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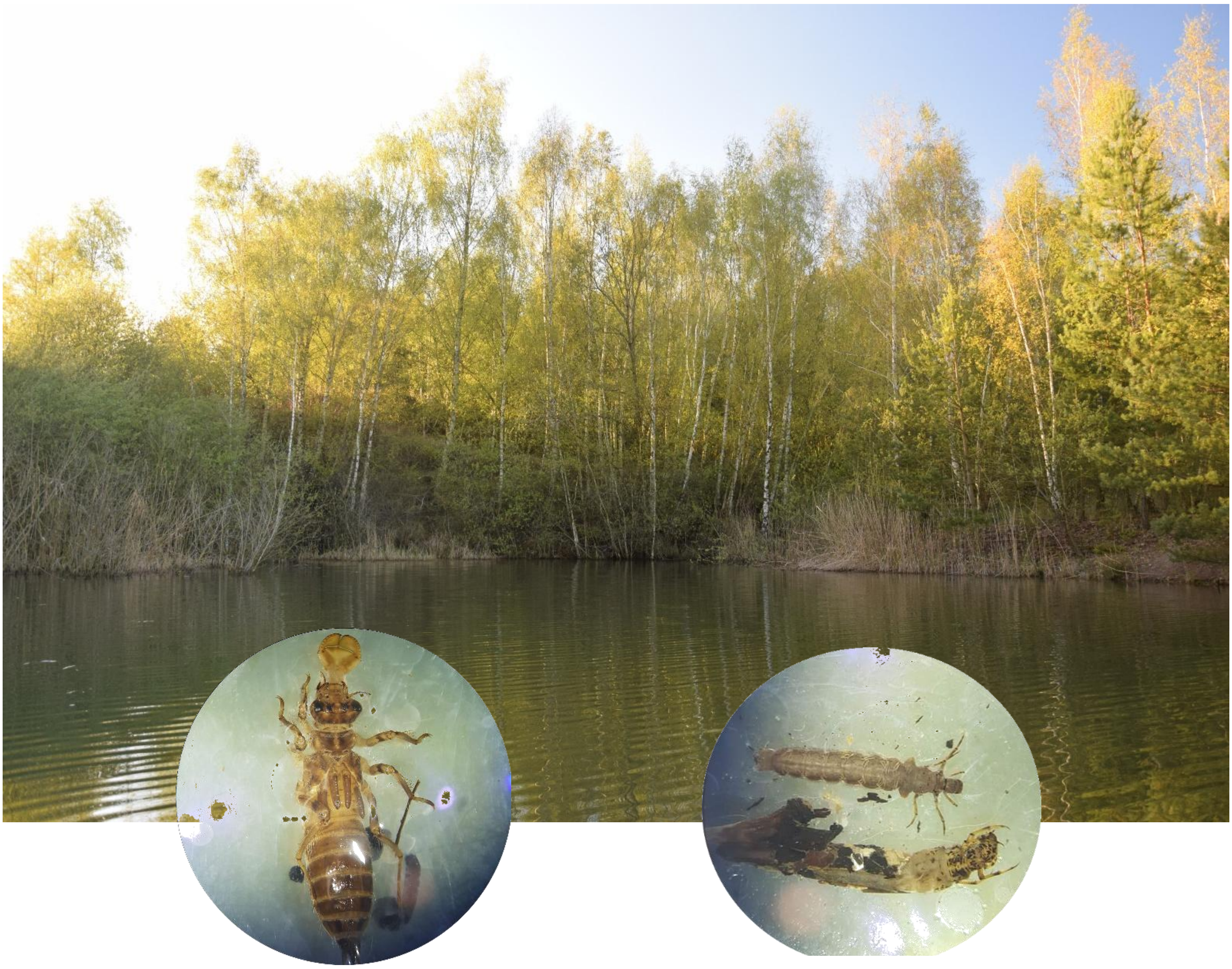

MUTEL Stephane

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Resume

This report is a part of a larger study lead by the Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB) and conducted within the "Baggersee Project". The main aim of the project is to assess freshwater biodiversity in gravel pit lakes in Lower Saxony region in Germany. Biodiversity of fish, birds, amphibians, dragonflies and macroinvertebrate communities was assessed to gain important information about sustainable management conducted in an ecological way which can improve ecosystem biodiversity. The project included 20 lakes which were managed or owned by angling clubs. Of the 20 lakes, four lakes are characterized by fish stocking, four are untouched, but used for fishing while other four lakes are untouched and preserved from any anthropic pressure including fishing. In other four lakes dead wood (as cut trees) will be introduced close to the shoreline. Finally in the last group of four lakes dead woods will be introduced in combination with the creation of shallow areas. In 5 years from now a new sampling campaign will be performed in order to assess the effect of the increased habitat diversity on the animal biodiversity. The study described in this report assessed macroinvertebrate communities in the gravel pit lakes before management was performed. This was done to have an idea of the initial condition of the macroinvertebrate communities. AESHNA/LIMCO micro-habitat sampling method was used to collect macroinvertebrate samples. In this study we analysed three lakes selected from the 20 lakes based on environmental and morphometric conditions of the lakes. Principal Component Analysis (PCA) was used to select the three lakes. Consequently, we used analysis of the variance (ANOVA) and permutational multivariate analysis of the variance (perMANOVA) and non-parametric multidimensional scaling (nMDS) plots to compare abundance and composition among lakes and microhabitats, respectively. Our results showed that higher number of macroinvertebrates was found in mineral (e.g. sand) microhabitats compared to biotic (e.g. submerged, emerged macrophytes etc.) microhabitats. In contrast, taxa richness was higher in the biotic microhabitats. Community composition analysis showed that different taxa were inhabiting different microhabitats and different lakes giving important insight on animal distribution at different spatial scales. This study represent an important starting point for a consequent larger scale analysis including the all 20 lakes assessed in the project and two phases: before and after the introduction of the habitat improvement modifications. Analysis performed in this study can be used similarly upscaling to larger spatial and temporal scales.

Key Words: macroinvertebrates; macroinvertebrate communities ; gravel pit lakes ; ecology ; biodiversity; habitat enhancement; monitoring

Version abrégée

Mon stage s'est déroulé au « Liebniz-Institute of Freshwater Ecology and Inland Fisheries » (IGB) à Berlin dans l'équipe du Docteur Robert Arlinghaus qui dirige entre autres le « Baggersee Project ». Ce projet a comme terrain d'étude des lacs artificiels issus d'anciennes exploitations minières. Ces gravières (gravel pit lakes) sont de « petits » lacs (inférieurs à 50ha) qui ne sont pas pris en compte dans les actions du gouvernement allemand concernant la directive européenne cadre sur l'eau. Ces lacs sont pourtant d'importants puits de biodiversité notamment pour les amphibiens, oiseaux migrateurs, poissons et macroinvertébrés.

Le but global de l'étude est d'estimer la biodiversité globale de ces lacs avec des relevés et suivis des espèces animales et végétales. En partenariat avec les clubs de pêche qui possèdent et/ou gèrent ces lacs, des aménagements vont être réalisés et des pratiques de gestion vont être mises en place afin d'évaluer leur impact sur la biodiversité. Sur les 20 lacs de l'étude, quatre bénéficient d'un « statut de protection » et resteront vierges de toute pression anthropique (pêche et accès interdit). Quatre ne subiront aucune modification mais la pêche continuera d'y être autorisée. Ces huit lacs sont les lacs témoins de notre étude. Dans quatre autres, des poissons seront ajoutés aux stocks actuels. Enfin les huit derniers lacs bénéficieront de diversifications des habitats. Ces diversifications constituent à la mise en place de bois noyés sur la zone littorale pour quatre d'entre eux, et à un ajout de bois ainsi que la création de zones de faible profondeur propice au développement de macrophytes et à la reproduction des poissons pour les quatre derniers.

Mon travail s'est focalisé sur l'inventaire des communautés de macroinvertébrés, de la phase de terrain et prélèvements jusqu'à l'analyse des premières données en passant par la phase de laboratoire et l'identification des individus. Pour la collecte des individus, nous avons suivi le protocole AESHNA/LIMCO, utilisé en Allemagne pour le prélèvement des macroinvertébrés dans les zones littorales lacustres. Cette méthode est basée sur l'échantillonnage des micro-habitats. Sur chaque site (nombre de sites variable par lacs) nous échantillonnions les différents micro-habitats disponibles avec un filet pourvu de mailles de 500µm. Les échantillons étaient ensuite stockés individuellement dans de l'alcool à 90% en attendant d'être triés et identifiés.

