Macrozoobenthos in the Lower Seine: a survey from the perspective of the European Water Framework Directive

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ecoconsult report: 200703

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Summary

In the framework of Seine Aval's "Action II-2005-03", entitled: "Etat des peuplement benthiques dans la partie amont de l'estuaire" macroinvertebrates of the tidal freshwater section of the river Seine were sampled in the period June 16-20, 2006. Samples were taken in the three zones distinguished for the European Water Framework Directive (WFD), in downstream direction indicated as T_1^A, T_1^B and T_2. The most diverse community was found in the upstream most zone T_1^A, in which also some sensitive species were present. Water quality in this part of the river is mediocre and the tidal currents are relatively low. In the zone, T_1^B, the macroinvertebrate diversity had sharply dropped and sensitive species had disappeared. Water quality is insufficient, due to the industrial activities in the vicinity of Rouen and the tidal currents are relatively strong. The macroinvertebrate diversity in the zone T_2 was much lower compared with the second zone. Only a few species maintain detectable populations in this harsh environment in which water quality is also insufficient and the tidal currents contain relatively high loads of suspended solids. Most important species was the gammarid *Gammarus salinus* comprising 25.4% of the total number of animals found in all samples from the tidal freshwater section of the river Seine. The species was found in 52% of the samples The chironomids *Dicrotendipes nervosus* and *Polypedilum scalaenum* were the most common species occurring in 71% and 54% of the samples respectively. Based on the TWINSPAN (Hill & Šmilauer, 2005) cluster analysis longitudinal distribution of the taxonomic groups present in the tidal freshwater section of the river Seine was made visible.
The ecological quality, assessed with the IGBA, revealed that with one exception, all values of the IGBA metrics IF and IFD, including the $IGBA_{total}$ values, gradually decrease in downstream direction. At a specific exceptional location, the relatively high IFD and, as a consequence, the $IGBA_{total}$ value was caused by the presence of four specimens of *Neuroclips bimaculata* (Polycentropidae) wich were found in one of the samples from the deep river bed. Differences between the different metrics were relatively small. They were mainly caused by few specimens of higher indicator taxa, if present in sufficient numbers in some of the samples. This means those taxa meet marginal conditions for their existence and the presence of one or two extra specimens can result in a higher IBGA value as, for example, shown at the exceptional location.

The development of ecological assessment and classification systems is considered one of the most important and technically challenging parts of the implementation of the WFD. However, assessment methods for tidal freshwater sections of large rivers still appeared to be under development in relevant European countries. Since member states are free to develop their information strategy including monitoring and assessment procedures, most obvious policy for Seine Aval is to pass jointly, with similar organisations for river management in France, through the iterative process of matching information needs with possibilities for information supply.

Although for most of the current assessment methods it is not needed to identify the macroinvertebrates at species level, a higher

identification resolution recommended to detecting differences between reference and test sites, and is required for detecting the presence of rare or threatened species, which in turn is important to identify protection areas for nature conservation. For these reasons species level identifications of macroinvertebrates was advocated by several authors. Species within a genus or family also can have different biological attributes, such as tolerances to and preferences for abiotic conditions (e.g., flow velocity, substrate composition, temperature, dissolved oxygen concentration), different food resource requirements and different life history strategies. When individuals from different species are aggregated into genera or families, information that is potentially valuable in discriminating between sites may be lost. Whether this is acceptable or not depends on the extent to which patterns expressed by the species in assemblages can be represented by the information retained at the resolution of genus or family level.

River pollution and lack of habitats seem to be the most important factors preventing macroinvertebrates to recolonise the tidal freshwater section of the river Seine. Data by courtesy of D.I.R.E.N. revealed the recolonisation potential from the vicinity of the Lower Seine and from upstream parts. Recolonisation can take place by means of drifting and/or flying. On the other hand, due to the interconnection of river basins and the presence of major ports at the mouth of larger European rivers, an increasing number of nonindigenous species can be expected taking into account the assumption that community vulnerability to invasions can be ascribed to combinations of several factors like the presence of vacant niches, habitat modification and disturbance before and during invasions. A major corridor for nonindigenous species to enter the river Seine is the Marne-Rhine Canal.

Prospects for river rehabilitation must be developed for defining the Maximum Ecological Potential (MEP) and Good Ecological Potential (GEP). Description of both potentials is prescribed in the WFD. Starting point for discussions and decision making could be the ecotope approach. In The Netherlands an antropogenic component was introduced in the ecotope definition, which was defined as "a physically limited ecological unit, of which composition and development are determined by abiotic, biotic and anthropogenic aspects together". The use of ecotopes in studies and scenario's has several advantages making changes better visible for water managers and politicians. One has to realise that natural riverine landscapes are dynamic, and biologically and spatially complex. They are characterised by often extensive flood plains, a natural flow regime, high hydraulic connectivity, a successional landscape mosaic with high habitat heterogeneity, and a complex land-water coupling and exchange.
The interplay between landscape elements has a direct bearing on the generation, distribution and maintenance of riverine biodiversity. On the other hand, the riverine fauna provides important feedbacks that can influence spatio-temporal dynamics of the landscape over long time periods. All these aspects should be considered in the process of river rehabilitation.

Résumé

1. Introduction

On October 3, 2005, Ecoconsult was asked to present a tender for monitoring and assessment activities in the field of Seine Aval's "Action II-2005-03", entitled: "Etat des peuplement benthiques dans la partie amont de l'estuaire". The tender for the project was accepted in March 2006 and the convention signed on May 18, 2006. The first activity planned in the project, being a quick scan of the freshwater tidal part of the river Seine, was performed already in the period December 6-8, 2005. This activity was considered to be important to get an impression of the main fluvial biotopes present, including the dominant macrofauna present in it, previous to the design of a sampling program. The results of the quick scan were summarized in a separate report (Paalvast *et al.*, 2006).
Next step in the project was the design of a sampling protocol followed by a sampling campaign in the period June 16-20, 2006. The results of this sampling campaign are discussed in this report and related to the conditions prescribed in the European Water Framework Directive (WFD). For this purpose an inventory was made of WFD related methods used for the classification and for ecological quality assessment, based on macroinvertebrates, of freshwater tidal river sections in the rivers Rhine, Scheldt and Elbe. All these rivers were identified as heavily modified, which means their ecological quality should at least meet the so-called "Good Ecological Potential" (GEP) in 2015. This status is derived from the "Maximum Ecological Potential" (MEP), which is the highest ecological status for heavily modified or artificial water bodies in the European Union.

The WFD establishes a framework for the protection of all water types (including inland surface waters, transitional waters, coastal waters and groundwater) in order to achieve a good (ecological) quality status by 2015. Important ecological aims are:

- to protect and enhance the status of water resources;
- to prevent further deterioration of water bodies;
- to promote sustainable water use,
- improvement of the aquatic environment through specific measures for the progressive reduction of discharges, emissions and losses of priority substances.

The ecological status is assessed from results of monitoring programs covering several so-called "Water Quality Elements" (WQE's). The lists of WQE's for each surface water category are subdivided into 3 groups:

a. biological elements, to which the element "macroinvertebrates" belongs;
b. hydromorphological elements supporting the biological elements;
c. chemical and physico-chemical elements also supporting the biological elements.

Although monitoring of all prescribed WQE's is obliged, member states are free to exclude some of them from their monitoring program, if well argued. However, composition of macroinvertebrate communities in freshwater tidal river section is an accepted WQE for all European rivers.

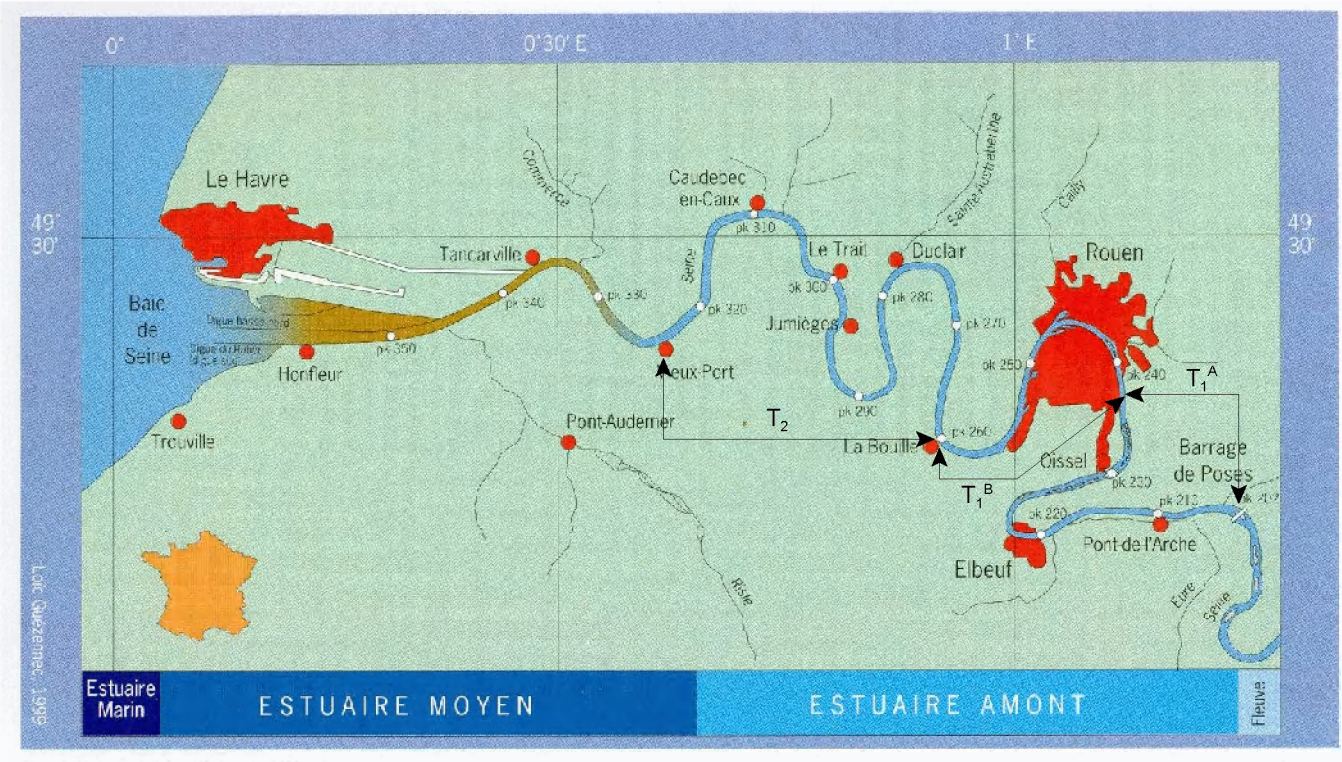
For each biological water quality element described in the WFD, assessment of the ecological status should be based on a comparison between the actual situation and reference conditions described for each water body type (European Union, 2003[A]). Since the Lower Seine was classified as a heavily modified water body, two reference conditions must be taken into account: the Maximum Ecological Potential (MEP) and Good Ecological Potential (GEP). Both conditions should be derived from the natural status. Prior to the description of reference communities for a MEP or GEP, insight is needed into possibilities for flora and fauna to colonise or recolonise the tidal freshwater section of the Lower Seine. Possibilities for macroinvertebrates are discussed.

In relation to the WFD, two sections, T_1 and T_2, were distinguished in the freshwater tidal part of the River Seine downstream of the weir near Poses. T_1 is the section between Poses (rk[1] 202) and La Bouille (rk 260), T_2 between La Bouille (rk 260) and Vieux Port (rk 325). In

[1] rk = river kilometer

section T_1 there is a big difference in water quality and river management between the parts up- and downstream of Rouen. Those are the reasons for splitting up this section into two subsections (Figure 1): T_1^A from Poses (rk 202) to Rouen (rk 236), T_1^B from Rouen (rk 236) to La Bouille (rk 260). This division into sections was the basis for the monitoring strategy.

Figure 1
Map of the fresh water tidal part of the river Seine with the devision into sections (see text) (after Guézennec et al, 1999).



1.1 Aim

Aim of the project was to develop a monitoring and assessment strategy for macroinvertebrates in the Seine aval, that meets the conditions prescribed in the WFD. This strategy should be based on practical experiences in that river section.

2. Material and Methods

Prior to the monitoring campaign performed in the period 16-20 June 2006 as part of the project, sampling methods planned to be used were elaborated. The methods are summarized in Annex 1. In Table 1 an overview is given of sampling devices used for the different biotopes.

Table 1
Overview of sampling devices used.

Biotope	Method
Littoral vegetation	Handnet
Stones in the littoral zone	Manually picked up
Littoral zone	Handnet (also used for kick samples) Eckman dredge
Small woody debris	Manually picked up and sawed into pecies
Deeper river bed	Hamon grab (Benne Hamon) Triangular dredge Van Veen grab Artificial substrate

Downstream of Rouen a Hamon grab (Photo 1) was used for sampling the deeper parts of the main channel. Because the exploring vessel could not pass the bridges in Rouen, this device could not be used upstream of this city. In that case a triangular dredge (Photo 2) was mainly used to sample the deeper parts, and in some cases also a Van Veen grab.

Photo 1
The Hamon grab (Benne Hamon).

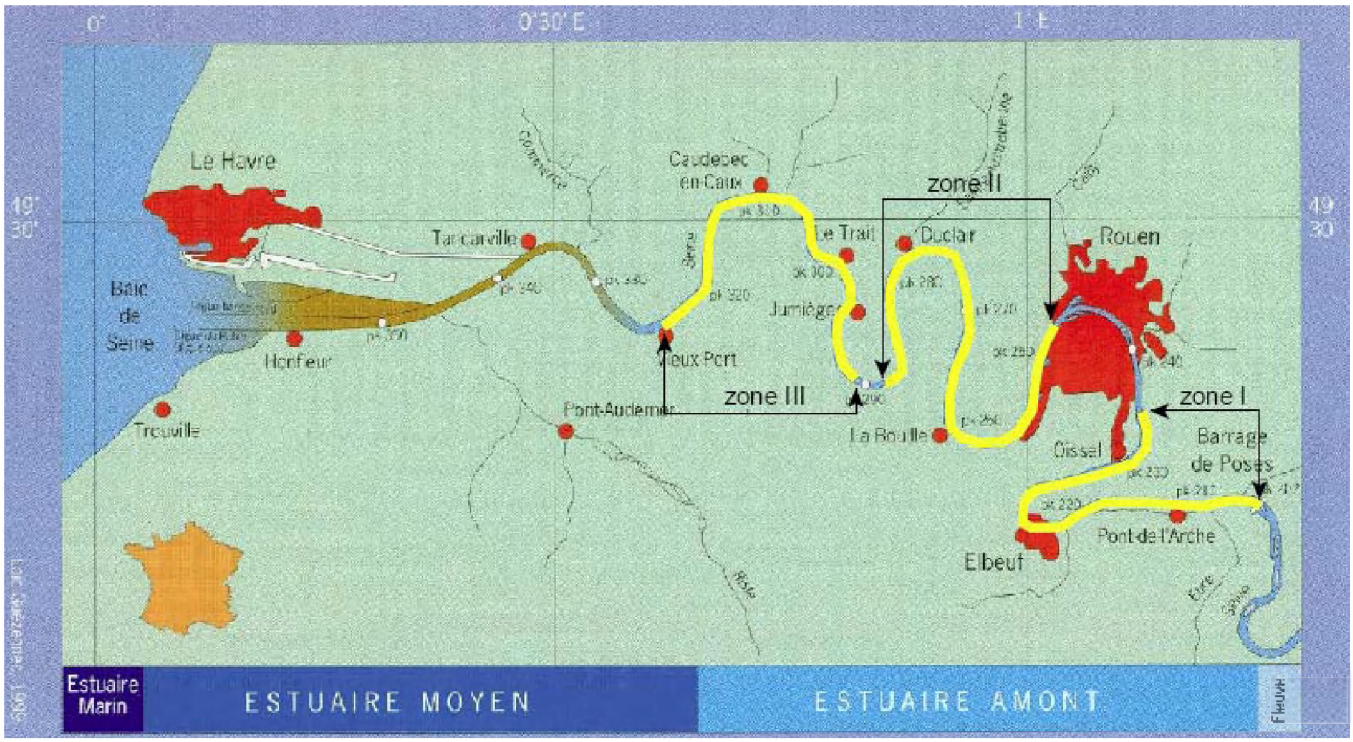
In addition to the samples from the deeper parts of the river, an artificial substrate was applied consisting of nettings with each three broken bricks (each brick 21 x 10 x 6.5 cm) in it. Total weight of each netting was about 7 kg (range 6.5 to 7.5 kg) (individual weights: 2 x 6.5 kg; 9 x 7.0 kg and 1 x 7.5 kg). They were suspended in the main channel in duplo at six locations. However, during retrieval it appeared that two of them were lost and one was found completely dried out on the riverbank.
For collecting the animals from the samples the procedures given in Annex 1 were used. The animals were preserved in ethanol directly after sampling, except several Hamon grab samples taken on June 17th which were processed the day after.
The macrozoobenthos was identified as much as possible to species level. However, it should be noticed that the identification level of several taxonomic groups of macrozoobenthos strongly depends on their developmental stage. Especially for the younger stages identification was not always possible at species level. Literature for identification of the organisms is given in Annex 2.
TWINSPAN (Hill & Šmilauer, 2005) was used for cluster analysis.

Photo 2
The triangular dredge.

3. Results

Taxa found during the June 2006 monitoring campaign are listed in the Annexes 3 and 4. A cluster analysis (TWINSPAN) showed that the major factor influencing the clustering was the river kilometre. Three ecological zones were identified, comprising the zones (T_1^A, T_1^B and T_2) distinguished in the framework of the WFD. The upstream most ecological zone is the river section confined by the Poses weir (river km 203) and river km 230 (in the vicinity of the town of Oissel), the second one between the river km's 247 and 288 (between Petit Quevilly and Yville sur Seine), and the downstream most zone between the river km's 292 and 324 (le Landin and Vieux Port respectively) (Figure 2). Since the ecological zones and the zones identified for the WFD are approximately equal, the ecological zones are indicated in this report according to their WFD classification.

Figure 2
Map of the fresh water tidal part of the river Seine with indicated the three identified ecological zones (see text) (after Guézennec et al, 1999).



The most diverse community of the whole tidal freshwater section was found in the upstream most zone T_1^A including some (pollution) sensitive species. Water quality in this part of the river is mediocre and the tidal currents are relatively low. In the second zone, T_1^B, the macroinvertebrate diversity had sharply dropped and sensitive species had disappeared. Water quality is insufficient, due to the industrial activities in the vicinity of Rouen and the tidal currents are relatively strong. The macroinvertebrate diversity in the zone T_2 was much lower compared with the second zone. Only a few species

maintain detectable populations in this harsh environment in which water quality is also insufficient and the tidal currents contain relatively high loads of suspended solids (Table 2).

Table 2
Number of taxa per taxonomic group found in three ecological zones distinguished in the Seine aval.

Taxonomic group	Number of taxa		
	zone T_1^A	zone T_1^B	zone T_2
Tricladida	3	3	1
Polychaeta	1	1	
Oligochaeta	15	14	10
Hirudinea	10	10	1
Mollusca	26	16	4
Crustacea	8	5	2
Ephemeroptera	3	1	
Plecoptera	1		
Heteroptera	2	1	
Coleoptera	3	3	1
Trichoptera	5	1	
Chironomidae	47	27	7
Total number of taxa	124	82	26

Most important species was the gammarid *Gammarus salinus* comprising 25.4% of the total number of animals found in all samples from the Seine aval. The species was found in 52% of the samples (Table 3). If the contribution of a species is >5% of the total number of animals, it is considered being dominant, and the same percentage of a species in the total number of animals ***minus*** the number of dominant species is considered being subdominant, four dominant and also four subdominant species could be identified comprising 68.9% of all animals found.

Table 3
Dominant and subdominant species in the Seine aval (P = percentage of the samples in which the species was found).

Dominant			
Family	Species	%	P
Gammaridae	*Gammarus salinus*	25.4	52
Oligochaeta	*Psammoryctides barbatus*	11.8	37
Chironomidae	*Dicrotendipes nervosus*	11.0	71
Bithyniidae	*Bithynia tentaculata*	7.9	39
Subdominant			
Asellidae	*Asellus aquaticus*	8.1	39
Chironomidae	*Glyptotendipes pallens*	7.5	45
Chironomidae	*Polypedilum scalaenum*	7.3	54
Sphaeriidae	*Sphaerium solidum*	5.9	2

The chironomids *Dicrotendipes nervosus* and *Polypedilum scalaenum* were the most common species occurring in 71% and 54% of the samples respectively. All dominant and subdominant species were found in >35% of the samples, except the mollusc *Sphaerium solidum*. This species was mainly found at one location (Petit Quevilly, rk 247.7) in a relatively high number in the deep river bed. Some species were only dominant or subdominant in either the littoral zones or in the deeper parts of the main channel (Table 4). The oligochaete *Psammoryctides barbatus*, the caddish fly *Hydropsyche contubernalis* and the molluscs *Corbicula fluminea* and *S. solidum* (Photo 3) were dominant or subdominant in the deeper parts of the main channel; the chironomid *Limnophyes* species, the isopod *Asellus aquaticus* and the mollusc *Sphaerium corneum* in the littoral zones.

Table 4
Dominant and subdominant species (%) in the littoral zone and in the deeper parts of the main channel

		dominant		subdominant	
Family	Species	littoral	deep	littoral	deep
Gammaridae	*Gammarus salinus*	36.9	12.3		
Oligochaeta	*Psammoryctides barbatus*		24.7		
Chironomidae	*Dicrotendipes nervosus*	11.0	11.1		
Bithyniidae	*Bithynia tentaculata*	12.4			6.2
Sphaeriidae	*Sphaerium solidum*		5.5		
Asellidae	*Asellus aquaticus*	5.3			
Chironomidae	*Limnophyes* species			12.7	
Chironomidae	*Polypedilum scalaenum*			9.1	6.9
Chironomidae	*Glyptotendipes pallens*			8.3	8.2
Sphaeriidae	*Sphaerium corneum*			8.0	
Corbiculidae	*Corbicula fluminea*				8.8
Hydropsychidae	*Hydropsyche contubernalis*				6.7

Based on the TWINSPAN cluster analysis longitudinal distribution of the taxonomic groups present in the Seine aval can be made visible. Three triclad taxa were found in the zones T_1^A and T_1^B; *Dugesia tigrina* was only present in samples from zone T_2 (Table 5[2]). All three species are common inhabitants of solid substrates in lager lentic and lotic water bodies.

Table 5
Longitudinal distribution of the triclads (Tricladida).

	zone T_1^A	zone T_1^B	zone T_2
Dendrocoelum lacteum	+[1]	++	
Dugesia lugubris/polychroa	++	++	
Dugesia tigrina	++	++	+

[2] Tables 5 – 14. Frequency: + = <10%; ++ = 10-25%; +++ = >25% (of the samples)

The bristle worm *Hypania invalida* (Polychaeta), which was very common in the zones T_1^A and T_1^B, is an invasive species from the Ponto-Caspian area. The species was able to expand its distribution range in westward direction after opening of the Danube-Main-Rhine Canal in September 1992 (Bij de Vaate *et al.*, 2002). *H. invalida* was found in >25% of the samples taken in both zones mentioned mentioned above, but not in zone T_2.

Of the oligochaetes at least 21 taxa were found (Table 6). Some of them could not be identified to species level, including the juveniles. According to Fomenko (1980), *Nais pardalis* and *Potamothrix moldaviensis* belong to mesorheophilic group of species, while *Psammoryctides barbatus* and *Tubifex ignotus* are limnophilic species. However, the other species are typical limnophilic as well. Most of the species found are abundant in the Lower Rhine as well, except *Tubifex ignotus*, *Haplotaxis gordioides* and *Peloscolex velutinus* which are rare species.

Table 6
Longitudinal distribution of the oligochaetes (Oligochaeta).

	zone T_1^A	zone T_1^B	zone T_2
Aulodrilus pluriseta	+		
Limnodrilus udekemianus	+		
Nais pardalis	+		
Peloscolex multisetosus	+		
Enchytraeidae species	++	++	
Lumbricidae species	+	+	
Ophidonais serpentine	+	+	
Stylaria lacustris	+	++	
Eiseniella tetraedra		+	
Chaetogaster diaphanous		+	
Tubifex ignotus		+	
Haplotaxis gordioides		+	+
Peloscolex velutinus			+
Stylodrilus heringianus			+
Nais ellinguis	+		+
Branchiura sowerbyi	+	++	++
Limnodrilus claparedeianus	+++	+++	+++
Limnodrilus hoffmeisteri	+++	++	++
Lumbriculidae species	+	+++	+
Potamothrix moldaviensis	++	++	+
Psammoryctides barbatus	+++	+++	++

Nine species of leeches were collected (Table 7). Those in the in the zones T_1^A and T_1^B hardly differentiate. In zone T_2 only *Glossiphonia complanata* was found. *Cystobranchus respirans* is the only rheophilic leech. It is an ectoparasite on cyprinids and salmonids. *Trocheta riparia* lives semi-aquatic (Nesemann, 1997).

Photo 3
A sample of *Sphaerium* spec. and oligochaetes.

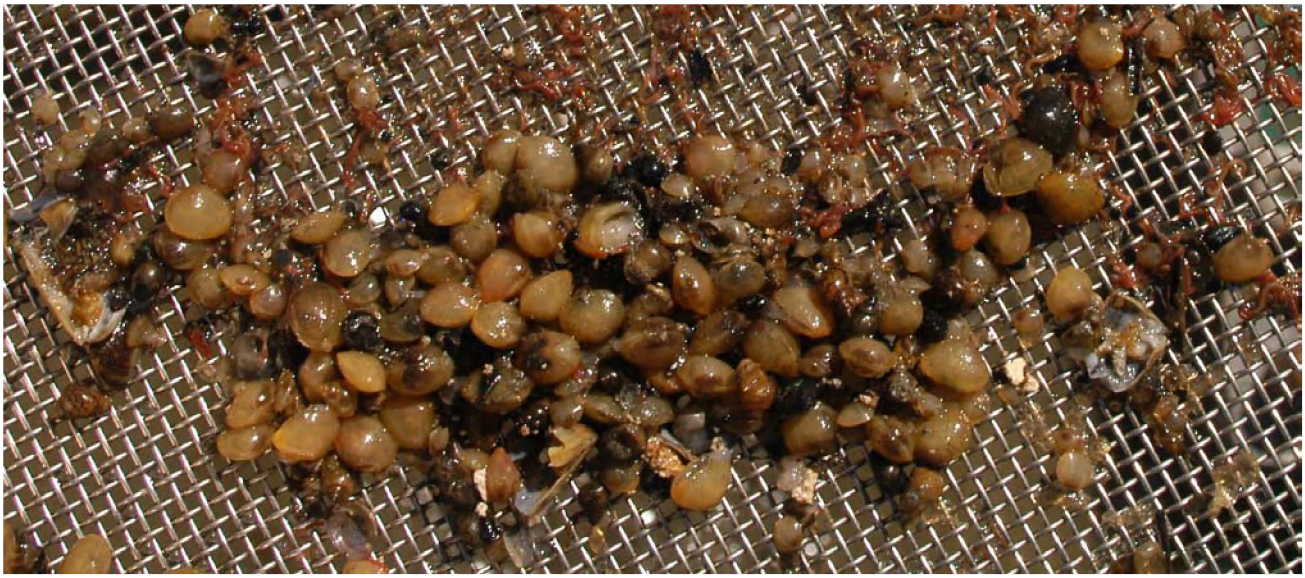


Table 7
Longitudinal distribution of the leeches (Hirudinea).

	zone T_1^A	zone T_1^B	zone T_2
Cystobranchus respirans	+		
Erpobdella octoculata	+++	+++	
Erpobdella testacea	+	++	
Glossiphonia concolor	++	+++	
Glossiphonia heteroclite	++	+	
Helobdella stagnalis	+	++	
Hemiclepsis marginata	+	+	
Trocheta riparia	+	+	
Piscicola geometra		+	
Glossiphonia complanata	+++	+++	++

The group of the molluscs clearly differentiate in the three zones (Table 8). The zone T_1^A inhabits many rheophilic species (e.g. *Ancylus fluviatilis*, *Pisidium amnicum*, *P. henslowanum*, *P. supinum*, *Sphaerium rivicola* and *S. solidum*). In the middle zone T_1^B *S. solidum* is the only rheophilic species that was left. Only four species were found in zone T_2: *Bithynia tentaculata*, *Dreissena polymorpha*, *Corbicula fluminea* and *Radix ovata*. *Galba truncatula*, found in zone T_1^A only, is a characteristic inhabitant of the intertidal mud areas. Both *Corbicula fluminea* and *C. fluminalis* are invasive species originating from East-Asia. They entered France using at least seven different main waterways, among which the Marne-Rhine canal connecting the rivers Rhine, Meuse and Marne had a dominant role (Vincent & Brancotte, 2002). *C. fluminea* was found for the first time in the river

Seine in the vicinity of Paris in 1997 (Vincent & Brancotte, 2000), three years later downstream of the weir at Poses (Vincent & Brancotte, 2002).
According to T. Vincent (Muséum d'Histoire Naturelle, le Havre, pers. comm.) the finding of *C. fluminalis* in June 2006 in some of the samples from the zone T_1^A was the first observation of this species in the river Seine. Some remarks on the occurrence of both *Corbicula* species are given in Annex 6.

