Framework for the definition of an oil spill impact assessment and monitoring programme for estuarine environments

I. PHASES AND ACTIONS OF THE SCIENTIFIC RESPONSE

Following an oil spill, the decision to implement environmental impact studies (drafting of specifications) depends on the definition of monitoring programmes designed to be launched at various points in time and extending over various periods:

- From initial observations of visible signs of impact (e.g. qualitative data) to the design and establishment of more or less long term scientific studies, such monitoring programmes should be considered, in the same way as the operational response (e.g. initial clean-up, final clean-up), as **successive steps of the scientific response**.
- These steps, designed to fulfil different objectives, require **different levels of accuracy**, and therefore variable **definition and implementation procedures**.

⇨ I.1) IMMEDIATE POST-SPILL ACTIONS

Emergency/short term estimations:

- Implementation time/duration (approx.): established in **the days** following the spill, these estimations may be occasional (simple observations/surveys) or extend over a few weeks, according to the situation: they generally coincide with the **spill spreading phase** (beaching of floating pollutant and/or remobilisation of beached oil).
- Aims: they aim to rapidly and roughly characterise all the visible impacts and their extent. They are undeniably **beneficial – or even necessary – for the subsequent design phase of medium/long term monitoring programmes** (definition of priorities and programme content; Cf. below section **I.2**).
- Procedures: they are based on **relatively simple methods which are rapidly implemented** (in terms of sampling, scientific expertise, data processing and analysis, etc.), and mainly involve the **decision, in an emergency situation, to collect (operational) data and/or conduct field surveys** relating to:
    - the spread/distribution and typology of the pollution (e.g. light to severe, surface/infiltrated),
    - the collection, in an emergency situation, and referencing of samples (e.g. sediment, biota) from beyond the edge of the pollution ("pre-spill reference condition"),
    - the characterisation/mapping of exposed habitats and biological components,
    - the characterisation of obvious signs of impact (e.g. visible phenomena such as mortalities, beachings, etc.), the species concerned (birds, invertebrates, etc.) and their extent (counts, photographs, etc.).

➯ I.2) SPILL STABILISATION PHASE

Medium term monitoring:

- **Implementation time/duration (approx.):** initiated within **a few weeks** after the spill, such monitoring programmes generally take place **once the pollution has stopped spreading**. When they are defined/launched, initial clean-up operations are generally well underway (if not completed). They generally last **around a few months to a year** (# completion of a seasonal/reproduction cycle).
- **Aims:** they ideally aim to **quantify the impact and/or identify the implementation and expected time scale of rehabilitation processes**. They therefore require more elaborate approaches than early estimations.

  Procedures: the establishment of specifications in theory includes a selection of a few components and parameters reasonably liable to enable the identification of the share of fluctuations caused by the spill. Within this context, this project, based on experience, predefines the minimum components to be considered (Cf. § *II.1*). The decision to include them within the specifications should be based on the advice of **an expert group of scientists** (the aim being to come to a consensus on the importance/relevance of monitoring these main components). Other components, which have not been selected, may nevertheless appear to be of interest given the spill context. In this case, their assessment should be based on as objective criteria as possible, which should be able to be adapted to the variability of spill components and contexts; a list of such criteria is provided below (Cf. § *II.2*).

  As part of a subsequent phase, the monitoring protocols for the components selected in the specifications should be optimised, in terms of targets (e.g. species, communities, etc.), parameters measured, sampling strategy (spatial, temporal comparisons...) etc. In this respect, recommendations have been produced as part of the project in terms of the minimum recommended components (Cf. § *III*).