Nous avons ensuite procédé à un test statistique (Analyse en Composante Principale – PCA) afin de sélectionner les lacs les plus semblables sur différents critères tels que : niveau trophique, micro-habitat, âge etc. Une premier groupe de 7 lacs s'est révélé présenter suffisamment de similarités. En raison de la durée de mon stage, nous avons concentré nos efforts sur l'identification des échantillons collectés sur trois lacs afin d'être en mesure de réaliser l'analyse des communautés et de servir de base à un futur article scientifique. Concernant l'identification des individus, nous avons utilisé différents guides et clés

dichotomiques afin d'identifier les individus jusqu'à l'espèce (exception faite des diptères pour lesquels nous nous sommes arrêtés à la famille). Au total, plus de 100 taxas et 16000 individus ont été triés, identifiés et stockés. Ces listes de taxa, une par échantillon (donc reliées à un micro-habitat), ont ensuite été utilisées pour déterminer les facteurs influençant la composition des communautés de macroinvertébrés.

Des tests statistiques (ANOVA) nous ont permis de montrer une corrélation du type de macro-habitat avec la richesse taxonomique et l'abondance. Les micro-habitats biotiques rassemblent la plus grande diversité taxonomique, tandis que les substrats minéraux abritent la plus grande abondance. La réalisation d'une perMANOVA en utilisant les distances de Bray-Curtis nous a ensuite permis d'expliquer les origines des différences entre les communautés. Les communautés semblent d'abord se différencier à cause de la différence de macro-habitats puis sont différentes à l'échelle du lac également. Le fort pourcentage de similarités entre les communautés de ces trois lacs abonde dans ce sens également. En parallèle de ces analyses statistiques et en partenariat avec le Docteur Oliver Miler, nous avons procédé au calcul des différentes métriques permettant le calcul des notes et l'obtention des classes écologiques des deux méthodes AESHNA et LIMCO. Ces résultats ont mis en évidences des peuplements perturbés. En effet, le faible nombre de taxas sensibles (éphéméroptères, trichoptères et odonates), leur faible abondance et une forte proportion de diptères et oligochètes révèlent une dégradation des peuplements, ou tout du moins une différence avec les communautés qui seraient présentes dans un bon état écologique.

Cette différence peut trouver différentes explications : les activités anthropiques ont effectivement causé des perturbations en termes d'habitat, d'hydromorphologie ou de pollutions qui ont affaibli les espèces les plus sensibles. Mais également, nous pouvons supposer qu'étant donné la relativement courte vie de ces écosystèmes (moins de 40 ans) et leur caractère déconnecté, la biodiversité n'a pas encore atteint sa pleine maturité. Nous pouvons également nous interroger sur la complète fiabilité de ces résultats puisque les outils utilisés (AESHNA et LIMCO) n'ont pas été créés ni pour cette région géographique ni pour ce type de lacs. Le faible nombre de lacs analysés jusqu'à présent ainsi que l'inexistence d'outils complètement adaptés à notre terrain d'étude nous empêche de pouvoir donner une explication irréfutable sur la situation actuelle de nos lacs.

L'analyse des 17 lacs restant devrait nous permettre de répondre à ces questions et permettra également d'améliorer les outils tels qu'AESHNA et LIMCO pour le suivi et l'analyse des lacs artificiels. De plus, la littérature scientifique concernant l'établissement des communautés de macroinvertébrés dans les lacs artificiels, leurs réactions à des modifications ainsi que les facteurs régissant leur composition est actuellement assez pauvre et nous espérons que cette étude pourra lever des zones

d'ombres sur ces différentes dynamiques. Il faut également garder à l'esprit que cette étude ne traite que de la première phase (état actuel des communautés avant modifications) d'une étude planifiée sur six ans et que la comparaison des états initiaux et finaux sera potentiellement la plus riche en informations et en compréhension du fonctionnement de ces écosystèmes.

Summary

Summary 1
Introduction 2
1. Material and methods 4
 1.1 Area of study 4
 1.2 Animal collection 6
 1.3 Data Analysis 8
  1.3.1 Lake Selection 8
  1.3.2 Macroinvertebrate analysis 10
2. Results 12
3. Discussion 16
Conclusion 19
References 20
Figures and tables 23
Annexs 24

Introduction

Freshwater ecosystems represent roughly 1% of the earth's surface and hold 10% of the earth's total biodiversity (Strayer & Dudgeon, 2010) making them one of the richest ecosystems. However, freshwater ecosystems are increasingly threatened by anthropogenic activities. There is an emergency to obtain a better understanding of those ecosystems and to develop protection measures. The EU Water Framework Directive (EU WFD) legally requires EU member states to perform an assessment of the ecological structure functioning and of aquatic ecosystems based on biological quality elements (BQEs), i.e. fish, phytoplankton, macrophytes, phytobenthos and benthic macroinvertebrates (Miler et al, 2013). The main objective of the EU WFD is to reach a "good ecological shape" for all of the waters bodies (Directive 2000/60/EC) which considering physico-chemical, morphological and biological parameters. Every country is required to setup programs based on management and monitoring to maintain such a "good ecological shape" of their water bodies. In Germany, water bodies smaller than 50 ha such as small natural lakes, ponds for agricultural or recreational purposes including gravel pit lakes are not considered by those EU WFD programs.

Gravel pit lakes have been created after World War Two during an intense period of mining exploitation to rebuild Germany. These lakes are artificially created from the flooding of abandoned open-cut sand and lignite mines (Blanchette & Lund 2016). Central Germany and western Germany host thousands of gravel pit lakes (Geller et al, 2012), and their number might increase in the near future due to the cessation of mining. Most the gravel pit lakes are used for human activities such as recreational fishing and other water-linked activities such as bathing, boating and bird watching. (Strayer & Dudgeon 2010). In such a recently created ecosystems (less than 40 years), anthropic overexploitation may have important consequences for the establishment of animal and plant communities (Evans & Warrington, 1996). Gravel pit lakes represent an important hot spot of biodiversity for many amphibians, dragonflies and birds (Frochot & Grodreau 1995). Despite this, studies analyzing gravel pit lakes animal and plant communities are still lacking. Moreover, since gravel pit lakes are isolated and not linked with any other water flow they are disconnected from most natural corridors and must have developed their own biodiversity. Therefore we can expect a large diversity of animal and plant communities among the different lakes.

The Baggersee Project held by the Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB) and several Lower Saxon fishing clubs aims for a better understanding of the biodiversity of gravel pit lakes. The main objective of The Baggersee Project is to monitor the biodiversity and investigate its response to different management interventions. In partnership with the angling clubs of Lower Saxony,

twenty gravel pit lakes have been selected as experimental lakes to investigate how management interventions implemented with the aim of improving fish stocks can affect the whole biodiversity. The twenty lakes will be divided into five different experimental treatments with four lakes per treatment. The treatments will consist of 1) untouched control lakes with no fishing allowed, 2) untouched control lakes with fishing allowed, 3) stocked lakes, 4) habitat enhancement lakes and 5) habitat enhancement lakes with shallow zones created. The habitat diversity enhancement will consist of introducing dead. The Baggersee Project will then assess the richness of the biodiversity of the study lakes, focusing on birds, dragonflies, fishes, aquatic macrophytes and algae and macroinvertebrates and their responses to the treatments

In this study we focuse on benthic macroinvertebrates, as they have a key role in freshwater ecosystems. Macroinvertebrates are an important link between primary producers, detrital deposits and higher trophic levels in lake ecosystems in freshwater food webs (Covich & all, 1999). As key communities, macroinvertebrates are widely used in the assessment of water quality in many European assessment programs (see EU WFD). Furthermore, many studies reference macroinvertebrate community assessments in both rivers and natural lakes but assessments of macroinvertebrate communities in artificial lakes are scarce (Calow et al, 1994).

The Baggersee Project is supported by the IGB in Berlin and the Anglerverband Niedersachsen which is a Regional Sport Fishing Association, federating several fishing clubs in Lower Saxony. The IGB is an independent research institute, partly funded by the German government and state of Berlin in addition to some private companies. With approximately twenty millions euros budget and over 250 employees the institute leads research in freshwater ecology with one major aim: to improve the fundamental understanding of freshwater systems. The leitmotiv of the institute is: “research for the future of our freshwaters”. In order to do so, research programs are divided in six different departments such as “Ecohydrology”, “Ecophysiology and Aquaculture” or “Chemical Analytics and Biogeochemistry”. The Baggersee Project, is a project under the “Ecology and Biology of Fishes” department and the project is led and supervised by Prof. Dr. Robert Arlinghaus.