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Table 8
Longitudinal distribution of the molluscs (Mollusca).

	zone T_1^A	zone T_1^B	zone T_2
Acroloxus lacustris	+		
Ancylus fluviatilis	+		
Corbicula fluminalis	++		
Galba truncatula	++		
Gyraulus albus	+		
Lithoglyphus naticoides	+		
Pisidium amnicum	+		
Pisidium casertanum plicatum	+		
Pisidium henslowanum	+		
Pisidium pulchellum	+		
Pisidium supinum	+		
Sphaerium rivicola	+		
Succineidae species	+		
Physa fontinalis	+	+	
Pisidium casertanum	+	+	
Pisidium nitidum	++	+	
Pisidium subtruncatum	+	+	
Potamopyrgus antipodarum	++	+	
Sphaerium corneum	++	+++	
Sphaerium solidum	+	+	
Valvata piscinalis	++	+	
Viviparus viviparus	+		
Physella acuta		+	
Radix peregra		+	
Valvata cristata		+	
Bithynia tentaculata	+++	+++	++
Corbicula fluminea	++	+++	+
Dreissena polymorpha	+	+++	++
Radix ovata	++	++	+

Another bivalve present, *Dreissena polymorpha* (Photo 4), is a much older invader, originating from the Ponto-Caspian region. It reached The Netherlands as early as 1827 (Van Bentum Jutting, 1922). Striking is the absence of living Unionidae in the samples, especially from the deeper parts of the main channel. Few empty shells were recorded downstream of Rouen, while subfossil shells were found everywhere along the river banks and in the deeper river bed. Also many empty shells of *Theodoxus fluviatilis* were recorded, but none with a living animal in it. Wolff (1968) attributed the absence of

Unionidae in the tidal freshwater section of the river Rhine in the 1960's to severe water pollution which reached rock bottom in the 1970's (Bij de Vaate *et al.*, 2006). After water quality improved, Unionidae recolonized the Rhine delta again. Recently five species are common in that area: *Unio tumidus*, *U. pictorum*, *Anodonta anatina*, *A. cygnea* and *Pseudanodonta complanata*.

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Photo 4

Dreissena polymorpha on a stone from the bottom of the Seine.

Of the nine crustaceans found in the three zones (Table 9), *G. salinus* belongs to the dominant species and has been wide spread in whole the Seine aval (Tables 3 and 4). In the Rhine-Meuse estuary this species is confined to brackish waters and to coastal areas with freshwater influences (Pinkster & Platvoet, 1986). *G. salinus* is the dominating macroinvertebrate community of solid substrates in the zone T_2.

Of the species found in the upstream section T_1^A, both *Crangonyx pseudogracilis* and *Dikerogammarus villosus* (Photo 5) belong to the group of nonindigenous invasive species. The former originates from America and the latter is a Ponto-Caspian invader. In The Netherlands both species are a menace to the indigenous gammarids (e.g. *Gammarus pulex* and *G. fossarum*). The crayfish *Orconectes limosus*, collected in the zones T_1^A and T_1^B, is also an American invader. Densities of this species increased considerably in the Rhine delta when water quality improved. Their main habitat in this river is the riprap along the river banks and groynes.

Photo 5
Dikerogammarus villosus mature male.

Table 9
Longitudinal distribution of the crustaceans (Crustacea).

	zone T_1^A	zone T_1^B	zone T_2
Crangonyx pseudogracilis	+		
Dikerogammarus villosus	+		
Echinogammarus berilloni	+		
Orchestia species	+		
Asellus aquaticus	+++	+++	
Orconectes limosus	+	+	
Proasellus coxalis		+	
Proasellus meridianus	+++	+++	+
Gammarus salinus	++	+++	+++

Only three mayflies species were collected: *Caenis macrura*, *Ephemerella ignita* (Photo 6) and *Heptagenia sulphurea*. *E. ignita* is a characteristic inhabitant of smaller rivers and streams. *H. sulphurea* and *C. macrura* are true potamal species. Mayflies were mainly found in the zone T_1^A (Table 10). Of *H. sulphurea* only one specimen was found. Both other species were more wide spread, but still rare. *E. ignite* was represented with totally nine specimens at three locations in the zone T_1^A, while *C. macrura* was represented with totally eight and three specimens at two locations in the zones T_1^A and T_1^B. Typical burying mayflies like species of *Ephemera*, *Ephoron* and *Palingenia* seem to be lacking in the Seine aval.

Table 10
Longitudinal distribution of the mayflies (Ephemeroptera).

	zone T_1^A	zone T_1^B	zone T_2
Ephemerella ignita	+		
Heptagenia sulphurea	+		
Caenis macrura	+	+	

Photo 6
Ephemerella ignita.

Table 11
Longitudinal distribution of the water bugs (Heteroptera).

	zone T_1^A	zone T_1^B	zone T_2
Aphelocheirus aestivalis	+		
Micronecta minutissima	+		
Sigara striata		+	

Stoneflies were present in the samples with one specimen of *Leuctra fusca* found just downstream of the Poses Weir (at rk 203), which might be the result of drift from upstream. The species is an inhabitant of streams and small rivers.

Notable was the finding of the water bug *Aphelocheirus aestivalis*. It is an inhabitant of large rivers and very sensitive to low oxygen concentrations since it breaths through diffusion of oxygen from the surrounding water. The two other water bugs found, *Micronecta minutissima* and *Sigara striata*, are not typical for large rivers (Table 11). Only one specimen was found of all three species.

Table 12
Longitudinal distribution of the water beetles (Coleoptera).

	zone T_1^A	zone T_1^B	zone T_2
Haliplus fluviatilis	+		
Elmis species	+	+	
Limnius species		+	
Esolus species	+	+	+

Also the group of water beetles was almost absent in the Seine aval (Table 12). Main reason is the lack of suitable habitats like small shallow ponds with lush vegetation. The relatively few larvae of *Haliplus fluviatilis*, *Elmis*, *Limnius* and *Esolus* found are indicators of a good oxygen content in the water, since these larvae do not breath air but provide themselves with sufficient oxygen by diffusion from the water column (like *Aphelocheirus*).

A total of only five species of caddis flies were collected. Almost exclusively in the upstream most zone. *Lepidostoma hirtum* is a rare and sensitive species from streams and rivers. Only on km 203 the larvae have been found. In the upstream section the potamal species *Hydropsyche contubernalis* (Photo 7) and *Neureclepsis bimaculata* were relatively abundant on solid substrates.

Photo 7
Hydropsyche contubernalis.

Table 13
Longitudinal distribution of the caddis flies (Trichoptera).

	zone T_1^A	zone T_1^B	zone T_2
Ecnomus tenellus	+		
Hydroptila species	+		
Lepidostoma hirtum	+		
Hydropsyche contubernalis	+++		
Neureclipsis bimaculata	+++	+	

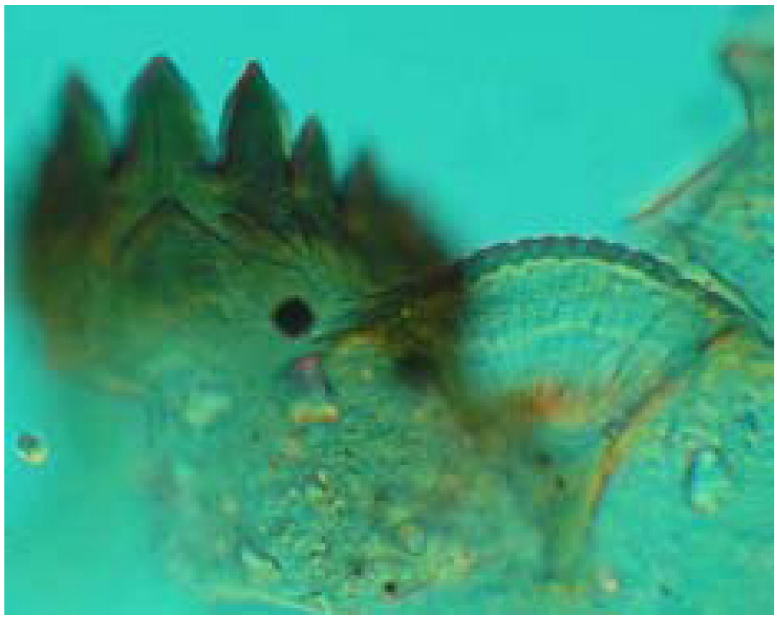
By far the most divers group of invertebrates are the midges (Table 14), of which 48 taxa were collected. Most taxa were found in the zone T_1^A. Species richness declines very rapidly in downstream direction. In zone T_2 the species number had reduced to seven taxa only: *Thalassosmittia thalassophila*, *Procladius* species, *Limnophyes* species, *Dicrotendipes nervosus*, *Parachironomus longiforceps*, *Polypedilum scalaenum* and *Cladotanytarsus mancus* group. Rheophilic species are the solid substrates inhabiting taxa *Paratrichocladius rufiventris*, ***Tvetenia calvescens***, ***Polypedilum convictum***, *Rheocricotopus chalybeatus* and *Rheotanytarsus* species, the sandy substrate inhabiting taxa *Chironomus acutiventris*, *Harnischia* species and *Microchironomus tener*, the shifting sand inhabiting species *Polypedilum scalaenum*, the woody debris inhabiting species ***Polypedilum cultellatum*** and *Microspectra atrofasciata* a non habitat selective species. Mentioned in bold are the most critical species, which also live in foothill streams. The majority of all rheophilic taxa mentioned above is confined to the zone T_1^A. An exception is *P. scalaenum*, one of the few species that also inhabits

Table 14
Longitudinal distribution of the midges (Chironomidae).

	zone T_1^A	zone T_1^B	zone T_2
Tanypus kraatzi	+		
Tanypus punctipennis	+		
Prodiamesa olivacea	+		
Bryophaenoicladius group *muscicola*	+		
Paratrichocladius rufiventris	+		
Tvetenia calvescens	+		
Chironomus acutiventris	+		
Chironomus bernensis	+		
Chironomus nudiventris	++		
Chironomus plumosus aggregate	+		
Cladopelma laccophila group	+		
Cryptochironomus defectus	+		
Dicrotendipes lobiger	+		
Endochironomus albipennis	+		
Harnischia species	++		
Microchironomus tener	+		
Microtendipes chloris group	+		
Phaenopsectra species	+		
Polypedilum convictum	+		
Polypedilum cultellatum	+		
Polypedilum sordens	+		
Cricotopus bicinctus	+++	++	
Cricotopus intersectus	+++	++	
Cricotopus sylvestris	+++	++	
Nanocladius bicolor aggregate	++	++	
Rheocricotopus chalybeatus	+++	++	
Cryptochironomus supplicans	+	+	
Cryptochironomus species	+	+	
Glyptotendipes pallens	+++	+++	
Glyptotendipes paripes	+++	+++	
Parachironomus arcuatus group	++	+	
Parachironomus species Kampen	+	+	
Paratendipes albimanus	++	+	
Polypedilum nubeculosum	++	+	
Xenochironomus xenolabis	++	+	
Micropsectra atrofasciata	+	+	
Paratanytarsus dissimilis aggregate	+	+	
Rheotanytarsus species	+	+	
Clinotanypus nervosus		+	
Pseudosmittia species		+	
Tanytarsus species		+	
Thalassosmittia thalassophila		+	+
Procladius species	+	+	+
Limnophyes species	+++	++	+
Dicrotendipes nervosus	+++	+++	+++
Parachironomus longiforceps	++	++	+
Polypedilum scalaenum	+++	+++	++
Cladotanytarsus mancus group	+++	++	+

the sandy substrates in the zones T_1^B and T_2. *Parachironomus* species Kampen is confined to colonies of Bryozoa, while *Xenochironomus xenolabis* inhabits freshwater sponges. Semi-aquatic taxa found are *Bryophaenocladius* group *muscicola*, *Pseudosmittia* species, *Thalassosmittia thalassophila* en *Limnophyes* species.
One of the most pollutant tolerant species is *Dicrotendipes nervosus* (Photo 8) It was among the first midge that recolonized the Lower Rhine in the late 1970's, after a period the river was nearly dead.

Photo 8
Detail of head of a *Dirotendipes nervosus* larva.



From the June 2006 monitoring results it can be concluded that the macroinvertebrates exhibit a strict differentiation between the three zones. Zone T_1^A is the most divers with rheophilic potamal and rhitral species. Zone T_1^B is deprived from rheophilic species and only trivial species are able to find a habitat. The situation in zone T_2 is even worse, reflected by the relatively low taxa richness and densities.

3.1. IGBA calculations

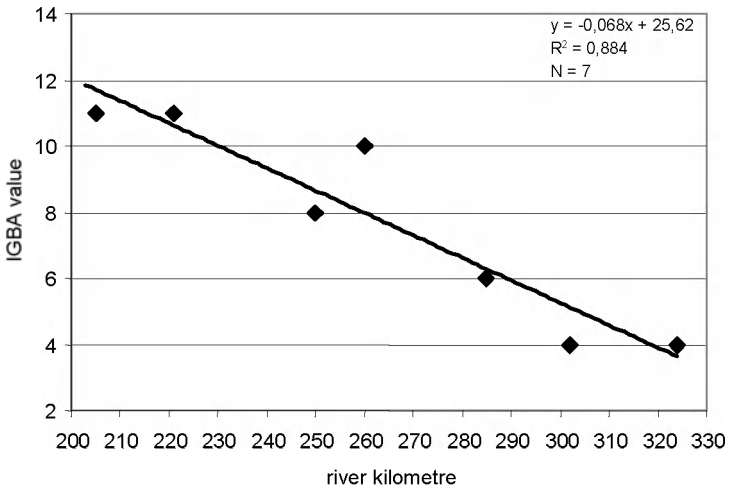
According to the general French monitoring and assessment practice for streams and rivers the IGBA was calculated for assessment of the ecological quality of the Seine aval. An overview of the results obtained for all locations sampled is given in table 15. Additional metrics are given in Annex 5.
With one exception, all values of the IGBA metrics IF and IFD, including the $IGBA_{total}$ values, gradually decrease in downstream direction (Figure 3).The relatively high IFD and, as a consequence, the $IGBA_{total}$ value at rk 260 is caused by the presence of four specimens of *Neuroclips bimaculata* (Polycentropidae) wich were found in one of the samples from the deep river bed. If this species was absent or present with less than three specimens the $IGBA_{total}$ value at that location should not exceed eight, which is the same value as at rk 250.

Table 15
Overview of IGBA values
(IF = indice filet, IFD = indice filet et drague and IS = indice substrat artificiel)

rk	IF	IFD	IS	Total
203		9		
205	8	9	6	11
221	7	10	6	11
227		8		
247.7		6		
250	7	7	6	8
260	6	9	6	10
278		5		
288	6	5	6	6
294		3		
302	4	3	3	4
324	4	2	3	4

Differences between the different metrics are relatively small. They were mainly caused by few specimens of higher indicator taxa, if present in sufficient numbers in some of the samples. This means those taxa meet marginal conditions for their existence in the Seine aval and the presence of one or two extra specimens can result in a higher IBGA value.

Figure 3
Development of the $IGBA_{total}$ value in the Seine aval.



4. Discussion

The development of ecological assessment and classification systems is one of the most important and technically challenging parts of the implementation of the WFD. It is the first time such systems have been required under community legislation and all member states are in a position of needing to significantly expand their technical knowledge and experience. Consequently, the development and improvement of appropriate systems will involve a learning process. The guidance documents of the EU (http://forum.europa.eu.int/Public/irc/env/wfd/library?l=/framework_directive&vm=detailed&sb=Title) provide a starting point for this learning process. It sets out some key principles and ideas on practical approaches. The aim is to help member states to build on their existing expertise to develop practical and reliable systems for assessment and classification that satisfy the requirements of the WFD (European Union, 2003[B]).

Guidance document no 5 (European Union, 2003[A]) describes the typology, reference conditions and classification systems for transitional and coastal waters.

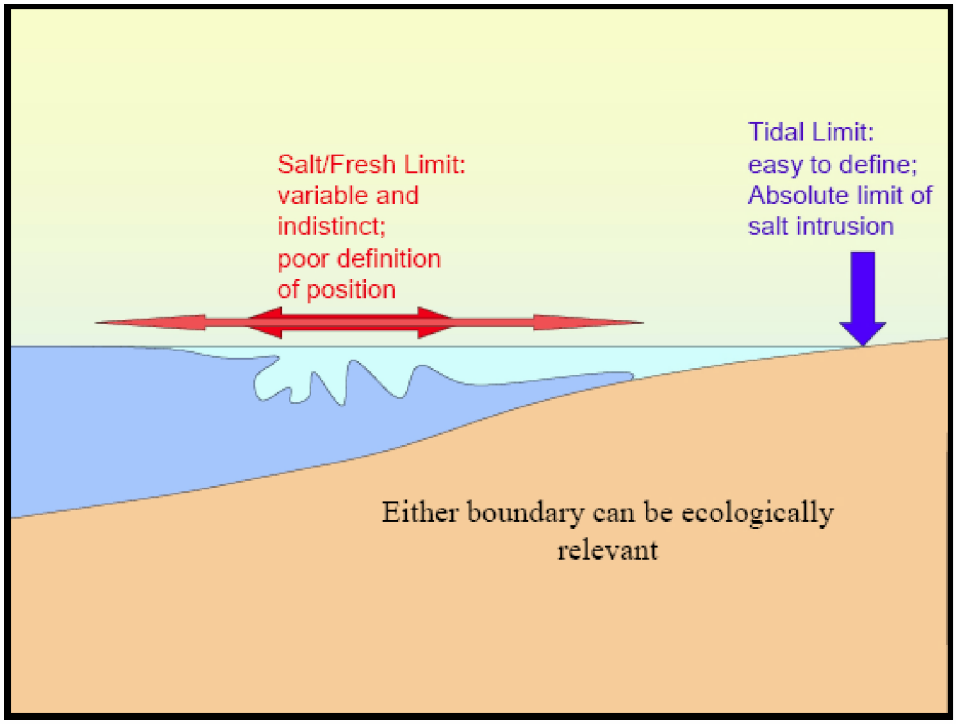
Transitional waters are usually characterised by their morphological and chemical features in relation to the size and nature of the inflowing rivers. Many different methods might be used to define them but the method should be relevant ecologically. This will ensure reliable derivation of type-specific biological reference conditions.The WFD defines transitional waters as: "*bodies of surface water in the vicinity of river mouths which are partly saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows*". When defining transitional waters for the purposes of the WFD, it is clear that the setting of boundaries between transitional waters, freshwaters and coastal waters must be ecologically relevant. From the definition it can be concluded that transitional waters are close to the end of a river where it mixes with coastal waters, that their salinity is generally lower than in the adjacent coastal water, and there is a change to salinity or flow (European Union, 2003[A]).

For the purpose of defining the seaward boundary of transitional waters four strategies are recommended:

1. the use of boundaries defined under other European and national legislation such as the European Urban Waste Water Treatment Directive;
2. estimation of the salinity gradient;
3. use of physiographic features;
4. modelling.

The upstream boundary can be defined by either the fresh /salt boundary or the tidal limit (Figure 4). Member states are free to make their choice. However, from international point of view, this possibility causes confusion in the classification of tidal freshwater zones; they are either part of the transitional zone or the lower section of the river.

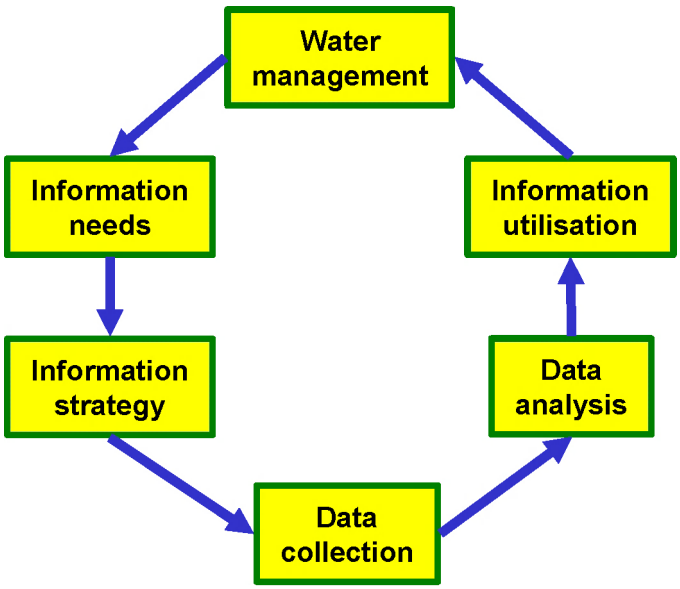
Figure 4
Two definition possibilities for the upstream boundary of transitional waters (European Union 2003[A]).



For the comparison of classification and assessment methods of European tidal freshwater zones it was aspired to compare rivers from the same size and ecoregion. In practice it was only possible to obtain information from three rivers in the North Sea region; de rivers Rhine, Elbe and Scheldt, but assessments procedures for the tidal freshwater section of the rivers Rhine and Scheldt are still under development. Much effort was put in obtaining information from other rivers in the same ecoregion and in the Atlantic Ocean ecoregion, however, without result. In general, tidal freshwater zones are the last river sections receiving attention for monitoring and assessment in most of the EU countries.

What can be learned for the Seine aval from what has been done for other European rivers in the framework of the WFD? Looking at the information cycle (Figure 5) (Timmerman *et al.*, 2000) most of the member states are not yet or in the beginning of the process for development of an information strategy for their tidal freshwater zones. This should mean an iterative process matching information needs with possibilities for information supply. The needs are clear (described in the WFD), the strategy to collect and to analyse data is in different stages of elaboration. Since member states are free to develop their information strategy including monitoring and assessment procedures, most obvious policy for Seine-Aval is to pass jointly, with similar organisations for river management in France, through the above mentioned iterative process for tidal freshwater zones.

Figure 5
The information cycle (Timmerman *et al.*, 2000).



The current situation is that Seine-Aval can not take advantage of knowledge developed for other tidal freshwater zones in similar transitional zones of rivers in the EU due to lack of information. Important for assessment, and therefore also for the development of an information strategy, are descriptions of the GEP and MEP for macroinvertebrates. These ecological potentials should be considered leading factors in this strategy.

4.1. Sampling methods

According to the WFD, methods used for the monitoring of type parameters must conform to the international standards listed below or such other national or international standards which will ensure the provision of data of an equivalent scientific quality and comparability. For the water quality element "Macroinvertebrates" sampling should follow next standards:

1. ISO 5667-3 (1995) Water quality. Sampling. Part 3: Guidance on the preservation and handling of water samples.
   Most recent version of this norm was published in 2003. For counting and identification of benthic macroinvertebrates next preservation techniques are recommended:
   a. add ethanol to the sample (if needed after decanting the clear supernatant) till the concentration is > 70% (volume fraction);
   b. add 37% neutralized formaldehyde to obtain in the sample a final concentration of 3.7% (formaldehyde is neutralized with sodium tetraborate or hexamethylene-tetramine).

2. EN 27828 (1994). Water quality. Methods for biological sampling. Guidance on handnet sampling of benthic macroinvertebrates. See norm ISO 7828 (1985).

3. EN 28265 (1994). Water quality. Methods of biological sampling. Guidance on the design and use of quantitative samplers for benthic macroinvertebrates on stony substrata in shallow waters. See norm ISO 8265 (1988).

4. EN ISO 9391 (1995). Water quality. Sampling in deep waters for macroinvertebrates. Guidance on the use of colonisation, qualitative and quantitative samplers.
   In this norm five sampling devices are described:
   a. *Colonization sampler*
      This sampler is a standardized artificial substrate consisting of a coarse mesh polyamide bag filled with approximately 40 pieces of a biological filter medium as used in sewage treatment. An alternative version of this sampler is the colonization unit in which the biological filter medium is assembled into a cylindrical shape. Colonization period is four weeks.
   b. *Naturalist's dredge*
      Two versions of this dredge are recommended; a small one with an opening of 46x19 cm, a bigger one with an opening of 61x20 cm. The supporting collecting net is about 35 cm in length; its mesh size depends on the objective of the sampling.
   c. *Birge-Ekman grab*
      The pole-operated version of this grab is recommended for water bodies with a depth of <3 m. In deeper waters without flow a rope-operated grab can be used. Sampling surface of the grab is 225 cm^2.
   d. *Ponar grab*
      The weighted version of this grab is recommended; sampling area 560 cm^2 and weight about 23 kg.
   e. *FBA air-lift sampler*
      The air-lift sampler recommended in the norm has a sampling area of 415 cm^2. It can be used to take quantitative samples on substrata ranging from fine gravel to stones of about 13 cm long. It is not recommended for use on mud. Water depth at the sampling location is modifying the length of the riser. It is impracticable to apply the sampler from a boat.

5. EN ISO 8689-1 (1999). Biological classification of rivers, part 1: Guidance on the interpretation of biological quality data from surveys of benthic macroinvertebrates in running waters.
   Most recent version of this norm was published in 2000. The norm does not prescribe sampling procedures previous to the biological classification.

6. EN ISO 8689-2 (1999). Biological classification of rivers, part 2: Guidance on the presentation of biological quality data from surveys of benthic macroinvertebrates in running waters.
   The norm prescribes that sampling of the macroinvertebrates should be in accordance with the norms ISO 5667-3, ISO 7828, ISO 8265 and ISO 9391.

7. ISO 7828 (1985). Water quality. Methods for biological sampling. Guidance on handnet sampling of benthic macroinvertebrates.
   This norm describes a handnet and the way to use it in different water types. The net is recommended to have an opening of 20-40 cm width and 20-30 cm height, and a length of 40-55 cm. Mesh size 0.25-0.75 mm depending on the survey objective.

