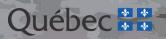
Comparative study of protocols used by Réseau de suivi du benthos of Quebec Governme and by Canadian aquatic biomonitoring of Government of Canada





Cover page photograph: Alain Armellin, Environment Canada

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# Foreword

The comparative study of protocols used by the *Réseau de suivi du benthos* (RSBenthos) and by the Canadian Aquatic Biomonitoring Network (CABIN) was initiated in 2008. However, synchronization of field teams in the fall of 2008 was made impossible due to constraints linked to the planning of the sampling period. Nonetheless, sampling was carried out, but according to each team's agenda. Eight stations were sampled (Annex 1). Unfortunately, in the fall of 2008, unexpected meteorological events, namely heavy rainfalls causing rivers to flood, occurred during the period of sampling for RSBenthos at the beginning of September and for CABIN at the end of September. In the end, four seasons have been excluded from the beginning of the statistical analyses because the number of weeks between the sampling of the two teams at the same station was too high (more than four weeks). It was the case for the stations on the Blanche, Jaune, le Renne and Yamachiche rivers sampled in 2008 (Annex 1).

Another monitoring event was carried out in 2009 after ensuring the synchronization of the two sampling teams. Seven stations were visited (Table 1). A clustering analysis with the four remaining stations from the 2008 sampling event (namely the Jacquot, Mékinac, du Valet and Ferrée rivers), and the seven stations sampled in 2009, highlighted a possible bias linked to the sampling dates of the 2008 stations; these data have therefore been excluded from subsequent analyses. Hence, only the data collected in 2009 were analyzed and are presently reported. However, the community variables and Hilsenhoff's Biotic Index were computed for the stations on the Jacquot, Mékinac, du Valet and Ferrée rivers sampled in 2008, and these results are presented in Annex 2. Annex 3 also shows the dendrogram of the complete linkage clustering of the four stations sampled in 2008 according to the two controls.

## **Summary**

In 2008 and 2009, a comparative study of results from the biomonitoring carried out by RSBenthos and CABIN was performed in order to verify if the differences between protocols used have an impact on the description of the specific assemblage of the sampled benthic communities and on the assessment of the integrity of benthic communities. Statistical analyses performed on the community variables and indexes, and multivariate analyses, have highlighted a large similarity between the two monitoring protocols. It appears that the different procedures used for the sampling and in the laboratory have a weak impact, chiefly on the taxonomic richness variables such as the number of taxa, the number of Ephemeroptera, Trichoptera and Plecoptera (EPT) taxa, Hilsenhoff's Biotic Index (FBI) (identification at the family level), the Benthos Health Index (ISBg) and the description of benthic assemblages. However, there are noticeable differences in the assemblage of some taxonomic groups, such as EPT. These differences can lead to poor diagnoses of the status of the integrity of streams.

# **Table of Contents**

1.	Intro	duction		1
2.	Stud	ied Sites		2
3.	Mate	erial and N	Methods	5
	3.1	MDDE	FP's Réseau de suivi du benthos	5
	3.2	EC's C	anadian Aquatic Biomonitoring Network	7
4.	Data	Analysis		8
	4.1	Prepara	tion of data matrices	8
	4.2	Statisti	cal analyses	8
5.	Resu	lts		11
	5.1	Comm	unity variables and biotic indexes approach	11
		5.1.1	Comparison of community variables	11
		5.1.2	Comparison of Hilsenhoff's Biotic Index (FBI) and of the Benthos Health Index (ISB <sub>8</sub> ) between the two monitoring programs	13
	5.2	Multiva	ariate approach	16
		5.2.1	Clustering analysis	16
		5.2.2	Non-metric multi-dimensional scaling	17
6.	Disc	ussion		20
7.	Conc	clusion		23
8.	Bibli	iography .		25

# List of Tables

Table 1.	List of stations sampled in 2009.	.2
Table 2.	Biophysical description of stations sampled in 2009.	.3
Table 3.	Summary of field and laboratory procedures of the RSBenthos and CABIN protocols.	5
Table 4.	Community variables and indexes used to compare monitoring protocols	.9
Table 5.	Common reference values (CABIN and RSBenthos) for identification at the family level and standardization formulas of each of the $ISB_g$ variables.	10
Table 6.	Frequency of occurrence of the families sampled in 2009 according to the protocols of both monitoring programs	12

Table 7.	Comparison of mean values (standard deviation) of the variables computed from the data collected by the two monitoring programs in 2009. $(n = 7)$	3
Table 8.	Standardized values according to formulas in Table 5 and ISBg values1	5
Table 9.	Comparison of ISB <sub>g</sub> values computed from the reference values produced with MDDEFP's database (genus) and those from this study (family)1	6
Table 10.	Comparison of the relative abundances at the family level for each of the monitoring programs and contribution to the dissimilarity between benthic assemblages in 2009.	9

# **List of Figures**

Figure 1.	Location of sampling stations	3
Figure 2.	Land use at a reference station, à la Pêche River (A), and at a test station, le Renne River (B)	4
Figure 3.	Example of the distribution of the twenty sampled plots for RSBenthos.	6
Figure 4.	Standardized kick net (D-net) from RSBenthos	6
Figure 5.	Standardized kick net (filet troubleau) from CABIN	7
Figure 6.	Example of zigzag pattern during CABIN sampling.	7
Figure 7.	Comparison of FBI values, at the family level, according to the two monitoring programs CABIN and RSBenthos	14
Figure 8.	Comparison of $ISB_g$ 's values, according to the two monitoring programs CABIN and RSBenthos.	15
Figure 9.	Dendrogram of the complete linkage clustering of stations sampled in 2009 according to the two monitoring programs.	17
Figure 10.	Non-metric multi-dimensional scaling of stations sampled in 2009 according to the two monitoring programs.	18

# List of Annexes

Annex 1.	List of stations discarded from the analyses	28
Annex 2.	Presentation of community variables and indexes from stations sampled in 2008	29
Annex 3.	Presentation of results from multivariate analyses from stations sampled in 2008	30
Annex 4.	Comparison of material and methods used for RSBenthos and CABIN	31

Annex 5.	Reference values of the six variables included in the Benthos Health Index (ISB <sub>g</sub> ) of streams with coarse-textured substrate according to the genus level of identification (MDDEFP 2012).	32
Annex 6.	Relative density from each of the monitoring programs, RSBenthos and CABIN	.33
Annex 7.	Values of community variables and indexes from each of the monitoring programs, RSBenthos and CABIN	.35

# **List of Initials**

AAFC	Agriculture and Agri-Food Canada
CABIN	Canadian Aquatic Biomonitoring Network
CWS	Canadian Wildlife Service
DUC	Ducks Unlimited Canada
EC	Environment Canada
EEM	Environmental Effects Monitoring
ESRI	Environmental Systems Research Institute inc.
$ISB_{g}$	Benthos Health Index – streams with coarse substrate
OBBN	Ontario Benthos Biomonitoring Network
MAPAQ	Ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec
MDDEFP	Ministère du Développement durable, de l'Environnement, de la Faune et des
	Parcs
MRNFP	Ministère des Ressources naturelles, de la Faune et des Parcs
RSBenthos	Réseau de suivi du benthos
SLC	St. Lawrence Centre

# 1. Introduction

For several years, the popularity of biomonitoring based on benthic macroinvertebrates has been increasing. The reasons behind this interest are multiple: biomonitoring is a complementary approach to traditional monitoring of the physical and chemical characteristics of water quality; because it uses living organisms to integrate effects over time, biomonitoring allows the observation of the cumulative effects of contaminants and of the maximum values that they can potentially reach beyond the physico-chemical grab sampling events; and biomonitoring enables the assessment of other pressures on aquatic ecosystems, such as water quantity, invasion of exotic species, and degradation of habitat. In Canada, several biomonitoring programs exist: at the federal level, the Canadian Aquatic Biomonitoring Network (CABIN) or the Environmental Effects Monitoring Program (EEM), and at the provincial level, the Ontario Benthos Biomonitoring Network (OBBN) in Ontario or the Réseau de suivi du benthos (RSBenthos) in Quebec. Some studies have focused on comparing the different existing monitoring programs (Brua et al. 2010; Bennett 2004, 2007; Page and Sylvestre 2006; Borisko et al. 2007). These studies have compared the selectivity of the collection devices with different mesh sizes (Surber net, kick net [D-net and kick net] and U-shaped net), and its effects on the specific assemblage of the benthic communities and on the different biotic indexes. These comparative studies demonstrate that, in general, benthic communities and biotic indexes are quite similar, regardless of the collection apparatus (Surber net, kick net [D-net and kick net] and U-shaped net).

In Quebec, Environment Canada (EC) with CABIN and the ministère du Développement durable, de l'Environnement, de la Faune et des Parcs (MDDEFP) with RSBenthos coordinate and carry out the monitoring of the integrity of small streams. These two monitoring programs show many differences in the collection and processing in laboratories of macroinvertebrate samples and in the assessment of the biological integrity of streams (Moisan and Pelletier 2008; Environnement Canada 2010; McDermott et al. 2010; cf. section 2.1). The main differences are the mesh sizes of the collection devices, the collection methodology for organisms (stationary sampling versus sweeping), the number of organisms to be identified, and the type of the fractionating apparatus for samples. Despite these differing methods, the possibility of sharing the data would be beneficial so that the biomonitoring programs could contribute to each other. Hence, a comparison of material and methods was undertaken in order to respond to the two following questions:

- 1) Are we observing differences in the specific assemblage of the benthic communities sampled according to the two types of monitoring protocols?
- 2) Is the assessment of the integrity of the benthic communities determined by the usual biotic indexes similar for the two sampling programs?

The comparison between the two monitoring programs will not be performed in regards to the description of the sites, the characterization of the section studied and the assessment of habitat. The 2009 monitoring event was carried out after ensuring the synchronization of the two sampling teams along with the comparability of the aquatic habitat in the sections of the rivers studied.

