Impact of multiple disturbances on microbiological quality of urban and peri-urban lakes

Adélaïde Roguet, Françoise S. Lucas

Impact of multiple disturbances on microbiological quality of urban and peri-urban lakes

Adélaïde ROGUET and Françoise S. LUCAS

Université Paris-Est Créteil, Laboratoire Eau Environnement Systèmes Urbains (Leesu) UMR MA 102 - AgroParisTech, 61 avenue du Général de Gaulle, 94000 Créteil, France
(E-mail: adelaide.roguet@leesu.enpc.fr and lucas@u-pec.fr)

Abstract

This project will focus on global changes impact on the microbiological quality of urban and peri-urban lakes in Ile-de-France (France). The goal is to better understand the impact of anthropogenic pressures and changes of land use on bacterial populations in urban lakes, to identify indicators of quality and ecological status and to better evaluate the ecology of emerging waterborne pathogens such as nontuberculous mycobacteria. The study will include an annual survey of forty eight lakes and a monthly monitoring of the Créteil Lake to assess the temporal and spatial variations of bacterial communities, faecal contamination indicators and nontuberculous mycobacteria. A supplementary study will be undertaken to identify the favorable habitats of mycobacteria in urban lakes.

Keywords

Microbial diversity; faecal bacterial indicators; nontuberculous mycobacteria; global changes; urban lakes

INTRODUCTION

Lakes are very common ecosystems in the urban and peri-urban landscape. More than 900 are listed in the Ile-de-France area (Catherine *et al.*, 2008)). These ecosystems have been studied for a long time because they provide many ecosystem services, such as important economical, recreational and educational services (Bolund and Hunhammar, 1999). Since, these ecosystems are coupled with watersheds, they appear to be good indicators of the general state of the landscape because they are particularly vulnerable to environmental change and they represent a complex set of environments (Williamson *et al.*, 2008). Moreover, these ecosystems are sensitive to a wide variety of environmental changes, which act at the same time at the local level (agricultural, industrial or urban pollution, eutrophication, etc.) and at global scale (increasing intensity of the rains and the heatwaves, etc.) (Poff *et al.*, 2002; Peters *et al.*, 2008). Anthropogenic disturbances mainly lead to the degradation of aquatic biological systems, which can impact the ecosystem functioning or the sanitary quality aspects (MEA, 2005). For these reasons, it is important to understand these ecosystems.

Microorganisms have a major importance for the functioning of aquatic ecosystems (Linderman, 1942). Indeed, these microorganisms play a critical role in biogeochemical cycles, by degrading and mineralizing the organic compounds to their inorganic forms (Cole *et al.*, 1988; Cotner *et al.*, 2002). In addition, these organisms constitute preys for eukaryotic predators, which by fueling the food web, has a profound impact on elemental fluxes and water quality in these ecosystems (Pernthaler and Amann, 2005). However, it is well documented (Williamson *et al.*, 2008) that deterioration of water quality because of land use or anthropogenic contaminants can significantly impact the functioning of aquatic ecosystems, by creating new ecological niches that could encourage the survival of certain bacteria species or pathogens (Johnson *et al.*, 2010).

In spite of their ecological importance, the composition of microbial communities in freshwater ecosystems still remained a black box and little is know about their response to the variations in their environment. So, molecular biology techniques since a decade provide us with unprecedented access to the identification and composition of microorganisms of freshwater lake bacterial communities and have for the first time enabled microbial ecology to identify the numerically dominant organisms in these ecosystems and to learn much about their distributions in time and space. Yet, the impact of physico-chemical properties of lakes related to land occupation on the diversity of bacteria is still unclear and need to be investigated.