8. ISO 8265 (1988). Water quality. Design and use of quantitative samplers for benthic macro-invertebrates on stony substrata in shallow freshwaters.
   In this norm two sampling devices are described which are both applicable in shallow fordable water bodies only:
   a. *Surber sampler*
      Several modifications of this sampler are described. The sampling surface is 0.09 m2 but can be changed to fulfil the objective of the sampling. Length of the net is about 70 cm long; its mesh size also depends on the objective of the sampling.
   b. *Cylinder sampler*
      Essentially an open ended cylinder having the lower edge serrated with 100 mm teeth. Diameter of the cylinder is equal to a cross-sectional area of 0.05 or 0.1 m^2. An oval aperture in the cylinder wall, fitted with a 1mm mesh screen, allows water to enter the cylinder. At the opposite site a second aperture to which a detachable net can be mounted to collect the benthic animals. Mesh size of this net is not prescribed.

      Sampling methods to be used in macroinvertebrate monitoring programs strongly depends on the size of rivers and streams. Wide deep river sections ask for additional methods including equipments (e.g., appropriate exploring vessels) than wadable streams which simply can sampled with a handnet.
      Most important aspects for a sampling methodology and strategy for deep rivers are:
   1. The way of using the monitoring results. What assessment method is used and for what purpose?
   2. The size of the area taken into account in the assessment procedure. Is each location assessed and the results combined for the regarding subsection or are the results of all locations in a subsection combined followed by an assessment of the subsection? In the later case the influence of absence of a biotope at one or more locations or sampling failures will play a minor role in the assessment procedure.
   3. The importance attributed to the various biotopes. In the case of the Seine aval, what should be the "weight" of the intertidal biotopes in the assessment procedure and of which of these biotopes?

4.2. Sampling sites

During the June 2006 monitoring campaign a secondary channel in zone T_1^A was sampled in addition to the samples taken in the main channel. When the results are compared it seemed that of the 76 taxa found 13 were unique for the secondary channel. Most of them were present in relatively low numbers (Table 16). From point of view of IGBA calculation meant two extra taxa, however, without indication value.

Table 16
Comparison of taxa richness in a secondary channel at rk 229-230 (8 samples) and in the littoral zones at rk 221.3 (8 samples) and rk 204-205 (11 samples). Single specimens in a group of samples were excluded (- = absent or 1 specimen present, + = 2-10, ++ = 11-100, +++ = 101-1000 and ++++ = > 1001). In blue taxa exclusively found in the secondary channels.

Taxa IGBA	Taxa identified	River kilometre 229-230	221,3	204-205
Tricladida	*Dugesia tigrina*	-	-	+
Oligochaeta	*Branchiura sowerbyi*	+	+	-
Oligochaeta	Enchytraeidae species	+++	+	-
Oligochaeta	*Limnodrilus claparedeianus*	++	+	+
Oligochaeta	*Limnodrilus hoffmeisteri*	+++	-	+
Oligochaeta	*Limnodrilus udekemianus*	+	-	-
Oligochaeta	Lumbricidae species	+	-	+
Oligochaeta	Lumbriculidae species	+	-	+
Oligochaeta	*Ophidonais serpentina*	-	+	-
Oligochaeta	*Peloscolex multisetosus*	++	-	-
Oligochaeta	*Potamothrix moldaviensis*	+	+	-
Oligochaeta	*Psammoryctides barbatus*	+	++	-
Oligochaeta	Tubificidae with hairs juvenile	++	+	+
Oligochaeta	Tubificidae without hairs juvenile	++++	++	++
Polychaeta	*Hypania invalida*	-	++	++
Erpobdellidae	*Erpobdella octoculata*	+	+	+
Erpobdellidae	*Erpobdellidae juvenile*	-	+	++
Glossiphonidae	*Glossiphonia complanata*	-	-	++
Glossiphonidae	*Glossiphonia concolor*	-	+	+
Glossiphonidae	*Helobdella stagnalis*	-	+	+
Asellidae	*Asellus aquaticus*	-	-	++
Asellidae	*Proasellus meridianus*	-	-	++
Gammaridae	*Dikerogammarus villosus*	-	-	+
Talitridae	*Orchestia species juvenile*	+	-	-
Bithyniidae	*Bithynia tentaculata*	+	++	+
Corbiculidae	*Corbicula fluminalis*	-	+	+
Corbiculidae	*Corbicula fluminea*	-	+	+
Hydrobiidae	*Potamopyrgus antipodarum*	++	++	+
Lymnaeidae	*Galba truncatula*	++	+	-
Lymnaeidae	*Radix ovata*	+	++	+
Planorbidae	*Gyraulus albus*	-	+	-
Sphaeriidae	*Pisidium amnicum*	+	-	-
Sphaeriidae	*Pisidium casertanum*	+	-	-
Sphaeriidae	*Pisidium henslowanum*	+	-	+
Sphaeriidae	*Pisidium nitidum*	-	+	++
Sphaeriidae	*Pisidium subtruncatum*	+	-	-
Sphaeriidae	*Sphaerium corneum*	-	-	+
Succineidae	*Succineidae species*	+	-	-
Valvatidae	*Valvata piscinalis*	+	++	-
Caenidae	*Caenis macrura*	-	+	-
Hydropsychidae	*Hydropsyche contubernalis*	-	-	++
Elmidae	*Esolus* larvae	-	+	-
Chironomidae	*Chironomus acutiventris*	+	-	-
Chironomidae	*Chironomus bernensis*	+	-	-
Chironomidae	*Chironomus nudiventris*	+	-	+
Chironomidae	*Chironomus plumosus* aggregate	+	-	-
Chironomidae	*Chironomus* species		-	-

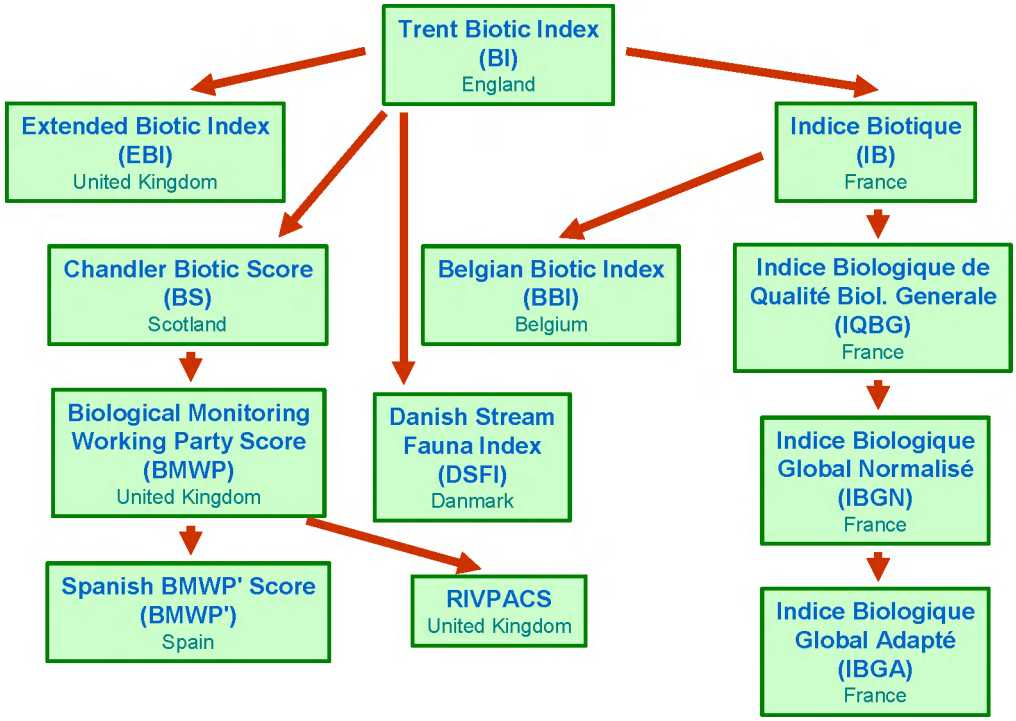
Taxa IGBA	Taxa identified	River kilometre 229-230	221,3	204-205
Chironomidae	*Cladopelma laccophila* group	++	-	-
Chironomidae	*Cladotanytarsus* juvenile	-	++	-
Chironomidae	*Cladotanytarsus mancus* group	++	++	+++
Chironomidae	*Cricotopus bicinctus*	-	++	++
Chironomidae	*Cricotopus intersectus*	+	+++	+++
Chironomidae	*Cricotopus sylvestris*	++	+++	++
Chironomidae	*Cryptochironomus supplicans*	++	-	+
Chironomidae	*Dicrotendipes lobiger*	-	-	++
Chironomidae	*Dicrotendipes nervosus*	++	++++	++++
Chironomidae	*Glyptotendipes pallens*	++	+++	+++
Chironomidae	*Glyptotendipes paripes*	+	+++	++
Chironomidae	*Harnischia* species	++	+	-
Chironomidae	*Limnophyes* species	+++	++++	++
Chironomidae	*Microchironomus tener*	+	-	-
Chironomidae	*Microtendipes chloris group*	-	-	+
Chironomidae	*Nanocladius bicolor aggregate*	-	-	+
Chironomidae	*Parachironomus arcuatus group*	-	-	+
Chironomidae	*Paratanytarsus dissimilis aggregate*	-	++	+
Chironomidae	*Paratendipes albimanus*	+	+	-
Chironomidae	*Paratrichocladius rufiventris*	-	-	+
Chironomidae	*Polypedilum cultellatum*	-	++	-
Chironomidae	*Polypedilum nubeculosum*	++	+	++
Chironomidae	*Polypedilum scalaenum*	++	+++	+++
Chironomidae	*Potthastia longimanus*	-	-	+
Chironomidae	*Procladius species*	++	-	-
Chironomidae	*Prodiamesa olivacea*	-	-	+
Chironomidae	*Rheocricotopus chalybeatus*	-	++	+
Chironomidae	*Tanypus punctipennis*	++	-	-
Limoniidae	*Limoniidae* species	+	-	-
	Number of taxa	47	40	47

With the intention to catch mobile macroinvertebrates, which easily can be overlooked when grabs or dredges are used for sampling, filter screens in the river water intake system of a Shell plant at Rouen (rk. 253.2) were sampled by scraping them off. Surprisingly hardy any mobile macroinvertebrates were found. Of the 1974 macro-invertebrate specimens sampled 58.8% were *Sphaerium corneum* (mollusc), 19.4% *Dicrotendipes nervosus* (chironomid) and 13.2% *Bythinia tentaculata* (mollusc) comprising 91.4% of the total catch. *Sciomyzidae* species present in the sample with one specimen was the only taxon not found in the river.

4.3. Assessment procedures

A thorough overview on different macroinvertebrate assessment procedures was made within the European AQEM-project (www.aqem.de). In this project most of the commonly used European metrics for calculating the ecological quality of individual stream types were described (Figure 6). These procedures intend to study the structure of the community assigning values according with natural status versus stress. One of the procedures, the RIVPACS approach, represents a different point of view because this is a predictive model that offers a prediction of expected fauna at a given site. Thus by comparing the existing fauna with the potential (predicted) one it is possible to know the degree of deviation and thereafter to establish the degree of alteration and/or goals for restoration (Wright et al., 2000). Such approach, not for predicting but for assessing the degree of deviation from target situations, is also obliged in the WFD. For heavily modified rivers, like the river Seine, references are the so-called "Good Ecological Potential" (GEP) and the "Maximum Ecological Potential" (MEP). The GEP is derived from the MEP, which is the highest ecological status for heavily modified and artificial water bodies in the European Union (European Union, 2003[B]).

Figure 6
Development of frequently used indices in Europe (modified after Goethals, 2002).



4.3.1. Identification level

A list of 172 macroinvertebrate taxa found during the June 2006 monitoring campaign is given in Annex 3. The number of taxa exceeds about three times the number found in 1997-1998 when totally 65 taxa were found in the three zones distinguished in the Seine aval (Table 6.2) (Costil, 1998[A,B]; Lasnier, 1998). The difference can be explained by the identification level which was at a higher resolution compared to the 1997-1998 monitoring.
Remarkable is the absence of Bryozoa, Coelenterata and Porifera in the samples of the June 2006 monitoring campaign. However, presence of these taxa is in general of minor importance in indices. In contrast to the June 2006 monitoring results the highest number of taxa was found in the zone T_2 in the period 1997-1998. The most important species in the June 2006 samples was the gammarid *Gammarus salinus* comprising 25.4% of the total number of animals found in all samples and in 52% of the individual samples. *G. salinus* species was not found in the period 1997-1998.

.....

Table 17
Macroinvertebrate taxa found in the period 1997-1998 (Costil, 1998[A,B]; Lasnier, 1998).

Taxa	Zone T_1[A]	Zone T_1[B]	Zone T_2
Spongilla species	+	+	+
Hydra species		+	+
Cordylophora caspia			+
Paludicella articulata	+		
Fredericella sultana	+	+	+
Plumatella fungosa	+		
Dendrocoelum lacteum	+	+	+
Dugesia gonocephala	+		+
Dugesia lugubris/polychroa	+	+	+
Dugesia tigrina	+	+	+
Dugesia species			+
Oligochaeta species	+	+	+
Limnodrilus claparedeianus		+	+
Limnodrilus hoffmeisteri		+	+
Limnodrilus udekemianus		+	
Aulodrilus pluriseta			+
Branchiura sowerbyi		+	+
Psammoryctides barbatus		+	+
Spirosperma velutinus			+
Haplotaxis gordioides			+
Lumbriculus variegatus			+
Stylaria lacustris	+	+	+
Stylodrilus heringianus			+
Trichodrilus species			+
Erpobdella octoculata	+	+	+
Erpobdella testacea	+	+	+
Erpobdella species juvenile		+	+
Glossiphonia complanata	+	+	+
Glossiphonia heteroclita	+	+	+
Helobdella stagnalis	+	+	+
Hemiclepsis marginata	+		+

Taxa	Zone T_1^A	Zone T_1^B	Zone T_2
Piscicola geometra	+	+	+
Acroloxus lacustris	+	+	+
Ancylus fluviatilis	+		
Anisus rodontatus			+
Bithynia tentaculata	+	+	+
Lymnaea peregra	+	+	+
Lymnaea ovata		+	+
Physa fontinalis	+	+	+
Physella acuta	+		+
Potamopyrgus antipodarum	+	+	+
Valvata cristata	+		
Valvata piscinalis	+		
Viviparus viviparus	+	+	+
Dreissena polymorpha	+	+	+
Pisidium supinum	+		+
Sphaerium corneum	+		+
Sphaerium species		+	
Asellus aquaticus	+	+	+
Asellus meridianus			+
Gammarus lacustris	+		
Gammarus species	+		+
Orconectes limosus	+		
Coenagrion species	+	+	
Ischnura elegans		+	
Platycnemis pennipes	+		
Sigara falleni			+
Caenis species	+		
Ephemerella ignita	+		
Ecnomus tenellus	+		
Hydropsyche species	+		
Diamesinae species			+
Chironomidae species	+	+	+
Orthocladinae species	+	+	+
Tanytarsini species			+
Number of taxa	42	34	49

Species have particular traits, preferences and tolerances which are important determinates of landscape patterns in their occurrence and abundance. This means the assemblages and communities respond to environmental gradients through these characteristics of single species in it (Poff, 1997). Consequently, in studies using macroinvertebrates as indicators for assessing the ecological quality of streams and rivers, species level identifications in comparison with lower resolution identifications can have greater information content and result in more reliable site classifications (e.g., Furse *et al.*, 1984), can give greater resolution to detecting differences between reference and test sites (e.g., Barton, 1996), and are required for detecting the presence of rare or threatened species (Lenat, 1988), which in turn is important to identify protection areas for nature conservation (Furse *et al.*, 1984). For these reasons species level identifications of macroinvertebrates was advocated by several authors (e.g., Resh & Unzicker, 1975; Lenat & Penrose, 1980; Lenat

1988, 1993). Species within a genus or family also can have different biological attributes, such as tolerances to and preferences for abiotic conditions (e.g., flow velocity, substrate composition, temperature, dissolved oxygen concentration), different food resource requirements and different life history strategies. When individuals from different species are aggregated into genera or families, information that is potentially valuable in discriminating between samples may be lost. Whether this is acceptable or not depends on the extent to which patterns expressed by the species in assemblages can be represented by the information retained at the resolution of genus or family level. In some studies it was demonstrated that loss of information due to lower resolution identification is negligible (Gayraud *et al.*, 2003), relatively low (Marshall *et al.*, 2006) or will lead to misinterpretations (Guerold, 2000), as opposed to species level identification.

Assessment procedures in many monitoring programs require specimens identification to lower resolutions, like genus, family and sometimes order level. Although the appropriate taxonomic resolution for a particular monitoring program should be determined by the information required to address its objectives, choice of this resolution can mostly be considered as a compromise between the costs of obtaining data and loss of information accepted by taxa identification at lower taxonomic resolution. From the development of abiotic monitoring programs, which have a much longer history than the biological monitoring programs, it can be concluded that the accuracy of determinations continuously increases due to increased knowledge of processes. The same can be expected for biotic parameters which is an extra argument for identification at high taxonomic resolution (species level as much as possible). Such a strategy also increases knowledge of habitat requirements and tolerances of species which is important for founding their classification values as assigned in several indices.

Important in assessment procedures is that the results can be linked to ecological stress factor. After a comparison of macroinvertebrate indices, Sandin & Hering (2004) concluded that only the ASPT (Average Score Per Taxon) (Armitage *et al.*, 1983) was well correlated with stress gradients in most stream types investigated. The IBGA (Anonymous, 1996) was not included in this comparison but the BBI (Belgian Biotic Index) (De Pauw & Hawkes, 1992) which belongs to the same "index family" (Figure 6). It should be noted that the ASPT is also based on scores assigned to families and not to taxa with a higher resolution (Armitage *et al.*, 1983).

4.3.2 Indices for tidal freshwater zones

A. The German AeTI

In Germany the so-called Aestuar-Typie-Index (AeTI) was developed for transitional zones. This index is a modification of the Potamo-Typie-Index (PTI) (Schöll & Haybach, 2001; Schöll *et al.*, 2005). The AeTI has also been proposed to use for the assessment of the water quality element "macroinvertebrates" as prescribed in the WFD (Krieg 2005, 2006).
The index is based on species which have their centre of existence in the estuarine part of North-German rivers. An ecological value was assigned to each species which indicates its indicator value. The more characteristic the species is for estuary, the higher its indicator

value assigned. For the calculation of the AeTI the relative abundance of the species is used as a weighting factor. After that the mean of all "weighted" indicator values is the result of the AeTI calculation. Its range is between 1.0 and 5.0. Quality classes were derived by a non-equidistance division of the range into five classes (Krieg, 2005).
Application of the AeTI for the transitional zone in the river Seine was considered to be impossible due to the presence of a relatively high number of taxa without indicator value assigned and relatively high number of specimens without indicator value in the zones T_1^A and T_2 (Table 18).

Table 18
The number of appropriate samples in the zones distinguished in the river Seine for calculation of the AeTI, the number of taxa and percentage of specimens with and without

	Zone		
	T_1^A	T_1^B	T_2
Appropriate samples	12	9	18
Taxa with indicator value	33	24	13
Taxa without indicator value	38	23	13
% specimens with indicator value	26	84	30
% specimens without indicator value	74	16	70

B. The Belgian index for tidal freshwater zones

For the assessment of the ecological status of water bodies in Belgium the macroinvertebrates are sampled by means of kick sampling with a handnet. For deeper parts of rivers an artificial substrate is used as described by De Pauw *et al*. (1986, 1994). A multimetric index for assessment of the ecological status was proposed for different types of water bodies, however, a good combination of metrics for larger rivers like the river Scheldt was not found due to the limited size of the dataset (Gabriels *et al*., 2004). For larger brooks, for example, next 11 metrics (out of 48) were proposed:

a. total number of taxa
b. number of Hemiptera taxa
c. number of insecta taxa
d. sum of Crustacea and Mollusca taxa
e. % specimens of the two most dominant taxa
f. % Hirudinea taxa
g. % Odonata taxa
h. % EPT taxa (Ephemeroptera, Plecoptera, Trichoptera)
i. % sensitive (or intolerant) taxa
j. the Shannon-Wiener index
k. the STS (Sum of Tolerance Score)

Since metrics were still not proposed for larger rivers, an assessment of the tidal freshwater part of the river Seine according to the Belgian prescription could not be made.

C. The Dutch index for tidal freshwater zones

For description of the ecological status of a water body based on the occurrence of macrozoobenthos, a multimetric index of groups of indicator species is used in The Netherlands (Knoben & Kamsma, 2004). Indicator species distinguished for each water body type are:

a. characteristic species,
b. positive dominant species,
c. negative dominant species.

Ascription of species to these three groups took place on the basis of their traits. Characteristic species have their centre of existence in specific water body types. Positive dominant are species occurring dominantly in the reference situation, while negative dominant species dominantly occur in water bodies with a moderate ecological status or lower. Traits of the species were derived from auto-ecological information of species, historical data, the Handbook for Nature Target Types (Bal *et al.*, 2001) and expert judgement (Van der Molen & Pot, 2006).
The multimetric index combines next metrics:

1. percentage of characteristic species;
2. percentage of individuals belonging to the group of positive dominant and characteristic species (relative abundance).
3. percentage of individuals belonging to the group of negative dominant species (relative abundance);

Based on these multimetrics the index calculation for tidal freshwater zones in rivers is (Van der Molen & Pot, 2006):

$$Value = (200*(CS/CS_{max}) + 200*(1-(ND/ND_{max})) + (CS+PD))/500$$

in which:

CS = percentage of characteristic species
CS_{max} = percentage of characteristic species in the reference situation
ND = percentage of negative dominant species
ND_{max} = percentage of negative dominant species in the reference situation
PD = percentage of positive dominant species

Classification of species into the three groups mentioned above must still be done for tidal freshwater zones. This is also the case for the classification of index values into quality classes according to the WFD because reference conditions (MEP and GEP) for tidal freshwater bodies are still lacking (situation March 2007).

4.4. Monitoring protocol

A monitoring protocol for the Seine aval is given in annex 7. From practical point of view two sampling locations per zone are recommended. They can be considered as duplicates and should be chosen in such a way that they are located in areas with average physical and chemical quality conditions in the biotopes sampled. This means that, e.g., point sources of pollutants or temporary river engineering activities should be avoided. Unacceptable deviations between assessment results of both locations should be a reason to reject them in reporting the monitoring results. The number of samples to be taken depends on the assessment procedure adopted.

In order to apply the IGBA, 13 samples are prescribed to be taken at each location. This number of samples from the main biotopes present in the river seems to be sufficient if other assessment procedures are taken into account. At least two sampling campaigns are recommended in each monitoring year to sample most of the macroinvertebrates (especially insect larvae) present in the river, in May after the relatively high spring discharge and in August/September.

4.5. Recolonisation potentials

In order to improve the ecological quality of the river, first objective must be the reduction of waste water discharges by expanding the water purification capacity to such extend that the oxygen demand of the discharges does not significantly affect the oxygen content in the river. Physical restraints for macroinvertebrates are loss of intertidal habitats due to the embankments. During and after rehabilitation works in the Lower Seine species will colonize or recolonize the restored areas.
Colonisation and recolonisation by aquatic macroinvertebrates strongly depends on three main processes of dispersal: drift, flying (for insects only) and human mediated dispersal.

- **Drift**
  The downstream displacement of macroinvertebrates through the water column. It is a natural process that can lead to massive displacement, especially at high discharges. Peak discharges in the Lower Rhine and Lower Meuse in February 1995 were among the highest of the 20th century. In erosion gullies formed along the main channel of both rivers a total number of 565 living aquatic macroinvertebrate taxa were collected of which some had drifted for 500 km or more prior to stranding in the floodplain (Klink, 1999). These peak discharges reintroduced, for example, the dragonfly *Gomphus flavipes* which was lastly observed in the Netherlands in 1901.

- **Flying**
  The East and West European lowland rivers contain a very similar insect fauna. Most groups of insects are good flyers that disperse very well by flying. Most vulnerable groups amongst them are the stone- and mayflies (Plecoptera and Ephemeroptera respectively) because of their sensitivity to pollution and habitat degradation. In addition, these insects are poor flyers. All these traits contributed to their extinction from the river Rhine in the late 19th century (Geijskes, 1948; Mol, 1985[a,b]), while reintroductions are very rare after improvement of the ecological quality of the river from the 1980's (Bij de Vaate *et al.*, 1992).

- **Navigation**
  Navigation is the main vector in human mediated dispersal of aquatic animals in rivers. Most successful are species that are able to attach to ship's hulls. Navigation is also an important vector for the introduction of nonindigenous species. Intercontinental dispersals of these species mainly occur through transport in ballast water. Continental dispersals are mainly the result of interconnections of European rivers by shipping canals (Bij de Vaate *et al.*, 2002). Also in this case animals are transported from one river basin to the other if attached to a ship's hull or as a result of water management in these canals. For

example, in the Main-Danube Canal, connecting the Rhine and Danube basins, water level in the upper section is maintained with water supply from the Danube basin, resulting in an annual flow of 150 million m^3 water from the Danube basin into the river Rhine (Tittizer, 1997). This especially facilitates dispersal of mobile animals (e.g., crustaceans) from the Danube basin towards the Rhine basin. The canal has already been successfully traversed by some amphipod species such as *Dikerogammarus haemobaphes* (Schleuter et al. 1994), *D. villosus* (Bij de Vaate & Klink 1995), *Echinogammarus trichiatus* (Prodraza *et al.* 2001) and *Obesogammarus obesus* (Nehring, 2006), the isopod *Jaera istri* (Schleuter & Schleuter 1995), the mysid *Limnomysis benedeni* (Reinhold & Tittizer 1998), the polychaete *Hypania invalida* (Klink & Bij de Vaate 1996), as well as the planarian *Dendrocoelum romanodanubiale* (Schöll and Behring 1998). All these Ponto-Caspian species including all other nonindigenous species occurring in the river Rhine are able to colonize the Seine basin directly through the Rhine-Marne Canal or through the existing European network of shipping canals.

Data by courtesy of D.I.R.E.N. enable us getting insight in the recolonisation potential from the vicinity of the Seine aval, which could happen by means of drifting and/or flying. Table 19 gives an overview of sensitive taxa found in the main and secondary channels of the Seine aval, its tributaries and the neighbouring upstream section. The group of *Chironomidae*, the most divers group of invertebrates has been left out since they were not identified.

Totally 46 sensitive taxa (probably >100 species) were found in the tributaries and the neighbouring upstream section. From the results of the June 2006 sampling it was concluded that ten of these taxa have a marginal existence in the Seine aval. Of these taxa the mollusc *Theodoxus fluviatilis* is missing in the Seine aval. Also *Hydracarina* have not been found in the river. This group mainly contains of predators and their occurence depend on the presence of vegetation as their habitat. Of the mayflies, only *Ephemerella ignita* and *Heptagenia sulphurea* live in very small numbers in the upper section of the Seine aval. *Baetidae*, *Ephemera* and *Ecdyonurus* were only collected in the confluents. Of the two stoneflies, *Leuctra fusca* was found in the Seine aval near the confluence of the Andelle while *Nemoura* was only found in the Rancon and the Sainte Gertrude. However, both species are no characteristic inhabitants of large lowland rivers. They are inhabitants of smaller streams. The dragonflies *Calopteryx* and *Platycnemis* live close to the Seine aval. The waterbug *Aphalocheirus aestivalis* was collected in the Seine aval and *Velia* lives in the Robec. Three genera of the critical beetles of the family *Elminthidae* live a marginal live in the Seine aval and five other genera live close by in the confluents (*Macronychus*, *Normandia*, *Oulimnius*, *Riolus* and *Stenelmis*).
The sensitive caddis flies (Trichoptera) are hardly able to develop viable populations in the Lower Seine. In the tributaries, however, many taxa have been found able to live in the river as well. The rivers Andelle and Eure accomodate the richest Trichoptera fauna compared with the other tributaries taken into account. Of the dipterans the black flies (Simuliidae) are very common in the tributaries, but absent in the river where they can live under natural circumstances. The same is the case for snipe flies (Athericidae) which were only observed in the river Oison.

