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Impacts of river habitat quality on the conservation of endangered target species

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Summary

In Central Europe and other densely populated areas, running water bodies are subject to various human impacts. Fine sediment introduction due to intensive landuse in catchment areas belongs to the most common and deleterious impacts and particularly affects organisms adapted to coarse substrata, characterized by high exchange rates between free-flowing water and interstitial zone. Though negative impacts of fine sediments on aquatic environments have been described extensively across the literature, detailed information on spatio-temporal variations, species- and life-stage specific differences are still rare. However, these aspects are necessary for the development of effective conservation programs.

Main objective of this thesis was the analysis of habitat quality dynamics and their scale dependent effects on aquatic biota and conservation efforts. As target species, two freshwater bivalves and two lithophilic fish species were selected. Implications of the case studies for conservation practice are discussed and a general scheme for the implementation of target-specific conservation projects is presented.

In a first step, a case study investigating riverbed dynamics and fine sediment deposition in relation to erosion hotspots and illuviation pathways was carried out to quantify spatio-temporal variations in sediment deposition of three different pearl mussel streams. The effects on abiotic habitat quality were assessed. The results revealed extreme spatio-temporal heterogeneity of fine sediment deposition in dependence on catchment landuse, discharge and flow velocity. In streams with degraded catchments and higher average fine sediment deposition, abiotic habitat quality reached unfavorable conditions for mussels in the interstitial zone, particularly during low flow times. The stream with the most intact catchment and lowest fine sediment deposition hosted the healthiest pearl mussel population underlining the sensitivity of the freshwater pearl mussel to habitat modifications.

Habitat requirements of the thick shelled river mussel were assumed to be similar to those of the freshwater pearl mussel. However, with 19.4 kg m^{-2} $month^{-1}$ a higher fine sediment deposition was detected in successfully recruiting thick shelled river mussel streams than in already non-recruiting pearl mussel populations (3.7 kg m^{-2} $month^{-1}$). Furthermore, redox potentials in the interstitial zone varied around 300 mV and were much lower than in functional pearl mussel streams. This confirms the need for detailed analyses of species habitat requirements and of habitat deficits before the implementation of restoration measures.

In addition, such results have to be interpreted life-stage specifically. For instance, the ecological state of a lacustrine brown trout spawning stream was analysed with the objective of identifying life-stage specific limitations to successful recruitment, attributable to deficiencies in (i) spawning migration, (ii) spawning habitat quality, and (iii) juvenile habitat quality. The results of physicochemical measurements and texture analysis indicated the availability of high quality spawning and juvenile habitats. Consequently, the presence of migration barriers hampering spawning runs of adults was likely to be the main factor for recruitment failure. This underlines the high relevance of river continuity.

Bioindication studies with fish eggs and juvenile mussels revealed that survival and recruitment success varies intraspecifically among genetically distinct stocks and is significantly influenced by life history traits. In a cross experiment, growth and survival of juvenile pearl mussels were correlated to water temperature and detritus composition, with higher temperatures and C/N ratios being generally favorable for juvenile performance. However, stock-specific differences in both endpoints were detected, which makes consideration of juvenile origin obligatory for the interpretation of bioindication experiments and for the selection of captive rearing habitats.

Furthermore, the resistance of species and stocks against habitat degradation are influenced by life history traits. In particular, increases of maximum water temperature during egg incubation reduced hatching rates of fall-spawners (resident and migratory brown trout), but were positively correlated to spring-spawner (Danube salmon) hatching rates. Significantly longer incubation periods of fall-spawners coincided with relatively low stream substratum quality induced by colmation processes at the end of the egg incubation. Spring-spawners seemed to avoid low oxygen concentrations in the hyporheic zone by faster egg development, favoured by higher water temperatures.

In conclusion, the case studies presented in this thesis revealed the strong impact of increased fine sediment deposition on riverbed conditions and on aquatic organisms, which induce scale-specific, strongly variable effects. As a consequence, target-specific restoration concepts are necessary to make conservation efforts successful. For that purpose, conservation concepts need to consider spatio-temporal, life-stage specific and evolutionary aspects.

Zusammenfassung

In Mitteleuropa und anderen dicht besiedelten Gebieten sind Fließgewässer einer Vielzahl menschlicher Einflüsse ausgesetzt. Feinsedimenteintrag aufgrund intensiver Landnutzung in den Gewässereinzugsgebieten zählt zu den häufigsten und schädlichsten Einflüssen. An grobkörnige Substrate angepasste Organismen, die hohe Austauschraten zwischen Freiwasser und Interstitial benötigen, sind hiervon am stärksten betroffen. Obwohl negative Auswirkungen von Feinsedimenten auf aquatische Systeme in der Literatur ausgiebig beschrieben wurden, sind die Kenntnisse über räumlich-zeitliche Unterschiede sowie art- und altersklassenspezifische Auswirkungen nach wie vor limitiert. Derartiges Detailwissen stellt jedoch eine Grundvoraussetzung für die Umsetzung effektiver Schutzkonzepte dar.

Hauptziel der vorliegenden Dissertation war die Analyse sich räumlich-zeitlich verändernder Habitatbedingungen und deren skalenabhängige Auswirkungen auf aquatische Organismen und Managementkonzepte. Als Zielorganismen für die jeweiligen Fallstudien wurden je zwei Süßwassermuschelarten sowie kieslaichende Fischarten ausgewählt. Die Resultate der einzelnen Fallstudien werden insbesondere im Hinblick auf ihre Auswirkungen auf Renaturierungs- und Managementkonzepte diskutiert. Zusätzlich werden Leitlinien für die Planung und Durchführung fallspezifischer Renaturierungsmaßnahmen entwickelt.

In einem ersten Schritt wurde am Beispiel dreier Perlmuschelbäche die Sedimentdynamik und Feinsedimentdeposition in Relation zu Erosionshotspots und Sedimenteintragspfaden untersucht, mit dem Ziel die räumlich-zeitlichen Unterschiede dieser Parameter zu quantifizieren. Die Auswirkungen auf die abiotische Habitatqualität wurden durch parallele Untersuchung physikochemischer Parameter erfasst. Die Feinsedimentdeposition wies, abhängig von der jeweiligen Landnutzung sowie der Abflussmengen und Strömungsgeschwindigkeiten, ein räumlich-zeitlich äußerst heterogenes Muster auf. In Gewässern mit degradierten Einzugsgebieten und erhöhten Feinsedimentdepositionsraten war die Habitatqualität im Interstitial zumindest während Niedrigwasserphasen für juvenile Flussperlmuscheln ungenügend. Das Gewässer, dessen Einzugsgebiet die geringste Nutzungsintensität und Feinsedimentdeposition aufwies, beherbergte den intaktesten Muschelbestand, wodurch die besondere Sensitivität der Flussperlmuschel gegenüber Habitatmodifikationen verdeutlicht wird.

Bis dato wurde angenommen, dass die Habitatansprüche der Bachmuschel denen der Flussperlmuschel ähneln. Mit 19.4 kg m^{-2} Monat^{-1} war die mittlere Feinsedimentdeposition in Gewässern mit natürlich reproduzierenden Bachmuschelbeständen deutlich höher als in

reproduktionsfreien Flussperlmuschelgewässern (3.7 kg m^{-2} $Monat^{-1}$). Außerdem schwankte das Redoxpotential im Interstitial stets um einen Wert von 300 mV und war somit erheblich niedriger als an funktionalen Perlmuschelstandorten. Dies verdeutlicht die Notwendigkeit einer eingehenden Untersuchung artspezifischer Habitatansprüche sowie der fallspezifischen Identifizierung von Habitatdefiziten vor der Umsetzung von Renaturierungsmaßnahmen.

Gleichzeitig müssen derartige Ergebnisse immer altersspezifisch interpretiert werden. So wurde der ökologische Zustand eines Seeforellenlaichgewässers analysiert, um altersspezifisch wirksame Faktoren für den Fortpflanzungserfolg im Hinblick auf Laichwanderung, Laichplatzqualität oder Juvenilhabitatqualität festzustellen. Die Ergebnisse der physikochemischen Messungen wiesen auf das Vorhandensein qualitativ hochwertiger Laich- und Juvenilhabitate hin. Der Hauptgrund für den mangelnden Fortpflanzungserfolg waren somit die vorhandenen Wanderhindernisse, die den Adulttieren keinen Zugang zu den Laichplätzen ermöglichten. Dies unterstreicht die Bedeutung der Gewässerdurchgängigkeit.

Bioindikationsstudien mit Fischeiern und Jungmuscheln wiesen auf einen deutlichen Einfluss der innerartlichen genetischen Konstitution sowie der Fortpflanzungsstrategie hin. Ein Kreuzexperiment deutete auf eine Korrelation von Wachstum und Überlebensrate juveniler Perlmuscheln mit der Wassertemperatur sowie der Detrituszusammensetzung hin. Höhere Wassertemperaturen und C/N-Verhältnisse begünstigten Wachstum und Überleben. Trotzdem waren bei beiden Parametern herkunftsspezifische Unterschiede feststellbar, die eine Berücksichtigung der Herkunft bei der Auswertung von Bioindikationsstudien sowie der Auswahl von Aufzuchtstandorten notwendig erscheinen lassen.

Darüber hinaus wird die Störungsanfälligkeit von Arten oder einzelnen Populationen substantiell durch deren Fortpflanzungsstrategie beeinflusst. Beispielsweise verringerte eine Zunahme der maximalen Wassertemperatur während der Eiinkubation die Schlupfraten von Herbstlaichern (Bachforelle und Seeforelle), wogegen eine positive Korrelation mit den Schlupfraten von Frühjahrslaichern (Huchen) nachgewiesen wurde. Die signifikant längeren Inkubationszeiten der Herbstlaicher führten aufgrund von Kolmationsprozessen zu vergleichsweise stark verringerter Substratqualität am Ende der Eiinkubation. Frühjahrslaicher schienen dagegen geringe Sauerstoffkonzentrationen im Interstitial durch eine schnellere Eientwicklung zu vermeiden, was durch höhere Wassertemperaturen begünstigt wird.

Zusammenfassend lässt sich festhalten, dass in den präsentierten Fallbeispielen substantielle Auswirkungen einer verringerten Substratqualität auf die Zielorganismen nachgewiesen wurden, die sich jedoch skalenspezifisch deutlich unterscheiden können. In der Folge sind individuell abgestimmte Renaturierungskonzepte für erfolgreiche Naturschutzprogramme notwendig. Zu diesem Zweck müssen räumlich-zeitliche, altersspezifische und evolutionäre Aspekte berücksichtigt werden.

1 Introduction

1.1 General background

Freshwater ecosystems are recognized as biodiversity hotspots and a most valuable resource for mankind. At the same time, their value makes freshwaters susceptible to overexploitation, documented by the fact, that many freshwater organisms are currently ranked among the most imperiled taxa worldwide (Dirzo & Raven 2003; Geist 2011; Lydeard et al. 2004). Especially running water bodies and their catchments in densely populated areas like Central Europe are often targets for various human activities, like hydro-power generation, water abstraction and intensive land-use (Deitch et al. 2009; Friberg et al. 2010; Mueller et al. 2011). In contrast, several national and international legislative documents like the Water Framework Directive (WFD) of the European Union or the Clean Water Act in the United States demand water bodies to be protected (Bundesministerium der Justiz 2009; European Parliament 2000; United States Congress 1972). The assessment of water and habitat quality is based on different criteria, such as chemical water quality, structural habitat quality and the status of biological communities and indicator species. Over the last decades improvements in sewage treatment have resulted in considerable increases of water quality, by which the goals of these guidelines are often met with respect to biochemical oxygen demand. However, biological communities in many places have not recovered as structural deficits frequently persist, e.g. structural riverbed degradation and poor habitat connectivity.

Functional riverbeds in the rhithral zone are described as clean, gravel dominated substrates, which are periodically flushed during major floods, possessing an interstitial zone characterized by a good water exchange with the free-flowing water. Fine particles have been identified as a main degradation cause for this riverbed type (e.g. Boulton et al. 1998; Geist & Auerswald 2007; Izagirre et al. 2009; Kemp et al. 2011; Wood et al. 2005). It has to be noted here, that in the literature the definition of fine particles is inconsistent as the size fractions, which are referred to as fine, vary between 0.063-4 mm in diameter (see also Table 7.1). Furthermore, the terms sediment (a mass of organic or inorganic solid fragmented material, or the solid fragment itself, that comes from weathering of rock and is carried by, suspended in, or dropped by air, water, or ice; McGraw-Hill Dictionary of Scientific & Technical Terms 2003) and substrate or substratum (the foundation to which a sessile organism is attached; McGraw-Hill Dictionary of Scientific & Technical Terms 2003) frequently are not clearly separated. Throughout this work, these terms are used according to the definitions given above and fine particles have a grain size < 0.85 mm.

The increased mobilization of fine particles is triggered by intensive land-use and elevated erosion rates and surface runoffs in catchment areas (Hümann et al. 2011; Scholz et al. 2008; Walling et al. 2006). After introduction into riverbeds, the fine sediment clogs the interstitial macropores in the substratum and reduces exchange rates between the free-flowing water and the interstitial zone (Malcolm et al. 2010). Modified flow regimes with lower discharge and flow velocities due to water abstraction and river dams often maintain this process (Osmundsen et al. 2002). If fine sediment introduction exceeds the intake capacity of the interstitial zone, a continuously moving fine sediment layer may be developed on the substratum surface. Substrate instability can be the consequence of sealed soils in the catchment and river straightening resulting in short, but unnaturally frequent, high peak flows. In contrast to increased sediment input and bed–load transport, dams often prevent sediment transport leading to a lack of substratum downstream and a surplus of material upstream (Gupta et al. 2012; Habersack et al. 2013). The lack of material downstream of dams generates scouring water, i.e. water with strongly reduced sediment load expending the excess energy on bank and riverbed erosion (Kondolf 1997). The scouring water then results in incision of the river channel until an armor layer, which cannot be moved by the flows, is produced. All of these processes not only influence hydrologic and abiotic processes but also associated biological communities, which are often highly dependent on intact substrates.

1.2 The target species

The riverbed is a key habitat for many aquatic organisms. As described in chapter 1.1, the most severe degradation processes usually occur in the rhithral zone, where many sensitive and highly specialized species are found. The target organisms in this study, salmonid fishes and freshwater bivalves, were chosen according to the following criteria: The target species should be

i) typical inhabitants of the rhithral zone depending on clean substrates with a well oxygenated interstitial zone.
ii) sensitive indicators for riverbed and habitat degradation
iii) long (unionid mussels) and short time (salmonid fishes) indicators for riverbed quality
iv) keystone species in their ecosystems

Salmonid target species

The fish family Salmonidae has colonized a Holarctic distribution area and is subdivided into three subfamilies, the Coregoninae, Thymallinae and Salmoninae (Nelson 2006). The two target species, brown trout (*Salmo trutta*) and Danube salmon (*Hucho hucho*), belong to the Salmoninae. The Danube salmon is endemic in the Danube drainage, whereas the brown trout is native to several other European basins as well (Kottelat & Freyhof 2007; Lelek 1987). The brown trout was also introduced to different countries in Asia, Africa and America (Kottelat & Freyhof 2007). Furthermore, three different ecotypes of the species are known, resident brown trout (*Salmo trutta fario*), lacustrine brown trout (*Salmo trutta lacustris*) and sea trout (*Salmo trutta trutta*), of which the first two were included in this thesis. The Danube salmon is listed endangered, brown trout is a species of least concern (Freyhof 2011; Freyhof & Kottelat 2008). However, locally also the brown trout and especially its migratory ecotypes can be subject to population declines.

Both target species are lithophilic, i.e. they depend on suitable gravel banks for reproduction where the females dig redds, in which the eggs are deposited. Brown trout is a typical fall spawner with egg development over winter and fry emergence in spring (Crisp 1996). Danube salmon spawns in spring exhibiting much shorter egg incubation periods with fry emergence in late spring to early summer (Holcik 1988). *S. trutta fario* lives stationary in the most upstream regions of the river continuum. In contrast, *H. hucho* and *S. trutta lacustris* are migratory. Juveniles migrate to feeding habitats, where they stay until maturation. As adults they perform spawning runs, usually returning to their native spawning grounds (Nordeng & Bratland 2006; Quinn et al. 2006).

Mussel target species

The two mussel target species, the freshwater pearl mussel (*Margaritifera margaritifera*) and the thick shelled river mussel (*Unio crassus*), belong to the families Margaritiferidae and Unionidae, respectively. They are currently seen as members of the subclass Paleoheterodonta and the order Unioniformes (Bogan 2008). Strayer (2008) further mentions both families belonging to the superfamily Unionoidea. Generally, taxonomic classification of unionoid species is still precarious and has undergone several revisions in the last years, mainly due to new molecular information (e.g. Campbell at al. 2005; Lydeard et al. 1996). Further, the Unionoid life cycle comprises a larval stage, the glochidium, which was believed to be the common larval type of the Unionoidea. However, Barnhart (2013) identified a differing growth mechanism of *Margaritifera falcata* larvae. These findings suggest that in case of the Margaritiferidae the larvae do not conform to the glochidial larva. Consequently, further adjustments in unionoid taxonomy can be expected.

Unionoid mussels have a more complex life cycle than salmonids, additionally involving a parasitic stage on suitable host fish. As mentioned above, mature mussels release larvae into the water column, which have to attach to suitable host fish. In case of the freshwater pearl mussel (*Margaritifera margaritifera*) only brown trout is a suitable host in the study area. In areas of the Atlantic basin the Atlantic salmon (*Salmo salar)* is the second potential host (Young & Williams 1984). The host fish range of the thick shelled river mussel (*Unio crassus*) is wider and consists of up to 16 fish species, with chub (*Squalius cephalus*), European minnow (*Phoxinus phoxinus*), ide (*Leuciscus idus*) and stickleback (*Gasterosteus aculeatus*) serving as primary hosts (Taeubert et al. 2012 a, b). At the end of the parasitic stage the juveniles drop off their hosts and bury into the substratum for up to five years (Buddensiek et al. 1993), where they live as peddle feeders. Finally they return to the substratum surface and start filter feeding. Hastie et al. (2001) and Strayer (1999) demonstrated the importance of long-term riverbed stability for freshwater mussels, emphasizing their strong, lifelong dependence on suitable substratum conditions.

The distribution area of both species is restricted to the northern hemisphere with an overlap in Central and Northern Europe. The freshwater pearl mussel further occurs on the British Isles, in South Western Europe and the west coast of North America. The thick shelled river mussel is absent in these areas, but its distribution is extended to the south east comprising areas of the Balkan and Anatolia. In spite of their broad distribution areas, both target species have severely declined in the past decades and are therefore listed as endangered (Mollusc Specialist Group 1996; Van Damme 2011).

The link between target species and ecosystem health

The life cycles of the target species are strongly connected to riverbed quality as well as to each other (Fig. 1.1), which makes them sensitive indicator species. Due to their active influence on biodiversity, ecosystem functioning and the provision of valuable ecosystem services they are further considered as keystone species. For instance, dense mussel beds are able to stabilize the riverbed, influence substratum texture and improve the habitat for benthic invertebrates and juvenile fish (Gutierrez et al. 2003; Hastie & Cosgrove 2001; Spooner & Vaughn 2006). By their filtering activity they transfer suspended particles and nutrients from the free-flowing water to the interstitial zone, stimulating microbial and macrozoobenthos communities (Vaughn et al. 2004; Vaughn & Spooner 2006). The latter are a dominant food source for most fish species. As a consequence, the mussels improve habitat conditions and increase food availability for their host fish. On the other hand fish do not only host glochidia and serve as vectors in mussel distribution. In case of salmonids, they may serve as ecological engineers and prepare suitable juvenile habitats for mussels by redd digging, which loosens substrates and results in a mobilization of fine sediments (Ziuganov & Nezlin 1988).

By the provision of ecosystem services, the target species contribute to healthy river ecosystems. The filtering action of mussels and the activity of associated microbial communities increase the self-cleaning power of rivers, by which clean freshwater resources are allocated. Freshwater fish, particularly salmonids, regionally form a considerable part of the human diet and are popular target species for recreational fishing. Despite their relevance, numerous aspects about the correlation between riverbed dynamics and associated species remain unclear.

1.3 Objectives

Fine sediment introduction into river habitats is increasingly recognized as a major degradation cause in stream ecosystems and information on correlations between fine sediment and several abiotic and biotic parameters is available. Yet to date, there is still a gap of knowledge concerning spatio-temporal dynamics of sediment transport and deposition and its influence on abiotic habitat parameters. Additionally, the life-stage and stock-specific analysis of habitat conditions is still in its infancy, neglecting the importance of local adaptations and life-stage specific shifts in habitat requirements. Consequently, the main objective of this thesis was the analysis of riverbed dynamics, fine sediment deposition and their scale dependent effects on habitat quality, aquatic organisms and conservation efforts. For this purpose, a representative set of study streams was chosen, covering typical Central European geological areas, stream types and sizes. Four model organisms were selected according to objective criteria described in chapter 1.2. In detail, this thesis had the following objectives:

(1) Linking of stream sediment deposition and aquatic habitat quality in pearl mussel streams.
(2) Identification of reasons for the current lack of natural reproduction in lacustrine brown trout in Lake Walchensee and its former spawning tributary, the river Obernach.
(3) Assessment of physicochemical properties in *Unio crassus* habitats.

(4) Identification of species- and stock-dependent variability of physicochemical impacts on reproductive success of salmonids and freshwater pearl mussels

(5) Investigation of the suitability of juvenile freshwater pearl mussels as bioindicators

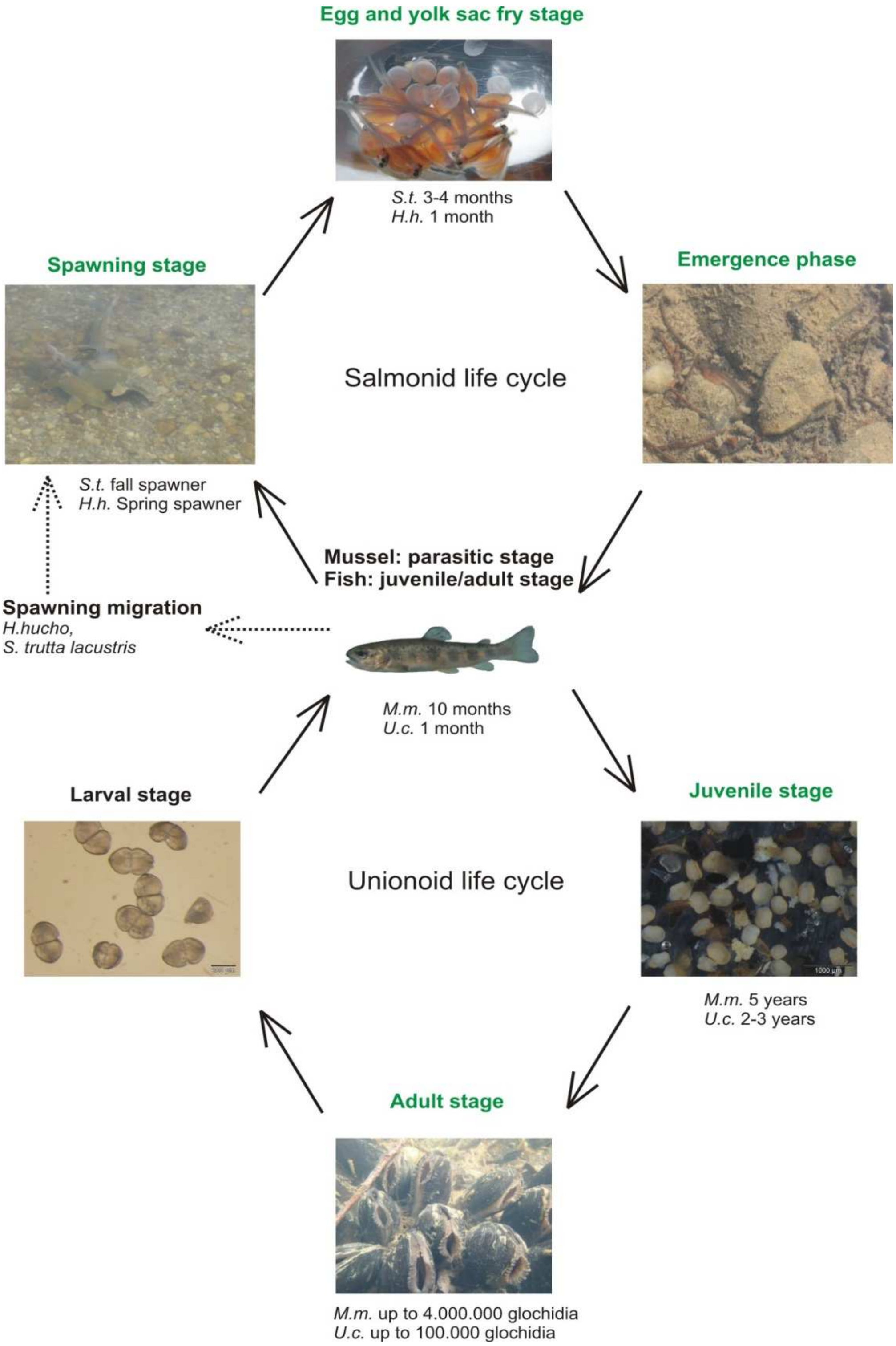


Figure 1.1: Unionid and salmonid life cycles including specific information for the target species of the study. Green life-stages depend on riverbed quality. Dotted arrows signify an additional step in the life cycle of migratory salmonids compared to resident species (*M.m.* = *Margaritifera margaritifera*; *U.c.* = *Unio crassus*; *H.h.* = *Hucho hucho*; *S.t.* = *Salmo trutta*).

2 Linking stream sediment deposition and aquatic habitat quality in pearl mussel streams: implications for conservation

A similar version of this chapter was published: Denic M & Geist J (2014). Linking stream sediment deposition and aquatic habitat quality in pearl mussel streams: implications for conservation. River Research and Applications: in press.

2.1 Abstract

The introduction of fine sediments into streams is considered to have a major effect on habitat quality affecting the reproduction of sensitive species like unionid mussels and salmonid fishes. To date, there is a lack of information on the magnitude and spatio-temporal resolution of sediment introduction. This study aimed to quantify the spatio-temporal deposition of fine sediments in headwater streams in relation to the status of *Margaritifera margaritifera* and *Salmo trutta*. Fine sediment deposition was linked to physicochemical conditions of the adjacent stream bed. The mean observed deposition of fine sediments over the study period was 3.4 kg m^{-2} $month^{-1}$ with a high spatio-temporal variation ranging from < 0.01-20.3 kg m^{-2} $month^{-1}$. Discharge had the strongest influence on deposition rates. Mean differences in redox potential between free-flowing water and the interstitial zone were 90 mV. The spatio-temporal variability of physicochemical parameters increased with degree of degradation. High-quality reaches had more constant conditions. Our results indicate that monitoring of sediment quality and deposition in streams has to comprise several time points and study reaches, or should at least be conducted during periods with the most adverse habitat conditions, to allow valid assessments of habitat quality. In streams with increased fine sediment deposition, instream restoration measures are insufficient for the enhancement of pearl mussel habitats due to rapid clogging of interstitial pores. Only integrative catchment management based on detailed habitat analysis can ensure sufficient habitat quality for species sensitive to siltation.

2.2 Introduction

In the northern hemisphere, running water bodies are intensively used and therefore often heavily modified. This results in a reduction of water quality and structural degradation (Bauer 1988; Douda 2010; Friberg et al. 2010). Weirs for discharge regulation and hydropower production typically reduce river continuity, hamper bed load discharge and change flow regimes (Crisp 1996; Denic & Geist 2010; Garcia de Leaniz 2008; Mueller et al. 2011). These features together with intensified land-use and elevated surface runoff in catchment areas can result in a strongly reduced substratum quality, e.g. high siltation rates and substratum compaction, especially in the rhithral zone (Larsen et al. 2011; Österling et al. 2010; Soulsby et al. 2001).

Typical inhabitants of the rhithral zone are the freshwater pearl mussel *Margaritifera margaritifera* L. and brown trout *Salmo trutta* L., which are adapted to cool, clean streams with low nutrient content. Due to their complex life cycles, including an obligate phase in the interstitial system, both species are highly sensitive to increased levels of fine sediments that reduce stream bed quality (Cosgrove et al. 2000; Meyer 2003) and fitness of susceptible species. High quality substrata necessary for successful reproduction of pearl mussels and brown trout are characterized by stable but well perfused gravel beds, i.e. coarse, well sorted substrata with a content of fine sediments (< 1 mm) constituting less than 20% (Geist & Auerswald 2007; Hastie et al. 2000; Sternecker & Geist 2010). Furthermore, high redox potentials of at least 400 mV and oxygen concentrations of 6.9 mg L^{-1} are prerequisites for successful development of juvenile mussels as well as salmonid eggs and larvae (Armstrong et al. 2003; Geist & Auerswald 2007; Ingendahl 2001; Sternecker et al. 2013 a, b).

The key role of high substratum quality for pearl mussel and brown trout reproduction has been increasingly recognized, triggering intensive research (Greig et al. 2007; Malcolm et al. 2004) and restoration efforts in this field, e.g. cleaning of gravel beds, gravel addition, river dam removal and the installation of sand/silt traps (Garcia de Leaniz 2008; Jähnig et al. 2010; Pander & Geist 2013). However, there is still a lack of quantitative data concerning sediment deposition and its impact on substratum quality throughout the year. Detailed information on such basic parameters is crucial for sustainable restoration and river management methods, particularly since the efficiency of commonly applied substratum restoration measures has not yet been systematically evaluated.

Our study aimed to i) quantify (fine) sediment deposition into the riverbed at a spatio-temporal scale, ii) assess spatio-temporal variability of physicochemical substratum conditions, and iii) recomend restoration and monitoring practices using the example of three Bavarian streams with pearl mussel and brown trout.