## 2. Studied Sites

The sites studied are located within the watersheds of the Sud, Etchemin, Montmorency, Sainte-Anne, Saint-Maurice and Yamaska rivers (Table 1 and Figure 1). The drainage area at the sites (Table 2) varies from 15.5 km<sup>2</sup> (Petite rivière Sainte-Marguerite) to 99.3 km<sup>2</sup> (Mauvaise River), and their altitude spans from 109 m (Jaune River) to 356 m (des Fleurs River). A geographic information system (GIS) (ArcGIS, version 9.3.1; ESRI Redlands, California) was used to determine the land use (as a percentage) upstream of each sampling site: urban setting, agriculture, forest and wetlands. Land use statistics originate from Landsat-7 classified images from Southern Quebec for 1999-2003 (CWS, Faune Québec, DUC, MRNFP, MAPAQ, AAFC, SLC) and Landsat-5 classified images for 1993-1996 (MAPAQ). The des Fleurs, Mauvaise, Ferrée, à la Pêche Rivers and the Petite rivière Sainte-Marguerite drain areas with forest cover exceeding 85% of their watershed. These are called "reference stations" because of the high proportion of forest in the watershed and the low anthropogenic, urban and agricultural pressures (Table 2). MDDEFP's RSBenthos uses quantitative criteria such as the area of the watershed upstream of the station with forest cover greater than 50 %, the area of the watershed upstream of agricultural activities less than 30 %, and the concentrations of total nitrogen less than 1.5 mg/l and of total phosphorus less than 0.03 mg/l in order to select the reference stations. For the stations of these five rivers, the set of criteria has been respected (MDDEFP 2012). The watersheds at the Jaune and le Renne river stations show an agro-forestal suitability with, respectively, 61 % and 44 % of the area upstream of the sampling stations being forested, while agriculture covers, respectively, 28% and 47% of the land (Table 2). These two will be called "test stations" because the pressures from agricultural activities are greater than those at the reference stations. Figure 2 illustrates the land use at a reference station and at a test station.

River	MDDEP's Station Number	BDMA Number <sup>1</sup>	Watershed	Sampling Year	Sampling Date		Natural Province <sup>2</sup>
		Rumber			RSBenthos	RCBA	
des Fleurs	FLEU0109	02330041	Etchemin	2009	2009-09-16	2009-09-16	Appalachians
Petite rivière Sainte-	PSMA0109	02310038	Rivière du Sud	2009	2009-09-16	2009-09-16	Appalachians
Marguerite							
Mauvaise	MAUV0109	05040190	Saint-Anne	2009	2009-09-15	2009-09-15	St. Lawrence Lowlands
Ferrée	FERR0109	05100032	Montmorency	2009	2009-09-16	2009-09-16	St. Lawrence Lowlands
Jaune	JAUN0109	03030339	Yamaska	2009	2009-09-14	2009-09-14	Appalachians
le Renne	RENN0109	03030341	Yamaska	2009	2009-09-14	2009-09-14	St. Lawrence Lowlands
à la Pêche	PECH0109	05010541	Saint-Maurice	2009	2009-09-15	2009-09-15	Meridional Laurentians

Table 1. List of stations sampled in 2009.

<sup>1</sup> BQMA : Banque de données sur la qualité du milieu aquatique

<sup>2</sup> Li et Ducruc (1999)

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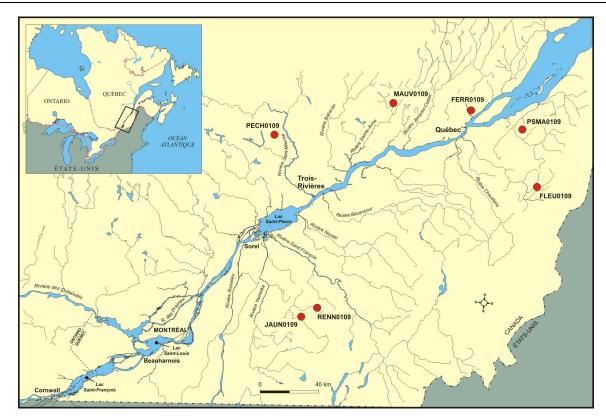


Figure 1. Location of sampling stations.

Table 2.	Biophysical	description	of stations	sampled in 2009.

Station Number	River	Year	Drainage Area	Altitude	Strahler Order <sup>1</sup>	Flow Velocity	Dominant Substrate			Land use	2
								Urban Setting	Forest	Wetland and Bog	Agriculture
			km <sup>2</sup>	m		cm/s				%	
FLEU0109	des Fleurs	2009	44.7	356	3	0.25	Cobbles	1.8	87.9	0.8	7.9
PSMA0109	Petite rivière Sainte- Marguerite	2009	15.5	152	2	0.24	Cobbles	0.9	98.0	0.4	0.4
MAUV0109	Mauvaise	2009	99.3	154	4	0.46	Cobbles	0.1	91	0.8	0.3
FERR0109	Ferrée	2009	22.9	139	2	0.46	Bedrock	2.8	92.8	0.1	2.9
JAUN0109	Jaune	2009	45.7	109	3	0.36	Blocks	2.7	60.5	8.0	28.2
RENN0109	le Renne	2009	79.6	117	2	0.30	Blocks	3.6	43.6	5.6	46.8
PECH0109	à la Pêche	2009	60.2	228	3	0.26	Blocks	0.00	87.1	0.6	0

<sup>1</sup>1/20 000 scale.
 <sup>2</sup> Percentage of the watershed area upstream of stations covered by the category.
 Source: non published data. MDDEFP's database on aquatic wildlife and its environment.

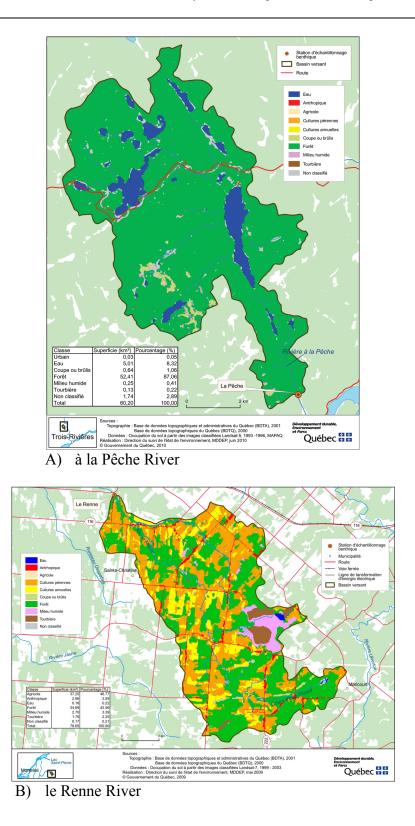


Figure 2. Land use at a reference station, à la Pêche River (A), and at a test station, le Renne River (B).

## 3. Material and Methods

The 2009 monitoring event was carried out after ensuring the synchronization of the two sampling teams along with the comparability of the aquatic habitat in the sections of the rivers studied (Table 1). Sampling of macroinvertebrates for CABIN was done immediately upstream of the 100-m station sampled for RSBenthos.

The following paragraphs briefly describe the methods of collection and laboratory analyses for macroinvertebrates used by each of the monitoring protocols. Table 3 summarizes the similarities and the divergences of the RSBenthos and CABIN protocols (Moisan and Pelletier 2008; Environment Canada 2010; McDermott et al. 2010). Annex 4 shows a complete comparative picture of the different components of the two monitoring protocols.

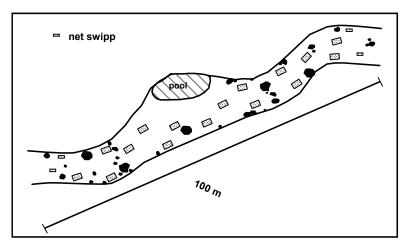
	RSBenthos	CABIN
Collection of Invertebrates		
a) Type of device	a) Kick net (30 cm wide; D- net)	a) Kick net (38 cm wide; filet troubleau)
b) Mesh of device	b) 600 μm	b) 400 μm
c) Area	c) $3 m^2$	c) n.d.
d) Mode of collection	d) Hand and occasionally foot	d) Foot occasionally hand
e) Technique	e) 20 net passes ( $30 \text{ cm} \times 50 \text{ cm}$ ; 30  seconds)	e) Continuous zigzag with net for 3 minutes
f) Field QA/QC	f) No	f) Yes
Processing of macroinvertebrate		
samples in laboratory		
a) Preparation of samples	a) Yes	a) Yes
<ul><li>b) Fractionation</li><li>c) Number of organisms</li><li>d) Types of organisms</li><li>e) Level of identification</li></ul>	<ul> <li>b) Yes, Caton tray</li> <li>c) &gt; 200</li> <li>d) Epibenthic</li> <li>e) 3 levels: level 1, family</li> </ul>	<ul> <li>b) Yes, Marchant box</li> <li>c) &gt; 300</li> <li>d) Epibenthic</li> <li>e) 2 levels: family (genus or</li> </ul>
f) Sorting & identification QA/QC	and genus f) Yes, performed at MDDEFP laboratory since 1989	species for reference sites) f) Yes

 Table 3.
 Summary of field and laboratory procedures of the RSBenthos and CABIN protocols.

#### 3.1 MDDEFP's Réseau de suivi du benthos

Sampling of streams with mainly coarse-textured substrate is carried out in riffles and strait run (Moisan and Pelletier 2008); this is qualified as a "monohabitat" method. Each station stretches 100 m and includes 20 randomly selected  $30 \text{ cm} \times 50 \text{ cm}$  plots which are sampled with a standardized kick net (D-net) with a 600 µm mesh size (Figures 3 and 4). In each plot, the

substrate is disturbed manually during 30 seconds and dislodged organisms are collected with the net. The set of samples collected in the 20 plots produces a composite sample from a total area of  $3 \text{ m}^2$ .



Source: Moisan and Pelletier 2008.

Figure 3. Example of the distribution of the twenty sampled plots for RSBenthos.



Photo: Julie Moisan, MDDEFP. Figure 4. Standardized kick net (D-net) from RSBenthos.

Collected samples are preserved in ethyl alcohol 95% and brought to the laboratory. Samples are then sub-sampled with a 30 cm  $\times$  36 cm Caton fractionating tray (Caton 1991), with the aim of attaining a minimum number of 200 organisms. Sorting is done with a stereomicroscope at the 10x to 100x magnification level in a "Bogorov" sorting tray, and most of the organisms are identified at the genus level. For insects, the final taxonomy is done according to Merritt et al. (2008), and for the other invertebrates (herein called "non-insects") the final taxonomy follows Smith (2001).