Table 19

Sensitive fauna elements in the tidal freshwater section of the river Seine, its tributaries and the neighbouring upstream section.

Taxonomic group	Taxa	Seine aval	Andelle	Oison	Eure	Robec	Aubette	Cailly	Austreberthe	Rancon	Ste-Gertrude	Seine amont
Mollusca	Acroloxus											+
	Ancylus	+	+	+	++	+++	++	+++	++	+++	++	
	Theodoxus		++		+++				+++	++	++	
	Pisidium											++
	Pseudanodonta											+
Hydracarina			+	++	+	++	++	++	+++	++	+++	
Ephmeroptera	Baetidae		+++	+++	+++	+++	+++	+++	++++	+++	+++	++
	Ephemerella	+	+++	++	++	+	++	+++	+++	++	+++	
	Ephemera		+		++			+				
	Ecdyonurus				+							
	Heptagenia	+		+	++							
Plecoptera	Leuctridae	+	++									
	Nemouridae									+	+	
Odonata	Calopteryx			+	+							
	Coenagron											++
	Orthretrum											+
	Platycnemis				+							+
Heteroptera	Aphelocheirus	+			++			+				
	Velia					+						
Coleoptera	Elmis	+	++	+++	+++		+	+	+	++	+	
	Esolus	+		++	++					+		
	Limnius	+	+++	+++	+++		+			+		
	Macronychus				+							
	Normandia			+								
	Oulimnius			+	++			+				
	Riolus		+	+	+		+			+		
	Stenelmis				+							
Trichoptera	Glossosomatidae					+	+++	+		+	++	
	Agapetus			+++	+		+++					
	Glossosoma				+							
	Beraeidae				+							
	Goeridae		+	+	+				+	+++		
	Hydroptilidae	+	++	++	++	+++	+++	++	++++	+	+++	
	Ithytrichia				+							
	Lepidostomatidae	+	+	+	++							
	Polycentropodidae											+
	Leptoceridae		+	+	++							
	Athripsodes				++							
	Mystacides				++							
	Triaenodes				+							
	Limnephilidae		+	++	+	+	+++	+	++			
	Odontocerum albicorne		+								+	
	Psychomyidae		+		+							
	Rhyacophila		+	+	+	+	+	+	++	+		
	Sericostomatidae		++		+		+		+	+		
Diptera	Simuliidae		++	++++	++++	+++	+++	++	+++	+++	++++	
	Athericidae			+								
Total number of taxa		10	20	21	33	10	14	13	12	16	11	8

When water quality of the Seine aval improves, a lot of pollution sensitive taxa present upstream and in the tributaries are able to return by drifting or flying. However, for several taxa it will be hard to find a suitable habitat. For instance *Macronychus quadrituberculatus* (Coleoptera) and *Atherix ibis* (Athericidae) depend on the presence of dead wood (snag) being their habitat; a rare phenomenon in the current lower Seine.
Also a number of species that used to live in the river Seine will not return because they became extinct in Western Europe and are no good flyers in the case of insects. A well documented example is the mayfly *Prosopistoma foliaceum* (Photo 9), that has become extinct in Western Europe in the 20th century and seems to disappear in Eastern Europe as well (Landa & Soldan, 1985). The same has also been observed for a number of stoneflies.

The data analysed do not allow getting insight into what species recently invaded the Seine aval. However, most of the nonindigenous species found during the June 2006 monitoring activities are recent invaders (Table 20). Their number will increase mainly due to introductions from other river basins. Water and bottom quality improvement including nature development in the remaining floodplain will facilitate population development of these species.

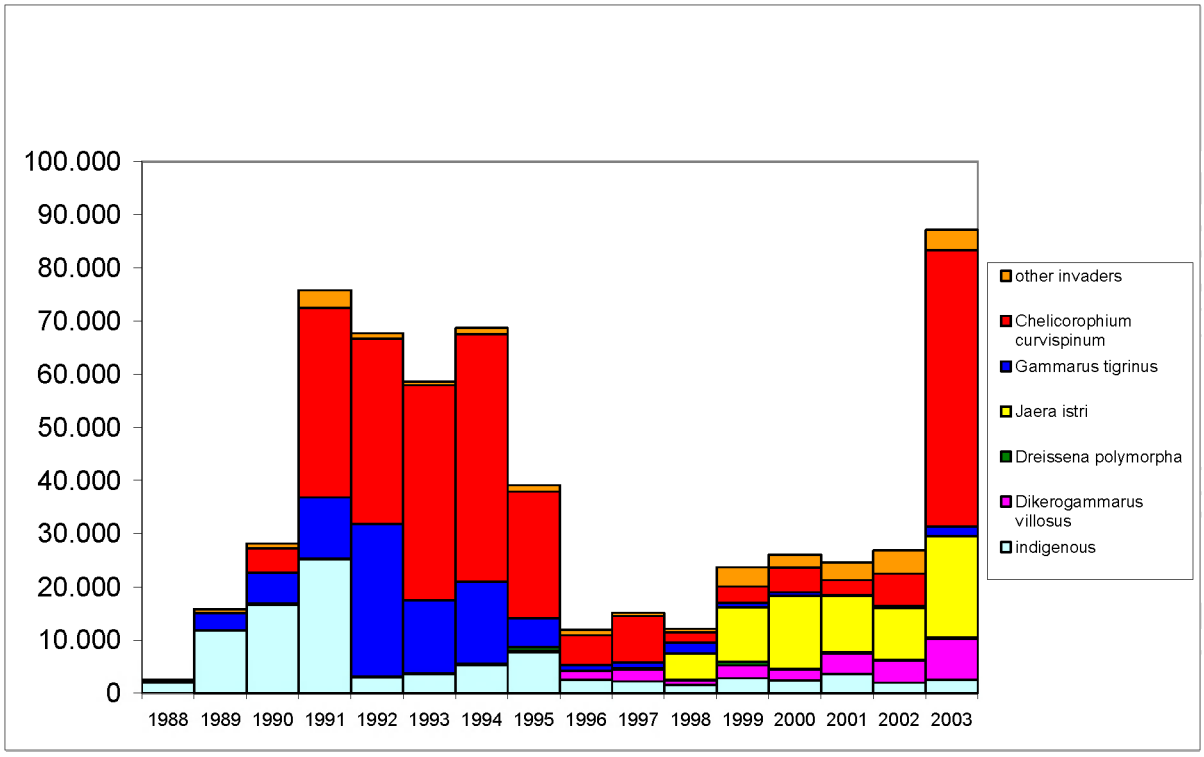
Table 4
Nonindigenous macroinvertebrates found during the June 2006 monitoring activities.

Taxonomic group	Species	Origin
Polychaeta	Hypania invalida	Ponto-Caspian area
Oligochaeta	Branchiura sowerbyi	probably East Asia
Tricladida	Dugesia tigrina	North America
Mollusca	Dreissena polymorpha	Ponto-Caspian area
	Corbicula fluminalis	East Asia
	Corbicula fluminea	East Asia
	Lithoglyphus naticoides	Eastern Europe
	Orconectes limosus	North America
Amphipoda	Crangonyx pseudogracilis	North America
	Dikerogammarus villosus	Ponto-Caspian area

The presence of only ten disturbance sensitive macroinvertebrate taxa in the Seine aval, having a marginal existence in it, reflects the poverty of natural biotic and abiotic processes present in that part of the river. Recolonisation potential is present in the tributaries and upstream sections. The most important supply of recolonizers is expected to arrive from upstream parts as was clearly demonstrated in the rivers Rhine and Meuse after peek discharges (Klink, 1999). However, the paradox is that with the ameliorating water quality, chances for nonindigenous nuisance species increase dominating the macroinvertebrate community (Den Hartog *et al.*, 1992). Examples for the river Rhine are the Ponto-Caspian species *Chelicorophium curvispinum* and *Dikerogammarus villosus* (Rajagopal et al., 1999; Van der Velde et al., 2000; Van Riel et al., 2006[a], 2006[b]). In Figure 7

recent developments of dominant species on an artificial substrate in the river Rhine at the Dutch-German border is shown for the period 1988-2003. Until 1991 the invaders did not seem hampering the colonisation of indigenous species. However, after that year clear impact has been demonstrated on indigenous species to colonize the substrate.

Figure 7
Density of invaders and indigenous invertebrates on artificial substrate in the Lower Rhine at the Dutch-German border from 1988-2003.



In general, community vulnerability to invasions is ascribed to combinations of several factors like the presence of vacant niches, habitat modification and disturbances before and during invasions. Although the link between the biodiversity of communities and their vulnerability to invasions remains to be proved, invasibility is known to increase if a community lacks certain species, which ought to be present under normal conditions. A new hypothesis linking the various explanations of increased invasibility is that of fluctuating resource availability such as an increased amount of unused resources (Davis *et al.*, 2000).

The river Rhine is a good example of all these related factors. Pollution over a long period weakened the original communities and caused the loss of certain species, creating open niches for pollution-tolerant invaders. Water quality improvement led to a partial recovery of the original communities together with the establishment of previously disappeared and new invaders. A major disturbance like the Sandoz accident in 1986 subsequently led to invasions by many new species, which reached unprecedented densities. The fact that filter feeders are particularly abundant can be attributed to intense

phytoplankton blooms due to eutrophication. Hardly any macrophytic vegetation is present in the Rhine channel to compete with phytoplankton for nutrients. Recolonisation after partial reduction of pollution in rivers modified by human activities seems to favour invaders more than indigenous species. These invaders then suppress the development of populations of indigenous species, although biodiversity increases (Van der Velde *et al.*, 2002).

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Photo 9

Prosopistoma foliaceum www.liis.lv/aizsargajamie/viendienites.htm.

Severe pollution can function as a barrier to the dispersal of invaders. An example is the Chicago connection between the Great Lakes and the Mississippi river, where the 1972 Clean Water Act provided subsequent improvements in municipal waste treatment. This resulted in improved water quality to such an extent that the zebra mussel (*Dreissena polymorpha*) and six other non-native "pest" species were able to spread from the Great Lakes to the Mississippi River (Stoeckel et al., 1996). *D. polymorpha* returned to the river Rhine in the 1970's and 1980's, when cadmium concentrations in the water fell below 1 µg l^{-1} (accumulation in the mussel at that level was 40 µg g^{-1} DW) (Van Urk & Marquenie, 1989).

The present day invasions of Ponto-Caspian invaders in Western Europe via the Main-Danube canal increases the likelihood that they will reach other harbours in Europe via ballast water transport because of the presence of many major ports in Western Europe (Bruijs et al., 2001). These species tolerate high temperatures and brackish water. The future will bring continued invasions, resulting in

unstable communities with an accelerated turnover due to increasing propagule pressure combined with greater anthropogenic disturbance (Nilsson & Grelsson, 1995; Stylinski & Allen, 1999). This future scenario will cause a shift from battles between invaders and indigenous species towards battles among invaders of various origins.

4.6. Prospects of river rehabilitation

For defining the MEP and GEP of the tidal freshwater zone of the river Seine, prospects of river rehabilitation should be developed. Starting point for such development could be the ecotope approach.

An ecotope is an area with uniform environmental conditions and characteristic plants and animals. Ecotopes comprise the smallest ecologically-distinct landscape features in landscape mapping and classification systems.
In general an ecotope system consists of:

a. an ecosystem classification in which ecosystems are classified on the spatial scale of ecotopes on the basis of the vegetation structure present and its habitat conditions;
b. a corresponding classification of species which can be expected in each ecotope.

In The Netherlands an antropogenic component was introduced in the ecotope definition, which was defined as "a physically limited ecological unit, of which composition and development are determined by abiotic, biotic and anthropogenic aspects together" (Wolfert, 1996). In relation to the ecotope the term physiotope is used for a homogeneous unit in respect to abiotic conditions being important for biotic aspects. In other words, if management and stage of development are the same, then both the physiotope and ecotope are the same physical unit. The Dutch Water Ecotope Classification was developed for inland waters (rivers, streams, canals and lakes), transitional waters, coastal waters and the Dutch part of the North Sea (Maas, 1998; De Jong, 1999; Lorenz, 2001). Classification aspects were:

a. **hydrology**
The hydrological regime is determined by the combined action of tide and river discharge. Upstream river discharge is the dominant factor, downstream the tidal movements at sea. The zone in between was considered to be the transitional area where either the tidal magnitude or the river discharge determines which of both dominates. The distinctive and essential phenomena for specific tidal freshwater ecotopes are the daily changes of the water level. They determine the duration of flooding or drought and as a consequence the development of bottom structures, vegetations and faunal elements. Water depth, duration and frequency of floodings lead to next distinction of subunits in a water body:

1 *very deep tidal water*
the deeper river channel, depth at the mean low water level (MLW) >5 m;
2 *deep water*
permanently flooded area, depth at MLW between 1.5 and 5 m;
3 *shallow water*
permanently flooded area, very rare uncovered, depth at MLW between 1,5 and 5 m;
4 *low intertidal zone*
very frequently submerged amphibious area above MLW with an inundation duration of >50% during one tide;
5 *middle intertidal zone*
frequently submerged amphibious area above MLW with an inundation duration between 30 and 50% during one tide;
6 *high intertidal zone*
frequently submerged amphibious area above the mean water

(MW) level with an inundation duration of <30% during one tide and restricted to the mean springtide high water (MSHW) level;

7 *periodically flooded zone*
periodically submerged terrestrial area above MSHW level, 20-50 days per year inundated;

8 *rarely flooded zone*
rarely inundated terrestrial area above the MSHW level, <20 days per year inundated;

9 *high water free zone*
never inundated terrestrial area

b. **morphodynamics**

This term comprises all mechanical water forces (waves and currents) on bottom, vegetation and fauna in an ecotope. Erosion and sedimentation processes, and transport of sediments (gravel, sand and silt) are manifestations morphodynamics. In most of the tidal freshwater zone of the river Seine the existing morphodynamics are mainly determined by the daily change in tidal current directions. Only upstream of Rouen river discharge and sediment transport plays an significant role (Le Hir & Silva Jacinto, 2001, Lesueur & Lesourd, 1999).
Three classes were used for the classification of the aspect morphodynamics:

1 *low dynamic*
the sediment is hardly moved by waves and/or currents. With sufficient silt supply a soft sediment layer varying from a few millimetres till some decimetres will be present. This layer will be eroded when stream velocity is > 0,3 m/s. Examples are natural tidal levees along creeks.

2 *dynamic*
In dynamic areas erosion and sedimentation processes are cause changes in the top layer of a few centimetres till some decimetres on a regular basis. The sediment is mainly sandy and mostly mixed with empty shells Water currents vary between 0,3 and 1 m/s. Water movements either prevents the growth of plants or creates repeatedly new habitats. Examples of such environments are sandbanks that are uncovered at low tide, but because of the movement of the sediment pioneer vegetation settlement is prevented.

3 *high dynamic*
Environments with strong tidal currents and/or high river water velocities (>1 m/s). The sediment, mainly consisting of sand and gravel, is continuously and strongly moving at depths of a few decimetres till some metres. In these dynamic environments shape of the river may change continuously by the formation of banks and secondary channels. Examples are sandbanks that constantly change there shape and position, and the formation of sand and gravel megaripples.

c. **human use**

Within this aspect all deliberate and purposive infrastructural measures and management activities influencing habitat structure, plant and animal communities are put together. The aspect comprises both measures like habitat management by extensive deployment of grazers (e.g., cattle) as well as intensive agricultural use, recreation and dredging. Three categories are distinguished:

1 *natural*
no or slight anthropogenic influence on habitat development,

vegetation structure and faunal elements. Development in plant and animal communities are the result of natural processes.

2 *semi-natural*
slight anthropogenic influence on habitat development, vegetation structure and faunal elements (Photo 10). Human activities are directed towards maintenance of natural values or to restore them.

3 *multifunctional*
extreme anthropogenic influence on habitat development (Photo 11), vegetation structure and faunal elements for economic reasons, such as transport, industrialization, urbanization and exploitation.

.....

Photo 10
An example of slight anthropogenic influence on habitat development.

The ecotopes approach could be a helpful tool in studies for improvement of the Seine aval environment because it:

a. can be used in GIS to link biotic and abiotic information;
b. makes use of univocal classifications in relation to river dynamics;
c. is a tool to make effect predictions of measures proposed;
d. can be used to explain changes at community level;
e. is a practical tool to quantify changes as result of interventions;
f. can be used to make historical references based on old maps;
g. is a practical tool in Environmental Impact Assessment procedures.

Photo 11
An example of extreme anthropogenic influence on habitat development.

In order to restore the ecological integrity of large rivers, restoration including nature development has become an important issue from the end of the 1980's (Boon et al., 1992; Gore & Shields, 1995; Sparks, 1995; Nienhuis & Leuven, 1999; Pedroli & Postma, 1999). The Netherlands adopted a river management policy of habitat restoration by reconnecting floodplain habitats with the main stream through restored flood pulses. The general underlying assumption of this policy is that flood pulses (hydrodynamics) and morphological diversity arising from the flow pulse (morphodynamics) are the main driving forces for the formation of characteristic riverine habitats and associated life forms (e.g., Amoros & Roux, 1988; Junk *et al.*, 1989; Sedell *et al.*, 1989). Various habitat restoration projects have been developed with the aim of creating an ecological network along the Netherlands Lower Rhine and its tributaries, consisting of several large ecologically important reaches (1000-6000 ha each) with smaller areas in between. At present, about 7,500 ha of floodplain along the Lower Rhine and its tributaries have an important ecological function. The Netherlands river management policy aims to protect these areas including an additional rehabilitated area of 5,000 ha within the next 10-15 years (Van Dijk *et al.*, 1995).

For the recolonisation of riverine fauna one has to realise that natural riverine landscapes are dynamic, and biologically and spatially complex (Ward *et al.*, 2002). They are characterised by often extensive flood plains (e.g. Lewis *et al.*, 2000), a natural flow regime (Poff *et al.*, 1997), high hydraulic connectivity (Ward *et al.*, 1999), a successional landscape mosaic with high habitat heterogeneity

(Wissinger, 1999), and a complex land-water coupling and exchange (Stanley, Fisher & Grimm, 1997). The interplay between these landscape elements has a direct bearing on the generation, distribution and maintenance of riverine biodiversity (Junk, 2000; Tockner *et al.*, 2000[a]). The riverine fauna also provides important feedbacks that can influence spatio-temporal dynamics of the landscape over long time periods (Naiman *et al.*, 2000).

Recently, also the importance of natural discharge fluctuations have been recognised in stream ecology (e.g., Stanley *et al.*, 1977; Tockner *et al.*, 2000[b]). For example, the extent of wetted areas can increase by orders of magnitude during the annual flood (Tockner *et al.*, 2000[a]), with concomitant effects on the distribution of aquatic and terrestrial organisms (e.g., Kohler *et al.*, 1999). Kohler *et al.* (1999) found that fish and macroinvertebrates were redistributed among floodplain ponds (temporary and permanent) during high waters. Consequently, the postflood community was affected strongly by direct fish predation on invertebrate predators. This suggests that the mosaic of successional stages in flood plains may reflect deterministic biotic interactions a well as stochastic physical forcing.
However, the fauna, as ecological engineers, also engage in autogenic and allogenic processes that influence biodiversity (structural, functional, genetic), community assembly (life cycles, species traits, strategies), system functioning (nutrient cycling, energy flow), and consequent biotic feedbacks (dispersal, predator-prey interactions, migration) in riverine landscapes (Robinson *et al.*, 2002).

The complex life cycles of many fauna of intact riverine landscapes infers that species loss translates to a loss of evolved morphologies, physiologies, behaviours and complex life cycles; that is, a loss in evolutionary trajectories. Ward *et al.* (1999), summarising many conceptual models regarding biodiversity, suggested that maximum biodiversity is maintained at intermediate disturbance and resource availability, levels typically found in intact riverine landscapes (e.g., Naiman *et al.*, 1988). Angermeier & Winston (1999) emphasised the importance of key landscape-scale features in conservation biology; the idea being that most species respond to changes in key environmental factors (Keddy, 1999). For example, because high ecotone/floodplain area ratios strongly correlate with high biodiversity (Brown, 1998), it follows that as the number and diversity of ecotones increases in regulated rivers the dynamic nature, integrity and biodiversity of these systems also will increase.

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Annexes

Annex 1. Sampling protocol used in the period June 16-20, 2006

Introduction

The European Water Framework Directive (WFD) prescribes monitoring of several so-called water quality elements. One of these elements is the macrozoobenthos community. Assessments of the ecological quality of water bodies should be based on a set of standardized methods for sampling and identification of the animals in the samples. This sampling protocol is the first step in standardizing the monitoring and assessment of relevant macrozoobenthos communities in the fluvial part of the Seine estuary.

Sampling potential fixed locations

Two main sections, T_1 and T_2, are distinguished in the freshwater section of the River Seine downstream of the weir near Poses. T_1 is the section between Poses (rk[3] 202) and La Bouille (rk 260), T_2 between La Bouille (rk 260) and Vieux Port (rk 325). In section T_1 there is a big difference in water quality and river management between the part upstream and the part downstream of Rouen. Those are the reasons for splitting up this section into two subsections: T_1^A from Poses (rk 202) to Rouen (rk 236), T_1^B from Rouen (rk 236) to La Bouille (rk 260).

Two sampling locations are proposed in each section or subsection:

T_1^A :	between rk 202 and 204
	between rk 221 and 227
T_1^B :	between rk 241 and 254
	between rk 254 and 260
T_2 :	between rk 285 and 295
	between rk 318 and 320

Samples will also be taken in the mouth of the Eure tributary (rk 216-217) and at locations of special interest.

At each location next biotopes are distinguished:

1. the tidal zone:
    a. soft bottom (mud, sand)
    b. solid substrates (boulders, pebbles, stones, bricks, woody debris)
    c. vegetation
2. the subtidal zone:
    a. soft bottom (mud, sand)
    b. solid substrates (boulders, pebbles).

In addition, an artificial substrate (broken bricks in a coarse mesh size netting) will be used to sample the active migrating macrozoobenthos in the subtidal zone.
All biotopes will be sampled triple.

Apart from the samples taken at the locations mentioned above, also interesting (from point of view of nature conservation) biotopes will be sampled. Maximum number of locations to be sampled is 15.

One cooling water intake (preferably SMEDAR Rouen) is proposed to be sampled in order to get a better insight in the presence of mobile organisms (mainly crustaceans) in the River Seine.

Sampling period

Sampling will be performed in the period June 16– 25, 2006, and will take place ± 2 hours around low tide (table 1). The artificial substrates will be sampled after a colonization period of about 4 weeks, which means they must be suspended into the river between May 16 and 21, 2006.

Table 1. Calculated moment of low tide and corresponding water level (m) at four locations in the lower Seine.

[3] rk = river kilometer

		St. Léonard		Caudebec		Duclair		Rouen	
Date	Day	Time	Hight	Time	Hight	Time	Hight	Time	Hight
16-06-06	Friday	11:36	2,70	12:31	3,55	13:46	4,35	15:06	4,55
17-06-06	Saturday	12:20	2,75	13:20	3,55	14:35	4,30	15:55	4,50
18-06-06	Sunday	13:08	2,80	14:13	3,60	15:28	4,30	16:48	4,50
19-06-06	Monday	14:00	2,90	15:09	3,60	16:25	4,35	17:45	4,45
20-06-06	Tuesday	15:00	2,90	16:07	3,60	17:25	4,30	18:45	4,45
21-06-06	Wednesday	3:29	3,30	4:37	3,65	5:59	4,30	7:19	4,40
21-06-06		16:04	2,95	17:14	3,65	18:29	4,30	19:49	4,45
22-06-06	Thursday	4:43	2,95	4:48	3,65	7:08	4,30	8:28	4,45
22-06-06		17:16	2,90	18:21	3,60	19:41	4,30	21:01	4,45
23-06-06	Friday	5:42	2,90	6:58	3,60	8:17	4,30	9:37	4,45
23-06-06		18:17	2,90	19:25	3,60	20:42	4,30	22:02	4,45
24-06-06	Saturday	6:54	2,80	7:59	3,60	9:15	4,30	10:35	4,45
24-06-06		19:15	2,85	20:16	3,60	21:36	4,30	22:56	4,45

Sampling dates

Next sampling dates for the potential fixed locations are proposed:

T_1^A :	between rk 202 and 204	June 20, 2006
	between rk 221 and 227	June 19, 2006
T_1^B :	between rk 241 and 254	June 16, 2006
	between rk 254 and 260	June 17, 2006
T_2 :	between rk 285 and 295	June 17 and 18, 2006
	between rk 318 and 325	June 18, 2006
$T_{1 \text{ and } 2}$	additional sampling	June 21-23, 2006

Interesting biotops and the mouth of the Eure tributary are planned to be sampled in the mean time as well. If no time enough, sampling of these locations will be performed from June 21, 2006.
Retrieval and sampling of the artificial substrates will be done in the period June 16-20, 2006.
Sampling of a cooling water intake will take place during one day in the period June 16-23, 2006.
If necessary, additional will be performed after June 20, 2006.

Sampling methods

a. ***Solid substrates in the tidal and subtidal zone***
All organisms attached to at least five pieces of solid substrate are brushed off with help of a soft (washing-up) brush. The material attached to these stones produces one sample.

b. ***Subtidal bottom***
Sampling method used for the profundal bottom depends on size of the bottom particles. In the case of coarse material (gravel and coarser particles) a (triangular) dragnet is used. If the bottom consists of sand or mud a Van Veen grab will be used. The macrozoobenthos is separated from the bottom material by washing each sample (in portions) on a 500 µm mesh sieve. If a dredge is used to sample bottoms with coarse material, the pebbles and cobbles (and other particles of these sizes) are brushed off.

c. ***Aquatic vegetation***
For sampling the aquatic vegetation a handnet (mesh size 500 µm) is used.
Preconditions:
- Included in the aquatic vegetation sampling are: macrophytes, bryophytes, sessile macroalgae, (submerged) roots and vegetal litter (e.g. leaves).
- The sample in the littoral vegetation is taken at a minimum of 1 meter inside the vegetation seen from the waterside.
- Sampling should preferably take place in an unbroken stretch of vegetation.
- If circumstances allow, sampling should be done from the water side and not from the embankment side of the sampling site.

d. ***Tidal bottom***
The sandy or muddy bottom will be sampled by means of a core at low tide. The macrozoobenthos

in the samples is separated from the bottom material by washing each sample (in portions) on a 500 µm mesh sieve.

e. ***Artificial substrate***

The artificial substrate consists broken bricks (± 4-8 cm in size) in a polyethylene or nylon netting (38x48 cm). Each netting is filled with 4 litre of the substrate and firmly packed in order to prevent rolling of the material. Colonisation period is 30 ±1 days. The sampling procedure of the artificial substrate is the same as for solid substrates.

The nettings used during the 2006 monitoring campaign each contained three broken bricks (each brick 21 x 10 x 6.5 cm). Total weight of each netting 7 kg (range 6.5 to 7.5 kg) (individual weights: 2 x 6.5 kg; 9 x 7.0 kg and 1 x 7.5 kg).

f. ***Sampling of a cooling water intake***

The cooling water will be sampling the screens or by suspending a net in the water the intake flow.