2.3 Materials and methods

Study area and experimental design

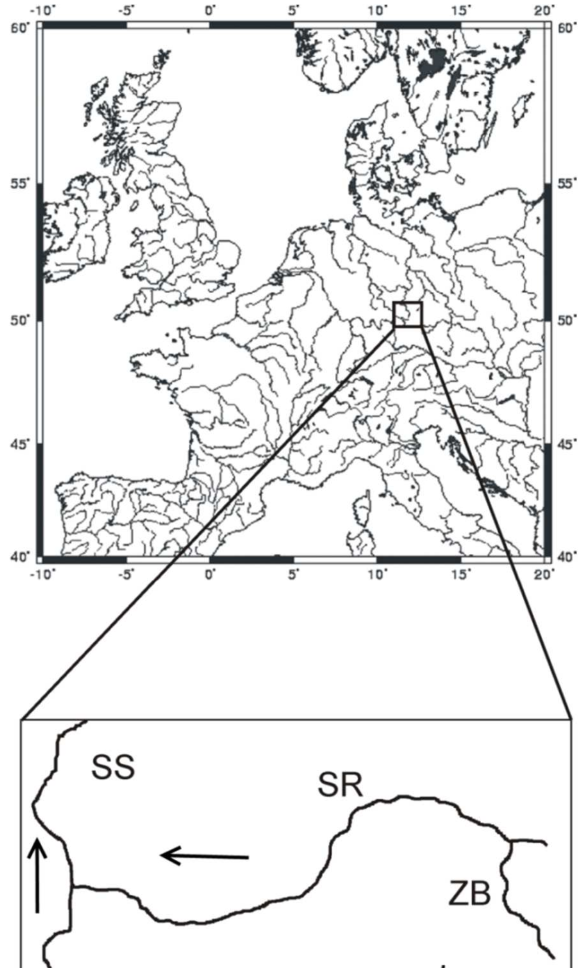


Figure 2.1: Schematic map of the study area. MB stands for Maehringsbach, ZB for Zinnbach, SR for Suedliche Regnitz, HB for Hoellbach and SS for Saechsische Saale which drains into the Elbe River. Arrows indicate stream flow direction.

The study area is situated in north-eastern Bavaria, Germany in the Saechsische Saale drainage, a subdrainage of the Elbe River (Fig. 2.1). The study streams were selected based on several catchment and stream parameters: To ensure the comparability of results, three study streams (Suedliche Regnitz, Zinnbach and Maehringsbach) within the same geological area and geographical region were selected. They are all oligotrophic, siliceous headwater streams with an average discharge of 0.4-0.7 m^3 s^{-1}, and an occurrence of freshwater pearl mussels and its host fish, the brown trout. Another criterion for selection was that study streams should cover the maximum possible range of ecological conditions for the target species. Stream status was determined by an assessment of the population status of *M. margaritifera* and *S. trutta* and land-use in the catchment. The Maehringsbach hosts a naturally recruiting pearl mussel population of about 30.000 individuals. Geist et al. (2006) found a total fish biomass of 133 kg ha^{-1} of which 91% were lithophilic brown trout. The catchment is dominated (90%) by forest and grassland. Pearl mussels still occur in the other two study streams but have not reproduced in these for several decades and populations have shrunk to about 2.000 individuals in each stream. In the Suedliche Regnitz, the fish biomass is high with 252 kg ha^{-1} but the percentage of brown trout was very low (20%). The Zinnbach shows a median fish biomass of 87 kg ha^{-1} and 89% brown trout biomass. Land-use in these two catchments is more intensive with 40% of the area covered by agricultural fields of wheat, barley and maize. In addition, several small villages and settlements are distributed across both catchments.

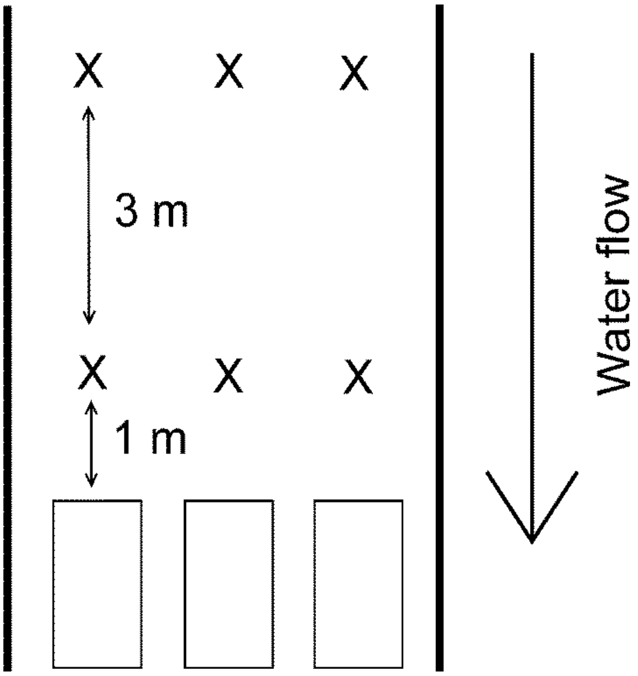


Figure 2.2: Schematic representation of a study reach, rectangles representing sediment traps and crosses representing sampling sites for physicochemical parameters.

Overall, fourteen study reaches were selected in the area, based on the location of possible erosion hot spots and illuviation pathways, such as intensively used fields or ditches. The study reaches were distributed downstream and upstream of such potential sediment sources. Nine reaches were situated in the Suedliche Regnitz (number 1-9), four in the Zinnbach (number 10-13) and one in the Maehringsbach (number 14), which was used as a reference reach due to its recruiting pearl mussel population. Study reaches were numbered in an upstream direction and every study reach comprised six study sites for physicochemical measurements and three sediment traps as shown in Fig. 2.2.

Sediment deposition

This study measured net sediment deposition rates (introduction of sediments minus re-mobilization). Sediment deposition was monitored using sediment traps consisting of plastic boxes with a volume of 5 l (33 x 19 x 11 cm) that were filled with round gravel of 16-32 mm in diameter. In May 2009, the traps were buried in the substratum to the depth of the substratum surface and emptied every four weeks from June 2009 to May 2011. This procedure was chosen because it mimics the introduction of clean gravel by spawning site restoration measures and since it allowed for a standardized comparison of net sediment deposition rates within and between streams. In the laboratory, samples were wet sieved (AS 200 digit, Retsch, Haan, Germany) to separate different grain sizes (mesh width: 20 mm, 6.3 mm, 2.0 mm, 0.85 mm). Representative aliquots of these fractions were dried at 80 °C for the determination of dry weight. The largest fraction was excluded from further analysis because of the restricted sample volumes (Sinowski and Auerswald, 1999). The deposition of particles < 0.85 mm is of particular relevance for substratum quality and is referred to as “fine sediment deposition” throughout the paper, whereas “sediment deposition” refers to the combined deposition of all size fractions.

Physicochemical parameters

Physicochemical parameters were investigated in three-monthly intervals from May 2009 to May 2011. Redox potential and penetration resistance were measured according to Geist and Auerswald (2007). Redox potential was recorded in the free-flowing water and in 5 and 10 cm substratum depth. Water depth was measured with an accuracy of ± 0.5 cm. Flow velocity was measured with a handheld flow meter (HFA, Höntzsch, Waiblingen, Germany) at 50 % water depth. Discharge values were obtained from a gauging station at the Suedliche Regnitz and used as an estimate for all sites. This was possible due to the close proximity and connection of streams that ensured similar discharge trends for the whole study area.

Data analysis

Data analysis was performed with PASW 18 and Excel 2010. Differences in sediment deposition and physicochemical parameters between the streams were analyzed using ANOVA with Tukey’s post-hoc test when variances were homogenous. If variances were not homogenous, Tamhanes-T2 test and the Mann-Whitney-U-test were used. To quantify the variability of physicochemical parameters at and detect the distribution pattern of the study reaches, Nonmetric Multidimensional Scaling (NMDS) was applied using the ALSCAL algorithm. Mean Euclidian distances based upon the NMDS matrix between the streams and reaches were calculated for a comparison of their differentiation. Principal component analysis (PCA) was used to reveal which variables accounted for inter-reach and inter-stream variations. Linear regression analysis showed a close relationship between the independent variable discharge and the dependent variable fine sediment deposition.

2.4 Results

Single factor analyses

During the study period, the mean sediment deposition over all streams and reaches was 8.8 kg m^{-2} month^{-1} of which 3.4 kg m^{-2} month^{-1} were fine sediments < 0.85 mm. At a mean river width of 2 m this equals 81.6 t of fine sediments per year and km stream length. Sediment deposition was highest during snow melt in February/March 2011 and in summer 2010 with a maximum of 31 kg m^{-2} month^{-1} (10.5 kg fine sediments m^{-2} month^{-1}) following a strong correlation with discharge independent of season (Fig. 2.3). The minimum values of net sediment deposition were observed at low flow in summer 2009. Mean sediment deposition in the Maehringsbach was only 4.3 kg m^{-2} month^{-1}, whereas it was 9.0 and 9.6 kg m^{-2} month^{-1} in the Suedliche Regnitz and the Zinnbach (Table 2.1). Average deposition of fine sediments was 2.6, 3.2 and 4.1 kg m^{-2} month^{-1}, respectively, and differed significantly between streams (Tamhane-T2 test, Suedliche Regnitz and Zinnbach p = 0.001, Suedliche Regnitz and Maehringsbach p = 0.015, Zinnbach and Maehringsbach p = 0.001). On the reach scale, the mean fine sediment deposition varied between 1.9 and 5.3 kg m^{-2} month^{-1} (Fig. 2.4) showing that local conditions can significantly influence sediment deposition even if the catchment generally supplies high amounts of material. No significant differences were detected between the study reaches upstream and downstream of sediment sources (Mann-Whitney-U-test, p = 0.350). No general increase of sediment deposition occurred in an upstream to downstream direction (Fig. 2.4).

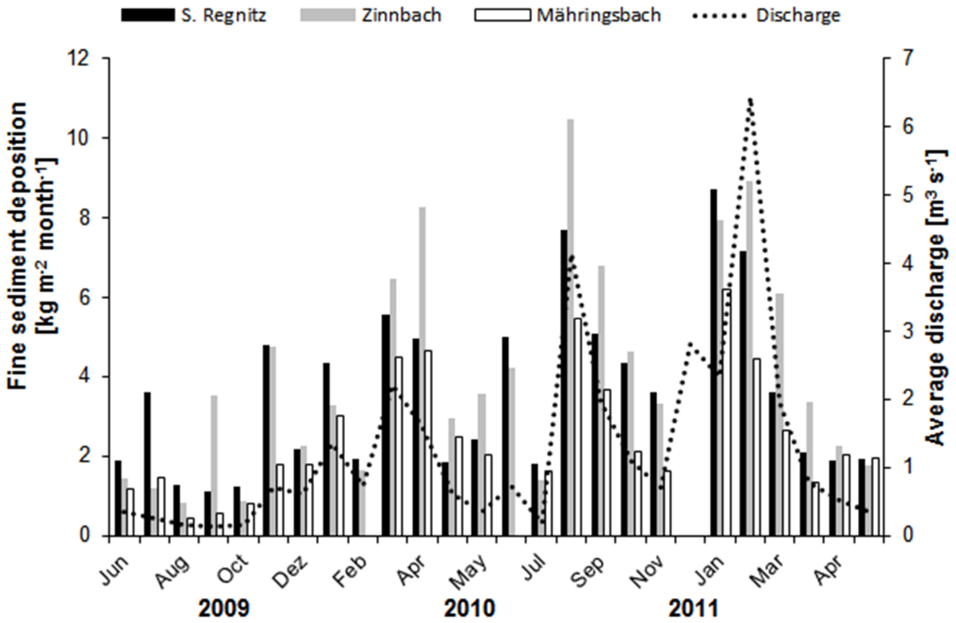


Figure 2.3: Fine sediment deposition (columns) and discharge of the Suedliche Regnitz (dotted line) from June 2009-May 2011.

Table 2.1: Mean and standard deviations, median and range of sediment deposition rates [kg m^{-2} $month^{-1}$] of different particle size fractions in the study streams.

Stream	Fraction size	Mean ± SD	Median	Range
Suedliche Regnitz	> 6.3 mm	1.7 ± 3.3	0.6	< 0.01 - 29.2
	6.3 - > 2.0 mm	2.6 ± 4.1	0.6	< 0.01 - 26.0
	2.0 - 0.85 mm	1.5 ± 2.1	0.4	< 0.01 - 12.6
	< 0.85 mm	3.2 ± 2.7	2.4	< 0.01 - 17.6
	Total	9.0 ± 9.6	4.0	< 0.01 - 85.4
Zinnbach	> 6.3 mm	1.5 ± 2.0	0.7	< 0.01 - 12.5
	6.3 - > 2.0 mm	2.5 ± 4.4	0.6	< 0.01 - 27.3
	2.0 - 0.85 mm	1.5 ± 2.3	0.4	< 0.01 - 18.8
	< 0.85 mm	4.1 ± 3.2	3.0	0.5 - 20.3
	Total	9.6 ± 10.2	4.7	0.5 – 78.9
Maehringsbach	> 6.3 mm	0.5 ± 0.7	0.3	< 0.01 - 2.8
	6.3 - > 2.0 mm	0.8 ± 1.4	0.2	< 0.01 - 8.6
	2.0 - 0.85 mm	0.4 ± 0.7	0.1	< 0.01 - 3.5
	< 0.85 mm	2.6 ± 1.7	2.1	0.3 - 7.9
	Total	4.3 ± 3.3	2.7	0.3 – 22.8

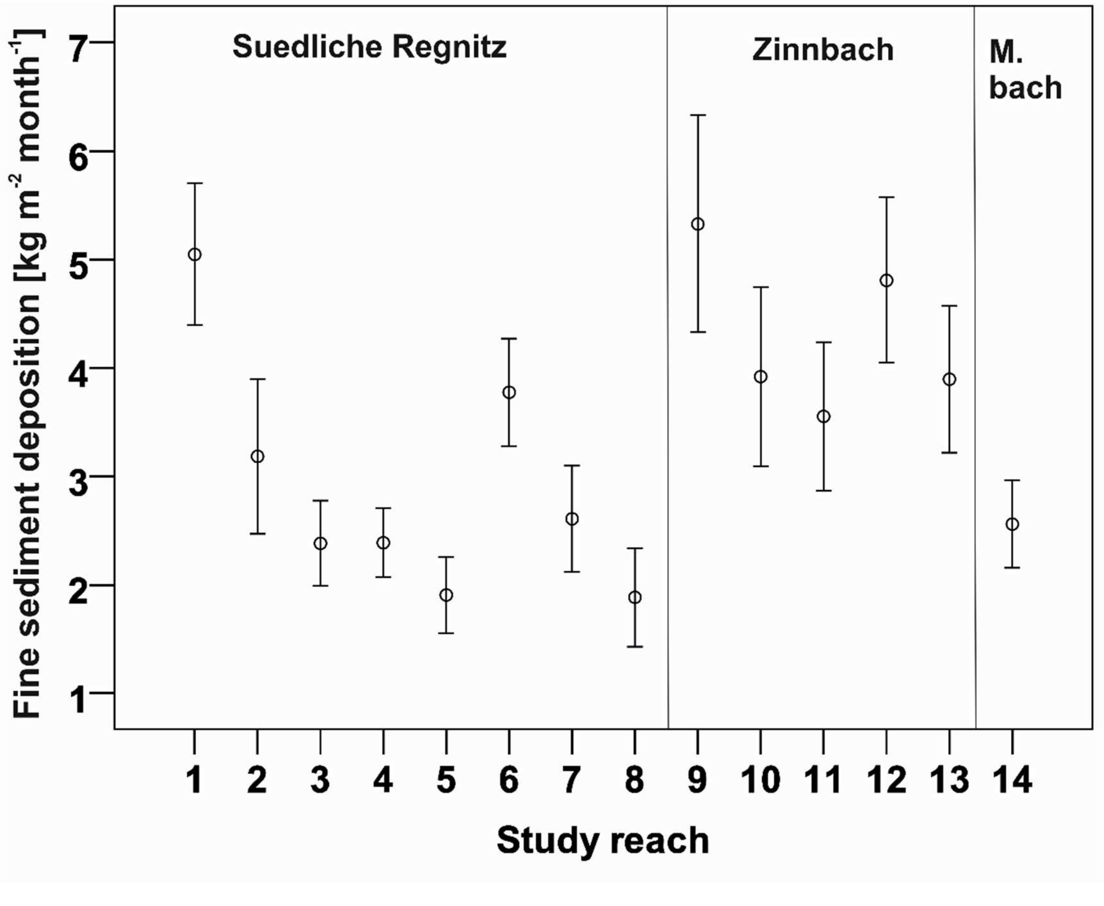


Figure 2.4: Mean fine sediment deposition kg m^{-2} $month^{-1}$ and standard errors at study reaches. Vertical lines separate study reaches in different study streams. M.bach is the abbreviation for Maehringsbach.

Mean redox potentials were 511 mV in free-flowing water, 420 mV in 5 cm and 346 mV in 10 cm substratum depth. The values were highly variable among streams, study reaches and seasons covering a range of 500 mV (Table 2.2). Maximum values occurred during colder seasons and after periods with elevated discharge. Lowest redox potentials in the interstitial zone and most pronounced differences between free-flowing water and interstitial water were found during periods of low flow in summer 2009 and May 2011 (Figure 2.5), when substratum conditions were stable. In association with elevated water temperatures leading to reduced oxygen solubility, maximal biological activity and respiration processes, redox potentials dropped even below 300 mV at certain study reaches, indicating anoxic conditions (Schlesinger 1991). Mean deltas of redox potentials between free-flowing water and the interstitial zone in 5 cm depth were 68 mV, 78 mV and 127 mV in the Maehringsbach, the Suedliche Regnitz and the Zinnbach, respectively. Deltas of redox potential were significantly different between the Maehringsbach and the Zinnbach (Tamhane-T2 test, p = 0.001) as well as between the Suedliche Regnitz and the Zinnbach (Tamhane-T2 test, p = 0.001), but not between the Maehringsbach and the Suedliche Regnitz (Tamhane-T2 test, p = 0.984).

Table 2.2: Mean and standard deviations, median and range of physicochemical parameters in the study streams. FW represents free-flowing water, 5 cm and 10 cm represent investigated substratum depths.

Stream	Parameter	Mean ± SD	Median	Range
Suedliche	Redox FW [mV]	504 ± 32	508	322 - 609
	Redox 5 cm [mV]	427 ± 85	449	142 - 650
	Redox 10 cm [mV]	360 ± 103	378	34 - 542
	Flow velocity [m s^{-1}]	0.55 ± 0.40	0.46	0.01 - 2.50
	Water depth [cm]	38 ± 22	33	3 - 110
	Penetration resistance [kg cm^{-2}]	1.74 ± 0.75	1.79	0.01 - 3.92
Zinnbach	Redox FW [mV]	521 ± 28	520	387 - 587
	Redox 5 cm [mV]	394 ± 90	411	182 - 600
	Redox 10 cm [mV]	303 ± 89	292	62 - 508
	Flow velocity [m s^{-1}]	0.28 ± 0.21	0.24	0.01 - 0.98
	Water depth [cm]	23 ± 10	22	5 - 51
	Penetration resistance [kg cm^{-2}]	1.48 ± 0.79	1.46	0.01 - 3.92
Maehringsbach	Redox FW [mV]	534 ± 29	530	462 - 594
	Redox 5 cm [mV]	462 ± 83	482	235 - 613
	Redox 10 cm [mV]	396 ± 92	396	195 - 567
	Flow velocity [m s^{-1}]	0.32 ± 0.19	0.34	0.01 - 0.66
	Water depth [cm]	14 ± 5	13	7 - 24
	Penetration resistance [kg cm^{-2}]	1.37 ± 0.68	1.31	0.36 - 3.36

Flow velocity ranged between 0.01 m s^{-1} and 2.50 m s^{-1}. Water depth ranged between 3 cm and 110 cm (Table 2.2). The variation was highest in the Suedliche Regnitz, whereas conditions were more uniform in the Zinnbach and the Maehringsbach. Penetration resistance exhibited the same pattern ranging from 0.01 kg cm^{-2} to 3.92 kg cm^{-2}. The study reaches with high flow velocities tended to exhibit high penetration resistances due to very coarse substratum.

Table 2.3: Extracted components of principal component analysis (PCA) and percentage of variation in the dataset, which is explained by these principal components.

Component	Extraction sums of squared loadings		
	Total	% of Variance	Cumulative %
1	3.605	32.777	32.777
2	1.993	18.120	50.807
3	1.301	11.827	62.724
4	1.076	09.782	72.506

Table 2.4: Rotated component matrix of principal component analysis (PCA) with contributions of single parameters to the extracted principal components.

	Component			
	1	2	3	4
Deposition of particles > 6.3 mm	0.716	-0.021	0.086	0.111
Deposition of particles 6.3 mm - > 2.0 mm	0.876	-0.037	-0.029	-0.045
Deposition of particles 2.0 mm – 0.85 mm	0.878	-0.070	-0.036	-0.129
Fine sediment deposition < 0.85 mm	0.777	0.097	-0.139	-0.133
Discharge	0.945	0.013	-0.069	0.015
Redox potential free-flowing water	0.200	0.257	-0.691	-0.185
Redox potential 5 cm substratum depth	0.051	0.886	-0.143	-0.034
Redox potential 10 cm substratum depth	0.003	0.874	-0.038	-0.072
Flow velocity	-0.059	0.559	0.284	0.164
Water depth	0.089	0.221	0.830	-0.162
Penetration resistance	-0.054	0.024	-0.001	0.966

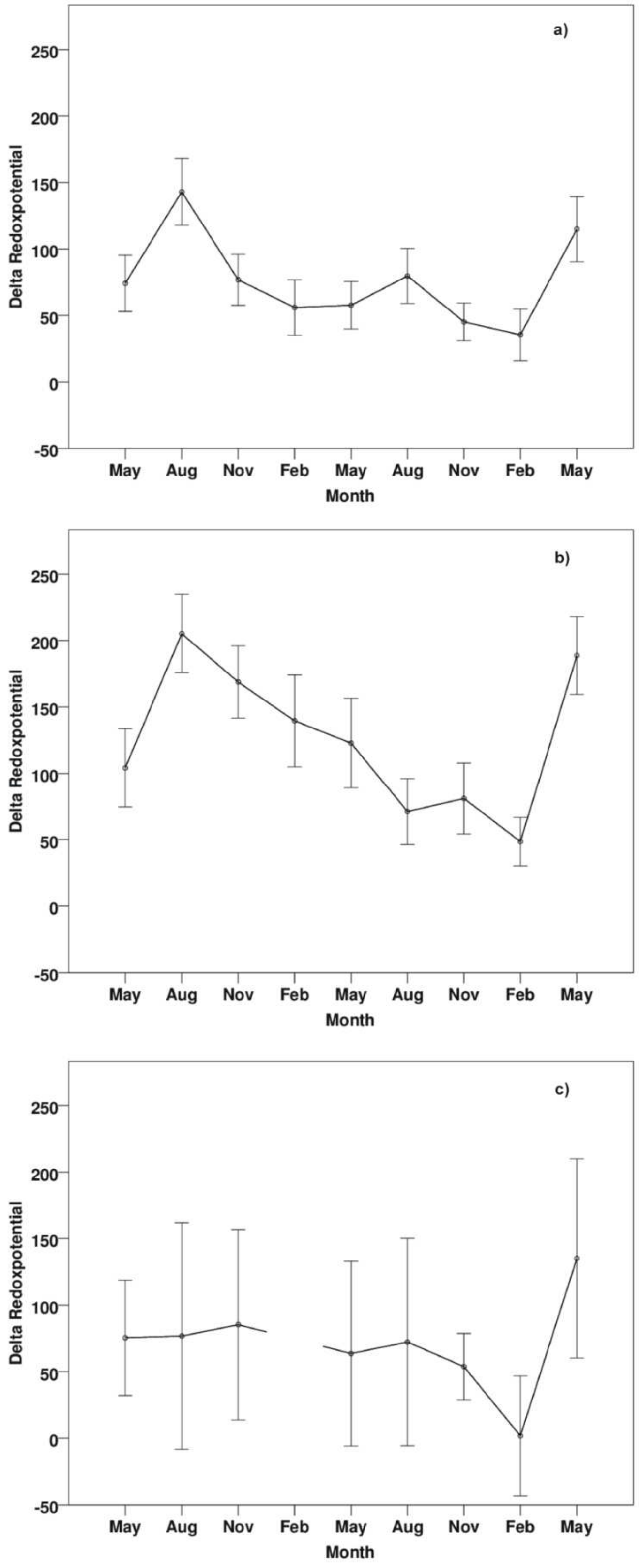


Figure 2.5: Average deltas of redox potentials between free-flowing water and 5 cm substratum depth from May 2009-May 2011 in a) the Suedliche Regnitz (n = 486), b) the Zinnbach (n = 216) and c) the Maehringsbach (n = 54).

Multivariate analyses

Nonmetric Multidimensional Scaling (NMDS) showed that the study reaches 10-13 in the Zinnbach were similar to each other (mean Euclidean distance 0.60). The Zinnbach was clearly separated from the two other streams with mean Euclidean distances of 2.52 to the Maehringsbach and 2.02 to the Suedliche Regnitz. The reaches 1-9 in the Suedliche Regnitz covered a wide range of conditions. The distances among the Suedliche Regnitz reaches were larger than between the Suedliche Regnitz and the Maehringsbach reaches with 1.77 compared to 1.57 (Figure 2.6). Principal component analysis (PCA) revealed sediment deposition and discharge as the main factors for differences in study reach conditions accounting for 30% of the variation. An additional 18% was explained by redox potentials in the interstitial zone and flow velocity (Tables 2.3 and 2.4).

Linear regression analysis revealed that discharge was the factor with the strongest influence on fine sediment deposition rates explaining 53% of the variance ($r = 0.730$; $r^2 = 0.532$; $p = 0.003$). The analysis of the delta values of discharge and fine sediment deposition indicated that high discharge events (with peak flows of Q by 3.0 - 3.5 $m^3 s^{-1}$ higher than average, i.e. 5-10 times greater than the mean discharge MQ) may result in slightly decreased net sediment deposition.

In degraded streams, low fine sediment deposition and high redox potentials in the interstitial zone were restricted to reaches with high flow velocities and substratum mobility resulting in a very coarse substratum texture, e.g. reaches 7 and 8 (fine sediment deposition: 2.6 and 1.9 kg m^{-2} $month^{-1}$; redox potentials at 5 cm: 478 and 480 mV; flow velocity: 0.86 and 0.82 m s^{-1}). At the reference reach in the Maehringsbach, similar conditions (fine sediment deposition: 2.6 kg m^{-2} $month^{-1}$; redox potential at 5 cm: 462 mV) were found, but at significantly lower flow velocities of only 0.32 m s^{-1} ($p < 0.001$, Tamhane-T2 test), suggesting reduced sediment influx rates from the catchment (see also Figure 6). Study reaches with comparable flow velocities in the Suedliche Regnitz or the Zinnbach were at least partially characterized by reduced habitat quality with low redox potentials in the interstitial zone such as reach 4 with 332 mV, high fine sediment deposition such as reach 1 with 5.0 kg m^{-2} $month^{-1}$ or both such as reach 10 with 383 mV and 3.9 kg m^{-2} $month^{-1}$.

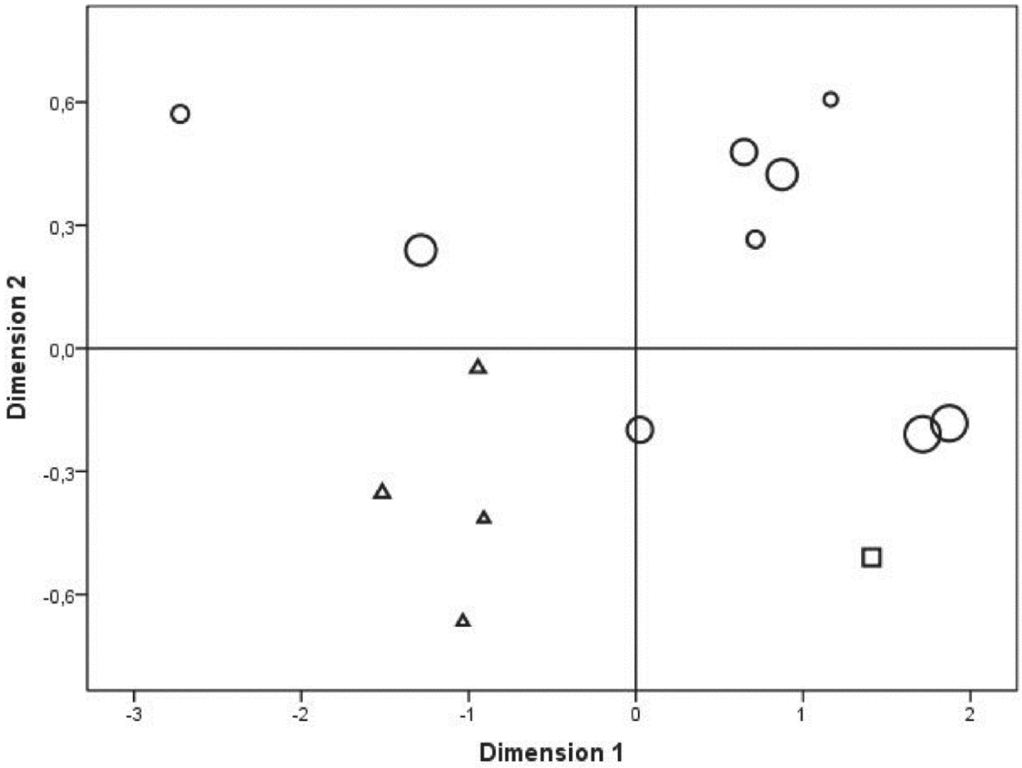


Figure 2.6: Similarity of study reaches in the Suedliche Regnitz (circles), the Zinnbach (triangles) and the Maehringsbach (square) expressed by NMDS analysis. Dot size indicates flow velocity at the study reaches.