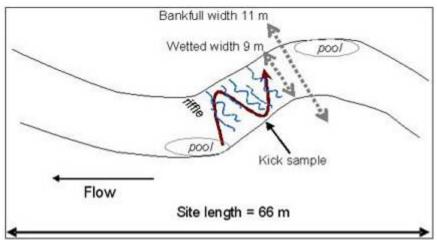
#### 3.2 EC's Canadian Aquatic Biomonitoring Network

CABIN's biomonitoring protocol was developed for the monitoring of small streams with a rocky substrate. A sampling station corresponds to six times the width of the stream who represents a complete pool/riffle/pool sequence (Newbury and Gaboury, 1993). Hence, for a stream with a 6-m width, the sampling station measures 36 m; the length of the station varies, therefore, according to the stream considered. The collection of invertebrates is done with a standardized kick net (filet troubleau) onto which is attached a bag-net with a 400-µm mesh size (Figure 5). The substrate is rubbed with the feet along a zigzag pattern performed from one shore of the river to the next in order to cover the maximum benthic microhabitats (Figure 6). The sampler collects the organisms by dragging a net in his wake; this operation last for three minutes. This type of sampling is qualified as semi-quantitative or as sampling by unit of effort (Environment Canada 2010). Collected organisms are preserved in a buffered formaldehyde 10% solution for a minimum period of 72 hours in order to bind the tissues. After this delay, they are transferred in ethyl alcohol 70%.



Photo: Alain Armellin, EC.

#### Figure 5. Standardized kick net (filet troubleau) from CABIN.



Source: Environment Canada 2010.

#### Figure 6. Example of zigzag pattern during CABIN sampling.

Once in the laboratory, the sample is sub-sampled using a Marchant box (Marchant 1989), which is made of a Plexiglas rectangular box that is subdivided into 100 cells. Afterwards, sorting and identification of organisms are done with the use of a stereomicroscope at the 10x to 80x magnification level or, otherwise with a microscope (60x to 1500x) for the identification of organisms mounted on a slide. The family level is the minimum taxonomic level; however, in the case of reference sites, identification at a finer taxonomic level is recommended, at the genus or species level. A minimum of 300 organisms have to be counted (McDermott et al. 2010). Identifications are done using recognized references quoted earlier (Merritt et al. 2008; Smith 2001). Quality assurance and quality control (QA/QC) procedures for the sorting and identification are described in McDermott et al. (2010).

# 4. Data Analysis

## 4.1 Preparation of data matrices

Comparison of the two monitoring protocols is performed using the data from seven stations sampled in 2009. Five of the seven stations are considered as weakly impaired or unimpaired sites and represent reference conditions (Ferrée, des Fleurs, à la Pêche, Mauvaise rivers and Petite rivière Sainte-Marguerite), while the two other stations are located on an agriculturally suitable land, Jaune and le Renne rivers.

Despite the fact that the taxonomic level chosen by CABIN and RSBenthos for the identification of macroinvertebrates (Table 3) is different, the identification level used for this study is the family. All values for abundance were hence compiled at the family level, except for Lepidoptera, Acarina, Oligochaeta and Nemertea, which are not families and which were maintained as in the analyses. Data on Ostracoda, Cladocera, Platyhelminthes and Nematoda were removed from the matrices.

### 4.2 Statistical analyses

Analysis of data and comparison between the monitoring protocols has been performed following two approaches, first with the community variables and indexes, and second using a multidimensional approach. The approach with the community variables and indexes (Table 4) is appropriate because its aim is to determine if the two monitoring protocols allow the description of the same biological integrity at a given station; in addition, it is recommended by several authors (Barbour et al. 1999; Karr 1998; WFD 2005; AQEM 2002).

Category	Variable or index	Definition or formula	Predicted response according to increase in disturbances
	Total number of taxa (NTAXTOT)	Total number of taxa	decreases
Taxonomic	Number of taxa EPT (NTAXEPT)	Number of EPT	decreases
richness (family)	Number of taxa E (NTAXEPH)	Number of Ephemeroptera taxa	decreases
	Number of taxa P (NTAXPL)	Number of Plecoptera taxa	decreases
	Number of taxa T (NTAXTRICH)	Number of Trichoptera taxa	decreases
	% insects (PINSE)	Abundance of insects / total abundance*100	decreases
	% non-insects (PNONINS)	Abundance of non-insects / total abundance*100	increases
	% EPT (PEPT)	Taxa abundance of EPT / total abundance*100	decreases
	% E (PEPH)	Taxa abundance of Ephemeroptera / total abundance*100	decreases
	% P (PPLE)	Taxa abundance of Plecoptera / total abundance*100	decreases
Taxonomic	% T (PTRICHO)	Taxa abundance of Trichoptera / total abundance*100	variable
assemblage	% EPT without H (PEPTSANHYDR)	Taxa abundance of Ephemeroptera, Trichoptera (excluding Hydropsychidae) and Plecoptera / total abundance*100	decreases
	% Chironomidae (PCHIRO)	Abundance of Chironomidae / total abundance*100	increases
	% Oligochaeta (POLIGOC)	Abundance of Oligochaeta / total abundance*100	increases
	% Hydropsychidae (PHYDRO)	Abundance of Hydropsychidae / total abundance*100	increases
	% molluscs (PMOLL)	Abundance of molluscs / total abundance*100	variable
	% Baetidae (PBAET)	Abundance of Baetidae / total abundance*100	increases
Taxonomic diversity	Shannon-Wiener Index (H') (SHANNWIENER)	H' = $-3,322 \sum_{i}^{s} p_{i} \log (p_{i})$	decreases
	% of two dominant taxa (family) (PTAXDOMDEUX)	Abundance of two most dominant taxa / total abundance*100	increases
Tolerance to	% tolerant (PTOL)	Abundance of organisms with tolerance score > 6 / total abundance*100	increases
pollution	Hilsenhoff's Biotic Index (FBI); tolerance score, at family level (HBI); tolerance score at genus level	$\begin{array}{l} \sum x_i \ t_i \ / \ n \\ x_i: \ number \ of \ individuals \ of \ the \ i^e \ taxon \\ t_i: \ tolerance \ of \ the \ i^e \ taxon \\ n: \ number \ of \ individuals \ included \ in \ the \ sample \end{array}$	increases

Table 4.	Community	variables and	indexes	used to	compare	monitoring	protocols.

The scale of interpretation of the results produced for Hilsenhoff's Biotic Index (FBI) (Table 4) is that of Hilsenhoff (1988) presented in Moisan and Pelletier (2008).

The Benthos Health Index (ISB<sub>g</sub>) for streams with coarse-textured substrate developed by the MDDEFP has also been computed and compared for each of the monitoring protocols. This multi-variable index (multi-metric) includes the six following variables: total number of taxa, number of EPT taxa, percentage of EPT without Hydropsychidae, percentage of Chironomidae, percentage of the two dominant taxa, and the FBI (MDDEFP 2012; Moisan and Pelletier 2008). Since the basic ISB<sub>g</sub> from the MDDEFP is calibrated with the data collected between 2003 and 2008 (83 samples) and for identification at the genus level (Annex 5; MDDEFP 2012), exceptional recalibration of the ISB<sub>g</sub> for the identification at the family level was necessary. Therefore, the reference values of each of the six ISB<sub>g</sub> variables used in the present study were established with the 95<sup>th</sup> or 5<sup>th</sup> percentile of the data from the 14 samples collected for RSBenthos and CABIN in 2009 (Table 5). Usage of the 95<sup>th</sup> percentile is reserved to the variables decreasing with increasing disturbances, such as the number of EPT taxa, while the 5<sup>th</sup> percentile is used for the variables increasing with the disturbances, such as the FBI (Table 4).

The computation of the  $ISB_g$  at a station is first performed by standardizing the values of the six variables to a common scale (0 to 100) using the formulas given in Table 5. Afterwards, the unique value of the  $ISB_g$  is obtained by averaging the computed values using the formulas for the six variables (Table 5). The  $ISB_g$  follows a scale of 0 to 100 units, 100 being the best biological integrity of the environment. The inter-annual variability of this index, or precision, is valued at 10 units (MDDEFP 2012).

Variable decreasing with disturbance	Reference values	Formulas
Total number of taxa	30	$(X \div 30) \times 100$
Number of EPT taxa	19	$(X \div 19) \times 100$
% EPT without Hydropsychidae	82.9	$(X \div 82.9) \times 100$
Variable increasing with disturbance	Reference values	
% Chironomidae	4.6	$[(100 - X) \div (100 - 4.6)] \times 100$
% of two dominant taxa	30.1	[(100 - X) ÷ (100 - 30.1)] × 100
FBI	3.18	[(10 - X) ÷ (10 - 3.18)] × 100

Table 5.	Common reference values (CABIN and RSBenthos) for identification at the
	family level and standardization formulas of each of the ISBg variables.

Comparison of the values of community variables and indexes (FBI and  $ISB_g$ ) computed for the different stations and for each of the monitoring protocols is done with a *t*-test (Page and Sylvestre 2006; Brua et al. 2010). When necessary, the data are transformed with the use of the

equation  $\log_{10} (x + 1)$  or  $\log_{10}$  in order to standardize them. Statistical analyses were performed with the SYSTAT software (SYSTAT 2004).

Multivariate analysis was done using the PRIMER 6 software (Clarke and Gorley 2006). Since the sampling effort was not comparable between methods (the area sampled versus the sampling effort by unit), results were expressed as the relative abundance for comparison purposes (Brua et al. 2010). In order to run the clustering analysis, the data for relative abundance were standardized using the equation  $\log_{10} (x + 1)$ . Afterwards, the Bray-Curtis Similarity Index was computed and the resulting matrix was submitted to a complete linkage clustering analysis (UPGMA routine). In order to interpret the differences between benthic communities, a similarity analysis (ANOSIM routine) was then performed (Brua et al. 2010). This statistical analysis allows comparison of the similarity between replicates, which correspond to the samples collected for each of the two monitoring programs, and is analogous to an analysis of variance. The R statistic produced by the similarity analysis ranges from zero (0) (the values of the Bray-Curtis Similarity Index between the sites and the replicates are similar), to one (1) (there is dissimilarity between and within the sites). Afterwards, the similarity matrix was submitted to a non-metric multi-dimensional scaling (NMS) and, finally, the SIMPER routine was applied to compare the contribution of each taxon to the average of dissimilarities. Therefore, the taxa that contribute the most to the differences between assemblages produced with both methods can be determined (Brua et al. 2010; Clarke and Warwick 2001).