Annex 2. Identification literature macroinvertebrates

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Annex 3. Identification of macroinvertebrates

Macroinvertebrates in the samples taken during the June 2006 campaign were identified as much as possible at species level. Some specimens were identified at higher taxonomic levels due to unclear identification marks. The 172 taxa found are summarized below, including the order they belong to, the identification level prescribed for assessing the value of the IGBA and the IGBA indicator taxa.

Order	Taxa	Identification level IGBA	Indicator taxa IGBA
Eucestoda	Caryophyllaeus species	Caryophyllidae	
Tricladida	Dendrocoelum lacteum	Tricladida	
Tricladida	Dugesia lugubris/polychroa	Tricladida	
Tricladida	Dugesia tigrina	Tricladida	
Polychaeta	Hypania invalida	Polychaeta	
Oligochaeta	Aulodrilus pluriseta	Oligochaeta	Oligochaeta
Oligochaeta	Branchiura sowerbyi	Oligochaeta	Oligochaeta
Oligochaeta	Chaetogaster diaphanus	Oligochaeta	Oligochaeta
Oligochaeta	Eiseniella tetraedra	Oligochaeta	Oligochaeta
Oligochaeta	Enchytraeidae species	Oligochaeta	Oligochaeta
Oligochaeta	Haplotaxis gordioides	Oligochaeta	Oligochaeta
Oligochaeta	Limnodrilus claparedeianus	Oligochaeta	Oligochaeta
Oligochaeta	Limnodrilus hoffmeisteri	Oligochaeta	Oligochaeta
Oligochaeta	Limnodrilus udekemianus	Oligochaeta	Oligochaeta
Oligochaeta	Lumbricidae species	Oligochaeta	Oligochaeta
Oligochaeta	Lumbriculidae species	Oligochaeta	Oligochaeta
Oligochaeta	Nais ellinguis	Oligochaeta	Oligochaeta
Oligochaeta	Nais pardalis	Oligochaeta	Oligochaeta
Oligochaeta	Ophidonais serpentina	Oligochaeta	Oligochaeta
Oligochaeta	Peloscolex multisetosus	Oligochaeta	Oligochaeta
Oligochaeta	Peloscolex velutinus	Oligochaeta	Oligochaeta
Oligochaeta	Potamothrix moldaviensis	Oligochaeta	Oligochaeta
Oligochaeta	Psammoryctides barbatus	Oligochaeta	Oligochaeta
Oligochaeta	Stylaria lacustris	Oligochaeta	Oligochaeta
Oligochaeta	Stylodrilus heringianus	Oligochaeta	Oligochaeta
Oligochaeta	Tubifex ignotus	Oligochaeta	Oligochaeta
Oligochaeta	Tubifex tubifex	Oligochaeta	Oligochaeta
Oligochaeta	Tubificidae with hairs	Oligochaeta	Oligochaeta
Oligochaeta	Tubificidae without hairs	Oligochaeta	Oligochaeta
Hirudinea	Cystobranchus respirans	Piscicolidae	Hirudinea
Hirudinea	Erpobdella octoculata	Erpobdellidae	Hirudinea
Hirudinea	Erpobdella testacea	Erpobdellidae	Hirudinea
Hirudinea	Erpobdellidae species	Erpobdellidae	Hirudinea
Hirudinea	Glossiphonia complanata	Glossiphonidae	Hirudinea
Hirudinea	Glossiphonia concolor	Glossiphonidae	Hirudinea
Hirudinea	Glossiphonia heteroclita	Glossiphonidae	Hirudinea
Hirudinea	Helobdella stagnalis	Glossiphonidae	Hirudinea
Hirudinea	Hemiclepsis marginata	Glossiphonidae	Hirudinea
Hirudinea	Piscicola geometra	Piscicolidae	Hirudinea
Hirudinea	Trocheta riparia	Erpobdellidae	Hirudinea
Mollusca	Acroloxus lacustris	Acroloxidae	Mollusca
Mollusca	Ancylus fluviatilis	Ancylidae	Mollusca
Mollusca	Bithynia tentaculata	Bithyniidae	Mollusca
Mollusca	Corbicula fluminalis	Corbiculidae	Mollusca
Mollusca	Corbicula fluminea	Corbiculidae	Mollusca
Mollusca	Dreissena polymorpha	Dreissenidae	Mollusca
Mollusca	Galba truncatula	Lymnaeidae	Mollusca

Order	Taxa	Identification level IGBA	Indicator taxa IGBA
Mollusca	Gyraulus albus	Planorbidae	Mollusca
Mollusca	Lithoglyphus naticoides	Hydrobiidae	Mollusca
Mollusca	Physa fontinalis	Physidae	Mollusca
Mollusca	Physella acuta	Physidae	Mollusca
Mollusca	Pisidium species	Sphaeriidae	Mollusca
Mollusca	Pisidium amnicum	Sphaeriidae	Mollusca
Mollusca	Pisidium casertanum	Sphaeriidae	Mollusca
Mollusca	Pisidium casertanum plicatum	Sphaeriidae	Mollusca
Mollusca	Pisidium henslowanum	Sphaeriidae	Mollusca
Mollusca	Pisidium moitessierianum	Sphaeriidae	Mollusca
Mollusca	Pisidium nitidum	Sphaeriidae	Mollusca
Mollusca	Pisidium pulchellum	Sphaeriidae	Mollusca
Mollusca	Pisidium subtruncatum	Sphaeriidae	Mollusca
Mollusca	Pisidium supinum	Sphaeriidae	Mollusca
Mollusca	Potamopyrgus antipodarum	Hydrobiidae	Mollusca
Mollusca	Radix species	Lymnaeidae	Mollusca
Mollusca	Radix ovata	Lymnaeidae	Mollusca
Mollusca	Radix peregra	Lymnaeidae	Mollusca
Mollusca	Sphaerium corneum	Sphaeriidae	Mollusca
Mollusca	Sphaerium rivicola	Sphaeriidae	Mollusca
Mollusca	Sphaerium solidum	Sphaeriidae	Mollusca
Mollusca	Succineidae species	Succineidae	Mollusca
Mollusca	Valvata cristata	Valvatidae	Mollusca
Mollusca	Valvata piscinalis	Valvatidae	Mollusca
Mollusca	Viviparus viviparus	Viviparidae	Mollusca
Isopoda	Asellus aquaticus	Asellidae	Asellidae
Isopoda	Proasellus coxalis	Asellidae	Asellidae
Isopoda	Proasellus meridianus	Asellidae	Asellidae
Decapoda	Orconectes limosus	Cambaridae	
Amphipoda	Crangonyx pseudogracilis	Crangonyctidae	
Amphipoda	Dikerogammarus villosus	Gammaridae	Gammaridae
Amphipoda	Dikerogammarus species	Gammaridae	Gammaridae
Amphipoda	Echinogammarus berilloni	Gammaridae	Gammaridae
Amphipoda	Gammaridae species	Gammaridae	Gammaridae
Amphipoda	Gammarus species	Gammaridae	Gammaridae
Amphipoda	Gammarus salinus	Gammaridae	Gammaridae
Amphipoda	Orchestia species	Talitridae	
Ephemeroptera	Caenis macrura	Caenidae	Caenidae
Ephemeroptera	Ephemerella ignita	Ephemerellidae	Ephemerellidae
Ephemeroptera	Heptagenia sulphurea	Heptageniidae	Heptageniidae
Plecoptera	Leuctra fusca	Leuctridae	Leuctridae
Odonata	Coenagrionidae species	Coenagrionidae	
Odonata	Orthetrum species	Libellulidae	
Heteroptera	Aphelocheirus aestivalis	Aphelocheiridae	Aphelocheiridae
Heteroptera	Micronecta minutissima	Nepomorpha	
Heteroptera	Sigara striata	Corixidae	
Coleoptera	Elmis species	Elmidae	Elmidae
Coleoptera	Esolus species	Elmidae	Elmidae
Coleoptera	Haliplus fluviatilis	Haliplidae	
Coleoptera	Limnius species	Elmidae	Elmidae
Coleoptera	Oulimnius species	Elmidae	Elmidae
Trichoptera	Ecnomus tenellus	Ecnomidae	
Trichoptera	Hydropsyche contubernalis	Hydropsychidae	Hydropsychidae

Order	Taxa	Identification level IGBA	Indicator taxa IGBA
Trichoptera	Hydropsyche species	Hydropsychidae	Hydropsychidae
Trichoptera	Hydroptila species	Hydroptilidae	Hydroptilidae
Trichoptera	Lepidostoma hirtum	Lepidostomatidae	
Trichoptera	Neureclipsis bimaculata	Polycentropodidae	Polycentropodidae
Diptera	Ceratopogonidae species	Ceratopogonidae	
Diptera	Clinotanypus nervosus	Chironomidae	Chironomidae
Diptera	Procladius species	Chironomidae	Chironomidae
Diptera	Tanypus kraatzi	Chironomidae	Chironomidae
Diptera	Tanypus punctipennis	Chironomidae	Chironomidae
Diptera	Potthastia longimanus	Chironomidae	Chironomidae
Diptera	Prodiamesa olivacea	Chironomidae	Chironomidae
Diptera	Bryophaenoicladius group muscicola	Chironomidae	Chironomidae
Diptera	Cricotopus bicinctus	Chironomidae	Chironomidae
Diptera	Cricotopus intersectus	Chironomidae	Chironomidae
Diptera	Cricotopus sylvestris	Chironomidae	Chironomidae
Diptera	Limnophyes species	Chironomidae	Chironomidae
Diptera	Limnophyes pumilio	Chironomidae	Chironomidae
Diptera	Nanocladius bicolor aggregate	Chironomidae	Chironomidae
Diptera	Nanocladius bicolor	Chironomidae	Chironomidae
Diptera	Paratrichocladius rufiventris	Chironomidae	Chironomidae
Diptera	Pseudosmittia species	Chironomidae	Chironomidae
Diptera	Rheocricotopus chalybeatus	Chironomidae	Chironomidae
Diptera	Thalassosmittia thalassophila	Chironomidae	Chironomidae
Diptera	Tvetenia calvescens	Chironomidae	Chironomidae
Diptera	Chironomus acutiventris	Chironomidae	Chironomidae
Diptera	Chironomus bernensis	Chironomidae	Chironomidae
Diptera	Chironomus nudiventris	Chironomidae	Chironomidae
Diptera	Chironomus plumosus aggregate	Chironomidae	Chironomidae
Diptera	Chironomus species	Chironomidae	Chironomidae
Diptera	Cladopelma laccophila group	Chironomidae	Chironomidae
Diptera	Cryptochironomus defectus	Chironomidae	Chironomidae
Diptera	Cryptochironomus supplicans	Chironomidae	Chironomidae
Diptera	Cryptochironomus species	Chironomidae	Chironomidae
Diptera	Cryptotendipes species	Chironomidae	Chironomidae
Diptera	Dicrotendipes lobiger	Chironomidae	Chironomidae
Diptera	Dicrotendipes nervosus	Chironomidae	Chironomidae
Diptera	Endochironomus albipennis	Chironomidae	Chironomidae
Diptera	Glyptotendipes pallens	Chironomidae	Chironomidae
Diptera	Glyptotendipes paripes	Chironomidae	Chironomidae
Diptera	Glyptotendipesspecies	Chironomidae	Chironomidae
Diptera	Harnischia species	Chironomidae	Chironomidae
Diptera	Kiefferulus tendipediformis	Chironomidae	Chironomidae
Diptera	Microchironomus tener	Chironomidae	Chironomidae
Diptera	Microtendipes chloris group	Chironomidae	Chironomidae
Diptera	Parachironomus arcuatus group	Chironomidae	Chironomidae
Diptera	Parachironomus longiforceps	Chironomidae	Chironomidae
Diptera	Parachironomus species Kampen	Chironomidae	Chironomidae
Diptera	Paratendipes albimanus	Chironomidae	Chironomidae
Diptera	Phaenopsectra species	Chironomidae	Chironomidae
Diptera	Polypedilum convictum	Chironomidae	Chironomidae
Diptera	Polypedilum cultellatum	Chironomidae	Chironomidae
Diptera	Polypedilum nubeculosum	Chironomidae	Chironomidae
Diptera	Polypedilum scalaenum	Chironomidae	Chironomidae
Diptera	Polypedilum sordens	Chironomidae	Chironomidae
Diptera	Polypedilum species	Chironomidae	Chironomidae
Diptera	Xenochironomus xenolabis	Chironomidae	Chironomidae

Order	Taxa	Identification level IGBA	Indicator taxa IGBA
Diptera	Cladotanytarsus mancus	Chironomidae	Chironomidae
Diptera	Cladotanytarsus mancus group	Chironomidae	Chironomidae
Diptera	Cladotanytarsus species	Chironomidae	Chironomidae
Diptera	Micropsectra atrofasciata	Chironomidae	Chironomidae
Diptera	Paratanytarsus dissimilis aggregate	Chironomidae	Chironomidae
Diptera	Paratanytarsus dissimilis	Chironomidae	Chironomidae
Diptera	Rheotanytarsus species	Chironomidae	Chironomidae
Diptera	Tanytarsus group lestagei/medius	Chironomidae	Chironomidae
Diptera	Tanytarsus species	Chironomidae	Chironomidae
Diptera	Limoniidae species	Limoniidae	
Diptera	Muscidae species	Muscidae	
Diptera	Psychodidae species	Psychodidae	
Diptera	Sciomyzidae species	Sciomyzidae	
Diptera	Tipulidae species	Tipulidae	

Annex 4. Results identification macroinvertebrates

An overview is given of the results of the macroinvertebrates identifications from the samples taken during the June 2006 campaign:

Annex	Location	rk[1]	Sampling date	
4.1	Pîtres	203	June 19, 2006	
4.2	le Manoir	204	June 19, 2006	
4.3	Île du Motillon	205	June 19, 2006	
4.4	Île de Freneuse	215.1	June 20, 2006	
4.5	Caudebec	216.5	June 20, 2006	(mouth river Eure)
4.6	Orival	221-223	June 19, 2006	
4.7	Bédanne	227	June 19 & 20, 2006	
4.8	Oissel	229-230.8	June 20, 2006	
4.9	Petit Quevilly	247.7	June 16, 2006	
4.10	Grand Quevilly	250	June 16, 2006	
4.11	la Bouille	258.3-260	June 16 & 18, 2006	
4.12	Duclair	278	June 17, 2006	
4.13	Yville sur Seine	288	June 17, 2006	
4.14	le Landin	292 & 294	June 17, 2006	
4.15	Côte de Caveaumont	302	June 17, 2006	
4.16	la Vaquerie	319.5-322	June 17 & 18, 2006	
4.17	Vieux Port	324	June 17, 2006	
4.18		artificial substrates		

[1] river kilometre

Annex 4.1. Pîtres

Location:	Seine, vicinity of Pîtres
River kilometre:	203.0-203.3
Sampling date:	June 19, 2006

		Sample code:					
		2	7	17	10	11	9
Sampling device:	Van Veen grab				●	●	●
	Triangular dredge	●	●	●			
Sampling surface:	(dm^2, - = unknown)	-	-	-	8.4	8.4	8.4
Cross section:	main channel right	●			●		
	middle		●			●	
	left			●			●
Substrate:	pebbles	●					●
	gravel	●	●	●	●	●	●
	sand				●		
	small woody debris	●					
	organic matter		●			●	

Co-ordinates sampling sites:

	Sample code: 2	7	17	10	11	9
X	370822	370891	371130	370544	370550	370559
Y	5463547	5463511	5463537	5464524	5464479	5464417

Results (number per taxon):

Taxa	Sample code: 2	7	17	10	11	9
Dugesia lugubris/polychroa	1		3			
Dugesia tigrina	2	5	3			
Hypania invalida			1	10		
Limnodrilus claparedeianus				1		
Limnodrilus hoffmeisteri		2		1		
Potamothrix moldaviensis				2		
Psammoryctides barbatus		2	9	11		
Stylaria lacustris	1	1			1	
Tubificidae with hairs juvenile		2	1			
Tubificidae without hairs juvenile		3	4	19	1	
Erpobdella octoculata	1	4	13	3		2
Erpobdellidae species juvenile	7	29	6	20	3	1
Glossiphonia complanata	13	132	13	11	1	5
Glossiphonia concolor		16				
Glossiphonia heteroclita					1	

Taxa	Sample code:					
	2	7	17	10	11	9
Helobdella stagnalis			4			
Acroloxus lacustris		1				
Ancylus fluviatilis		1				
Bithynia tentaculata		3		1		
Corbicula fluminalis				2		
Sphaerium corneum				1		
Sphaerium rivicola				1		
Viviparus viviparus	1					
Asellus aquaticus	2	17	12	7		2
Proasellus meridianus	13	21				4
Echinogammarus berilloni	5			2		
Gammaridae species juvenile	6					
Gammarus species juvenile				5		
Gammarus salinus		1				
Ephemerella ignita	5				1	
Heptagenia sulphurea	1					
Leuctra fusca	1					
Elmis species larvae	1					
Hydropsyche contubernalis	432	178	56	25	21	9
Lepidostoma hirtum	7					
Neureclipsis bimaculata	249	120	18	22	11	5
Prodiamesa olivacea				1		
Cricotopus bicinctus	6	6				
Cricotopus bicinctus pupae					1	
Cricotopus intersectus	3	3	1		1	
Cricotopus sylvestris			1			
Nanocladius bicolor agg.	3	3				
Rheocricotopus chalybeatus	3					
Tvetenia calvescens	3					
Chironomus nudiventris				1	1	
Dicrotendipes nervosus	193	233	96	66	9	44
Dicrotendipes nervosus pupae	6	22	9	1	1	4
Glyptotendipes pallens	6	41	32	43	1	9
Glyptotendipes paripes				3		
Glyptotendipes species juvenile		3				
Microtendipes chloris gr	3					
Parachironomus arcuatus gr.		28	7	3	1	1
Parachironomus longiforceps						7
Paratendipes albimanus				1		
Phaenopsectra			1			
Polypedilum cultellatum	54					
Polypedilum nubeculosum				1		
Polypedilum nubeculosum pupae				1		
Polypedilum scalaenum		13	3	3	1	1
Polypedilum scalaenumpupae		9				
Polypedilum sordens	3					
Polypedilum species pupae	6					
Cladotanytarsus mancus gr.		3	3		1	1
Micropsectra atrofasciata		3				
Paratanytarsus dissimilis agg	3				1	
Number of specimens	1041	906	297	269	58	95
number of taxa	30	28	21	28	17	13

Annex 4.2. Ie Manoir

Location:	Seine, vicinity of Ie Manoir
River kilometre:	204
Sampling date:	June 19, 2006

		Sample code: 1	73	74
Sampling device:	Handnet	•		•
	Manual		•	
Sampling surface:	(dm^2, - = unknown)	150	66	150
Cross section:	left bank	•	•	•
	subtidal zone	•	•	
	intertidal zone			•
Substrate:	sand	•		•
	small woody debris		•	

Co-ordinates sampling sites:

	Sample code: 1	73	74
X	369760	369760	369760
Y	5463442	5463442	5463442

Results (number per taxon):

Taxa	Sample code: 1	73	74
Dugesia tigrina		2	
Hypania invalida	1	1	4
Aulodrilus pluriseta			1
Enchytraeidae			1
Limnodrilus claparedeianus	2		
Limnodrilus hoffmeisteri	2		
Nais ellinguis			1
Tubificidae with hairs juvenile	1		2
Tubificidae without hairs juvenile	5	2	1
Erpobdella octoculata		1	
Erpobdellidae species juvenile	3	1	1
Glossiphonia complanata	3	1	
Glossiphonia concolor	1		
Bithynia tentaculata		1	
Galba truncatula			1
Potamopyrgus antipodarum		1	
Viviparus viviparus		1	
Asellus aquaticus	2	13	

Taxa	Sample code:		
	1	73	74
Proasellus meridianus		3	
Crangonyx pseudogracilis		1	
Dikerogammarus villosus		4	
Orchestia species juvenile		1	
Hydropsyche contubernalis	5	2	
Cricotopus bicinctus			5
Cricotopus intersectus		50	13
Cricotopus sylvestris		6	3
Limnophyes		12	
Limnophyes species pupae			2
Nanocladius bicolor agg.		6	
Chironomus	6		
Cryptochironomus supplicans	3		
Dicrotendipes nervosus	39	384	41
Dicrotendipes nervosus pupae	3	12	5
Glyptotendipes pallens		68	2
Glyptotendipes paripes pupae			2
Microtendipes chloris gr.	3		
Parachironomus arcuatus gr.	3		
Polypedilum nubeculosum	3		
Polypedilum scalaenum	244	25	64
Polypedilum scalaenumpupae	3		23
Cladotanytarsus mancus pupae			5
Cladotanytarsus mancus gr.	9		3
Paratanytarsus dissimilis pupae			2
Number of specimens	341	599	180
number of taxa	18	23	17

Annex 4.3. Île du Motillon

Location:	Seine, vicinity of Île du Motillon
River kilometre:	205
Sampling date:	June 19, 2006

		Sample code:										
		94	80	116	84	95	79	5	81	85	114	149
Sampling device:	Handnet (kick)				•			•	•			•
	Triangular dredge	•	•	•								
	Manual					•	•			•	•	
Sampling surface:	(dm^2, - = unknown)	-	-	-	60	35	35	150	30	35	35	150
Cross section:	main channel right	•										
	middle		•									
	left			•								
	right bank				•	•		•			•	
	left bank						•		•	•		•
	subtidal zone	•	•	•	•	•	•		•			
	intertidal zone							•		•	•	•
Substrate:	pebbles		•									
	gravel	•	•	•	•			•	•			•
	stones					•	•			•	•	

Co-ordinates sampling sites:

	Sample code:										
	94	80	116	84	95	79	5	81	85	114	149
X	368847	368893	368857	368731	368731	368898	368731	368898	368898	368731	368898
Y	5463384	5463341	5463296	5463381	5463381	5463301	5463381	5463301	5463301	5463381	5463301

Results (number per taxon):

Taxa	Sample code:										
	94	80	116	84	95	79	5	81	85	114	149
Dugesia lugubris/polychroa				1							
Dugesia tigrina	3		1	6		1					
Hypania invalida				31		1	1	1		1	
Limnodrilus hoffmeisteri							1				
Lumbricidae				3						1	
Lumbriculidae							1	1			
Psammoryctides barbatus				1							
Tubificidae with hairs juv.				1			1				
Tubificidae without hairs juv.			1	2		1	2	2	1	1	
Erpobdella octoculata	1	5		2		2		2			

Taxa	Sample code:										
	94	80	116	84	95	79	5	81	85	114	149
Erpobdella testacea							1				
Erpobdellidae species juv.	5	6	4	16					1		
Glossiphonia complanata	13	12	2	6	1			4			
Glossiphonia concolor		2						1			
Glossiphonia heteroclita	1	1									
Helobdella stagnalis				2		1					
Hemiclepsis marginata	1										
Trocheta riparia					1						
Acroloxus lacustris	1										
Ancylus fluviatilis		1									
Bithynia tentaculata				4	1					2	
Corbicula fluminalis				5		1					
Corbicula fluminea				3							
Dreissena polymorpha	3	5									
Physa fontinalis	2			1							
Pisidium species juv.				6							
Pisidium casertanum				1							
Pisidium casertanum plicatum				1							
Pisidium henslowanum				2							
Pisidium nitidum				15		1				1	
Pisidium pulchellum				1							
Pisidium subtruncatum				1							
Potamopyrgus antipodarum						1					
Radix species juv.					4						
Radix ovata		1		2						7	
Sphaerium corneum	1			4							
Viviparus viviparus		1									
Asellus aquaticus	9	4	12	23		2		2	1		
Proasellus meridianus	24	4	2	19				2			
Dikerogammarus species juv.										1	
Gammaridae species juv.				1							
Gammarus species juv.										1	
Gammarus salinus	4										
Caenis macrura				1							
Ephemerella ignita	1										
Micronecta minutissima								1			
Ecnomus tenellus					1						
Hydropsyche contubernalis	337	185	6	8		9	1	3	1		
Hydropsyche species juv.										1	
Neureclipsis bimaculata	200	55	7		1						
Potthastia longimanus					6						
Prodiamesa olivacea					6						
Cricotopus bicinctus		7		16		7	1			10	
Cricotopus bicinctus pupae				8							
Cricotopus intersectus					26	9	14		3	52	
Cricotopus sylvestris	8	1			13	2	6	3	2	10	
Limnophyes									22		
Nanocladius bicolor agg.		1						2			
Paratrichocladius rufiventris						2		2			
Rheocricotopus chalybeatus	16	4				2					
Rheocricotopus chalybeatus pupae	8			8							
Chironomus nudiventris								2			
Dicrotendipes lobiger									12		
Dicrotendipes nervosus	571	87	9	301	438	151	47	47		266	
Dicrotendipes nervosus pupae	33	1		16		7	6			31	

Taxa	Sample code:										
	94	80	116	84	95	79	5	81	85	114	149
Endochironomus albipennis		1									
Glyptotendipes pallens	24	24	3	16	90	5	8		3	16	
Glyptotendipes paripes				16		5	7	2		5	
Harnischia	8										
Microtendipes chloris gr.							1				
Parachironomus arcuatus gr.	8	6									
Parachironomus longiforceps	49	1									
Polypedilum convictum	8										
Polypedilum nubeculosum	8			8							
Polypedilum scalaenum	16	1		253	45	24	25	60	5	42	
Polypedilum scalaenumpupae				32			3	18			
Cladotanytarsus mancus pupae								5			
Cladotanytarsus mancus gr.				150		2		10			
Micropsectra atrofasciata							1				
Paratanytarsus dissimilis agg.	16										
Tipulidae										1	
Number of specimens	1381	418	47	993	634	237	126	168	51	450	0
number of taxa	28	23	10	38	13	20	16	19	10	17	0

Annex 4.4. Île de Freneuse

Location:	Seine, vicinity of Île de Freneuse
River kilometre:	215.1
Sampling date:	June 20, 2006

		Sample code: 58	64
Sampling device:	Handnet	●	●
Sampling surface:	(dm^2, - = unknown)	30	150
Cross section:	right bank	●	●
	intertidal zone	●	●
Substrate:	coarse sand		●
	mud	●	

Co-ordinates sampling sites:

	Sample code:	
X	359063	359050
Y	5463161	5463150

Results (number per taxon):

Taxa	Sample code: 58	64
Hypania invalida	1	
Limnodrilus claparedeianus	9	8
Limnodrilus hoffmeisteri	5	48
Peloscolex multisetosus	2	
Tubifex tubifex	2	
Tubificidae with hairs juvenile		8
Tubificidae without hairs juvenile	80	88
Neureclipsis bimaculata	1	
Procladius species	3	
Cricotopus intersectus	3	8
Rheocricotopus chalybeatus	3	
Chironomus acutiventris	14	
Dicrotendipes nervosus	8	8
Glyptotendipes paripes	3	8
Kiefferulus tendipediformis		8
Paratendipes albimanus	5	
Polypedilum scalaenum	73	24
Cladotanytarsus mancus pupae	3	8
Cladotanytarsus mancus gr.	38	16
Tanytarsus gr. lestagei/medius	5	
Number of specimens	258	232
number of taxa	17	11

Annex 4.5. Caudebec

Location:	mouth of the river Eure, vicinity of Caudebec
River kilometre:	216.5
Sampling date:	June 20, 2006

		Sample code:			
		33	35	63	100
Sampling device:	Eckman grab	•	•	•	
	Handnet				•
Sampling surface:	(dm^2, - = unknown)	11,25	11,25	11,25	150
Cross section:	main channel middle	•			
	left		•		
	right bank			•	•
	subtidal zone	•	•		
	intertidal zone			•	•
Substrate:	coarse sand	•			
	mud		•	•	
	vegetation				•

Co-ordinates sampling sites:

	Sample code:			
	33	35	63	100
X	357003	358007	357986	357986
Y	5462170	5462136	5462182	5462182

Results (number per taxon):

Taxa	Sample code:			
	33	35	63	100
Caryophyllaeus species		1		
Hypania invalida		8		
Branchiura sowerbyi	2	4		
Limnodrilus claparedeianus	3	11	64	
Limnodrilus hoffmeisteri	5	15	16	
Lumbricidae species		4		
Psammoryctides barbatus	2			
Tubifex ignotus	15	4		
Tubificidae with hairs juvenile	3	19		
Tubificidae without hairs juvenile	66	153	112	3
Helobdella stagnalis		1		
Bithynia tentaculata		1		
Corbicula fluminalis		1	1	
Corbicula fluminea	1	4	1	
Galba truncatula				29

Taxa	Sample code:			
	33	35	63	100
Pisidium species juvenile		1		
Pisidium amnicum		2	2	
Pisidium casertanum		4		
Pisidium casertanum plicatum		2		
Pisidium henslowanum		5		
Pisidium moitessierianum	1			
Pisidium nitidum		1		
Pisidium subtruncatum			1	
Pisidium supinum			2	
Potamopyrgus antipodarum		18	4	40
Radix species juvenile		1		
Valvata piscinalis			1	
Viviparus viviparus		2		
Caenis macrura	4	8		1
Haliplus fluviatilis			4	12
Limnius species larvae		1		
Oulimnius species larvae	1			
Hydropsyche contubernalis		1		
Ceratopogonidae	6		2	
Procladius species	3	1		
Limnophyes species	1			
Nanocladius bicolor agg.				1
Chironomus nudiventris	1			
Chironomus species juvenile	1			
Cladopelma laccophila gr.				1
Cryptochironomus species		1		
Cryptotendipes species	3			
Dicrotendipes nervosus	3			1
Harnischia species	20	7		
Paratendipes albimanus			1	
Polypedilum cultellatum		1		17
Polypedilum nubeculosum		1		
Polypedilum scalaenum	55	14	13	
Polypedilum scalaenum pupae			1	
Cladotanytarsus mancus gr.	28	2	1	
Rheotanytarsus species	1			
Muscidae species				1
Number of specimens	225	301	226	106
number of taxa	23	33	16	10

Annex 4.6. Orival

Location:	Seine, vicinity of Orival
River kilometre:	221-223
Sampling date:	June 19, 2006

		Sample code: 57	37	55	113	69	31	98	99	101	102	34	61	104
Sampling device:	Van Veen grab					•								
	Handnet						•							
	Handnet (kick)													•
	Triangular dredge	•	•	•	•									
	Manual							•	•	•	•	•	•	
Sampling surface:	(dm^2, - = unknown)	-	-	-	8	8	150	35	23	35	35	35	35	150
Cross section:	main channel right	•				•								
	middle		•											
	left			•	•									
	right bank						•	•	•					
	left bank									•	•	•	•	•
	subtidal zone	•	•	•	•	•	•	•	•	•	•			•
	intertidal zone											•	•	
Substrate:	pebbles		•	•										
	gravel	•	•	•										•
	sand		•	•		•	•							
	mud					•								
	stones							•		•	•	•	•	
	shell remnants	•												
	small woody debris			•	•				•					
	organic matter		•											

Co-ordinates sampling sites:

	Sample code: 57	37	55	113	69	31	98
X	355036	355009	354882	354882	355809	354383	354632
Y	5464199	5464322	5464252	5464252	5464790	5463434	5463764

	Sample code: 99	101	102	34	61	104
X	354383	354165	354385	354165	354165	354165
Y	5463434	5463385	5463825	5463385	5463385	5463385

Results (number per taxon):

Taxa	Sample code:												
	57	37	55	113	69	31	98	99	101	102	34	61	104
Dendrocoelum lacteum			1										
Dugesia lugubris/polychroa	1												
Dugesia tigrina			1										
Hypania invalida	17	1	96				18		3				8
Branchiura sowerbyi						1	1						
Enchytraeidae species												2	
Limnodrilus claparedeianus					10	2							
Limnodrilus hoffmeisteri						1							
Lumbricidae species									1				
Lumbriculidae species							1						
Ophidonais serpentina							4						
Potamothrix moldaviensis						2	1				1		
Psammoryctides barbatus	7	1			13	1	28						
Stylaria lacustris							1						
Tubificidae with hairs juvenile	13		160		3		6				1		1
Tubificidae without hairs juvenile	15		64		23	26	24	1			8		2
Cystobranchus respirans			1										
Erpobdella octoculata	136	3	3				3						
Erpobdellidae species juvenile	192	4	3						1			1	2
Glossiphonia complanata	130	12	10	1									1
Glossiphonia concolor	87	5	1				2						1
Glossiphonia heteroclita	56	1	2										1
Helobdella stagnalis													3
Hemiclepsis marginata			3										
Acroloxus lacustris	1												
Bithynia tentaculata	2		32						4	16			6
Corbicula fluminalis			3				3	1					
Corbicula fluminea	6		11				1	1			1		1
Dreissena polymorpha	2		1										
Galba truncatula											2	3	
Gyraulus albus									1				2
Lithoglyphus naticoides						1							
Physa fontinalis	1												
Pisidium species juvenile	1												3
Pisidium nitidum	3		1						1				1
Potamopyrgus antipodarum	1								37	1			7
Radix species juvenile							1			1	1	4	2
Radix ovata									76				
Sphaerium corneum	1			1						1			
Valvata piscinalis							1			1			13
Asellus aquaticus	246	2	77						1				
Proasellus meridianus	30	7	51	2						1			
Orconectes limosus	1	1											1
Gammarus salinus	62	31	38	10									
Caenis macrura	2		1										4
Ephemerella ignita	2												
Aphelocheirus aestivalis													1

Taxa	Sample code:												
	57	37	55	113	69	31	98	99	101	102	34	61	104
nymph													
Esolus species larvae							1						1
Ecnomus tenellus				1									
Hydropsyche contubernalis	8	2		5									
Hydropsyche species juvenile			3										
Hydroptila species	1												
Neureclipsis bimaculata	22	3	61	11									
Cricotopus bicinctus	12	7						5		9			
Cricotopus bicinctus pupae	12												
Cricotopus intersectus		2					15	5	9		142	52	
Cricotopus sylvestris								5	39	26	284	17	1
Limnophyes species							8	14		9	206	1477	
Nanocladius bicolor agg.		2											
Rheocricotopus chalybeatus	25	18	15	4			23	5	4				
Chironomus nudiventris			31		2								
Cryptochironomus supplicans						1							
Cryptochironomus species						1							
Dicrotendipes nervosus	370	127	399	145		5	453	172	216	299	28	206	17
Dicrotendipes nervosus pupae	12			2			8	5	9	18			
Glyptotendipes pallens	543	38	292	32		1	69	226	43	308		103	6
Glyptotendipes pallens pupae										9			
Glyptotendipes paripes	148	4	553		2		69	5	73	158		17	40
Glyptotendipes paripes pupae		2	15							26			
Harnischia species			15		7	2							
Parachironomus arcuatus gr.				2		1							
Parachironomus longiforceps		2		8									
Parachironomus longiforceps pupae		2											
Paratendipes albimanus			15		5	1							1
Polypedilum cultellatum											36		
Polypedilum nubeculosum					2						7		1
Polypedilum scalaenum	25		246	4	134	44	69	5	30		7	17	13
Polypedilum scalaenum pupae							8						1
Xenochironomus xenolabis	25	11	15	38									
Cladotanytarsus mancus gr.	12	9	15				31						2
Cladotanytarsus species juvenile												17	
Paratanytarsus dissimilis agg.							15						
Rheotanytarsus species	25			2									
Number of specimens	2256	297	2237	268	203	90	864	446	548	883	724	1916	143
number of taxa	37	23	33	17	10	15	25	12	16	12	13	12	28

Annex 4.7. Bédanne

Location:	Seine, vicinity of Bédanne
River kilometre:	227
Sampling date:	June 19 & 20, 2006 (sample 65 and 66 on June 20, 2006)

		Sample code:					
		65	66	3	131	4	89
Sampling device:	Van Veen grab	●	●				
	Triangular dredge			●	●	●	
	Manual						●
Sampling surface:	(dm^2, - = unknown)	8	8	-	-	-	15
Cross section:	main channel right		●	●			
	middle				●		
	left					●	
	left bank						●
	secondary channel	●					
	subtidal zone	●	●	●	●	●	●
Substrate:	solid bottom						
	pebbles						
	gravel			●	●	●	
	sand	●				●	
	mud	●	●[1]				
	small woody debris						●
	organic matter			●			

[1] contaminated with oil

Co-ordinates sampling sites:

	Sample code:					
	65	66	3	131	4	89
X	359530	359905	359240	359244	359267	359267
Y	5465647	5465792	5465898	5465868	5465801	5465801

Results (number per taxon):

Taxa	Sample code:					
	65	66	3	131	4	89
Dugesia lugubris/polychroa				4		
Dugesia tigrina				2		
Hypania invalida				1	9	
Branchiura sowerbyi	6					
Enchytraeidae species					1	

Taxa	Sample code:					
	65	66	3	131	4	89
Limnodrilus claparedeianus	44	23			1	
Limnodrilus hoffmeisteri	17				1	
Lumbricidae species					1	
Potamothrix moldaviensis	6				3	
Psammoryctides barbatus		85	1		16	
Tubificidae with hairs juvenile		5				
Tubificidae without hairs juvenile	177	89		2	9	
Erpobdella octoculata	1					
Erpobdellidae species juvenile				1	1	2
Glossiphonia complanata			4	16	9	
Bithynia tentaculata	1		2	353		3
Corbicula fluminalis	2		1	1		
Corbicula fluminea	26		1	1		
Dreissena polymorpha						1
Physa fontinalis			1			
Pisidium species juvenile	2			1		
Pisidium amnicum	1					
Pisidium casertanum	2					
Pisidium henslowanum	3					
Pisidium subtruncatum	5					
Pisidium supinum	3					
Potamopyrgus antipodarum	1					
Radix species juvenile			1	11		
Sphaerium corneum				5		
Sphaerium rivicola				1		
Sphaerium solidum	2		1			
Valvata piscinalis	1					
Asellus aquaticus			4	3	14	
Proasellus meridianus	1		4	20	56	2
Gammarus salinus			490	126	206	12
Ecnomus tenellus			3			
Hydropsyche contubernalis			100		8	80
Neureclipsis bimaculata			1	5	18	68
Nanocladius bicolor agg.				3	4	
Nanocladius bicolor				3		
Paratrichocladius rufiventris		2				
Rheocricotopus chalybeatus				2		
Chironomus nudiventris	8	4				
Chironomus species juvenile	11					
Chironomus species pupae					4	
Cladopelma laccophila gr.	6					
Cryptochironomus species	3					
Dicrotendipes nervosus		2	177	80	234	674
Dicrotendipes nervosus pupae			10	6	13	19
Glyptotendipes pallens			263	20	68	243
Glyptotendipes paripes		13	10	2	13	9
Harnischia species	19					
Microchironomus tener	11					
Parachironomus longiforceps			5	12	34	140
Parachironomus longiforceps pupae					9	
Parachironomus spec. Kampen			5		4	9
Paratendipes albimanus	6	2				
Polypedilum convictum						9
Polypedilum scalaenum	243	107		5	38	
Polypedilum scalaenum pupae	6			2		

Taxa	Sample code:					
	65	66	3	131	4	89
Xenochironomus xenolabis				2	17	
Cladotanytarsus mancus gr.	17	7		2	9	
Paratanytarsus dissimilis agg.				2		
Rheotanytarsus species				2	4	
Number of specimens	628	340	1088	693	809	1272
number of taxa	29	11	21	32	29	14

Annex 4.8. Oissel

Location:	Seine, vicinity of Oissel
River kilometre:	229-230.8
Sampling date:	June 20, 2006

		Sample code:									
		147	106	60	70	68	67	59	72	56	62
Sampling device:	Eckman grab			•	•	•					
	Van Veen grab	•	•								
	Handnet						•	•	•		
	Manual									•	•
Sampling surface:	(dm^2, - = unknown)	8	8	11.3	11.3	11.3	150	150	150	23	16
Cross section:	main channel middle		•		•						
	left	•									
	right bank			•		•	•	•	•	•	•
	subtidal zone	•	•		•						
	intertidal zone			•		•	•	•	•	•	•
Substrate:	sand	•									
	mud		•	•	•	•	•				
	vegetation							•	•		
	small woody debris									•	•

Co-ordinates sampling sites:

	Sample code:									
	147	106	60	70	68	67	59	72	56	62
X	363165	355160	361895	361744	361760	362358	362464	362358	361760	362361
Y	5466823	5466803	5466449	5466408	5466386	5466509	5466423	5466509	5466386	5466405

Results (number per taxon):

Taxa	Sample code:									
	147	106	60	70	68	67	59	72	56	62
Branchiura sowerbyi			4		4					
Enchytraeidae species						45	253	8		10
Limnodrilus claparedeianus	31	2	20	26	26	18		1	1	
Limnodrilus hoffmeisteri	21		4	10	26	161		1		
Limnodrilus udekemianus					4					
Lumbricidae species						9				
Lumbriculidae species							3			
Nais pardalis species								1		
Peloscolex multisetosus			8	3						
Potamothrix moldaviensis				3						

Taxa	Sample code:									
	147	106	60	70	68	67	59	72	56	62
Psammoryctides barbatus	10			5						
Tubificidae with hairs juvenile	14				22	9				
Tubificidae without hairs juvenile	237	2	132	148	243	500		9	16	3
Erpobdella octoculata							1	1		
Glossiphonia complanata	1	1								
Glossiphonia heteroclita							1			
Bithynia tentaculata							1	1	1	
Corbicula fluminea	18				1					
Galba truncatula						6	16	9	2	1
Pisidium amnicum			5		5					
Pisidium casertanum					1	1				
Pisidium henslowanum			2							
Pisidium nitidum			1							
Pisidium subtruncatum			1		1					
Pisidium supinum					1					
Potamopyrgus antipodarum						4	11			
Radix species juvenile							1			
Radix ovata						2				
Succineidae species								6		
Valvata piscinalis			3			6	1			
Gammaridae species juvenile		1								
Orchestia species juvenile										3
Haliplus fluviatilis						1				
Ceratopogonidae						3				
Procladius species			3	8	18				2	
Tanypus kraatzi									1	
Tanypus punctipennis			23	6	26					
Tanypus punctipennis pupae					4					
Bryophaenoicladius gr. muscicola									1	
Cricotopus bicinctus						1				
Cricotopus intersectus								2		3
Cricotopus sylvestris						2	3		2	3
Limnophyes species	1		3			42	308	4	6	112
Limnophyes species pupae							10	1		
Rheocricotopus chalybeatus								1		
Chironomus acutiventris					4					
Chironomus bernensis					4					
Chironomus nudiventris				6						
Chironomus plumosus agg.			6							
Chironomus species juvenile			29	27	7				1	
Chironomus species pupae					4					
Cladopelma laccophila gr.			6	2	7	1				
Cryptochironomus defectus	2									
Cryptochironomus supplicans			6	2	11					
Cryptochironomus species					4					
Dicrotendipes nervosus	1					2		1	1	26
Dicrotendipes nervosus pupae							7			
Glyptotendipes pallens						5	10			26
Glyptotendipes paripes	2					2				
Glyptotendipes species juvenile			3						2	
Harnischia species			3	8	7					
Microchironomus tener					4					
Paratendipes albimanus					7	1				
Polypedilum nubeculosum			6	4	4					
Polypedilum scalaenum	86	2	32	17	7				3	

Taxa	Sample code:									
	147	106	60	70	68	67	59	72	56	62
Polypedilum scalaenum pupae	4		3							
Cladotanytarsus mancus gr.	2		9	2		5				
Limoniidae species						3		1		
Number of specimens	430	8	311	276	452	827	625	48	39	189
number of taxa	13	5	22	16	26	23	13	16	13	9

Annex 4.9. Petit Queville

Location:	Seine, vicinity of Petit Queville
River kilometre:	247.7
Sampling date:	June 16, 2006

		Sample code:		
		39	51	22
Sampling device:	Hamon grab	•	•	•
Sampling surface:	(dm^2, - = unknown)	25	25	25
Cross section:	main channel right	•		
	middle		•	
	left			•
Substrate:	gravel	•	•	
	sand	•	•	•
	mud			•

Co-ordinates sampling sites:

	Sample code:		
	39	51	22
X	356595	356660	356724
Y	5476592	5476588	5476515

Results (number per taxon):

Taxa	Sample code:		
	39	51	22
Dugesia lugubris/polychroa	1		
Dugesia tigrina	1		
Hypania invalida		8	
Branchiura sowerbyi	83	20	
Limnodrilus claparedeianus	165	60	1
Limnodrilus hoffmeisteri		201	1
Lumbriculidae species		181	
Potamothrix moldaviensis	83		
Psammoryctides barbatus	3469	181	
Tubificidae with hairs juvenile	743	281	11
Tubificidae without hairs juvenile	1900	763	5
Erpobdella octoculata	1	3	1
Erpobdella testacea		1	
Erpobdellidae species juvenile	19	8	
Glossiphonia complanata	20	9	
Glossiphonia concolor	10	1	
Glossiphonia heteroclita	1		

Taxa	Sample code:		
	39	51	22
Helobdella stagnalis	8		
Bithynia tentaculata	400	1	
Corbicula fluminea	600	41	
Dreissena polymorpha	1		
Pisidium casertanum		1	
Sphaerium corneum		10	
Sphaerium solidum	2510		
Viviparus viviparus	2	7	
Asellus aquaticus	19	4	
Proasellus coxalis	24		
Proasellus meridianus		11	
Gammarus salinus	3	17	2
Esolus species larvae		1	
Cryptochironomus species		1	
Dicrotendipes nervosus	40	41	1
Dicrotendipes nervosus pupae		2	
Glyptotendipes pallens	1	4	
Glyptotendipes paripes	2	8	
Glyptotendipes species juvenile		5	
Cladotanytarsus mancus gr.		1	
Psychodidae species		1	
Number of specimens	10107	1885	22
number of taxa	26	31	7

Annex 4.10. Grand Queville

Location:	Seine, vicinity of Grand Queville
River kilometre:	250
Sampling date:	June 16, 2006

		Sample code:												
		52	121	53	19	12	41	21	38	49	16	47	86	122
Sampling device:	Hamon grab	•	•	•	•	•	•							
	Van Veen grab													
	Handnet									•	•	•	•	
	Triangular dredge													
	Manual							•	•					•
Sampling surface:	(dm^2, - = unknown)	25	25	25	25	25	10	35	35	150	150	30	30	0.8
Cross section:	main channel right	•	•											
	middle			•										
	left				•	•	•							
	right bank							•	•	•				
	left bank										•	•	•	•
	subtidal zone	•	•	•	•	•	•							•
	intertidal zone							•	•	•	•	•	•	
Substrate:	cobbles		•											
	pebbles	•			•									
	gravel	•		•	•					•	•			
	sand	•		•		•				•	•			
	mud	•										•	•	
	stones							•	•					
	small woody debris						•							
	synthetic cable													•

Co-ordinates sampling sites:

	Sample code:						
	52	121	53	19	12	41	21
X	355747	355747	355808	355932	355865	355865	355715
Y	5474205	5474205	5474245	5474502	5474267	5474267	5474236

	Sample code:					
	38	49	16	47	86	122
X	355714	355714	355931	355985	355986	355916
Y	5474238	5474238	5474503	5474634	5474634	5474504

Results (number per taxon):

Taxa	Sample code:												
	52	121	53	19	12	41	21	38	49	16	47	86	122
Caryophyllaeus species				1									
Dendrocoelum lacteum				1						1			
Dugesia lugubris/polychroa		1		2				1					
Dugesia tigrina	1	1		1				1					
Hypania invalida			5	64		2	19	37	34	4			
Branchiura sowerbyi			13		15						14	3	
Chaetogaster diaphanus										1			
Eiseniella tetraedra				3									
Enchytraeidae species												3	
Limnodrilus claparedeianus			13	4		3	1		12	6	172	5	
Limnodrilus hoffmeisteri				1	10	1		2			37		
Lumbriculidae species	21			4	26	2	1	2	6	1			6
Ophidonais serpentina								2		1			
Potamothrix moldaviensis					41	5				2			
Psammoryctides barbatus	1660	5	850	28	98	10	6	36	102	5	5	3	7
Stylaria lacustris				1								3	
Tubifex ignotus										1			
Tubificidae with hairs juvenile	42		53	5	41	10			18	14	46	108	1
Tubificidae without hairs juvenile	84		66	49	150	37	28	15	306	34	144	97	2
Erpobdella octoculata	1		2	67	1	1		1		5			1
Erpobdella testacea	1		1										
Erpobdellidae species juvenile	7	12	6	55			88	78	17	5			2
Glossiphonia complanata	6	5	2	28	1		4	6	3	3			
Glossiphonia concolor	3	1	3	10	1	1	1	1					
Glossiphonia heteroclita				2									
Helobdella stagnalis		1		12				1		4	1		
Hemiclepsis marginata								1					
Piscicola geometra				2									
Bithynia tentaculata	306	68	4					7	45	1			
Corbicula fluminea	99	1	320	10			1	1	7	1			
Dreissena polymorpha	34	3	1	2			5	42	63				1
Pisidium subtruncatum										1			
Radix species juvenile	2								227	23			
Radix ovata				71			374	130					
Sphaerium corneum	2			2				5	27	1			
Valvata cristata		1											
Viviparus viviparus	1	1											
Asellus aquaticus	16	8	1	118	1	5	567	1183	214	18			38
Proasellus meridianus	9	5	1	24					26	7			6
Orconectes limosus								1		1			
Gammarus salinus	281	102	230	47	27	3				20	1		

Taxa	Sample code:												
	52	121	53	19	12	41	21	38	49	16	47	86	122
Caenis macrura				2					1				
Orthetrum species juvenile				1									
Esolus species larvae											1		
Ceratopogonidae species pupae												1	
Procladius species				5					3				
Cricotopus bicinctus				5						1			
Cricotopus intersectus							1	1					
Cricotopus sylvestris							4	4				2	
Limnophyes species								1				2	
Nanocladius bicolor agg.			2					3		2			
Nanocladius bicolor						1							
Rheocricotopus chalybeatus					1								
Thalassosmittia thalassophila							5			1			
Chironomus species juvenile											1		
Cryptochironomus supplicans										3			
Dicrotendipes nervosus	14	10	47	302	41	49	82	82	93	39	1	4	648
Dicrotendipes nervosus pupae		1		16		1				5			
Glyptotendipes pallens		2		21		3		12		2			35
Glyptotendipes paripes			2		1	3						1	21
Glyptotendipes species juvenile	4		7	16	1				8	7	1		
Parachironomus longiforceps							7						
Parachironomus longiforceps pupae							4						
Parachironomus spec. Kampen								1					
Paratendipes albimanus			2				1						
Polypedilum nubeculosum					2				3				
Polypedilum scalaenum	3		175	233	67	1			15	49	1	18	14
Polypedilum scalaenum pupae				5		1						1	
Xenochironomus xenolabis	4	4											
Cladotanytarsus mancus gr.			2							6		1	
Number of specimens	2603	237	1809	1226	526	139	1198	1662	1228	274	425	250	782
number of taxa	24	19	24	36	18	17	18	29	21	34	13	15	13

Annex 4.11. la Bouille

Location:	Seine, vicinity of la Bouille
River kilometre:	258.3-260
Sampling date:	June 16 & 18, 2006 (sample 13 and 20 on June 16)

		Sample code[1]:												
		20	13	25	91	46	23	126	28	110	26	140	108	129
Sampling device:	Hamon grab	•	•	•	•	•								
	Handnet (kick)													•
	Handnet								•	•			•	
	Triangular dredge						•	•						
	Manual										•	•		
Sampling surface:	(dm^2, - = unknown)	25	25	25	25	25	-	-	150	150	35	35	150	150
Cross section:	main channel right	•			•		•							
	middle		•			•		•						
	left			•										
	right bank								•	•				
	left bank										•	•	•	•
	subtidal zone	•	•	•	•	•	•	•				•		
	intertidal zone								•	•	•		•	•
Substrate:	gravel	•	•			•	•						•	•
	sand	•	•	•	•	•		•					•	•
	mud	•			•									
	clay			•										
	stones										•	•		
	shell remnants			•										
	steel dam								•	•				

[1] sample 123 was left out of consideration (Hamon grab sample taken on a solid bottom) because few animals was present in the sample

Co-ordinates sampling sites:

	Sample code:						
	20	13	25	91	46	23	126
X	351440	351448	349597	349746	349633	349471	349381
Y	5468621	5468524	5468895	5468954	5468964	5469317	5469442

	Sample code:					
	28	110	26	140	108	129
X	349471	349471	349110	349110	349110	349110
Y	5469317	5469317	5469486	5469486	5469484	5469484

Results (number per taxon):

Taxa	Sample code:												
	20	13	25	91	46	23	126	28	110	26	140	108	129
Caryophyllaeus species		3				2	1						
Dugesia lugubris/polychroa											1		
Dugesia tigrina					1				1		1		
Hypania invalida	16		3		5						1	9	1
Branchiura sowerbyi					1	2	10						
Enchytraeidae species										8			1
Haplotaxis gordioides	70												
Limnodrilus claparedeianus						2	91	1					
Limnodrilus hoffmeisteri			6										
Lumbricidae species					3								
Lumbriculidae species	70	2				16		1					
Ophidonais serpentina						2							
Potamothrix moldaviensis		1				2							
Psammoryctides barbatus	4524	21	9		88	43	51					1	
Stylaria lacustris									2				
Tubifex ignotus		1											
Tubificidae with hairs juvenile		7	3		4	8							
Tubificidae without hairs juvenile	557	19		1	4	47	172	3		3		3	2
Erpobdella octoculata	32	3			5	3							1
Erpobdella testacea	4											1	
Erpobdellidae species juvenile	72	1			6	26	2	1	2		9	7	4
Glossiphonia complanata	16	3			10	5				1	2	2	1
Glossiphonia concolor	16	1			1	7	1					6	2
Helobdella stagnalis		1			1	4					1	1	1
Hemiclepsis marginata					1								
Trocheta riparia												2	
Bithynia tentaculata			48		14						13	5	1
Corbicula fluminea	464				260	23	3						
Dreissena polymorpha						1		13	12		2		
Physa fontinalis	4												
Pisidium species juvenile											1	28	
Pisidium nitidum											1		
Potamopyrgus antipodarum											1		
Radix species juvenile			6					1		3			
Radix ovata									3		35	69	30
Radix peregra						1							
Sphaerium corneum			3		1						15	57	10
Valvata piscinalis											2	1	
Asellus aquaticus	36	9	9		7	33	1	4	46		3	11	16
Proasellus meridianus	24	5	3		22	3			1		2	4	
Gammaridae species							15						

