SSD

SCIENCE FOR A SUSTAINABLE DEVELOPMENT

TOWARDS A SUSTAINABLE MANAGEMENT OF POND DIVERSITY AT THE LANDSCAPE LEVEL (PONDSCAPE)

SD/BD/02A

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SCIENCE FOR A SUSTAINABLE DEVELOPMENT (SSD)

Biodiversity

FINAL REPORT PHASE 1

TOWARDS A SUSTAINABLE MANAGEMENT OF POND DIVERSITY AT THE LANDSCAPE LEVEL (PONDSCAPE)

SD/BD/02A

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1. EXECUTIVE SUMMARY

The aim of the PONDSCAPE project is to provide scientifically underpinned recommendations for a sustainable management approach, considering not only the protection and increase of biodiversity levels of ponds, but also economic activities and growth.

A detailed protocol for sampling and sample analyses was compiled (WP1, Task 1.1), which allows us to compare data in a straightforward way. Analyses of extant databases (Task 1.2) are completed and related publications are in preparation.

Sampling of 125 ponds was performed for the multi-scale analysis of pond biodiversity (Task 1.3). Currently, the samples are being processed. Detailed analyses will be performed during phase 2 of the project. The same dataset will be used for the execution of WP2 (biodiversity and pond age) and WP3 (biodiversity and ecosystem functioning).

The case study of the Tommelen pond complex (Task 1.4) is completed. In spite of the fact that all ponds have the same age and similar size, are located very close to each other and share a similar environmental context, they show pronounced differences in biodiversity and ecology. During the summer of 2007, samples have been collected for several taxonomic groups (bacteria, phyto- and zoobenthos, phyto- and zooplankton, macro-invertebrates, amphibians, fish and macrophytes), while also the microbial functions were assessed. Most taxonomic groups had a relatively high (gamma) diversity and between-pond (beta) diversity, suggesting that it is essential to safeguard the area. For all investigated taxonomic groups, the differentiation amongst ponds in community structure was linked to purely spatial variables, which is possibly related to dispersal limitation, priority effects, source-sink dynamics, stochasticy or undetected latent environmental gradients. Also purely environmental variables appeared important for the communities of several of the taxonomic groups, which can result from processes such as species sorting.

Three field campaigns have been completed for the study of the links between taxon diversity and specific ecosystem functions (WP3). The microbial activities (primary production, bacterial production and nitrification rate) were highly inter-correlated and discriminated the sites very well. No clear relationship could be established with the physical and chemical variables or with the bacterial diversity, but the algal community changed significantly along the primary production gradient. Phytoplankton production and community composition varied significantly according to eutrophication gradients. Dredging (for management purposes) did not have a profound impact on the microbial functioning in the short term.

The impact of these management practices was assessed in 14 ponds that were dredged in 2008 (WP 4). The post-dredging community composition and biodiversity will be compared to the pre-dredging situation and to the situation in nine neighbouring control ponds. All 23 ponds have also been sampled for biotic and abiotic characteristics in the summers of 2007 and 2008.

Water sampling was performed in fifteen ponds for the evaluation of the dynamics of pollutants by agricultural activities (WP 5). The results show that some insecticides, phenol compounds and PAHs do not represent a high risk of pollution. However, attention should be given to herbicides, especially over the periods of massive pulverisations, such as in autumn. These results are useful to provide advice to the stakeholders of ponds for safety management actions.

During the socio-economic survey (WP6), we observed that ponds were subjects of debate and that there seemed to be dissimilarities on the environmental perceptions. Field observations indicate that management programs for ponds need to take into account local conditions. Historical analysis revealed that most ponds are artificial objects. Moreover, it appears that the recollection of past pond uses is needed to better preserve them. Preliminary analyses of interviews show that childhood memories concerning ponds help stakeholders to maintain a preservation centered attitude.

All genera of the mollusc family Lymnaeidae known as common intermediate hosts of the liver fluke, were found during the sampling campaign in Belgium and Luxembourg. Especially the species *Galba truncatula* was common, which, in Europe, is the principal intermediate host of *Fasciola hepatica*. Apparently, soil conditions in Flanders (sand/silt) are not unfavourable for the development of lymnaeid snails.

In conclusion, during phase 1 of the PONDSCAPE project, we have completed the Tommelen case study, and also performed sampling, sample processing and some preliminary analyses concerning the large scale survey, the assessment of microbial functions, the evaluation of management practices and the determination of pollution levels. Also for the study of the history of social and economic relevance of ponds to stakeholders, much progress has been made. There are no major delays in the execution of any of the work packages, although for logistic reasons, the order of execution has been modified (see previous reports for justification of these changes).

In Annex I, the present summary has been translated to Dutch (COO language).

2. DESCRIPTION OF THE PROJECT

2.1 Context

Water is needed in all aspects of life (article 18.2 of Agenda 21). The biota of freshwater habitats constitute a large component of overall biodiversity: more than 8% of all described species occur in only 0.01% of total surface area of the planet (Balian et al. 2008). Recent research (Biggs et al. 2005, Davies et al. 2008) has pointed out that ponds, despite their small size, contribute significantly to the aquatic biodiversity at regional scales. In comparison to lakes, rivers, streams and ditches, ponds were found to harbour relatively a high local species richness (alpha diversity) when sampling is standardized for area. Furthermore, and even more importantly, ponds harbour a significant proportion of the total species richness of plants and macro-invertebrates that are present at larger spatial scales. Finally, up to 60% of all rare freshwater species in the UK are found in ponds.

2.2 Objectives and expected outcomes

PONDSCAPE will provide scientifically underpinned recommendations for a sustainable management approach (Göteborg strategy) that will reconcile desires to protect and increase biodiversity levels at various spatial scales (CBD, RAMSAR convention on wetlands, EU Water Framework Directive) with the need to sustain economic activities and ensure economic growth (Lisbon strategy with renewed impetus from the European Council meeting in Brussels 2005).

Operational general objectives are:
• To study the organization of pond biodiversity, including ecosystem functioning, in Belgium and Luxembourg at multiple spatial scales and relate it with important driving variables, with special reference to pond age
• To quantify the effects of management strategies on local and regional pond biota biodiversity and to broaden our knowledge on the prevalence of pollutants and the effects they have on pond biota
• To obtain insights into the way stakeholders value risks and benefits of ponds, and to investigate how the creation and maintenance of ponds can be promoted in a sustainable way.

Various deliverables have been identified. The sampling protocol developed by MANSCAPE will be updated for the present project. Databases with measurements on abiotic variables, biodiversity measurements, stakeholder interviews etc will be drafted and made available to the BBPF. Results of analyses will be published in primary scientific literature, but will also be disseminated through popular press, general PONDSCAPE meetings and through the Follow-Up Committee.

2.3. Scientific methodology

WP1: Biodiversity at multiple spatial scales: patterns and driving variables

Task 1.1: Fine tuning of existing sampling and sample analysis protocol (P2)

An existing protocol for the sampling of ponds and sample analysis (MANSCAPE protocol) is adapted to the research questions of this project and is attached as Annex II. This protocol will be applied for all of the sampling performed within the PONDSCAPE project.

Task 1.2: Analyses of extant databases (C)

The data obtained within the MANSCAPE project have been analyzed with the aim of (1) comparing species richness and composition of cladoceran communities between two sets of ponds with different age (De Bie et al., in press.) and (2) exploring patterns of congruency in the diversity of a wide variety of pond organisms and identifying a gradient of overall diversity common to the majority of these groups (Declerck et al., in prep.). Within the framework of the PONDSCAPE project, the MANSCAPE data were re-analyzed in order to check for multi-group biodiversity patterns at different spatial scales (Declerck et al., in prep.).

Task 1.3: Multi-scale survey (C, P2, P3)

A selection of 125 ponds was made according to a strict a priori defined spatial design. We selected in total five ecoregions in Belgium/Luxembourg: Gutland, chalk region, sand region, dune region and loam region (Fig. 1). In each of these ecoregions, we defined five 30 km² circular areas and in each of these areas five ponds were randomly selected. This will allow us to assess biodiversity at four different spatial scales (Fig. 2): the scale of individual ponds (level 1), the scale of clusters of ponds within 30 km² areas corresponding with natural subregions (level 2), the scale of groups of pond clusters corresponding to the scale of landscapes (level 3), and the scale encompassing five different biogeographic areas of Belgium/Luxembourg (level 4). In Annex III we give an overview of the locations of the sampled ponds for each of the five bio-geographic regions, with indication of the date of sampling.

The sampling campaign for the nation-wide survey was done during the summer of 2008: biotic and abiotic characteristics were sampled. Details on the sampled variables, the sample collection and the sample processing can be found in Annex II.

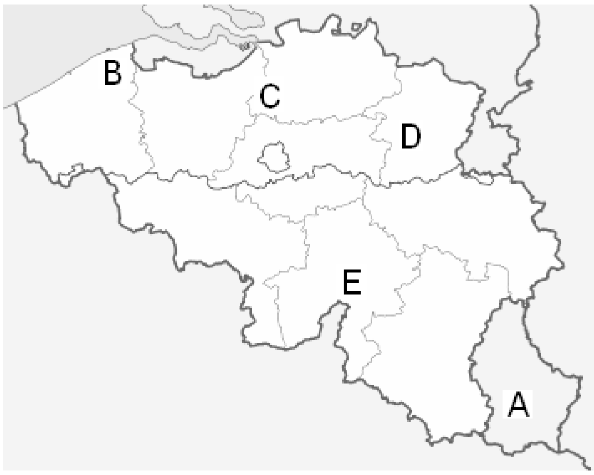

Figure 1. The five ecoregions in Belgium/Luxembourg that were studied in the multi-scale survey: A: Gutland; B: polders; C: sand region; D: loam region; E: chalk region.

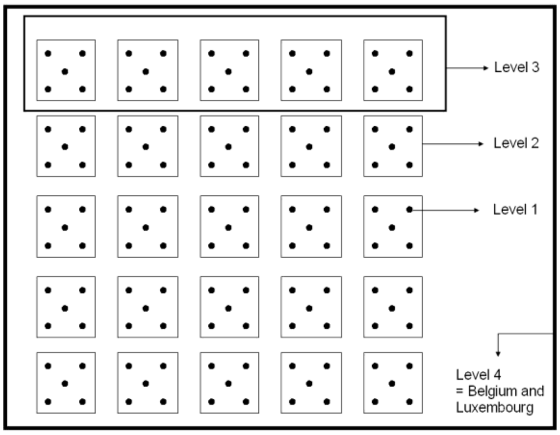

Figure 2. Schematic overview of the different levels of clustering applied within the multi-scale survey.

Task 1.4: Detailed case study of a high density pond cluster: the bomb crater survey at Tommelen (C, P2, P3)

We studied a high-density pond cluster: the bomb crater complex at Tommelen (Hasselt, Belgium). This area was bombed in the Second World War (1944), an event that gave birth to a unique landscape of 110 ponds with a high biodiversity level. The study of the biota in these ponds is of high scientific relevance. It allows us to assess the importance of spatial organization and very local environmental gradients for the local richness and community structure, without having the interference of geographical gradients, such as temperature gradients and pond age. Also, the study is of relevance for the Tommelen area, as it provides a very comprehensive list of species present in this unique area.

In the bomb crater field of Tommelen, we sampled the biotic and abiotic characteristics in a set of 49 ponds during spring and summer of 2007 (Figs. 3 & 4). This set holds almost all permanent ponds (i.e. ponds that keep water during the summer period). Most of the other ponds dry out each summer.
In all of the selected ponds, we applied the standardized PONDSCAPE protocol (see Task 1.1; Annex II). Details on the sample collection and sample processing can be found in Annex II. The next few paragraphs summarize the ecological data that have been gathered.
In each of the selected ponds primary characteristics such as water depth and transparency, water colour, and thickness of the mud layer were noted, as well as the position of the water level in relation to the soil level of the surrounding vegetation. Vegetation and flora were separately noted for the open water, the (inundated) transition zone near the shore, the wetter terrestrial part of the shore, the dryer terrestrial part of the shore and the immediate surroundings of the pond (a zone of some 5 m wide). The general aspect of each pond (seen from on top of a pair of steps) and incidental details of the vegetation were photographed. The contour and position of each pond were determined by using a GPS with accuracy at the level of <0.5 m (Mobilemapper). This gives a good idea of the whole of the studied area (see Fig. 4). The calculation of the circumference and area of all ponds based on the GPS-data gave extra habitat characteristics.

Figure 3. Aerial picture of the bomb crater field in Tommelen (Hasselt, Belgium).

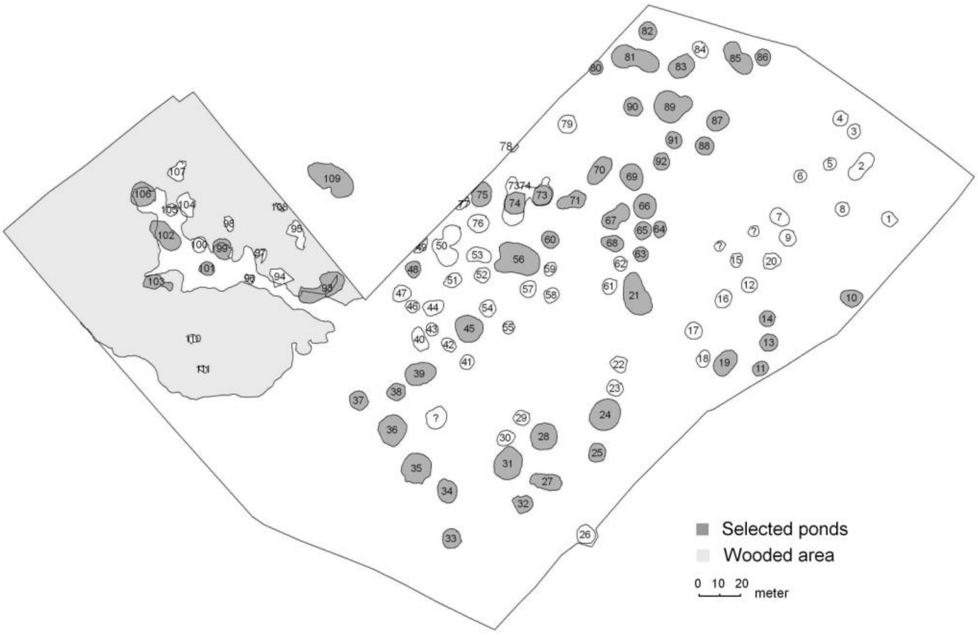

Figure 4. Overview of the selected ponds (dark grey) in Tommelen (Hasselt, Belgium).

Apart from the physical, chemical and morphometric variables, we also sampled the communities of a selection of organism groups. Bacteria, phytoplankton, phytobenthos, macrobenthos and the aquatic vegetation were sampled once during summer 2007. Zooplankton was sampled in spring, summer

and late summer. Species richness was calculated after rarefaction to 100 individuals for all samples. Macroinvertebrates were sampled twice, once in spring and once in summer. The amphibians were sampled with two different methods: sampling of adults was done in early spring by means of fykes and juveniles were sampled with a sweep net during three subsequent months (May, June and July 2007). Densities were expressed by catch per unit effort. DNA extraction, PCR and DGGE (denaturing gradient gel electrophoresis) has been done for all bacterial samples. More than 50 bands were cut out the DGGE gels and have been processed. Enumeration of phytoplankton taxa was done using an inverted microscope for 40 samples. If possible, at least 200 individuals were counted and identified up to genus level. The biomass (µg C/L) is calculated using conversion factors. All phytobenthos samples have been processed. Relative abundance of individual benthic diatom taxa was determined from counts of 500 valves along randomly chosen transects. Additional taxa were detected by scanning one or more slides, using a stopping rule to standardize relative effort, and given a marginal abundance (0.1 individuals). Concerning the chironomids, priority is given to the study of the macro-invertebrate samples instead of the zoobenthos samples, in order to integrate this taxon in the global multivariate analysis of the other taxa. All samples have meanwhile been processed.
Relations between environmental variables and species composition were examined by means of ordination analyses (DCA, CCA, RDA). Cluster analysis (relative Euclidean distance, Ward's group linkage) and non-metric multidimensional scaling (NMS) of samples based on selected environmental variables, Mantel tests and Indicator Species Analysis were carried out with PC-ORD 5 (McCune & Mefford 1999). All taxa included within the counts were used in these analyses. Nestedness was determined for all observed taxa according to the NODF metric (Almeido-Neto et al. 2008) using ANINHADO 3.0 (Guimaraes & Guimaraes 2006; 1000 simulations with Er and Ce null models); however, the number of idiosyncratic taxa was determined as those with a temperature (*T*; Atmar & Paterson 1993) above the mean for all taxa, calculated using the same program. Diversity partitioning was carried out with the program Partition (Veech & Crist 2007). For principal coordinates of neighbor matrices analysis (PCNM; method described in: Borcard & Legendre 2002), starting from the X and Y coordinates, a matrix of PCNM variables was created with a truncation distance of 56m. This critical distance was obtained by running a single linkage clustering on a matrix of Euclidean distances among the sites, and computing the chain of primary connections. The largest of these connections is the shortest distance to retain in the truncation process of the PCNM method to maintain the graph of the entire object connected. The procedure yielded 29 PCNM variables. The first PCNM variables represent coarse patterns and the last ones finer-scale patterns (Borcard et al. 2004). Prior to the application of the PCNM variables, the response data was checked for linear trends, because PCNM variables are unsuitable to cover linear trends. For this we performed a preliminary redundancy analysis (RDA) involving only the X and Y geographic coordinates as explanatory variable and the community of interest as response variable.

WP2: Biodiversity and pond age

Task 2.1: Selection of ponds belonging to different age classes (P2)

The original plan was to select ponds belonging to different known age classes within a spatially restricted area. Despite elaborate efforts, no such area was found in which ponds of markedly different age co-occur in reasonable numbers. Therefore, it was agreed between the project partners and the members of the Follow-Up committee to relax the spatial restriction, which permits the inclusion of a larger number of ponds. Also, this way we can re-use the data obtained for many of the ponds selected for Task 1.3. Thus far, the age of about 60 of these ponds has been assessed, and an additional 5 ponds were selected in order to also include ponds that are less than five years old. Together, this selection of ponds will allow us to compare biodiversity levels and community structures amongst ponds belonging to four different age classes: 0-5 years, 5-10 years, 10-20 years and >20 years.

Task 2.2: Sampling and sample analysis (C, P2, P3)

As in tasks 1.1 & 1.3. Sampling of the selected ponds was performed in the summer of 2008. Currently, the samples are being processed.

Task 2.3: Data analysis (C)

The data analyses are planned for phase 2.

WP3: Biodiversity and ecosystem functioning

Task 3.1: In situ and ex-situ assessment of microbial ecosystem functions (IP)

This work package aims to reveal key aspects of the links between taxon diversity in focal organism groups and specific ecosystem functions. As it is impractical to measure all ecosystem functions, this work package focused on microbial processes. Planktonic bacteria and algae are in close and direct relation with the cycle of organic matter and nutrients in the ponds. They are found in every pond and can be isolated and analysed in a standard way allowing comparison between ponds. Moreover, they are likely to respond to eutrophication and chemical pollution.

These measures of functionality take the whole microbial community into consideration, and therefore do not reveal which taxa engage in which transformation. This is intrinsic to the limitation that most microbial organisms cannot be cultured. However, as we also quantify bacterial diversity using molecular techniques as part of WP1, WP2 and WP4, we are able to directly relate functional characteristics and functional diversity of the bacterial community with taxon diversity of the bacterial community.

Task 3.1. includes the in situ and ex situ assessment of microbial ecosystem functions. This encompasses the field campaigns and the laboratory work that must take place rapidly after the sampling. As samples for the analysis of the microbial activities must be incubated for 2 to 24 hours immediately after sampling, this limits the number of ponds that can be analysed during a field campaign. As a consequence, the number of ponds analysed for the microbial activities is generally lower than that analysed for the taxon diversity. Forty ponds were sampled in 2007 in Tommelen for the microbial activity monitoring. Seventy-five ponds on 125 were sampled in 2008 during the multi-scale survey, and 23 ponds were sampled after the dredging in Tommelen in autumn 2008. The sampling for the assessment of the microbial activities was dissociated from the sampling for the assessment of the biodiversity and was performed before the latter.

The following functional variables were monitored in the studied ponds: microbial primary production, bacterial secondary production, nitrification rate and microbial respiration. Moreover, a Community Level Physiological Profiling (CLPP) was performed in each pond. From a methodological point of view, the whole water column was sampled in a central point in the pond using a 2-L Ruttner bottle. Water sampled from one pond was pooled and stored in autoclaved glass bottles that were kept in the dark and were immediately brought back to the laboratory. The major limnological variables of the pond were measured simultaneously to the microbial activities: water temperature, conductivity, pH, dissolved oxygen concentration and turbidity. Microbes were isolated from the larger organisms (zooplankton, insects) by filtration of the water on a Nylon plankton net with a mesh size of 40 µm. Filamentous algae are thus excluded from the samples. Methodological details can be found in the Annex II.

Task 3.2: Data analysis (P3, IP)

Task 3.2 concerns the analysis of the microbial data in relation to the abiotic and biotic data available from the other WP. Using different multivariate analyses, these variables will be combined with the DGGE data of bacterial community composition and diversity as well as with the diversity and community data of other organism groups and of vegetation. Furthermore, we will explore the spatial structure of microbial community composition and functioning (cf. WP1), its succession with pond age (WP2) and its response to management measures (WP4).

WP4: Assessment of management techniques

Task 4.1: Inventory of planned management by 3rd parties and pond selection (P2)

A list of planned management practices in ponds is being compiled based on interviews with stakeholders and conservators. Already more than 20 sites are known to be managed. More extensive data is being gathered in phase 2 of the project.