# 5. Results

### 5.1 Community variables and biotic indexes approach

#### 5.1.1 Comparison of community variables

A total of 43 taxa for RSBenthos and 40 taxa for CABIN are included in the database of the seven stations sampled in 2009; 22 families with occurrence greater than 50 % are common to both monitoring programs (in Table 6 [in grey]). The families with occurrence simultaneously equal to or greater than 86 % in the two monitoring programs are the Baetidae, Ephemerellidae, Heptageniidae, Hydropsychidae, Perlidae, Chironomidae, Elmidae, Rhyacophilidae, Oligochaeta, Tipulidae, Acarina, Philopotamidae, Capniidae and Empididae. The less common families, namely those with an occurrence of less than 15%, are numbered at 9 for RSBenthos and 10 for CABIN. When occurring, these families also show a very low relative abundance (Annex 6). The Gastropoda Ancylidae, Diptera Ceratopogonidae and Trichoptera Brachycentridae appear to be collected more frequently in RSBenthos than in CABIN (Table 6). As for the occurrence, the similarity between the two types of monitoring protocols is good (Table 6).

		RSBenthos	CABIN
		Frequency of	Frequency of
ORDER	FAMILY	occurrence (%)	occurrence (%)
TRICHOPTERA	RHYACOPHILIDAE	100	86
OLIGOCHAETA	OLIGOCHAETA	100	86
EPHEMEROPTERA	BAETIDAE	100	100
EPHEMEROPTERA	EPHEMERELLIDAE	100	100
EPHEMEROPTERA	HEPTAGENIIDAE	100	100
TRICHOPTERA	HYDROPSYCHIDAE	100	100
PLECOPTERA	PERLIDAE	100	100
DIPTERA	CHIRONOMIDAE	100	100
COLEOPTERA	ELMIDAE	100	100
TRICHOPTERA	PHILOPOTAMIDAE	86	86
PLECOPTERA	CAPNIIDAE	86	86
DIPTERA	EMPIDIDAE	86	86
DIPTERA	TIPULIDAE	86	100
ACARI	ACARI	86	100
DIPTERA	CERATOPOGONIDAE	71	29
TRICHOPTERA	GLOSSOSOMATIDAE	71	57
DIPTERA	SIMULIIDAE	71	57
EPHEMEROPTERA	LEPTOPHLEBIIDAE	71	71
PLECOPTERA	TAENIOPTERYGIDAE	71	71
PLECOPTERA	PERLODIDAE	71	86
TRICHOPTERA	BRACHYCENTRIDAE	57	29
PLECOPTERA	CHLOROPERLIDAE	57	71
PLECOPTERA		57	71
EULAMELLIBRANCHIA		57	71
LIMNOPHILA	ANCYLIDAE	43	14
TRICHOPTERA	HYDROPTILIDAE	43	29
COLEOPTERA	PSEPHENIDAE	43	43
TRICHOPTERA	PSYCHOMYIIDAE	43	57
TRICHOPTERA	LIMNEPHILIDAE	29	0
TRICHOPTERA	POLYCENTROPODIDAE	29	14
DIPTERA	NYMPHOMYIIDAE	29	14
MEGALOPTERA	CORYDALIDAE	29	14
TRICHOPTERA	LEPIDOSTOMATIDAE	29	29
DIPTERA	ATHERICIDAE	29	43
EPHEMEROPTERA	ISONYCHIIDAE	14	43
TRICHOPTERA	LEPTOCERIDAE	14	0
DIPTERA	TABANIDAE	14	0
ODONATA	AESHNIDAE	14	0
ODONATA	GOMPHIDAE	14	0
HIRUDINEA	HIRUDINEA	14	0
TRICHOPTERA	APATANIIDAE	14	14
PLECOPTERA	PELTOPERLIDAE	14	14
TRICHOPTERA	HELICOPSYCHIDAE	14	29
DIPTERA	MUSCIDAE		29 14
DIPTERA	PSYCHODIDAE	0 0	14
LEPIDOPTERA	LEPIDOPTERA	0	14
	NEMERTEA		14
NEMERTEA	INEIVIERTEA	0	14

# Table 6.Frequency of occurrence of the families sampled in 2009<br/>according to the protocols of both monitoring programs.

Most of the variables linked to the specific richness and tolerance do not show any significant difference between the two monitoring protocols according to a paired *t*-test (Table 7). Almost one third of the assessed variables indicate a significant difference, namely the number of taxa for Trichoptera, the percentages of EPT without Hydropsychidae, the percentages of Ephemeroptera, Plecoptera, Baetidae and Oligochaeta, and the total abundance of organisms. Among the variables showing significant differences between the two monitoring programs, average values of the percentage of EPT without Hydropsychidae, of the percentage of Ephemeroptera, of the percentage of Plecoptera and of the percentage of Baetidae are higher for CABIN than for RSBenthos. RSBenthos indicates the highest mean values (Table 7) for the following variables: the number of taxa for Trichoptera, the percentage of Oligochaeta and the total abundance. Annex 7 presents the values of the different variables and indexes computed for each of the stations sampled in 2009.

Category	Variable or index	Va	alue	Paired <i>t</i> -test
		CABIN	RSBenthos	Value of <i>p</i>
	Number of taxa	23.3 (2.4)	24.7 (4.2)	0.261
	Number of EPT taxa	14.0 (2.3)	14.7 (3.0)	0.253
	Number of Ephemeroptera <sup>1</sup> taxa	3.7 (0.5)	3.9 (0.7)	0.667
	Number of Trichoptera taxa	5.3 (1.6)	6.3 (1.7)	0.018
	Number of Plecoptera <sup>1</sup> taxa	5.0 (2.1)	4.6 (2.1)	0.380
	% EPT	73.5 (17.9)	67.7 (10.6)	0.414
	% EPT without	59.2 (21.5)	47.9 (15.7)	0.02
Richness and	Hydropsychidae			
taxonomic	% Ephemeroptera	37.1 (15.6)	29.6 (12.9)	0.035
assemblage	% Plecoptera	9.5 (5.6)	6.2 (4.9)	0.013
	% Trichoptera	26.9 (14.1)	31.9 (16.9)	0.334
	% Hydrospychidae	14.3 (9.7)	19.8 (13.4)	0.216
	% Baetidae	17.3 (10.8)	7.3 (4.2)	0.009
	% insects	95.6 (1.6)	96.5 (1.9)	0.357
	% non-insects	4.4 (1.6)	3.5 (1.9)	0.357
	% Chironomidae	12.5 (13.5)	18.2 (9.7)	0.356
	% Oligochaeta <sup>2</sup>	0.6 (0.7)	1.7 (1.6)	0.025
	% molluscs <sup>2</sup>	0.7 (0.7)	1.2 (1.3)	0.394
	% tolerant <sup>1</sup>	13.1 (13.6)	19.9 (10.7)	0.081
Tolerance	FBI <sup>1</sup>	3.79 (0.68)	4.03 (0.59)	0.343
	% of two dominant taxa	43.4 (6.2)	42.9 (10.1)	0.874
Taxonomic	H'	3.4 (0.3)	3.5 (0.3)	0.577
diversity				
Abundance	Total abundance of organisms	1361	7778	0.000

Table 7. Comparison of mean values (standard deviation) of the variables computed from the data collected by the two monitoring programs in 2009. (n = 7)

Highlighted values: significant, <sup>1</sup>transformation to  $\log_{10}$ ; <sup>2</sup>transformation to  $\log_{10}(x + 1)$ .

5.1.2 Comparison of Hilsenhoff's Biotic Index (FBI) and of the Benthos Health Index (ISB<sub>g</sub>) between the two monitoring programs

The FBI, which provides information on the level of organic pollution, uses an inverse scale of assessment which ranges from 0 to 10. On this scale, the greater the value, the greater the

disturbance of the environment by organic pollution. The values of the FBI computed from the CABIN data are usually lower, which indicates a better integrity than those from RSBenthos (Figure 7). However, these differences are not significant according to the paired *t*-test (Table 7).

Considering the scale of interpretation for the tolerance scores at the family level, we typically observe that both monitoring protocols indicate values of the FBI of the same class of quality, or show a difference of one class, except for the stations MAUV0109 and RENN0109, where the difference is of 3 and 2 classes of quality, respectively (Figure 7). These differences in the FBI values are caused by the strong proportion of Chironomidae. In the computation of the FBI (Table 4), when this taxon is abundant, it has a great weight due to its high tolerance score (valued at 8 over a maximum of 10). Therefore, at station MAUV0109, the Chironomidae are occurring at a greater rate (36.4%) than for RSBenthos, while for station RENN0109, Chironomidae are represented at a higher rate (41.7%) for CABIN. The FBI does not, however, show any significant difference between the two monitoring protocols according to the paired *t*-test (Table 7).

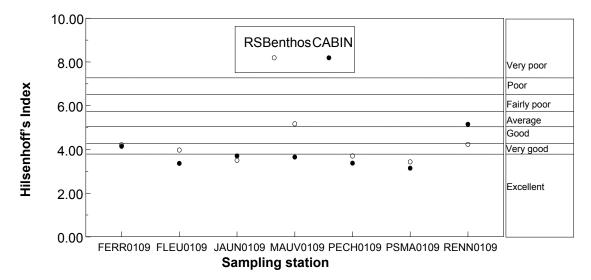


Figure 7. Comparison of FBI values, at the family level, according to the two monitoring programs CABIN and RSBenthos.

