.3 Fish species interactions

The research questions on fish interaction were the following:

a) Does the presence of in-stream habitat structure effect the interaction strength between trout and bullhead?

b) Is the dietary overlap between bullhead and brown trout influenced by revitalization measures?

The bullhead / brown trout ratio calculated for each stream reach indicates where the antagonistic interaction exerted by brown trout on bullhead was expected to be more important. The ANOVA and Tukey HSD test showed that Scheidgraben had the lowest ratio, which means that predation rate in this stream should be the highest. Furthermore, in this stream the presence of other potential predators is fairly high compared to the other streams. In Lochrütibach, although not significantly different from Mühlebach, were the highest values for the ratio, which should denote a lower predation rate. The non-significance of differences in Fulton's condition factor among stream reaches means that, overall, the fishes had a similar state of health.

The distance between the peaks of the normal distributions fitted for fish length distributions in each reach showed an interesting result in the case of bullhead. These measures, which should be considered as a proxy for fish growth, were significantly greater in Scheidgraben compared to Lochrütibach. As suggested by the significant correlation between this proxy of growth and the bullhead to brown trout ratio, it might be that bullheads in Scheidgraben have adapted to the higher predation rate by increasing their growth rate. Individuals that grow faster will reach sexual maturity earlier and likely will reproduce earlier than individuals that have smaller growth rate. In an environment like Scheidgraben, where predators are abundant, individuals that procreate early will be able to pass their genes to the next generation before being eaten, while other strategies will more likely fail.

In the case of Lochrütibach, predation risk is less and the bullheads might be selected toward a strategy that is more effective for intraspecific competition. Faster growth and earlier maturity age is usually constrained in other characteristics by trade-offs (Mangel & Stamps 2001). It would be interesting to see what kind of trade-offs occur among the populations of Scheidgraben and Lochrütibach. Nevertheless, it is important to take these results carefully, since the normal distributions fitted for the S2 and S3 reaches relied on very few fish length data. It is possible that the growth values are an overestimate of reality.

The ANOVA and Tukey's HSD test found no significant difference in the dietary overlap values between brown trout and bullhead among streams. As showed in figure 9, the food importance indices of both fish species for each macroinvertebrate order are highly variable across

streams. This means that brown trout and bullheads are both generalists in their prey selection (as confirmed by Tomlinson & Perrow 2000 and Belica 2007) and that the competition for food items between the two species might not be so relevant. The main food items found in the gut contents are mostly represented by Ephemeroptera larvae, Crustacea (mainly gammarids), Diptera larvae and Trichoptera larvae. These are among the most abundant macroinvertebrates taxa in the examined streams, therefore there is no risk of food shortage during spring and summer. It would be interesting to see if the interactions between brown trout and bullhead change during autumn and winter, when the macroinvertebrate species composition and abundance might be different.

The idea that dietary overlap might increase with decreasing macroinvertebrates biodiversity was not confirmed by the correlation test, but the trend seems to be present in Scheidgraben. The results obtained from the correlation test suggest that the presence of in-stream habitat structures has little role in determining the dietary overlap between the two fish species.

In summary, hypothesis 3a was rejected since changes in the bullhead-brown trout interaction are inconsistent with the revitalization of streams. The two restored streams showed two opposite situations of bullhead-brown trout interaction. The results suggested interspecific competition for food items was less than expected, while bullhead predation by brown trout might be responsible for a change in life history traits of the bullhead population. ∂^{15}N contents of brown trout were higher only in Scheidgraben and were considered the result of a generally higher content of ∂^{15}N in the whole food web. Hypothesis 3b was rejected because the dietary overlaps did not differ significantly between the three streams.

4.4 Conclusion

The rejection of essentially all the hypotheses formulated for this project shows how inaccurate the predictions might be if based on the assumption that revitalization measures will automatically create better conditions than those of channelized streams. We showed that the outcome of revitalizations on similar streams might be significantly different and that the effect of in-stream habitat structures on fish populations might be overrated, while other stream morphology characteristics, like in-stream vegetation and substrate composition, seems to be more relevant for habitat improvement.