Task 4.2: Sampling and sample analyses (P2, P3)

Ponds need to be managed; otherwise they fill in due to natural succession and disappear. The research on management techniques will be focused on the bomb crater field in Tommelen (Hasselt). The high number of ponds, similar age and similar environmental context (e.g. soil, bottom water, surrounding land use) give us the ideal opportunity to evaluate the effects of management techniques on a number of

replicated systems. The effect of management on 23 ponds of the Tommelen bomb crater complex is currently being followed up.
One of the most widely used management techniques applied to ponds is dredging (sludge removal). To assess the effect of dredging on the composition and diversity of biota, 14 ponds in Tommelen were dredged in October 2008. We already sampled these ponds and nine control ponds for biotic and abiotic characteristics in the summer of 2007 and 2008 (just before the dredging). In addition, the managed ponds were sampled a few weeks after dredging (autumn 2008), to check for remnants of the communities still present after this management practice. In the spring and summer of 2009, these ponds will be sampled again in order to study the response of pond characteristics (physical, chemical and biodiversity-related variables) to dredging and evaluate them against the dynamics in non-manipulated ponds. The dredging of the ponds was carried out by the city of Hasselt, in close co-operation with ‘Natuurpunt vzw’ and the manager of the nature reserve (Mr Rik Jacobs).
A second objective is to compare the effect of restoration measures on ponds with different trophic state. The ponds in the southern part of Tommelen are more eutrophic than those of the northern part due to historical contamination. We want to compare the rate and degree of recovery between impaired ponds (southern part) and non-impaired ponds (northern part). The selection of ponds to be dredged therefore consists of four impaired ponds and ten non-impaired ponds (Fig. 5).
Originally, it was planned to sample these ponds in autumn 2007 and to do the dredging in winter. However, the winter period was too humid, so it was impossible to deploy the bulldozers needed for the dredging. The actual work was thus postponed until October 2008.

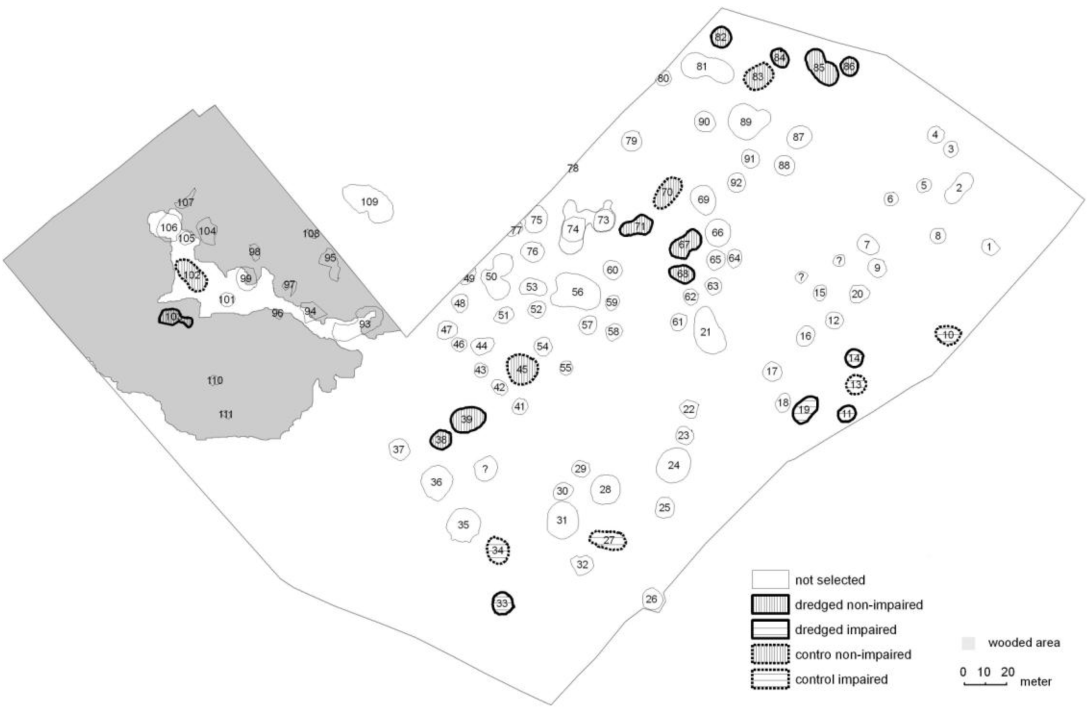

Figure 5. Overview of the ponds in Tommelen (Hasselt, Belgium) sampled for the assessment of management techniques.

Task 4.3: Data analysis: evaluation of pond management practices (P2)

The data analyses are planned for phase 2.

Task 4.4: Evaluation of management practices as a tool for increasing beta and gamma diversity

The study on the impact of dredging will allow us to evaluate the potential of dredging management schemes to increase beta and gamma diversity in entire pond complexes. The evaluation is planned for phase 2.

WP5: Pond biodiversity, management and pollution

Task 5.1: Evaluation of pesticide pollution (P4)

The objectives of task 5.1 are (1) to determine whether there are peak levels of widely used pesticides in water of selected ponds in relation to pesticide applications scheduled by farmers and (2) to verify the occurrence of estrogenicity in the case of agricultural ponds by in vitro screening assay of water and by in vivo aromatase activity and vitellogenin induction in fish.

A. Dynamics in the pesticide loads: Fifteen small ponds were selected in Flanders (six) and Wallonia Region (nine) in areas under intensive or semi-intensive anthropogenic activities (roads, agricultural activities, etc) as well as in natural reserves. Water was sampled three times during the year 2007: mid-April, beginning of July and mid-October.

Evaluation of the dynamics of problematic pesticides focussed on some widely new pesticide molecules already reported as problematic pesticides in Belgium and other European countries, namely (1) herbicides: atrazine and its metabolites (terbuthylatrazine, etc), simazine, diuron, isoproturon, and glyphosates and its surfactant AMPA and (2) insecticides, polycyclic-aromatic hydrocarbons (PAHs) and other associate molecules: aldrine, chlorfenvinphos, α-endosulfan, dicofol, benzo(b)fluoranthène and benzo(a)pyrene, octylphenol and 4-(para)-nonylphenol.

The water samples were stored at -20°C until filtrations and solid-phase extraction (SPE). After the SPE process, the extracts were stored in acetonytrile solution at -20°C. Then, triazines, isoproturon and diuron were assayed by HPLC methods already standardized in our laboratory. Isoproturon and diuron were re-analysed by GC-MS methods together with other pesticide molecules (glyphosates, insecticides, etc) in collaboration with the Centre of Analysis of Residues in Traces (CART) and the laboratory of Food Science of the University of Liège.

B. Evaluation of pollution incidence by estrogen-like properties*:* To test the eventual occurrence of estrogenicity activity in the water pond, we used in vivo and in vitro approaches.

(1) In vivo assays*:* Six intensive or semi-intensive ponds of the fifteen ponds already sampled for pesticide loads were selected. Ten juvenile (15 – 65g) fish (*Carassius auratus*) from each of these ponds were captured in July 2008, and sampled for blood, brains and gonads. For each pond, five females and five males were compared for vitellogenin induction and aromatase activity. Plasma vitellogenin (VTG) levels were determined by ELISA method, while brain or gonad aromatase activity was assayed by radioimmunoassay.

(2) In vitro assays: (a) Estrogenic compounds (octylphenol, nonylphenol, endosulfan and PAHs; see 5.1.A) were assayed in all the samples used for the dynamics in pesticides loads (15 ponds sampled three times in 2007). Moreover, the six ponds in which fish were captured for the in vivo test were sampled twice for water in July and October 2008 for the analysis of the same estrogenic compounds. (b) Evaluation of in vitro estrogenicity by cell proliferation: It was planned that in case of high concentrations of potential estrogenic compounds, water samples from the ponds in which fish were captured will be assayed for in vitro estrogenicity by cell proliferation. For this screening, water samples are first clarified by micro-filtrations before SPE extraction. Then, estrogen-like properties in water samples are revealed by MCF-7 cell proliferation assay (E-screen). The first step of this E-screen method is the cell proliferation by seeding the MCF-7 cells on a black 96-well microtiter plates after removal of all estrogenic compounds. Cells are incubated for 72 hr to make them estrogen responsive. As a second step, cells are exposed to the extract samples including standard solutions containing different level of estrogen. Then, the cells are incubated at 37°C, and on the sixth day of incubation, the physiological responses are determined by cell proliferation analysis using flow cytometric methods.

Task 5.2: Effects of pond management on the bio-availability of pollutants (P4)

Task 5.2 aims at studying the bio-availability of pollutants in relation to pond management actions (see WP4). The effects of the management actions will be confirmed by ecotoxicological biotests in which a sedentary amphibian *Rana temporaria* will be submitted to Roundup and isoproturon challenges in laboratory conditions.

All the ponds which have been submitted to the management actions (see WP4) were sampled in June 2008 for water and sediments before the practices, and will be sampled over 2009 for evaluation of changes in heavy metal loads, and some problematic pesticides as well as their eventual toxicity on aquatic organisms.

Heavy metals will be determined by mass spectrometry methods using a HR-ICP-MS integrated system, while pesticides will be analysed by HPLC and GC-MS.

Fertilized eggs will be sampled in early March 2009 on both control and treated ponds, and transferred to the laboratory conditions of URBO in Namur. Then, spawns and tadpoles of the same developmental stages will be monitored for decontamination kinetics based on multiple physiological stress response, such as detoxication enzymes, thyroid hormones, and proteome stress.

WP6: History of social and economic relevance of ponds to stakeholders

Task 6.1: Evolution of social and economic relevance of pools (P4)

The objective of this task is to assess in a qualitative (collection of relevant archives on pond history) and a quantitative way (cartographic approach), how ponds gained or lose socio-economic value along a recent historic period (typically the 20th century).

The documentary approach consists in the collection and analysis of historical documents. Information on history of pond use and value has been gathered by research on the basis of historical documents collected from different sources: local libraries, local historians, and local history groups.

To get insights on pond density, distribution and evolution, an analysis of maps has been performed (cartographic approach). The methodology consists in an inventory and a comparison of ponds based on 1:20 000 or 1:25 000 maps from the National Geographic Institute (NGI) at four different periods (late 18th, early/mid/late 20th century). As an exhaustive inventory of ponds of the whole area is not possible in the available time, representative areas of 80 km² belonging to one of the "ecoregions[1]" and delimited by the NGI maps limits were chosen.

Task 6.2: Analysis of present day perception of social and economic value of ponds by different sectors (P4)

This task aims at understanding stakeholders perceptions concerning ponds values, and consists of different sections.

The exploratory phase consisted in:

- contacting resource persons whose personal experience could enlighten the subject,
- analysing general and scientific papers, and
- discussing with experts in the field.

This gave us a general overview of the present day situation of ponds in Belgium. This exploration is a necessary phase to discover and to delimit the object "pond", but also to establish the basis of the following research phases.

A method based on the analysis of half-directive interviews was used to study the perception and representation of stakeholders. In total 48 interviews (27 in Wallonia and 21 in Flanders) were performed. Half-directive means that the interviewee is guided by the interviewer, throughout selected topics, but the scope and the direction of the discussion are allowed to follow the associations identified by the interviewee. This means that questions asked by the interviewer are supposed to be open (and not fixed) questions, giving free place to the interviewee in the formulation of his/her answer. This method has demonstrated its relevancy to reveal less visible aspects of stakeholders' ways of thinking or acting.

An extensive protocol can be found in Annex IV.

Task 6.3: Risk assessment regarding *Fasciola* infection using different watering systems (P4)

Fasciolosis is a food-borne trematode infection, which has a worldwide distribution. It is responsible for marked economic losses in livestock due to reduced weight gain, milk production, fertility, and condemnation of the livers (Genicot et al., 1991). The parasite is prevalent in Europe and a recent study from Belgium reported that 17.3% of cattle were serologically positive (Lonneux et al., 2000). This disease is transmitted by freshwater snails of the family Lymnaeidae (Mollusca : Gastropoda : Basommatophora) (Boray, 1982; Malek, 1984). In Europe, the principal intermediate host for *Fasciola hepatica* is *Galba truncatula* O.F. Müller, 1774.

[1] Belgium is divided into 13 areas (Dunes, Polders, Sandy, Campine, Silty and Sandy, Silty, Hennuyere, Famenne, Condroz, Grassland, Ardenne, High Ardenne and Jurassic) called ecoregions, differing from each other in soil composition, culture type, and geographic and climatic conditions.

The main objective of the multi-scale survey is to study patterns of diversity within different spatial scales and this for a « random » set of ponds that are present within and typical for each location. This work (Task 6.3) aims to study the epidemiology of the different Lymnaeidae intermediate hosts of *Fasciola hepatica* in Belgium. The snails will be morphologically identified and classified. PCR analyses will be used to detect the trematode DNA in snails to assess the epidemiological weight of the different snail species collected. Secondly, all the Lymnaeidae snails collected will be phylogenetically identified with the internal transcribed spacer 2 sequences (ITS2).
During summer 2008 (from the first of July until the 8th of August), 125 ponds were sampled to collect lymnaeidae snails in Belgium/Luxembourg in five ecoregions (A - Gutland, B – polders, C – sand region, D – loam region, E – chalk region) (Fig. 1). In each ecoregion (or supercluster), five clusters were selected each containing five ponds. Within each pond, four sampling areas (black, red, green and yellow) were defined to facilitate the sampling protocol (Fig. 6). For each area, snails were collected during a maximum of 15 minutes in spots separated by 4-5 meters all around the ponds (irrespective of the fact that snails were present or not). Several variables were also measured, for example: size of the ponds, temperature, GPS coordinates, pond type (natural, extensive, intensive), soil type (clay, silt, stone, sand), fence, cattle, … For a more detailed protocol, we refer to Annex II.
The snails were morphologically separated into 4 genera (*Galba sp*., *Lymnaea sp*., *Radix sp*., *Stagnicola sp*.) with a binocular microscope (x10), measured and sorted in several size classes depending on the sampling area.

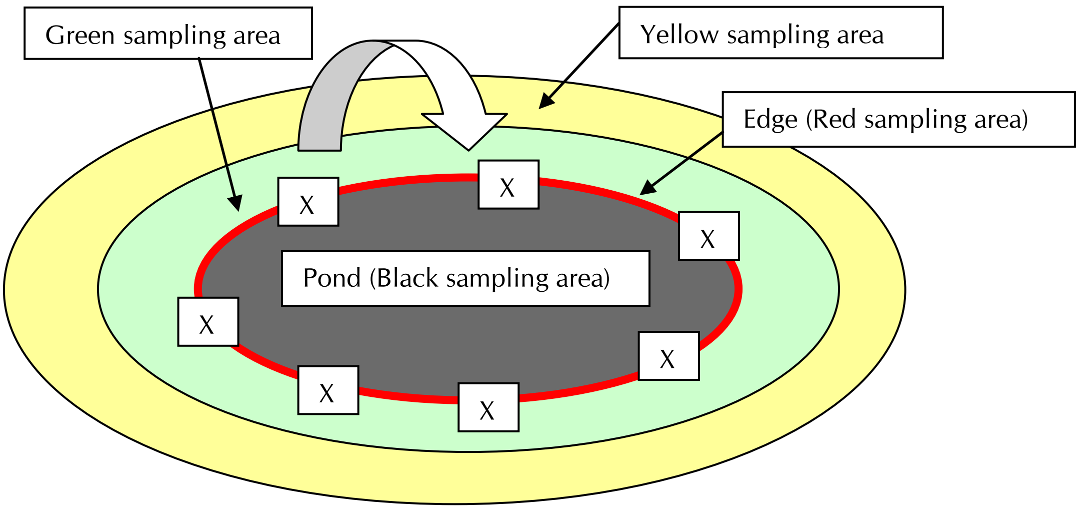

Figure 6. Within each pond, four sampling areas were sampled (black, red, green and yellow area).

WP7: Valuation of results, policy measures, management recommendations

WP7 implies an integrative analysis of all PONDSCAPE data and is planned for phase 2 of the project. Some preliminary results are already presented in this report.

4. RESULTS

WP1: Biodiversity at multiple spatial scales: patterns and driving variables

Task 1.1: Fine tuning of existing sampling and sample analysis protocol (P2)

The protocol is included in Annex II.

Task 1.2: Analyses of extant databases (C)

Three publications related to the Manscape data are submitted at the moment. More detailed analyses are in progress.

Task 1.3: Multi-scale survey (C, P2, P3)

125 ponds have been sampled in the summer of 2008 (see Annex III). Analyses are currently ongoing. Further analyses and data analysis are planned for phase 2.

The 125 sampled ponds cover a wide range of ecological conditions (Table 1). Most are almost pH neutral, but vary substantially in oxygen content, alkalinity, conductivity, water transparency, sludge content and macrophyte abundance.

The samples of phytoplankton, phytobenthos, zoobenthos, microbes, zooplankton, macrophytes, amphibians and macro-invertebrates are currently being analysed. For example, about one fourth of the macro-invertebrate samples are already counted and identified, while more than 70% of the samples have been sorted. No analyses have as yet been performed, since an insufficient number of samples from all regions have been processed, which could lead to misleading results.

Table 1. Average, range (minimum (min) and maximum (max)) and quartiles (first (1st quart) and third (3d quart)) for some key ecological variables measured in 125 ponds over Belgium and Luxembourg during summer 2008.

	Average	Min	1st quart	3d quart	Max
Alkalinity (mg CaCO3/l)	179.8	1.6	99.5	236.5	469.0
O2 (%)	62.3	0.0	25.2	83.2	200.0
Temperature (°C)	20.2	10.9	18.0	22.1	30.3
pH	8.1	6.5	7.6	8.6	10.7
Conductivity (µS/cm)	471	31	239	595	3340
Sneller (cm)	20	1	12	28	62
N total (ppm)	6.2	1.1	2.5	5.7	42.7
P total (ppm)	1.2	0.04	0.2	1.3	13.7
Maximum depth (m)	0.8	0.03	0.5	1.0	2.0
Sludge (cm)	30	0	5	45	150
Emerse macrophytes (%)	21	0	5	30	100
Submerse macrophytes (%)	22	0	0	45	100
Floating macrophytes (%)	30	0	0	60	100
Open water (%)	42	0	5	80	100
Infestation (%)	19	0	1	30	100
Dead plant material (0/1/2)	1	0	0	2	2

Task 1.4: Detailed case study of a high density pond cluster: the bomb crater survey at Tommelen (C, P2, P3)

During spring and summer 2007, 49 ponds were sampled in the Tommelen pond complex. Here, we present the abiotic and biotic data (bacteria, phytoplankton, phytobenthos, macrobenthos, macroinvertebrates, zooplankton, aquatic vegetation and amphibians).

1. Abiotic environment, macrophytes, cyanobacteria and chlorophyll a

The 49 sampled ponds cover a wide range of ecological conditions (Table 2). Most of them are almost pH neutral, shallow (< 1 m maximum depth) and small (< 100 m²), but they vary substantially in chlorophyll a content, oxygen content, alkalinity, conductivity, water transparency, sludge content and macrophyte abundance (see Table 2).

Several of the environmental variables were significantly correlated with each other, for example: conductivity with pH and amount of sludge, total nitrogen with water transparency, while also the variables pH, conductivity, hardness, chloride and calcium were interrelated with each other (Pearson correlations, $p < 0.001$).

Table 2. Average, range (minimum (min) and maximum (max)) and quartiles (first (1st quart) and third (3d quart)) for some key ecological variables measured in 49 ponds in Tommelen during summer 2007.

	Average	Min	1st quart	3rd quart	Max
Suspended solids (µg/l)	19.0	0.3	6.9	23.1	127.0
Cyanobacteria (RFU)	1405	1281	1349	1433	1751
Chlorophyll a (µg/l)	222	41	76	207	3288
Alkalinity (mg $CaCO_3$/l)	34	4	14	53	93
O_2 (%)	54	5	20	75	144
Temperature (°C)	17.0	15.1	16.4	17.6	19.6
pH	6.8	5.4	6.3	7.3	8.4
Conductivity (µS/cm)	146	31	65	187	418
Sneller (cm)	20.6	3.0	15.0	28.0	42.0
N total (mg/l)	3.0	0.69	1.59	3.05	20.51
P total (mg/l)	0.60	0.02	0.08	0.67	7.00
Maximum depth (cm)	56	10	39	73	150
Sludge (cm)	24	2	10	30	110
Shadow (%)	5	0	0	0	80
Emerse macrophytes (%)	24	2	10	40	70
Submerse macrophytes (%)	32	0	5	60	80
Floating macrophytes (%)	51	0	10	85	100
Open water (%)	22	0	0	30	90
Infestation (%)	27	0	10	45	80
Tree/shrub (% cover)	6	0	0	0	60
Dead plant material (0-3)	1.6	0	1	3	3
Infilment (0-3)	0.7	0	0	1	3

2. Bacteria and phytoplankton

The main environmental variables influencing phytoplankton community composition identified by a forward-selection redundancy analysis were: oxygen (explaining 5%, $p = 0.002$), sulphate (4%, 0.029) and emergent plants (4%, 0.043) (Fig. 7). The spatial configuration can be observed in the environmental RDA because of a significant relation between environmental variables and spatial variables (Fig. 8).

Forward-selection redundancy analysis revealed that the following variables significantly explained the variation in the bacterioplankton community: oxygen (O2 mg/l, explaining 6%, $p = 0.001$), water volume (5%, $p = 0.001$), alkalinity (4%, $p = 0.002$), total nitrogen (3%, $p = 0.028$), and the absence of water vegetation (OPEN, 3%, $p = 0.046$) (Fig. 9).

Variation partitioning of phytoplankton communities (relative abundances, only species contributing on average >1% to total biomass) revealed that 9% ($p = 0.062$) of the variation can be attributed to pure environmental variables, 16.1% ($p = 0.006$) to pure spatial factors and 4.4 % to common (spatial and environmental) effects (Fig. 10A). Only significant variables in a forward selection RDA were used for the analysis (environmental: O_2, the amount of emergent plants, SO_4, spatial: v1, v3, v11, v16, v21). A large amount of variation (70.5%) was not explained by the environmental and spatial variables.

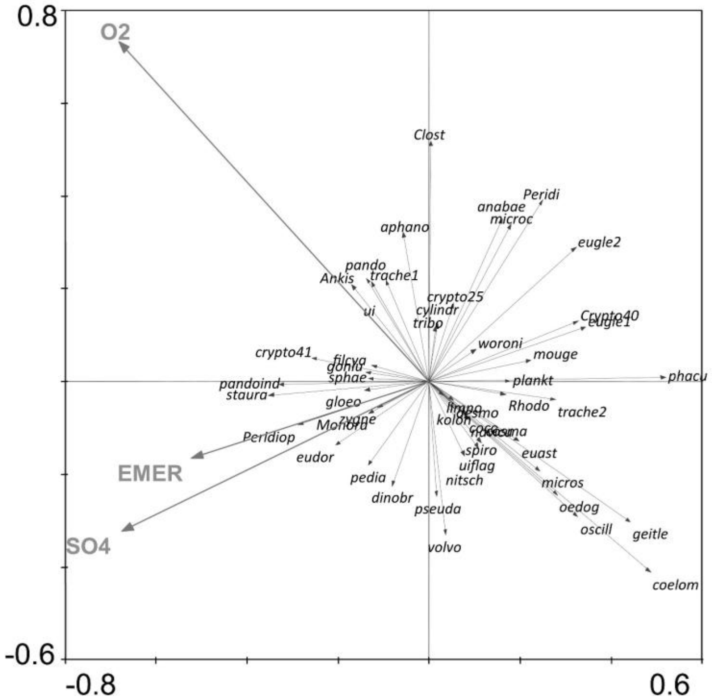

Figure 7. Redundancy analysis of the phytoplankton communities (O2 = oxygen; EMER = emergent vegetation; SO4 = sulphate). Only significant variables are drawn.