Among services provided by urban lakes, recreational activities by practicing recreational activities such as bathing, fishing or practice of nautical sports, humans are often directly in contact with freshwaters. Yet, lakes waters can be contaminated by waterborne pathogens, which are originated from animals, humans or environment. Therefore, to assess and characterize the sanitary quality of bathing water, various indicators of faecal contamination are usually used. The abundance of these indicators is supposed to be correlated to the density of pathogenic microorganisms from faecal origin and thus to be an indication of the sanitary risk associated with the various water use (bathing, shellfish harvesting, etc.). For years, the group of faecal coliforms (FC) has been the most widely used as faecal indicator. In recent years, some organizations (USEPA, 1999; WHO, 2004) have proposed to use *Escherichia coli* and intestinal enterococci as indicators of faecal pollution: *E. coli* is used to detect recent contamination, whereas intestinal enterococci detect faecal is employed to assess a old pollution (PIREN-Seine, 2009). However, these bacteria can survive in natural environment (Rivera *et al.*, 1988; Hardina and Fujioka, 1991; Muller *et al.*, 2001; Whitman *et al.*, 2002), consequently the amount of these indicators do not always reflect an ancient or recent contamination. For instance, according to Whitman *et al.*, (2003), in Lake Michigan, *E. coli* can persist several months in the algae *Clodophora*. Nevertheless, these two indicators are the most studied in the world to characterize the quality of bathing waters.

In addition to not be often correlated with enteric cases, faecal indicators do not correlate at all with environmental pathogens such as nontuberculous mycobacteria (NTM). These organisms have been recognized as emergent opportunistic pathogens since 2004 by the World Health Organization and it is generally accepted that environmental water is the main source of most human infections caused by NTM, such as severe human skin, pulmonary, digestive or urinary infections (Falkinham III, 2002; Primm *et al.*, 2004; Griffith *et al.*, 2007). On NTM, despite numerous studies, to date their diversity, their environmental sources and reservoir and their persistence in freshwater are still poorly understood. Consequently, because NTM are emerging pathogens for humans and domestic animals, it is important to identify these aspects.

Thus, even if many studies focus on pathogens in lakes and that few multilake studies have shown that differences in bacterial community composition between lakes can be quite large and in relation with lakes properties (Lindström, 2000; Yannarell *et al.*, 2004; Yannarell *et al.*, 2005; Jones *et al.*, 2009; Newton *et al.*, 2011), no study has described and analyzed jointly the sanitary and the environmental statutes of lakes, especially in small urban lakes which are often less studied than the great lakes in microbial ecology. Thus, our study aims to combine these two approaches in urban and peri-urban lakes in Ile-de-France over three years by focusing on the bacterial assemblages. Firstly, in order to identify factors controlling the pathogens and faecal indicators densities and the bacterial diversity to characterize the relative impact of global change and other anthropogenic pressures. Secondly, we aim to identify sensitive areas and factors aggravating the ecological and sanitary status of these ecosystems in order to optimize the management practices of urban lakes.

This study is part of the research project PULSE (Peri-Urban Lakes, Society and Environment) supported by ANR (National Research Allowance) funds and which involve seven laboratories. To fulfill our objectives two complementary approaches will be undertaken: at a larger scale for a global vision and at a smaller temporal and spatial scale for a finer analysis of the processes and factors involved. The first is an inter-annual comparison of the sanitary and ecological status of 48 lakes, representative of the land occupation in the Ile-de-France region (Catherine *et al.*, 2008) for three consecutive years. The Ile-de-France region constitutes a good model for studying the impact of multiples pressures on waterbodies, because it lies within a single, first-order hydro-ecoregion as defined by Wasson *et al.* (2002) and thus, its geology is considered to be homogeneous at all the sampling sites. Moreover, this region displays a diversity of anthropogenic pressures and global changes in terms of their nature and intensity, and it includes a wide range of urban and peri-urban lakes. This first approach will be coupled with a more detailed analysis by monthly monitoring the lake of Créteil (Val de Marne, France). Two transects will be sampled, one vertical and the one horizontal in order to assess the temporal and spatial variations of bacterial communities, indicators of faecal contamination and NTM.

In addition, a study will be conducted in the spring and summer on two contrasted lakes, one oligotrophic and the other one eutrophic, in order to study the spatial distribution of NTM. We aim to identify which habitats are favourable to MNT within urban lakes by investigating the densities of NTM in the sediment, water column, neuston, and in biofilm covering plants (epiphytic) and submerged rocks (epilithic).