An equivocal response to revitalization and in-stream habitat structures also was found among the macroinvertebrate communities of the different streams. The biodiversity indices were not significantly different across Mühlebach, Lochrütibach and Scheidgraben, while taxa composition and the higher importance of a particular functional feeding group (different for each stream) reflected the dissimilarities in habitat characteristics.

The combination of stable isotope analysis and gut content analysis resulted in a powerful tool for the examination of food web dynamics and the explanation of fish feeding habits. With stable isotopes the primary source of carbon was easily identified and the trophic structure of the food web was explained. Gut contents, on the other hand, clarified aspects of the $\partial^{15}N$ values of fishes and gave indications on how strong the interactions between bullhead and brown trout are.

The fish interactions also seemed inconsistently influenced by the presence of in-stream habitat structures. Interspecific competition for food, based on the dietary overlap, was found less important than expected, while the direct predation of brown trout on bullhead appeared as a strong influence on the bullhead population.

These analyses reported some results that might need further investigation. Firstly, from the $\partial^{13}C$ values, Mühlebach seemed to rely more on terrestrial primary production than on in-stream production, although the main functional feeding group were represented by scrapers. Secondly, in Scheidgraben, an overall higher $\partial^{15}N$ content of the food web was observed and, in general, (although statistically not significantly different from the other streams) lower values for macroinvertebrate biodiversity were measured. Although the water parameters were very good, the influence of the nearby airport might be a relevant factor. The last matter is the suggested adaptation of life history traits in bullhead to the higher predation rate of Scheidgraben, which may be over-estimated since the data obtained for the bullhead population in this stream were particularly low in two of the analysed reaches.

More value would have been added to this project if data previous to stream restoration on the food web from stable isotopes and gut content analyses were available. Concrete data on how the situation changed during the revitalization projects would have given much more clear results on the efficiency of the implemented measures. An objective for future restoration should be a more thorough monitoring of the project before and after the revitalization works in order to clearly assess their effectiveness and to recognize where the benefits are in case of success.