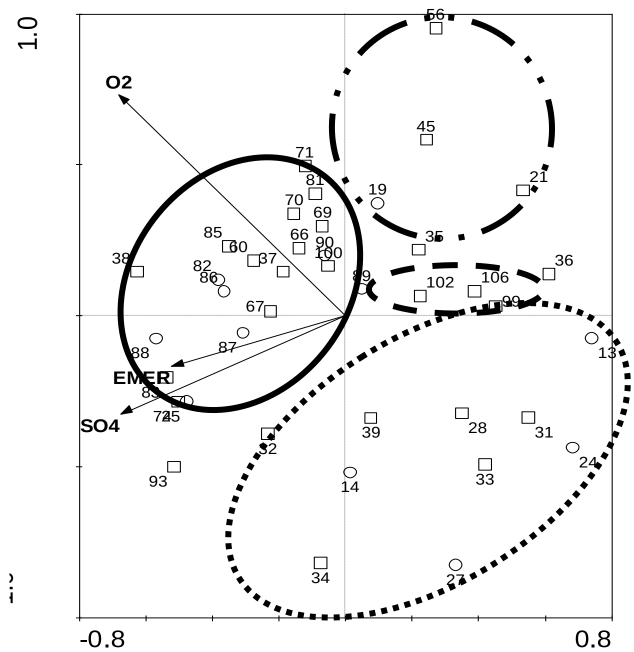

Figure 8. Correlation of environmental drivers of phytoplankton community structure and phytoplankton community structure itself with the spatial configuration of the ponds. Ponds have been clustered according to the spatial position. Solid line: ponds from Northeast area; Dotted line: Southeast area; Discontinuous line: West area; and Dotted-solid line: Centre area. Only significant variables are drawn.

Results of a variation partitioning of the bacterioplankton communities between significant environmental variables and spatial variables yielded 14% of total variance significantly explained by pure spatial variables, 18% by pure environmental variables, and 3% by common effects. A large amount of variation (65%) was not explained by the environmental and spatial variables (Fig. 10B). The environmental variables that significantly explained the bacterioplankton communities patterns were as follows: oxygen concentration, water volume, alkalinity, total nitrogen and the lack of vegetation (OPEN) (Fig.9).

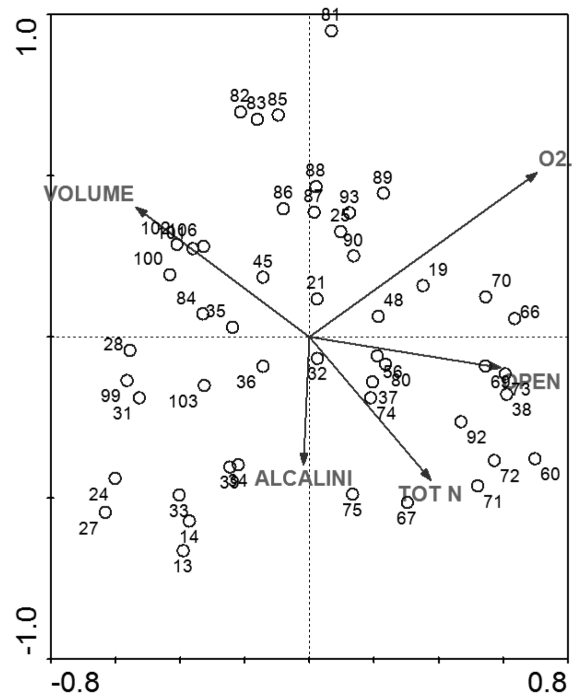

Figure 9. Biplot of a forward-selection redundancy analysis of the bacterioplankton community in the bomb crater complex Tommelen (O2 = oxygen; ALCALINI = alkalinity; TOT N = total nitrogen; OPEN = absence of water vegetation). Only significant variables are drawn.

The main environmental gradient structuring both the phytoplankton and bacterioplankton communities appears to be the spatially correlated eutrophication gradient present in the pond complex. Phytoplankton, associated with these eutrophied ponds, are euglenophyte and cyanobacterial taxa, typical of organically enriched and eutrophic water bodies, respectively. Moreover, the amount of sludge seems to limit bacterioplankton diversity. Also, clear-water ponds seem to have a higher diversity of these planktonic microbial organisms. Together, this suggests that management of the most eutrophied ponds aimed at reducing eutrophication would benefit their phyto- and bacterioplankton communities.

Spatial structure independent of environmental variation seems more important for the phytoplankton than for the bacterioplankton communities, possibly reflecting differences in dispersal capacities or in the strength of species sorting in these communities.

Shannon-Wiener diversity of the phytoplankton communities ranged from 0.25 to 3.1 (median 2.28). Pearson correlation analysis revealed that phytoplankton diversity was enhanced by the increase in transparency ($R^2 = 0.36$, $p < 0.05$) and by the amount of dead plant material ($R^2 = 0.32$, $p < 0.05$). The number of taxa in a local bacterioplankton community was restricted by the amount of sludge present ($R^2 = -0.36$, $p < 0.05$), while the presence of fish had a negative ($R^2 = -0.44$, $p < 0.05$) and a high temperature a positive influence ($R^2 = 0.34$, $p < 0.05$) on the Shannon-Wiener diversity.

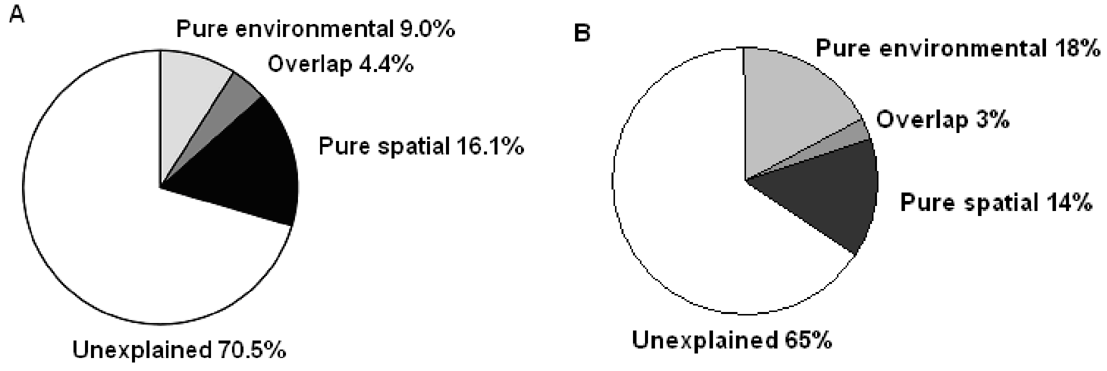

Figure 10. Results of a variation partitioning of the (A) phytoplankton and (B) the bacterioplankton communities between significant environmental variables and spatial variables.

Diversity partitioning of phytoplankton and bacterioplankton revealed that the beta diversity was relatively high (Table 3). For the phytoplankton, relative biomass values (%, multiplied by 100 to remove most commas) were used. Values still lower than 1 after multiplication by 100 were set to an artificial value of 1. Also for the bacterioplankton, relative abundances were multiplied by 100 to avoid using commas.

Table 3. Diversity partitioning of phytoplankton and bacterioplankton in ponds of the bomb crater complex Tommelen 2007 and associated *p*-values.

Dataset	Diversity	Species Richness	Simpson	Shannon
Phytoplankton % 40 samples	α	23.1 (1)	0.6836 (1)	1.671 (1)
	β	72.9 (0)	0.2552 (0)	1.577 (0)
	γ	96	0.9388	3.248
Bacterioplankton % 49 samples	α	11.2 (1)	0.845 (1)	2.122 (1)
	β	43.8 (0)	0.1015 (0)	1.312 (0)
	γ	55	0.9465	3.434

3. Phytobenthos

Overall, 319 taxa (including phenodemes) were distinguished, with an average of 58.3 ± 20.2 per sample (median 55.5; minimum 25, maximum 121). These represent at least 49 genera. The biraphid, mostly epipelic, genera *Pinnularia* (50 taxa) and *Nitzschia* (39 taxa) were most speciose, followed by the biraphid and mainly epiphytic genus *Gomphonema* (31 taxa). *Navicula* (23 taxa, 32 with inclusion of *Navicula* s.l.), *Sellaphora* (possibly 19 taxa), *Stauroneis* (17 taxa) and *Craticula* (13 taxa), all biraphid and motile, were also well represented, but only 10 centric taxa were observed.

The more abundantly represented taxa are listed in Annex V. *Achnanthidium minutissimum*, *Eolimna minima*, *Eunotia bilunaris, Gomphonema parvulum*, *Mayamaea atomus* var*. permitis*, *Nitzschia acidoclinata*, *N. palea* and *N. palea* var. *debilis* were nearly omnipresent. *Eolimna minima and Achnanthidium minutissimum* were represented by the highest numbers of individuals; *A. saprophilum*, *Eunotia bilunaris*, *Mayamaea atomus* var*. permitis*, *Navicula cryptocephala*, *N. s.l. arvensis*, *Nitzschia acidoclinata*. *N. archibaldii*, *N. palea*, *N. palea* var. *debilis*, *Planothidium frequentissimum* and *Sellaphora seminulum* reached a very high relative abundance in some ponds. Many of the more abundantly represented taxa are considered to be tolerant to organic pollution and severe eutrophication. Overall, there appear to be few sensitive species present.

219 taxa occurred in the counts and were included in exploratory ordination analyses (sqrt transformation). Species gradient lengths (DCA, detrending by segments, downweighting of rare taxa) were relatively short (2.1 and 2.2 SD for axes 1 and 2, respectively). RDA provided the best explanation of species-environment relations and forward selection suggested that a model including alkalinity (ALK, F = 8.0, $p \leq 0.001$), presence of fish (FISH, F = 2.5, $p = 0.004$), percentage of bare substrate (OPEN, F = 3.5, $p \leq 0.001$) and total nitrogen (TN, F = 2.3, $p = 0.005$) explained 1/4th of the variation in species composition ($R^2_{adj.}$ 0.26). Additionally, oxygen saturation (O_2%) was selected (F = 1.8, $p = 0.025$), but this variable was dropped after adjustment of α to multiple testing. The inclusion of FISH was rather unexpected. This variable serves as a proxy for regular inundation by water from the ditch at the south-eastern border of the reserve. Figure 11 shows how the selected variables correlate to the principle axes and other measured variables, demonstrating that diatom assemblage composition is influenced mainly by gradients in the concentration of dissolved minerals (axis 1) and vegetation abundance (axis 2). Higher vegetation abundance relates to lower nutrient concentrations, but also to larger dimensions. The latter appears to be associated with a lower ratio of marginal drying area to a permanently inundated centre, or stronger permanency, as suggested by field observations and the opposite vector for sulphate. Sulphate concentrations are likely to peak when oxidative soil conditions develop temporarily. The higher nutrient concentrations also observed in these ponds may result from lower vegetation biomass, allowing concentrations to remain high in the water column or more intensive mineralization, and do not result from flooding with nutrient-rich water. Alternatively, nitrogen could be associated to abundant leaf litter from the formerly adjacent poplar stand, but this seems unlikely given the prevailing wind direction and the distribution pattern of group 4 ponds.

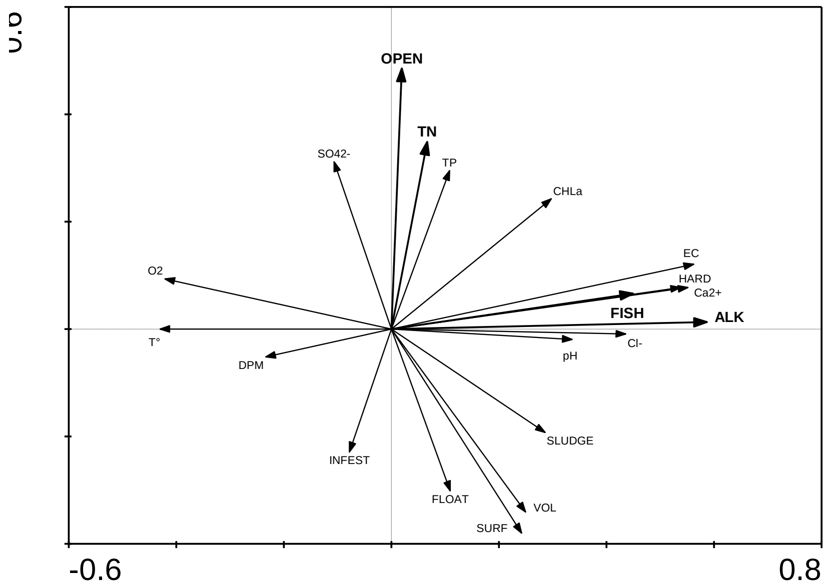

Figure 11. RDA ordination plot of selected environmental variables (bold) and passive variables with interset correlations ≥ 0.3.

More dilute, less alkaline ponds were characterized by *Gomphonema exilissimum*, various species of *Eunotia*, *Mayamaea* and *Pinnularia*, *Navicula s.l. arvensis* and *Nitzschia acidoclinata*, with *Gomphonema exilissimum* and *Nitzschia acidoclinata* growing better in vegetation-rich conditions (Fig. 12). *Mayamaea* spp. and *Eunotia botuliformis* were most abundant where larger areas of bare/drying substrate occurred. A larger number of taxa was typical of the ponds subjected to influx of mineral and nutrient rich surface water. Among these, *Achnanthidium saprophilum*, *Gomphonema gracile* and *Navicula cryptocephala* were associated with abundant vegetation and/or thicker sapropels, whereas species such as *Nitzschia amphibia* and the planktonic *Cyclostephanos invisitatus* and *Cyclotella meneghiniana* were found mostly in ponds with higher chlorophyll levels.

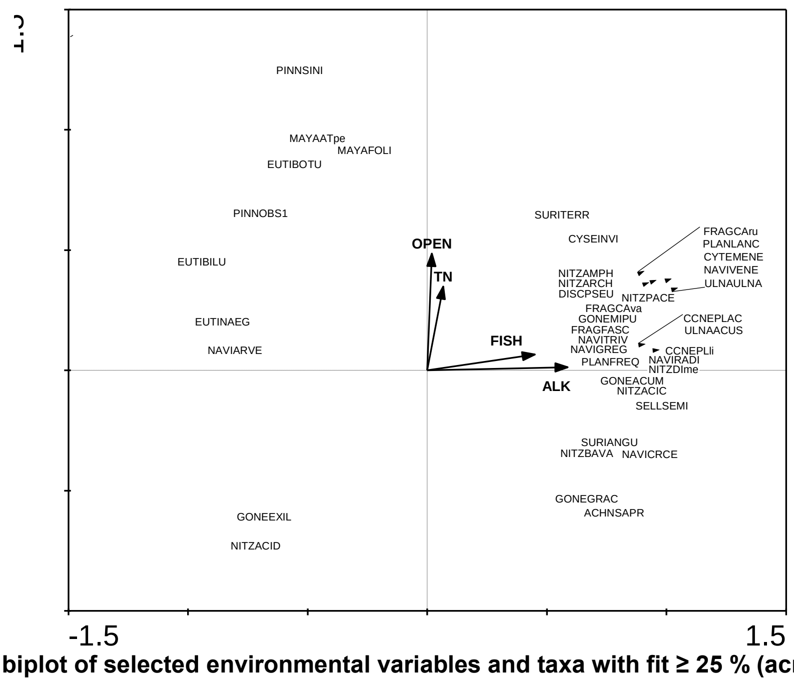

Figure 12. RDA biplot of selected environmental variables and taxa with fit ≥ 25 % (acronyms: FRAGFASC - *Fragilaria fasciculata*, GONEMIPU - *Gomphonema micropus*, NAVICRCE - *Navicula cryptocephala*, NAVIGREG - *N. gregaria*, NAVIRAD - *N. radiosa*, NAVITRIV - *N. trivialis*, NITZACIC - *Nitzschia acicularis*, NITZAMPH - *N. amphibia*, NITZBAVA - *Nitzschia bavarica*, SURRITERR - *Surirella terrestris*, ULNAACUS - *Ulnaria acus*, see Annex V and Table 5 for remaining ones).

The match between environmental similarity of ponds and their position in the RDA was checked by means of NMS ordination and clustering, using only the selected variables. Both indicated very similar groupings. Figure 13 shows the position of the four sample groups suggested by a two-dimensional NMS (stress 10.7 %, p = 0.01). The position of one sample (nr. 75) was awkward, indicating a discrepancy between environmental and species data; if left unconsidered, the groups were well-defined. Their position in the plot identified group 1 (8 ponds) as being more strongly influenced by inundation with (polluted) ditch water, group 3 (7 ponds; nr. 75 excluded) as moderately influenced, group 2 (5 ponds) as hydrologically isolated, but rich in nutrients and poor in vegetation and group 4 (28 ponds) as isolated, but rich in vegetation and spanning a gradient from more to less minerotrophic conditions, without suffering from eutrophication.

Table 4 describes the environmental conditions for these groups relative to the observed range, based on visual examination of percentile box-plots and between-group Kruskal-Wallis tests. In contrast to group 1, groups 2 and 4 were characterized by the absence of inundation by ditch water, as indicated by the lack of fish, low EC, chloride, hardness, etc. The position of group 3 was more intermediate. This concurred with the situation of most group 1 and 3 ponds in the south-western part of the reserve (a few ponds in these groups are close to the entrance), whereas group 2 occurred in the central part; group 4 ponds were distributed from the northwest to the northeast. The higher temperature and oxygen levels for groups 2 and 4 possibly relates to the somewhat smaller dimensions of these ponds.

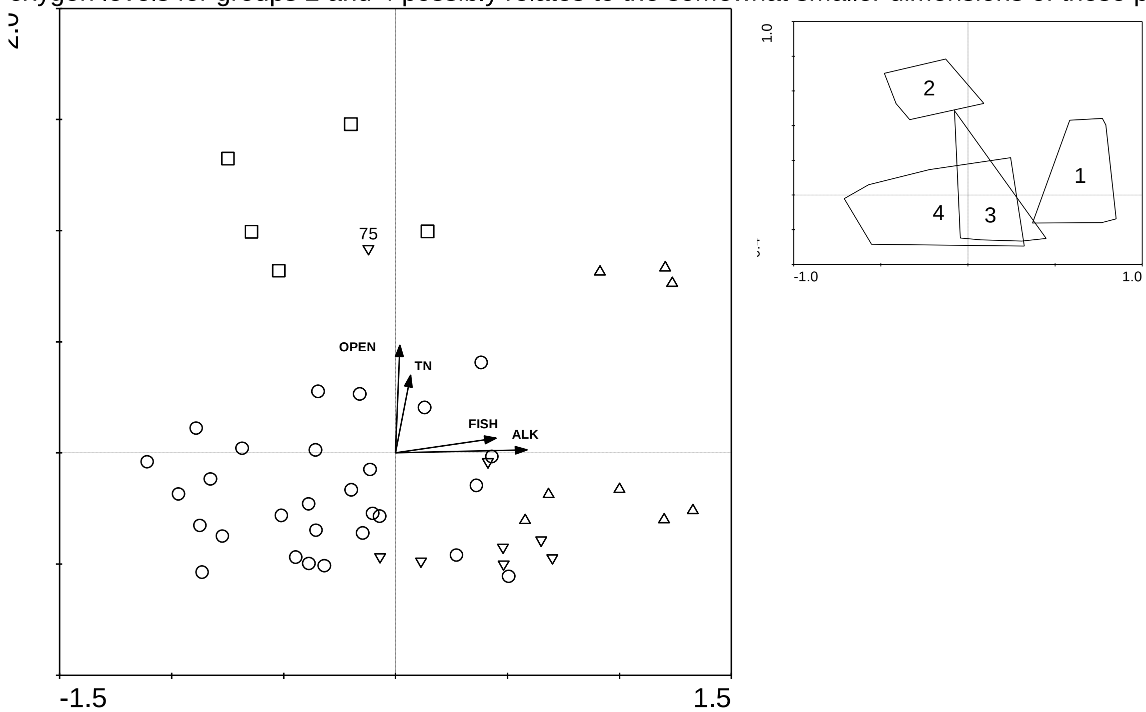

Figure 13. Left: RDA biplot of selected environmental variables and samples, with indication of NMS groups based on the environmental variables (group 1 upward triangles – rich in minerals, strongly influenced by ditch; group 2 squares – poor in vegetation, moderately influenced by ditch; group 3 downward triangles – rich in vegetation, moderately influenced by ditch; group 4 circles - rich in vegetation, slightly to moderately influenced by ditch). Right: *idem*, envelopes of the four groups.

Table 5 gives the results of the Indicator Species analysis for these groups (p ≤ 0.05, 4999 randomizations). Note that group 3 in particular presents high scores for subaerial taxa (*Mayamaea atomus* var. *permitis* and *Pinnularia* spp.), in agreement with the presumably lower water retention.

As expected, only ALK, TN and OPEN remained significant after exclusion of groups 1 and 3, with some effect of SLUDGE emerging in case only group 4 was retained (Annex VI). In these ponds, the amount of organic sediment correlated positively with vegetation and surface area.

