Universität Koblenz-Landau
Campus Landau
Institut für Umweltwissenschaften

Biologische Bewertung der Grundwasserökosysteme in Deutschland: Untersuchungen zum Auftreten der Fauna auf unterschiedlichen räumlichen Skalen

Dissertation zur Erlangung des akademischen Grades eines Doktors der Naturwissenschaften,
Fachbereich 7: Natur- und Umweltwissenschaften
Universität Koblenz-Landau
Campus Landau

vorgelegt von:
Diplom-Biologin Heide Stein

Tag der mündlichen Prüfung:

1. Berichterstatter: PD Dr. H. J. Hahn, Univ. Koblenz-Landau, Campus Landau

2. Berichterstatter: Prof. Dr. K. Schwenk, Univ. Koblenz-Landau, Campus Landau

Inhaltsverzeichnis

1 Zusammenfassung

Die Beeinflussung des Grundwassers durch Schadstoffeintrag und Raubbau beeinträchtigt langfristig nicht nur seine Qualität, sondern auch seine Funktionsfähigkeit. Die biologische Aktivität der Grundwasserorganismen leistet einen wichtigen Beitrag zur Reinigung und Speicherung des unterirdischen Wassers, wodurch die Qualität stark vom gesunden Zustand dieses Ökosystems und seiner Biozönosen abhängt. Der nachhaltige Grundwasserschutz erfordert demnach ein Bewertungssystem, das Aussagen über den Ökosystemzustand zulässt. Folglich sollten, zusätzlich zu physikochemischen Analysen, auch ökologische Kriterien bei der Grundwasserbewertung berücksichtigt werden. Dies schließt sowohl die Erfassung subterraner aquatischer Invertebratengemeinschaften (= Stygofauna) ein, deren oberirdische Vertreter schon seit Jahrzehnten erfolgreich bei der Bewertung von Oberflächengewässern eingesetzt werden, als auch die Erfassung mikrobiologischer Parameter.

Um die Anwendbarkeit und Eignung faunistischer und mikrobiologischer Organismen für eine qualitative Grundwasserbewertung zu überprüfen, wurden stygofaunale und bakterielle Verbreitungsmuster unterschiedlicher Grundwasservorkommen Deutschlands untersucht. Die Datensätze umfassen insgesamt 1423 Proben, aus 542 Grundwassermessstellen, die zwischen 2002 und 2009 gesammelt wurden.

Der Schwerpunkt der Untersuchungen lag in der Überprüfung des Indikatorpotenzials von Invertebratengemeinschaften im Grundwasser gegenüber Umwelteinflüssen und insbesondere gegenüber Oberflächenwasser-Grundwasser-Interaktionen. Im Fokus stand ebenfalls die Analyse stygofaunaler Verbreitungsmuster unter Berücksichtigung unterschiedlicher räumlicher Skalen, als Basis und Referenz für ein faunistisches Bewertungssystem für Grundwasserlebensräume. Vor diesem Hintergrund wurden – erstmals für Deutschland – umfassende, methodisch vergleichbare Untersuchungen der Grundwasserfauna durchgeführt. Die Auswahl der Untersuchungsgebiete konzentrierte sich auf Grundwassersysteme, die sich biogeografisch, physiogeografisch und geomorphologisch voneinander unterscheiden. Dieser Untersuchungsansatz folgt dem Modell eines hierarchischen Gliederungssystems für Grundwasserhabitate und Gemeinschaften nach HAHN (2009). Das Modell berücksichtigt drei räumliche Ebenen, die als prägend für die rezente Invertebratenverbreitung im Grundwasser angesehen werden: die *biogeografische*, die *regionale* und die *lokale* Ebene.

Die Ergebnisse zeigen deutlich den Einfluss aller drei Ebenen auf die faunistische Zusammensetzung im Grundwasser. Demzufolge bildet das hierarchische Drei-Ebenen-Modell eine geeignete Grundlage für ein faunistisches Grundwasserbewertungssystem.

Die stygofaunalen Verbreitungsmuster auf *regionaler* und *lokaler* Ebene, wurden exemplarisch anhand unterschiedlicher Grundwassersysteme im Umkreis von Köln (Erftgebiet) und Ulm (Alb-Donau-Kreis) untersucht. Im Fokus stand die Erfassung hydrologischer Oberflächenwasser-Grundwasser-Interaktionen unter dem Einfluss von Landnutzung und der hydraulischen Konnektivität von Grundwasserleitern.

Anhand von Faunadaten aus quartären Lockergesteinsleitern im Erftgebiet konnte die Sensibilitiät der Grundwasserfauna gegenüber Landnutzungs-effekten aufgezeigt werden. Korrelationsanalysen ergaben positive Zusammenhänge von organischem Material (geschätzter Detritus, TOC) und Nitrat mit der faunistischen Abundanz, Artenzahl und Diversität sowie dem Anteil grundwasserfremder Arten (= Nicht-Stygobionte). Diese Korrelationen spiegeln im Wesentlichen einen erhöhten hydrologischen Oberflächeneinfluss wider. Dabei scheint Nitrat keinen direkten Effekt auf die Grundwassertiere auszuüben, sondern ist vielmehr als eine Folge des erhöhten

Oberflächeneintrags durch intensive Landnutzung anzusehen, mit dem sowohl Nitrat als auch Nahrung (organisches Material) und Nicht-Stygobionte ins Grundwasser gelangen. In regionaler Hinsicht ergab der Vergleich der Grundwassergemeinschaften, die in zwei unterschiedlichen Grundwassersystemen (Erft-, Rurscholle) gesammelt wurden, keine signifikanten Besiedlungsunterschiede. Folglich scheint zwischen der Erft- und Rurscholle ein hydrologischer Austausch zu bestehenden.

Die Grundwasseruntersuchungen im Alb-Donau-Kreis berücksichtigen ebenfalls lokale und regionale Verhältnisse. Die Invertebratenbesiedlung wurde unter dem Aspekt des Naturraums (Lonetal-Flächenalb, Donauried), des Grundwasserleitertyps (Karst-, Lockergestein), der hydrogeologischen Bezugseinheit (Kalksteine des Oberen Jura, Schotter und Moränen des Alpenvorlandes) sowie kleinräumiger Verhältnisse im Bereich einzelner Messstellen betrachtet. Dabei reflektierte die Fauna die Stärke des hydrologischen Austauschs auf unterschiedlichen räumlichen Skalen und zeigte auch die Überlagerung regionaler und lokaler Effekte an. Die Gemeinschaften wiesen keine Unterschiede hinsichtlich des Grundwasserleitertyps und der Hydrogeologie auf, was auf die hohe Konnektivität der untersuchten Grundwassersysteme zurückgeführt wird. Stattdessen zeigten sich geringe naturräumliche Besiedlungsunterschiede, die als Effekte der Landnutzung, abschirmender Deckschichten sowie auf ein unterschiedliches Grundwasseralter, durch das Gradienten im Nahrungsangebot entstehen, angesehen werden. Einen negativen Effekt auf die Gemeinschaften (Diversität, Abundanz) hatte auch die Messstellentiefe, da mit zunehmender Tiefe das organische Material abnimmt.

Die Untersuchung biogeografischer Besiedlungsmuster der Grundwasserfauna erfolgte anhand von Daten, die in verschiedenen biogeografischen Regionen, Naturräumen und Grundwasserleitertypen erhoben wurden. Die Analysen ergaben signifikante biogeografische Unterschiede zwischen den subterranen

Gemeinschaften, die deutlich von bestehenden Gliederungssystemen für oberirdische Landschaften oder Fließgewässerzönosen abweichen. Die größten Abweichungen zwischen den stygofaunalen Verbreitungsmustern und den oberirdischen Gliederungssystemen wurden vor allem in den eiszeitlich überprägten Gebieten Nord- und Süddeutschlands sowie in den Vorgebirgsregionen der zentralen Mittelgebirge beobachtet. Anhand der erfassten Daten konnten vier Stygoregionen definiert werden, die deutlich unterschiedliche Faunengemeinschaften aufweisen: 1) das *„Nördliche Tiefland“*, 2) die *„Zentralen Mittelgebirge“*, 3) die *„Südwestlichen Mittelgebirge“* sowie 4) die *„Südlichen Mittelgebirge und die Alpen“*.

Die Untersuchung mikrobieller Gemeinschaften orientierte sich im Gegensatz zum faunistischen Untersuchungsansatz eher an hydrochemischen und hydrogeologischen Verhältnissen, da diese als prägend für die Besiedlung subterraner Mikroorganismen angesehen werden. Grundlage für die Analysen waren die Gliederung von KUNKEL *et al.* (2004), nach der die Grundwassersysteme in Deutschland in 17 hydrogeologische Bezugseinheiten (HBE) gegliedert sind, sowie lokale hydrochemische Verhältnisse. Die Untersuchungen der Bakteriengemeinschaften wurden ausschließlich im Erftgebiet und Alb-Donau-Kreis durchgeführt.

Im Gegensatz zur Fauna, zeigten die mikrobiologischen Untersuchungen im Erftgebiet signifikante Unterschiede der mittleren bakteriellen Abundanz (BA) der Erft- und Rurscholle. Die mittlere BA der Rurscholle war dabei doppelt so hoch wie im Grundwasser der Erftscholle, was aber vor allem auf zwei organisch belastete Messstellen zurückgeführt werden konnte. Die bakterielle Diversität der Bruchschollen unterschied sich dagegen nicht. Im Vergleich zu oligotrophen Grundwassersystemen, weisen die hohe BA und Diversität im Grundwasser des Erftgebiets auf Effekte der Landnutzung hin. Dies zeigen auch positive Korrelationen zwischen den Bakteriengemeinschaften und Nitrat und Phosphat. Dabei können vor allem die Korrelationen mit Nitrat als indirekte Korrelationen von Oberflächeneinfluss durch z. B. Landwirtschaft betrachtet werden. Zwischen den bakteriellen Gemeinschaften und der Stygofauna wurden keine interpretierbaren Korrelationen gefunden. Im Allgemeinen ergaben die faunistischen und mikrobiologischen Daten aber ein ähnliches Bild.

Die Bakteriengemeinschaften im Grundwasser des Alb-Donau-Kreises zeigten keine Unterschiede hinsichtlich des Grundwasserleitertyps, des Naturraums und der hydrogeologischen Bezugseinheit (HBE). Dieses Ergebnis spiegelt die Zugehörigkeit aller Messstellen zu einem Grundwassersystem wider. Unterschiede gab es jedoch in der Besiedlung einzelner Messstellen, wobei sich die Bakteriengemeinschaften der nah beieinander stehenden Messstellen (*lokal verteilt*) insgesamt ähnlicher waren, als Gemeinschaften aus flächig verteilten Messstellen (*regional verteilt*). Die bakterielle Diversität im Alb-Donau-Kreis war typisch für oligotrophe, oligoalimonische Grundwässer. Demzufolge scheint sich die Diversität als Indikator für anthropogene Belastungen im Grundwasser zu eignen. Generell korrelierten die Bakteriengemeinschaften kaum mit der Fauna und Abiotik. Positive Zusammenhänge wurden zwischen der bakteriellen Kohlenstoffproduktion (BCP) und Ammonium, Gesamtkohlenstoff, Sauerstoff und der Temperatur gefunden. Mit der Fauna korrelierten, wie auch im Erftgebiet, bestimmte Bakteriengruppen mit einzelnen Invertebratenarten. Diese Zusammenhänge konnten aber im Rahmen der Untersuchungen nicht geklärt werden.

Insgesamt leisten diese Untersuchungen einen wichtigen Beitrag für die Entwicklung eines ökologischen Grundwasser-Erfassungsprogramms, das auch die faunistische und mikrobiologische Bewertung integriert. Die Ergebnisse zeigen die generelle Anwendbarkeit und Eignung der Stygofauna und der Bakteriengemeinschaften für die qualitative Bewertung von Grundwasser-ökosystemen.

Die faunistische Zusammensetzung bestätigte, dass das hierarchische Drei-Ebenen-Modell als Grundlage für ein faunistisches Grundwasserbewertungs-system geeignet ist. Insbesondere verdeutlichen die Untersuchungen das gute Indikatorpotenzial stygofaunaler Grundwassergemeinschaften hinsichtlich Oberflächenwasser-Grundwasser-Interaktionen. Weiterhin ist die Definition von Stygoregionen ein entscheidender Schritt für die Entwicklung eines eigenständigen Referenzsystems für Grundwasserlebensräume. Die definierten Stygoregionen gilt es in Zukunft zu überprüfen und zu ergänzen. Die im Erftgebiet und Alb-Donau-Kreis erhobenen Daten können als regionale Referenzen bei zukünftigen Untersuchungen genutzt werden, um eventuelle Abweichungen vom erfassten Zustand zu entdecken.

Hinsichtlich der mikrobiologischen Kriterien scheinen vor allem die bakterielle Diversität und Abundanz geeignete Indikatorgrößen für die Grundwasser-bewertung zu sein. Hier ist aber noch zu klären welche taxonomische Einheit (Arten, funktionelle Gruppen) angemessen ist, um Grundwasser zu bewerten. Demgegenüber scheint bei der Stygofauna das Artniveau und die Einteilung in Stygobionte und Nicht-Stygobionte ausreichend für eine Bewertung zu sein.

2 Einleitung

Der Schutz des Grundwassers ist in Anbetracht des steigenden Wasserverbrauchs durch den Menschen, z. B. als Trinkwasser, Kühlwasser oder zur Bewässerung, wichtiger denn je. Zwei Drittel der gesamten Trinkwasserversorgung werden in Deutschland aus Grundwasser gewonnen (PREUß & SCHMINKE, 2004; AVRAMOV *et al.*, 2010). Gleichzeitig sind die wichtigsten Trinkwasserressourcen jedoch durch Schadstoffeintrag und die Entnahme großer Mengen Wasser langfristig bedroht (DANIELOPOL *et al.*, 2003).

Grundwasserökosysteme mitsamt ihren mikrobiellen und faunistischen Gemeinschaften leisten wichtige Ökosystemdienstleistungen (TOMLINSON *et al.*, 2007; AVRAMOV *et al.*, 2010). Zu den bedeutendsten zählen die Reinigung und Speicherung sauberen Grundwassers, die biologische Eliminierung von Schadstoffen und pathogenen Mikroorganismen sowie die Versorgung grundwasserabhängiger oberirdischer Ökosysteme (HERMAN *et al.*, 2001; BOULTON, 2005; HUMPHREYS, 2006). Die Grundwasserqualität hängt demnach stark vom gesunden Zustand, d.h. der funktionellen und strukturellen Intaktheit dieses Ökosystems ab (GIBERT & DEHARVENG 2002; DANIELOPOL *et al.*, 2004; HAHN, 2006; BOULTON *et al.*, 2008).

Vor diesem Hintergrund wächst der Druck auf Entscheidungsträger in Politik und Wasserwirtschaft, biologische Kriterien in die Qualitätsprüfung von Grundwasser zu integrieren (DANIELOPOL *et al.*, 2003; 2004). Im Gegensatz zu Oberflächengewässern existiert für Grundwassersysteme bis heute kein ökologisch orientiertes Erfassung- und Bewertungssystem (DANIELOPOL *et al.*, 2008; KORBEL & HOSE, 2011). Der aktuelle europäische Grundwasserschutz ist auf physikochemische und quantitative sowie wenige mikrobiologische Kriterien beschränkt (EG-WRRL, 2000; EG-GWRL, 2006). Ein umfassender Grundwasserschutz erfordert aber zusätzlich die Berücksichtigung biologischer Kriterien, die bekanntermaßen Störungen über einen längeren Zeitraum integrieren (BALKE & GRIEBLER, 2003; HOSE, 2005). Gestresste Ökosysteme weisen z. B. Veränderungen in ihrer Strukturqualität und Funktionalität auf, was eine veränderte Zusammensetzung der Biozönosen bewirkt (FRÄNZLE, 2003; DANIELOPOL *et al.*, 2008; AVRAMOV *et al.*, 2010). Aktuell besteht somit ein großer Bedarf an geeigneten Kriterien, standardisierten Methoden und Referenzen, um Grundwasserlebensräume und ihre Gemeinschaften ökologisch zu erfassen und zu bewerten. Biologische Kriterien, die sich im Monitoring von Oberflächengewässern bewährt haben, können nicht direkt auf das Grundwasser übertragen werden, da große Unterschiede zwischen diesen Systemen bestehen (DEHARVENG *et al.*, 2009). So zeigt die Grundwasserfauna vor allem eine starke Anpassung an sauerstoff- und nährstofflimitierte Bedingungen, wie sie, im Gegensatz zu den meisten Oberflächengewässern (HAHN, 2009), häufig in oligotrophen Grundwassersystemen auftreten.

Das Grundwasser wird durch hydrologische Wechselwirkungen mit Oberflächenwasser (Gewässer, infiltrierende Niederschläge) stark beeinflusst. Die Stärke der Wechselwirkungen hängt dabei von den hydrogeologischen Verhältnissen ab, die sowohl räumlich als auch zeitlich stark variieren können (BRUNKE & GONSER, 1999; DATRY *et al.*, 2005). Dabei stellt der Kontakt mit Oberflächenwasser und insbesondere ein vermehrter allochthoner Stoffeintrag stets ein Risiko für die Grundwasserqualität dar (BORK *et al.*, 2009).

Die Verbreitung und Zusammensetzung der Grundwasserfauna ist vor allem von der Verfügbarkeit von Sauerstoff und Nahrung, in Form von partikulärem organischem Material (POM, Detritus), abhängig (BRUNKE & GONSER, 1999; MÖSSLACHER, 2003; DATRY *et al.*, 2005). Die Menge an Sauerstoff und Detritus sowie die Anteile echter Grundwasserarten (= Stygobionte) und Arten, die vorwiegend in Oberflächengewässern oder im Hyporheos vorkommen (= Nicht-Stygobionte), hängen oft direkt mit der Stärke des hydrologischen Oberflächenaustauschs zusammen (MALARD, *et al.*, 1996; HAHN, 2006; HUMPHREYS, 2006). Auch die Zusammensetzung mikrobieller Gemeinschaften verschiebt sich bei einem verstärkten hydrologischen Oberflächeneinfluss von Gruppen, die an grundwassertypische oligotrophe Bedingungen angepasst sind, zu Gruppen, die in Böden und Oberflächengewässern auftreten (GRIEBLER & LUEDERS, 2009). Somit lassen die Grundwassergemeinschaften Rückschlüsse auf veränderte hydrologische Bedingungen durch Eingriffe wie z. B. Grundwasserentnahmen zu (DATRY *et al.*, 2005; SCHMIDT *et al.*, 2007; BORK *et al.*, 2009; GRIEBLER & LUEDERS, 2009, STEUBE *et al.*, 2009). Ebenso haben verschiedene Studien gezeigt, dass sich die Besiedlungsmuster der Grundwasserfauna grundsätzlich zur Abschätzung der Richtung und Stärke der Oberflächenwasser-Grundwasser-Interaktionen und damit zur Abschätzung der Vulnerabilität des Grundwassers eignen (z. B. MÖSSLACHER, 1998; MATZKE *et al.*, 2005; DANIELOPOL *et al.*, 2008; BERKHOFF *et al.*, 2009; BORK *et al.*, 2009; SCHMIDT & HAHN, 2012). Bakteriengemeinschaften scheinen sich dagegen vor allem für den Nachweis verschiedener Schadstoffe zu eignen, da sie durch erhöhte Stoffumsatzaktivitäten den aktiven biologischen Abbau anzeigen (BRIELMANN *et al.*, 2009; GRIEBLER & LUEDERS, 2009).

Während subterrane, aquatische Mikroorganismen ubiquitär verbreitet sind und vor allem geologische und mineralogische Faktoren die Besiedlungsstruktur prägen (GOLDSCHEIDER *et al.*, 2006; GRIEBLER & LUEDERS, 2009), erfordert die faunistische Grundwasserbewertung die Berücksichtigung biogeografischer, physiogeografischer und geomorphologischer Faktoren (HAHN, 2009). Die Verbreitung der Invertebratenarten ist einerseits von klimatischen und erdgeschichtlichen Bedingungen sowie vom verfügbaren Lückenraum und dem Einzugsgebiet abhängig, andererseits aber auch von kleinräumigen, lokalen Strukturen, die die hydrologischen und trophischen Verhältnisse im Grundwasser prägen (STRAYER *et al.* 1997; MÖSSLACHER, 1998; DUMAS *et al.*, 2001; HAHN & FUCHS, 2009). Da die kleinräumigen Strukturen innerhalb eines Grundwassersystems stark variieren können, muss die faunistische Bewertung von Grundwasserhabitaten auf lokaler Ebene, d.h. auf kleinräumiger Skala (< 1 km^2), erfolgen (HAHN, 2006).

Voraussetzung für die Bewertung auf lokaler Ebene ist jedoch die Definition biogeografischer Referenzen (Stygoregionen) sowie regionaler Referenzen (DANIELOPOL *et al.*, 2003; HAHN & FUCHS, 2009; HAHN, 2009). Dies erfordert eine großräumige Erfassung der Grundwasserfauna. Die meisten Untersuchungen sind jedoch regional beschränkt und eine aktuelle, flächendeckende Erfassung der Grundwasserfauna fehlt bislang in Deutschland.

Den Schwerpunkt der vorliegenden Arbeit bilden Untersuchungen faunistischer Verbreitungsmuster im Grundwasser auf biogeografischer, regionaler und lokaler Ebene. Vor diesem Hintergrund wurden die Invertebratengemeinschaften verschiedener Grundwassersysteme Deutschlands analysiert. Die Proben (1423), aus insgesamt 542 Grundwassermessstellen, wurden zwischen 2002 und 2009 in Schleswig-Holstein, Niedersachsen, Sachsen-Anhalt, Nordrhein-Westfalen, Baden-Württemberg, Rheinland-Pfalz und Bayern gesammelt. Dem Verfahren von FUCHS (2007) folgend, wurden alle Tierproben mit einem Netzsammler direkt vom Boden der Grundwassermessstellen entnommen.

Die Erfassung lokaler und regionaler Muster erfolgte exemplarisch in quartären Lockergesteinsleitern im Umkreis von Köln (Erftgebiet) sowie in fluviatilen Lockergesteins- und Karstleitern bei Ulm (Alb-Donau-Kreis). Im Fokus dieser Untersuchungen stand die Erfassung hydrologischer Oberflächenwasser-Grundwasser-Interaktionen unter dem Einfluss von Landnutzung und der hydraulischen Konnektivität von Grundwassersystemen. Ebenso wurde der Einfluss des Grundwasserleitertyps, des Naturraums und der hydrogeologischen Bezugseinheit auf die Fauna untersucht. Parallel zu den faunistischen Erhebungen wurden im Erftgebiet und Alb-Donau-Kreis die Besiedlungsmuster von Bakteriengemeinschaften untersucht.

Biogeografische Verbreitungsmuster der Grundwasserfauna wurden anhand von Datensätzen analysiert, die in verschiedenen biogeografischen Regionen, Naturräumen und Grundwasserleitertypen erhoben wurden. Die Analysen konzentrierten sich auf die Ermittlung biogeografischer Unterschiede in der Artenzusammensetzung der Grundwasserfauna. Ziel war die Abgrenzung faunistischer Gemeinschaften und die Definition von Stygoregionen. Darüber hinaus sollte überprüft werden, ob sich die subterranen Verbreitungsmuster mit bestehenden Gliederungssystemen für oberirdische Landschaften oder Fließgewässerzönosen decken und welche Faktoren für die Grundwasserfauna prägend sind.

Der überwiegende Teil der Untersuchungen wurde im Rahmen des Forschungsprojektes „Entwicklung biologischer Bewertungsmethoden und –kriterien für Grundwasserökosysteme“ durchgeführt, welches vom Umweltbundesamt (UBA) initiiert und von Januar 2007 bis Dezember 2010 gefördert wurde. Zusätzlich förderte die Länderarbeitsgemeinschaft Wasser (LAWA) zwischen 2009 und 2010 dieses Projekt. Ziel des UBA-/LAWA-Forschungsprojektes war die Entwicklung eines integrativen ökologischen Konzepts für die Erfassung und Bewertung der Grundwasserqualität sowie dem Gesundheitszustand von Grundwasserökosystemen. Dabei lag der Schwerpunkt des Promotionsvorhabens in der Ausarbeitung der faunistischen Fragestellungen, die diese Untersuchungen beinhalteten. Die Projektdurchführung erfolgte in Zusammenarbeit mit der Arbeitsgruppe Grundwasserökologie des Helmholtz Zentrums München, die für die Bearbeitung der mikrobiologischen Aspekte zuständig war. Die vollständige Bearbeitung und Analyse der UBA-/LAWA-Untersuchungen sind dem entsprechenden UBA-Forschungsbericht (GRIEBLER *et al.*, in Druck), zu entnehmen.

Um die Datenbasis hinsichtlich der Analysen biogeografischer Verbreitungsmuster der Grundwasserinvertebraten zu erweitern, wurden die im UBA-

/LAWA-Projekt untersuchten Gebiete mit vergleichbaren Faunadaten weiterer Gebiete kombiniert. Die zusätzlichen Daten stammen aus 431 Messstellen, die zwischen 2002 und 2009 in Sachsen-Anhalt (MATZKE *et al.*, 2009), Baden-Württemberg (FUCHS, 2007), Rheinland-Pfalz (MATZKE *et al.*, 2005) und Bayern (FUCHS & HAHN, unveröffentl.) erhoben worden sind (Kapitel 7, Artikel 3).

Alle Befunde zur Mikrobiologie wurden aus STEIN *et al.* (2010) und GRIEBLER *et al.* (2010) übernommen.

2.1 Gliederung der Arbeit

Die Einleitung, gliedert sich in mehrere Kapitel. Die Hypothesen und Fragestellungen, die dieser Arbeit zugrunde liegen, werden in Kapitel 2.2 aufgeführt.

In Kapitel 2.3 und 2.4 wird der aktuelle der Stand der Grundwasserpolitik und der Forschung hinsichtlich einer biologischen Bewertung für Grundwasserlebensräume erläutert.

Kapitel 2.5 gibt eine Übersicht der Anpassungen von Invertebraten an die Verhältnisse im Grundwasser und ihre Gemeinschaftsstrukturen.

In Kapitel 2.6 folgt die Beschreibung des Konzepts der hierarchischen räumlichen Gliederung von Grundwasserhabitaten und ihren Gemeinschaften nach HAHN (2009). An diesem Ansatz orientieren sich die Fragestellungen und die Wahl der Untersuchungsgebiete der vorliegenden Arbeit.

In Kapitel 3 folgt die Darstellung, Beschreibung und Diskussion der wichtigsten Ergebnisse der Untersuchungen. Diese werden thematisch in einzelnen Kapitel (3.1-3.3) abgehandelt. Jedes Kapitel enthält eine Zusammenfassung sowie eine Einleitung in die Thematik und Methodik der speziellen Untersuchungen in deren Anschluss die Ergebnisse präsentiert und diskutiert werden. Die Kapitel 3.1.2, 3.2.2 und 3.3.2 sind in englischer Sprache verfasst, da diese Ergebnisteile bereits veröffentlicht, bzw. zur Veröffentlichung eingereicht sind. In Kapitel 3.2

wurden die Ergebnisse durch ein Schema des Untersuchungsgebiets und der entsprechenden Beschreibung ergänzt (Ergänzung zu 3.2).

Abschließend folgen in Kapitel 4, die Diskussion der Ergebnisse sowie ein kurzer Ausblick auf die ökologisch orientierte Grundwasserbewertung.

2.2 Hypothesen und Fragestellungen der Arbeit

Für die ökologische Bewertung von Grundwasserhabitaten und ihren Lebensgemeinschaften ist die Betrachtung der räumlichen Skala höchst relevant (GIBERT & DEHARVENG 2002). Grundwasserlebensräume lassen sich drei hierarchischen räumlichen Ebenen zuordnen, welche die Verbreitung und Zusammensetzung der Invertebratengemeinschaften im Grundwasser prägen (HAHN 2009).

Diese sind:

1) die biogeografische Ebene
2) die regionale Ebene
3) die lokale Ebene.

Vor allem die lokale, kleinräumige Ebene ist für die Bewertung relevant. Hier sind der Sauerstoffgehalt und die Verfügbarkeit organischen Materials, als Konsequenz des hydrologischen Austauschs, Schlüsselparameter für das Auftreten der Fauna im Grundwasser (DATRY *et al.*, 2005; HAHN, 2006; SCHMIDT *et al.*, 2007). Die übergeordnete, regionale und biogeografische Ebene sind dabei ausschlaggebend für das potenzielle Arteninventar der lokalen Ebene. Dieses wird beispielsweise durch klimatische Bedingungen, die hydraulischen Eigenschaften verschiedener Grundwasserleitertypen oder naturräumliche Besonderheiten geprägt. Das Konzept der hierarchischen räumlichen Ebenen für Grundwasserhabitate und ihre Gemeinschaften wird in Kapitel 2.6 ausführlich beschrieben.

Der Schwerpunkt der Untersuchungen lag in der Überprüfung der Invertebratengemeinschaften hinsichtlich ihrer Eignung und Anwendung für die ökologische Bewertung von Grundwasserlebensräumen. Zentrale Fragestellungen der Untersuchungen waren:

- Welche faunistische Parameter eignen sich für die Bewertung von Grundwasserhabitaten?
- Welche Rolle spielt die räumliche Ebene bei der faunistischen Bewertung von Grundwasserhabitaten?

Die *lokale und regionale Ebene* wurde exemplarisch anhand grundwasserfaunistischer Daten aus dem Erftgebiet und dem Alb-Donau-Kreis untersucht. In Anlehnung an die Eigenschaften dieser Gebiete wurden spezielle Fragestellungen bearbeitet.

Erftgebiet: *Die Untersuchung der Effekte von Landnutzung auf die Invertebratengemeinschaften des Grundwassers.*

- Spiegeln die Invertebratengemeinschaften des Grundwassers lokale Landnutzungseffekte wider?
- Welche biotische und abiotische Parameter eignen sich für die Bewertung der Grundwasserqualität?
- Weisen die Invertebratengemeinschaften zweier hydrologisch unabhängiger, benachbarter Lockergesteinsleiter (Erft- und Rurscholle) faunistische Unterschiede auf?

Alb-Donau-Kreis: *Die Untersuchung der Invertebratengemeinschaften eines dynamischen Karstsystems und der angrenzenden fluviatilen Lockergesteinsleiter hinsichtlich der Effekte des Grundwasserleitertyps, des Naturraums und der hydrogeologischen Eigenschaften.*

- Unterscheidet sich die Fauna hinsichtlich des Grundwasserleitertyps (Karst-, Lockergestein), der Zugehörigkeit zum Naturraum (Lonetal-Flächenalb, Donauried) oder beeinflusst die hydrogeologische Bezugseinheit (Kalksteine des Oberen Jura, Schotter und Moränen des Alpenvorlands) die faunistische Zusammensetzung?
- Spiegelt die Fauna die hydrologischen Verhältnisse wider und welche Rolle spielen regionale Besonderheiten wie z. B. die ausgeprägte

Dynamik und die Konnektivität der Grundwasserleiter oder die Zugehörigkeit zu demselben hydrologischen System?

Die Untersuchung der *biogeografischen Ebene* basiert auf Faunadaten aus unterschiedlichen Grundwassersystemen Deutschlands.

Biogeografische Untersuchungen: *Die Evaluierung biogeografischer Verbreitungsmuster von Invertebratengemeinschaften im Grundwasser und der Vergleich mit bestehenden oberirdischen Gliederungssystemen.*

- Unterscheiden sich die Invertebratengemeinschaften im Grundwasser biogeografisch voneinander und können anhand dieser Verbreitungsmuster Stygoregionen definiert werden?
- Decken sich die Verbreitungsmuster im Grundwasser mit bestehenden Gliederungssystemen, die sich an naturräumlichen Kriterien (SSYMANK 1994) bzw. an Fließgewässertypen (POTTGIEßER *et al.* 2004) orientieren?

2.3 Aktueller Stand der gesetzlichen Grundwasserbewertung

Das Grundwasser wurde bisher vor allem aus wasserwirtschaftlicher und nutzungsorientierter Perspektive betrachtet (DANIELOPOL *et al.*, 2008). Daher beinhalten nur wenige nationale und internationale Regelungen zum Grundwasserschutz ökologische Kriterien, die bei der Erfassung und Überwachung von Grundwasserökosystemen eingesetzt werden. Aktuell werden die europäischen Grundwasserkörper durch quantitative und physikochemische Analysen sowie vereinzelte hygienische Parameter, die auf pathogene Keime testen, bewertet und überwacht (EG-WRRL, 2000; EG-GWRL, 2006).

Im Gegensatz zum Management von Oberflächengewässern fehlt für unsere Grundwasserressourcen bis heute eine angewandte ökologische Zustandsbewertung (DANIELOPOL & GRIEBLER, 2008). Ursache dafür ist das noch immer lückenhafte Verständnis komplexer Zusammenhänge und Vorgänge der extrem heterogenen Grundwasserlebensräume. Diese Wissensdefizite führen oft zu einer kritischen Betrachtung im Grundwassermanagement, da ein finanzieller Mehraufwand befürchtet wird, während die Vorteile eines ökologischen Bewertungssystems noch unklar scheinen (STEUBE *et al.*, 2008).

Insgesamt wächst bei Fachbehörden und politischen Entscheidungsträgern jedoch die Anerkennung der Ökosystemdienstleistungen, die von gesunden und intakten Grundwasserökosystemen erbracht werden (QUEVAUVILLER, 2005; DANIELOPOL & GRIEBLER, 2008). Grundsätzlich wird davon ausgegangen, dass gestresste Ökosysteme Veränderungen in ihrer Strukturqualität, wie z. B. eine veränderte Zusammensetzung der faunistischen und mikrobiologischen Gemeinschaften, aufweisen (BOULTON *et al.*, 2008, KORBEL & HOSE, 2011). Diese strukturellen Veränderungen können sich auch auf die Funktionalität, wie z. B. dem Abbau organischen Materials, und somit auf die Ökosystemdienstleistungen auswirken (KEPPNER, 2005; 2006). Diese Erkenntnisse haben zu einem größeren Verständnis gegenüber der Bedeutung der Erfassung biologischer und ökologischer Kriterien für den nachhaltigen Grundwasserschutz geführt.

Obwohl bis heute ein angewandter ökologisch orientierter Grundwasserschutz fehlt, ist auf politischer Ebene seit Ende der 90iger Jahre ein gewisser Trend zu einem nachhaltigen Grundwasserschutz zu vermerken. Während die Schweizer Gewässerschutzverordnung (GSchV) schon seit 1998 ökologische Ziele hinsichtlich der Wasserqualität formuliert, hält auch die Grundwasserrichtlinie (EG-GWRL) seit 2006 fest, dass Grundwasserleiter und Grundwasser nicht nur Rohstoffe und Trinkwasser liefern, sondern eigenständige Lebensräume sind (DANIELOPOL *et al.*, 2004; 2008). Weiterhin wird in der EG-GWRL (2006) die Relevanz von Schutzmaßnahmen für Grundwassersysteme festgehalten und gefolgert, dass ein erhöhter Forschungsbedarf besteht, um bessere Kriterien für die Sicherstellung eines guten Zustands der Grundwasserökosysteme bereitzustellen. Die Grundwasserverordnung (GRWV, 2010) verlangt sogar den gleichzeitigen und gleichrangigen Schutz aquatischer Ökosysteme und erkennt die Biodiversität im Grundwasser als eigen und schützenswert an.

Im Vergleich zu Europa integriert die australische Grundwasserpolitik bereits ökologische Kriterien in verschiedenen Ansätzen. Der Bundesstaat New South Wales (NSW) schützt seine staatlichen Grundwasserressourcen durch den Erlass eines Grundsatzpapiers für grundwasserabhängige Ökosysteme (NSW-SGDEP, 2002). In diesem Papier wird die Relevanz des Erhalts der ökologischen Prozesse und der Biodiversität im Grundwasser betont. Darüber hinaus besteht im Falle von Grundwasserförderung eine Richtlinie zur Erfassung und Bewertung von Umweltfaktoren, einschließlich der Fauna in Grundwasser und Höhlen (EPA, 2003).

Die oben aufgeführten Veränderungen auf politischer Ebene regten Diskussionen über die Notwendigkeit und Perspektiven für ökologische Zustandskriterien zukünftiger Grundwasserüberwachungsprogramme an (QUEVAUVILLER, 2005). Eine ökosystemare Zustandserfassung sowie ein umfassendes Bewertungssystem erfordern die Berücksichtigung ökologischer Kriterien, die zusätzlich zu abiotischen auch biotische Parameter integrieren (CAIRNS *et al.*, 1993; HANCOCK *et al.*, 2005; TOMLINSON *et al.*, 2007). Letztere sind für Grundwassersysteme nicht vorhanden (DANIELOPOL & GRIEBLER, 2008). Da im Grundwasser eine stark angepasste Fauna lebt, können Bioindikationsverfahren, die sich in Oberflächengewässern bewährt haben, nicht direkt auf das Grundwasser übertragen werden (DEHARVENG *et al.*, 2009). Weiterhin problematisch sind fehlende Referenzen weitgehend natürlicher Grundwasserleiter, um zeitliche Trends zu erfassen und die Basis für ein Frühwarnsystem für Ökosystemgefährdung zu bilden. Darüber hinaus fehlen standardisierte Methoden für die Erfassung biologischer Kriterien (HANCOCK *et al.*, 2005; TOMLINSON *et al.*, 2007; DANIELOPOL *et al.*, 2008).

Die Ergebnisse und Erfahrungen der aktuellen Untersuchungen fließen in den Entwurf eines ökologischen Bewertungssystems für Grundwasserlebensräume ein (siehe GRIEBLER *et al.*, in Druck).

2.4 Aktueller Stand der biologischen Grundwasserbewertung

Die Entwicklung eines ökologischen Bewertungssystems für Grundwasser erfordert neben physikochemischen und einfachen mikrobiologischen Analysen auch geeignete biologische Kriterien, um den ökologischen Zustand sowie zeitliche Veränderungen erfassen und bewerten zu können (STEUBE *et al.*, 2010). In dieser Hinsicht können faunistische und mikrobiologische Kriterien als Frühwarnsystem dienen, da Grundwassergemeinschaften relativ schnell auf hydrologische Veränderungen reagieren (Cairns *et al.*, 1993). Voraussetzung für die Zustandsbewertung sind die Erfassung und Definition faunistischer und mikrobieller Referenzen sowie die Klassifizierung und Typisierung von Grundwassersystemen, um Abweichungen von einem definierten Sollzustand detektieren zu können (STEUBE *et al.*, 2008). Diese Referenzen sind für weitgehend natürliche Grundwassersysteme bisher jedoch nicht verfügbar.

In Fachkreisen besteht kein Zweifel, dass sich Grundwassergemeinschaften als direkte oder indirekte Indikatoren für die Erfassung natürlicher und anthropogener Einflüsse und somit zur Evaluierung des Ökosystemstatus und der Wasserqualität eignen (TOMLINSON *et al.*, 2007; KORBEL & HOSE, 2011). Die ökologisch orientierten Grundwasseruntersuchungen haben in den letzten zwei Jahrzehnten zugenommen. Trotzdem besteht ein verstärkter Forschungsbedarf, um für ein Bewertungssystem geeignete faunistische und mikrobiologische Kriterien, einschließlich sensitiver Bioindikatoren, zu evaluieren und auf ihre Anwendbarkeit zu testen. Ebenso müssen die Methoden zur Erfassung und Analyse biotischer Daten standardisiert werden, um eine vergleichbare Bewertung zu ermöglichen (BOULTON *et al.*, 2008; DANIELOPOL *et al.*, 2008).

Bisher ist bekannt, dass die Zusammensetzung der Invertebraten-gemeinschaften im Grundwasser die Stärke und Richtung hydrologischer Wechselwirkungen von Grund- und Oberflächenwasser reflektieren (DUMAS *et al.*, 2001; HAHN, 2006; BERKHOFF *et al.*, 2009). Auch mikrobielle Gemeinschaften oberflächennaher Grundwässer scheinen durch den hydrologischen Oberflächenaustausch geprägt zu sein (GRIEBLER & LUEDERS, 2009). Die natürlichen Grundwassergemeinschaften sind auf einen kontinuierlichen Oberflächeneintrag angewiesen, da dieser Sauerstoff und organisches Material in die meist Energie limitierten Grundwassersysteme einbringt (HAHN, 2006; FERREIRA *et al.*, 2007). Ein vermehrter Oberflächeneinfluss ist aber sowohl ein Risiko für die Grundwasserqualität (BORK, 2008), als auch für die Gemeinschaften, da mit infiltrierendem Oberflächenwasser, neben Nährstoffen und Sauerstoff, z. B. auch Schadstoffe oder grundwasserfremde Organismen wie z. B. pathogene Mikroorganismen oder konkurrierende Oberflächenarten in den Grundwasserleiter gelangen können (GRIEBLER *et al.*, 2010). So wird ein starker Oberflächeneinfluss zwar nicht immer durch physikochemische Parameter detektiert, aber er wird meist durch die Anwesenheit grundwasserfremder Organismen angezeigt (HAHN & PREUß, 2005). In dieser Hinsicht liefern die Grundwassergemeinschaften Informationen für die Bewertung des Grundwassers. Anhand der Zusammensetzung der Grundwassergemeinschaften kann z. B. die hydraulische Konnektivität zu einem Oberflächengewässer und somit die Vulnerabilität des Grundwassersystems festgestellt werden (SCHMIDT *et al.*, 2007).

Für die Grundwassergemeinschaften ist der Eintrag von Nahrung und gelöstem Sauerstoff essentiell (HAHN, 2006; FERREIRA *et al.*, 2007). Verschiedene Untersuchungen haben gezeigt, dass die Präsenz von Grundwassertieren direkt und hauptsächlich von Detritus (POM) und Mikroorganismen abhängt (SKET, 1999; MALARD *et al.*, 1999; DATRY *et al.*, 2005). Bei ausreichenden Sauerstoffkonzentrationen (> 1 mg/l), scheint es zwischen der verfügbaren Menge organischen Materials direkte positive Korrelationen mit Arten- und Individuenzahlen zu geben (HAHN, 2006). Das Artenspektrum verschiebt sich bei zunehmendem Oberflächeneinfluss von stygobionten zu nicht-stygobionten (= stygophile, stygoxene, euryöke) Arten (CLARET *et al.*, 1999; DUMAS *et al.*, 2001;

THULIN & HAHN, 2008). Ebenso zeigen Untersuchungen, dass die mikrobielle Besiedlung nicht nur von den chemischen Verhältnissen im Grundwasser abhängt, sondern auch durch die Verfügbarkeit organischen Materials kontrolliert wird (GOLDSCHEIDER *et al.*, 2006; GRIEBLER & LUEDERS, 2009). Bei einem erhöhten organischen Eintrag verändern sich die Gemeinschaften von Organismen, die an oligotrophe Verhältnisse angepasst sind, zu solchen aus Böden und Oberflächengewässern (GRIEBLER & LUEDERS, 2009).