This report is focused on the pre-management intervention assessment of the macroinvertebrates. I will present the assessment methods, fieldwork and lab-work protocols and an analysis of the macroinvertebrate communities from the three lakes which the sorting and identification of macroinvertebrate samples have been completed thus far. The goal of this first work phase is to obtain a clear view of the states of the lakes in term of macroinvertebrate communities, including the diversity and abundance on a micro-habitat level. The objective is to see how macroinvertebrate communities in gravel pit lakes (which may be quite different: depth, size, age, trophic level, number of fish species,

chemistry and physicals, uses etc.) are more affected by anthropic stressors or microhabitat whether the lake characteristics.

1. Material and methods

1.1 Area of study

All lakes are located in Lower-Saxony (Niedersachsen) (figure 1) and are the results of former mining exploitation.

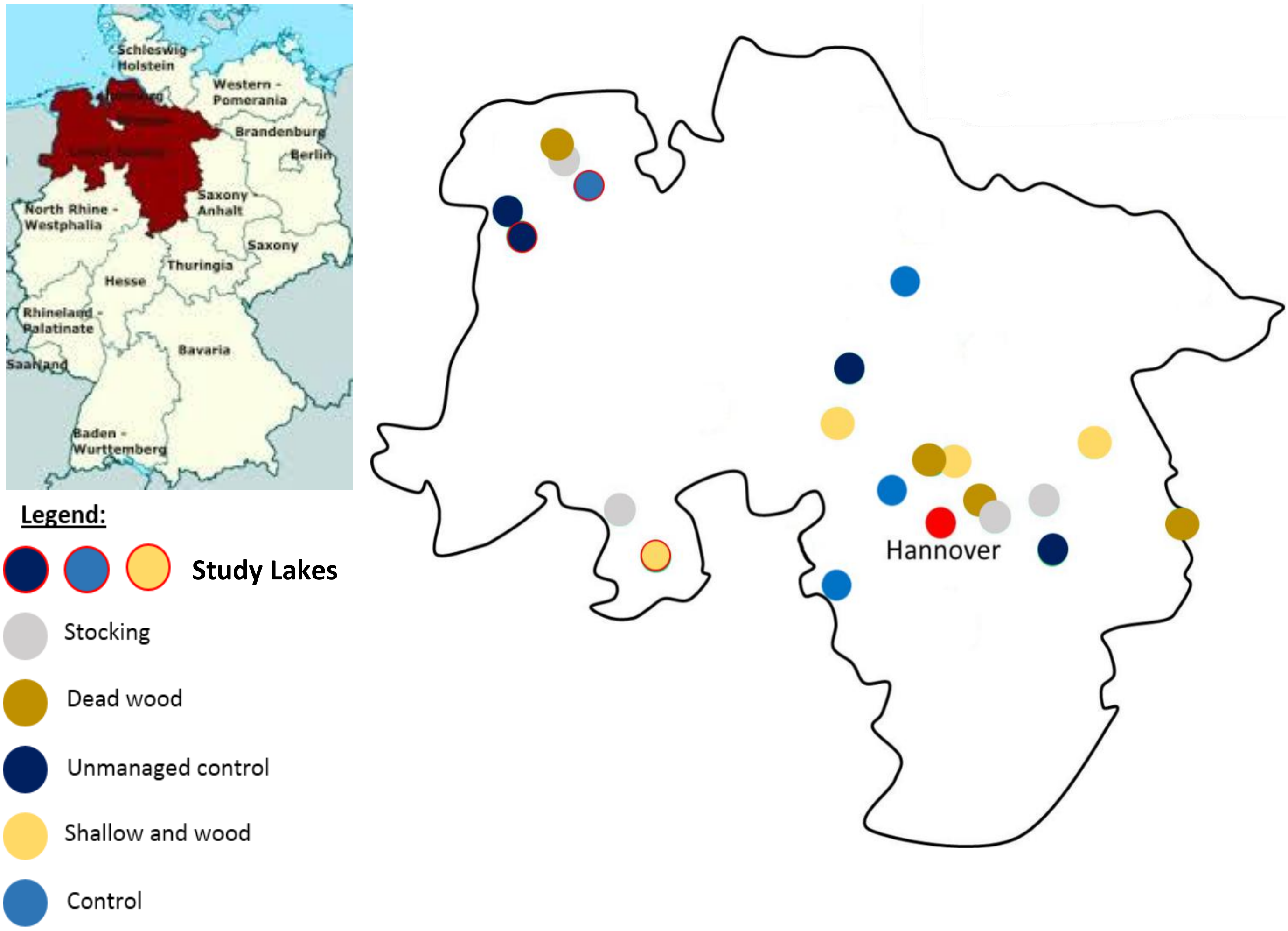

Figure 1: Localization and lake typology of the Baggersee Project

The mining of the study lakes stopped between 25 and 35 years ago (i.e., lakes are between 25 and 35 years old). All study lakes are classified as mesotrophic or eutrophic lakes (except for two lakes: one oligotrophic and one polytrophic) (figure 2)

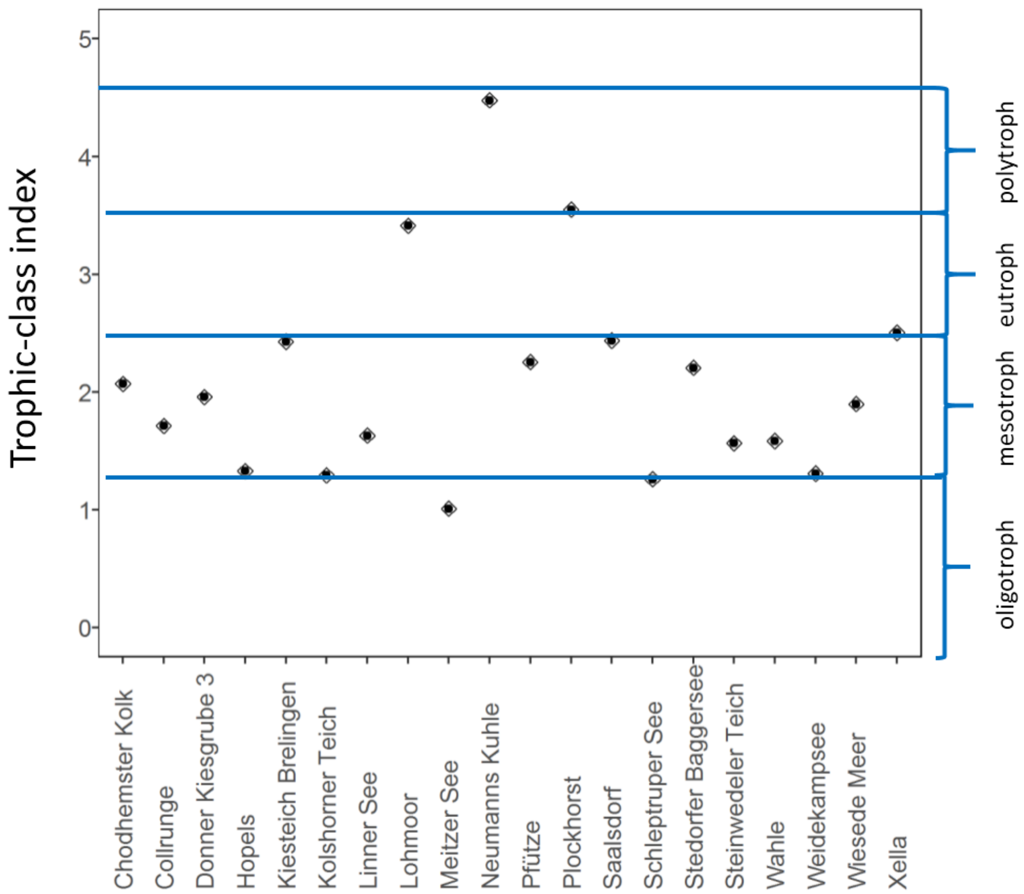

Figure 2: Trophic level of Baggersee Project's lakes (Source: Baggersee Project workshop)

The lakes intrinsic characteristics are highly variable in term of surface area, shoreline, depth, number of fish species etc. (table 1). The surrounding of the lakes are also diverse (farmland, pastures, close to villages, natural reserve etc.) leading to a high variability of the vegetation around the lakes.