Assessment of the level of organic pollution using the FBI indicates that all but two stations show a water quality ranging from good to excellent, i.e., showing little or no organic pollution, for both CABIN and RSBenthos. As opposed to the other stations, CABIN's RENN0109 station shows a fairly significant organic pollution, while this station would be affected by a possible organic pollution under RSBenthos' monitoring protocol. The same conclusion is drawn at station MAU0109: the difference between the two monitoring protocols is significant, while RSBenthos indicates a fairly important organic pollution and CABIN does not show any. For this set of stations, the quality of the aquatic and riparian habitat and the quality of the riparian strip were very good (Unpublished data). The values of the  $ISB_g$  and of the different variables included are shown in Table 8. Generally, the  $ISB_g$  of the CABIN stations results in greater values than those for RSBenthos, except for two stations, PSMA0109 and RENN0109 (Figure 8). Observed differences for the  $ISB_g$  between the two monitoring protocols are fairly weak, and these differences do not significantly affect the assessment of the quality of the ecosystem, except for stations on the Mauvaise (MAUV0109) and le Renne (RENN0109) rivers. Assessments of the biological integrity of these two stations differ by more than 10 units on the  $ISB_g$  scale, depending on the monitoring program carried out (Figure 8; Table 8). Such differences between the two monitoring programs in the assessment of the quality of the ecosystem at these two same stations have also been observed with the FBI (Figure 7). For station MAUV0109, the community assemblage variables, chiefly the percentage of EPT without Hydropsychidae and the percentage of Chironomidae, are responsible for these differences between the two monitoring rograms (Table 8).

Overall, the ISB<sub>g</sub> does not, however, show any significant difference between the two methods, according to the paired *t*-test (p = 0.486). The average ISB<sub>g</sub> is 82.5 for CABIN and 80 for RSBenthos.

Table 8. Standardized values according to formulas in Table 5 and ISBg values.

Station	Numb	er of Taxa	Numb Taxa	er of EPT		EPT without opsychidae	% Ch	ironomidae		wo Dominata xa		FBI		ISBg
	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos
PSMA0109	90	100	89.5	100	88.5	80	100	92.3	93.4	100	100	96.3	93.6	94.8
FLEU0109	83.3	93.3	89.5	94.7	96	73.5	100	81.9	77.1	92.4	97.4	88.4	90.5	87.4
JAUN0109	70	60	57.9	52.6	68.6	57.8	99.7	97.7	80.5	77	92.4	95.3	78.2	73.4
RENN0109	66.7	83.3	63.2	78.9	27.1	34.9	61.1	82.2	64.5	70.7	71.1	84.6	59	72.4
PECH0109	76.7	86.7	73.7	68.4	100	76.2	97.2	90.9	83.5	94.8	97.2	92.4	88	84.9
MAUV0109	76.7	73.3	73.7	73.7	61	38.4	96.5	66.7	82	62.4	93.1	70.8	80.5	64.2
FERR0109	80	76.7	68.4	73.7	57.2	44.1	87.5	88.5	85.4	73.1	85.9	84.9	77.4	73.5

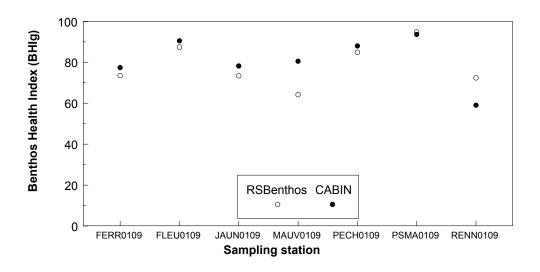


Figure 8. Comparison of ISB<sub>g</sub>'s values, according to the two monitoring programs CABIN and RSBenthos.

Table 9 shows the results for the basic  $ISB_g$  calibrated with the reference values based on identification at the genus level (Annex 5), and the results for the  $ISB_g$  calibrated with the data at the family level from this current study (Table 5). Results are fairly comparable, despite the calibration done with a larger database for the basic  $ISB_g$ , namely MDDEFP's data at the genus level. Classes of quality from the basic  $ISB_g$  are presented in Table 9's legend. According to this classification, results for the  $ISB_g$  for the two protocols are not different by more than one class of quality, and it is also the case when results are compared with those for the basic  $ISB_g$ .

Index	ISB	ISB	ISB
Level of identification	genus	family	family
STABIO	RSBenthos	RCBA	RSBenthos
PSMA0109	96,2	95,2	95,3
FLEU0109	86,1	92,3	89,2
JAUN0109	75,4	79,6	74,7
RENN0109	69,7	60,1	73,9
PECH0109	93	89,4	86,5
MAUV0109	71,7	82	65,5
FERR0109	73,3	78,9	74,9

Table 9.Comparison of ISBg values computed from the reference values produced with<br/>MDDEFP's database (genus) and those from this study (family).

Legend: Class of quality for basic ISBg (MDDEFP 2012)

Very poor 0 - 24,1	Poor 24,2 - 48,3	Fair 48,4 - 72,6	Good 72,7 - 89,1	Very good 89,2 - 100
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#### 5.2 Multivariate approach

#### 5.2.1 Clustering analysis

Values of the Bray-Curtis Similarity Index are in general greater than 75% and less than 82%. We observe that the similarity of communities is higher within a station than it is between stations (Figure 9). We note that the "agricultural" test stations, namely RENN109 and JAUN109, indicate greater similarity between themselves than with the reference stations. The dissimilarity between the reference stations and the "agricultural" test stations was previously observed in the case of the biotic indexes for station RENN109. Results from comparisons of the data collected at stations from the Ferrée, Jacquot, Mékinac and du Valet Rivers by the two monitoring programs in 2008 are presented in Annex 2.

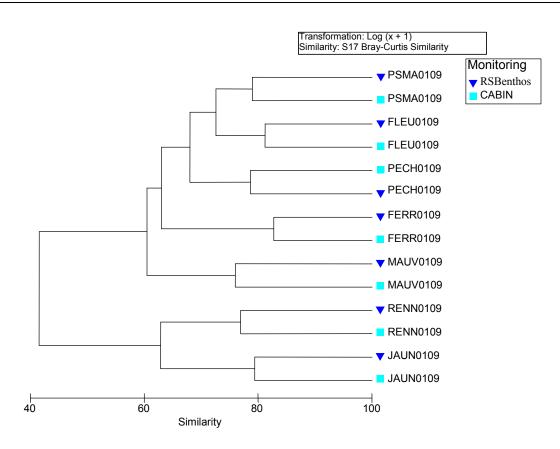


Figure 9. Dendrogram of the complete linkage clustering of stations sampled in 2009 according to the two monitoring programs.

#### 5.2.2 Non-metric multi-dimensional scaling

Non-metric multi-dimensional scaling (NMS) allows a better visualization of the association, similarity or distance between stations (objects) by ordering them in a bi-dimensional or tridimensional space. However, there is a distortion or stress between a similarity and its distance in an ordination plot; stress increases with decreasing dimensional representativeness of the ordination. Generally speaking, in a two-dimensional space, a distortion value (or stress) of NMS lower than 0.1 indicates a good graphical representation, explained by two axes or dimensions, and does not lead to a poor interpretation of the association between the objects (Clarke and Warwick 2001). The NMS of the CABIN and RSBenthos stations produces a stress value of 0.09, and therefore the graphical representation of Figure 10 illustrates well the similarity between the stations in the two monitoring programs. The R value from the analysis of similarity (ANOSIM) in the case of the test on the differences between monitoring programs is 0.015 (p = 0.46) and, in the case of the test on the differences between the reference stations and the (agricultural) test stations, it is 0.845 ( $p \le 0.02$ ). A value of 0.015 indicates that the benthic assemblages are similar between monitoring protocols, but not identical, while the second value of 0.845 shows significant differences ( $p \le 0.02$ ) between benthic assemblages from the reference and the agricultural test stations.

Average dissimilarity and percentage of contribution to this dissimilarity produced with the SIMPER analysis show that the differences between benthic assemblages are not due to the individual contribution of any taxon; 13 of 30 taxa contribute 50.92% of the dissimilarity between assemblages (Table 10). In fact, benthic communities collected using either method are very similar in regards to taxonomy, which is represented by the dendrogram (Figure 9) and the NMS (Figure 10). The main difference originates from the average relative abundance of each of the taxa in both monitoring programs. The taxa contributing the most to the dissimilarity belong to Ephemeroptera (Leptophlebiidae, Baetidae and Heptageniidae), Trichoptera (Glossosomatidae, Psychomyiidae, Philopotamidae and Rhyacophilidae) and Plecoptera (Capniidae, Perlodidae, Taeniopterygidae and Chloroperlidae). Also, we note that the relative abundance of taxa varies according to the monitoring protocol considered.

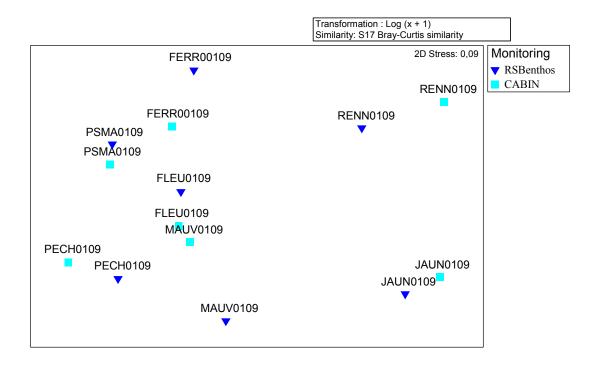


Figure 10. Non-metric multi-dimensional scaling of stations sampled in 2009 according to the two monitoring programs.

Table 10. Comparison of the relative abundances at the family level for each of the monitoring programs and contribution to the dissimilarity between benthic assemblages in 2009.