2.5 Discussion

In this study, quantitative data on sediment deposition, physicochemical habitat quality and their spatio-temporal variability in pearl mussel streams are presented with a special focus on fine sediment deposition. Fine sediment deposition and variability in physicochemical habitat quality were generally high in streams with catchments subject to increased soil erosion. Yet, the interpretation of these parameters is scale-dependent, with clear spatio-temporal differences occurring from the stream to the study site scale, as hydrologic conditions can significantly shape local conditions (Seydell et al. 2009; Sternecker et al. 2013a).

Temporal variations of sediment deposition were found to be strongly linked to discharge variations but independent of season. The correlation to discharge is supported by previous findings (Acornley & Sear 1999; Zimmermann & Lapointe 2005), whereas the independence of sediment deposition and season contradicts the general assumption that vegetation cover during summer prevents high surface runoffs in temperate catchment areas (Herringshaw et al. 2011). The highest sediment deposition rates occurred during high flows in August 2010, when vegetation cover is at its peak. This indicates that land-use rather than season controls sediment erosion and transport in the catchment. Agricultural fields are more susceptible to erosion than forest or grassland areas due to reduced ground cover (Allan 2004; Wasson et al. 2010). In a study of Collins & Walling (2007), between 81 and 85% of deposited fine sediments were eroded from cultivated land and pasture areas. These results are also confirmed in our study with generally lower fine sediment deposition (2.6 kg m^{-2} $month^{-1}$) in the Maehringsbach, where over 90% of the catchment consists of forest and grassland. Though forests are still dominated by spruce (Picea abies), forest modification to mixed woodland has occurred in recent years and especially areas adjacent to the stream are already dominated by deciduous trees. The grassland mainly consists of hay meadows and wet meadows and is free of pasture. In comparison, fine sediment deposition was about 1.5 times higher in the Suedliche Regnitz (3.2 kg m^{-2} $month^{-1}$) and the Zinnbach (4.1 kg m^{-2} $month^{-1}$) where forest and grassland only cover 40-50% of the catchment area, respectively. The rest of the catchment is dominated (> 40%) by arable land. The main crops are different cereals including wheat, barley and maize. Catchment topography and soil structure also influence the intensity of erosion with steep slopes and loose soils enhancing soil loss (Wood & Armitage, 1997). Slopes, especially in the Suedliche Regnitz catchment, are steeper than in the Maehringsbach catchment.

Elevated fine sediment deposition influenced physicochemical conditions in the interstitial zone but became apparent only after a time lag. In particular, this meant high water flows not only resulted in elevated fine sediment delivery but also in movement of coarser matrix particles, therefore allowing high exchange rates between free-flowing water and upper substratum layers. During low flow times interstitial pores were clogged by fine sediments, which induces a reduction of exchange rates between the free-flowing water and the interstitial zone (Arntzen et al. 2006; Rehg et al. 2005), resulting in reduced oxygen supply to the interstitium. Especially in summer, when temperatures and biological activity are high, this can result in severe oxygen depletion posing a major threat for highly sensitive and stationary species such as *Margaritifera margaritifera*, which depend on stable substrata with a well oxygenated interstitial zone for several years (Geist & Auerswald 2007; Greig et al. 2005; Hastie et al. 2003; Larsen et al. 2011). The only study reach where redox potentials in the interstitial zone were high during the complete study period without elevated flow was the reference in the Maehringsbach, where juvenile pearl mussels still occur. These results may also give a suitable explanation for some aspects of the millrace phenomenon. It refers to the observation that populations have managed to persist in mill channels, even better than in connected streams, which is probably due to low fine sediment supply and stable flow conditions. In the past, the regular maintenance of silted channel stretches may even have provided suitable substrates for juvenile recruitment. Today, many mills are abandoned and channels are no longer maintained, causing recruitment failure as in silted natural streams. Yet, mussels moved by elevated flow may be washed into millraces and be able to persist in these constant flow habitats. In many cases, mussels reveal reduced growth rates in these areas, which can be erroneously interpreted as juveniles.

In addition to the pearl mussel, brown trout and other salmonid populations are impacted by fine sediments as well (Ingendahl 2001; Julien & Bergeron 2006; Kemp et al. 2011; Rubin 1998; Sternecker et al. 2013 a, b; Sternecker & Geist 2010), yet not as strongly as the pearl mussel due to life cycle differences. The time spent by eggs and juveniles of *S. trutta* in the interstitial zone is much shorter and does not exceed six months. Moreover adult fish actively choose suitable spawning grounds and remove fine sediments from gravel banks through redd digging activities (Crisp & Carling 1989; Nika et al. 2011). Additionally, spawning periods of most species are in colder seasons, where biological activity is lower and oxygen solubility in the water is higher.

Conclusions and recommendations for management and monitoring

This is to our knowledge the first study providing quantitative, continuous data on sediment deposition and basic habitat parameters in streams over a longer period of time (2 years). The data on fine sediment deposition in this study and in the literature indicate that the reduction of fine sediment deposition to natural levels is the key to preserve or restore functional habitats for the pearl mussel (Altmueller & Dettmer 2006; Moorkens 2010). However, these references provide evidence that the reduction of fine sediment deposition is a time consuming task, no matter if sediments are trapped on their pathway to the river (Altmueller & Dettmer 2006) or catchments are managed and landuse is extensified (Moorkens 2010). Consequently, captive breeding of juvenile pearl mussels, which has been increasingly implemented in the last few years, is necessary to preserve the many overaged populations until their habitats are restored (Geist 2010; Gum et al. 2011).

Management and restoration efforts generally have to comprise three major steps, which are i) analysis of ecological demands of target species and habitat deficits, ii) identification and implementation of the most effective restoration measures, and iii) monitoring the effects of implemented measures. Furthermore, the results in this study underline that specific sampling designs which consider the spatio-temporal heterogeneity of river habitats are necessary for the provision of valuable and accurate data, as was also suggested by Bolland et al. (2010) and Braun et al. (2012). For the first step, this means that an assessment of sediment deposition rates has to be integrated over an extended period of time (at least half a year) and several study reaches. It also needs to comprise different flow conditions for obtaining meaningful results. In addition, the development of new methods for fine sediments and detritus analyses, e.g. for “sediment fingerprinting” is important for a more exact determination of the origin of sediments (Walling et al. 2003), the suitability of its organic compounds as nutrients for mussels and effective reduction of erosion.

In contrast, analysis of physicochemical habitat quality can often be reduced to shorter time frames as long as it is ensured that worst case conditions are covered. In case of the pearl mussel, the recommended period is during (summer) low flow, as juveniles depend on constant oxygen supply all year round. Yet, other species with deviating life cycles may require differing sampling schemes, such as salmonid species, which only spend a comparably short period in the interstitial zone. Consequently, in case of the pearl mussel's host fish, the brown trout, investigations have to be carried out during the egg incubation period in autumn and winter.

Restoration measures can be divided into immediate, short-term efficient measures such as gravel addition or cleaning and long-term measures such as land-use modification and extensification, which should be implemented considering their practicability and utility. For instance, assuming that 1 m^3 of round gravel of 16-32 mm diameter with a pore volume of about 35% is added as a 10 cm thick layer to study reach 9 and study reach 14, the timespan until interstitial pores are clogged with fine sediments widely differs. At study reach 9 with a median deposition of 4.9 kg m^{-2} $month^{-1}$, a complete filling of interstitial voids will occur after 14 months and at study reach 14 with a median deposition of 2.1 kg m^{-2} $month^{-1}$ after 33 months. These numbers illustrate that instream restoration will often be ineffective and unsustainable for enhancement of freshwater pearl mussel habitats due to the species' life cycle. Yet, the method appears suitable to quickly improve spawning ground conditions for brown trout (Meyer et al. 2008), but has to be repeated regularly to keep habitat quality in a suitable range.

For long-term improvement of habitat quality in running waters degraded by increased sediment deposition, the only appropriate solution is integrative catchment management. This often comprises mitigation of land-use, which can have various positive effects. Where possible, alluvial forests and wetlands should be restored as they are known for flood protection due to prolonged water retention (Hümann et al. 2011; Reinhardt et al. 2011; Wahren et al. 2007). Moreover, they act as silt and nutrient traps, thus protecting rivers from surface runoffs in intensively used areas (Braskerud 2001; Braskerud 2002). Also, benefits for terrestrial and semiaquatic flora and fauna are widely recognized, elevating the recreational value for humans as well (Lasne et al. 2007; Tockner et al. 2006; Ward et al. 1999). If land-use modifications cannot be achieved, other solutions like sediment traps or buffer strips are possible alternatives with their suitability depending on catchment properties.

After an implementation of restoration measures, the third major step is monitoring the effects, which is oftentimes neglected. However, it has to be stressed that monitoring the impacts of restoration efforts is a necessary control whether the project aims were achieved and provides valuable information for the planning of future projects. The use of the same methods as during the monitoring of initial conditions is obligatory to ensure comparability of results.

3 Habitat suitability analysis for lacustrine brown trout (*Salmo trutta*) in Lake Walchensee, Germany: Implications for the conservation of an endangered flagship species

A similar version of this chapter was published: Denic M & Geist J (2010). Habitat suitability analysis for lacustrine brown trout (*Salmo trutta*) in Lake Walchensee, Germany: Implications for the conservation of an endangered flagship species. Aquatic Conservation: Marine and Freshwater Ecosystems 20: 9-17.

3.1 Abstract

The lacustrine brown trout (*Salmo trutta*) is endangered and of high conservation relevance. In the only spawning habitat of the population in the Bavarian Lake Walchensee, the river Obernach, a substantial decrease in spawning runs has been reported. In this study, the present ecological state of the spawning stream was analysed with the objective of identifying life-stage specific limitations to successful recruitment of lacustrine brown trout attributable to deficiencies in (i) spawning migration, (ii) spawning habitat quality, and (iii) habitat quality for juveniles. Structural stream analysis showed that discharge and several migration barriers – particularly near the river outlet into the lake – prevent successful spawning migrations at regular water levels. Migration barriers are likely the main limiting factor for reproduction of lacustrine brown trout, whereas structural variability of the Obernach meets the habitat requirements of both spawners and juveniles. Spawning site quality was suitable for trout, as indicated by stream substratum texture and high exchange rates between free-flowing water and interstitial zone in physico-chemical parameters (redox potential, dissolved oxygen, pH, temperature and conductivity). Analyses of fish community structure revealed dominance of lithophilic species, in particular of riverine brown trout (*Salmo trutta*). Its density and intact demographic population structure suggest that spawning and juvenile habitat quality for salmonids is not limiting. Recapture of stocked lacustrine trout juveniles also indicates habitat suitability for the juvenile stage. In conclusion, the results show that the methodology used in this study is suitable for the identification of life-stage specific habitat deficiencies in lacustrine brown trout and other fish species. Availability of habitat data throughout the species´ distribution range is a first crucial step for the development of an effective recovery plan.

3.2 Introduction

The polytypic species *Salmo trutta* was formerly described to consist of the three subspecies *Salmo trutta fario*, *Salmo trutta lacustris*, and *Salmo trutta trutta* (Behnke 1972; Elliott 1994; Muus & Dahlström 1981). These are currently considered varieties, forms or ecophenotypes (Kottelat & Freyhof 2007) and will therefore be referred to as riverine brown trout, lacustrine brown trout and sea trout in this paper. Lake Walchensee in Bavaria, Germany, is regarded as an important habitat for the lacustrine brown trout (Taller 2007), but its population is highly endangered at present. As no natural reproduction has been recorded for several years, the survival of the population has been maintained exclusively by stocking measures (Böhm pers.). In recent years, the decline of the lacustrine brown trout has even posed limitations to the sampling of sufficient numbers of spawners for artificial reproduction.

Throughout the whole distribution range, which covers most large and cool European lakes, particularly in the Alps, Scandinavia, northern England and Ireland (Lelek 1987), lacustrine brown trout face similar problems as in Lake Walchensee (Gosset et al. 2006; Rustadbakken et al. 2004; Schulz 1994). However, Lake Constance seems to be the only area, where intensive research on lacustrine brown trout and habitat restoration efforts have been carried out (Caviezel 2006; Mendez 2007; Rulé et al. 2005; Schulz 1994). Effective management of lacustrine brown trout is important, particularly when considering that it does not only play an important ecological role, but is also significant economically because of its fisheries impact. These features make lacustrine trout a popular symbol and leading element of conservation campaigns which match the concepts of ´flagship species´.

The lacustrine brown trout depends on migration between habitats to complete its life cycle. Adults spawn in streams, where the offspring spend their juvenile life-stage. After one or two years, they migrate to the lakes and return for spawning after two or three years. Migratory populations are generally vulnerable, as they are simultaneously threatened by negative influences in lakes, along their migration routes and in streams (Rulé et al. 2005). Due to their complex life-cycle and their demanding habitat requirements, such as spawning grounds with little fine sediment and good oxygen supply, many salmonid species are endangered (Acornley & Sear 1999; Julien & Bergeron 2006). As a consequence of intensive land use and high surface runoffs many rivers carry increased amounts of fine sediment (Soulsby et al. 2001b), which may lead to a reduced oxygen supply in the interstitial system and consequently to higher mortality rates among eggs and larvae (Crisp 1996; Greig et al.

2007a; Hendry et al. 2003). In addition, the return of adult fish to their spawning grounds is often impaired or prevented due to migration barriers (Gosset et al. 2006).

The objective of this study was to identify the reasons for the current lack of natural reproduction in lacustrine brown trout in Lake Walchensee and its former spawning tributary, the river Obernach. We tested three alternative hypotheses: (i) presence of structural deficiencies which prevent migration of spawners to their spawning sites in the Obernach, (ii) low interstitial habitat quality at spawning sites or (iii) unsuitable and missing habitat for the juvenile stage are the main limiting factors for successful recruitment of lacustrine brown trout. A structural stream analysis was carried out to examine the present state of the stream connectivity and habitat structure. Spawning habitat quality of the Obernach was analysed by investigating substratum texture and gradients between free-flowing water and the interstitial zone in redox potential, dissolved oxygen, pH, electrical conductivity and temperature. Analyses of fish community structure and demography were used to assess spawning success of salmonids and salmonid juvenile habitat quality. This study may serve as an example of the identification of life-stage specific key habitat deficiencies in lacustrine brown trout and for the conservation of other endangered salmonids. The methodology used in this study appears to be suitable for a comparative monitoring of habitats in other areas as well.

3.3 Material and Methods

Study site

Lake Walchensee and its only natural tributary, the Obernach River, are located in the Bavarian Limestone Alps, Germany, about 100 km south of Munich (Fig. 3.1). The river outlet is situated at 047° 34’ 14’ N, 011° 18’ 22’ E. The river is about 3.5 km long, has a width of 5 to 20 m and a depth ranging from a few centimetres to 2 m. The river source is a waterfall and a second tributary from the Lake Sachen merges in about 1 km downstream. Water discharge of the Obernach between 1977 and 2006 was on average 1.3 $m^3 s^{-1}$ but had a high variability (range between 0.1 and 25 $m^3 s^{-1}$) due to highly variable water withdrawals for a hydropower station. From the source to the river outlet, a gradient in land use can be observed. In the headwater area the river catchment is dominated by forest. Further downstream, the catchment of the river is mainly used for cattle grazing.

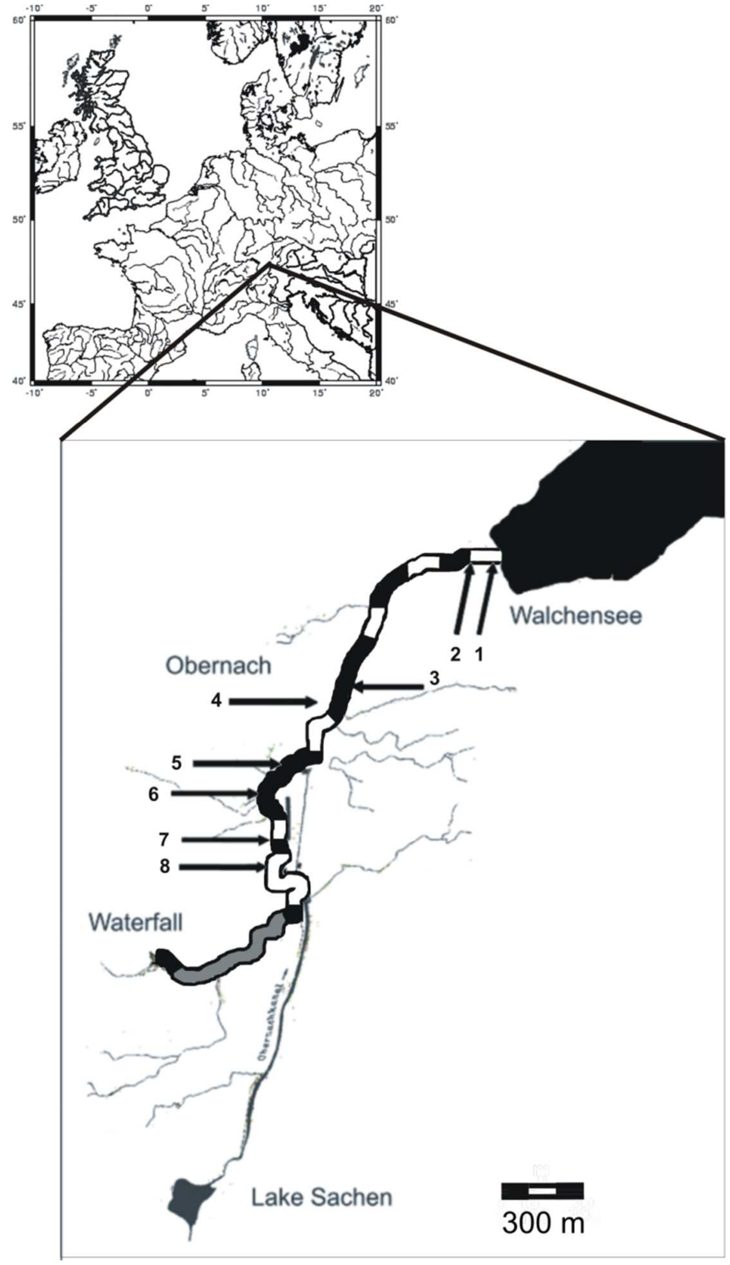


Figure 3.1: Map of the study area, arrows 1-8 indicate positions of migration barriers, colours refer to the results of the structural stream analysis (grey, black and white refer to structural class 2, 3, 4 respectively).

Habitat analysis

Habitat analysis was divided into three categories in order to identify whether poor stream connectivity, degradation of spawning and juvenile habitat, or a combination of these factors is most limiting for the lack of natural reproduction in lacustrine brown trout of Lake Walchensee.

A structural stream analysis of the Obernach was carried out according to the German standardized method for the assessment of structural integrity of streams and rivers (LFU 2002) with the main objective of assessing habitat structure for spawners and juveniles. Briefly, this model combines the evaluations of 21 single habitat factors (including flow characteristics and structural variability into one value for the assessment of structural integrity of each section from 1 (natural) to 7 (highly modified)). Habitat factors included variability in water depth, stream width, substratum diversity, diversity of flow velocities, diversity of bank vegetation, introduction of lateral and longitudinal obstacles and weirs, as well as other anthropogenic habitat alterations and adjacent landuse. In a first step, the Obernach was subdivided into 31 sections with equal length of 100 m each. Within these sections, habitat quality was assessed based on the LFU criteria (LFU 2002).

Based on the information from the structural stream analysis, six potential spawning areas have been selected for an assessment of spawning habitat quality. Since salmonid hatching success and stream bed functionality were shown to strongly depend on substratum composition (Rubin, 1998), oxygen supply (Greig et al. 2005) and on the exchange rates between free flowing water and interstitial zone (Geist & Auerswald 2007; Pander et al. 2009; Rubin & Glimsäter 1996), we analysed texture and gradients in physico-chemical variables (redox potential, dissolved oxygen, pH, electrical conductivity, temperature) between free-flowing water and the interstitial zone. At each potential spawning ground, a substratum sample of approximately 10 kg dry weight from the upper 10 cm of the stream bed was sampled with a gravel sledge. In the laboratory the samples were wet-sieved with a sieving machine (EML 400, Haver and Boecker, Germany) to separate the different grain sizes (mesh width: 20 mm, 6.3 mm, 2.0 mm, 0.85 mm). Representative aliquots of these fractions were dried at 100 °C for 24 h for determination of dry weight. Texture lines and the mean geometric diameter d_g were determined according to Sinowski & Auerswald (1999). The largest fraction was excluded from further analysis because of the restricted sample volumes. Differences in physico-chemical variables (redox potential, dissolved oxygen, pH, electrical conductivity and temperature) between free-flowing water and at 5 cm and 10 cm substratum depth were investigated at potential spawning sites, as they have a critical influence on the development of eggs and larvae. The investigations were carried out according to Geist & Auerswald (2007) at two representative timepoints during trout spawning and egg incubation periods (24/10/2007 and 21/11/2007). Conductivity and redox potential were investigated only at the second date.

Fish community structure and stocking experiment

Electro fishing surveys at the Obernach were carried out to record the present fish community in the study area as it is a strong indicator of habitat quality. Two representative wading electro fishings (25/09/2007 and 21/01/2008) were conducted from the river mouth upstream to the fifth barrier (Fig. 3.1), which represents about 50% of the total stream length. During the first survey, a stationary generator (FEG 5000, EFKO, Germany, 5 kW, D.C., not pulsed) was used to generate the electric field. Due to the low water level a backpack generator was used in the second survey (FEG 3000, EFKO, Germany, 3 kW, D.C., not pulsed). Fish were removed from the electric field as quickly as possible with a landing net. Caught fish were identified, weighed (± 1 g) and measured (± 1 cm). Different trout forms were discriminated according to body shape, size and colour (Muus & Dahlström 1981). The distinctive red spots of riverine trout and the larger x-shaped black spots of lacustrine brown trout were used as main criteria for discrimination between the two forms. Reliability of this discrimination was supported by the fact that all of the elastomer-tagged lacustrine trout were correctly classified three months after a stocking experiment. Total numbers of individuals per species, and total biomass per species (kg) were calculated and related to a catch per unit effort (CPUE) of individuals and biomass per ha water surface (at average annual discharge) and per 100 m stream length. Demographic structure and recruitment of salmonids were assessed by computing length frequency distribution diagrams.

A stocking experiment with 500, one year old lacustrine brown trout was carried out in order to assess the Obernach's quality as a juvenile habitat for the target species. The fish, obtained from a local fish hatchery, were marked by elastomer-tagging (Northwest Marine Technology, Seattle, WA, USA) behind the left eye on 14/11/2007. Before their release into the Obernach all fish were kept in a tank for one week to recover from the marking procedure. On 20/11/2007 the fish were distributed over the whole stream in ten groups of 50 individuals. The distance between release sites varied between 200 and 400 meters. Three months after stocking, an electro fishing survey was carried out to check the survival and physical condition of the stocked fish.

Statistical analysis

ONEWAY ANOVA was used to test for significant differences between mean values in the free flowing water, at 5 and 10 cm substratum depth of redox potential, pH, dissolved oxygen, electrical conductivity and temperature if the data showed normal distribution and homogeneity of variances. The Duncan test was used as a post-hoc test.

If prerequisites of normal distribution and equality of variances were not fulfilled, nonparametric KW-ANOVA and Mann-Whitney U-tests were carried out. All statistical analyses were done with SPSS 15.0.

3.4 Results

Habitat structure and stream connectivity

From the 31 stream sections evaluated in the structural stream analysis, six were assessed grade 2 (slightly changed), fifteen grade 3 (moderately changed) and ten grade 4 (clearly changed). All sections assessed grade two were exclusively located in the headwaters of the stream (Fig. 3.1). Structural variability and stream substratum diversity were generally high in all sections, indicating good conditions for both spawning adults and juvenile fish. In contrast, embankment and the use of the riparian area were often evaluated negatively.

The stream connectivity of the Obernach was found to depend on two major factors, obstacles and water level. The most downstream first obstacle (obstacle numbers according to Fig. 3.1) is located at the Obernach outlet into Lake Walchensee, but remains below the water surface as long as the lake's water level stays above 8 m at the gauging station of Lake Walchensee. Due to the regular lowering of the lake's water level in autumn and winter, the obstacle is usually the first migration barrier for spawners of lacustrine brown trout trying to ascend the Obernach for spawning. The second obstacle is situated 100 m upstream and designed as a rockfill ramp. The third one located about 1 km further upstream could be passed by bigger fish as it is comparatively low (about 40 cm above the surface) at periods of average water flow. Fifty metres upstream from this point, there is an insurmountable barrier with a height of 1.5 m. Directly below the barrier, horiontally fixed wooden piles (´Floßfedern´) with a length of 4-5 m are installed, preventing successful running jumps of fish. The main purpose of the ´Floßfedern´ is to protect the barriers from erosion of substratum directly below the dams. At distances of 300 to 500 m upstream four more barriers follow which are constructed the same way as obstacle number four and consequently function as effective migration barriers for aquatic organisms.

In addition, the highly variable water levels over the year, caused by the dependence of water discharge on precipitation and water demand of the adjacent hydropower station, crucially influence the connectivity and habitat availability. Water demand of the hydropower station often leads to unnaturally low discharge, resulting in sections of several hundred meters drying out. This is especially problematic during the spawning season of lacustrine brown trout.

Physico-chemical parameters

The substratum at potential spawning sites was dominated by gravel with a diameter of at least 6.3 mm and a mean geometric diameter d_g of 7.6 mm. The percentage of fine sediment (< 2.0 mm in diameter) was low (less than 10% of total mass) in all samples. Except for two samples, fine sediments contributed less than 2% to the total sample mass (Fig. 3.2).

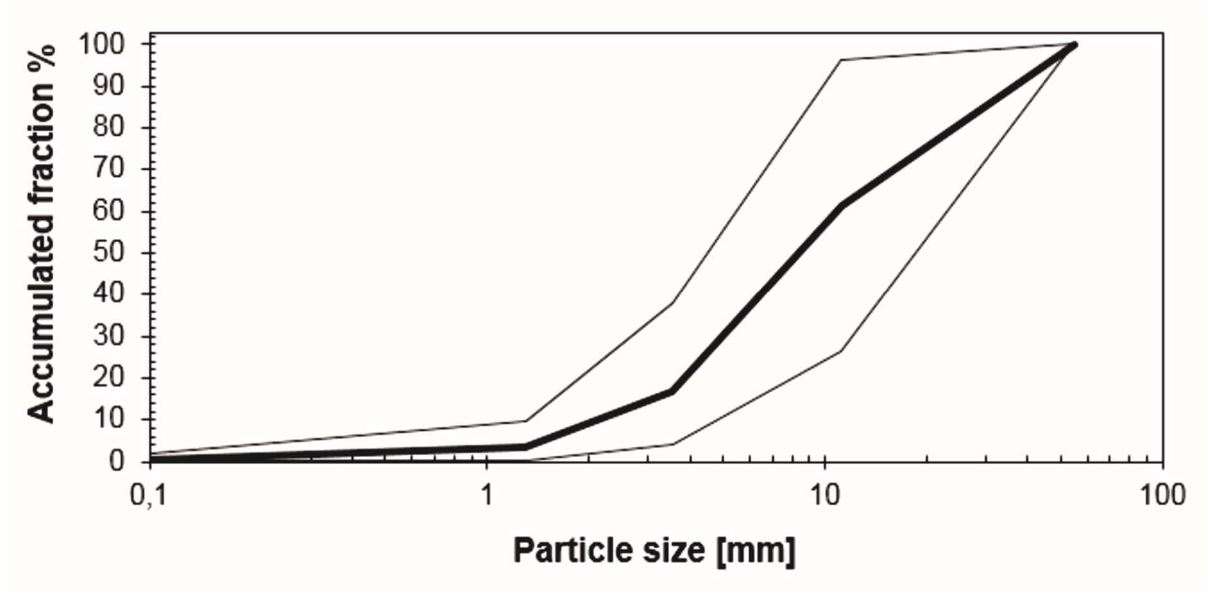


Figure 3.2: Texture of the stream bed at potential spawning sites (n = 6); thick line indicates arithmetic mean; thin lines represent minimum and maximum values.

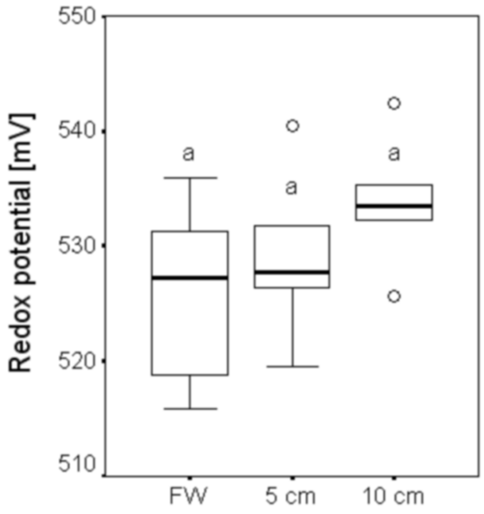


Figure 3.3: Box-Whisker plots (Whisker: 0.05 and 0.95 percentiles; Box: lower quartile, median and upper quartile; circles: outliers) of redox potential at potential spawning sites in the free flowing water (FW, n = 18) and at 5 cm (n = 18) and 10 cm substratum depth (n = 18); no significant difference at $p = 0.05$ was detected between depth levels.

Redox potential was high in the free flowing water with a mean of 526 mV and a standard deviation (SD) of 8 mV as well as in the substratum at 5 cm (529 mV; SD = 7 mV) and at 10 cm (534 mV; SD = 5 mV), indicating high exchange rates between the interstitial zone and free flowing water (Fig. 3.3). Differences between mean values at different depths were not significant.

Measured oxygen concentration supported the findings of the redox potential measurements, as all results except one single value were above the minimum concentration of 7 mg L^{-1} required by salmonids (Turnpenny

& Williams 1980; Peterson & Quinn 1996a). On 24 October the mean oxygen content was 10.8 mg L^{-1} (SD = 0.6 mg L^{-1}) in the free flowing water, 8.8 mg L^{-1} (SD = 1.4 mg L^{-1}) at 5 cm and 8.7 mg L^{-1} (SD = 1.4 mg L^{-1}) at 10 cm substratum depth. Due to the lower water temperature at the second measuring date, values were slightly higher in this case (Fig. 3.4).

