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# The impact of stream substratum quality on salmonid reproduction

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Danube salmon (*Hucho hucho* L.) and grayling (*Thymallus thymallus* L.) spawning in the river Lech.

No one steps into the same river twice.

Heraclitus of Ephesus (c. 535 - c. 475 BCE)

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# Summary

Anthropogenic impacts like river regulation or intensive land-use have strong effects on freshwater ecosystems. In many Central European rivers, natural flow regimes are regulated in the contexts of flood control and hydropower generation. Increasing erosion of agricultural soil causes higher fine sediment contents within rivers. Interrupted sediment transport and increasing fine sediment deposition combined with missing river dynamics induce the degradation of functional stream substratum, which is the key habitat for salmonid reproduction (e.g. endangered Danube salmon and grayling). In particular, the decrease of natural salmonid reproduction in colmated stream substratum has different reasons. On the one hand, the egg and fry development is affected indirectly by the substratum caused by changes in physicochemical conditions as a result of reduced water exchange. On the other hand, the stream substratum composition, which can build a migration barrier between the open water and the interstitial zone, has direct effects on the emergence of fry. The impact of physical (e.g. sediment texture, penetration resistance of substratum surface) and physicochemical factors (e.g. oxygen, redox, nitrite, nitrate, ammonium, pH, SC, T) is specific for the individual development stages. Hence, the reproduction of salmonids has to be split up in five stages: 1. identification and acceptance of spawning grounds, 2. digging of 'redds', 3. egg deposition, 4. egg and yolk sac fry development and 5. emergence of fry.

In this thesis, a combination of standardized laboratory experiments and field validations under natural conditions was conducted to analyze direct and indirect effects of stream substratum composition on recruitment success in brown trout (*Salmo trutta*) and Danube salmon (*Hucho hucho*) at different development stages. Additionally, stream substratum restoration as a typical tool to improve habitat quality was addressed. Short-time studies of positive and negative effects within and downstream of the restoration area as well as long-time monitoring of restored stream substratum were conducted in a holistic evaluation approach.

In a first step, the physical effects of different substratum textures on the emergence success as well as on the post-emergence survival and growth of salmonid fry (brown trout and Danube salmon) were tested under standardized physicochemical water conditions. Physical effects of substratum compaction by fines alone could strongly reduce the number of emerging fry. The emergence chronology, the post-emergence survival and the post-emergence growth of brown trout as well as of Danube salmon strongly depend on the stream substratum composition. Fry of both salmonid species benefited from coarser sediment, whereas fine-textured substratum (5 - 8 mm) formed a physical barrier for the upmigrating fish. The time period of emergence was shortened by finer substratum compositions. The emergence peak was higher in treatments with coarser sediment resulting in an overall higher emergence rate. Post-emergence survival as well as growth was affected by the sediment texture depending on the life-history strategy of the respective species.

For validation of egg development and survival in natural stream substrates, standardization of egg exposures is most crucial. To accomplish this task, a new tool for exposing eggs at different substratum depths, the 'egg sandwich' (ES), was developed and established. This tool is suitable for linking a biotic factor (salmonid hatching rates) and abiotic factors in stream substratum. An egg exposure unit and a measurement unit were combined to monitor the interstitial water condition during the egg development within stream substratum. Individual eggs were exposed separately in chambers (L: 15 mm, W: 15 mm and D: 15 mm) of an upright aluminum grid. Perforated PVC tubes were attached horizontal at defined substratum depths (e.g. 0 - 50 mm, 50 - 100 mm, 100 - 150 mm) to extract interstitial water by vacuum. The volume of the extracted water was calculated according to the volume of the egg chambers containing to the sediment depth. Hatching rates and physicochemical parameters were measured with this tool at different sediment depths under laboratory conditions as well as at field sites.

The effects of different stream substratum quality on salmonid egg development (e.g. brown trout) at different temporal and spatial scales under natural conditions were studied using the ES. ESs were exposed in three different rivers in Southern Germany (river Moosach, river Wiesent and river Lech) during three spawning seasons. A bimodal distribution of very high and very low egg hatching success was detected within the sediment (contrary to a unimodal distribution in hatchery and open water references). Hatching success of brown trout decreased with increasing sediment depth. Statistical models (discriminant analysis and generalized linear model) were carried out to identify the impact of physicochemical factors on egg development. The impact of physicochemical water conditions within the hyporheic zone are strongly time and space depended (macro-scale, river-specific scale or micro-scale) of the study. This is presumably a result of variations in watershed characteristics between rivers and fluctuating river discharge between years. Hence, a linear prediction of the reproduction success by physicochemical parameters within different study sites over the whole study period was not reliable with respect to the stream substratum. Nevertheless, it was possible to demonstrate the strong impact of water exchange within the hyporheic zone by comparing discriminant analysis at different scales.

The effects of stream substratum restoration by loosening colmated substratum as well as by the reduction of fine sediment content were studied in a next step. The comprehensive evaluation of short-time effects on stream habitats and the monitoring of long-time success of the restoration were focused on two separate projects.

Abiotic as well as biotic indicators were used to study the improvement of spawning habitat quality for salmonids by excavating colmated stream substratum. Depth-specific brown trout hatching success as well as the fluctuation of macroinvertrebrate abundance and diversity were screened to evaluate the impact of the restoration on the aquatic biota. Potential effects on downstream habitats were analyzed additionally. One day after the treatment, the sediment compaction and the fine sediment content were reduced. The decrease was persistent three months after the restoration. The habitat improvement increased brown trout hatching success after the restoration significantly. Even though a strong decrease of macrozoobenthos abundance was detected within the sediment directly after the disturbance, three months later the number of species and abundance were higher than before the restoration. Nevertheless, short-term increases of fine sediment deposition caused by stream substratum restoration were observed, which may have negative impact on downstream habitats.

The sustainability of salmonid spawning habitat was studied after stream substratum restoration of seven test sites (restored by sediment cleaning and gravel addition). The effects of sediment restoration on the acceptance of spawning habitat, on salmonid hatching success and on brown trout populations as well were monitored during this study period. Strong enhancements of habitat conditions were detected after the restoration of highly degraded brown trout spawning sites with an increase of the relative number of young-of-the-year brown trout. Even though the short-term results of the small-scale restoration of stream substratum was beneficial for the brown trout reproduction, the long-term monitoring of the restored sites demonstrated that highly suitable conditions for brown trout egg development in the interstitial zone lasted for only two years (hatching success >50 %). Unsuitable conditions for salmonid reproduction were expected after 5 to 6 years.

In conclusion, substratum characteristics have both direct and indirect effects on the salmonid reproduction success. The sediment texture had strong effects on the emergence success of salmonids. This indicates a crucial impact of the substratum compositions on salmonid populations. Indirect effects (impacted physicochemical parameters within the interstitial water) suggest that the stream substratum represents a limiting factor for hatching success and consequently for reproductive success in all of the streams investigated in this thesis. The great success of small-scale habitat restoration with short lag-time for target species (e.g. salmonids) should not overshadow the fact that the improvements are limited in space and time. The consideration of physical stream bed characteristics and additionally the approach of integrating potential negative downstream effects of stream substratum restoration into catchment-based management plans may provide great benefits for biodiversity conservation in stream ecosystems. Holistic approaches like the reduction of fine sediment input in river systems or the regeneration of natural river dynamics are necessary to extend the success of stream substratum restoration.

# Zusammenfassung

Anthropogene Eingriffe, wie die Regulation der Flüsse oder intensive Landnutzung, haben einen starken Einfluss auf Gewässerökosysteme. In vielen zentraleuropäischen Flüssen ist der natürliche Abfluss im Rahmen des Hochwasserschutzes oder aufgrund von Stromerzeugung durch Wasserkraft reguliert. Bodenerosion, die durch intensive landwirtschaftliche Nutzung verstärkt wird, führt zu erhöhten Feinsedimentraten in den Gewässern. Die Behinderung des Sedimenttransports und ansteigende Ablagerung von Feinsediment erzeugen zusammen mit unzureichender Fließgewässerdynamik eine Degradierung von funktionellem Fließgewässersubstrat. Lockeres kiesiges Substrat ist unter anderem das Schlüsselhabitat für die Reproduktion gefährdeter Salmoniden (z.B. Huchen und Äsche).

Kolmatiertes Gewässersubstrat führt aus verschiedenen Gründen zu einem Rückgang der natürlichen Vermehrung der Salmoniden. Auf der einen Seite ist die Ei- und Larvalentwicklung indirekt vom Gewässersubstrat abhängig, da verminderte Durchströmung die physikochemischen Bedingungen verändert. Auf der anderen Seite hat die Zusammensetzung des Substrats auch direkten Einfluss auf die Emergenz der Larven, da sie eine Barriere bei der Wanderung aus dem Interstitial in das Freiwasser bilden kann. Die Einflüsse physikalischer (z.B. Sedimentzusammensetzung, Penetrationswiderstand der Substratoberfläche) und physikochemischer Faktoren (z.B. Sauerstoff, Redoxpotential, Nitrit, Nitrat, Ammonium, pH-Wert, Leitfähigkeit, Temperatur) sind für die einzelnen Entwicklungsstadien spezifisch. Deshalb wird die Reproduktion von Salmoniden in fünf Stadien unterteilt: 1. Identifizierung und Akzeptanz von Laichplätzen, 2. Graben einer Laichgrube, 3. Eiablage, 4. Ei- und Larvalentwicklung und 5. Emergenz der Larven.

In dieser Arbeit wurden sowohl standardisierte Laborversuche als auch Felderhebungen unter natürlichen Bedingungen durchgeführt, um die direkten und indirekten Effekte von Substratzusammensetzungen auf den Reproduktionserfolg von Bachforelle (*Salmo trutta*) und Huchen (*Hucho hucho*) in unterschiedlichen Entwicklungsstadien zu untersuchen. Zusätzlich wurden Substratrestaurierungen zur Verbesserung der Substratqualität durchgeführt. Es wurden kurzzeitige Untersuchungen zu positiven und negativen Effekten innerhalb und stromabwärts von restaurierten Flächen angesetzt. Für eine möglichst umfassende Beurteilung der Maßnahme wurde an Versuchsflächen mit restauriertem Gewässersubstrat außerdem ein Langzeit-Monitoring durchgeführt.

In einem ersten Schritt wurden die physikalischen Effekte von unterschiedlichen Substratzusammensetzungen sowohl auf den Erfolg der Emergenz als auch auf das Überleben und das Wachstum von Salmonidenlarven (Bachforelle und Huchen) nach der Emergenz unter stabilen physikochemischen Bedingungen untersucht. Die Verdichtung von Substrat aufgrund von feinkörnigem Material reduzierte die Anzahl der aufsteigenden Larven. Der zeitliche Ablauf und auch das Überleben sowie das Wachstum der Bachforellen- und Huchenlarven nach der Emergenz waren stark von der Substratzusammensetzung abhängig. Die Larven beider Arten profitierten von gröberem Material (16 - 32 mm), wohingegen feines Substrat (5 - 8 mm) eine physikalische Barriere für die aufsteigenden Fische bildetete. Die Dauer der Emergenz war bei feiner Körnung verkürzt. Der Höhepunkt der Emergenz war in gröberem Substrat ausgeprägter, was zu einer insgesamt besseren Emergenzrate führte. Die Überlebensrate und das Wachstum nach der Emergenz wurden von der Substratzusammensetzung beeinflusst, sie waren jedoch abhängig von der "life-history strategy" der jeweiligen Art.

Um die Eientwicklung und Überlebensrate in natürlichem Gewässersubstrat zu bewerten, ist es wichtig die Eier unter standardisierten Bedingungen auszubringen. Um das zu erreichen wurde ein neues System, das 'egg sandwich' (ES), entwickelt und etabliert, mit dessen Hilfe Eier in unterschiedlicher Substrattiefe ausgebracht wurden. Jeweils eine Einheit für die Messung eines biotischen Faktors (Schlupfrate von Salmoniden) und für die Messung abiotischer Faktoren wurden miteinander verbunden, um die Bedingungen im Substrat während der Eientwicklung zu untersuchen. Einzelne Eier wurden separat in Kammern (L: 15 mm, B: 15 mm und H: 15 mm) eines Aluminiumgitters ausgebracht. Durchlöcherte PVC-Röhren wurden horizontal in unterschiedlichen Höhen (z.B. 0 - 50 mm, 50 - 100 mm, 100 - 150 mm) befestigt, um über ein Vakuum Interstitialwasser zu entnehmen. Die Menge des entnommenen Wassers wurde anhand der Volumina berechnet, die durch die Eikammern in der jeweiligen Sedimenttiefe eingenommen wurden. Schlupfraten und physikochemische Parameter wurden mit dem ES sowohl unter Laborbedingungen als auch unter Freilandbedingungen in unterschiedlichen Tiefen gemessen.

Die Effekte unterschiedlicher Substratqualität auf die Entwicklung von Salmonideneiern wurden mit dem ES in verschiedenen zeitlichen und räumlichen Skalen unter natürlichen Bedingungen untersucht. Dazu wurden ESs in drei verschiedenen Flüssen in Süddeutschland (Moosach, Wiesent und Lech) während drei Laichzeiten ausgebracht. Es wurde eine bimodale Verteilung von sehr gutem und sehr schlechtem Schlupferfolg, im Gegensatz zu einer unimodalen Verteilung unter Laborbedingungen und in Freiwasser-Referenzen, festgestellt. Der Schlupferfolg der Bachforelle sank mit ansteigender Sedimenttiefe. Statistische Modelle (Diskriminanzanalyse und Generalisierte Lineare

Modelle) wurden angewendet, um den Einfluss von physikochemischen Faktoren auf die Eientwicklung zu ermitteln. Der Einfluss der physikochemischen Bedingungen im Interstitialwasser hing stark von der zeitlichen und räumlichen Ebene der Studie ab (Makro-Ebene, Gewässer-Ebene oder Mikro-Ebene). Die Gewässercharakteristik und Fluktuationen der Abflüsse zwischen den Jahren verursachen wahrscheinlich diese Abhängigkeit. Dadurch ist ein linearer Zusammenhang zwischen Reproduktionserfolg und physikochemischen Parametern im Substrat verschiedener Versuchsflächen über den gesamten Untersuchungszeitraum nicht möglich. Durch den Vergleich der Diskriminanzanalysen auf verschiedenen Ebenen konnte dennoch gezeigt werden, dass ein starker Einfluss der Durchflussrate innerhalb des Interstitials besteht.

In einem weiteren Schritt wurden die Effekte der Substratrestaurierung durch Lockerung von kolmatiertem Substrat und die Verminderung des Feinsedimentanteils getestet. Die umfassende Beurteilung von kurzzeitigen Effekten auf die Gewässerhabitate und das Langzeit-Monitoring des Restaurierungserfolgs wurde in zwei Projekten getrennt untersucht.

Sowohl abiotische als auch biotische Indikatoren wurden für die Untersuchung der Laichhabitatqualität für Salmoniden und deren Verbesserung durch das Umgraben von kolmatiertem Gewässersubstrat herangezogen. Der Schlupferfolg und die Veränderung der Abundanz und der Diversität von Makrozoobenthos wurden abhängig von der Tiefe untersucht, um den Einfluss der Restaurierung auf weitere aquatische Organismen zu ermitteln. Potentiell negative Effekte auf Habitate, die flussabwärts der restaurierten Fläche lagen, wurden ebenfalls berücksichtigt. Einen Tag nach der Restaurierungsmaßnahme waren die Verfestigung des Substrats und der Feinsedimentanteil deutlich reduziert. Ein Effekt, der auch noch drei Monate später messbar war. Die habitatverbessernde Maßnahme erhöhte den Schlupferfolg der Bachforelle signifikant. Obwohl eine starke Dezimierung des Makrozoobenthos innerhalb des Sediments unmittelbar nach der Restaurierung festgestellt wurde, stiegen die Anzahl der Arten und die Abundanz drei Monate später über den Referenzwert, der vor der Restaurierung gemessen wurde. Dennoch muss berücksichtigt werden, dass ein kurzzeitiger Anstieg der Ablagerung von Feinsediment, der durch die Restaurierungsmaßnahme verursacht wurde, negative Effekte auf Habitate unterhalb der restaurierten Fläche haben kann.

Die Nachhaltigkeit von restauriertem Laichhabitat für Salmoniden wurde an weiteren sieben Untersuchungsflächen, die durch Umbaggerung oder Zugabe von Kies wiederhergestellt wurden, geprüft. Während des gesamten Untersuchungszeitraums wurden die Effekte der Substratrestaurierung auf die Akzeptanz der Laichplätze ebenso wie der Schlupferfolg in der Bachforellen-Population untersucht. Große Verbesserungen der Habitatbedingungen nach der Restaurierung von stark degradierten Laichplätzen gingen einher mit einem Anstieg der relativen Anzahl einsömmriger Bachforellen. Auch wenn die kurzzeitigen Ergebnisse der mikroskalierten Restaurierung des Gewässersubstrats die Vermehrung der Bachforelle förderte, zeigten Langzeit-Beobachtungen, dass sehr gute Bedingungen für die Eientwicklung im Interstitial nur zwei Jahre anhielten (Schlupferfolg >50 %). Ungeeignete Bedingungen werden bereits nach 5 bis 6 Jahren erwartet.

Zusammenfassend kann gesagt werden, dass das Gewässersubstrat sowohl direkte als auch indirekte Effekte auf den Reproduktionserfolg von Salmoniden hat. Die physikalischen Substrateigenschaften haben starke Effekte auf die Emergenz der Salmoniden. Der Erfolg der Emergenz hat wiederum einen großen Einfluss auf die Populationen. Indirekte Effekte (z.B. veränderte physikochemische Parameter im Interstitialwasser) deuten an, dass das Gewässersubstrat einen limitierenden Faktor für den Schlupferfolg und somit für den Reproduktionserfolg der Salmoniden darstellt. Der große Erfolg von klein angelegten Habitat-Restaurierungen mit nur kurzer zeitlicher Verschiebung für Zielarten (z.B. Salmoniden) sollte nicht darüber hinwegtäuschen, dass die Verbesserungen zeitlich und räumlich eingeschränkt sind. Die Berücksichtigung der physikalischen Eigenschaften von Gewässersubstrat und zusätzlich der Einbezug von negativen Effekten, die flussabwärts von restaurierten Flächen auftreten können, sind in zukünftigen Management-Plänen der Einzugsgebiete nötig. Daraus kann großer Nutzen für den Erhalt der Biodiversität in Gewässerökosystemen gezogen werden. Ganzheitliche Ansätze wie die Reduzierung von Feinsedimenteintrag in die Gewässersysteme oder die Wiederherstellung von natürlichen Abflussregimen sind notwendig, um den Erfolg der Restaurierung von Gewässersubstrat zu erhöhen.

# 1 Introduction

## 1.1 Degradation of river sediment and associated consequences to the river ecosystem

Freshwater ecosystems are highly affected by anthropogenic impacts such as over-exploitation, water pollution, flow modification and changes in land use (Dudgeon et al., 2006; Denic and Geist, 2009; Kemp et al., 2011; Geist, 2011). These impacts have not only single effects on functional habitats; they also have cumulative or even synergistic effects on the habitat degradation (Lake et al., 2000). In turn, the advancing degradation of functional freshwater habitats has pronounced consequences on the occurrence, abundance and population dynamics of biological communities (Beard and Carline, 1991; Boulton et al., 1998; Burkhardt-Holm et al., 2005). In particular, key habitats like the riverbed are crucial for freshwater organisms (Kondolf, 2000a; Palmer, 1997; Boulton, et al. 1998; Geist and Auerswald, 2007). Microbes, macroinvertebrates (e.g. insects, mussels) and rheophilic fishes are typical groups of organism of the stream substratum and the functionality of the hyporheic zone is of fundamental importance, especially for their reproduction (Williams and Hynes, 1974; Bauer 1979, Stanford and Ward, 1988; Hendricks, 1993; Elliott, 1994; Buddensiek, 1995; Palmer et al., 1997). The decline of biological communities in the stream substratum caused by riverbed degradation has effects on the whole freshwater ecosystem, e.g. freshwater mussels have an impact on the clearance of the water body and the nutrient content of the free-flowing water. Additionally, macrozoobenthos represents basic food items e.g. for freshwater fish. Food webs are disturbed or ultimately changed as a result of stream substratum degradation and consequently, the biodiversity of the river ecosystems decreases (Suttle et. al., 2004; Geist, 2011).

Stream substratum with flow-through of oxygenated surface water characterizes the reproduction habitat for several lithophilic species, e.g. freshwater mussels and freshwater fish (Bauer et al., 1980; Ottaway et al., 1981; Crisp and Carling, 1989; Soulsby et al., 2001a; Klemetsen et al., 2003; Louhi et al., 2008). The degradation of this key habitat has negative effects on the egg and larval development of lithophilic fishes and invertebrates. Consequently, the natural reproduction, crucial component to the conservation of these species, often decreases (Turnpenny and Williams, 1980; Brunke and Gonser, 1997; Acornley and Sear, 1999; Soulsby et al., 2001a; Soulsby et al., 2001b; Malcolm et al., 2003).

Deficits in salmonid reproduction are caused by colmation of river sediment and a lack of stream substratum within anthropogenic manipulated rivers. Reduced mobility of stream substratum, e.g. because of hydropower or flood control, and high fine sediment loads because of intensive agriculture in catchment areas, are crucial for the colmation of river sediment and the clogging of the hyporheic zone (e.g. Sutherland et al., 2002; Opperman et al., 2005; Kemp et al., 2011). Furthermore the depletion of gravel and erosion of stream substratum as a result of modified flow regimes downstream of dams (hungry water) additionally decreases areas with high quality sediment, which is necessary for natural reproduction of salmonids (Kondolf and Wolman, 1993).

## 1.2 About salmonids and their strong connection to the riverbed

Salmonids, which are a regionally important part of the human diet (about 600,000 t / year; Muus and Dahlström, 1981) and include very popular species for commercial and recreational fisheries like Danube salmon and brown trout, are heavily affected by the degradation of their spawning habitat. Currently, all lithophilic fish are listed as endangered species in Central Europe (Jungwirth et al., 2003).

To date, salmonid research is focused on the impact of the stream substratum quality on salmonid reproduction success, but it remains to be completely understood (Grost et al., 1991; Rubin and Glimsäter, 1996; Acornley and Sear, 1999; Milan et al., 2000; Soulsby et al., 2001a; Heywood and Walling, 2007; Fudge et al., 2008). It is known that increased fine sediment deposition and changes in physicochemical conditions, especially the reduction of oxygen supply, within the interstitial zone decreases the hatching success and also the emergence of fry (Olsson and Persson, 1986; Chapman, 1988; Rubin and Glimsäter, 1996; Ingendahl 2001; Jonsson and Jonsson, 2011). Nevertheless, studies of physical effects of different stream substratum composition on the salmonid reproduction are still missing, but have to be considered for a comprehensive approach.

The impact of different substratum qualities on the reproduction success of salmonids can be split up into five important stages:

a) identification and acceptance of spawning grounds by spawners,

b) digging of ‘redds’ by females,

c) egg deposition into substratum voids,

d) egg and yolk sac fry development and

e) emergence of fry.

The knowledge of factors with positive and negative effects on the success of salmonid reproduction specific to all five stages in sediment is critical for the evaluation of the stream substratum quality and their impacts (Elliott, 1994; Kondolf 2000a; Denic and Geist, 2010). This would allow the effectiveness of habitat restoration methods, which have to be considered in conservation management, to be controlled and improved.

Microscale restorations are useful to sustain or rebuild key habitats and to enhance the functionality of the whole ecosystem (e.g. Kondolf et al., 1996; Kondolf, 2000b; Pander and Geist, 2010). In Bavaria, the restoration of river sediment is a commonly used in-stream restoration (e.g. sediment cleaning, addition of gravel), to improve the stream substratum quality for salmonid reproduction (Shackle et al. 1999; Pulg, 2007; Sear and DeVries, 2008). Overall, the reduction of fine sediment particles in the river system is becoming a core topic in river and catchment management (Greig et al., 2005; Palm et al., 2007).

## 1.3 Aim of thesis and approaches in this study

To study the effects of different sediment compositions and interstitial water conditions on the reproductive success of salmonids at different development stages, field exposures and laboratory experiments were carried out.

In detail, five questions were addressed:

(1) What are the physical effects of different substratum compositions on the reproduction success of salmonids?

(2) What are the indirect effects of different substratum compositions on the reproduction success of salmonids considering physicochemical conditions in the interstitial zone?

(3) To which degree do spatial and / or temporal variations of stream substratum quality exist?

(4) Which kinds of negative and positive effects of small-scaled salmonid reproduction habitat improvements (e.g. ‘excavation of stream substratum’) can be observed locally in river ecosystem?

(5) How effective and sustainable are within river restorations and what kind of impact do they have on salmonid populations (e.g. increase number of young-of-the-year)?

Physical effects of different substratum composition on the emergence of fry were tested under standardized conditions in the laboratory. Overall 2880 brown trout eggs and 2880 Danube salmon eggs close to hatch were incubated within four treatments with 3 different gravel sizes (5 - 8 mm, 8 - 16 mm, 16 - 32 mm and reference). After the successful emergence, the post-emergence survival and growth were studied.

A new tool was applied, called the ‘egg sandwich’, to directly connect biotic factors (salmonid hatching success) and abiotic parameters (e.g. oxygen, redox potential. temperature, specific conductance, pH, ammonium, nitrate and nitrite concentration) in field conditions. In three spawning seasons, a total of 110 egg sandwich boxes were exposed in 19 study sites located in three rivers (River Moosach, River Wiesent and River Lech) of two river catchments. Statistical models (discriminant analysis and generalized linear model) were evaluated to identify the influence of physicochemical water conditions in the interstitial zone on the hatching success at different spatial and temporal scales.

Short-term positive as well as negative effects of a microscaled in-stream restoration were analyzed by linking biotic (hatching success of brown trout and macrozoobenthos abundance) and abiotic factors (sediment texture, physicochemical parameters in the interstitial zone and downstream fine sediment load). Finally, a long-term monitoring (4 years) was conducted after spawning ground restoration (gravel addition and sediment cleaning) for studying the sediment quality, egg survival as well as fish population structure (brown trout) and river morphology in a highly regulated chalk stream (River Moosach).

Brown trout (*Salmo trutta*), grayling (*Thymallus thymallus*) and Danube salmon (*Hucho hucho*) were used as indicator species for habitat quality. These model species are endangered target species for conservation and hence they are adequate representative species for the research of this key habitat. All three species have overlapping distribution areas, but different spawning seasons. The comparison of different life history strategies highlights different requirements to the stream substratum quality.

# 2 Salmonids

## 2.1 Systematics and distribution

Salmonidae are distributed holarctic in North America, Europe and Asia. Drastic climate changes like glaciations in the last million years did not force salmonids to change significantly since the origin of the first salmonids. After the last glaciations (between 8,000 and 14,000 years ago), the northern rivers of Europe and North America were recolonized from southern and northern refuges (Ferguson, 2006). The long isolation and also postglacial geographic isolation resulted in distinct ancestral lineages (Bernachez, 2001). These lineages were mixed up later by interbreeding and introgressive hybridization e.g. due to environmental perturbations, but also by anthropogenic interference like aquaculture, commercial and recreational fisheries (Hendry and Stearns, 2004; Gum et al., 2006). Consequently, the phylogeographic structure e.g. of brown trout is heavily influenced by stocking (Kottelat and Freyhof, 2007).

The family Salmonidae is subdivided into three subfamilies, the Coregoninae, the Thymallinae and the Salmoninae (Nelson, 2006). The Thymallinae and the Salmoninae are closer related to each other than to the Coregoninae (Figure 2-1; Yasuike et al., 2010). One of the major differences between the Coregoninae and the two sister groups Salmoninae and Thymallinae is the different spawning behavior.

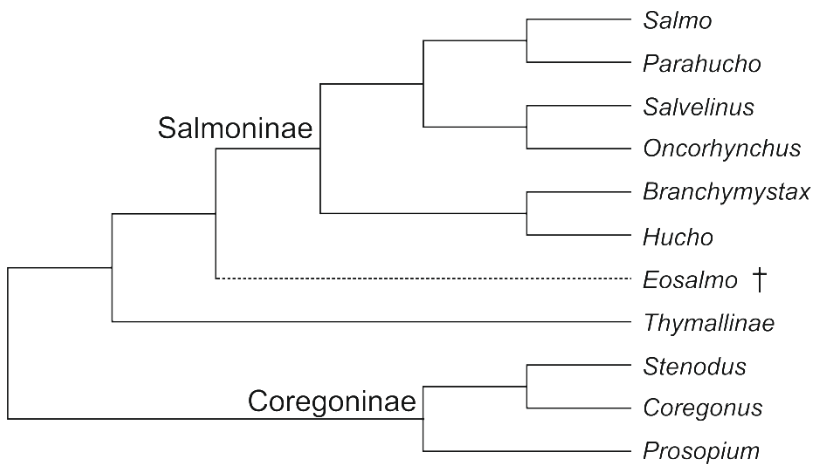


**Figure 2-1: Phylogeny of Salmonidae (modified after Ramsden et al., 2003).**

Whereas the Salmoninae and the Thymallinae build ‘redds’ and the egg development is located in the hyphoreic zone of the substratum, the eggs of the Coregoninae develop in the pelagic zone.

The Thymallinae comprise 10 - 20 species including the European grayling (*Thymallus thymallus*). The conservation status of *Thymallus thymallus* is least concern (IUCN Red List of Threatened Species), but it is locally threatened (Freyhof, 2011a).

The Salmoninae comprise 30 species in six genera: *Salmo* (including *Salmo salar* and *Salmo trutta*), *Oncorhynchus, Salvelinus, Branchymystax, Parahucho and Hucho* (including *Hucho hucho*). *Hucho* is endemic to the Danube basin and occurs in freshwater exclusively. *Parahucho* is endemic in northern Asia (Phillips et al., 2004). *Brachymystax* is also spread in Asia (Xia et al., 2006). *Oncorhynchus* is distributed in the North Pacific basin and includes freshwater as well as anadromous fish (Esteve and McLennan, 2007). *Salvelinus* and *Salmo* are also freshwater and anadromous fish. Whereas *Salvelinus* is spread circumpolar in the northern hemisphere, *Salmo* is endemic to the North Atlantic basin (Behnke, 1980; Stearley and Smith, 1993).

Two target species of this study, the brown trout (*Salmo trutta*) and the Danube salmon (*Hucho hucho*) have overlapping distribution in the Danube basin, where the Danube salmon is endemic. The brown trout is additionally native in the Atlantic, the North, and the White and Baltic Sea basins. It is also introduced throughout Europe, North and South America, Africa, Pakistan, India, Nepal, Japan, New Zealand and Australia (Kottelat and Freyhof, 2007). Even though the brown trout is widely spread, declines of populations are locally observed (Freyhof, 2011b). Anthropogenic modifications of rivers, e.g. artificial flow discharges, and water pollution are important causes of endangerment simultaneous to over-stocking and numerous electrofishing. The distribution of the Danube salmon is very fragmented due to anthropogenic alterations of the flow-regime by dams and additionally the natural reproduction is very limited. Hence, the conservation status of the Danube salmon is endangered, basically due to spawning habitat degradation (Freyhof and Kottelat, 2008).

## 2.2 Life history

The salmonids include fish that are freshwater residents, freshwater migrants and anadromous migrants. The Atlantic salmon (*Salmo salar*), the Pacific salmon (*Oncorhynchus* spp.) and the sea trout (*Salmo trutta*) are well known representatives of anadromous salmonids. It is a fact that some species like the brown trout are comprised by freshwater resident lineages as well as freshwater migratory lineages. In particular, the lake trout (*Salmo trutta lacustris*) migrates from freshwater lakes to spawning areas in influent rivers.