TAXON	Order/ Class	Average relative abundance		% contribution	Cumulative % contribution
		CABIN	RSBenthos		
Leptophlebiidae	EPH	1.81	2.05	5.56	5.56
Glossosomatidae	TRI	0.86	1.79	4.66	10.22
Acarina	ARC	2.19	0.84	4.51	14.73
Baetidae	EPH	3.61	2.98	4.30	19.02
Psychomyiidae	TRI	1.00	0.83	4.05	23.08
Heptageniidae	EPH	2.74	3.12	3.95	27.03
Philopotamidae	TRI	2.07	1.98	3.91	30.94
Capniidae	PLE	1.40	1.16	3.62	34.56
Perlodidae	PLE	1.62	0.96	3.48	38.04
Taeniopterygidae	PLE	1.44	1.25	3.34	41.39
Elmidae	COL	2.59	2.67	3.25	44.63
Rhyacophilidae	TRI	1.80	1.41	3.14	47.78
Chloroperlidae	PLE	0.88	0.92	3.14	50.92
Lepidostomatidae	TRI	0.67	0.55	3.13	54.05
Chironomidae	DIP	3.39	3.92	2.89	56.94
Sphaeriidae	BIV	0.91	0.85	2.86	59.80
Tipulidae	DIP	1.79	1.90	2.83	62.63
Simuliidae	DIP	0.80	0.87	2.80	65.43
Oligochaeta	OLI	0.87	1.54	2.79	68.22
Hydroptilidae	TRI	0.53	0.58	2.75	70.97
Leuctridae	PLE	0.99	0.75	2.65	73.62
Ephemerellidae	EPH	2.82	3.09	2.62	76.24
Hydropsychidae	TRI	3.60	3.85	2.61	78.86
Brachycentridae	TRI	0.41	0.76	2.52	81.38
Perlidae	PLE	1.89	1.62	2.29	83.67
Empididae	DIP	0.92	1.01	2.24	85.91
Ancylidae	GAS	0.10	0.61	1.96	87.87
Psephenidae	COL	0.49	0.35	1.80	89.67
Ceratopogonidae	DIP	0.31	0.55	1.74	91.41

Legend: EPH – Ephemeroptera; TRI – Trichoptera; PLE –Plecoptera; COL – Coleopters; DIP – Diptera; GAS – Gastropoda; BIV – Bivalves; ARC – Arachnida; OLI – Oligochaeta.

# 6. Discussion

Comparison of results for taxonomic richness and percentages of occurrence of families shows a large similarity between the two monitoring programs. Although observed differences are not significant, it seems that RSBenthos collects, on average, a greater number of taxa than CABIN, in particular the taxa EPT. The observed difference for this last variable seems related to the order Trichoptera. The collection procedure for RSBenthos appears to sample, on average, more Trichoptera from the Hydropsychidae family (non-significant). Only the number of Trichoptera taxa is significantly higher for RSBenthos. CABIN would favour the collection of Trichoptera without shell, while RSBenthos would favour Trichoptera with shell. The attachment mode of these organisms could be responsible of this bias due to the collection method. As a matter of fact, using the RSBenthos method, the collection is done with the hands, which enables a more efficient collection of the organisms bound to cobbles, blocks and other coarse-textured substrates such as Trichoptera, and therefore is akin to the use of the Surber net. Meanwhile, the CABIN method, which uses only the feet to dislodge the invertebrates, would be less efficient to collect organisms that are solidly attached to the substrate.

There are significant differences between monitoring protocols in regards to the variables of taxonomic assemblage, such as the percentages of Ephemeroptera, Plecoptera and EPT without Hydropsychidae and Baetidae. Results from this study highlight a greater selectivity of the CABIN protocol towards Ephemeroptera (when expressed as relative abundance), in particular for the family Baetidae, and it is also the case with Plecoptera; Baetidae are known as good swimmers occurring in swift waters. The CABIN method is likely, under some river conditions, to favour the collection in swift sections where the collection of a greater number of Plecoptera and Ephemeroptera is more highly probable, because of the continuous sampling. In opposition to the Brua et al. (2010), Bennett (2004, 2007) and de Page and Sylvestre (2006) studies concluding that, in general, the standard CABIN net captures more benthic organisms than the U-shaped and Surber nets, the RSBenthos procedure collects more individuals than CABIN's. As for the taxonomic richness, Brua et al. (2010) and Page and Sylvestre (2006) indicate that the U-shape net allows the collection of more taxa than the standard CABIN net. Bennett (2004) reached the same conclusion and observed a significantly greater taxonomic richness in the composite from three samples collected with the Surber net. Although the observed difference is not significant, the RSBenthos procedure also collects more taxa than CABIN's.

However, these differences are not important enough to discriminate the benthic communities described by either monitoring protocol. Clustering analyses and NMS confirm the strong similarity between benthic communities, regardless of the sampling method. Three orders, Ephemeroptera, Trichoptera and Plecoptera, contribute almost 50% of the dissimilarity between benthic communities, as shown by the clustering analysis and the NMS. Therefore, the variability within a station is lower than between stations. According to Brua et al. (2010), multivariate analyses, in particular the index of comparability of sampling methods according to their ordering power (classification strength-sampling-methods comparability [CS-SMC]), also highlight a strong similarity between macroinvertebrate assemblages. The clustering analyses allow a good discrimination of each of the sites, the intra-site variability, and subsequently between sampling devices, being weaker than the inter-site variability. According to Brua et al. (2010), these results

show that regardless of the sampling device used (standard CABIN net or U-shaped net), the same benthic community is sampled. In the Page and Sylvestre (2006) study, the NMS reveals light differences between the Surber net and the kick net, with sites in urban settings showing a lesser variability than the less impacted sites.

Four potential sources of variability between monitoring protocols could be responsible for the observed significant differences, namely the sampling effort, the sub-sampling aiming for 200 or 300 organisms (fractionation), the dimension of stations and the contagious distribution of macroinvertebrates. The sampled area for RSBenthos is important (3 m<sup>2</sup>) and the duration of the collection is 600 seconds, while for CABIN the sampling effort is 180 seconds. This could explain the greater abundance observed with RSBenthos. The fixed length of an RSBenthos (100 m) sampling station shelters a greater diversity of benthic microhabitats than a CABIN station, which corresponds to six times the width of the stream represents a complete pool/riffle/pool sequence (Newbury and Gaboury, 1993), which is lesser than this number for the small streams of a Strahler order of 1, 2 or 3. The influence of microhabitats on the specific assemblage of benthic communities does not need to be further demonstrated. The type of material in place and the flow velocity or the type of flow have an impact on the spatial distribution of benthic invertebrates (Thorp et Covich 2001).

In addition to the causes linked to the collection of organisms, the processing of samples in the laboratory is added to the potential sources of difference between the CABIN and RSBenthos methods. First, the minimum number of organisms to reach for the count during the fractionation is different, namely 300 and 200 organisms, respectively, for CABIN and RSBenthos. Intuitively, the taxonomic richness should increase with the counted number of organisms due to the increasing probability of observing rarer and less abundant taxa. This effect on the number of taxa was not observed in this study because the taxonomic richness does not differ significantly between CABIN and RSBenthos samples. The second possible cause of variability could originate from the fractionating apparatus used, namely the Marchant box and the Caton tray. Results from this present study do not allow determination of the existence of biases inherent to these two devices. However, Lester et al. (2009) report in their study comparing these two devices that the median percentage of similarity was 83.6% for the taxonomic richness, 86.6% for the percentage of sub-sampling, and 95.9% for the biological integrity index. The authors concluded that either fractionating devices could be used for biomonitoring. We have to specify that the variables of taxonomic assemblage have not been analyzed in this study, therefore limiting its scope.

The contagious distribution of benthic organisms could be a source of bias between the two monitoring protocols. The distribution of benthic organisms is associated with the hydrological conditions which, by combining the conditions of the flow velocity, the depth and the rugosity of the substrate, create microhabitats (Brooks et al. 2005). The composite sample from RSBenthos (namely, 20 plots selected randomly in a 100-m station), would be more likely to reduce the collection of some taxa displaying a contagious distribution than a CABIN sample.

#### Assessment of the biological integrity of streams

Even though some differences were observed between the benthic communities studied for each of the monitoring programs, it was important to determine if these dissimilarities could lead to different diagnoses of the biological integrity of the streams. First, the multivariate analyses (clustering analysis and NMS) have made it possible to differentiate between the reference and agricultural test stations, although the similarity between these two types of stations remains elevated and the number of stations is low. However, the two test stations showed different benthic communities, and this is chiefly obvious with the NMS (Figure 10).

Comparison of the FBI and ISB<sub>g</sub> between the two monitoring programs shows fairly similar results, and the observed differences are relatively weak and do not significantly change the assessment of the biological integrity of the aquatic ecosystem, except for two stations. Depending on the monitoring protocol used, stations RENN0109 (test station) and MAUV0109 (reference station) show different assessments greater than 10 units for the ISB<sub>g</sub>, and of two to three classes of quality for the FBI. These differences could be linked to the sampling itself, and not to the computation of the indexes, and would be due to the proportions of Chironomidae in each of the samples. This assumption appears to be confirmed by the fact that station MAUV0109 (reference) produced an unexpected result for RSBenthos in 2009. As a matter of fact, the 2007 monitoring event at this station showed a community in good condition (MDDEFP 2012). Using only two test stations, a good assessment of the comparison of the diagnoses of the biological integrity of streams between the two monitoring protocols is difficult to achieve.

# 7. Conclusion

In Quebec, EC with the Canadian Aquatic Biomonitoring Network, as well as MDDEFP, coordinate and carry out biomonitoring for small streams. These two monitoring programs show numerous differences in the collection and processing in the laboratory of macroinvertebrate samples and in the assessment method for the biological integrity of their streams. This report compared results produced by the two monitoring protocols while targeting the taxonomic level of the family.

Results from this comparison are rewarding. The multivariate analyses and the biotic indexes demonstrate that the benthic communities are similar in the majority of cases; the different sampling methods and laboratory procedures have a limited influence. However, noticeable differences in the assemblage of some taxonomic groups are observed, notably for EPT, which can lead to poor diagnoses of the biological integrity of streams if these indexes are used on their own. Furthermore, this is crucial due to the fact that these taxonomic groups are currently used in the biomonitoring programs, and are therefore very important.

Even though these results are rewarding, a major constraint hampers the sharing of databases at this time. This constraint is mainly linked to the fact that the minimum number of organisms counted in the laboratory that are required for each of the monitoring protocols, namely 200 organisms for RSBenthos and 300 organisms for CABIN, is not the same.