Values of pH varied between 7.5 and 8.5 and were lower in the substratum than in the free flowing water. Differences were statistically significant ($p < 0.05$) between free flowing water and the interstitial zone at the first measuring date but not at the second one. Conductivity was constant in the free flowing water (300 µS cm^{-1}; SD = 5 µS cm^{-1}), but maximum values of 600 µS cm^{-1} were found in the interstitial zone. During the measurements in October water temperature varied between 7 and 9 °C and was about two degrees lower in November, ranging between 5-6 °C.

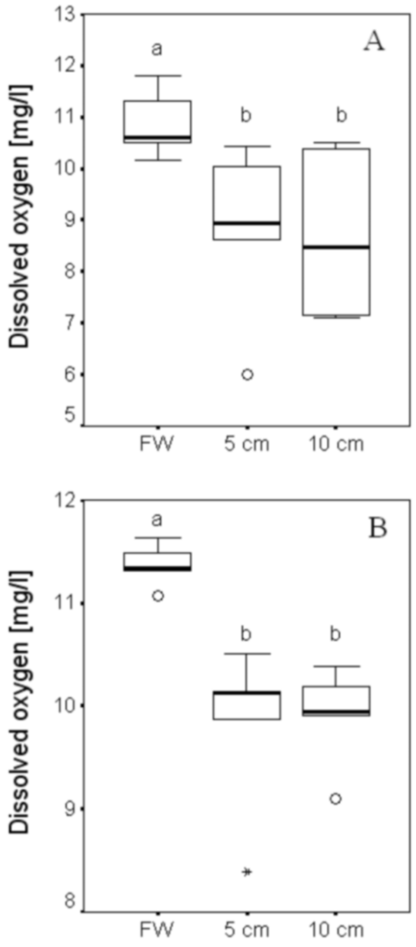


Figure 3.4: Box-Whisker plots (Whisker: 0.05 and 0.95 percentiles; Box: lower quartile, median and upper quartile; circles, star: outliers) of dissolved oxygen at potential spawning sites on 24/10/2007 (A, n = 18, 18, 18 respectively) and on 21/112007 (B, n = 18, 18, 18 respectively) in the free flowing water FW, at 5 cm and 10 cm substratum depth; different letters indicate significant differences at p < 0.05.

Fish community structure and stocking success

Six species were recorded during the electro-fishing surveys in the Obernach (Tab. 1 and 2): Riverine brown trout and lacustrine brown trout (*Salmo trutta*), bullhead (*Cottus gobio*), burbot (*Lota lota*), grayling (*Thymallus thymallus*), brook trout (*Salvelinus fontinalis*) and northern pike (*Esox lucius*). Only one mature lacustrine brown trout (70 cm, 6.100 g, male), trying to ascend the Obernach, was caught at the base of the most downstream weir. The species composition was dominated by riverine brown trout, representing 67% and 62% of the individuals and a CPUE of 30.7 kg ha^{-1} and 19.1 kg ha^{-1} on the two electro fishing dates, respectively. Bullhead showed the second highest abundance with 22% and 26% of all individuals, respectively. The biomass contribution of the other species only ranged between 0.8% and nearly 6%. The length frequency distribution of riverine brown trout showed that the majority of individuals were young-of-the-year. The total length of this age group slightly increased between the electro-fishing dates (Fig. 3.5). However, fish of all length classes up to a maximum size of 50 cm were detected. This population structure indicates a good natural reproduction as no stocking measures were carried out in the past few years. Out of the 500 specimens of the elastomer-tagged lacustrine brown trout juveniles, which had been stocked three months before the second electro fishing survey, thirteen were re-captured on 21/01/2008 (re-capture rate 3%). All of them were in good physical condition (no injuries, intact fins, well fed), indicating that the Obernach provides a suitable juvenile habitat.

Table 3.1: Number of individuals, biomass, catch per unit effort (CPUE) and abundance of each fish species caught during the first electro fishing survey on 25/09/2007.

fish species	**catch numbers**		**biomass**		**CPUE**			
	individuals	%	kg	%	kg ha^{-1}	kg 100 m^{-1}	ind ha^{-1}	ind 100 m^{-1}
***Salmo trutta* (riverine form)**	350	67.0	47.8	65.5	30.7	2.5	224	18
***S. trutta* (lacustrine form)**	2	0.4	8.7	11.9	5.6	0.4	1	0
Thymallus	31	6.0	8.2	11.2	5.3	0.4	20	2
Lota lota	16	3.1	5.5	7.4	3.5	0.3	10	1
Salvelinus	4	0.8	2.2	3.0	1.4	0.1	3	0
Cottus gobio	114	21.9	0.6	0.9	0.4	0	73	6
Esox lucius	4	0.8	0	0.1	0	0	3	0
Total	521	100	73.1	100	46.8	3.7	334	27

Table 3.2: Number of individuals, biomass, catch per unit effort (CPUE) and abundance of each fish species caught during the second electro fishing survey on 21/01/2008; * indicates elastomer-tagged lacustrine brown trout stocked on 20/11/2007.

fish species	**catch numbers**		**biomass**		**CPUE**			
	individuals	%	kg	%	kg ha^{-1}	kg 100 m^{-1}	ind ha^{-1}	ind 100 m^{-1}
***Salmo trutta* (riverine form)**	243	62.4	29.8	70.0	19.1	1.5	156	12
***S. trutta* (lacustrine form)**	7	1.8	3.2	7.6	2.1	0.2	5	0
S. trutta* (lacustrine form)	13	3.3	1.7	4.0	1.1	0.1	8	1
Thymallus	7	1.8	2.6	6.0	1.6	0.1	4	0
Lota lota	11	2.8	3.2	7.6	2.1	0.2	7	1
Salvelinus	7	1.8	1.6	3.6	1.0	0.1	4	0
Cottus gobio	102	26.1	0.5	1.2	0.3	0.0	65	5
total	391	100	42.5	100	27.2	2.2	251	20

3.5 Discussion

Suitability of the Obernach for spawning migration of lacustrine brown trout

In the Obernach, as well as in other habitats of lacustrine brown trout, spawning migrations are often prevented by impassable obstacles and occasional insufficient water discharge (Gosset et al. 2006; Rustadbakken et al. 2004). Spawning runs for lacustrine brown trout usually take place between October and December, when water level frequently drops in the Obernach as well as in Lake Walchensee, which is used as a water reservoir for the Lake Walchensee hydropower station. Depending on water supply rate, the lake's water-level may drop under the critical level of 8 m (gauging station Lake Walchensee) as early as late October. Thus, the structure at the Obernach outlet becomes an impassable migration barrier, where the conditions for successful running jumps are not met due to the lack of deep pools below the barrier (Stuart 1962). This is also true for the obstructions number 4, 5, 6, 7 and 8 (height varying between 0.5-2 m) where the 'Floßfedern' prevent the formation of deep pools. In addition, factors such as absolute height of the barrier, water temperature or fish size influence the surmountability of obstacles (Crisp 1996; Turnpenny 1989) and are problematic in the study area as well. Consequently, removal or reconstruction of these barriers is an essential measure for the restoration of the Obernach's connectivity and of a functioning lacustrine brown trout juvenile habitat.

Frequently, the river's low water level prevents lacustrine brown trout from ascending the stream since the river bed falls completely dry over longer stretches or at least does not exceed depths of a few centimetres at times with low flow. Often this is not a natural phenomenon but it is caused by the water being used for electricity generation at the Obernach hydropower station. Therefore, an ecologically sufficient water supply to the Obernach should be secured at periods of low water discharge.

Suitability of the Obernach as spawning habitat for lacustrine brown trout

Assuming that migration barriers are removed, the Obernach is likely to be a suitable spawning habitat for lacustrine brown trout. This fact is supported primarily by the results of the physico-chemical measurements at potential spawning sites, as well as by the fish community structure and its demography.

The quality of the spawning substratum depends on the substratum composition, and its physical and chemical parameters (Malcolm et al. 2003; Rubin & Glimsäter 1996). The data from this study show that the Obernach contains suitable spawning substratum, as the portion of fine sediment was consistently below 10%, which several authors consider the threshold value above which the mortality rate of eggs and alevins rises due to reduced exchange between the free flowing water and the hyporheic zone (Geist & Auerswald 2007; Heywood & Walling 2007; Jungwirth et al. 2003; Soulsby et al. 2001b). Moreover, the maximum gravel size in the Obernach rarely exceeded 70 or 100 mm respectively, which are considered as maximum sizes for spawning substratum used by lacustrine brown trout (Caviezel 2006; Jungwirth et al. 2003).

The most important physico-chemical parameters, dissolved oxygen and redox potential, also matched the requirements of lacustrine brown trout. The redox potential permanently stayed above 500 mV suggesting constantly sufficient oxygen supply both in the free flowing water and in the interstitial zone, as values above 300 mV indicate oxic conditions (Schlesinger 1991). The measurements of dissolved oxygen confirmed this conclusion, as only one outlier value below 7 mg L^{-1} was found, which has been determined to be the minimum oxygen concentration for salmonids by various authors (Crisp 1996; Ingendahl 2001). Exchange between the free flowing water and the interstitial zone seems to function well, even though peaks in conductivity indicated a reduced exchange rate in certain areas. Consequently, substratum quality is likely to meet the habitat requirements of lacustrine brown trout.

Suitability of the Obernach as a juvenile habitat for lacustrine brown trout

The results of the structural stream analysis, of the electro-fishing surveys, and of the stocking experiment indicate that the Obernach is a suitable juvenile habitat for lacustrine brown trout.

Structural variability was high throughout the stream. Shallow areas with low water velocity alternating with deep pools and stronger current provide suitable habitats for fish of different size classes (Armstrong et al. 2003; Bardonnet & Heland 1994; Crisp 1996). The high availability of cover, mainly provided by riparian vegetation and undercut banks, protects fish from predators (Eklöv & Greenberg 1998) and may even increase the habitat's carrying capacity due to the visual isolation of individuals (Kallenberg 1958).

The results of the electro-fishing surveys support these conclusions as they revealed a well reproducing population of riverine brown trout, which require environmental conditions similar to those of lacustrine brown trout (Lelek 1987). The trout population surveyed consisted mainly of young-of-the-year individuals but also included all other age classes. Several studies showed that catch efficiency for salmonids can vary between 20 and 70%

depending on equipment and environmental conditions (e.g. Bateman et al. 2005; Crozier & Kennedy 1994; Reid et al. 2009). Our data from standardized previous electrofishing runs in similar streams from the same area and with a similar fish community structure (data not shown) revealed a catch efficiency of around 30% for *Salmo trutta*, which is therefore also likely for brown trout in the Obernach. This leads to an estimated riverine brown trout biomass of 102.2 kg ha^{-1} and 63.6 kg ha^{-1}, respectively, exceeding the trout density found in studies of Lobon-Cervia & Penczak (1984) in the Spanish Jarama River but being comparable to the results of Geist et al. (2006) and Lobon-Cervia et al. (1986) from oligotrophic, middle-European watersheds. These findings suggest a great potential for lacustrine brown trout recovery of the Walchensee population since intact, naturally reproducing trout populations have become rare due to anthropogenic influences (Kuenzli 2005).

The stocking experiment also supports the conclusion that the Obernach provides an adequate juvenile habitat. Considering the catch efficiency of about 30%, the fact that only half of the watershed was electro-fished and that some individuals may already have migrated into the lake, considerably more juvenile lacustrine brown trout than the 13 individuals found can be expected in the stream.

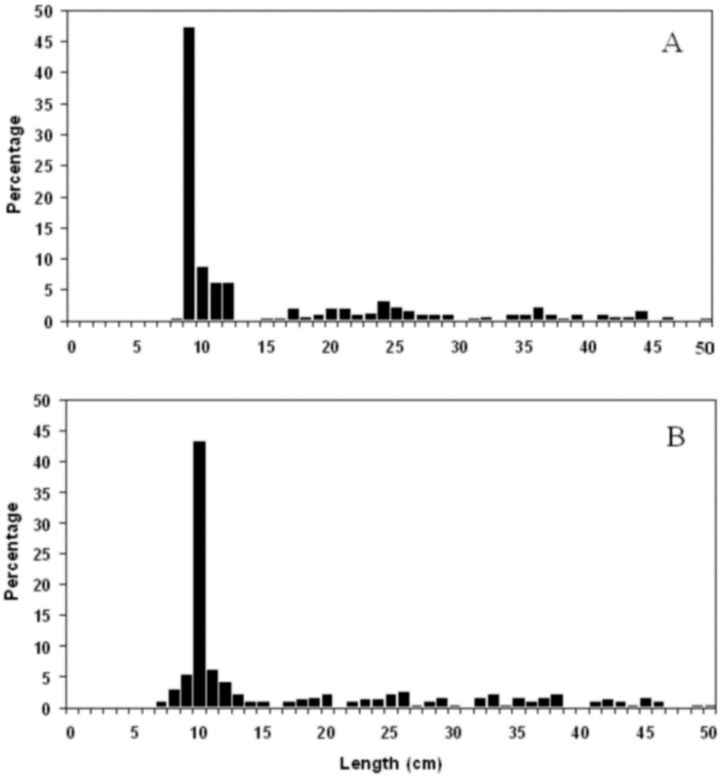


Figure 3.5: Length-frequency distribution of riverine brown trout in the Obernach during electro fishing surveys on 25/09/2007 (A, n = 315) and on 21/01/2008 (B, n = 243).

Implications for conservation

The combination of migration barriers and water discharge turned out to be the main problems for reproduction of lacustrine brown trout at the study site. Removal or at least remodelling of obstacles and ensuring a minimum water level will be of highest importance for the conservation of the Lake Walchensee lacustrine trout population. According to the European Water Framework Directive connectivity of running waters should be achieved until 2015 which implies the necessity to find solutions for modification of obstacles regardless of the specific case discussed here. It is recommendable to begin with obstructions like those at the Obernach, where the ecological need for habitat restoration is obvious and where this aim can also be achieved quite easily due to size and function of the constructions. Successful habitat restoration of the Obernach is possible if all stakeholders from fisheries, water authorities, nature conservation and from the hydropower company cooperate in this restoration process.

There are several possibilities to make obstructions passable for aquatic organisms, of which the conversion into rockfill ramps is the most preferable one in the case of the Obernach, as they are most natural constructions for various reasons (RRC 2002). They provide connectivity on the whole river width and are consequently easily located by all organisms. Moreover, different water velocities are caused by the construction's rough surface, which gives animals of different genera the possibility to pass those constructions (Wang 2008). The ramps may be constructed in different ways, but they should be built in a curved form to maintain connectivity during periods of low flow.

In order to solve the problem of water discharge, it is important to ensure a minimum discharge that prevents the Obernach from partly falling dry at times, which can easily be achieved due to the possibility of regulating water outflow from Lake Sachen. Based upon measurements of water depths and flow velocities, a minimum water discharge in the order of 1.5 $m^3 s^{-1}$ can be recommended. To optimize conditions for lacustrine brown trout, the establishment of a pulse-flow concept, shortly before spawning runs take place, would be useful, as elevated discharge often induces upstream migration (Gosset et al. 2006; Ovidio et al. 1998; Svendsen et al. 2004). Moreover, higher discharge would guarantee sufficient water depths for wandering fish. It may be possible to reduce discharge in other periods, at least to a certain degree.

After restoration of habitat connectivity, monitoring of spawning runs will be crucial for the further management of lacustrine brown trout. If the current lake population is below the limits of a minimum viable population or if no sufficient number of spawners is able to locate the river Obernach, initial stocking may be necessary. Since homing behaviour, imprinting and conservation genetics aspects are important in this context, successful management also requires further research.

As juvenile lacustrine and riverine brown trout occupy a similar ecological niche, competition may reduce riverine brown trout densities if lacustrine brown trout populations are enhanced. This can lead to a target conflict in conservation which will likely set a priority for the more critically endangered lacustrine brown trout. However, many conservation measures for lacustrine brown trout, such as the suggested improvements of connectivity, will also have positive effects for other species in the ecosystem.

The results of this study show that recognition of life-stage specific habitat deficiencies in lacustrine brown trout and other salmonids is a first crucial step for the development of an effective recovery plan which should integrate genetic and ecological data throughout the distribution range.

4 Physicochemical assessment of *Unio crassus* habitat quality in a small upland stream and implications for conservation

A similar version of this chapter was published: Denic M, Stoeckl K, Gum B & Geist J (2014). Physicochemical assessment of *Unio crassus* habitat quality in a small upland stream and implications for conservation. Hydrobiologia 735: 111-122.

4.1 Abstract

The abundance of *Unio crassus* (Philipsson 1788) has declined over the last decades. Despite the high conservation status of this species, knowledge on its ecological requirements is scarce. The objective of this study was to identify key habitat characteristics in areas with recent recruitment of *Unio crassus* in a small upland stream. Furthermore, we investigated stretches where the species is presently absent. Sediment deposition, redox potential, flow velocity, water depth, nitrogen and phosphorus load were investigated. Fine sediment deposition was high with 19.4 kg m^{-2} $month^{-1}$ at colonized and 13.3 kg m^{-2} $month^{-1}$ at non-colonized stretches of the Sallingbach. At all study stretches redox potentials in the interstitial zone varied around 300 mV, which constitutes the boundary value between oxic and anoxic conditions. Results of chemical water analyses indicated high nitrogen loads. Nitrate nitrogen concentrations averaged between 4.1 and 6.5 mg NO_3-N l^{-1} at all study stretches, significantly exceeding the currently proposed threshold value of 2.0 mg L^{-1} for functional *U. crassus* streams. The results in this study suggest that *U. crassus* is more tolerant to eutrophic habitat conditions than previously expected. Our findings show that currently considered physicochemical parameters and high fine sediment deposition cannot mechanistically explain the occurrence of *U. crassus* in the stream.

4.2 Introduction

Worldwide, freshwater ecosystems experience high biodiversity losses (e.g. Abell 2002; Dirzo & Raven 2003; Dudgeon 1992; Geist 2011), with species extinction rates significantly exceeding those of terrestrial ecosystems (Revenga & Kura 2003; Sala et al. 2000). Non-marine mollusks are particularly affected, with species extinction rates exceeding 40% during the last millennium (Lydeard et al. 2004). According to literature from the beginning of the 20th century, the thick-shelled river mussel (*Unio crassus)* was regarded as the most common unionoid species in Central Europe (Geyer 1927; Israel 1913), reaching densities >700 individuals m^{-2} (Tudorancea & Gruia 1968). Due to significant declines within the last 50 years (e.g. Bless 1980; Jungbluth et al. 1988; Reischuetz & Sackl 1991), the species is now strictly protected and considered “critically endangered” in several European countries, since only few successfully reproducing populations remain (von Proschwitz & Lundberg 2004; Zettler & Jueg 2007). Although potentially harmful effects of anthropogenic activities, including water pollution and flow modification, have been reduced in Central Europe over the last decades (Aarts et al. 2004; Bogan 2008), *U. crassus* populations are not recovering accordingly.

The sensitivity of *U. crassus* to unfavorable conditions is presumably closely linked to the complex life cycle, during which different life-stages have specific ecological requirements. After maturation, adult mussels release glochidial larvae, which need to attach to the gills of suitable host fish (Taeubert et al. 2012b; Waechtler et al. 2001). After their parasitic life-stage, juvenile mussels live buried in the interstitial system of rivers for a period of up to five years (Hochwald 1997). This post-parasitic phase is generally considered the most vulnerable of all life-stages. Based on information from related species, it is assumed that unionids constantly need stable physicochemical conditions in the stream substratum over a relatively long period of time (Geist 2010; Hastie et al. 2000; McRae et al. 2004). For example, sites with natural *M. margaritifera* recruitment were found to be dominated by coarse, well–sorted substrata and a low fraction of fines, providing well-oxygenated water, nutrients and organic matter to the interstitial zone. Increased fine sediment loads, e.g. caused by agricultural land-use, can reduce hyporheic exchange rates by clogging the interstitial macropores (Schaelchli 1992), resulting in reduced oxygen supply.

Due to their similar life cycle and co-occurrence in the same habitat in several German streams (Björk 1962), it is often assumed that functional *U. crassus* populations have similar requirements concerning substratum quality as *M. margaritifera* (Buddensiek 1993; Engel 1990; Zettler et al. 1994). However, the validity of these assumptions has not yet been tested, and information on physicochemical conditions in the interstitial system in functional *U. crassus* streams is still scarce. Although there is still a lack of peer-reviewed information on key habitat parameters of *U. crassus* (Koehler 2006), various conservation efforts targeting habitat restoration for this species have been initiated (Perez-Quintero 2007). In order to improve conservation strategies, profound knowledge about species-specific habitat requirements with regard to substratum quality is a main prerequisite.

The objective of this study was to identify habitat characteristics in a *Unio crassus* stream with recent recovery of *Unio crassus*. In particular, we characterized the physicochemical conditions in the free-flowing water and in the hyporheic zone in areas with recent increase of mussel population densities and recruitment of *U. crassus.* Furthermore, we also analyzed areas where the species is currently absent. The study comprises analyses of sediment deposition and physicochemical variables such as redox potential, flow velocity, water depth, pH, specific conductance, ammonia, nitrite, nitrate and phosphate, which were previously assumed to be important key factors for the recruitment of juvenile *U. crassus* (Buddensiek et al. 1993; Engel 1990; Hochwald 1997).

4.3 Material and Methods

Study area and design

The study area is located in the Bavarian tertiary hills in the north-east of Munich, Germany (Fig. 4.1). The study stream Sallingbach belongs to the upper catchment of the Danube and has a total length of 8.8 km and a width of 0.5 – 2 m. Substratum texture is dominated by fine gravel and sand. The mean discharge in the downstream area of the stream is 0.082 m^3 s^{-1}. The region is highly impacted by agricultural land-use. In the Ilm, a stream similar to the Sallingbach regarding its geomorphological and hydrological characteristics, a reference study stretch was chosen for validating the results of physicochemical parameters from the Sallingbach in a comparable environment. Here, a self-sustaining functional *U. crassus* population had been discovered by the Bavarian Mussel Coordination in 2010 (Gum pers. comm.). The stream hosts a dense host fish population of European minnow (*Phoxinus phoxinus*).

Due to the occurrence of *Unio crassus* and several other endangered aquatic species, the river Sallingbach was target to a statewide restoration program conducted from 1988 to 2001 (ABSP 1991). Distribution, population size and recruitment of juvenile mussels of *U. crassus* were thoroughly analyzed in several surveys from 1988 to 2009 (Ansteeg 2010; Ansteeg 1999; Ansteeg 1994; Colling 2007; Hochwald 1988). The monitoring data show that

U. crassus has a core distribution area in the downstream area, where highest mussel densities and natural recruitment of juvenile mussels were found. The survey in 2009 (Ansteeg 2010) showed that mussel densities had increased in this area for the first time since 1989. Findings of spent shells and data from over 20 years ago (Colling 2007; Seitz 1988) document that also upstream stretches represented the natural habitat of *U. crassus* in the past.

In order to representatively cover all areas of the Sallingbach, 8 stretches were defined for analyses of physicochemical parameters. Four stretches are currently colonized with *U. crassus* and recent juvenile recruitment was observed in 2009 (distance between stretch 1 and 4: 1.7 km). In addition, four stretches were determined upstream, where *U. crassus* currently is absent (stretch number 5 and 6), and where *U. crassus* occurrence is documented (stretch 7 and 8) (total distance between stretch 5 and 8: 3.5 km). Depending on the occurrence of *U. crassus*, the stretches are referred to as colonized (C) and as non-colonized (NC) throughout the text. The reference stretch in the Ilm is classified as colonized, due to the presence of *U. crassus* in different age classes.

Within each stretch, four transects were analyzed as shown in Fig. 4.2. In each transect, two measuring spots in the middle and two at either side of the stream were defined (n = 6). After the physicochemical measurements in a transect were completed, the substratum was screened for the presence of mussels to validate the results of the survey in 2009 (Ansteeg 2010). For this purpose, the substratum was removed to check for buried mussels. In order to avoid that investigations at one time point change habitat conditions and consequently introduce a bias to the data, sampling in the following month was carried out in a previously assigned transect, moving from downstream to upstream transects (t_1-t_4) within sampling stretches. Measurements at the reference stretch located in the stream Ilm were carried out once in October 2010. In each sampling period all measurements were conducted within the same day.

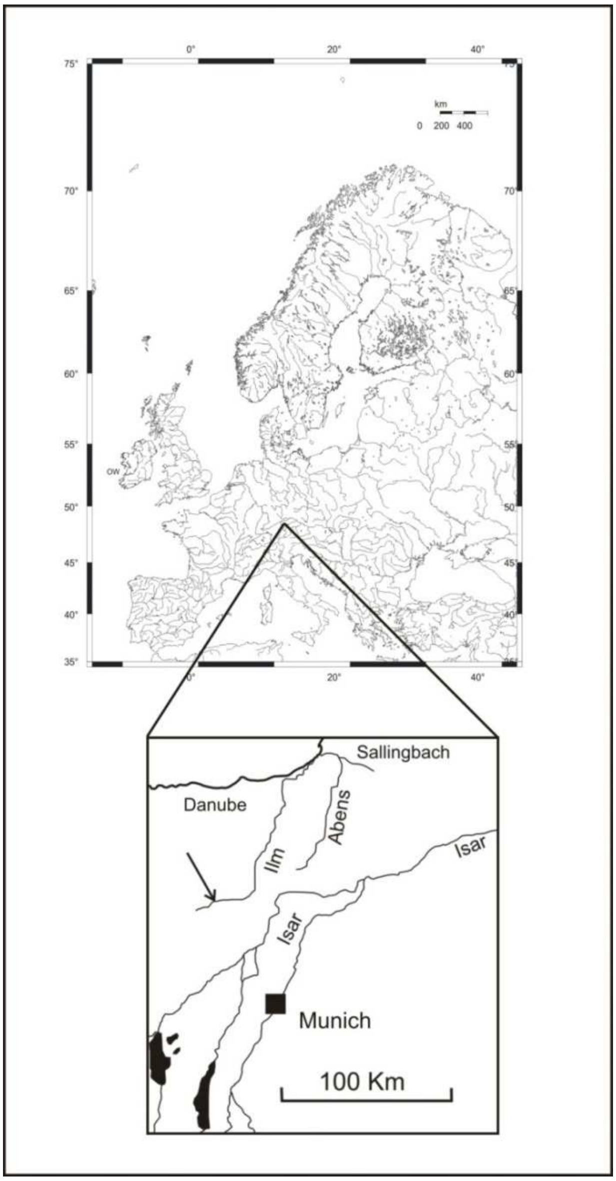


Figure 4.1: Map of the study area, arrow indicating position of the reference stretch in the River Ilm.

Sediment deposition

Sediment net deposition in the Sallingbach was investigated using sediment traps. The traps consisted of plastic boxes that contained a volume of 5 l (33 (length) x 19 (width) x 11 cm (height)) and had been filled with clean gravel with a standardized particle size between 16-32 mm in diameter. Prior to the physicochemical analysis in the stream, a total of 24 sediment traps (3 traps per study stretch) was deployed in the substratum such that the top of the traps was flush with the riverbed. The 3 sediment traps at each of the 8 stretches were horizontally aligned and evenly distributed across the streambed. After 28 days, the traps were removed and the deposited material was collected. The sediment traps with washed gravel were reinserted for further sediment collection in the following month. Thus, a total of 96 sediment samples were collected during August – November 2010. The deposited grain size fractions were separated at the laboratory by wet sieving (AS 200 digit, Retsch, Haan, Germany). Mesh width was 20 mm, 6.3 mm, 2.0 mm and 0.85 mm, respectively. The fractions retained on each sieve were dried at 80 °C and weighed. The largest fraction was excluded from further analysis due to the restricted sample volumes (Sinowski & Auerswald 1999).

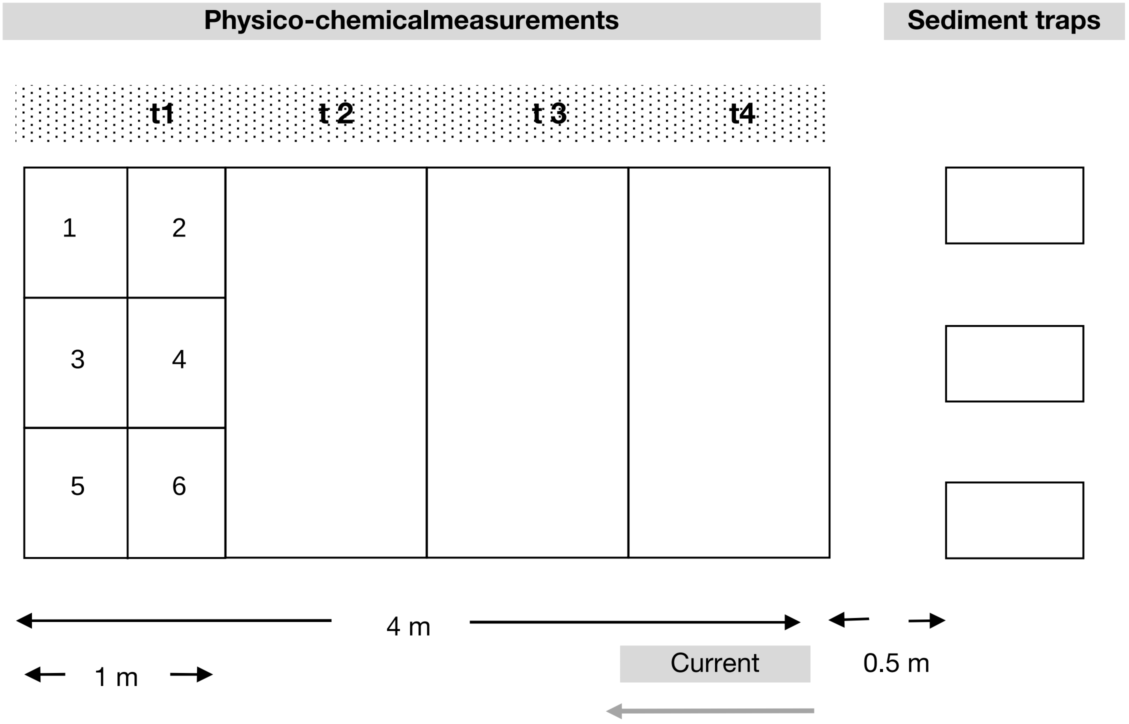


Figure 4.2: Sampling design at each study stretch (n = 8). Physicochemical parameters in the free-flowing water and in the interstitial zone were measured monthly from August until November within 1 m transects (t_1-t_4) at six sampling spots (n = 6, sampling spots shown for first month). Sediment traps (n = 3 per stretch) were placed upstream in order to avoid additional sediment deposition during sampling.