Salmoninae and Thymallinae have similar stages of reproduction (Figure 2-2; after Elliott, 1994). Typically, after sexual maturation, the adults head for spawning habitats, which are characterized by a well-oxygenated interstitial zone (7 - 10 mg $L^{-1}$) in the stream substratum (Crisp, 1996; Rubin and Glimsäter, 1996; Kondolf, 2000a; Ingendahl, 2001; Malcolm et al., 2003). It is assumed that salmonid populations return to the same spawning sites over generations.

Potential spawning areas vary between species as well as within species. Whereas the preferred spawning habitats e.g. for Atlantic salmon are shallow areas in large rivers with high flow-velocity from 0.35 m $s^{-1}$ to 0.80 m $s^{-1}$, brown trout spawns not only in large rivers, but also in tributaries and small streams with lower flow-velocity down to 0.15 m $s^{-1}$ (Ottaway et al., 1981; Witzel and MacCrimmon, 1983; Heggberget et al., 1988; Crisp and Carling, 1989; Kondolf et al., 1993; Leclerc et al., 1996; Mills, 1989; Zimmer and Power, 2006).

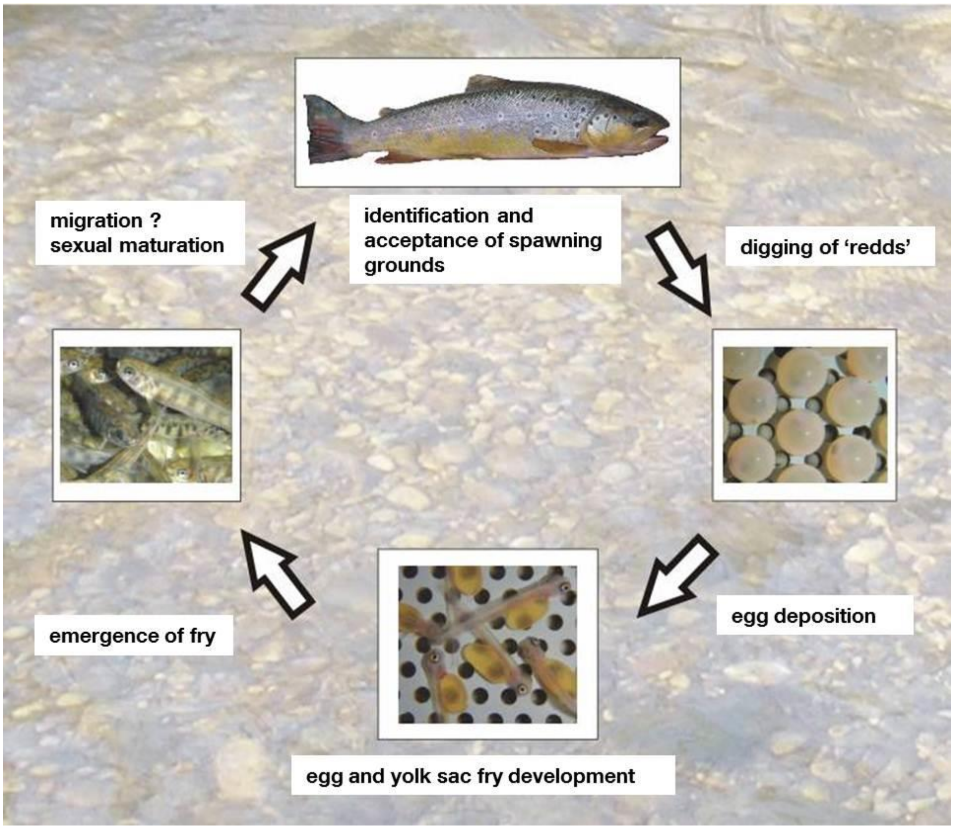


**Figure 2-2: The reproduction - cycle of salmonids.**

Generally, the favored flow-velocity, and furthermore the gravel size, depends on the size of the spawner. However, the Atlantic salmon was also found to spawn in inlets and outlets of lakes, even lake-spawning populations are known (Kondolf et al., 1993; Gibbins et al., 2002; Verspoor and Cole, 2005).

If the spawners accept a potential spawning ground, they start to build a nest called 'redd' (Figure 2-3). During 'redd'-building, the spawner hits the substratum surface to raise gravel. For instance Atlantic salmon and brown trout typically prefer particles with the diameter 6 - 128 mm (Ottaway et al., 1981; Shirvell and Dungey, 1983; Olsson and Persson, 1986; Heggerbet et al., 1988; Chapman, 1988; Crisp and Carling, 1989; Kondolf et al., 1993; Leclerc et al., 1996; Moir et al., 1998; Moir et al., 2002). Fine sediment particles are washed out of the substratum and drift with the stream flow, whereas coarser gravel settles downstream of the pit. The eggs are deposited by the spawner in the hill consisting of well-sorted and loosen substratum with an efficient exchange between the interstitial water and the open water. The modification of the spawning habitat by the adult fish induces an enhancement of the hatching success. The size of the 'redd', and hence the area of stream substratum with improved interstitial water conditions for egg and larval development, depends on the body size of the spawner as well as on physical factors like flow-velocity and gravel size (Ottaway et al., 1981; Crisp and Carling, 1989; Kondolf et al., 1993; Fleming, 1996; Bayliss, 2006).

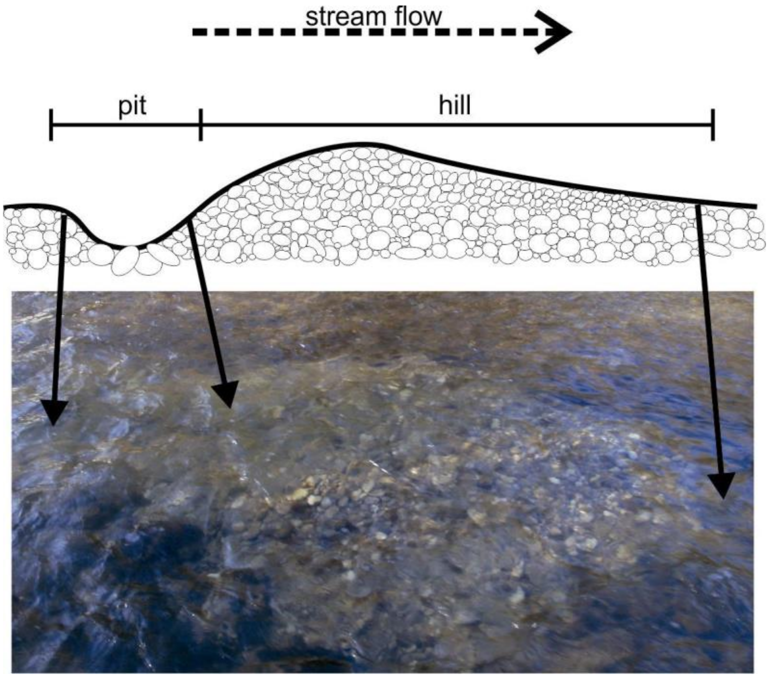


**Figure 2-3: The design of a typical salmonid 'redd'.**

Eggs and yolk-sac fry develop within the sediment until the fry emerge through the stream substratum into the open water. The flow-through within the hyporheic zone decrease during the development caused by the accumulation of fine particles after the ‘redd’ was built (Scott et al., 2005; Greig et al., 2007). The hatching success and the subsequent emergence of fry are affected by the decreased in substrate permeability and the assumed lack of oxygen (Witzel and McCrimmon, 1981; Kondou et al., 2001; Malcolm et al., 2003; Heywood and Walling, 2006; Julien and Bergeron, 2006).

Even though the reproduction process is very similar, the life history of salmonids can differ remarkably e.g. anadromous fish versus resident fish or spring spawner versus fall spawner.

In the northern hemisphere, the spawning season of brown trout is between September and January (starting with falling temperatures in fall) and lasts about 3 - 4 weeks for individual populations. The spawning period of e.g. Atlantic salmon occurring in the same regions starts typically 1 month later and is usually twice as long as the spawning period of brown trout. However, the duration of the spawning season also depends on the geographical latitude. In particular, southern populations of European Atlantic salmon were observed to breed until March (Garcia de Leániz et al., 1987; Heggberget, 1988).

Whereas the Atlantic salmon is typically anadromous, three different life history forms of brown trout are known: an anadromous form, a lake form and a resident form. However, brown trout populations in the Danube basin are land-locked. The resident stream individuals are usually smaller (~ 200 mm - 300 mm standard length) than the anadromous or lacustrine individuals (450 mm - 600 mm standard length). They live in clean streams with good oxygen conditions, where temperature does not exceed 20 °C - 22 °C for a long period. Resident trouts spawn for the first time when they are 2 - 3 years old. They are generally repetitive spawners (iteroparous; 2 - 3 seasons). After choosing the spawning sites, the female spawning adults start digging the ‘redds’. At the end of ‘redd’-building, the eggs are covered by gravel (~ 1500 eggs per kg fish).

In contrast to the Atlantic salmon or the brown trout, the Danube salmon is typically a spring spawner, (usually March - April; Holčik, 1988). The males spawn for the first time at the age of 2 - 4 years; females become sexually mature one year later. The Danube salmons stay their whole life in freshwater, typically in montane and submontane reaches of large streams and swift rivers with low temperatures (<15 °C). Spawners migrate to the upper reaches of the tributaries (generally small river tributaries), where the male and the female dig the ‘redd’. The eggs are covered with gravel after finishing the ‘redd’ (~ 1000 eggs per kg fish). The largest documented Danube salmon was 1650 mm standard length and accordingly, the ‘redd’ reached sizes of 1.2 m - 3.0 m in diameter. The maximum age of the Danube salmon is estimated to be more than 20 years, which implies more than 15 spawning seasons for reproduction by a single fish.

For Danube salmon and brown trout, the availability and quality of all habitats, including spawning habitat, juvenile fish rearing habitat, and habitat for the fully-grown fish, are crucial for the natural life-cycle. Spawning grounds with clean gravel, riffles, deeper pools, hiding places, overgrown stream banks and small tributaries are necessary for healthy populations with natural reproduction.

# 3 The effects of stream substratum composition on the emergence of salmonid fry

A similar version of this chapter was published: Sternecker Katharina, Geist Jürgen. 2010. The effects of stream substratum composition on the emergence of salmonid fry. Ecology of Freshwater Fish. 19: 537-544.

## 3.1 Abstract

Salmonid fishes are target species for the conservation of freshwater habitats, but their natural reproduction is often insufficient. The emergence of fry is a crucial phase in the life cycle of salmonids and the stream substratum is the key habitat which regulates the emergence success. In this study, brown trout (*Salmo trutta*) and Danube salmon (*Hucho hucho*) eggs were exposed to different sediment textures and the emergence and the postemergence survival and growth were observed under constant water chemical conditions in the laboratory. In both species, textural effects on emergence rate, chronology of emergence, survival rate after emergence and growth after emergence were detected. Fine-textured substratum (5 - 8 mm) formed a physical barrier to the posthatch migration of salmonids from the interstitial zone to the open water. The time period between the first and the last emerged fish was shorter in treatments with fine texture compared with coarse substratum. The survival rate was higher in treatments of coarser sediment. The effects of different textures on the growth of fry after emergence differed between brown trout and Danube salmon, which can be explained by different life history strategies. These results suggest that physical characteristics of substratum texture can have strong effects on salmonid emergence, and ultimately on the persistence of salmonid populations. They also suggest that biodiversity conservation in stream ecosystems can greatly benefit from an inclusion of the physical characteristics of the stream bed into catchment-based management plans.

## 3.2 Introduction

The completion of the salmonid life-cycle depends on habitat quality and availability at all development stages (Elliott, 1994; Denic and Geist, 2010). The river bed is the key habitat for the reproduction of lithophilic spawners. At spawning time, salmonid females dig their nests in the gravel bed, eggs are deposited into the substratum and subsequently develop from eggs to larvae and eventually to emerging fry. The substratum requirements of salmonids change during different phases of the reproduction process and the substratum suitability must be specifically assessed in the period from the nest digging by females to the emergence of fry (Kondolf, 2000a). For instance, during egg-development, a porous gravel overlay with an exchange between oxygen-rich water from the surface and water from the hyporheic zone is necessary. Several authors have stressed the importance of stream substratum characteristics for the hatching success of salmonids (Rubin and Glimsäter, 1996; Acornley and Sear, 1999; Soulsby et al., 2001b; Heywood and Walling, 2007). In this context, deposition of fine sediment resulting in oxygen depletion is a well-known problem for organisms living in the interstitial zone (Wood, 1997; Geist and Auerswald, 2007; Pander et al., 2009). The effects of high fine sediment quantities on the first habitat migration in the salmonid life-cycle, i.e., the emergence from interstitial to open river water, however, seems to be governed by other factors and is not yet fully understood (Phillips et al., 1975; Hausle and Coble, 1976; Chapman, 1988). Methods to detect the emergence-rate in the field have been explored and the success of alevin emergence has been in the focus of conservation studies (Phillips and Koski, 1969; Weaver and Fraley, 1993).

To date, most experiments on the effects of fine sediments have been carried out in the field and do not distinguish the effects related to physical and chemical factors. In contrast, this study was intended to exclusively focus on the physical effects of fines in the process of fry emergence under otherwise constant conditions, because a better understanding of the emergence-process is still required (Fudge et al., 2008). Sublethal effects of textures on posthatch survival and growth are also important but have previously not been considered. At the same time, a comparison between fall-spawning salmonids (e.g., brown trout, *Salmo trutta* L.) and spring-spawning salmonids (e.g., Danube salmon, *Hucho hucho* L.) can reveal links between life history strategies and spawning habitat quality requirements. This variation of emergence strategies between different salmonid species and the consequences in the life history strategy success are relevant for conservation studies (Beer and Anderson, 2001; Klemetsen et al., 2003). The objective of this study was to test the physical effects of texture on fry emergence and to study the variation in the emergence strategies between a spring- and a fall-spawning salmonid. The emergence success was tested by exposing brown trout and Danube salmon eggs to three different textures and a control treatment without sediment. These two model species were selected since: (i) they represent a spring-spawning and a fall-spawning species with overlapping distribution, (ii) they represent one of the largest and one of the smallest European salmonids and (iii) both species are currently in the focus of conservation in Europe, with the need to provide data on habitat restoration.

## 3.3 Material and methods

### *3.3.1 Experimental setup*

To test the effect of stream substratum texture on the emergence success of brown trout and Danube salmon, four different treatments were set up in modified salmonid egg incubation trays (Figure 3-1): a treatment without substratum as control (D), a treatment with 5 - 8 mm rounded gravel (treatment A), a treatment with 8 - 16 mm rounded gravel (treatment B) and a treatment with 16 - 32 mm rounded gravel (treatment C).

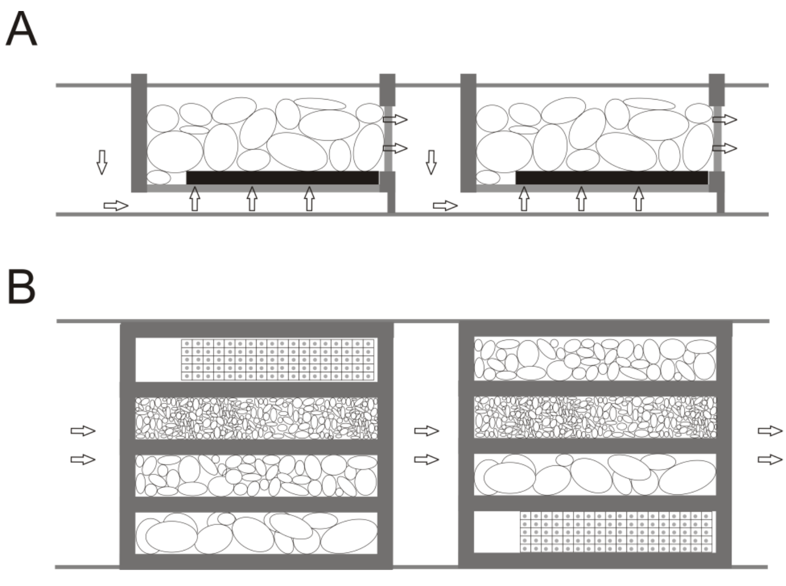


**Figure 3-1: Emergence experiment design; (A) side view of two incubation boxes in a laboratory flume filled up to 10 cm with rounded gravel; an aluminum grid is placed on the bottom of each incubation box (perforation bore diameter: 2 mm); (B) top view of two incubation boxes, four compartments (L: 410 mm, W: 105 mm and D: 145 mm) per box are shown with 5 - 8 mm, 8 - 16 mm, 16 - 32 mm gravel and a reference (random order of treatments within boxes); in the reference compartment, the aluminum grid with 90 chambers (each L: 15 mm, W: 15 mm and D: 15 mm) filled up with eggs is shown.**

Five brown trout females and three males (Landesfischzuchtanstalt Mauka, Germany) along with two Danube salmon females and three males (Fischereilicher Lehr- und Beispielbetrieb Lindbergmühle, Germany) were used as spawners and a well-mixed batch of eggs was created for each species. A total of 2880 brown trout eggs and 2880 Danube salmon eggs in a late phase of 'eyed egg stage' were incubated with ground water in a flow-through system (discharge: 0.1 l $s^{-1}$). Eight replicate incubation boxes (AGK Kronawitter GmbH, Germany) were equally distributed over two different flumes. Each incubation box contained the four treatment compartments (L = 410 mm, W = 105 mm and D = 145 mm) in random order and was filled with 4 x 90 eggs. In each compartment, eggs were separately incubated in chambers of an aluminium grid (size of chamber: L = 15 mm, W = 15 mm and D = 15 mm) to avoid the direct contact of eggs. To mimic natural egg incubation, all compartments (except for the reference) were filled up to 10 cm with gravel.

Daily emergence success was measured by capturing the emerged alevins every 12 h by dip net or vacuum using a glass tube. The experiment was terminated 3 days (brown trout) and 8 days (Danube salmon) after the last emerged fry was observed. Then the sediment was removed and physically blocked fry were counted in the sediment. Daily measures of temperature at the inlet and at the outlet of both laboratory flumes revealed a mean temperature of 11.4 °C (SD = 0.1) for brown trout and 11.8 °C (SD = 0.3) for the Danube salmon experiment. Mean oxygen concentration was 8.8 mg $L^{-1}$ (SD = 0.7) in the brown trout exposure and 8.6 mg $L^{-1}$ (SD = 0.9) in the Danube salmon exposure.

Additionally, pH (mean = 7.7; SD = 0.0), specific conductance (mean = 984 µS $cm^{-1}$; SD = 36), redox potential (mean = 488 mV; SD = 17), nitrate (mean = 14.9 mg $L^{-1}$; SD = 2.7), nitrite (mean = 0.04 mg $L^{-1}$; SD = 0.02) and ammonium (mean = 0.10 mg $L^{-1}$; SD = 0.06) were measured at the beginning and at the end of the experiment. Due to the comparatively long emergence period in brown trout, measurements were also taken at two additional time points during the emergence experiment. Except for texture, none of the physicochemical variables showed significant differences between treatments after Bonferroni correction ($\alpha$ = 0.0063), indicating that the observed effects on fry emergence were not influenced by these variables.

After emergence of fry, the alevins were fed on commercial trout chow (F-0.5 GR Pro Aqua; Trouw nutrition Deutschland GmbH, Burkheim) ad libitum for 67 days (brown trout) and 82 days (Danube salmon) to test for post-emergence effects resulting from the different texture treatments.

Ninety-eight days (brown trout) and 100 days (Danube salmon) after detecting the first hatched fish, 10 fish of every compartment were sacrificed (if less than 10 fish emerged, all available fish were taken) and total length (±1.0 mm) was measured immediately. Dry weight (±0.001 mg) was determined after 48 h at 60 °C in a drying chamber.

### *3.3.2 Statistical analyses*

The timing of the emergence between the treatments was compared using the sum of day degrees (dd) using the first fish hatched in the reference replicates as a starting point for normalization. The 0.10 percentile ($H_{10}$), the 0.50 percentile ($H_{50}$) and the 0.90 percentile ($H_{90}$) of emerged fry was calculated on the basis of the total emergence count per treatment. Homogeneity and normal distribution of data were tested by Levene-test and Shapiro-Wilk test.

Differences in $H_{50}$ between the species were tested with one sample t-test (P*). Differences in the emergence success, the survival rates after emergence and sublethal effects (weight and length) between treatments and replicates were tested with Kruskal-Wallis (KW) test and multiple U-test applying Bonferroni corrections (p) since these data did not follow normal distribution and homogeneity of variances. All statistical analyses were performed using R version 2.7.0 [Copyright (C) 2008, The R Foundation for Statistical Computing, Vienna, Austria; program available free of charge at http://www.r-project.org].

## 3.4 Results

### *3.4.1 The effects of texture on emergence rate and survival*

Over all treatments, a total of 1279 brown trout fry (59 %) successfully emerged within 807 dd. A similar emergence rate within a shorter emergence period (483 dd) was found in Danube salmon with 1378 fry (64 %). Only completely developed Danube salmon fry emerged, whereas brown trout fry with residual yolk sac were detected until 375 dd. Texture had a strong effect on the emergence rate per day (KW: $P < 0.001$). The number of emerged fry in treatment A was significantly lower compared to the daily emergence rate in the coarser textured treatments B and C for brown trout (KW: $P < 0.001$) as well as for Danube salmon (KW: $P < 0.001$). No significant differences of emergence counts per day were found between replicates of each treatment.

The total emergence rate of brown trout fry was lowest in the fine-textured treatment A where only 7 % of the fry emerged (mean emergence per day = 0.1; SD = 0.4). Emergence rates were higher in treatments B and C, with 78 % (mean emergence per day = 1.3; SD = 2.7) and 92 % emergence (mean emergence per day = 1.5; SD = 3.0) in treatments B and C, respectively (Figure 3-2). A similar trend was observed in the Danube salmon experiment where the emergence rate was 3% in treatment A (mean emergence per day = 0.2; SD = 0.5), 91 % in treatment B (mean emergence per day = 3.8; SD = 4.7), and 96 % in treatment C (mean emergence per day = 4.5; SD = 5.0). The hatching rate in the reference without substratum was 96 % (SD = 2) for brown trout and 100 % (SD = 0) for Danube salmon.

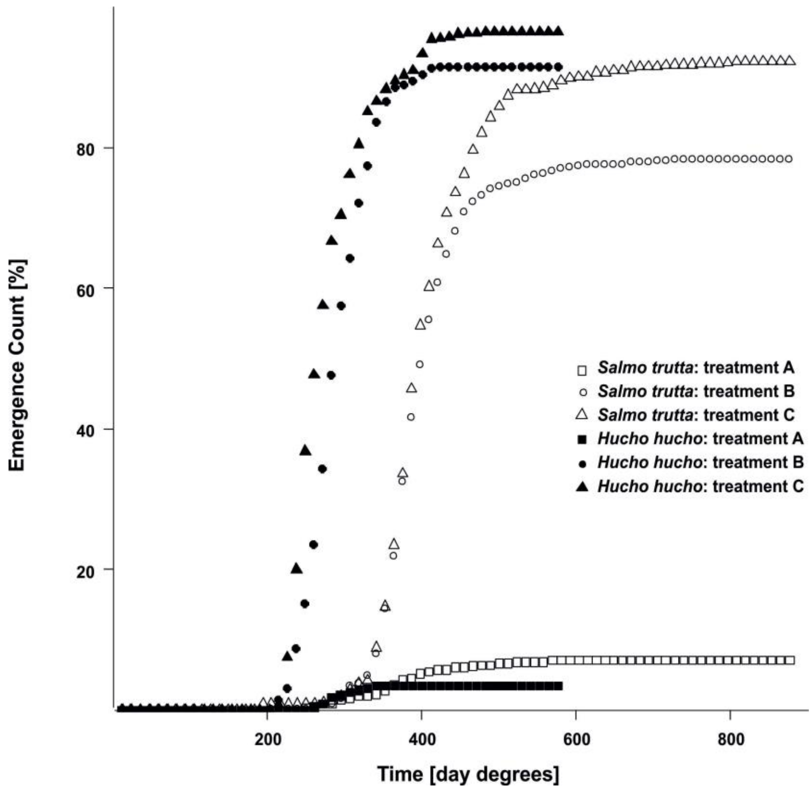


**Figure 3-2: Time series of cumulative emergence counts (%) in brown trout (N = 1279) and Danube salmon (N = 1378); treatment A, B and C represent the gravel sizes 5 - 8 mm, 8 - 16 mm and 16 - 32 mm for brown trout (white) and Danube salmon (black), respectively. The sum of day degrees (dd) was calculated using the first fish hatched in the reference replicates as a starting point for normalization. The number of initially incubated eggs per treatment was 720.**

The hatching rate was consistently higher than the emergence rate. In treatment A, and to a lesser extent in treatment B and C, fry which did not manage to emerge to the substratum surface were detected. Three days (brown trout) and 8 days (Danube salmon) after the last observed emergence of fry, 30 % (SD = 9) of initially incubated brown trout eggs and 18 % (SD = 4) of Danube salmon eggs had completely developed into fry but were physically blocked in the finest substratum (treatment A). In treatment B, as well as in treatment C, 3 % (SD = 2 and SD = 1, respectively) of hatched brown trout did not emerge. Zero percent (0 %) (SD = 1) and 1 % (SD = 1) of dead Danube salmon fry were found in the sediment of treatments B and C.

### *3.4.2 The effects of texture on the chronology of emergence*

The chronology of emergence was similar in all treatments of brown trout and Danube salmon (Figure 3-2). At first, individual alevins emerged until fry emergence number reached a peak, subsequently the emergence activity decreased. The $H_{50}$ of all treatments was not significantly different within the brown trout experiment and within the Danube salmon experiment, respectively (Figure 3-3).

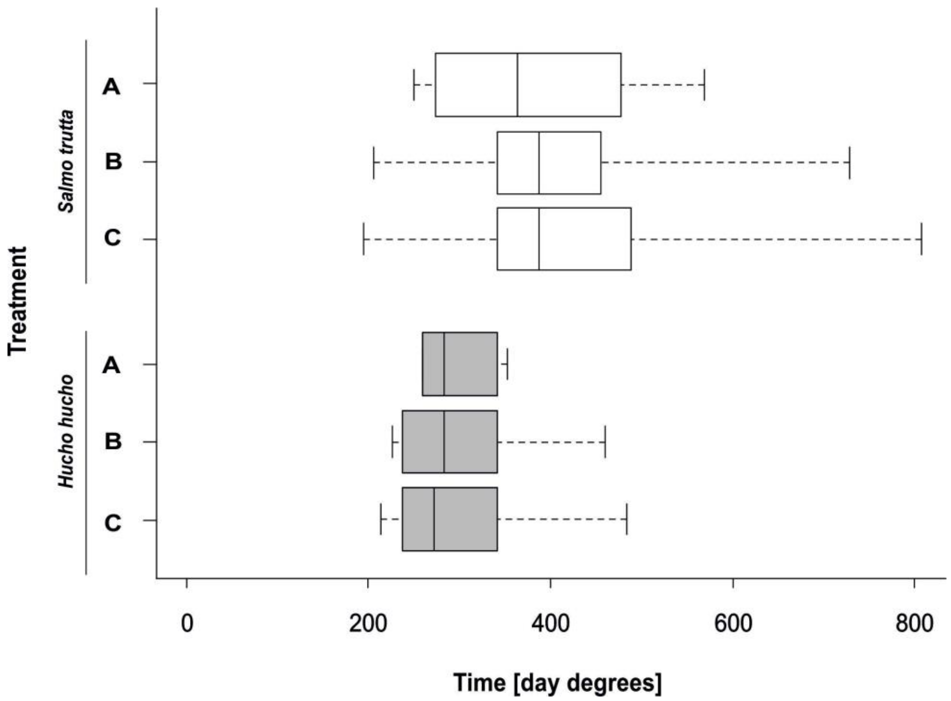


**Figure 3-3: Emergence count of brown trout (white, N = 1279) and Danube salmon (grey, N = 1378) in relation to day degrees. A, B, C refer to the treatments 5 - 8 mm, 8 - 16 mm, 16 - 32 mm, respectively. Boxes: 0.90 percentile is conform to 90 % ($H_{90}$), the 0.10 percentile is conform to 10 % ($H_{10}$) of total emergence of each treatment. The median shows 50 % of emerged fry ($H_{50}$) calculated on the total emergence of each treatment. Whisker: first and last detected emerged fry.**

However, effects of different gravel sizes in the emergence progression were observed in the amplitude of the emergence peak and in the duration of the time period between the emergence of the first and the last fry with the variability in the emergence time span being highest in treatment C and lowest in treatment A (Figure 3-3). This effect was mainly caused by the lower absolute number of fry that emerged from the finest sediment.

Species-specific differences in the timing of emergence were evident. The spring-spawning Danube salmon emergence peaked after 283 dd ($H_{50}$ = 281; SD = 3.9). The $H_{50}$ of the fall-spawning brown trout was 384 dd (SD = 11.9) and significantly higher than in Danube salmon (*p < 0.001). The emergence period range was also much shorter in Danube salmon, with a difference of 79 dd between $H_{90}$ and $H_{10}$, compared with 139 dd in brown trout.

### *3.4.3 Chronic effects on survival and growth*

Pronounced differences in survival rates after emergence were observed between the two species (Figure 3-4) with a mean survival rate of 83 % (SD = 15.0) in brown trout and 8% (SD = 9.3) in Danube salmon. Different treatments within species did not result in significant differences of mean survival rates (all P > 0.05), but a decrease in survival variability was observed in both species from fine-textured treatment A to coarse substrata (B, C), to the reference treatment without substratum. The mean survival rate after emergence was 76% (SD = 25) for brown trout in treatment A, 82 % (SD = 9) in treatment B, 85 % (SD = 9) in treatment C and 89% (SD = 3) in the reference. Danube salmon had a mean survival rate of 8 % (SD = 13) in treatment A, 8 % (SD = 7) in treatment B, 11 % (SD = 9) in treatment C and 4 % (SD = 4) in the reference. Median values for survival indicated a similar pattern (Figure 3-4).

Significant species-specific chronic differences in growth of brown trout and Danube salmon were detected after emergence from the different substratum treatments (Figure 3-5). The length of brown trout fry that had emerged from finer sediment (treatment A) was higher than the length of the fry that emerged from sediment of coarser gravel (treatment B and C; KW: all P > 0.05). Fry from treatment C was significantly smaller than fry from treatment A (KW: P < 0.004). Similarly, the weight of the brown trout fry from treatment A was significantly higher than from treatment B (KW: P = 0.036) as well as from treatment C (KW: P < 0.001). Between the coarser-textured treatments B and C, the weight of fry that emerged from the coarsest substratum, was also significantly lower (KW: P = 0.031). Danube salmon fry from treatment C were significantly heavier (KW: P = 0.013) than fry from treatment B.