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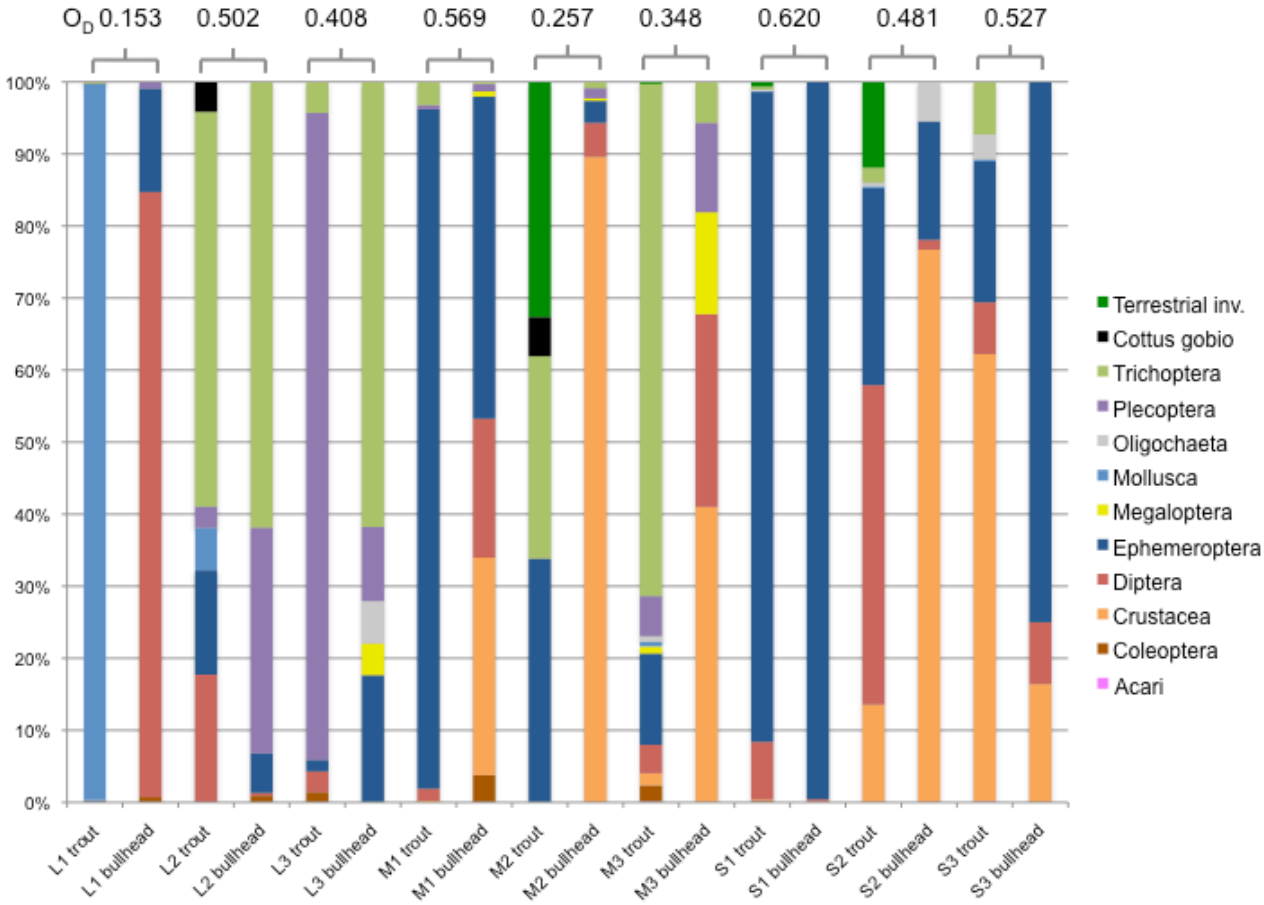
Appendix:

All the data sampled for this project are collected in a Microsoft Excel file, which is attached to this thesis on a compact disc.



Appendix 1: Bar plot representing the food importance indices I_{FI} for prey items found in the fish gut contents from different streams reaches (L stands for Lochrütibach, M for Mühlebach and S for Scheidgraben). The dietary overlap O_D values, calculated between brown trout and bullhead individuals from the same stream, are also reported.

Appendix 2: Summary of the stable isotope values per each stream pooled for food web components.

Stream	Type	Mean 15N	Sd 15N	Mean 13C	Sd 13C
Lochrütibach	Algae	1.465	1.421	-35.735	0.742
	Bullhead	6.038	1.253	-36.387	2.842
	Collectors	1.869	0.798	-38.209	2.790
	Macrophyte	0.810	0.697	-32.168	1.957
	Moss	-1.175	0.134	-44.005	2.058
	Scrapers	1.965	0.407	-38.403	1.714
	Shredders	1.816	0.526	-40.190	2.053
	Trout	6.784	0.479	-35.784	1.351
Mühlebach	Algae	1.540	1.556	-38.845	3.514
	Bullhead	8.679	0.568	-35.440	1.099
	Collectors	3.169	1.171	-36.854	4.458
	Macrophyte	3.754	1.433	-31.739	1.607
	Moss	2.770	2.231	-41.923	3.344
	Predators	3.625	2.241	-36.440	3.369
	Scrapers	5.003	0.833	-36.567	1.784
	Sediment	2.393	0.946	-24.490	2.423
	Shredders	2.826	1.175	-37.796	3.707
	Trout	7.708	0.956	-37.258	2.054
Scheidgraben	Algae	3.687	0.492	-40.773	1.217
	Bullhead	8.336	0.805	-41.284	2.148
	Collectors	5.427	1.574	-41.497	2.127
	Macrophyte	3.866	1.237	-31.877	1.736
	Predators	4.210	0.000	-41.270	0.000
	Scrapers	4.233	0.701	-40.710	1.268
	Sediment	2.140	2.845	-21.597	8.275
	Shredders	5.990	1.004	-41.985	1.506
	Trout	9.126	0.897	-38.537	2.431

Appendix 3: Summary of the macroinvertebrate taxa encountered in each stream reach during the first biodiversity assessment.