Physicochemical parameters

Physicochemical parameters were analyzed in 28 day intervals over a period of 4 months from August to November 2010. Flow velocity was measured with a handheld flow meter (HFA, Höntzsch, Waiblingen, Germany) and water depth was measured with a pocket rule (exactness ± 0.5 cm). Redox potentials (Eh) were analyzed according to Geist & Auerswald (2007). At each measuring spot within a transect, Eh was first measured in the free-flowing water, and then at depths into the substratum of 5 and 10 cm. Values above 300 mV imply oxic conditions, whereas values below indicate anoxia (Schlesinger 1991).

Water samples from both the interstitial system (n = 192 per parameter) and from the open water body (n = 96 per parameter) in the Sallingbach were drawn for the determination of ammonium nitrogen, nitrite nitrogen, nitrate nitrogen, phosphate phosphorous (NH_4-N, NO_2-N, NO_3-N, dissolved PO_4-P), pH and specific conductance. Samples of interstitial water at a substratum depth of 5 cm were collected with a fixed PVC tube attached to a flexible plastic hose in combination with a 100 ml syringe (Braun, Melsungen, Germany), which was used for creating a vacuum. Water samples were also drawn from the reference stream (n = 3 for free-flowing water and n = 6 for intersitital water, respectively).

For each sample, 30 ml of free-flowing or interstitial water were extracted and transferred to 50 ml falcon tubes (Roth, Karlsruhe, Germany). Water samples were stored on ice until analysed. Prior to each analysis, water samples were filtered using paper filters (MN 615 ¼, diameter: 125 mm, Macherey-Nagel, Düren, Germany) in order to remove coarse particles. Concentrations of chemical variables were determined photometrically (Photometer: Photolab S12, WTW, Weilheim, Germany; testkits: Spectroquant, Merck KGaA, Darmstadt, Germany) according to German standard norm protocols (DIN standards). Specific conductance and pH were measured using handheld 315i conductivity and 315i pH-meters (WTW, Weilheim, Germany) in the free-flowing water and in the interstitial water from 5 cm substratum depth.

Statistical Analysis

Whilst the main focus of the study was an analysis of mean values, variation and range of physicochemical data from the sites where *U. crassus* is abundant and recruiting, we also compared C and NC stretches in the Sallingbach using pairwise Student's t-test or the Mann-Whitney U-test. Discriminant analysis was used to identify discriminating parameters between C and NC stretches of the Sallingbach. In a stepwise approach, seven parameters were incorporated in the model (NH_4-N, pH and specific conductance in 5 cm substratum depth, pH and specific conductance in free-flowing water, deposition of particles >6.3 mm, water depth). All tests were implemented in PASW 18 (IBM Statistics 20, SPSS Inc., NY, USA).

4.4 Results

Analysis of water chemistry

At C stretches in the Sallingbach, the mean nitrate nitrogen concentration in the free-flowing water was 6.39 ± 1.98 mg NO_3-N l^{-1} (mean and SD), whereas NO_3-N concentrations in the interstitial were significantly lower (4.13 ± 2.65 mg NO_3-N l^{-1}, Mann-Whitney-U-test, $p < 0.05$). At the colonized stretch in the reference, a mean concentration of 5.00 ± 1.06 mg NO_3-N l^{-1} in the free-flowing water was measured compared to 2.61 ± 1.33 mg NO_3-N l^{-1} in 5 cm substratum. With 7.05 ± 3.07 mg NO_3-N l^{-1} in free-flowing water, concentrations at NC stretches of the Sallingbach were also similar to concentrations at C stretches, whereas interstitial nitrate nitrogen concentrations at NC stretches (5.62 ± 3.46 mg NO_3-N l^{-1}) were significantly different from C stretches (Mann-Whitney U-test, $p < 0.05$). Mean NH_4-N concentrations averaged 0.09 ± 0.16 mg L^{-1} in the free-flowing water at C stretches in the Sallingbach (Table 4.1), whereas maximum values reached 3.12 mg NH_4-N l^{-1}. Mean NH_4-N concentrations in the substratum at C sites were higher (0.39 ± 0.56 mg NH_4-N l^{-1}). Measured NH_4-N substratum values at NC stretches (0.22 ± 0.21 mg NH_4-N l^{-1}) were significantly lower than at C stretches of both the study stream and the reference stream (Mann-Whitney-U-test; $p < 0.05$). Nitrite nitrogen concentrations generally ranged between 0.03 and 0.07 mg NO_2-N l^{-1} in the free-flowing water of all study stretches, whereas higher variations were found in the substratum. Highest nitrite nitrogen concentrations reaching a maximum of 0.49 mg NO_2-N l^{-1} were measured at C stretches of the Sallingbach. Arithmetic mean values of the dissolved PO_4-P concentration in the free-flowing water were between 0.02 and 0.11 mg PO_4-P l^{-1} (Table 4.1); high values between 0.8 and 1.0 mg PO_4-P l^{-1} were measured both in the free-flowing water and in the interstitial water at C and at NC stretches.

Table 4.1: Arithmetic mean values and ranges of chemical parameters phosphate phosphorous (PO4-P), nitrite nitrogen (NO2-N), nitrate nitrogen (NO3-N), and ammonium nitrogen (NH4-N) in free-flowing water (FW) and at 5 cm substratum depth (substratum) at colonized (C) and non-colonized (NC) stretches of the Sallingbach (n = 36 for FW, n = 96 for substratum) and the reference stream Ilm (n = 3 for FW, n = 6 for substratum).

Parameter	Study group	FW (mean [range])	Substratum (mean [range])
PO_4-P (mg L^{-1})	C	0.06 [0.01 – 0.83]	0.08 [0.01 – 0.82]
	NC	0.11 [0.01 – 0.98]	0.07 [0.01 – 0.90]
	Ilm (C)	0.02	0.03
NO_2-N (mg L^{-1})	C	0.04 [0.03 – 0.05]	0.09 [0.02 – 0.49]
	NC	0.04 [0.03 – 0.07]	0.05 [0.01 – 0.24]
	Ilm (C)	0.04	0.01
NO_3-N (mg L^{-1})	C	6.39 [3.62 – 15.68]	4.13 [0.59 – 12.58]
	NC	7.05 [1.61 – 13.67]	5.62 [0.75 – 15.29]
	Ilm (C)	5.00	2.60
NH_4-N (mg L^{-1})	C	0.09 [0.01 – 0.32]	0.39 [0.01 – 3.12]
	NC	0.15 [0.02 – 1.41]	0.22 [0.03 – 1.44]
	Ilm (C)	0.09	0.37

Physical stream bed characteristics

Redox depth profiles exhibited significant differences between the free-flowing water and the substratum (Mann-Whitney U-tests, $p < 0.001$). Mean values in the free-flowing water of the Sallingbach were 450 ± 23 mV (C) and 445 ± 35 mV (NC), respectively. In 5 cm substratum depth, mean values in the Sallingbach were close to 300 mV (C: 305 ± 93 mV, NC: 317 ± 88 mV) (Fig. 4.3). In 10 cm substratum depth, means were constantly below 300 mV. In the reference stream, depth profiles were similar: high delta values were found between the free-flowing water (446 ± 33 mV) and the interstitial zone (295 ± 46 mV) in 5 cm substratum depth and 292 ± 57 mV in 10 cm). Redox potentials showed high microscale variability between study stretches and even between sampling spots at single study transects with ranges up to 350 mV in 10 cm substratum depth.

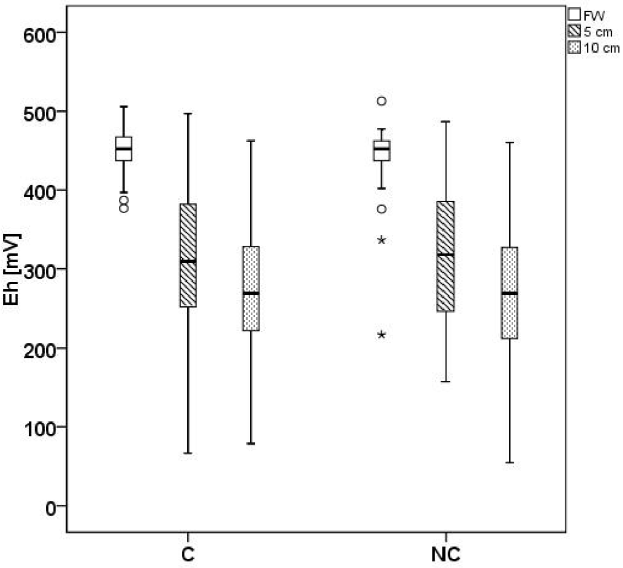


Figure 4.3: Depth profiles of redox potentials in the free-flowing water and in 5 and 10 cm substratum depth at stretches with Unio crassus presence (= colonized; C; n = 102, 102, 102) or absence (= non-colonized; NC; n = 96, 96, 96) in the Sallingbach (Whisker: 0.05 and 0.95 percentiles; Box: 0.25 quartile, median and 0.75 quartile; circles: outliers beyond 1.5 times of the interquartile range (IQR), asterisks: extreme outliers (more than 3 times the IQR).

Specific conductance in the free-flowing water deviated from substratum values at both study streams. C stretches of the Sallingbach exhibited lower values in the free-flowing water (773 ± 125 µS cm^{-1} compared to 810 ± 144 µS cm^{-1} in the substratum) whereas at NC stretches, lower values were found in the substratum (810 ± 96 µS cm^{-1} compared to 753 ± 68 µS cm^{-1}). In the Sallingbach values in the free-flowing water differed significantly between C and NC stretches (Student's t-test, $p = 0.001$). Variations in pH were small, ranging from 7.8 to 8.0 (free-flowing water) and from 7.5 to 8.0 (5 cm substratum depth) in the

Sallingbach. In the reference, pH in the free-flowing water was 7.6, whereas pH in the substratum was 7.7.

Sediment deposition in the Sallingbach was dominated by fine sediments with grain sizes < 0.85 mm. Fine sediment deposition was significantly higher at colonized stretches with a mean of 19.4 ± 10.2 kg m^{-2} $month^{-1}$ than at NC stretches with 13.3 ± 6.6 kg m^{-2} $month^{-1}$, respectively (Mann-Whitney U-test, $p = 0.003$) (Fig. 4.4). For larger particles, mean deposition rates were lower and did not exceed 2.0 kg m^{-2} $month^{-1}$. Fine sediment deposition increased in downstream direction reaching its maximum at downstream stretches 1 and 2 (mean: 28.2 kg m^{-2} $month^{-1}$) (Fig. 4.5), where mussel density was highest. Maximum values were detected at study stretch 1 with 38.7 kg m^{-2} $month^{-1}$ and the minimum was observed at stretch 2 (1.7 kg m^{-2} $month^{-1}$).

Water depth in the Sallingbach increased in a downstream direction and from NC to C stretches with means of 13 and 22 cm respectively, whereas flow velocity decreased from upstream to downstream (mean: 0.27 m s^{-1} at NC stretches and 0.18 m s^{-1} at C stretches). The hypothesis that lower flow velocity may influence sedimentation rates of fines at the downstream stretches was not supported by regression analysis (data not shown).

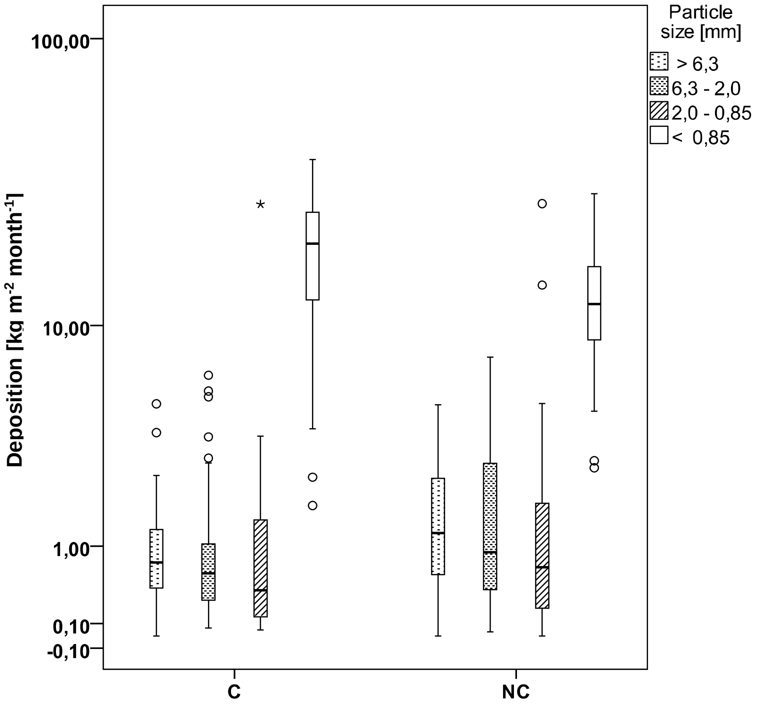


Figure 4.4: Deposition of particle size fractions >6.3 mm, 6.3–2.0 mm, 2.0-0.85, and < 0.85 mm (n = 90, 93, 87, 91 respectively) at stretches with Unio crassus presence (= colonized; C) or absence (= non-colonized; NC) in the Sallingbach (Whisker: 0.05 and 0.95 percentiles; Box: 0.25 quartile, median and 0.75 quartile; Circles: outliers beyond 1.5 times of the interquartile range (IQR), asterisks: extreme outliers (more than 3 times the IQR) in kg m^{-1} $month^{-1}$.

Multivariate analysis of physicochemical habitat parameters

In the multivariate discriminant analysis, physicochemical parameters were used to characterize C and NC stretches of the Sallingbach. The discriminant analysis correctly assigned 91.7% of the cases. The most important parameters for the discrimination between C and NC stretches were interstitial NH_4-N concentration (Canonical Coefficient: 0.676), Specific Conductance in the free-flowing water (-0.637) and pH in the substratum (0.666) (Table 4.2).

Table 4.2: Standardized canonical coefficients of physicochemical parameters incorporated as variables in a discrimant analysis for the discrimination of C (n = 4) and NC (n = 4) stretches in the Sallingbach.

Variable	Standardized Canonical Discriminant Function Coefficients
NH4-N (mg L^{-1}) in 5 cm substratum	0.676
Specific Conductance in free-flowing	-0.637
Specific Conductance in 5 cm	0.492
pH in free-flowing water	0.460
pH in 5 cm substratum depth	0.666
Deposition of particles >6.3 mm	-0.324
Water depth	0.516

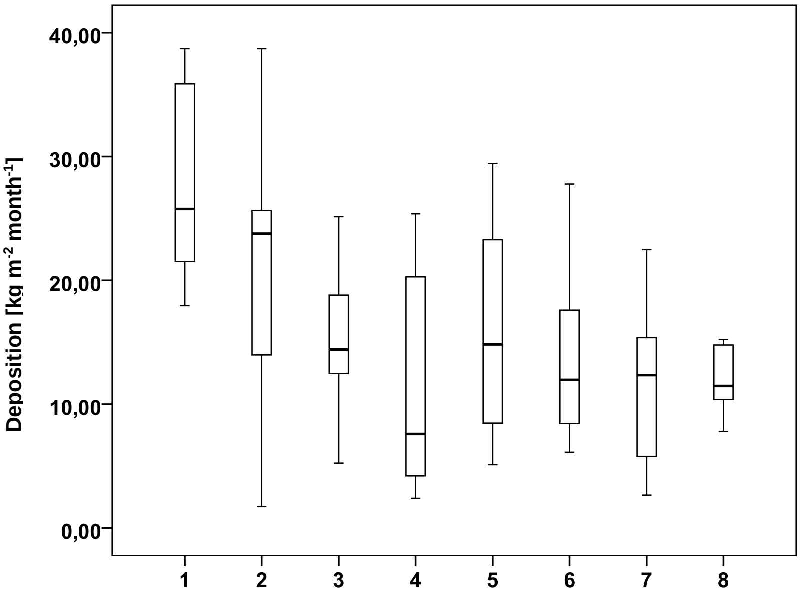


Figure 4.5: Net fine sediment deposition (particle size < 0.85 mm) at 8 sampling stretches (n = 12 per study stretch) in the Sallingbach. Sampling stretches are numbered in ascending order in an upstream direction (Whisker: 0.05 and 0.95 percentiles; Box: 0.25 quartile, median and 0.75 quartile). The stretches 1- 4 are colonized with U.crassus, whereas at 5 -8 the species is absent.

4.5 Discussion

The aim of this study was to characterize the habitat of the endangered unionid species *Unio crassus* by determining physicochemical key parameters of the free-flowing water and the interstitial zone in a small upland stream.

Chemical water quality

In the Sallingbach, recovery of the *Unio crassus* population in C stretches (Ansteeg, 2010) occurred under elevated NO_3–N mean concentration regimes of 4.1 mg L^{-1} in the substratum and 6.4 mg L^{-1} in the free-flowing water. Furthermore, nitrate nitrogen concentrations in the reference stream hosting a self-sustaining *U. crassus* population also averaged values >2.5 mg NO_3–N l^{-1} both in the free-flowing water and in the hyporheic zone. This finding is in contradiction to other studies, where impaired vitality and reproduction of unionid mussel populations was linked to elevated nitrate nitrogen concentrations in streams (Douda 2010; Hochwald 1997; Hochwald & Bauer 1990; Hus et al. 2006; Zettler et al. 1994). Hochwald (1997) reported that successful reproduction of *U. crassus* populations is reduced at 3.6 mg NO_3-N l^{-1} and completely ceases at concentrations higher than 5.0 mg NO_3-N l^{-1}. Consequently, nitrate nitrogen concentrations below 2.2 mg NO_3-N l^{-1} are commonly accepted as a threshold value for streams hosting functional populations of *U. crassus* (Hochwald 2001; Koehler 2006; Zettler & Jueg 2007). In contrast to these observations in the field and in line with the results described herein, laboratory experiments also indicate an absence of direct negative effects of nitrate on juvenile mussels. Acute nitrate nitrogen toxicity tests (96 h) with juvenile *U. crassus* showed extraordinarily high LC_{50} values of >1200 mg NO_3-N l^{-1}, placing the species amongst the least sensitive groups of freshwater animals (Douda 2010). It is therefore assumed that elevated nitrate nitrogen concentrations might rather act as an indirect indicator for contamination with other nitrogen compounds such as nitrite and ammonia, which are released during nitrogen transformation pathways (Douda 2010; Mueller et al. 2013; Patzner & Mueller 2001).

Previous nitrate (NO_3) measurements in the Sallingbach show that this mussel population was exposed to elevated concentrations during the last 20 years. According to Hochwald (1990), nitrate concentrations averaged a mean of around 35 mg NO_3 l^{-1} in 1990 (≈ 7.7 mg NO_3–N l^{-1}, n = 4) and Brandner (1997) measured a mean nitrate nitrogen concentration of 6.3 ± 2.6 mg NO_3–N l^{-1} (n = 15) in 1997. Unpublished data of the regional watershed authorities show that there is no significant seasonal variation in the nitrate nitrogen concentration, which ranged around 6.9 ± 0.9 mg NO_3–N l^{-1} (n = 32) from 2007 to 2010. Therefore, the measured elevated nitrate nitrogen concentration in the Sallingbach as well as in the reference stream did not prevent juvenile recruitment, and thus cannot be used as an indicator for a functional population in this study.

Mean concentrations of nitrite and ammonium nitrogen in the substratum, which may be formed from nitrate through respiratory denitrification and in nitrate reduction processes to ammonium (Burgin & Hamilton 2007), were in general higher at C than at NC stretches. Ammonia is considered to be of minor toxicity to unionid mussels in its ionic form NH_4^+, which is mainly prevalent at a pH below 7 (Augspurger et al. 2003). PH values in the substratum ranging from 7.5 up to 8.0 at C and NC stretches did not significantly differ from those measured in the free-flowing water. Based on our data, it is yet not possible to determine the actual toxicity of ammonia depending on pH and temperature. Therefore, further analyses should focus on determining the causal mechanisms that explain the influence of nitrogen compounds on the chemical water quality in the interstitial depending on physical parameters such as temperature and pH.

Mean phosphate phosphorous concentrations in the free-flowing water as well as in the substratum are in line with other PO_4-P values reported from functional *U. crassus* streams (Buddensiek et al. 1993). Like Engel (1990) and Fleischauer-Roessing (1990), we did not find a correlation between the occurrence of young *Unio* and the concentration of phosphate phosphorous within the sediment water.

Physical stream bed characteristics

Both C and NC stretches in the Sallingbach are characterized by high fine sediment deposition, reaching a maximum of 38.7 kg m^{-2} $month^{-1}$in study stretch 1. The increased levels of fines seem to have a strong effect on the exchange rates between the free-flowing water and the interstitial system, which is indicated by the pronounced difference of measured redox potentials between the free-flowing water and the interstitial zone. Since increased fine sediment loads and low interstitial redox potentials were also recorded at stretches with highest mussel densities in the study stream (Ansteeg 2010), differences in the occurrence and distribution of the *U. crassus* population at C and NC stretches within the Sallingbach cannot be explained by these habitat factors. Although other studies point out the general importance of substratum quality for the recruitment of juvenile mussels of the Unionoidea occurring in Central Europe (Buddensiek et al. 1993; Engel 1990; Fleischauer-Roessing 1990; Geist & Auerswald 2007), we observed relatively low mean interstitial exchanges at river stretches with juvenile *U. crassus* recruitment. High Eh values at several measuring spots however indicate the existence of interstitial sections with better oxygen supply, which are not evident when only comparing mean values.

Measured specific conductance values are in line with results from other studies on functional *U. crassus* streams (Hus et al. 2006), though they varied widely between stream stretches Elevated specific conductance at non-colonized stretches may reflect runoffs from agricultural surfaces as extensive land use is mainly found in the area of colonized stretches in the Sallingbach.

The lower water levels in NC stretches of the Sallingbach may expose mussels in this area to an elevated risk of draughts. Yet, it seems unlikely that draughts caused the population decline in NC reaches, as water authorities did not observe a drying out of the stream in the last decade.

The multivariate analysis for the discrimination between C and NC stretches proposed that the most important discriminant parameters were ammonium nitrogen concentration in the interstitial water, specific conductance and pH, where higher values were measured at C stretches, respectively. The variables that were found for the group assignment were chemical water compounds neglecting physical parameters which are often found to discriminate between functional and nonfunctional habitats in other studies on freshwater mussel ecology (Buddensiek et al. 1993; Geist & Auerswald 2007; Hastie et al. 2000).

Concluding from our results, we propose that functional *U. crassus* populations have a higher tolerance to poor substratum quality on a population level compared to other native endangered stream dwelling unionoid species such as *Margaritifera margaritifera* than previously thought, because i) it has a higher dispersal ability, resulting from a prolonged spawning period lasting up to 4 months, from multiple breeding events per season (Hochwald 1997), as well as from species-specific specialities such as the spurting behavior (Vicentini 2005). These behavioural traits may increase the chances of a successful host fish infestation which ii) is also supported by a higher number of suitable fish species, which occupy different habitats, such that *U. crassus* is able to spread glochidia over a wider range of habitats within a stream (Douda et al. 2012; Eroes et al. 2003; Pander & Geist 2010; Taeubert et al. 2012b). This will in turn increase the probability of finding high quality substrates within the stream for the post-parasitic phase (Waechtler et al. 2001). iii) The post-parasitic phase of *U. crassus* is relatively short and can be completed after two years (Hochwald 1997). Therefore, microsites of high physicochemical stream bed quality and substratum stability are only required during a significantly shorter period of time compared to juvenile freshwater pearl mussels, which are known to live buried within the substratum for at least five years.

Implications for conservation

The presented results suggest that previously assumed analogies on habitat requirements of different unionoids such as high exchange rates between the free-flowing water and the interstitial system have to be questioned critically, even if the species have a comparable life cycle. At least for the stream investigated herein, fine sediment deposition and nitrogen load could not mechanistically predict recruitment of *U. crassus*. However, both factors can also be indicative of other pollution problems and should thus not be generally overlooked. The results of this study also suggest that other factors such as predation or host fish availability (Douda et al. 2012; Taeubert et al. 2012b; Strayer et al. 2004) as well as other chemical (e.g. pesticides, TOC, heavy metals) and hydrological factors (e.g. changes of discharge regimes) not investigated herein need to be considered for an effective conservation of the species.

5 Influence of stock origin and environmental conditions on the survival and growth of juvenile freshwater pearl mussels (*Margaritifera margaritifera*) in a cross-exposure experiment

A similar version of this chapter was published: Denic M, Taeubert JE, Lange M, Thielen F, Scheder C, Gumpinger C & Geist J (2014). Influence of stock origin and environmental conditions on the survival and growth of juvenile freshwater pearl mussels (*Margaritifera margaritifera*) in a cross-exposure experiment. Limnologica: in press.

5.1 Abstract

The freshwater pearl mussel (*Margaritifera margaritifera*) is a highly specialized and sensitive freshwater bivalve, whose survival in the juvenile phase is indicative of high quality habitats. This contribution investigates the use of juvenile freshwater pearl mussels as bioindicators, considering the influence of mussel stock and study stream conditions on juvenile performance, as described by survival and growth rates. A standardized cross experiment was carried out investigating juvenile performance in four different pearl mussel stocks originating from the Rhine, Danube and Elbe drainages, representing distinct genetic conservation units. The juveniles were exposed in five study streams which were selected to integrate pearl mussel streams with different water qualities and recruitment status of the mussel population. Per study stream, five standard mesh cages containing an equal number of 20 (10 x 2) juvenile pearl mussels per stock in separate chambers were installed. Survival and growth rates of juveniles were checked after three months (i.e. before their first winter) and after nine months (i.e. after their first winter). Mussel stock and study stream conditions significantly influenced juvenile performance. Growth rates were determined by study stream conditions and increased with stream water temperature, organic carbon and C/N ratios. Survival rates varied stock-specifically, indicating different levels of local adaptation to their native streams. Due to the detection of stream-specific differences in juvenile performance, freshwater pearl mussels appear suitable as bioindicators. However, a careful of consideration of stock-specificity is necessary to avoid false interpretation of bioindication results. The comparison of stock-specific survival in native versus non-native streams implicates that exposure of juveniles outside their native habitats is able to increase breeding success or else serve for risk spreading in breeding programs.

5.2 Introduction

The freshwater pearl mussel (*Margaritifera margaritifera*) is a highly specialized and sensitive freshwater bivalve, inhabiting oligotrophic, high quality stream habitats (Geist & Auerswald 2007; Hastie et al. 2000; Oesterling et al. 2010). The species has a complex life cycle including an obligate parasitic stage on suitable host fish and a juvenile phase in the interstitial zone (Taeubert et al. 2010; Young & Williams 1984). The most sensitive life stage appears to be the early post-parasitic phase, during which the juveniles need a stable, but well oxygenized interstitial for up to 5 years (Buddensiek et al. 1993). At present, land-use changes have resulted in severe siltation of pearl mussel rivers degrading juvenile habitats by clogging of macropores with subsequent reduction of oxygen supply to the interstitial zone (Denic & Geist 2014; Leitner et al. 2014; Oesterling et al. 2008; Scheder et al. 2014). As a consequence, many populations are on the brink of extinction, as they lack juveniles and have not recruited for decades (Geist 2010).

As catchment restoration is time consuming, (semi-)artificial propagation and captive breeding are currently implemented as short term conservation action to preserve the overaged populations (Gum et al. 2011). In semi-artificial breeding, juveniles are kept in mesh cages and exposed in the free-flowing water of rivers, serving as bioindicators for ambient water quality at the same time. The captive breeding effort in endangered mussel species such as the freshwater pearl mussel has led to an increasing availability of artificially bred juvenile mussels for reintroduction and bioindication studies. However, the variable survival rates of these mussels and the unknown reasons for this observation have led to a controversy about the suitability of juvenile pearl mussels as bioindicators (Gum et al. 2011; Schmidt & Vandre 2010).

It is known that different stocks of the freshwater pearl mussel can show high rates of genetic differentiation even at small spatial scales (Geist & Kuehn 2005; Geist et al. 2010; Karlsson et al. 2013), suggesting that specialization and local adaptation may occur. However, the phenomenon of local adaptation is controversially discussed in the literature with an approximately equivalent amount of studies demonstrating or disconfirming local adaptation (Jones 2013; Lajeunesse & Forbes 2002). Kaltz & Shykoff (1998) proposed that local adaptation and its detection depend on the spatial scale on which experiments are conducted. Jones (2013) argued that local populations may not always be optimally adapted to their native habitats, especially in degraded environments. Experiments investigating the correlation between genetic differentiation, local adaptation and ecological performance are mainly restricted to terrestrial plants or parasites and are often based on theoretical models exclusively (e.g. Bennington et al. 2012; Gandon & Michalakis 2002; Jones 2013; Leimu & Fischer 2008; Malagnini et al. 2013). Knowledge on the interaction between genetics and performance may help to increase conservation success for endangered species such as the freshwater pearl mussel. In this contribution, we therefore tested the influence of freshwater pearl mussel stock and study stream conditions on juvenile mussel performance. A standardized cross experiment was carried out investigating growth and survival of four different pearl mussel stocks from three large Central European drainage systems, the Rhine, Elbe and Danube. Specifically the following hypotheses were tested: i) environmental factors determine growth and survival rates independent of mussel stock, ii) the probability of winter survival increases with mussel shell length, iii) stocks are locally adapted and juvenile mussels from native stocks exposed to the streams of parental origin exhibit higher growth and survival rates than non-native stocks and iv) freshwater pearl mussel juveniles are suitable bioindicators separating high and low quality habitats.