Andere Untersuchungen befassten sich mit einzelnen Grundwasserarten, die auf verschiedene Belastungen, wie z. B. Pestizide, Kohlenwasserstoffe, organisches Material oder Schwermetalle, Sauerstoff- und Redoxbedingungen sowie Temperaturveränderungen getestet wurden (z. B. MALARD *et al.*, 1994; 1996; NOTENBOOM *et al.*, 1995; MÖSSLACHER, 2000; MÖSSLACHER *et al.*, 2001; SCARSBROOK & FENWICK, 2003; HOSE, 2005; MATZKE *et al.*, 2005; BRIELMANN, 2009; DOLE-OLIVIER *et al.*, 2009). Auch wurden die Invertebratengemeinschaften in Zusammenhang mit der Sedimentstruktur und Porosität und vereinzelt auf biogeografische Aspekte untersucht (WARD & PALMER, 1994; DOLE-OLIVIER *et al.*, 2009; HAHN & FUCHS, 2009). Weiterhin wurde der Einfluss von Oberflächengewässern und Sickerwasser sowie Landnutzung auf das Grundwasser untersucht (z. B. DUMAS *et al.*, 2001; MALARD *et al.*, 2004; HAHN, 2006; SCHMIDT *et al.*, 2007; BERKHOFF *et al.*, 2009; BORK *et al.*, 2009; SCHMIDT & HAHN, 2012). Anhand von Mikroorganismen kann z. B. der biologische Abbau verschiedener Schadstoffe nachgewiesen werden, der durch erhöhte Stoffumsatzaktivitäten angezeigt wird (Brielmann et al., 2009; Griebler & Lueders 2009). Die genannten Studien zeigen die Sensibilität von Invertebraten und Mikroorganismen gegenüber Störungen und Veränderungen im Grundwasser.

Aktuell bestehen noch immer erhebliche Wissensdefizite hinsichtlich taxonomischer, ökologischer und biogeografischer Aspekte im Grundwasser, im Vergleich zu den traditionell viel länger untersuchten

Oberflächengewässern (FERREIRA *et al.*, 2007). Dies ist in Anbetracht der Größe und Heterogenität von Grundwassersystemen sowie der schlechten Zugänglichkeit und auch der geringen Anzahl ökologischer Grundwasserstudien nicht verwunderlich (STEUBE *et al.*, 2008). Aktuelle Untersuchungen zu biogeografischen Aspekten gibt es für Deutschland kaum (HAHN & FUCHS, 2009). Die meisten Grundwasseruntersuchungen konzentrieren sich häufig auf gestörte Standorte und sind regional begrenzt. Biotische Daten aus weitgehend unbelasteten Standorten sowie flächendeckende Studien zur Grundwasserbioindikation fehlen bislang (TOMLINSON & BOULTON, 2009).

Das Wissen über die Ökologie und Taxonomie von Invertebraten im Grundwasser ist noch immer unzureichend. Im Grundwasser gibt es eine große Anzahl von Reliktarten und Endemiten, als Konsequenz der Fragmentierung und Isolation vieler Grundwasservorkommen sowie der geringen Ausbreitungsfähigkeit vieler Grundwasserarten (DEHARVENG *et al.*, 2009; STOCH & GALASSI, 2010). Hinzu kommen die inhomogene Verteilung der Grundwassertiere, die Seltenheit vieler Arten sowie die geringe standörtliche Diversität (2-3 Arten pro Messstelle in Deutschland). Diese Verhältnisse erfordern eine große Anzahl von Grundwasserstandorten, um den Artenbestand eines Gebietes zu ermitteln (GIBERT & DEHARVENG, 2002; CASTELLARINI *et al.*, 2005; HAHN & FUCHS, 2009; MARTIN *et al.*, 2009). So kann die Abwesenheit von Grundwassertieren durchaus natürlichen Charakters sein und weist nicht zwangsläufig auf eine Belastung hin (FUCHS, 2007; HAHN & FUCHS, 2009). Diese Faktoren erschweren die Vorhersage faunistischer Muster sowie die Untersuchungen hinsichtlich der Indikatorwerte von Arten und Gemeinschaften gegenüber Störungen (THULIN & HAHN, 2008).

Ein biologisches Bewertungssystem für Grundwasser erfordert neben geeigneten biotischen Kriterien, Bioindikatoren, standardisierten Erfassungs- und Analysemethoden auch die Definition von Grenz- und Hintergrundwerten. Dafür sind umfangreiche Datenerhebungen erforderlich. Im Gegensatz zu physikochemischen Kriterien können für biologische Kriterien keine Standards der Trinkwasser- und Umweltqualität genutzt, sondern es müssen die Grenzwerte und die natürlichen Hintergrundbereiche verschiedener Grundwasservorkommen berücksichtigt werden (STEUBE *et al.*, 2008).

Ein erstes Bewertungssystem zur Erfassung des Gesundheitszustands von Grundwasserökosystemen ("Tiered framework for assessing groundwater ecosystem health"), dass neben abiotischen Kriterien auch biotische berücksichtigt, haben die Australier KORBEL & HOSE (2010) entwickelt. Dieses wurde anhand der aktuellen Untersuchungen getestet und entsprechend angepasst (siehe GRIEBLER *et al.*, in Druck).

2.5 Die Invertebratengemeinschaften des Grundwassers

Weltweit wurden bisher mehr als 7000 Grundwasserarten beschrieben (BOTOSANEANOU, 1986). Diese Angabe scheint aber in Anbetracht der Heterogenität, Größe und wenigen Untersuchungen von Grundwasserlebensräumen die Diversität im Grundwasser weit zu unterschätzen (DANIELOPOL & GRIEBLER, 2008; HAHN & FUCHS, 2009). Typische Vertreter der Grundwasserfauna sind Crustaceen, Anneliden, Acari, Turbellarien, Nematoden und Gastropoden, wobei die Crustacea besonders artenreich sind (STOCH, 1995, GIBERT & DEHARVENG, 2002; DEHARVENG *et al.*, 2009.). Echte Grundwassertiere (= Stygobionte) zeigen ausgeprägte Adaptationen an die besonderen Verhältnisse im Grundwasser und zeichnen sich vor allem durch ihre Toleranz gegenüber Nahrungsknappheit und Sauerstoffmangel aus. Ein verlangsamter Metabolismus hilft ihnen Hungerperioden zu überstehen. In Anpassung an die Dunkelheit sind stygobionte Arten blind und unpigmentiert. Die lang gestreckte Körperform und die geringe Körpergröße der meisten Arten sind als Anpassung an ein Leben in engen Lückenräumen entstanden (GIBERT & DEHARVENG, 2002; MÖSSLACHER, 2003).

Im Allgemeinen sind die Umweltbedingungen im Grundwasser relativ stabil und Schwankungen der abiotischen Verhältnisse sind meist auf den Einfluss von Oberflächenwasser zurückzuführen (BORK, 2008). Aufgrund ihrer ausgeprägten Anpassung an die ausgeglichenen Verhältnisse im Grundwasser, führen veränderte Umweltbedingungen direkt zu Strukturveränderungen der stygobionten Gemeinschaften. Gegenüber oberirdischen, hyporheischen und euryöken Arten, die hier als Nicht-Stygobionte zusammengefasst werden, sind stygobionte Arten hinsichtlich ihrer Reproduktions- und Ausbreitungsraten konkurrenzschwach (MÖSSLACHER, 2003). Dem Konkurrenzdruck entgehen die Stygobionten durch das Ausweichen in unwirtliche Grundwasserhabitate. Verbessern sich bei stärkerem Oberflächeneinfluss die Bedingungen im Grundwasser, können nicht-stygobionte Arten die Stygobionten verdrängen (SCHMIDT & HAHN, 2012). Mit zunehmender Stärke des Oberflächeneinflusses verschiebt sich die Artenzusammensetzung im Grundwasser von einer rein stygobionten, individuenarmen Gemeinschaft in Richtung einer individuenreichen Gemeinschaft, die vor allem von nicht-stygobionten Arten geprägt ist (HAHN, 2006; THULIN & HAHN, 2008; KORBEL & HOSE, 2011). Die Zusammensetzung der Invertebratengemeinschaften im Grundwasser reflektiert demnach die Stärke und Richtung der Grundwasser-Oberflächenwasser-Interaktionen. Aufgrund dieser Eigenschaften ist die Grundwasserfauna für die ökologische Bewertung von Grundwassersystemen sowie zur Risikoabschätzung durch Oberflächeneinfluss geeignet (DANIELOPOL *et al.*, 2004; TOMLINSON *et al.*, 2007, HUMPHREYS, 2009; DANIELOPOL & GRIEBLER, 2008, SCHMIDT & HAHN, 2012).

2.6 Klassifizierung und Typisierung von Grundwassersystemen und ihren Invertebratengemeinschaften

Voraussetzung für die Klassifizierung von Grundwasserökosystemen ist die Auswahl von Zustandskriterien, die z. B. den Grundwasserleitertyp, die Gesteinsart und das Einzugsgebiet berücksichtigen, da diese Faktoren die hydrochemischen und hydrologischen Verhältnisse im Grundwasser prägen (HEATH, 1982). Um Grundwasserressourcen und Aquifere auf unterschiedlichen Skalen und nach bestimmten Kriterien einzuteilen, wurden bisher verschiedene nationale und internationale Bemühungen unternommen, (CEC, 1982; GILBRICH, 2000; IGRAC, 2005; STRUCKMEIER *et al.*, 2006). Eine erste Typologie von Aquiferen in Europa wurde von WENDLAND *et al.*, (2008) vorgenommen. Hier werden Hauptklassen von Aquiferen hinsichtlich der Gesteinseigenschaft, der Hydrologie und der Hydrodynamik definiert. KUNKEL *et al.*, (2004) entwickelten diese Typologie weiter und gliederten Deutschland in 17 so genannte hydrogeologische Bezugseinheiten (HBE). Da nach bisherigem Kenntnisstand die Verbreitung von Mikroorganismen vor allem von geologischen und mineralogischen Verhältnissen geprägt zu sein scheint (GRIEBLER & LUEDERS, 2009), wurde die Gliederung der HBE als Ansatz für die Untersuchung mikrobieller Verbreitungsmuster gewählt.

Im Gegensatz dazu sind die genannten Gliederungssysteme aus grundwasserökologischer Sicht nicht für die Interpretation faunistischer Muster ausreichend. Einem hierarchischen Modell für die Klassifizierung von Grundwasserhabitaten und –gemeinschaften nach HAHN (2009) folgend, müssen drei räumliche Ebenen Beachtung finden, die die Verbreitung und Gemeinschaftsstruktur von Grundwasserinvertebraten prägen (siehe auch Abbildung 1):

1. Die biogeografische Ebene, auch kontinentale Ebene, Makroskala oder Megaskala (GIBERT *et al.*, 1994; HAHN, 2009) genannt, auf der klimatische

Bedingungen und erdgeschichtliche Ereignisse wie z. B. die Hydrographie oder die Eiszeiten ausschlaggebend für das rezente Vorkommen verschiedener Grundwasserarten sind. Diese unterirdischen biogeografischen Einheiten werden als Stygoregionen bezeichnet (GIBERT *et al.*, 2005; HAHN, 2009; STOCH & GALASSI, 2010).

2. Die regionale Ebene, auch Landschaftsebene oder Mesoskala (GIBERT *et al.*, 1994; HAHN, 2009) genannt, auf der die Grundwasserfauna von naturräumlichen Besonderheiten sowie vom hydrogeologischen Grundwasserleitertyp (Karst, Kluft, Locker, Kompakt) geprägt wird. Vom Grundwasserleitertyp hängt sowohl der verfügbare Lebensraum für die Invertebraten ab, als auch die hydraulische Leitfähigkeit und Konnektivität eines Grundwassersystems (GIBERT *et al.*, 1997; HAHN, 2009; HAHN & FUCHS, 2009).

3. Die lokale Ebene, auch „habitat-level" oder Mikroskala (GIBERT *et al.*, 1994; HAHN, 2009) genannt, umfasst kleinräumige, lokale Faktoren innerhalb eines Grundwasserleiters. Die Grundwasserbiozönosen sind durch den hydrologischen Austausch mit Oberflächensystemen (Niederschlag, Sickerwasser, Oberflächenwasser) geprägt. Die Stärke und Richtung des hydrologischen Austauschs bestimmt die Menge der verfügbaren Nahrung (POM, Biofilme) und des Sauerstoffs sowie dem Eintrag konkurrierender Oberflächenarten. Dies spiegelt sich in der Artenzusammensetzung, Abundanz und Diversität der Gemeinschaften wider. Die lokalen hydrologischen Bedingungen werden demnach als Schlüsselfaktoren angesehen (HAHN, 2009) und mittels des Grundwasserfauna-Index (GFI) abgeschätzt. Die Indexwerte werden aus dem Sauerstoffgehalt, der Detritusmenge sowie der Standard-abweichung der Temperatur berechnet Der GFI erlaubt so die Vorhersage der faunistischen Gemeinschaftsstruktur (HAHN, 2006). Je nach Menge des organischen Materials unterscheidet HAHN (2006) drei

Typen von Habitaten, die von unterschiedlichen Gemeinschaften geprägt sind. Die Habitate werden nach geringem, mittlerem und starkem Oberflächenwassereinfluss klassifiziert.

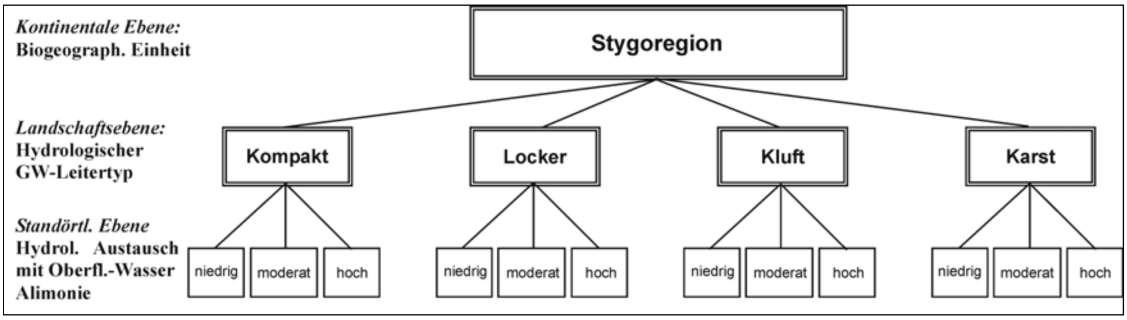

Abbildung 1: Hierarchisches Modell zur Typologie von Grundwasserhabitaten nach HAHN, 2009.

Nur auf der lokalen Ebene (Messstellenebene) kann eine ökologisch orientierte Bewertung von Grundwasserlebensräumen stattfinden. Das vorgestellte Konzept der hierarchischen Gliederung von Grundwasserlebensräumen und Gemeinschaften nach HAHN (2009) war der theoretische Ansatz auf dem die Vorgehensweise und die Wahl der Untersuchungsgebiete beruhte.

2.7 Literatur

AVRAMOV M., SCHMIDT S.I., GRIEBLER C., HAHN H.J. & BERKHOFF S. (2010) Dienstleistungen der Grundwasserökosysteme. Korrespondenz Wasserwirtschaft 3(2), 75-81.

BALKE K.D. & GRIEBLER C. (2003) Grundwassernutzung und Grundwasserschutz. In: GRIEBLER C. & MÖSSLACHER F. (Hrsg.). Grundwasserökologie – eine Einführung. Wien.

BERKHOFF S.E., BORK J. & HAHN H.J. (2009) Grundwasserfauna als Indikator für Oberflächenwasser-Grundwasser-Interaktionen im Bereich einer Uferfiltrationsanlage. Grundwasser 14, 3–20.

BORK J., BERKHOFF S.E. & HAHN H.J. (2008) Testing unbaited stygofauna traps for sampling performance. Limnologica 38, 105-115.

BORK J., BERKHOFF S.E., BORK S., HAHN H.J. (2009) Using subsurface metazoan fauna to indicate groundwater-surface water interactions in the Nakdong River floodplain, South Korea. Hydrogeol. J. 17, 61–75.

BOTOSANEANU L. (1986) Stygofauna Mundi. A faunistic, distributional and ecological synthesis of the world fauna inhabiting subterranean waters (including the marine interstitial). Brill, Backhuys, Leiden.

BOULTON A.J. (2005) Chances and challenges in the conservation of groundwaters and their dependent ecosystems. Aqua. Conserv. Marine Freshwater Ecosyst. 15, 319–323.

BOULTON A.J., FENWICK G.D., HANCOCK P.J. & HARVEY M.S. (2008) Biodiversity, functional roles and ecosystem services of groundwater invertebrates. Invertebrate Syst. 22, 103–116.

BRIELMANN H., GRIEBLER C., SCHMIDT S.I., MICHEL R. & LUEDERS T. (2009) Effects of thermal energy discharge on shallow groundwater ecosystems. FEMS Microbiol. Ecol. 68, 273–286.

BRUNKE M. & GONSER T. (1999) Hyporheic invertebrates – the clinal nature of interssitial communities structured by hydrological exchange and environmental gradients. J. North Am. Benth. Soc. 18(3), 344-362.

CAIRNS JR. J., MCCORMICK P. V. & NIEDERLEHNER B. R. (1993) A proposed framework for developing indicators of ecosystem health, Hydrobiologia, 263, 1–44.

CASTELLARINI F., DOLE-OLIVIER M.-J., MALARD F. & GIBERT J. (2005) Improving the assessment of groundwater biodiversity by exploring environmental heterogeneity at a regional scale. In: Processings of an International Symposium of World Subterranean Biodiversity. Gibert, J. (ed). Villeürbanne: University of Lyon, 83-88.

CEC (1982) Groundwater resources of the European Community Synthetical report. Brussels, Luxemburg.

CLARET C., MARMONIER P., DOLE-OLIVIER M. J., CRUEZÈ DES CHÂTELLIERS M., BOULTON A. J. & CASTELLA E. (1999) A functional classification of interstitial invertebrates: supplementing measures of biodiversity using species traits and habitat affinities, Arch. Hydrobiol. 145, 385–403.

DANIELOPOL D.L., GRIEBLER C., GINATILAKA A. & NOTENBOOM J. (2003) Present state and future prospects for groundwater ecosystems. Environ. Conserv. 30, 104–130.

DANIELOPOL D., GIBERT J., GRIEBLER C., GUNATILAKA A., HAHN H.J., MESSANA G. NOTENBOOM J. & SKET B. (2004) Incorporating ecological perspectives in European groundwater management policy. Environ. Conserv. 31, 1–5.

DANIELOPOL D.L. & GRIEBLER C. (2008) Chaniging paradigms in groundwater ecology – from the ´living fossils` tradition to ´new groundwater ecology`. Int. Rev. Hydrobiol. 93, 565-577.

DANIELOPOL D., GRIEBLER C., GUNATILAKA A., HAHN H.J., GIBERT J., MERMILLOD-BLONDIN G., MESSANA G., NOTENBOOM J. & SKET B. (2008) Incorporation of groundwater ecology in environmental policy. In: Quevauviller, P. (Ed.), Groundwater Science and Policy – An International Overview. RSC Publishing, The Royal Society of Chemistry, London.

DATRY T., MALARD F. & GIBERT J. (2005) Response of invertebrate assemblages to increased groundwater recharge rates in a phreatic aquifer. J. North Am. Benthol. Soc. 24, 461–477.

DEHARVENG L., STOCH F., GIBERT J., BEDOS A., GALASSI D., ZAGMAJSTER M., BRANNCELJ A., CAMACHO A., FIERS F., MARTIN P., GIANI N., MAGNIEZ G. & MARMONIER P. (2009) Groundwater biodiversity in Europe, Freshw. Biol. 54(4), 709–726.

DOLE-OLIVIER M.-J., MALARD F., MARTIN D., LEFÉBURE T. & GIBERT J. (2009) Relationships between environmental variables and groundwater biodiversity at the regional scale. Freshw. Biol. 54, 797–813.

DUMAS P., BOU C. & GIBERT J. (2001) Groundwater Macrocrustaceans as Natural Indicators of the Ariège Alluvial Aquifer, International Review of Hydrobiology 86(6), 619-633.

EPA (2003). Guidance for the assessment of environmental factors: consideration of subterranean fauna in groundwater and caves during environmental impact assessment in Western Australia. In: ´Guidance Statement for the Assessment of environmental Factors no. 54 Environmental Protection Authority, Perth, Australia.

EG-GWRL (Grundwasserrichtlinie) (2006) Richtlinie 2006/118/EG Des Europäischen Parlaments und des Rates. Amtsblatt der Europäischen Union L 372 (19).

EG-WRRL (Wasserrahmenrichtlinie) (2000) Richtlinie 2000/60/EG Des Europäischen Parlaments und des Rates vom 23 Oktober 2000 zur Schaffung eines Ordnungsrahmens für Maßnahmen der Gemeinschaft im Bereich der Wasserpolitik. Official J. European Comm. Brüssel, Belgien. L327(1).

FERREIRA D., MALARD F., DOLE-OLIVIER M.J. & GIBERT J. (2007) Obligate groundwater fauna of France: diversity patterns and conservation implications. Biodivers. Conserv. 16, 567–596.

FRÄNZLE O. (2003) Bioindicators and environmental stress assessment. In: Markert, B.A., Breuie, A.M., Zechmeister, H.G. (Eds.), Bioindicators and Biomonitors. Elsevier Science Ltd.

FUCHS A. (2007) Erhebung und Beschreibung der Grundwasserfauna in Baden-Württemberg. In: Natur- und Umweltwissenschaften. Universität Koblenz-Landau, Landau.

FUCHS A. & HAHN H.J. (unveröffentl.) Untersuchung der Grundwasserfauna in Bayern, Bayrisches Landesamt für Umwelt (LFU).

GIBERT J., STANFORD J.A., DOLE-OLIVIER M.-J. & WARD J.V. (1994) Basic attributes of groundwater ecosystems and prospects for research. In: Gibert, J., Danielopol, D.L., Stanford, J.A. (Eds.), Groundwater Ecology. Academic Press, San Diego.

GIBERT J., MATTHIEU J. & FOURNIER F. (Hrsg.) (1997): Groundwater / Surface water ecotones: Biological and hydrological interactions and management options. Cambridge: Cambridge University Press.

GIBERT J., DEHARVENG L. (2002) Subterranean ecosystems: a truncated functional biodiversity. BioScience 52, 473–481.

GIBERT J., BRANCELJ A., CAMACHO A., CASTELLARINI F., DE BROYER C., DEHARVENG L., DOLE-OLIVIER J.-M., DOUADY C., GALASSI D.M., MALARD F., MARTIN P., MICHEL G., SKET B., STOCH F., TRONTELJ P., VALDECASAS A.G. (2005) Groundwater biodiversity. Protocols for the ASsessment and Conservation of Aquatic Life In the Subsurface (PASCALIS): Overview and main results. In: Proceedings of an International Symposium on World Subterranean Biodiversity (Ed. J. Gibert). University of Lyon, Lyon, Villeurbanne. 8-10 December.

GILBRICH W.H. (2000) International hydrogeological map of Europe Feature article, Waterway no. 19, Paris.

GRIEBLER C. & LUEDERS T. (2009) Towards a conceptual understanding of microbial biodiversity in groundwater ecosystems, Freshw. Biol., 54(4), 649–677.

GRIEBLER C., STEIN H., KELLERMANN C., BRIELMANN H., SCHMIDT S. I., SELESI D., STEUBE C., BERKHOFF S.E., FUCHS A. & HAHN H. J. (2010), Ecological assessment of groundwater ecosystems – Vision or illusion? Ecol. Engeneer. 36, 1174-1190.

GRIEBLER C., STEIN H., KELLERMANN C., BRIELMANN H., BERKHOFF S.E., STEUBE C., FUCHS A. & HAHN H.J. (in Druck) Entwicklung biologischer Bewertungsmethoden und -kriterien für Grundwasserökosysteme. Endbericht. Umweltbundesamt No FKZ 3708 23 200, Dessau.

GRWV (2010) Verordnung zum Schutz des Grundwassers vom 9. November 2010.

GSCHV (1998) Gewässerschutzverordnung (Swiss Water Ordinance) 814.201. Der Schweizer Bundesrat, Bern, Switzerland.

HAHN H.J. (2006) The GW-Fauna-Index: a first approach to a quantitative ecological assessment of groundwater habitats. Limnologica 36, 119–137.

HAHN H.J. (2009) A proposal for an extended typology of groundwater habitats. Hydrogeol. J. 17, 77–81.

HAHN H.J. & MATZKE D. (2005) A comparison of stygofauna communities inside and outside groundwater bores. Limnologica 35, 31–44.

HAHN H.J. & PREUß G. (2005) Anwendungsmöglichkeiten grundwasser-ökologischer Forschung. GWF Wasser-Abwasser 146, 568-572.

HAHN H.J. & FUCHS A. (2009) Distribution patterns of groundwater communities across aquifer types in south-western Germany. Freshw. Biol. 54, 848–860.

HANCOCK P. J., BOULTON A. J. & HUMPHREYS W. F. (2005) Aquifers and hyporheic zones: towards an ecological understanding of groundwater, Hydrogeol. J. 13(1), 98–111.

HEATH R.C. (1982) Classification of groundwater systems of the United States. Ground Water 20, 393–401.

HERMAN J. S., CULVER D. C. & SALZMANN J. (2001) Groundwater ecosystems and the service of water purification, Stanford. Environ. Law. J. 20, 479–495.

HOSE G.C. (2005) Assessing the need for groundwater quality guidelines for pesticides using the species sensitivity distribution approach. Human and Ecological Risk Assessment 11, 951-966.

HUMPHREYS W. F. (2006) Aquifers: the ultimate groundwater-dependent ecosystems, Aust. J. Bot. 54, 115–132.

HUMPHREYS W.F. (2009) Hydrogeology and groundwater ecology: does each inform the other? Hydrogeol. J. 17, 5–21.

IGRAC (2005) International Groundwater Resources Assessment Centre: Proposed groundwater provinces. info@igrac.nl.

KEPPNER L. (2005) Der Lebensraum Grundwasser braucht eine Lobby. GWF Wasser Abwasser 146, 527.

KEPPNER L. (2006) Groundwater – a valuable resource for drinking water: strategies of groundwater protection under the European legislative framework in Germany. European Groundwater Conference, Vienna 22-23 June 2006, 47-49.

KORBEL K. L. & HOSE G. C. (2011) A tiered framework for assessing groundwater ecosystem health. Hydrobiologia 661, 329-349.

KUNKEL R., WENDLAND F., VOIGT H. J. & HANNAPPEL S. (2004) Die natürliche, ubiquitär überprägte Grundwasserbeschaffenheit in Deutschland, Schriften des Forschungszentrums Jülich, Reihe Umwelt / Environment 47.

MALARD F., REYGROBELLET J.-L., MATHIEU J. & LAFONT M. (1994) The use of invertebrate communities to describe groundwater flow and contaminant transport in a fractured rock aquifer. Arch. Hydrobiol. 131, 93-110.

MALARD F., PLENET S. & GIBERT J. (1996) The use of invertebrates in ground water monitoring: A rising research field, Ground Water Monit. Rem. 16, 103–113.

MALARD F., TOCKNER K. & WARD J.V. (1999) Shifting dominance of subcatchment water sources and flow paths in a glacial flood plain (Val Roseg, Switzerland). Arct. Antarct. Alp. Res. 31, 135–150.

MALARD F., CASTELLARINI F. & OLIVIER J. (2004) Statistical analyses and identification of indicators. In: Stoch, F. (Ed), PASCALIS (Protocols for the assessment and conservation of aquatic life in the subsurface) Deliverable 8, 1–156.

MATZKE D., HAHN H.J., RAMSTÖCK A. & ROTHER K. (2005) Bewertung von Altlasten im Grundwasser anhand der Meiofaunagemeinschaften - erste Ergebnisse. Grundwasser 10, 25-34.

MÖSSLACHER F. (1998) Subsurface dwelling Crustaceans as indicators of hydrological conditions, oxygen concentrations, and sediment structure in an alluvial aquifer, Int. Rev. Hydrobiol. 83, 349–364.

MÖSSLACHER F. (2000) Sensitivity of groundwater and surface water crustaceans to chemical pollutants and hypoxia: implications for pollution management, Arch. Hydrobiol. 149, 51–66.

MÖSSLACHER F. (2003) Evolution, Adaption und Verbreitung. -In: Griebler C. & Mösslacher F. (2003) Grundwasser - eine ökosystemare Betrachtung. In: Grundwasserökologie. Griebler C. & Mösslacher F. (eds). Wien: UTB-Facultas-Verlag, 253-310.

MÖSSLACHER F., GRIEBLER C. & NOTENBOOM J. (2001) Biomonitoring of groundwater systems: methods, applications and possible indicators among the groundwater biota. In: Groundwater ecology. a tool for management of water resources (Griebler, C., Danielopol, D.L., Gibert, J., Nachtnebel, H.-P. & Notenboom, J., eds.). Official Publication of the European Communities, 173-182.

NOTENBOOM J., SERRANO R., MORELL I. & HERNÁNDEZ F. (1995) The phreatic aquifer of the ‘Plana de Castellón’ (Spain): relationships between animal assemblages and groundwater pollution. Hydrobiologia 297, 241–249.

NSW-SGDEP (2002) The NSW State Groundwater Dependent Ecosystems Policy. Department of Land and Water Conservation, NSW Government.

POTTGIEßER T., KAIL J., SEUTER S. & HALLE M. (2004) Abschließende Arbeiten zur Fließgewässertypisierung entsprechend den Anforderungen der EU-WRRL – Teil II, Endbericht zur „Karte der biozönotisch bedeutsamen Fließgewässer-typen Deutschlands“ (Stand Dezember 2003). Forschungsprojekt im Auftrag der LAWA.

PREUß G. & SCHMINKE H.K. (2004) Ein globales Ökosystem – Grundwasser lebt!. Chemie Unserer Zeit 38, 340-347.

QUEVAUVILLER P. (2005) Groundwater monitoring in the context of EU legislation: reality and integration needs. J. Environ. Monit. 7, 89.

SCARSBROOK M. R. & FENWICK G. D. (2003) Preliminary assessment of crustacean distribution patterns in New Zealand groundwater aquifers. N.Z.J. Mar. Freshw. Res. 37, 405–413.

SCHMIDT S. I., HAHN H. J., HATTON T. & HUMPHREYS W. F. (2007) Do faunal assemblages reflect the exchange intensity in groundwater zones? Hydrobiologia 583, 1–19.

SCHMIDT S.I. & HAHN H.J. (2012) What is groundwater and what does this mean to fauna? – An opinion. Limnologica 42, 1-6.

SKET B. (1999): The nature of biodiversity in hypogean waters and how it is endangered. - Biodiversity and Conservation 8, 1319-1338.

STEIN H., KELLERMANN C., SCHMIDT S.I., BRIELMANN H., STEUBE C., BERKHOFF S. E., FUCHS A., HAHN H. J., THULIN B. & GRIEBLER C. (2010) The potential use of fauna and bacteria as ecological indicators for the assessment of groundwater ecosystems. J. Environ. Monit. 12, 242-254.

STEUBE C., RICHTER S. & GRIEBLER C. (2009) First attemps towards an integrative concept for the ecological assessment of groundwater ecosystems. Hydrogeol. J. 17, 23–35.

STOCH F. (1995): The Ecological and historical determinants of crustacean diversity in Groundwaters, or why are there so many species? Mémoires de Biospéologie, Tome XXII. 139-160.

STOCH F. & GALASSI M.P. (2010) Stygobiotic crustacean species richness: a question of numbers, a matter of scale. Hydrobiologia 653, 217-234.

STRAYER D. L., MAY S. E., NIELSEN P., WOLLHEIM W. & HAUSAM S. (1997) Oxygen, organic matter, and sediment granulometry as controls on hyporheic animal communities. Arch. Hydrobiol. 140, 131–144.

STRUCKMEIER F.W., GILBRICH W.H., GUN J.V.D., MAURER S., PURI S., RICHTS A., WINTER P. & ZAEPKE M. (2006) WHYMAP and the groundwater resources map of the world at the scale of 1:50,000,000. Special ed. on for the 4th world water forum. Mexico City, March 2006. BGR Hannover/UNESCO, Paris.

THULIN B. & HAHN H.J. (2008) Ecology and living conditions of groundwater fauna. In: Stockholm: Technical Report (TR-08-06) of the Svensk Kärnbränslehantering (SKB).

TOMLINSON M., BOULTON A. J., HANCOCK P. J. & COOK P. G. (2007) Deliberate omission or unfortunate oversight: Should stygofaunal surveys be included in routine groundwater monitoring programs? Hydrogeol. J. 15(7), 1317–1320.

TOMLINSON M., BOULTON A.J. (2009) Subsurface groundwater dependent ecosystems: a review of their biodiversity, ecological processes and ecosystem services. In: Commission, N.W. (Ed). Waterlines Occasional Paper No. 8, National WaterCommission, Canberra.

WARD J. V. & PALMER M. A. (1994) Distribution patterns of interstitial freshwater meiofauna over a range of spatial scales, with emphasis on alluvial river-aquifer systems. Hydrobiologia 287(1), 147–156.

WENDLAND F., BLUM A., COETSIERS M., GOROVA R., GRIFFIOEN J., GRIMA, J., HINSBY K., KUNKEL R., MARANDI A., MELO T., PANAGOPOULOS A., PAUWELS H., RUISI M., TRAVERSA P., VERMOOTEN J. S. A. & WALRAEVENS K. (2008) European aquifer typology: a practical framework for an overview of major groundwater composition at European scale. Environ. Geol. 55, 77–85.

3 Ergebnisse und Diskussion

3.1 Die Untersuchung lokaler und regionaler Effekte auf die Invertebratengemeinschaften quartärer Lockergesteinsleiter im Erftgebiet (Nordrhein-Westfalen)

3.1.1 Zusammenfassung

Das Erftgebiet wurde für die Untersuchungen ausgewählt, um die Verbreitungsmuster der Grundwasserfauna exemplarisch für die lokale Ebene, auf der eine qualitative Bewertung der Grundwasserqualität erfolgen kann, zu analysieren. Gleichzeitig konnte der Aspekt der regionalen Ebene bei den Untersuchungen berücksichtigt werden, da die untersuchten Grundwassermessstellen in zwei hydrologisch unabhängigen Grundwasserleitern liegen.

Die oberflächennahen quartären Lockergesteinsleiter des Erftgebiets, ca. 20 km südlich von Köln, sind den Einflüssen einer intensiven Landnutzung ausgesetzt. In der Regel geht Landnutzung mit einem erhöhten Oberflächeneinfluss auf das Grundwasser einher, durch den sowohl Schadstoffe als auch grundwasserfremde Organismen ins Grundwasser gelangen können. Das stark landwirtschaftlich genutzte und dicht besiedelte Gebiet wurde ausgewählt, um exemplarisch das Indikatorpotenzial der Grundwasserfauna hinsichtlich lokaler Landnutzungseffekte zu analysieren. Zu diesem Zweck wurden im quartären Lockergestein insgesamt 20 Grundwassermessstellen im Frühjahr und Herbst 2007 untersucht. Regional betrachtet, sind die Messstellen auf zwei Lockergesteinsleiter (Erft- und Rurscholle) verteilt, die hydrologisch teilweise getrennt sind. Der Vergleich der Invertebratengemeinschaften im Grundwasser der Erft- und Rurscholle ergab jedoch keine signifikanten Unterschiede. Dieses Ergebnis weist darauf hin, dass ein hydrologischer Austausch zwischen den beiden angrenzenden Lockergesteinsleitern besteht.

Die Datensätze wurden auf bestehende Korrelationen zwischen faunistischen Parametern, wie der Taxazahl, der Abundanz und dem Verhältnis stygobionter zu nicht-stygobionter Arten mit abiotischen Kriterien wie Nitrat, Sauerstoff und organischem Material sowie mit bakteriellen Parametern getestet.

Die Ergebnisse zeigen signifikante Unterschiede zwischen Messstellen, die von Stygobionten bzw. von Nicht-Stygobionten dominiert wurden. Dabei unterlagen die Messstellen, die überwiegend von Nicht-Stygobionten besiedelt waren, offensichtlich einem ausgeprägten hydrologischen Oberflächeneinfluss. In diesen Messstellen wurden deutlich höhere Nitratkonzentrationen gemessen, im Vergleich zu Messstellen, in denen Stygobionte die Gemeinschaften dominierten. Weiterhin wurden positive Korrelationen zwischen Sauerstoff, bakterieller Abundanz und Detritus mit dem Auftreten nicht-stygobionter Arten gefunden. Diese Zusammenhänge zwischen der Fauna, den bakteriellen Gemeinschaften und der Abiotik resultieren wahrscheinlich aus dem stärkeren Oberflächeneintrag ins Grundwasser, der eine Folge von intensiver Landnutzung ist. Wie auch in anderen Grundwasserstudien beobachtet, wurden insgesamt nur wenige belastbare Korrelationen zwischen der Grundwasserfauna und abiotischen Parametern gefunden. Während Sauerstoff, Detritus und vermutlich auch die bakterielle Abundanz einen direkten Einfluss auf die faunistische Zusammensetzung haben, ist ein direkter Effekt von Nitrat auf die Fauna bisher nicht bekannt.

Weiterhin zeigen die Daten, dass die von Oberflächenwasser beeinflussten Grundwasserhabitate, mit einem hohen Anteil nicht-stygobionter Arten, heterogener besiedelt sind als Messstellen, die überwiegend Stygobionte aufweisen. Letztere konnten in zwei Untergruppen (Stygobionte 1, Stygobionte 2) unterteilt werden, wobei angenommen wird, dass hier Substrat-unterschiede eine Rolle spielen.

Die Daten zeigen, dass faunistische Kriterien bei der Qualitätsbewertung von Grundwasser generell anwendbar sind. Dabei ist der Einfluss von

Oberflächenwasser ein treibender Faktor für die faunistischen Besiedlungs muster. Die fehlenden bis schwachen Korrelationen zwischen der Fauna und organischem Material (z. B. geschätzter Detritus, Gesamtkohlenstoff) weisen auf einen Optimierungsbedarf bei der methodischen und analytischen Erfassung hin.

3.1.2 The potential use of fauna and bacteria as ecological indicators for the assessment of groundwater quality

3.1.2.1 Abstract

The use of ecological criteria for the assessment of aquatic ecosystem status is routine for surface waters. So far no ecological parameters are considered for the assessment and monitoring of groundwater quality. It has been well known for decades that aquifers are ecosystems harbouring a vast diversity of invertebrates and microorganisms. The growing knowledge on groundwater microbial and faunal communities as well as the molecular and statistical tools available form a solid ground for the development of first ecologically sound assessment schemes. The sensitivity of groundwater communities towards impacts from land use and surface waters is exemplarily demonstrated by a data set of two geologically similar but hydrologically partially separated aquifer systems. Subgroups of the fauna in groundwater (stygobites vs. stygophiles and stygoxenes) successfully indicated elevated nitrate impacts linked to land use activities. Within the microbial communities, impacts from land use are mirrored by high bacterial biodiversity values atypical for pristine groundwater of comparable systems. The data show that there is legitimate hope for the application of ecological criteria for groundwater quality assessment in the future.

3.1.2.2 Environmental impact

Aquifers are hidden ecosystems. Compared to surface waters, groundwaters have received little attention from an ecological point of view. Today, these most important drinking water reservoirs are subject to frequent and ongoing threats caused by the introduction of contaminants and overexploitation. In the long-term, these anthropogenic impacts will not only affect groundwater quality but will also impair ecosystem functioning. Since biological activities of groundwater organisms provide water purification and storage, groundwater quality highly depends on ecosystem health. It is time to think about ecological criteria for the assessment of groundwater quality, since for surface waters this is routine for decades. In this study the sensitivity of biological variables (microbial and faunal) to environmental impacts was evaluated, constituting a first step towards an integrative groundwater assessment scheme.

3.1.2.3 Introduction

In Europe, drinking water originates, to a large extent, from groundwater. Safeguarding drinking water resources thus necessarily means protection of groundwater ecosystems. Besides the service of drinking water provision, groundwater ecosystems sustain the integrity of most surface aquatic and terrestrial ecosystems. Consequently, all ecosystems connected to aquifers depend on groundwater in sufficient quality and quantity. Groundwater quality issues are almost exclusively addressed by abiotic, physicochemical criteria (TOMLINSON *et al.*, 2007. These are complemented only by routine bacteriological tests (plate counts) to check for the putative presence of pathogenic microorganisms. There are several quality and quantity aspects related to groundwater which cannot be addressed satisfactorily by abiotic criteria alone (HANCOCK *et al.*, 2005; BOULTON *et al.*, 2008; DANIELOPOL *et al.*, 2008; TOMLINSON & BOULTON, 2009). Since the anthropogenic stressors can be physical (e.g. temperature), chemical (e.g. pollutants), and biological (e.g. pathogens), the assessment of ecosystems should consider all of them. Moreover, physicochemical changes directly impact the biological compartment of an ecosystem with a back coupling. An integrative assessment thus needs a set of abiotic and biotic criteria to assess the environmental status, to monitor trends over time, to provide early warning of ecosystem deterioration, and to diagnose the cause of an existing impact (CAIRNS *et al.*, 1993). Fortunately, the increasing public and political recognition of ecological sustainability (e.g. QUEVAUVILLER, 2005) clearly calls for the consideration of ecological aspects in modern monitoring schemes, triggering a paradigm change in groundwater management. The use of organisms as bioindicators has a long-standing history and tradition in surface waters. With a delay of several decades, 'biological criteria' have made their way into most national and international water-related directives. A powerful example is the European Water Framework Directive (EC-WFD, 2000), in which several biological indices form an important basis for the assessment of water quality and ecosystem status. Although continental groundwater ecosystems are the world's largest freshwater domain, being essential reservoirs for drinking water, agriculture and industry, they have not received comparable attention so far. Indeed, there is no doubt that aquifers are ecosystems (HUMPHREYS, 2006) inhabited by a vast diversity of microorganisms (GOLDSCHEIDER *et al.*, 2006; GRIEBLER & LUEDERS, 2009) and groundwater fauna (DEHARVENG *et al.*, 2009). It is exactly the living component of the aquifers that are responsible for groundwater purification and storage (HERMAN *et al.*, 2001; BOULTON *et al.*, 2008; TOMLINSON & BOULTON, 2009). In the recent past, it has been frequently argued that a paradigm shift in groundwater management is due (QUEVAUVILLER, 2005). In fact, new integrative assessment schemes and groundwater management concepts, based on the knowledge and experience from decades of ecological research are available. But yet it turns out difficult to implement these measures because of significant uncertainties about the distribution, taxonomy and ecology of groundwater microbes and fauna. Still, sensitive ecological parameters and reliable bioindicators await identification. Moreover, a multitude of sampling techniques is available but standardised protocols are lacking (TOMLINSON *et al.*, 2007, BOULTON *et al.*, 2008; DANIELOPOL *et al.*, 2008). Due to the different organismic repertoire and environmental constraints, existing surface water protocols and indices cannot just be transferred and adapted for aquifers. They need to be carefully developed and complemented. Without doubt groundwater communities have the potential to function as indicators of ecosystem health and water quality (BOULTON *et al.*, 2008, TOMLINSON & BOULTON, 2009). Organisms integrate impacts over space (their habitat) and time, extending the information obtained from abiotic monitoring. Groundwater fauna is suggested to be very sensitive to environmental changes. This is mainly related to the relatively stable environmental conditions prevailing in aquifers. And indeed, a number of studies have demonstrated the applicability of fauna for monitoring purposes related to groundwater quality. Groundwater invertebrates have already been applied so far in the evaluation of surface water infiltration to aquifers (DUMAS *et al.*, 2001; HAHN, 2006; MALARD *et al.*, 1996; SCHMIDT *et al.*, 2007; BERKHOFF *et al.*, 2009; BORK *et al.*, 2009). There have been studies performed on the impact of organic nutrients and pollution on groundwater (MALARD *et al.*, 1996; NOTENBOOM *et al.*, 1995; SCARSBROOK *et al.*, 2003) oxygen and redox conditions (MÖSSLACHER, 1998; MÖSSLACHER, 2000), sediment structure and porosity (MÖSSLACHER, 1998; PARAN *et al.*, 2004) and biogeographical aspects (WARD & PALMER, 1994). Moreover, stygofauna have been explored as biomonitors of heavy metal contamination (CANIVET *et al.*, 2001), as well as heat discharge to an aquifer (BRIELMANN *et al.*, 2009). In contrast to the patchy distribution of invertebrates, microorganisms are omnipresent in groundwater and aquifers. Nevertheless, so far groundwater microorganisms and/or microbial measures such as microbial biomass, activity and diversity have hardly been considered as water quality criteria (Griebler *et al.*, 2010; Promk *et al.*, 2009, Steube *et al.*, 2009). Following the line of argument that microbes are involved in almost every geochemical process occurring in the subsurface and are therefore directly linked to prevailing abiotic conditions it seems worthwhile to consider them for the development of integrated assessment schemes, complementing the assessment of stygofauna. Moreover, microbes are characterised by short generation times and comparably high metabolic rates providing a fast reaction to environmental changes, while the stygofauna is predestined for time-integrated patterns because of their longevity. The central working hypothesis is that in low dynamic habitats microbial communities nicely reflect the in situ environmental conditions. In this sense, the abundance, biomass, and structure of microbial communities and their physiological status (activity) may serve as sensitive ecological criteria for short-term dynamics in aquifers. The general applicability of microbes as indicators for the assessment of aquatic ecosystem change has been proven for certain aspects (PEARL *et al.*, 2003; SOLÉ *et al.*, 2008; LEAR *et al.*, 2009). Within the framework of a current German research project funded by the Federal Environmental Agency (UBA) several aquifer systems located in geographically distinct areas of Germany are being investigated. The focus of the project is to develop an ecologically sound and integrative concept for the assessment of groundwater quality and ecosystem health. This attempt is distinguished by several steps including (i) the selection of appropriate biological and ecological parameters, (ii) the typology of groundwater ecosystems from an ecological point of view, (iii) the derivation of natural background values of biological parameters and definition of a reference status (''Leitbild'') for aquifers, (iv) the identification of potential bioindicators and deduction of threshold values, and, (v) the development of an assessment model. In particular, this paper intents to discuss difficulties and perspectives in using groundwater fauna and bacteria as criteria for water quality and ecosystem health. Groundwater fauna and bacterial communities' data from two geologically similar aquifer systems (the Rur and Erft block) impacted by extensive land use (agriculture) are exemplarily exercised. A selected set of physicochemical variables was analysed in comparison to the abundance and diversity of fauna and bacteria with respect to spatiotemporal similarities and differences.