MATERIAL & METHODS

Study of multiple disturbances on the microbial assemblages in urban lakes

Impact of land use

<u>Sampling area</u>

The 48 lakes surveyed are localized in the Ile-de-France region (Figure 1), an area covering 12,011 km^2 in north-central France, in the center of Paris basin which concentrated 18.3% of the French population in 2011 (INSEE[1]). This fertile sedimentary basin contains many large industrial cities, residential suburbs and agricultural areas which represent half of the total area of the region (INSEE). Forested areas cover over 24% of the region (INSEE). The characteristics of lakes are presented in Table 1 (Catherine *et al.*, 2008).

<u>Sampling design</u>

For three years (2011-2013), between July and August, the 48 lakes will be monitored. For each lake, the water column is sampled at three depths on three different locations. Water samples are then pooled together in order to obtain one average sample per lake. Various biotic and abiotic parameters are measured by four different laboratories (Table 2).

Faecal indicator densities will be estimated by the most probable number (MPN) method using microplates according to the standards NF EN ISO 9308-3 and 7899-1 for *E. coli* and intestinal enterococci respectively. We use these two indicators, in particular in the goal to be able to compare our data with other studies, such as those of the Conseil Général du 94. Total bacterial densities will be measured by flow cytometry. To characterize the genetic structure of the bacterial community from lakes, bacterial DNA will be extracted to analyze the diversity of genes coding for the 16S rRNA (gene essential for the survival of species) by dHPLC profiling

[1] INSEE (Institut National de la Statistique et des Études Économiques) : http://www.insee.fr/

(Denaturing High-Performance Liquid Chromatography) (Danger *et al.*, 2008). A high throughput sequencing method will be performed for one of the three campaigns, to define the composition of the bacterial assemblages in order to link diversity indices and main taxonomic groups with the characteristics of the lakes. Finally, the density and the community structure of NTM will be analyzed by real time PCR (Radomski *et al.*, 2010) and dHPLC respectively. Statistical analysis will be conducted with R software (R development Core Team 2012) by combining biological results with land occupation data of watershed. These will be obtained using the ArcGis (vs 9.3 ESRI), an geographic information system software that uses layers of vector geo-referenced and describing land occupation of an area (crops, forests, cottage homes, buildings, industries, etc.), to isolate only the information related to the lake watersheds.

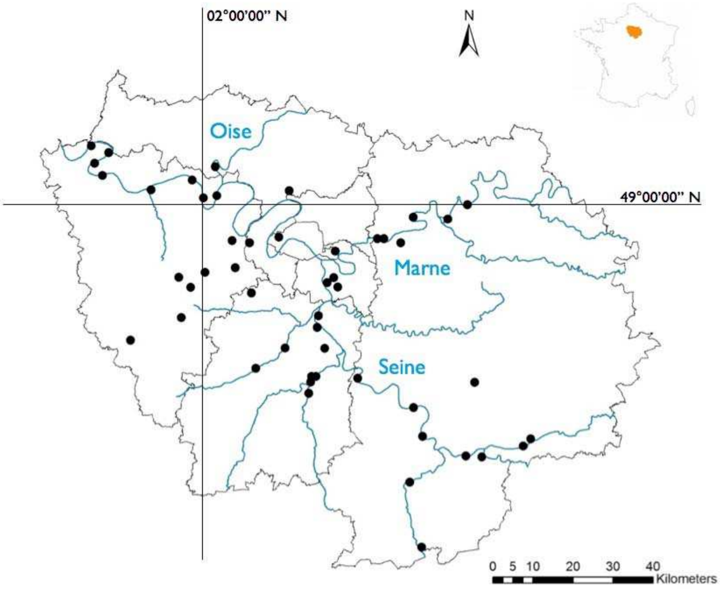

Figure 1: Localization of the studied 48 lakes in the Ile-de-France region.

Temporal and spatial variability

<u>Sampling area</u>

Créteil Lake located in Ile-de-France region (48°46’N 02°27’E) originates from an excavation of alluvial sediments near the confluence of the Rivers Seine and Marne. Its surface is 40.8 ha and has an average depth of 4.5 meters. The lake is mainly supplied with anoxic phreatic waters circulating through alluvial deposits and diverse filling materials. It is also supplied by a rainwater sewer. Well exposed to wind, the lake stratified only weakly and intermittently from May to October.