5.3 Material and methods

Study area

Mussel stocks and study streams were selected to cover three large Central European drainage systems of the Rivers Rhine, Elbe and Danube. Their location, names and codes, which are referred to throughout the text, are visualized and explained in Fig. 5.1. Mussel stocks were selected to represent distinct genetic conservation units (Geist & Kuehn, 2005). The study streams were chosen according to the following criteria: The streams had to be pearl mussel streams of different status with respect to water chemistry and the mussel population. Basic parameters of the study streams are summarized in Table 5.1. Three of the study streams (DG, ER, RO) were native streams with non-recruiting mussel stocks used in the experiment. In addition, one stream with a recruiting pearl mussel population (DW) and one stream where the freshwater pearl mussel is considered extinct (EH) were included.

Study design

Infestation of host fish was carried out by on site collection of glochidia (Gum et al. 2011) and subsequent preparation of an infestation bath to which host fish were exposed. As host fish, local brown trout (*Salmo trutta*) strains were used to ensure high infestation rates (Taeubert et al. 2010). Infestation measures were performed in areas of mussel stock origin. Before the start of the experiment, infested host fish were transferred to the Aquatic Systems Biology laboratories at Technische Universitaet Muenchen, where collection of juvenile mussels was carried out. Juvenile excystment occured from 04 June 2012 to 13 July 2012, with peak collection between 26 June 2012 and 06 July 2012 following the procedure described by Taeubert et al. (2013). Only juveniles collected during the peak collection period were used in the experiment to avoid the introduction of a bias by using incompletely developed juveniles. Since not all mussels excysted at the same day, freshly dropped off juveniles were maintained for a maximum of 14 days and supplied with food following Eybe et al. (2013) until they were randomly transferred to the mesh cages. Per study stream, five standard mesh cages, so-called *Buddensiek cages* (Buddensiek 1995), were installed. Each cage contained an equal number of 20 (10 x 2) juvenile pearl mussels per stock in separate chambers. Due to the restricted number of individuals from the Elbe populations, the mesh cages exposed in the ER did not contain mussels of stock EW and cages exposed in RO did contain juveniles from RO and DG only. Performance of juvenile mussels (described by growth and survival rates) was checked after three (before the first winter) and nine months (after the first winter) of exposure under a binocular microscope. Growth was defined as the ratios of the pooled maximal shell lengths of all living individuals of specific stocks and at specific sites at different time points. To minimize the potential effects of the measurements on the mussels, they were always kept covered by original stream water and handling times between retrieval and replacement in the streams were kept minimal. Juvenile mussel size was determined by measuring the maximum total shell length (+/- 2 µm) using a binocular microscope connected to the cell D software program.

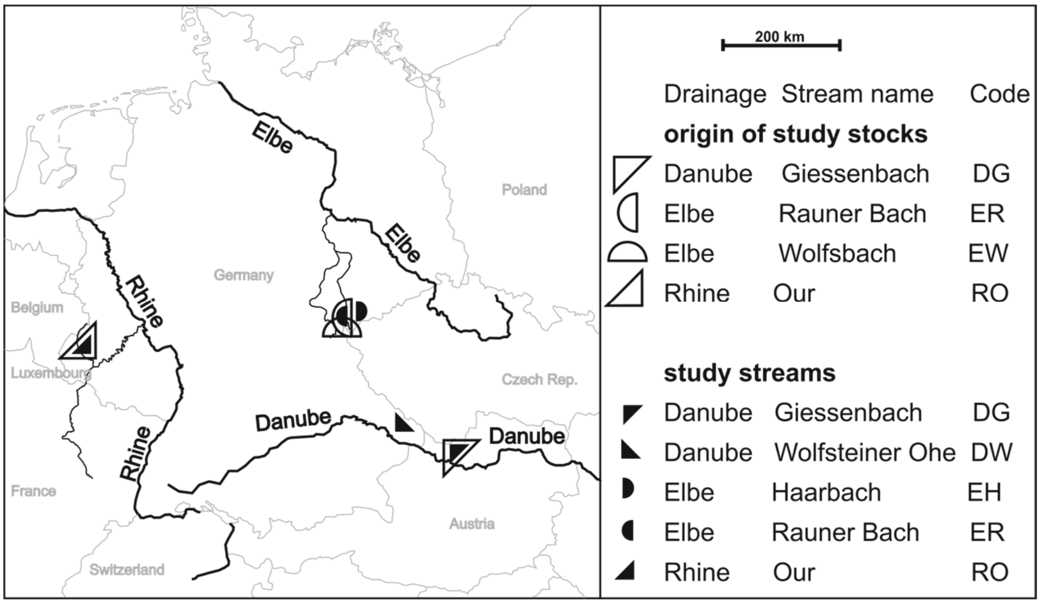
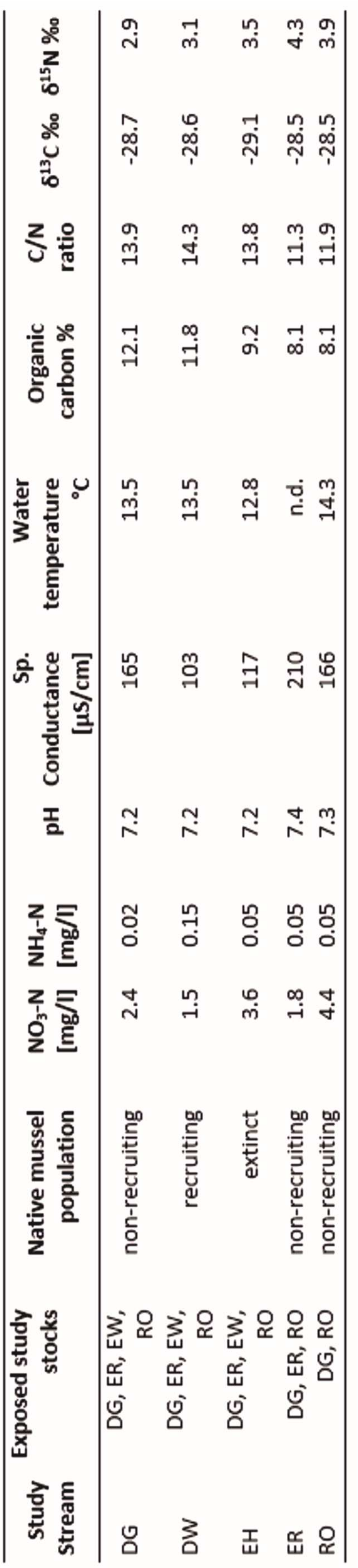


Figure 5.1: Location of mussel stock origin and study streams in relation to major Central European drainage areas. Codes are composed of initial letters of major drainage (first code letter) and mussel stock origin or study stream, respectively (second letter).

Table 5.1: Basic characteristics of the study streams used in the cross exposure experiments: Exposed study stocks, recruitment status of the native mussel populations and arithmetic mean values of NO_3-N, NH_4-N, pH, specific conductance, water temperature, organic carbon, C/N ratios, $\delta^{13}C$ and $\delta^{15}N$ are provided.



Study Stream	Exposed study stocks	Native mussel population	NO_3-N [mg/l]	NH_4-N [mg/l]	pH	Sp. Conductance [µS/cm]	Water temperature °C	Organic carbon %	C/N ratio	$\delta^{13}C$ ‰	$\delta^{15}N$ ‰
DG	DG, ER, EW, RO	non-recruiting	2.4	0.02	7.2	165	13.5	12.1	13.9	-28.7	2.9
DW	DG, ER, EW, RO	recruiting	1.5	0.15	7.2	103	13.5	11.8	14.3	-28.6	3.1
EH	DG, ER, EW, RO	extinct	3.6	0.05	7.2	117	12.8	9.2	13.8	-29.1	3.5
ER	DG, ER, RO	non-recruiting	1.8	0.05	7.4	210	n.d.	8.1	11.3	-28.5	4.3
RO	DG, RO	non-recruiting	4.4	0.05	7.3	166	14.3	8.1	11.9	-28.5	3.9

Water temperature at the study sites was measured continuously once per hour with temperature loggers (EL-USB-1, Lascar Electronics, Salisbury, UK). Detritus samples were collected from the streams every three months and stored at -20 °C until analysis. In the laboratory, organic carbon was determined by burning the samples for 5 h at 550 °C in a muffle oven. Stable isotope analysis ($\delta^{13}C$ and $\delta^{15}N$) was carried out for determination of detritus origin and quality as a food source for juvenile mussels. Samples were dried for 48 h at 40 °C, ground to fine powder and packed into tin capsules. $\delta^{13}C$ and $\delta^{15}N$ were measured with an isotope ratio mass spectrometer (IRMS, Delta plus, Finnigan MAT, MasCom GmbH, Bremen, Germany). The IRMS was connected to (via ConFlo II, Finnigan MAT, MasCom GmbH, Bremen, Germany) an elemental analyser (EA 1108, Carlo Erba, Thermo Fisher SCIENTIFIC, Milan, Italy). Stable isotope ratios are expressed in delta (d) notation, as parts per thousand (‰) relative to a Vienna-PeeDee Belemnite (V-PDB) standard for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$. Analyses of a solid internal laboratory standard (bovine horn, run after each ten samples) were used to calibrate C and N isotope determination revealing maximum standard deviations of 0.19‰ for $\delta^{13}C$ and 0.17‰ for $\delta^{15}N$. Deltas were calculated as follows: $\delta X = [(R_{sample}/R_{standard})-1]*1000$, where δX is $\delta^{13}C$ or $\delta^{15}N$, and R is the respective $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio.

Statistical analysis

Relative survival and growth rates were calculated per stock and study stream. Univariate general linear models (GLM) were calculated to quantify the contribution of study stream (i.e. environmental factors) and mussel stock to the variation in survival and growth rates, with separate models for the total, before winter and over winter periods: $Y = \mu + A + B + C + \varepsilon$ and $Z = \mu + A + B + C + \varepsilon$ where Y is the survival rate and Z the growth rate. μ represents the intercept and ε the random error term. Study stream (A) and mussel stock (B) are fixed factors and C is their interaction. Differences of survival and growth rates between streams and mussel stocks were analyzed with one-way ANOVA and Tukey's post-hoc test in case of normal distribution and homogeneity of data. Kruskal-Wallis test and Tamhane-T2 test were used in case of non-homogenous data. Stream specific differences in RO were tested by Student's t-test, as only two groups were compared. Linear regression analyses were carried out to assess the dependence of survival on size parameters (growth rates, shell length). Relative comparisons of stock specific survival and growth rates were expressed as differences between survival and growth of stocks in native versus non-native study streams. Analogously, stream-specific survival and growth rates (differences in survival and growth rates of the non-native versus native mussel stocks in a study stream) were computed, with positive values indicating higher growth or survival of non-native versus native stocks, or of stocks in non-native versus native streams (i.e. where mussel stocks originated from). All statistical analyses were performed with IBM SPSS 20.

5.4 Results

After nine months of exposure, mean survival of juveniles was 16.1%, mean growth rate was 79.4% and mean shell length was 0.77 mm. Mean values of stock and stream specific total survival and growth rates ranged from 1 (stock RO in ER) to 33% (stocks DG and EW in DG) and from 60 (stock ER in EH) to 102% (stock ER in DW), respectively.

Analysis of univariate general linear models revealed that study stream and mussel stock contributed significantly to the variation in juvenile performance, though model contributions varied by date and variable. Significant contributions of models to the variation in total, before winter and over winter survival were detected with $p < 0.001$ and $r^2 = 0.536$, 0.802 and 0.665, respectively. In contrast, the model parameters only contributed significantly to total and before winter growth rates ($p < 0.001$, $r^2 = 0.588$ and $p = 0.01$, $r^2 = 0.412$) but not to over winter growth ($p = 0.799$). In all models, variation in growth rates was explained mainly by study stream, whereas mussel stock had a stronger influence on variation in survival rates. For instance, mussel stock explained 40.9% of the variation in total survival ($p < 0.001$) whereas study stream accounted only for 15.0% ($p = 0.034$).

Multiple comparisons of stock and stream-specific performance supported the results of GLM analyses. Overall, the highest growth rates were found in warmer study streams with mean water temperatures of at least 13.5 °C during summer (DW, DG, RO), whereas cooler water temperatures (12.8 °C) as in the EH resulted in reduced growth (Table 5.1). The highest growth rate and shell length before winter were detected in the DW, where high water temperatures and the highest C/N ratios in detritus samples were found. However, differences for C/N ratios were not significant between streams (Kruskal-Wallis test, $p = 0.284$). $\delta^{13}C$ values of detritus samples ranged closely around -28‰ and $\delta^{15}N$ values varied around 3‰ in all streams.

A comparison of stream-specific total growth rates of single study stocks revealed significant differences for all stocks except RO (one-way ANOVA, $p < 0.04$ and $p = 0.612$). Total growth of the stocks DG, ER and EW was highest in the study stream DW. However, in case of stock DG, total growth was similarly high in the native stream DG and RO (Tukey-HSD, $p = 0.119$ and 0.931, respectively). The highest total survival rates were detected in the streams DW and the DG (Fig. 5.2; one-way ANOVA $p = 0.003$). Differences were significant between DW and ER (Tukey-HSD, $p = 0.022$) as well as between DG and ER and RO, respectively (Tukey-HSD, $p = 0.005$ and 0.028). Over-winter survival rates in the DW (94.1%) were significantly higher than in the other study streams (Tukey-HSD, $p < 0.001$) suggesting a correlation between winter survival and shell length before winter. This finding is supported by regression analysis revealing highly significant, positive relationships of winter and total survival rates to shell lengths and growth rates before winter. In contrast, survival before winter did not depend on these factors (Fig. 5.3). Mussels of the RO stock had the lowest growth rates and shell lengths before winter. Consequently, over-winter and total survival rates were significantly lower in the Rhine stock compared to the Danube and Elbe stocks (Tukey-HSD, $p = 0.02$ and Tamhane-T2 test, $p < 0.001$). This result remained the same, no matter if streams were analyzed simultaneously or separately. Stock-specific total survival did not differ between study streams (one-way ANOVA, $p \geq 0.133$).

Strongest indications of local adaptation were found in the stock DG. This stock revealed highest total survival rates of 33% in its native environment compared to 13-27% in the other streams (one-way ANOVA, $p = 0.135$). Total growth rates were highest in DW with 101%, but were not significantly lower in the native stream DG (81%, Tukey-HSD, $p =$

0.119). Furthermore, non-native stocks did not perform better in the DG than the native stock,

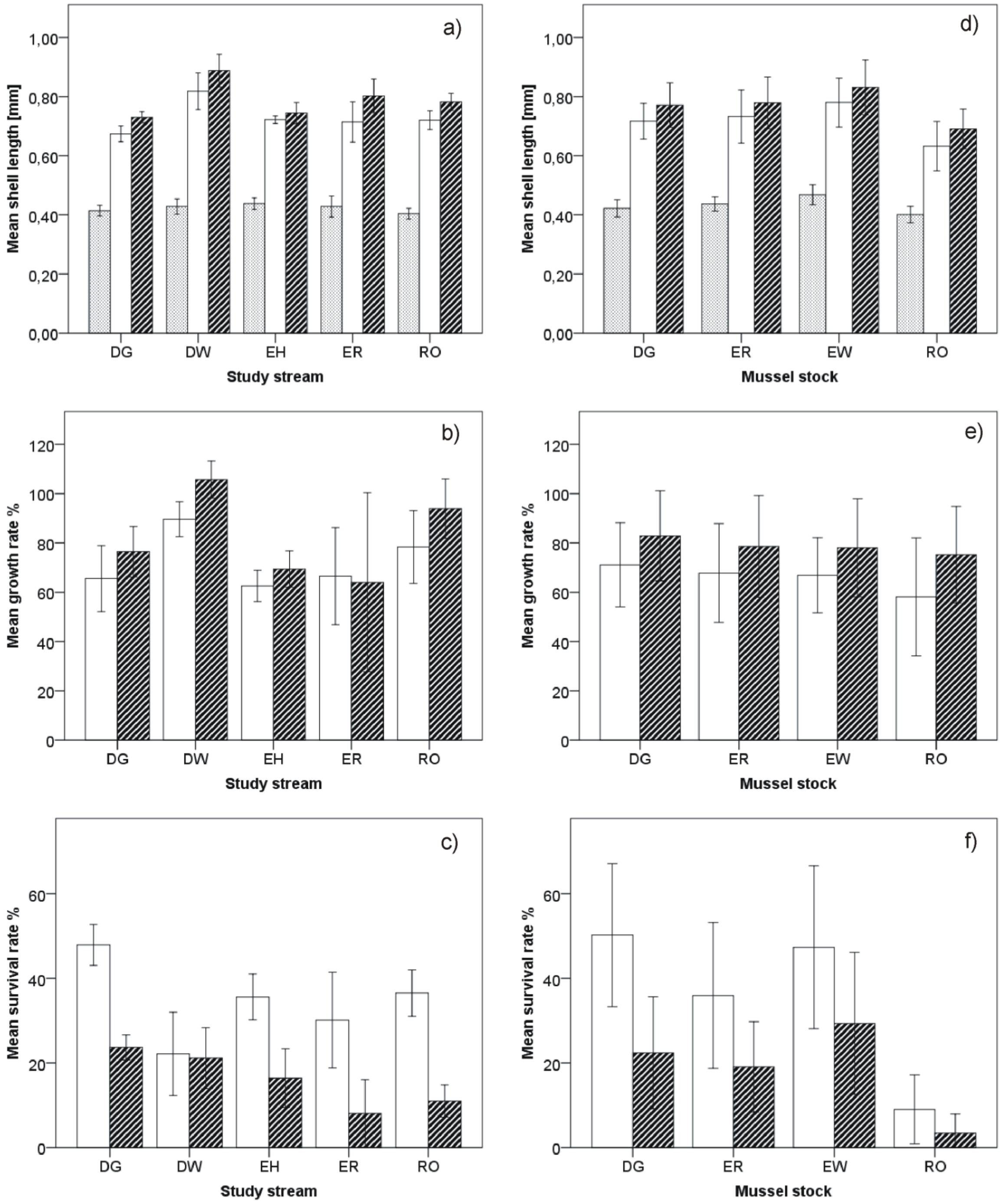


Figure 5.2: Study stream specific (a-c) and mussel stock-specific (d-f) initial (dotted bars), before winter (white bars) and total (shaded bars) mean shell lengths, growth rates and survival rates with standard deviations.

though performance of DG was not significantly increased, except for the comparison of total survival to RO (Tukey-HSD, p = 0.025). In contrast, stock RO rather showed tendencies of maladaptation, as in study stream RO total survival of RO was significantly lower than of DG with 3% compared to 19%, respectively (Student's t-test, p = 0.001). Total growth rate was also lower with 76% compared to 96%, though not significantly (Student's t-test, p = 0.245). It is important to note that time can substantially influence the relative performance of study stocks (Fig. 5.4). For instance, before winter survival rates of stock DG were significantly lower in stream DW than in stream DG (29% compared to 60%; Tukey-HSD, p = 0.009), but total survival rates became similar to each other with 27% and 33% (Tukey-HSD, p = 0.935).

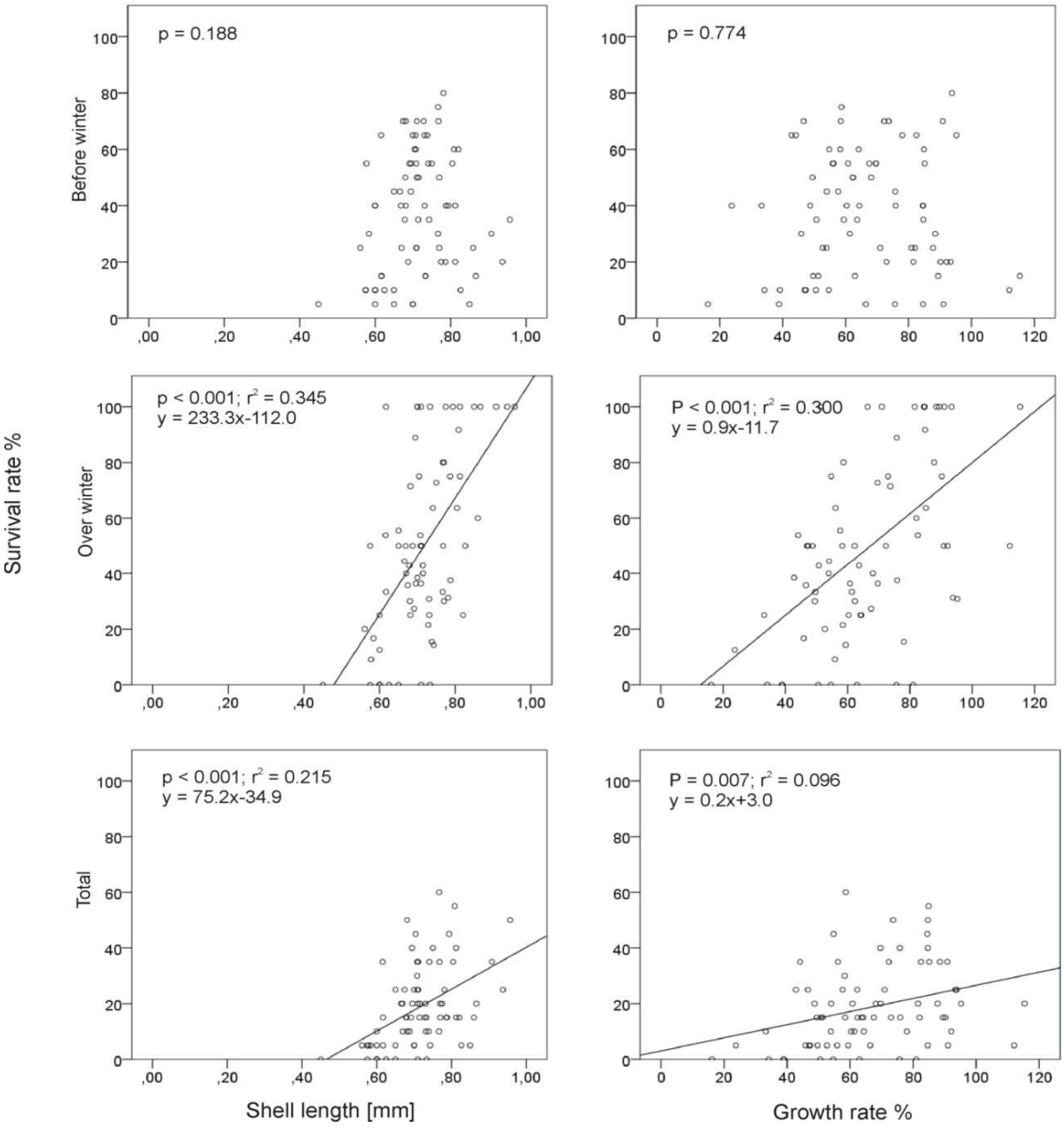


Figure 5.3: Before winter, over winter and total survival rates as a function of juvenile mussel shell length and growth rate.

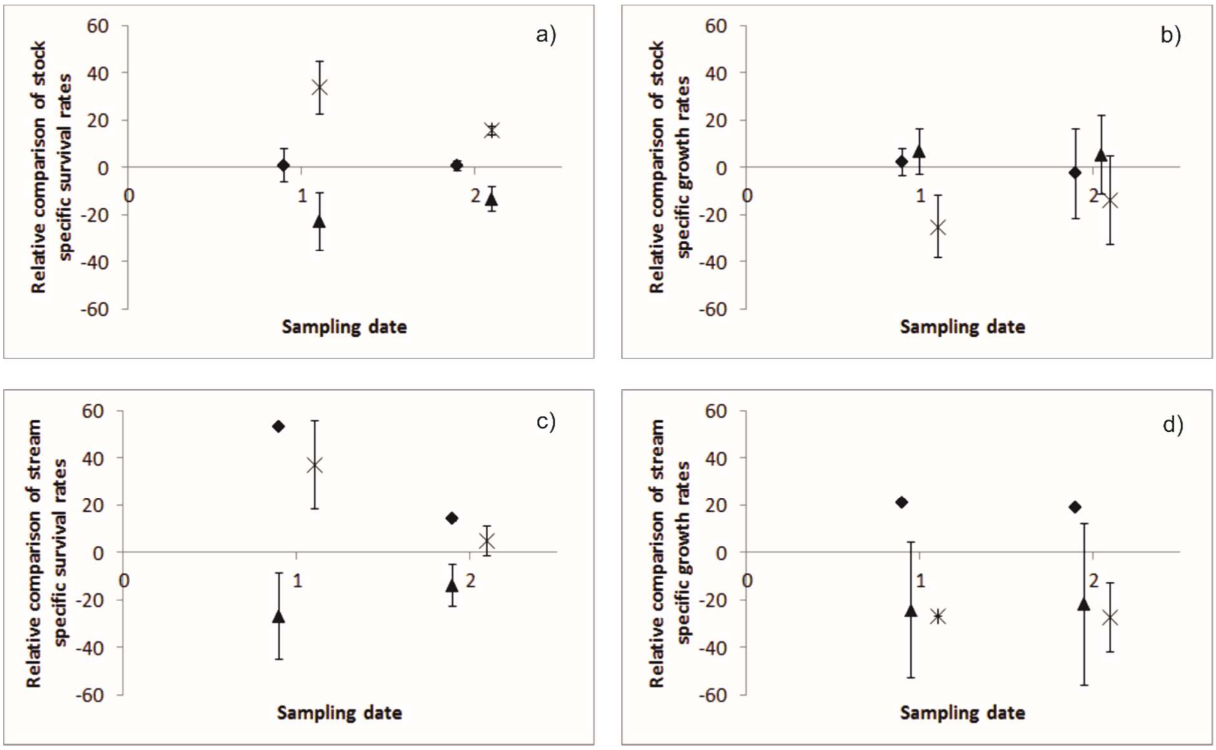


Figure 5.4: Relative comparison of mussel stock specific a) survival and b) growth rates (i.e. differences between survival and growth of stocks in native versus non-native study streams) as well as study stream specific c) survival and d) growth rates (i.e. differences of survival and growth rates between native versus non-native mussel stocks in a study stream) after three and nine months of exposure (sampling dates 1 and 2). Quadrats = RO; Triangles = DG; Stars = ER. Note that positive values indicate higher growth or survival of non-native versus native stocks, or of stocks in non-native versus native streams (i.e. where mussel stocks originated from).

5.5 Discussion

This study tested the influence of freshwater pearl mussel stock and study stream conditions on juvenile mussel performance with a special focus on local adaptation and the use of juvenile *M. margaritifera* as bioindicators for stream habitat quality. The results indicate a significant influence of both, mussel stock and study stream conditions, on juvenile performance and point to a variable amount of local adaptation among pearl mussel stocks. This specificity makes a careful consideration of mussel stock obligatory to avoid bias in bioindication studies.

Stream specific influences on juvenile mussel performance

Several studies indicate that key habitat parameters determine survival and growth rates of juvenile freshwater pearl mussels regardless of mussel stock. For instance, Buddensiek (1995) and Hruska (1992) found a correlation between juvenile growth and water temperature during the summer growing season. Shell length is considered as an indicator for juvenile mussel fitness and higher shell lengths are believed to increase the probability of winter survival (Lange & Selheim 2011). These results were confirmed in this study, as juveniles exposed in the warmest study streams grew fastest over summer and a correlation between shell length and winter survival was detected. In addition to water temperature, food quality (e. g. detritus composition) is important for the performance of juveniles. In our study, organic carbon and C/N ratio tended to be higher in streams with higher survival and growth rates, though differences were not significant. In contrast, Geist & Auerswald (2007) did not observe any separation between functional and non-functional pearl mussel streams in terms of detritus composition on a European scale, indicating that this factor may only locally differentiate high and low quality streams. Organic carbon content and C/N ratios were generally lower in their study with mean values of 4.01% and 11.28, respectively. The $\delta^{13}C$ values of approximately -28‰ indicated that all streams are heterotrophic systems and detritus originated mainly from terrestrial material, as this signature is typical for C_3 plants (Troughton et al. 1974). The $\delta^{15}N$ values of about 3‰ cannot be as clearly matched, as ^{15}N compounds are processed in various transformation pathways and can therefore deviate from the signatures of the source materials (Kellman & Hillaire-Marcel 2003). Furthermore, signatures of different nitrogen compounds derived from the same source material can vary (Bedard-Haughn et al. 2003). Nevertheless, low $\delta^{15}N$ values, as found in this study, are usually associated with forested catchments and low human influence (Harrington et al. 1998). Agricultural catchments, especially with intensive livestock farming (due to manure and septic waste production) are characterized by elevated levels of $\delta^{15}N$ values of 6-20‰ (Harrington et al. 1998; Lefebvre et al. 2007; Ohte 2013; Peterson and Fry 1987) and are unlikely to be of high importance for the river stretches analyzed herein. Yet, nitrate input from artificial fertilizers is known to have $\delta^{15}N$ signatures corresponding to the values in this study (Bedard-Haughn et al. 2003; Ohte 2013) and therefore cannot be excluded to play a role in some of the investigated catchments.