3.1.2.4 Materials and methods

Sampling area

Data presented originate from two sampling surveys in the area of the town of Euskirchen located southwest of Cologne in North Rhine Westphalia, Germany (Fig. 1). The aquifers in that region belong to the hydrogeological unit termed 'The Sands and Gravels of the Lower Rhine'; see the classification by KUNKEL *et al.*, 2004). The area further belongs to the bioregion of 'central lowland' (Zentrales Flachland, ILLIES, 1978) and the River Rhine catchment. On a smaller scale it is associated with the natural geographic region (Naturraum) 'Kölner Bucht' (Bay of Cologne). Although part of one huge aquifer type, the investigation area is separated into various hydrogeological subunits (blocks) formed by Tertiary and Quaternary tectonic movements (SCHLIMM, 1988) (Fig. 1). Two of these blocks, the Rur and Erft block, have been investigated in this study. At each of these blocks ten wells ranging from 5 to 31 meters in depth were sampled in spring (April 2007) and autumn (October 2007). At the Rur block, three wells grouped together on a local scale (distance < 1 km; referred to as lumped wells) and seven wells distributed over a regional scale (≥ 1 km; referred to as dispersed wells). At the Erft block, the wells grouped into five lumped wells and five wells regionally dispersed. All wells were situated in the shallow Quaternary aquifer.

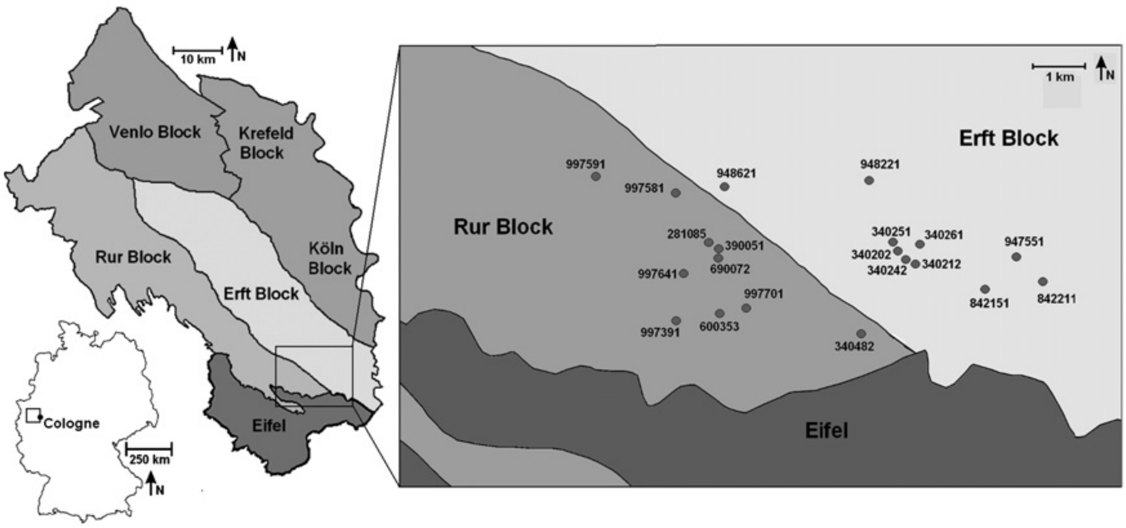

Fig. 1: Study area southwest of Cologne, Germany. The area is separated in various blocks. 10 groundwater monitoring wells were investigated in the southern parts of the Rur and Erft block, respectively. All wells are situated in the shallow Quaternary porous aquifer.

Groundwater sampling

The selected sites were sampled in a different way for fauna and microorganisms. The faunistic samples along with sediment and detritus were extracted from the monitoring wells using a phreatic net sampler (HAHN & MATZKE, 2005). Previous studies have shown that the groundwater fauna inside the well is representative of the adjacent aquifer (DUMAS & FONTANINI, 2001; HAHN & MATZKE, 2005). To examine possible correlations between the groundwater fauna and prevailing physicochemical conditions at the monitoring well, well water was collected using a bailer (Bürkle Aquasampler 0.75 L) and subsequently bottled into prepared vials. The water was sampled close to the bottom of the well, right before the faunal collection had occurred. After having sampled fauna, well water and sediment, a submersible pump (MP1, Grundfos Corp.) was used to replace the well water and then to pump aquifer water according to the guidelines of the German Industrial Norm (DIN 38402-A 13, 1985). Aquifer water was filled into appropriate glass and plastic bottles for further analyses of physicochemical and microbiological parameters. Samples were filtered on-site and/or preserved and transported to the lab and stored under cooled conditions (4°C) until further processing.

Physicochemical analyses

General physicochemical parameters, such as temperature, electric conductivity (EC), pH, redox potential (EH), and dissolved oxygen (DO) were measured in water from the aquasampler as well as from pumped groundwater using field sensors (WTW, Weilheim, Germany). Well water and aquifer water samples were filtered through a 0.45 µm pore size filter (Millipore) for analyses of major ions and soluble reactive phosphate (SRP) while TOC measurements included particle ≤ 200 µm. Major anions (chloride, nitrate, sulfate) and cations (calcium, magnesium, potassium, sodium, ammonium) were measured by means of ion chromatography (Dionex Model DX 100). TOC was determined using high temperature combustion with infrared detection of CO_2 (Shimadzu, TOC-5050). Orthophosphate was analysed colorimetrically as soluble reactive phosphorus (SRP) by the ammonium-molybdate method according to MURPHY & RILEY, 1962). Additionally, the relative amount of detritus (particulate organic matter), as part of the sediment sample collected from the well bottom, was estimated via an ordinal scale following Hahn (2006). Stable isotopes of oxygen ($^{18}O/^{16}O$) and hydrogen ($^{2}H/^{1}H$) were determined by isotope ratio mass spectrometry. The $\delta^{18}O$ values in samples were analysed via equilibration with CO_2 at 25°C for 24 h (EPSTEIN & MAYEDA, 1953) and for d^2H values via reaction with Cr at 850°C (COLEMAN *et al.*, 1982). Both, $\delta\,^{18}O$ and $\delta\,^{2}H$ values, were determined relative to internal standards that were calibrated using IAEA SMOW standards. Data were normalised following COPLEN (1988) and are expressed relative to V-SMOW. Samples were measured at least in duplicates with a precision of ± 0.1 ‰ for $\delta^{18}O$ and ± 1.0 ‰ for $\delta^{2}H$.

Analyses of groundwater fauna

Prior to further processing, fauna samples were fixed with 4 % formaldehyde. In the laboratory the organisms were separated from sediments, counted and pre-sorted into taxonomic groups (crustaceans, oligochaets, water mites, nematodes, turbellarians). All crustacean groups, which constitute the major part of the animals in groundwater samples, and oligochaets were determined to species level, whilst the remaining taxonomic groups were identified to order or higher level. For statistical analyses the crustaceans and oligochaets were distinguished into two ecological groups. One group comprising obligate groundwater organisms referred to as stygobites. The other group is characterised by non-stygobites including organisms frequently found in groundwater and able to survive considerable times in aquifers (stygophiles) as well as foreigners to aquifers originating from surface waters or soil (stygoxenes).

Microbiological analyses

Total bacterial counts were determined from 15 mL of glutardialdehyde fixed (2 % f. conc.) sample aliquots by epifluorescence microscopy of SYBR Green I (Molecular Probes) stained cells following a protocol of GRIEBLER *et al.* (2001). For microbial community structure analysis, approximately 2.5 L of groundwater was filtered through 0.22 µm sterile membrane filters (Neolab). Filters were subsequently frozen and maintained at -20°C. Later, freshly thawed filters were aseptically cut into small pieces (c. 2 mm^2), which were then transferred into beadbeating cups and total DNA was extracted as described in WINDERL *et al.* (2008). After extraction and precipitation, DNA pellets were resuspended in 25 µL of EB buffer (10 mM Tris-HCl [pH 8.5], Qiagen) and stored frozen (-20°C) until further analyses. T-RFLP analysis of bacterial 16S rRNA gene amplicons was performed as described previously (WINDERL *et al.* 2008) with the primers Ba27f-FAM/907r and MspI digestion. Primary electropherogram analysis was conducted using the GENEMAPPER 5.1 software (Applied Biosystems) excluding peaks < 50 bp. Identification of baseline threshold of true peaks over noise and the alignment of terminal restriction fragments (T-RFs) were conducted with the help of the T-REX software.47 The software implements a procedure developed by ABDO *et al.* (2006) where true peaks are iteratively identified as those whose height exceeds (in our case one) standard deviation computed over all peaks. This way, T-RFs with a relative fluorescence signal of ≤ 100 were excluded from further analysis. Peak alignment in T-REX can be done automatically (SMITH *et al.*, 2005). A clustering threshold of ± 0.5 bp was specified for the grouping of peaks into a common T-RF. The Shannon–Wiener index H′ was calculated as $H' = -\sum p_i \ln p_i$, whereas p_i is the relative abundance of single T-RFs in a given fingerprint (HILL *et al.*, 2003). The reproducibility of our workflow and of T-RFLP analysis was exemplarily verified via replicate fingerprinting analyses of duplicate DNA extracts from 36 of the 38 samples collected during the two campaigns. AMMI analysis (Additive Main Effects and Multiplicative Interaction model, GAUCH 1992) within T-REX suggested high reproducibility of replicate fingerprints (pattern: 90.6 % of total interaction, noise: 9.4 % of total interaction).

Statistical analyses

The conducted sampling approach resulted in two environmental datasets (well water and aquifer water) comprising each 16 quantitative physicochemical variables and two quantitative isotopic variables: water temperature, pH, EC, EH, DO, TOC, orthophosphate, major anions (HCO_3^-, Cl^-, NO_3^-, SO_4^{2-}), major cations (Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+), $\delta^{18}O$, and $\delta^{2}H$. For statistical analyses, variables were preliminarily log-normalized (except for pH) adding a translation constant when necessary and z-score standardised, resulting in dimensionless quantitative variables. In addition, two sets of biotic population data were generated: bacterial community data were expressed as relative abundance of detected T-RFs, while the counts of recovered faunal populations in wells were fourth root-transformed (CLARKE & WARWICK, 1994). Several statistical analyses were performed to search for patterns in bacterial community structures and faunal assemblages in relation to the hydrogeolocial subunits, season and the spatial distribution of groundwater wells. In addition, they were applied to attribute community patterns to the measured environmental variables. Generally, the analyses of faunal assemblages and bacterial communities were related to the physicochemical datasets of the groundwater, since no significant differences between well water and groundwater were found. To assess the trophic conditions at the well bottom, which constitutes the food basis for the stygofauna, faunal data were additionally compared with TOC values and the relative amount of detritus of well sediment. In a first step, the correlation between the two environmental datasets (well water, aquifer water) was evaluated by a Mantel Test, a multivariate permutational procedure (MANTEL & VALAND, 1970). Principal component analysis (PCA) was conducted for each hydrogeological subunit (Rur and Erft block) to reduce the measured abiotic variables to meaningful components describing the major physicochemical and isotopic gradients encountered in the field. The impact of the two hydrogeological subunits (Rur and Erft block), seasons (spring and autumn), and the distribution of wells (lumped and dispersed) on the aquifer water dataset was tested with ADONIS (multivariate analysis of variance based on dissimilarity matrices, ANDERSON, 2001) based on Euclidian distances. Patterns in the bacterial community structure and faunal assemblages were explored by Nonmetric Multi Dimensional Scaling (referred to as MDS) based on Bray-Curtis dissimilarities. Additionally, for bacterial community data the impact of the two hydrogeological subunits, seasons, and the distribution of wells was also tested with ADONIS while for the faunal assemblages this was evaluated by the ANOSIM (analysis of similarities) procedure (CLARKE & GREEN, 1988). Correlations of faunal data and physicochemical data were analysed by the Spearman rank test. Mann-Whitney U-test was used to detect differences of physicochemical parameters between wells dominated by stygobites and non-stygobites. Moreover, canonical correspondence analysis (CCA), a direct constrained ordination method that uses linear combinations of environmental (explanatory) variables to optimally explain the observed variance in T-RFs and faunal species (LEGENDRE & LEGENDRE, 1998) were applied. All statistical analyses were performed either with the R software, version 2.8.1, using the packages MASS (VENABLES & RIPLEY, 2002), reshape 0.8.0 and vegan 1.8–8 (for the latter package the recommendations of the author were followed, OKSANEN, 2008), or with Primer v6, Plymouth Laboratories, Plymouth, UK) or SPSS v15 (SPSS Inc.).

3.1.2.5 Results

Physicochemical characteristics

The composition of well water and aquifer water correlated significantly ($r = 0.805$, $p = 0.001$, Mantel Test) in its physicochemical conditions. In Fig. 2 selected physicochemical parameters are separately depicted for the Erft and Rur block, distinguished into lumped and dispersed wells as well as spring and autumn samples. Multivariate Analysis of Variance (ADONIS) revealed small but highly significant differences in abiotic variables for the two blocks ($R^2 = 0.13$, $p = 0.005$). Differences between the Rur and Erft block were detected especially for DO and nitrate. At the Rur block groundwater from two wells, i.e. 997391 and 281085, were characterised by reducing conditions, as can be seen from the absence of DO and nitrate (Fig. 2). Moreover, groundwater from a few other wells displayed low DO concentrations ≤ 4 mgL^{-1} (Fig. 2). With exception of the two wells yielding reduced groundwater, sites of both blocks showed nitrate concentrations distinctly elevated and exceeding by far the natural background values (NBV) described for this groundwater landscape (KUNKEL *et al.*, 2004). In case of EC, only water of well 390051 considerably exceeded the NBV accompanied by the highest $\delta^{18}O$ values (Fig. 2). TOC values ranged from 0.7 to 1.5 $mg\,L^{-1}$ (data not shown) and concentrations of soluble reactive phosphate (SRP) were generally below 50 mg L^{-1} with up to 180 mg L^{-1} in individual samples (Fig. 2).

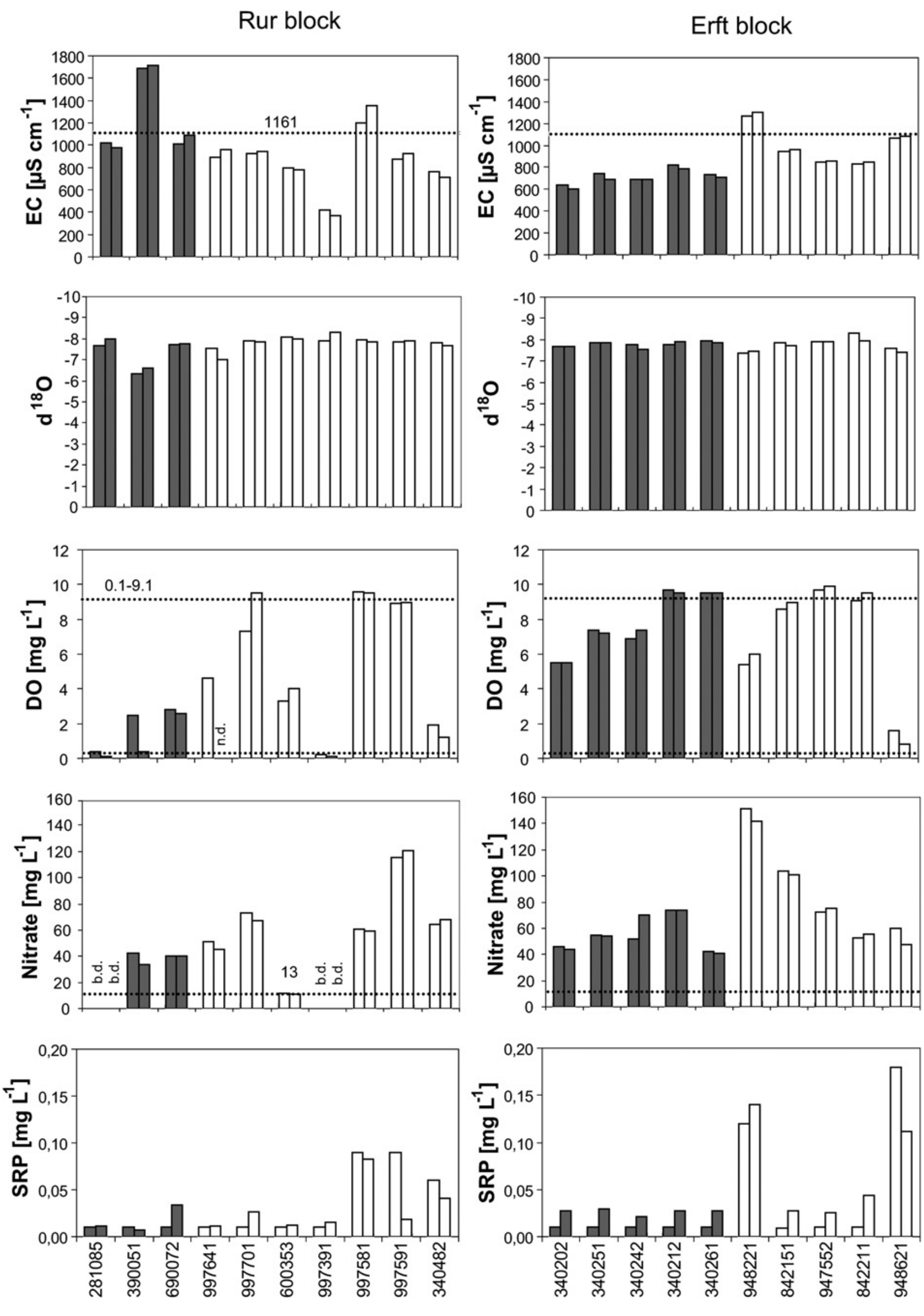

Fig. 2: Selected physicochemical parameters determined in groundwater from wells distributed over the Rur and the Erft block, respectively. The first bar of each pair depicts the spring (April 2007) and the second the autumn (October 2007) measurements. Grey bars represent locally lumped and white bars regionally dispersed wells. Dashed lines and nearby values give the mean natural background values as defined by Kunkel *et al.*, 2004. EC: electrical conductivity, $\delta^{18}O$: in water, DO: dissolved oxygen, SRP: soluble reactive phosphate.

The physicochemical composition of groundwater from individual wells did not display any significant variation with season (spring and autumn) ($R2 = 0.01$, $p > 0.9$, ADONIS) (Fig. 2). When the groundwater data is pooled for lumped and dispersed wells, the abiotic data was found dissimilar in both, the Rur block ($R2 = 0.2$, $p = 0.01$, ADONIS) and the Erft block ($R2 = 0.31$, $p = 0.005$, ADONIS). For a further evaluation of these detected differences, PCA were conducted for the abiotic datasets of the two blocks individually (Fig. 3). For the Erft block, groundwater samples evidently clustered into two groups (lumped/dispersed), separated mainly along the PC1-axis. PC1 explained 59 % of the total variance and correlated with bicarbonate (-0.497) and calcium (-0.387). PC2 accounted for another 32 % of the total variance and correlated mainly with DO (-0.674) (Fig. 3). For the Rur block, the distinction between groundwater samples from lumped and dispersed wells was less clear (Fig. 3). However, two of the lumped wells separated from all the other samples (in spring and in autumn) along PC2 that explained 27 % of total variance and correlated with nitrate (-0.555), sodium (0.442) and bicarbonate (0.355). However, the main part of total variance was explained by PC1 (59 %) strongly associated with nitrate (-0.621) (Fig. 3). The PCA plots support the results from ADONIS separating the lumped wells from the dispersed ones. Further, at the Erft block the physicochemical conditions of the lumped wells were more similar when compared to the dispersed ones, while at the Rur block the lumped wells displayed a considerably high variation (Fig. 3).

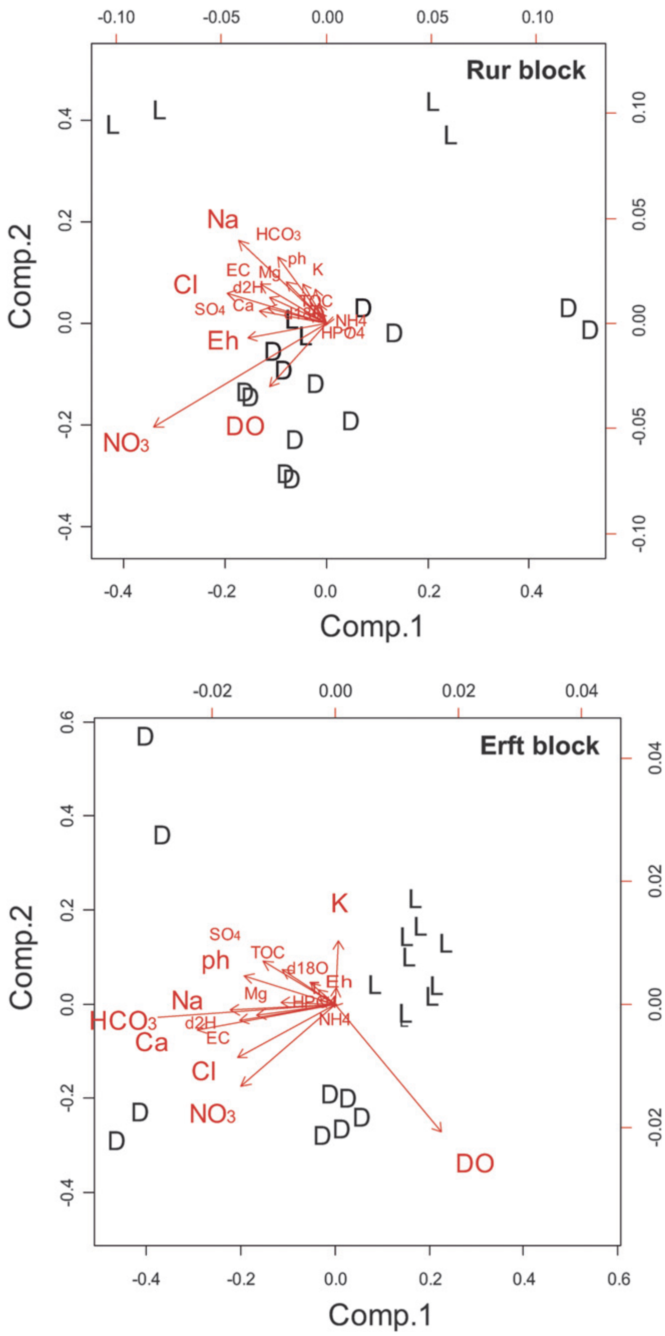

Fig. 3: Principal component analysis (PCA), performed for the Rur and the Erft block, taking into account selected physicochemical parameters in relation to the spatial distribution of the sampled wells, either locally lumped (L) or regionally dispersed (D).

Diversity, abundance and distribution patterns of groundwater fauna

In total, 22 species comprising crustaceans (73 %) and oligochaets (27 %) as well as five higher metazoan taxa (acari, insects, nematodes, macro-, and microturbellarians) were found in the 20 monitoring wells distributed to the Erft and Rur block, respectively. In all sampling sites metazoans were present, but at five wells invertebrates were found at only one sampling occasion, either spring or autumn. Amongst the metazoans that were determined to species level (crustaceans and oligochaets), 10 stygobiontic and 12 non-stygobiontic species were identified (Tab. 1). Most species and taxa (> 50 %) occurred in low frequencies and were found in less than three wells. *Diacyclops languidoides* (Cyclopoida), as the most abundant species, was only found in six wells. No faunal differences were found between the wells of the Erft and the Rur block (Tab. 1) (ANOSIM $R = 0.089$). According to the faunal composition of the monitoring wells, two ecological groups, either dominated by non-stygobiontic (stygophiles and stygoxenes) or stygobiontic species, could be distinguished (Fig. 4A, ANOSIM $R = 0.467$). Furthermore, the monitoring wells harbouring stygobiontic biocoenoses can be divided in two subgroups. One subgroup, referred to as stygobiontic 1 in the MDS-plot (wells 948621, 997641) was dominated by the copepod *Diacyclops languidoides* (Cyclopoida), while amphipods (*Niphargus aquilex*, *Niphargellus nolli*, *Crangonyx subterraneus*) were the prevailing organisms in the other subgroup, labelled as stygobiontic 2 (wells 947552, 842211, 390051, 340482) (Fig 4A). In general, the wells dominated by non-stygobiontic species were characterised by a relatively heterogeneously composed community, resulting in the scattering observed in the MDS-plot (Fig. 4A).

Table 1: Taxa-well-matrix of metazoan species recorded in groundwater monitoring wells at the Erft and Rur block. Data include samples of two sampling occasions (April and October 2007). Only faunal taxa that could be classified to ecological groups are shown.

		Erft block										Rur block									
	Ecological group	**340251**	**340202**	**340242**	**340212**	**842211**	**842151**	**947552**	**340261**	**948221**	**948621**	**340482**	**997581**	**281085**	**390051**	**690072**	**600353**	**997701**	**997391**	**997641**	**997591**
Group	**Stygobites**																				
Amphipoda	*Niphargus aquilex*				2						2										
	Niphargus spec.																			2	
	Crangonyx subterraneus										7									3	
	Niphargellus nolli										10									1	
Cyclopoida	*Acanthocyclops sensitivus*																			35	
	Diacyclops languidoides			3		182		65				89			18					2	
Harpacticoida	*Bryocamptus typhlops*									2											
Syncarida	*Bahtynella freiburgensis*																				3
	Pseudantrobathynella husmanni								1												
Ostracoda	*Fabaeformiscandona breuili*									3											
	Fabaeformiscandona spec.									5	1										
	Non-Stygobites																				
Cyclopoida	*Diacyclops bicuspidatus*				11																
	Diacyclops bisetosus			23						251	1	1									5
	Diacyclops languidus							1												16	1
	Paracyclops fimbriatus																	14			143
Ostracoda	*Pseudocandona albicans*									1											
Oligochaeta	*Amphichaeta leydigii*									1						11					
	Cernovsvitoviella atrata						5		9	1						1					
	Dorydrilus michaelseni					12		1													
	Haplotaxis gordioides													1							9
	Marionina riparia																				8
	Mesenchytraeus armatus																				7

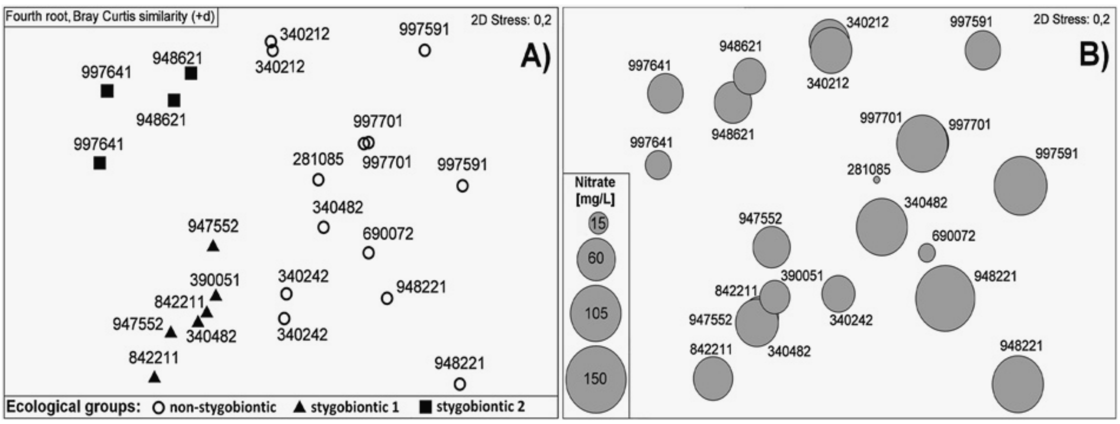

Fig. 4: (A) MDS (Multidimensional scaling) of metazoan assemblages (mean values) recorded in groundwater monitoring wells at two sampling occasions in April and October 2007. Only wells populated by metazoan taxa, which could be classified to ecological groups, are included. (B) MDS overlaid by concentrations of nitrate.

Bacterial community patterns

Mean values of bacterial abundance (BA) differed within the two blocks investigated. Groundwater from the Rur block aquifer contained $5.7 \pm 4.5 \times 10^4$. bacterial cells mL^{-1} and $4.8 \pm 5 \times 10^4$ bacterial cells mL^{-1} in spring and autumn, respectively. The high standard deviations are caused by extraordinary high BA values of individual wells, especially well 390051 (1.6×10^5 in autumn) and 340482 (1.7×10^5 in spring). The Erft block displayed considerable lower numbers with 2.4 ± 0.7 and $2.5 \pm 0.5 \times 10^4$ cells mL^{-1} in spring and autumn (data not shown). Groundwater bacterial communities, as analysed via T-RFLP fingerprinting, displayed no statistical differences between the two blocks ($R2 = 0.039$, $p = 0.055$, ADONIS). When pooling the data of both blocks, no differences were found between spring and autumn samples ($R2 = 0.024$, $p = 0.72$, ADONIS). Similarly, when looking at effects of the season for the individual blocks, no seasonal effects within the Rur block samples ($R2 = 0.042$, $p = 0.78$, ADONIS) and the Erft block ($R2 = 0.044$, $p = 0.88$, ADONIS) were found. This is best illustrated by a Multidimensional scaling (MDS) plot (Fig. 5). Moreover, the MDS analysis revealed a weak but significant difference between bacterial community composition of lumped and dispersed wells; for the Rur block ($R2 = 0.11$, $p < 0.005$) and for the Erft block ($R2 = 0.092$, $p = 0.005$). A total of 611 different T-RFs were identified in 38 groundwater samples, of which 20 were collected in spring and 18 in autumn (for two of the autumn samples no T-RFLP data are available). In spring, 441 different T-RFs were detected for the wells of the Rur block, of which only 2 were found in all of the samples collected (Tab. 2). Eleven out of 441 T-RFs occurred in 9 of the 10 samples and 25 % of the T-RFs were found in 50 % ($n = 5$) of the samples taken. The picture was quite similar with the Erft block samples. Five out of the 433 T-RFs occurred in all samples, already 18 in 9 samples and 27 % of the T-RFs were common to 50 % of the groundwater samples. The patterns with autumn samples were found similar with respect to the individual blocks. Detailed data are summarized in table 2. When looking at the presence and absence of individual T-RFs in the samples of the two blocks distinguished into spring and autumn, changes in the presence or distribution of individual T-RFs are visible (Fig. 6). Clearly, there are a considerable number of common T-RFs, indicating common components of natural bacterial communities in these aquifer systems. The biodiversity within the bacterial communities as expressed by the Shannon-Wiener index (H´) was found considerably high. Bacterial diversity of samples from the Rur block exhibited mean values of H´ = 4.1 ± 0.7 (min 2.75, max 4.82) and 4.1 ± 0.4 (min 3.24, max 4.78) in spring and autumn, respectively. For the Erft block values of H´ = 4.2 ± 0.4 (min 3.66, max 4.70) and 4.0 ± 1.2 (min 3.59, max 4.82) in spring and autumn were found.

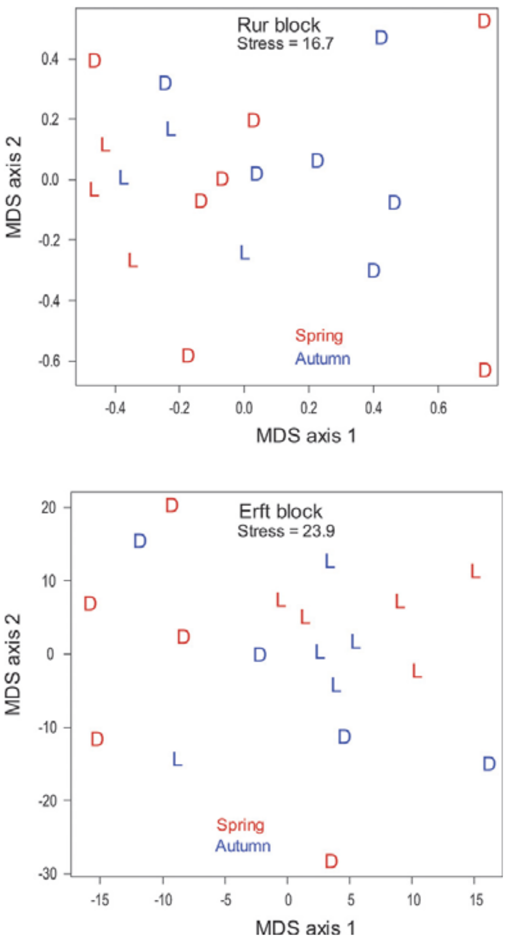

Fig. 5: MDS plot for the bacterial communities (T-RFs) of the two hydrogeological units (Rur and Erft block) discriminating between samples from lumped (L) and dispersed (D) wells. Furthermore, spring and autumn samples are distinguished.

Table 2: Frequency of T-RF occurence in samples as distinguished for the Rur and Erft block and the season. The bottom lines give the total number of T-RFs detected for the individual groups of samples.

No. of wells	*Rur spring*	*Rur autumn*	*Erft spring*	*Erft autumn*
0	170	166	178	181
1	140	153	148	131
2	82	81	72	73
3	62	59	49	64
4	45	56	47	41
5	28	34	34	33
6	35	19	27	33
7	19	22	17	22
8	17	9	16	20
9	11	12	18	13
10	2		5	
total/block	441	445	433	430
total	611	611	611	611

Relationship between chemistry, fauna and microorganisms

According to the data on physicochemical variables a few reasonable but weak correlations with groundwater fauna were found. The nitrate concentrations were positively correlated with the proportion (Spearman: $R = 0.488$, $p = 0.002$, $n = 38$), the taxa richness ($R = 0.524$; $p = 0.001$; $n = 38$) and abundance ($R = 0.516$, $p = 0.001$, $n = 38$) of non-stygobiontic species. Similarly, oxygen was positively correlated with the abundance of nonstygobiontic invertebrates (Spearman: $R = 0.319$, $p = 0.045$, $n = 40$). It was found that nitrate concentrations in groundwater were slightly higher at sites dominated by a non-stygobiontic biocoenosis (Mann-Whitney: $p = 0.041$, $n = 24$), as shown in Fig. 4B. With respect to the non-stygobiontic taxa richness, weak correlations were found also with the relative amount of detritus (Spearman: $R = 0.329$, $p = 0.038$, $n = 40$). Furthermore, the groundwater bacterial abundance (BA) correlated weakly positive with the proportion ($R = 0.374$, $p = 0.017$, $n = 40$), the abundance ($R = 0.331$, $p = 0.037$, $n = 40$) and the taxonomic richness ($R = 0.317$, $p = 0.046$, $n = 40$) of non-stygobiontic invertebrates. Additionally, the extraordinary high EC value at well 390051 was accompanied by a high BA in autumn, and the lowest bacterial diversity was found for well 997391 characterised by the lowest EC values and reduced conditions. Canonical correspondence analyses (CCA) were performed to further evaluate the relationship between selected physicochemical parameters and the presence of T-RFs. The CCA plots show that, for the Rur block, abiotic parameters tend to be correlated with certain sampling sites, and although they do not exhibit very clear patterns, all of them have high explanation power. There are numerous T-RFs which orientate along the gradients formed by orthophosphate and $\delta^{18}O$ (Fig. 7). Moreover, certain T-RFs exhibit a negative correlation with EC. The CCA plot for the Erft block shows more pronounced patterns. Numerous T-RFs orientate along the gradients spanned by individual parameters to certain sampling sites. An example is again the PO_4^{3-} gradient (Fig. 7) which is positively correlated to that of NO_3^- and EC. In a CCA analysis applying the pooled data set of fauna and T-RFs it could be seen that the large majority of T-RFs does not have high explanation power and is not correlated well with the patterns of faunal distribution. While some of the T-RFs were highly correlated with some of the invertebrate species (data not shown) however, the taxa explaining the majority of the variance, e.g. macroturbellarians, nematodes, harpacticoids, could not be related to any ecological group among the microbiological data.

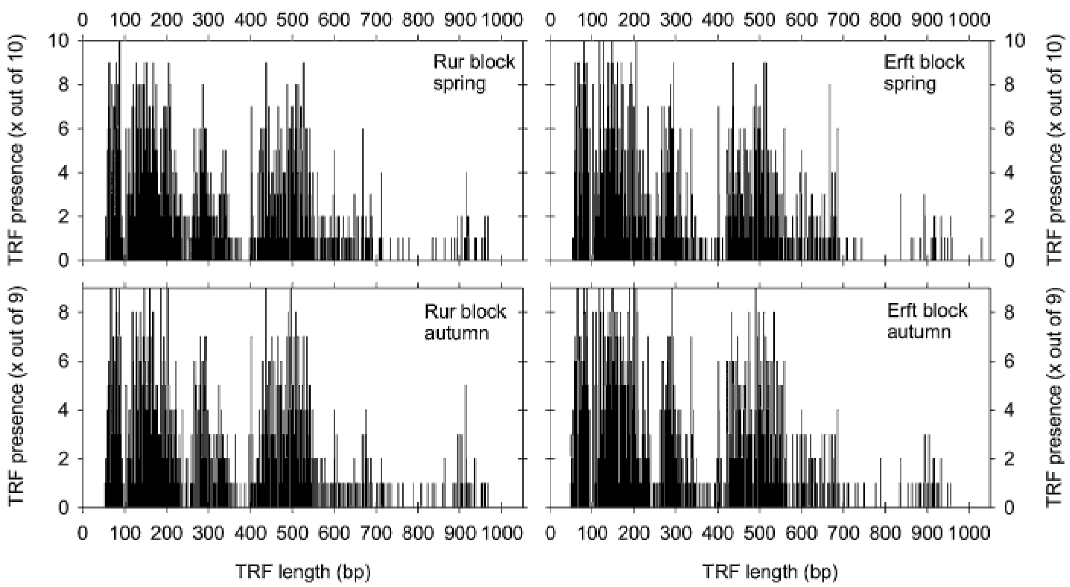

Fig. 6: Frequency of appearance of individual T-RFs in samples from the Rur and Erft block in spring and autumn.

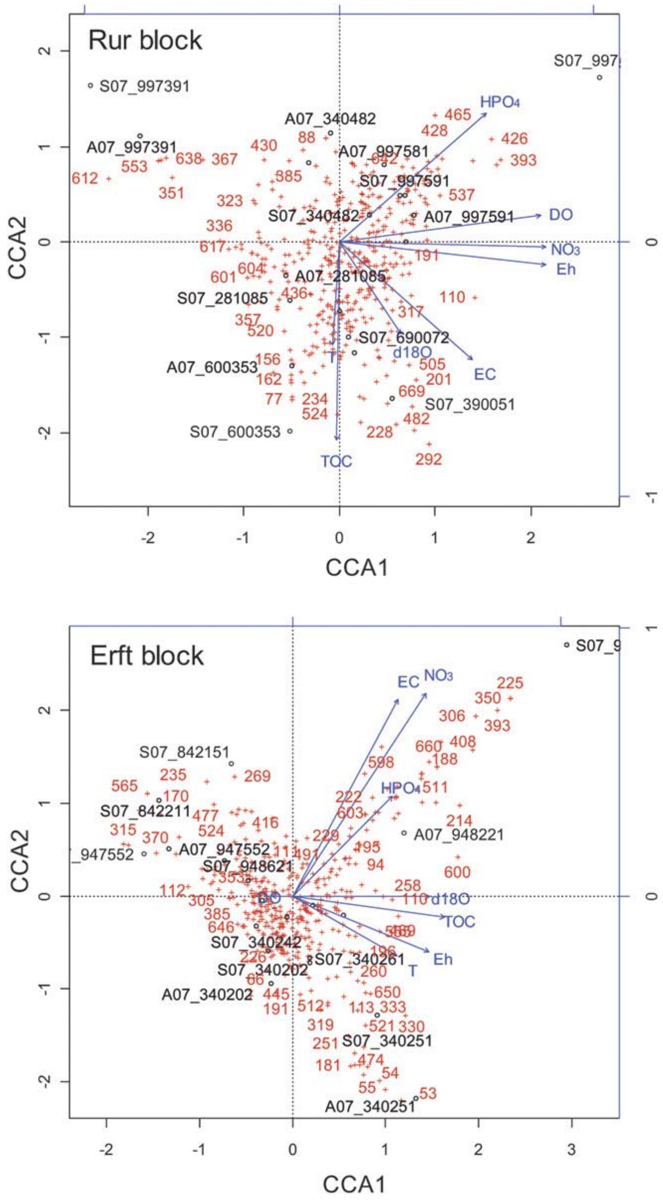

Fig. 7: CCA ordination diagram of bacterial community data for the Rur (top) and Erft block (bottom) against environmental variables. Biplot representing the species (cross symbols), sites (black circles + label) and environment variables (arrows). The length of the arrow indicates the degree of correlation with the presented ordination axes. Axis CCA1 represents 20 % and 19 % of the inertia (weighted variance) for the Rur and Erft block, respectively. Axis CCA2 accounts for 18 % and 17 % of the inertia (weighted variance) for the Rur and Erft block, respectively.