	Minimum value	**Maximum value**
Area (ha)	1	23
Depht (m)	4	20
Shoreline (m)	415	2660
Fish species	3	12
Biovolume plant	15,1	52,9
Biovolume All water	2,2	49,2
TP spring [µg/L]	8	160
secchi depth (m)	0,45	5,5
Chlorophyll a [µg/L]	1,6	112,39

Table 1: Range of the lake characteristics

1.2 Animal collection

The sampling campaign took place between the 25th of April and the 1st of June across the Lower-Saxony (lands). Every lake was monitored following the official macroinvertebrate Lake Assessment (AESHNA) used in Germany (Schreiber & Brauns 2010, Miler et al 2013). For our study some adjustments were needed due to the specificities of our lakes. The main difficulty was to find accessible sampling sites. Indeed, gravel pit lakes have steep slopes and the littoral zone (less than 1.2m) was sometime narrow (less than one meter wide). In the beginning of the sampling period, access to the shore was required to sample properly and bottle every sample, but later we had access to a boat, which allowed us to find and access sampling sites more easily. (Figure 3)

Figure 3: Sample with shore access (left panel) / Sample from the boat (right panel

The number of sampling sites per lake was variable. The lakes without any habitat enhancement treatment each held four sampling sites. The habitat enhancement only held six sampling sites: three replicates as control and three in the exact same location where the dead wood will be added.

For the four lakes receiving both wood and shallow zones nine sampling sites were used: three control sites, wood implement sites and shallow area sites. At every site, GPS coordinates were recorded with a three meter precision device (GPSMAP62, Garmin, Taiwan) ensure accuracy during the addition of the wood. (Figure 4)

Figure 4: Flooded wood addition

On every site, the first step was to identify and quantify the micro-habitats available. The different micro-habitats found were classified as: sand, submerged macrophytes, emerged macrophytes, reeds, coarse particle of organic matter (CPOM) dead wood and roots according to AESHNA sampling protocol (see Miler et al. 2013). Next for every micro-habitat representing at least 10% of the site, 0.75 m² were sampled with a 500 µm mesh hand net (25 cm wide). To cover the 0.75m² area, we performed three sweeps of one meter transects on the substrate with the 25 cm wide net (1cm deep in the substrate). Every sample (one per micro-habitat, per site) was bottled with 90% ethanol and stored identification. For the analyses microhabitats were classified as biotic (CPOM, emerge macrophyte, submerge macrophyte etc.) and Mineral (Sand, cobbles,) (see Hering et al. 2003). We also collected additional microhabitat-specific environmental data such as: pH, water temperature, oxygen concentration and saturation and conductivity. Furthermore, information about riparian structure (type of trees, size and land) was collected as it is a requirement of the LIMCO/AESHNA programs. (Annex I) Such additional data facilitated the calculation of an anthropic pressure score. Macroinvertebrates from the lakes were then identified in the laboratory using a light microscope. Every taxa from every microhabitat of every sampling site was counted and individually stored (one taxa per vials) into vials with 70% ethanol. We dichotomous used keys and field guides (Tachet et al etc.[1]) to reach the species, genus or family level according to the Aeshna protocol (Brauns et al, 2011) (Annex II)

[1] Please see the keys and guides part on the references

1.3 Data Analysis

1.3.1 Lake Selection

We first classified the lakes according to environmental similarity (table 2). Environmental parameters considered for the selection included: proportion (%) of sample microhabitats at each lake, age, size, Phosphorous concentration, chlorophyll-a, depth (average and maximum) and secchi depth. The data related to every lake came from the monitoring of the lake from previous field work. The environmental variables were used to discriminate lakes among each other by their physical, chemical and trophic level characteristics.

Lake Name	Acronym	Lake Type
Chodhemster Kolk	CHK	Control
Collrunge	COL	dead wood
Donner Kiesgrube 3	DK3	shallow water and dead wood
Hopels	HOP	unmanaged control
Kiesteich Brelingen	KTB	dead wood
Kolshorner Teich	KHT	dead wood
Linner See	LNS	shallow water and dead wood
Lohmoor	LG	unmanaged control
Meitzer See	MTS	shallow water and dead wood
Neumanns Kuhle	MMK	Control
Pfütze	PFÜ	unmanaged control
Plockhorst	PLO	Fish stocking
Saalsdorf	SAA	dead wood
Schleptruper See	SCHS	Fish stocking
Stedorfer Baggersee	SB	Control
Steinwedeler Teich	SWT	Fish stocking
Wahle	WAH	Control
Weidekampsee	WKS	shallow water and dead wood
Wiesede Meer	WM	Fish stocking
Xella	XEL	unmanaged control

Table 2: List of lakes and lake types

To classify the lakes we performed a Principle Component Analysis (PCA) which ordinates the multivariate environmental parameters in just two dimensions and can be visualized in a biplot (figure 5). Environmental parameters were standardized (z-transformed) prior to the PCA (Wold et al, 1987). The principle of a PCA is to transform correlated variables (here the different variables of every lakes) into uncorrelated variables. This allowed for the creation of a multi-dimensional space (one axis for every variable) within which all of the lakes in this new space can be positioned, according to multi variable components. Using the PCA results we were able to see which lakes were the most similar between one another. From the group of lakes found to have similar environmental conditions (red circle in the PCA) we selected three lakes (Chodhemster Kolk, Hopels and Linner See) in which we conducted our analyses of macroinvertebrate communities within the time constraints of my internship.

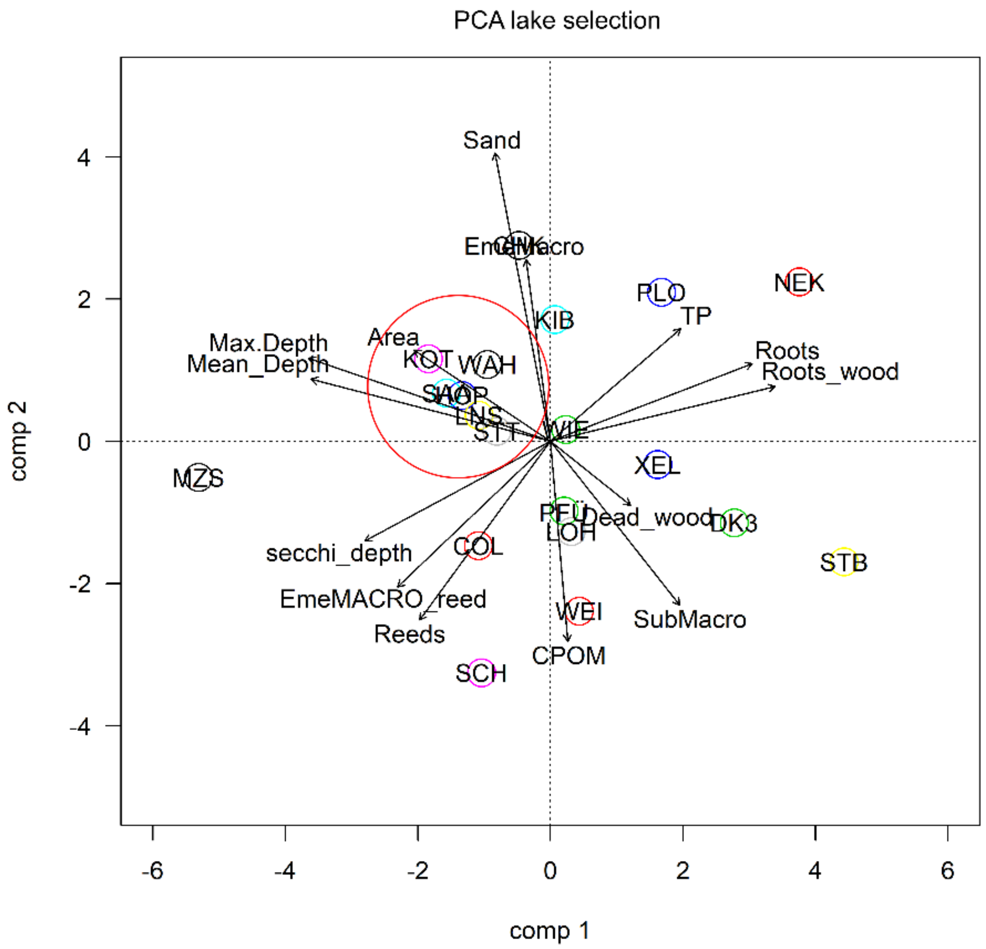

Figure 5: PCA results (Source: Manfrin A and Mutel S)

1.3.2 Macroinvertebrate analysis

1.3.2.1 AESHNA and LIMCO assessment

For all analyses of the macroinvertebrates abundance was standardized to one square meter surface area and to the proportion of microhabitat (see Miler et al. 2013).