Stock-specific influences on juvenile mussel performance

In contrast to these obviously generally valid correlations between abiotic conditions and juvenile performance, univariate GLM indicated a significant influence of mussel stock as well, particularly on survival rates. This observation points to local adaptation of mussel stocks and is in accordance with previous observations of strong genetic population structuring and host fish specificity down to the subpopulation level (Geist & Kuehn 2005; Geist et al. 2010; Karlsson et al. 2013). However, the relative comparison of stock and stream specific survival and growth rates suggests generally low levels of local adaptation as well as different adaptation levels of the study stocks. There are various explanations for these inconsistencies in adaptation levels. It was shown that local adaptation is scale-dependent and in some species can be detected on the microscale, whereas in others at least a regional scale has to be considered (Kaltz & Shykoff 1998). Furthermore, population size and intra-population genetic variability influence the ability to adapt to specific or changing environmental conditions. Due to the small population sizes of the mussel stocks used in this study, it cannot be excluded that this hypothesis only applies to some of the study stocks. Jones (2013) further proposed that species can only show local adaptation to intact, but not to degraded habitats. Nearly all Central European freshwater pearl mussel streams are presently considered more or less degraded as evident from a lack of natural recruitment in most of them (Geist 2010; Sousa et al. 2014). Additionally, differences during the parasitic stage may also contribute to variation, not only on mussel stock but even on an individual level, as unionid juveniles receive nutrients from their host fish (Fritts et al. 2013). Depending on the fitness of host fish specimen and the intensity of their immune response, excysting juvenile mussels may start at different energetic levels. In addition, there are indications that excystment timing may influence size and fitness of juvenile mussels (Jung et al. 2013). Some experiments also found a correlation between glochidial densities on the host fish and their size after excystment, though the majority of studies did not confirm this result (Jung et al. 2013; Taeubert et al. 2010). To reduce individual differences as far as possible, locally infested host fish were kept under identical conditions for juvenile mussel excystment and juveniles were further kept under identical conditions until start of the experiment. Nevertheless, the stock-specific results should be interpreted with caution until they are being confirmed in a greater dataset.

Conclusions for bioindication and conservation

Freshwater mussels are considered target species for conservation, at the same time matching the concepts of flagship, keystone, umbrella and indicator species (Geist 2010).

Suitable bioindicators are characterized by their relevance, reliability, robustness, responsiveness and reproducibility. The freshwater pearl mussel fulfils all of these criteria, but bioindication experiments with endangered juvenile pearl mussels appear only useful in potential habitats (i.e. oligotrophic, silicate streams) and in situations in which the success of restoration measures or chances of reintroduction are to be assessed. Generally, the freshwater pearl mussel is notably one of the most sensitive freshwater organisms, reacting highly sensitive to changes in abiotic habitat conditions (Bauer 1988; Geist 2010; Geist & Auerswald 2007; Hastie et al. 2000; Oesterling et al. 2008; Taskinen et al. 2011). Due to the high conservation status and mostly small population sizes, the availability of adult individuals is low. However, the availability of juveniles is continuously increasing due to intensive breeding efforts throughout Europe (Gum et al. 2011), during which juveniles are already most often exposed in Buddensiek cages in streams for rearing purposes, as was the case in our study. Consequently, bioindication would just increase the benefit of a system already in use, without sacrificing an additional number of juvenile mussels. The rearing success of juvenile mussels in Buddensiek cages was previously controversially discussed in the literature (Gum et al. 2011; Schmidt & Vandre 2010). Based on the obvious suitability of the exposure setup of our study as well as results from Gum et al. (2011) and Spisar (pers. comm.), this system appears appropriate for use in bioindication studies that aim at testing water and nutrient quality of streams. The survival rates in our experiment were comparable to or higher than survival under natural conditions, which is estimated to range around 5% during the juvenile phase (Young & Williams 1984) and revealed stream specific variation with better juvenile performance in study streams hosting the most intact mussel populations. However, the fact that significant stock-specific differences in juvenile performance were detected, demands a careful consideration of this parameter in the interpretation of bioindication results. The direct comparison between bioindication results using different mussel stocks should be avoided. Furthermore, cages were exposed to the free-flowing water in this experiment. Exposure to the substratum will alter bioindication results with probably further reduction of survival rates.

Different study stocks showed different levels of local adaptation, but the specific cause for this observation remains unclear. Here, further research including completely intact habitats and mussel stocks may help to further clarify the picture. Comparisons of stock-specific survival in native versus non-native streams implicate that exposure of juveniles outside of native habitats is able to increase success of breeding programs, although rearing of juveniles in the native stream is still the preferable method. Consequently, rearing of juveniles in non-native habitats is recommendable in case of low breeding success in native habitats to bridge the time which is needed to restore the stream and for the purpose of risk spreading.

6 Timing matters: Species-specific interactions between spawning time, substrate quality and recruitment success in three salmonid species

A similar version of this chapter was published: Sternecker K, Denic M, Geist J (2014). Timing matters: Species-specific interactions between spawning time, substrate quality and recruitment success in three salmonid species. Ecology and Evolution 4 (13): 2749-2758.

6.1 Abstract

Substratum quality and oxygen supply to the interstitial zone are crucial for reproductive success of salmonid fishes. At present, degradation of spawning grounds due to fine sediment deposition and colmation are recognized as main factors for reproductive failure. In addition, changes in water temperatures due to climate change, damming, and cooling water inlets are predicted to reduce hatching success. We tested the hypothesis that the biological effects of habitat degradation depend strongly on the species-specific spawning seasons and life history strategies (e.g. fall- vs. spring-spawners, migratory vs. resident species) and assessed temperature as an important species-specific factor for hatching success within river substratum. We studied the species-specific differences in their responses to such disturbances using egg-to-fry survival of Danube Salmon (*Hucho hucho*), resident brown trout (*Salmo trutta fario*) and migratory brown trout (*Salmo trutta lacustris*) as biological endpoint. The egg incubation and hatching success of the salmonids and their dependence on temperature and stream substratum quality were compared. Hatching rates of Danube salmon were lower than of brown trout, probably due to higher oxygen demands and increased interstitial respiration in spring. Increases in maximum water temperature reduced hatching rates of resident and migratory brown trout (both fall-spawners), but were positively correlated to hatching rates of Danube salmon (a spring-spawner). Significantly longer incubation periods of resident and migratory brown trout coincided with relatively low stream substratum quality at the end of the egg incubation. Danube salmon seem to avoid low oxygen concentrations in the hyporheic zone by faster egg development favoured by higher water temperatures. Consequently, the prediction of effects of temperature changes and altered stream substratum properties on gravel-spawning fishes and biological communities should consider the observed species-specific variances in life history strategies to increase conservation success.

6.2 Introduction

Salmonid fishes are adapted to cool, oligotrophic rivers and lakes. Throughout their distribution range, they are considered target species in conservation with a high ecologic and socio-economic value. For instance, species such as the Danube salmon (*Hucho hucho* L.) are top predators of their ecosystems with important regulatory functions (Geist et al. 2009). In general, salmonids are main target species for aquaculture and recreational fishing (Denic & Geist 2010). Some species like brown trout (*Salmo trutta* L.) or Atlantic salmon (*Salmo salar* L.) are obligate hosts for the larvae of the freshwater pearl mussel (*Margaritifera margaritifera* L.) (Geist et al. 2006; Taeubert et al. 2010; Young & Williams 1984). Consequently, there is a high interest in stable and healthy salmonid populations, resulting in a diversity of habitat assessment and restoration efforts globally (Denic & Geist 2010; Pander & Geist 2013; Sternecker et al. 2013b). Habitat restoration is required, because most salmonid species have severely declined in recent decades and are currently endangered (e.g. Geist 2011; Jungwirth et al. 2003; Kemp et al. 2011; Kondolf 1997; Thorstad et al. 2008).

Salmonids are lithophilic fish species, i.e. they deposit their eggs in the interstitial zone of suitable gravel banks. For successful reproduction, all salmonid species depend on clean stream substratum (i.e. with low fine sediment content) with an intact and well-oxygenated interstitial zone for egg and larval development (Crisp 1996; Ingendahl 2001; Kondolf 2000; Malcolm et al. 2003; Greig et al. 2007b; Rubin & Glimsäter 1996; Sternecker & Geist 2010; Sternecker et al. 2013a, b).

Several studies recognized fine sediment introduction and colmation (i.e. blockage of streambed interstitial spaces by the ingress of fine sediments and organic material; Buss et al. 2009) of spawning gravels as major factors for reproductive failure (Acornley & Sear 1999; Franssen et al. 2012; Jungwirth 1978; Levasseur et al. 2006; Soulsby et al. 2001b). Due to the influence of temperature on oxygen solubility in water and on salmonid egg development, rising water temperatures in the course of global warming, damming, and cooling water inlets are expected to further reduce salmonid reproduction rates (Battin et al. 2007; Jonsson & Jonsson 2009; Lake et al. 2000).

However, different species and evolutionary significant units (Moritz 1994) of salmonids use different time periods for spawning and may thus be differently affected by changes in their spawning habitats. Such differences among populations of Pacific Chinook salmon (*Oncorhynchus tshawytscha*) have already been observed in previous studies (e.g. Crozier &

Zabel 2006), but remain untested for Atlantic salmonids. Such species-specific differences of life history strategies are well-known (e.g. Sternecker & Geist 2010), but the potentially resulting differences in the population-level responses in spring- versus fall-spawning salmonids have not been considered in the context of habitat degradation. In Germany, the Danube Salmon (*Hucho hucho*) is a migratory spring-spawner, whereas the resident brown trout (*Salmo trutta fario*) and the migratory brown trout (*Salmo trutta lacustris*) are typical fall-spawners. As a consequence of their individual life history strategies, the reproductive periods of the study species do not overlap in time. Thus all three salmonid species face different environmental conditions during reproduction.

In the current study, we investigated to what extent different physical impacts on reproductive success are species-dependent by comparing data of monitoring experiments in three native European salmonids: the Danube Salmon, resident brown trout and migratory brown trout. We tested the hypotheses that i) Danube salmon as a spring-spawner is less affected by increased fine sediment deposition and colmation than fall-spawning migratory and resident brown trout due to shorter egg development periods and ii) increasing water temperatures reduce hatching rates of the study species.

6.3 Material and methods

Study area

Three typical alpine salmonid streams in Bavaria, Germany, inhabited by all three study species, were selected for this case study (Fig. 6.1). The study streams were the rivers Lech, Moosach and Obernach (mean annual discharges: 82.9 at water gauge Landsberg, 2.6 and 1 $m^3 s^{-1}$, respectively). They are all anthropogenically-manipulated limestone streams within the Danube catchment, with regulated flow regimes and migration barriers due to dams and hydropower generation. In all three rivers, a decline of salmonid recruitment during the last decades was observed. Spawning grounds, where natural reproduction of at least one of the study species was observed, were chosen as study sites and could be natural or man-made gravel banks (Pulg et al. 2013, Sternecker et. al 2013b).

Microhabitat assessment

Differences in spawning habitat quality were analysed using egg-to-fry development success (active bioindication) as a biological endpoint (for details, see Sternecker et al. 2013b). A total of 77 egg sandwich boxes (ES; Pander et al. 2009) were exposed during 3 consecutive years and spawning seasons (2009 - 2011). Of these ES, 37 were filled with

Danube salmon eggs (16 x River Lech, 21 x River Moosach), 29 with eggs of resident brown trout (10 x River Lech, 19 x River Moosach) and 12 with eggs of migratory brown trout (River Obernach). Each ES was filled with 90 salmonid eggs in separate chambers and buried in the substratum to line up with the substratum surface, i.e. 30 eggs were exposed in substratum depths 0-50mm, 50-100mm and 100-150mm, respectively. The ESs were placed haphazardly in the gravel banks in the first studied spawning season. During the consecutive spawning seasons, the location of the ESs were chosen on the basis of the first season. For winter bioindication, 5-12 female and 3-6 male resident brown trout (RBT) of a hatchery stock (Landesfischzuchtanstalt Mauka, Germany) and 2 females and 3 males of autochthonous migratory brown trout (MBT) from Lake Walchensee (River Obernach is a tributary of Lake Walchensee) were used as spawners. For spring bioindication, 2 female and 3 male Danube Salmon (DS) of the hatchery stock of the “Fischereilicher Lehr- und Beispielbetrieb Lindbergmühle” were used as spawners. In each experiment, fertilized eggs of different spawners were mixed and randomly distributed to ES. ES were exposed considering local stream bed variability and the avoidance of spatially autocorrelated datapoints (Braun et al. 2012). Hatching (egg-to-fry development) success of substratum exposures was analysed after hatching in field references (anchored floating box with 3 x 100 eggs each study site) was observed (according to Sternecker et al. 2013b). Field references allowed to observe egg development without disturbing ES experiments and to detect hatching success under river water conditions without the effects of stream substratum during the egg incubation.

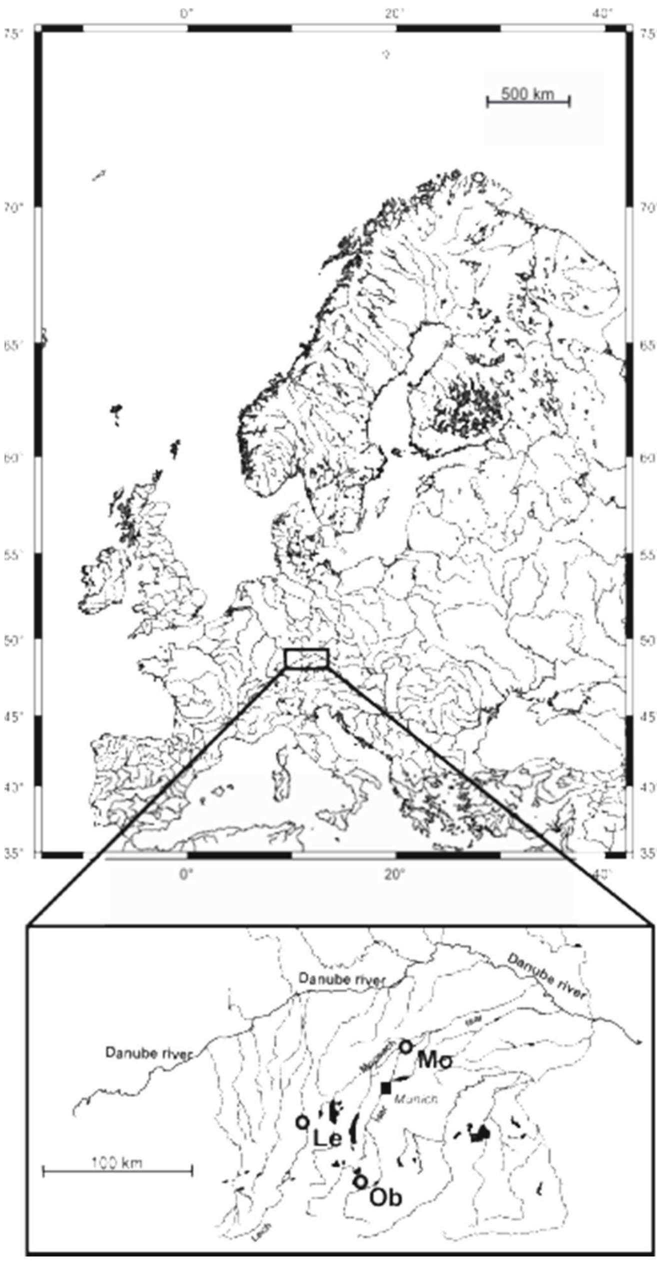


Figure 6.1: Location of the study sites in the rivers Lech (Le), Moosach (Mo) and Obernach (Ob).

Interstitial water samples for water quality analysis were taken from the measurement unit of each ES at 50 mm, 100 mm, and 150 mm substratum depth. Interstitial water conditions were characterized by analysing dissolved oxygen concentration (mg L^{-1}), pH, specific conductance (corrected to 20°C), redox potential (mV) within water samples using handheld oxygen-, conductivity- and pH-meters (WTW, Weilheim, Germany). Nitrate NO_3^- (mg L^{-1}), nitrite (NO_2^-, mg L^{-1}), and ammonium (NH_4^+, mg L^{-1}) were determined by using analytical kits (Spectroquant, Merck, Germany) and a PC spectrometer (photoLab S12, WTW, Weilheim, Germany). Next to every ES, redox potential was measured in three substratum depths (50mm, 100mm, and 150mm) according to Geist & Auerswald (2007). Water temperature during egg incubation was continuously (one data-point per hour) monitored by data loggers (EL-USB-1, Lascar Electronics, Salisbury, UK). All other parameters were measured three times, i.e. at the beginning of egg exposure, after eggs reaching the eyed stage and after hatching of juveniles. Habitat quality in the hyporheic zone is determined by the exchange rate with the free-flowing water, which is reduced by fine sediments clogging interstitial macropores. This causes deviances in physicochemical parameters between the hyporheic zone and the free-flowing water. Differences of exchange rates were evaluated by additional measurements of all physicochemical parameters in the free-flowing water proximal to each ES (Sternecker et al. 2013b).

Statistical analysis

Differences in hatching success (egg-to-fry survival) between species, as well as between rivers, were analysed using time and water temperature as determining variables. Duration of the incubation period was expressed in absolute values of days (d) and using sums of degree-days (dd). Dd were calculated as the product of mean water temperature during egg incubation and absolute incubation period in days. Bivariate correlations were calculated between hatching rate, mean and maximum water temperature during the incubation period, respectively. Relative hatching rate (calculated for each ES as a proportional hatching rate of eggs) was used for the following calculations as proposed in Sternecker et al. (2013b). Delta values were calculated by the difference between the value in the free-flowing water and in the interstitial water, respectively. For analysing the differences of interstitial water conditions between the rivers Moosach, Obernach and Lech during different spawning seasons, discriminant analyses (DCA) were conducted to separate groups of high and low hatching success for the individual rivers and individual salmonid species, respectively.

For the evaluation of the interstitial flow-through, the angles between discriminant functions (considering absolute and delta values separately) were calculated according to Batschelet (1979). High and low hatching success of every individual species was determined for DCA using cluster analyses since the distribution of hatching success varied between species. Differences between species, spawning season, study river and study year were calculated using Mann-Whitney U-tests. All statistical analysis was performed using the software IBM SPSS Statistics 20.

6.4 Results

Egg incubation period, degree-days until hatch, and mean water temperature during egg incubation varied with species, spawning season, study river and study year (Fig. 6.2). The differences between spring-spawning DS and fall-spawning MBT as well as RBT were highly significant (Mann-Whitney U-tests, $p < 0.001$ for each parameter, respectively), whereas the conditions of egg development of RBT and MBT were comparable with similar egg incubation periods, degree-days until hatch and water temperatures (Table 6.1).

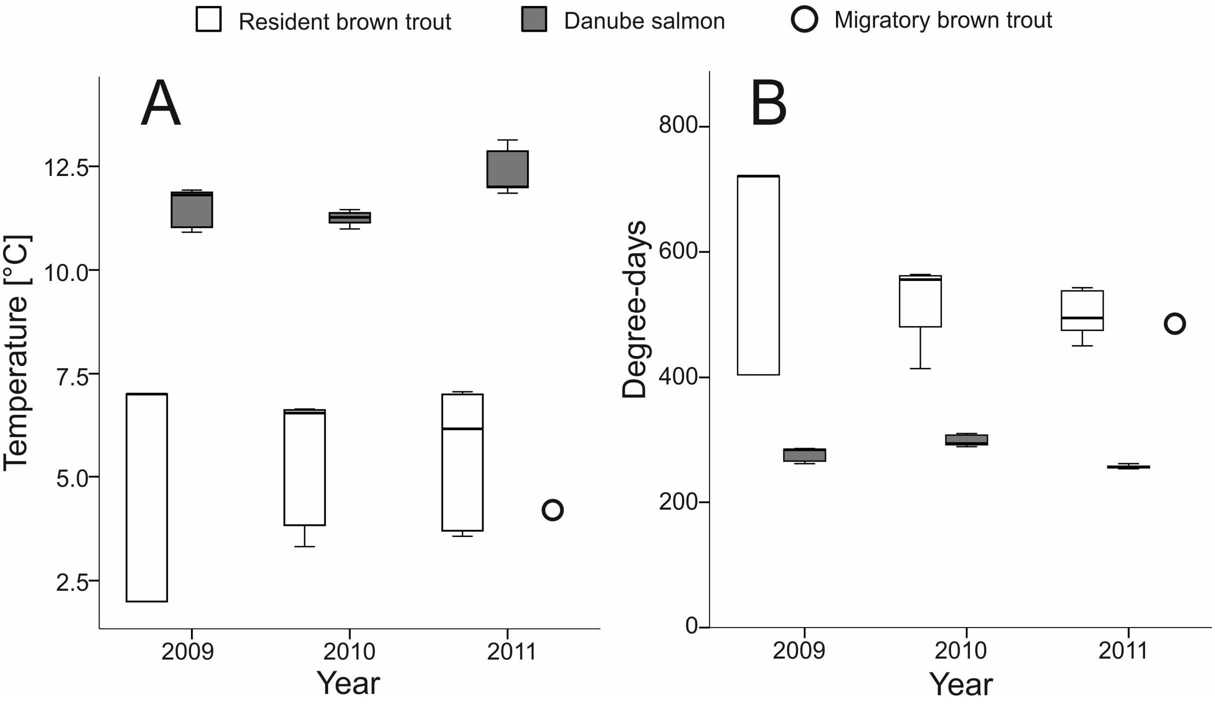


Figure 6.2: A) Mean temperature [°C] during salmonid egg development, and B) period of egg development [sum of degree-days] in three spawning seasons for Danube salmon (*Hucho hucho*) in the rivers Lech ($n_{2009} = 6$, $n_{2010} = 7$ and $n_{2011} = 6$) and Moosach ($n_{2009} = 7$, $n_{2010} = 8$ i and $n_{2011} = 8$), for resident brown trout (*Salmo trutta fario*) in the rivers Lech ($n_{2009} = 6$, $n_{2010} = 4$ and $n_{2011} = 3$) and Moosach ($n_{2009} = 8$, $n_{2010} = 7$ and $n_{2011} = 6$) and for migratory brown trout (*Salmo trutta lacustris*) in river Obernach ($n_{2011} = 1$), respectively (Box-Whisker plots; Whiskers: maximum, minimum; Box: 0.25 quartile, median and 0.75 quartile).

Table 6.1:Temperature (mean, minimum and maximum), sum of degree-days as well as incubation period (days) and hatching rate of resident brown trout (*Salmo trutta fario*), Danube salmon (*Hucho hucho*) and migratory brown trout (*Salmo trutta lacustris*); means are provided with standard deviations; the number of data loggers for measuring temperature was n = 15 (river Moosach) and n = 8 (river Lech) for resident brown trout, n = 18 (river Moosach) and n = 22 (river Lech) for Danube salmon and n = 1 (river Obernach) for migratory brown trout.

River	Species	Mean temperature [°C]	Minimum temperature [°C]	Maximum temperature [°C]	Mean sum of degree-days [°C]	Mean incubation period	Hatching rate [%]
Moosach	*Salmo trutta fario*	7 ± 0.3	3	15	554 ± 55.3	84 ± 5.8	31 ± 34.9
Lech	*Salmo trutta fario*	3 ± 0.6	1	9	453 ± 36.5	126 ± 32.5	56 ± 22.6
Moosach	*Hucho hucho*	12 ± 0.3	8	17	277 ± 17.2	24 ± 2.0	37 ± 27.1
Lech	*Hucho hucho*	12 ± 0.8	9	16	280 ± 23.1	24 ± 2.0	18 ± 19.3
Obernach	*Salmo trutta lacustris*	4	2	8	485	116	75 ± 25.8

Brown trout hatching success in field references was 38%-89% in the river Moosach (RBT), 71%-98% in the river Lech (RBT) and 96% in the river Obernach (MBT). The DS hatching success in field references exposed to open water, i.e. excluding substrate effects investigated with the ES exposures, was 70%-83% in the river Moosach and 41%-84% in the river Lech. Overall, ES exposure hatching rates of MBT (75%) were significantly higher than the RBT hatching rates (42%) and DS hatching rates (30%). When ES exposure hatching rates were analysed river specifically, RBT hatching rates in the River Lech were similar to MBT hatching rates. DS hatching rates in the River Lech were significantly lower than in all other groups, except for RBT in the River Moosach. Regarding the studied species, the impact of stream substratum quality acted longer during the fall spawning season than in spring, as evident from the extended egg incubation periods of RBT as well as MBT versus DS. Mann-Whitney U-tests revealed significant differences for RBT ($p < 0.001$), but not for DS ($p > 0.569$) concerning egg incubation periods, sums of degree-days until hatching and mean water temperatures between the rivers Moosach and Lech. These patterns remained highly similar between study years.

High rates of correct classification of hatching rates by DCA into high and low ES exposure hatching success groups indicates that a linear combination of interstitial water parameters successfully separates the two groups (Table 6.2). In the discriminant function, positive coefficients with high values were evident for oxygen concentration and redox potential, stressing the importance of these two variables. The weight and direction of action (positive or negative effect) of other coefficients varied by species. For instance, coefficients of fall-spawning MBT and RBT were similar to each other whereas coefficients of specific conductance varied among DS compared to MBT and RBT (Table 6.3). The correlation between discriminant functions of absolute and delta values (differences between interstitial and free-flowing water) was mostly low. The angle calculated between discriminant functions of absolute and delta values was highly variable with respect to species. An angle of $\theta = 78.3°$ indicated that differences between free-flowing and interstitial water were most substantial for RBT. The angles for functions of MBT and DS were smaller with $\theta = 53.1°$ and $\theta = 46.2°$, respectively. Yet, similarity of conditions of free-flowing and interstitial water differed substantially on the river scale, e.g. the angle of DS discriminant functions were $\theta = 66.7°$ in the River Moosach and $\theta = 48.7°$ in the River Lech.

The correlation between mean water temperature and hatching rate was weak, but the correlation between maximum water temperature during egg incubation and hatching rate was significant. There was a strong negative correlation for MBT and RBT (Spearman-Rho -0.711; $p < 0.001$) and a positive one for DS (Spearman-Rho 0.529; $p = 0.001$).

Table 6.2: Classification of the discrimination analysis (DCA); DCAs refer to the dependency of physicochemical parameters [O_2 = dissolved oxygen concentration (mg L^{-1}), pH, specific conductance (µS cm^{-1}; corrected to 20 °C), Eh = redox potential (mV), NO_3^- = nitrate (mg L^{-1}), NO_2^- = nitrite (mg L^{-1}) and NH_4^+ = ammonium (mgL-1)] on hatching success (relative rate) in the river Moosach (2009-2011), the river Lech (2009-2011), in both rivers (macro-scaled level) and the river Obernach (2011); absolute values (AV) and delta values (DV) of the physicochemical parameters were considered separately; delta values of the physicochemical parameters were calculated by the difference between interstitial and free-flowing water.

			Abs. Values		**Delta Values**	
RBT		Predicted class				
Moosach and Lech			> 50	< 50	> 50	< 50
	Actual class	> 50	95.1	4.9	89.4	10.6
		< 50	21.2	78.8	41.7	58.3
		% explained variance	87.8		75.9	
Moosach		Predicted class				
			> 50	< 50	> 50	< 50
	Actual class	> 50	92.0	8.0	88.0	12.0
		< 50	22.6	77.4	29.0	71.0
		% explained variance	83.9		78.6	
Lech		Predicted class				
			> 50	< 50	> 50	< 50
	Actual class	> 50	93.8	6.2	90.9	9.1
		< 50	0.0	100.0	0.0	100.0
		% explained variance	94.4		92.6	
DS		Predicted class				
Moosach and Lech			> 50	< 50	> 50	< 50
	Actual class	> 50	56.1	43.9	38.6	61.4
		< 50	32.1	67.9	11.3	88.7
		% explained variance	61.8		62.7	
Moosach		Predicted class				
			> 50	< 50	> 50	< 50
	Actual class	> 50	80.8	19.2	69.2	30.8
		< 50	29.7	70.3	40.5	59.5
		% explained variance	74.6		63.5	
Lech		Predicted class				
			> 50	< 50	> 50	< 50
	Actual class	> 50	61.3	38.7	74.2	25.8
		< 50	6.2	93.8	12.5	87.5
		% explained variance	72.3		78.7	
MBT		Predicted class				
Obernach			> 50	< 50	> 50	< 50
	Actual class	> 50	90.0	10.0	75.0	25.0
		< 50	0.0	100.0	0.0	100.0
		% explained variance	91.2		77.8	

Table 6.3: Discriminant analysis (DCA) referring to the dependency of physicochemical parameters on hatching success (relative hatching rate) of RBT, DS and MBT in the rivers Moosach (2009-2011), Lech (2009-2011) and Obernach (2011), respectively. Groups were defined by cluster analysis (high versus low hatching success), discriminatory power of absolute values as well as delta values of the physicochemical parameters (difference between interstitial and free-flowing water) were compared.

Variables	**RBT**		**DS**		**MBT**	
	Abs.	Delta	Abs.	Delta	Abs.	Delta
	Moosach and Lech				**Obernach**	
Redox potential	0.722	0.779	0.586	0.729	0.457	0.279
Oxygen	0.427	0.223	0.352	0.770	0.374	0.523
Specific	-0.435	0.422	0.640	0.267	0.183	-0.386
pH	0.338	0.362	-0.144	0.701	0.397	0.151
NO_3^- concentration	-0.440	0.292	0.606	0.307	0.275	0.089
NO_2^- concentration	-0.332	0.637	0.142	0.074	-0.114	-0.143
NH_4^+ concentration	0.045	0.097	-0.529	0.408	-0.089	0.172
	Moosach					
Redox potential	0.596	0.755	-0.387	0.614		
Oxygen	0.783	0.532	-0.494	0.375		
Specific	0.386	-0.025	0.472	0.268		
pH	0.407	0.245	-0.222	-0.056		
NO_3^- concentration	0.250	-0.130	0.277	0.385		
NO_2^- concentration	0.439	-0.301	0.393	0.284		
NH_4^+ concentration	-0.051	0.195	0.602	0.103		
	Lech					
Redox potential	0.287	0.438	0.354	0.376		
Oxygen	0.091	-0.079	0.868	0.667		
Specific	0.235	-0.397	-0.304	-0.245		
pH	0.394	-0.105	0.737	0.613		
NO_3^- concentration	0.030	-0.115	0.119	0.660		
NO_2^- concentration	-0.304	0.077	-0.070	0.075		
NH_4^+ concentration	-0.387	0.473	-0.280	0.257		

6.5 Discussion

The results of this study suggest that the differences in life history strategies of salmonid fish species results in different susceptibilities to substrate degradation and temperature change. Habitat degradation is thus likely to exert diverse mechanisms and directions of selection on different salmonid species. There is evidence that substratum degradation lowered reproductive success in all study species. In contrast, temperature increases reduce hatching rates of fall-spawning MBT and RBT, but are positively correlated to spring-spawning DS hatching rates. Consequently, the precise analysis of spawning habitat deficits (e.g. lack of stream substratum, substratum colmation or migration barriers) with respect to species-specific variances in life history strategies is crucial for the success of conservation management.