3.1.2.6 Discussion and conclusion

Physicochemical conditions

The Rur and Erft block have a similar geologic background but have been described hydrologically separated (SCHLIMM, 1988). There is some recent evidence that this isolation is incomplete (N. Cremer pers. comm.). Fact is that both blocks generally displayed statistically similar physicochemical patterns. Although for individual parameters such as DO and nitrate differences were found. The investigation area of both blocks is shaped by extensive agricultural land use. While, based on concentrations of total organic carbon (TOC) and orthophosphate (SRP), the shallow Quaternary porous aquifers of the Rur and Erft block would still match the definition of oligotrophic and oligoalimonic conditions, nitrate concentrations clearly rank the aquifers as contaminated; see for comparison proposed nitrate natural background values (NBV) of this groundwater landscape (KUNKEL *et al.*, 2004) (Fig. 2). The lacking correlation between nitrate and electric conductivity (EC) as a sum parameter for the major ions, as well as with individual major ions such as sulfate, chloride and calcium (data not shown) underline that the elevated nitrate concentrations are not a natural component in this system. In future, a combined analysis of $^{18}O/^{16}O$ and $^{15}N/^{14}N$ of nitrate could allow the specific origin of the nitrate detected. Outstanding low nitrate concentrations found at the wells 281085 and 997391 were linked to hypoxic/anoxic conditions and subsequent denitrification. Unfortunately, there is no clear explanation for the anoxic conditions in groundwater of the wells of the Rur block. It could not at all be explained by the TOC values which never exceeded 2 mgL^{-1} in the spring samples. Stable isotope measurements proved a comparable recharge/evaporation history for all waters sampled, with one exception. Groundwater from well 390051 at the Rur block exhibited considerably heavier $\delta^{18}O$ and $\delta^{2}H$ values accompanied by profoundly higher EC values, pointing at a higher degree of mineralization or evaporation.

Groundwater fauna

A comparably low α-biodiversity, small number of specimen and the heterogeneous spatiotemporal distribution of fauna are features typical for pristine groundwater ecosystems (BORK *et al.*, 2009; GALASSI *et al.*, 2009; HAHN & FUCHS, 2009; MARTIN *et al.*, 2009). This picture is confirmed by the data presented. A total of 22 species were found in 20 wells investigated. This 'one well to one species' ratio, seems to be a general tendency when recording regional groundwater biodiversity from a small number of wells and limited sampling surveys. Data from south-western Germany supposed that regional taxonomic diversity is directly correlated with the number of wells sampled, and does not necessarily reflect real subsurface diversity (HAHN & FUCHS, 2009). On the other hand, individual wells occasionally contain an extraordinary high number of specimens. As shown in our study, more than 182 stygobite copepods were found in well 997641 at the Rur block and up to 250 non-stygobite copepods in well 842211 at the Erft block (Tab. 1), underlining the patchy distribution of groundwater fauna and the heterogeneity of aquifers. Exactly this inhomogeneous distribution patterns somehow limits the use of groundwater invertebrates as bioindicators. However, the more data are available the clearer the picture becomes. There is strong evidence, for example, that the irregular distribution of fauna is mainly governed by the availability of organic matter (STRAYER *et al.*, 1997; GIBERT & DEHARVENG, 2002; DATRY *et al.*, 2005). Consequently, influence from land use and from surface waters is a driving factor for shaping fauna in shallow aquifers. Habitats, hydrologically influenced by surface water are characterised by diverse and heterogeneous communities, often dominated by stygophiles and stygoxenes (HAHN & FUCHS, 2009). The invertebrate communities found in the aquifers of the Rur and Erft block could be distinguished into two main groups of which one was characterised by a high proportion of stygobites (obligate groundwater fauna) and the other one by a high proportion of non-stygobiontic species. The stygobiontic group could further be distinguished into two sub-groups, one dominated by the copepod *Diacyclops languidoides* and the other by three species of amphipods, forming two relative homogenous clusters in a multidimensional scaling (MDS) analysis (Fig. 4A). The data indicate that the samples dominated by non-stygobites are affected by a pronounced land surface impact. It is also known, that the space available for living strongly shapes aquifer communities. With respect to the two subgroups of stygobite-dominated samples, it is to be supposed that the key species, copepods in subgroup 1 and amphipods in subgroup 2, reflect different grain and pore sizes of the aquifers embedding the wells. However, besides these local differences, the two hydrogeological subunits (Erft and Rur block) could not be distinguished based on the faunistic data set.

Groundwater bacterial communities

Assuming that the fundamental assumption that 'everything is everywhere: but the environment selects' (BAAS-BECKING, 1934) holds true for shallow porous aquifers, which are well connected to the surface and other ecosystems, then we may expect to find some common features in groundwater bacterial communities of an aquifer exhibiting common hydrogeochemical conditions. This indeed would be very useful with respect to the assessment of ecosystem status. Shifts in the microbial community could then be related to deviations from a reference status. However, so far the information on bacterial communities in 'pristine' and moderately contaminated shallow aquifers is scarce and does not allow to draw this conclusion. The data obtained in our study are convincing. Statistically, the bacterial communities of the two blocks could not be differentiated, nor pronounced differences were found for the spring and autumn samples. This means the composition of aquifer bacterial communities in the sampling areas is to a significant extent governed by the prevailing hydrogeochemical situation. The geologically similar aquifers of the Rur and Erft block thus harbour bacterial communities which contained numerous identical bacterial groups and species (T-RFs) (Tab. 2; Fig. 6). For both blocks a weak, but significant difference was observed between the bacterial communities from the locally distributed (lumped) and regionally distributed (dispersed) wells. Communities of the lumped wells were more similar to each other (Fig. 5). Certainly, a next step must be to identify the dominating T-RFs by cloning and sequencing, to obtain an idea on the phylogenetic composition of typical bacterial communities for these aquifer systems. Moreover, T-RFs related to individual stressors, such as phosphate (Fig. 7), need to be identified and approved to possibly reveal first indicator bacterial species. Besides the information on bacterial community composition, other integrating microbial parameters such as the bacterial abundance, biomass, activity and biodiversity may serve as ecological criteria (STEUBE *et al.*, 2009). The bacterial abundances (BA) found in groundwater samples from the two blocks matched the range typical for 'pristine' and moderately impacted groundwaters (GRIEBLER & LUEDERS, 2009; BRIELMANN *et al.*, 2009; GRIEBLER *et al.*, 2010), i.e. a mean cell number of 2.5×10^4 mL^{-1} in the Erft block aquifer and 5×10^4 cells mL^{-1} in the Rur block aquifer. However, groundwater from individual wells at the Rur block displayed BA values increased by a factor of 3 to 10. STEUBE *et al.* (2009) related elevated BA values in groundwater samples from the Rur block to high DOC concentrations. However, DOC values given in STEUBE *et al.* (2009) have to be interpreted with caution. There is indication that these are overestimated by a factor of 3–5. No direct correlation could be found between BA and TOC in this study. If the frequently low DO concentrations may stand for a stronger impact at the Rur block when compared with the Erft block, this is reflected by BA values which are on average two times higher in

Rur block groundwater. Also the bacterial diversity was found to be a sensitive parameter for the impact from land use. Groundwater of both blocks exhibited a considerably high bacterial biodiversity in comparison to data from other oligotrophic shallow aquifers (BRIELMANN *et al.*, 2009; GRIEBLER *et al.*, 2010). A temperate impact from organic carbon and nutrients is known to increase microbial biodiversity, while a strong impact generally reverses this trend (GRIEBLER & LUEDERS, 2009). According to this 'intermediate disturbance hypothesis' bacterial diversity data underline a moderate impact on aquifers from land use. Concerning all microbiological analyses one must keep in mind that only bacterial communities suspended in pore water could be considered in this study. The major part of bacterial biomass in porous aquifers, however, is attached to the sediment particles (ALFREIDER *et al.*, 1997; GRIEBLER *et al.*, 2002).

Relations between groundwater organisms and environmental conditions?

One of the results of this study is a positive correlation found for the non-stygobiontic organisms with both detritus (particulate organic matter) and the BA. The stronger the impact from the surface, the better the food base and the higher the proportions of non-stygobites (MALARD *et al.*, 1996; MAUCLAIRE *et al.*, 2000; DATRY *et al.*, 2005; HAHN, 2006). Also elevated numbers of bacteria may directly originate from infiltrating water (CHO & KIM, 2000) or are a result of increased organic carbon and nutrients availability (GRIEBLER & LUEDERS, 2009). Furthermore, non-stygobiontic organisms were found to be positively correlated with nitrate. But elevated nitrate concentrations must be seen as an indicator for an increased impact from agricultural land use, leading to a higher proportion of non-stygobites. DUMAS *et al.* (2001) investigating the impacts of agricultural pollutants in aquifers have found that microcrustaceans constitute natural indicators of aquifer hydrodynamics. Consequently, a dominance of stygobites indicated low hydrological connectivity to the surface (CLARET *et al.*, 1999). With respect to bacteria, an effect of nitrate is generally linked to the simultaneous presence of organic carbon. Under well oxygenated conditions and in the absence of sufficient amounts of organic material nitrate hardly influences bacterial activity and biomass. Thus the correlations of nitrate with organismic variables must be seen as an indirect correlation with groundwater-surface water interactions and the impact from land surface activities (e.g. agriculture). Positive correlations between bacterial abundance (BA) and biomass with nitrate were found in an unconsolidated sandy area of the Donana aquifer system (SW Spain) (VELASCO *et al.*, 2009). Not surprisingly, CCA analysis of T-RFLP data and fauna revealed the correlation of several bacterial species/groups with individual invertebrates (data not shown). Whether the correlations found for taxa such as *Diacyclops languidoides* or oligochaets with some bacterial species (T-RFs) are meaningful, has to be elucidated in further, more detailed studies.

Perspectives for the use of groundwater ecological indicators

The lack of multitude simple correlations between abiotic and biotic variables underline that ecosystem processes and services cannot sufficiently be evaluated on a hydrological and geochemical basis. Thus, the assessment of water quality and ecosystem health needs ecological criteria. The primary role of such criteria is to provide information on the response of the ecosystem to anthropogenic disturbances. Ecological criteria are not necessarily useful to identify specific anthropogenic stress(es) causing the impairment (NIEMI & MCDONALD, 2004). Based on the data currently available, no single indicator fulfils the expected purposes of (i) detection of an impact, (ii) determination of its effect on the system, and (iii) identification of its origin. Thus, a set of indicators is required for the development of an assessment scheme for groundwater ecosystems. In case of ecosystem impairment, more refined investigations are needed to link specific causes with the biotic responses observed (NIEMI *et al.*, 2009). Our study could show that from the set of biological and ecological variables tested, some proved to have a clear indicative value with respect to anthropogenic impacts mainly from land use. Community composition of fauna in groundwater successfully reflected the impact from agricultural activities, indirectly displayed by the elevated nitrate concentrations (Fig. 4). In a similar way, bacterial diversity mirrored the impact from land surface. Although the data presented underline the great potential of groundwater fauna and bacteria to serve as ecological indicators, to date still several restrictions exist. First to mention is the difficult accessibility of groundwater ecosystems, the patchy distribution of its fauna, the high number of endemic species therein and the limited data on fauna and microbiology in general. And, there are a number of further obstacles including: shortage of taxonomists, lack of knowledge of reference conditions, lack of data from replicated surveys, incomplete coverage of habitat types, lack of standardised protocols for sample collection and processing, and a need for a central database adhering to nationally-agreed standards (TOMLINSON & BOULTON, 2009).

It will need additional strategies in the future. With respect to the fauna, we have to improve our data sets. This asks for a higher sampling frequency and the investigation of more wells (EBERHARD *et al.*, 2009; HANCOCK & BOULTON, 2009). With regard to microbial communities, we have to get a better understanding on the functional role and redundancy of dominant species/groups. Today, molecular methods provide the research tools required to analyse shifts in microbial patterns in relation to changes in environmental conditions (GRIEBLER & LUEDERS, 2009).12 Instead of total community composition, the analysis of functional units (physiological groups, functional guilds and genes) or individual marker organisms seems promising. Moreover, in further studies pathogenic microorganisms as well as viruses should be considered as complementing indicators for anthropogenic impacts to groundwater ecosystems. The current national research project funded by the German Federal Environmental Agency provides a first ground for the development of a databank taking into account physicochemical, microbial and faunal properties of groundwater ecosystems. It allows having a closer look at non- or moderately contaminated areas which did not receive much attention in the past. An improved data basis of the composition of natural faunal and microbial communities and its driving forces, provide the prerequisite for the identification of ecological criteria. In conclusion, although there is still a considerable lack of knowledge on the distribution and autecology of groundwater organisms which makes it difficult to identify ecological indicators and criteria ready to use, the scientific outcome of the past two decades provides the basis for the development of modern assessment schemes including ecological criteria originating from groundwater fauna and microbes.

3.1.2.7 References

ABDO Z., SCHUETTE U.M.E., BENT S.J., WILLIAMS C.J., FORNEY L.J. & JOYCE P. (2006) Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. Environ. Microbiol. 8, 929–938.

ALFREIDER A., KRÖSSBACHER M., PSENNER R. et al. (1997) Groundwater samples do not reflect bacterial densities and activity in subsurface systems. Water Res. 31, 832–840, 86(6), 619–633.

ANDERSON M. J. (2001) A new method for non-parametric multivariate analysis of variance. Austral. Ecology 26, 32–46.

BAAS-BECKING L. G. M. (1934) Geobiologie of inleiding tot de milieukunde, W. P. van Stockumand Zoon N. V.

BERKHOFF S.E., BORK J. & HAHN H.J. (2009) Grundwasserfauna als Indikator für Oberflächenwasser-Grundwasser-Interaktionen im Bereich einer Uferfiltrationsanlage. Grundwasser 14, 3–20.

BORK J., BERKHOFF S.E., BORK S., HAHN H.J. (2009) Using subsurface metazoan fauna to indicate groundwater-surface water interactions in the Nakdong River floodplain, South Korea. Hydrogeol. J. 17, 61–75.

BOULTON A.J., FENWICK G.D., HANCOCK P.J. & HARVEY M.S. (2008) Biodiversity, functional roles and ecosystem services of groundwater invertebrates. Invertebrate Syst. 22, 103–116.

BRIELMANN H., GRIEBLER C., SCHMIDT S.I., MICHEL R. & LUEDERS T. (2009) Effects of thermal energy discharge on shallow groundwater ecosystems. FEMS Microbiol. Ecol. 68, 273–286.

CAIRNS JR. J., MCCORMICK P. V. & NIEDERLEHNER B. R. (1993) A proposed framework for developing indicators of ecosystem health, Hydrobiologia, 263, 1–44.

CANIVET V., CHAMBOM P. & GIBERT J. (2001) Toxicity and bioaccumulation of arsenic and chromium in epigean and hypogean freshwater macroinvertebrates, Arch. Environ. Contam. Toxicol. 40, 345–354.

CHO J.C. & KIM S.J. (2000) Increase in bacterial community diversity in subsurface aquifers receiving livestock wastewater input, Appl. Environ. Microbiol. 66, 956–965.

CLARET C., MARMONIER P., DOLE-OLIVIER M.J., CRUEZÈ DES CHÂTELLIERS M., BOULTON A.J. & CASTELLA E. (1999) A functional classification of interstitial invertebrates: supplementing measures of biodiversity using species traits and habitat affinities, Arch. Hydrobiol. 145, 385–403.

CLARKE K.R. & GREEN R.H. (1988) Statistical design and analysis for a 'biological effects' study. Biological effects of pollutants. Results of a practical workshop, Mar. Ecol.: Prog. Ser. 46(1–3), 213–226.

CLARKE K.R. & WARWICK R.M. (1994) Similarity-based testing for community pattern: the 2-way layout with no replication, Mar. Biol. 118, 167–176.

COLEMAN M.L., SHEPHERD T.J., DURHAM J.J., ROUSE J.E. & MOORE G.R. (1982) Reduction of water with zinc for hydrogen isotope analysis. Anal. Chem. 54, 993–995.

COPLEN T.B. (1988) Normalization of oxygen and hydrogen isotope data. Chem. Geol. 72, 293–297.

CULMAN S.W., BUKOWSKI R., GAUCH H.G., CADILLO-QUIROZ H. & BUCKLEY D.H. (2009) TRex: software for the processing analysing of T-RFLP data. BMC Bioinformatics 10, 171.

DANIELOPOL D.L. & GRIEBLER C. (2008) Chaniging paradigms in groundwater ecology – from the ´living fossils` tradition to ´new groundwater ecology`. Int. Rev. Hydrobiol. 93, 565-577.

DANIELOPOL D., GRIEBLER C., GUNATILAKA A., HAHN H.J., GIBERT J., MERMILLOD-BLONDIN G., MESSANA G., NOTENBOOM J. & SKET B. (2008) Incorporation of groundwater ecology in environmental policy. In: Quevauviller, P. (Ed.), Groundwater Science and Policy – An International Overview. RSC Publishing, The Royal Society of Chemistry, London.

DATRY T., MALARD F. & GIBERT J. (2005) Response of invertebrate assemblages to increased groundwater recharge rates in a phreatic aquifer. J. North Am. Benthol. Soc. 24, 461–477.

DEHARVENG L., STOCH F., GIBERT J., BEDOS A., GALASSI D., ZAGMAJSTER M., BRANNCELJ A., CAMACHO A., FIERS F., MARTIN P., GIANI N., MAGNIEZ G. & MARMONIER P. (2009) Groundwater biodiversity in Europe, Freshw. Biol. 54(4), 709–726.

DIN (German industry norm) 38402-A 13, (1985) EN-German standard methods for the examination of water waste water and sludge; general information (group A); sampling from aquifers. 13.060.10

DUMAS P., BOU C. & GIBERT J. (2001) Groundwater Macrocrustaceans as Natural Indicators of the Ariège Alluvial Aquifer, International Review of Hydrobiology 86(6), 619-633.

DUMAS P. & FONTANINI G. (2001) Sampling fauna in aquifers; a comparison of net-sampling and pump-sampling, Arch. Hydrobiol. 150(4), 661–676.

EBERHARD S.M., HALSE S.A., WILLIAMS M.R., SCANLON M.D., COCKING J. & BARRON H.J. (2009) Exploring the relationship between sampling efficiency and short-range endemism for groundwater fauna in the Pilbara region, Western Australia, Freshw. Biol. 54(4), 885–901.

EPSTEIN S. & MAYEDA T. (1953) Variation of O^{18} content of waters from natural sources. Geochim. Cosmochim. Acta 4, 213–224.

EU-WFD, 2000. Directive 2000/60/EC of the European Parliament and of the Council of the 23 October 2000: establishing framework for the community action in the field of water policy. Official J. European Comm. L327, 321–371.

GALASSI D.M.P., STOCH F., FIASCA B., DI LORENZO T. & GATTONE E. (2009) Groundwater biodiversity patterns in the Lessinian Massif of northern Italy. Freshw. Biol. 54, 830–847.

GAUCH JR. H.G. (1992) Statistical analysis of regional yield trials: AMMI Analysis of factorial designs. Elsevier, Amsterdam.

GIBERT J. & DEHARVENG L. (2002) Subterranean ecosystems: a truncated functional biodiversity. BioScience 52, 473–481.

GOLDSCHEIDER N., HUNKELER D. & ROSSI P. (2006) Review: Microbial biocenoses in pristine aquifers and an assessment of investigative methods. Hydrogeol. J. 14, 926–941.

GRIEBLER C., MINDL B. & SLEZAK D. (2001) Combining DAPI and SYBR Green II for the enumeration of total bacterial numbers in aquatic sediments, Int. Rev. Hydrobiol. 86, 453–465.

GRIEBLER C., MINDL B., SLEZAK D. & GEIGER-KAISER M. (2002) Distribution patterns of attached and suspended bacteria in pristine and contaminated shallow aquifers studied with an in situ sediment ecposure microcosm, Aquat. Microb. Ecol. 28, 117–129.

GRIEBLER C. & LUEDERS T. (2009) Towards a conceptual understanding of microbial biodiversity in groundwater ecosystems, Freshw. Biol., 54(4), 649–677.

GRIEBLER C., STEIN H., KELLERMANN C., BRIELMANN H., SCHMIDT S. I., SELESI D., STEUBE C., BERKHOFF S.E., FUCHS A. & HAHN H. J. (2010) Ecological assessment of groundwater ecosystems – Vision or illusion? Ecol. Engeneer. 36, 1174-1190.

HAHN H.J. (2006) The GW-Fauna-Index: a first approach to a quantitative ecological assessment of groundwater habitats. Limnologica 36, 119–137.

HAHN H.J. & MATZKE D. (2005) A comparison of stygofauna communities inside and outside groundwater bores. Limnologica 35, 31–44.

HAHN H.J. & FUCHS A. (2009) Distribution patterns of groundwater communities across aquifer types in south-western Germany. Freshw. Biol. 54, 848–860.

HANCOCK P.J., BOULTON A.J. & HUMPHREYS W.F. (2005) Aquifers and hyporheic zones: towards an ecological understanding of groundwater, Hydrogeol. J. 13(1), 98–111.

HANCOCK P.J. & BOULTON A.J. (2009) Sampling groundwater fauna: efficiency of rapid assessment methods tested in bores in eastern Australia, Freshw. Biol. 54(4), 902–917.

HERMAN J.S., CULVER D.C. & SALZMANN J. (2001) Groundwater ecosystems and the service of water purification, Stanford. Environ. Law. J. 20, 479–495.

HILL T.C.J., WALSH K.A., HARRIS J.A. & MOFFETT B.F. (2003) Using ecological diversity measures with bacterial communities. FEMS Microbiol. Ecol. 43, 1–11.

HUMPHREYS W.F. (2006) Aquifers: the ultimate groundwater-dependent ecosystems, Aust. J. Bot. 54, 115–132.

ILLIES J. (1978) Limnofauna Europaea. Gustav Fischer. Stuttgart.

KUNKEL R., WENDLAND F., VOIGT H.J. & HANNAPPEL S. (2004) Die natürliche, ubiquitär überprägte Grundwasserbeschaffenheit in Deutschland, Schriften des Forschungszentrums Jülich, Reihe Umwelt / Environment 47.

LEAR G., BOOTHROYD I.K.G., TURNER S.J., ROBERTS K. & LEWIS G.D. (2009) A comparison of bacteria and benthic invertebrates as indicators of ecological health in streams, Freshw. Biol. 54(7), 1532–1543.

LEGENDRE P. & LEGENDRE L. (1998) Numerical Ecology. Elsevier Science BV, Amsterdam.

MALARD F., PLENET S. & GIBERT J. (1996) The use of invertebrates in ground water monitoring: A rising research field, Ground Water Monit. Rem. 16, 103–113.

MANTEL N. & VALAND R.S. (1970) A technique of nonparametric multivariate analysis, Biometrics, 26, 547–55.

MARTIN P., DE BROYER C., FIERS F., MICHEL G., SABLON R. & WOUTERS K. (2009) Biodiversity of Belgian groundwater fauna in relation to environmental conditions. Freshw. Biol. 54(4), 814–829.

MAUCLAIRE L., GIBERT J. & CLARET C. (2000) Do bacteria and nutrients control faunal assemblages in alluvial aquifers? Arch. Hydrobiol. 148, 85–98.

MÖSSLACHER F. (1998) Subsurface dwelling crustaceans as indicators of hydrological conditions, oxygen concentrations, and sediment structure in an alluvial aquifer, Int. Rev. Hydrobiol. 83, 349–364.

MÖSSLACHER F. (2000) Sensitivity of groundwater and surface water crustaceans to chemical pollutants and hypoxia: implications for pollution management, Arch. Hydrobiol. 149, 51–66.

MURPHY J. & RILEY J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.

NIEMI G. J. & MCDONALD M. E. (2004) Application of ecological indicators, Annu. Rev. Ecol. Syst. 35, 89–111.

NIEMI G.J., BRADY V.J., BROWN T.N., CIBOROWSKI J.J.H., DANZ N.P., GHIOCA D.M., HANOWSKI J.M., HOLLENHORST T.P., HOWE R.W., JOHNSON L.B., JOHNSTON C.A. & REAVIE E.D. (2009) Development of ecological indicators for the U.S. Great Lakes coastal region – A summary of applications in Lake Huron, Aquat. Ecosyst. Health Manage. 12(1), 77–89.

NOTENBOOM J., SERRANO R., MORELL I. & HERNÁNDEZ F. (1995) The phreatic aquifer of the 'Plana de Castellón' (Spain): relationships between animal assemblages and groundwater pollution. Hydrobiologia 297, 241–249.

OKSANEN J. (2008) Multivariate analysis of ecological communities, in R: vegan tutorial. available at http://cc.oulu.fi/◎jarioksa/opetus/metodi/ vegantutor.pdf.

PARAN F., MALARD F., MATHIEU J., LAFONT M., GALASSI D.M.P. & MARMONIER P. (2004) Distribution of groundwater invertebrates along an environmental gradient in a shallow water-table aquifer, in World Subterranean Biodiversity. J. Gibert (Ed.), Villeurbanne, France.

PEARL H.W., DYBLE J., MOISANDER P.H., NOBLE R.T., PIEHLER M.F., PINCKNEY J.L., STEPPE T.F., TWOMEY L. & VALDES L.M. (2003) Microbial indicators of aquatic ecosystem change: current applications to eutrophication studies. FEMS Microbiol. Ecol. 46, 233–246.

PRONK M., GOLDSCHEIDER N. & ZOPFI J. (2009) Microbial communities in karst groundwater and their potential use for biomonitoring. Hydrogeol. J. 17, 37–48.

QUEVAUVILLER P. (2005) Groundwater monitoring in the context of EU legislation: reality and integration needs. J. Environ. Monit. 7, 89.

TOMLINSON M., BOULTON A.J., HANCOCK P.J. & COOK P.G. (2007) Deliberate omission or unfortunate oversight: Should stygofaunal surveys be included in routine groundwater monitoring programs? Hydrogeol. J. 15(7), 1317–1320.

TOMLINSON M., BOULTON A.J. (2009) Subsurface groundwater dependent ecosystems: a review of their biodiversity, ecological processes and ecosystem services. In: Commission, N.W. (Ed). Waterlines Occasional Paper No. 8, National Water Commission, Canberra.

SCARSBROOK M.R. & FENWICK G.D. (2003) Preliminary assessment of crustacean distribution patterns in New Zealand groundwater aquifers. N.Z.J. Mar. Freshw. Res. 37, 405–413.

SCHLIMM W. (1988) Geologie am Niederrhein, Hilden H. D. (ed) Geologisches Landesamt Nordrhein-Westfalen, Krefeld.

SCHMIDT S.I., HAHN H.J., HATTON T. & HUMPHREYS W.F. (2007) Do faunal assemblages reflect the exchange intensity in groundwater zones? Hydrobiologia 583, 1–19.

SMITH C.J., DANILOWICZ B.S., CLEAR A.K., COSTELLO F.J. & WILSON B., MEIJER W.G. (2005) T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. Fems Microbiol. Ecol. 54, 375–380.

SOLÉ M., FETZER I., WENNRICH R., SRIDHAR K.R., HARMS H. & KRAUSS G. (2008) Aquatic hyphomycete communities as potential bioindicators for assessing anthropogenic stress, Sci. Total Environ. 389, 557–565.

STEUBE C., RICHTER S. & GRIEBLER C. (2009) First attemps towards an integrative concept for the ecological assessment of groundwater ecosystems. Hydrogeol. J. 17, 23–35.

STRAYER D.L., MAY S.E., NIELSEN P., WOLLHEIM W. & HAUSAM S. (1997) Oxygen, organic matter, and sediment granulometry as controls on hyporheic animal communities. Arch. Hydrobiol. 140, 131–144.

VELASCO AYUSO S., GUERRERO M.C., MONTES C. & LÓPEZ-ARCHILLA A.I. (2009) Spatiotemporal distribution of microbial communities in a coastal, sandy aquifer system (Donana, SW Spain). Geobiology 7, 66–81.

VENABLES W.N. & RIPLEY B.D. (2002) Modern applied statistics with S. Springer, Heidelberg.

WARD J.V. & PALMER M.A. (1994) Distribution patterns of interstitial freshwater meiofauna over a range of spatial scales, with emphasis on alluvial river-aquifer systems. Hydrobiologia 287(1), 147–156.

WINDERL C., ANNESER B., GRIEBLER C., MECKENSTOCK R.U. & LUEDERS T. (2008) Depthresolved quantification of anaerobic toluene degraders and aquifer microbial community patterns in distinct redox zones of a tar oil contaminant plume. Appl. Environ. Microbiol. 74, 792–801.

Acknowledgements

The authors gratefully acknowledge project funding by the German Federal Environment Agency (UBA-project 'Biologische Bewertung von Grundwasserökosystemen'). We further thank S. Richter for continuous discussion and support, N.Cremer, F. Wilhelms, U. Lack, E. Hömberg and M. Trimborn of the 'Erftverband' in Bergheim, Germany, for logistical help during sampling and provision of selected chemical analysis. A. Mösch and M. Stöckl from the Institute of Groundwater Ecology at the HMGU in Neuherberg, Germany, are acknowledged for sampling assistance and K. Euringer and T. Lueders are acknowledged for helpful ideas concerning data analysis. The Helmholtz Center Munich (HMGU) is acknowledged for support and providing ideal working conditions.

3.2 Einfluss regionaler und lokaler Effekte auf die Invertebraten-gemeinschaften eines dynamischen Karstsystems und angrenzender fluviatiler Lockergesteinsleiter (Baden-Württemberg)

3.2.1 Zusammenfassung

In den Grundwassersystemen des Alb-Donau-Kreises wurden die Besiedlungsmuster der Invertebratengemeinschaften vor allem auf regionaler, aber auch auf lokaler Ebene untersucht. Zu diesem Zweck wurden im Frühjahr und Herbst 2007 insgesamt 40 Grundwassermessstellen, die in demselben hydrologischen System liegen, faunistisch beprobt. Das Untersuchungsgebiet kann in verschiedene regionale Einheiten gegliedert werden. Die Messstellen liegen im Übergangsbereich zweier Naturräume (Lonetal-Flächenalb, Donauried). In beiden Naturräumen wurden Karst- und Lockergesteinsleiter beprobt. Das Gebiet lässt sich gleichzeitig in zwei unterschiedliche hydrogeologische Bezugseinheiten (HBE) gliedern (Kalksteine des Oberen Jura, Schotter und Moränen des alpinen Vorlands).

Zentrale Fragestellung auf *regionaler Ebene* war, ob sich die Besiedlung der Grundwasserfauna hinsichtlich des Naturraums, des Grundwasserleitertyps oder der HBE unterscheidet. Zur Untersuchung der *lokalen Ebene* wurden die Gemeinschaften einzelner Messstellen miteinander verglichen und auf Korrelationen mit abiotischen Parametern geprüft.

Die *regionalen Besiedlungsmuster* weisen geringe aber signifikante naturräumliche Unterschiede auf. Im Grundwasser der Lonetal-Flächenalb wurde eine höhere faunistische Artenzahl, Abundanz und Diversität als im Donauried erfasst. Darüber hinaus traten einzelne Arten in nur einem der beiden Naturräume auf. Im Gegensatz dazu zeigen die Gemeinschaften keine signifikanten Unterschiede hinsichtlich des Grundwasserleitertyps und der Hydrogeologie. Die insgesamt geringen Besiedlungsunterschiede sind auf die Zugehörigkeit aller Messstellen zu demselben Grundwassereinzugsgebiet

zurückzuführen. Darüber hinaus sind die untersuchten Lockergesteinsleiter im Alb-Donau-Kreis großlumig und weisen eine starke hydrologische Anbindung an die Karstleiter auf. Dadurch besteht eine relativ große hydrologische Dynamik im Untersuchungsgebiet, die auch zu einer starken Überlagerung naturräumlicher und grundwasserleitertypischer Effekte führt. Gleichwohl zeigen die Besiedlungsmuster auch geringe Unterschiede in der Landnutzung und der Geomorphologie an. Während die Lonetal-Flächenalb eher durch extensive Nutzung und Karstleiter geprägt ist, wird das Donauried intensiv landwirtschaftlich genutzt und ist von Lockergesteinsleitern geprägt.

Ebenso zeigen die Analysen, dass kleinräumige hydrologische Verhältnisse die regionalen Effekte überlagern. Dadurch waren die *lokale und regionale Ebene* nicht immer klar voneinander abzugrenzen und führten zu komplexen Besiedlungsmustern, die eine Interpretation der Verhältnisse erschwerten. Die auffallend hohen Anteile stygobionter Arten sind eigentlich typisch für Grundwassersysteme, die kaum durch Oberflächenwasser beeinflusst sind. Gleichzeitig sind die relativ hohe faunistische Diversität und Abundanz typisch für offene Karstsysteme und oberflächennahe Lockergesteinsleiter. Die untersuchten Gemeinschaften scheinen vor allem von Karstwasser geprägt zu sein das in darüber liegende Lockergesteinsschichten exfiltriert, wie auch durch organisches Material, das durch Oberflächenwasser ins Grundwasser gelangt. Dabei weist das vereinzelte Auftreten grundwasserfremder Arten eher auf einen Eintrag durch infiltrierendes Niederschlags- und Sickerwasser hin, als auf den Eintrag durch Oberflächengewässer, mit dem auch Nicht-Stygobionte ins Grundwasser gelangen. Im Donauried sind zusätzlich zu den genannten Effekten die wenigen Nicht-Stygobionten durch tonige Deckschichten zu erklären, die das Grundwasser weitgehend abschirmen. Die niedrigere faunistische Abundanz im Donauried ist vermutlich auf ein höheres Grundwasseralter zurückzuführen. Das Grundwasser fließt von der Lonetal-Flächenalb in Richtung Donauried, wobei organisches Material biologisch abgebaut wird. Diesen Gradienten zeigen positive Korrelationen zwischen der faunistischen Besiedlung mit dem partikulären organischen Material (Detritus, POM), der Standardabweichung der Temperatur sowie dem Grundwasserfauna-Index (GFI) an. Ebenso hat die Messstellentiefe einen negativen Einfluss auf die Besiedlung, wie die negativen Korrelationen mit dem organischen Material und der Fauna zeigen. Dieser Gradient war im Donauried besonders deutlich ausgeprägt. Wie schon in anderen Studien beobachtet, hatte Ocker einen negativen Einfluss auf die Besiedlung.

Die Ergebnisse zeigen, dass Invertebratengemeinschaften generell als Anzeiger der hydrologischen Verhältnisse auf regionaler und lokaler Skala geeignet sind. Die insgesamt ähnlichen Besiedlungsmuster reflektieren im Wesentlichen die ausgeprägte hydrologische Konnektivität der Naturraum überschreitenden Grundwassersysteme. Die Fauna zeigt ebenfalls Gradienten hinsichtlich des organischen Materials an, die auf die Stärke des hydrologischen Oberflächeneintrags hinweisen. Die Ergebnisse verdeutlichen aber auch, dass eine ausreichende Einschätzung und Bewertung nur erfolgen kann, wenn die regionalen und lokalen Einflüsse auf das Grundwasser bekannt sind. Darüber hinaus wurden im Alb-Donau-Kreis Arten gefunden, die typisch für das Donaueinzugsgebiets sind und biogeografische Charakteristika anzeigen. Demnach ist das hierarchische Drei-Ebenen-Modell für die Typologie von Grundwasserhabitaten geeignet und ein viel versprechender Ansatz für die faunistische Grundwasserbewertung.

Ergänzung zu Kapitel 3.2.1

Die hier vorgestellten Befunde, die auf den vollständigen Datensätze (2007 und 2009) beruhen, bestätigen die Ergebnisse des Kapitels 3.2. Abbildung 2 verdeutlicht die Zusammenhänge zwischen der Invertebratenbesiedlung und den hydrologischen Verhältnissen im Untersuchungsgebiet Alb-Donau-Kreis.

Das angefügte Schema zeigt einen geologischen Schnitt durch das Untersuchungsgebiet (Abbildung 2). Die Besonderheiten liegen in der Naturraum überschreitenden Charakteristik des untersuchten Grundwassers, dass einerseits ein zusammenhängendes System bildet, andererseits aber unterschiedlichen regionalen und lokalen Einflüssen unterliegt. Eingezeichnet sind die abnehmenden Gradienten des Oberflächeneintrags (großer blauer Pfeil) und des organischen Materials (großer brauner Pfeil) von der Lonetal-Flächenalb zum Donauried. Deutlich wird auch das durch Deckschichten abgeschirmte Grundwasser im Donauried und der offen liegende Karst der Lonetal-Flächenalb. Die faunistischen Besiedlungsmuster spiegeln im Wesentlichen diese hydrologischen Bedingungen wider. Die farbig markierten Schilder stellen Hauptgruppen von Messstellen dar (Gruppe 1-3), die anhand ähnlicher Besiedlungsmuster zusammengefasst wurden (Untergruppen a-c siehe in GRIEBLER *et al.* in Druck). Die Gruppierung basiert auf statistischen Gemeinschaftsanalysen durch die die Gemeinschaften einzelner Messstellen nach autökologischen Aspekten gruppiert wurden. Auf die Fauna der jeweiligen Messstellengruppen wirken unterschiedliche Effekte und hydrologische Bedingungen. Demnach lassen sich Standorte mit leichtem Oberflächeneinfluss (grün), abgeschirmte bzw. tiefe Standorte (gelb) und stark verockerte Standorte, die durch Sickerwasser beeinflusst sind (rot), unterscheiden. Dabei ist Gruppe 1 von höheren Abundanzen sowie dem Auftreten Nicht-Stygobionter und Gruppe 2 durch relativ geringe Abundanzen von ausschließlich Stygobionten geprägt, während in Gruppe 3 nur Oberflächenarten auftraten. Diese unterschiedlichen lokalen Einflüsse sind schematisch durch die kleinen Pfeile dargestellt: Niederschlags- (blau) und Sickerwasser (orange). Die Messstellentiefe ist schematisch durch die Schilderlänge dargestellt und zeigt, dass auch mit der Tiefe die Besiedlung abnimmt. Weitere Informationen und Bezeichnungen sind dem UBA-Forschungsbericht zu entnehmen (GRIEBLER *et al.* in Druck).

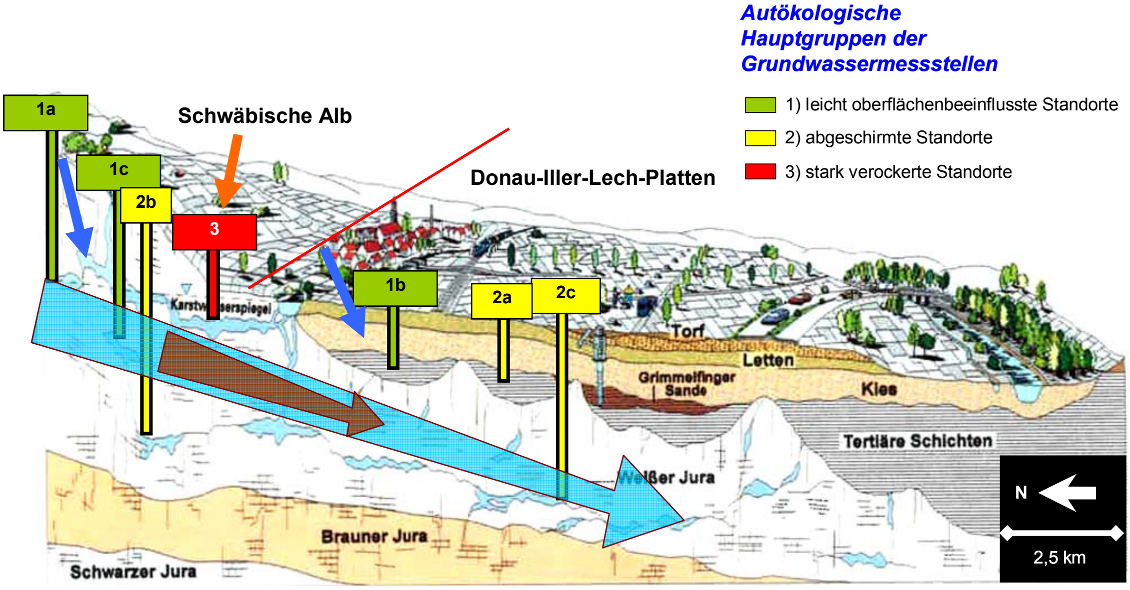

Abbildung 2: Geologischer Schnitt durch das Untersuchungsgebiet (nach LW-Baden-Württemberg 2003). Schematisch eingezeichnet sind sieben autökologische Gruppen von Messstellen, die anhand faunistischer Ähnlichkeiten (Abundanz, Artzahl) gebildet wurden. Die Gruppen sind zu drei Hauptgruppen zusammengefasst und entsprechend der Legende farbig markiert. Die kleinen Pfeile stellen den lokalen Einfluss von Niederschlags- (blau) und Sickerwasser (orange) auf das Grundwasser dar. Der große blaue Pfeil zeigt die Fließrichtung des Grundwassers an, der braune Pfeil den abnehmenden Gradienten des organischen Materials im Grundwasser mit zunehmender Verweilzeit. 1a) Alb: Stygobionte/Stygophile, 1b) DIL: Stygobionte/Stygophile, 1c) Alb: Stygobionte, 2a) DIL: Stygobionte A, 2b) Alb) Stygobionte B, 2c) DIL: Stygobionte B (GRIEBLER *et al*. in Druck).

3.2.2 Ecological assessment of groundwater ecosystems – Vision or illusion?

3.2.2.1 Abstract

Environmental policy and in particular the European water legislation, in the framework of the EU Groundwater Directive, has started to consider groundwater not only as a resource but as a living ecosystem. A precondition for comprehensive groundwater protection is thus the assessment of the biological and ecological state. The assessment of ecosystems requires consideration of ecological criteria, which so far are not available for groundwater systems. In the framework of a national project, the German Federal Environment Agency (UBA) supports a consortium of scientists and stakeholders from water boards and regional environmental authorities to develop a first concept for an ecological assessment scheme for groundwater ecosystems. The attempts towards an integrative concept include the following steps: (i) selection of appropriate biological and ecological parameters, (ii) typology of groundwater ecosystems, (iii) derivation of a reference status (Leitbild) and natural background values for biological variables, (iv) identification of potential bioindicators and definition of threshold values, and (v) development of an assessment model. These proposed steps are discussed on the basis of a data set from two groundwater landscapes in Southern Germany. Investigations considered three different spatial units, i.e. the habitat unit at the local scale, and the aquifer type unit as well as the landscape unit at the regional scale. Fauna as well as bacterial communities could provide valuable ecological information on the ecosystems status. The paper reviews 'state of the art' knowledge and evaluates the near future perspectives for the development and implementation of groundwater ecosystems assessment programmes.