Table 4. Environmental characteristics of the 4 assemblage-environment groups (exl. pond 75; only variables with significant difference between medians ($p < 0.05$, Kruskal-Wallis; - indicates a broad range).

variable	group 1	group 3	group 2	group 4
temperature	low	low	high	high
oxygen saturation	low	low	high	intermediate
EC	high	low	low	low
alkalinity	high	high	low	-
pH	high	intermediate	low	intermediate
hardness	high	high	low	low
calcium	high	high	low	intermediate
sulphate	low	low	high	low
chloride	high	high	low	low
chlorophyll	high	low	low	low
TN	low	low	high	low
TP	low	low	high	low
transparency	intermediate	low	intermediate	-
sludge	high	low	low	-
submerged	intermediate	high	low	-
floating	-	high	low	-
open	low	low	high	low
infestation	intermediate	intermediate	low	-
depth	intermediate	intermediate	low	-
surface	-	-	low	-
volume	-	-	low	-
fish	yes	no	no	no

Table 5. Selected indicator species for the 4 assemblage-environment groups (IndVal values; only taxa with IndVal ≥ 50 shown).

taxon	acronym	group 1	group 3	group 2	group 4
Hippodonta capitata		63	-	-	-
Discostella pseudostelligera	DISCPSEU	56	-	-	-
Cyclostephanos invisitatus	CYSEINVI	50	-	-	-
Encyonema vulgare complex		56	1	-	3
Gomphonema subclavatum		58	2	-	-
Fragilaria capucina var. *vaucheriae*	FRACAva	57	3	-	-
Nitzschia dissipata var. *media*	NITZDIme	52	6	-	1
Cocconeis placentula var. *euglypta*		51	6	-	1
Cocconeis placentula	CCNEPLAC	81	6	-	1
Navicula veneta	NAVIVENE	71	5	-	1
Surirella angusta	SURIANGU	68	9	-	-
Cyclotella meneghiniana	CYTEMENE	67	3	-	-
Neidium productum		64	4	-	10
Ulnaria ulna	ULNAULNA	63	8	-	-
Gomphonema acuminatum	GONEACUM	64	16	-	1
Cocconeis placentula var. *lineata*	CCNEPLIi	62	16	-	-
Planothidium lanceolatum	PLANOLANC	60	12	1	-
Craticula ambigua		59	12	-	-
Lemnicola hungarica		52	25	4	5
Achnanthidium saprophilum	ACHNSAPR	58	35	-	4
Sellaphora pupula urban elliptical		3	54	-	-
Achnanthidium minutissimum		17	51	2	30
Eunotia botuliformis	EUTIBOTU	-	-	73	4
Pinnularia sinistra	PINNSINI	1	-	89	6
Pinnularia obscura MT1	PINNOBS1	-	-	81	11
Pinnularia schoenfelderi		10	6	60	16
Gomphonema cymbelliclinum		4	-	57	16
Mayamaea atomus var. *permitis*	MAYAATpe	8	7	66	17
Eunotia naegelii	EUTINAEG	-	-	60	21
Eunotia bilunaris	EUTIBILU	4	4	59	32
Nitzschia acidoclinata	NITZACID	12	16	14	54
Gomphonema exilissimum	GONEEXIL	-	9	6	73

In spite of notoriously strong relations to habitat conditions, only a limited proportion of diatom assemblage composition could be explained by measured environmental data, and c. 2/3 of their variation remained unexplained, even with inclusion of spatial variables. There are several plausible reasons for this: intra- and inter-annual variation in habitat conditions, important variables that remained unaccounted for (cf. scale-issues for micro-organisms; Azovsky 2002), biotic interactions (with other diatoms, meiofauna, fungi, viruses,…), undetected diversity and noise in detecting rare taxa (although less here than in the majority of diatom studies), stochasticity… Among the measured variables, differences in alkalinity, nutrients, vegetation abundance and associated variables are the most influential habitat characteristics in the study area. This aligns well with the bulk of published observations on lakes and ponds as well as with the results of a previous survey of ponds throughout Belgium carried out within the MANSCAPE project (Denys 2008). In comparison with the more varied and on average larger water bodies in lower Belgium investigated by Denys (2006), however, the role of vegetation abundance in structuring sediment assemblages appears to be more prominent in the ponds of Tommelen. Most likely, this is due to the relative importance of suitable habitat for plant-associated taxa. Preliminary analyses identified a number of taxa as potential indicators to assess external influences on pond condition within the reserve. This can serve as a basis for developing a scheme that can be used to guide future management and evaluate its results.

A Mantel test of distance matrices for species (Bray-Curtis) and X-Y coordinates (Euclidean) indicated a significant positive relation between both ($r = 0.18$, $p \leq 0.001$, 999 randomized runs), so the influence of spatial configuration on assemblage composition was examined further. RDA with forward selection of the X-Y and P coordinates (retaining all variables at $p \leq 0.05$) suggested that Y and 4 Ps made up a minimal model explaining 18.3 % of the species variation in the entire data set (Annex VI). After accounting for the measured environmental heterogeneity, pure spatial configuration explained only c. 6 % of the variation (Annex VI). If variation partitioning was carried out with the ponds of groups 2 + 4, less of the total variation could be explained due to a decrease of spatially structured environmental variation (interaction term), but the unique spatial component remained unchanged. Less than 1/5th of the total variation could be explained for ponds of group 4 and no unique spatial effect remained, in case only subset-specific Ps were included. Similar analyses were made with the pond subsets, providing (A) only the overall P coordinates or (B) overall as well as all subset Ps as explanatory variables. More variation (c. 1/3rd of the total) could be explained than with the subset-specific or the overall Ps, only, if all possible configurations were included (C). In this case, and especially for the most similar ponds of group 4, the unique contribution of measured environmental variables declined and was even surpassed by the unique spatial component. *Achnanthidium minutissimum*, *Eolimna minima*, *Eunotia minor*, *Frustulia marginata*, *Gomphonema cymbelliclinum*, *G. exilissimum*, *Mayamaea atomus*, *M. atomus* var. *permitis*, *Lemnicola hungarica*, *Navicula cryptotenelloides*, *N. s.l. difficilima*, *N. s.l. pseudoarvensis*, *Nitzschia archibaldii*, *N. gracilis*, *N. palea*, *N. palea* var. *debilis*, *N. paleaeformis*, *Pinnularia subcapitata* var. *elongata*, *Stauroneis gracilis*, *S. muriella* and *Tabellaria flocculosa* were the most strongly related to the principal axes (fit ≥ 20 %) in this constrained ordination. Most of these taxa are motile and have a small cell volume, but other shared features are not very apparent. *Lemnicola hungarica*, for instance, is a specialized epiphyte on lemnids, and its spatial distribution probably reflects the occurrence of this substrate. Some of the other species are often reported as inhabiting periodic water bodies or subaerial habitats, whereas *Achnanthidium minutissimum* is notorious for its widespread distribution and opportunistic, weedy behaviour. If only the P coordinates for the entire set of ponds were taken into account (B), the amount of explained variation was intermediate to A and C for group 4 but similar to A with groups 2 +4. The relative proportions of the unique environmental and spatial components were intermediate between the least (A) and most complex (C) configurations.

The results of variation partitioning do not confirm that pond diatom communities develop independently from each other and merely reflect habitat suitability. They appear to suggest that the relative influence of nearby ponds on species composition increases as environmental conditions are more similar within a given set of ponds. If further analysis confirms that the observed differences between certain fractions of explained variation are statistically significant, this relation might be the result of distance and/or density-dependent dispersal rates (e.g. higher influx from nearby habitat patches) or other spatial dynamics,…). Elimination of groups 1 and 3 progressively shortens measured environmental gradients and also allows to discount dispersal by flooding water. However, true spatial information may still be confounded by unmeasured sources of environmental variation that significantly influence diatom distribution and are spatially structured. This study does not allow to dismiss this possibility. Yet, it is important to emphasize that the environmental gradients largely accounted for (i.e. alkalinity/pH, nutrients, vegetation abundance,…) are the ones that are widely recognized to influence the distribution of freshwater diatoms most. To explain our observations by environmental heterogeneity, other significant and independently structured environmental gradients – either in space or time (e.g. temporal variability, memory effects) – would need to be present. Given current knowledge, this does not appear more plausible *a priori* than the hypothesis of a dispersal-driven mechanism. Some of the taxa showing a more distinct relation to the spatial structure of the study area are also among those that are the most abundantly represented (*Achnanthidium* *minutissimum*, *Eolimna minima*) and the influence of mass effects would not be unexpected in this case. Evidence for the latter, however, requires quantifying diffusion and growth rates (Amarasekare 2003) and cannot be established from abundance data. Nevertheless, results from similar small-scale studies may possibly provide additional circumstantial indications for the importance of inter- vs. intra-patch processes and habitat quality. This would be important, because it would help to unravel whether certain pond configurations may be more or less appropriate to sustain maximum diversity of micro-organisms according to landscape properties.

Taxa distribution was significantly nested for the entire matrix ($NODF_{total}$ 34.5, $p \leq 0.001$ for Er and Ce); nestedness was greater among ponds ($NODF_{sites}$ 58.6.) than among taxa ($NODF_{taxa}$ 34.0). According to their *T*, 30.3 % of the taxa might be considered as idiosyncratic. *Eunotia botuliformis*, *E. naegelii*, *Frustulia marginata*, *Gomphonema anjae*, *G. cymbelliclinum*, *Hantzschia abundans*, *H. amphioxys*, *Luticola acidoclinata*, *Mayamaea fossalis*, *Navicula s.l. arvensis*, *N. s.l. difficilima*, *N. s.l. obsoleta*, *Pinnularia interuptiformis*, *P. obscura* MT1, *P. viridiformis* MT1 and *Ulnaria ulna* var. *angustissima* were the taxa with temperatures in the highest 5 %. A number of these are typically common in subaerial habitats (*Hantzschia* spp., Mayamaea fossalis, *Navicula s.l. obsoleta*, *Pinnularia obscura*) or widespread overall (*Pinnularia viridiformis*, *Ulnaria*). The degree of nestedness did not change if only the samples from groups 2 and 4 were considered ($NODF_{total}$ 34.8, $p \leq 0.001$; $NODF_{sites}$ 60.6, $NODF_{taxa}$ 34.3).

A few taxa occurred in nearly all ponds (Annex V), but most were found in only very few (57 % in 1 pond only; Fig. 14). Only 23 taxa attained a maximum abundance of more than 10 %, and the average abundance did not exceed 2 % for 96 % of all taxa (Fig. 14). All resulting frequency distributions were unimodal, suggesting a very high proportion of 'specialists' against very few 'generalists'.

Nestedness may have particular consequences in relation to conservation, for instance with regard to a relative independence of regional diversity from local habitat loss due to succession, management,… In addition to passive sampling, area, habitat nestedness and isolation are the factors commonly considered in explaining the colonization and extinction dynamics leading to nestedness (Gaston & Blackburn 2000). On the other hand, strong dispersion would tend to homogenize nested patterns. Although presumably widespread (cf. McAbendroth et al. 2005), the prevalence of nested patterns may have been considerably overestimated so far, due to methodological issues (Almeida-Neto 2008) and the few reports on its presumed occurrence in diatoms refer to stream communities at considerably larger geographical scales; truly comparable observations are unknown to us. The observation of significant nestedness in the Tommel data will allow to investigate possible correlates in more detail. Strong significance in comparison with the Er null model, which accounts for the probability of cell occupation on the basis of a taxon's total frequency, makes it unlikely that we are dealing merely with an effect of passive sampling. At the scale of the study area, nestedness does not seem to break down with increasing hydrological isolation. Nevertheless, idiosyncratic taxa make up a considerable proportion of the observed flora. It appears that at least part of them, i.c. the more drought resistant diatoms commonly found in soils, are taxa that are particularly well adapted to disperse by other means than water.

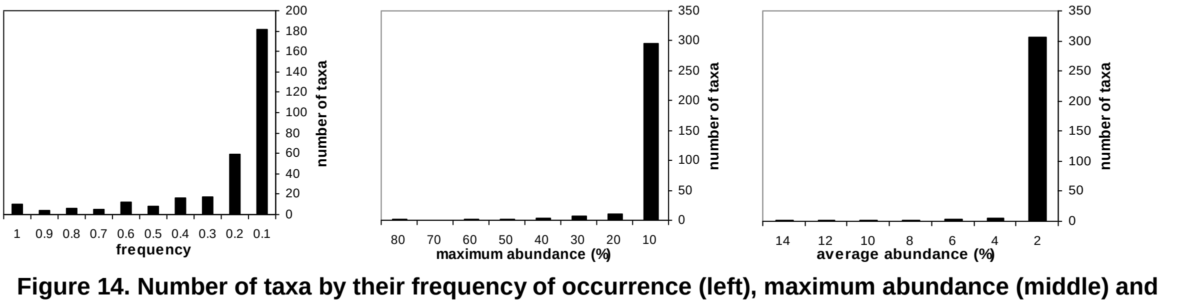

Figure 14. Number of taxa by their frequency of occurrence (left), maximum abundance (middle) and average abundance (right) in Tommelen.

Total taxonomic diversity was slightly higher for the 16 periodically flooded ponds (groups 1 and 3) than for the 33 hydrologically more isolated ponds (groups 2 and 4), but measures of structural γ diversity were very similar (Table 6). Diversity partitioning indicated a higher proportion of β (and consequently lower α) diversity than expected from a random distribution of individuals throughout. Taxonomic β diversity among ponds was almost 9 % higher for the more isolated ponds than for those experiencing flooding, but structural diversity was unaffected.

Somewhat surprisingly perhaps, taxonomic β diversity was not lower, even somewhat higher, for the more isolated ponds, which are less influenced by eutrophication and appear environmentally more homogenous, than for those with stronger hydrological connectivity to linear surface waters. The MANSCAPE data (unpublished) showed no difference in taxonomic β between more or less impacted ponds.

Table 6. Results of diversity partitioning (%; 10000 individual-based randomizations; all β values greater than expected, $p < 0.0001$; α diversity is complementary).

	all ponds		groups 1 + 3		groups 2 + 4		group 4	
	total (γ)	β (%)	total (γ)	β (%)	total (γ)	β (%)	total (γ)	β (%)
taxa	319	81.9	254	70.2	237	79.0	220	76.8
Simpson	0.99	10.1	0.99	10.4	0.99	10.7	0.99	9.8
Shannon	5.48	47.5	4.92	42.4	5.08	45.5	4.96	43.1

4. Macrobenthos

Screening of the samples revealed that chironomid larvae are present in high densities and species richness in the macro-invertebrate samples (compared to the benthos samples of the former Manscape project).

5. Macro-invertebrates

In total, 117 macro-invertebrate taxa have been found in the samples of the 49 ponds of Tommelen (data from spring and summer 2007). None of the taxa occurred in all sites. The most frequently observed macro-invertebrates are Chironomidae, which were found in all of the processed samples. More than half of the taxa were found in less than 20% of the sites, and 10% of the species were detected in only 1 site.
Ponds differ substantially in macro-invertebrate taxon richness, with a minimum of 20 and a maximum of 51 taxa (combined data from spring and summer 2007). At first sight, taxon richness is not associated with the location of the pond within the pond complex (Fig. 15).

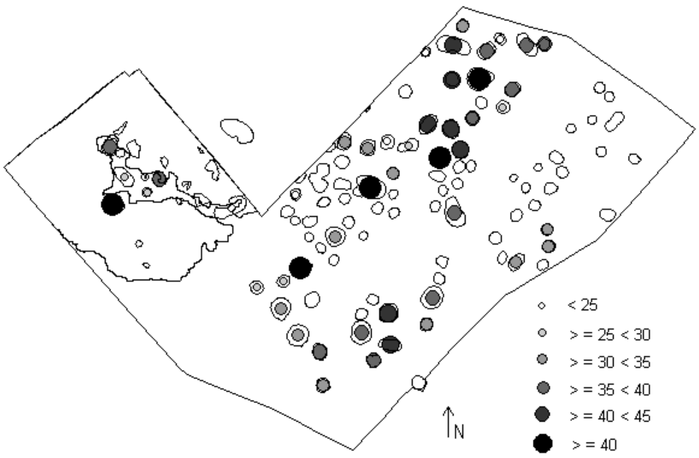

Figure 15. Map showing the ponds in the Tommelen pond complex, with indication of the number of macro-invertebrate taxa present.

To determine which environmental factors shape the trophic composition (predators, omnivores, filter feeders, detritivores, grazers and other herbivores), we performed redundancy analyses (Monte Carlo permutation tests on the summer 2007 data; Fig. 16). These analyses revealed that the amount of oxygen, the water temperature, the water transparency and the presence of fish contribute significantly to the explanation of the variation in trophic structure. Detritivores tend to be more abundant in ponds that contain fish, while grazers are associated with ponds that have higher temperatures and oxygen levels. These patterns were relatively weak, since the total amount of explained variation is 32%. The association between detritivores and the presence of fish is not surprising, since the ponds that contain fish were mostly located on a former ditch track that used to transport household water. Those ponds were also characterised by large amounts of sludge.
The average observed macro-invertebrate taxon richness in ponds (summer data; alpha: 22.8) was considerably smaller than expected based on random permutations of individuals (40.7). Beta diversity among ponds (41.2), on the other hand, was substantially larger than expected (23.3). Beta diversity still represented approximately 64% of the average total richness (gamma; 64) of the studied ponds.
A forward-selection redundancy analysis with the macro-invertebrate community as dependent variable and spatial variables as explanatory variables yielded seven spatial variables (X, Y, v2, v3, v5, v15 and

v24). The pure spatial factors (after correction for environmental variables) significantly explained 19.8% (F = 1.793; p = 0.001) of the variation in the macro-invertebrate community. Results of a variation partitioning of the macro-invertebrate community between the forward-selected spatial and environmental variables are summarized in table 7.

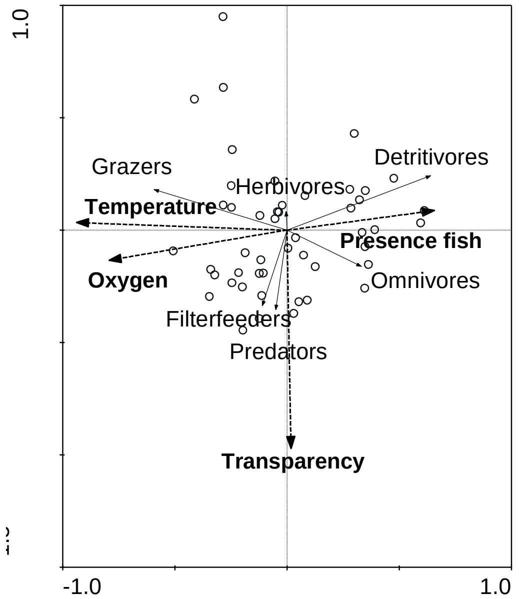

Figure 16. Visualisation of a forward-selection redundancy analysis on the macro-invertebrate community in the bomb crater complex of Tommelen. Only the significant environmental variables are drawn.

Table 7. Variation partitioning of the macro-invertebrate community matrix into different components and associated *P*-values. The components are [E + S] total explained variation; [E] variation explained by environmental variables; [S] variation explained by spatial variables; [E I S] pure environmental variation; [S I E] pure spatial variation; [E ∩ S] explained variation shared by the environmental and spatial data; 100 - [E + S] unexplained variation.

Variation	Variation explained	*P*-value
[E + S]	46.4	0.001
[E]	27.9	0.001
[S]	30.2	0.001
[E I S]	17.6	0.001
[S I E]	19.8	0.001
[E ∩ S]	10.4	-
100 - [E + S]	53.6	-

6. Zooplankton

Overall, 19 cladoceran species were distinguished, with an average of 5 species per sample (minimum 1, maximum 9). *Simocephalus vetulus*, *Chydorus sphaericus, Ceriodaphnia reticulata* and *Scapholeberis ramneri* were the most prominent cladoceran species with regard to overall relative abundance. Cyclopoid copepods occurred in all samples, whereas calanoid copepods were only observed sporadically (four ponds). A number of cladocerans (e.g. *Alona quadrangularis*, *Alonella nana*, *Oxyurella tenuicaudis* and *Daphnia ambigua*) were found in less than three sites. The average number of cladoceran species per pond was not exceptional, but comparable with the average species richness that was found in the surveyed set of ponds of the MANSCAPE-project.

Multiple regression results with a diversity index (species richness/Shannon-Wiener) as dependent variable and forward selected environmental variables as independent variable revealed a negative effect of the percentage of shadow (beta = -3.3; p = 0.002) and a positive effect of water transparency (beta = 2.9; p < 0.01) and the percentage of open water (beta = 2.1; p < 0.05) on zooplankton species richness. Shannon-Wiener diversity was positively related with the degree of open water (beta = 0.45; p = 0.004), submersed vegetation (beta = 0.34; p = 0.03) and water transparency (beta = 0.24; p = 0.08). These most parsimonious models explained respectively 27.2% and 15% of the variation in the diversity index. Our results indicate that cladoceran diversity was not only determined by the presence of aquatic vegetation, but also by the availability of open water. The important role of submerged macrophytes in ponds on the aquatic invertebrate community structure and diversity, and on the total food web in general, is already well discussed in the literature (e.g. Jeppesen et al. 1998, Scheffer, 1998; Van Donk et al. 2002). The fact that also the presence of open water is included as an important additional variable could reflect the importance of the successional stage of the pond. Ponds that lack open water are often filled in to a large extent with sludge ($r = -0.37$, $p < 0.01$) and infested with a lot of vegetation ($r = -0.50$ $p < 0.001$). These habitat conditions are inferior for a number of pelagic zooplankton species like *Daphnia* ($r = -0.31$, $p = 0.02$).

The average observed cladoceran species richness in ponds (alpha: 4.7) was smaller than expected based on random permutations of individuals (7.3). In contrast, beta diversity among ponds (14.3) was substantially larger than expected (11.7). Beta diversity represented approximately 75% of the average total richness (gamma; 19) of the studied ponds.

The main environmental variables influencing the zooplankton community composition as identified by a forward-selection redundancy analysis are maximum depth, density of *Hydra*, a zooplankton predator, the concentration of total phosphorus and the degree of infilment (i.e. the relative degree of desiccation) (Explained variation = 21.3 %; $F = 2.96$; $p = 0.001$; Fig. 17). Zooplankton community structure was hence mainly governed by structural pond characteristics, like the maximal depth and the degree of infilment, but also by total phosphorus, which is often associated with the potential primary productivity of freshwater systems (Chase & Ryberg, 2004). Furthermore, the presence of predators, like *Hydra*, appeared to have an important effect on the community structure. Conform to the experimental results of Schwarzt & Hebert (1989), we found a negative relationship between the density of *Hydra* and *Daphnia* ($r = -0.31$, $p = 0.02$) and a positive between *Hydra* and *Simocephalus* ($r = 0.30$, $p = 0.03$). The reason for this is because *Simocephalus* spends most of its time attached to substrates, contrary to the more active *Daphnia* species, which have a higher risk of encountering the sessile predator, *Hydra*. The effect of Hydra on the zooplankton community remained significant (5.7%; $p = 0.01$) after controlling for the effect of floating vegetation, which was related to the density of Hydra.

No linear gradient in the data was found. A forward-selection redundancy analysis with the zooplankton community as dependent variable and the 29 spatial variables as explanatory variables yielded only three spatial variables (v4, v10, v20). These variables explained 18.4 % ($F = 3.4$; $p = 0.001$) of the variation in the zooplankton community. Results of a variation partitioning of the zooplankton community between the forward-selected spatial and environmental variables are summarized in table 8.

Table 8. Variation partitioning of the zooplankton community matrix into different components and associated *p*-values. The components are [E + S] total explained variation; [E] variation explained by environmental variables; [S] variation explained by spatial variables; [E I S] pure environmental variation; [S I E] pure spatial variation; [E ∩ S] explained variation shared by the environmental and spatial data; 100 - [E + S] unexplained variation.