Differences in evolutionary effects of stream substratum and temperature on salmonid recruitment

The period of egg and larval development within the substrate is crucial for reproductive success. Re-degradation processes (e.g. fine sediment introduction) after redd-building induce compaction of substratum, which affects interstitial water conditions, especially towards the final stages of the egg and larval development (Glimsäter & Jarvi 2004; Jensen et al. 2009; Peterson & Quinn 1996a; Rubin et al. 2006; Sternecker et al. 2013a; Zeh & Doenni 1993). We showed that the egg development of DS is significantly faster than of RBT and MBT. Consequently, the period of time in which DS hatching success is affected by stream substratum degradation is shorter than of MBT and RBT. A shorter period of development in substratum by DS compared to RBT in the subsequent life stage, i.e. the emergence of fry, was shown previously (Sternecker & Geist 2010). Cumulative effects of shorter development times are important to consider, because it has also been shown that an earlier emergence of fry increases competitiveness of species and individual fish, respectively (Skoglund et al. 2013). Since both life stages (egg-to-fry and emergence of fry) benefit from an accelerated development, the total effect is likely to be even stronger than the one described herein. The hypothesis that DS is adapted to high quality substratum was confirmed by the smaller differences between interstitial and free-flowing water conditions compared to MBT and RBT at the end of egg development. The hatching success of spring-spawning DS was lower than the hatching success of fall-spawning brown trout at the same gravel banks. The reduced hatching rates of DS in the hyporheic zone suggest that DS have even higher spawning habitat quality requirements than RBT or MBT. That spring-spawners are more strongly affected by substratum colmation is also supported by the more serious decline of spring runs compared to fall runs in California Chinook salmon populations (Fisher 1994).

Although high DCA coefficients for oxygen content and redox potentials indicated that oxygen availability is important for all studied species, coefficients were highest for DS suggesting either extremely high oxygen demand of DS or increased oxygen depletion due to higher water temperatures and biological activity in spring. However, a high number of eggs and big redd sizes caused by the large body size of the female DS likely increase the probability of eggs within high substratum quality pockets on the micro-scale, because the eggs are distributed within a bigger area in the substratum. A high micro-scale variability of interstitial water conditions during egg development was previously detected (Malcolm et al. 2009; Sternecker et al. 2013a) and seems to be characteristic of the hyporheic zone of most stream ecosystems (Braun et al. 2012). Such variation at small spatial scales was recently described to buffer temporal fluctuations in early juvenile survival in Pacific Chinook salmon (Thorson et al. 2014). Substratum depth in our study was limited to 150mm, hence deeper zones with a potential flow-through of oxygen rich ground water was not considered resulting in a possible underestimation of hatching success (Peterson & Quinn 1996b). Furthermore, individual adaptation can moderate the effects of increasing fine sediment input or other consequences of climate change, as e.g. the burial depth of salmonid eggs is known to vary between individuals and populations within salmonid species (DeVries 1997). Hendry & Day (2003) hypothesized that a large egg size is advantageous during low oxygen supply within stream substratum. As a consequence, smaller fish with smaller eggs should be subject to stronger selective pressure than larger fish. Consequently, larger size of the DS females compared to brown trout females may be an evolutionary result of more adverse interstitial water conditions in spring.

The results of this study indicate that resident and migratory brown trout (both fall-spawners) depend more strongly on cool water temperatures, which was suggested by the strong negative correlation between maximum water temperatures and hatching success in our study. This makes them more susceptible to the effects of climate change, which is expected to cause more frequent temperature peaks in winter time in the future (Mauser et al. 2008). Additionally, these winter temperature peaks result in untimely snow melts and increased fine sediment mobilization after rain events due to low vegetation cover in catchments (Herringshaw et al. 2011). As a consequence, the risks of elevated fine sediment input into spawning grounds and of river bed scouring increase, which both negatively affect hatching success of fall-spawners (Battin et al. 2007; Goode et al. 2013; Jonsson & Jonsson 2009; Wedekind & Küng 2010).

Altogether, the spring- and fall-spawners in this study seem to be equally affected by temperature changes and reduced water quality in the interstitial zone. However, DS is currently more endangered than brown trout, most likely due to anthropogenic impacts that additionally affect this species (IUCN 2013). In particular, DS spawning migrations are hampered by habitat fragmentation, e.g. due to dams and hydroelectric power stations. A separate consideration of brown trout evolutionary significant units RBT and MBT further supports this hypothesis. MBT, which is a migratory form of brown trout, is endangered throughout most of its distribution range, whereas RBT lives stationary and shows the most stable populations of the studied species/evolutionary significant units (Denic & Geist 2010; Kottelat & Freyhof 2007; Lelek 1987).

Management implications

Selective forces on egg burying fish species and habitat degradation factors often have synergistic effects (Lake et al. 2009; Parrish et al. 1998). Such an adverse synergistic effect of stream substrate degradation and increased water temperature on salmonids was detected in our study and found to be species-specific. It is likely to be driven by variable and scale-dependent physicochemical and genetic impacts (Sternecker et al. 2013a; Thorson et al. 2014). Consequently, there is no general solution for the support of declining salmonid populations, as restoration concepts have to consider specific conditions of the river (stretch) to be restored as well as specific demands of the target species.

The examples in this study reveal that reasons for the lack of suitable spawning substrates can vary, which requires the use of different restoration techniques. In rivers like the Moosach, where substrates are clogged with fine material and often colmated, cleaning and loosening of substrates is an efficient restoration method (Pulg et al. 2013; Shackle et al. 1999; Sternecker et al. 2013a). On the other hand, in rivers with interrupted bedload transport such as the river Lech, the addition of clean gravel is recommended (Pulg et al. 2013). It has to be noted, that in the course of spawning ground restoration, species-specific habitat preferences have to be considered as suitable gravel size, flow velocity and water depth at spawning grounds are fish size and species dependent (Crisp & Carling 1989; Kondolf & Walman 1993; Louhi et al. 2008). Thus, systematic comparisons of different types of substrate restoration are mandatory for the assessment of their sustainability (Mueller et al. 2014). Substratum in the river Obernach proved to be of high quality, hosting a healthy RBT population but allowing no recruitment of MBT (Denic & Geist 2010), which underlines the importance of habitat connectivity for migratory species. In case of migratory species like MBT and DS, our study clearly shows that river continuity is to be set as a first priority, in order to make suitable spawning habitats accessible. The migration to the spawning habitat is often interrupted by barriers that impair the return of mature adults. Where possible, barrier removal should be preferred, otherwise, the construction of fishways is recommended (De Leaniz 2008; Gosset et al. 2006; Ovidio & Philippart 2002).

The widespread practice of breeding all of the studied fish species in aquaculture facilities and their consecutive stocking is likely to reduce fitness of individuals with possible negative effects on the natural recruitment of those species. The study of Geist et al. (2009) showed a high variance in the success of DS stocking measures indicated by a strongly variable contribution of hatchery stocks to the identified genetic clusters. Milot et al. (2013) demonstrated a reduced reproductive success of hatchery born Atlantic salmon (*Salmo salar*) compared to wild individuals. Furthermore, the later fish were released to natural habitats, the lower was their contribution to natural reproduction and a selection for other biological characteristics than such that are crucial for natural reproduction, e.g. the egg and larval development within stream substratum was suggested. Genetic fitness of spawners may also have influenced the results of this study, as spawners from a wild stock (MBT) and from hatchery stocks were used (DS and RBT). However, the significant difference of DS hatching rates in the Rivers Moosach and Lech and the similarity between MBT hatching rates in the River Obernach and RBT hatching rates in the River Lech corroborate a dominant influence of habitat conditions on hatching success.

7 General discussion

The case studies presented in this thesis revealed the strong impact of increased fine sediment deposition on riverbed conditions and on aquatic organisms. The findings corroborate the results of other studies, which identified unnaturally high amounts of fine sediments as the main cause for population declines of unionoid mussels and salmonid fishes (e.g. Geist & Auerswald 2007; Sear et al. 2008). However, this study adds a new dimension to the complex interactions between the riverbed and the aquatic fauna considering spatio-temporal, life-stage specific and evolutionary aspects and their respective impacts on conservation management.

7.1 Fine sediments and their impacts on abiotic riverbed quality

A vast amount of literature deals with fine sediment introduction and its impacts on riverine environments and the aquatic fauna (e.g. Acornley & Sear 1999; Cover et al. 2008; Geist & Auerswald 2007; Julien & Bergeron 2006; Levasseur et al. 2006; Malcolm et al. 2010; Sear et al. 2008). Typically, riverbed quality is adversely affected by increased fine sediment deposition as fine sediments clog the interstitial macropores, hamper exchange of interstitial and free-flowing water and result in e.g. reduced oxygen contents of interstitial water (Greig et al. 2005; Ingendahl 2001). The negative influence of fine sediments was confirmed in chapters 2 and 6, where an increase of fine sediments resulted in lower redox potentials in the interstitial zone. Reduced oxygen supply due to fine sediments was identified as a main cause for the decline of various sensitive faunistic groups such as macroinvertebrates and lithophilic fish. In particular, the decline of riverine freshwater mussels and salmonid fish species is closely linked to the amount of fine sediment introduced into the interstitial zone (Greig et al. 2005; Kondolf 2000; Österling et al. 2010). Consequently, threshold values are often defined to separate between functional and non-functional substrates. However, the functionality of substrates and maximally tolerable amounts of fine sediment are difficult to define. Threshold values and definitions of fine sediment often vary considerably among studies (Table 7.1). More important however, is the complexity of parameters shaping riverbed quality and sediment dynamics. The drivers controlling sediment transport and deposition patterns are various such as channel morphology, flow velocities, up- or downwelling zones and substratum texture (Lisle 1989; Rehg et al. 2005; Seydell et al. 2009). In addition, chapters 2 and 4 indicate an extremely high spatio-temporal variability of riverbed conditions. For instance, the highest fine sediment deposition rates in chapter 2 were found during elevated water levels, but the effects on physicochemical conditions (e.g.

lower redox potentials) could only be detected after a certain period of low flow. The results support previous findings that instream variations in sediment deposition are caused by flow conditions to a high degree (St. Hilaire et al. 2005). Soil erosion in the catchment was mainly controlled by vegetation type as suggested by El Kateb et al. (2013). In contrast, sheer vegetation cover seemed to play a minor role, as highest fine sediment deposition in chapter 2 occurred during summer.

Table 7.1: Examples of different definitions of fine sediment including target species and threshold values, if specified.

Target Species	Particle size mm	Critical proportion %	Reference
Fish			
Oncorhynchus gorbusha	0.83	15	McNeil & Ahnell 1964
Oncorhynchus keta	4.00	-	Peterson & Quinn 1996a
Oncorhynchus kisutch	0.85	10	Reiser & White 1988
Oncorhynchus kisutch	0.50	10	Meyer 2003
Oncorhynchus kisutch; Oncorhynchus mykiss	2.00	20	Lisle 1989
Oncorhynchus mykiss; Salmo salar; Salmo trutta	1.00	15	Crisp & Carling 1989
Salmo salar	2.00	9	Heywood & Walling 2007
Salmo salar	1.00	-	Julien & Bergeron 2006
Salmo trutta	4.00	-	Acornley & Sear 1999
Salmo salar; Salmo trutta	2.00	-	Soulsby et al. 2001b
Salmonids	-	-	Greig et al. 2007a
Freshwater Bivalves			
Margaritifera margaritifera	1.00	38	Geist & Auerswald 2007
Margaritifera margaritifera	gravel (4-63)	-	Hastie et al. 2000
Margaritifera margaritifera	1.00	-	Jung et al. 2013
Margaritifera margaritifera	-	-	Bolland et al. 2010
Unio crassus	0.85	-	Denic et al. 2013
Macroinvertebrates			
Macroinvertebrates	2.00	30 -50	Descloux et al. 2013
Macroinvertebrates	2.00	-	Extence et al. 2013
Macroinvertebrates	2.00	-	Jones et al. 2012a
Macroinvertebrates	0.20	-	Larsen et al. 2011
Periphyton and Macrophytes			
Periphyton	-		Izagirre et al. 2009
Periphyton	2.00	-	Magbanua et al. 2013
Microorganisms	0.85	-	Mueller et al. 2013
Macrophytes	2.00	-	Jones et al. 2012b
Unspecific			
	0.063	-	Belmont et al. 2011
	-	-	Mueller et al. 2011

Furthermore, riverbeds are often characterized by extreme microscale variability (Braun et al. 2012), resulting in significant differences of interstitial water qualities within a few centimeters, e.g. in different substratum depths of single egg-sandwich units (chapter 6). Chapter 6 as well as Sternecker et al. (2013a) confirmed that the importance of single variables and their effects can be strongly scale-dependent. Consequently, fine sediment deposition may be an important driver in a river or river stretch, but be negligible on the microscale or vice versa, which can be explained by river stretch specificity of spatial variability in physicochemical parameters (Braun et al. 2012). Despite intensive research, there is still considerable lack of knowledge concerning the exact interactions of single parameters and their importance for sediment dynamics.

7.2 Fine sediments and their impacts on aquatic fauna

As the assessment of riverbed quality remains incomplete without the link between abiotic and biotic components, numerous studies try to relate riverbed conditions to the population status and reproductive success of target organisms (e.g. Acornley & Sear 1999; Crisp 1996; Geist & Auerswald 2007; Greig et al. 2007a; Greig et al. 2005). However, the majority of these studies focused on the same target species which are usually widely distributed, easily available or popular flagship species. With respect to the target species in this thesis, these attributes apply to the brown trout and the freshwater pearl mussel. At the same time, other imperiled species are neglected due to restricted distribution areas (Danube salmon) or lower popularity (thick shelled river mussel compared to pearl mussel) leading to their underrepresentation in research and literature. As a consequence, results are often generalized, especially in conservation, for closely related or co-occurring species. As revealed in this thesis, such analogies frequently do not withstand a scientific examination as the species or life-stage specific effects of fine sediments depend on two factors: the general autecological needs, which are often closely linked to life cycle characteristics, and the ability to utilize microscale habitat variability. The comparison of the results in chapters 2 and 4 reveals successful recruitment of the thick shelled river mussel in streams with considerably higher sediment deposition and lower redox potentials than were detected in non-functional pearl mussel streams indicating lower habitat demands of *U. crassus*. Furthermore, it is quite common that stable brown trout populations exist in streams where reproduction of pearl mussels fails (Geist et al. 2006), though Österling (2014) gives examples where both species are equally affected by forest clear-cuts. An explanation to this observation is that brown trout actively choose their spawning grounds and therefore are able to avoid the most unsuitable sites for reproduction. In the course of redd digging, substrates are loosened and cleaned of fine sediments. Both aspects do not apply for pearl mussels, which are unable to influence the place of their drop-off from the host fish and manipulate riverbed conditions subsequently. In addition, with a few months the residence time of brown trout eggs and alevins in the substratum is comparably short compared to several years in case of the pearl mussel. In case of the two studied fish species in chapter 6, a higher susceptibility against fine sediment deposition was detected for Danube salmon compared to brown trout. However, there are indications that autecological characteristics like shorter residence time in the interstitial zone and bigger redd sizes allow Danube salmon to avoid adverse conditions and to profit from microscale variabilities thus compensating for elevated habitat demands.

The topic of local adaptation is controversially discussed in the literature and several authors expect it to be dependent on certain parameters, such as study scale, population size or habitat status (Gandon & Michalakis 2002; Jones 2013; Kaltz & Shykoff 1998; Lajeunesse & Forbes 2002). Despite these controversies, there is increasing evidence of local adaptations within target species, thus forming evolutionary significant units. The results presented in chapter 5 indicate intraspecific specialization concerning water quality and detritus composition in some freshwater pearl mussel stocks. The potential of pearl mussel and thick shelled river mussel to reach high levels of adaptation is further corroborated by Douda et al. (2014), Karlsson et al. (2013), Österling & Larsen (2013) and Taeubert et al. (2010), who discovered differences in host compatibility down to the level of mussel and host fish subpopulations and strains. DeVries (1997) reported deviating egg burial depths for different populations of the same salmonid species, probably due to varying scour risks in different habitats. Similar adaptation processes are imaginable with respect to fine sediment loads and substrate composition, as they naturally differ between different streams.

As demanded by Nislow & Armstrong (2012) the correct assessment of abiotic impacts on target organisms requires the consideration of life-stages and evolutionary significant units, as illustrated by the case study in chapter 3. Here, two evolutionary significant units, the resident and the migratory brown trout, shared the same habitat. However, only the migratory form was affected by reduced habitat connectivity and migration barriers due to a pronounced habitat shift between life-stages. Specifically, migrating spawners that could not return to their spawning grounds were affected, whereas habitat conditions for other life-stages remained suitable.

7.3 Consequences for conservation and monitoring programs: a general concept for individual solutions

The variability of habitat demands between species, evolutionary significant units and life-stages as well as the spatio-temporal heterogeneity of habitat conditions requires individual solutions for each conservation project. Various contributions already demanded the inclusion of different levels and basic steps into conservation projects (e.g. Geist 2010; Malcolm et al. 2012; Nislow & Armstrong 2012; Palmer at al. 2005). Yet, there is still a trend to neglect the development of specific solutions and consequent monitoring programs of conservation success (Greig et al. 2005; Malcolm et al. 2012). Bernhardt et al. (2007) and Kondolf et al. (2007) stated that in more than half of the restoration projects carried out in the U.S.A., the absence of measurable objectives complicates an assessment of project success. In addition, practitioners often lack methodological, financial and personnel resources for the implementation of adequate monitoring programs. Indeed, several examples in the literature show that the assessment of restoration success is difficult already starting with the definition of success. Frequently, the success of ecologically motivated projects is evaluated by public opinion and post-project appearance, parameters which are not related to ecology (Bernhardt et al. 2007). Furthermore, the analysis of different parameters or endpoints may produce contradictory results concerning success/efficiency of specific restoration measures (Lepori et al. 2005; Mueller et al. 2014). As a consequence, Mueller et al. (2014) propose a multi-scale evaluation concept.

Here, I present an attempt to build up a universal guide for the development of individual conservation concepts incorporating ideas and experiences from other studies (Fig 7.1). A prerequisite for the development of individual, target specific concepts is detailed information on the present status of the habitat and biological communities, ideally incorporating different scales from micro- to macrohabitats and from the individual/life-stage to the species or even community level. For species with a wide distribution area this process may also include the prioritization of target populations. The assessment of the status quo combined with the reference conditions to reach, forms the basis for the formulation of specific restoration aims and the choice of measures. For instance, reproductive failure of streambed associated species can have various reasons, which may not be obvious at a first glance. Often fine sediment introduction causes riverbed degradation, but in case of the River Lech it was the absence of substratum due to impaired bedload transport (chapter 6). In other cases, the identification of bottlenecks revealed reasons independent from riverbed conditions, e.g. in case of migratory brown trout (see chapter 3; Deitch et al. 2009; Gosset et al. 2006) or *U. crassus* where a lack of host fish frequently hampers reproduction (Engel & Wächtler 1989; Stoeckl et al. 2014). Obviously, suitable restoration measures will widely differ for the given examples. Gravel addition or substratum raking are widely accepted measures for mitigation of riverbed degradation (Pulg et al. 2013; River Restoration Centre 2002), though chapter 2 and Mueller et al. (2014) revealed that instream restoration is highly ineffective in rivers where fine sediment introduction constitutes the main problem. Nevertheless, it may be effective for mitigating a lack of substratum. At this level, the impact of measures on adjacent areas has to be considered following the demand of Palmer et al. (2005) that "during the construction phase, no lasting harm should be inflicted on the ecosystem". For that purpose as well as for the modification and adaptation of restoration concepts, the consecutive monitoring of restoration activities is necessary. Substratum raking proved to adversely affect downstream river sections (Mueller et al. 2014; Sternecker et al. 2013b). It has to be noted that the monitoring period has to comply with the generation time of the target species to detect biological effects. Further it is recommended that even intact or successfully restored habitats should be monitored regularly due to the dynamic of river systems.

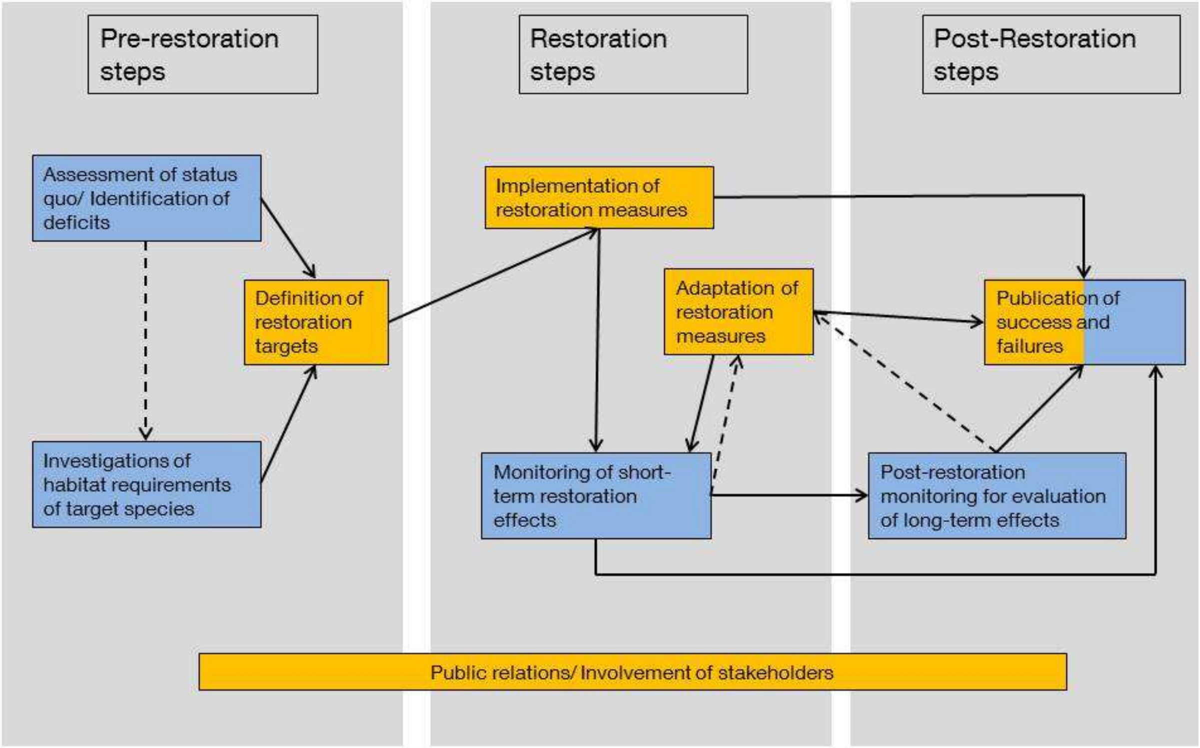


Figure 7.1: Restoration project scheme; blue boxes represent research steps and orange boxes represent practical conservation steps; drawn through lines are obligate and dashed lines are optional pathways.

Finally, the involvement of stakeholders, landowners and public awareness are crucial factors in conservation and restoration projects in general and riverbed restoration in particular. As the source of problems is often located in the catchment, usually a high number of people with different interests and information status are affected (Linke et al. 2011). Their involvement at an early stage, at latest during the choice of measures and definition of conservation aims, increases the chance for active support of the projects.

7.4 Outlook

The increasing number of publications dealing with riverbed ecology illustrates the relevance of the riverbed for aquatic ecosystem functioning. In densely populated and intensively used catchments, riverbed degradation due to fine sediment deposition constitutes a main threat for riverine ecosystems. Simultaneously, the possibilities for extensification of catchment use are limited in such areas and the results in chapter 2 make clear that instream restoration is ineffective to resolve siltation problems. Consequently, future research activities need to focus on the development and advancement of innovative land-use concepts such as precision farming, which allow optimal human land-use to be combined with nature conservation targets securing ecosystem functioning and services. For this purpose an intensification of interdisciplinary research is necessary. Biology and ecology can contribute to this target as follows:

i) Knowledge on autecological demands of species is the basis for the assessment of human impacts on ecosystems or threatened species and many studies on this topic are already available in the literature. However, research was focused on very few flagship species so far, but information on less popular organisms is still lacking. In addition, the importance of life-stage and evolutionary significant units has been neglected and needs to be considered in future investigations.

ii) Development of suitable monitoring methods allowing the assessment of human impacts or the success of restoration measures is required. Monitoring is often neglected not only due to financial but methodological restrictions as well. Tools like the egg-sandwich, which establish a direct link between abiotic parameters and biological endpoints, are of highest relevance. For instance, the development of a biomonitoring tool for the assessment of juvenile mussel habitats is urgently needed with respect to worldwide captive rearing efforts for reintroduction of mussels into native habitats.

8 Publication list

Papers included in this thesis:

Denic M, Geist J (2014) Linking stream sediment deposition and aquatic habitat quality in pearl mussel streams: implications for conservation. River Research and Applications: in press.

Denic M, Geist J (2010) Habitat suitability analysis for lacustrine brown trout (*Salmo trutta*) in Lake Walchensee, Germany: implications for the conservation of an endangered flagship species. Aquatic Conservation: Marine and Freshwater Ecosystems 20: 9-17.

Denic M, Stoeckl K, Gum B, Geist J (2013) Physicochemical assessment of *Unio crassus* habitat quality in a small upland stream and implications for conservation. Hydrobiologia735: 111-122.

Denic M, Tauebert JE, Lange M, Thielen F, Scheder C, Gumpinger C, Geist J (2014): Influence of stock origin and environmental conditions on the survival and growth of juvenile freshwater pearl mussels (*Margaritifera margaritifera*) in a cross-exposure experiment. Limnologica: in press.

Sternecker K, **Denic M**, Geist J (2014): Timing matters: Species-specific interactions between spawning time, substrate quality and recruitment success in three salmonid species. Ecology and Evolution: 4 (13): 2749-2758.

Papers not included into this thesis:

Taeubert JE, **Denic M**, Gum B, Lange M, Geist J (2010) Suitability of different salmonid strains as hosts for the endangered freshwater pearl mussel (*Margaritifera margaritifera* L.). Aquatic Conservation: Marine and Freshwater Ecosystems 20: 728–734.

Oral contributions related to this thesis:

Denic M (2013) Muschelschutz und Gewässerunterhaltung. Gewässernachbarschaftstag Fürstenfeldbruck, Puchheim, Germany, September 2013.

Denic M, Geist J. (2013) Suitability of different *Margaritifera* stocks for bioindication studies. World Congress of Malacology 2013, Ponta Delgada, Azores, Portugal, July 2013.

Denic M (2013) Muschelschutz in Bayern und Europa – Status quo. Fachtagung Muschelschutz, Freising, Germany, March 2013.

Denic M (2012) Muschelschutz in Bayern. Gewässernachbarschaftstag Hof, Selbitz, Germany, October 2012.

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Stoeckl K, **Denic M** (2012) Gewässerunterhaltung und Auswirkungen auf die heimischen Muschelbestände - Fallbeispiele und Erfahrungen. Niederbayerischer Fischereitag, Seyboldsdorf, Germany, September 2012.

Denic M (2012) Biologie heimischer Großmuscheln und ihre Bedeutung im Arten- und Gewässerschutz. Vortragsreihe des LBV Freising, Freising, Germany, June 2012.

Denic M, Gum B, Geist J (2011). Integriertes Sedimentmanagement in Einzugsgebieten von Fließgewässern. Meeting of the borderline commission, Hof, Germany, October 2011.

Denic M, Gum B, Geist J (2011) Integriertes Sedimentmanagement in Einzugsgebieten von Fließgewässern. Jahrestagung der Deutschen Gesellschaft für Limnologie. Freising, Germany, September 2011.

Denic M (2011) Redoxmessung in Fließgewässern. Workshop zur In-situ Redoxpotentialmessung in Fließgewässern, Wels, Austria, June 2011.

Denic M (2011) Measurement of redox potentials in freshwater environments. Nordisk workshop Stormusslor, Storabränna, Sweden, June 2011.

Denic M (2011) Substratrestaurierung in Fließgewässern. Habitat Restoration Seminar, Heinerscheid, Luxemburg, May 2011.

Denic M (2011) Erfahrungen vom Sallingbach. Fachtagung Muschelschutz, Freising, Germany, March 2011.

Denic M (2010) Die Bedeutung des Gewässersubstrats im Muschelschutz – Fragestellungen und Untersuchungsmethoden. Fachtagung Muschelschutz, Freising, Germany, March 2010.

Denic M, Geist J (2010) Biology and ecology of mollusca. Slovak-German Expert Workshop on Methodological competence of experts on river / species assessment (according to European WFD). Banská Bystrica, Slovakia, February 2010.

Denic M, Strohmeier P, Gum B, Geist J (2009) Integrated Sediment Management in River Catchments. International Seminar - Increased sedimentation, a widespread problem leading to degradation of freshwater communities and habitats. Clervaux, Luxemburg, November 2009.

Poster contributions related to this thesis:

Denic M, Stoeckl K, Geist J (2013) Conservation of Freshwater Mussels in Bavaria, Germany, With a Focus on the Critically Endangered Freshwater Pearl Mussel (*Margaritifera margaritifera*) and Thick Shelled River Mussel (*Unio crassus*). International Meeting on Improving the Environment of the Freshwater Pearl Mussel. Weinberg Castle, Austria, November 2013.

Denic M, Strohmeier P, Gum B, Geist J (2010) Integriertes Sedimentmanagement in Einzugsgebieten von Fließgewässern. Jahrestagung der Deutschen Gesellschaft für Limnologie. Bayreuth, Germany, September 2010.

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