3.2.2.2 Introduction

The ecological status of groundwater ecosystems constitutes an important role in provision of good quality water for human needs (e.g. drinking water). Apart from that, the integrity of most surface waters and wetlands depends on an intact connectivity to aquifers and groundwater in sufficient quantity and quality (BOULTON, 2005). Aquifers are complex ecosystems harbouring a vast and almost unrecognized diversity of microorganisms and invertebrates (GRIEBLER & LUEDERS, 2009; HAHN & FUCHS, 2009). Recently, environmental policy has started to consider groundwater not only as a resource for high quality water but as a living ecosystem. In particular, the Swiss Water Protection Ordinance (GSCHV, 1998) and the EU Groundwater Directive (EU-GWD, 2006) mention ecological objectives to keep the biocoenosis in a natural state and to foster research in groundwater ecology, respectively. This situation clearly demands biological and ecological assessment criteria, which so far, are not available for groundwater ecosystems. Thus, the aim of a current research project funded by the German Federal Environment Agency (UBA) is to work out a conceptual framework for an ecological assessment of groundwater ecosystems and to take first steps in its development.

National and international policy

To our knowledge, so far only a few national and international regulations include ecological criteria for groundwater ecosystems. An often cited example is the Swiss Water Protection Ordinance released in 1998 (GSCHV, 1998), which implicates ecological objectives with respect to water quality: 'the biocoenosis should be in a natural state adapted to the habitat and characteristic of water that is not or only slightly polluted' (GOLDSCHEIDER *et al.*, 2006; HUNKELER *et al.*, 2006). However, it is worth mentioning that information on the natural status as well as adequate methods for the assessment of an aquifer's current ecological status is still missing. The European Union Groundwater Directive (EU-GWD, 2006), a daughter directive of the European Union Water Framework Directive (EU-WFD, 2000), forced the discussion about the necessity and perspectives in using 'ecological status criteria' in future groundwater monitoring schemes (DANIELOPOL *et al.*, 2004, 2008). Released in December 2006, the EU-GWD states the importance of protective measures for groundwater ecosystems in its introductory section and it further notes: 'Research should be conducted in order to provide better criteria for ensuring groundwater ecosystem quality'. In Australia, several attempts to incorporate ecological criteria into groundwater policy were made. In 2002, the Australian state New South Wales (NSW) released the 'NSW State Groundwater Dependent Ecosystems Policy' as a guidance paper specifically designed to manage the State's groundwater resources in regard to sustain environmental, social and economic uses. It highlights that wherever possible, the ecological processes and biodiversity shall be maintained or restored, for the benefit of present and future generations (NSW-SGDEP, 2002). In 2003, the Western Australian Environmental Protection Authority published a guideline for the assessment of environmental factors, including subterranean fauna in groundwater and caves, in case of groundwater exploitation (EPA, 2003). Summing up, there is a growing recognition of the ecosystem services provided by healthy and intact groundwater ecosystems. Consequently, the evaluation of groundwater microorganisms and representatives of the metazoan fauna may serve as bioindicators, which constitutes an important basis for the development of a groundwater ecosystem status assessment concept, is right in time.

Five steps to an ecologically focused assessment scheme

As a first outcome of the current UBA project, the conceptual framework suggests five consecutive steps to be followed in the development of an ecological assessment scheme for groundwater ecosystems. These are partly in accordance with established assessment schemes developed for surface waters, which successfully entered official water protection and management directives; i.e. the European Water Framework Directive (EU-WFD, 2000). The suggested steps are (i) the selection of appropriate biological and ecological parameters, (ii) the typology of groundwater ecosystems based on biological and ecological criteria, (iii) the derivation of a reference status and natural background values for biological variables, (iv) the identification of potential bioindicators and definition of threshold values, and (v) the development of an assessment model (e.g. index).

Classification and typology of groundwater ecosystems

Basic to a classification and typology of groundwater ecosystems is the selection of 'state' or key factors. In groundwater systems, the classification is generally build up on several key criteria or sets of conditions, such as the type of aquifer (compact, porous, fissured, karst), with its mineralogical composition, its porosity and hydraulic permeability, and the origin of recharge (JÄCKLI, 1970; HEATH, 1982). Several national and international efforts have been undertaken to map groundwater resources and aquifers considering different spatial scales and criteria such as the origin of groundwater recharge, porosity, productivity petrographical or hydrochemical properties (CEC, 1982; GILBRICH, 2000; IGRAC, 2005; STRUCKMEIER *et al.*, 2006). However, these classification schemes are not sufficient for the monitoring of groundwater quality at European scale nor for the assessment of the ecological status. A first appropriate step towards the classification of aquifers was undertaken by WENDLAND *et al.* (2008) who developed a European aquifer typology, including major classes of aquifers, based on MÜLLER *et al.* (2006). This typology is founded on common petrographic properties (geology and mineralogy) and hydrological and hydrodynamic conditions, since these factors decisively determine the natural composition of groundwater. A refined version was established for Germany by

KUNKEL and coworkers (KUNKEL *et al.*, 2004), who classified 17 hydrogeological units. Certainly, ecologists are not satisfied with a hydrogeological classification since the driving forces for the distribution of groundwater organisms are more complex.

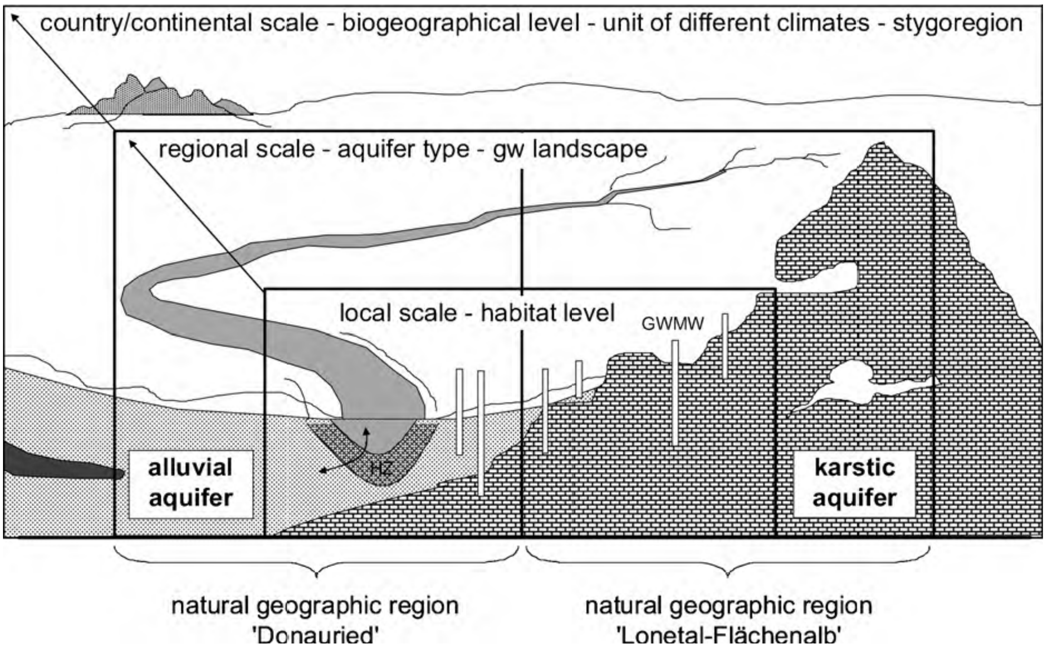

Fig. 1: Scale-based, ecologically focused hierarchical typology with respect to groundwater systems. The country/continental scale contains all various types of groundwater systems. At this scale, biogeography and climate are driving forces shaping distribution patterns of groundwater fauna. The regional scale accounts for areas up to several hundreds of km in radius. It is also the scale of individual groundwater landscapes and aquifer types. At this scale geology, groundwater chemistry and porosity are driving forces. The local scale refers to a radius of 1 km, typically a subsection of an aquifer. Hydrological connectivity to the surface and input of POM and oxygen as well as temperature variations shape the composition of groundwater communities. Additionally, the bottom of groundwater monitoring wells (GWMW), where fauna is sampled from, may represent the micro scale environment. With respect to the data shown in this paper, the investigation areas were not only distinguished by aquifer types but also into geographic regions, i.e. the 'Donauried' and the 'Lonetal-Flächenalb', as indicated at the bottom of schematic drawing.

According to HAHN (2009) the distribution and structure of groundwater communities occurs at different spatial scales. Hence, he suggests a hierarchical model for the typology of groundwater habitats considering three spatial scales: (1) On the continental or macro scale, metazoan communities are shaped by biogeographic factors such as e.g. continental shifts, ice ages, hydrography, transgression and regression of seas and climatic conditions (Fig. 1). A typology of these biogeographical units may also be termed stygoregions (HAHN, 2009) or mega scale (GIBERT *et al.*, 1994). (2) On the landscape or meso scale the metazoan community structure is formed by the hydrogeological type of aquifer (porous, fractured, karst, compact), which determines the living space available (size of pores and voids) and the hydraulic conductivity and connectivity within habitats (GIBERT *et al.*, 1997; HAHN, 2009; HAHN & FUCHS, 2009). The meso scale, which is also termed aquifer/macro scale by GIBERT *et al.* (1994) is similar to the regional scale or at least does include it (Fig. 1). (3) On the local scale or habitat-level species composition, faunal abundance and diversity is influenced by the hydrological connection to the surface (precipitation, seepage water, surface water). Since in groundwater systems food (e.g. particulate organic matter, biofilms) and oxygen supply depend on water as carrier phase. HAHN (2009) proposed the hydrological conditions to be the integrating key factor at the local scale. The Groundwater Fauna-Index (GFI), developed by HAHN (2006) describes the strength of the hydrological impact from the surface. GIBERT *et al.* (1994) use the micro scale as an additional lower level in their typology, which ranges in its size from 0.1 to 100m^3 and can be seen as part of the habitat of an invertebrate species. It is also worth taking a brief look at the existing classification systems and typologies from a microbiological perspective. Similar to stygofauna, the distribution of microbes is found to be controlled by the availability of organic matter (GOLDSCHEIDER *et al.*, 2006; GRIEBLER & LUEDERS, 2009). However, according to their small size and metabolic flexibility microorganisms may reveal heterogeneous distribution patterns already at the µm to cm scale, i.e. the habitat scale of microbes, differing in abundance by orders of magnitude within centimetres of aquifer sediment (KÖLBEL-BOELKE *et al.*, 1988; BROCKMAN & MURRAY, 1997). At the local scale and close to the surface, microbial communities are indeed shaped by the hydrological exchange, which means the import of organic matter and energy but also the import of allochthonous microbes (GRIEBLER & LUEDERS, 2009).

While pore space is unlikely a limiting factor for the occurrence of individual microbes, geochemistry is suggested to be important for microbial community composition (GRIEBLER & LUEDERS, 2009). So far hardly any studies have been published on the meso and macro scale distribution of microorganisms in aquifers and groundwater and factors related (STEIN *et al.*, 2010). It seems also likely that part of the groundwater (micro) biocoenosis and certain ecological features are not directly linked to groundwater chemistry. The current project will show if a different kind of typology is needed for microbial components. The strategy chosen in the current UBA project is to take the classification into hydrogeological units with a common natural groundwater chemical signature (the authors propose the term groundwater landscapes) as defined for Germany by KUNKEL *et al.* (2004) as a starting point for an ecologically based typology of groundwater ecosystems. Sub-sections of selected hydrogeological groundwater units are currently investigated on the local and regional scale. Where necessary, the typology will be further refined into smaller units.

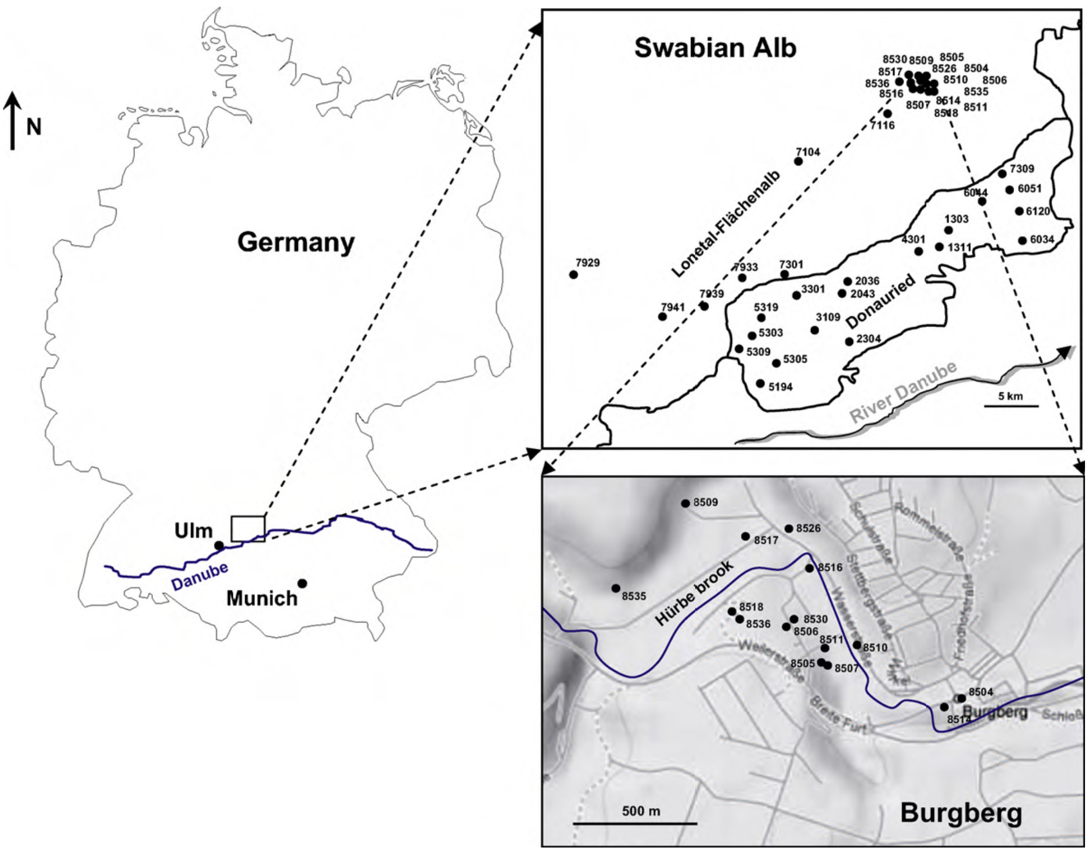

Fig. 2: Study area.

Criteria for the ecological status assessment

To date, groundwater ecosystems are evaluated and monitored analysing for selected physical–chemical parameters. Additionally, selected hygienic aspects are tackled to examine for the potential of contamination with pathogenic microbes via standard assays testing for the presence of *Escherichia coli* and coliforms. So far, no biological and ecological criteria are routine and standard in groundwater status assessment. Moreover, research in the past decades, without doubt, focused on contaminated sites. Thus, there is still a lack of microbial and faunal data for groundwater ecosystems, which have not been seriously anthropogenically impacted. Consequently, at the current stage, it is very difficult to select the 'right' biological and ecological criteria informing about the ecological status of aquifers. For surface waters, the use of organisms as bioindicators has a long standing history and tradition and 'biological criteria' have made its way into most national and international water related regulations. A powerful example is the European Water Framework Directive (EU-WFD, 2000) in which several biological indices form an important basis for the assessment of water quality and ecosystem status. However, due to the different repertoire of organisms and environmental constraints, existing surface water protocols and indices cannot just be adapted for groundwater habitats. Criteria need to be carefully selected and tested for its applicability. In a first attempt, we complemented physical–chemical routine analyses of groundwater samples by focussing on the abundance, taxonomic richness (biodiversity) and community composition of organisms, i.e. bacteria and metazoans, in groundwater (Table 1). Additionally, we analysed groundwater samples for bacterial activity, determined as bacterial carbon production (BCP), and for the amount of detritus (POM), which is suggested to have a crucial influence on the patchy distribution patterns of groundwater fauna (HAHN, 2006, 2009). An extension of this set of parameters is currently realized in the second phase of the UBA project (Table 1). A serious problem related to microbiological features is that non-impacted groundwater systems are characterized by low carbon and nutrient concentrations and thus exhibit a low productivity (GOLDSCHEIDER *et al.*, 2006; GRIEBLER & LUEDERS, 2009). In consequence, the number of organisms found is significantly lower when compared to surface aquatic ecosystems and methods routinely applied for the measurement of bacterial biomass and activity in surface waters are at or even below its limit of sensitivity. Part of the efforts in the UBA project is therefore dedicated to optimize methods for its applicability with natural groundwaters.

Physical-chemical parameters	pH, Temp, EC, K+, Na+, Ca^{2}+, Mg^{2}+, NH_4+, Cl-, NO_{3}-, $SO_4{}^{2}$-, $PO_4{}^{3}$-, alkalinity, stable water isotopes (^{2}H, ^{3}H, ^{18}O)
Microbial parameters	Bacterial abundance, bacterial biomass, BCP, Viral abundance*, BOD*, CFU´s, coliforms, T-RFLP fingerprinting, DNA microarrays*
Groundwater fauna parameters	abundance, Community composition, diversity, Groundwater fauna index

* Parameters additionally considered since start of the UBA-project phase II (spring 2009)

Table 1 Abiotic and biotic variables chosen to be tested within the UBA project (phase I and II) for their use in assessing the ecological status of groundwater ecosystems. EC = electric conductivity, EH = redox, DO = dissolved oxygen, DOC = dissolved organic carbon, TOC = total organic carbon (including particles ≤ 200 μm), AOC = readily assimilable DOC, POM = particulate organic matter, COD = chemical oxygen demand, BCP = bacterial carbon production, BOD = biological oxygen demand, CFU = viable cell counts via colony forming units on R2A agar.

Natural background values and definition of a 'reference status'

As already mentioned, natural groundwater quality is mainly determined by the geological and hydrological properties of an aquifer or a hydrogeological unit and its catchment area. The composition of originally pristine groundwater underwent a continuous change in the past centuries and faces ongoing pressures (DANIELOPOL *et al.*, 2003). Due to forest clearance, agriculture, atmospheric deposition, groundwater extraction and waste water discharge to rivers etc., natural groundwater in shallow aquifers is ubiquitary affected and altered (KUNKEL *et al.*, 2004). Consequently, natural groundwater that recharges aquifers today reflects its geogenic plus its anthropogenic impacts. Thus, instead of a pristine reference status, which to some extent may be evaluated in deep aquifers containing groundwater that has been infiltrated the subsurface in pre-industrial times (HINSBY & RASMUSSEN, 2008), we have to define a natural target or nominal status, which must still equal a good or only moderately influenced chemical and ecological status with respect to the given conditions. The definition of a target status requires the evaluation of the natural background levels of the parameters of interest. Individual models have been developed in the past for the derivation of natural background concentration ranges (KUNKEL *et al.*, 2004; WENDLAND *et al.*, 2005; MÜLLER *et al.*, 2006; CUSTODIO & MANZANO, 2008; EDMUNDS & SHAND, 2008; HINSBY *et al.*, 2008). KUNKEL *et al.* (2004) introduces two approaches for the derivation of the natural groundwater composition, which have been applied to the 17 hydrogeological groundwater units in Germany. The first approach is based on the univariate statistical analysis of each individual physical parameter after pre-elimination of data/wells exhibiting anthropogenic impacts (KUNKEL *et al.*, 2004). A second approach was developed to reproduce the distribution patterns of the 'natural' and the 'influenced' components by two statistical distribution functions (WENDLAND *et al.*, 2005). The range of natural groundwater concentrations is characterized by the 10th and 90th percentile of the distribution function of the natural component. Currently these two approaches are considered for the derivation of natural background ranges of selected microbial parameters, such as the total number of bacteria and bacterial carbon production rates. Based on this information we will try to derive the characteristics of a (nominal) good microbiological status (see discussion below).

Bioindicators and threshold values

The ecological status is an expression of the quality of structure and functioning of ecosystems. Ecosystems under stress undergo changes in both. Changes in structure are manifested by modifications of the composition of the various biocoenoses. Changes in function are reflected, for example, in changes in the organic matter production and degradation on a trophic or ecosystem level (CAIRNS & NIEDERLEHNER, 1993; FRÄNZLE, 2003). This latter definition may be extended to changes in the physiological status of populations or functional groups (guilds) within communities. In summary, changes on a functional level may equal changes in ecosystem services (BOULTON *et al.*, 2008). Without doubt groundwater fauna (stygofauna) and microbes have the potential to offer a service as indicators of ecosystem status and the integrity of some of the fundamental ecological processes occurring in aquifers. We hypothesise that due to the relative invariant and predictable physical–chemical conditions in groundwater ecosystems communities or individual organisms should be highly sensitive to environmental changes. Structural variables chosen in our study are the composition of microbial and faunal communities and the taxonomic richness. Functional variables are represented by bacterial activity measures focusing on microbial turnover of carbon. The direct link of groundwater fauna and its functional importance for maintaining ecosystem processes is still in its infancy. A stimulating paper on that topic was recently released by BOULTON *et al.* (2008). Here, exemplified for the shallow alluvial aquifer of a subtropical Australian river, the invertebrate fauna was categorized into 'ecosystem service providers' (ESPs) and analysed for their functional importance, which is defined as a product of abundance and biomass as well as efficiency. As soon as systematic information exists on the natural background range of individual biological and ecological parameters for the different types of aquifers one can think about the derivation of threshold values. In case of physical-chemical parameters, generally drinking water standards and environmental quality standards are used for the derivation of threshold values. Some common methodology has been developed in the frame of the European BRIDGE project (MÜLLER *et al.*, 2006; HINSBY *et al.*, 2008). In case of biological and ecological parameters this will not be an easy task, as these may not necessarily be linked to results from (eco)toxicology.

The Swabian Alb case study – transition of two groundwater landscapes

To test if the proposed hierarchical typology summarized in Fig. 1 is appropriate for an ecologically driven groundwater ecosystem assessment, current field work concentrates on several aquifer systems in geographically distinct areas of Germany. In the following, first data are presented, which have been collected in the area of the Swabian Alb, South Germany. Here, two types of aquifers, karst and alluvial, which belong to two distinct hydrogeological units, i.e. the 'Limestones of the upper Jurassic' and the 'Gravels and moraines of the Alpine foothills' (KUNKEL *et al.*, 2004), merge. Moreover, the area can be distinguished into two natural geographic regions ('Naturraum'), the 'Lonetal-Flächenalb' and the 'Donauried' (Table 2), differently impacted by land use activities, both containing wells in karst and alluvial groundwater bodies. Ideally, wells could be selected in both aquifer types that are located closely together, and groundwater community patterns could be investigated and compared at the local and regional scale. The results are discussed in conjunction with the different steps proposed for the development of an ecologically sound groundwater ecosystem assessment scheme. Additionally, the paper provides some useful prospects on promising strategies and methods to be used in future research.

Table 2: Geographical, geological and hydrological assignment of the study area.

Study area	'Alb-Donau-District'				
Biogeographic scale	Biogeographic region (according to Illies, 1978)	'central German uplands' (Zentrales Mittelgebirge)			
	River catchment area	Danube			
Landscape scale	Natural geographic region (Naturraum) (Meynen et al., 1962)	'Lonetal-Flächenalb'		'Donauried'	
	Hydrogeological unit (according to Kunkel et al., 2004)	'Limestones of the upper Jurassic'			'Gravels and moraines of the Alpine foothills'
	Regional geology (georeg) (according to Hahn and Fuchs, 2009)	Lonetal-Flächenalb – alluvial	Lonetal-Flächenalb – karst	Donauried – karst	Donauried – alluvial

3.2.2.3 Materials and methods

Investigation area

Sampling sites are located in the 'Alb-Donau'-district, an area near the city of Ulm in the state of Baden-Wuerttemberg, Southern Germany (Fig. 2). The data presented originate from physical–chemical, microbiological and faunal samples that were collected in 40 groundwater monitoring wells, taken in spring and autumn 2007. Table 2 shows in detail the geographical, geological and hydrological assignment of the area that was investigated. The study area belongs to the bioregion 'Central German uplands' ('zentrales Mittelgebirge') and the River Danube catchment (ILLIES, 1978). On a smaller scale, the wells are spread over two natural geographic regions ('Naturraum') – 'Lonetal-Flächenalb' (22 wells) and 'Donauried' (18 wells), according to MEYNEN *et al.* (1962). In each 'Naturraum', two types of aquifers (alluvial quaternary and karst) were sampled. At the Lonetal-Flächenalb six wells are situated in alluvial and 16 wells in the karst aquifer, while in the Donauried 14 wells are located in alluvial sediments and 4 wells in karst. The aquifers of the Lonetal-Flächenalb and the karst aquifers of the Donauried belong to the hydrogeological unit termed 'Limestones of the upper Jurassic' ('Kalksteine des Oberen Jura'); and the alluvial aquifer of the Donauried belong to the 'Gravels and morains of the Alpine foothills' ('Schotter und Moränen des Alpenvorlands'), according to the hydrogeochemical classification that was introduced by KUNKEL et al (2004). Additionally, in the Lonetal-Flächenalb 11 karst and four alluvial wells are located in a distance of approximately 1 km, referred to as lumped wells. The remaining 25 wells are located in a distance of 20 km (referred to as dispersed wells). Finally, all wells of the study area are situated in the same groundwater catchment area (Fig. 2).

Groundwater sampling and analyses

Faunal samples were collected from the bottom of the monitoring wells by using a modified phreatic net sampler, originally described by CVETKOV (1968). This method supplies semiquantitative data. The net sampler was adapted to small well diameters (5–18 cm) and had an aperture of 4.9 cm in diameter and a mesh size of 74 µm (see HAHN & MATZKE, 2005). Previous studies of HAHN & MATZKE (2005) have shown the macroinvertebrate diversity inside the well to be representative for the surrounding aquifer, though the abundance usually is higher inside of the well. Directly, after the faunistic sampling, well water was extracted close to the well bottom using a bailer (Buerkle Aquasampler 0.75 L). Subsequently to the extraction of fauna and well water, a submersible pump (MP1, Grundfos Corp.) was used to replace the well water and pump aquifer water. Depending on further physical-chemical and microbiological analyses, the aquifer water was filtered and/or fixed in the field, transferred to appropriate pre-cleaned vials, and transported to the laboratory. Prior to further processing, samples were stored under cooled conditions (4°C). Temperature, pH, electric conductivity (EC) and dissolved oxygen (DO) were recorded in groundwater samples of the well and the aquifer, respectively by means of field sensors (WTW, Weilheim, Germany). For analyses of major ions, soluble reactive phosphate, and total organic carbon (TOC), the samples were filtered (0.45 µm pore size). Major anions like nitrate, chloride, sulphate and cations like calcium, magnesium, potassium, sodium were analysed by ion chromatography (Dionex, Model DX 100). Orthophosphate was determined colorimetrically as soluble reactive phosphorus by the ammonium-molybdate method according to MURPHY & RILEY (1962). TOC was measured by using high temperature combustion with infrared detection of CO_2 (Shimadzu, TOC-5050). Furthermore, the amount of detritus (particulate organic matter) that was removed together with the sediments from the bottom of the well was quantified by combustion at 450°C for 5 h, following DIN (EN) 12879 (2000) norm method. Additionally, the relative amount of detritus, the proportion of ochre, silt, fine sand, and coarse sand of the faunal samples were estimated on an ordinal scale. The stable isotopes of oxygen ($^{18}O/^{16}O$) and hydrogen ($^{2}H/^{1}H$) of water were determined by isotope ratio mass spectrometry. The $\delta^{18}O$ values in samples were analysed via equilibration with CO_2 at 25°C for 24 h (EPSTEIN & MAYEDA, 1953) and for $\delta^{2}H$ values via reaction with Cr at 850°C (COLEMAN *et al.*, 1982). Both $\delta^{18}O$ and $\delta^{2}H$ values were determined relative to internal standards that were calibrated using IAEA SMOW standards. Data were normalised following COPLEN (1988) and are expressed in delta ‰ notation relative to V-SMOW. Samples were measured at least in duplicates with a precision of ± 0.1 ‰ for $\delta^{18}O$ and ± 1.0 ‰ for $\delta^{2}H$. All faunal samples were preserved with formaldehyde (4 % f. conc.). The fauna was pre-sorted into taxonomic groups (crustaceans, oligochaets, water mites, insects, nematods, tardigrada) and counted. All crustaceans were determined to species level, while the remaining taxonomic groups were identified to order or higher level. Furthermore, crustaceans were assigned to two different ecological groups (stygobites and non-stygobites), in accordance to their ecological preferences (EINSLE, 1993; JANETZKY *et al.*, 1996; MEISCH, 2000; SCHMINKE, 2006). The stygobites refer to the obligate groundwater species. The non-stygobites include metazoans commonly found in groundwater that are capable to survive certain times in aquifers (stygophiles) and species accidentally deriving from surface water or soil into groundwater habitats (stygoxenes). For measurement of bacterial carbon production (BCP), triplicates of 30 ml groundwater samples were incubated for 5 h with 10 nM tritium labelled thymidine ([methyl–^{3}H]-thymidine, 85 Ci mmol^{-1}, GE Healthcare) on site at in situ temperature (± 1°C) in thermoboxes. Groundwater samples immediately fixed with 3 ml formaldehyde (3.7 % f. conc.) were run as blanks. Incubation of the samples was stopped with 3 ml of formaldehyde (37 %). Later, samples were treated with

1.5 ml of 100 % TCA and placed on ice for 15 min prior to filtration. Samples were filtered through cellulose nitrate filters (0.2 µm, Whatman), repeatedly rinsed with ice-cold 5 % TCA solution and ice-cold MQ-water. Filters were then place into scintillation vials, first dried at room temperature and then dissolved in 1 ml ethylacetate. After addition of a scintillation cocktail (Ultima Gold XR) samples were radioassayed by liquid scintillation counting on a Tri-carb 1600 TR liquid scintillation counter (Perkin Elmer). Quench curves were computed by the external standard method. For microbial community analysis, approximately 2.5 L of groundwater was filtered through 0.22 µm sterile membrane filters (Neolab). Filters were subsequently frozen and maintained at −20°C. Later, freshly thawed filters were aseptically cut into small pieces (c. 2 mm^2), which were then transferred into bead-beating cups and total DNA was extracted as described in WINDERL *et al.* (2008). After extraction and precipitation, DNA pellets were resuspended in 25 µL of EB buffer (10 mM Tris–HCl [pH 8.5], Qiagen) and stored frozen (−20°C) until further analyses. T-RFLP analysis of bacterial 16S rRNA gene amplicons was performed as described previously (WINDERL *et al.*, 2008) with the primers Ba27f-FAM/907r and MspI digestion. Primary electropherogram analysis was conducted using the GENEMAPPER 5.1 software (Applied Biosystems) excluding peaks < 50 bp. Identification of baseline threshold of true peaks over noise and the alignment of terminal restriction fragments (T-RFs) were conducted with the help of the T-REX software (CULMAN *et al.*, 2009). The software implements a procedure developed by ABDO *et al.* (2006) where true peaks are iteratively identified as those whose height exceeds (in our case one) standard deviation computed over all peaks. This way, TRFs with a relative fluorescence signal of ≤ 100 were excluded from further analysis. Peak alignment in T-REX can be done automatically following SMITH *et al.* (2005). A clustering threshold of ± 0.5 bp was specified for the grouping of peaks into a common T-RF. The Shannon–Wiener index H’ was calculated as $H' = -\sum p_i \ln p_i$, whereas p_i is the relative abundance of single T-RFs in a given fingerprint (Hill *et al.*, 2003). The reproducibility of our workflow and of T-RFLP analysis was exemplarily verified via replicate fingerprinting analyses of duplicate DNA extracts from 36 of the 38 samples collected during the two campaigns. AMMI analysis (Additive Main Effects and Multiplicative Interaction model; GAUCH, 1992) within T-REX suggested high reproducibility of replicate fingerprints (pattern: 90.6 % of total interaction, noise: 9.4 % of total interaction).

Statistical analyses

The conducted sampling approach resulted in five different environmental datasets. With respect to the physical–chemical variables we distinguished between groundwater (aquifer water) and well water data. Groundwater samples furthermore comprised microbial activity (BCP) and community (T-RFs) data. Last but not least, the groundwater invertebrates collected in the monitoring wells resulted in a fauna data set. Groundwater and well water data of 40 samples collected twice, in spring and autumn, comprised each 19 quantitative physical–chemical variables. As most physical–chemical parameters did not show normal distribution (Kolgomorov-Smirnov test, Shapiro-Wilks test, $p < 0.05$), for statistical analyses, variables were preliminarily log-normalized (except for pH) adding a translation constant when necessary and z-score standardized, resulting in dimensionless quantitative variables. For the purpose of comprehensive multivariate statistical analysis, we filled individual gaps in the two physical–chemical data sets with proxy values which were obtained from analysing (i) external data sets, (ii) time series data, and (iii) calculation of mean values for closely related groups of wells. This kind of data preparation included a plausibility check, evaluating the performance of closely related parameters. Moreover, variables known to perform dynamically with season (e.g. temp, oxygen, redox) have been excluded from this approach. Bacterial community data were expressed as relative abundance of detected operational taxonomical units (OTUs), i.e. T-RFs. Faunal data were fourth root-transformed to reduce any influence of high abundant species. Several statistical analyses were performed to search for patterns in bacterial community structures and faunal assemblages in relation to the hosting hydrogeological or geographical subunits, season and the spatial distribution (local vs. regional) of groundwater wells. In addition, they were applied to attribute community patterns to the measured environmental variables. If not specifically mentioned, faunal assemblages were always related to the well water and aquifer water data sets, while bacterial communities were only analyzed in comparison to the aquifer water data. In a first step, the correlation between the two aquifer water and well water datasets was evaluated by a Mantel Test, a multivariate permutational procedure (MANTEL & VALAND, 1970). Alternatively, the non-parametric Wilcoxon-Test and Spearman rank test were used for correlation analysis. For the analysis of differences between unpaired groups the Mann–Whitney U-test and the Kruskal-Wallis One Way ANOVA on Ranks were applied. Principal component analysis (PCA, Euclidian distance) were conducted to reduce the list of abiotic variables to meaningful components responsible for the major physical–chemical gradients encountered in the field, as well as to identify spatial patterns. To prove the statistical quality of patterns that were found discriminant analyses (DA) were performed. Patterns in the bacterial community structure and faunal assemblages were explored by cluster-analyses (Average linkage, Bray-Curtis similarity), and non-metric Multi Dimensional Scaling (referred to as MDS) based on Bray-Curtis dissimilarities. Additionally, for bacterial community data the impact of the two hydrogeological subunits (karst and alluvial aquifer), natural geographical regions (Lonetal-Flächenalb and Donauried), seasons (spring and autumn), and the distribution of wells (local vs. regional) was also tested with ADONIS (ANDERSON, 2001) while for the faunal assemblages this was evaluated by the ANOSIM (analysis of similarities) procedure (CLARKE &

GREEN, 1988) and DA. Both approaches were based on Bray-Curtis dissimilarities. Moreover, a regression analysis was applied to examine which environmental data correlate with the faunal distribution pattern depicted in the MDS plot. Finally, canonical correspondence analysis (CCA), a direct constrained ordination method that uses linear combinations of environmental (explanatory) variables to optimally explain the observed variance in T-RFs and faunal species (LEGENDRE & LEGENDRE, 1998) were applied. We chose CCA because it is known to be robust towards the absence of TRFs or faunal species in certain samples of a data set (RAMETTE, 2007), and towards relatively high numbers of TRFs compared with sampling locations (LEGENDRE & LEGENDRE, 1998). Prior to CCA, any variable or sampling site containing missing values were removed from the dataset. All statistical analyses were performed either with the R software, version 2.8.1, using the packages MASS (VENABLES & RIPLEY, 2002), reshape 0.8.0 and vegan 1.8–8 (for the latter package the recommendations of the author were followed: OKSANEN, 2008), or with Primer v6, Plymouth Laboratories, Plymouth, UK) and SPSS v15 (SPSS Inc.). Several faunal Indices – Margalef species richness ($d = (S - 1) / \log(N)$), Shannon-Wiener, ($H' = -\sum p_i \ln p_i$), and Pielouís Evenness ($J' = H' / \log(S)$) were calculated for each cluster (hydrogeological unit, geographical unit, etc.) for faunal and microbial assemblages. Moreover, the groundwater fauna index ($GFI = \sqrt{DO\ (mgL^{-1})} \times \sqrt{\text{relative amount of detritus}} \times SD$ temperature), developed by HAHN (HAHN, 2006) was calculated.

3.2.2.4 Results and Discussion

Physical–chemical characteristics of the karst and alluvial aquifer

According to our proposed typology, we tested if the two hydrogeological units (aquifer types) can be separated from each other by their hydrochemistry, which was not the case. Statistically, no significant difference in the composition of groundwater was observed between samples from karst and alluvial wells (DA: $p = 0.201$). This is also expressed by a very low ANOSIM value ($r = 0.051$), indicating a low similarity between wells of the same aquifer type, compared to wells located in a different type of aquifer. However, looking at the individual data, it is obvious that groundwater samples from the alluvial aquifer displayed a higher variability within sampling sites (Fig. 3).

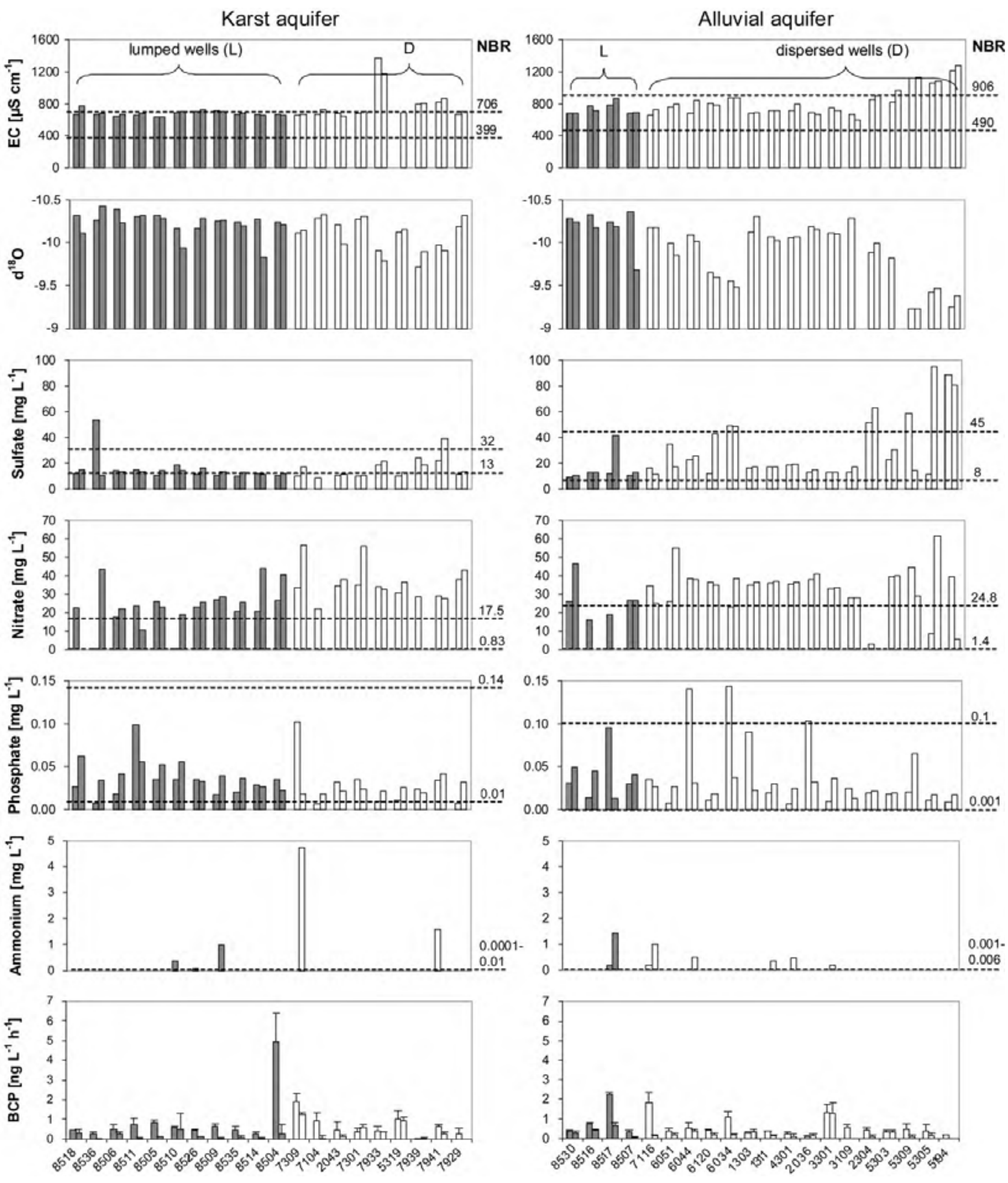

Fig. 3: Selected physicochemical and microbial parameters determined in groundwater from monitoring wells in karst ($n = 20$) and alluvial ($n = 20$) aquifers at the Swabian Alb, South Germany. The first of the bar pairs depicts the spring (May/June 2007) and the second the autumn (October 2007) samples. Grey bars represent locally (lumped) and white bars regionally (dispersed) distributed wells. Values are either means of duplicate measurements or means of triplicates ± SD. Dashed lines and nearby values give the natural background range (NBR; 10th and 90th percentile) as reported in KUNKEL *et al.* (2004). BCP = bacterial carbon production, EC = electric conductivity.