Variation	Variation explained	*P*-value
[E + S]	34.5	0.001
[E]	21.3	0.001
[S]	18.5	0.001
[E I S]	16.1	0.002
[S I E]	13.3	0.001
[E ∩ S]	5.2	
100 - [E + S]	65.5	

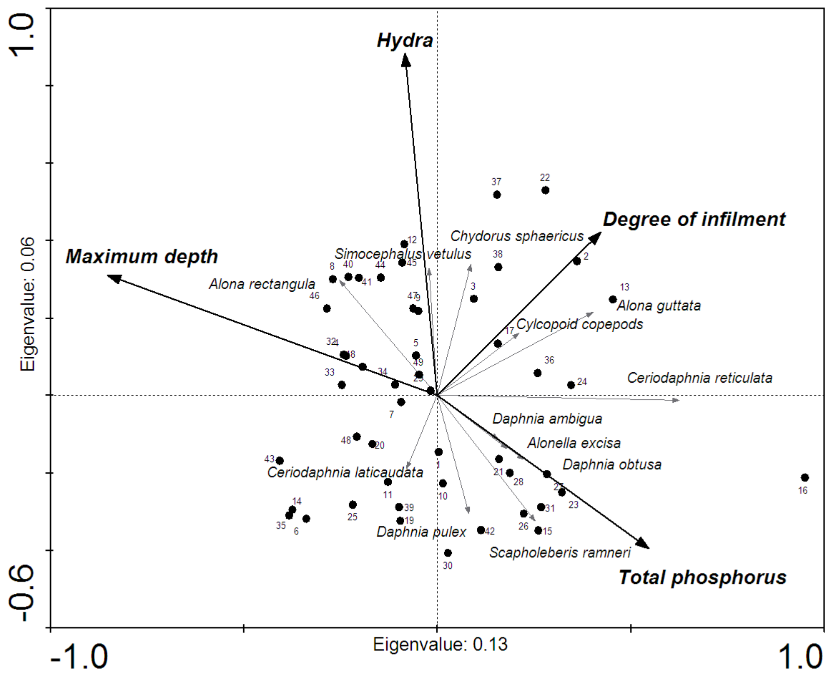

Figure 17. Biplot of a forward-selection redundancy analysis of the zooplankton community in the bomb crater complex Tommelen. Significant variables are drawn and include maximum depth, total phosphorus, the degree of infilment and the density of *Hydra*, a zooplankton predator. The different ponds are indicated with a black dot.

7. Amphibians

In the selected ponds of Tommelen we found in total seven species of amphibians: three newts (*Triturus cristatus*, *Triturus alpestris* and *Triturus vulgaris*), *Rana esculenta*, *Rana ridibunda*, *Rana temporaria* and *Bufo bufo*. Two spin off projects will deal with the conservation genetics of selected species of rare Amphibia in Belgium and Luxembourg (student theses at Brussels, starting September 2008).

The presence of fish appeared to be the most important variable in predicting the densities of the salamander larvae, although this relation was rather weak (trace = 4.6%; F-ratio= 3.39; p = 0.047). This can be explained by an increased predation risk in ponds containing fish compared to fishless ponds. However, the ponds that contain fish in Tommelen are largely located on the former ditch track which used to transport household water. Those ponds are also characterised by large amounts of sludge and high water turbidity. The combination of these factors can explain low densities of amphibians.

The average amphibian species richness within ponds (alpha: 3.6) was similar to what was expected based on random permutations of individuals (4.1). Also beta diversity among ponds (2.4) was only slightly higher than what was expected (1.9). Beta diversity consists of about 40% of the total richness (gamma).

Table 9. Variation partitioning of the amphibian community matrix into different components and associated P-values. The components are [E + S] total explained variation; [E] variation explained by environmental variables; [S] variation explained by spatial variables; [E I S] pure environmental variation; [S I E] pure spatial variation; [E ∩ S] explained variation shared by the environmental and spatial data; 100 - [E + S] unexplained variation.

Variation	Variation explained	*P*-value
[E + S]	26.9	0.003
[E]	11.1	0.001
[S]	25.5	0.001
[E I S]	1.4	> 0.05
[S I E]	15.8	0.001
[E ∩ S]	9.7	-
100 - [E + S]	73.1	-

A forward-selection redundancy analysis with the amphibian presence absence data as dependent variable and spatial variables as explanatory variables yielded five spatial variables (X, Y, v2, v4 and v25). The pure spatial variables (after correction for environmental variables) explained 15.8% (F = 3.230; p = 0.001) of the variation in the amphibian community. Results of a variation partitioning of the amphibian community between the forward-selected spatial and environmental variables are summarized in table 9.

8. Macrophytes

Floristic, as well as structural, the water and transitional vegetations are poor. Widespread species, such as the hydrophytes *Lemna minor* and *Potamogeton natans*, and the helophytes *Glyceria fluitans* and *Eleocharis palustris* are both most frequent and most often in a dominating position. More interesting species, such as *Utricularia australis* and *Hydrocharis morsus-ranae*, as well as the liverwort-with-a-macrophytic-appearance *Riccia fluitans*, occasionally reach predominant presences. Most surprising, however, is that lots of species only occur very locally, e.g. in one or two ponds (such as *Carex rostrata*, *C. vesicaria*, *Hottonia palustris*, *Hypericum elodes*, *Lemna gibba*, *Equisetum fluviatile*, *Hippuris vulgaris*, *Sparganium emersum*, *Schoenoplectus lacustris*, *Typha latifolia*). We have some evidence that at least some of those have been deliberately introduced in the past. The presence of the alien neophytes *Ludwigia grandiflora* and *Sparganium latifolium* do enforce this hypothesis: both anecdotic information and their situation in the field demonstrate a wilful introduction. On the other hand, those cases also illustrate the high degree of isolation between the ponds mutually. After their introduction, the neophytes and introduced native species did not spread at all or very poorly. The presence of one unit of Galloway-cattle cannot be considered as a successful vector for dispersal. These findings are in accordance with the diversity partitioning analyses (alpa, beta and gamma). The average observed open water vegetation taxon richness in ponds (alpha: 3.4) was similar to what was expected based on random permutations of individuals (2.8). Also beta diversity among ponds (17.8) was relatively high, but similar to what was expected (18.2). Beta diversity consists of approximately 84% of the total richness (gamma; 21) of the studied ponds. The sub-habitats open water vegetation and vegetation from the shallow marshy transition zone together contain in total 32 species of vascular plants (gamma diversity, aquatic mosses not included). When also the plants of the wet embankment zone (also in direct contact with the pond water, and being the preferential zone for many amphibians) are taken into consideration, 60 vascular plant species were found (excluding water mosses).

Lemna minuta, a rather recently discovered and invasive neophyte in Belgium was mentioned for Tommelen by local botanists, but could not be observed. We stated that in many of its contemporary pond-populations *L. minor* plants were very small and we therefore suppose that some identification in the past should be reconsidered. The problem was studied in some more detail and material of the Tommelen *Lemna*'s was compared to material of different *Lemna*-species from elsewhere in Flanders.

Structurally, especially the water vegetation was poorly developed. Only *Potamogeton natans* was present as a submerged aquatic plant, limiting the number of submerged plant life forms to one. *Hottonia palustris* (life form of the myriophyllids) was only present in two ponds and with extremely poor numbers. Floating plants at and near the water surface (respectively *Lemna*'s, *Hydrocharis morsus-ranae* and *Riccia fluitans*) very often dominated the water vegetation adding only one or two more life forms to the total spectrum. Near the ponds' shores helophytes created some additional structural habitat differentiation.

Table 10. Variation partitioning of the macrophyte community matrix into different components and associated P-values. The components are [E + S] total explained variation; [E] variation explained by environmental variables; [S] variation explained by spatial variables; [E I S] pure environmental variation; [SI E] pure spatial variation; [E ∩ S] explained variation shared by the environmental and spatial data; 100 - [E + S] unexplained variation.

Variation	Variation explained	*P*-value
[E + S]	35.53	0.001
[E]	21.14	0.001
[S]	19.94	0.001
[E I S]	15.60	0.001
[S I E]	14.40	0.001
[E ∩ S]	5.54	-
100 - [E + S]	64.46	-

The following variables were significantly associated with the open water vegetation communities (presence absence data, same data were used as for the diversity partitioning; CCA; trace = 21.1%; F-ratio= 2.682; p = 0.001): pH, maximum water depth, amount of sludge and cyanobacteria (Fig. 18).

A forward-selection redundancy analysis with the water vegetation community (presence absence data) as dependent variable and spatial variables as explanatory variables yielded four spatial variables (X, Y, v2 and v13). The pure spatial variables (after correction for environmental variables) explained 14.4% (F = 2.01; p = 0.001) of the variation in the macrophyte community. Results of a variation partitioning between the forward-selected spatial and environmental variables are summarized in table 10.

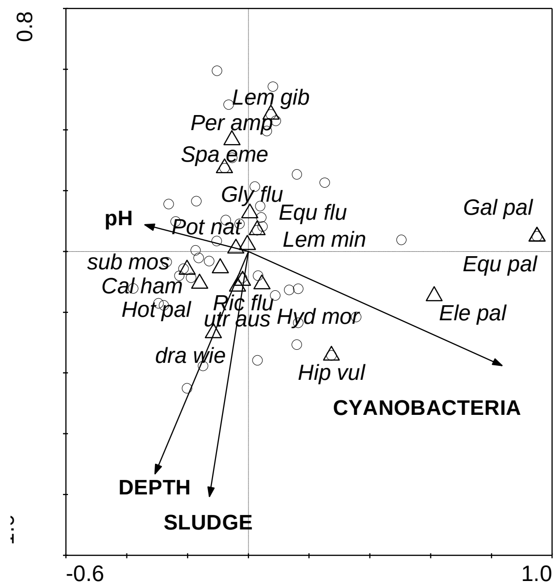

Figure 18. Visualisation of a forward-selection redundancy analysis of the water vegetation community in the bomb crater complex Tommelen. Ponds are visualized by circles, species by triangles.

10. General analyses

An overview of the relative contribution of alpha & beta diversity to gamma diversity based on taxon richness is presented in Figure 19. For several groups, beta diversity was higher than expected based on random permutations of individuals. This indicates that taxa that are present in one pond, are often absent in other ponds and vice versa.

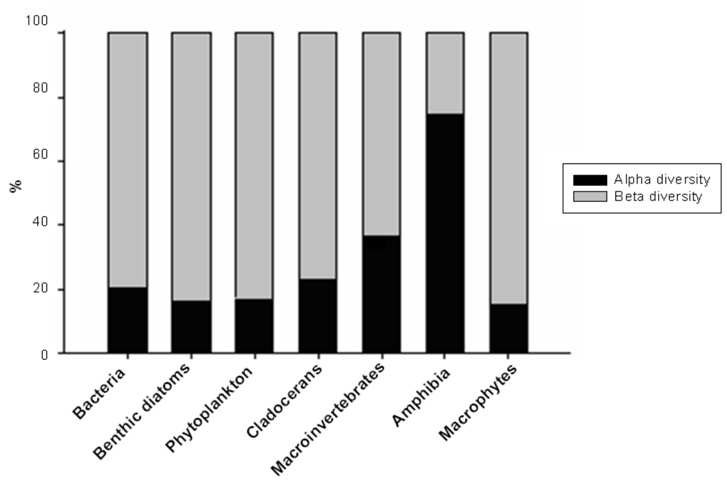

Figure 19. Relative contribution of alpha and beta diversity (based on taxon richness) of the investigated taxonomic groups in the bomb crater complex of Tommelen.

A general multivariate analyses on the taxon richness of all investigated groups, indicated that the presence of fish, the amount of total phosphorous and the conductivity of the water significantly affected the general richness of the ponds in Tommelen (RDA; trace = 36.2; F-value = 6.25, p = 0.001). Eutrophication hence possibly plays an important role in these ecosystems (negative association with most of the groups). The presence of fish was in our dataset associated with the connectivity between ponds. This suggests that, in terms of taxon richness of most investigated groups, digging trenches between ponds is probably not a good conservation strategy. Further investigations on this dataset, as well as in WP4, will reveal more concerning this topic.

For the investigated groups, we have partitioned the observed variation in the pond communities into four components: (1) variation explained by gradients in the local environmental conditions, (2) spatially explained variation (3) spatially structured environmental variation and (4) unexplained variation. For most of the investigated groups, there was a significant pure environmental effect, which suggests that factors such as species sorting take place in the investigated systems. For the phytoplankton and amphibian communities there was no significant pure environmental effect (due to intercorrelation with the spatial effects). For all the groups, we did find significant spatial relations, which could result for processes such as dispersal limitation, priority effects, sink source dynamics, but this could also be caused by hidden environmental variables or stochastic processes. We can not assess the relative contribution of these alternative mechanisms, but there is evidence that dispersal limitation is probably not of major importance in this pond complex, at least for some of the studied organism groups such as the zooplankton, since the distance between ponds is small and migration rates are expected to be high.

WP2: Biodiversity and pond age

Task 2.1: Selection of ponds belonging to different age classes (P2)

The age of about 70 ponds has been assessed.

Task 2.2: Sampling and sample analysis (C, P2, P3)

As in tasks 1.1 & 1.3. Sampling was conducted in the summer of 2008. Analyses are ongoing.

Task 2.3: Data analysis (C)

This is postponed to phase 2.

WP3: Biodiversity and ecosystem functioning

Task 3.1: In situ and ex-situ assessment of microbial ecosystem functions (IP)

3.1.1. Microbial activities at Tommelen

Among the 40 ponds analysed for microbial activities, the hourly primary production ranged from 3.7 to 419.5 µgC.l^{-1}.h^{-1}. The bacterial production ranged from 0.06 to 56.29 µgC.l^{-1}.h^{-1}, the nitrification rate from 0.01 to 6.35 µgN.l^{-1}.h^{-1} and microbial respiration from 7.5 to 213.8 µmolO2.l^{-1}.h^{-1}.

Spatial maps of bacterial production and primary production are presented in Figure 20 and 21. There is no clear spatial separation of productive and unproductive ponds. However, the most productive ponds from the primary production point of view are generally those with the highest bacterial production.

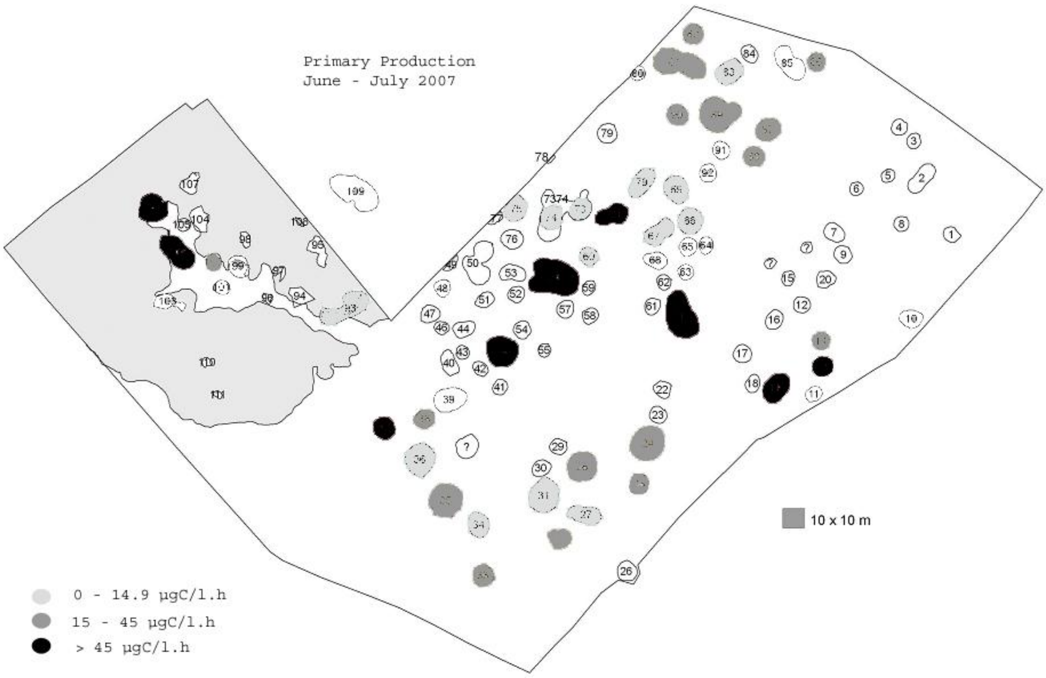

Figure 20. Map of primary production measured in June-July 2007 in a selection of 40 ponds at Tommelen.

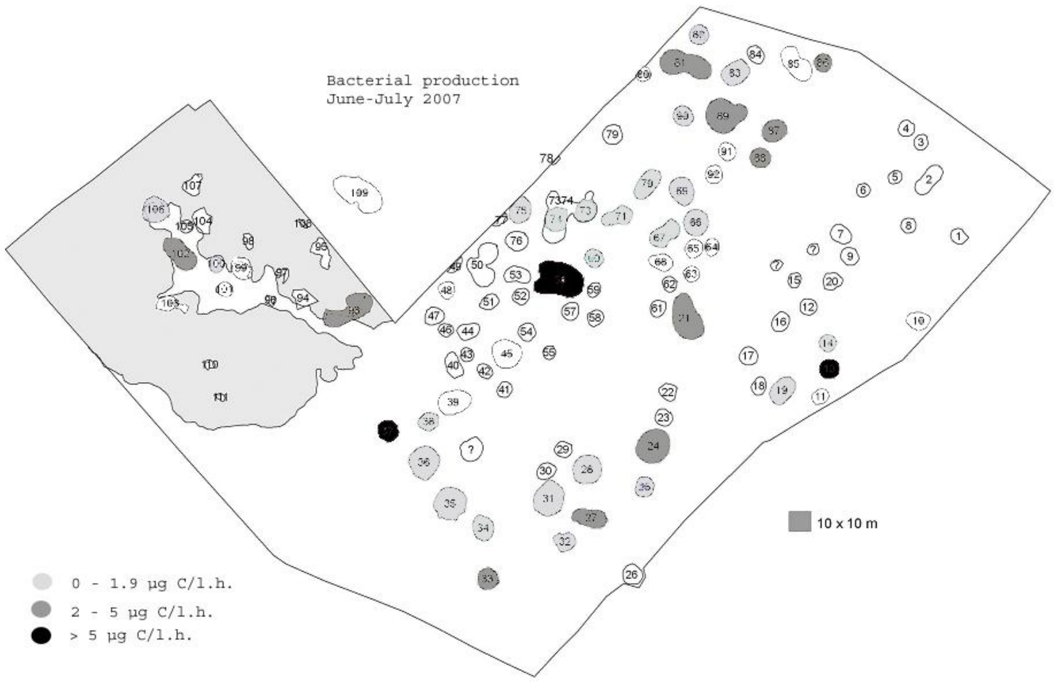

Figure 21. Map of bacterial production measured in June-July 2007 in a selection of 40 ponds at Tommelen.

Primary production, secondary bacterial production and nitrification rate presented right-skewed distributions while respiration values were normally distributed. Some data were thus transformed prior to statistical analyses in order to fit normal distributions. A separation of the 40 sites in Tommelen was possible using a Principal Component Analysis. The resulting correlation biplot is shown in Figure 22. The first principal axis strongly discriminated sites with low activities (e.g. 69, 70 and 83) from those with high activities (e.g. 37 and 45). Primary production, bacterial production and nitrification rate appeared to be highly inter-correlated while respiration appeared to be independent from the other variables.

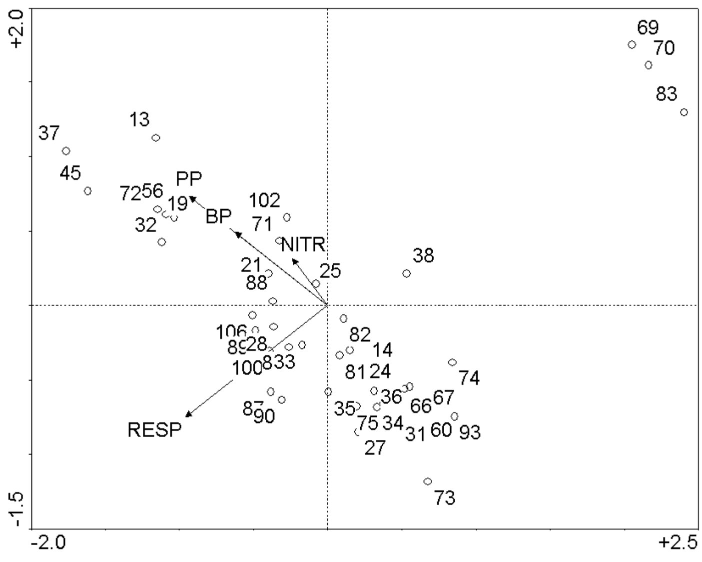

Figure 22. Correlation biplot of the Principal Component Analysis of the microbial activity dataset. PP = primary production, BP = bacterial production, NITR = nitrification rate, RESP = respiration rate. Principal Axis 1 = 63.5% of explained variance, principal axis 2 = 23.6% of explained variance.

3.1.2. Impact of dredging on the microbial activity at Tommelen

The microbial activities have been monitored in 23 ponds in autumn 2008. Fourteen ponds have been dredged in October 2008 (ponds 11, 14, 27, 33, 38, 39, 67, 68, 71, 82, 84, 85, 86, 103). Vegetation has been re-inoculated in one of this dredged pond (pond 103). Nine ponds were sampled and served as controls (ponds 10, 13, 19, 34, 45, 70, 83, 87, 102). Total phosphorus and total nitrogen were also measured in the ponds. Results are compiled in Table 11.

Table 11. Water temperature, total Nitrogen (TN) concentration, total Phosphorus (TP) concentration, microbial respiration, primary production and nitrification rate in a selection of ponds at Tommelen in November 2008.