Table 1: Location, morphological characteristics of the 48 lakes and total chlorophyll a observed in August 2006 (Catherine *et al.*, 2008)

Hydrographical zones are based on land use index (urban impervious, non-impervious, cropland surfaces), on morphological index (altitude and surface) and on drainage index of the watershed. Within each cluster the number of lakes is proportional the representativeness of lakes in the region.

Name	Localisation	Clusters (defined by hydrographical zones)	Linked to rivers and streams	Surface area (ha)	Mean depth (m)	Total Chl *a* ($\mu g.L^{-1}$)
Isles-les Villenoy Pond	48°54' N 02°50' E	1	-	41.6	2.6	10.1
Fontenay/Vic Pond	48°33' N 02°23' E	1	+	9.8	1.2	357.3
Vert-le-Petit Pond	48°33' N 02°22' E	1	-	5.0	2.0	123.1
Fleuri Pond	48°32' N 02°22' E	1	+	8.4	1.3	67.6
Courcouronnes Pond	48°37' N 02°25' E	1	+	6.5	1.4	123.5
Delomez Pond	48°24' N 03°07' E	1	-	6.7	4.2	9.6
Leclerc Pond	48°24' N 03°05' E	1	-	5.4	2.8	6.1
Maurepas Reservoir	48°46' N 01°55' E	2	+	7.6	3.4	138.7
Noés Reservoir	48°45' N 01°58' E	2	+	24.0	0.7	73.0
Vaux-de-Cernay Pond	48°41' N 01°56' E	2	+	6.5	1.4	101.6
Grande Paroisse Pond	48°22' N 02°54' E	2	+	52.7	3.7	191.5
Grand Marais Pond	48°22' N 02°57' E	2	-	16.5	2.4	27.1
Villefermoy Reservoir	48°32' N 02°56' E	2	+	21.7	2.1	54.8
Freneuse Cove	49°04' N 01°35' E	3	+	14.0	2.4	202.3
Grand Lavacourt Pond	49°03' N 01°41' E	3	-	111.5	4.3	25.3
Ilon Cove	49°01' N 01°37' E	3	+	7.4	3.8	84.5
ASM Club Cove	49°00' N 01°40' E	3	+	9.2	2.5	181.4
Bout du Monde Pond	48°58' N 01°50' E	3	-	27.6	1.2	3.1
Port-Sud Pond	48°34' N 02°11' E	3	+	6.5	1.7	118.8
Clarette Pond	48°19' N 02°42' E	3	-	13.9	1.4	129.7
Souppes/Loing Pond	48°10' N 02°45' E	3	+	21.1	2.9	39.7
Saint-Blaise Pond	48°31' N 02°22' E	4	+	5.2	1.4	68.6
Gazeran Pond	48°38' N 01°46' E	5	+	8.2	0.7	45.7
Samoreau Pond	48°25' N 02°45' E	5	-	10.2	2.4	2.4
Grosse Pierre Pond	49°00' N 01°58' E	6	-	46.2	4.3	34.2
Gaule Achéroise Pond	48°58' N 02°03' E	6	-	5.3	6.2	7.8
Triel Pond	48°57' N 02°00' E	6	-	34.1	3.9	61.4
Jabline-Anet Lake	48°54' N 02°43' E	6	-	77.1	7.0	6.5
Saclay Reservoir	48°44' N 02°10' E	6	+	30.2	1.5	77.0
La Veyssière Pond	48°41' N 02°24' E	6	+	27.1	1.6	143.5
Noues de Seine Pond	48°40' N 02°23' E	6	+	29.1	3.4	49.9
Seine Port Pond	48°33' N 02°32' E	6	-	5.9	2.9	176.5
Saint-Quentin Reservoir	48°47' N 02°01' E	6	+	120.1	1.6	103.7
Bois-le-Roi pond	48°29' N 02°43' E	6	+	8.3	2.6	23.3
Les Galets Pond	49°01' N 02°03' E	7	-	10.6	2.2	15.0
Epinoche Pond	48°37' N 02°17' E	7	+	5.7	2.4	110.4
Loy Pond	48°51' N 02°41' E	8	+	6.9	1.3	225.3
Créteil Lake	48°46' N 02°27' E	9	+	40.8	4.5	20.7
Choisy Pond	48°46' N 02°25' E	9	-	21.6	2.6	39.6
Plage Bleue Pond	48°45' N 02°28' E	9	-	9.3	3.1	27.4
Lake Inférieur	48°51' N 02°16' E	10	+	9.5	1.2	255.3
Les Pâtis Pond	48°52' N 02°36' E	10	-	10.3	2.6	35.8
UCPA Centre Pond	48°52' N 02°37' E	10	-	92.0	5.3	1.6
Saint-Cucufa Pond	48°51' N 02°10' E	10	+	5.4	1.8	85.0
UTE Louveciennes	48°51' N 02°06' E	10	-	7.5	2.5	< d.l.
Lake Minimes	48°50' N 02°27' E	10	+	5.8	1.2	194.4
Swiss Pond	48°48' N 02°07' E	10	-	14.4	1.9	32.5
Enghien Lake	48°58' N 02°18' E	n.d.	n.d.	n.d.	1.0	n.d.