Macroinv. order	Genus/ species	Functional feeding group	M1	M2	M3	S1	S2	S3	L1	L2	L3
Trichoptera	Agapetus sp.	scraper	0	0	0	0	0	0	0	81	32
	Allogamus sp.	shredder	0	0	1	0	0	0	5	0	0
	Annitella sp.	shredder	0	15	9	0	0	0	0	0	0
	Chaetopterix sp.	shredder	5	10	3	0	0	0	0	0	0
	Drusus sp.	shredder	0	0	0	0	0	0	0	0	1
	Halesus sp.	shredder	1	5	2	1	1	0	0	0	0
	Hydropsyche sp.	collector	0	0	0	0	0	0	10	23	3
	Hydroptila sp.	scraper	10	0	7	2	2	5	5	6	4
	Limnephilus sp.	shredder	4	6	16	0	0	1	2	0	0
	Melampophylax sp.	collector	0	1	0	0	0	0	0	0	0
	Odontocerum sp.	scraper	2	1	6	6	4	2	0	1	0
	Potamophylax sp.	shredder	0	0	0	0	0	0	0	1	0
	Rhyacophyla sp.	predator	1	0	0	3	0	2	1	3	3
	Sericostoma sp.	shredder	0	0	0	0	0	0	0	1	1
	Silo sp.	scraper	0	0	0	1	1	0	0	0	0
Plecoptera	Chloroperla sp.	predator	0	0	0	0	0	0	0	1	0
	Isoperla sp.	predator	2	0	1	0	0	0	5	6	8
	Leuctra sp.	shredder	8	3	82	0	0	0	0	6	8
	Nemoura sp.	shredder	1	10	12	0	0	0	0	0	0
	Protonemoura sp.	shredder	0	0	0	0	0	0	10	26	28
Coleoptera	Elmis sp.	scraper	17	44	41	3	4	68	133	69	299
	Hydraena sp.	predator	0	0	0	0	0	0	0	0	2
	Hydroporus sp.	predator	1	1	12	0	0	0	0	4	0
	Limnius sp.	scraper	0	0	0	0	0	0	0	1	14
	Riolus sp.	scraper	19	2	0	0	0	0	0	0	12
Ephemeroptera	Baetis sp.	collector	13	37	23	132	106	69	66	30	16
	Ecdyonurus sp.	scraper	0	0	0	0	0	0	39	46	8
	Ephemerella sp.	collector	70	13	24	23	28	66	364	198	58
	Paraleptophlebia sp.	collector	0	0	2	0	0	0	2	2	0
Mollusca	Ancylus fluviatalis	scraper	0	10	7	5	3	1	102	49	11
	Bythinella sp.	scraper	0	0	0	1	0	0	0	0	0
	Gyraulus sp.	scraper	0	1	7	0	0	0	0	0	0
	Pisidium sp.	collector	0	6	2	1	0	1	1	1	0
	Planorbarius sp.	scraper	2	3	0	0	0	0	0	0	0
	Radix sp.	scraper	1	0	3	12	46	19	133	0	1
Diptera	Anthomyiidae	predator	0	0	0	0	0	1	0	0	0
	Ceratopogonidae	predator	3	4	9	1	4	12	0	1	1
	Chironomini	collector	1	20	0	4	0	0	0	9	0
	Empididae	predator	0	0	0	1	0	1	0	5	3
	Hemerodromiinae	predator	1	0	6	0	0	0	0	0	0
	Orthocladinae	collector	24	216	113	902	252	48	209	367	204
	Pediciini	collector	0	2	5	0	1	0	0	0	0
	Simulinii	collector	2	1	0	58	43	57	1	1	5
	Stratiomyidae	collector	0	0	0	0	0	0	0	1	0
	Tanypodinae	collector	51	54	49	108	71	11	46	102	38
	Tanytarsini	collector	6	131	24	301	134	84	4	14	7
	Tipulidae	shredder	0	0	0	3	0	2	0	0	0
Crustacea	Asellus aquaticus	shredder	0	0	0	1	0	14	0	0	0
	Gammarus sp.	shredder	34	416	392	38	87	23	5	59	137
Megaloptera	Sialis sp.	predator	0	0	1	0	0	0	2	0	0
Hirudinea	Erpobdella sp.	predator	0	0	1	0	0	0	0	0	0
	Glossiphonia sp.	predator	0	0	0	0	0	1	0	0	0
	Helobdella stagnalis	predator	0	0	0	0	0	4	0	0	0
Heteroptera	Microvelia sp.	predator	0	1	0	0	0	0	0	0	0