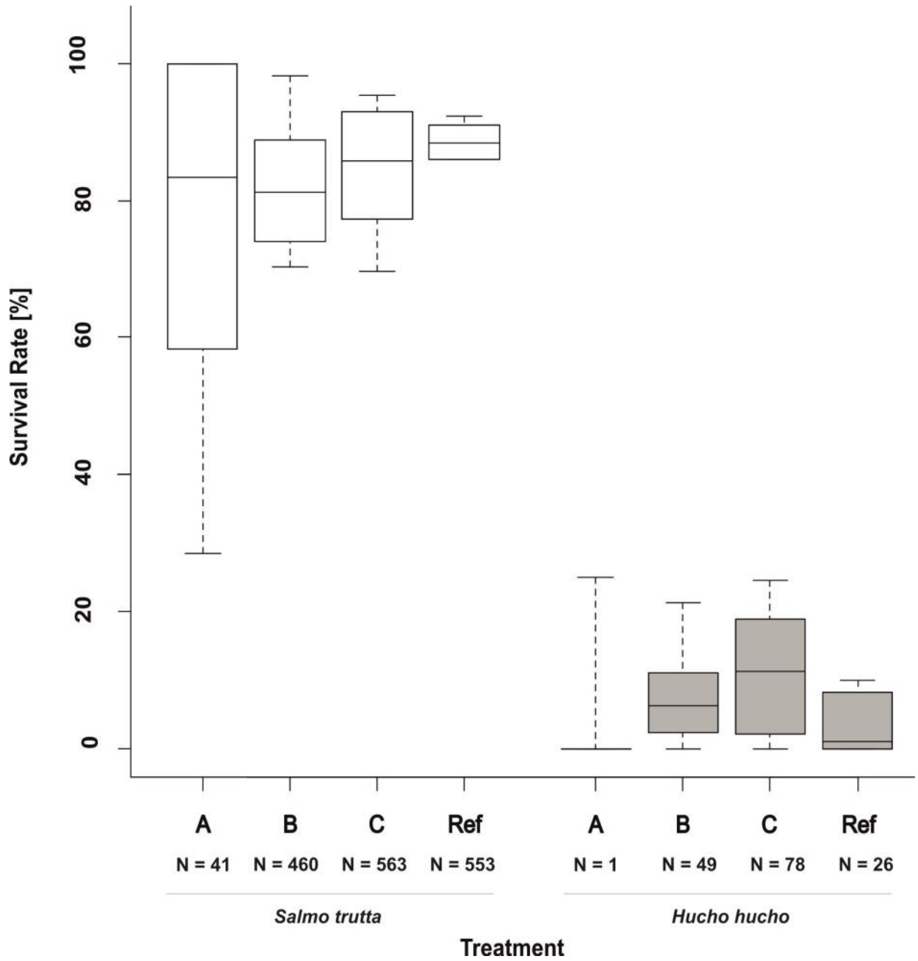


**Figure 3-4: Survival rate of brown trout (white) and Danube salmon (grey) after 98 days (*brown trout*) and 100 days (*Danube salmon*) of detecting the first hatched fish; A, B, C refer to the treatments 5 - 8 mm, 8 - 16 mm, 16 - 32 mm (eight replicates each), respectively; only emerged fish from treatments with sediment and fish from the references (Ref) were included. Boxes are 0.75 and 0.25 percentiles and median; whiskers represent the maximum and the minimum survival rate.**

Similarly, total length of these fish in treatment C was on average 4 mm greater than in treatment B, although mean total lengths were not statistically significant (KW: $P = 0.085$). The weight and length of fry from treatment A was similar to treatment C (KW: all $p > 0.05$) but the survival of only one specimen in this treatment limits further interpretation. The fry of Danube salmon reference was significantly heavier (KW: $P = 0.05$) and significantly longer than the fry of the brown trout reference (KW: $P < 0.001$). The reference in both species showed similar growth to fish in the coarse textured treatment B (KW: all $p > 0.05$).

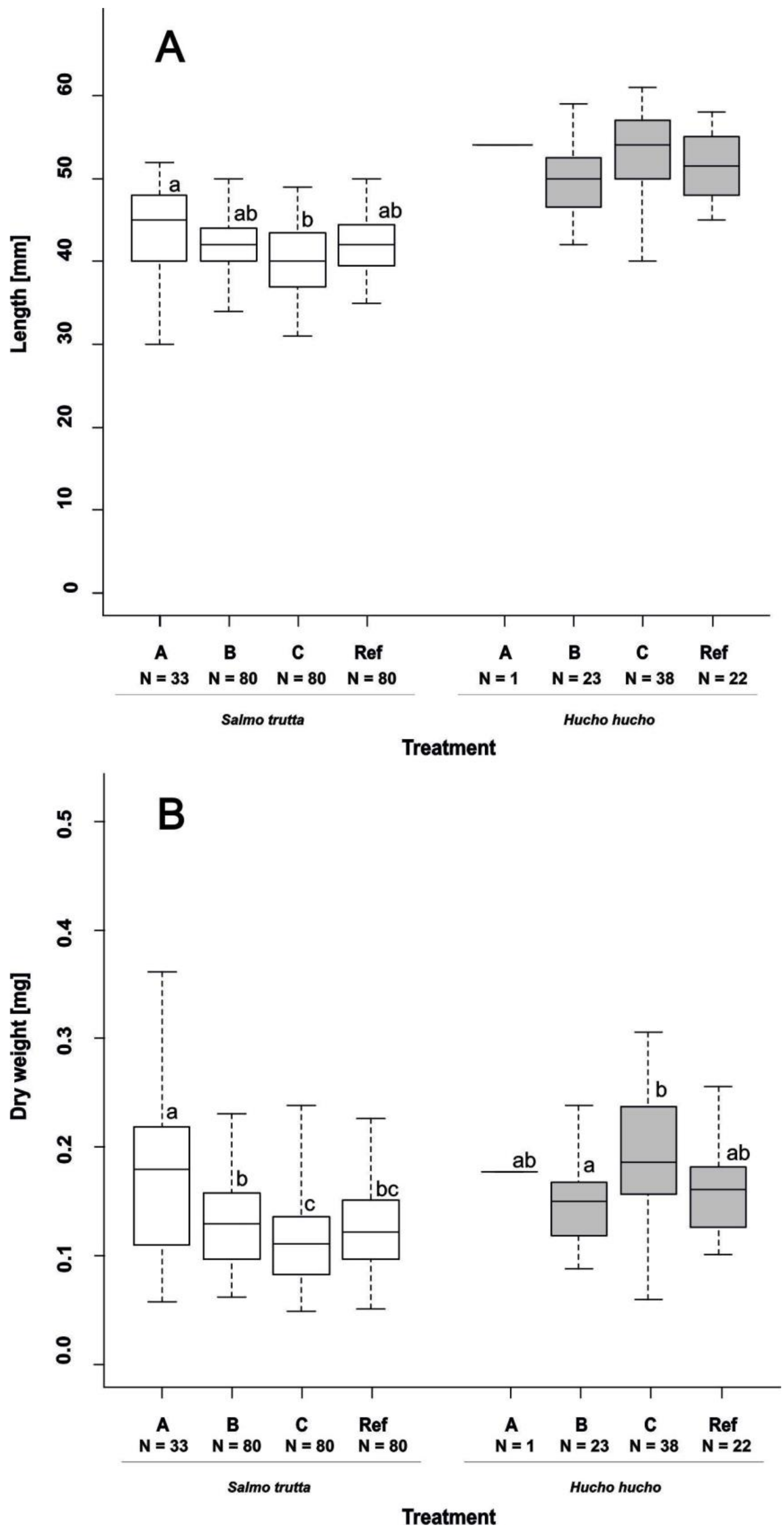


**Figure 3-5: (A) Length (mm) of brown trout (white) and Danube salmon (grey), (B) Dry weight (mg) of brown trout (white) and Danube salmon (grey) measured 98 days (brown trout) and 100 days (Danube salmon) after detection of the first hatched fish. A, B, C refer to the treatments 5 - 8 mm, 8 - 16 mm, 16 - 32 mm, respectively; only emerged fish from treatments with sediment and fish from the references (Ref) were included. Boxes are 0.75 and 0.25 percentiles and median. Whisker: maximum and minimum length and weight, respectively. Lower case letters (a, b, c) indicate significant differences (tests were performed for both species separately).**

## 3.5 Discussion

The results of this study show that stream substratum composition has a significant effect on the timing of emergence, survival of emerged fry and growth of brown trout and Danube salmon after emergence. Previous studies have mostly explained the effects of texture on salmonid egg and fry development by the water chemistry in the interstitial zone (e.g. Rubin, 1998). Since water-chemical variables were kept constant between treatments in the study presented here, it is likely that physical effects of the substratum on the emergence process are of similar importance. Under natural conditions, it is likely that adverse effects of fines on hatching and emergence success of salmonids result from both direct physical barrier effects and the indirect effects of altered interstitial water chemistry and oxygen depletion due to a limited exchange with the free-flowing water.

It is of evolutionary relevance that the spread of emergence time between spring-spawning Danube salmon and fall-spawning brown trout differed and that the texture had different effects on the post-emergence survival rate and growth of both species.

Within both species, fry from the coarsest sediment emerged most efficiently and fry that had to migrate through the finest sediment were often totally blocked by this physical barrier. The strong clogging effect is not only evident from the emergence rate, but also from the dead fry found in the substratum after termination of the emergence experiment. Surprisingly, texture did not result in time shifts of emergence in this experiment. Hausle and Coble (1976) showed that in brook trout (*Salvelinus fontinalis*) the emergence was shifted in time in treatments with higher proportions of sand (<2 mm). In a study on sea trout (*Salmo trutta*), fry emergence was only observed in substratum with mean particle diameters >15 mm and <6 mm, with emergence occurring earlier in the fine-textured sediment (Rubin 1998). Negative effects of fine sediments on the survival of salmonid eggs (e.g. Soulsby et al., 2001b; Julien and Bergeron, 2006) and on juvenile mussels inhabiting the interstitial zone (Geist and Auerswald, 2007) have been previously reported. Most studies, however, link the adverse effects of fines to chemical effects on interstitial water quality such as depleted oxygen levels and ⁄ or followed a study design which did not allow for separation of the physical and chemical effects of texture during egg development and emergence (Witzel and MacCrimmon, 1983; Rubin, 1998; Malcolm et al., 2003; Heywood and Walling, 2007; Pander et al., 2009). The results of this study suggest that the physical barrier effects caused by fine sediments should be equally considered.

The emergence counts in both species clearly show that the migration of fry from the hyporheic zone to the open water is a highly synchronized short-time event with a high emergence peak for the brown trout as well as for the Danube salmon. This phenomenon has already been observed in other salmonid species. For instance, in rainbow trout and sea trout, the emergence peak was explained by a rapid change in the phototactic direction (Carey and Noakes, 1981; Rubin, 1998). Brännäs (1995) showed that a highly synchronized emergence peak is beneficial for individual survival if predation pressure is present and if territory is limited.

The brown trout fry started to emerge with yolk sac, which however, was depleted at the peak of emergence. It is known that the early emerging salmonid fry has a higher survival rate in the first days after migration to the open water than later emerged fry (Einum and Fleming, 2000). Hence, it is possible that a part of the yolk sac fry of brown trout risk the obstacle of a yolk sac to use the advantage of an early emergence. In addition, unfavorable conditions in the interstitial water can induce emergence of yolk sac fry (Olsson and Persson, 1986). This explanation is unlikely for our dataset, however, since interstitial water chemistry did not differ among treatments. In contrast to brown trout, the fry of Danube salmon were completely developed, even though the emergence of Danube salmon begins earlier. The Danube salmon has a high growth rate which may be linked to an earlier food intake (Vøllestad and Lillehammer, 2000). A nutritional insufficiency in interstitial water may force the fish to emerge before their energy reserves are depleted.

The high growth rate of brown trout fry that emerged from the finest sediment may be explained by the fact that only the strongest and the largest fish were able to emerge, particularly in the treatment with the finest sediment. The assumption of a higher selection rate in fine-textured treatments is also supported by the lower survival rates in these treatments. The lower weight and length of brown trout after emergence in the coarser sediment compared to the finest sediment can be an effect of limited space after emergence due to a higher number of emerged fish. This fact simulates the limited territories in a natural river habitat.

Effects of texture resulted in opposite patterns for the Danube salmon exposure. The fish that emerged from treatments with the biggest gravel size were the heaviest and the longest. The peak of emergence time was more distinctive and the fish were completely developed during emergence throughout the experiment. This may result in optimal conditions for the fry, which can emerge from the sediment with the biggest gravel size, because migration requires less energy and the fry can immediately use nutrients for growth and not for regeneration. It has to be noted that the Danube salmon is known as a fish with a mortality rate of more than 90% in aquaculture, particularly during early life stages (Jungwirth, 1978; Geist et al., 2009). Hence, the low survival rate, especially in the reference, is not unusual for

Danube salmon held in captivity and differences in mortality and growth among treatments under hatchery conditions can thus not directly be transferred to the situation in the wild.

The observed differences in the emergence patterns of both species can also be explained by the different size of the emerging fry and by their different developmental stages. The fry of brown trout emerge with yolk sac, however, the fry of Danube salmon are completely developed at emergence and the emergence activity occurs within a shortened time period. The variations may result from adaptation to their different spawning seasons. The emergence of fully developed fry may be a strategy that compensates deficits in development for the later hatching date of Danube salmon.

This study shows that the influence of differently textured sediment on the emergence rate and on the chronology of emergence and additionally the post-emergence effects on survival rate and growth of fry are of evolutionary importance, even though the effects have to be considered species-specific.

This study also suggests that restoration of functional salmonid populations in general, and of brown trout and Danube salmon in particular, requires the restoration of functional stream substrates with low colmation and clogging by fine sediments. Management in the upper catchment areas requires supply of coarse sediment and control of unnatural and excessive siltation from the surrounding landuse into stream ecosystems. In addition, the restoration of flow regimes which govern the processes of erosion and sedimentation in the stream bed is required.

Biodiversity conservation in stream ecosystems can greatly benefit from an inclusion of the physical characteristics of the stream bed into catchment-based management plans.

# 4 The ‘egg sandwich’ - a method for linking spatially resolved salmonid hatching rates with physicochemical habitat variables in stream ecosystems

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## 4.1 Abstract

This paper describes the development of the ‘egg sandwich’, a system for assessing stream substratum quality by linking measurements of depth-specific salmonid egg hatching success and physicochemical water variables from the same sites within the interstitial zone.

## 4.2 Introduction

Biodiversity in freshwater ecosystems is critically threatened globally (Ricciardi and Rasmussen, 1999; Jenkins, 2003) with stream ecosystems being most heavily affected (Stein and Flack, 1997; Pimm et al., 2001; Gleick, 2003). Increasing evidence suggests that the properties of the stream substratum have a strong effect on the overall health of stream ecosystems (Palmer et al., 1997; Geist and Auerswald, 2007). Conservation efforts in salmonid habitats have traditionally focused on stream substratum and spawning site restoration (Grost et al., 1991; Acornley and Sear, 1999; Milan et al., 2000; Soulsby et al., 2001b). In light of the strong interest in restoration and assessment of stream substrata quality and salmonid spawning grounds, there is a need to provide tools for integratively assessing physicochemical and biological indicators. Here, a method for assessing stream substratum quality by measuring depth-specific salmonid egg hatching success, and physicochemical water variables from adjacent sites within the interstitial zone is presented. The applicability of this ‘egg sandwich’ (ES) was successfully tested in the laboratory and in natural and artificially constructed spawning sites of brown trout (*Salmo trutta* L.) and grayling (*Thymallus thymallus* L.).

## 4.3 Material and methods

The ES is composed of two principal subunits: an egg exposure unit and a unit for extracting interstitial water samples from the same substratum depth layers in which the eggs are exposed (Figure 4-1). The egg exposure unit consists of an aluminum grid and two perforated aluminum plates on the outside, creating 10 x 13 dice-like chambers. Each chamber has a volume of 3.375 $cm^3$, providing sufficient space for the hatched fry. In the test, one fertilized egg per chamber was exposed, resulting in 10 - 13 replicates distributed over different depth horizons, and a total of 112 exposed eggs per box.

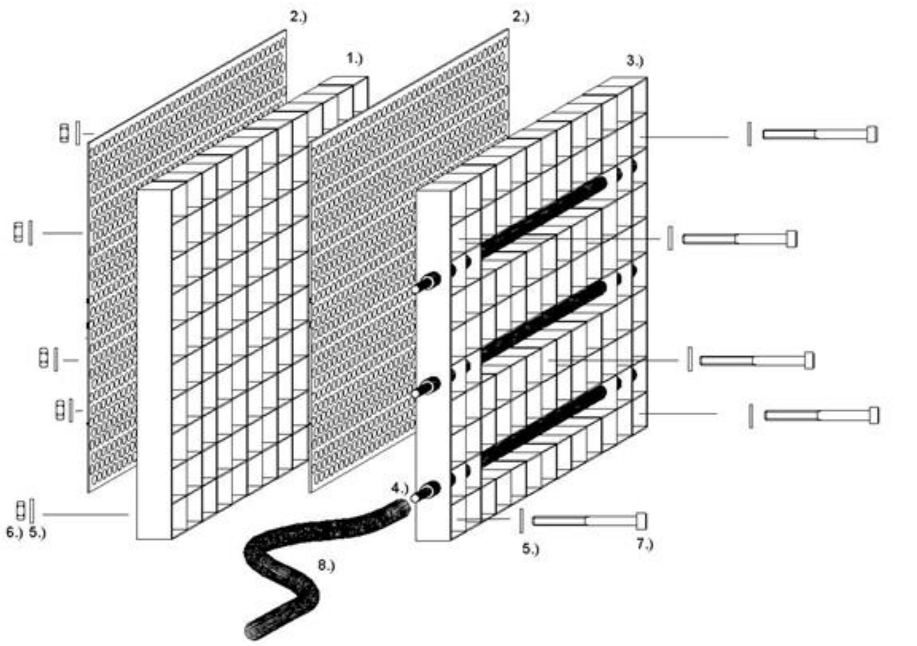



**Figure 4-1: Construction scheme of the ‘egg sandwich’. 1, aluminum grid [naturally anodized, length (L): 195 mm, width (W): 150 mm, depth (D): 15 mm, material thickness (MT): 0.5 mm, 130 chambers, L: 15 mm, W: 15 mm and D: 15 mm]; 2, perforated aluminum plate (L: 198 mm, W: 140 mm and MT: 1 mm, perforation bore diameter: 2 mm, partition: 3.5 mm diagonally lined); 3, aluminum grid (like 1, bore diameter for PVC tubes 8 mm); 4, perforated PVC tube [L: 220 mm, inner diameter (ID): 5.5 mm, outer diameter (OD) 7.5 mm, one end with sliding socket OD: 5 mm and ID: 3.5 mm, other end sealed]; 5, flat washer (stainless steel, M4, OD: 19.5 mm and MT: 1.2 mm); 6, socket-head screw (stainless steel, M4, L: 45 mm, length of thread: 20 mm, thread lead: 1.5); 7, hexagonal nut or wing nut (stainless steel, M4, thread lead: 1.5); 8, PVC flexible hose (OD: 8 mm, MT: 1.5 mm and L: 1200 mm).**

Five chambers are penetrated by stainless steel socket-head screws for fixing both units and thus cannot house eggs. The upper horizontal row serves as a visual indicator for monitoring the exposure depth of the box.

A second unit for extracting interstitial water is attached to the egg exposure unit. Its construction resembles that of the egg exposure unit, but the grid is penetrated by three perforated PVC tubes for sampling interstitial water at pre-defined depth horizons. One end of the tubes is equipped with sliding sockets to which flexible hoses with a length of 1.2 m are attached. The other end is closed with an elastic joint seal. Hoses can be sealed and individually marked with different color codes to ensure correct depth assignments of water samples extracted through the hoses. In practical tests, sampling at 20, 70 and 115 mm proved successful, but this system can be easily adapted for sampling in even more horizons or in different depths. ES size, the size of the chambers and the number of eggs exposed in each chamber can be varied according to the research question addressed.

The ES is loaded with fertilized eggs by placing it into a shallow water tank. Ideally, the water level in the tank barely covers the grid. Individual chambers can be filled with fertilized eggs using a large core pipette or a turkey baster. After closing the lid, boxes should permanently stay immersed in cool, oxygenated water to prevent damage to the eggs. For the assessment of substratum conditions in a typical field setting, the egg-filled ‘sandwich box’ is vertically inserted into the stream substratum of the study site. For comparisons of conditions between free-flowing water and different substratum depths, it is recommended that the box is buried at a depth, which ensures that the upper box surface layer stays exposed to the free-flowing water conditions above the stream bed level. To ensure minimal disturbance of the native stream bed characteristics, a spade is used to create a small gap within the substratum into which the ES can be inserted (Figure 4-2). Reference exposure of eggs in the free-flowing water (e.g. within a swimming box) allows for determination of specific development stages and hatching dates, which vary depending on the temperature day degree sum at the study sites.

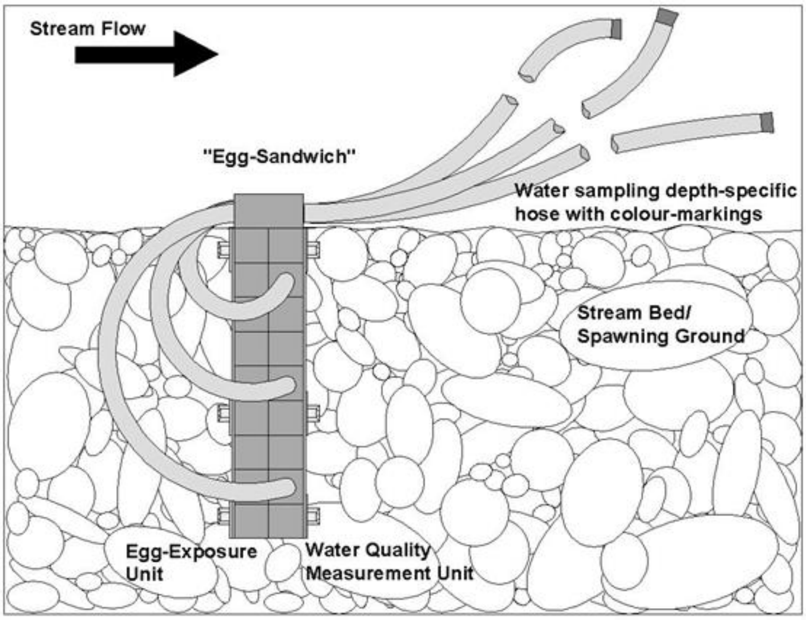
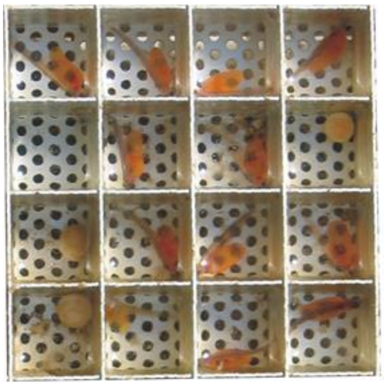



**Figure 4-2: Schematic side view of the exposed ‘egg sandwich’ in the stream bed; note that the egg exposure unit is situated upstream of the water quality measurement unit.**

During egg exposure, water samples are extracted by attaching a mobile 100 ml syringe to the hoses and by creating a vacuum, similarly to the procedure of sampling interstitial water in the stream substratum as described in Geist and Auerswald (2007). In a first step, the water volume entrapped inside the hose needs to be sampled and discarded before interstitial water from the defined substratum depths can be collected. The water volume has to be calculated or measured by the length and inner diameter of the hose. In the present exposures, water samples were analyzed for pH, electric conductivity, temperature, dissolved oxygen, nitrite, nitrate, ammonium and redox potential.

After hatching, the ES can be excavated and re-opened. Hatching success can be assessed according to the following criteria: (a) living fry, indicating favorable substratum conditions, (b) dead fry, indicating favorable substratum conditions during egg development but unfavorable conditions in the final exposure stage, (c) dead egg, indicating non-fertilized eggs or unfavorable conditions during early development and (d) missing egg due to predation, decomposition or erroneous loading of the chamber. An example evaluation is shown in Figure 4-3. Further variables, such as siltation or clogging of chambers, can also be assessed at this stage, e.g. by taking photographs of the chambers and using computer-based image processing applications.



| | | | |
|---|---|---|---|
| a | a | a | a |
| d | a | a | c |
| c | a | a | a |
| c | b | a | a |

**Figure 4-3: Proposed evaluation key for the ‘egg sandwich’; assessment of salmonid egg development (a, living fry; b, dead fry; c, undeveloped egg and d, chamber empty).**

Applicability of the ES was tested by field and laboratory tests, including the following aspects: (1) mean hatching rates ($H_R$) were compared between ‘egg sandwich’ exposure and reference egg exposure in regular upflow incubation trays (as typically used in salmonid hatcheries) under otherwise identical conditions (2) $H_R$ in the ES exposure were compared to the most commonly used field egg-exposure system, the modified Whitlock-Vibert boxes (WV-box; Whitlock, 1979; Mackenzie and Moring, 1988) at the same sites in the River Moosach (38°23’93’’90 N; 11°43’92’’60 E), (3) hatching rates of (1) and (2) were compared to ES substratum exposed in a laboratory flume. Detailed descriptions of numbers of replicates are provided in Fig. 4. One-way ANOVA and Tukey post hoc tests with SPSS 11 (SPSS Inc., Chicago, IL, U.S.A.) were used to compare treatments. The spatial resolution of the ES exposure was resolved by testing pair-wise differences in $H_R$ between three different depth layers of stream substratum (20, 70 and 115 mm) exposed ES boxes in the River Moosach. All investigations were carried out in winter 2007 to 2008.

## 4.4 Results

The results of these comparisons (Figure 4-4) revealed that $H_R$ of eggs exposed in the ES (mean ± S.D. = 80 ± 13 %) did not differ (Tukey HSD, $p > 0.05$) from those in the upflow incubation trays (84 ± 5 %), suggesting no systematic correction factor must be applied when using an ES exposure instead of direct egg exposure.

Hatching periods in the ES closely (± 2 days) matched the hatching periods of the reference samples. Mean $H_R$ did not differ ($p > 0.05$) between ES and WV-box in the field exposure, indicating that an assessment of stream substratum conditions will deliver similar results in both cases. Variability in $H_R$ was higher in the ES compared to the WV-box, however, since $H_R$ and physicochemical water variables differed markedly in different depth horizons within ES boxes (Figure 4-5). In the field test set, concentrations of dissolved oxygen, nitrite and nitrate, redox potential and pH value were the most determining factors for egg survival, whereas temperature, concentration of ammonium and electric conductivity explained little, or no variation in hatching success.

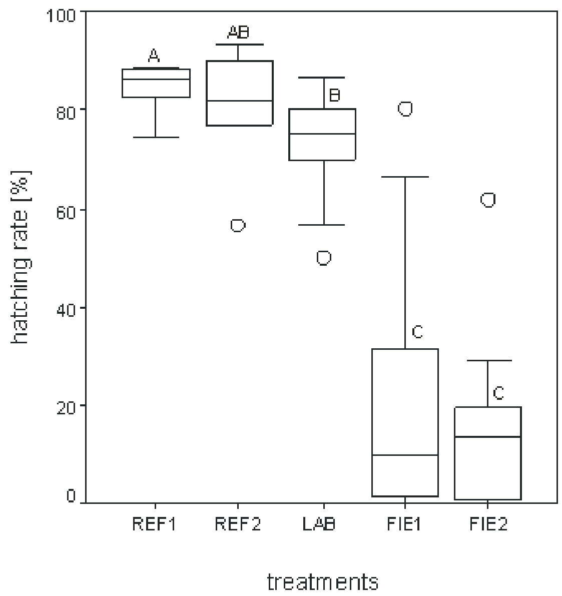



**Figure 4-4: Comparison between different egg exposure treatments: REF1, reference exposure in upflow incubation trays under regular hatchery conditions (n = 4 replicates, each with 1000 eggs); REF2, reference exposure in the 'egg sandwich' under the same conditions as REF1 (n = 6 replicates, each with 30 eggs); LAB, 'egg sandwich' exposure in substratum within a laboratory flume (n = 18 replicates, each with 3 x 30 eggs at depths 1, 2 and 3); FIE1, 'egg sandwich' exposure to natural stream substrata in the River Moosach (n = 25 replicates, each with 3 x 30 eggs at depths 2.0, 7.0 and 11.5 cm); FIE2, Whitlock-Vibert box exposure (encased with 1 mm gauze to avoid the escape of fry) at the same sites like FIE1 (n = 25 replicates, each with 200 eggs). Different upper case letters indicate significant differences at p < 0.05; box plots show the median and the interquartile range; O, outliers.**

Considering the significant sampling site effect ($p < 0.001$) on $H_R$ in ANOVA with interactions, the relation between exposure depth and hatching success became significant ($p < 0.001$). This indicates that an assessment of habitat quality at a high spatial resolution on a microhabitat scale is advantageous.

Hatching rates in the laboratory flume resembled those of the reference exposures in the upflow incubation trays but were significantly lower in both field exposures. This result can most probably be explained by the adverse effects of high fine sediment loads and low oxygen values in the stream substratum of the River Moosach compared to the flume exposure in coarse substratum with high oxygen saturation. Thus, the salmonid egg development in the ES unit is not significantly different from that under natural conditions if water quality is sufficient, which proves the suitability of the ES for assessing stream substratum quality.

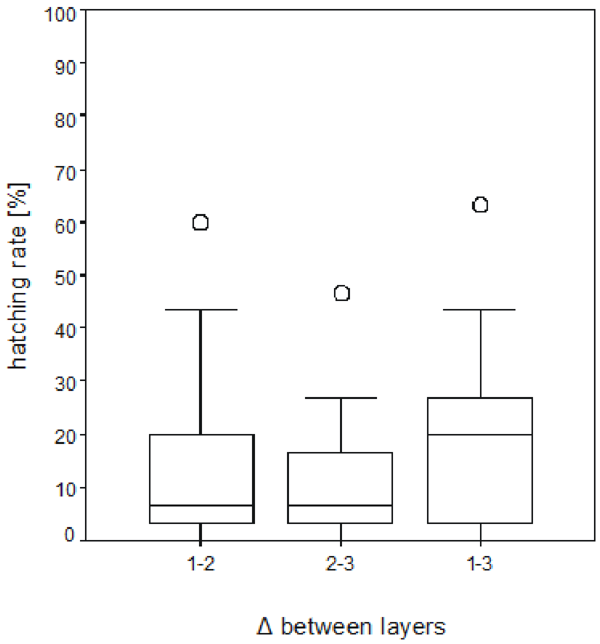



**Figure 4-5: Pair-wise differences in hatching rates ($\Delta H_R$) between three different depth layers of stream substratum exposed 'egg sandwich' boxes in the River Moosach (n = 25 for each depth layer); box plots show the median and the interquartile range; O, outliers; note that D is greatest between the most distant depth layers 1 and 3, although overall differences are not significant (ANOVA, p > 0.05).**

## 4.5 Discussion

Different alternative systems for hatching salmonid eggs in streams have been previously described (Vibert, 1949; Whitlock, 1979). Most of these systems, however, were primarily designed for salmonid propagation with the purpose of directly releasing hatched fishes into the stream. These systems are of limited use for assessing $H_R$ and for linking these with stream substratum quality variables, although some authors describe the use of modified Whitlock- Vibert boxes suitable for assessing hatching success (Mackenzie and Moring, 1988). Systems specifically designed for the assessment of $H_R$ under natural conditions both during exposure to the free-flowing water (Rubin, 1995; Donaghy and Verspoor, 2000) and in the stream substratum (Harris, 1973; MacCrimmon et al., 1989; Pauwels and Haines, 1994; Rubin, 1995; Donaghy and Verspoor, 2000; Bernier-Bourgault et al., 2005; Dumas and Marty, 2006) have been developed. Most of these methods, however, have not been designed to allow an assessment of spatial variation at different substratum depths (Harris, 1973; Pauwels and Haines, 1994; Rubin, 1995; Bernier-Bourgault et al., 2005), which appears to be crucial at least in the stream investigated in this study. As far as is known, however, none of these systems is coupled with a measurement unit, which allows linking the biological effect of hatching success with adjacent water variables. Also, exposure of single eggs in separate chambers is more difficult with other systems compared to the ES, which can be a crucial factor if infection and transmission of fungi is a major problem. Due to the compact slight design of the ES and the planting technique of creating a small gap in the riverbed substratum, the disruption of the interstitial zone is marginal compared to the planting of other systems (Donaghy and Verspoor, 2000).