Moreover, groundwater from the alluvial quaternary aquifer showed slightly elevated concentrations for most chemical species, which is in accordance with the natural geochemical background (Fig. 3; KUNKEL *et al.*, 2004). It is striking that nitrate concentrations in almost every sample exceeded the upper natural background concentrations of the karst (17.5 mgL^{-1}) and alluvial system (24.8 mgL^{-1}) of that region. Karst groundwater systems are generally characterized by high seasonal hydraulic dynamics (BAKALOWICZ, 2005). This is partly reflected in the variation of individual physical–chemical parameters determined in spring and autumn samples, such as nitrate and orthophosphate (Fig. 3), as well as temperature and dissolved oxygen (data not shown). Nevertheless, these parameters displayed also a considerable seasonal dynamics within the alluvial aquifer. We also compared water pumped from the aquifer (groundwater) which was used for microbiological analysis with water from the according wells where the fauna originated from (see materials and methods section). A striking correlation ($r = 0.90$, $p = 0.001$, Mantel test) was revealed. Looking at individual parameters, only pH, ammonium, orthophosphate and ^3H showed significant differences between aquifer and well water (data not shown). A principal component analysis (PCA) of physical-chemical parameters underlined the lack of separation between the two aquifer types but indicated a slight segregation between the two natural geographic regions Lonetal-Flächenalb and Donauried (Fig. 4). Compared to Donauried samples, the Lonetal-Flächenalb and especially wells at Burgberg (located close to each other) are characterized by a more homogeneous groundwater composition, except for the outlier wells 7939, 7941 and 7933 where high EC values were recorded. The samples of the Donauried showed generally a higher variability in its chemical composition. Compared to the Lonetal-Flächenalb, Donauried wells exhibited slightly higher concentrations of nitrate, chloride and orthophosphate. However, the differences between the hydrochemistry of groundwater from the Lonetal-Flächenalb and Donauried were small and not statistically significant. This is emphasized by PCA axis 1 and 2 that explain only 55.2 % of the variation of the data set examined. For PCA axis 1 the factors with the strongest influence are electric conductivity (EC) (Eigenvector: 0.448) and sulphate (Eigenvector: 0.440), while for function 2 nitrate (Eigenvector: 0.696) and chloride (Eigenvector: 0.681) are the most influencing factors (Fig. 4). Especially function 2 of the PCA is responsible for the separation of the two natural geographic regions (Fig. 4). The small differences in groundwater composition of the two aquifer types are ascribed to the strong hydraulic connectivity. According to SCHNECK *et al.* (2004), the alluvial quaternary groundwater bodies of the Donauried and Lonetal-Flächenalb are mainly supplied by karst groundwater from the Lonetal-Flächenalb. The connection between karst and alluvial aquifer seems pronounced especially at Burgberg, where the numerous wells are situated in a close spatial proximity (Fig. 2). Data from karst and alluvial wells exhibited a high similarity in groundwater composition. Looking at the natural groundwater background composition, as defined by KUNKEL *et al.* (2004) for both hydrogeological units, i.e. the 'Limestones of the upper Jurassic' and the 'Gravels and moraines of the Alpine foothills', it basically displays some pronounced differences for individual chemical variables. Groundwater from the alluvial aquifers is characterized by higher anion and cation concentrations. Worth to mention is the elevated concentration of carbonate, sulphate, nitrate, and consequently an increased conductivity (KUNKEL *et al.*, 2004). In contrary, natural orthophosphate concentration and pH was found a little higher for the karst waters. However, not all samples reflected natural conditions. The slightly higher concentrations of chloride, nitrate, orthophosphate, sodium, potassium, calcium, magnesium and sulphate determined for samples of the Donauried indicate some impact from agriculture (STREBEL *et al.*, 1989; BÖHLKE, 2002; SAFFIGNA & KEENEY, 2006). In fact, the Donauried is intensively used for agriculture (HAAKH, 2002; SCHNECK *et al.*, 2004) and most wells are located beside agricultural crop lands. In contrast, land of the Lonetal-Flächenalb located in the area of Burgberg (lumped wells) is characterized by pastures. Nevertheless, differences between the two natural geographic regions are statistically weak most probably caused by sharing one groundwater catchment area (KOLOKOTRONIS *et al.*, 2002).

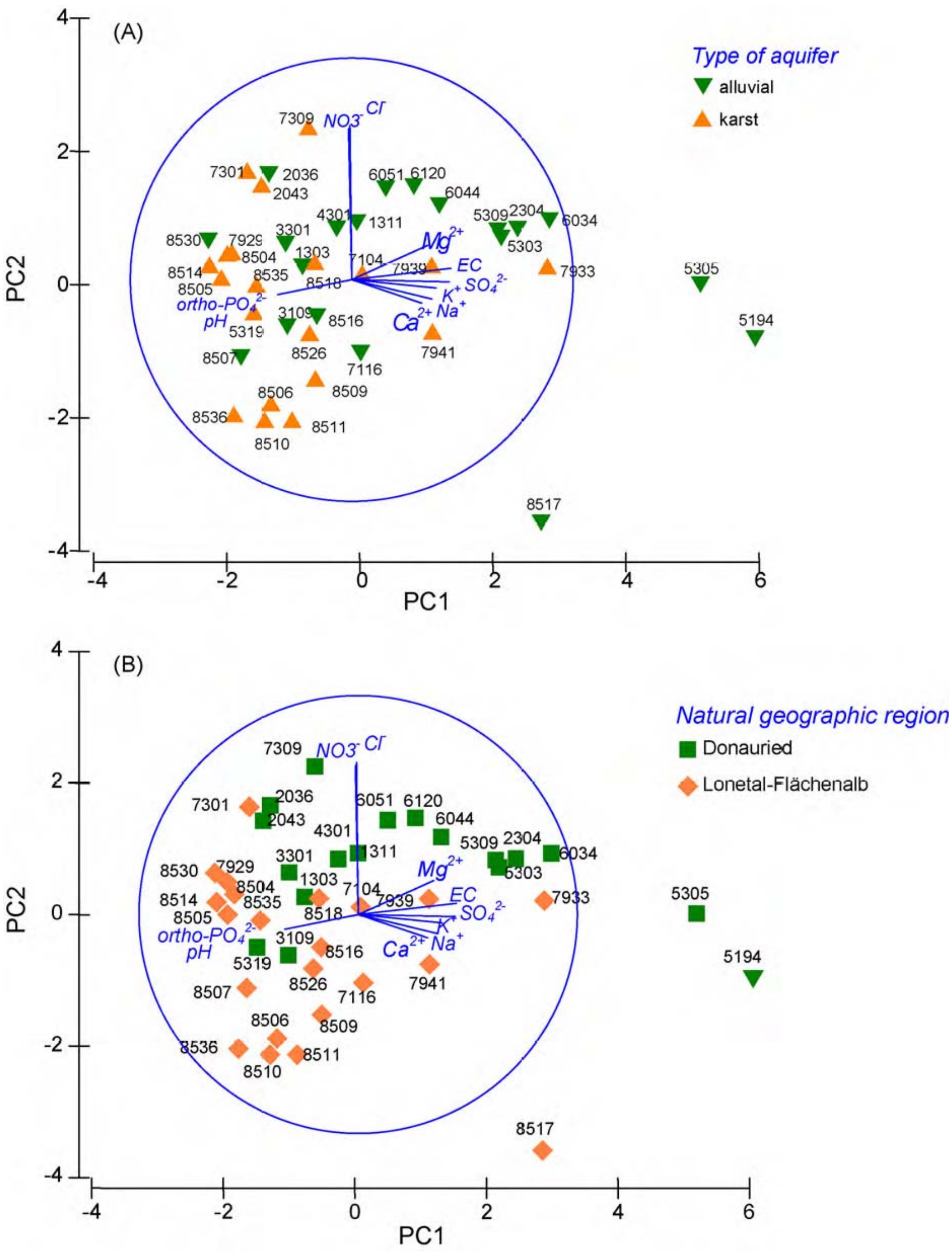

Fig. 4: Principal component analysis (PCA) performed for all samples collected in the study area taking into account all physical–chemical variables in relation to the spatial distribution of (A) aquifer types and (B) natural geographic regions.

Groundwater fauna

In total, 1332 animals were found, constituting 32 taxonomic groups of crustaceans, oligochaets, nematods, tardigrads, gastropods, water mites and insects (Collembola). Approximately 71 % of the faunal samples were composed of crustaceans, of which 631 animals were identified to species level, comprising 22 species. Furthermore, all crustacean species were assigned to two ecological groups – stygobites (= true groundwater organisms) and non-stygobites. The proportion of stygobiontic crustaceans was very high (98.7 %) compared to the proportion of non-stygobiontic ones (Table 3). Out of 40 groundwater monitoring wells sampled, fauna was absent in only three wells of the Lonetal-Flächenalb pertaining two karst (8511, 8535) and one alluvial (8517) well. The abundance and taxa richness found in this study are typical for groundwater communities (CASTELLARINI 2005, MARTIN *et al.*, 2009, HAHN & FUCHS, 2009). However, the high proportion of stygobiontic crustacean species (98.7 %) was surprising. Such a high proportions of stygobiontic species as well as low frequencies and abundances of non-stygobites are referred to groundwater systems that are hardly affected by surface water impacts (STRAYER, 1994; MÖSSLACHER *et al.*, 1996; BRUNKE *et al.*, 2003; DATRY *et al.*, 2005; HAHN, 2006). Since shallow quaternary aquifers, and especially karst aquifers, as were examined in this study, are characterized as open systems with a high connectivity to the surface and adjacent water bodies (HAHN & FUCHS, 2009), usually high proportions of non-stygobiontic species can be expected (BERKHOFF *et al.*, 2009; BORK *et al.*, 2009). However, for the Lonetal-Flächenalb exfiltration of karst water into the alluvial aquifers and associated surface waters seem to be of much higher importance then surface water infiltration, as documented for the Hürbe brook at the Burgberg (KOLOKOTRONIS *et al.*, 2002). This way, non-stygobiontic species probably are temporarily prevented from entering the groundwater system. In contrast, stygobiontic metazoans are dwelling from deeper karst into the alluvial groundwater bodies. Another aspect is the comparatively low number of rivers at the Lonetal-Flächenalb, which is caused by quick and extensive infiltration of precipitation into the karstified underground (KOLOKOTRONIS *et al.*, 2002). In the following, only wells (34 wells) populated by crustaceans that could be determined to species level, were included into analysis (see Table 3). Taking into account the close connectivity of the two hydrogeological units, it is not surprising that no distinct differences in the metazoan community structure were found between the alluvial and karst aquifer (ANOSIM: $r = 0.077$; DA: $p = 0.281$, $n = 34$). Except for the amphipods *C. subterraneus* and *N. casparyi* as well as the cyclpoid *D. bisetosus* (karst aquifer), no other species that was found at more than one site was recorded exclusively in either one of the aquifer types (Table 3). Moreover, this relation to one type of aquifer probably results in the low repetition of sampling, since in other studies (e.g. FUCHS, 2007) these species have been recorded in alluvial aquifers. A similar result was obtained when comparing indices of biodiversity. Almost no difference between the type of aquifer and species richness (karst: $d = 3.27$; alluvial: $d = 3.89$), diversity (karst:$H' = 2.03$; alluvial: $H` = 2.26$) and evenness (karst: $J' = 0.66$; alluvial: $J' = 0.71$) became apparent. However, the diversity was slightly higher in the alluvial wells, whereas in the same number of samples more than twice as many animals were found in karst wells (617) compared to wells in the alluvial groundwater bodies (287). When comparing locally distributed wells versus regionally distributed wells, no difference in faunal assemblages was found. The same result became apparent when testing separately for each natural geographic region (ANOSIM for all combinations < 0.3). Interestingly, faunal data revealed differences of metazoan assemblages on the level of the two natural geographic regions, Donauried and Lonetal-Flächenalb. The multidimensional scaling analysis displayed in Fig. 5 was generated with all crustacean species and the taxonomic group of oligochaets. In the MDS plot the groundwater monitoring wells of both natural geographic regions are distinguished by faunal data. Exceptions are a few outliers from the Lonetal-Flächenalb, well 8509, 7929 and 7939, which were assigned to the Donauried and vice versa well 3301 presenting an assemblage more similar to the Lonetal-Flächenalb communities (Fig. 5). Statistically, these differences between the natural graphic regions are weak (ANOSIM, $r = 0.426$, $n = 34$) but significant. Compared to the Donauried, a higher species richness (18 species, $d = 2.78$), abundance (453 specimen) and diversity ($H' = 1.65$) was recorded for the Lonetal-Flächenalb.Onthe opposite, 13 crustacean species ($d = 2.32$), composing 178 individuals and a slightly lower diversity ($H' = 1.51$) were found at the Donauried. It is notable, that several species, namely *P. nolli*, *A. sensitivus*, *A. venustus* and *C. subterraneus* were mostly or only recorded in wells of the Lonetal-Flächenalb. Another observation is that *D. languidoides* was found more frequently (14 wells, 245 specimen) at the Lonetal- Flächenalb, compared to the Donauried (7 wells, 45 individuals). In contrast, *A. rhenanus* seems to have its main range at the Donauried (11 well, 88 specimen), while it was recorded less frequently at the Lonetal-Flächenalb (7 wells, 34 specimen). Oligochaets were recorded more frequently in wells of the Donauried. But the proportion of non-stygobiontic species was very low in both regions. Faunistic groundwater studies of Baden-Württemberg underlined biogeographic patterns in faunal assemblages (FUCHS, 2007). For example, the groundwater species *P. nolli*, *N. laisi* and *A. rhenanus* where exclusively found in groundwaters of the Danube River catchment. These patterns are confirmed by the results of this study, supporting the hierarchical classification concept of groundwater ecosystems by HAHN (2006, 2009) as proposed in our study. There is information that the seasonal changes in groundwater table at the area of Burgberg (Lonetal-Flächenalb) are moderate. One reason is that a surplus of groundwater from the karst directly exfiltrates into the Hürbe brook. Another explanation is the constant extraction of groundwater by the communal waterworks (KOLOKOTRONIS *et al.*, 2002). At the Donauried, groundwater level fluctuations are reduced, due to a layer of silt and clay reducing surface water-groundwater interactions. Furthermore, the amplitude of groundwater level fluctuations is attenuated, since the maingroundwater recharge takes place in the north-western parts of the Swabian Alb and thus the drainage to the Donauried has a long retention time (KOLOKOTRONIS *et al.*, 2002). In contrast to our early expectations, no differences in the faunal assemblages were found when comparing the two types of aquifer but for the natural geographic regions. According to HUMPHREYS (2009), groundwater fauna is significantly shaped by hydrological interactions. The karst groundwater is frequently dwelling into the alluvial aquifer, and contributes to a mixture of faunal communities. Besides that, the matrix of the unconsolidated groundwater bodies at the Lonetal-Flächenalb originates from karstified and weathered rocks and is characterized by large interstices. Thus at the Lonetal-Flächenalb, the high similarity of faunal communities clearly reflects the strong hydrological connectivity of the alluvial and karst aquifer. For the Donauried, also no faunal differences between the alluvial and karst aquifer were found. It is assumed that the similarity of metazoan communities recorded in this region of the study site is caused by, (1) the karst groundwater dwelling from the Lonetal-Flächenalb into the Donauried, and (2) the low hydraulic connectivity of the aquifer to the surface. The latter is mainly determined by a distinct silt and clay layer overlying the quaternary aquifer of the Donauried. Exceptions are the area of the wells 2304 and 7929 (Fig. 2). In summary, faunal data revealed differences on the regional scale (meso scale). However, due to the similar groundwater composition of the karst and alluvial aquifers and its extraordinary interconnectivity, no separation of aquifer types was revealed, but a separation by natural geographic regions. Consequently, the separation is more driven by regional aspects and anthropogenic impacts, such as land use practices. Still, with respect to the intensity of land use in the Donauried, the impacts from surface waters and agriculture to groundwater fauna are low which is reflected by the low proportion of non-stygobites, again hinting towards a low direct hydraulic connectivity to the surface.

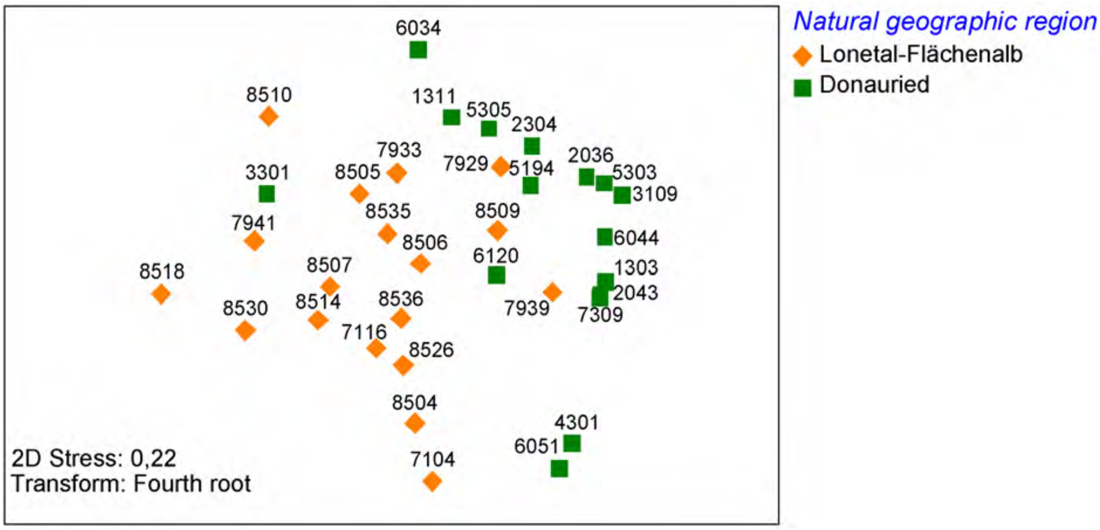

Fig. 5: MDS plot based on Bray-Curtis similarity plus dummy variable (= 1) calculated for faunal assemblages of crustaceans and oligochaets recorded in 34 groundwater monitoring wells. The symbols and colouring refer to the natural geographic regions (Lonetal-Flächenalb and Donauried). ANOSIM: R = 0.312.

Table 3: Taxa-well-matrix of metazoan species recorded in groundwater monitoring wells at the federal state Baden-Württemberg, south-western Germany. Data include samples of two sampling campaigns (spring and autumn 2007). Only wells where crustaceans were recorded are shown and the crustacean species are assigned to ecological groups (stygobites and non-stygobites). A= alluvial aquifer, K= karstic aquifer.

Natural geographic region	Donauried																Lonetal-Flächenalb																	
Type of aquifer	A	A	A	A	A	A	A	A	A	A	A	A	A	A	K	K	A	A	A	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K
Well depth (m)	12	148	8	9	10	7	10	13	8	9	15	14	8	8	91	21	7	6	8	38	10	11	15	50	45	17	30	10	39	41	27	48	15	50
Well no.	3301	5303	5305	2304	5194	3109	2036	1303	6120	6044	4301	1311	6034	6051	2043	7309	8507	8530	7116	7941	8518	8510	8505	8535	8514	8506	7939	8504	7933	7929	8509	8536	7104	8526
Stygobites																																		
Proasellus nolli	1																1	3		8	22	5	6	2	3	2	1	3						
Acanthocyclops sensitivus	18																		2	5	4	1	7						11				2	
Niphargus laisi							1											2		1	3	1		2		1			1			1		
Acanthocyclops venustus																		1			4	3												1
Niphargopsis casparyi																					1				2									
Crangonyx subterraneus																				27	2									7				
Fabaeformiscandona breuili																	1																	
Fabaeformiscandona wegelini																					2													
Schellencandona schellenbergi																					11													
Schellencandona insueta																						1												
Echinocamptus pilosus	1																																	
Niphargus fontinalis																		1																
Fabaeformiscandona bilobata													5					2						1										
Niphargus auerbachi											1							2			1													
Niphargus aquilex			3																															
Graeteriella unisetigera														1																				
Niphargus kieferi	1	1	3									3		1								1												
Acanthocyclops rhenanus		7	11	1	13	33	3	13	1	1					3	2							4	1		1	8		9	4	7			
Diacyclops languidoides	4		8	1	2				1			2	27				10	1	1	6	1		159	5	2	1			44	4	9	1		1
Non-stygobites																																		
Diacyclops bisetosus	1			1																													2	
Diacyclops languidus				1									2																					
Cypria ophtalmica																															1			
Other taxonomic groups																																		
Acari						1		2				2	2				5				1			5	1		1				2	1	8	1
Gastropoda																		6			26			98										
Insekten		2			1			6		10	1	8	1	7		6			1	7		1	6				2	1	5	3	2		1	
Microturbellaria													1																					
Nematoda													1	2																1				
Oligochaeta		2		2	5	2	4			1		1	8					1				5								1				
Tardigra													2									1												
Juvenile & indettaxa	3	4	6	1	9	13	5	5	4	1		2	29		2	4	4	2	2	7	25	1	178	9		3	2	1	60	25	7	2	3	1

Microbial communities in groundwater

The bacterial communities in groundwater from the Swabian Alb were found to differ significantly in their taxonomic richness. While a total of 468 TRFs (operational taxonomic units; OTUs) were identified, the maximum diversity was 166 TRFs which were detected in a karst water sample from well 5319. At the same time, samples with lowest number of TRFs were represented by karst samples, i.e. only 5, 10 and 12 TRFs were detected in water from the wells 8526, 7929, and 7939, respectively. On average, karst water samples contained 62 TRFs and groundwater from the alluvial aquifer system 65 TRFs (Table 4). Bacterial diversity, expressed by the Shannon-Wiener Index (H′) was found comparably low, as it is typical for oligotrophic and oligoalimonic groundwaters. The alluvial aquifer system displayed slightly higher values than the karst waters (Table 4). Highest biodiversity and lowest deviation, i.e. $H' = 2.98 \pm 0.5$ SD, within a cluster of samples were detected for the locally distributed wells of the alluvium in the area of Burgberg. Although hydrological tightly connected, the karst waters in this area exhibited on average a lower diversity. However, no significant differences in biodiversity where found in between the different cluster according to aquifer types ($p = 0.945$; Kruskal-Wallis One Way ANOVA on ranks) and natural geographical regions ($p = 0.671$). When compared to results from other studies, bacterial biodiversity at the Swabian Alb was found in the lower range. BRIELMANN *et al.* (2009) reported H′ values between 2.6 and 3.4 for an oligotrophic quaternary alluvial aquifer in the area of Munich, Southern Germany. A much higher bacterial biodiversity was found in two gravel and sandy aquifer systems seriously impacted by agriculture, in the area of the city of Cologne, Germany. Here, values of $H' = 2.75$ to 4.77 have been determined (STEIN *et al.*, 2010). These results indicate that the bacterial diversity as inferred by the Shannon-Wiener index may be a first indicator of anthropogenic impacts (see discussion below). With respect to community composition, a significant difference was found only for the samples of Burgberg when karst and alluvial samples were pooled together and compared with the regionally distributed wells at the Lonetal-Flächenalb. No difference was found between the karst and alluvial aquifer ($R^2 = 0.023$, $p = 0.6$, ADONIS) as well as for the two regions Lonetal-Flächenalb and the Donauried ($R^2 = 0.0236$, $p = 0.555$, ADONIS). A canonical correspondence analysis (CCA) revealed that microbial communities grouped according to the local versus regional distribution (Fig. 6). In brief, this means that microbial communities were found more similar within groundwater samples of wells joining closely. However, it has to be mentioned that for microbial community analysis only data from the spring sampling survey were available and a slightly reduced number of wells (those with a complete data set) were used for the CCA analysis. To sort out in the future if microbial communities display a typical composition with aquifer type or natural geographic region, a higher temporal and spatial resolution is needed. Bacterial carbon production (BCP) activity was generally found higher with spring samples (Table 4). This may be related to the late autumn sampling in November (beginning of winter). Highest activities were found on average in samples from the Burgberg wells. Opposite to the biodiversity, spring samples displayed higher activities in karst water samples than in samples from the alluvial aquifer. In autumn, activities were generally found decreased by a factor of 2–3 and no significant difference was found between karst samples and groundwater from the alluvial system (Table 4).

Table 4 Bacterial carbon production, biodiversity and taxonomic richness for different clusters.

Cluster	Taxonomic richness [no. TRFs] spring 2007			Bacterial biodiversity [H'] spring 2007		Bacterial activity (BCP) [ng L^{-1} h^{-1}]			
						Spring 2007		Autumn 2007	
	MW	Minimum	Maximum	MW	SD	MW	SD	MW	SD
Karst all	62.25	5	166	2.58	1.10	0.82	1.05	0.29	0.34
Karst local	62.91	5	157	2.69	1.06	0.93	1.34	0.17	0.15
Karst regional	61.44	10	166	2.45	1.20	0.68	0.55	0.46	0.45
Alluvial all	65.4	18	131	2.85	0.79	0.63	0.57	0.29	0.28
Alluvial local	70.50	44	130	2.98	0.50	0.92	0.91	0.35	0.25
Alluvial regional	64.0	18	131	2.81	0.88	0.56	0.47	0.27	0.30
Lonetal-Fl all	61.7	5	157	2.62	1.04	0.84	1.05	0.23	0.20
Lonetal-Fl local	64.93	5	157	2.77	0.94	0.93	1.21	0.22	0.19
Lonetal-Fl regional	54.71	10	156	2.3	1.25	0.65	0.59	0.24	0.22
Donauried all	66.63	18	166	2.83	0.9	0.58	0.46	0.37	0.40

Comparable low activities have been found in an oligotrophic alluvial aquifer in Southern Germany (BRIELMANN *et al.*, 2009). No simple correlation was found between BCP and other abiotic variables. However, as can be seen from Fig. 3 increased BCP values coincided in most cases with the appearance of ammonium in groundwater and extraordinary high TOC concentrations, which unfortunately have been determined only in well water (data not shown).

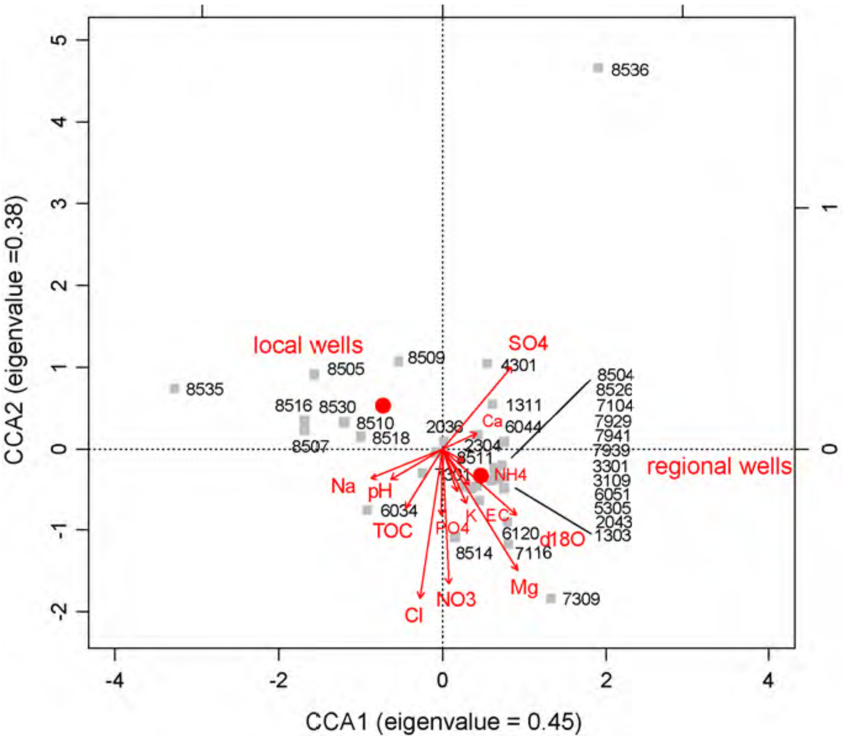

Fig. 6: Species-conditional ordination diagram based on the canonical correspondence analysis (CCA) of TRF data from 28 wells displaying 12 % of the inertia (= weighted variance) in the relative TRF abundances and 27 % of variance in the weighted averages and class totals of species (TRFs) with respect to the environmental variables. Single TRFs are not shown but sites (squares) clearly group along the spatial gradient indicated by the class variable 'distribution" given by labeled 'local wells" and 'regional wells" (red points). Quantitative environmental variables are indicated by arrows.

Correlations between fauna, microbes and physical–chemical conditions

For faunal data and environmental parameters mostly weak and inconsistent correlations were found. Amongst the trophic parameters the relative amount of detritus was positively correlated to species richness (Spearman: $r = 0.462$, $p = 0.003$, $n = 40$) and abundance ($r = 0.469$, $p = 0.002$, $n = 40$). Furthermore, detritus correlated positively with the standard deviation of temperature ($r = 0{,}346$, $p = 0.031$, $n = 39$), while a negative correlation was found with the depth of wells ($r = -0.436$, $p = 0.01$, $n = 34$). Another trophic parameter correlating with faunal data was the amount of particulate organic matter (POM). It correlated positively with the species richness ($r = 0.390$, $p = 0.033$, $n = 30$), abundance ($r = 0.433$, $p = 0.017$, $n = 30$) and H′ diversity ($r = 0.459$, $p = 0.011$, $n = 30$). In contrast, weak negative correlations were found between ochre and species richness ($r = -0.389$, $p = 0.013$, $n = 40$) and abundance ($r = -0.378$, $p = 0.016$, $n = 40$). The groundwater fauna index (GFI) developed by HAHN (2006), which indicates the strength of ecologically relevant hydrologic connectivity to surface ecosystemswaspositively correlated with invertebrate abundance ($r = 0.374$, $p = 0.018$, $n = 40$) and species richness ($r = 0.320$, $p = 0.044$, $n = 40$), indicating an increase in abundance and species richness with an enhanced influence from the surface (HAHN, 2009). With an increase of depth, the direct influence from the surface (precipitation, seepage water, surface water) decreases, what is underlined by the negative correlations between the GFI and the depths of the groundwater monitoring wells ($r = -0.572$, $p = 0.000$, $n = 40$). As confirmed by a number of other groundwater studies, tight correlations between faunal and physical–chemical variables are generally rare and inconsistent (e.g. HAHN, 2006, HUMPHREYS, 2009; TOMLINSON & BOULTON, 2009). However, the frequently found correlations between trophic parameters (relative amount of detritus and POM) with species richness, diversity and abundance demonstrate the importance of organic matter for the distribution of groundwater fauna (GIBERT & DEHARVENG, 2002; MALARD *et al.*, 2004; DATRY *et* *al.*, 2005; HAHN, 2006; STEIN *et al.*, 2009). Negative correlations between ochre, abundance and species richness was also found in previous groundwater studies (FUCHS, 2007). As true for the fauna, microbial parameters do hardly exhibit simple correlations with individual physical–chemical parameters. Extensive research is still needed to identify and better understand factors controlling microbial biodiversity and activity in the subsurface. Potentially important factors include the local hydrogeochemistry (such as e.g. nitrate pollution, STREBEL *et al.*, 1989), availability of oxidizable substrates, dispersal of microbes from overlying unsaturated zones, and the simple food-web structure in aquifers (GRIEBLER & LUEDERS, 2009). In our study, spearman rank analyses revealed some correlations between microbial parameters and hydrochemistry. Bacterial carbon production (BCP) activity was related to dissolved oxygen (DO) ($R = 0.298$, $p < 0.05$, $n = 64$) and temperature ($r = 0.255$, $p = < 0.05$, $n = 67$). BCP also exhibited a strong correlation to the TOC concentration ($r = 0.521$, $p = < 0.001$, $n = 73$) in well water; unfortunately no DOC/TOC data were available for the aquifer water. Bacterial diversity [H´], available only for spring samples, showed a negative correlation with DO ($r = -0.470$, $p = < 0.05$, $n = 26$). In spring BCP additionally correlated with the concentration of ammonium ($r = 0.312$, $p < 0.05$, $n = 40$). So far, there is only little information for direct relationships between fauna and microorganisms. Our data set revealed first evidence by multivariate statistical analyses (CCA) that individual metazoans group together with certain microbes (TRFs) (data not shown). Whether these correlations between individual bacterial strains and invertebrates are meaningful, has to be elucidated in further, more detailed studies.

3.2.2.5 Prospects and conclusions

There is no doubt that groundwater microorganisms and invertebrates may serve as sentinels for anthropogenic impacts. Unfortunately, our knowledge on the natural composition of groundwater ecosystems communities is still limited. Our study could show that from the set of biological and ecological variables tested, some proved to have a clear indicative value. On one hand groundwater metazoan communities could be separated according to the two natural geographic regions investigated, reflecting the anthropogenic impacts from land use rather than the structural settings (aquifer type). Moreover, the low percentage of non-stygobiontic metazoans proved a mean hydraulic impact to these aquifers from the surface. In a similar way, the relatively low bacterial diversity mirrored conditions of low energy and productivity (no simultaneous availability of DOC and nutrients) and low degree of disturbance (intermediate disturbances cause an increased biodiversity; GRIEBLER & LUEDERS, 2009). However, we still have not identified bioindicators related to individual stressors present, such as nitrate. Whereas with metazoans the 'species' is most probably the taxonomic unit of choice, this is not clear at all with respect to microbes. The immense microbial biodiversity and the 'artificial' species definition (GRIEBLER & LUEDERS, 2009) provide no simple solution in searching for bioindicative 'units'. On one hand, we look for high resolution analysis combined with high-throughput methods, while on the other hand, we intent to keep the analysis and resolution as simple as possible. A selection of molecular methods available for microbial community analysis is briefly discussed in the following before current statistical methods for the identification of sentinels are introduced. In a final paragraph we conclude on the progress for the development of an ecological assessment scheme for groundwater ecosystems.

High-throughput analysis of microbial communities

The discrepancy between culturable and in situ diversity has highly encouraged the development of culture-independent molecular methods to analyse microbial diversity in ecosystems. Predominantly, the 16S rRNA coding gene is used to assess microbial diversity and abundance. At the same time, genes involved in specific metabolic pathways are increasingly used to study physiological capabilities of microorganisms in environmental samples. Today, a number of community analysis methods are available and have been successfully applied in microbial ecology; i.e. terminal restriction fragment length polymorphism (T-RFLP) (AVANISS-AGHAJANI *et al.*, 1994; CLEMENT *et al.*, 1998), denaturing gradient gel electrophoresis (DGGE) (MUYZER *et al.*, 1993), single strand conformation polymorphism (SSCP) (LEE *et al.*, 1996), automated ribosomal intergenic spacer analysis (ARISA) (Fisher and Triplett, 1999), and the randomly amplified polymorphic DNA (RAPD) approach (WILLIAMS *et al.*, 1990) to name the most popular ones. Recently, few molecular approaches have been developed whose technique is based on the recovery of short DNA sequences tags from microbial communities (SARD: serial analysis of rRNA genes, SARST-V1/V6: Serial analysis of V1/V6 ribosomal sequence tags, SARST) (NEUFELD *et al.*, 2004; KYSELA *et al.*, 2005; YU *et al.*, 2006; ASHBY *et al.*, 2007). Serial analysis of ribosomal sequence tags (RSTs) employs a set of enzymatic reactions to amplify RSTs from variable regions of the 16S rRNA gene and to legate them into concatemers that are subjected to cloning and sequencing. It provides a considerable high-throughput method because up to 20 RSTs can be obtained from one sequencing reaction. Of course, the shortage in sequence length is accompanied by a much lower resolution. About ten years ago, first microarrays for application in microbial ecology were developed that have the capability to provide a simultaneous detection of different genes (GUSHIN *et al.*, 1997). There are two different kinds of microarrays that differ in their gene specificity. Phylochips employ 16S rRNA gene sequences of specific microorganisms within a community and functional arrays target genes encoding enzymes of specific metabolic pathways such as biogeochemical cycling of carbon, nitrogen and sulphur (ZHOU, 2003; RHEE *et al.*, 2004; HE *et al.*, 2007; WAGNER *et al.*, 2007). At this point, microarrays provide a meaningful high-throughput method to describe the enormous microbial diversity. Nevertheless, there are still limitations of this application concerning specificity and sensitivity. The appropriate method, however, has to be chosen based on aspects such as the complexity of the community, resolution required and desired, or simply by money and time available. Without doubt, a common goal of our studies must be the identification of microbial sentinels, which will build the ground for the development of a 'groundwater ecostatus' microarray.

Identification of ecological criteria and bioindicators (sentinels)

Linking biodiversity patterns with environmental data is an ongoing challenge for ecologists (e.g. MARTINY *et al.*, 2006) and it takes the application of multivariate statistics to analyse the usually complex data sets. In multivariate statistics, a variety of explorative techniques such as PCA (principal component analysis), PcoA (principal coordinate analysis), MDS (nonmetric multidimensional scaling), and CA (correspondence analysis) are available to search for community patterns. Further, hypothesis-driven techniques such as redundancy analysis (RDA), canonical correspondence analysis (CCA) or matrix correlations (Mantel test) allow an ecological interpretation of the data sets and can help to detect gradients or correlations between biotic and abiotic variables (see LEGENDRE & LEGENDRE, 1998, RAMETTE, 2007). Several of these techniques have been successfully applied in the current study, and helped to (i) reveal correlations between biotic and abiotic variables, (ii) to separate and characterize investigation areas by organismic assemblages (i.e. MDS), and (iii) allowed first identification of candidate sentinels indicative for individual stressors such as nutrients (i.e. CCA). A new multivariate approach, which recently became popular, is niche analysis (Outlying Mean Index; OMI; DOLÉDEC *et al.*, 2000) and has been successfully applied to relate environmental variables to groundwater faunal biodiversity (DOLE-OLIVIER *et al.*, 2009; GALASSI *et al.*, 2009; MARTIN *et al.*, 2009). This method gives equal weight to all sites whatever their species richness is. It positions sampling units in a multidimensional space defined by the environmental variables. Hence, the distribution of species along these environmental gradients represents their realized niche. Here, we propose the application of multivariate regression trees (MRT) and aggregated boosted trees (ABT) as promising powerful alternatives for the directed community analysis of species-environment data (DE'ATH, 2002, 2007). MRT combine the advantages of multivariate regression and constrained clustering resulting in groups that are similar in a chosen measure of species dissimilarity but are also defined by a set of environmental variables. Hence, each cluster defines an assemblage type characterized by certain indicator species while the environmental values additionally define an associated habitat type (DE'ATH, 2002). ABT analysis helps to overcome two weaknesses associated with MRT, namely (1) the poor predictive power and (2) the difficulty in interpretation of large trees (DE'ATH, 2007). So far, MRT and ABT have been used to assess the influence of land management on soil and forest floor microbial communities (GE *et al.*, 2008; SWALLOW *et al.*, 2009). It is planned to apply these methods for further data analysis. We think these will help to define natural reference conditions and to identify bioindicators for the different groundwater units under investigation.

Appropriate scale and needs for the assessment of groundwater ecosystems

In a recent review, TOMLINSON & BOULTON (2009) raised the question of the appropriate scale for an ecologically-relevant groundwater management. For an assessment of groundwater ecosystems the complexity of subterranean aquatic environments needs to be distinguished and organized into simplified manageable units. In the past, lots of efforts have been put into first ecologically driven classification systems of groundwater ecosystems (HUSMANN, 1967; GIBERT *et al.*, 1994; HAHN, 2006). The data presented, as well as comparable studies from the literature (e.g. GIBERT *et al.*, 2009; HAHN, 2009; HAHN & FUCHS, 2009; HUMPHREYS, 2009; TOMLINSON & BOULTON, 2009) underline that in groundwater ecological investigations different scales have to be considered

(Fig. 1). Without doubt the data show that the approach of the three scaled hierarchical model for the typology of groundwater habitats, proposed by HAHN (2006), generally is an appropriate way to assess groundwater communities. However, since the faunistic assessment only included study sites in one biogeographic region ('Central German Uplands') this spatial scale could not be examined appropriately. Nevertheless, some of the species recorded can be considered as typical faunal elements of the Danube River catchment area (FUCHS, 2007). And, species composition as found for the Swabian Alb differed when compared to faunal assemblages collected in the bioregion 'Central German Lowlands' (STEIN *et al.* 2010, MATZKE *et al.* unpublished report). However, one has to be aware that the 'bioregion' (above surface) is not necessarily accordant with the 'stygoregion' (below surface). Hence, future studies should be directed to the delineation of distinct stygoregions. The importance of biogeographical aspects often overriding the influence of the aquifer type was demonstrated by FUCHS (2007) and HAHN & FUCHS (2009). At the meso scale, our data show that the groundwater fauna of the Swabian Alb distributes according to natural geographic regions (Lonetal-Flächenalb, Donauried), rather than according to the type of aquifer (alluvial, karst). The similarity of faunal assemblages found in alluvial and karstic aquifers indicates the strong hydraulic connectivity of both systems. Similarly, the microbial communities, which are generally suggested to be strongly determined by the geology and hydrochemistry (GRIEBLER & LUEDERS, 2009) displayed no distinct compositions, which could be attributed to the aquifer types. In contrast to the fauna, no separation of the two natural geographic regions based on microbial community composition was obvious. However, while the fauna did not reveal different patterns between locally and regionally distributed wells, microbial communities did. Results from a comparable study focusing on the distribution and diversity of fauna and microbes in two hydrologically separated but geologically similar shallow quaternary aquifer systems revealed similar patterns for the distribution and structure of microbial and faunal communities, although the physical–chemical conditions slightly differed (STEIN *et al.*, 2010). These patterns were strongly supported by the data from hydrochemistry. Although the two aquifer types were expected to exhibit some minor differences in physical–chemical conditions with respect to their geological background (KUNKEL *et al.*, 2004), the strong interconnection of the alluvial groundwater bodies at the Lonetal-Flächenalb with the karst system suspended these small differences. The slight hydrochemical segregation on landscape scale (Lonetal-Flächenalb, Donauried) reflects the different anthropogenic impacts such as land use activities and regional aspects as well as the differences in groundwater mean transit time. At the local scale, the absence of non-stygobiontic crustaceans indicated the hydrological situation in the wells sampled at both natural geographic regions. At the Lonetal-Flächenalb, the lack of non-stygobionts could be related to the low number of surface waters and the dominance of exfiltrating karst waters. The comparatively high diversity and abundance of stygobionts indicated surface influence governed by precipitation rather than by surface water infiltration. At the Donauried, the absence of non-stygobites, together with the generally low diversity and abundance of metazoans again indicated a reduced influence from the surface. However, here this can be explained partly by a silt and clay layer overlying the alluvial shallow aquifer. Concluding the results of this study, the three-scaled hierarchical model for the typology of groundwater habitats (HAHN, 2006) is a promising approach. However, comprehensive data sets of groundwater communities occurring in different groundwater systems of Germany and Europe are needed for verification. A common objective should be to define and distinguish stygoregions in all European countries. At the meso scale it is of special interest to agree on a unified sampling protocol taking regional conditions (natural geographic region, hydrogeological unit, aquifer type etc.) into account. Finally, at the local scale, ecologically relevant drivers needs to be identified and the relevance of the small scale heterogeneity for the groundwater ecosystem assessment and protection of groundwater biodiversity needs to be elaborated.

The groundwater ecosystem assessment scheme

The final step subsequent to the various steps and efforts mentioned above is the synthesis to one groundwater ecosystem assessment model. Starting with individual physical–chemical, microbiological and faunal variables measured, individual indices may be calculated for the different groups of variables, and a final ranking will deliver the 'ecological status' of the groundwater ecosystem under investigation. Due to a limited number of ecological studies on natural groundwater ecosystems not highly anthropogenically impacted and a general lack of ecological databases (TOMLINSON & BOULTON, 2009) this is still a long way to go. The selection of useful and sensitive parameters, the identification of natural reference conditions for individual groundwater ecosystem units – the appropriate scale for this still awaits decision–and the identification of bioindicators will constitute a big challenge. It will need an improved knowledge on the autecology of groundwater organisms as well as the application of sophisticated methods (e.g. multivariate statistics). However, data recently collected are promising.

3.2.2.6 References

ABDO Z., SCHUETTE U.M.E., BENT S.J., WILLIAMS C.J., FORNEY L.J. & JOYCE P. (2006) Statistical methods for characterizing diversity of microbial communities by analysis of terminal restriction fragment length polymorphisms of 16S rRNA genes. Environ. Microbiol. 8, 929–938.

ANDERSON M.J. (2001) A new method for non-parametric multivariate analysis of variance. Austral. Ecology 26, 32–46.