Pond number	Sampling date	Temperature °C	TP ppm	TN ppm	respiration $\mu molO_2.l^{-1}.d^{-1}$	Primary production $\mu gC.l^{-1}.h^{-1}$	Nitrification $\mu gN.l^{-1}.h^{-1}$
10	5-Nov-08	8.2	1.00	4.11	115.6	0.0	0.000
11	5-Nov-08	8.2	0.10	1.39	55.3	26.6	0.012
13	6-Nov-08	7.8	0.42	4.33	74.8	66.9	0.148
14	6-Nov-08	8.1	0.16	2.33	0.0	42.6	0.042
19	6-Nov-08	7.5	1.07	4.71	20.0	7.7	0.229
27	4-Nov-08	10.9	0.13	1.68	28.8	25.1	0.046
33	4-Nov-08	8.4	0.36	2.62	0.0	54.2	0.080
34	4-Nov-08	9.1	1.09	4.30	53.2	224.2	0.076
38	4-Nov-08	10.7	0.24	2.69	0.0	59.7	0.065
39	4-Nov-08	9.7	0.10	1.36	57.3	21.3	0.003
45	6-Nov-08	7.8	0.08	1.71	71.4	65.5	0.006
67	6-Nov-08	8.2	0.28	2.27	40.7	117.2	0.022
68	6-Nov-08	8.3	0.27	2.10	60.8	86.1	0.032
70	6-Nov-08	8.2	0.12	1.69	39.0	24.4	0.045
71	6-Nov-08	8.4	0.11	1.37	23.0	38.1	0.014
82	5-Nov-08	8.4	0.16	1.23	28.1	117.4	0.046
83	5-Nov-08	7.9	0.20	2.64	54.9	9.6	0.070
84	5-Nov-08	8	0.31	1.86	44.5	78.4	0.037
85	5-Nov-08	8.6	0.08	0.85	20.3	17.5	0.031
86	5-Nov-08	8.4	0.22	2.00	37.0	111.9	0.050
87	5-Nov-08	7.9	0.12	1.07	28.7	44.8	0.083
102	4-Nov-08	9.6	0.16	1.40	0.0	128.0	0.033
103	4-Nov-08	7.9	1.12	4.46	23.2	257.2	0.016

For 16 ponds, data on microbial activity and physico-chemistry are available for both Year 2007 and Year 2008. As observed in most water bodies, total phosphorus and total nitrogen concentrations varied significantly between the field spring-summer and the autumn campaigns. This is mainly the consequence of the seasonal variation of biological activity. The dynamics in each pond appear however to be quite different from the others. Interesting information is that the behaviour of dredged ponds did not differ significantly from that of the control ponds. Primary production in these ponds was most of the time notably higher in autumn 2008 from that measured in early summer 2007 (Fig. 20 and 23). This can be the result of a decrease in the predation by phytoplankton as temperature decreased.

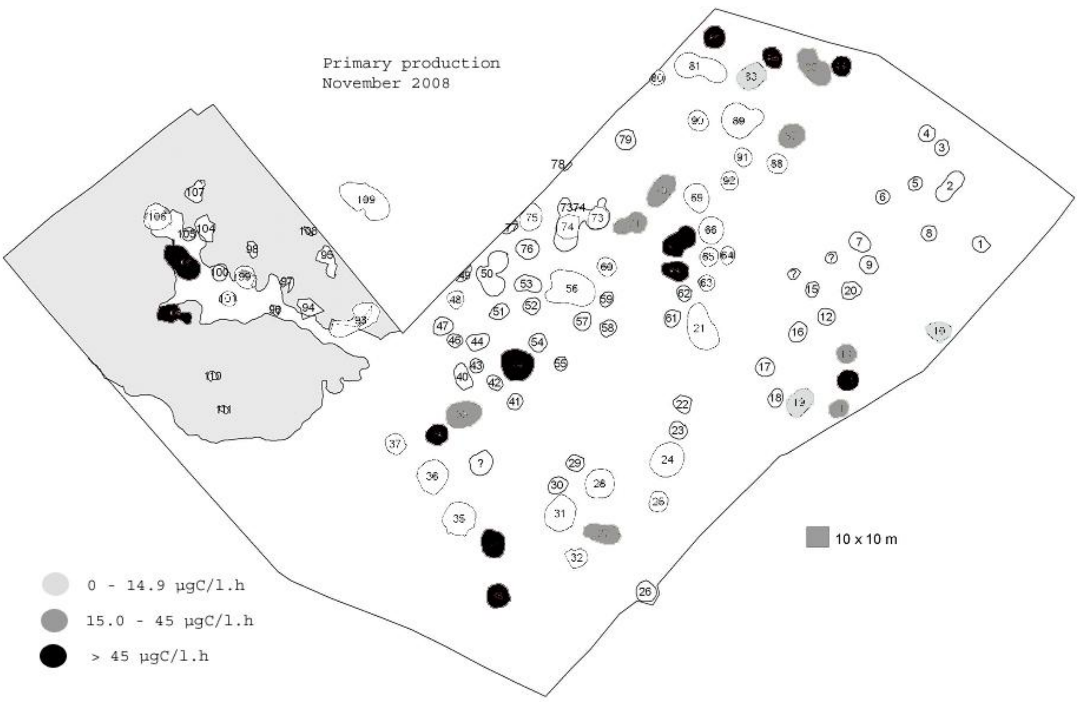

Figure 23. Map of primary production measured in November 2008 in a selection of ponds (see text for details) after dredging.

Microbial respiration values were significantly lower in November 2008 compared to June-July 2007, except in two "control" ponds (70 and 83). This decrease in respiration is certainly linked to differences in temperature. Samples taken in June-July 2007 presented water temperatures of 17.3°C on average when samples taken in November 2008 presented water temperature of 8.5°C. Respiration is known to be highly temperature-dependent (del Giorgio & Williams, 2005) and is therefore most probably not adequate to monitor the effect of management techniques at different seasons. To allow comparisons between the respiration of the ponds before and after management, the microbial respiration will be measured once again in Spring 2009 in the selected Tommelen ponds.

3.1.3. Multi-scale survey (2008)

Three ponds were sampled and analysed for microbial activities in each of the 25 pond clusters. Among the 75 studied ponds, the hourly primary production ranged from 1.5 to 7,264.9 $\mu gC.l^{-1}.h^{-1}$. The bacterial production ranged from 0.38 to 39.00 $\mu gC.l^{-1}.h^{-1}$, the nitrification rate from 0.01 to 4.21 $\mu gN.l^{-1}.h^{-1}$ and microbial respiration from 1.0 to 239.2 $\mu molO2.l^{-1}.h^{-1}$. These ranges are similar to those observed in Tommelen, except for primary production that displayed some extremely high values in some ponds included in the multi-scale survey.

An exploration of the relationships between microbial activities and physico-chemical variables (water temperature, pH, conductivity, dissolved oxygen concentration, total nitrogen concentration, total phosphorus concentration, dissolved inorganic carbon concentration, chlorophyll a concentration) has been conducted using Redundancy Analysis. Temperature, total nitrogen and total phosphorus concentrations as well as chlorophyll a concentrations appeared to be environmental variables with a significant correlation with the microbial activities ($p<0.05$, Monte-Carlo Permutation Test, CANOCO). Temperature was subsequently used as covariables. The resulting triplot is displayed in Figure 24.

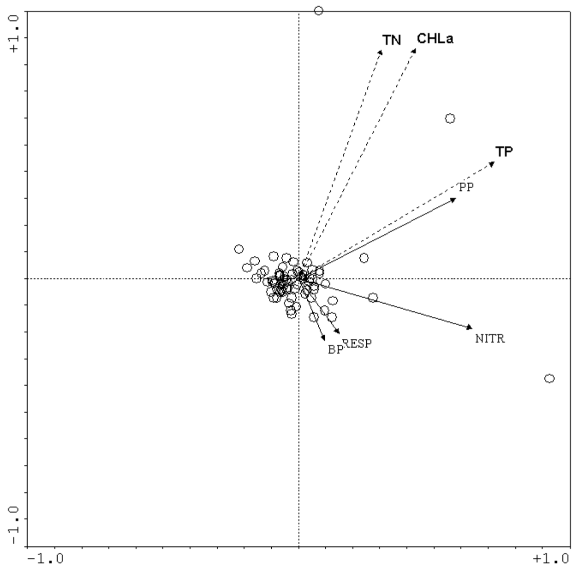

Figure 24. Triplot showing relationships between microbial variables (primary production: PP, bacterial production: BP, microbial respiration: RESP, nitrification rate: NITR) and total nitrogen concentration (TN), total phosphorus concentration (TP) and chlorophyll a concentration (CHLa). Percentage variance of species-environment relations: axis 1 = 71.4%, axis 2 = 20.2%.

Planktonic primary production appears to be highly related to total phosphorus, that ranged from 0.05 to 15.05 $mgP.l^{-1}$, and to a lesser extent to total nitrogen. Similarly to what has been observed in Tommelen at a different spatial scale, phytoplankton production and community composition appears to vary significantly according to eutrophication gradients. On the contrary, at the regional scale, bacterial production and microbial respiration are not correlated with primary production while it was the case in Tommelen. The often observed link between primary production and bacterial production has not been detected in the multi-scale survey dataset. This indicates that the decomposition processes are not only dependent on the primary production of the plankton but certainly depends in a large part on the primary production of the macrophytes and on the input of organic material from the outside of the ponds.

Task 3.2: Data analysis (P3, IP)

3.2.1. Relations of microbial activities with the taxonomic diversity and the physicochemical characteristics of the ponds at Tommelen

The relationships between the microbial activities and the other biotic and abiotic variables have been investigated using multivariate analyses (Principal Component Analysis – PCA and Redundancy Analysis – RA). Microbial activities appeared to be significantly associated to a very limited number of biotic variables. The relationship between the activity and the taxonomical diversity of the algae and the bacteria was analysed using redundancy analyses with a forward selection of the significant variables. Only the primary production appeared to be significant correlated with the algal species ordination (using phytoplankton relative abundance, $F = 1.874$, $p = 0.016$; using phytoplankton absolute abundance, $F = 1.929$, $p = 0.013$). The composition of the algal community appeared thus to change along the primary production gradient. Nitrification rate and bacterial production appeared to be significantly correlated with the absolute as well as relative abundance of detritivores. Such correlation suggests that the bacterial production and nitrification rates could be increased in the presence of detritic organic material that favours detritivores. The exact nature of the interaction has to be ascertained with experiments that are beyond the scope of the present project.

The operational taxonomical units detected using DGGE were also analysed. Sites were not well separated using a Principal Component Analysis (total variance explained by the two first axis = 22.2%) nor with a non-metric multidimensional scaling (stress = 0.24). A selection of the "environmental" variables best explaining the activity pattern was also determined by maximizing a rank correlation between their respective similarity matrices based on normalised Euclidian distances (BIO-ENV procedure, PRIMER software). Chlorophyll a concentrations, dissolved oxygen concentration, pH and sulphate concentrations appeared as the variables that explained the best the variations in the microbial activity dataset. The Spearman correlation between this subset of environmental variables and the microbial variables remained however very low (Spearman rho=0.18). To sum up, the microbial activities were highly inter-correlated and discriminated very well the sites. No clear relationship was established with the physico-chemical variables or with the bacterial diversity. On the contrary, the algal community changed in a significant way along the primary production gradient.

WP4: Assessment of management techniques

Task 4.1: Inventory of planned management by 3rd parties and pond selection (P2)

A list of planned management practices in ponds is being compiled based on interviews with stakeholders and conservators.

Task 4.2: Sampling and sample analyses (P2, P3)

14 ponds in Tommelen were dredged in October 2008. These ponds and a number of control ponds were sampled for biotic and abiotic characteristics in the summer of 2007 and in spring 2008. Also just before (summer 2008) and right after (autumn 2008) the dredging samples were taken, to look at the communities still present after this management practice.

Task 4.3: Data analysis: evaluation of pond management practices (P2)

This is due during phase 2.

Task 4.4: Evaluation of management practices as a tool for increasing beta and gamma diversity

The evaluation is planned for phase 2.

WP5: Pond biodiversity, management and pollution

Task 5.1: Evaluation of pesticide pollution (P4)

Pesticide loads and seasonal variations Water was sampled three times over the year 2007 in fifteen small ponds selected in Walloon Region and Flanders in accordance with the average peak periods of

pesticide pulverisations by farmers and other users.
Only isoproturon was observed at high levels in the selected ponds. This herbicide was present over the various periods of the year either at low concentrations or at high peak levels, especially in October (Table 12). These peak levels were comparable among the types of ponds (Figure 25) since isoproturon herbicide is often used anywhere in agricultural areas and other landscape sectors. For six of the sampled small ponds, peak levels were close to values reported for surface water in Belgium and other European countries (Region Walloon, 2005-2007). The presence of peak levels of isoproturon observed in the selected ponds also corroborates with different reports that this herbicide is one of the problematic pesticides in surface water in spring and autumn in Belgium and other European countries. High accumulation of isoproturon in water may interfere with the welfare of aquatic living animals since high level of isoproturon can affect the detoxication enzymatic system in tadpoles of various amphibian species (Greulich et al., 2002).
Glyphosate and its surfactant AMPA were detected in 33 water samples collected from eleven small ponds, but levels were very low (concentrations closely to the detection limits of the GC-MS determinations). Both for glyphosate and AMPA, levels were less than 10 ppb whatever the sampling period and the type of pond, so any peak period could not be identified. High concentrations of glyphosates have been found in pond environment of some European and American countries with deleterious effects on aquatic living animals, namely disturbance in the metamorphosis process, tail damages, endocrine disruption, and gonad abnormalities (Howe et al., 2004, Brausch and Smith, 2007, Dinehart et al., 2009). The long term-effects of low concentrations of glyphosates and their surfactants are not well described. This issue will be investigated in the ecotoxicological protocol planned during the second phase of the project.

Table 12. Levels of isoproturon in water sampled from ponds located in intensive or semi-intensive agricultural areas and in natural reserves.

Type	Pond	Month	[ng/L]
Intensive	Diest-Tielt-Winge	October	17 ± 12
	Zoutleeuw	October	3810 ± 1235
	Molenbeek	October	628 ± 345
	Morialmée-Manège	October	1256 ± 158
	Sorée A	April	1541 ± 786
	Sorée C	October	154 ± 66
Semi-intensive	Focant A	April	897 ± 456
	Morialmée 2	October	569 ± 42
	Moriaz 2	October	2130 ± 230
	Focant B	July	897 ± 176
Natural	Zand Straat	October	3607 ± 2033
	Tommelen	October	36 ± 32
	Driestlinter	October	11 ± 11
	Morialmée 3	April	1189 ± 79

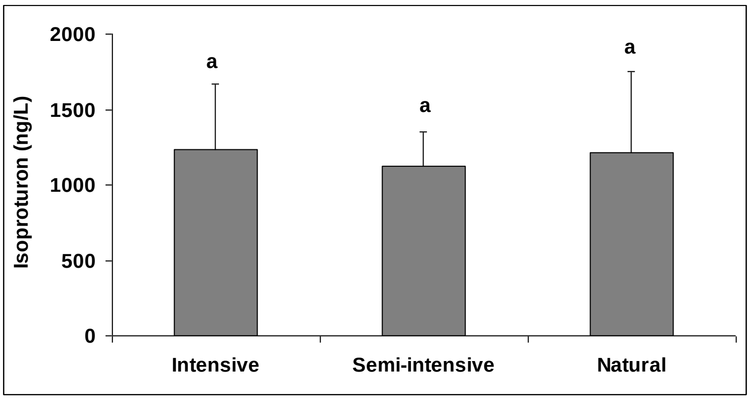

Figure 25. Mean levels of isoproturon in water sampled from intensive agricultural use (n = 6), semi-intensive (n = 4) and natural (n = 4) small ponds.

Except for the presence of isoproturon and glyphosate herbicides, no other pesticides could be identified in all the selected small ponds. Therefore, these results confirmed the previous survey on 125 small ponds during the Manscape project that the Belgian water ponds do not display high levels of triazine herbicides. It is interesting to note that other widely relevant pesticides, such organo-chlorinated and phenolic compounds as well as HAPs are not released in the pond environment. In fact, such compounds are widely used as insectides, fungicides or co-factors of various types of products. They are nowadays found at high concentrations in other water surfaces (Region Wallonne, 2007), and some of them are often associated with a high risk of physiological failures including endocrine disruption for most the aquatic living animals (Lintelmann et al., 2003; Jobling et al., 2006).

Occurrence of estrogenicity a. In vivo test The occurrence of in vivo estrogenicity activity was evaluated by two sensitive physiological indicators, namely the induction of hepatic vitellogenin (VTG) evidenced by plasma vitellogenin level, and the brain aromatase activity in male juvenile fish.
Both for female and male fish, plasma VTG levels (Figure 26) were similar to values already reported for juveniles fish reared in circulating water system by our previous study (Spano et al., 2004). As expected, VTG values were markedly higher in juvenile female fish than in males (Figure 26). Except lower values in one of the five selected ponds, VTG levels in females were comparable between ponds without abnormal elevated VTG levels (Figure 27). No abnormal increase in VTG level was also observed in male fish (Figure 28) confirming the lack of estrogenicity incidence in all the fish from the selected ponds.

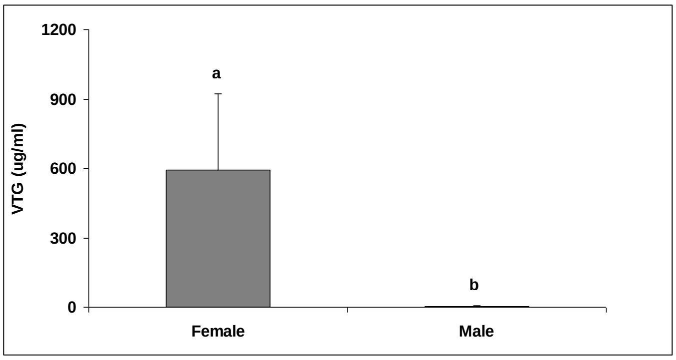

Figure 26. Plasma VTG levels in juvenile females (n = 25) and males (n = 24) of *Carassius auratus* collected in intensive or semi-intensive ponds.

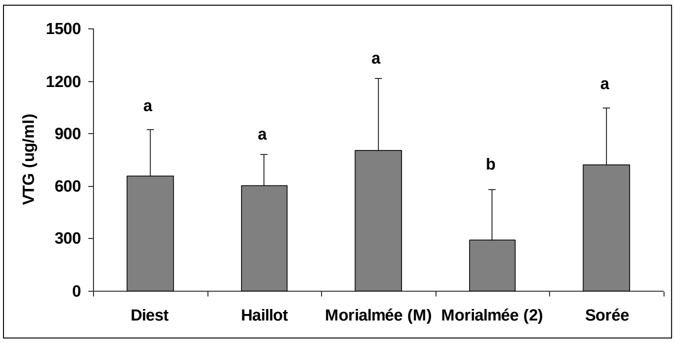

Figure 27. Plasma VTG levels in juvenile females (n = 5) of *Carassius auratus* collected in intensive or semi-intensive ponds.

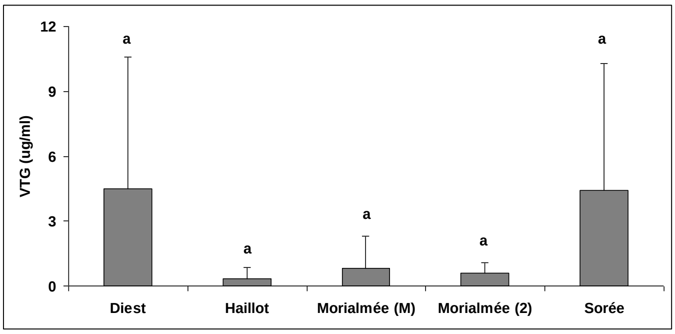

Figure 28. Plasma VTG levels in juvenile males (n = 4-5) of *Carassius auratus* collected in intensive or semi-intensive ponds.

The lack of estrogenic occurrence evidenced by the normal synthesis of VTG was confirmed by the brain aromatase activity. Indeed, aromatase activity was markedly lower in males than in females (Figure 29), as expected. As for VTG level in female fish, aromatase activity was comparable among the selected ponds and no abnormal values were observed for females (Figure 30). Only four males of the 30 tested male fish displayed relatively higher aromatase activity, but lower than values observed in females (Figure 31).
The occurrence of increased estrogenicity is often associated with an increased ratio of intersexes in fish populations. Such effect was not observed in the selected ponds since only two intersexes were observed of a total of 60 collected fish (3.3%). That abnormality may be related to intrinsic features of the fish populations, not to the presence of estrogenic compounds, even if high VTG (317-809 µg/ml) and aromatase (19-23 fmol/mg prot/min) levels in the two intersexes were similar to values observed in female fish.

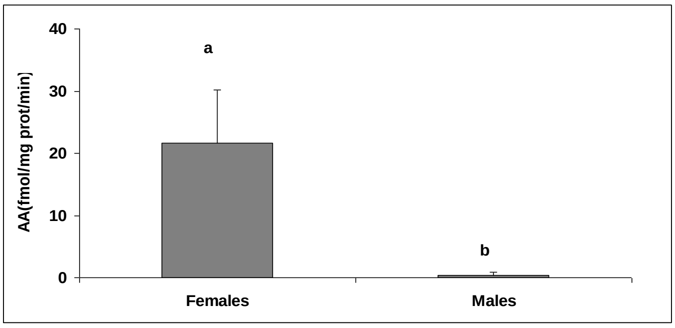

Figure 29. Brain aromatase activity in juvenile females (n = 30) and males (n = 30) of *Carassius auratus* collected in intensive or semi-intensive ponds.

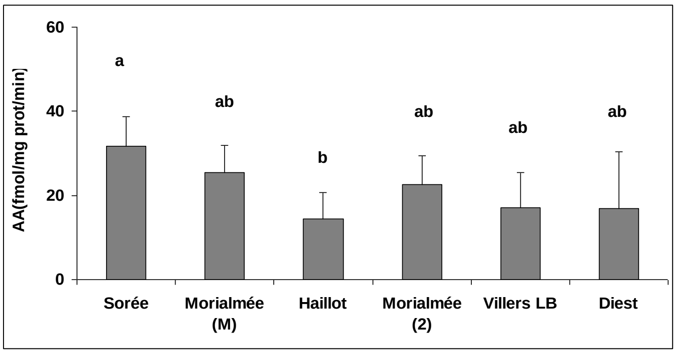

Figure 30. Brain aromatase activity in juvenile females (n = 5) of *Carassius auratus* collected in intensive or semi-intensive ponds.

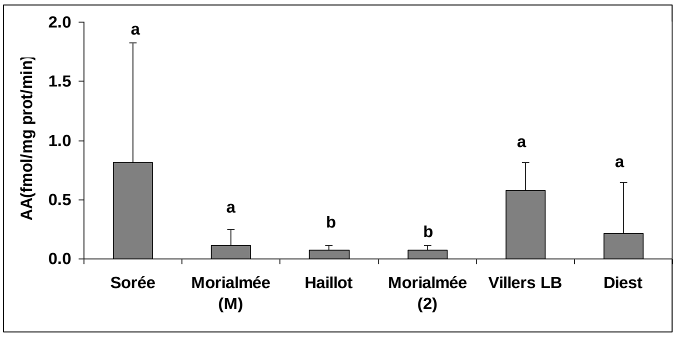

Figure 31. Brain aromatase activity in juvenile males (n = 5) of *Carassius auratus* collected in intensive or semi-intensive ponds.

b. In vitro test The first step for the evaluation of the in vitro estrogenicity was to determine the levels of estrogenic compounds in water sampled from the selected ponds. This evaluation was concerned with the fifteen ponds sampled three times in 2007 and with the six ponds sampled twice in 2008. No estrogenic compounds were observed whatever the period of sampling and the pond. Therefore, water samples were not assayed for in vitro cell proliferation. The absence of estrogenic compounds and low levels of glyphosates confirm the result of in vivo test which demonstrated no estrogenicity occurrence in juvenile fish collected in six selected ponds.