In conclusion, practical experience with the use of the ES suggests that this technique provides an easy tool with high operational reliability for assessing stream substratum quality by linking spatially resolved salmonid egg survival and physicochemical water variables from the same sites within the interstitial zone. This system may also be used for incubation of other species, such as juvenile freshwater bivalves, for which assessment of stream substratum quality is of great importance (Buddensiek et al., 1990; Geist and Auerswald, 2007).

# 5 Factors influencing the success of salmonid egg development in river substratum

A similar version of this chapter was published: Katharina Sternecker, David E. Cowley and Jürgen Geist. 2012. Factors influencing the success of salmonid egg development in river substratum. Ecology of Freshwater Fish. doi: 10.1111/eff.12020.

## 5.1 Abstract

Interstitial water conditions in the hyporheic zone of the stream bed are determinants of salmonid egg hatching success. We used standardized egg exposures to develop and validate discriminant analysis and generalized linear model models linking the hatching success of brown trout (*Salmo trutta*) with physicochemical factors of the interstitial zone (e.g., oxygen, specific conductance, nitrate, nitrite, ammonium, pH and redox potential). Interstitial water quality was identified as a limiting factor for egg development (median of relative hatching rates = 0.23). Hatching success was unimodal in hatchery and field references incubated in free-flowing water, but bimodal (very high or very low hatching success) in natural sediment exposures. The effects of physicochemical factors on the hatching success of *Salmo trutta* strongly depended on both the time and spatial scale analyzed. The variables retained in the models differed between the macro-scale (over all rivers), the river-specific scale (within a river) and the micro-scale (at different sediment depths). Egg hatching success decreased with increased substratum depth (decrease of 26 % in 150 mm compared with 50 mm). Increasingly more variable interstitial water conditions (e.g., oxygen) throughout the egg incubation period suggest progressive degradation rates in the stream substratum during the incubation period at the micro-scale level. Consequently, consideration of different spatial and temporal scales is necessary for the evaluation of habitat quality in salmonid conservation and catchment management plans.

## 5.2 Introduction

Ecologically functional stream substratum is a key habitat in river ecosystems (Geist, 2011). The interstitial zone is essential for the ‘self-purification’ of rivers and is also an important habitat for many aquatic organisms (Orghidan, 1959; Boulton et al., 1998), including microbes, macroinvertebrates (e.g., insects, mussels) and rheophilic fishes (Williams and Hynes, 1974; Stanford and Ward, 1988; Hendricks, 1993; Elliott, 1994; Buddensiek, 1995; Palmer et al., 1997). In particular, degradation of this habitat, due to anthropogenic or natural disturbance, results in a decline of habitat quality for egg and larval development of lithophilic fishes and invertebrates, and consequently for the natural reproduction of these species (Brunke and Gonser, 1997; Acornley and Sear, 1999; Soulsby et al., 2001b; Malcolm et al., 2003; Kemp et al., 2011). Endangered species such as freshwater pearl mussel (*Margaritifera margaritifera*, L.), European grayling (*Thymallus thymallus*, L.) and Danube salmon (*Hucho hucho*, L.) are highly affected by the degradation of functional gravel banks (Jungwirth, 1978; Zeh and Dönni, 1993; Geist and Auerswald, 2007). Hence, the effects of stream substratum degradation have to be considered in conservation management (Denic and Geist, 2009; Sternecker and Geist, 2010; Pulg et al., 2011).

Recently, several restoration measures were adapted to improve the quality of river sediments. This includes reduction in fine sediment input from the watershed, creation of artificial gravel banks, or agitation and cleaning of embedded sediment (Shackle et al., 1999; Hendry et al., 2003; Rubin et al., 2004; Owens et al., 2005; Sear and DeVries, 2008; Pedersen et al., 2009). To assess the efficiency of such conservation actions, powerful tools are required for evaluating the ecological functionality of the hyporheic zone (Maddock, 1999). To date, the effects of stream substratum on the physicochemical and biological properties are often addressed separately and thus poorly integrated into habitat assessments (Acornley, 1999; Kondolf, 2000; Malcolm et al., 2003; Meyer, 2003; Sternecker and Geist, 2010).

The objective of this study was to bridge this gap and establish links between abiotic (i.e., physicochemical) stream substratum properties and biological effects on egg development of brown trout (*Salmo trutta*) using standardized egg exposure systems. Models were used to identify the influence of interstitial physicochemical water conditions on hatching success. In three spawning seasons, a total of 110 egg sandwich boxes (ES; Pander et al., 2009) were exposed in 19 study sites, located in three rivers of two river catchments.

## 5.3 Materials and methods

### *5.3.1 Study sites*

Three typical brown trout rivers in Bavaria, Germany, were selected for studying the effects of physicochemical parameters in the stream substratum on salmonid egg hatching success. (Figure 5-1). To include a wide range of water and substratum conditions, the rivers were chosen according to substantial differences in flow discharge and stream bed characteristics. The river Moosach (mean annual discharge of 2.6 $m^3 s^{-1}$) and the river Lech (mean annual discharge of 82.9 $m^3 s^{-1}$, water gauge Landsberg) belong to the Danube river catchment. The river Wiesent (mean annual discharge of 7.5 $m^3 s^{-1}$; water gauge Muggendorf) is part of the river Main/Rhine catchment. In all rivers, naturally spawning brown trout and 'redds' were observed in the study areas.

In recent years, several restoration measures, like restoration of gravel beds, were implemented in the river Moosach, the river Lech and the river Wiesent. Fifteen of the study sites were located in restored areas. Two study sites in the river Moosach and one in the river Lech were located in natural gravel bars formed by the movement of the river bed (Table 5-1).

### *5.3.2 Microhabitat assessment*

Microhabitat conditions in the hyporheic zone were studied by analyzing the upper layer (150 mm) of the stream substratum, because this fraction was found to be of greatest biological relevance in previous studies (Palmer et al., 1997; Geist and Auerswald, 2007). During the study period (2007 - 2010), a total of 110 ES were used as bioindication tools as described in Pander et al. 2009 (Table 5-1). Briefly, the ES combines an egg exposure unit with 90 separate egg chambers and a unit to extract water samples from three substratum depths (50 mm; 100 mm; 150 mm) within the ES.

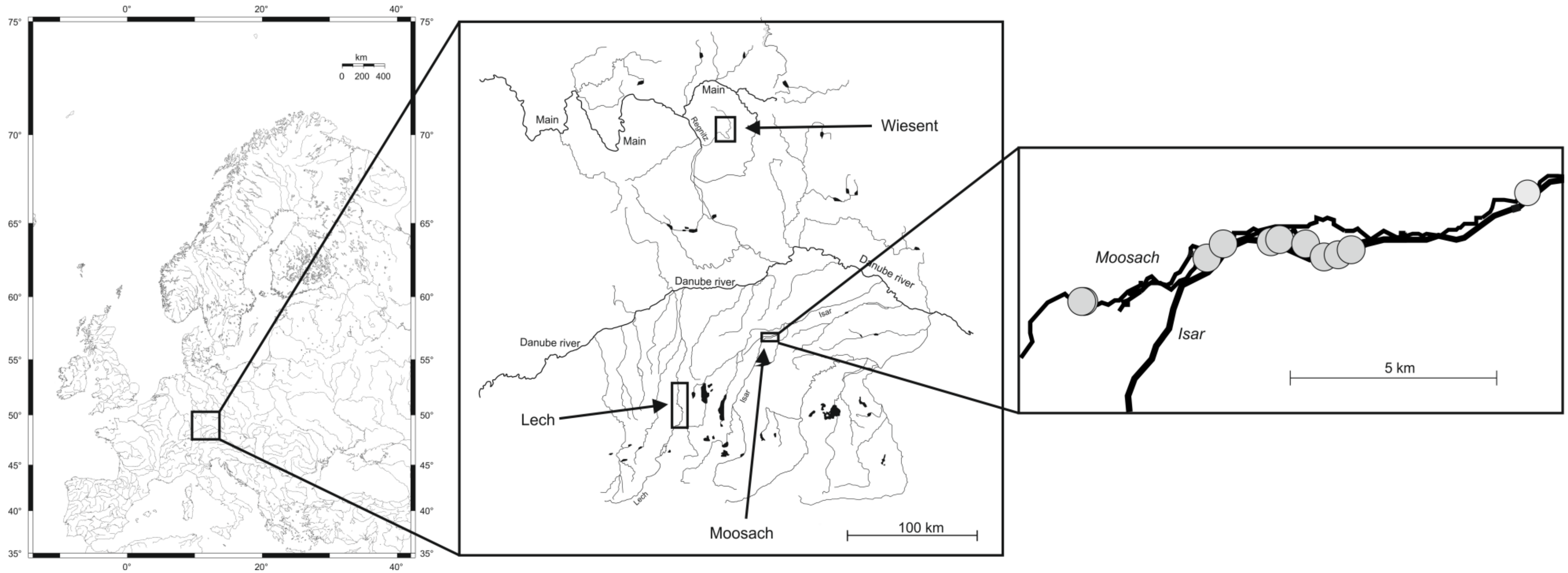



**Figure 5-1: Location of the study sites**

**Table 5-1: Experimental setup for three winter spawning periods including the hatching rates (H) of the hatchery and field references**

| | | *Moosach* | | | *Lech* | | *Wiesent* |
|---|---|---|---|---|---|---|---|
| | | 2007 | 2008 | 2009 | 2008 | 2009 | 2008 |
| Experimental setup | $N_s$ | 11 | 7 | 7 | 3 | 3 | 5 |
| | $N_0$ | 36 | 23 | 12 | 15 | 9 | 15 |
| | $N_e$ | 36 | 20 | 12 | 9 | 8 | 12 |
| Hatchery reference | H [%] | 88 ± 1.2 [1)] | 82 ± 1.1 [1)] | 57 ± 4.9 [2)] | 69 ± 1.1 [1)] | 68 ± 1.2 [2)] | 54 ± 4.9 * [1)] |
| | $D_e$ | 19 | 21 | 20 | 19 | 20 | 29 |
| | $D_h$ | 34 | 33 | 35 | 34 | 34 | 56 |
| | $T_e$ [°C] | 11 | 11 | 11 ± 0.2 | 11 ± 0.2 | 12 ± 0.0 | 8 ± 0.2 |
| | $T_h$ [°C] | 11 | 11 | 11 ± 0.1 | 11 ± 0.2 | 11 ± 0.1 | 8 ± 0.2 |
| Field reference | H [%] | 80 ± 4.8 * [1)] | 81 ± 3.6 * [1)] | 43 ± 4.9 * [2)] | 75 ± 3.6 * [1)] | 83 ± 1.5 [2)] | 39 ± 5.4 [1)] |
| | $D_e$ | **45** | **41** | **36** | **82** | **67** | **43** |
| | $D_h$ | **60** | **62** | **72** | **120** | **89** | **112** |
| | $T_e$ [°C] | 8 ± 0.6 | 7 ± 1.5 | 7 ± 1.3 | 2 ± 0.8 | 4 ± 2.1 | 6 ± 1.5 |
| | $T_h$ [°C] | 7 ± 0.6 | 7 ± 1.3 | 6 ± 0.8 | 2 ± 1.2 | 3 ± 0.9 | 4 |

$N_s$ represents number of study sites, $N_0$ represents number of exposed egg sandwich boxes (ES) and $N_e$ represents number of recovered boxes at the end of exposure; the study sites were the same across years. Exposures in the hatchery (ground water; *river water) and in the field (river water) are shown separately. Superscript numbers 1 and 2 represent number of eggs $n_1$ = 1000 and $n_2$ = 100, respectively; $D_e$ represents number of days from fertilization until eyed egg stage, $D_h$ represents number of days from fertilization until hatch, $T_e$ represents mean temperature from fertilization until eyed egg stage, $T_h$ represents mean temperature from eyed egg stage until hatch; ± represents standard deviation; days determining physicochemical measurement related to the year are bold.

The locations of the ESs in the gravel banks were chosen haphazardly in the first year. The visually apparent embeddedness of the substratum surface was not considered for the placement locations of ES. In subsequent years, the ESs were placed at the same locations as precisely as possible. The studies started every year after natural spawning of brown trout was observed in each of the rivers.

For bioindication, 90 fertilized brown trout eggs from 5 - 12 female and 3 - 6 male hatchery fish (Landesfischzuchtanstalt Mauka, Germany and Lehranstalt für Fischzucht des Bezirks Oberfranken, Germany) were incubated in every ES. Both hatchery strains originate from locally adapted wild stocks (from the river Main drainage for Lehranstalt für Fischzucht des Bezirks Oberfranken and from the river Danube drainage for Landesfischzuchtanstalt Mauka). Both hatchery stocks were established at least eight generations ago, but are frequently spawned with sperm from wild fishes. Eggs from these locally adapted strains were used to avoid a reduction in hatching rates due to possible maladaptation with local conditions. The ESs were left in river sediments until eggs hatched. To evaluate the effects of physicochemical properties of interstitial water during egg development, two reference groups were used: (i) fertilized eggs incubated in oxygenated ground water in a hatchery provided a ‘hatchery reference’ (Table 5-1) and (ii) eggs contained within an ES were incubated in free-flowing water in each of the studied rivers (‘field reference’).

Physicochemical parameters [temperature (°C), dissolved oxygen concentration (mg $L^{-1}$), pH, specific conductance (corrected to 20 °C), redox potential (mV), nitrate (mg $L^{-1}$), nitrite (mg $L^{-1}$) and ammonium (mg $L^{-1}$)] were measured for three interstitial water samples withdrawn from each in situ ES; samples represented upper, middle and lower zones within an ES as described above. Water samples taken from each of the three depths within an ES were analysed three times during exposure: 1 day after placing the ES in the stream bed, at the eyed fish-egg stage and before removal of the ESs from the substrate. The second and third measurement dates were determined by the developmental stages of the field reference in respective rivers. Temperature, dissolved oxygen, pH and specific conductance were measured by handheld oxygen, conductivity and pH meters (WTW, Weilheim, Germany). Nitrate, nitrite and ammonium concentrations were quantified using a photoLab S12 (WTW). Redox potential was measured in 50 mm, 100 mm and 150 mm depth in direct proximity to the ESs according to Geist and Auerswald (2007). All physicochemical measurements were also conducted in the free-flowing water proximal to an ES to evaluate differences with the interstitial zone.

### 5.3.3 Statistical analysis

Relative hatching rate for each ES was calculated by dividing the proportional hatching rate of eggs exposed in the ES by the hatching rates of the respective field reference. This procedure normalized for differences introduced by varying egg quality or fertilization rates.

Differences in egg hatching rates between rivers and between the three depths within an ES across samples were analysed by Kruskal-Wallis test and Mann-Whitney U-test (Conover, 1999) with Bonferroni adjustment for multiple comparisons (Gotelli and Ellison, 2004).

Delta values of physicochemical parameters were calculated by subtracting values of the interstitial zone from values of the free flowing water above the respective ES. Differences in physicochemical parameters in the hyporheic zone at different sediment levels and at different times during egg development were also analyzed by Kruskal-Wallis test and Mann-Whitney U test (Conover 1999) with Bonferroni adjustment for multiple comparisons (Gotelli and Ellison, 2004).

Delta values of physicochemical parameters were calculated by subtracting values of the interstitial zone from values of the free-flowing water above the respective ES. Differences in physicochemical parameters in the hyporheic zone at different sediment levels and at different times during egg development were also analyzed by Kruskal-Wallis test and Mann-Whitney U-test (Conover, 1999) with Bonferroni adjustment for multiple comparisons (Gotelli and Ellison, 2004).

Discriminant analysis (DCA) was used to find a linear combination of physicochemical variables, which maximized the separation of ES groups with high hatching success (0.80 - 1.00) versus low hatching success (0 - 0.20), because these were the most frequently detected hatching rates. The discriminant function was used to classify the remaining samples of intermediate values of hatching success (> 0.20 and < 0.80) into either high or low egg hatching success to group the more rarely observed hatching rates. Error rates of misclassification of the high and low hatch samples were obtained along with the classification rates of intermediate hatch success samples into the two categories (high or low). Three different data sets were used for the DCA:

1. A data set including data of the river Moosach from the years 2007/2008 (Moosach 2007) and 2008/2009 (Moosach 2008) and the river Lech from the years 2008/2009 (Lech 2008) and 2009/ 2010 (Lech 2009).
2. A data set including Moosach 2007 and Moosach 2008.

3. A data set including Lech 2008 and Lech 2009.

The analyses were focused on these data, because they had the lowest variations between individual references within different batches of fertilized eggs and field exposures. Data of hatching rates in sediment with low hatching rates in the field references (< 75 %) were excluded.

The discrimination functions separating high and low hatching rates were used to predict intermediate hatching rates. The hatching success of river Moosach 2009/2010 (Moosach 2009) and river Wiesent 2008/2009 (Wiesent 2008) was predicted with the discrimination functions calculated using data set (1).

The influences of physicochemical parameters and local scale-dependent factors on hatching success were tested by performing a generalized linear model (GLM) at the macro-scale (including data of the river Moosach 2007 and 2008 as well as data of the river Lech 2008 and 2009):

$$Y_{ijklmn} = \mu + A_i + B_{i(j)} + C_{k(j(i))} + D_{l(k(j(i)))} + \varepsilon_{ijklmn}$$

The respective year (A), the river (B) within years and the sites (C) within rivers are fixed effects. ESs (D) are random effects, because different shapes of the gravel banks do not enable an exact repeat of the study design. The physicochemical parameters were included in the model as continuous covariates ($\beta_p * x_p$).

Relative hatching rates were used as the response variable for the DCA and the GLM. To determine detectable and random effects of the interstitial water conditions, the GLM and the DCA were performed two ways using (i) absolute values and (ii) delta values (differences between interstitial and free-flowing water) of the physicochemical parameters. Data measured at the end of egg exposure were used for the DCA and the GLM because the values of the interstitial water conditions showed highest variability at this time. Due to its diurnal and annual variability, temperature was not included in the calculations (Acornley, 1999). The angles between discriminant functions were calculated according to Batschelet, 1979. Analyses were performed using the statistical software SAS (© 2002–2008 by SAS Institute Inc., Cary, NC, USA) and PASW Statistics 18 (Version 18.0.0, 30.07.2009).

## 5.4 Results

### *5.4.1 Differences between reference and field exposures*

Field conditions, and in particular interstitial water conditions in the stream bed, had a strong impact on the length of time to egg hatching, as well as on the variability and success of egg development. The distribution of hatching success showed a unimodal (normal distribution) curve under reference conditions (low standard deviations not exceeding 5.4 in any of the open water field and hatchery references; Table 5-1), but a distinct bimodal distribution with much greater variation under natural interstitial water conditions in the field substratum exposure (Figure 5-2). In this study, 25 % of all measured hatching rates in sediment were 0, and in 13 % of the hatching units, the relative hatching rates were 100 %. Overall the medians of hatching in sediment were shifted to very low hatching success (median of relative hatching rates = 0.23).

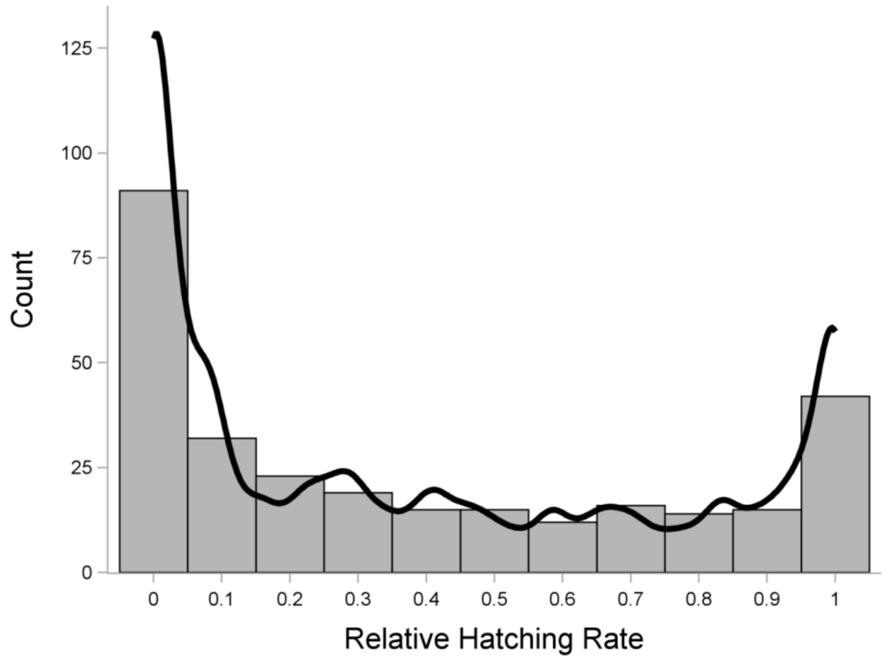



**Figure 5-2: Distribution and Kernel Density (▬) for relative hatching rates in the river Moosach, Lech and Wiesent (N = 291); relative hatching rates were calculated by dividing the proportional hatching rate of eggs exposed in the ES by the hatching rates of the field references.**

The variability of the reference hatching rates within years and rivers was overall very low (variance ≤ 0.003). The relative hatching rates in the sediment exposures (overall variance: 0.14) showed higher variability than the reference exposures.

Between the rivers, egg hatching success differed significantly ($P < 0.001$; Figure 5-3). The relative egg hatching rates, adjusted to field reference values, were highest in the river Lech and lowest in the river Wiesent. The relative egg hatching rates in the river Moosach ranged from 0 to 100 % and thus had the highest variation in egg hatching success.

Under hatchery reference conditions, egg development from fertilization to hatching was completed after 374 - 385 degree days (dd) at 11 °C. The time to egg hatching in the rivers ranged from 240 - 334 dd (2 - 4 °C) in the river Lech to 434 - 468 dd (6 - 8 °C) in the river Moosach.

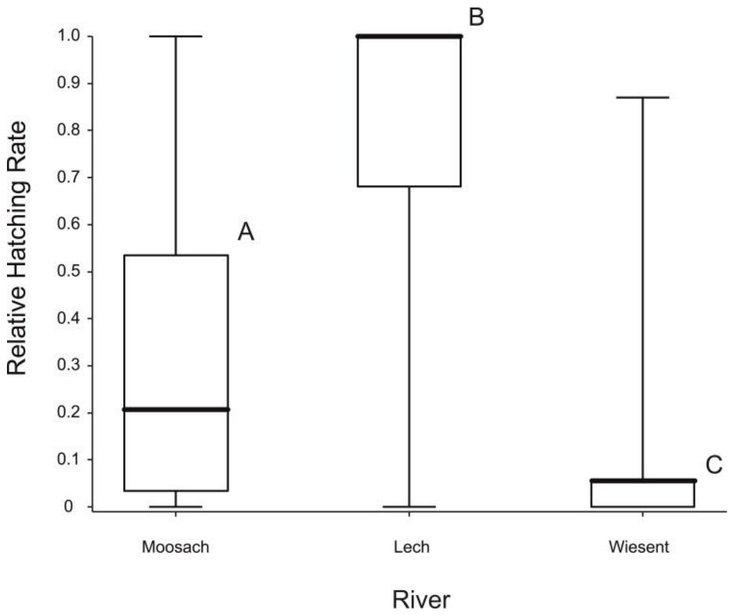



**Figure 5-3: Box-Whisker plots (Whiskers: maximum, minimum; Box: 0.25 quartile, median and 0.75 quartile) for relative hatching rates in rivers; different letters indicate significant difference (Mann-Whitney U test with Bonferroni correction: $p < 0.017$), $n = 291$; relative hatching rates were calculated by dividing the proportional hatching rate of eggs exposed in the ES by the hatching rates of the field references.**

Pronounced interannual variation of the hatching success was observed in all study sites. The interstitial water quality for brown trout egg hatch within the rivers (e.g. Moosach) changed from year to year, but the trends were not predictable (Figure 5-4). There was no directional trend in the spawning ground quality as evident from increase (e.g. MO05) and decrease (e.g. MO10) in the hatching rates in consecutive years.

### 5.4.2 Factors affecting hatching rates at the macro-scale

The high rate of correct classification in discriminant analysis of ES hatching rates into high and low hatching success groups indicates that a linear combination of interstitial water parameters successfully separates the two groups (Table 5-2).

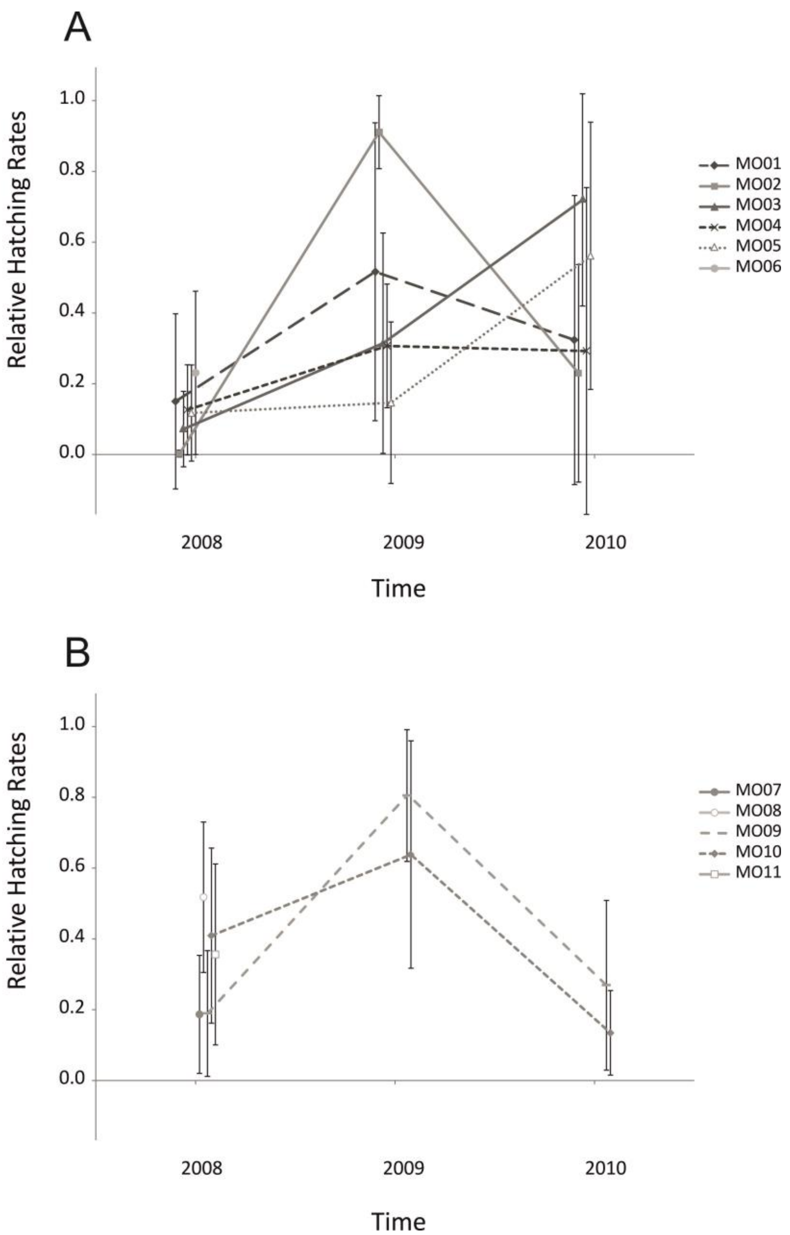



**Figure 5-4: A and B represents means and standard deviations of the relative hatching rates within the study sites of the river Moosach over the years 2007, 2008, and 2009. The area MO02 was restored before the brown trout spawning season 2008; relative hatching rates were calculated by dividing the proportional hatching rate of eggs exposed in the ES by the hatching rates of the field references.**

**Table 5-2: Effects of physicochemical parameters on hatching success at different study scales**

| Study level | variables | DCA Absolute values | DCA Delta values | GLM Absolute values | GLM Delta values |
|---|---|---|---|---|---|
| Macro-scaled differences | *Moosach and Lech* | | | | |
| | year | | | < 0.001 | < 0.001 |
| | river | | | 0.04 | 0.06 |
| | site | | | < 0.001 | < 0.001 |
| | egg sandwich box | | | < 0.001 | < 0.001 |
| | oxygen-concentration | - 0.34 | 0.37 | 0.15 | 0.03 |
| | pH | - 0.32 | <u>0.94</u> | 0.16 | 0.19 |
| | specific conductance (corrected to 20° C) | <u>0.71</u> | 0.67 | 0.95 | 0.89 |
| | redox potential | - 0.35 | 0.25 | 0.82 | 0.94 |
| | nitrite-concentration | 0.37 | - 0.55 | 0.48 | 0.47 |
| | nitrate-concentration | 0.58 | 0.18 | 0.55 | 0.58 |
| | ammonium-concentration | - 0.08 | - 0.09 | 0.18 | 0.15 |
| River-specific differences | *Moosach* | | | | |
| | year | | | < 0.001 | < 0.001 |
| | site | | | < 0.001 | < 0.001 |
| | egg sandwich box | | | < 0.001 | < 0.001 |
| | oxygen-concentration | 0.41 | 0.80 | 0.64 | 0.65 |
| | pH | 0.34 | <u>0.86</u> | 0.24 | 0.25 |
| | specific conductance (corrected to 20 °C) | 0.52 | 0.48 | 0.56 | 0.57 |
| | redox potential | <u>0.59</u> | 0.25 | 0.46 | 0.44 |
| | nitrite-concentration | - 0.44 | - 0.39 | 0.23 | 0.23 |
| | nitrate-concentration | - 0.16 | 0.06 | 0.91 | 0.93 |
| | ammonium-concentration | 0.34 | 0.02 | 0.35 | 0.37 |
| | *Lech* | | | | |
| | year | | | 0.22 | 0.48 |
| | site | | | 0.04 | 0.34 |
| | egg sandwich box | | | 0.06 | 0.20 |
| | oxygen-concentration | 0.17 | 0.17 | 0.09 | 0.02 |
| | pH | 0.28 | 0.11 | 0.54 | 0.66 |
| | specific conductance (corrected to 20 °C) | - 0.24 | - 0.04 | 0.14 | 0.09 |
| | redox potential | 0.43 | 0.22 | 0.40 | 0.40 |
| | nitrite-concentration | - 0.42 | - 0.38 | 0.87 | 0.79 |
| | nitrate-concentration | <u>0.54</u> | <u>0.46</u> | 0.32 | 0.21 |
| | ammonium-concentration | - 0.22 | - 0.37 | 0.12 | 0.42 |

Discriminant Analysis (DCA) and Generalized Linear Model (GLM) concerning the dependency of physicochemical parameters on hatching success (relative hatching rate) in the river Moosach (2007 and 2008) and the river Lech (2008 and 2009); DCA: groups were defined by hatching success (0 - 20 % and 80 - 100 %), variables with highest impact are underlined, the discriminatory power of absolute values as well as delta values of the physicochemical parameters (difference between interstitial and free-flowing water) were compared; GLM: correlations (p-values) of absolute values as well as delta values of physicochemical parameters (difference between interstitial and free-flowing water) on hatching success were compared.

At a macro-scale of two rivers (Moosach and Lech combined), the discriminant function had positive coefficients for specific conductance, nitrite, and nitrate and negative coefficients for dissolved oxygen, pH, redox potential, and ammonium. Considering delta values (differences between interstitial and free flowing water) of the physicochemical parameters, a similarly high significance of discrimination was found, but the discriminant function was nearly uncorrelated with that for the absolute values. Here the discriminant function had negative coefficients for nitrite and ammonium concentrations and positive coefficients for the remaining physicochemical parameters. The angle between the two discriminant functions was θ = 94.6º, indicating that differences in physicochemical parameters between interstitial and free flowing water were substantial at a macro-scale of two rivers. The confusion matrix (Table 5-3) shows that the predictive power of the discrimination function tended to be higher if the calculation was based on delta values. The classifications of intermediate hatching rates by physicochemical parameters with the discrimination function show balanced results at macro-scaled level when delta values are considered (Table 5-4).