ASHBY M.N., RINE J., MONGODIN E.F., NELSON K.E. & DIMSTER-DENK D. (2007) Serial analysis of rRNA genes and the unexpected dominance of rare members of microbial communities. Appl. Environ. Microbiol. 73, 4532–4542.

AVANISS-AGHAJANI E., JONES K., CHAPMAN D. & BRUNK C. (1994) A molecular technique for identification of bacteria using small subunit ribosomal RNA sequences. Biotechniques 17, 144–146, 148-149.

BAKALOWICZ M. (2005) Karst groundwater: a challenge for new resources. Hydrogeol. J. 13, 148–160.

BERKHOFF S.E., BORK J. & HAHN H.J. (2009) Grundwasserfauna als Indikator für Oberflächenwasser-Grundwasser-Interaktionen im Bereich einer Uferfiltrationsanlage. Grundwasser 14, 3–20.

BÖHLKE J.K. (2002) Groundwater recharge and agricultural contamination. Hydrogeol. J. 10, 153–179.

BORK J., BERKHOFF S.E., BORK S., HAHN H.J. (2009) Using subsurface metazoan fauna to indicate groundwater-surface water interactions in the Nakdong River floodplain, South Korea. Hydrogeol. J. 17, 61–75.

BOULTON A.J. (2005) Chances and challenges in the conservation of groundwaters and their dependent ecosystems. Aqua. Conserv. Marine Freshwater Ecosyst. 15, 319–323.

BOULTON A.J., FENWICK G.D., HANCOCK P.J. & HARVEY M.S. (2008) Biodiversity, functional roles and ecosystem services of groundwater invertebrates. Invertebrate Syst. 22, 103–116.

BRIELMANN H., GRIEBLER C., SCHMIDT S.I., MICHEL R. & LUEDERS T. (2009) Effects of thermal energy discharge on shallow groundwater ecosystems. FEMS Microbiol. Ecol. 68, 273–286.

BROCKMAN F.J. & MURRAY J. (1997) Microbiological heterogeneity in the terrestrial subsurface and approaches for description. In: Amy, P.S., Haldeman, D.L. (Eds.), The Microbiology of the Terrestrial Subsurface. Lewis Publishers, Boca Raton, FL.

BRUNKE M., HOEHN E. & GONSER T. (2003) Patchiness of river-groundwater interactions within two floodplain landscapes and diversity of aquatic invertebrate communities. Ecosystems 6, 707–722.

CAIRNS JR. J. & NIEDERLEHNER B.R. (1993) Ecological function and resilience: neglected criteria for environmental impact assessment and ecological risk analysis. Environ. Prof. 15, 116–124.

CEC (1982) Groundwater resources of the European Community Synthetical report. Brussels, Luxemburg.

CLEMENT B.G., KEHL L.E., DEBORD K.L. & KITTS C.L. (1998) Terminal restriction fragment patterns (TRFPs), a rapid PCR-based method for the comparison of complex bacterial communities. J. Microbiol. Methods 31, 135–142.

COLEMAN M.L., SHEPHERD T.J., DURHAM J.J., ROUSE J.E. & MOORE G.R. (1982) Reduction of water with zinc for hydrogen isotope analysis. Anal. Chem. 54, 993–995.

COPLEN T.B. (1988) Normalization of oxygen and hydrogen isotope data. Chem. Geol. 72, 293–297.

CULMAN S.W., BUKOWSKI R., GAUCH H.G., CADILLO-QUIROZ H. & BUCKLEY D.H. (2009) TRex: software for the processing analysing of T-RFLP data. BMC Bioinformatics 10, 171.

CUSTODIO E. & MANZANO M. (2008) Groundwater quality background levels. In: Quevauviller, P. (Ed.), Groundwater Science and Policy. RSC Publishing, Cambridge.

CVETKOV L. (1968) Un filet phreatobiologique. Bull. Inst. Zool. Mus. Acad. Bulgare. Sci. 27, 215–218.

DANIELOPOL D.L., GRIEBLER C., GINATILAKA A. & NOTENBOOM J. (2003) Present state and future prospects for groundwater ecosystems. Environ. Conserv. 30, 104–130.

DANIELOPOL D., GIBERT J., GRIEBLER C., GUNATILAKA A., HAHN H.J., MESSANA G. NOTENBOOM J. & SKET B. (2004) Incorporating ecological perspectives in European groundwater management policy. Environ. Conserv. 31, 1–5.

DANIELOPOL D., GRIEBLER C., GUNATILAKA A., HAHN H.J., GIBERT J., MERMILLOD-BLONDIN G., MESSANA G., NOTENBOOM J. & SKET B. (2008) Incorporation of groundwater ecology in environmental policy. In: Quevauviller, P. (Ed.), Groundwater Science and Policy – An International Overview. RSC Publishing, The Royal Society of Chemistry, London.

DATRY T., MALARD F. & GIBERT J. (2005) Response of invertebrate assemblages to increased groundwater recharge rates in a phreatic aquifer. J. North Am. Benthol. Soc. 24, 461–477.

DE'ATH G. (2002) Multivariate regression trees: a new technique for modeling species–environment relationships. Ecology 83, 1105–1117.

DE'ATH G. (2007) Boosted trees for ecological modeling and prediction. Ecology 88, 243–251.

DOLE-OLIVIER M.-J., MALARD F., MARTIN D., LEFÉBURE T. & GIBERT J. (2009) Relationships between environmental variables and groundwater biodiversity at the regional scale. Freshw. Biol. 54, 797–813.

DOLÉDEC S., CHESSEL D. & GIMARET-CARPENTIER C. (2000) Niche separation in community analysis: a new method. Ecology 81, 2914–2927.

EINSLE U. (1993) Crustacea Copepoda, Calanoida und Cyclopoida. In: Süßwasserfauna von Mitteleuropa. Gustav Fischer, Stuttgart.

EDMUNDS W.M. & SHAND P. (Eds.) (2008) Natural Groundwater Quality. Wiley-Blackwell.

EPA (2003). Guidance for the assessment of environmental factors: consideration of subterranean fauna in groundwater and caves during environmental impact assessment in Western Australia. In: ´Guidance Statement for the Assessment of environmental Factors no. 54 Environmental Protection Authority, Perth, Australia.

EPSTEIN S. & MAYEDA T. (1953) Variation of O^{18} content of waters from natural sources. Geochim. Cosmochim. Acta 4, 213–224.

EU-GWD, 2006. Directive 2006/118 of the European Parliament and the Council of the 12 December 2006. Official J. European Comm. L372, 371–319.

EU-WFD, 2000. Directive 2000/60/EC of the European Parliament and of the Council of the 23 October 2000: establishing framework for the community action in the field of water policy. Official J. European Comm. L327, 321–371.

FISHER M.M. & TRIPLETT E.W. (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl. Environ. Microbiol. 65, 4630–4636.

FRÄNZLE O. (2003) Bioindicators and environmental stress assessment. In: Markert, B.A., Breuie, A.M., Zechmeister, H.G. (Eds.), Bioindicators and Biomonitors. Elsevier Science Ltd.

FUCHS A. (2007) Erhebung und Beschreibung der Grundwasserfauna in Baden-Württemberg. In: Natur- und Umweltwissenschaften. Universität Koblenz-Landau, Landau.

GALASSI D.M.P., STOCH F., FIASCA B., DI LORENZO T. & GATTONE E. (2009) Groundwater biodiversity patterns in the Lessinian Massif of northern Italy. Freshw. Biol. 54, 830–847.

GAUCH JR. H.G. (1992) Statistical analysis of regional yield trials: AMMI Analysis of factorial designs. Elsevier, Amsterdam.

GE Y., HE J.Z., ZHU Y.G., ZHANG J.B., XU Z.H., ZHANG L.M. & ZHENG Y.M. (2008) Differences in soil bacterial diversity: driven by contemporary disturbances or historical contingencies? ISME J. 2, 254–264.

GIBERT J., STANFORD J.A., DOLE-OLIVIER M.-J. & WARD J.V. (1994) Basic attributes of groundwater ecosystems and prospects for research. In: Gibert, J., Danielopol, D.L., Stanford, J.A. (Eds.), Groundwater Ecology. Academic Press, San Diego.

GIBERT J., DEHARVENG L. (2002) Subterranean ecosystems: a truncated functional biodiversity. BioScience 52, 473–481.

GIBERT J., CULVER D.C., DOLE-OLIVIER M.J., MALARD F., CHRISTMAN M.C. & DEHARVENG L. (2009) Assessing and conserving groundwater biodiversity: synthesis and perspectives. Freshw. Biol. 54, 930–941.

GILBRICH W.H. (2000) International hydrogeological map of Europe Feature article, Waterway no. 19, Paris.

GOLDSCHEIDER N., HUNKELER D. & ROSSI P. (2006) Review: Microbial biocenoses in pristine aquifers and an assessment of investigative methods. Hydrogeol. J. 14, 926–941.

GRIEBLER C. & LUEDERS T. (2009) Towards a conceptual understanding of microbial biodiversity in groundwater ecosystems, Freshw. Biol., 54(4), 649–677.

GSCHV, 1998. Gewässerschutzverordnung (Swiss Water Ordinance) 814.201. Der Schweizer Bundesrat, Bern, Switzerland. GUSHIN, D.Y., Mobarry, B.K., Proudnikov, D., Stahl, D.A., Rittmann, B.E., Mirzabekov, A.D., 1997. Oligonucleotide microchips as genosensors for determinative and environmental studies in microbiology. Appl. Environ. Microbiol. 63, 2397–2402.

HAAKH F. (2002) Die Kartierung der Realnutzung als Grundlage für Flächenmanagement im Wasserschutzgebiet. LW-Schriftenreihe 21, 34–46.

HAHN H.J. (2006) The GW-Fauna-Index: a first approach to a quantitative ecological assessment of groundwater habitats. Limnologica 36, 119–137.

HAHN H.J. (2009) A proposal for an extended typology of groundwater habitats. Hydrogeol. J. 17, 77–81.

HAHN H.J. & MATZKE D. (2005) A comparison of stygofauna communities inside and outside groundwater bores. Limnologica 35, 31–44.

HAHN H.J. & FUCHS A. (2009) Distribution patterns of groundwater communities across aquifer types in south-western Germany. Freshw. Biol. 54, 848–860.

HE Z., GENTRY T.J., SCHADT C.W., WU L., LIEBICH J., CHONG S.C., HUANG Z., WU W., GU B., JARDINE P., CRIDDL C. & ZHOU J. (2007) GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. ISME J. 1, 67–77.

HEATH R.C. (1982) Classification of groundwater systems of the United States. Ground Water 20, 393–401.

HILL T.C.J., WALSH K.A., HARRIS J.A. & MOFFETT B.F. (2003) Using ecological diversity measures with bacterial communities. FEMS Microbiol. Ecol. 43, 1–11.

HINSBY K. & RASMUSSEN E.S. (2008) The Miocene sand aquifers, Jutland, Denmark. In: Edmunds and Shand (Eds.), Natural Groundwater Quality.

HINSBY K., CONDESSO DE MELO M.T. & DAHL M. (2008) European case studies supporting the derivation of natural background levels and groundwater threshold values for the protection of dependent ecosystems and human health. Sci. Total Environ. 401, 1–20.

HUMPHREYS W.F. (2009) Hydrogeology and groundwater ecology: does each inform the other? Hydrogeol. J. 17, 5–21.

HUNKELER D., GOLDSCHEIDER N., ROSSI P. & BURN C. (2006) Biozönosen im Grundwasser – Grundlagen und Methoden der Charakterisierung von mikrobiellen Gemeinschaften. Bundesamt für Umwelt, Bern.

HUSMANN S. (1967) Klassifizierung mariner, brackiger und limnischer Grundwasserbiotope. Helgoländer wissenschaftliche Meeresuntersuchungen 16, 271–278.

IGRAC, 2005. International Groundwater Resources Assessment Centre: Proposed groundwater provinces. info@IGRAC.nl. ILLIES, J., 1978. Limnofauna Europaea. Stuttgart.

IGRAC (2005) International Groundwater Resources Assessment Centre: Proposed groundwater provinces. info@igrac.nl.

ILLIES J. (1978) Limnofauna Europaea. Gustav Fischer. Stuttgart.

JÄCKLI H. (1970) Kriterien zur Klassifizierung von Grundwasservorkommen. Eclogae. Geol. Helv. 63, 389–434.

JANETZKY W., ENDERLE R. & NOODT W. (1996) Crustacea, Copepoda, Gelyelloida und Harpacticoida. In: Süßwasserfauna von Mitteleuropa. Gustav Fischer Stuttgart.

KÖLBEL-BOELKE J., ANDERS E.M. & NEHRKORN A. (1988) Microbial communities in the saturated groundwater environment. II. Diversity of bacterial communities in a pleistocene sand aquifer and their in vitro activities. Microb. Ecol. 16, 31–48.

KOLOKOTRONIS V., PLUM H., PRESTEL R., SCHOLZ W. & RAUSCH R. (2002) Hydrogeologische Karte von Baden-Württemberg, Ostalb: Erläuterungen: Landesamt für Geologie, Rohstoffe und Bergbau Baden-Württemberg, Freiburg i. Br. Landesamt für Umweltschutz Baden-Württemberg, Karlsruhe.

KUNKEL, R., Voigt, H.-J., WENDLAND, F., Hannappel, S., 2004. Die natürliche, ubiquitär überprägte Grundwasserbeschaffenheit in Deutschland (The natural, ubiquitary determined composition of groundwaters). Forschungszentrums Jülich, Jülich.

KYSELA D.T., PALACIOS D. & SOGIN M.L. (2005) Serial analysis of V6 ribosomal sequence tags (SARST-V6): a method for efficient, high-throughput analysis of microbial community composition. Environ. Microbiol. 7, 356–364.

LEE D.H., ZO Y.G. & KIM S.J. (1996) Nonradioactive method to study genetic profiles of natural bacterial communities by PCR-single-strand-conformation polymorphism. Appl. Environ. Microbiol. 62, 3112–3120.

LEGENDRE P. & LEGENDRE L. (1998) Numerical Ecology. Elsevier Science BV, Amsterdam.

MALARD F., CASTELLARINI F. & OLIVIER J. (2004) Statistical analyses and identification of indicators. In: Stoch, F. (Ed), PASCALIS (Protocols for the assessment and conservation of aquatic life in the subsurface) Deliverable 8, 1–156.

MANTEL N. & VALAND R.S. (1970) A technique of nonparametric multivariate analysis, Biometrics, 26, 547–55.

MARTIN P., DE BROYER C., FIERS F., MICHEL G., SABLON R. & WOUTERS K. (2009) Biodiversity of Belgian groundwater fauna in relation to environmental conditions. Freshw. Biol. 54(4), 814–829.

MARTINY J.B.H., BOHANNAN B.J.M., BROWN J.H., COLWELL R.K., FUHRMAN J.A., GREEN J.L., HORNER-DEVINE M.C., KANE M., ADAMS KRUMINS J., KUSKE C.R., MORIN P.J., NAEEM S., ØVREÅS L., REYSENBACH A.-L., SMITH V.H. & STALEY J.T. (2006). Microbial biogeography: putting microorganisms on the map. Nat. Rev. Micro. 4, 102–112.

MEISCH C. (2000) Freshwater Ostracoda of Western and Central Europe. Spektrum Akademischer Verlag, Heidelberg.

MEYNEN E., SCHMITHÜSEN J., GELLERT J., NEEF E., MÜLLER-MINY H. & SCHULTZE H.J. (1962) Handbuch der naturräumlichen Gliederung Deutschlands. Selbstverlag der Bundesanstalt für Landeskunde, Remagen.

MÖSSLACHER F., POSPISIL P. & DREHER J. (1996) A groundwater ecosystem in the "Lobau" wetland (Vienna), reflecting the interactions between surface water and groundwater. Arch. Hydrobiol. (Suppl. 10), 451–455.

MÜLLER D., BLUM A., HART A., HOOKEY J., KUNKEL R., SCHEIDLEDER A., TOMLIN C. & WENDLAND F. (2006) Final proposal for a methodology to set up groundwater threshold values in Europe. Deliverable 18 of the BRIDGE project. In: www.wfd-bridge.net. 1–63.

MURPHY J. & RILEY J.P. (1962) A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36.

MUYZER G., DE WAAL E.C. & UITTERLINDEN A.G. (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes-coding for 16S ribosomal-RNA. Appl. Environ. Microbiol. 59, 695–700.

NEUFELD J.D., YU Z., LAM W., MOHN W.W. (2004) Serial analysis of ribosomal sequence tags (SARST): a high-throughput method for profiling complex microbial communities. Environ. Microbiol. 6, 131–144.

NSW-SGDEP (2002) The NSW State Groundwater Dependent Ecosystems Policy. Department of Land and Water Conservation, NSW Government.

OKSANEN J. (2008) Multivariate analysis of ecological communities, in R: vegan tutorial. available at http://cc.oulu.fi/◎jarioksa/opetus/metodi/ vegantutor.pdf.

RAMETTE A. (2007) Multivariate analyses in microbial ecology. FEMS Microbiol. Ecol. 62, 142–160.

RHEE S.K., LIU X., WU L., CHONG S.C., WAN X. & ZHOU J. (2004) Detection of genes involved in biodegradation and biotransformation in microbial communities by using 50-mer oligonucleotide microarrays. Appl. Environ. Microbiol. 70, 4303–4317.

SAFFIGNA P.G. & KEENEY D.R. (2006) Nitrate and Chloride in Ground Water Under Irrigated Agriculture in Central Wisconsina. Ground Water Monit. Remedia. 15, 170–177.

SCHMINKE K. (2006) Copepoda (Ruderfußkrebse). In: Schminke K. (ed), Grundwasserfauna Deutschlands, pp. 61–134.

SMITH C.J., DANILOWICZ B.S., CLEAR A.K., COSTELLO F.J. & WILSON B., MEIJER W.G. (2005) T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. Fems Microbiol. Ecol. 54, 375–380.

SCHNECK A., HAAKH F. & LANG U. (2004) Multikriterielle Optimierung der Grundwasserbewirtschaftung – dargestellt am Beispiel des Wassergewinnungsgebiets Donauried: Eigenverlag des Zweckverbandes Landeswasserversorgung

STEIN H., KELLERMANN C., SCHMIDT S.I., BRIELMANN H., STEUBE C., BERKHOFF S. E., FUCHS A., HAHN H. J., THULIN B. & GRIEBLER C. (2010) The potential use of fauna and bacteria as ecological indicators for the assessment of groundwater ecosystems. J. Environ. Monit. 12, 242-254.

STRAYER D.L. (1994) Limits to biological distributions in groundwater. In: Gibert J., Danielopol D.L., Stanford J.A. (Eds). Groundwater Ecology. New York.

STREBEL O., DUYNISVELD W.H.M., BÖTTCHER J. (1989) Nitrate pollution of groundwater in Western Europe. Agri., Ecosyst. Environ. 26, 189–214.

STRUCKMEIER F.W., GILBRICH W.H., GUN J.V.D., MAURER S., PURI S., RICHTS A., WINTER P. & ZAEPKE M. (2006) WHYMAP and the groundwater resources map of the world at the scale of 1:50,000,000. Special ed. on for the 4th world water forum. Mexico City, March 2006. BGR Hannover/UNESCO, Paris.

SWALLOW M., QUIDEAU S.A., MACKENZIE M.D. & KISHCHUK B.E. (2009) Microbialcommunity structure and function: the effect of silvicultural burning and topographic variability in northern Alberta. Soil Biol. Biochem. 41, 770–777.

TOMLINSON M., BOULTON A.J. (2009) Subsurface groundwater dependent ecosystems: a review of their biodiversity, ecological processes and ecosystem services. In: Commission, N.W. (Ed). Waterlines Occasional Paper No. 8, National WaterCommission, Canberra.

WAGNER M., SMIDT H., LOY A. & ZHOU J. (2007) Unravelling microbial communities with DNA-microarrays: challenges and future directions. Microb. Ecol. 53, 498–506.

WENDLAND F., HANNAPPEL S., KUNKEL R., SCHENK R., VOIGT H.J. & WOLTER R. (2005) A procedure to define natural groundwater conditions of groundwater bodies in Germany. Water Sci. Technol. 51, 249–257.

WENDLAND F., BLUM A., COETSIERS M., GOROVA R., GRIFFIOEN J., GRIMA, J., HINSBY K., KUNKEL R., MARANDI A., MELO T., PANAGOPOULOS A., PAUWELS H., RUISI M., TRAVERSA P., VERMOOTEN J. S. A. & WALRAEVENS K. (2008) European aquifer typology: a practical framework for an overview of major groundwater composition at European scale. Environ. Geol. 55, 77–85.

WILLIAMS J.G.K., KUBELIK A.R., LIVAK K.J., RAFALSKI J.A. & TINGEY S.V. (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18, 6531–6535.

WINDERL C., ANNESER B., GRIEBLER C., MECKENSTOCK R.U. & LUEDERS T. (2008) Depthresolved quantification of anaerobic toluene degraders and aquifer microbial community patterns in distinct redox zones of a tar oil contaminant plume. Appl. Environ. Microbiol. 74, 792–801.

YU Z., YU M. & MORRISON M. (2006) Improved serial analysis of V1 ribosomal sequence tags (SARST-V1) provides a rapid, comprehensive, sequence-based characterization of bacterial diversity and community composition. Environ. Microbiol. 8, 603–611.

ZHOU J. (2003) Microarrays for bacterial detection and microbial community analysis. Curr. Opin. Microbiol. 6, 288–294.

Acknowledgements

The authors gratefully acknowledge the financial support by the German Federal Environment Agency (UBA-project ‘Biologische Bewertung von Grundwasserökosystemen’), the help from Mr. Heisele and Mr. Fischer of the

‘Landeswasserversorgung’ in Langenau, Germany, and from Mr. R. Scheck from the ‘Landeswasserversorgung’ in Stuttgart, Germany. Mr B. Schneider and Mr. K.-P. Barufke from the LUBW (Landesanstalt für Umwelt, Messungen und Naturschutz Baden-Württemberg) are acknowledged for continuous support. A. Mösch, M. Stöckl, P. Seibel and W. Stichler from the Institute of Groundwater Ecology at the HMGU in Neuherberg, Germany, are acknowledged for sampling assistance and analyses. The Helmholtz Zentrum München (HMGU) is acknowledged for support and providing ideal working conditions.

3.3 Die Evaluierung biogeografischer Verbreitungsmuster der Invertebraten-gemeinschaften im Grundwasser und der Vergleich mit bestehenden oberirdischen Gliederungssystemen

3.3.1 Zusammenfassung

Zur Überprüfung der biogeografischen Ebene des hierarchischen Gliederungsmodells für Grundwasserhabitate wurde die Zusammensetzung der Grundwasserfauna aus unterschiedlichen Teilen Deutschlands untersucht.

Die faunistischen Analysen sollten klären, ob die Invertebratengemeinschaften im Grundwasser biogeografische Unterschiede aufweisen und anhand dieser Verbreitungsmuster so genannte Stygoregionen definiert werden können, die auf einer eigenständigen Gliederung von Grundwassersystemen basieren. Weiterhin wurden die im Grundwasser erfassten Verbreitungsmuster mit bestehenden Gliederungseinheiten für oberirdische Systeme verglichen, wie die naturräumliche Einteilung (SSYMANK 1994) oder die Einteilung der Fließgewässertypen (POTTGIEßER *et al.* 2004). Letztendlich wurde die Relevanz der räumlichen Ebene bei der faunistischen Bewertung von Grundwasserhabitaten evaluiert.

Die Ergebnisse zeigen, dass die Verbreitung subterraner Invertebraten signifikant von bestehenden bioregionalen Klassifizierungssystemen, die für Oberflächengewässer entwickelt wurden, abweichen.

Die rezenten Verbreitungsmuster im Grundwasser unterscheiden sich deutlich von oberirdischen Klassifikationen und spiegeln erdgeschichtliche Ereignisse wie z. B. Vereisungsgrenzen und pliozäne hydrogeografische Verhältnisse wider. Dem entsprechend sind die größten Abweichungen von oberirdischen Gliederungssystemen im Norden und Süden Deutschlands sowie im Grundwasser der Mittelgebirgsvorländer gefunden worden. Anhand der biogeografischen Verbreitungsmuster im Grundwasser, werden vier Stygoregionen für die Grundwasserhabitate in Deutschland vorgeschlagen, die als Basis für ein Referenzsystem gelten könnten: 1) das *„Nördliche Tiefland“*, 2) die *“ Zentralen Mittelgebirge“*, 3) die *„Südwestlichen Mittelgebirge“* sowie 4) die *„Südlichen Mittelgebirge und die Alpen“*. Die Stygoregionen weisen deutliche Unterschiede im Arteninventar und in der Abundanz ihrer Faunengemeinschaften auf. Diese Ergebnisse verdeutlichen die Notwendigkeit eines eigenständigen Gliederungssystems für Grundwasserlebensräume. Die vorgeschlagenen Stygoregionen sind erste biogeografische Referenzen für faunistische Verbreitungsmuster im Grundwasser Mitteleuropas. In weiteren Untersuchungen sollten diese Muster flächendeckend ergänzt und überprüft werden. Die Definition von Stygoregionen ist eine wichtige Grundlage für ein ökologisches Bewertungssystems, das Voraussetzung für ein nachhaltiges Grundwassermanagement ist.

3.3.2 The sustainable groundwater management requires the definition of stygoregions

3.3.2.1 Abstract

Clean groundwater is mainly the result of biological processes. This is the most important ecosystem service of its communities. Currently, the protection of our groundwater resources only refers to their good physicochemical and quantitative state. However, a sustainable groundwater management means to maintain its ecological integrity and to assess groundwater ecosystem health also considering biological criteria, such as invertebrates.

As a first step towards an ecological groundwater assessment scheme, which has not yet been defined for groundwater ecosystem health, we examined regional and biogeographical distribution patterns of groundwater dwelling invertebrates aiming to define references for Central Europe. Our results reveal that the distribution of subsurface invertebrates differs significantly from existing bioregional classification systems established for surface waters,

emphasising the need of an independent classification system for groundwater habitats. We propose four biogeographical regions, socalled stygoregions, for the Central European groundwaters forming the basis of assessment and sustainable management.

3.3.2.2 Introduction

Groundwater systems are diversely populated by microbes and invertebrates. World wide, the number of obligate groundwater invertebrate species was described to be around 7000 (BOTOSANEAUNU, 1986). Since groundwater has not been studied to a great extend, the true species richness is assumed to exceed this number by factor (e.g. HANCOCK & BOULTON, 2008; DOLE-OLIVIER *et al.*, 2009). Commonly found are oligochaetes, nematods, acari, but also molluscs and even vertebrates such as fish or cave salamanders. However, the major taxonomic group of groundwater dwelling animals, the so-called stygofauna, are small crustaceans less than 1 mm to several centimetres in body size (Fig. 1). This high diversity is to some degree the consequence of habitat heterogeneity, but in particular it is the result of the exceptional large endemism in groundwater due to the high fragmentation of subterranean habitats, with most species being rare (GIBERT & DEHARVENG, 2002).

Fig. 1) Stygobiontic invertebrates (here *Proasellus slavus*, Isopoda, Crustacea) are perfectly adapted to groundwater conditions. As a convergent evolutionary response to darkness, obligate groundwater animals (stygobites) are translucent, blind, exhibit enhanced tactile sense organs and lack circadian periodicity. Their bodies are elongated to vermiform facilitating locomotion in habitats with limited pore spaces. As an adaption to low and patchy food supply stygobites have slow metabolic, low reproduction rates and they exhibit longevity, compared to related surface species. (Photo: K. Grabow).

The ecosystem services of groundwater (e.g. self-purification potential, provision of water for surface systems, resistance and resilience towards anthropogenic impacts) are essential for humanity (DANIELOPOL & GRIEBLER, 2008). Along with microbiological degradation processes, invertebrates play an essential role in the purification of subterranean water and thus, the maintainance of high water quality (MURRAY *et al.*, 2006; HUMPHREYS, 2006). Feeding, burrowing and bioturbating activities of the fauna prevent the clogging of sediment interstices and thereby, maintain the hydraulic connectivity of the aquifer (HUMPHREYS, 2006). The fragmentation of organic matter and the grazing of biofilms foster microbiological decomposition processes and contribute to nutrient cycling and the degradation of pollutants (GIBERT & DEHARVENG, 2002; TOMLINSON & BOULTON, 2008). These ecosystem services can only be provided by groundwater communities with structural and functional integrity (STEIN *et al.*, 2010; GRIEBLER *et al.*, 2010) Moreover, these ecosystem services are not limited to aquatic subsurface habitats, but affect groundwater dependant ecosystems such as most surface freshwater and terrestrial systems (BOULTON, 2005; HUMPHREYS, 2006; GRIEBLER *et al.*, 2010).

The urgent need for groundwater management that sustains the ecological integrity of groundwater systems has become increasingly recognised by political authorities (DANIELOPOl *et al.*, 2008; STEUBE *et al.* 2009). The implementation of the Swiss Water Protection Ordinance (GSCHV, 1998) and the European Union Groundwater Directive (EU-GWD, 2006) emphasize the importance of protective measures for groundwater ecosystems. Consequently, an increased research with a focus on criteria ensuring groundwater ecosystem quality, which is based on an integrative assessment of the ecological state of groundwater systems, is needed (DANIELOPOL & GRIEBLER, 2008; STEUBE *et al.* 2009). As the ecosystem functioning is not sufficiently reflected by abiotic criteria alone, its assessment must be complemented by biological criteria (STEUBE *et al.* 2009). Moreover, the advantage of using groundwater fauna as bioindicators is an assessment on the overall impact (e.g. pollutants, heavy metals, water exploitation, aquifer recharge) (MALARD *et al.*, 1994, NOTENBOOM *et al.*, 1995, MÖSSLACHER 2000, DATRY *et al.*, 2005, MATZKE *et al.*, 2005). Further, do faunal assemblages integrate impairments over time and thus, do not reflect just a particular sampling date but some short term history of environmental influences (KORBEL & HOSE, 2005). over an extended time period. However, ecological assessment criteria and protection measures are still not available for European groundwater. In contrast, Australia has become a pioneer incorporating ecological criteria and guidelines in groundwater policies and ecological management (NSW-SGDEP, 2002; EPA, 2003). Furthermore, a first approach of an ecosystem health assessment of groundwater, which defines criteria and tresholds for physico-chemical, microbiological and faunistic conditions, has been developed (KORBEL & HOSE, 2011). On a local scale (< 1 km²) an Index that quantifies the hydrological exchange with surface systems has been approached (HAHN, 2006).

The recent classification systems of freshwater in Central Europe are based on biogeographical, physiogeographical and hydrological aspects but they do not regard groundwater ecosystems and their assessment. For European groundwaters an aquifer typology map has beeen developed (WENDLAND *et al.*, 2008) based on hydrogeological criteria, which, for Germany, has been further refined by KUNKEL *et al.* (2004). The official European groundwater assessment is focussed on the good chemical and quantitative state only, and disregards biogeographical and ecological aspects.

For groundwater, few zoogeographic approaches such as the classifications suggested by BOTOSANEANU (1986) or HUSMANN (1966) are available, but proved inapplicable in practice. More recent studies, like the PASCALIS project (Protocols for the ASsessment and Conservation of Aquatic Life In the Subsurface; GIBERT *et al.*, 2005; GIBERT & CULVER, 2009), focussed on European groundwater biodiversity, but did not have the objective of a biogeographical classification of groundwater – defining so-called stygoregions, though. Currently, STOCH and GALASSI (2010) discussed the term of stygoregions based on invertebrates. However, this study is limited to the edge of the Southern Alps only comprising the stygoregion ´North Eastern Italy`. For the groundwater fauna of Western Australia, HUMPHREYS (2006) has shown very ancient distribution patterns, but he did not pursue the definition of stygoregions.

Assessment and classification systems of freshwater *surface* ecosystems show the way: On a biogeographical scale the EU-WFD (2000) applies zoogeographic regions derived from Illies (1978), complemented by geomorphological features. Accordingly, Central Europe is divided into five Ecoregions: The

Central and Western European Lowlands, the Central and Western Mountain Ranges, and the Alps. Generally, the assessment of freshwater surface systems is based on bioregional references with the good ecological state being the key criterion (EU-FWD, 2000). However, it does not seem suitable for groundwater ecosystems.

In order to clarify this issue we examined the groundwater fauna of the main ecoregions of Central Europe. Exemplarily, our studies focus on biogeographic distribution patterns of groundwater invertebrates across Germany. Germany encompasses three of the major central European landscapes (Fig. 2). On a regional scale (several 100-1000 km²), it is divided into 69 major physiographic units (MPU), e.g. the Black Forest, the Lower Rhine Valley or the Pflälzerwald Mountains (SSYMANK, 1994). The MPU consider surface and subsurface features such as climate, geology, physical geography, morphology (SSYMANK, 1994). MPU are the official German landscape units on a regional scale (BFN, 1994).

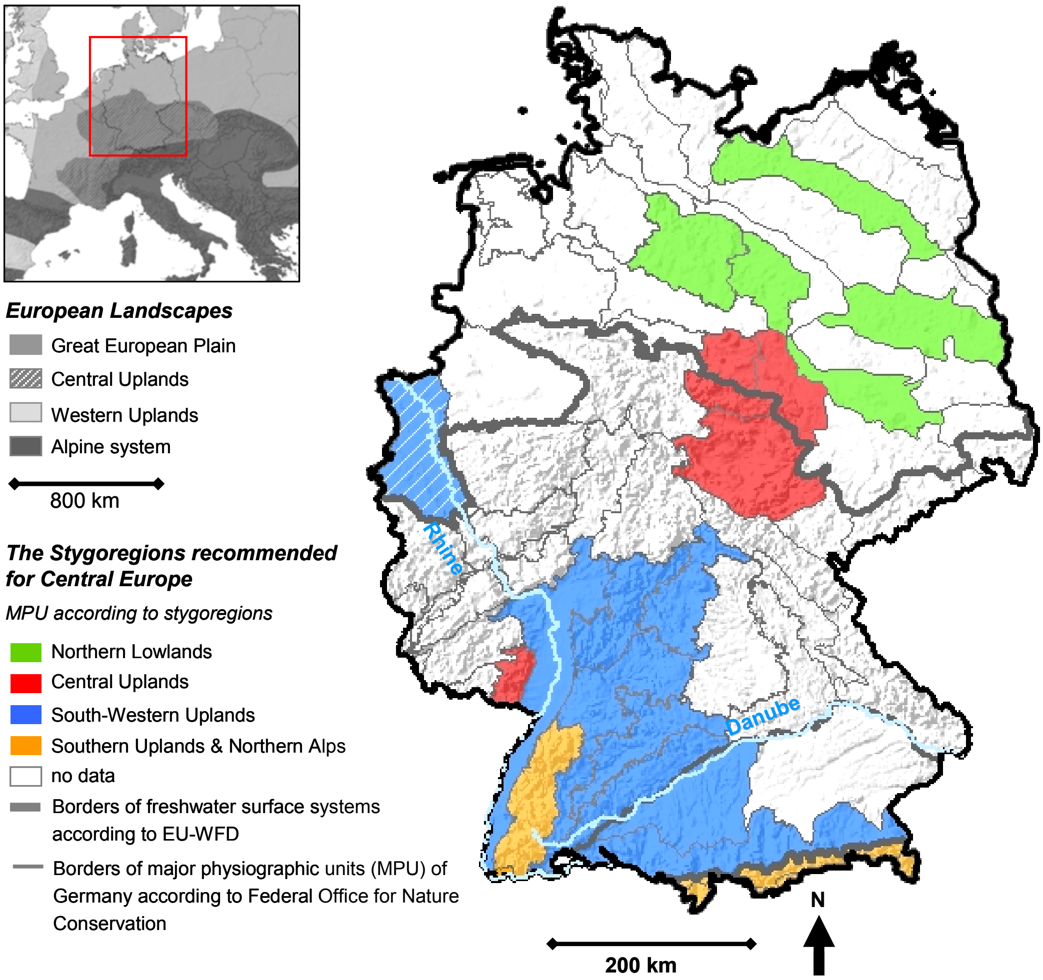

Fig. 2) European physical landscapes and a topographic map of Germany exhibiting recommended stygoregions: Coloured areas show the major physiographic units (= MPU), which were studied. The white areas indicate MPU were no data was available. The colours refer to stygoregions, which were defined according to invertebrate distribution patterns found in groundwater. The stygoregions base on MPU, which were the level of data analyses. The affiliation of the Lower Rhine Valley is not clear yet, and thus blue-white hatched. Topographic map / GIS: http://www.eea.europa.eu/legal/copyright.

3.3.2.3 Materials and methods

Study regions

The faunal data presented originate from sampling surveys conducted in a wide variety of aquifers across Germany (Fig. 2), including unconsolidated porous aquifers, karst and fractured aquifers. A total of 515 groundwater monitoring wells were repeatedly examined (2-5 sampling surveys) between 2002 and 2009. Most samples were obtained from near-surface groundwater in approximately 10-80 m depth. The major selection criterion of the choice of sampling areas was the comparability of faunal datasets.

Fauna sampling and taxonomic identification

We extracted the groundwater dwelling invertebrates from the bottom of the monitoring wells by using a phreatic net sampler (75 µm mesh size), according to HAHN and FUCHS (2009). All faunal samples were immediately stored in a refrigerator box and subsequently fixed with 4 % formaldehyde before further processing. The taxonomic identification was based on morphological characteristics. Crustaceans, oligochaetes, polychaetes and gastropods were target groups and were determined by morphological characteristics to species level according to specialist literature (EINSLE, 1993, JANETZKY *et al.*, 1996, MEISCH, 2000, SCHMINKE, 2006). Specimens of other taxonomic groups were excluded from analyses. Species were classified as ´stygobites` (obligate groundwater species) and ´non-stygobites` (facultative or foreign groundwater dwelling surface species). This classification was based on ecological preferences according to DATRY *et al.*, 2005 Hand specialist literature (EINSLE, 1993, JANETZKY *et al.*, 1996, MEISCH, 2000, SCHMINKE, 2006).

Data analyses

Prior to statistical analysis, the counts of faunal populations collected in wells were averaged for each major physiographic unit (MPU) and subsequently fourth root-transformed. The data aggregation was conducted to reduce the data scattering of individual wells, as result of the inhomogeneous faunal distribution that is naturally found in groundwater. Stygofaunal assemblages are extremely heterogeneous and usually 30 % of the wells are unpopulated. Therefore, faunal data collected in wells that were located in the same MPU were pooled and averaged, respectively. We use MPU as the smallest scale for data aggregation. This is in contrast to HAHN & FUCHS (2009), who used ´georegs` (regional geology = aquifer type and naturraum) for data aggregation. Previously, we have tested various spatial units (georegs, naturraum, HBE, MPU), whereas the averaging via the MPU obtained best results (see table 1). The reason for this is that on biogeographical scale, the influence of the aquifer type is overriding the physiographic effects.

Since the faunal data did not show a normal distribution, even after fourth root-transformation, we used exclusively non-parametrical methods for our analyses. Patterns in faunal community structure were explored by Nonmetric Multi-Dimensional Scaling (MDS), Permutational Multivariate Analysis of Variance (PERMANOVA) and ANalysis Of SIMilarity (ANOSIM) based on Bray-Curtis distance. The measure of Bray-Curtis was chosen because it does consider zero values but does not consider joint absences where both samples have zeroes (CLARKE & GORLEY, 2006). Before generating the MDS, a dummy variable (d=1) was added to each group (physiographic unit), for a better interpretation and graphical presentation of the community patterns found. This dummy variable suggests an additional virtual species that is shared by each group, and thus reduces the differences, without changing its proportions. In the PERMANOVA the number of permutations was set to 9999 using the reduced model and type III sums of squares (ANDERSON *et al.*, 2001) to obtain the *P*-values. In addition, we used an Analysis of similarities (ANOSIM) to test overall differences between groups. This test is non-parametric analogue to the ANOVA and is adequate for data that shows no normal distribution (ANDERSON *et al.*, 2001). We performed all statistical analyses with the PRIMER v.6 computer programme[26] and the add-on package PERMANOVA+ (ANDERSON *et al.*, 2001).

3.3.2.4 Results

Our results reveal distinct biogeographic distribution patterns of groundwater invertebrates, when averaged on the major physiographic units (SSYMANK, 1994) (Fig. 3 a,b). These patterns differ strongly from the spatial units classified by the EU-WFD (2000), emphasising that this surface water classification system is not an adequate tool for the ecological assessment of groundwater. We found four major groups of groundwaters, which are characterized by significantly distinct faunal communities ($p < 0.05$) (Fig. 3 a,b, Tab.2).

Accordingly, we propose four stygoregions for Central Europe:

1*) The Northern Lowlands* comprise groundwater systems that were strongly affected by pleistocene ice shields, and as a result these groundwaters are naturally unpopulated or stygofauna is scant (HAHN & FUCHS, 2009) (Fig. 3b, Tab. 2).

2) *The Central Uplands* comprise the groundwater habitats of the Central Mountain Ranges and the adjacent sub-mountainous forelands, including the Pfälzerwald Mountains. Invertebrate groundwater communities are mainly characterized by ubiquist species, so-called post-glacial recolonisers (GIBERT & DEHARVENG, 2002; S TOCH & GALASSI, 2010) with few endemic species (Fig. 3b, Tab. 2).

3) The *South-Western Uplands* are generally characterized by a highly diverse groundwater fauna that have endured the periods of glaciation. The proportion of stygobites (obligate groundwater species) is high and the fauna generally reflects the pleistocenious Danube catchment area with a diverse amphipod and ostracod fauna (HAHN & FUCHS, 2009) (Fig. 3b, Tab. 2). So far we included the Lower Rhine Valley in this stygoregion because of major overlaps in species composition (data not shown). Nevertheless, the groundwater dwelling invertebrate fauna of the Lower Rhine Valley exhibits a lower diversity.

4) The *Southern Uplands and Northern Alps* comprise those areas that were covered by the pleistocenious ice shields of the Alps and the Black Forest. The species composition is similar to those of the *South-Western Uplands*, but generally less diverse (Fig. 3b, Tab. 2).