Chl a: clorophyll a; n.d.: not defined

Table 2: Biotic and abiotic parameters analysed in the 48 lakes by each laboratory.

LEESU UMR MA 102 University of Paris-Est	**MCAM** UMR 7245 CNRS/MNHN	**ESE** UMR CNRS 8079 University of Paris-Sud	**BIOEMCO** UMR CNRS 7618 ENS
pH*	% Transmitted signal*	Eukaryotic Phytoplankton	Seston
Light*	Depth max*	Cyanobacteria	Zooplankton
Temperature*	NH_4^+*	Heterotrophic bacteria	
Thermal stratification*	N_{total}*	Heterotrophic protists	
Extinction coefficient*	PO_4^{3-}*	Viruses	
Conductivity*	P_{total}*	Haptophytes	
O_2 concentration*	Total Chlorophyll a *		
O_2 saturation (%)*	Green algae		
NO_3- *	Cyanobacteria		
NO_2-*	Diatoms		
TSS (Total Suspended Solid)*	Crypto algae		
TOC*	Yellow substance		
DOC*	Total phytoplankton		
POC*	Microcystis : toxic blue-green algae		
Metals**			
PAH (Polycyclic Aromatic Hydrocarbon)**			
E. coli			
Intestinal enterococci			
NTM			
Bacterial diversity			

* Correspond to the abiotic parameters analyzed for the monthly campaigns of Créteil Lake and the NTM study.
** Correspond to parameters analyzed only for Créteil lake monthly campaigns.

<u>Sampling design</u>

A monthly monitoring is carried out on this lake since December 2011 on a horizontal transect consisting of three points between the storm sewer outlet and the outlet: the « S » point is close to the storm sewer outlet, the « R » point is located next to a rich organic reedbed area and the central point « C », is located between S and R (Figure 2a). The water is collected at three depths for each of the points at about 0.5, 1.5 and 3.5 meters deep (Figure 2b). For each campaign an average sample, named « M » is achieved by pooling the water from the three depths at the three points.
This monitoring will be supplemented by punctual campaigns during particular events (storms, phytoplankton blooms, etc.).

Furthermore, only three of the four laboratories presented in Table 2 participated in the field campaigns of Créteil Lake: LEESU, BIOEMCO and ESE. The parameters analyzed by these laboratories are the same as for the 48 lakes campaign. The details of the parameters investigated are shown in Figure 2b and Table 2.

Microbial assemblages (Figure 2b) are surveyed on the horizontal surface transect (S1, C1 and R1) in order to compare these data with the survey of the Conseil Général du Val de Marne four times per year. Moreover, on the central point, microbial parameters are also measured on the vertical transect (C1, C2, C3) and in the average sample (M).