We assessed AESHNA and LIMCO (Littoral Invertebrate Multimetric Index based on Composite Sampling) indices for the three lakes. The AESHNA index is calculated based on macroinvertebrate metrics such as abundance, taxa richness, sensitivity of the species. The AESHNA index was developed using macroinvertebrates data collected from German environmental state agencies (Miler et al 2013). By contrast, the LIMCO index is currently under development as a potential standardized method for Central European macroinvertebrate lake assessments (Miller et al, 2012). This LIMCO could be used for the EU WFD as an official lake macroinvertebrate assessment method. The database to create the LIMCO's indices and metrics is supported by a larger scale study, with data from different European countries, creating different eco-regions and different standards used to implement the program in charge of metrics' calculations. Ultimately, both methods are similar, and are composed by metrics and indices that describe macroinvertebrate communities (e.g., sensitivity, diversity, balance). The AESHNA and LIMCO methods are based on habitat-specific sampling designed for sampling all available habitats at up to 1.2 m depth of water by hand net 500 µm (mesh-size of net) (Poikane, 2009).

Habitat-specific sampling is an effective way to reduce the inherent spatial variability of littoral macroinvertebrate communities. A sampling area of 0.63 m² per habitat is sufficient to represent a habitat's dominant and subdominant elements (Schreiber & Brauns 2010). After sampling, the individuals macroinvertebrates caught were sorted and identified to species level (diptera were identified to just family level). If the species could not be identified because of degradation of their bodies, the highest level of identification was recorded (genus or family). The abundance of every taxa was then used to calculate indices that quantify the perturbations of the macroinvertebrates communities. The multimetric indices are standardized from 0 (bad status) to 1 (= reference value). This range is divided evenly into the five quality classes (very good 1-0.8; good 0.8-0.6; moderate 0.6-0.4; poor 0.4-0.2; bad 0.2-0) along a pressure gradient from undisturbed to disturbed (Poikane, 2009) linked with the ecological status class from 1 to 5 where 1 refers to the very good status and 5 the bad status. I used both LIMCO and AESHNA indices and metrics as they are quite similar. However, coefficients and parameters may vary among the methods and lead to slightly different results. For example, different AESHNA indices exist for three natural and four artificial lake types in Germany and therefore AESHNA was calculated several times assuming different lake types to understand which lake type the gravel pits in Niedersachsen are best represented by. Also, methods for sampling macroinvertebrate

communities in rivers exist such as PERLODES (Kail et al, 2012), however, they are likely not the most appropriate for our lake systems and are constantly being updated. An additional motivation to use the LIMCO assessment is that our data will support the development of the LIMCO index (with Oliver Miller's assistance) as our data will reveal if LIMCO index can also be adapted for a larger range of lake types (e.g. natural, artificial etc.) (O.Miller. personal communication). Ultimately, both the AESHNA and LIMCO methods aim to describe the macroinvertebrate communities by their richness and sensitivity and how disturbed they may be from anthropogenic stressors. See Table 3 for detailed list of AESHNA' metrics and table 4 for LIMCO metrics and please see Miller et al., 2012 for full details and information.

AESHNA'S METRICS	DETAILS
FI_NORM01	saprobic index based on hydromorphological disturbance
GATHER_AC_NORM01	AC of gather and collector
LIT_AC_NORM01	AC of lithal related taxa
ASPT_NORM01	indix related to macroinvertebrate pollutant tolerance
NO_EPTCBO_NORM01	average number of species per sampling
ODO_AC_NORM01	AC of odonata
ETO_AC_NORM01	AC of Epheroptera, Trichoptera and Odonata
CHIRINAE_NORM01	AC of chirinae
MMI	Multi Metric Index: give the AESHNA notation [0;1]
ECOLOGICAL_STATUT_CLASS	[1;5]

Table 3: AESHNA's metrics (Source: Miler O and Mutel S)

LIMCO'S METRICS	DETAILS
PERCGATHERERSCOLLECTORS_AC	refers to the feeding groups
CHIROPERC_AC	chironomidae percentage_abundance_class
EPTCBO	average number of species per sampling
DIVERSITYMARGALEFINDEX	$d = (S - 1) / \ln N$
LIMCO	Limco notation: average of the previous metrics
ECOLOGICAL_STATUS_CLASS	[1;5]

Table 4: LIMCO's metrics (Source: Miler O and Mutel S)

1.3.2.2 Macroinvertebrate communities analysis

Community metrics (Abundance, Shannon diversity index, taxa richness)

Abundance, taxa richness (number of taxa) and Shannon diversity index[2] were examined using linear regression models. The factors "Microhabitat" (Biotic, Minerals) and "Lakes" were considered in each model. The selection of the model factors was done using AIC (Akaike, 1987). The distribution of residuals was assessed using Wilk-Shapiro tests (Shapiro and Wilk 1965) and quantile-plots (Wilk and Gnanadesikan 1968). The aim was to investigate relationships between abundance, Shannon diversity and taxa richness with factors such as micro-habitat, lake identification and anthropic pressure (sum of the different anthropogenic impairment – see page 4 for the field protocol).

Community composition

Compositional differences among samples were computed as Bray-Curtis dissimilarities (Beals 1984). Differences in taxonomic composition between micro-habitats and lakes were visualized using non-metric multidimensional scaling (nMDS). A Permutational Multivariate Analysis of Variance (perMANOVA) was used to test for compositional dissimilarity among "Microhabitats" and "Lakes" using the function Adonis in the package Vegan (Oksanen et al, 2007) for R. The aim was to investigate the driver of the taxa composition of a sample: lake, micro-habitat or anthropogenic pressure. More specifically regarding, the dissimilarities between two random samples we could ask whether the differences are mostly due to micro-habitat, lake specificities or anthropogenic pressure?

2. Results

In total we analyzed 31 samples from the three lakes (Annex III). We found 16857 individuals belonging to 45 taxonomic families totaling of 102 taxa identified (see taxa list annex II).

Community metrics' results

The linear regression models show that the factor explaining the differences in macroinvertebrate abundance and taxa richness was micro-habitat (table 5). However, we observed a dissimilarity between

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[2] $$H = \sum_{i=1}^{s} - (P_i * \ln P_i)$$

the mineral and biotic micro-habitats. The first mineral habitat (sand) held the higher abundance (figure 6), but the number of taxa was higher in the "biotic" group than in the mineral (86 and 54).

Variable	Model factors	F-stat	p
Abundance	Microhabitats	$F_{1,26} = 7.287$	**0.012**
	Anthropogenic pressures	$F_{2,26} = 1.845$	0.178
	Lake	$F_{1,26} = 3.824$	0.061
Shannon (H)	Microhabitats	$F_{1,26} = 1.1983$	0.283
	Anthropogenic pressures	$F_{2, 26} = 2.7449$	0.082
	Lake	$F_{1,26} = 0.0803$	0.176
Richness	Microhabitats	$F_{1,26} = 14.7124$	**0.000716**
	Anthropogenic pressures	$F_{2,26} = 0.5695$	0.572
	Lake	$F_{1,26} = 0.2517$	0.620

Table 5: Results of linear regression between Abundance, Shannon and Richness according to chosen parameters [Micro-habitat; Lake; Anthropogenic index] (Source : Manfrin A and Mutel S)