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River	Watershed	BQMA Number	Sampling Year	Sampling D	ate
				RSBenthos	CABIN
Ferrée	MONTMORENCY	05100032	2008	2008-09-23	2008-09-30
Ruisseau du Valet	SAINT-CHARLES	05090071	2008	2008-09-24	2008-09-30
Jacquot	SAINT-ANNE	05040197	2008	2008-09-22	2008-09-30
Mékinac	BATISCAN	05030215	2008	2008-09-12	2008-10-01
Yamachiche	YAMACHICHE	05300013	2008	2008-09-09	2008-10-01
Blanche	MASKINONGÉ	05260034	2008	2008-09-10	2008-10-01
Jaune	YAMASKA	03030339	2008	2008-09-04	2008-10-24
le Renne	YAMASKA	03030341	2008	2008-09-04	2008-10-24

Annex 2. Presentation of community variables and indexes from stations sampled in 2008

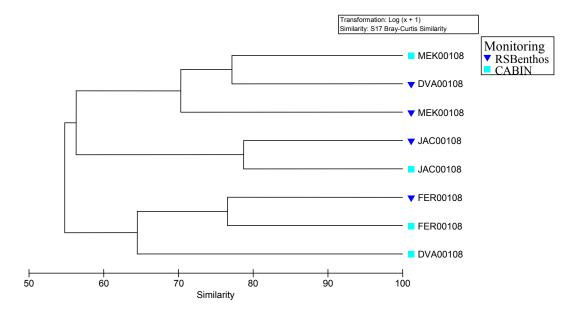
Comparison of the values of Hilsenhoff's Biotic Index (FBI) produced at stations sampled in 2008 for CABIN and RSBenthos

Station		CABIN		RSBenthos
	Index	Status	Index	Status
FERR0108	3.78	Very good	4.84	Good
JACQ0108	3.85	Very good	4.62	Good
MEKN0108	4.03	Very good	4.21	Very good
VALE0108	3.81	Very good	4.29	Good

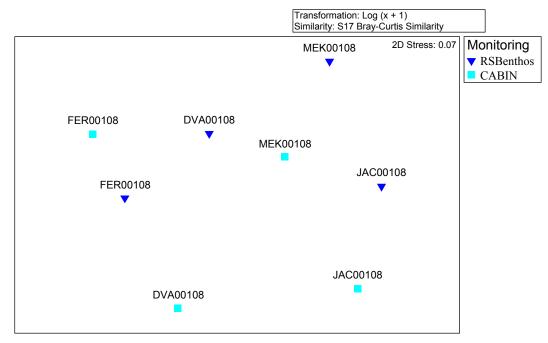
Values of community variables and indexes measured for each monitoring program in 2008

Station	MEK	N0108	JAC	Q0108	VAL	E0108	FERR0108		
BQMA	05030215	05030215	05040197	05040197	05090071	05090071	05100032	05100032	
Date	2008-10-01	2008-09-12	2008-09-30	2008-09-22	2008-09-30	2008-09-30 2008-09-24		2008-09-23	
Monitoring program	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	
Ntaxtot	27	28	21	22	28	28	25	21	
NTaxEph	5	5	2	3	4	4	4	4	
NTaxPl	4	4	5	4	5	5	5	4	
NTaxTrich	4	6	6	5	4	7	8	6	
NTaxEPT	13	15	13	12	13	16	17	14	
ShannWiener	3,67	3,25	3,34 3,05		3,71	3,50	2,74	3,49	
Peph	24,8	18,8	18,8 4,6		21,9	22,9	66,2	28	
Pple	5,6	2,3	25,5	9,9	19,3	6	4,4	5,8	
Ptricho	24,1	51,9	29,1	43,7	18,3	27,5	11,2	15,2	
РЕРТ	54,5	72,9	59,3	55,6	59,5	56,4	81,9	49	
PEPTwithoutHydr	36,6	31,2	46	24,2	46,4	41,1	77,8	40,3	
Pinse	96,7	94,7	96,4	95,9	88,6	98,2	95,7	86	
Pbaet	0,3	5,6	0	0,3	1,3	1,2	55,5	15,2	
Phydro	17,8	41,7	13,2	31,4	13,1	15,2	4,1	8,6	
Pchiro	20,1	11,7	24,2	27,3	16,3	27,5	5,3	26,7	
Poligoc	0,7	3,4	3,3	4,1	1	1,4	0	7,8	
Pmoll	1	1,9	0,3	0	7,2	0,5	1,3	3,3	
PTaxdomTWO	38	53,4	40,7	58,7	34,6	42,7	62,8	42	
Ptol	21,1	15	27,5	31,4	18,3	28,9	5,3	34,6	
FBI	4,03	4,21	3,85	4,62	3,81	4,29	3,78	4,84	
Abundance	303	266	302	293	306	433	607	243	

#### Annex 3. Presentation of results from multivariate analyses from stations sampled in 2008



Dendrogram of the complete linkage clustering of stations sampled in 2008 according to the two monitoring programs.



Non-metric multidimensional scaling (NMS) of stations sampled in 2008 according to the two monitoring programs.

	RSBenthos	CABIN
Method		
a) Reference conditions	a) Yes	a) Yes
b) Habitat	b) Monohabitat (riffle	b) Multi-microhabitat (must
	and flat flow)	include riffle and strait run)
Collection of invertebrates	· · · · · · · · · · · · · · · · · · ·	
a) Station	a) 100 m	a) Variable (6 times the width of
		stream)
b) Area	b) $3 m^2$	b) n.d.
c) Device	c) Kick net ( <i>D-net</i> )	c) Kick net (kick net)
d) Mesh of device	d) 600 μm	d) 400 μm
e) Collection	e) Hand and	e) Foot and occasionally hand
	occasionally foot	
f) Technique	f) 20 net passes	f) 1 net pass for 3 minutes
<i>y</i> 1	$(30 \text{ cm} \times 50 \text{ cm}; 30)$	, 1
	seconds)	
g) $QA/QC - field$	g) No	g) Yes
	6,	
River order	1/20 000	1/50 000
Habitat characteristics (at station)		
a) Type of flow	a) OK classes	a) OK class
b) Velocity of flow	b) OK cm/s	b) OK cm/s
c) Forest cover	c) %	c) 5 categories
d) Transparency	d) 3 categories	d) No
e) Riparian vegetation	e) 2 banks OK	e) OK
f) Aquatic vegetation	f) No	f) Presence/absence
g) Substrate	g) OK 6 categories	g) OK 8 categories and 3 classes
h) Embeddedeness	h) 4 categories	h) No
i) Sedimentation	i) 4 categories	i) No
j) Periphyton	j) No	j) 5 categories
k) In situ physico-chemistry	k) OK	k) OK
1) In vitro physico-chemistry	1) OK	I) OK
m) Tide range	m) Yes	m) No
n) Stream modification	n) Yes	n) No
o) Freq. of riffles	o) Yes	o) No
p) Stability of banks	p) Yes	p) No
q) Vegetal protection of banks	q) Yes	q) No
r) Width vegetal band	r) Yes	r) No
s) Quality of habitat Index	s) Yes	s) No
Laboratory – invertebrates		
a) Processing of samples	a) Yes	a) Yes
b) Fractionation	b) Yes, Caton tray:	b) Yes, Marchant box:
-,	$30 \text{ cm} \times 36 \text{ cm} (30)$	$35 \text{ cm} \times 25 \text{ cm} \times 10 \text{ cm}$
	squares of 6 cm $\times$ 6	$(100 \text{ equal cells of } 1 \text{ cm} \times 1)$
	1	
c) Number of organisms	$\begin{array}{c} cm)\\ c) > 200 \end{array}$	cm) c) $> 300$
d) Types of organisms	c) > 200 d) Epibenthic	<i></i>
e) Level of identification	1 1 1 1 1 I	
-, Lever et rachtinearten	e) 3 levels: novice, family and genus	e) 2 levels: family and genus or species in reference sites
f) QA/QC – sorting and		
identification	f) Yes	f) Yes
Analyses and interpretation		
a) Database	a) Yes	a) Yes
b) Metric and multimetric	b) Yes	b) Yes
c) Clustering	c) No, possibly	c) Yes
d) Ordination	d) No, possibly	d) Yes
u) Orumation	$u_j$ into, possibly	u) 105

Annex 4. Comparison of material and methods used for RSBenthos and CABIN

Annex 5. Reference values of the six variables included in the Benthos Health Index (ISBg) of streams with coarse-textured substrate according to the genus level of identification (MDDEFP 2012).

Variable decreasing with disturbance	Reference value X <sub>95</sub>	X <sub>min</sub>	Standardization formula
Total number of taxa	35	0	$(X \div 35) \times 100$
Number of EPT taxa	22.4	0	$(X \div 22.4) \times 100$
% EPT without Hydropsychidae	72.5	0	$(X \div 72.5) \times 100$
	Reference value		
Variable increasing with disturbance	Χ <sub>5</sub>	$X_{\text{max}}$	
% Chironomidae	4.1	100	$[(100 - X) \div (100 - 4.1)] \times 100$
% of two dominant taxa	32	100	$[(100 - X) \div (100 - 32)] \times 100$
HBI	2.53	10	$[(10 - X) \div (10 - 2.53)] \times 100$

Table of classes of quality of MDDEFP's basic ISB<sub>g</sub> (identification at genus level).