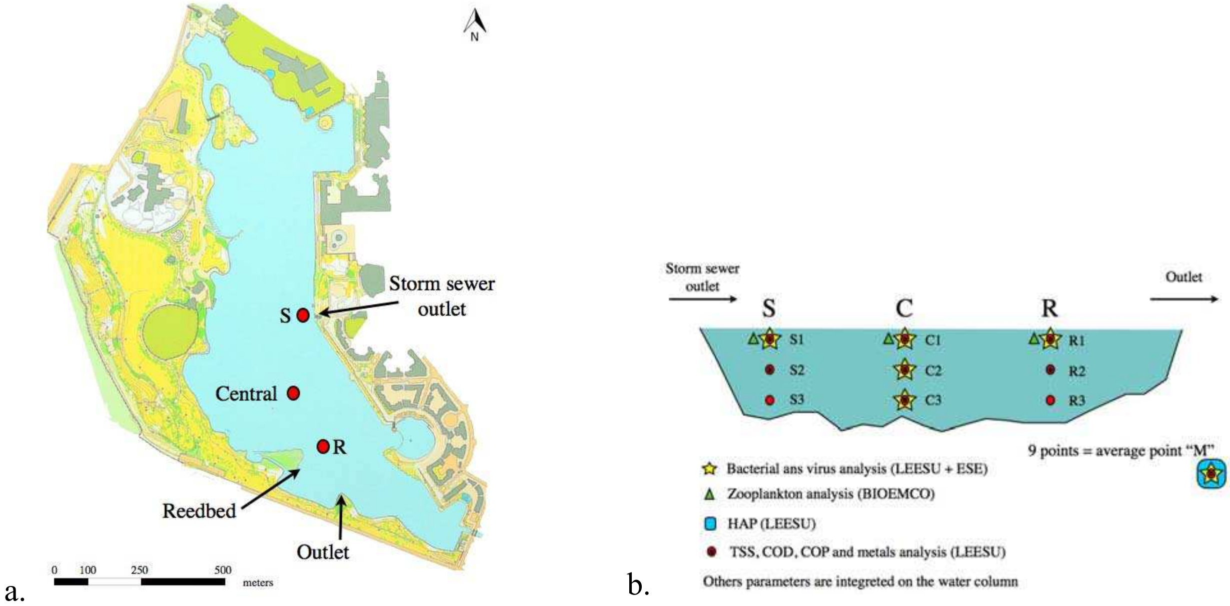

Figure 2: a. Distribution of the three sampling points on the lake of Créteil. b. Biotic and abiotic parameters analyzed according to the sampling points and depths.

Study of the distribution of NTM in urban lakes

<u>Sampling area</u>

This study is conducted on two urban lakes: Daumesnil and Créteil. Daumesnil Lake is located in Bois de Vincennes, in the suburbs of Paris. The water supply of this lake comes from a network of non-potable water of Charonne reservoir, itself fed by the Ourcq canal (fed by the Marne River). It varies in depth from 1 to 1.40 meters for most of its surface area (12 ha). This lake is considered as eutrophic with the following characteristics: 0.1 $mg.L^{-1}$ of total phosphorous, 0.1 $mg.L^{-1}$ of total phosphate, <1.0 $mg.L^{-1}$ of nitrogen (Moulin, 2011). Créteil Lake has an oligotrophic lake status.

<u>Sampling design</u>

On each site, five points will be followed once in the spring and once in the summer (Figure 3). On these points, NTM present in the sediment, water column, neuston, epiphytic and epilithic biofilms will be characterized by quantitative PCR, dHPLC profiles and by sequencing. Abiotic parameters surveyed during Créteil Lake monthly campaigns, except metals, will be determined for each points. Humic acids will be analyzed too in sediments.