**Table 5-3: Confusion matrix of discrimination analysis**

| | | | AV | | DV | |
|---|---|---|---|---|---|---|
| Macro-scaled level | | **Predicted class** | | | | |
| | | | *0 - 0.20* | *0.80 – 1.00* | *0 - 0.20* | *0.80 – 1.00* |
| | **Actual class** | *0 – 0.20* | 95.3 | 4.7 | 80.2 | 19.8 |
| | | *0.80 – 1.00* | 41.8 | 58.2 | 15.5 | 84.5 |
| | | % explained variance | 42.8 | | 31.8 | |
| River Moosach | | **Predicted class** | | | | |
| | | | *0 - 0.20* | *0.80 – 1.00* | *0 - 0.20* | *0.80 – 1.00* |
| | **Actual class** | *0 – 0.20* | 97.5 | 2.5 | 90.0 | 9.9 |
| | | *0.80 – 1.00* | 76.0 | 24.0 | 56.0 | 44.0 |
| | | % explained variance | 22.6 | | 25.8 | |
| River Lech | | **Predicted class** | | | | |
| | | | *0 - 0.20* | *0.80 – 1.00* | *0 - 0.20* | *0.80 – 1.00* |
| | **Actual class** | *0 – 0.20* | 50.0 | 50.0 | 80.0 | 20.0 |
| | | *0.80 – 1.00* | 3.3 | 96.7 | 0.0 | 100.0 |
| | | % explained variance | 35.3 | | 53.9 | |

Classification of the discrimination analysis (DCA); DCAs concern the dependency of physicochemical parameters [$O_2$ = dissolved oxygen concentration (mg $L^{-1}$), pH, SC = specific conductance (corrected to 20°C), Eh = redox potential (mV), $NO_3^-$ = nitrate (mg $L^{-1}$), $NO_2^-$ = nitrite (mg $L^{-1}$), and $NH_4^+$ = ammonium (mg $L^{-1}$)] on hatching success (relative rate) in the river Moosach (2007 and 2008), the river Lech (2008 and 2009) and in both rivers (macro-scaled level); 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; relative hatching rates were calculated by dividing the proportional hatching rate of eggs exposed in the ES by the hatching rates of the field references.

If absolute values were used to classify the hatching success, the predicted hatching success of 0 - 20 % was 32.9 % higher. Analysis of the GLM indicated that significant differences ($p < 0.05$) in hatching rates occurred between years, rivers within the years, sites within rivers and the ESs within the sites. No consistently significant covariates were identified among the physicochemical parameters (Table 5-2). In terms of oxygen, significant effects were only observed using delta values between free-flowing water and interstitial zone, but not for absolute oxygen concentrations.

**Table 5-4: Prediction of hatching success**

| | **AV** | | **DV** | |
|---|---|---|---|---|
| | *0 – 0.20* | *0.80 – 1.00* | *0 – 0.20* | *0.80 – 1.00* |
| Macroscaled level | 87.0 | 13.0 | 54.1 | 45.9 |
| Moosach | 100.0 | 0.0 | 73.8 | 26.2 |
| Lech | 12.5 | 87.5 | 30.8 | 69.2 |
| Moosach 2009* | 91.4 | 8.6 | 55.5 | 45.5 |
| Wiesent 2008* | 88.9 | 11.1 | 88.9 | 11.1 |

Predicted classification [%] of intermediate hatching success (> 0.20 - < 0.80 of relative hatching rate) in the river Moosach (2007 and 2008), the river Lech (2008 and 2009) and in both rivers (macro-scaled level); 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. * The prediction of 0 - 100 % hatching success.

### *5.4.3 Factors affecting hatching rates at river-specific scale*

On a smaller scale, the effects of the factors on the hatching success depended on the respective river. The discriminant analysis by river indicated differences in the importance of specific physicochemical parameters on egg hatching success in the two rivers. In the river Moosach, the difference in discriminant functions for absolute versus delta values of the physicochemical parameters (Table 5-2), as represented by the angle between the discriminant functions ($\theta = 37.8°$), was larger than that for the river Lech ($\theta = 23.8°$).

In the river Moosach, high versus low hatching success groups were separated most by dissolved oxygen concentration, pH, specific conductance, and nitrite (Table 5-2). In the river Lech redox potential, nitrite, nitrate, and ammonium were more important in separating the high and low hatching success groups. Comparing the functions between two rivers, the difference between the functions calculated using absolute values ($\theta = 72.3°$) is slightly bigger than the difference between the functions calculated with delta values ($\theta = 65.5°$).

Classifying the intermediate hatching success by physicochemical parameters (Table 5-4), the predicted hatching success was more balanced between high and low success considering delta values compared to the use of absolute values. On the macro-scale level, 87 % of datapoints were predicted to reveal low (0 - 20 %) hatching success and 13 % were predicted to have high (80 - 100 %) hatching success using absolute values, whereas this ratio was more balanced (54 % and 46 %) for delta values. The prediction of the hatching success in the river Wiesent resulted in low hatching success both for absolute and delta values between free flowing water and interstitial, matching the field observation of low hatching success from this river (Figure 5-3).

### 5.4.4 Factors affecting hatching rates at the micro-scale

Water conditions in the interstitial zone of specific study areas within a river differed over space and time. The mean hatching rate at 50 mm sediment depth was 43 % and it significantly decreased to 32 % in the 150 mm depth ($p < 0.05$; Figure 5-5).

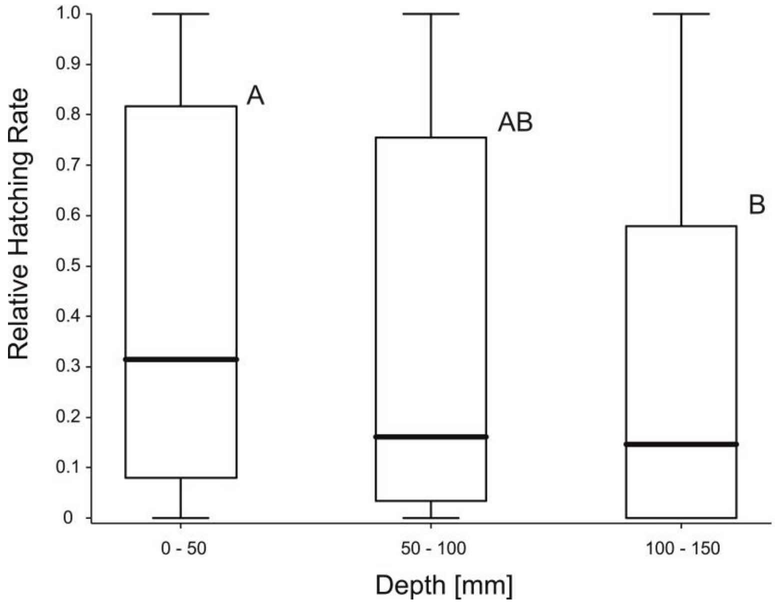



**Figure 5-5: Box-Whisker plots (Whiskers: maximum, minimum; Box: 0.25 quartile, median and 0.75 quartile) for relative hatching rates in 0 - 50 mm, 50 - 100 mm and 100 - 150 mm sediment depth pooled for all sites; different letters indicate significant differences (Mann-Whitney U test with Bonferroni correction: $p < 0.017$), $n = 291$; relative hatching rates were calculated by dividing the proportional hatching rate of eggs exposed in the ES by the hatching rates of the field references.**

This is supported by extreme water conditions, which limit egg survival such as minimum oxygen concentrations (< 2.7 mg $L^{-1}$) or maximum ammonium concentrations (> 1.5 mg $L^{-1}$), that were generally detected in the deepest analyzed sediment zone at the end of the egg exposure period. Interstitial water conditions (e,g., dissolved oxygen, nitrite concentrations) in deeper sediment zones differed significantly to conditions at the sediment surface when the larvae hatch (Figure 5-6). The interstitial water conditions showed a trend to higher delta values in deeper sediment zones particularly at the end of the egg exposure. Only in few cases (28 % of observations) were hatching rates in the deepest portion of an ES higher than in the shallower zones (data not shown).

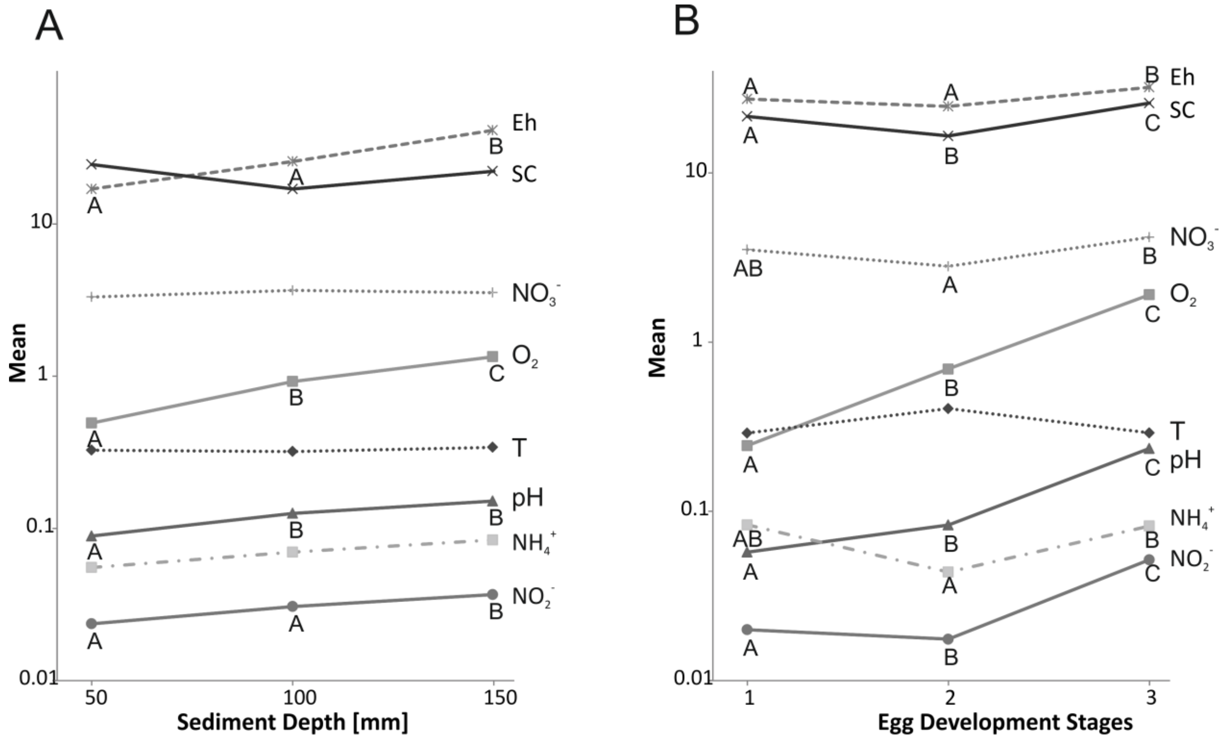



**Figure 5-6: Delta mean (absolute values) of physicochemical parameters [T = temperature (C), $O_2$ = dissolved oxygen concentration (mg $L^{-1}$), pH, SC = specific conductance (corrected to 20°C), Eh = redox potential (mV), $NO_3^-$ = nitrate (mg $L^{-1}$), $NO_2^-$ = nitrite (mg $L^{-1}$), and $NH_4^+$ = ammonium (mg $L^{-1}$)] between interstitial water and free-flowing water pooled for all sites; A represents physicochemical parameters in free-flowing water (FW), 50 mm, 100 mm and 150 mm sediment depth; B represents physicochemical parameters in the interstitial zone at three development stages of brown trout eggs (*Salmo trutta*): after fertilization (1), at eyed fish-egg stage (2) and after hatching (3); different letters indicate significant differences (Mann-Whitney U test with Bonferroni correction: $p < 0.017$).**

During egg development, variability and delta values changed and several parameters showed significant differences over time. In particular, dissolved oxygen concentration continuously decreased with increasing exposure time. Particular parameters (e.g. specific conductance, nitrate and ammonium concentrations) were most variable at the beginning of the egg incubation, after disturbing the sediment to place the ES, and showed higher differences to the free-flowing water at this time point. The example of nitrate shows that the consideration of absolute concentrations and thresholds of single parameters can be misleading. Even though nitrate was lowest in the substratum of the river Lech (8 mg $L^{-1}$; SD

= 3 mg $L^{-1}$) in comparison to the Moosach (23 mg $L^{-1}$; SD = 4 mg $L^{-1}$) and the river Wiesent (24 mg $L^{-1}$; SD = 6 mg $L^{-1}$), it was a good indicator for hatching success in the Lech but not in other rivers (Table 5-2).

## 5.5 Discussion

The evaluation of the stream substratum and interstitial water quality is an essential step in river management procedures aimed at successful restoration of salmonid spawning grounds. As evident from this study, the ecological functionality of the substratum depends strongly on spatial and temporal scales, even within the same location of a river. Substantial heterogeneity in egg hatching success was observed at the scale of placement.

### *5.5.1 Differences between reference and field exposures*

This study revealed pronounced differences in brown trout egg hatching rates between hatchery and natural in situ conditions in the stream bed, as well as between different streams and different sediment depths. The quality of the free-flowing water in rivers afforded high hatching success similar to the hatchery references. The results indicate that interstitial water quality is a potentially limiting factor for salmonid egg development. The distribution of hatching success was rather unimodal in the reference groups as compared with a bimodal distribution in the sediment exposures. Consequently, water conditions of the interstitial zone determine an ‘all or nothing’ response, which can also explain results from other studies (e.g., Kirkland, 2012).

The observed hatching success strongly varied by river, and this variation was driven by differences in local environmental factors, which also determined the time period of egg development under field conditions. Different annual environmental conditions are crucial determinants for hatching success as well as for the evaluation of substratum quality at potential spawning grounds (Lisle and Lewis, 1992; Kondolf, 2000; Meyer, 2003; Sear and DeVries, 2008). This is particularly important for hydrological patterns, including peaks of high or low stream discharge, or sediment transport. Eggs incubated in the river Lech hatched after a lower sum of day degrees than eggs from the hatchery incubated under controlled conditions, even though egg development lasted four times the number of days under field condition. Probably, the time of egg development under natural conditions is limited and could only be expanded by low temperature to natural hatch (Crisp, 1981;

Acornley, 1999). On the other hand, higher sums of day degrees indicate potentially unfavourable conditions for salmonid egg development in the river Moosach, which probably did not relate to temperature. Similarly, for Pacific salmon and trout, Quinn (2005) found that egg development is primarily dependent on temperature, with important secondary effects of dissolved oxygen.

### *5.5.2 Factors affecting hatching rates at the macro-scale*

The DCA clearly showed that the influence of physicochemical parameters on hatching success strongly depends on the scale of consideration. Factors that were crucial on the river-scale level (e.g., nitrate concentration) were overlaid by strong effects of factors differing between rivers (e.g., specific conductance). This is particularly evident for nitrate, which was identified as an important factor explaining hatching success in the Lech, where lowest concentrations were observed, but not in any of the other rivers. Sensitivity of aquatic organisms to nitrate still remains controversial (Kincheloe et al., 1979; Camargo et al., 2005) with indirect effects resulting from reduction to nitrite and ammonium being probably more ecotoxicologically relevant in the interstitial zone than absolute nitrate values (Mueller et al., 2012).

The comparison of the discriminant functions at different spatial scales showed that the exchange of the interstitial water is crucial for the evaluation of hatching success, especially if more than one river is included into a model. The differences between the functions at river scale confirm that egg development was negatively or positively influenced by different parameters within the interstitial water of the different rivers. The hyporheic zone is a layer comprising different ecological patches and consists of surface water, ground water, alluvial aquifer and parafluvial zones. The impact of different systems creates a heterogeneous ecosystem with complex interactions depending on discharge (Naiman et al., 1988; Boulton et al., 1998).

Different results of the DCA may also be caused by cross-reactions and interrelated effects of the physicochemical parameters, which also depend on the water exchange processes in the hyporheic zone. The rate of the flow through the sediment tended to be typical for each river depending on stream substratum composition - an observation that confirms previous results (e.g., Malcolm et al. 2002). The heterogeneity within rivers originated from a high variability of water exchange within and between the study sites as well as between different sediment depths. It is known from previous studies that physical conditions determining exchange between free-flowing water and interstitial zone are crucial for the overall quality and functionality of the habitat (Geist & Auerswald 2007; Jensen et al. 2009; Pulg et al. 2011).

Even though the oxygen concentration is often considered to be a good indicator for salmonid egg development (Wickett, 1954; Rubin and Glimsäter, 1996; Greig et al., 2007), it was not the main factor contributing to high hatching success in this study. Our results indicate that oxygen concentration in the hyporheic zone strongly depends on the hydraulic exchange (in accordance with the differences of the oxygen supply between the interstitial and the free-flowing water). Consequently, changed permeability induces a river-specific correlation in combination with different minimum oxygen concentration and supply during the egg development (Malcolm et al., 2002; Meyer, 2003; Louhi et al., 2008). Furthermore, sheltered regions within the substratum can induce anoxic microhabitats even though well-oxygenated water flows through the substratum (Boulton et al., 1998). It is also possible that even with good oxygen supply of the interstitial water, a silt layer around an individual egg could cause oxygen deficits and hence affect egg development (Greig et al., 2005; Levasseur et al., 2006).

### *5.5.3 Factors affecting hatching rates at the micro-scale*

The high variance of hatching rates on micro-scale level indicates that water conditions in the stream substratum are highly variable on a very small spatial scale. In this study, a strong trend of decreasing hatching success and higher differences of water conditions in deep sediment regions compared with the free-flowing water were observed. This resulted in a negative gradient of conditions for egg development in deeper zones of the river substratum. However, high hatching success in deeper substratum zones was occasionally also observed at locations where the physical structure of the stream substratum causes a reversal of the gradient. Accumulated fine sediment in the surface of the interstitial zone resulted in low hatching success at these sites, whereas more favorable conditions for egg development were found in deeper substratum layers. This can likely be explained by infiltration of high-quality ground water (e.g., Malcolm et al., 2003). Observed high variability of specific conductance and ammonium concentration at the sediment surface indicates a high metabolic rate in shallow hyporheic zones. It is known that microbial activity is concentrated in the near-surface zones of the stream substratum because of the supply of labile organic molecules (Battin et al., 2003). In our study, this did not seem to have negative effects on the egg development within the substratum.

The physicochemical water conditions in the river bed were not only highly variable within the interstitial zone at time points, but also changed significantly with exposure times. Similar interstitial and free-flowing water conditions (e.g., oxygen concentration) at the beginning of egg incubation indicate optimum water flow-through in the substratum. The burying of the ESs resulted in well-mixed sediments with low amounts of fine sediment immediately around the ESs. This procedure imitates the digging of 'redds' by gravel spawners, who modify the stream substratum as ecosystem engineers (Kondolf et al., 1993; Moore, 2006).

High variability of the specific conductance and ammonium concentrations indicates that organic matter was probably infiltrated at the beginning of the egg exposure in the substratum. The variability of ammonium concentration decreased over time, possibly due to its sorption by clay sediment (Triska et al. 1993).

The colmation of the substratum over time indicates that the physicochemical properties of interstitial water at late stages of egg development and hatching are probably most crucial. This is supported by the highest differences between interstitial water and free-flowing water conditions at the end of the egg exposure, likely resulting from less intense exchange between free-flowing water and interstitial water (Grost et al., 1991; Soulsby et al., 2001a; Julien and Bergeron, 2006).

Decreasing oxygen concentrations during the egg exposure could be explained by decomposition of organic matter, in particular dead eggs, by saprophytic fungi, but also by oxygen consumption of the salmonid eggs themselves (Rubin, 1995). Oxygen supply at the beginning of the egg development seems to be often sufficient for successful hatching, considering that after premature initial hatch, it is possible for salmonid fry to move through the substratum voids (Rubin and Glimsäter, 1996; Sternecker and Geist, 2010).

Even though the locations for the ES exposure were chosen carefully, natural hatching success is possibly underestimated. Salmonids seek specific areas within spawning grounds, for example, with upwelling oxygen-rich groundwater (Witzel and MacCrimmon, 1983; Mull and Wilzbach, 2007; Guillemette et al., 2011). High variability of interstitial water conditions impedes the detection of high-quality spawning grounds.

Sites where ESs were lost during the egg exposure due to high flow events likely reflect areas prone to scouring and can thus be considered nonfunctional spawning sites. However, the ES is a measurement tool that could be easily used for the evaluation of stream substratum functionality and measuring the effects of colmation. Therefore, the success of restoration measures and long-time monitoring of the hyporheic zone could be evaluated using ESs.

## 5.6 Conclusions

The results of this study suggest that the physicochemical properties of the stream bed strongly influence salmonid hatching success. Both model-predicted and observed hatching rates in the substratum followed a bimodal ‘all or nothing response’ (i.e., resulting in low or high survival), which typically did not match the unimodal hatching success of salmonid eggs exposed to the free-flowing water of the same rivers or to hatchery conditions. Different variables were important in the models of hatching success on the macro-scale (over all rivers), the river-specific scale (within a river) and the micro-scale (at different sediment depths). The finding that salmonid hatching rates are governed by river-specific factors suggests the consideration of multiple scales of resolution, as well as of multiple timepoints for accurate assessment and prediction of hatching rates. The properties of the stream bed are crucial for the recruitment of salmonids, as well as for many other aquatic taxa, and have been heavily modified by changes in land use, flow regulation and structural corrections. Consequently, the restoration of functional stream beds should be an important target in salmonid conservation and in the conceptual design of catchment management plans.

# 6 Effects of stream substratum restoration on habitat quality in a subalpine stream

A similar version of this chapter was submitted: Katharina Sternecker, Romy Wild and Jürgen Geist. Effects of stream substratum restoration on habitat quality in a subalpine stream.

## 6.1 Abstract

Stream substratum restoration is a widely applied tool to improve spawning habitat quality for salmonid fishes. However, there is a lack of studies which comprehensively assess effects of the restoration on site, as well as on downstream habitats. Our study addressed effects at both locations and compared abiotic (analyses of texture, penetration resistance, oxygen concentration, redox, nitrite, nitrate, ammonium, pH, electric conductivity, temperature) with biotic (depth-specific macroinvertrebrate abundance and diversity, brown trout hatching success) indicators before and after excavation of the substratum in a highly colmated brown trout spawning site. Strong improvements of hyporheic water conditions (increased oxygen supply and redox potential, reduced concentrations of nitrite and ammonium) as well as ~50 % reductions of substratum compaction and fine sediment content were observed one day after the restoration measure. Improvements of habitat quality were still detectable three months after treatment. Consequently, the hatching success of *Salmo trutta* eggs increased from 0 % to 77 % after the restoration. Short-term decrease of macroinvertebrate abundance (from 13.1 to 3.9 macroinvertebrates / kg substratum) was observed within the hyporheic zone of the restoration site, but after three months, the number of taxa increased from 13 to 22 taxa and abundance reached 17.9 macroinvertebrates / kg. Significantly increased fine sediment deposition was detected within 1 km downstream of the restoration site and may negatively affect these habitats. Trade-offs between positive effects at restored sites and negative effects in downstream habitats need to be considered for a comprehensive evaluation of stream substratum restoration.

## 6.2 Introduction

A functional riverbed is a crucial habitat component of freshwater ecosystems. Many vertebrates and invertebrates depend on dynamic substratum movements that facilitate transport of oxygen-rich surface water into the hyporheic zone (Kondolf, 2000a; Geist and Auerswald, 2007). The reduction of substratum quality can alter the occurrence, abundance and population dynamics of biological communities (Boulton et al., 1998). In particular, salmonid reproduction is often curtailed as a result of colmation or depletion of river substratum (Kondolf and Wolman, 1993; Soulsby et al., 2001; Denic and Geist, 2009; Pulg et al., 2011). Anthropogenic modifications of flow regimes (e.g. induced by dam or diversions) and higher fine sediment loads in rivers (e.g. induced by changes in land use) can decrease stream bed quality by increasing compaction and cementation of stream substratum (Kondolf 1997; Kemp et al. 2011). Furthermore, reductions in flood frequency and increases in fine sediment production increase clogging of salmonid spawning gravels (Schälchli, 1992; Acornley and Sear, 1999; Owens et al., 2005). As a result, the reduced hydraulic connectivity between surface water and the hyporheic zone leads to a degradation of physicochemical conditions within the riverbed (Brunke, 1999). Both the chemical and physical effects of high concentrations of fine sediment were shown to reduce survival of early life stages in salmonids (Olsson and Persson, 1986; Chapman, 1988; Rubin and Glimsäter, 1996; Ingendahl, 2001; Sternecker and Geist, 2010; Jonsson and Jonsson, 2011). High amounts of fine sediment also affect the invertebrate community in rivers and hence could reduce prey availability for juvenile salmonids (Suttle et. al., 2004). Therefore, the reduction of fine sediment loads and restoration of a functional riverbed have become core topics in stream restoration management (Greig et al., 2005; Palm et al., 2007; Geist, 2011; Pulg et al., 2011).

The restoration of key habitats such as spawning grounds is often considered a practical option to compensate for deficits in habitat quality in highly altered rivers, but only few studies provide systematic guidance to conservationists (e.g. Kondolf et al., 1996; Kondolf, 2000b; Pander and Geist, 2010). In particular, the effects of the restoration measures on downstream habitats are often ignored, and suitable tools for monitoring the success of restoration measures are needed (Shackle et al., 1999; Palmer et al., 2005; Roni et al., 2010). Furthermore, evaluations of restoration effects in the restored area and on downstream sections are necessary for an objective assessment.

The overall objective of this study was to restore a highly colmated gravel bar in a subalpine stream by excavating the substratum before the brown trout spawning season, studying the effects on the abiotic and biotic environment within the restoration site as well as on downstream habitats. The main motivation for restoration was the improvement of habitat conditions for brown trout (*Salmo trutta*). We hypothesize an increase in the hatching success of brown trout eggs as a result of the improved hydraulic exchange of the interstitial zone after the substratum restoration, as well as negative effects of the restoration on downstream habitat quality. Analyses of texture and penetration resistance were used to determine the direct effects on substratum characteristics. Additional abiotic parameters such as oxygen concentration and redox potential were analyzed as they are important indicators of substratum habitat quality (Wickett, 1954; Rubin and Glimsäter, 1996). Measurements of nitrite, nitrate, ammonium and pH reflect the accumulation of decomposition products within the substratum. Consequently, combined with EC, they are indicative of the hydraulic exchange within the substratum (Geist and Auerswald, 2007). As an additional biological endpoint to brown trout hatching success, the effects of the spawning ground restoration on macroinverebrate community composition was monitored. To detect downstream effects of the restoration, the deposition of fines was measured up to 1000 m downstream of the restoration site.

## 6.3 Materials and methods

### *6.3.1 Study area*

We selected a gravel bar (5 m length x 6 m width) within the river channel to study the effects of the restoration method ‘excavation’ on the restored area, and within a 1000 m section downstream of the manipulated site. The study area is located downstream of a weir in the River Moosach, a highly regulated subalpine calcareous stream (mean annual discharge of 2.6 $m^3 s^{-1}$, mean flow velocity of 0.3 $m s^{-1}$) in Bavaria, Germany. During the study period (November 07$^{th}$ - March 09$^{th}$), the mean discharge was 1.9$m^3 s^{-1}$ (SD = 0.6, range = 1.8 $m^3 s^{-1}$ - 2.8 $m^3 s^{-1}$).

In 2008, one year before the restoration, 360 brown trout eggs in four ‘egg sandwich’ - boxes (Pander et al., 2009) had 0 % (SD = 0 %) hatching success at this site, whereas the hatching rate in a reference box upstream of the restoration area was 80 % (± 5 %) in the same year. Therefore, the gravel bar was an ideal target for streambed restoration.

### *6.3.2 Restoration method*

We carried out a commonly applied substratum restoration technique for salmonid fishes, using a walking excavator, on November 24th, 2008. Substratum within the riverbed was mixed up for one hour with the excavator shovel to a depth of 0.5 - 0.6 m. The excavator shovel loosened colmated and sclerotic substratum and dropped it from a height of approximately one meter. Consequently, the accumulated fine sediment was washed downstream.

### *6.3.3 Effects within the restoration area*

The success of the restoration was quantified at four points within the restoration area using abiotic and biotic parameters as indicators (Figure 6-1). At each site, we measured physicochemical variables (oxygen concentration, temperature, redox potential, specific conductance, pH, and concentrations of nitrate, nitrite and ammonium) 18 days before, as well as 1 day and 101 days after excavation. We collected water samples (50 ml) from 50 mm, 100 mm, 150 mm substratum depth and water column using a syringe with a 120 mm PVC tube and a 200 mm aluminum tube (diameter = 5 mm) as described in Geist and Auerswald (2007). Substratum penetration resistance was measured in five replicates per measuring point using a modified pocket-penetrometer (Eijelkamp Agrisearch Equipement, Giesbeek, Netherlands).

Abundance and diversity of benthic macroinvertebrates were quantified using a modified freeze-core method (Pugsley and Hynes 1983; Pulg et al. 2011). We collected freeze-core samples one day after the measurement of physicochemical parameters. For this purpose liquid nitrogen (-196 °C) was injected into a 100 mm copper tube that we inserted 300 mm into the substratum. We then loosened the surrounding substratum with a shovel and lifted the frozen substratum core. Afterwards the different depth levels of the core (0 - 50 mm, 50 - 100 mm, 100 - 150 mm and > 150 mm) were melted and we collected the depth levels of the substratum in separate samples. The samples were stored at 4 °C until the substratum was searched for macroinvertebrates and they were removed for identification. Texture of the substratum samples was analyzed according to Sinowski and Auerswald (1999).

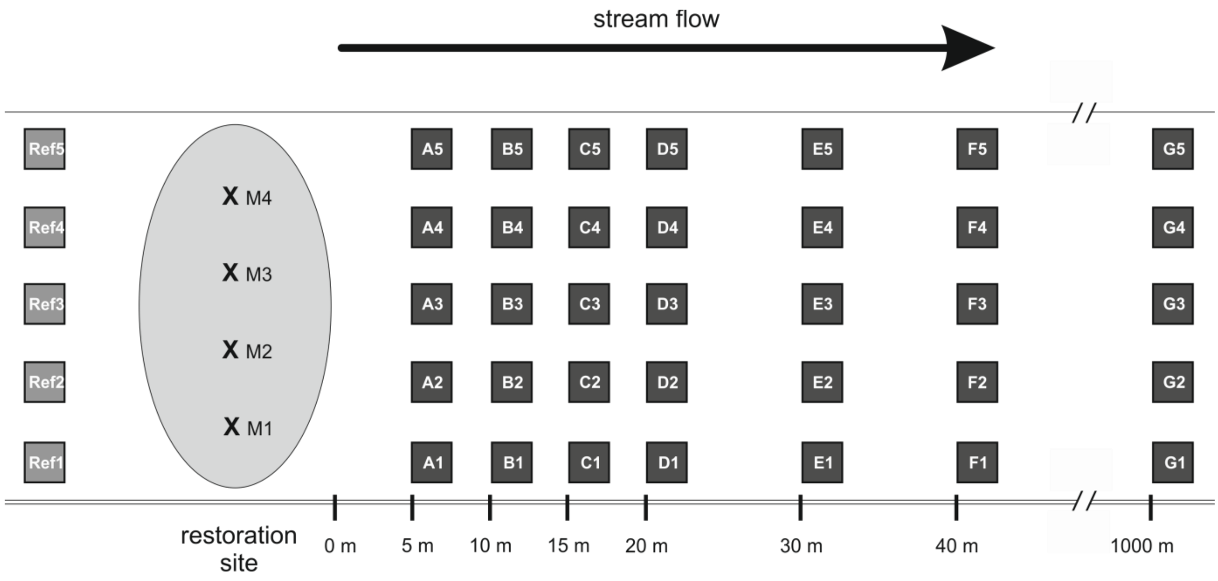



**Figure 6-1: Sampling design at the restoration site including upstream and downstream points: measuring points (M1 - M4) of the physicochemical parameters in the interstitial water (50 mm, 100 mm and 150 mm) within the restoration site 18 days before, 1 day after and 101 days after the excavation; A1 - G5 represent the sediment trap arrangement downstream of the restoration site for the measurement of the fine sediment deposition within 24 h after the beginning of the excavation; each sediment trap (length = 290 mm, width = 180 mm, height = 34 mm, filled with washed round gravel of 16 - 32 mm grain size) was exposed 24 h from the beginning of the excavation; Ref1 - Ref5 represent reference sediment traps upstream of the restoration site.**

We analyzed abundance and diversity of collected macroinvertebrates to assess depth distribution of organisms in the substratum. Macroinvertebrates were identified to species wherever possible using identification keys from Nagel (1989) and Schmedtje and Kohmann (1992). Since gammarids (*Gammarus pulex and Gammarus roeseli*) were the dominant invertebrate species in the investigated river contributing >90 % to the brown trout diet in this stream (own observations), we particularly considered effects of the restoration on *Gammarus spp*.