WP6: History of social and economic relevance of ponds to stakeholders

Task 6.1: Evolution of social and economic relevance of pools (P4)

Origins The origin of a pond can be natural, for example when water accumulates in depression. It can also be created by the change in the course of rivers caused by erosion and leaving in the landscape old meanders as ponds. Another natural phenomenon is the emergence of small cups by collapse of the basement after the dissolution of carbonates in the marls. It is a typical phenomenon from the Lorraine area, and ponds are there called "mardelles".

Nevertheless, the main origin of ponds is anthropogenic. Many ponds are the work of men. It can be either through direct action, i.e. by creating water point for a specific use, or through an indirect action, i.e. caused by an action of man on the environment, resulting in the unintentional appearance of a pond in the landscape (extraction activities or bombing, for instance).

First man-made ponds probably appeared during the Neolithic period when human beings settled down and began to develop the first primitive forms of agriculture. Ponds were used as a solution for water supply (for their own consumption, for watering their livestock) in areas where water was absent or scarce.

Uses The various uses of ponds can be divided into 3 main categories. (1) First, ponds played a role in the domestic life of people. In the absence of other sources of water nearby (river source...), men used ponds as reservoirs of water for household tasks: cooking, cleaning, laundry. (2) Ponds for agricultural purposes represent another type: drink for cattle, duck ponds (breeding of ducks and geese). (3) Ponds were also used as water reservoirs in case of fire and were used in craft industry (water reservoir for mills, fish ponds).

Evolution Until the 19th century, ponds were appreciated mainly in rural areas. They started to loose their value after the emergence of a new concept: hygiene (1848). At this period, public health development was of main importance because of the emergence of many epidemics (a.o. cholera) across Europe. Ponds then suffered from hygiene campaigns aiming at removing all stagnant water, which was supposed to be the vector of many diseases. Afterwards, in the early 20th century, distribution of running water expanded in villages and removed some uses of traditional ponds. After the 1950s, agricultural development and intensification (increase in livestock density, reallotment) and habitat extension let fewer places for ponds in the landscape.

The high sensitivity of these elements makes quantification of their disappearance almost impossible. Those who have tried have been able to establish local losses are between 50 and 90% between the late 19th century and now.

Fortunately, after many recent studies pointing out their ecological, hydraulic and also their educational interest, there is currently a renewal of interest for ponds, supported by increasing environmental awareness in the population.

Cartography Analyses of NGI maps at four different time periods turned out to be not so accurate. Indeed, maps conception evolved through time and gained continuously in precision from the first map until now. Ponds being sensible and dynamic elements of our landscape, they couldn't be caught as precisely as now in the first maps. Even with the help of satellites and imagery, ponds are still difficult to grasp. Thus, ponds evolution analysis through NGI maps were abandoned after some attempts on representative areas.

However, NGI maps analysis can bring really interesting results if analyses focus only on particular types of ponds that are man-made and/or depend on human activities. This is the case for moated sites in Western Hainaut and for clay extraction sites in Condroz. Moated sites are ditches surrounding farms and medieval castles. These structures provided defence for seigniories, watering places for cattle and sometimes were used as fish ponds. Condroz is well-known for its clayey subsoil which was exploited for a few hundred years. Subsoil extraction of clay creates soil subsidence that was filled up naturally with water. Finally many ponds coming from these activities appeared in the condrusian landscape.

These sites, thanks to their social and economics importance, were precisely mapped in the first NGI (MCI at this time, for Military Cartographic Institute) maps. It is easy to study their evolution through time, as we can observe below (Fig. 32).

For these particular types of pond we can observe, thanks to the maps analysis, contrasting influences of man on ponds. Moated sites (•), after they lost their traditional use, were progressively badly-kept and abandoned by man. In consequence, we observe a continuous decline in density since their first appearance on NGI maps. Oppositely, we observe an increase in the density of ponds (**x**) coming from clay extraction. Indeed, this activity of extraction survived until the middle of the 20th century. After the cessation of activities, exploited areas were abandoned and are filled with water, creating important pond networks in the area. This tends to prove the large influence, positive as well as negative, of man on small water bodies.

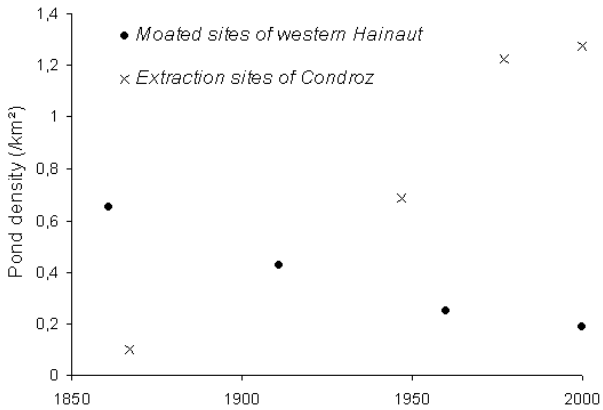

Figure 32. Ponds life history of two different areas in Belgium.

Task 6.2: Analysis of present day perception of social and economic value of ponds by different sectors (P4)

Semi-interviews have just been done for Flanders. Analyses have started but are not yet completed. The first step of classification of speeches into categories is already done, but a sharp analysis still needs to be made in phase 2.

Task 6.3: Infectious capacity of the Lymnaeidae intermediate hosts of *Fasciola hepatica* in small aquatic surfaces and epidemiological impact on fasciolosis in Belgium (P4).

One hundred twenty five ponds and sixteen other Lymnaea biotopes were sampled. Eight thousand eight hundred eighty five (8885) snails were collected. Snails were found in 77 ponds (61.6%). The four genera were found (Fig. 33): 4545 *Radix* sp. (51%), 2557 *Galba* sp. (*G. truncatula*) (28.7%), 1038 *Stagnicola* sp. (11.6%) and 745 *Lymnaea* sp. (*L. stagnalis*) (8.3%). The genus *Radix* is the most represented genus in this sampling campaign.

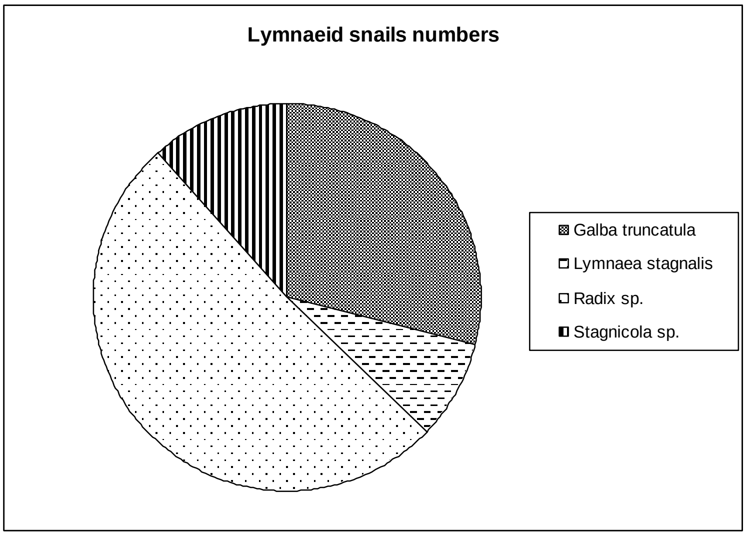

Figure 33. Lymnaeid snails numbers.

We can see (Fig. 34) that except *Stagnicola* sp. in ecoregion B, all the 4 genera were present. *Galba truncatula* was principally sampled in ecoregions C, D et E (83.2%) as well as *Stagnicola* species (97.8%). Most of the *Radix* species were found in Polders (57.2%). *Lymnaea stagnalis* was more or less evenly represented in the different ecoregions.

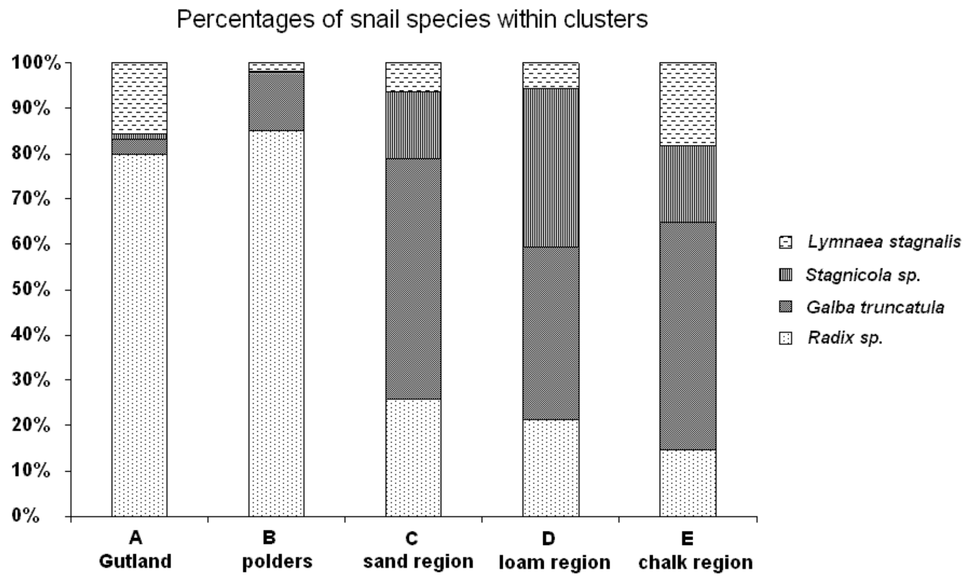

Figure 34. Lymnaea snail numbers in the five investigated ecoregions (Gutland, polders, sand region, loam region and chalk region).

WP7: Valuation of results, policy measures, management recommendations

Task 7.1: Identification of stakeholder communities

Below, we give a shortlist of stakeholder parties that have already been contacted:
• Charentes-nature – French association of nature conservation
• Marc de Toffoli – Framing agent in agri-environnement
• Axelle Dierstein – Life project about rehabilitation of peat and wet habitats on the Saint-Hubert Plateau
• Marc Dufrêne – MRW – Centre de Recherche Nature Forêt Bois (CRNFB)
• Eric Graitson and Serge Rouxhet - Acrea Ulg
• Jean-Yves Paquet - Aves Natagora
• Thierry Paternoster - CRNFB in Harchies, Natura 2000 agent
• Marc Thirion and Christian Mulders – MRW – DGA - Direction de l'espace rural
• Rudi Vanherck – NATAGORA
• Guido Waajen – Waterschap Brabantse Delta – Platform Meren en Plassen

A more comprehensive overview of the Follow-Up committee is provided in point 7.1.

Task 7.2: Organisation of topical workshops with various stakeholder communities

Thus far, already eight meetings have been organised with members of the Follow-Up Committee. For details, see point 7.2.

Task 7.3: Final policy and management recommendations documents

This requires an integrative analysis of all PONDSCAPE data and is planned for phase 2 of the project. Preliminary recommendations are already provided in point 5 (Conclusions and recommendations).

5. CONCLUSIONS AND RECOMMENDATIONS

The bomb crater complex in Tommelen was studied in detail: apart from measuring the most ecologically relevant abiotic variables, we also assessed the main biological communities (bacteria, phyto- and zooplankton, phyto- and zoobenthos, macro-invertebrates, macrophytes, amphibians and fish). Despite the similar age of the ponds, a high variability in abiotic conditions (i.e. oxygen content, alkalinity, conductivity, water transparency, total phosphorous, sludge content, etc) was detected among ponds.
The total biodiversity observed in the Tommelen bomb crater complex (gamma diversity) was high for several organism groups (e.g. 319 taxa for phytobenthos alone). On the other hand, total diversity of the cladocerans and the macrophytes was not high (19 cladoceran species and 21 species of macrophytes). For several groups, also beta diversity was relatively high and higher than expected for random distribution, which can result from variation in environmental factors or isolation. Management strategies should therefore be based on the principle of maximal complementarity of ponds in order to preserve a maximal regional diversity. The high species richness of most groups suggests that it is essential to safeguard the area. The best way to manage ponds, however, is still not clearly understood. Therefore we will evaluate the effect of restoration measurements (clearing of ponds; i.e. dredging) during the second phase of this project.
The Tommelen bomb crater complex is quite isolated from other areas containing ponds, due to its location close to urban area and roads and railways. This isolation may have severe consequences for the biodiversity of the pond complex in the longer term, especially for amphibians, but potentially also for other organism groups with weak dispersal abilities or low regional abundance, due to suboptimal extinction-colonisation dynamics. Currently, discussions are going on about the creation of a corridor for amphibians to improve the connectivity with the neighbouring populations. Furthermore, maintenance of existing ponds and creation of new ponds in the wider area may also alleviate the effects of isolation.
A general analysis on the taxon richness of all investigated organism groups indicated that the general richness of the ponds in Tommelen is significantly related to the presence of fish, the amount of total phosphorous and the conductivity of the water. Eutrophication and fish predation hence possibly play an important role in these ecosystems (negative association with most of the groups). The presence of fish was in our dataset associated with the connectivity between ponds. Most fish only occurred in those ponds that were strongly connected with each other by a ditch. This indicates that one should be very careful about connecting ponds by means of a permanent hydrological connection. In addition, a very detailed study on phytobenthos revealed different ecological patterns when taking into account dissimilar groups of ponds (differing in influence of inundation with the ditch water, isolation, nutrients and vegetation).
For the investigated groups, we have partitioned the observed variation in community structure and species richness into four components: (1) variation explained by gradients in the local environmental conditions, (2) spatially explained variation (3) spatially structured environmental variation and (4) unexplained variation. The results of this very local study will be compared with those at larger scales at the end of phase 2 of the PONDSCAPE project.
The environmental factors influencing communities differ amongst trophic groups. The main environmental variables influencing the bacterial community composition were oxygen, total nitrogen, volume, alkalinity and amount of open water, while oxygen, amount of emergent vegetation and sulphate were associated with gradients in phytoplankton community composition. Benthic diatoms were associated with alkalinity, the presence of fish, the percentage of bare substrate and the amount of total nitrogen in the water. Macro-invertebrate communities were primarily determined by oxygen content, temperature, water transparency and the presence of fish. Zooplankton on the other hand was mainly affected by pond depth, infillment, total phosphorous and by the density of *Hydra,* a zooplankton predator. Amphibians showed a relation with the presence of fish, while water vegetation was mainly related to Cyanobacteria, pH, amount of sludge and pond depth. For most investigated groups (macro-invertebrates, bacterioplankton, zooplankton and macrophytes), environmental variables also independently of spatial variables significantly affected the communities (pure environmental effect), indicating species sorting mechanisms.
The main environmental gradient, however, structuring several of the investigated organism groups (both the phytoplankton and bacterioplankton communities) appears to be the spatially correlated eutrophication gradient present in the pond complex. Phytoplankton associated with these eutrophied ponds are euglenophyte and cyanobacterial taxa typical of organically enriched and eutrophic water bodies respectively. Moreover, the amount of sludge seems to limit bacterioplankton diversity. Also, clear-water ponds seem to have a higher diversity of these planktonic microbial organisms. Together, this suggests that management of the most eutrophied ponds aimed at reducing eutrophication would benefit their phyto- and bacterioplankton communities.

We also found a significant spatial effect within the investigated communities. A part of the spatial effect could be accounted for by environmental variables. The remaining part could be due to undetected latent environmental gradients, dispersal limitation, priority effects or source-sink dynamics. We can not assess the relative contribution of these alternative mechanisms, but there is evidence that dispersal limitation is probably not of major importance in this pond complex, at least for some of the studied organism groups such as the zooplankton, since 1) the distance between ponds is small because all ponds (>100) lay within a restricted area of approximately 10 hectares, 2) migration rates of resting stages between ponds are expected to be high due to wind-mediated transport or transport by animals (like invertebrates, amphibians, birds, cattle) which are omnipresent in the area, and 3) also active dispersal is probably high in such a small area, at least for some of the organisms groups, like several macroinvertebrate taxa and amphibians.

Microbial activities discriminated the sites very well. The algal primary production, the bacterial secondary production and the nitrification rate were highly inter-correlated. The often observed link between primary production and bacterial production has not been detected in the multi-scale survey dataset. This indicates that the decomposition processes are not only dependent on the primary production of the plankton but certainly depends in a large part on the primary production of the macrophytes and on the input of organic material from the outside of the ponds. Therefore, when looking at these ecosystems, for example for management purposes, several factors must be taken into account. Preliminary results indicate that the microbial functioning of dredged ponds did not differ significantly from that of the control ponds. This management practice thus appears not to have a profound impact on the microbial functioning in the short term.

We did not find a high risk of pollution by the investigated insecticides, phenol compounds and PAHs in the studied ponds. However, attention should be given to herbicides (e.g. isoproturon), especially over the periods of massive pulverisations, such as in autumn. The results are useful to address advices to the stakeholders of ponds for safety management actions in the areas around the ponds, such as using herbicides only at a larger distance from the ponds and creating a buffer area to mitigate contamination.

For the socio-economic survey, all the contacted persons agreed to talk freely about ponds. However, simultaneously, we also observed that ponds were subjects of debate and that there were dissimilarities regarding the environmental perceptions. Often interviewees used the discussion on ponds to reveal conflicts existing between the owner/manager and other local stakeholders. These conflicts could be linked to ponds but also to more general aspects. Ponds are used as starting point to reveal a conflict. Going deeply in the understanding of these antagonisms will be necessary to find out solutions that could mitigate them. Moreover, field observations indicate that future conservative management programs for ponds will have to take into account local particularities. Local conditions, and thus local pond history, seem to play an important role in the perception of stakeholders.
Historical analysis revealed that the majority of the ponds are artificial objects. These elements are currently well integrated into the natural landscape, even if people have often forgotten the human involvement behind many of them. At this step of our work, we are convinced of the need for remembrance of past uses of ponds to better preserve them for the future. Preliminary analyses of interviews show that childhood memories concerning ponds or stories coming from older relatives help stakeholders to have a good opinion about the pond, or at least a preservation attitude towards it. As well as cultural monuments or ruins, ponds represent traces of our past that according to our interviews have a high value for most of the stakeholders and must be valued in this way. In addition to this patrimonial view, we will need to take into account and promote new pond uses: ponds as biodiversity reservoir, ponds as tools for environmental education and ponds as hydraulic tools (storm basin, water reservoir…).

The presence of parasites is also important information for pond management. We found all the genera of the mollusk family Lymnaeidae during the sampling campaign in Belgium, particularly *Galba truncatula*, which is in Europe the principal intermediate host for *Fasciola hepatica*. Soil conditions in Flanders (sand/silt) are not unfavorable for the development of Lymnaeids snails. Molecular analyses will detect to which extend these snails are infected with this parasite in Belgian ponds.

Several recommendations concerning pond management can already be formulated at this stage of the project

- The Tommelen bomb crater complex should be conserved, since it contains a relatively high species diversity for several organism groups. Given that the pond complex is isolated, maintenance of existing ponds and the creation of new ponds in the area may reduce the effect of isolation on the communities.

- A ditch connecting ponds may strongly influence communities, for example by spreading fish predators. The creation of hydrological connections must therefore be considered with care.
- The pond's phyto- and bacterioplakton communities could benefit from management strategies that reduce eutrofication.
- Herbicides are preferably used at a larger distances (> 50m) from the ponds, hence creating a buffer area to mitigate contamination.
- Local conditions, pond history and new pond uses should be taken into account when developing conservation strategies.

6. PERSPECTIVES FOR PHASE 2

WP1: Biodiversity at multiple spatial scales: patterns and driving variables

Tasks 1.1 (sampling and sample analyses protocol), Task 1.2 (analyses of extant databases) and Task 1.4 (Tommelen case study) was completed during phase 1, as well as the sampling and preliminary sampling processing of the 125 ponds selected for the multi-scale survey (Task 1.3). During phase 2, abiotic (water chemistry) and biotic samples (bacterio-, phyto- and zooplankton, phyto- and zoobenthos, macro-invertebrates, amphibians, fish and macrophytes) collected in each of the 125 ponds will be further processed, the data will be analysed and the results disseminated.

WP2: Biodiversity and pond age

Workpackage 2 has been partially integrated in Task 1.3 (multi-scale survey; see above). We broadened the geographic range, and included a higher number of sites than originally planned in these analyses. We acquired accurate age estimates for more than half of the 125 sites selected in Task 1.3 (> 5 years old), and selected an additional 10 sites of recent origin that are spatially scattered among the ponds of older age classes (Task 2.1 completed: 'Selection of ponds belonging to different age classes'). Not only the age of the ponds, but also the time since last management will be assessed, mainly through interviews with stakeholders (see WP6). This is because we assume that certain management practices like dredging or an increase of the groundwater level slows down or even reverses the natural succession typical of ponds. The sampling was conducted in 2008. Most of the sample processing and data analyses will be done during year 3 of the project.

WP3: Biodiversity and ecosystem functioning

In 2009 and 2010, the analysis of the relationships between the microbial functional responses and the taxonomical diversity will be continued and will need the human resources indicated in the contract (taking into account the amendment stating the actual commitment of the Centre de Recherche Public-Gabriel Lippmann). On the basis of our experience in the first phase of the project, the human resources will however be slightly differently used for this work package.

Task 3.1 (In situ and ex-situ assessment of microbial ecosystem functions) will be continued in order to relate the taxonomical diversity to the basic functionalities of the ponds, i.e. the primary production, the bacterial secondary production, the nitrification rate and the microbial ecto-enzymatic activities after the management of the ponds. The microbial activities will be measured as described in phase 1. All the manpower will be used in 2009 (and not in 2010 as previously scheduled) as the monitoring of the effects of pond dredging will take place in 2009. Pond management will not only change the taxonomical diversity but will also result in different functionalities. Nutrients and/or pollutants will be released from the sediment or the soil and will increase or decrease the microbial metabolic rates. These measures are therefore good indicators for changes occurring in ponds after a short period of time after the management actions.

A dataset about the microbial activities will be added to the taxonomical databases set up in the PONDSCAPE project. Extensive analyses will ascertain the relationships between functional and taxonomical diversity (Task 3.2. Data analysis). The preliminary exploration of the datasets on the Tommelen and the multi-scale survey will be extended, using not only classical ordination techniques such as PCA, CA, RA and CCA but also non linear techniques and generalized linear models or generalized additive models.