Taxa	Sample code:												
	20	13	25	91	46	23	126	28	110	26	140	108	129
juvenile													
Gammarus species juvenile											1		
Gammarus salinus	2432	26	192	7	17	244		2	24	3	428	349	119
Coenagrionidae species juvenile													1
Sigara striata									1				
Esolus species larvae						2							
Limnius species larvae						1							
Clinotanypus nervosus		3											
Cricotopus bicinctus									1	2	26	3	3
Cricotopus intersectus										5	13	1	1
Cricotopus intersectus pupae													1
Cricotopus sylvestris					1					3	18	2	4
Limnophyes species										2			1
Nanocladius bicolor agg.											3		
Rheocricotopus chalybeatus			10			1						1	1
Dicrotendipes nervosus	156	163	374		54	62	4	68	8	83	156	29	59
Dicrotendipes nervosus pupae	4	5				5		5		3	8		
Glyptotendipes pallens		5	15		1	2		3		1	5	5	3
Glyptotendipes paripes	4	3	5		7				2		3	1	
Parachironomus longiforceps						2							
Parachironomus longiforceps pupae						1							
Paratendipes albimanus					1								
Polypedilum nubeculosum					1								2
Polypedilum scalaenum	160	90	74		83	2				2	8	2	2
Polypedilum scalaenum pupae					1							1	
Xenochironomus xenolabis						1							
Cladotanytarsus mancus gr.			34							1		1	
Micropsectra atrofasciata												1	1
Tanytarsus species										1			
Number of specimens	8664	372	794	8	602	551	351	102	103	121	758	603	268
number of taxa	19	20	16	2	26	28	11	10	12	14	26	27	25

Annex 4.12. Duclair

Location:	Seine, vicinity of Duclair
River kilometre:	278
Sampling date:	June 17, 2006

		Sample code:		
		15	115	142
Sampling device:	Hamon grab	●	●	●
Sampling surface:	(dm^2, - = unknown)	25	25	25
Cross section:	main channel right	●		
	middle		●	
	left			●
	subtidal zone	●	●	●
Substrate:	solid bottom	●[1]		
	sand			●
	mud		●	
	clay		●	
	shell remnants			●

[1] including part of a steel cable

Co-ordinates sampling sites:

	Sample code:		
	15	115	142
X	345979	345977	346002
Y	5482981	5482907	5482825

Results (number per taxon):

Taxa	Sample code:		
	15	115	142
Dendrocoelum lacteum	1		
Dugesia lugubris/polychroa	1	1	
Dugesia tigrina	2		
Hypania invalida	21		
Branchiura sowerbyi			1
Enchytraeidae species			1
Lumbriculidae species	1		
Psammoryctides barbatus		1	1
Glossiphonia complanata	18	2	2
Glossiphonia concolor	27		3
Radix species juvenile	1		
Cricotopus sylvestris		1	
Dicrotendipes nervosus	42		

Taxa	Sample code:		
	15	115	142
Glyptotendipes pallens	1		
Parachironomus longiforceps	2	1	
Parachironomus spec. Kampen	2		
Polypedilum scalaenum			3
Number of specimens	250	26	25
number of taxa	21	8	9

Annex 4.13. Yville sur Seine

Location:	Seine, vicinity of Yville sur Seine
River kilometre:	288
Sampling date:	June 17, 2006

		Sample code:									
		143	133	127	107	117	112	128	124	24	118
Sampling device:	Hamon grab	•	•	•							
	Handnet (kick)				•		•				
	Handnet					•		•			
	Manual								•	•	•
Sampling surface:	(dm^2, - = unknown)	25	25	25	150	150	150	150	35	35	35
Cross section:	main channel right	•									
	middle		•								
	left			•							
	right bank				•	•		•		•	
	left bank						•		•		•
	subtidal zone	•	•	•	•	•	•		•		
	intertidal zone							•		•	•
Substrate:	solid bottom	•		•							•
	pebbles										
	gravel				•	•	•				
	sand		•				•				
	mud							•			
	clay		•								
	stones								•	•	•

Co-ordinates sampling sites:

	Sample code:									
	143	133	127	107	117	112	128	124	24	118
X	344681	344692	344698	344534	344700	344534	344700	344534	344700	344534
Y	5473518	5473692	5472542	5473354	5473694	5473354	5473694	5473354	5473694	5473354

Results (number per taxon):

Taxa	Sample code:									
	143	133	127	107	117	112	128	124	24	118
Dugesia lugubris/polychroa		1								
Dugesia tigrina		1								
Branchiura sowerbyi							1			
Enchytraeidae species							3			
Haplotaxis gordioides						1				
Limnodrilus claparedeianus		1								
Limnodrilus hoffmeisteri							1			
Lumbricidae species									2	
Ophidonais serpentina								1		
Potamothrix moldaviensis		2								
Psammoryctides barbatus			1				5			
Stylaria lacustris		1								
Tubificidae with hairs juvenile							15	1		
Tubificidae without hairs juvenile		2		2		1	17		6	
Glossiphonia complanata		12		1				1		
Bithynia tentaculata		5	4	4				37		
Corbicula fluminea		1								
Dreissena polymorpha	5									
Pisidium nitidum		1								
Radix species juvenile								1		
Asellus aquaticus		2								
Gammaridae species juvenile		1					1	7		
Gammarus salinus	42		36	8		456				
Cricotopus intersectus								1		
Limnophyes species									1	3
Pseudosmittia species										1
Thalassosmittia thalassophila									8	1
Dicrotendipes nervosus	2	4	1	1				2	1	
Polypedilum scalaenum				2			1			
Muscidae species				1						
Number of specimens	49	34	42	19	0	458	44	51	18	5
number of taxa	3	13	4	7	0	3	8	8	5	3

Annex 4.14. le Landin

Location:	Seine, vicinity of le Landin
River kilometre:	292 and 294 (at 292 sample 136)
Sampling date:	June 17, 2006

		Sample code:			
		119	136	138	130
Sampling device:	Hamon grab	•	•	•	•
Sampling surface:	(dm^2, - = unknown)	25	25	25	25
Cross section:	main channel right	•			
	middle		•	•	
	left				•
Substrate:	solid bottom		•		
	pebbles				
	gravel			•	•
	sand			•	•
	mud	•			
	clay	•			

Co-ordinates sampling sites:

	Sample code:			
	119	136	138	130
X	340914	341328	340862	340792
Y	5476273	5474488	5476251	5476256

Results (number per taxon):

Taxa	Sample code: 119	136	138	130
Branchiura sowerbyi			1	
Haplotaxis gordioides			1	
Limnodrilus claparedeianus				2
Potamothrix moldaviensis				19
Psammoryctides barbatus	1		4	38
Tubificidae with hairs juvenile				17
Tubificidae without hairs juvenile	1		1	71
Glossiphonia complanata		1	1	4
Bithynia tentaculata		2		6
Gammarus salinus	2	422		200
Dicrotendipes nervosus				3
Polypedilum scalaenum	1			1
Number of specimens	5	425	8	360
number of taxa	4	3	5	10

Annex 4.15. Côte de Caveaumont

Location:	Seine, vicinity of Côte de Caveaumont
River kilometre:	302
Sampling date:	June 17, 2006

		Sample code:										
		50	93	48	27	42	43	77	54	29	90	87
Sampling device:	Hamon grab	•	•	•								
	Handnet				•	•	•	•	•			
	Manual									•	•	•
Sampling surface:	(dm^2, - = unknown)	25	25	25	15	75	30	30	15	35	35	35
Cross section:	main channel right	•										
	middle		•									
	left			•								
	right bank				•	•	•	•	•	•	•	
	left bank											•
	subtidal zone	•	•	•	•				•			•
	intertidal zone					•	•	•		•	•	
Substrate:	cobbles	•										
	sand		•									
	mud		•	•	•	•	•	•	•			
	clay	•										
	stones									•	•	•

Co-ordinates sampling sites:

	Sample code:										
	50	93	48	27	42	43	77	54	29	90	87
X	339732	329888	339458	339275	339275	339275	339275	339274	339275	339274	339130
Y	5480164	548020	5492982	5483424	5483424	5483424	5483424	5483426	5483424	5483424	5483093

Results (number per taxon):

Taxa	Sample code:										
	50	93	48	27	42	43	77	54	29	90	87
Branchiura sowerbyi			1	5					1		
Limnodrilus claparedeianus	2		32	6		228		3	9	1	
Limnodrilus hoffmeisteri				6	1	186					
Lumbriculidae species						8					
Nais ellinguis species					1				1		
Psammoryctides barbatus	2		2					4	1		
Tubificidae with hairs juvenile		3	8	1	1			4	1		

Taxa	Sample code:										
	50	93	48	27	42	43	77	54	29	90	87
Tubificidae without hairs juvenile	2		23	55		321	13	32	26	8	
Bithynia tentaculata	1	3									1
Corbicula fluminea	3										
Dreissena polymorpha											526
Radix species juvenile									3		2
Gammaridae species juvenile					11						
Gammarus salinus		5	4	4		5		15		190	7020
Esolus species larvae				1							
Procladius species								1			
Limnophyes species					2				1		
Dicrotendipes nervosus		1	1		2				5	25	29
Dicrotendipes nervosus pupae										1	
Glyptotendipes species juvenile											1
Parachironomus longiforceps										1	
Polypedilum scalaenum			1		1			1			2
Cladotanytarsus mancus gr.					1						
Number of specimens	10	12	72	78	20	749	13	60	48	226	7581
number of taxa	5	4	8	7	8	5	1	7	9	5	7

Annex 4.16. la Vaquerie

Location:	Seine, vicinity of la Vaquerie
River kilometre:	319.5-322
Sampling date:	June 17 & 18, 2006 (samples 92 and 134 on June 17)

		Sample code:							
		146	105	132	120	145	144	92	134
Sampling device:	Hamon grab	•	•	•	•	•	•		
	Handnet							•	
	Manual								•
Sampling surface:	(dm^2, - = unknown)	25	25	25	25	25	25	150	35
Cross section:	main channel right	•							
	middle		•		•	•			
	left			•			•		
	right bank								
	left bank							•	•
	subtidal zone	•	•	•	•	•	•		
	intertidal zone							•	•
Substrate:	solid bottom		•	•			•		
	pebbles								
	gravel				•	•		•	
	sand	•	•			•			
	mud								
	stones								•

Co-ordinates sampling sites:

	Sample code:							
	146	105	132	120	145	144	92	134
X	329,692	330622	330728	329711	329,711	329781	328832	328832
Y	5480084	5480946	5480880	5479974	5479974	5479920	5479108	5479108

Results (number per taxon):

Taxa	Sample code:							
	146	105	132	120	145	144	92	134
Haplotaxis gordioides					1			
Peloscolex velutinus							4	
Stylodrilus heringianus							1	
Tubificidae without hairs juvenile							1	
Glossiphonia complanata	1			1				
Dreissena polymorpha								1
Gammarus salinus	11	8	21	21	34	5	975	8
Number of specimens	12	8	21	22	35	5	981	9
number of taxa	2	1	1	2	2	1	4	2

Annex 4.17. Vieux Port

Location:	Seine, vicinity of Vieux Port
River kilometre:	324
Sampling date:	June 17, 2006

		Sample code:								
		125	141	135	8	82	30	83	103	76
Sampling device:	Hamon grab	•	•	•						
	Handnet					•	•	•	•	
	Manual				•					•
Sampling surface:	(dm^2, - = unknown)	25	25	25	35	90	30	30	30	35
Cross section:	main channel right	•	•							
	left			•						
	right bank					•	•	•	•	•
	left bank				•					
	subtidal zone	•	•	•	•	•				
	intertidal zone						•	•	•	•
Substrate:	solid bottom	•	•	•						
	pebbles				•					
	gravel	•		•						
	mud					•	•	•	•	
	stones									•

Co-ordinates sampling sites:

	Sample code:								
	125	141	135	8	82	30	83	103	76
X	327176	327233	327187	327920	327920	327920	327920	327920	327920
Y	5478100	5478201	5477964	5478750	5478749	5478750	5478749	5478749	5478750

Results (number per taxon):

Taxa	Sample code:								
	125	141	135	8	82	30	83	103	76
Limnodrilus claparedeianus								1	
Limnodrilus hoffmeisteri					1	2		6	
Tubificidae with hairs juvenile								1	
Tubificidae without hairs juvenile	1				8	1	9	19	1
Bithynia tentaculata			8						
Dreissena polymorpha				28					
Radix ovata				1					
Proasellus meridianus					1				
Gammarus salinus	5	4	62	1					
Thalassosmittia thalassophila				1					294
Dicrotendipes nervosus				2				1	9
Dicrotendipes nervosus pupae				1					
Polypedilum scalaenum					1				
Number of specimens	6	4	70	34	11	3	9	28	303
number of taxa	2	1	2	5	4	2	1	5	3

Annex 4.18.

Location:	Seine, artificial substrates[1]
River kilometre:	207-306

Sample code	109	111	148	139	45	137	14	88
River kilometre	207,6	207,6	230,4	265,6	278,1	278,1	305,9	305,9
Suspension day	17-05	17-05	17-05	16-05	16-05	16-05	16-05	16-05
Retrieval day	19-06	19-06	20-06	18-06	18-06	18-06	17-06	17-06
Number of days	33	33	34	33	33	33	32	32
Sampling surface[2]	0.48	0.48	0.48	0.48	0.48	0.48	0.48	0.48

[1] Nettings with each three broken bricks (each brick 21 x 10 x 6.5 cm) in it. Total weight of each netting 7 kg (range 6.5 to 7.5 kg) (individual weights: 2 x 6.5 kg; 9 x 7.0 kg and 1 x 7.5 kg).
[2] estimated

Co-ordinates sampling sites:

	Sample code:							
	109	111	148	139	45	137	14	88
X	366285	366285	362703	349205	345904	345904	338778	338778
Y	5463312	5463312	5466551	5474288	5482950	5482950	5486306	5486306

Results (number per taxon):

Taxa	Sample code:							
	109	111	148	139	45	137	14	88
Dendrocoelum lacteum		5			3			
Dugesia lugubris/polychroa		79			5			
Dugesia tigrina		45			1		2	
Hypania invalida					61			
Enchytraeidae species			1					
Lumbricidae species			1					
Lumbriculidae species			1					
Peloscolex multisetosus			1					
Psammoryctides barbatus			2					
Tubificidae with hairs juvenile					1			
Tubificidae without hairs juvenile			10		5			
Erpobdella octoculata			3	2	10	3		
Erpobdella testacea					1			
Erpobdellidae species juvenile		3			19	6		
Glossiphonia complanata		1			29	8		
Glossiphonia concolor					31	2		
Glossiphonia heteroclita						1		
Hemiclepsis marginata		2						
Trocheta riparia						1		
Bithynia tentaculata	9	43	1		2476	594	1470	8
Corbicula fluminea					10			
Dreissena polymorpha					25	11	3	
Physa fontinalis		4						
Physella acuta			1					
Pisidium species juvenile						1		

Taxa	Sample code:							
	109	111	148	139	45	137	14	88
Potamopyrgus antipodarum	2	15	4					
Radix species juvenile			2		1	11		
Radix ovata	36	68						
Sphaerium corneum					37	1		
Viviparus viviparus		6						
Asellus aquaticus		322		6	54	7		
Proasellus meridianus		10			29	4		
Dikerogammarus villosus		9						
Echinogammarus berilloni		1						
Gammaridae species juvenile						1		
Gammarus salinus				708	1358	1555	3583	2138
Elmis species larvae						1		
Hydropsyche contubernalis			1					
Cricotopus bicinctus		7		4		1		
Cricotopus intersectus	5	7						
Cricotopus sylvestris	1							
Limnophyes species	1		8					
Nanocladius bicolor agg.				1		1		
Rheocricotopus chalybeatus				1				
Dicrotendipes nervosus	17	335	24	83	16	33	9	1
Dicrotendipes nervosus pupae				1				
Glyptotendipes pallens	30	282	52	8	1	7		
Glyptotendipes paripes			10					
Glyptotendipes species juvenile							1	
Parachironomus arcuatus gr.				3				
Parachironomus longiforceps				3	1	3		
Parachironomus spec. Kampen						1		
Polypedilum cultellatum	2							
Polypedilum scalaenum	1		1			1	1	
Paratanytarsus dissimilis agg.				2				
Rheotanytarsus species						1		
Number of specimens	104	1243	123	822	4174	2255	5069	2147
number of taxa	10	19	17	11	22	24	7	3

Annex 5. Results of IGBA calculations

In order to apply the indice biologique global adapté aux grands cours d'eau et aux rivières profondes, protocole expérimental (IGBA) (Anonymous, 1996) on monitoring results, next samples are prescribed to be taken at each location:

a. Eight different habitats in the littoral zone (depth <1 m) with a Surber or handnet (Haveneau type), sampling surface 0.05 m^2, net opening width 250 mm, height 200 mm, 0.5 mm mesh net. The results are used to calculate the **IF** (indice filet).
b. Three different locations in the main channel with a triangular or cylindrical-conical dredge to sample three different bottom substrates. The results are used to calculate the **IFD** (indice filet et drague).
c. Four to six artificial substrates, placed in the littoral zone of both river banks, of which two with the highest species diversity are used. The results are used to calculate the **IS** (indice substrat artificiel).

Basis for the calculation of the three metrics is a score table which is derived from on a taxa list in which 38 of them were identified being indicator taxa. The calculation of each metric is made with the combined results of all samples per category.

Assesses are:

a. **the taxonomical variety**, which is equal to the total number of taxa found in the samples;
b. **the class variety**;
c. **the faunistic indicator**; the taxon with the highest indicator value (the number of specimens in this taxon must be 3 or 10 at minimum);
d. **the number of faunistic indicators**;
e. **the index value**, the IGBA for each metric and for all samples

Calculation of the IS (artificial substrates)

Two nettings with artificial substrates were suspended at six locations (Table 1). During retrieval it appeared that two of them were lost and one was found completely dried out on the riverbank at rk 230. Because macroinvertebrate densities in one substrate from the site at rk 207 (sample 109) and in the other from the site at rk 230 (sample 230) were relatively low, the results of both substrates were joined together. With the result from the second artificial sample from the site at rk 207 (sample 111) the IS was calculated for all locations in the zone T_1^A. Results from the artificial substrate samples 18 and 139 were used for the IS calculation in the zone T_1^B, those obtained from the artificial substrates retrieved at rk 278.1 (sample no. 45 and 137) and rk 305.9 (sample no. 14 and 88) for the IS calculation in the up- and downstream part of the zone T_2 respectively.

Table 1. Artificial substrates retrieved from the suspension sites (rk = river kilometre) and the macroinvertebrate density in these substrates

rk	Sample no.	Density
207.6	109	104
207.6	111	1234
230.4	148	123
258.3	18	2395
265.6	139	822
278.1	45	4174
278.1	137	2255
305.9	14	5069
305.9	88	2147

Table 2. IS calculation for combinations of artificial substrates (for sample numbers see table 1)

Sample no.	109, 111 & 148	18 & 139	45 & 137	14 & 88
IGBA value	6	6	6	3
Number of taxa	13	13	14	5
Class variety	5	5	5	2
Number of indicator taxa	7	5	5	3
Indicator taxon	Gammaridae	Gammaridae	Gammaridae	Gammaridae

Overview of metrics

Table 3. Location Pîtres, rk: 203

	IFD
IGBA value	9
Number of taxa	19
Class variety	6
Number of indicator taxa	8
Indicator taxon	Polycentropidae

Table 4.Location Île du Motillon, rk 205

	IF	IFD	IS	Total
IGBA value	8	9	6	11
Number of taxa	19	17	13	25
Class variety	6	6	5	8
Number of indicator taxa	6	6	7	8
Indicator taxon	Hydropsychidae	Polycentropidae	Gammaridae	Polycentropidae

Table 5. Location Orival, rk 221

	IF	IFD	IS	Total
IGBA value	7	10	6	11
Number of taxa	17	22	13	27
Class variety	6	7	5	8
Number of indicator taxa	5	8	7	9
Indicator taxon	Canidae	Polycentropidae	Gammaridae	Polycentropidae

Table 6. Location Bédanne, rk 227

	IFD
IGBA value	8
Number of taxa	16
Class variety	5
Number of indicator taxa	8
Indicator taxon	Polycentropidae

Table 7. Location Petit Quevilly, rk 247.7

	IFD
IGBA value	6
Number of taxa	15
Class variety	5
Number of indicator taxa	5
Indicator taxon	Gammaridae

Table 8. Location Grand Quevilly, rk 250

	IF	IFD	IS	Total
IGBA value	7	7	6	8
Number of taxa	18	20	13	23
Class variety	6	6	5	7
Number of indicator taxa	6	6	5	6
Indicator taxon	Gammaridae	Gammaridae	Gammaridae	Gammaridae

Table 9. Location la Bouille, rk 260

	IF	IFD	IS	Total
IGBA value	6	9	6	10
Number of taxa	16	17	13	21
Class variety	5	6	5	7
Number of indicator taxa	7	8	5	9
Indicator taxon	Gammaridae	Polycentropidae	Gammaridae	Polycentropidae

Table 10. Location Duclair, rk 278

	IF	IFD	IS	Total
IGBA value		5		
Number of taxa		12		
Class variety		4		
Number of indicator taxa		5		
Indicator taxon		Gammaridae		

Table 11. Location Yville sur Seine, rk 288

	IF	IFD	IS	Total
IGBA value	6	5	6	6
Number of taxa	7	10	14	15
Class variety	3	4	5	5
Number of indicator taxa			5	
Indicator taxon	Gammaridae	Gammaridae	Gammaridae	Gammaridae

Table 12. Location le Landin, rk 294

	IFD
IGBA value	3
Number of taxa	5
Class variety	2
Number of indicator taxa	3
Indicator taxon	Gammaridae

Table 13. Location Côte de Caveaumont, rk 302

	IF	IFD	IS	Total
IGBA value	4	3	3	4
Number of taxa	7	5	5	8
Class variety	3	2	2	3
Number of indicator taxa	4	2	3	4
Indicator taxon	Gammaridae	Mollusca	Gammaridae	Gammaridae

Table 14. Location Vieux Port, rk 324

	IF	IFD	IS	Total
IGBA value	4	2	3	4
Number of taxa	6	3	5	8
Class variety	2	1	2	3
Number of indicator taxa	4	2	3	4
Indicator taxon	Gammaridae	Gammaridae	Gammaridae	Gammaridae

Table 15. Location Caudebec, mouth river Eure, rk 216.5

	IF
IGBA value	8
Number of taxa	19
Class variety	6
Number of indicator taxa	5
Indicator taxon	Hydropsychidae

IF based on four samples

Table 16. Secondary channel at rk 229-230

	IF
IGBA value	4
Number of taxa	15
Class variety	5
Number of indicator taxa	3
Indicator taxon	Mollusca

IF based on four samples

Annex 6. *Corbicula* in the Seine aval

The invasion history of *Corbicula fluminea* in the river Seine was well documented by Brancotte & Vincent (2001, 2002) and Vincent & Brancotte (2000, 2002). The species was supposed to reach the river through the Marne-Rhine Canal. In the river Rhine the first observations of both *C. fluminea* and *C. fluminalis* were made in its delta (Bij de Vaate & Greijdanus-Klaas, 1990). From these observations it was concluded that *C. fluminea* had colonized the delta in 1986 or before. Based on observations in Germany in 1990, Bij de Vaate (1991) concluded that the species colonized the river Rhine in upstream direction with a speed of at least 85-115 km per year.

According to T. Vincent (Muséum d'Histoire Naturelle, Le Haver, pers. comm.) the finding of *C. fluminalis* in June 2006 was the first observation of this *Corbicula* species in the river Seine. The species was restricted to the zone T_1^A while *C. fluminea* was found in all three zones (T_1^A, T_1^B and T_2) (Table 1). Spread of both species is similar to that the river Rhine. *C. fluminalis* seems to prefer coarser substrates than *C. fluminea*. However, densities of *C. fluminalis* were relatively low.

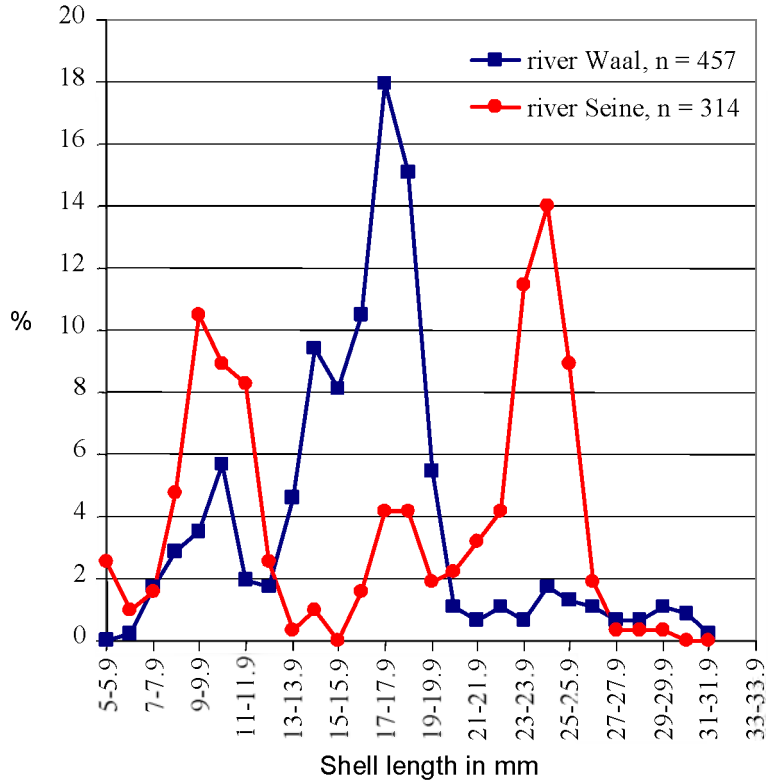
Table 1. Occurrence of *C. fluminea* and *C. fluminalis* in the Seine aval. Sampling dates: June 16-20, 2006

river kilometre	habitat	substrate	C. fluminea	C. fluminalis
203.3	river bed	sand	+	
205	river bed	gravel	+	+
216.5	mouth river Eure	mud	+	+
221	river bed	sand + gravel	+	
221	subtidal littoral	gravel		+
221	subtidal littoral	stones		+
221.5	subtidal littoral	stones	+	
226.8	bed secondary channel	sandy mud	+	+
227	river bed	sand + gravel	+	+
230.8	river bed	sand		+
247.7	river bed	sand + gravel		+
249.8	river bed	gravel		+
249.8	subtidal littoral	sand + gravel		+
250	river bed	sand + gravel		+
250	subtidal littoral	sand + gravel		+
258	river bed	mud + sand + gravel		+
260	river bed	sand + gravel		+
265	river bed	bricks[1]		+
288	river bed	sand		+
302	river bed	mud		+

[1] artificial substrate

Relatively high densities of *C. fluminea* were found in the deeper river bed between the river kilometers 245 and 260. Population structure of the specimens found at river kilometer 258.3 on June 16, 2006, was compared with the river Rhine population sampled on June 23, 2006, in the river Waal (the main distributary of the river Rhine) at river kilometer 910.9 (Fig. 1). In both rivers, three generations can be distinguished. The second generation in the river Waal and the third generation in the river Seine dominated the populations, while the third generation in the river Waal and the second generation in the river Seine played a minor role.

Figure 1. Population structure of *C. fluminea* in the rivers Waal and Seine.



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Annex 7. Elaboration of a sampling methodology for the study of macroinvertebrates in the tidal freshwater section of the river Seine

Contents

1. Introduction

In the framework of Seine-Aval's "Action II-2005-03", entitled: "Etat des peuplement benthiques dans la partie amont de l'estuaire" macroinvertebrates of the tidal freshwater section of the river Seine (Seine aval) were sampled in the period June 16-20, 2006. Based on the experiences obtained during this monitoring campaign, a monitoring strategy is discussed for that part of the river. It is an elaboration of activity D in the project entitled: "Elaboration of a sampling methodology to study the macroinvertebrates, including sampling protocols, sampling vessels and equipment, sampling sites and related subjects".

2. Existing protocols

Since macroinvertebrate monitoring is part of the French National Monitoring Program and the EU Water Framework Directive (WFD) (Anonymous, 2000), a monitoring protocol for the Seine aval should take into account the sampling conditions prescribed in those programs.