Hatching success in the restored area was tested using 360 brown trout eggs incubated in four ‘egg sandwich’ - boxes (ES) as described in Pander et al., 2009. We inserted the boxes four days after the excavation (Figure 6-1) and assessed them 101 days after the restoration of the gravel bar. The hatching success was determined by counting living and dead larvae within the aluminum grid of the boxes. The hatching success and egg development in the boxes was compared to that in an anchored floating reference box (egg incubation tray) with three separate compartments (3 x 1000 eggs) in which eggs were exposed to the surface water conditions of the study site.

### 6.3.4 Downstream effects

In order to measure the introduction of fine sediment into downstream areas, we installed solid sediment traps (length = 290 mm, width = 180 mm, height = 34 mm) in the river bed which were filled with washed gravel (16 - 32 mm). The sediment traps were rowed in equal lateral distance to each other at 5 m, 10 m, 15 m, 20 m, 30 m, 40 m and 1000 m downstream of the restoration area (Figure 6-1). In addition, we placed five control traps (references) upstream of the restoration site in order to measure the background deposition rate. We closed all traps with a solid lid during the installation and opened them shortly before the start of the excavation. Twenty-four hours after the beginning of the excavation, sediment traps were closed and removed from the river. Contents of the sediment traps were washed and separated from the gravel. For texture analysis, we wet sieved the substratum (using AS 400 control sieving equipment, Retsch, Haan, Germany) and fractioned the sediment into coarse (≥ 2.0 mm), medium (2.0 - 0.85 mm) and fine (< 0.85 mm) fraction sizes.

### 6.3.5 Statistical analysis

We tested differences in physicochemical parameters, substratum texture, penetration resistance and gammarid abundance at different time periods (before and after the restoration) using Repeated Measures ANOVA with subsequent pairwise comparison tests in SPSS v. 18. We carried out multivariate analyses of all physicochemical parameters, including fine sediment content (≤ 0.85 mm) and penetration resistance, for comparisons of different time periods and substratum depth zones using PRIMER V6 (Plymouth Marine Laboratories, UK). The data were normalized in order to create a comparable scale across the differing units of physicochemical parameters (Clarke & Warwick, 2001). The computation of the overall Euclidean distance between the sampling times was derived from a resemblance matrix based on individual Euclidean distances and quantified using the CLUSTER function.

In order to test for significant differences between the sampling times, the ANOSIM procedure (Analysis of Similarity; Clarke, 1993) was applied with the sampling time as fixed factor. We identified the contribution of the individual variables to the ordination using Principal Component Analysis (PCA) based on the normalized data matrix (PRIMER V6). PRIMER V6 software was used to identify the factors underlying the similarity of the properties “substratum depth” and “time point” in the PCA. As a means to analyze changes in macroinvertebrate community composition in response to the restoration, we carried out an ANOSIM procedure between the three sampling times based on the square-root transformed abundance data and the Bray-Curtis similarity measure.

We identified differences in sedimentation rates between upstream (reference) and downstream sediment traps using paired t-tests with subsequent Bonferroni corrections to account for false positive errors as a result of multiple comparisons, using the open source software R (http://www.r-project.org/). The underlying assumptions of the t-test, normality of distribution and equality of variances, were tested using the Shapiro-Wilk normality test and the F-test.

## 6.4 Results

### *6.4.1 Effects within the restoration site*

Post excavation, the substratum composition at the restoration site changed from consolidated and clogged to loose gravel with a significantly lower fraction of fines ($\leq 0.85$ mm; $p = 0.002$). The fraction of fine sediment (arithmetic means ± SD) decreased from 6.73 % ± 2.73 % to 3.35 ± 2.04 % after the restoration (Figure 6-2). After three months, the proportion of fine sediment in the substratum decreased even further to 2.28 ± 0.83% and variability across the measurements in the fine sediment fraction was notably lower than before the excavation. The variance of the fine sediment proportion among treatments was highest directly after the restoration (CV = 0.61) and lowest after three months (CV = 0.36). A significant reduction of penetration resistance after the restoration was detected (Repeated Measures ANOVA subsequent pairwise test $p < 0.001$; Figure 6-2). Although penetration resistance increased slightly after three months, the difference to pre-excavation conditions was still significant ($p < 0.001$).

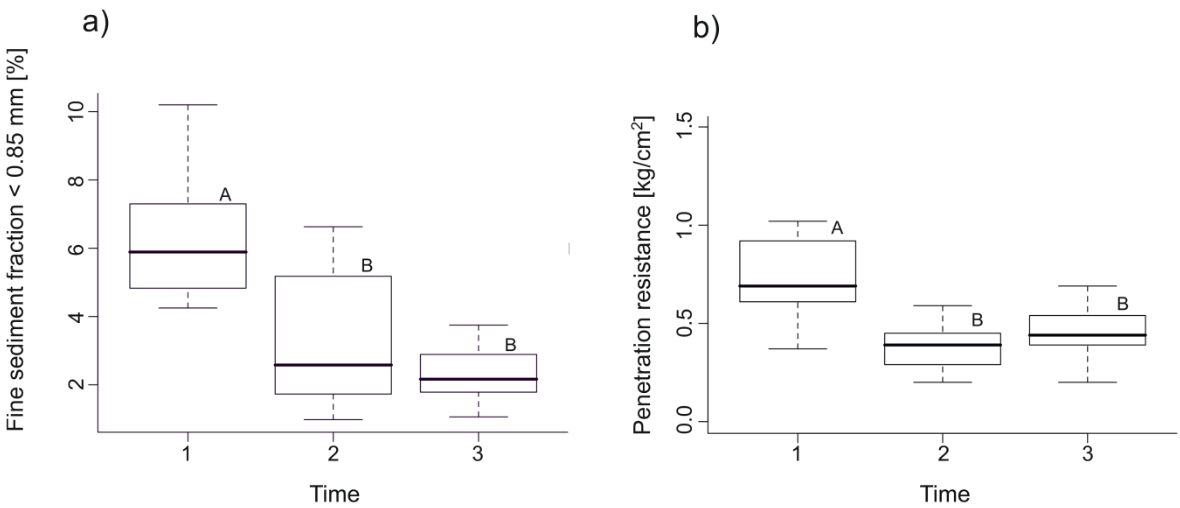



**Figure 6-2: Box-Whisker plots (Whiskers: maximum, minimum; Box: 0.25 quartile, median and 0.75 quartile) for a) percentage of fine sediment fraction (< 0.85 mm; n = 16 per sampling) and b) penetration resistance (n = 20 per sampling) at the restoration site 18 days before excavation (1), 1 day after excavation (2) and 105 days after excavation (3); different letters indicate significant differences (Repeated Measures ANOVA, subsequent pairwise test p < 0.05).**

The collective analysis of physicochemical parameters indicated significant differences in abiotic habitat conditions between all three sampling times. The difference in physicochemical habitat conditions was greatest between pre-restoration state and 101 days later (ANOSIM R = 0.4, $p \leq 0.001$), followed by the pairwise comparison of pre-restoration state and the day after restoration (ANOSIM R = 0.3, $p \leq 0.001$), and the comparison of the two past restoration time points (ANOSIM R = 0.2, $p = 0.003$). Comparing the differences of the physicochemical water conditions in the hyporheic zone based on Euclidian distances, similar differences were observed between pre-restoration state and 1 day after the excavation (overall Euclidean distance = 4.86), as well as between pre-restoration state and 101 days later (overall Euclidian distance = 4.77). The gravel bar colmated slightly after 101 days, indicated by the small Euclidean distance between 1 day and 101 days after the restoration (0.94) as well as by the ANOSIM result. As important effects of the restoration, a strong increase of oxygen concentration and a decrease of ammonium concentration within the substratum were evident (Table 6-1).

Effects of the restoration were also detected by a change in variation of physicochemical parameters over time. The variability of physicochemical parameters was highest before restoration, indicating high spatial heterogeneity. In particular, conditions in the deeper substratum layers (100 and 150 mm) strongly differed from conditions closer to the substratum surface and the surface water (Figure 6-3). After the excavation, variability decreased and physicochemical water conditions in the deeper layers resembled the conditions measured at the substratum surface.

**Table 6-1: Physicochemical parameters of the interstitial water within the restoration site 18 days before the restoration, 1 day after the restoration and 101 days after the restoration**

| Physicochemical parameters | 18 days before restoration | 1 day after restoration | 101 days after restoration |
|---|---|---|---|
| Temperature [°C] | 12.2 (SD = 0.5)$^{A}$ | 7.7 (SD = 0.3)$^{B}$ | 6.6 (SD = 0.4)$^{C}$ |
| Specific conductance [µS $cm^{-1}$] | 761 (SD = 24)$^{AB}$ | 769 (SD = 5)$^{A}$ | 758 (SD = 5)$^{B}$ |
| Redox potential [mV] | 380 (SD = 107)$^{A}$ | 502 (SD = 14)$^{B}$ | 490 (SD = 70)$^{B}$ |
| pH | 7.6 (SD = 0.1)$^{A}$ | 7.8 (SD = 0.1)$^{B}$ | 7.7 (SD = 0.1)$^{C}$ |
| Oxygen [mg $L^{-1}$] | 7.3 (SD = 2.6)$^{A}$ | 9.0 (SD = 0.5)$^{B}$ | 9.4 (SD = 0.7)$^{B}$ |
| Ammonium [mg $L^{-1}$] | 0.91 (SD = 0.41)$^{A}$ | 0.11 (SD = 0.02)$^{B}$ | 0.19 (SD = 0.04)$^{C}$ |
| Nitrate [mg $L^{-1}$] | 23.3 (SD = 5.3)$^{A}$ | 24.6 (SD = 3.6)$^{AB}$ | 25.4 (SD = 0.7)$^{B}$ |
| Nitrite [mg $L^{-1}$] | 0.19 (SD = 0.08)$^{A}$ | 0.10 (SD = 0.02)$^{B}$ | 0.10 (SD = 0.03)$^{B}$ |

Mean and standard deviation (SD) of the physicochemical parameters (temperature, specific conductance (25°C), redox potential, pH, oxygen, ammonium, nitrate, nitrite concentrations) measured in the interstitial water within the sediment at the restoration site 18 days before the restoration, 1 day after the restoration and 101 after the restoration. Samples of 3 depths (50 mm, 100 mm and 150 mm) at four sampling points were analyzed; different letters indicate significant differences within the parameters between different time points (Repeated Measures ANOVA, subsequent pairwise test $p < 0.05$).

The PCA analysis explained a total of 89.4 % of the variation. With the greatest share of variation being explained by the first PC axis (42.2 %, Eigenvalue: 3.38), the separation between samples taken in the substratum depth > 10 cm before the restoration and all other samples accounts for most of the variability of physicochemical parameters in the data set. PC1 is defined as a linear combination of the correlation coefficients of following variables: + 0.15 temperature - 0.48 dissolved oxygen - 0.46 redox potential - 0.27 pH - 0.22 specific conductance + 0.48 nitrite - 0.16 nitrate + 0.39 ammonium. Whilst high positive PC scores of dissolved oxygen and redox potential correlated strongly with past restoration abiotic conditions and conditions in substratum depths 0 - 50 mm, strongly negative scores of nitrite and ammonium correlated with pre-restoration conditions in deeper substratum layers > 50 mm. PC 2 accounted for a further 16.4 % of the explained variation (Eigenvalue: 1.31) displaying a linear combination of following parameters: + 0.70 temperature - 0.06 dissolved oxygen - 0.09 redox potential + 0.42 pH + 0.54 specific conductance + 0.14 nitrite - 0.12 nitrate - 0.08 ammonium. Along the second axis, samples were further separated between pre-restoration 0 - 50 mm substratum depth and between 1 day after and 101 days after restoration. Parameters correlating most strongly along this axis were temperature, specific conductance and pH.

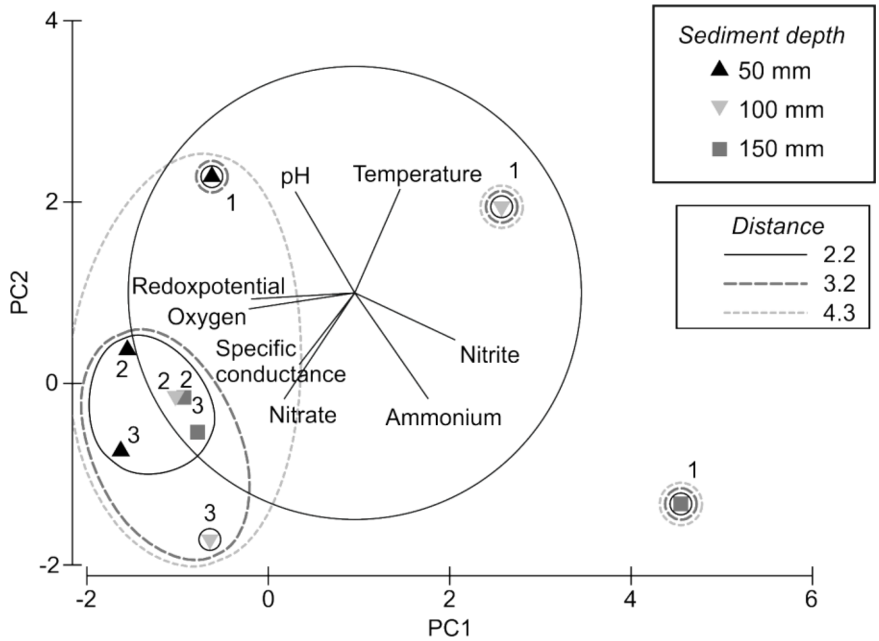



**Figure 6-3: Similarity of interstitial water conditions at the restoration site 18 days before the restoration (1), 1 day after the restoration (2) and 101 days after the restoration (3) shown by Principal Component Analysis (PCA) within the different substratum depths; the correlations of the physicochemical parameters (temperature, specific conductance (25°C), redox potential, pH, oxygen, ammonium, nitrate, nitrite concentrations) within the substratum are presented. Each point displays the mean value of parameters measured at the four sampling locations for each of the different substratum depths (50 mm, 100 mm, 150 mm)**

The mean abundance of macroinvertebrates in the freeze-cores was highest in the upper substratum layer from 0 - 50 mm and lowest in >150 mm before the gravel bar restoration, similar to the trend in abiotic parameters (Figure 6-4). Directly after the excavation, the overall mean abundance decreased with very similar numbers of individuals in all substratum layers. Three months later, the mean abundance of macroinvertebrates had recovered, with a particularly strong increase in abundance in the layer of 50 - 150 mm substratum depth. Similarly, number of taxa was highest three months after the excavation (Figure 6-4). In the 0 - 50 mm substratum layer, the number of taxa was highest before the restoration, decreased rapidly after the excavation and was equal to pre-excavation conditions three months after the excavation. The number of taxa in the substratum layer 50 - 100 mm was clearly enhanced by the restoration measures with more than double the amount of taxa 3 months after the restoration than before. A similar but less pronounced pattern was also detected in the substratum layers <100 mm. Differences in macroinvertebrate abundances were significant between all sampling points (ANOSIM $R > 0.6$, $p = 3$). The abundance of *Gammarus spp.* as the dominant and most important diet component of trout in the Moosach slightly increased directly after the restoration. The number of individuals was significantly higher three months after excavation than before the excavation or 1 day after the excavation (Repeated Measures ANOVA subsequent pairwise test ANOVA $p < 0.001$).

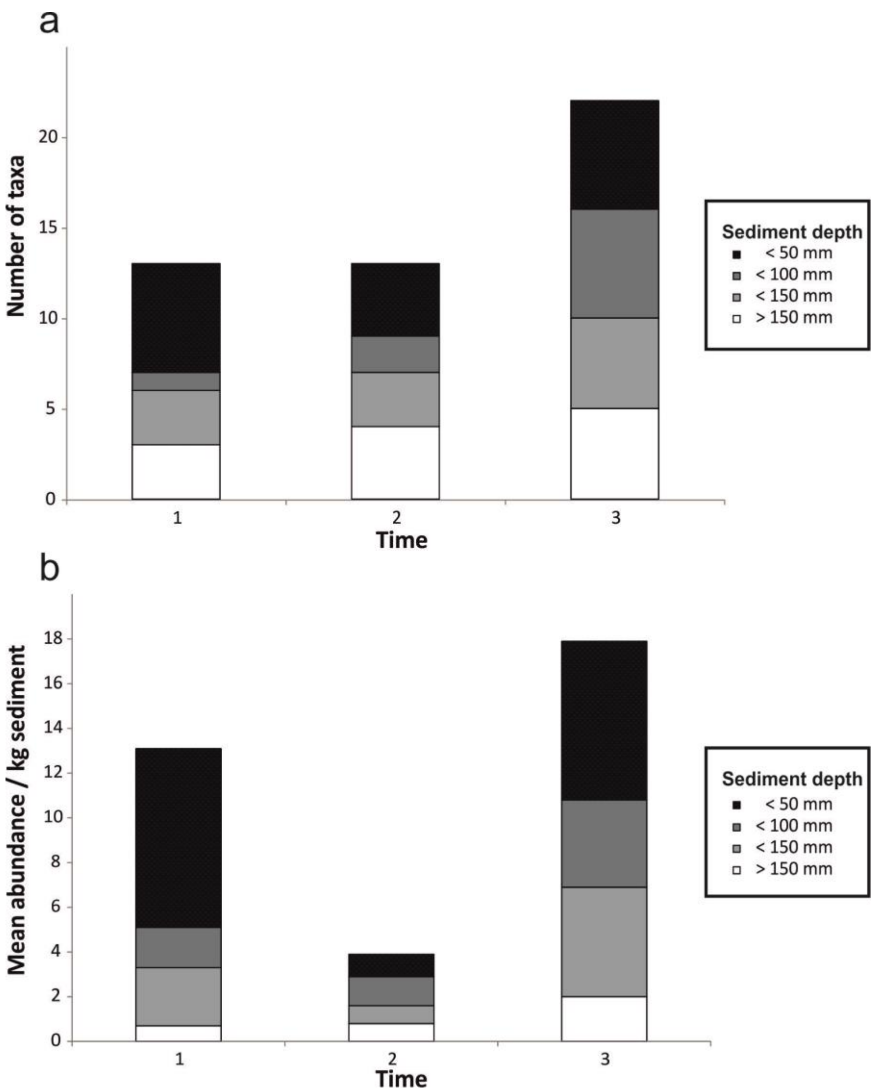



**Figure 6-4: Number of species (a) and mean abundance of macroinvertebrates / kg substratum (b) in substratum samples (n = 4 per sampling) at the restoration site 17 days before (1), 2 days (2) and 102 days (3) after the stream substratum restoration.**

The hatching success of brown trout eggs in the restored gravel bar increased from 0 % (± 0 %) in the spawning season one year before the restoration to 77 % (± 6 %) after the restoration. The hatching rate of the reference in the water column (hatching rate: 81 % ± 4) differed marginally compared to the hatching success within the substratum.

### *6.4.2 Downstream effects*

The deposition of fine sediment (<0.85 mm) downstream of the restored area was significantly higher than in the upstream reference ($p < 0.05$; Figure 6-5). Fine sediment deposition was highest in sediment traps closest to the excavation and decreased exponentially after the first 10 m. Even though fine sediment deposition was marginal after 1000 m, it was still significantly higher than fine sediment deposition at the reference upstream (t-test $p = 0.007$).

The sediment loads of particles ≥0.85 mm and ≥2.00 mm were also higher downstream of the excavated area, and decreased with increasing distance from the excavation area, although these effects were less pronounced than those for fine sediment (<0.85 mm). The highest amount of both particle sizes, ≥0.85 mm and ≥2.00 mm, was detected in traps 10 m downstream the excavation area.

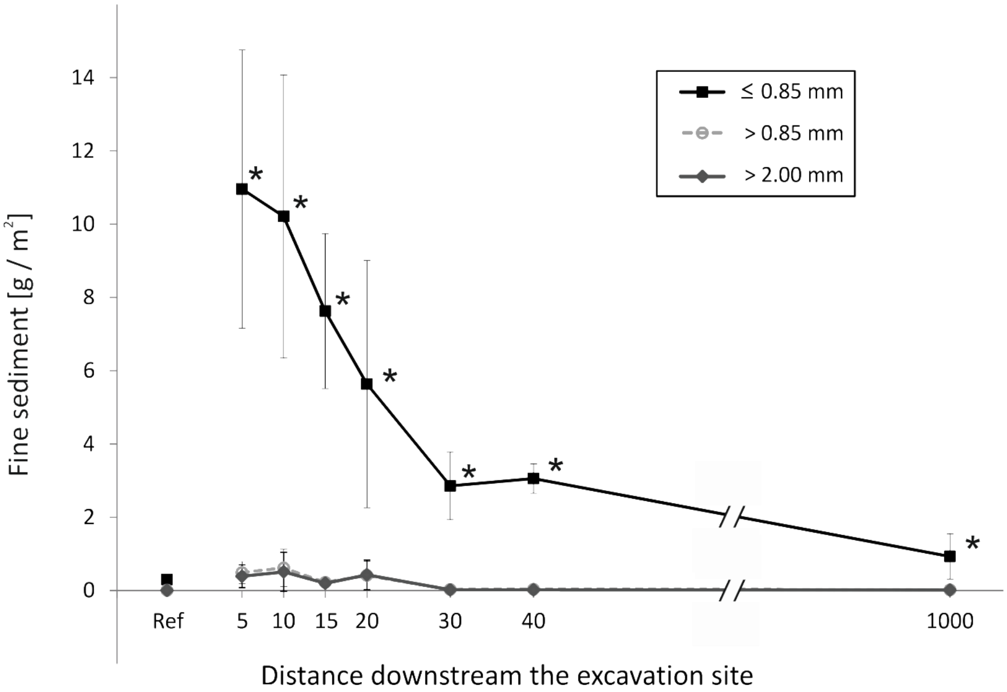



**Figure 6-5: Sediment deposition in sediment traps (length = 290 mm, width = 180 mm, height = 34 mm) downstream of the excavation site (5 m, 10 m, 15 m, 20 m, 30 m, 40 m and 1000 m) after 24 - hour exposure; sediment fractions are fine (≤0.85 mm), medium (>0.85 - 2.0 mm) and coarse (>2.0 mm; n = 5 per distance); Ref = reference upstream the excavation (n = 4), * indicates significant difference (t-test p < 0.006) compared to the reference site.**

## 6.5 Discussion

The excavation method assessed herein clearly reduced stream bed colmation and increased exchange rates between surface water and the hyporheic zone. Positive effects on salmonid hatching success, as well as on macroinvertebrate diversity and abundance were detected. However, the observed high siltation rates downstream of the successfully restored site suggest pronounced negative impacts of this in-stream substratum restoration.

The restoration of stream substratum is widely recommended as an in-stream restoration measure by public authorities and fisheries associations alike (Pulg, 2007; Hanfland, 2010). It is thus surprising that the effects and sustainability of this restoration technique have to our knowledge not been comprehensively assessed. Despite the necessity to rely on an unreplicated study design, the strong trends observed in this study suggest that the conclusions and the sampling design proposed herein can also be transferred to other small-scale substratum restoration measures in streams. This also includes measures such as the introduction of gravel or coarse material for the creation of new spawning sites (Zeh and Dönni, 1994).

Physical effects of the remobilization of the substratum such as the significant reduction of the fine sediment fraction and the loosening of compact substratum clearly resulted in an improvement of interstitial water conditions by increasing the exchange with surface water. Consequently, the high variability of physicochemical conditions in the interstitial water before the restoration along with the low numbers and densities of macroinvertbrates were indicative of low stream bed quality. The excavation method reduced fine sediment content, increased porosity of the interstitial zone (as evident from the decrease in penetration resistance) and resulted in a greater similarity of interstitial conditions with the surface water. These effects resulted in higher hatching rates, which was expected according to previous studies, e.g. by Greig et al., 2007, Heywood and Walling, 2007, and Pulg et al., 2011. It is likely that the increased survival rates of salmonid eggs also correlate with improved survival of the following fry stage (Hausle and Coble, 1976; Levasseur et al., 2006; Jensen et al., 2009; Sternecker and Geist, 2010). The significant changes in oxygen concentrations and the reduction of fish-toxic nitrite and ammonium are probably the most important single parameters that indicate improved habitat quality for salmonid eggs and larvae (Lewis and Morris, 1986; Chapman, 1988; Louhi et al., 2008; Kemp et al., 2011).

The functionality of the riverbed as key habitat increased not only salmonid hatching success, but also improved habitat quality for macroinvertebrate communities. The abundance of macroinvertebrates was initially heavily reduced by the excavation. However, the number of macroinvertebrates recovered quickly after the restoration, likely caused by recolonization from upstream refugia (Palmer et al., 1995). Positive effects on macroinvertebrate abundance could have been even more pronounced if the restoration had been carried out in a different season since dispersal and colonization is typically low at low water temperatures during the winter months (Reice, 1980; Pöckl et al., 2003). The higher abundance of species and the higher number of taxa 101 days after the excavation in all substratum depths suggest that deeper substratum zones became functional macroinvertebrate habitat after restoration (Coleman and Hynes, 1970). The observed impact of fine sediment on number of taxa and abundance of invertebrates is similar to previous studies that have shown an alteration in feeding group diversity (e.g., reduction of feeding group densities and species richness) due to increasing deposits of fine sediments (Wood and Armitage, 1997; Muotka et al., 2002; Rabení et al., 2005). Macroinvertebrates, preferred salmonid prey, especially benefit from low amounts of fine sediment (Suttle et al., 2004; Cover et al., 2008). The increase of gammarid and overall macroinvertebrate abundance after restoration confirm the positive effects on salmonid populations by increasing hatching success as well as by increasing prey availability for the following life stages.

The further decrease of the fine sediment content 101 days after the restoration was unexpected and suggests that the excavation method induced higher mobility of the gravel bar. This hints at a possible regeneration of self-perpetuating riverbed dynamics and the recovery of self-cleansing properties of the restored habitat. Surprisingly, this ongoing regeneration even occurred without the presence of peak flow events which are untypical for the well-buffered Moosach system. In the study period, Moosach discharge varied between 1.8 $m^3$ $s^{-1}$ and 2.8 $m^3$ $s^{-1}$ with no changes greater than 0.5 $m^3$ $s^{-1}$ within a month. Consequently, the sustainability of restoration is likely to be much more pronounced in other streams where peak flows are more intense. Hence the otherwise typically observed accumulation of fine sediment during egg incubation (Kondolf, 2000a, Scott et al., 2005) was not detected in this study. However, the long-term effectiveness of substratum cleansing also depends on fine sediment transportation in antropogenically manipulated river catchment and flow regime (Zeh and Dönni, 1994; Rubin et al., 2004; Meyer et al., 2008).

The negative effects downstream of the restoration site suggest that the success of small-scale stream substratum restoration is restricted to the restoration area itself. Restoration activities released a large quantity of fine sediment which can cause considerable impacts on downstream river habitats, and hence also on the biota (Bilotta and Brazier, 2008). It should be noted that siltation downstream of the restored area was only measured within a limited time period (24h) - so subsequent re-suspension of fine particles is likely with delayed effects on sites further downstream.

*Management implications*

This study shows that the restoration of a gravel bar with the widely applied ´excavation method´ is successful in removing fine sediment and improving interstitial habitat. The excavation of stream substratum is a low-cost (~ 100 €/h) and small-scale management tool for highly anthropogenic manipulated river stretches, where natural flow of substratum cannot be restored, e.g. due to flood control or monetary constraints. The benefits of this restoration method on the site where it is being applied are quickly realized and can easily be adapted to target species (e.g. gravel-spawning fishes, macroinvertebrates).

However, negative effects of fine sediment mobilization caused by the excavation have to be considered carefully and in a holistic approach. Although the transport of fine sediment is mainly a short-term impact, the extent of sedimentation in relation to sensitive habitats downstream and hydrological regimes have to be considered concurrently in order to lower the risk of undesirable side-effects. This can be easily achieved by adapting the timing, duration, and scope of excavation. For example, excavation can be done at higher water discharge, over a longer time period, or after covering up sensitive downstream areas. This is especially valid for restoration measures that target larger areas and therefore have the potential to create sediment loads that exceed the natural carrying capacity of the stream ecosystem (e.g. Milan et al., 2000; Merz and Setka, 2004).

Ultimately, the reconstitution of functional processes in rivers needs to consider holistic catchment approaches including the reduction of fine sediment erosion by sustainable land use, and the restoration of natural flow regimes for sustaining aquatic biodiversity on the long run (Soulsby et al., 2001; Dudgeon et al., 2006; Merz et al., 2006; Einum et al., 2008; Sear et al., 2009; Geist, 2011). The limitations of the in-stream substratum restoration clearly indicate that it cannot replace catchment management approaches and the restoration of natural flow regimes. Whilst both measures can be considered sustainable in the long-run, they have the disadvantage of comparatively long lag-times before an effect on the riverbed quality can be detected. Consequently, these measures are often not as popular as band-aid measures such as the ´excavation technique´ or the addition of gravel to streams.

# 7 Restoration of spawning habitats of brown trout (*Salmo trutta*) in a regulated chalk stream

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## 7.1 Abstract

Gravel bed spawning grounds are essential for the reproduction of salmonids. Such spawning grounds have been severely degraded in many rivers of the world because of river regulation and erosive land use. To reduce its effects on salmonid reproduction rates, river managers have been restoring spawning grounds. However, measures of effectiveness are lacking for the restored spawning sites of brown trout (*Salmo trutta*).

In this study, two methods were used to restore gravel bed spawning grounds in the Moosach River, a chalk stream in Southern Germany: the addition of gravel and the cleaning of colmated gravel. Seven test sites were monitored in the years 2004 to 2008, focusing on sediment conditions. Furthermore, brown trout egg survival and changes in the brown trout population structure were observed.

Both gravel addition and gravel cleaning proved to be suitable for creating spawning grounds for brown trout. Brown trout reproduced successfully at all test sites. The relative number of young-of-the-year brown trout increased clearly after the restoration. Sediment on the test sites colmated during the 4 years of the study. In the first 2 years, highly suitable conditions were maintained, with a potential egg survival of more than 50 %. Afterwards, the sites offered moderate conditions, indicating an egg survival of less than 50 %. Conditions unsuitable for reproduction were expected to be reached 5 to 6 years after restoration.