Very poor 0 - 24,1	Poor 24,2 - 48,3	Fair 48,4 - 72,6	Good 72,7 - 89,1	Very good 89,2 - 100
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	PECH01	09	FLEU01	09	FERR01	109	MAUV0	109	PSMA01	09	
	05010541		023300	41	051000		050401	90	02310038		
FAMILY	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	
BAETIDAE	3.5	13.4	13.5	33.7	9.9	21.6	2.5	8.9	8.9	20.1	
EPHEMERELLIDAE	7.7	0.3	9.8	10.2	10.3	9.4	3.4	6.6	5.3	6.2	
HEPTAGENIIDAE	15.1	28	12.5	12.4	3.4	1	8.9	12.3	12.2	6.2	
ISONYCHIIDAE	0	0	0	0	0.4	0	0	0	0	0	
LEPTOPHLEBIIDAE	18.6	7.6	4	3.1	3.1	2.3	0	0.6	16.9	14.6	
TRICHOPTERA	0.4	0	0	0	0	0	0	0	0	0	
APATANIIDAE	0	0	2	0.6	0	0	0	0	0	0	
BRACHYCENTRIDAE	0	õ	0.3	0.0	1.5	0	0	õ	0.4	1.6	
GLOSSOSOMATIDAE	0	0	5.7	1.2	0	0	2.1	0.6	0.9	0	
HELICOPSYCHIDAE	0 0	0	0	0	0 0	0	0	0.0	0	0	
HYDROPSYCHIDAE	9.5	3	5.4	5	33.2	18.7	19.9	30.4	4.9	8.8	
HYDROPTILIDAE	0	0	0	0	0.4	0	0	0	4.5 0	0.0	
	5.3	10.9	0	0.6	0.4	0	0.8	0	0	0	
LEPTOCERIDAE	0	0	0	0.0	0	0	0.8	0	0	0	
	0	0	0.3	0	0	0	0	0	0.2	0	
PHILOPOTAMIDAE	7	10.9	0.3 3.4	1.5	0.4	0.6	5.5	5.4	2.6	3.9	
POLYCENTROPODIDAE	0	0	0.7	0	0.4	0.0	0	0	0.8	0.3	
PSYCHOMYIIDAE	-										
	0	0	0	0.6	0	0	0	0	0.6	0.3	
RHYACOPHILIDAE	0.4	3.6	1.3	0.9	2.3	3.5	1.7	4.1	1.5	3.2	
PLECOPTERA	0	0	0	0	0	0	0	0	0.2	0	
CAPNIIDAE	0.4	0.3	0.7	0.3	0.8	0.3	0.4	2.2	5.6	7.1	
CHLOROPERLIDAE	0.4	0.3	1.7	2.8	0	0.3	0.4	0.6	4.7	1	
	0.7	0.6	0.7	0.3	0	1.3	0.8	1.9	1.1	1.3	
PELTOPERLIDAE	0	0	0	0	0	0	0	0	0.4	1	
PERLIDAE	1.8	4.6	2.4	4.6	0.8	1	2.5	3.2	1.5	1	
PERLODIDAE	2.1	1.8	0.7	5	1.1	1.3	0.4	1.6	0.8	3.6	
TAENIOPTERYGIDAE	0	0.9	1.3	1.5	2.3	4.8	2.1	2.5	1.7	1.9	
ATHERICIDAE	0	0	0	0.3	0	0	0	0.3	0.2	0	
CERATOPOGONIDAE	0.4	0	0.3	0.6	0	0	0	0	0.2	0.6	
CHIRONOMIDAE	13.3	7.9	21.9	4.6	15.6	16.5	36.4	7.9	11.9	4.5	
EMPIDIDAE	0.7	0.3	0.3	0.6	0.4	0.3	3	2.5	0.2	0	
MUSCIDAE	0	0	0	0	0	0.3	0	0	0	0	
NYMPHOMYIIDAE	0	0	0	0	0.4	0.3	0	0	0.6	0	
SIMULIIDAE	0.4	0.6	0	0	2.7	1.3	0	0	1.1	2.6	
TABANIDAE	0.4	0	0	0	0	0	0	0	0	0	
TIPULIDAE	1.8	0.9	2.7	2.2	0	0.3	1.7	1.6	1.9	1	
ELMIDAE	3.5	1.5	6.4	4.3	5.3	9.4	0.8	0.6	10.7	5.2	
PSEPHENIDAE	0.4	0	0	0	0	0	0.8	0	0	0.3	
AESHNIDAE	0	0	0	0	0	0	0.4	0	0	0	
GOMPHIDAE	0.7	0	0	0	0	0	0	0	0	0	
LEPIDOPTERA	0	0	0	0	0	0	0	0	0	0.3	
CORYDALIDAE	0.4	0	0.3	0	0	0	0	0.6	0	0	
ACARI	0.4	0.3	0.7	2.5	0.4	2.9	0.4	4.1	0	1.6	
SPHAERIIDAE	2.8	1.5	0	0	0.4	0.3	0	0.3	1.1	1.6	
ANCYLIDAE	0	0	0.7	0	2.7	0.3	0	0	0.4	0	
HIRUDINEA	0	0	0	0	0	0	0	0	0.2	0	
OLIGOCHAETA	2.5	0.3	0.3	0.3	2.3	1.9	4.7	0.9	0.4	0	
NEMERTEA	0	0.3	0	0	0	0	0	0	0	0	

Annex 6.	Relative density	from each of t	he monitoring programs	, RSBenthos and CABIN.

# Annex 6 (cont'd). Relative density from each of the monitoring programs, RSBenthos and CABIN.

	RENN01	109	JAUN01	09		
	030303	41	03030339			
FAMILY	RSBenthos	RCBA	RSBenthos	RCBA		
BAETIDAE	3.1	0.3	9.7	22.8		
EPHEMERELLIDAE	11.5	12.7	4.7	2.8		
HEPTAGENIIDAE	2.2	1.5	5.5	0.6		
ISONYCHIIDAE	0	0	0	0.0		
LEPTOPHLEBIIDAE	0.6	0	0	0		
	0.3	0	0	0		
APATANIIDAE	0.0	0	0	0		
BRACHYCENTRIDAE	1.7	0.6	0	0		
GLOSSOSOMATIDAE	4.8	0.6	9.3	2.5		
HELICOPSYCHIDAE	4.0 1.1	0.9	0	0.3		
HYDROPSYCHIDAE	28.9	13.3	36.4	20.9		
HYDROPTILIDAE	0.6	0.6	3.8	4		
	0.0	0.0	0	0		
	0.3	0	0	0		
	0.0	0	0	0		
	0	0	4.2	3.7		
	0	0	4. <u>2</u> 0	0		
PSYCHOMYIIDAE	0.8	0.6	8.5	19.1		
RHYACOPHILIDAE	0.3	0.6	0.8	0		
PLECOPTERA	0.5	0.0	0.0	0		
	0.6	3.4	0.4	0		
	0.0	0	0	0		
	0	0	0	0		
PELTOPERLIDAE	0	0	0	0		
PERLIDAE	0.6	0.6	0.8	0.9		
PERLODIDAE	0.0	0.0	0.0	0.3		
TAENIOPTERYGIDAE	0.6	0	0	0.0		
	0.3	0.3	0	0		
CERATOPOGONIDAE	0.6	0.0	0.4	0		
CHIRONOMIDAE	21.6	41.7	6.8	4.9		
EMPIDIDAE	1.4	0.3	0.0	0.6		
MUSCIDAE	0	0.0	0	0.0		
NYMPHOMYIIDAE	0	0	0	0		
SIMULIIDAE	0.3	0	0.4	0.3		
TABANIDAE	0.0	0	0	0.0		
TIPULIDAE	5.3	3.4	3.8	4.3		
ELMIDAE	10.1	13	2.1	3.7		
PSEPHENIDAE	0	0.6	0.4	1.2		
AESHNIDAE	0	0.0	0.4	0		
GOMPHIDAE	0	0	0	0		
	0	0	0	0		
CORYDALIDAE	0	0	0	0		
ACARI	1.1	4.6	0.8	5.5		
SPHAERIIDAE	0.6	4.0	0.8	0.9		
ANCYLIDAE	0.0	0	0	0.9		
HIRUDINEA	0	0	0	0		
OLIGOCHAETA	0.8	0.3	0.8	0.3		
NEMERTEA	0.8	0.5	0.8	0.3		
	0	U	U	U		

Station	PSMA01	109	FLEU0	109	JAUN0	109	RENN01	109	PECH01	09	MAUV01	109	FERRO	109
Date	2009-09-	-16	2009-09	-16	2009-09	-14	2009-09-	-14	2009-09-	15	2009-09-	15	2009-09	-16
BQMA	0231003	38	023300	41	030303	39	0303034	03030341 05010541		05040190		05100032		
Monitoring	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN	RSBenthos	CABIN
Ntaxtot	31	27	28	25	18	21	25	20	26	23	22	23	23	24
NTaxEph	4	4	4	4	3	3	4	3	4	4	3	4	5	4
NTaxPl	7	7	6	6	1	2	3	2	5	6	6	6	4	6
NTaxTrich	8	6	8	7	6	6	8	7	4	4	5	4	5	3
NTaxEPT	19	17	18	17	10	11	15	12	13	14	14	14	14	13
ShannWiener	3.9	3.9	3.7	3.5	3.2	3.3	3.2	2.8	3.7	3.4	3.2	3.6	3.3	3.4
Peph	43.3	47.1	39.7	59.4	19.9	26.2	17.4	14.5	44.9	49.5	14.8	28.5	27.1	34.2
Pple	16	16.9	7.4	14.6	1.3	1.2	1.7	4	5.3	8.6	6.8	12	5	9
Ptricho	11.9	18.2	19.2	10.5	63.1	50.5	38.8	17.3	22.5	28.7	30.1	40.5	37.8	22.9
PEPT	71.2	82.1	66.3	84.5	84.3	77.8	57.9	35.8	72.6	86.8	51.7	81	69.8	66.1
PEPTwithoutHydr	66.3	73.4	60.9	79.6	47.9	56.9	28.9	22.5	63.2	83.8	31.8	50.6	36.6	47.4
Pinse	97.9	96.8	98.3	97.2	98.3	93.2	97.5	95.1	94.4	97.6	94.9	94.6	94.3	94.5
Pbaet	8.9	20.1	13.5	33.7	9.7	22.8	3.1	0.3	3.5	13.5	2.5	8.9	9.9	21.6
Phydro	4.9	8.8	5.4	5	36.4	20.9	28.9	13.3	9.5	3.1	19.9	30.4	33.2	18.7
Pchiro	11.9	4.5	21.9	4.6	6.8	4.9	21.6	41.7	13.3	7.3	36.4	7.9	15.6	16.5
Poligoc	0.4	0	0.3	0.3	0.8	0.3	0.8	0.3	2.5	0.3	4.7	0.9	2.3	1.9
Pmoll	1.5	1.6	0.7	0	0	0.9	0.6	0	2.8	1.5	0	0.3	3.1	0.6
PNonIns	2.1	3.2	1.7	2.8	1.7	6.8	2.5	4.9	5.6	2.4	5.1	5.4	5.7	5.5
PTaxdomTWO	29.2	34.7	35.4	46.1	46.2	43.7	50.6	54.9	33.7	41.6	56.4	42.7	48.9	40.3
Ptol	12.2	4.5	22.2	5	7.6	5.5	22.5	42	15.8	7.6	41.1	8.9	17.9	18.4
FBI	3.43	3.14	3.97	3.36	3.5	3.7	4.23	5.15	3.7	3.37	5.17	3.65	4.21	4.14
Abundance	531	308	297	323	236	325	356	324	285	327	236	316	262	310

# Annex 7. Values of community variables and indexes from each of the monitoring programs, RSBenthos and CABIN.



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