WP4: Assessment of management techniques

The inventory of planned management practices (Task 4.1) was completed in autumn 2008, thereby also including information obtained during the interviews with stakeholders (June-August 2008; see WP6). The effect of management practices on the local fauna and flora was assessed in a subset (n=14) of the Tommelen ponds (Task 4.4). These ponds were sampled before (spring and summer 2008) and right after (autumn 2008) sludge removal. To integrate the seasonal variability in community structure, it is planned to sample in spring and summer 2009 (phase 2) (Task 4.2). The analyses of the data (Task 4.3) and the evaluation of management practices to increase biodiversity will be completed in 2010.

WP5: Pond biodiversity, management and pollution

The investigations during the second phase of the project will focus on the influence of pond management on the bio-availability of pollutants, and its potential toxicity on the welfare of living organisms in the targeted small ponds by management actions:

For the evaluation of the loads of heavy metals and problematic herbicides before the management actions, water and sediment samples were taken at the end of June 2008 from all the ponds selected as controls and from ponds that will be managed.

For the study of bio-availability effects after management, ecotoxicological biotests will be performed. Also water and sediments will be sampled from control ponds and from ponds submitted to management actions for the investigation of heavy metals and some herbicide loads.

As no fish was present in the ponds before management, we will transfer fertilized eggs of *Rana temporaria* from control and treated ponds at the end of February or the beginning of March of 2009 in the conditions of laboratory rearing in Namur. Then, spawns and tadpoles will be investigated for survival, growth and malformation ratio, as well as physiological status, including oxidative stress, thyroid hormones and proteome stress. Some groups will be challenged with different doses of Roundup and isoproturon or a mix of these herbicides and investigated for the stress physiolical response.

WP6: History of social and economic relevance of ponds to stakeholders

During phase 2, the analysis on the cartographic evolution of ponds in Flanders will be completed. This analysis will allow us to point out possible variations between the two territories (Wallonia and Flanders) and to get a global viewpoint on the past and present situation of ponds in Belgium. Such an analysis has been found to be very useful to establish the link between the perception/representation/management practices of stakeholders and the local situation of ponds, taking into account that the ecoregion conditions play an important role on pond value. This cartographic analysis for relevant areas of Flanders will have to take into account the limits of NGI maps.

A thematic analysis of the interviews will be performed during phase 2. The themes revealed by a deep reading of the interviews' contents will constitute the framework for analysis of the entire dataset. Organised into an analytical table, the thematic analysis will help to observe the differences in perception of various stakeholders and answer our research questions.

In phase 2, a large quantitative survey (closed questionnaire) will be sent to pond stakeholders across Belgium. This survey will be based on the qualitative results obtained during phase 1, and will complement the studies on perception/representation and historical aspects of ponds. This questionnaire has two main objectives: (1) to obtain quantitative results on particular but relevant findings made with semi-direct interviews, and (2) to quantify the effect of historical changes and management practices documented by the qualitative survey.

This task includes an exhaustive inventory of all actors involved in pond management. This inventory was already initiated during phase 1, but the plethora of work is planned for the first year of phase 2, together with the validation and testing of the closed questionnaire. For this, the questionnaire will be distributed to all identified actors, either through direct phone contact or courier services. Afterwards, analysis of the results will be performed.

Finally, in the last year of phase 2, contacts with international experts will be made in order to identify practices and initiatives already successfully implemented in other countries.

During phase 2 of the project, snail DNA will be extracted (Chelex ®) and amplified with a PCR that targets *Fasciola* sp. DNA. Taking into account the huge number of snails collected (8885) a statistical study will be done to determine an appropriate size of sampling for PCR analysis. The huge number of snails explains the fact that the molecular biology analysis have not started yet. Positive snails will be phylogenetically identified using ITS2 sequences by means of cloning and sequencing, multiplexing or real-time PCR (Duffy et al., 2009).

WP7: Valuation of results, policy measures, management recommendations

Although we already identified a wide variety of stakeholder communities (Task 7.1), we will continue this task also during phase 2. Identified stakeholders will be invited to join the Follow-Up Committee and attend topical workshops at least twice per year (Task 7.2). The final policy and management recommendations documents (Task 7.3) will be compiled during year 4.

7. FOLLOW-UP COMMITTEE

7.1. Composition of the Follow-Up Committee

List of members of the Follow-Up Committee:

- Pauwel Bogaert - Regionaal Landschap Houtland
- Pierre Gerard - Ministère de la Région Wallonne
- Paul Haustraete - Regionaal Landschap Vlaamse Ardennen
- Louis-Joan Lemmer - Stad Hasselt
- Hendrik Segers - Royal Belgian Institute of Natural
- Thierry Vercauteren - Provinciaal Instituut voor Hygiëne
- Dominique Aerts - INBO
- Tom Andries - Natuurpunt, Cel Beheerplanning en –evaluatie
- Philippe Collard - vet
- Jos Coteur - Natuurpunt Hasselt-Zonhoven
- Luc Crevecoeur - Provincie Limburg
- Olivier Dochy - INBO and Provincie West-Vlaanderen
- Édmée Engel - Institute National Museum of Natural History
- Emmanuel Gilissen - Afrikamuseum
- Rik Jacobs - Natuurpunt Hasselt - Zonhoven
- Claudine Junck - Biologische Station SICONA/Wissenschaftliche Abteilung
- Alain Le Roi - GIREA-UCL (Interuniversitary Group for Applied Ecology Research) UCL - Unité d'Ecologie
- Anne Loncin - Crie of Anlier Forest
- Gerald Louette - INBO
- Christian Mulders - Direction générale de l'Agriculture, Ministère de la région Wallonne
- An Rekkers - Regionaal Landschap Zenne, Zuun & Zoniën vzw
- Herman Reynders - Mayor of the city Hasselt
- Wim Slabbaert - Agentschap voor Natuur en Bos
- Joeri Steeno - Regionaal Landschap Dijleland
- Marc Thirion - Direction générale de l'Agriculture, Ministère de la région Wallonne
- Luc Vandenbosch - Natuurpunt Hasselt-Zonhoven
- Jeroen van Gemert - Natuurpunt Zuidoost Limburg
- Henk Schaut - Regionaal Landschap Ijzer & Polder
- Bert Vanholen - AMINAL
- Gaby Verhaegen - VMM
- Patrice Verscheure - Maison de l'Eau de l'Attert asbl, Luxembourg
- Ivo Adam - Gemeentebestuur Merelbeke
- An Devroey - Regionaal Landschap Dijleland
- An Digneffe - Regionaal Landschap Haspengouw & Voeren
- Luk Dombrecht - Regionaal Landschap West-Vlaamse Heuvels
- Machteld Grijseels - Institut Bruxellois pour la Gestion de l'Environnement
- Yvan Hayez - Fédération Wallonne de l'Agriculture
- Pierre Hubau - AMINAL
- Filip Jonckheere - Regionaal Landschap Meetjesland
- Marc Meyer - National Museum of natural History, Luxembourg
- Bart Paesen - Regionaal Landschap Meetjesland
- Rudi Vanherck - NATAGORA
- Paul Vos - Stad Hasselt - Dienst Leefmilieu, cel Landbouw

7.2. Follow-up committee

During the first phase of the project, several meetings with members of the Follow-Up committee were organized:

- 3 January 2007, Hasselt: On this meeting the ecological, cultural and scientific importance of the pond complex in Tommelen was discussed. An invited speaker, prof. A. Hull, stressed that this pond complex represents a unique landscape whose conservation is highly significant, even at the European level. As a result of this meeting, we compiled the historical, ecological and scientific information into one brochure: 'Tommelen: Scars of war contain a significant wildlife resource'.

Already 500 copies were distributed.

- 27 March 2007, Natural History Museum, Luxembourg: Dr Engel gave at this occasion information on the past studies of ponds in Luxembourg, transferred the scientific reports from these studies and gave recommendations concerning the choice of the ponds to be studied in the PONDSCAPE project.
- 14 June 2007, Tommelen: On this meeting we discussed the possibility of a 'management' experiment in the pond complex in Tommelen. Different ponds in the complex will disappear due to natural succession. The conservator, managers and the owner (city of Hasselt) strongly support the idea of combining the management (dredging) of these ponds with a scientifically underpinned experiment.
- 28 November 2007, Scientific Station SICONA, Olm, Luxembourg: Mrs Claudine Jung and Mr. Fernand Schoos gave us at this occasion an overview of the work of SICONA-OUEST concerning ponds in Luxembourg. They gave us fundamental information about the ponds they manage in the western and the southern parts of Luxembourg (exact location, pond age, date of the last management, dominant plants and presence of amphibians). Mrs Jung also presented the philosophy of SICONA concerning the management of ponds.
- 5 February 2008, RBINSc, Brussels: On this meeting, all PONDSCAPE partners presented their first results to the Follow-Up Committee, after which the committee was given the opportunity to ask for additional information and to formulate suggestions, critics, etc.
- 12 June 2008, Centre of Agronomic Technologies (CTA), Strée (Modave), GIREA-UCL Louvain-La-Neuve: This meeting was organized by the Walloon Ministry of Agriculture (DGA) and the Interuniversity Group on Applied Ecology Research. The main objective of the meeting was to define the sustainable strategies for promoting the protection of biotopes and biodiversity related to agricultural ponds in Walloon Region. The members of the University of Namur presented a summary of the current research conducted within the PONDSCAPE project.
- 15 September 2008, RBINSc, Brussels: Presentations were given concerning the PONDSCAPE activities of the past few months. A discussion was held on the management experiment in the Tommelen pond complex. In addition, the replies that were formulated by the project partners to the questions raised by the reviewers of the mid-term evaluation report were presented to the Follow-Up Committee members and discussed.
- 7 October 2008, Tommelen, Hasselt: Meeting with Rik Jacobs, Jos Coteur and Paul Vos: follow up in the field and evaluation of the management practices performed in the Tommelen pond complex (dredging).

The number of members of the Follow-Up Committee expanded throughout the first phase of the project. In total, there are 43 members coming from different institutions and backgrounds, so that a multi-disciplinary approach of the project is created. A website of the project is created (www.pondscape.be) and makes it possible for the Follow-Up Committee members to consult project related documents.

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9. PUBLICATIONS

9.1 Publications of the teams

9.1.1 Peer review

P2: Louette G., De Meester L. and Declerck, S., in press. Assembly of zooplankton communities in newly created ponds. *Freshwat. Biol. IS UIT*

P2: Louette G., De Bie T., Vandekerkhove J., Declerck S. and De Meester L. 2007. Analysis of the inland cladocerans of Flanders (Belgium) - Inferring changes over the past 70 years. *Belg. J. Zool.*, 137: 117-123.

P4: Fatima M., Mandiki S.N.M., Douxfils J., Silvestre F., Coppe P. and Kestemont P. 2007. Combinatorial effects of herbicides on biomarkers reflecting immune-endocrine interactions in goldfish-Part I: Immune and antioxidant effects. *Aq. Toxicol.* 81: 159-167.

P4: Douxfils J., Mandiki S.N.M., Frédéric S. Arnaud B., Delphine L., Jean-Pierre T. and Kestemont P. 2007. Do sewage treatment plant discharges substantially impair fish reproduction in polluted rivers? *Sci. Total Environ.* 372: 497-514.

P4: Gillardin V., Silvestre F., Divoy C., Thomé J.P. and Kestemont P. 2009. Effects of Aroclor 1254 on oxidative stress in developing Xenopus tadpoles. *Ecotoxicological and Environmental Safety* 72: 546-551.

P4: Gillardin V., Silvestre F., Dieu M., Delaive E., Raes M., Thomé J.P. & Kestemont P., 2009. Protein expression profiling in the African clawed frog *Xenopus laevis* tadpoles exposed to polychlorinated biphenyl mixture Aeroclor 1254. *Molecular and Cellular Proteomics*, in press.

SC4: Caron Y., Lasri S. and Losson B. 2007. *Fasciola hepatica*: an assessment on the vectorial capacity of *Radix labiata* and *R. balthica* commonly found in Belgium. *Vet. Parasitol.* 149: 95-103.

9.1.2 Others

C: Lamatsch D.K., Schön I., Martens K. 2007. Hybridization as a source for clonal diversity in asexual non-marine ostracods. Abstracts of the symposium on "Hybridization in Animals – Extent, Processes and Evolutionary Impact".

C: Schön I. and Martens K. 2007. Did Pleistocene glaciations shape genetic patterns of European ostracods? A phylogeographic analysis of two species with asexual reproduction. 5th International Symposium on Ecological Genetics, Leuven.

C: Schön I. and Martens K. 2007. Evolution and speciation in ancient lake ostracods – a comparison between Tanganyikan and Baikalian species flocks. Abstracts of the SIL 2007 symposium, Montreal.

C: Schabetsberger R., Drozdowski G., Rott E., Stoch F., Lenzenweger R., Martens K., Kotov A., Reiff N., Traunspurger W. and Schatz H. 2007. Losing the bounty? - Investigating biodiversity in isolated freshwater ecosystems of Oceania. Abstracts of the SIL 2007 symposium, Montreal.

C: Balian E., Leveque. C., Segers H. and Martens K. 2007. Freshwater Animal Diversity Assessment (FADA). Abstracts of the SIL 2007 symposium, Montreal.

C: Eggermont H., Cocquyt C., Van Damme K., Martens K., Dumont H.J. and Verschuren D. 2007. Aquatic biodiversity of unique tropical high-elevation lakes in the Rwenzori Mountains (Uganda-DR Congo). Abstract meeting "Biodiversity and Climate Change", BBP.

C: Verheyen E., Martens K., Schön I., Irvine K., Paradis E., Sturmbauer C., Väinölä R., Michel E., Todd J., Verschuren D., Sherbakov D. and Ngatunga B. 2007. Molecular archives of climatic history: exploring patterns of genomic differentiation in endemic species. Radiations of ancient lakes (MOLARCH). Abstract meeting "Biodiversity and Climate Change", BBP.

C: Martens K., Segers H. and Balian E. 2007. Freshwater Animal Diversity Assessment (FADA). European Ostracod Meeting, Frankfurt.

C: Savatenalinton S., Borgonie G. and Martens K. 2007. Biodiversity of non-marine ostracods in Thailand. European Ostracod Meeting, Frankfurt.

C: Schön I. and Martens K. 2007. Is there molecular evidence for sex in *Vestalenula cornelia*? European Ostracod Meeting, Frankfurt.

C: Vandermeeren T., Ito E., Almendinger J., Khand Y. and Martens K. 2007. Recent Ostracoda of Western Mongolia: diversity, ecology and paleolimnological applications. European Ostracod Meeting, Frankfurt.

C: Vanblaere S., Schön I. and Martens K. 2007. Transposable elements in Ostracoda (Crustacea). Open project meeting of the SexAsex EU RTN project "Paradox of sex, theory and data", Zürich.

C: Van den Broeke L. Schön, I. and Martens K. 2007. DNA repair in the asexual ostracods *Darwinula stevensoni* and *Eucypris virens*. Open project meeting of the SexAsex EU RTN project "Paradox of sex, theory and data", Zürich.

C: Major T., Rossetti G., Vandekerkhove J., Martins M.J.F., Bode S., Schön I. and Martens K. 2007. Valve morphology in sexual and clonal lineages of *Eucypris virens*. Open project meeting of the SexAsex EU RTN project "Paradox of sex, theory and data", Zürich.

P2: Declerck S. 2007. Biodiversiteit in poelen: algemene patronen en relatie tot agrarisch landgebruik. Oral presentation, Congres Watersysteemkennis: Aquatische biodiversiteit, Ghent.

P2: Louette G., De Bie T., Vandekerkhove J., Declerck S., and De Meester, L. 2007. Watervlooien in Vlaanderen: verspreiding, status en trends. *Water* 29: 49-51.

P2: Louette G., De Bie T., Vandekerkhove J., Declerck S. and De Meester L. 2007. Onderzoek naar het voorkomen van watervlooien in Vlaanderen – Trends over de laatste 70 jaar. Poster, Congres Watersysteemkennis: Aquatische biodiversiteit, Ghent.

P2: Declerck S. 2007. Geëutrofieerde vijvers en meren: ecologische achtergronden en beheer. Oral presentation, Congres Watersysteemkennis: Mogelijkheden voor ecologisch herstel van watersystemen, Ghent.

P2: De Meester L. 2007. De wisselwerking tussen biodiversiteit en ecosysteemdiensten: van poel tot stroomgebied. Oral presentation, Congres Watersysteemkennis: Aquatische biodiversiteit, Antwerp.

P2: De Bie T. 2007. Tommelen: oorlogslittekens herbergen een schatrijke natuur. Brochure in collaboration with 'Natuurpunt Hasselt-Zonhoven' and The European Pond Conservation Network.

P3: Van Gremberghe I., Van der Gucht K., Van Wichelen J., De Keyser K., De Coster S., De Ruysscher F., D'hondt S., Wilmotte A. and Vyverman W. 2006. Co-variation between zooplankton community composition and cyanobacterial community turnover in lake Blaarmeersen (Belgium). Poster presentation, Conference on Peptides in Cyanobacteria (PEPCY), Berlin.

P3: Van Wichelen J. and Van Gremberghe I. 2007. Cyanobacterial blooms in Flanders: nature, occurrence, toxicity and potential threat to wildlife. Oral presentation, Belgian Wildlife Disease Society 2nd symposium, Neder-Over-Heembeek.

P4: Morelle K. and Graitson E., "Origine et diversité des mares agricoles en Région Wallonne", Workshop on « Ponds and Agro-environment », CTA Stree-Modave, organized by Groupe Interuniversitaire de Recherches en Écologie Appliquée (G.I.R.E.A.), 12 June 2008.

P4: Morelle K., "L'évolution des mares en Belgique : entre Histoire humaine et Histoire de l'environnement", First Environmental History Meeting: Belgium-Luxemburg-Congo, Rwanda, Burundi, organized by the Department of Historical Research, FUNDP, 11-13 December 2008.

SC4: Caron Y. 2004. Une étude de la faune malacologique associé aux petites surfaces aquatiques en Belgique et évaluation préliminaires par des techniques de biologie moléculaire du rôle potentiel de différentes espèces dans le maintien du cycle de *Fasciola hepatica* (Trematoda, Linnaeus 1758) (Liège, Université de Liège), p. 35.

9.2 Co-publications

9.2.1. Peer review

C & P2: De Bie T., Declerck S., Martens K., De Meester L. and Brendonck L., in press. A comparative analysis of cladoceran communities from different water body types: patterns in community composition and diversity. *Hydrobiologia* 597:19-27.. DOI: 10.1007/s10750-007-9222-y.

P2 & P3: Vanormelingen P., Cottenie K., Michels E., Muylaert K., Vyverman W. and De Meester L., in press. The relative importance of dispersal and local processes in structuring phytoplankton communities in a set of highly interconnected ponds. *Freshwater Biology*.

9.2.2 Others

C & P2: De Bie T, Declerck S., Louette G., Martens K., De Meester L. and Brendonck L. 2007. Zooplankton diversity in relation to pond age and isolation. Poster, Congres Watersysteemkennis: Aquatische biodiversiteit, Ghent.

P2 & P3: Vanormelingen P., Vyverman W., De Bock D., Van der Gucht K. and De Meester L. 2007. Local genetic adaptation to grazing pressure of the green alga *Desmodesmus armatus* in a strongly connected pond system. Oral presentation, First EURODiversity Symposium, Paris.

P2 & P3: Van Gremberghe I., Vanormelingen P., Van der Gucht K., Souffreau C., D'hondt S., De Meester L. and Vyverman W. 2007. Population build-up of *Microcystis*: importance of priority effects and interaction with *Daphnia* grazing. Oral presentation, First EURODiversity Symposium, Paris.

P2 & P3: Vyverman W., Van Gremberghe I., Van Wichelen J., Van der Gucht K., Vanormelingen P., De Meester L. and Wilmotte A. 2007. Bloom incidence, population build-up and toxicity of *Microcystis* in Flemish surface waters. Oral presentation, Vito Workshop: Toxicity of natural agents, Mol.

C, P2 & P3 & SC1: Declerck S., De Bie T., Ercken D., Hampel H., Van Wichelen J., Van de Meutter F., Van Hecke L., Denys L., Vyverman W., Goddeeris B., Van der Gucht K., Brendonck L., Martens K. and De Meester L. 2007. Soortenrijkdom in veedrinkpoelen: patronen van congruentie en potentieel

voor biodiversiteitsindicatoren. *Water* 29: 21-25.

9.3 Other activities

C-P2-P3-P4-IP-SC1-SC2-SC3-SC4: Creation of a website (www.pondscape.be)

C: Organisation of project meetings and meetings with the Follow-Up Committee

C: Vandekerkhove J., Namiotko T., Rossetti G., Martins M.J.F., Mezquita F., Schmit O., Butlin R.K., Schön I. and Martens K. 2008. Sex and stochasticity: insights from the geographic parthenogen *Eucypris virens* (Ostracoda). Oral presentation at the 3rd European Pond Workshop, Valencia.

P2: Declerck S. 2007. Biodiversiteit in poelen: algemene patronen en relatie tot agrarisch landgebruik. Oral presentation, Congres Watersysteemkennis: Aquatische biodiversiteit, Ghent.

P2: De Bie T., Declerck S., Louette G., Martens K., De Meester L. and Brendonck L. 2007. Zooplankton diversity in relation to pond age and isolation. Poster, Congres Watersysteemkennis: Aquatische biodiversiteit, Ghent.

P2: Louette G., De Bie T., Vandekerkhove J., Declerck S. and De Meester L. 2007. Onderzoek naar het voorkomen van watervlooien in Vlaanderen – Trends over de laatste 70 jaar. Poster, Congres Watersysteemkennis: Aquatische biodiversiteit, Ghent.

P2: Declerck S. 2007. Geëutrofieerde vijvers en meren: ecologische achtergronden en beheer. Oral presentation, Congres Watersysteemkennis: Mogelijkheden voor ecologisch herstel van watersystemen, Ghent.

P3: Co-organization of a meeting with members of the Follow-Up Committee, Centre of Agronomic Technologies (CTA), Strée (Modave), 12 June 2008.

P4: Morelle K. and Graitson E. 2008. Origins and diversity of farmland ponds in Wallonia. Oral presentation during workshop on «ponds and Agri-environment», CTA Stree-Modave (aCREA-ULg).

IP: Organization of meeting with members of the Follow-Up Committee (Scientific Station SICONA, Olm, 28 November 2007; Natural History Museum, Luxembourg, 27 March 2007).