## 7.2 Introduction

Brown trout (*Salmo trutta*) as well as many other salmonids require high-quality spawning grounds for reproduction. Brown trout are nest builders, burying their eggs in gravel. (Ottaway et al., 1981; Crisp and Carling, 1989; Klemetsen et al., 2003 and Louhi et al., 2008). The eggs develop within the gravel's interstitial space and need several months until they hatch. During this period, the eggs are dependent on suitable hydraulic conditions for the provision of oxygen and the evacuation of wastes (Greig et al., 2007). The eggs are sensitive to the erosion of gravel banks and to the deposition of fine sediments, which clog the interstitial space (colmation; Platts et al., 1989; Kondolf, 2000a; Greig et al., 2007). Colmation has become a widespread threat to gravel bed spawners (Acornley and Sear, 1999; Soulsby et al., 2000b). Damming and shoreline stabilization reduce or prevent gravel transport in rivers by affecting river bed dynamics and by preventing the natural formation of suitable spawning grounds. Furthermore, highly erosive forms of land use (e.g. maize farming) generate much fine sediments (fines, <0.85 mm), reducing the suitability of the remaining spawning grounds (Sutherland et al., 2002, Opperman et al., 2005). Migration barriers (weirs, dams and sills) prevent fish from moving to spawning grounds elsewhere (Jungwirth et al., 1998). Almost all gravel bed spawning (lithophilic) fish, including brown trout, are considered as endangered species in Central Europe (Jungwirth et al., 2003). A shortage of spawning grounds can restrict the population size (Beard and Carline, 1991) and can lead to secondary effects such as high concentrations of spawners on a few gravel banks resulting in superimposition and local high density-dependent mortality of fry (Elliot, 1994; Einum and Nislow, 2005; Sear and DeVries, 2008).

Some managers consider the restoration or construction of spawning grounds to be an appropriate method to increase fish reproduction success in affected rivers. In some areas, it is a popular tool for river management, and it has been attempted in many places for decades, especially in the rivers of North America, with a focus on the Oncorhynchus species (White, 1942; Wilson, 1976; Kondolf et al., 1996; Kondolf, 2000a; Sear and DeVries, 2008). However, monitoring artificial or restored spawning grounds for brown trout has been unsatisfactory, being short term or not studying the redegradation of the sediment. Most studies have focused on the spawning behavior and the eventual effects of improved spawning area on the number of young of the year (YOY) - but not on the development of sediment quality and the survival of eggs (Sømme, 1941; Madsen and Tent, 2000; Rubin et al., 2004; Gerken, 2006; Kuhr, 2006; Pedersen et al., 2009; Barlaup et al., 2008). Shackle et al. (1999) successfully attempted different methods to clean spawning gravel for brown trout, and Niepagenkemper and Meyer (2003) analyzed the degradation of cleaned sediments on restored gravel banks; however, both studies were short term not exceeding 1 year.

It was not clear for how long restored spawning grounds would offer suitable spawning conditions in regulated rivers with high suspended loads, for example, European chalk streams in densely populated and intensively agriculturally used areas that were formerly productive trout streams. To answer these questions, we chose the Moosach River, a regulated chalk stream with increased suspended load in Southern Germany, to test the success of spawning ground restoration through gravel addition and sediment cleaning. After restoration, the gravel banks and the fish community were monitored for 4 years, in terms of sediment quality, egg survival, fish population structure and river morphology.

## 7.3 Materials and Methods

### *7.3.1 River characteristics*

The Moosach River is a 35-km-long chalk stream (calcium ~114 mg $L^{-1}$; Stein, 1988) in the Danube drainage basin, flowing over tertiary and quaternary lime stone gravel (Figure 7-1). It has oxygen concentrations more than 9 mg $L^{-1}$ and no discharges of sewage. Between 2004 and 2008, the median water discharge at test site M1 (main channel) was 1.5 $m^3\ s^{-1}$ ($Q_{50}$) and varied between 1 and 7.4 $m^3\ s^{-1}$ (Pulg, 2009). The discharge exceeded 1.3 $m^3\ s^{-1}$ 90 % of the time ($Q_{90}$) and exceeded 2.2 $m^3\ s^{-1}$ 10 % of the time ($Q_{10}$). The river is groundwater fed in its upper part and has low discharge dynamics: the $Q_{90}/Q_{50}$ ratio was 0.87, and the $Q_{10}/Q_{50}$ ratio was 1.47. The river's natural gradient is 0.13 % (Burbach et al., 2006).

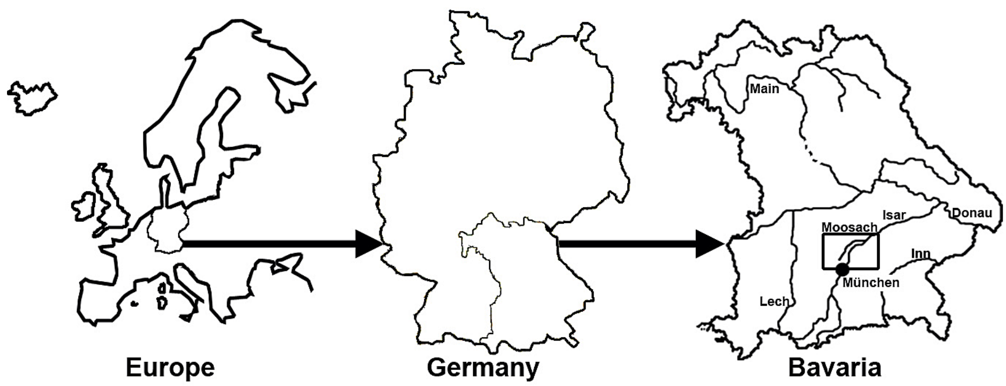


**Figure 7-1: Location of the study area.**

The stream was formerly known as a highly productive waterway in terms of lithophilic species, especially brown trout and grayling, and for the spawning migrations of lithophilic fishes from the River Isar (Helmsauer, 1846; Von dem Borne, 1882). As with many other rivers in European cultural landscapes, the Moosach River has been modified by human activities since the Middle Ages and systematically regulated in the 20th century. Gradient, flow velocity and shear stress were reduced because of impoundments more than approximately one third of its length. There are 11 major weirs, interrupting connectivity and gravel transport, and further several small ground sills stabilizing the sediment; the mean distance between the weirs is 3.2 km. The banks have been reinforced in the urban stretches (Burbach et al., 2006). Gravel bed spawning grounds have been severely reduced in the last few decades and have completely disappeared in some sections of the river because of the colmation and consolidation of the substrate. According to Stein (1988), the erosion of fines had increased since the 1970s, mainly because of the conversion of grassland into maize fields in hilly parts of the drainage basin. By the end of the 1980s, erosion had exceeded 8 t/ha/annum on maize fields, whereas it was estimated to be less than 1 t/ha/annum on grassland and forests (Jung, 1990). In the Moosach River, clogged and consolidated fluvial sediments remained undisturbed for many years and were, in parts, additionally stabilized by lime coagulations caused by blue-green algae (‘Onkoids’, Persoh, 1998). One test site (M1) and a control site were situated in the main channel of the Moosach, in a stretch bounded by weirs between milestone 13.4 and 14.4 (Fkm, distance [km] from the river mouth). Six test sites (M2 - M7) were situated in a side channel of the Moosach River, the Schleifermoosach (Figure 7-2). This river stretch is 5.6 km long and approximately 5 m wide. In the study period (2004 - 2008), the discharge varied between 0.1 and 10.9 $m^3\ s^{-1}$ and had a median of 0.3 $m^3\ s^{-1}$ ($Q_{50}$). $Q_{90}$ was 0.2 $m^3\ s^{-1}$, and $Q_{10}$ was 0.6 $m^3\ s^{-1}$. The discharge dynamics were slightly higher than those of the main channel ($Q_{90}/Q_{50}$ = 0.67, $Q_{10}/Q_{50}$ = 2).

Moosach River’s suspended load was 7 mg $L^{-1}$ on average, and in the Schleifermoosach, it was 10 mg $L^{-1}$ (mean, based on 60 water samples and turbidity monitoring in the years 2004 - 2008; Pulg, 2009). In both the side and the main channels, suspended loads could exceed 130 mg $L^{-1}$ under annual floods. High loads were mostly recorded after rain events in late winter and during river maintenance work in dammed stretches upstream (Stein, 1988; Jung, 1990; Pulg, 2009).

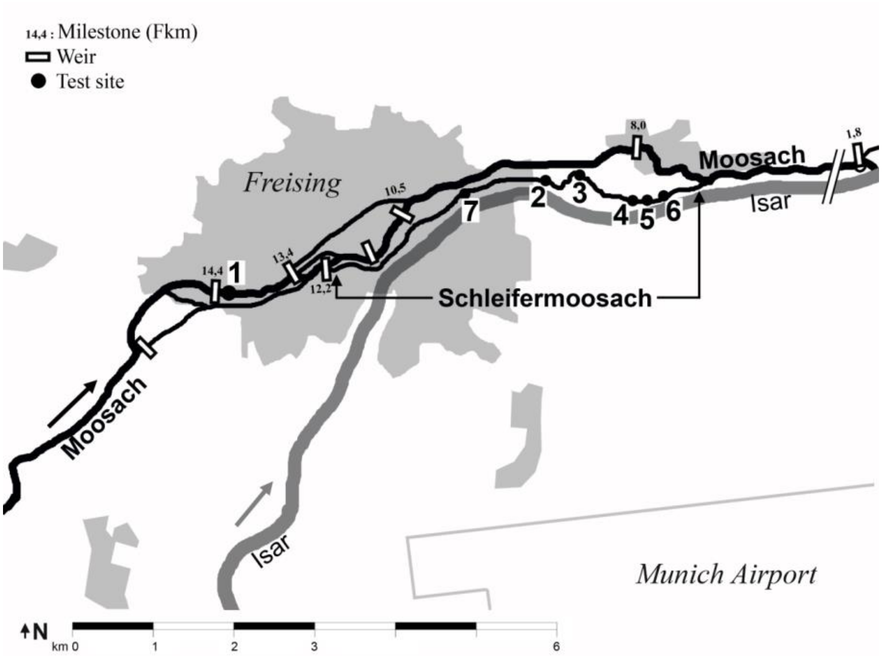


**Figure 7-2: Location of the test sites on which spawning grounds were restored.**

### *7.3.2 Restoration of spawning grounds*

The restoration design (Figure 7-3) was based on the description of spawning habitats by Crisp and Carling (1989), Walker (2007), Louhi et al. (2008) and our own measurements at natural spawning grounds (Pulg, 2009). Pool-riffle structures were established with flow velocities from 0.3 to 1 m $s^{-1}$, water depths from 0.1 to 0.6 m (median water flow), average grain sizes between 10 and 23 mm, percentage fines less than 6 % and a gradient between 0.3 % and 0.6 %. The main characteristics of the test sites are shown in Figure 7-3.

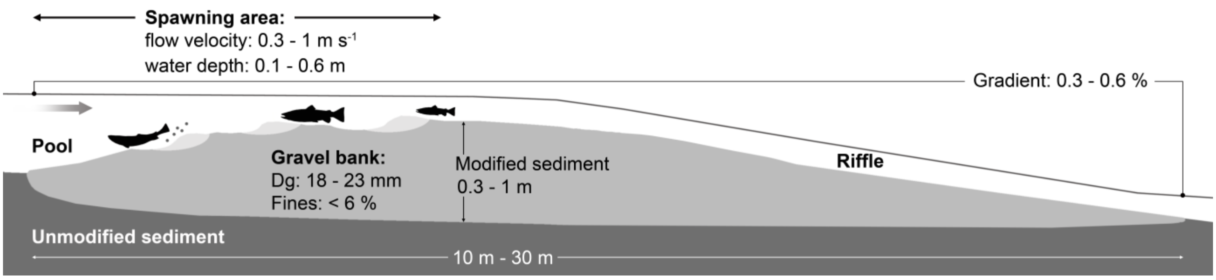


**Figure 7-3: Principal longitudinal section through a restored spawning ground with the characteristics that were established by restoration on all test sites.**

The following techniques were used to achieve the conditions:

(1) Cleaning and loosening an existing substrate with an excavator turning over the sediment in situ. The sediment of the area was lifted up and dropped in again, and fines were consequently washed out by the current. This was repeated twice in immediate succession and resulted in an average grain size ($D_g$) of 18 mm and a percentage of fines lower than 6 %.

(2) Introduction of additional gravel (16 - 32 mm, $D_g$=23 mm, percentage fines <1 %).

In total, seven test sites were established and monitored (see Figure 7-1 and Figure 7-2). The restoration of the test sites differed because of different construction permissions by the authorities. Permission was received in 2004 for sites 1 to 4, allowing monitoring more than four spawning seasons, and permission for sites 5 to 7 was received in 2005, allowing monitoring more than three spawning seasons. Test sites M1 - M4 were constructed through the addition of gravel (August 2004). Test sites M5 - M7 were on sediment that was cleaned and loosened (March 2005). In total, 12.5 % (3500 $m^2$) of the Schleifermoosach's surface area (28000 $m^2$) sediment was cleaned. Together with the residual area of loose and clean gravel banks (1.5 %), the Schleifermoosach offered gravel banks suitable for spawning on 14 % of the rivers area (~3900 $m^2$) after the restoration. The construction costs for the restoration works varied from 0.5 to 3 €/$m^2$ for sediment cleaning and from 3 to 7 €/$m^2$ for gravel addition.

### *7.3.3 Monitoring*

'Redds' were visually identified from November to January in the years 2004 to 2007 (Crisp and Carling, 1989) and were checked for eggs. Egg-to-alevin survival was measured following the technique of Rubin and Glimsäter (1996). Five Whitlock-Vibert boxes were incubated per control site and per test site in the river, each box containing 190 freshly fertilized brown trout eggs. The boxes were additionally covered by a fine mesh to prevent fry from escaping. On the test sites in the river, the boxes were incubated next to natural 'redds' if existent. The eggs were fertilized in the fish hatchery of the Landesfischereiverband Bayern from a brood stock originating from the Isar drainage basin. The eggs were mixed and distributed to the sites within 8 h after fertilization. The boxes were incubated under a 10 cm - layer of sediment; thus, the eggs were located between 10 and 15 cm under the sediment surface. Boxes were checked just before calculated emergence (based on temperature measurements, day degrees given in Geldhauser and Gerstner, 2003, and the development of the control group). The number of living individuals was counted for calculating survival rates. Survival was measured in the years 2004 to 2007 on all test sites, four control groups in the field (nonrestored, colmated gravel banks), four incubations in the laboratory and seven natural spawning grounds on remaining gravel banks in the rivers Moosach and Schleifermoosach. Not every incubation could be used because some of the boxes got lost because of a flood in February 2005 and other reasons. In the entire monitoring period, 33 survival measurements based on survival counts in 165 Whitlock-Vibert boxes could be used.

Sediment samples were collected using freeze-core technique. The sampler consisted of a copper tube that was partly introduced into the substrate and filled with liquid nitrogen (196 °C). The substrate froze to the tube and could be lifted (Ingendahl, 2001; Kondolf, 2000a). The samples were collected at the end of the incubation period, close to the incubated eggs to represent the sediment that the eggs were incubated in. Sediment samples were wet sieved. Each sample was divided in the following classes: < 0.85 mm (fines), 0.85 - 2 mm, 2 - 6.3 mm, 6.3 - 20 mm, 20 - 63 mm and 63 - 120 mm following Bahlburg (1998; see also Rubin and Glimsäter, 1996; Kondolf, 2000a). The upper 20 cm of the sediment was used for substrate analyses. The average grain size ($D_g$) was calculated after Rubin and Glimsäter (1996). The fraction of dry mass with diameters less than 0.85 mm was defined as percentage fines. Sediment consolidation was measured by kick samples (Zeh and Dönni, 1994). Gravel sediment that could be moved to a depth < 15 cm was categorized as loose, and all other sediment was categorized as consolidated. Interstitial oxygen concentration was measured by pulling interstitial water through sampling tubes made of rubber (Pusch and Schwoerbel, 1994; Ingendahl, 2001). The tubes were permanently fixed on the sample sites in a sediment depth of 15 cm. The upper end of each tube was closed with a stopper and lay on the sediment surface. After removing the stopper, the first 80 ml of water (volume of the rubber tube) was drawn out using a syringe and discarded, and the second 80 ml of interstitial water was used for in situ analysis with a portable instrument (WTW Multi 340). Samples were collected twice a week during each incubation period.

In the Moosach system, fish were stocked for recreational purposes, especially brown trout and grayling (Hanfland, 2002). In the surveyed stretch of the Moosach (main channel), all spawning banks were degraded. Trout and grayling could not reproduce and originated from stocking or downward migration from spawning grounds upstream (Stein, 1988). Upstream migration was prevented by weirs. Lithophilic fishes in the Schleifermoosach could reproduce on a few residual gravel banks and could immigrate from the lower Moosach and the River Isar. In the Schleifermoosach, brown trout were stocked until March 2004 (fry). Thereafter, there was no stocking in this stretch. Fish were sampled by semiquantitative electrofishing in the Schleifermoosach in winter after the spawning period (December 2004, December 2005 and January 2007) to observe the development of YOY (< 17 cm). The method followed the German standard for electrofishing (VDFF, 2000): wading upstream, 10 kW, 600 V, DC. A stretch of the Schleifermoosach (Fkm 0 - 4, A = 2.3 ha) was electrofished once per sample. The electrofishing efficiency was determined by mark and recapture in the same river with the same equipment used by Hanfland (2002) and was determined to be 33 % for YOY of brown trout. Captured fish were measured and released. A reference stretch of the Moosach between the milestones (Fkm) 14.4 and 17 was sampled with the same method in the years 2004 to 2007 by Oswald (2007).

## 7.4 Results

Brown trout spawned at all seven test sites. Sites 2 to 4 were not used by fish in the first spawning season; thereafter, all sites were used. On the smallest gravel banks (M3 and M4, each 30 $m^2$), no more than one 'redd' was recorded. On the bigger sites (M2, M5 and M6, each 60 $m^2$), up to three 'redds' were recorded. On the biggest site (M1, 250 $m^2$), up to 11 'redds' were found.

The egg survival of brown trout on the test sites varied between 0 % and 93 %. The statistical median of the egg survival at each site varied between 0 % and 80 % (n = 33; Figure 7-4 - Figure 7-6). The median of survival at the seven test sites in the river was 48 % (mean = 46 %); it varied between 11 % and 77 % at each site. Survival was significantly correlated ($p < 0.001$, linear regression) with average grain size (positively, $R^2 = 0.54$; Figure 7-4), percentage fines (negatively, $R^2 = 0.68$; Figure 7-5) and interstitial $O_2$ (positively, $R^2 = 0.46$; Figure 7-6). Field data for 2005 were excluded because the field experiment was spoiled by a flood in the incubation period 2004 - 2005.

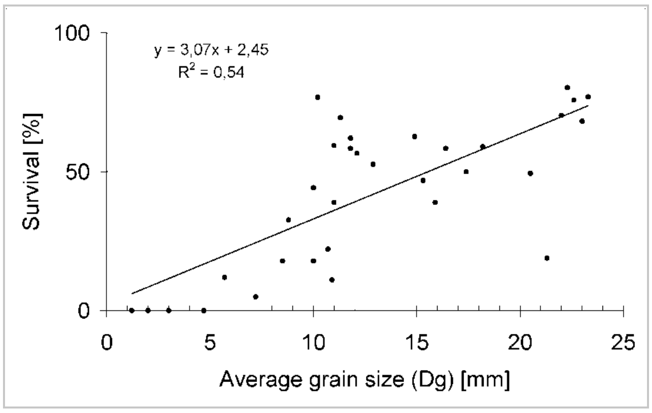


**Figure 7-4: Median egg survival and average grain size ($D_g$) at the test and control sites in the years 2004 - 2007 (n = 33, p < 0.001).**

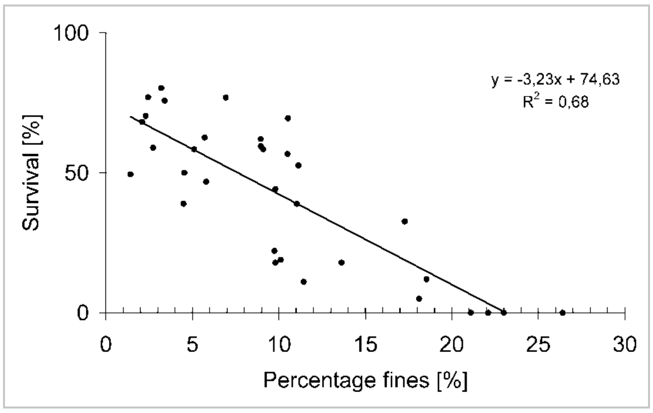


**Figure 7-5: Median egg survival and percentage fines at the test and control sites in the years 2004 - 2007 (n = 33, p < 0.001).**

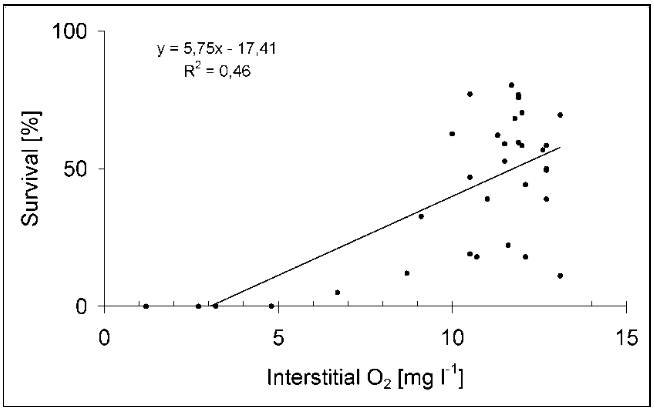


**Figure 7-6: Median egg survival and interstitial $O_2$ at the test and control sites in the years 2004 - 2007 (n = 33, p < 0.001).**

After 4 years, the arranged pool-riffle structures persisted. The gravel was still loose and could be moved by fish and stronger currents. Water depth, velocity and the pool-riffle structure were still in the range described in Figure 7-3. The average grain size ($D_g$) had reduced significantly from 22 mm to approximately 13 mm (median) within 4 years (p = 0.015, Wilcoxon test; Figure 7-7). The percentage of fines increased significantly (p = 0.015, Wilcoxon test; Figure 7-8) from 0 % to approximately 10 % (median). In all four incubation periods, the mean interstitial $O_2$ concentration varied between 8.9 and 13 mg $L^{-1}$, with a median of 11 mg $L^{-1}$. The concentrations seem to tend downward in the last 2 years, but the differences were too small to be significant.

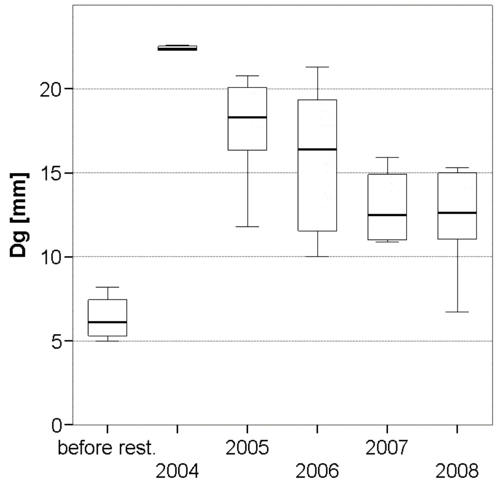


**Figure 7-7: Average grain ($D_g$) size at the test sites in the years 2004 - 2008. The graph for 2004 represents gravel addition alone.**

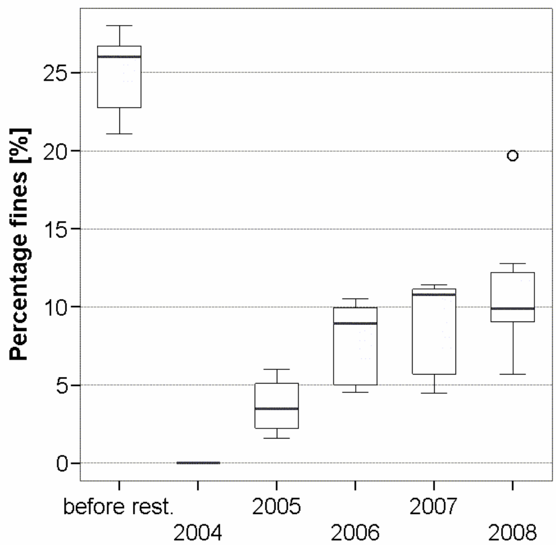


**Figure 7-8: Percentage fines at the test sites in the years 2004 - 2008. The graph for 2004 represents gravel addition alone.**

The sites with cleaned gravel had a higher percentage of fines directly after restoration (3.5 % - 5.3 %) and a lower $D_g$ (18 mm). They were thus on the same level of degradation directly after restoration as the sites with added gravel after 1 year (Table 7-1). In the following years, the sediment conditions at the sites with cleaned and added gravel were similar and in the same range. The differences between the groups were not significant, neither in the first year nor in the following years ($p > 0.125$, Wilcoxon test).

**Table 7-1: Thresholds for egg survival in the Moosach River based on a classification tree (QUEST) of the data shown in Figure 7-4 - Figure 7-6.**

| | No survival observed | Low survival observed | High survival observed | QUEST-analysis | |
|---|---|---|---|---|---|
| Survival [%] | 0 | < 50 | 50 - 100 | Significance* | d.f. |
| $D_g$ [mm] | < 5.7 | ≤ 12.9 | > 12.9 | p = 0.002 | F = 11.8 |
| Percentage fines [%] | > 18.5 | > 10.3 | ≤ 10.3 | p < 0.001 | F = 15.5 |
| Interstitial $O_2$ [mg/l] | < 6.7 | ≤ 10.4 | > 10.4 | p = 0.012 | F = 8 |

* Between the groups with high and low survival

At the control site, no significant changes were observed. The $D_g$ varied between 5 and 6 mm, the percentage fines was between 26 % and 27 % and the interstitial $O_2$ concentration less than 3 mg $L^{-1}$. The survival of incubated eggs was 0. No natural 'redds' were found on the area. Sediment shifts due to high discharge (>2 $m^3\ s^{-1}$ at the sites M2 - M7 and >3 $m^3\ s^{-1}$ at M1) were observed on all sites (in February 2005 and April 2006). The test sites' pool-riffle structures and the gradients did not change substantially.

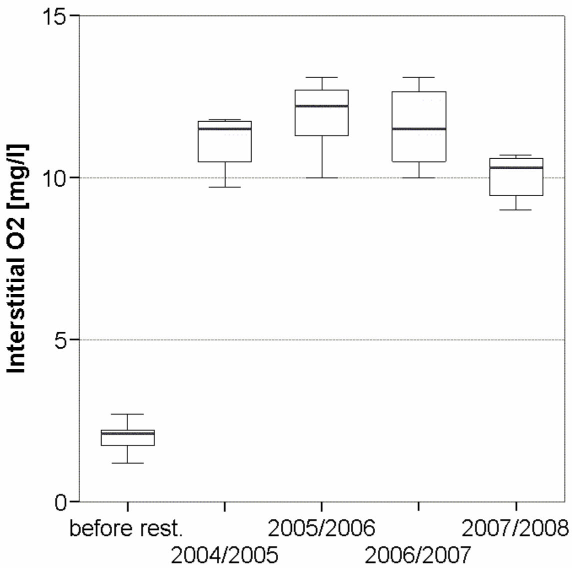


**Figure 7-9: Interstitial $O_2$ concentration at the test sites in the incubation periods in the years 2004 - 2008. The graph for 2004 / 2005 represents gravel addition alone.**

The number of brown trout, especially the juveniles, increased during the monitoring period (Figure 7-10). The catch per unit effort for brown trout YOY per 100 $m^2$ increased from 0.6 (2004, before restoration) to 3.2 (2005), 3.3 (2006) and 1.8 (2008). Oswald (2007) did not find any increase in the number of YOY of brown trout (or other lithophilic species) in the reference stretch in the main channel of the Moosach River.

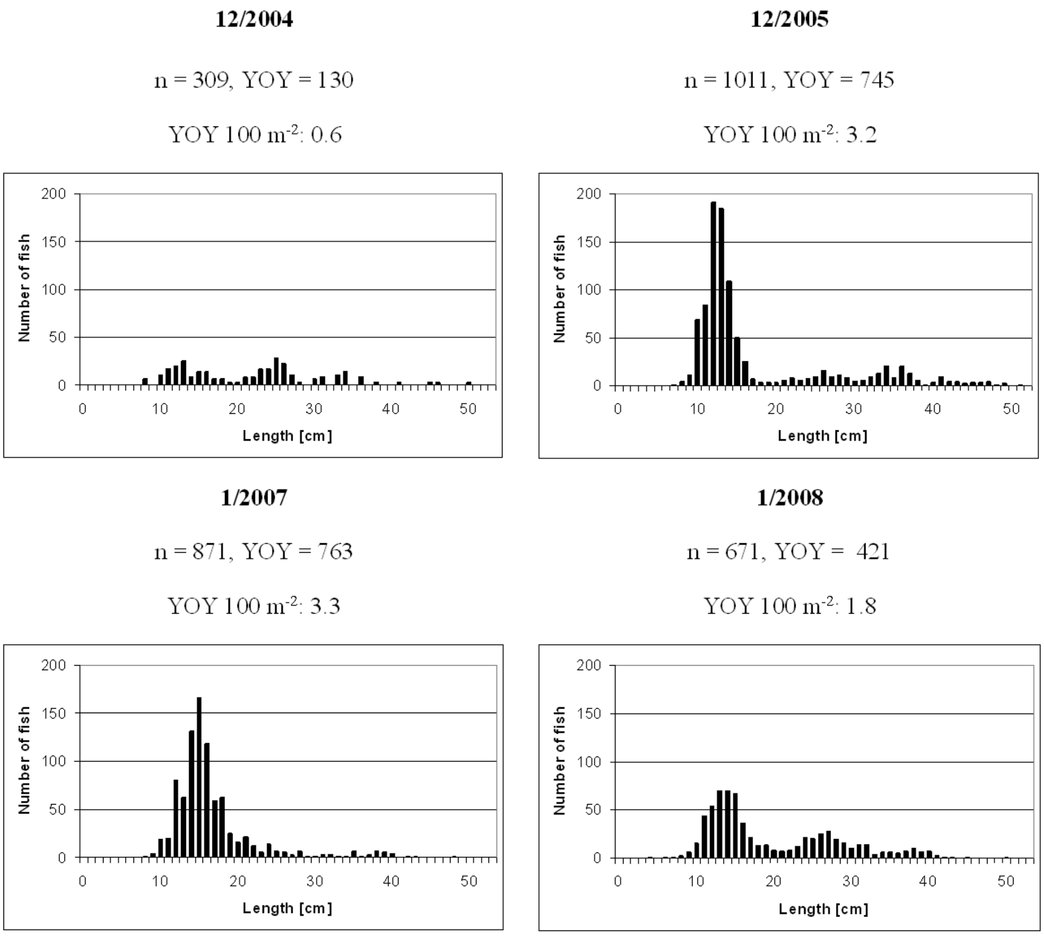


**Figure 7-10: Length-frequency diagrams of brown trout in the catches in the years 2004 - 2008.**

## 7.5 Discussion

The egg survival of brown trout (mean = 46 %, maximum = 93 %) in the Moosach River was higher than that in Central European streams affected by fines and sewage (Ingendahl, 1999; Rhine drainage, Germany, mean = 8 %, maximum = 45 %) but was in the same range as the survival in streams on agricultural land described by Rubin and Glimsaeter (1996; Gotland, Sweden, mean = 28 %, maximum = 91 %). However, the survival rate was lower than that in mountain streams unaffected by fines (Barlaup et al., 2008; West Norway, mean = 90 %, maximum = 100 %). As described by others (Rubin et al. 1996; Kondolf, 2000a; Greig et al., 2007), survival was correlated with average grain size ($D_g$), interstitial $O_2$ and percentage fines. Survival could only be observed in sediments with a Dg ≥ 5.7 mm, a percentage fines ≤ 18.5 % and an interstitial $O_2$ concentration ≥ 6.7 mg $L^{-1}$. A classification tree based on a QUEST algorithm (Loh and Shih, 1997) applied to two groups with high (> 50 %) and low median survival (≤ 50 %) demonstrated that differences between high and low survival were best significant for a $D_g$ of 12.9 mm ($p = 0.002$, $F = 11.8$), a percentage fines of 10.3 % ($p < 0.001$) and an interstitial $O_2$ concentration of 10.4 mg $L^{-1}$ ($p = 0.012$) (see Table 7-1 and Figure 7-7 - Figure 7-9). Thus, the sediment can be classified in three categories:

(1) Nonsuitable for the reproduction of brown trout (Dg < 5.7 mm, percentage fines > 18.5 %, interstitial $O_2$ < 6.7 mg $L^{-1}$).