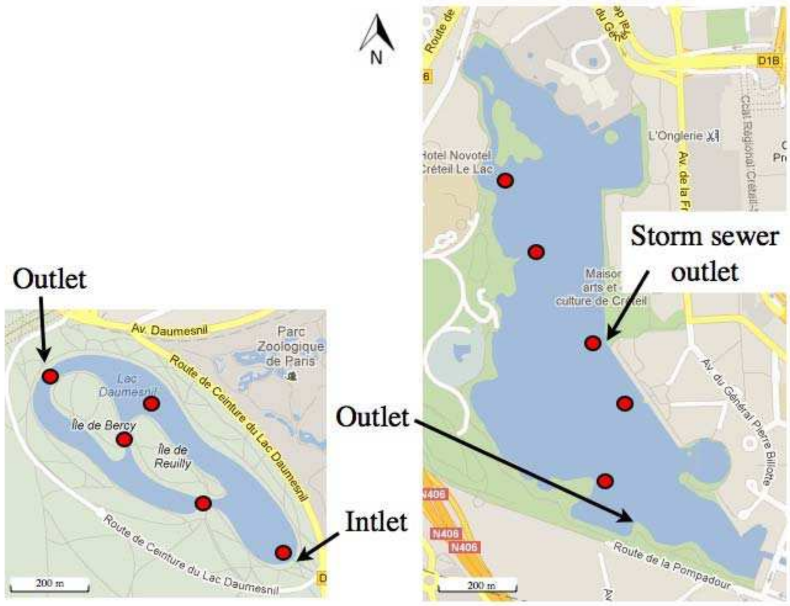

Figure 3: Distribution of five sampling spots on Daumesnil Lake (left) and Créteil Lake (right)

PRELIMINARY RESULTS

The first sampling campaign on the 48 lakes was conducted during the summer 2011. For now data cannot be analyzed since all laboratories, including ours, have not yet measured all their parameters. However, at first glance the concentration of faecal indicators has show that lakes with a storm sewer outlet or being connected directly to the Seine tend to display a water more contaminated water than the other lakes.

Moreover, during the monthly monitoring of Créteil Lake, the weather factor appears determining in the distribution of the indicators of faecal pollution. Indeed, the concentration of these bacteria is very abundant after rain events, especially in the surroundings of the storm sewer outlet. While their concentration is almost zero during dry weather in all the samples, including nearby the sewer outlet (data not shown).

REFERENCES

- BOLUND P. and Hunhammar S. (1999). Ecosystem services in urban areas. Ecological Economics, 29:293-301.
- CATHERINE A., Troussellier M. and Bernard C. (2008). Design and application of a stratified sampling strategy to study the regional distribution of cyanobacteria (Ile-de-France, France). Water Research, 42:4989-5001.
- COLE J.J., Findlay S. and Pace M.L. (1988). Bacterial production in fresh and saltwater ecosystems - a cross-system overview. Marine Ecology - Progress Series, 43:1-10.
- COTNER J.B. and Biddanda B.A. (2002). Small players, large role: microbial influence on biogeochemical processes in pelagic aquatic ecosystems. Ecosystems, 5:105–121.
- DANGER M., Daufresne T., Lucas F.S., Pissard S. and Lacroix G. (2008). Does Liebig's law of the minimum scale up from species to communities? Oikos, 117:1741-1751.
- FALKINHAM III J. O. (2002). Nontuberculous mycobacteria in the environment. Clinics in chest medicine, 23(3):529-551.