Table 1: List of major physiographic units of Germany (MPU) were sampling was conducted, according to (BFN, 1994; SSYMANK, 1994)

MPU code	MPU name
D35	Kölner Bucht & Niederrheinisches Tiefland
D60	Schwäbische Alb
D64	Donau-Iller-Lech-Platten
D04	Mecklenburgische Seenplatte
D28	Lüneburger Heide
D68	Nördliche Kalkalpen
D29	Wendland & Altmark
D20	Mitteldeutsches Schwarzerdegebiet
D10	Elbe-Mulde-Tiefland
D11	Fläming
D12	Mittelbrandenburgische Platten, Niederungen, Ostbrandenburgisches Heide- & Seengebiet
D09	Elbtalniederung
D33	Nördliches Harzvorland
D18	Thüringer Becken & Randplatten
D31	Weser-Aller-Tiefland
D37	Harz
D53	Oberrheinisches Tiefland & Rhein-Main-Tiefland
D69	Hochrheingebiet & Dinkelberg
D54	Schwarzwald
D57	Neckar- & Tauberland, Gäuplatten
D56	Mainfränkische Platten
D55	Odenwald, Spessart & Südrhön
D58	Schwäbisches Keuper-Lias-Land
D66	Voralpines Hügel- und Moorland
D67	Schwäbisch-Oberbayrische Voralpen
D51	Pfälzerwald & Haardtgebirge

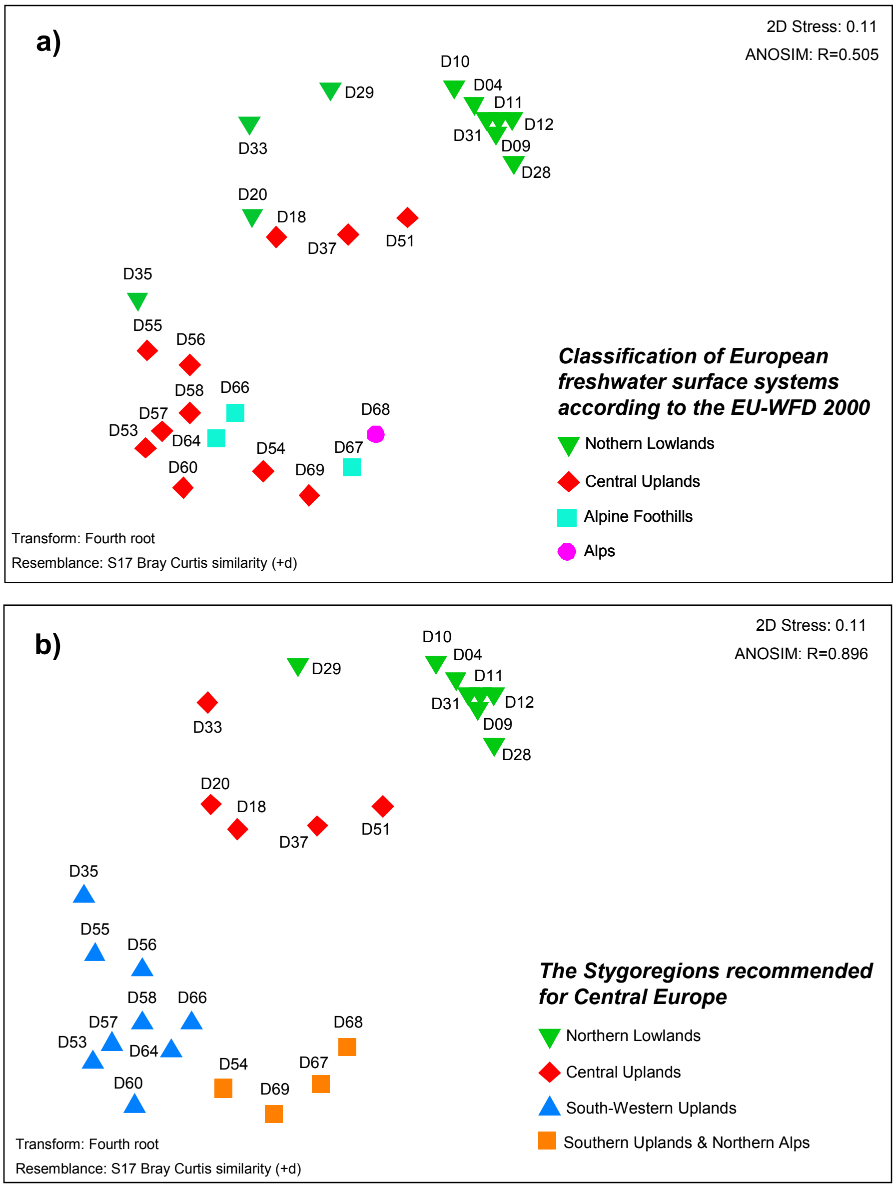

Fig. 3: Non-metrical Multi-Dimensional-Scaling plot (nMDS) of the distribution found in groundwater dwelling invertebrates. Symbols refer to a) freshwater surface systems according to the EU-WFD; b) stygoregions recommended for groundwater systems based on distribution patterns of groundwater dwelling invertebrates. Plotted are Bray-Curtis similarities of faunal means that were pooled for major physiographic units of Germany (MPU) (see table 1), respectively. Abbreviations: NL = Northern Lowlands, CU = Central Uplands, SWU = South-Western Uplands, SU&NA = Southern Uplands & Northern Alps. All stygoregiones differed significantly from each other (PERMANOVA: p=0,0001; PERMANOVA Pairwise-Test: NL - CU: p=0,0013; NL - SWU: p=0,0003; NL – SU&NA: p=0,0024; CU – SWU: p=0,001; CU - SU&NA: p=0,0087; SWU - SU&NA: p=0,0015).

Table 2: Species matrix of groundwater dwelling invertebrates collected in different stygoregions. Species are classified as stygobiontic and non-stygobiontic, according to their ecological preference. NL: Northern Lowlands, CU: Central Uplands, SWU: South-Western Uplands, SU & NA: Southern Uplands and Northern Alps. Species are classified as stygobites and non-stygobites, according to their ecological preferences., x = species present

Stygoregion		NL	CU	SWU	SU & NA
No. of GW-monitoring wells		40	60	376	38
No. of samples		116	223	821	81
Stygobiontic species	**Taxonomic group**				
Parastenocaris phreatica	Copepoda, Crustacea	x			
Parastenocaris phyllura	Copepoda, Crustacea	x	x		
Bogidiella albertimagni	Amphipoda, Crustacea		x	x	x
Crangonyx subterraneus	Amphipoda, Crustacea		x	x	x
Niphargellus nolli	Amphipoda, Crustacea		x	x	x
Niphargus fontanus	Amphipoda, Crustacea		x	x	x
Diacyclops languidoides	Copepoda, Crustacea		x	x	x
Graeteriella unisetigera	Copepoda, Crustacea		x	x	x
Proasellus cavaticus	Isopoda, Crustacea		x	x	x
Niphargus aquilex	Amphipoda, Crustacea		x	x	
Microniphargus leruthi	Amphipoda, Crustacea		x	x	
Parastenocaris germanica	Copepoda, Crustacea		x	x	
Chappuisius singeri	Copepoda, Crustacea		x	x	
Bathynella natans	Syncarida, Crustacea		x	x	
Fabaeformiscandona breuili	Ostracoda, Crustacea			x	
Fabaeformiscandona latens	Ostracoda, Crustacea			x	
Fabaeformiscandona wegelini	Ostracoda, Crustacea			x	
Parastenocaris psammica	Copepoda, Crustacea			x	
Schellencandona belgica	Ostracoda, Crustacea			x	
Schellencandona insueta	Ostracoda, Crustacea			x	
Schellencandona triquetra	Ostracoda, Crustacea			x	
Niphargopsis casparyi	Amphipoda, Crustacea			x	
Niphargus kochianus	Amphipoda, Crustacea			x	
Niphargus laisi	Amphipoda, Crustacea			x	
Niphargus puteanus	Amphipoda, Crustacea			x	
Niphargus tatrensis	Amphipoda, Crustacea			x	
Acanthocyclops gmeineri	Copepoda, Crustacea			x	
Acanthocyclops kieferi	Copepoda, Crustacea			x	
Bryocamptus typhlops	Copepoda, Crustacea			x	
Chappuisius inopinus	Copepoda, Crustacea			x	
Echinocamptus pilosus	Copepoda, Crustacea			x	
Elaphoidella elaphoides	Copepoda, Crustacea			x	
Graeteriella laisi	Copepoda, Crustacea			x	
Moraria fontinalis	Copepoda, Crustacea			x	
Nitocrella omega	Copepoda, Crustacea			x	
Parapseudoleptomesochra spec.	Copepoda, Crustacea			x	
Parastenocaris c.f. glacialis	Copepoda, Crustacea			x	
Anthrobathynella stammeri	Syncarida, Crustacea			x	
Bathynella freiburgensis	Syncarida, Crustacea			x	
Parabathynella c.f. ferdii	Syncarida, Crustacea			x	
Pseudantrobathynella husmanni	Syncarida, Crustacea			x	
Proasellus coxalis	Isopoda, Crustacea			x	
Proasellus walteri	Isopoda, Crustacea			x	x
Niphargus auerbachi	Amphipoda, Crustacea			x	x
Niphargus bajuvaricus	Amphipoda, Crustacea			x	x
Schellencandona schellenbergi	Ostracoda, Crustacea			x	x
Niphargus inopinatus	Amphipoda, Crustacea			x	x
Niphargus foreli	Amphipoda, Crustacea			x	x
Niphargus kieferi	Amphipoda, Crustacea			x	x
Parastenocaris c.f. moravica	Copepoda, Crustacea			x	x
Cryptocandona kieferi	Ostracoda, Crustacea			x	x
Fabaeformiscandona bilobata	Ostracoda, Crustacea			x	x
Mixtacandona laisi	Ostracoda, Crustacea			x	x
Acanthocyclops rhenanus	Copepoda, Crustacea			x	x
Proasellus slavus	Isopoda, Crustacea			x	x
Acanthocyclps venustus	Copepoda, Crustacea			x	x
Acanthocyclops sensitivus	Copepoda, Crustacea			x	x
Parastenocaris c.f. aedis	Copepoda, Crustacea				x
Bathynella chappuisi	Syncarida, Crustacea				x
Nitocrella hirta tirolensis	Copepoda, Crustacea				x
Niphargus strouhali	Copepoda, Crustacea				x

Table 2: continued

Stygoregion		NL	CU	SWU	SU & NA
No. of GW-monitoring wells		40	60	376	38
No. of samples		116	223	821	81
Non-stygobiontic species	**Taxonomic group**				
Pristina proboscidea	Oligochaeta	x			
Tubifex tubifex	Oligochaeta	x			
Diacyclops crassicaudis	Copepoda, Crustacea	x	x	x	x
Dorydrilus michaelseni	Oligochaeta	x	x	x	x
Marionina riparia	Oligochaeta	x	x	x	
Aelosoma hyalina	Oligochaeta	x		x	
Cernovsvitoviella atrata	Oligochaeta	x	x	x	
Bryocamptus minutus	Copepoda, Crustacea		x		
Paracyclops poppei	Copepoda, Crustacea		x		
Potamothrix/Tubifex	Oligochaeta		x		
Troglochaetus beranecki	Polychaeta		x	x	x
Paracyclops fimbriatus	Copepoda, Crustacea		x	x	x
Diacyclops bisetosus	Copepoda, Crustacea		x	x	
Diacyclops languidus	Copepoda, Crustacea		x	x	
Mesenchytraeus armatus	Oligochaeta		x	x	
Bryochamtus echinatus	Copepoda, Crustacea			x	x
Parastenocaris brevipes	Copepoda, Crustacea			x	x
Aelosoma niveum	Oligochaeta			x	x
Haplotaxis gordioides	Oligochaeta			x	x
Tubifex ignotus	Oligochaeta			x	x
Tubifex species A	Oligochaeta			x	x
Tubificidae bifurcata	Oligochaeta			x	x
Bythiospeum ssp.	Gastropoda			x	x
Acanthocyclops robustus	Copepoda, Crustacea			x	
Acanthocyclops vernalis	Copepoda, Crustacea			x	
Cyclops strenuus	Copepoda, Crustacea			x	
Cyclops vicinus	Copepoda, Crustacea			x	
Cypria ophtalmica	Ostracoda, Crustacea			x	
Diacyclops bicuspidatus	Copepoda, Crustacea			x	
Eucyclops serrulatus	Copepoda, Crustacea			x	
Eudiaptomus gracilis	Copepoda, Crustacea			x	
Macrocyclops albidus	Copepoda, Crustacea			x	
Megacyclops viridis	Copepoda, Crustacea			x	
Moraria brevipes	Copepoda, Crustacea			x	
Moraria pectinata	Copepoda, Crustacea			x	
Nitocra hibernica	Copepoda, Crustacea			x	
Thermocyclops crassus	Copepoda, Crustacea			x	
Tropocyclops prasinus	Copepoda, Crustacea			x	
Candona weltneri	Ostracoda, Crustacea			x	
Ancylus fluviatilis	Gastropoda			x	
Aelosoma hemprichi	Oligochaeta			x	
Aelosoma psammophylum	Oligochaeta			x	
Aelosoma quaternarium	Oligochaeta			x	
Amphichaeta leydigi	Oligochaeta			x	
Buchholzia appendiculata	Oligochaeta			x	
Eiseniella tetraedra	Oligochaeta			x	
Fridericia perrieri	Oligochaeta			x	
Marionina argentea	Oligochaeta			x	
Nais c.f. variabilis	Oligochaeta			x	
Phyllognathopus viguieri	Oligochaeta			x	
Potamothrix hammoniensis	Oligochaeta			x	
Pristinella bilobata	Oligochaeta			x	
Psammoryctides albicola	Oligochaeta			x	
Pseudocandona albicans	Oligochaeta			x	
Rhyacodrilus falciformis	Oligochaeta			x	
Uncinais uncinata	Oligochaeta			x	
Paracamptus schmeili	Copepoda, Crustacea				x
Cryptocandona vavrai	Ostracoda, Crustacea				x
Spirosperma velutinus	Oligochaeta				x
Vejdovskiella comata	Oligochaeta				x

Compared to surface bioregional classifiation systems, stygofaunal distribution patterns differ most notable in the southern groundwater habitats where assemblages differed markedly from those of the surface Central Uplands and the Alps. Another striking difference is the large expand of stygofauna, typically found in the *Central Uplands*, or in the case of the Lower Rhine Valley typically found in the *South-Western Uplands*, into the boundary areas of the surface Northern Lowlands (Fig. 2).

3.3.2.5 Discussion

The large scale distribution patterns found for groundwater dwelling invertebrates differ strongly from the classification systems of surface bioregions implying the need for an independent classification system of groundwater habitats, based on stygoregions. In Central Europe, the large scale distribution of groundwater fauna is highly dependent on quarternary glaciations, which have affected species richness and composition (STOCH & GALASSI, 2010) . Our data reflect the general latitudinal gradient of species richness, declining from south to north, which was observed in earlier studies and which is less pronounced in organisms from surface freshwaters (STOCH & GALASSI, 2010).

The groundwater faunal impoverishment, which is considered as typical of the *Northern Lowlands,* is a consequence of species extinction during glaciations and inhibited post-glacial recolonisation (HAHN & FUCHS, 2009): The reasons are glacially shaped underground habitats with insufficient living conditions for most invertebrates as are high amounts of fine sediments and extremely low oxygen concentrations (HUSMANN, 1966).

In comparision, the stygoregion of the *Central Uplands* was less affected by the periods of glaciation. However, most of the old tertiary stygofauna was extinct during the ice age. The prevailingly unglacified *Central Uplands* had served as refugial areas for few surviving groundwater species - although many aquifers are assumed of have dried out or have been affected by permafrost. After the end of the ice age, some of the old surviving groundwater species and many ubiquitous species recolonised the *Central Uplands* as well as the groundwater habitats of the formerly glacified sub-mountainous forelands where environmental conditions were appropriate (e.g. sufficient pore space and oxygen conditions) (HAHN & FUCHS, 2009). The low endemism of this stygoregion is a consequence of this post-glacial recolonisation.

In contrast, the highly diverse groundwater fauna characterizing the stygoregion of the *South-Western Uplands* is composed of ancient stygofauna, which outlasted the periods of glaciation (HAHN & FUCHS, 2009). The affiliation of the Lower Rhine Valley groundwater to the *South-Western Uplands* is not clear yet (Fig. 2). All classification systems, biological as well as geographical ones, consider the Lower Rhine Valley as a part of the Northern Lowlands. In contrast, the stygofauna of the Lower Rhine Valley were very different from those of the impoverished stygoregion *Northern Lowlands*. Our data suggest, however, that the groundwater of the Lower Rhine Valley is connected via the middle Rhine valley to the Pleistocenious Danube and Main catchments (HAHN & FUCHS, 2009).

The state of the stygoregion *Southern Uplands and Northern Alps* is uncertain yet. Although its fauna resembles to an impoverished *South-Western Uplands* fauna, some species, such as *Niphargus strouhali,* were unique to this stygoregion and are known from groundwaters located further east, e.g. from Austria. We suppose, that these eastern species re-colonised the *Southern Uplands and Northern Alps* both from the *South-Western Uplands* and from the East via the ´interstitial highways` of the Danube and its southern tributaries. Therefore, our results presented here seem to corroborate the earlier findings of HAHN & FUCHS (2009), that the *Southern Uplands and Northern Alps* form a distinct, individual stygoregion.

In conclusion, reference habitats and communities on different spatial scales have to be considered as fundamental to the ecological assessment and monitoring of the environment. In particular for the groundwater such references are still elusive (KORBEL & HOSE, 2011). As faunal distribution patterns in groundwater are clearly distinct from the biogeographic units defined for surface freshwaters, we strongly recommend the development of an independent classification system for groundwater habitats. Therefore, a comprehensive mapping of groundwater diversity and community structure is crucial for future groundwater protection programmes (HAHN & FUCHS 2009; SCHMIDT & HAHN 2012). Our study gives insights into the distribution patterns of stygofauna in Central Europe and may show the way to an ecological classification system of groundwater. The definition of stygoregions is an indispensable initial step towards the ecological assessment of groundwater leading to a sustainable groundwater management (SCHMIDT & HAHN 2012).

3.3.2.6 References

ANDERSON M.J., GORLEY R.N. & CLARKE K.R. (2008) PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E: Plymouth, UK.

BOTOSANEANU L. (1986) Stygofauna Mundi. A faunistic, distributional and ecological synthesis of the world fauna inhabiting subterranean waters (including the marine interstitial). Brill, Backhuys, Leiden.

BOULTON A.J. (2005) Chances and challenges in the conservation of groundwaters and their dependent ecosystems. Aqua. Conserv. Marine Freshwater Ecosyst. 15, 319–323.

BUNDESAMT FÜR NATURSCHUTZ (Hrsg.) (2008) Daten zur Natur 2008. – Münster (Landwirtschaftsverlag): 10-11.

CLARKE K. R. & GORLEY R. N. (2006) Primer v6: User Manual/Tutorial. Plymouth.

DANIELOPOL D., GIBERT J., GRIEBLER C., GUNATILAKA A., HAHN H.J., MESSANA G. NOTENBOOM J. & SKET B. (2004) Incorporating ecological perspectives in European groundwater management policy. Environ. Conserv. 31, 1–5.

DANIELOPOL D.L. & GRIEBLER C. (2008) Chaniging paradigms in groundwater ecology – from the ´living fossils` tradition to ´new groundwater ecology`. Int. Rev. Hydrobiol. 93, 565-577.

DANIELOPOL D., GRIEBLER C., GUNATILAKA A., HAHN H.J., GIBERT J., MERMILLOD-BLONDIN G., MESSANA G., NOTENBOOM J. & SKET B. (2008) Incorporation of groundwater ecology in environmental policy. In: Quevauviller, P. (Ed.), Groundwater Science and Policy – An International Overview. RSC Publishing, The Royal Society of Chemistry, London.

DATRY T., MALARD F. & GIBERT J. (2005) Response of invertebrate assemblages to increased groundwater recharge rates in a phreatic aquifer. J. North Am. Benthol. Soc. 24, 461–477.

DOLE-OLIVIER M.-J., MALARD F., MARTIN D., LEFÉBURE T. & GIBERT J. (2009) Relationships between environmental variables and groundwater biodiversity at the regional scale. Freshw. Biol. 54, 797–813.

EU-GWD (European Groundwater Directive): Directive 2006/118/ECEU GWD, of the European Parliament and of the Concil of 12 December 2006 - Official J. of the European Comm. L372, (2006).

EU-WFD, 2000. Directive 2000/60/EC of the European Parliament and of the Council of the 23 October 2000: establishing framework for the community action in the field of water policy. Official J. European Comm. L327, 321–371.

EINSLE U. (1993) Crustacea Copepoda, Calanoida und Cyclopoida. In: Süßwasserfauna von Mitteleuropa. Gustav Fischer, Stuttgart.

EPA (2003). Guidance for the assessment of environmental factors: consideration of subterranean fauna in groundwater and caves during environmental impact assessment in Western Australia. In: ´Guidance Statement for the Assessment of environmental Factors no. 54 Environmental Protection Authority, Perth, Australia.

GIBERT J., DEHARVENG L. (2002) Subterranean ecosystems: a truncated functional biodiversity. BioScience 52, 473–481.

GIBERT J., BRANCELJ A., CAMACHO A., CASTELLARINI F., DE BROYER C., DEHARVENG L., DOLE-OLIVIER J.-M., DOUADY C., GALASSI D.M., MALARD F., MARTIN P., MICHEL G., SKET B., STOCH F., TRONTELJ P., VALDECASAS A.G. (2005) Groundwater biodiversity. Protocols for the ASsessment and Conservation of Aquatic Life In the Subsurface (PASCALIS): Overview and main results. In: Proceedings of an International Symposium on World Subterranean Biodiversity (Ed. J. Gibert). University of Lyon, Lyon, Villeurbanne. 8-10 December.

GIBERT J. & CULVER D. (2009) Assessing and conserving groundwater biodiversity: an introduction. Freshw. Biol. 54, 639-648.

GRIEBLER C., STEIN H., KELLERMANN C., BRIELMANN H., SCHMIDT S. I., SELESI D., STEUBE C., BERKHOFF S.E., FUCHS A. & HAHN H. J. (2010) Ecological assessment of groundwater ecosystems – Vision or illusion? Ecol. Engeneer. 36, 1174-1190.

GSCHV (1998) Gewässerschutzverordnung (Swiss Water Ordinance) 814.201. Der Schweizer Bundesrat, Bern, Switzerland.

HAHN H.J. (2006) The GW-Fauna-Index: a first approach to a quantitative ecological assessment of groundwater habitats. Limnologica 36, 119–137.

HAHN H.J. & FUCHS A. (2009) Distribution patterns of groundwater communities across aquifer types in south-western Germany. Freshw. Biol. 54, 848–860.

HANCOCK P. J., BOULTON A. J. & HUMPHREYS W. F. (2005) Aquifers and hyporheic zones: towards an ecological understanding of groundwater, Hydrogeol. J. 13(1), 98–111.

HUMPHREYS W. F. (2006) Aquifers: the ultimate groundwater-dependent ecosystems, Aust. J. Bot. 54, 115–132.

HUSMANN S. (1966) Versuch einer ökologischen Gliederung des interstitiellen Grundwassers in Lebensbereiche eigener Prägung Arch. Hydrobiol. 62, 231–268.

ILLIES J. (1978) Limnofauna Europaea. Gustav Fischer. Stuttgart.

JANETZKY W., ENDERLE R. & NOODT W. (1996) Crustacea, Copepoda, Gelyelloida und Harpacticoida. In: Süßwasserfauna von Mitteleuropa. Gustav Fischer Stuttgart.

KORBEL K. L. & HOSE G. C. (2011) A tiered framework for assessing groundwater ecosystem health. Hydrobiologia 661, 329-349.

KUNKEL R., WENDLAND F., VOIGT H. J. & HANNAPPEL S. (2004) Die natürliche, ubiquitär überprägte Grundwasserbeschaffenheit in Deutschland, Schriften des Forschungszentrums Jülich, Reihe Umwelt / Environment 47.

MALARD F., REYGROBELLET J.-L., MATHIEU J. & LAFONT M. (1994) The use of invertebrate communities to describe groundwater flow and contaminant transport in a fractured rock aquifer. Arch. Hydrobiol. 131, 93-110.

MATZKE D., HAHN H.J., RAMSTÖCK A. & ROTHER K. (2005) Bewertung von Altlasten im Grundwasser anhand der Meiofaunagemeinschaften - erste Ergebnisse. Grundwasser 10, 25-34.

MEISCH C. (2000) Freshwater Ostracoda of Western and Central Europe. Spektrum Akademischer Verlag, Heidelberg.

MÖSSLACHER F. (2000) Sensitivity of groundwater and surface water crustaceans to chemical pollutants and hypoxia: implications for pollution management, Arch. Hydrobiol. 149, 51–66.

MURRAY B.R., HOSE C.G., EAMUS D. & LICARI D. (2006) Valuation of groundwater-dependent ecosystems: a functional methodology incorporating ecosystem services. Aust. J. Bot. 54, 221-229.

NOTENBOOM J., SERRANO R., MORELL I. & HERNÁNDEZ F. (1995) The phreatic aquifer of the 'Plana de Castellón' (Spain): relationships between animal assemblages and groundwater pollution. Hydrobiologia 297, 241–249.

NSW-SGDEP (2002) The NSW State Groundwater Dependent Ecosystems Policy. Department of Land and Water Conservation, NSW Government.

QUEVAUVILLER P. (2005) Groundwater monitoring in the context of EU legislation: reality and integration needs. J. Environ. Monit. 7, 89.

SCHMIDT S.I. & HAHN H.J. (2012) What is groundwater and what does this mean to fauna? – An opinion. Limnologica 42, 1-6.

SCHMINKE K. (2006) Copepoda (Ruderfußkrebse). In: Schminke K. (ed), Grundwasserfauna Deutschlands, pp. 61–134.

SSYMANK A. 1994 Neue Anforderungen im europäischen Naturschutz: Das Schutzgebietssystem Natura 2000 und die "FFH-Richtlinie der EU". Natur und Landschaft 69(9), 395-406.

STEIN H., KELLERMANN C., SCHMIDT S.I., BRIELMANN H., STEUBE C., BERKHOFF S. E., FUCHS A., HAHN H. J., THULIN B. & GRIEBLER C. (2010) The potential use of fauna and bacteria as ecological indicators for the assessment of groundwater ecosystems. J. Environ. Monit. 12, 242-254.

STEUBE C., RICHTER S. & GRIEBLER C. (2009) First attemps towards an integrative concept for the ecological assessment of groundwater ecosystems. Hydrogeol. J. 17, 23–35.

STOCH F. & GALASSI M.P. (2010) Stygobiotic crustacean species richness: a question of numbers, a matter of scale. Hydrobiologia 653, 217-234.

TOMLINSON M. & BOULTON A.J. (2009) Subsurface groundwater dependent ecosystems: a review of their biodiversity, ecological processes and ecosystem services. In: Commission, N.W. (Ed). Waterlines Occasional Paper No. 8, National WaterCommission, Canberra.

WENDLAND F., BLUM A., COETSIERS M., GOROVA R., GRIFFIOEN J., GRIMA, J., HINSBY K., KUNKEL R., MARANDI A., MELO T., PANAGOPOULOS A., PAUWELS H., RUISI M., TRAVERSA P., VERMOOTEN J. S. A. & WALRAEVENS K. (2008) European aquifer typology: a practical framework for an overview of major groundwater composition at European scale. Environ. Geol. 55, 77–85.

Acknowledgements

We thank the German Federal Environment Agency (Umweltbundesamt) and the German Working Group of the Federal States on Water Issues (Bund / Länder-Arbeitsgemeinschaft Wasser) whose efforts helped to render possible our studies. We are also indebted to the Federal Ministry of environmental measurments and nature conservation of Baden-Wurttemberg (Landesanstalt für Umwelt Messungen und Naturschutz Baden Württemberg), the Federal institution of flood protection and water management of Saxony-Anhalt (Landesbetrieb für Hochwasserschutz und Wasserwirtschaft Sachsen-Anhalt) and the Bavarian Enviroment Ageny (Bayerisches Landesamt für Umwelt) for the provision with information and groundwater monitoring wells. Martin Alt, Landau, kindly prepared the maps, and Karsten Grabow, Karlsruhe, provided the photography.

4 Schlussfolgerung und Ausblick

Die vorliegende Studie zeigt, dass subterrane, aquatische Invertebraten und Bakteriengemeinschaften geeignete Mittel zur Erfassung und Bewertung des ökologischen Zustands von Grundwassersystemen sind. Die Invertebratengemeinschaften eignen sich insbesondere als Anzeiger von Oberflächenwasser-Grundwasser-Interaktionen, wobei der Einfluss von Oberflächenwasser ein treibender Faktor für die Besiedlung im Grundwasser ist. Dabei geben vor allem die Präsenz und Abwesenheit grundwasserfremder Arten (Nicht-Stygobionte) sowie die Abundanz und die Diversität Aufschluss über die hydrologischen Verhältnisse. Ebenso können bakterielle Abundanzen und Aktivitäten auf Oberflächeneinträge hinweisen. Die Grundwassergemeinschaften sollten ergänzend zu physikochemischen Kriterien bei der Grundwasserbewertung eingesetzt werden, da sie Störungen unabhängig vom Zeitpunkt der Probennahme integrieren und Rückschlüsse auf den ökologischen Zustand zulassen. Die Gemeinschaften reagieren relativ schnell auf hydrologische Veränderungen und eignen sich somit als Frühwarnsystem bei der Grundwassermanagement.

Entscheidend für die faunistische Grundwasserbewertung ist die Berücksichtigung der räumlichen Skala. Das hierarchische Drei-Ebenen-Modell von HAHN (2009), das Grundwasserhabitate und ihre Gemeinschaften auf lokaler, regionaler und biogeografischer Ebene unterscheidet, erwies sich als ein geeignetes Gliederungssystem für Grundwasserökosysteme. Die eigentliche Grundwasserbewertung muss dabei auf lokaler Ebene erfolgen, während regionale und biogeografische Verhältnisse die Rahmenbedingungen für die Verbreitungsmuster vorgeben.

Geeignete Bewertungskriterien auf *lokaler Ebene* sind die Artenzahl, Abundanz, Diversität sowie das Verhältnis stygobionter zu nicht-stygobionten Arten. Der hydrologische Einfluss wird als Schlüsselparameter für die Fauna angesehen, da er allochthones organisches Material einbringt. Dies zeigen die positiven Korrelationen zwischen den oben genannten Kriterien und der Menge des organischen Materials (z. B. geschätzter Detritus, TOC) sowie dem Grundwasserfauna-Index (GFI). Die Korrelationen sind jedoch nur schwach ausgeprägt und weisen auf einen Optimierungsbedarf der quantitativen und qualitativen Kohlenstoff-Analysen hin. Dabei erscheint vor allem die Quantifizierung des biologisch verwertbaren Kohlenstoffs wichtig für eine verlässlichere Bewertung. Der im GFI bisher verwendete Parameter „geschätzter Detritus“ sollte durch eine gemessene Kenngröße des biologisch verwertbaren Kohlenstoffs ersetzt werden, um die Aussagekraft des Index zu verbessern. Indirekte Korrelationen zwischen der Fauna und dem Nitratgehalt weisen auf einen erhöhten Oberflächeneintrag hin, der mit intensiver Landnutzung einhergeht. Die meist fehlenden direkten Korrelationen zwischen der faunistischen Besiedlung und mikrobiologischen Parametern deuten ebenfalls auf eine notwendige Optimierung hinsichtlich der Erfassungs- und Analysemethoden hin. Einerseits muss geprüft werden, welche Auflösung des taxonomischen Niveaus der Bakterien für eine Bewertung ausreichend ist. Andererseits werden Methoden gebraucht, um neben den im Wasser suspendierten Bakterienanteilen auch die festsitzenden Gemeinschaften zu erfassen, die quantitativ bedeutender sind.

Die Interpretation der Besiedlungsmuster erfordert zudem die Erfassung der *regionalen Verhältnisse* anhand standardisierter Protokolle. Diese müssen vor allem die Beschaffenheit des Naturraums und des Grundwasserleitertyps beinhalten, da die Invertebratengemeinschaften maßgeblich durch diese geprägt werden. Dabei muss bedacht werden, dass lokale Effekte, wie z. B. kleinräumige heterogene Strukturen im Lückensystem die Hydrologie beeinflussen, wodurch regionale Effekte überlagert werden können. Die aufgenommenen Daten liefern für die untersuchten Gebiete erste Referenzen, die auch für weitere Gebiete benötigt werden, um eine Grundwasserbewertung durchzuführen.

Die Berücksichtigung der *biogeografischen Ebene* ist eine weitere Voraussetzung für ein faunistisches Bewertungssystem, da die biologische Vielfalt und das Auftreten bestimmter Arten meist von biogeografischen Faktoren abhängig sind. Die durchgeführten Analysen der Grundwasserfauna weisen deutliche biogeografische Verbreitungsmuster auf, welche nicht mit bestehenden Gliederungssystemen aquatischer, oberirdischer Zoozönosen oder naturräumlicher Einheiten übereinstimmen. Demzufolge ist für Grundwasserlebensräume ein eigenständiges Gliederungssystem erforderlich, für das erste faunistische Referenzen (Stygoregionen) abgeleitet wurden.

Die erhobenen Daten bilden eine solide Grundlage für die Erfassung ökologischer Referenzen einer Grundwasserbewertung. Die bestehende Datenbasis sollte in zukünftigen Untersuchungen erweitert werden, um die genannten Problemfelder vertiefend zu bearbeiten und die vorliegenden Ergebnisse zu überprüfen. So kann auch die inhomogene Verteilung der Stygofauna, die problematisch bei der Bewertung ist, mit einer erhöhten Datenmenge ausgeglichen werden. Ebenso können mithilfe multivariater Analysen Verbreitungsmuster besser erkannt und interpretiert sowie zur Klassifizierung von Gemeinschaften als auch zur Ableitung von Referenzen eingesetzt werden.

Schlussendlich ist vor allem ein Umdenken in Politik und Wasserwirtschaft erforderlich, das die Relevanz eines eigenständigen Bewertungssystems für Grundwasserhabitate gegenüber den Oberflächensystemen anerkennt. Auch die interdisziplinäre Zusammenarbeit von Hydrologen, Geologen und Ökologen kann zu einem besseren Verständnis der Funktionen und Gemeinschaften im Grundwasser beitragen. Da nur intakte Grundwasserökosysteme samt ihrer Biodiversität sauberes Trinkwasser liefern, ist die Analyse ihrer Lebensgemeinschaften, neben physikochemischen Kriterien, eine Vorraussetzung für den nachhaltigen Grundwasserschutz.

Danksagung

Die in dieser Arbeit vorgestellten Forschungsergebnisse wurden nur durch die Hilfe und Unterstützung zahlreicher Personen und Institutionen ermöglicht.

Mein besonderer Dank gilt Herrn PD Dr. Hans Jürgen Hahn, Universität Koblenz-Landau, Campus Landau für die Vergabe des Themas, die wissenschaftliche Betreuung während der Forschungsarbeiten sowie die Übernahme des Erstgutachtens. Seine stete Hilfsbereitschaft, Unterstützung und Förderung trugen sehr zur erfolgreichen Durchführung der Arbeit bei.

Ich bedanke mich herzlich bei Herrn Prof. Dr. Klaus Schwenk für seine Bereitschaft zur Erstellung des Gutachtens und für seine Unterstützung im Verlauf dieser Promotionsarbeit. Ebenso danke ich den Herren Prof. Ralf Schulz und Dr. E. Friedrich für ihre Unterstützung.

Ganz besonders möchte ich meinen KollegInnen von der grundwasserökologischen Gruppe an der Universität Koblenz-Landau, Campus Landau danken, die am Gelingen des Projektes sowie bei der Probennahme beteiligt waren. Vor allem danke ich Herrn Dr. Sven Berkhoff, Herrn Simon Gutjahr, Herrn Dr. Andreas Fuchs, Herrn Dr. Jörg Bork und Frau Cornelia Spengler.

Ebenso gilt mein Dank Stefanie Sachers, Phil Merkens, Alexandra Tresp für die Hilfe bei der Probennahme sowie Martin Alt für seine Hilfe Kartenerstellung.

Mein Dank gilt insbesondere auch Herrn Dr. Christian Griebler vom Helmholtz Zentrum München und Frau Dr. Claudia Kellermann für die sehr gute Zusammenarbeit während der Durchführung des UBA- und LAWA-Projektes.

Ich danke dem Umweltbundesamt (UBA) und der Länderarbeitsgemeinschaft Wasser (LAWA) für die Finanzierung des Forschungsprojektes „Entwicklung biologischer Bewertungsmethoden und –kriterien für Grundwasserökosysteme“. Insbesondere gilt mein Dank Frau Simone Richter (UBA) für die engagierte Projektkoordination sowie Herrn Dr. Michael Gierig als LAWA-Beauftragter und Mitglied des Begleitkreis des Projektes. Ebenso danke ich den anderen Mitgliedern des Begleitkreises, Herrn K.P. Barufke, Herrn Michael Sinreich, Herrn Lutz Keppner, Herrn Schenk, für die fachliche Betreuung und für die konstruktiven Diskussionen.

Dank geht auch an den Landesbetrieb für Hochwasserschutz und Wasserwirtschaft Sachsen-Anhalt und die Life-Science Stiftung. Beide Institutionen haben unter ihrer Förderung erhobene Daten für vergleichende Analysen zur Verfügung gestellt.

Für die zur Verfügung gestellten Daten, die ich für die biogeografischen Untersuchungen verwendet habe, danke ich Herrn Dr. Dirk Matzke sowie Herrn Dr. Andreas Fuchs.

Für die Durchführung der Laborarbeiten danke ich der LUBW, der Landeswasserversorgung-Langenau, dem Landesamt für Umweltschutz Bayern (LFU) und dem Erftverband, Bergheim.

Dem Erftverband und der Landeswasserversorgung danke ich auch für die zur Verfügung gestellten Messstellen und der Unterstützung bei der Probennahme im Erftgebiet. Namentlich danke ich vor allem Herrn N. Cremer, Herrn F. Wilhelms, Herrn U. Lack, Herrn E. Hönberg, Thomas und Harald (Erftverband) sowie Herrn R. Scheck, Herrn Heisele und Herrn Fischer (Landeswasser-versorgung).

[redacted]

Anhang

Folgende Kapitel wurden in Fachzeitschriften veröffentlicht bzw. wurden zur Veröffentlichung eingereicht:

Kapitel 3.1

STEIN H., KELLERMANN C., SCHMIDT S.I., BRIELMANN H., STEUBE C., BERKHOFF S. E., FUCHS A., HAHN H. J., THULIN B. & GRIEBLER C. (2010) The potential use of fauna and bacteria as ecological indicators for the assessment of groundwater ecosystems. J. Environ. Monit. 12, 242-254.

Kapitel 3.2

GRIEBLER C., STEIN H., KELLERMANN C., BRIELMANN H., SCHMIDT S. I., SELESI D., STEUBE C., BERKHOFF S.E., FUCHS A. & HAHN H. J. (2010) Ecological assessment of groundwater ecosystems – Vision or illusion? Ecol. Engeneer. 36, 1174-1190.

Kapitel 3.3

STEIN H., BERKHOFF S. E., MATZKE D., FUCHS A. & HAHN H.J. (...) The sustainable groundwater management requires the definition of stygoregions. Nature Scientific Reports (in Begutachtung)

Erklärung

Hiermit erkläre ich, dass ich die eingereichte Dissertation selbständig verfasst habe und alle für die Arbeit benutzten Hilfsmittel in der Arbeit angegeben sowie die Anteile etwaig beteiligter Mitarbeiter und anderer Autoren klar gekennzeichnet habe.

Ich erkläre, dass ich die Dissertation oder Teile hiervon nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Einrichtung eingereicht habe, und dass ich die gleiche oder eine andere Abhandlung nicht in einem anderen Fachbereich oder einer anderen wissenschaftlichen Hochschule als Dissertation eingereicht habe.

Landau, den 23.Mai 2012 Heide Stein

TABELLARISCHER LEBENSLAUF

PERSÖNLICHE DATEN

Name:	Heide Stein
Geburtsdatum/-ort:	[redacted]
Staatsangehörigkeit:	deutsch
Familienstand:	[redacted]

SCHULE

1984 - 1988	Grundschule „Otto Buchwitz“ Bieberstein / Sachs.
1988 - 1990	Realschule „Otto Buchwitz“ Reinsberg / Sachs.
1990 - 1998	Sekundarstufe I und II an der Intergrierten Gesamtschule Hannover- Linden Abschluss: Abitur

STUDIUM

Okt.1998 - Aug.2005	Studium der Biologie an der Universität Hannover Fachrichtung: Zoologie, Ökologie, Hydrobiologie Diplom-Arbeit: *“Investigation of anthropogenic influences on the Río Dos Novillos with the help of benthic macroinvertebrates for water analyses in Guácimo, province Limón, Costa Rica”*. Die Diplomarbeit war Teil des Projektes *„Monitoreo de la calidad de agua y el uso de bioindicadores acuáticos en la microcuenca del Río Dos Novillos, Guápiles, Limón, Costa Rica”* der Universitäten “Escuela de Agricultura de la Región Trópica Húmeda” (EARTH) und “Universidad de Costa Rica” (UCR). Abschluss: Diplom-Biologin (Dipl.-Biol.)
2001 - 2005	Studentische Hilfskraft am Institut für Tierökologie und Botanik bei der Durchführung verschiedener Kurse und Praktika (Niedere Pflanzen, Zoologisches Grundpraktikum, Bestimmungsübungen Invertebraten) an der Universität Hannover
Mai-Sept 2003	Projektarbeit: „Vergleich zweier Methoden und Analyse der benthischen Makrofauna des Río San Carlos in Alajuela, Costa Rica“, durchgeführt im Rahmen eines Projektes der Universidad de Costa Rica: „Estudio básico sobre la calidad de las aguas de los ríos Sarapiquí y San Carlos (Alajuela, Costa Rica)“

PROMOTION

Okt. 2006 – vorauss. Juni 2012	Promotion am Institut für Umweltwissenschaften der Universität Koblenz-Landau, Campus Landau bei PD Dr. Hans Jürgen Hahn zum Thema: „Ökologisch - faunistische Untersuchungen zur Typisierung und Bewertung von Grundwasserökosystemen“

2007 - 2010	Betreuung verschiedener Kurse und Praktika (Zoologisches Grundpraktikum, Zoologische Bestimmungsübungen) sowie Betreuung von Examenskandidaten am Institut für Umweltwissenschaften der Universität Koblenz-Landau, Campus Landau

AUSLANDSAUFENTHALTE

Aug.1994 - Juni 1995	Schüleraustausch (11./12. Klasse) an der Princeton Secondary School, British Columbia, Kanada
Juni 2002 - Sept. 2003	Integriertes Auslandsstudium der Tropischen Biologie an der Universidad Nacional Autonómica (UNA) in Heredia (Costa Rica), gefördert vom DAAD
Okt 2004 - April 2005	Datenerhebung für die Diplomarbeit in Limón, Costa Rica, gefördert vom DAAD

FREMDSPRACHEN

Englisch (fließend), Spanisch (fließend), Französisch (Schulkenntnisse)

MITGLIEDSCHAFTEN

Deutsche Gesellschaft für Limnologie (DGL)

VERÖFFETLICHUNGEN

Stein H., Springer M. & Kohlmann B. (2008): Comparison of two sampling methods for biomonitoring using aquatic macroinvertebrates in the Dos Novillos River, Costa Rica. EcoEng, 34(4), 267-275.

Stein H., Kellermann C., Schmidt S.I., Brielmann H., Steube C., Berkhoff S.E., Fuchs A., Hahn H.J., Thulin B. & Griebler C. (2010): The potential use of fauna and bacteria as ecological indicators for the assessment of groundwater quality. Environ. Monit.,12, 242-254.

Griebler C., Stein H., Kellermann C., Berkhoff S., Brielmann H., Schmidt S., Selezi D., Steube C., Fuchs A., Hahn H.J. (2010): Ecological assessment of groundwater ecosystems – Vision or illusion? EcoEng 36, 1174-1190.

Unterschrift: Landau, den 03. Mai 2012

[redacted]