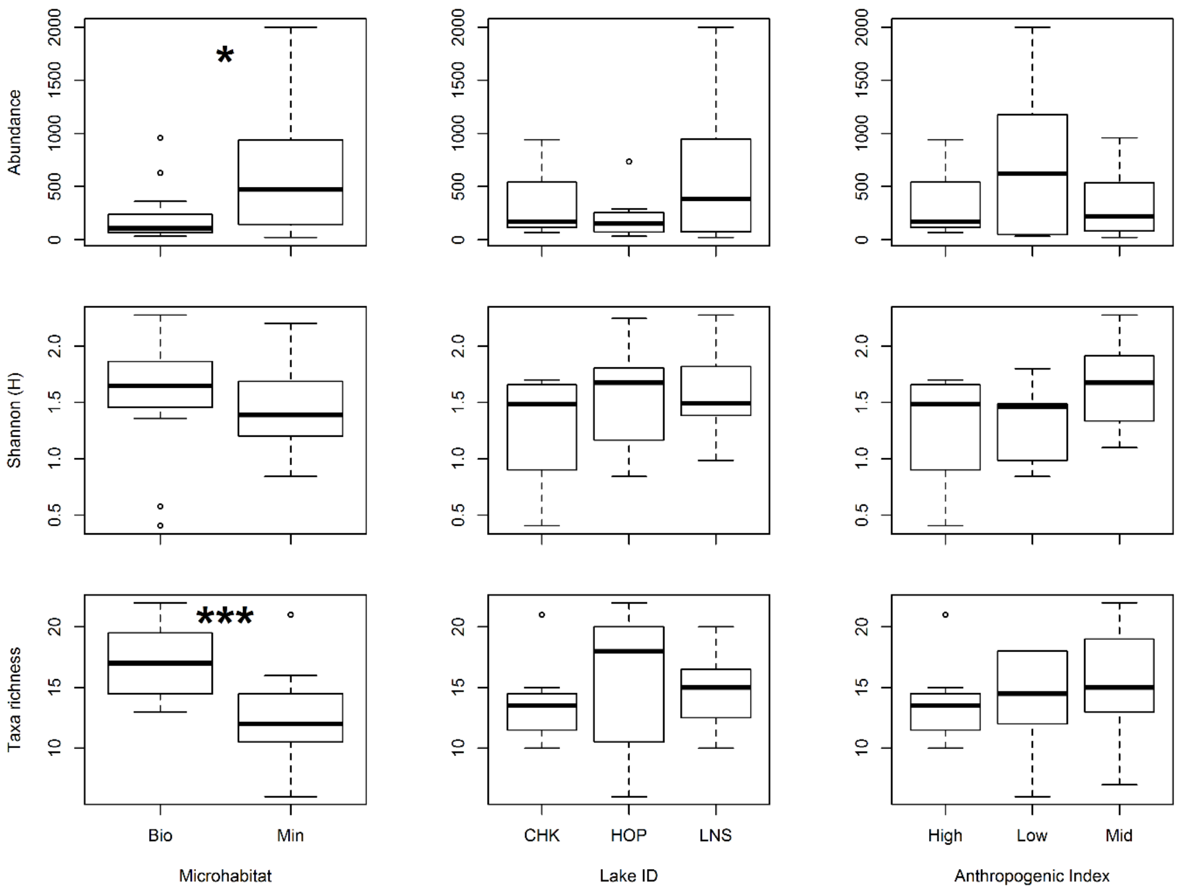

*Figure 6: Box-plot of Abundance, Shannon and Taxa richness according to chosen parameters [Micro-habitat; Lake; Anthropogenic index] - *refers to a p-value <0.05 - *** refers to a p-value< 0.001 (Source: Manfrin A and Mutel S)*

Community composition's results

The perMANOVA showed both micro-habitat (perMANOVA: $F_{1, 26} = 3.84$, $p<0.001$) and lake scale (perMANOVA: $F_{1, 26} = 3.96$, $p<0.001$) can explain the dissimilarities between two random samples. (Table 6)

	Degree of freedom	F Model	R^2	Pr(>F)
Lake	2	3,964	0,203	**0,001**
Micro-habitat	1	3,842	0,098	**0,001**
Anthropic Index	1	1,328	0,034	0,179
Residuals	26			
Total	30			

Table 6: perMANOVA's results showing the dissimilarities between two samples (Source: Manfrin A and Mutel S)

The figure 7 represents the three lakes based on the Bray-Curtis distances linked with presence and the abundances of every taxa sampled or not in the lakes. The non-metric MultiDimensional Scaling (nMDS) allowed us to represent macroinvertebrate communities in a two-dimensional space. The table 7 shows the similarities between the taxa list of every lake, based only on the presence. We see that LNS and CHK share more taxa than Hopels and CHK. The percentage of common taxa between every lakes seems high (>77) in the context that there is no connections among those lakes.

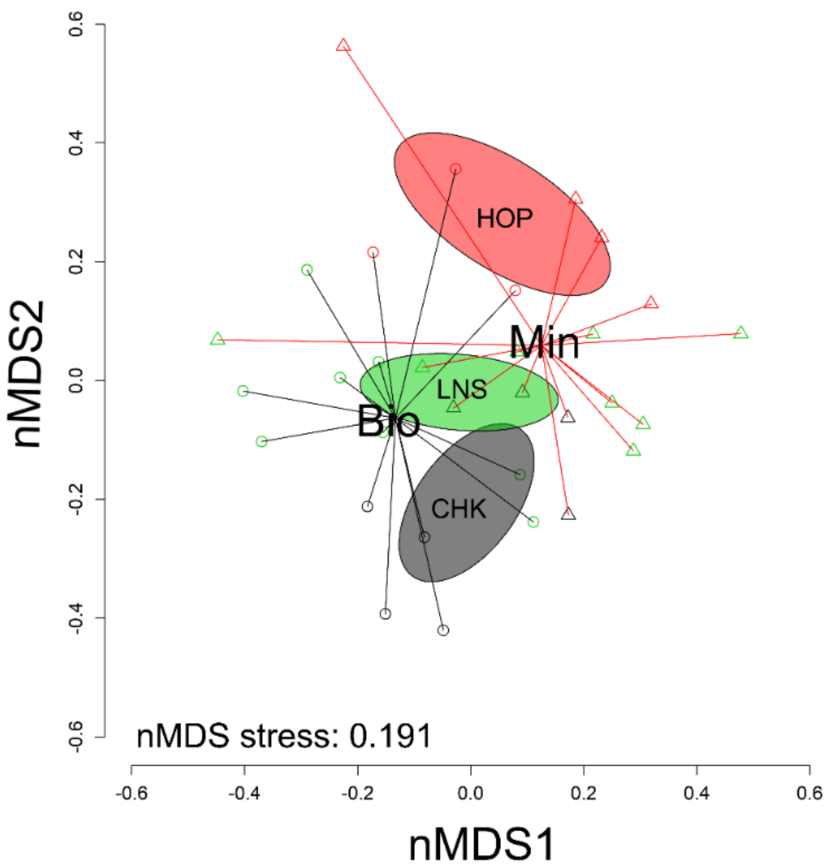

Figure 7: Lake's macroinvertebrate communities using Bray-Curtis distance with nMDS analysis (Source : Manfrin A and Mutel S)

	CHK	Hopels	LNS
CHK			
Hopels	77,4		
LNS	79,8	79,2	

Table 7: Percentage of similiraties at the lake level

<u>*AESHNA & LIMCO's results*</u>

The results for AESHNA and LIMCO were calculated at the site level and at the micro-habitat level. For the lake result, we calculated metrics for every sites (multi-habitat notation) and we calculated the mean value for every metrics based on the value of the site's metrics. The micro-habitat results (Mineral and Biotic) came from the assessment of every sampling, which was then regrouped by micro-habitat type. The results presented here are also the mean values of all the samples belonging to a same habitat type.

Even if the two indices are based on the same principle, we note important differences between them. AESHNA led to an ecological status from good to moderate (table 8) while LIMCO (table 9) suggested that the ecological status is generally bad (one poor value) for both lakes and micro-habitats. We can confirm that our lakes display some anthropogenic disturbances or at least that the population established (richness and abundance of taxa) did not fit with the supposed population of reference lakes.