- GRIFFITH D.E., Aksamit T., Brown-Elliott B.A., Catanzaro A., Daley C. *et al.* (2007). An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. American Journal of Respiratory and Critical Care Medicine, 175(4):367-416.
- JOHNSON P.T.J., Townsend A.R., Cleveland C.C., Glibert P.M., Howarth R.W. *et al.* (2010). Linking environmental nutrient enrichment and disease emergence in humans and wildlife. Ecological Applications, 20(1):16-29.
- JONES S. E., Newton R.J. and McMahon K.D. (2009). Evidence for structuring of bacterial community composition by organic carbon source in temperate lakes. Environmental microbiology, 11(9):2463-2472.
- HARDINA C.M. and Fujioka R.S. (1991). Soil: the environmental source of *Escherischia coli* and enterococci in Hawaii's stream. Environmental Toxicology Water Quality, 6:185-195.
- LINDERMAN R.L. (1942). The trophic-dynamic aspect of ecology. Ecology, 23: 399-418.
- LINDSTRÖM E.S. (2000). Bacterioplankton community composition in five lakes differing in trophic status and humic content. Microbial Ecology, 40:104-113.
- MEA – Millenium Ecosystem Assessment (2005). Ecosystems and human well-being: wetlands and water. Synthesis. World Resources Institute, Washington, DC.
- MOULIN L. (2011). Contrôle de la qualité de l'eau des lacs des bois de Boulogne et de Vincennes. Prepared for the City of Paris, Eau de Paris.
- MULLER T.A., Ulrich A., Ott E.M. and Muller M. (2001). Identification of plant-associated enterococci. Journal of Applied Microbiology, 91:268-278.
- NEWTON R.J., Jones S.E., Eiler A., McMahon K.D. and Bertilsson S. (2011). A guide to the natural history of freshwater lake bacteria. Microbiology and Molecular Biology Reviews, 75(1):14-49.
- PERNTHALER J. and Amann R. (2005). Fate of heterotrophic microbes in pelagic habitats: focus on populations. Microbiology and Molecular Biology Reviews, 69:440-461.
- PETERS D.P.C., Groffman P.M., Nadelhoffer K.J., Grimm N.B., Collins S.L. *et al.* (2008). Living in an increasingly connected world: a framework for continental-scale environmental science. Frontiers in Ecololy Environment, 6:229-237.
- PIREN-SEINE (2009). La contamination microbienne dans le bassin de la Seine. In : Servais P., Billen G., Garcia-Armisen T., Georges I., Goncalves A., Thibert S. Editors. Eau Seine Normandie. Nanterre, France.
- POFF N.L., Brinson M.M. and Day J.W. (2002). Aquatic ecosystems and global climate change: potential impacts on inland freshwater and coastal wetland ecosystems in the United States. www.pewtrusts.org/our_work_ektid30677.aspx. Viewed on January 2012.
- PRIMM T.P., Lucero C.A. and Falkinham III J.O. (2004). Health Impacts of Environmental Mycobacteria. Clinical Microbiology Reviews, 17(1):98-106.
- RADOMSKI N., Lucas F.S., Moilleron R., Cambau E., Haenn S. and Moulin L. (2010). Development of a real-time qPCR method for detection and enumeration of *Mycobacterium* spp. in surface water. Applied and Environmental Microbiology, 76:7348-7351
- RIVERA S.C., Hazen T.C. and Toranzos G.A. (1988). Isolation of fecal coliforms from pristine sites in a tropical rainforest. Journal of Applied Microbiology, 54:513-517.
- USEPA – United States Environmental Protection Agency (1999). Action plan for beaches and recreational waters. EPA 600-R-98/079. Washington DC.
- WASSON J.G., Chandesris A., Pella H. and Blanc L. (2002). Typology and reference conditions for surface water bodies in France: the hydro-ecoregion approach. In: Ruoppa, M., Karttunen, K. (Eds.), Typology and Classification of Lakes and Rivers, Symposium on Typology and Classification of Lakes and Rivers. Helsinki, Finland, October 24-26, 2002. Tema Nord 566, pp. 37–41.
- WHO – World Health Organization (2001). Water quality: guidelines, standards and health. Assessment of risk management for water-related infectious disease. In: Fewtrell L., Bartram J., editors. WHO Water series. London, UK: IWA Publishing.

- WILLIAMSON C.E., Dodds W., Kratz T.K. and Palmer M.A. (2008). Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Frontiers in Ecology and the Environment, 6(5):247-254.
- WHITMAN R.L., Fowler M., Shively D.A. and Byappanahalli M.N. (2002). Distribution and characterization of *E. coli* within the Dunes Creek watershed, Indiana Dunes State Park. Report to the Indiana Department of Natural Resources. U.S. Geological Survey, Porter, Ind.
- YANNARELL A.C. and Triplett E.W. (2004). Within- and between-lake variability in the composition of bacterioplankton communities: investigations using multiple spatial scales. Applied Environmental Microbiology, 70:214-223.
- YANNARELL A.C. and Triplett E.W. (2005). Geographic and environmental sources of variation in lake bacterial community composition. Applied Environmental Microbiology, 71:227-239.

09-WWW-YES-Roguet-Paper-DT-2012-06-12.doc