(2) Moderately suitable for the reproduction of brown trout ($D_g$ ≤ 12.9 mm, percentage fines > 10.3 %, interstitial $O_2$ < 10.4 mg $L^{-1}$).

(3) Highly suitable for the reproduction of brown trout ($D_g$ > 12.9 mm, percentage fines ≤ 10.3 %, interstitial $O_2$ > 10.4 mg $L^{-1}$).

This interpretation also matches data on brown trout found by Rubin and Glimsaeter (1996), Ingendahl (1999) and Sear et al. (2002) in other streams with different hydrological conditions.

In the Schleifermoosach, the total catch of brown trout, especially the number of YOY, strongly increased after the spawning area was increased from 1.5 % to 14 % of the river's area (Figure 7-10). Oswald (2007) reported no increase in the populations of brown trout, grayling or other lithophilic fishes in the main channel of the Moosach River above the restoration works. Causes for the changes in the population structure of the Schleifermoosach can be multifarious, and this study is not sufficient for their detailed analysis. However, the strong increase in YOY of brown trout at the restored sites seems to be a consequence of the restoration works because there was no such increase in the control channel. The increase in the restored area can be explained by higher reproduction rates due to better spawning conditions.

In addition to brown trout, other species could be observed spawning on the test sites: grayling (*Thymallus thymallus*), chub (*Squalius cephalus*), dace (*Leuciscus leuciscus*) and bullhead (*Cottus gobio*). A significant increase in the number of grayling at the restored sites, especially the number of YOY, was documented after the restoration (Pulg, 2009).

In addition, the data from the Schleifermoosach suggest that the remaining spawning grounds covering 1.5 % of the area (420 $m^2$ of 28,000 $m^2$) were a limiting factor for the reproduction of brown trout, as the number of fish increased clearly after the spawning area was enlarged to 14 % (3900 $m^2$). The reproduction after the restoration seems to be high enough to eliminate the bottleneck of restricted spawning habitat, as the proportion of juveniles substantially increased.

The extensive use of the restored sites by spawning brown trout demonstrates that the design of the sites (shown in Figure 7-3) was suitable to trigger spawning. In the river stretch with test site M1, there were no other spawning grounds available; therefore, spawning may have occurred because of the absence of alternatives. However, around test sites M2 - M7, there were alternative spawning grounds, and the fish reproduced both on the test sites and other gravel banks (both natural and restored).

For all sites, the sediment conditions ($D_g$, percentage fines, interstitial $O_2$) showed a clear trend to degradation caused by the accumulation of fines (Figure 7-7 - Figure 7-9).

The percentage fines increased and the Dg decreased significantly. Also, the interstitial $O_2$ concentration decreased, but by a small amount. In 2008, however, six of seven test sites still offered conditions suitable for low survival (<50 %; Table 7-2) or better. Only at test site 3 the percentage fines was higher than 18.5 %, probably because of the high sedimentation of fines caused by large woody debris on the site the year before. On the basis of the statistical medians that represent the conditions at all seven test sites, highly suitable sediment conditions (survival >50 %) were maintained in the first 2 years after restoration. In years 3 and 4, moderate conditions dominated indicating low survival (<50 %). If it is assumed that the degradation continues linearly, completely unsuitable conditions are reached at the sites with added gravel after 6 years, at the sites with cleaned gravel after 5 years (Figure 7-7 - Figure 7-9, linear trend of the statistical median of Dg and percentage fines).

Both the addition of gravel and the cleaning of sediment were adequate methods to establish the spawning sites. The added gravel was cleaner in the first year, but the differences were not statistically significant. This may be a consequence of low sample number, but because there were no differences between the groups after 2 and 3 years, it is concluded that local sediment dynamics are more important for the sediment conditions than the restoration method. The costs for sediment cleaning (0.5 - 3 €/$m^2$) were considerably lower than those for gravel addition (3 - 7 €/$m^2$). Therefore, sediment cleaning can be recommended in similar cases. In the long run, the sites may reach similar conditions as before the restoration ($D_g$, ~6 mm; interstitial $O_2$, ~2 mg $L^{-1}$; percentage fines, ~26 %). The degradation of the sites may be delayed if sediment dynamics led to cleaning effects (Kondolf, 2000a; Sear and

DeVries, 2008). Sediment shifts were observed on the test sites and potential cleaning effects could be recorded (i.e. Dg on M6 and M7 2005 - 2006; Table 7-2).

**Table 7-2 : Sediment conditions on the test sites in the years 2004 - 2008**

| Test site | Sediment conditions | Before restoration | 2004 | 2005 | 2006 | 2007 | 2008 |
|---|---|---|---|---|---|---|---|
| | Dg [mm] | 6.1 | 22.3 | 20.1 | 11.8 | 14.9 | 15.1 |
| **M 1** | Perc. fines [%] | 22.5 | 0.0 | 2.2 | 9.0 | 5.7 | 5.7 |
| | Interst. $O_{2\ [mg\ L^{-1}]}$ | 2.7 | | 10.6 | 11.8 | 10.0 | 9.0 |
| | Dg [mm] | 5.0 | 22.5 | 20.1 | 16.4 | 12.9 | 11.3 |
| **M 2** | Perc. fines [%] | 27 | 0.0 | 2.3 | 5.1 | 11.1 | 12.8 |
| | Interst. $O_{2\ [mg\ L^{-1}]}$ | 2.2 | | 11.8 | 12.7 | 11.5 | 9.9 |
| | Dg [mm] | 5.2 | 22.6 | 11.8 | 10.0 | ** | 6.7 |
| **M 3** | Perc. fines [%] | 26.4 | 0.0 | 6.0 | 9.8 | ** | 19.7 |
| | Interst. $O_{2\ [mg\ L^{-1}]}$ | 1.8 | | 11.7 | 12.1 | ** | 10.3 |
| | Dg [mm] | 5.4 | 22.3 | 20.8 | 21.3 | 12.1 | 15.3 |
| **M 4** | Perc. fines [%] | 28 | 0.0 | 1.6 | 5.0 | 10.5 | 11.6 |
| | Interst. $O_{2\ [mg\ L^{-1}]}$ | 1.2 | | 11.3 | 12.2 | 12.6 | 10.6 |
| | Dg [mm] | 8.2 | * | 18.0 | 11.3 | 11.0 | 12.6 |
| **M 5** | Perc. fines [%] | 23 | * | 5.3 | 10.5 | 11.0 | 9.6 |
| | Interst. $O_{2\ [mg\ L^{-1}]}$ | 1.7 | * | | 13.1 | 12.7 | 10.6 |
| | Dg [mm] | 7.1 | * | 14.7 | 17.4 | 10.9 | 14.9 |
| **M 6** | Perc. fines [%] | 26 | * | 3.5 | 4.5 | 11.4 | 8.5 |
| | Interst. $O_{2\ [mg\ L^{-1}]}$ | 2.1 | * | | 12.7 | 13.1 | 10.7 |
| | Dg [mm] | 7.8 | * | 18.3 | 21.3 | 15.9 | 10.8 |
| **M 7** | Perc. fines [%] | 21.1 | * | 4.9 | 10.1 | 4.5 | 9.9 |
| | Interst. $O_{2\ [mg\ L^{-1}]}$ | 2.2 | * | | 10.5 | 11.0 | 9.0 |
| | Dg [mm] | 6.1 | 22.4 | 18.3 | 16.4 | 12.5 | 12.6 |
| **Median M 1-7** | Perc. fines [%] | 26.0 | 0.0 | 2.9 | 9.0 | 10.8 | 9.9 |
| | Interst. $O_{2\ [mg\ L^{-1}]}$ | 2.1 | | 11.5 | 12.2 | 12.1 | 10.3 |

* non existant; ** covered by woody debris

The differences and the number of samples are too small to distinguish the cleaning effects of sediment shifts from methodological variation due to freeze core sampling (see Kondolf, 2000a). Therefore, no conclusions can be drawn on the cleaning effects of sediment shifts in this study. Other factors that may prolong the longevity of the spawning grounds are the reduction of fines in the river basin (i.e. due to less erosive land use; Opperman et al., 2005) and the modification of the gravel bed by the spawners. It is well known that the construction of 'redds' leads to the removal of fines (Young et al., 1989; Kondolf et al., 1993; Montgomery et al., 1996). As a result of successful spawning, population recruitment and subsequently the number of spawners are likely to increase. A high number of spawning fish in the same areas each autumn may contribute to a higher longevity of the spawning grounds. On the other hand, high loads of fines during a period with a stable gravel framework can increase the sediment degradation and shorten the longevity (Greig et al., 2007). A further observation of the sites was therefore recommended.

Clogged top layers ('seals') that could entomb fry (Kondolf, 2000a; Greig et al., 2007) were not observed. The typical fines of the Moosach system were too small to be a hindrance for emerging fry. The effects of clay-sized particles (0.5 - 4 mm) clogging the egg membrane (Greig et al., 2005) could not be detected with the approach used here.

This case study demonstrates that spawning grounds for brown trout could be restored in a regulated chalk stream with artificially reduced gravel transport, reduced sediment dynamics (weirs and bank stabilization) and increased suspended loads (mean = 10 mg $L^{-1}$ Schleifermoosach, 7 mg $L^{-1}$ main channel). Both restoration methods used, gravel cleaning and gravel adding, were considered as suitable to restore spawning grounds because there were no significant differences in the sediment conditions or the degradation. However, gravel cleaning was clearly cheaper. Although the river's suspended load regime was not reduced and clogging could be observed, the sites offered highly suitable conditions (median of $D_g$, percentage fines and interstitial $O_2$) for reproduction for 2 years. After 4 years, the conditions were moderately suitable, indicating low egg survival (<50 %). If the degradation continued linearly, unsuitable conditions would have been reached after 5 to 6 years.

Is restoration of gravel bed spawning grounds a tool that can be recommended for other rivers? The results of this case study cannot be transferred directly to other rivers. Different sediment dynamics, flow regimes and loads of fines can lead to other results. However, several studies focusing on fish demonstrated successful restoration of gravel bed spawning grounds elsewhere (e.g. Sear and DeVries, 2008; Barlaup et al., 2008; Pedersen et al., 2009). In the Moosach River, the restored spawning gravels colmated. Restoration has to be repeated after 5 to 6 years if spawning grounds are to be maintained. In rivers unaffected by fines, similar works may result in permanent improvement, as the study of Barlaup et al. (2008) indicates. The authors did not find a noteworthy colmation in some Norwegian mountain streams. The small amount of fines accumulating there was washed out when the spawning fish built their 'redds'.

The restoration works did not eliminate the causes for the degradation of gravel banks (damming, bank stabilization and fines). To achieve this, other methods like largescale river restoration with the removal of dams and bank stabilization as well as the reduction of pollution and discharge of fines are required (Jungwirth et al., 2003; Opperman et al., 2005). This implies works throughout the river and its drainage basin. Various studies demonstrate that such an approach can restore natural river habitats and fish assemblages successfully (Hendry et al., 2003; Jungwirth et al., 2003; Schnell and Pulg, 2007; Muhar et al., 2008).

Undoubtedly, local habitat improvements, such as the restoration of spawning grounds, do not address the root causes of the habitat degradation but address the symptoms, which is criticised by Hendry et al. (2003). However, for many regulated rivers in densely populated and intensively used areas of Europe, largescale restorations including drainage and changes in land use are not feasible in the short and medium term. For these rivers, local works, such as the restoration of spawning grounds, can be a tool to stabilize and to increase stocks of brown trout and probably other lithophilic species. Pedersen et al. (2009) came to similar conclusions after monitoring 32 restored spawning sites of anadromous brown trout with electrofishing in Denmark. The restoration of spawning grounds can be considered as a helpful tool to improve the environmental status of rivers according to the European Union's Water Frame Directive, especially but not exclusively in rivers categorized as 'heavily modified water bodies'.

The restoration of spawning areas can be especially effective for population management if recruitment is the limiting factor for the population size. As a management tool, it has to be applied with regard to other habitat features. Einum et al. (2008) demonstrated for the size of a modeled Atlantic salmon population that the increase of spawning grounds can be ineffective or even decrease population size if there is a low abundance of fry and parr habitat as well as a high-density-dependent mortality.

In contrast to other fish management practices, for example, stocking, which is still widely spread in parts of Central Europe (Arlinghaus, 2006; Siemens et al., 2008) and North America (Halvorsen, 2010), the restoration of spawning grounds can contribute to conserve wild stocks and genetic resources as well as to enhance natural reproduction and selection. Spawners can behave naturally on the restored sites (choice of mate, 'redd' construction), hatching fish can adapt to local conditions and also other lithophilic species than brown trout can benefit, many of which are red listed. Besides, the costs for restoration of spawning grounds are low compared with usual stocking programs (Siemens et al., 2008), even if it is considered that the restoration has a limited period of suitability and may have to be repeated after some years.

# 8 How to improve habitat quality with anthropogenic manipulations

## 8.1 Impact of altered stream substratum composition - why monitor interstitial water conditions?

The studies of this thesis show that the riverbed is an important key habitat with a complex structure which has a highly variable quality over space and time. Direct and indirect effects of different stream substratum compositions had strong impacts on the reproduction success of salmonids that also remained after the fry left the hyporheic zone. Consequently, salmonid populations correlate to the substratum quality.

The result of this study suggest that the success of substratum restoration needs to consider both physical and chemical properties of the restored site. Previous studies primarily focused on differences in egg and fry development as a result of variations in water chemistry within the substratum and not on the direct effects of different sediment textures. In some cases, the study design did not allow a clear separation between physical effects and effects of different water conditions like low oxygen supply (e.g. Rubin, 1998; Witzel and MacCrimmon, 1983; Rubin, 1998; Malcolm et al., 2003; Heywood and Walling, 2007; Pander et al., 2009).

The physical effects of different substratum compositions are as evolutionarily important on reproductive success as the indirect effects of the substratum (see chapter 3). It was shown under standardized water conditions that accumulated small grain sizes result in a physical barrier to emerging fry. Moreover, different substratum compositions result in different post-emergence effects (survival rate and growth) of salmonids (e.g. brown trout and Danube salmon). The timing of emergence, survival of emerged fry and growth of salmonids after emergence are significantly influenced by different substratum compositions. The negative effects of fine sediment on the reproduction success of salmonids were confirmed (e.g. Soulsby et al., 2001b; Julien and Bergeron, 2006). However a shift to later emergence due to increasing fine sediment levels, which was observed in other species (Hausle and Coble, 1976, Rubin, 1998), was not detected in this experiment. The emergences of both species were highly synchronized relatively quick events with high emergence peaks. Previous studies explained this trend by a rapid change in the phototactic direction that enhances the individual survival rate in case of predation pressure and limited territory (Carey and Noakes, 1981; Brännäs, 1995; Rubin, 1998; Skoglund et al., 2011). In this case, the impact of the substratum composition on the emergence period is crucial for post-emergence survival and growth. The experimental methods (observation of post-emergence growth and survival in separate incubation boxes for each treatment) imitated the situation of limited territory in nature; hence, low growth rates and high mortality rates in treatments with coarser gravel (and also within the reference treatment without substratum) can be explained by the repression of growth after emergence due to limited space (Armstrong and Nislow, 2006). Additionally, the selective pressure of fine sediment induces higher growth rates after emergence (only the strongest and largest fish are able to survive). In contrast, Danube salmon fry profited from incubation in the coarsest gravel size, most likely because they develop faster for a shorter time-period in the sediment, as compared to brown trout fry. A lower amount of energy is lost during the migration through the sediment. Different life history strategies (fall-spawning versus spring-spawning) differ in emergence patterns; these differences have to be considered in conservation management separately.

Beside direct effects, the sediment texture has an impact on interstitial water conditions (e.g. reduced water exchange within the hyporheic zone) which has an impact on egg and larvae development within the sediment. Use of the ‘egg sandwich’ (ES) provided observations of great heterogeneity in egg hatching success within the river sediment, especially on a small spatial scale. The ES, which directly link biotic factors (salmonid hatching success) and abiotic conditions (e.g. oxygen, temperature, specific conductance, pH, ammonium, nitrate and nitrite concentrations) offers detailed information of effects at a small spatial scale (see chapter 4). Advantages of using the ES are that the river bed need only be minimally disturbed (imitating the building of a ‘redd’ by a spawner), a single incubating egg can be separated in each chamber to minimize the risk of fungus infection, and the ability to directly measure interstitial water conditions. No previously developed system for hatching salmonid eggs can provide all of these attributes simultaneously (Vibert, 1949; Whitlock, 1979; Harris, 1973; MacCrimmon et al., 1989; Pauwels and Haines, 1994; Rubin, 1995; Harris, 1973; Pauwels and Haines, 1994; Rubin, 1995; Donaghy and Verspoor, 2000; Bernier-Bourgault et al., 2005; Dumas and Marty, 2006).

Using the ES, the quality of stream substratum was shown to be a potentially limiting factor for salmonid reproduction under natural conditions (see Chapter 5). Greatly varied interstitial water conditions results in highly variable hatching success, in both rivers as well as individual ESs. No linear correlations between physicochemical water conditions and salmonid hatching success were detected. However, the importance of the interstitial water exchange in the hyporheic zone was approved and the importance of stream substratum as a functional habitat was confirmed (Geist and Auerswald, 2007).

Even though water quality in open water of the rivers was similar to hatchery conditions, altered interstitial water conditions induced a shift from a unimodal distribution (control groups) to a bimodal distribution (sediment exposures) of hatching success within the sediment. Very high or very low hatching rates were most frequently detected.

Local environmental factors caused high variations in hatching success, observed at different time-periods of egg development. Furthermore, the comparisons of discrimination functions, which were evaluated at different spatial scales, suggested that crucial factors at river-scale (e.g. nitrate-concentration) are overlaid by strong river-specific effects (e.g. specific conductance). Different ecological patches created by different systems, e.g. ground water and surface water, characterize a very heterogenous ecosystem with complex interactions depending on flow discharge (Naiman et al., 1988; Boulton et al., 1998). This fact combined with cross-interactions and interrelated effects of the physicochemical parameters may have caused the different results from discriminant analysis at different spatial scales. Hence, every river suggests having a typical hydraulic exchange within the stream substratum. Even though oxygen concentration was not a good direct indicator for hatching success in this study unlike previous studies (Wickett, 1954, Rubin and Glimsäter, 1996), it was a good indicator for hydraulic exchange. However, the exchange of interstitial water with surface water and minimum oxygen supply are river specific (Meyer, 2003, Louhi et al., 2008). Sheltered regions probably induce anoxic pockets even though well-oxygenated water flows through the substratum (Boulton et al., 1998). It should also be noted that, even though the interstitial water had overall good oxygen supply, a silt layer around the individual egg could indicate oxygen deficits at the micro-scale and hence affect the egg development (Greig et al., 2005; Levasseur et al., 2006).

Highly variable hatching rates at the micro-scale indicates highly variable interstitial water conditions with a strong trend of decreasing hatching success in deeper sediment zones. Accumulations of fine sediment in the surface interstitial zone and high-dissolved oxygen from ground water could cause a reversal of this trend (Chapman, 1988; Lisle, 1989; Malcolm et al., 2003). The variability of physicochemical conditions in the interstitial water is crucial over space as well as over time. The exposure of ESs within the sediment imitates conditions in ‘redds’ and the procedure of digging the ESs cleans and loosen the substratum similar to 'redd' building by spawners (Chapman, 1988; Kondolf et al., 1993). The degradation of stream substratum quality by fine sediment deposition was observed during egg development. Depth-specific differences of interstitial water conditions are typically higher at the end of the egg exposure than at the beginning (Grost et al., 1991; Soulsby, 2001a; Julien and Bergeron, 2006). Incubating salmonids possibly compensate for oxygen-deficits at the end of development with premature initial hatch and early emergence (Rubin and Gilmsäter, 1996). Consequently, stream substratum composition has indirect

effects on the timing as well as on the periode of emergence. Both are important factors for the growth and the survival of juveniles (Einum and Lewis, 2000; Skoglund et al., 2012).
Knowledge about the range of annual environmental conditions (e.g. peaks of high or low stream discharge or sediment transport) of individual rivers is important for conservation management, because these factors affect the quality of substratum at potential spawning grounds (Lisle and Lewis, 1992; Kondolf, 2000; Meyer, 2003). The results presented in chapter 5 show that the ecological functionality of the sediment differs at spatial and temporal scales, even within the same location of a river. Therefore, monitoring of interstitial water conditions within potential salmonid spawning habitat together with consideration of the possible physical effects from different sediment texture are necessary to enhance the success of river restoration.

## 8.2 Implications for river management - small versus large scale restoration efforts

Riverbed restoration is an anthropogenic disturbance to the stream channel and should be considered critically and holistically. A commonly used method, which is also widely recommended by public authorities and fisheries associations alike, is the cleaning of the stream substratum through excavation of the sediment (Pulg, 2007; Hanfland, 2010). The comprehensive assessment the excavation of a highly degraded gravel bank showed that effects on the restored site as well as on downstream habitats are important for the evaluation of restoration success (see chapter 6).

Positive effects of the excavation method on oxygen-sensitive species were observed within the restorations site. These effects resulted from the significant reduction of fine sediment within the substratum and the remobilization of colmated substratum. Water exchange was improved and the heterogeneity of interstitial water conditions between different sediment depths decreased. Observed natural reduction of fine sediment within the restored area during the study period was likely caused by higher mobility of formerly colmated stream substratum. Self-cleansing processes were probably activated by the restoration measure. Surprisingly, typical accumulations of fine sediment during salmonid egg incubation were not observed (Kondolf, 2000a). Overall, the water chemistry of the hyporheic zone, in particular oxygen as well as nitrite concentrations, was more similar to the conditions in open water after the excavation. The higher numbers and densities of macrozoobenthos species at all analyzed sediment depths (50 mm, 100 mm and 150 mm) and the strong increase of brown trout hatching success within the restoration site approved the expected enhancement of habitat functionality even in deeper sediment zones (e.g. Coleman and Hynes, 1970; Greig et al., 2007; Heywood and Walling, 2007). The increased hatching rates of brown trout eggs likely indicate higher survival of subsequent fry stages (Hausle and Coble, 1976; Levasseur et al., 2006; Jensen et al., 2009; Sternecker and Geist, 2010).

Even though the macroinvertebrate species number and density dropped immediately after the excavation, the quick recovery of macroinvertebrate abundance suggests an improvement of the habitat quality. Changes in macroinvertebrate communities as a result in the reduction of fine sediment were similar to those seen by Wood and Armitage (1997), Muotka et al. (2002), Rabení et al. (2005) and Cover et al. (2008). It is crucial that the beneficial impact on preferred salmonid prey is considered in restoration management, because the food supply for salmonids is manipulated directly by this kind of restoration technique.

The overall success of the restoration at the excavated area should not hide the fact that the sedimentation rate was increased downstream of the restoration site. These negative effects demonstrate that benefits from small-scale substratum restoration are restricted to the restored area. The strongest impact, i.e. extremely high siltation rates, was located close to the excavation area. Large quantities of fine particles were expected to affect functional habitat and consequently freshwater communities (Bilotta and Brazier, 2008). Even though the high amount of fine sediment caused by the excavation downstream of the restoration area was spatially restricted, it is likely that sediments were remobilized further downstream at a later date. Restoration sites should be selected carefully to avoid the destruction of functional river habitat downstream of the restored area.

The pronounced positive effects are the great advantages of smallscale restorations, like the ‘excavation method’. They have short lag-times, which can easily be adapted to the target species (e.g. lithophilic fish, macroinvertebrates). It is crucial that the mobilized amount of fine sediment does not exceed the natural capacity of the river ecosystem (e.g. Merz and Setka, 2004). The effectiveness of substratum restoration in the long-run depends on the amount of transported fine sediment in river catchments as well as on the regional flow regime (Zeh and Dönni, 1994; Kondolf, 2000b; Rubin et al., 2004; Meyer et al., 2008; Sear and DeVries, 2008). In manipulated rivers, the redegradation of functional stream substratum after restoration is an often-disregarded problem. In particular, even though brown trout catch increased remarkably after improving spawning habitat with excavation and / or addition of gravel in a case study, the extrapolation of degradation data predicts non-functional sediment conditions after 5 to 6 years (see chapter 7).

It is ecologically significant, that the reproduction success of the target species (brown trout) increased along with that of grayling (*Thymallus thymallus*), chub (*Squalius cephalus*), dace (*Leuciscus leuciscus*) and bullhead (*Cottus gobio*) in the restored sites. The outstanding acceptance of the restored areas indicates that the number of suitable spawning grounds was a limiting factor for the reproduction of gravel-spawning fish. The low quality of spawning habitat may have acted as a bottleneck for population size; this bottleneck was temporarily alleviated by selective stream substratum restoration, as evident from the substantially increase of brown trout juveniles.

The accumulation of fines clearly degraded sediment functionality, as represented by significantly decreased oxygen concentration in the interstitial zone. Even though highly suitable conditions (survival success >50 %) were detected for the first two years, the following two years exhibited only moderate suitable conditions (survival success <50 %) in the restored areas. Restored sites were assumed to revert back to non-functional spawning habitat after 5 years ('excavation method') and 6 years ('addition of gravel'). Due to the higher costs of gravel augmentation, the 'excavation method' can still be recommended as a low cost but very effective restoration technique.

Reduction of fine sediment inputs from the watershed and creation of artificial gravel banks are often used to restore high quality river sediment (Wood, 1997; Shackle et al., 1999; Hendry et al., 2003; Rubin et al., 2004; Owens et al., 2005; Heywood and Walling, 2007). Due to the ephemeral nature of the restoration at spawning grounds, long-term monitoring of substratum quality and interstitial water conditions is necessary to evaluate degradation processes. Previous studies also demonstrated the success of stream substratum restoration for lithophilic fish, but the endurance of habitat improvements varied by river (e.g. Sear and DeVries, 2008; Barlaup et al., 2008; Pedersen et al., 2009).

Besides fish stocking, the restoration of spawning habitat is a suitable method for conservation of lithophilic fishes modified rivers (e.g. Hendry et al., 2003; Jungwirth et al., 2003; Muhar et al., 2008; Pedersen et al., 2009). The original causes for sediment degradation (e.g. colmation caused by high fine sediment content in the river system) and subsequently, the deficit of spawning habitats could not be eliminated by small-scale restoration methods. Most likely, the problem is only shifted downstream of the restored area. A more sustainable way to provide the functionality of the riverbed in freshwater ecosystems is to focus river management plans on the reduction fine sediment inputs into river systems (e.g. change of land use), especially in upper catchment areas. Additionally, the re-establishment of natural flow regimes by e.g. removing dams is crucial to provide a dynamic ecosystem (Jungwirth et al., 2003; Hendry et al., 2003; Opperman et al., 2005; Greig et al., 2007). A holistic approach is rarely considered in densely populated and intensively used areas like Central Europe, because other factors like flood prevention are of higher priority. The creation of a number of essential key habitats for reproduction, juvenile and adult phases, and connectivity among these habitats (e.g. river continuum concept by Vannote et al., 1980), may increase the abundance of endangered species (e.g. Danube salmon, grayling) to population sizes that are stable, even in years with low reproduction success (Cowley, 2008).

## 8.3 Outlook

The high variability of interstitial water conditions at different time and spatial scales have determining consequences for future research on the functionality of stream substratum habitat.

The evaluation of habitat conditions at different salmonid reproduction stages was confirmed to be crucial for a successful improvement of stream substratum as key habitat for reproduction. The results of this thesis support that the substratum has direct and indirect effects on the egg to fry development as well as on the emergence of fry. For an increase of natural reproduction in anthropogenic altered rivers, the acceptance of high quality substratum by spawners has to be addressed in a comprehensive approach. Studies under standardized conditions have to be conducted to analyze the preferences of spawning salmonids. In previous studies it was shown that e.g. salmons return to their breeding spots (e.g. Hasler et al., 1978; Stabell, 1984; Dittman and Quinn, 1996). Characteristics of salmonid spawning areas such as gravel sizes and flow velocities are often described (e.g. Crisp and Carling, 1989; Grost et al., 1991; Ingendahl, 2001; Mull and Wilzbach, 2007; Louhi et al., 2008). Nevertheless, it is important to know if salmonids, especially resident species like the Danube salmon or the non-migrating brown trout, return to their individual spawning territory or if they were seeking for high quality spawning habitat at the beginning of every spawning season. Heading for fixed spawning areas would mean that spawners would consequently hazard low reproduction habitat quality in case of stream substratum degradation. Genetic studies of spawners and their progeny within their ‘redds’ could highlight the spawning site selection and evolutionary effects of spawning ground acceptances of spawners. Then, recommendations about the restoration of degraded spawning grounds at established sites or the building of spawning grounds at more suitable sites are possible.

High variability of interstitial water conditions at river-scale as well as individual degradations of substratum at different study sites in time was observed. Subsequently, it should be tested under standardized conditions, if high numbers of small restoration areas are more efficient in habitat restoration than solitary largescale areas. Different flow discharges have probably different effects at the structure and water exchange of the substratum. Egg- and larval development may profit of intermediately compacted stream substratum during high flow discharge whereas loose substratum is required during low water periods. Information about river specific interstitial water conditions combined with the individual water regime has to be considered in research to give recommendation about the required variability of smallscale stream substratum restoration for management plans.

The variability of results using the DCA at different time and spatial scales showed that physicochemical factors are not universally usable indicators. The variation of interstitial water conditions during the egg development suggests that physicochemical factors are characterized by being unstable and more stable indicators are necessary for a comprehensive habitat quality evaluation of the interstitial zone. Consequently, biotic factors like macrozoobenthos and microbes have to be considered as holistic indicators for habitat quality. Especially microbes suggest being reliable, because they are not only affected by interstitial water conditions, but they also significantly generate interstitial water conditions (Mueller et al. 2012).

Another important question is whether the results of this thesis are transferable to other lithophic fishes or not. In one of these studies an increase of the reproduction success of grayling, chub, dace and bullhead was observed after salmonid spawning ground restoration (see chapter 7). Positive side effects of habitat restoration, not only for the target species, on non-commercial lithophilic fishes are crucial for many other endangered species (e.g. grayling, freshwater pearl mussel). Long time effects of habitat improvements could increase the freshwater biodiversity significantly, which would be an extraordinary effect of within-river restoration. The ES could be used to study the indirect effects of stream substratum composition on other species like the freshwater pearl mussel or grayling. Additionally, different effects on the reproduction success of species, which are burying eggs at different sediment depths, should be analyzed and compared.

Overall, the effects of habitat degradation on the stream substratum biodiversity are crucial for the river ecosystem. It is important to know the linkages between the interstitial water and the open water to preserve freshwater ecosystems.

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