Lake/Habitat	CHK	Hopels	LNS	Mineral	Biotic
FI_norm01	0,77	0,74	0,84	0,78	0,65
gather_AC_norm01	0,21	0,46	0,68	0,53	0,55
LIT_AC_norm01	0,89	0,89	0,88	0,88	0,79
ASPT_norm01	0,47	0,47	0,37	0,40	0,49
no_EPTCBO_norm01	0,63	0,66	0,35	0,38	0,59
Odo_AC_norm01	0,37	0,18	0,00	0,12	0,19
ETO_AC_norm01	0,62	0,53	0,15	0,32	0,42
Chirinae_norm01	1,00	1,00	1,00	1,00	1,00
MMI	0,64	0,63	0,57	0,58	0,59
Ecological_status_class	2	2	3	3	3

Table 8: AESHNA's results (Source: Miler O and Mutel S)

Lake/Habitat	CHK	Hopels	LNS	Mineral	Biotic
PercGatherersCollectors_AC	57,31	45,36	36,32	45,46	55,52
ChiroPerc_AC	18,76	18,49	18,70	20,49	10,12
EPTCBO	6,00	7,00	4,78	4,56	7,27
DiversityMargalefIndex	1,59	2,02	1,60	1,49	2,25
LIMCO	0,03	0,15	0,18	0,13	0,23
Ecological_status_class	5	5	5	5	4

Table 9: LIMCO's results (Source: Miler O and Mutel S)

3. Discussion

We observed that taxa richness is indeed highest in the more complex micro-habitat (biotic) but the overall abundance is highest in the mineral substrate. The sand was dominated by two orders of low ecological value (Skrobialowski et al, 2004) (diptera and oligochaeta) whereas biotic micro-habitat held a large proportion of the same two orders but also offered a larger proportion of other orders with a greater ecological value (ephemeroptera, trichoptera and odonata: (ETO)). While Kurt et al (1998) showed that more complex, three-dimensional artificial substrates, with a greater substrate heterogeneity, surface complexity, and interstitial space, will support a more diverse and abundant macroinvertebrate community in lakes. Support for this effect has also been found for natural substrates within rivers (Williams, 1980; Erman and Erman, 1984). The biotic micro-habitat presented a larger structural habitat diversity (e.g. food sources, substrate type, structure, cover) and therefore could likely hold a wider diversity of macroinvertebrates but on our lakes the abundance statement is not respected. What impede our biotic substrates to hold the highest taxa richness and abundance?

AESHNA/LIMCO indices showed that our samples were anthropically disturbed, ranging from highly disturbed (according to LIMCO) to moderate/low disturbance according to AESHNA (implemented with"artificial lakes" settings) and our observations about macroinvertebrate composition (high abundance of diptera and oligochaeta and a relatively low number of sensitive ETO taxa). There was a significant trend correlating anthropogenic pressure and community metrics (table 5), anthropic pressure may still impact the communities with respect of their richness and abundance. Indeed, it had been shown for rivers that anthropicc pressure can explain up to 30% the macroinvertebrate community composition (Marzin et al, 2012 and 2013) and we can expect similar effects on macroinvertebrate communities in lakes. When anthropogenic disturbances appear, they will first affect the most sensitive taxa. We found that biotic micro-habitat still sheltered a larger number of ETO taxa (24% more) but their abundance is lower than expected by of AESHNA/LIMCO assessment. The cause of such state might be explained by different factors:: i) macroinvertebrate communities are disturbed by anthropogenic

stressors (organic pollution, hydromorphological degradations etc); ii) the communities of gravel pit lakes are relatively of the formation (colonization) and colonization and successional processes might be still acting (richness and abundance). However, with the limited time and dataset we cannot formulate any strong conclusion about such hypothesis

We used both AESHNA and LIMCO method because they are the only available but none of them suit perfectly our field study and results. AESHNA has been developed for several type of lakes but none of them strictly fit with our lakes, the metrics where run with artificial lakes of central Germany. Moreover, the identification to perfectly run AESHNA needs to reach the species level (most of the time) and also the sub-family level for chironomids. We weren't able to reach this level due to the limited time which led to the under-estimation of our "chirinae" metrics. In fact our AESHNA MMI should be lower than what we get, and closer to the LIMCO one leading to worst ecological status class. Also, LIMCO is currently set for natural lakes of central Europe, and running artificial gravel pit lakes data might not offer completely accurate results. The data base from the twenty lakes at the end of the study will be a good asset to improve the accuracy of the method for artificial lakes, furnishing important basis for a further development and application of these indexes for the assessment of smaller, but still biologically important water bodies

Biotic habitats held a greater number of taxa but looking at the result of AESHNA/LIMCO indices for micro-habitat the ecological situation of those taxa is considered as "bad status". Biotic and mineral habitats are composed by 46% and 68% of diptera and oligochaeta, respectively. Despite the lack of effect of anthropogenic pressure measured at the site level on the abundance, shannon index and taxa richness, I cannot rule out an overall effect of anthropogenic disturbance on the macroinvertebrate communities. The poor ratings of the macroinvertebrate community status according to AESHNA and LIMCO suggest that a history of anthropogenic pressure may have already reduced or limited the taxa richness in our lakes. Therefore we may not have had a wide enough variation in macroinvertebrate diversity to detect effects of anthropogenic disturbance among our sampling sites. Alternatively, it may be that the young status of our lakes has not allowed enough time for a substantial biodiversity of macroinvertebrate taxa to establish.

We focused our analysis and interpreation on just three lakes along a gradient of anthropogenic disturbance. Although, this is not enough to reach ecological conclusion that we can generalize at larger scales, this study provides interesting insights on the importance of microhabitat in the distribution and composition of macroinvertebrate communities in gravel pit lakes. This first study is an important contribution to the scientific knowledge regarding gravel pit ecosystems for which many ecological

processes are still largely unknown (Minshall, 1984). Despite similar formation processes (flooded pit mines) that lead to a pit lake, how they evolve in term of animal biodiversity (e.g. colonization dynamics) is still largely obscure. These type of lakes evolved for years in an isolated state (no water inflow or outflow) and therefore may have been followed different ecological trajectories (Hastings et al, 1993) leading to important singularity in species composition. Our future work investigating the invertebrate communities of the remaining 17 lakes will shed light on these aspects.

Conclusion

My internship was conducted within the team of the Baggersee Project, which is a long time study (6 years) with the objective to monitor and enhance the gravel pit lakes' biodiversity. Gravel pit lakes are small water bodies which are currently not protected by the EU WFD and are currently understudied. I was part of the first step of the project, running the initial assessment of the macroinvertebrate communities on 20 study lakes. On this report we focused our work on three lakes and we aimed to determine the drivers of macroinvertebrate communities. We showed that macroinvertebrate abundance and taxa richness was correlated with micro-habitats type, where biotic habitats held the highest taxa richness and mineral habitats held the largest number of macroinvertebrate individuals. We also showed that the dissimilarities between sampling sites are explained by both the micro-habitat and the lake. This means that two random sampling sites are more likely to be the same if they share the same micro-habitat or if they are located in the same lake. The AESHNA and LIMCO indices allowed us to formulate hypothesis about the previous findings. The global "bad ecological status" at both lake and micro-habitat level reveal disturbances which are impacting the communities. Such disturbances might come from anthropogenic stressors or may be explained by the biodiversity development process of those lakes which are still young (mature and stable populations not established yet). Unfortunately, the small number of lakes analyzed prevents us from explaining the reasons for the community disturbance with certainty. In the future, the global study of the macroinvertebrate communities of the 20 gravel pit lakes will provide information about the response from communities to specific management, macroinvertebrate communities' establishment in gravel pit lakes and how parameters such as: micro-habitat, lake specificities or anthropogenic pressure drive macroinvertebrate communites. This will create a key dataset to improve monitoring method in this kind of artificial lakes.

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Others

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Figures and tables

Figure 1: Localization and lake typology of the Baggersee Project 4
Figure 2: Trophic level of Baggersee Project's lakes 5
Figure 3: Sample with shore access (left panel) / Sample from the boat (right panel........ 6
Figure 4: Flooded wood addition 7
Figure 5: PCA results........ 9
Figure 6: Box-plot of Abundance, Shannon and Taxa richness according to chosen parameters [Micro-habitat; Lake; Anthropogenic index] - *refers to a p-value <0.05 - *** refers to a p-value< 0.001 13
Figure 7: Lake's macroinvertebrate communities using Bray-Curtis distance with nMDS analysis...... 14

Table 1: Range of the lake characteristics........ 5
Table 2: List of lakes and lake types 8
Table 3: AESHNA's metrics 11
Table 4: LIMCO's metrics........ 11
Table 5: Results of linear regression between Abundance, Shannon and Richness according to chosen parameters [Micro-habitat; Lake; Anthropogenic index] 13
Table 6: perMANOVA's results showind the dissimilarities between two samples........ 14
Table 7: Percentage of similiraties at the lake level........ 15
Table 8: AESHNA's results........ 15
Table 9: LIMCO's results 16

Annexs

Annex I: Field Protocol

FIELD PROTOCOL FOR AESHNA/LIMCO

General Informations :

Lake Name ....

Lake Surface (ha) ....

Shoreline Length (km) ....

Maximum depth (m) ....

Lake type (abiotic LAWA typology) ....