Appendix 4: Summary of the macroinvertebrate taxa encountered in each stream reach during the second biodiversity assessment. Green are the taxa encountered in both biodiversity assessments, red are taxa not encountered during the second assessment and blue are the taxa encountered only in the second assessment.

Macroinv.	Genus/ species	M1	M2	M3	S1	S2	S3	L1	L2	L3
Trichoptera	Agapetus sp.							x	x	x
	Allogamus sp.						x		x	x
	Annitella sp.									
	Chaetopterix sp.	x	x	x			x			
	Drusus sp.									
	Halesus sp.	x	x	x	x	x	x			
	Hydropsyche sp.							x	x	x
	Hydroptila sp.				x					x
	Limnephilus sp.			x						
	Melampophylax sp.		x					x		
	Odontocerum sp.	x	x	x	x	x	x	x	x	x
	Potamophylax sp.								x	
	Rhyacophyla sp.	x	x	x	x	x	x	x	x	x
	Sericostoma sp.									
	Silo sp.	x	x	x	x	x				x
Plecoptera	Chloroperla sp.									
	Isoperla sp.									x
	Leuctra sp.	x	x	x					x	x
	Nemoura sp.	x	x	x			x			
	Protonemoura sp.							x	x	x
Coleoptera	Agabus sp.				x					
	Elmis sp.	x	x	x	x	x	x	x	x	x
	Haliplus sp.				x					
	Hydraena sp.									
	Hydroporus sp.	x	x	x				x	x	x
	Limnius sp.	x								x
	Platambus sp.			x	x			x		
	Riolus sp.	x								
Ephemeroptera	Baetis sp.	x	x	x	x	x	x	x	x	x
	Ecdyonurus sp.							x	x	x
	Ephemerella sp.	x	x	x	x	x	x	x	x	x
	Paraleptophlebia sp.							x	x	
Mollusca	Ancylus fluviatalis	x	x	x	x	x	x	x	x	x
	Bythinella sp.									
	Gyraulus sp.									
	Pisidium sp.		x	x	x	x	x	x	x	
	Planorbarius sp.									
	Radix sp.		x	x	x	x	x	x	x	x
	Theodoxus sp.			x						
Diptera	Anthomyiidae					x				
	Ceratopogonidae		x			x	x			
	Chironomini				x					
	Empididae				x	x				x
	Hemerodromiinae									
	Orthocladinae	x	x	x	x	x	x	x	x	x
	Pediciini	x	x	x						
	Simulinii	x	x	x	x	x	x	x	x	x
	Stratiomyidae									
	Tanypodinae	x	x	x	x	x	x	x	x	x
	Tanytarsini	x	x	x	x	x	x	x	x	x
	Tipulidae									
Crustacea	Asellus aquaticus				x	x				
	Gammarus sp.	x	x	x	x	x	x	x	x	x
Megaloptera	Sialis sp.									
Hirudinea	Erpobdella sp.		x							
	Glossiphonia sp.				x					
	Helobdella stagnalis									
Heteroptera	Microvelia sp.									