A. Documents for the national monitoring program

1. ***Indice Biologique Global Normalisé***
   The indice biologique global normalisé (IBGN) (AFNOR, 2004) prescribes sampling of macroinvertebrates with a Surber or handnet (Haveneau type), sampling surface 0.05 m^2, net opening width 250 mm, height 200 mm, 0.5 mm mesh net. At each location 0.05 m^2 of eight different habitats characterized by substrate type and water velocity. Identification of the animals at family level, except the Oligochaeta which are identified at class level.
   Frequency:
   Sampling should be performed in a period when the hydrological conditions allow sampling of all habitats. Unfavourable circumstances should be avoided.

2. ***Indice Biologique Global Adapté***
   In order to apply the indice biologique global adapté aux grands cours d'eau et aux rivières profondes, protocole expérimental (IGBA) (Anonymous, 1996) next samplings must be performed at each location:
   a. eight different habitats in the littoral zone (depth <1 m) with a Surber or handnet (Haveneau type), sampling surface 0.05 m^2, net opening width 250 mm, height 200 mm, 0.5 mm mesh net;
   b. three different locations in the main channel with a triangular or cylindrical-conical dredge to sample three different bottom substrates;
   c. four to six artificial substrates, placed in the littoral zone of both river banks, of which two with the highest species diversity are used.

   Frequency:
   It is recommended to sample at least two times under different conditions. Starting sampling large rivers it is recommended to sample three times per year in different periods, avoiding the high discharge period.

B. European Water Framework Directive

According to the EU Water Framework Directive (WFD), methods used for the monitoring of type parameters must conform to the international standards listed below or such other national or international standards which will ensure the provision of data of an equivalent scientific quality and comparability.
For the water quality element "Macroinvertebrates" sampling should follow next standards:
1. ISO 5667-3 (1995) Water quality. Sampling. Part 3: Guidance on the preservation and handling of water samples.

Most recent version of this norm was published in 2003. For counting and identification of benthic macroinvertebrates next preservation techniques are recommended:

a. add ethanol to the sample (if needed after decanting the clear supernatant) till the concentration is > 70% (volume fraction)
b. add 37% neutralized formaldehyde to obtain in the sample a final concentration of 3.7% (neutralize the formaldehyde with sodium tetraborate or hexamethylene-tetramine)

2. EN 27828 (1994). Water quality. Methods for biological sampling. Guidance on handnet sampling of benthic macroinvertebrates. See norm ISO 7828 (1985).

3. EN 28265 (1994). Water quality. Methods of biological sampling. Guidance on the design and use of quantitative samplers for benthic macroinvertebrates on stony substrata in shallow waters. See norm ISO 8265 (1988).

4. EN ISO 9391 (1995). Water quality. Sampling in deep waters for macroinvertebrates. Guidance on the use of colonisation, qualitative and quantitative samplers.
In this norm five sampling devices are described:
   a. *Colonization sampler*
   This sampler is a standardized artificial substrate consisting of a coarse mesh polyamide bag filled with approximately 40 pieces of a biological filter medium as used in sewage treatment. An alternative version of this sampler is the colonization unit in which the biological filter medium is assembled into a cylindrical shape. Colonization period is four weeks.
   b. Naturalist's dredge
   Two versions of this dredge are recommended; a small one with an opening of 46x19 cm, a bigger one with an opening of 61x20 cm. The supporting collecting net is about 35 cm in length; its mesh size depends on the objective of the sampling.
   c. *Birge-Ekman grab*
   The pole-operated version of this grab is recommended for water bodies with a depth of <3 m. In deeper waters without flow a rope-operated grab can be used. Sampling surface of the grab is 225 cm^2.
   d. Ponar grab
   The weighted version of this grab is recommended; sampling area 560 cm^2 and weight about 23 kg.
   e. FBA air-lift sampler
   The air-lift sampler recommended in the norm has a sampling area of 415 cm^2. It can be used to take quantitative samples on substrata ranging from fine gravel to stones of about 13 cm long. It is not recommended for use on mud. Water depth at the sampling location is modifying the length of the riser. It is impracticable to apply the sampler from a boat.

5. EN ISO 8689-1 (1999). Biological classification of rivers, part 1: Guidance on the interpretation of biological quality data from surveys of benthic macroinvertebrates in running waters.
Most recent version of this norm was published in 2000. The norm does not prescribe sampling procedures previous to the biological classification.

6. EN ISO 8689-2 (1999). Biological classification of rivers, part 2: Guidance on the presentation of biological quality data from surveys of benthic macroinvertebrates in running waters.
The norm prescribes that sampling of the macroinvertebrates should be in accordance with the norms ISO 5667-3, ISO 7828, ISO 8265 and ISO 9391.

7. ISO 7828 (1985). Water quality. Methods for biological sampling. Guidance on handnet sampling of benthic macroinvertebrates.
This norm describes a handnet and the way to use it in different water types. The net is recommended to have an opening of 20-40 cm width and 20-30 cm height, and a length of 40-

55 cm. Mesh size 0.25-0.75 mm depending on the survey objective.

8. ISO 8265 (1988). Water quality. Design and use of quantitative samplers for benthic macro-invertebrates on stony substrata in shallow freshwaters.
   In this norm two sampling devices are described which are both applicable in shallow fordable water bodies only:
   a. *Surber sampler*
      Several modifications of this sampler are described. The sampling surface is 0.09 m2 but can be changed to fulfil the objective of the sampling. Length of the net is about 70 cm long; its mesh size also depends on the objective of the sampling.
   b. *Cylinder sampler*
      Essentially an open ended cylinder having the lower edge serrated with 100 mm teeth. Diameter of the cylinder is equal to a cross-sectional area of 0.05 or 0.1 m2. An oval aperture in the cylinder wall, fitted with a 1mm mesh screen, allows water to enter the cylinder. At the opposite site a second aperture to which a detachable net can be mounted to collect the benthic animals. Mesh size of this net is not prescribed.

3. Sampling practice in the Seine aval

Macroinvertebrates in the Seine aval were sampled in June 2006. In the framework of the WFD, this river section was subdivided into three zones:

T_1^A :	between Poses (rk[1] 202) and Rouen (rk 236)
T_1^B :	between Rouen (rk 236) and La Bouille (rk 260)
T_2 :	between La Bouille (rk 260) and Vieux Port (rk 325)

In each zone next biotopes were distinguished:

1. the tidal zone:
   a. soft bottom (mud, sand)
   b. solid substrates (boulders, pebbles, stones, bricks, woody debris)
   c. vegetation
2. the subtidal zone:
   a. soft bottom (mud, sand)
   b. solid substrates (boulders, pebbles).

In addition, an artificial substrate (broken bricks in coarse mesh size netting) was used to sample the active migrating macrozoobenthos in the subtidal zone.

Samples were taken at low tide at several location locations in each zone. For sampling the zone T_1^A a service vessel and a Zodiac were used. The other zones (T_1^B and T_2) were sampled with an exploring vessel and the Zodiac as well. A combination of a fast travelling Zodiac and a bigger vessel for the heavier sampling equipment was an ideal combination. With the Zodiac sampling locations could be reached in time to sample the intertidal and

The service vessel used for sampling zone T_1^A

[1] rk = river kilometer (point kilométrique)

the shallow subtidal littoral zones at low tide.
However, the use of two different bigger vessels did not allow using the same sampling equipment for sampling the main channel in the zones. In the zones T_1^B and T_2 the Hammond grab was used, while in zone T_1^A a Van Veen grab was used for sampling mud and/or sandy substrates, and the triangular dredge for the coarser substrates.
In all zones the subtidal littoral zone and in intertidal zone biotopes the muddy and/or sandy substrates were sampled with a standard handnet (opening 30x20 cm, mesh size 0.5 mm). The handnet was also used for kick sampling in the coarse substrates in the subtidal littoral zone. Animals on pebbles, cobles and other coarse substrates (e.g. stones) in both the subtidal and tidal zones were sampled by brushing these substrates in a bucket, followed by collecting them on a 0.5 mesh sieve.

The exploring vessel "Cote d'Aquitaine", used for sampling zones T_1^B and T_2

The Zodiac, a very useful "device" for sampling littoral biotopes in a tidal river section

4. Sampling methodology

Development of a sampling program for monitoring macroinvertebrates must be part of the so-called monitoring cycle (Timmerman *et al.*, 1997). Filling up such a cycle should be the first step to develop a framework for the monitoring activities. In the case of the assignment, it must be clear for what purpose the monitoring results are used and what the assessment conditions are before the sampling strategy can be adopted. When the monitoring results are used for a WFD-proof assessment they should meet next conditions:

a. accurate enough to distinguish ranges (with sufficient width) for the given quality classes;
b. possibility of (relative) density assessment of taxa found in the samples.

The later condition means that all sampling techniques must be quantitative or at least semi-quantitative if absolute densities are used. If relative densities are used this pre-condition is of minor importance. In the case of absolute densities, attention should be paid to the way in which density is expressed. For a muddy, sandy or gravel bottom it is clear, the number of animals per unit of bottom surface can be used. However in the case of stones functioning as bank protection, cobbles, vegetation or artificial substrates one has to realize that all are three dimensional substrates which require clear definitions for their (calculated) surface units.

Most important aspects for a sampling methodology and strategy are:

1. The way of using the monitoring results. What assessment method is used and for what purpose?
2. The size of the area taken into account in the assessment procedure. Is each location in a river section separately assessed and the results combined for the whole section? Or are the results of all locations in a river section combined followed by an assessment of the section? In the later case the influence of the absence of samples from biotopes at one or more locations or sampling failures will play a minor role in the assessment procedure.
3. The importance attributed to the various biotopes. In the case of the Seine aval, what should be the "weight" of the intertidal biotopes in the assessment procedure and of which of these biotopes?

4.1. Sampling locations

From practical point of view two sampling locations per zone are recommended. They can be considered as duplicates and should be chosen in such a way that they are located in areas with average physical and chemical quality conditions in the biotopes sampled. This means that, e.g., point sources of pollutants or temporary river engineering activities should be avoided. Unacceptable deviations between assessment results of both locations should be a reason to reject them in reporting the monitoring results.

4.2. Sampling methods

The method of sampling macroinvertebrates depends on the biotope to be sampled. Important factors are water depth and the substrate composition. Main biotopes in the Seine aval are:

a. ***Solid substrates in the tidal and subtidal littoral zone***
   All organisms attached to at least five pieces of solid substrate are brushed off with help of a soft (washing-up) brush. The material attached to these stones produces one sample. For details of this sampling method see annex 7.2.

b. ***Subtidal channel bottom***

Sampling method used for the subtidal channel bottom depends on the size of the bottom particles. In the case of coarse material (gravel and coarser particles) a (triangular) dragnet or Hammond grab is used. The dragnet is for qualitative sampling only, the Hammond grab for quantitative sampling as well. If the bottom consists of sand or mud and the current velocity is relatively low a Van Veen grab can be used. If the current velocity is too high a Hammond grab or triangular dredge is recommended.

A triangular dragnet

The macrozoobenthos is separated from the bottom material by washing each sample (in portions) on a 0.5 mm mesh sieve. If a dredge is used to sample bottoms consisting of coarse material, like pebbles and cobbles (and other particles of these sizes), the particles should be brushed off.

c. ***Aquatic vegetation***

For sampling the aquatic vegetation a standard handnet (mesh size 0.5 mm) is used.

d. ***Intertidal bottom***

The sandy or muddy bottom is sampled with a standard handnet (mesh size 0.5 mm) at low tide. The macrozoobenthos in the samples is separated from the bottom material by washing each sample (in portions) on a 0.5 mm mesh sieve.

e. ***Intertidal solid substrates***

Sampling procedure the same as for solid substrates in the tidal and subtidal littoral zone (paragraph a).

f. ***Artificial substrate***

The artificial substrate consists of broken bricks (± 4-8 cm in size) in a polyethylene or nylon netting (about 35x50 cm). Each netting is filled with 4 litre of the substrate and firmly packed in order to prevent rolling of the material. Colonisation period is 30 ±2 days. The procedure for collecting the animals from the artificial substrate is the same as for solid substrates.

The artificial substrate

g. ***Sampling of a (cooling) water***

intake

A (cooling) water intake is sampled by suspending a driftnet (mesh size 1 mm) in the water at the intake flow for part of a night or by be sampling the screens (if present at appropriate mesh and if possible) manually.

A detailed description of the sampling methods including sampling conditions and methods to collect the animals is given in the annexes 7.1-7.6.

4.3. Number of samples

The number of samples to be taken depends on the assessment procedure adopted. In order to apply the IGBA (Anonymous, 1996), 13 samples are prescribed to be taken at each location (see paragraph 3). This number of samples from the main biotopes present in the river seems to be sufficient if other assessment procedures are taken into account.

4.4. Sampling period

In order to obtain a representative survey of the macroinvertebrates living in the river Seine (especially insect larvae), at least two sampling periods are recommended in each monitoring year (Van Helmond *et al.*, 1992):

a. in May (after the relatively high spring discharge);
b. in August/September.

4.5. Field collection records

All sampling activities are documented in the field on an field collection form. An example of such a form is given in annex 7.5.

5. Analysis of samples

The macrozoobenthos in the samples should be identified as much as possible to species level with the most recent identification literature. Building up a reference collection of species during the analysis is recommended since identification of several taxonomic groups demands a certain degree of specialism and to prove the right identification if there are some doubts afterwards. This is specially the case for rare and "unknown" specimens (not identified to species level). It should be noticed that the identification level of several taxonomic groups of macrozoobenthos strongly depends on the developmental stage.

Identification of the animals should meet two goals:

1. to assess the biological quality of the river;
2. to indicate biodiversity.

Identification level for each group of animals is summarized in annex 7.6.

For the purpose of goal 1, the total number of individuals in each sample will be indicated:

a. relatively small numbers are counted directly;
b. relatively large numbers are assessed from sub-samples.

For the purpose of goal 2 only an indication of the presence of the taxa is needed.

A list of literature used for identification of the animals is prepared during analysis of the samples and must be part of the identification results.

Analysis results of each sample must be used to supply information:

a. a taxa list;
b. the number of specimens per taxon found in each sample;
c. the calculated (relative) density of the taxa found.

6. Literature

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Annex 7.1
Sampling protocol

Introduction

Analysis of benthic macroinvertebrate communities is prescribed in the French National Monitoring Program and in the assessment of the ecological quality of water bodies according to the EU Water Framework Directive (Anonymous, 2000). Recommendations for a monitoring programme given in this document are based on experiences obtained during a sampling campaign in the Seine aval in June 2006.

Definitions

Biotope	An area uniform in environmental conditions and in its distribution of animal and plant life.
Habitat	The place where a certain species lives.
Handnet	A standard handnet has an opening of 25-30 cm width and 15-20 cm height. The net itself is 0.5 mm mesh and has a length of 50-60 cm.
Location	A sampling location is defined as a river stretch covered by the width of the river channel with a total length of about 1 km. In monitoring practice each sampling location is indicated by one point on a map and samples can be taken 500 m upstream and downstream of that point at maximum.
Site	A specific place within a sampling location. In each location different sampling sites can be distinguished depending on the place where the biotopes can be found which have to be sampled.

Part of the river bank with different habitats.

Sampling methods

a. *Stones in the subtidal littoral zone*

All organisms attached to at least five stones are brushed off with help of a soft (washing-up) brush. The sampling procedure is described in Annex 7.2.

Preconditions:

- Stones include blocks, bricks, pebbles, and rocky material. Size of each piece about 20x20 cm of the largest face. If smaller more than five pieces must be brushed off till an equivalent of above mentioned five pieces is obtained. The animals of all pieces of stones produce one sample.
- In areas with varying water levels it has to be determined from water level fluctuations over the last two months, at what depth the stones are to be sampled. They should have been under water for at least two months and are preferably taken at 30 cm under the lowest water level in that period.
- From the attached organisms (like zebra mussels/algae/sponges) it has to be concluded if a stone has been under water for enough time. The stone ought to be (partly) covered by fouling organisms.

b. *Subtidal channel bottom*

Sampling method used for the subtidal channel bottom depends on the size of the bottom particles. In the case of coarse material (gravel and coarser particles) a (triangular) dragnet or Hammond grab is used.

Preconditions:

- The dragnet is for (semi-)qualitative sampling only, the Hammond grab for quantitative sampling.
- If the bottom consists of sand or mud and the current velocity is relatively low a Van Veen grab is used. Sampling surface of the grab is about 400 cm2. At least five samples are taken at one site to produce one overall sample.
- If the current velocity is too high a Hammond grab is recommended.
- If a dragnet or dredge is used to sample bottoms with coarse material, the pebbles and cobbles (and other particles of these sizes) are brushed off (see annex 7.2).
- The macrozoobenthos is separated from the bottom material by washing each sample (eventually in portions) on a 0.5 mm mesh sieve.

c. *Aquatic vegetation*

For sampling the aquatic vegetation a standard handnet (mesh size 0.5 mm) is used. The sampling procedure is described in annex 7.3.

Preconditions:

- Included in the aquatic vegetation sampling are: macrophytes, bryophytes, sessile macro-algae, (submerged) roots and vegetal litter (e.g. leaves).
- The sample in the littoral vegetation is taken at a minimum of 1 meter inside the vegetation seen from the waterside.
- Sampling should preferably take place in an unbroken stretch of vegetation.
- If circumstances allow, sampling should be done from the waterside (use wading trousers or boat) and not from the embankment side of the sampling site.
- A new sampling site is chosen if better developed vegetation is observed within the location.

d. *Intertidal bottom*

The sandy or muddy intertidal bottom is sampled with a standard handnet (opening 25-30 cm width, height 15-20 cm; 0.5 mm mesh net, length of the net 50-60 cm) at low tide. The macrozoobenthos in the samples is separated from the bottom material by washing each sample (in portions) on a 0.5 mm mesh sieve.

Preconditions:
- The littoral bottom is defined as mineral substrates with a particle size equal or smaller than sand (e.g. sand and mud).

e. *Intertidal solid substrates*

The sampling procedure is the same as for solid substrates in the tidal and subtidal littoral zone (paragraph a).

f. *Artificial substrate*

The artificial substrate consists of broken bricks (± 4-8 cm in size) in a polyethylene or nylon netting (about 35x50 cm). Each netting is filled with 4 litre of the substrate and firmly packed in order to prevent rolling of the material. The procedure for collecting the animals from the artificial substrate is the same as for solid substrates (annex 7.2).

Preconditions:
- Colonization period is 30 ±2 days.
- Three nettings with artificial substrate are placed on the river bottom at different locations. It is recommended to choose locations where the substrate can be fixed to posts in the main channel. Locations with point sources of pollutants or temporary river engineering activities must be avoided.
- Each netting is sampled separately.
- Organisms from each netting produce one sample. Two samples are analysed, the third one, with the less animals, acts as a spare.
- Should one of the nettings have sunk into the riverbed (thus containing a great deal of sand or mud), or been washed away, the substrate is not sampled.

g. Sampling of a (cooling) water intake

A (cooling) water intake is sampled by suspending a driftnet (mesh size 1 mm) in the water at the intake for one night or by be sampling the screens (if present and if possible) manually.

Preconditions:
- Opening of the driftnet must be at least 30 cm.
- One night means an unbroken period of about 8-10 hours maximum between 18:00 p.m. and 08:00 a.m.
- If the net had silt up during that period a new sample must be taken by suspending the drift net during a shorter period in the intake.

Annex 7.2
Sampling macrofauna on stones

Five stones (e.g. bricks, concrete blocs, pebbles) in the littoral zone are sampled. Size of each stone is about 20x20 cm of the largest face. If smaller more than five pieces must be brushed off till an equivalent of above mentioned five pieces is obtained. The material attached to these stones produces one sample.

- Pick up stones manually from a depth of least 30 cm over the highest point (elbow depth).
- Have a washing-up bowl at hand and transfer the stones into it straight away, add water to prevent drying.
- Before brushing off the stone check if there are any sponges, Bryozoa and/or algae on the stone. Fill in this observation on the sampling form.
- If there are zebra mussels present scrape them with the help of a scalpel off the stone and transfer them to the 0.5 litre jar.
- Brush the rest of the material off the stone with the help of a washing-up brush, the stone should be brushed completely clean.
- Lift the stones out of the washing-up bowl and pour the remaining water with the material from the washing-up bowl over a 0.5 mm mesh sieve.
- Take any leeches off the sides of the bowl and put them in the 0.5 litre jar.
- Wash the material to a corner of the sieve; put the sieve on the jar and wash, with a siphon filled with 96% ethanol, the material into the jar.
- Preserve the material with 96% ethanol whereby the quantity of ethanol should be no less than twice the amount of the material sampled in the jar. Use larger or more jars if necessary.

If the river bank is a concrete wall, sampling can be done with a standard (0.5 mm mesh) handnet provided with a scrapper (see also annex 7.3).

Colonized stone from the subtidal zone of the river bank

Annex 7.3

Sampling with a standard macroinvertebrate handnet

Littoral bottom without aquatic vegetation

- A sample consists of one haul of 1.5-2.0 meter with the handnet through the bottom. Haul the net intermittently and against the water current. In doing this, organisms are collected that are found in toplayer of the river bottom and in the water column just above the bottom.
- Shake the net in the water in such a way that silt, sand or other fine particles are removed from the sample. If too much bottom material is left in the net after this procedure, put small portions of it into a bucket, and wash these carefully (see washing procedure).
- After all bottom material is washed transfer the material left in the net into a sampling jar.
- Preserve the material with 96% ethanol whereby the quantity of ethanol should be no less than twice the amount of the material sampled in the jar. Use larger or more jars if necessary.

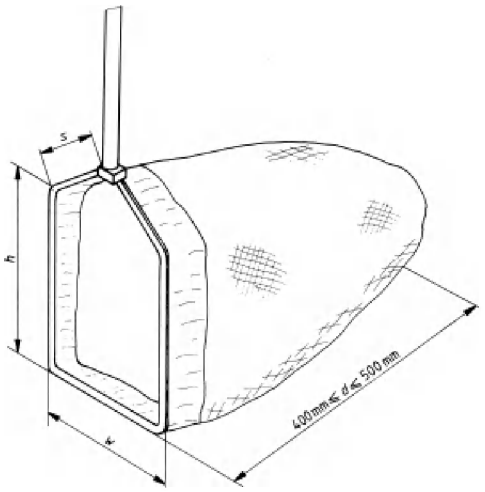
Littoral bottom with aquatic vegetation

- Determine the total cover and the cover of the most common (abundant) species and fill in on the sample form.
- Haul the net, over a length of 1.5-2.0 meter, intermittently and against the water current through the water plants.
- After that move the net immediately upwards in the opposite direction.
- Shake the net in the water in such a way that silt, sand or other fine particles are removed from the sample.
- After all bottom material is washed transfer the material left in the net into a sampling jar.
- Preserve the material with ethanol 96%, whereby the quantity of ethanol should be no more and no less than twice the amount of the material sampled in the jar. Use larger or more jars if necessary.

Washing procedure

This procedure should be followed if too much bottom material is left in the net after shaking it in the water to remove smaller particles.

- Put small portions (about 1 l) of the material collected with the handnet into a bucket.
- Add some river water (about 3-5 l) to each portion, mix thoroughly, and pour out the supernatant on a 0.5 mm mesh sieve immediately.
- Repeat these handlings until no animals release from the substrate (five times is mostly enough).
- Check the bottom material for larger snails and mussels before throwing away.
- Collect the material from the sieve and preserve it with ethanol 96%, whereby the quantity of ethanol should be no less than twice the amount of the material sampled in the jar. Use larger or more jars if necessary.



The standard handnet

Annex 7.4

Sampling the artificial substrate

- Retrieve the substrate carefully from the water by keeping the handnet under it to collect the animals that try to escape.
- Transfer it immediately into a bucket or a plastic container after lifting from the water surface to prevent further losses of organisms.
- Empty the netting in a bucket or container and add some river water.
- Empty the handnet in the same bucket.
- Clean the netting and the pieces of brick by means of a soft brush.
- If zebra mussels are present scrape them with help of a scalpel from the substrate and transfer them to a 0.5 litre jar.
- Pour the content from the bucket or container on a 0.5 mm mesh sieve.
- Take any leeches off the sides of the bucket or container and put them in the 0.5 litre jar.
- Wash the material to a corner of the sieve, put the sieve on the jar and wash, with a siphon filled with ethanol 96%, the material into the jar.
- Preserve the material with 96% ethanol whereby the quantity of ethanol should no less than twice the amount of the material sampled in the jar. Use larger or more jars if necessary.

Annex 7.5

Field Collection Form

Location : Sampling date: Time: hour

River section: River km:

Discharge : $m^3.sec^{-1}$ at reference location: (name ref. location)

Samples:

	Position	Sampling method					
River channel	left ☐ middle ☐ right ☐	Hammond grab ☐ Van Veen grab ☐ dragnet ☐ other ☐					
		Substrate:					
Subtidal bank	left ☐ right ☐	mud ☐ mud ☐	sand ☐ sand ☐	gravel ☐ gravel ☐	pebbles ☐ pebbles ☐	cobbles ☐ cobbles ☐	stones ☐ stones ☐
Intertidal bank	left ☐ right ☐	mud ☐ mud ☐	sand ☐ sand ☐	gravel ☐ gravel ☐	pebbles ☐ pebbles ☐	cobbles ☐ cobbles ☐	stones ☐ stones ☐
Bank vegetation[1])	left ☐ right ☐	subtidal ☐ subtidal ☐	intertidal ☐ intertidal ☐				
Artificial substrate	left ☐ right ☐	suspending date: retrival date: colonisation period: days					

[1]) Bank vegetation:

☐ fennal pondweed (*Potamogeton pectinatus*) ☐ loddon pondweed (*P. nodosus*)
☐ perfoliate pondweed (*P. perfoliatus*) ☐ hornwort (*Ceratophyllum demersum*)
☐ water crowfoot (*Ranunculus fluitans*) ☐ water milfoil (*Myriophyllum spicatum*)
☐ other species:

Stream velocity: $m.sec^{-1}$ (± 0.5 m above the river channel bottom)

Additional parameters:

Water temp.: °C Turbidity : Photograph no's:

Oxygen : $mg.l^{-1}$ Secchi disc depth: Map no's :

Conductivity: $\mu S.cm^{-1}$ Colour :

pH :

Bank condition:

General observations:

Annex 7.6

Identification level for macroinvertebrates

It should be noticed that identification level of larvae of insects, Araneida and Oligochaeta strongly depends on the developmental stage in which these animals are found. In general only (nearly) adult stages can be indentified to species level. Identification levels given below are considered to be target levels for (nearly) adult stages (S = Species level, G = Genus level and F = Family level, - = no identification).

Group	Identification level for:	
	Assessment	Biodiversity
Porifera	S	S
Coelenterata	S	S
Turbellaria	S	S
Nemertini	-	-
Nematoda, Nematomorpha	-	-
Polycheata	S	S
Oligochaeta	G	S
Hirudinea	S	S
Dreissenidae	S	S
Rest Bivalva	S	S
Gastropoda	S	S
Actinedida	F	S
Araneida	S	S
Cladocera	-	-
Ostracoda	-	-
Branchiura	S	S
Cirripedia	S	S
Decapoda	S	S
Mysidacea	S	S
Corophidae	S	S
Rest Amphipoda	S	S
Isopoda	S	S
Colembola	-	-
Ephemeroptera	S	S
Odonata	S	S
Plecoptera	S	S
Heteroptera	S	S
Coleoptera	S	S
Megaloptera	S	S
Neuroptera	S	S
Hymenoptera	S	S
Chironomidae	S	S
Rest Diptera	F	S
Trichoptera	S	S
Lepidoptera	S	S
Bryozoa	S	S