Shoreline Type

...............% Anthropized

...............% Cover stone bed

...............% Wood construction (e.g., palisades)

...............% Blocking (concrete / steel)

...............% Paving

...............% Steganlage

...............% Open

...............% Natural

NOTES

EULITORAL FIELD PROTOCOL

See	Site	People	Date

GPS Coordinates:

Collected Data

Habitat type	water depth (cm)	Nb replicate	surface sampled (m²)
Emerged macrophyte	....	....	....
Submerged macrophyte	....	....	....
Sand	....	....	....
FPOM	....	....	....
Concrete	....	....	....
Riprap	....	....	....
Docks	....	....	....
Stone	....	....	....
Submerge roots	....	....	....
Dead wood	....	....	....

Micro-habitats (sum=100%)

Submerged Macrophyte
Proportion (%) :........................
Species :.....................................
Coverage :
low medium important

Emerged Macrophyte
Proportion (%) :........................
Species :.....................................
Coverage :
low medium important

Stones
Proportion (%):........................
Grain diameter > 20mm
Algae coverage:
low medium important

Concrete
Proportion (%) :........................
Riprap
Proportion (%) :........................
Docks
Proportion (%) :........................

Sand
Proportion (%):........................
Grain diameter < 20mm
Dominant type (clay, silt, sand, gravel or organic material (finely particulate (FPOM) or coarse-particulate (CPOM))
Sediment (FPOM >90%)
Proportion (%):........................
(If sediments with a very high proportion of Fine particulate organic material (FPOM) these will be considered as independent habitat and sampled proportionately)

(Submerged)Roots
Proportion (%) :........................
Shape:
Gappy Tight Short Long

Deadwood
Proportion (%) :........................
Degree of decomposition:

EULITORAL FIELD PROTOCOL

See	Site	People	Date

Physic-chemistry

Conductivity ....
pH ....
Dioxygen (mg/L) ....
Dioxygen saturation(%) ....
Temperature ....
depth ....

Usage types (several possible)

☐ Atropized
- ☐ Cover stone bed
- ☐ Wood construction (e.g., palisades)
- ☐ Blocking (concrete / steel)
- ☐ Paving
- ☐ Steganlage

☐ Natural

☐ Open

Land Shore Structure (sum=100%)

(20m/100m inland)

Urban:......./.........% parking, Gardens

......../.........% buildings, roads/path/rails

......../.......% swimming area, Camping

......./.......% port facility, bridges, shore lining, pipes,ditches, clearing areas

Intensive farming: ..../.........% farmland

Extensive farming: ..../.........% grass, pastures

......../.........% orchards

Natural Vegetation:

......../.........% herbaceous vegetation

......../.........% bushes / shrubs / single trees

......../.........% deciduous forest

......../.........% coniferous forest

......../.........% waters

.... / ....% stones / boulders / dunes without vegetation or poor vegetation

.... / ....% riparian forest/flooded forest, reeds, moist meadows, high and low moors

.... / ....% Others

EULITORAL FIELD PROTOCOL

See	**Site**	**People**	**Date**

Structure of the vegetation (sum =100%) (First 20m from the shore)

> 5 m ....% Trees diameter

> = 0.3 m ....% Trees diameter <0.3 m

0.5 - 5 m ....% shrubs / young trees

........% tall grasses / herbaceous vegetation

<0.5 m ....% bushes / young trees

........% grasses, herbaceous vegetation, aquatic vegetation

........% emerse macrophytes

Anthropogenic impairments (20m 100m)

- ☐☐ Mining peat
- ☐☐ Mining
- ☐☐ Fruit gardens
- ☐☐ Stone quarry
- ☐☐ Farmland
- ☐☐ Grass / Pastures
- ☐☐ Roads, railways
- ☐☐ Buoys fields
- ☐☐ Residential buildings, schools etc.
- ☐☐ Camping / tent sites
- ☐☐ Bridges
- ☐☐ Coniferous forest plants
- ☐☐ Commercial use fences
- ☐☐ Deciduous forest plants
- ☐☐ Artificial tributaries,
- ☐☐ Water troughs
- ☐☐ Trample paths, footpaths
- ☐☐ Dikes, flood protection walls
- ☐☐ Filling / filling the shore
- ☐☐ Dredging of littoral substrates
- ☐☐ Parks, gardens, golf courses
- ☐☐ Control / section of macrophyte
- ☐☐ Ports, marinas
- ☐☐ Pontoons, ventilation systems, fish cages and nets, refuse collection, garbage etc.
- ☐☐ Capping of stones, earth, debris, landfill rubbish clearing
- ☐☐ Leisure activities (bathing site, fishing / hunting, water sports, boat activity etc.)
- ☐☐ Waves and sedimentation protection (groins, stone walls)
- ☐☐ Stone walls, pile walls, stalemate walls (hard shoring)
- ☐☐ Baskets, planted pastures, org. Material, plastic (soft shoring)
- ☐☐ Other

Annex II: Taxa list

acheta	**19**	**ephemeroptera**	**727**
acheta_hirudinea_juv	2	baetis_juv_sp	1
batracobdella_paludosa	1	baetis_rhodani	1
helobdella_stagnalis	16	caenis_horaria	308
bivalva	**1511**	caenis_juv_sp	20
bivalva_sphaeriidae_juv	127	caenis_luctuosa	170
galba_truncatula	3	caenis_robusta	178
pisidium_sp	1349	centroptilum_luteolum	5
sphaerium_corneum	2	cloeon_dipterum	17
sphaerium_juv_	30	cloeon_sp	20
cladocera	**3227**	ephemeroptera-baetidae	6
daphnia_sp	3227	procloeon_pennulatum	1
coleoptera	**52**	**gasteropoda**	**277**
agabus_larvae_sp	1	armiger_crista	2
agabus_sp	3	bithynia_tentaculata	4
coleoptera	1	galba_truncatula	76
coleoptera_dytiscidae_larvae	36	gyraulus_albus	87
coleoptera_elminthidae_larvae	1	gyraulus_juv_sp	25
coleoptera_hydroporinae_larvae	1	gyraulus_sp	22
haliplus_sp	5	hippeutis_complanatus	14
hydraena_sp	1	physa_fontinalis	2
laccophilus_ad_sp	1	physa_sp	3
laccophilus_larvae_	1	radix_balthica	9
rhanthus_pulverosus	1	segmentina_nitida	11
coleoptera_ad	**1**	stagnicola_palustris	2
coleoptera_ad	1	valvata_piscinalis	20
coleoptera_larvae	**1**	**heteroptera**	**80**
coleoptera_larvae	1	heteroptera_corixidae_juv	1
crustacean	**1381**	heteroptera_mesoveliidae	1
asellus_aquaticus	667	micronecta_larvae_sp	17
crustacean_copepoda	619	micronecta_sp	54
gammarus_juv_sp	4	ranatra_linearis	1
gammarus_pulex	90	sigara_sp	6
gammarus_sp	1	**hydracarina**	**152**
diptera	**3722**	hydracarina	152
diptera_ceratopogonidae	168		
diptera_chironomidae	3530		
diptera_limoniidae	1		
diptera_psychodidae	3		
diptera_tabanidae	9		
dipterachironomidae	11		

odonata	**62**
chalcolestes_viridis	1
coenagrion_juv_sp	3
coenagrion_sp	35
cordulegaster_sp	3
gomphus_flavipes	1
gomphus_sp	2
odonata_corduliidae	2
platycnemis_pennipes	8
pyrrhosoma_juv_sp	2
pyrrhosoma_nymphula	1
somatochlora_alpestris	1
somatochlora_sp	3
oligochaeta	**2304**
oligochaeta	2304
trichoptera	**127**
anabolia_furcata	7
anabolia_nervosa	1
athripsodes_aterrimus	2
cyrnus_trimaculatus	1
leptocerus_lusitanicus	2
limnephilus_auricula	1
limnephilus_flavicornis	3
limnephilus_germanus	4
limnephilus_lunatus	1
limnephilus_sp	8
molanna _albicans	2
molanna _angustata	3
molanna _sp	1
mystacides_azurea	19
mystacides_longicornis	60
oecetis_sp	1
oecetis_testacea	2
orthotrichia_costalis	1
sericostoma_sp	2
triaenodes_bicolor	4
trichoptera_leptoceridae_juv	1
trichoptera_limnephilidae	1
turbellaria	**21**
dugesia_sp	16
planaria_torva	2
turbellaria	3
zooplancton	**3193**
polyphemus_sp	3193

Annex III: Detail of micro-habitat samplings