Long term monitoring:

- **Implementation time/duration (approx.):** such monitoring programmes **follow on from medium term monitoring programmes** – according to the results obtained around 12 months after the spill. They involve continuing previously implemented actions (Cf. above), based on reasons and for a duration (possibly several years) closely linked to the medium term observations.
- **Aims:** they generally aim to **understand and determine the scale of the rehabilitation process** for the most lastingly affected components (biota, habitat) and/or that are the focus of specific concern(s) (e.g. regulatory status).
- **Procedures:** the decision to establish such monitoring programmes is based on **the scientific opinion of the prior established expert group** in terms of how appropriate the continuation of monitoring of the selected components is considered to be. In this case, the protocols are unchanged and only certain **adaptations** may be recommended on a case by case basis (reduced sampling frequency, reduced number of sampling stations, etc.).

II. MINIMUM COMPONENTS AND PRIORITIZATION OF OTHER PROPOSALS

⇨ II.1) MINIMUM "CANDIDATE" COMPONENTS FOR SPECIFICATIONS

Based on experience in impact assessment (real cases of spills), several environmental components have been identified as regularly – if not systematically – being the focus of monitoring programmes. The following "candidate" components are therefore selected as (i) they would appear to be indispensable (exposed) and applicable in the event of estuarine pollution and (ii) it has been possible, from past experience, to define protocols liable to determine the occurrence (or non-occurrence) of impacts[1]:

- subtidal soft substrate benthos
- intertidal soft substrate benthos
- intertidal hard substrate benthos
- ichthyofauna (water column + demersal fish)
- shorebirds living in estuarine mudflats and reed beds
- plant assemblages growing on banks (salt marshes, reed beds, wetlands)

This list constitutes a proposal of potential components, which should be considered as a minimum by the experts mobilised when drawing up specifications. It is not intended to be limiting, and other potential components considered to be of interest may also be included according to the specific context of the spill and on a case by case basis.

For each of those components to be considered *a minima*, indications[2] as regards to basic features of the studies (resources -species, communities, etc; biological parameters; etc.) are listed in table 1a et 1b.

[1] For these same reasons, certain other potential components have been eliminated, in particular mammals and the plankton component (phyto and zooplankton).

[2] drawn from Cedre's previous bibliographic studies (considering environmental impact assessment & monitoring after significant oil spill cases)

Table 1a

<table>
<tr><th colspan="3">COMPOSANTE</th><th>Exposition (cas général)</th><th>Fluctuations naturelles</th><th>Recommandations de cibles potentielles (d’après l’expérience)</th><th>Recommandations de critères/paramètres (d’après l’expérience)</th></tr>
<tr><td rowspan="11">Benthos</td><td rowspan="7">Médiolittoral</td><td rowspan="4">Sédimentaire</td><td rowspan="7">- Élevée (estran = zone naturelle d’arrivage de la pollution flottante)</td><td rowspan="4">Modérée
à
élevée</td><td rowspan="2"><u>Espèces :</u>
- Bivalves endogés à cycle long (ex: S. plana, M. balthica)
- Macrofaune représentative et/ou sensible (ex: amphipodes)</td><td>- Dyn. Populations (abond[ce], taille, etc.)
↳ Analyse : Classes d’âge, indices condition, etc.</td></tr>
<tr><td>- Biomarqueurs :
↳ Analyse : Effets (stab. lysosomale, histopathol.) // Exposition (AChE) // Génotoxicité (micronoyaux, tests comètes)</td></tr>
<tr><td><u>Communautés :</u>
- invertébrés macrobenthiques</td><td>- Descripteurs structure peuplements (SAB)
↳Indices?</td></tr>
<tr><td><u>Groupes indicateurs</u></td><td>- Abondances, dominances… (ex: polychète/amphipodes)?</td></tr>
<tr><td rowspan="3">Rocheux</td><td rowspan="3">Elevée
(selon complexité structurelle de l’habitat)</td><td rowspan="2"><u>Espèces</u>
- Gastéropodes, bivalves épigés à cycle long</td><td>- Dyn. Populations (Abondances, classes de taille, présence/absence, indices d’abondance semi quantitatifs, etc.)</td></tr>
<tr><td>- Biomarqueurs : effets, exposition, génotoxicité</td></tr>
<tr><td><u>Communautés :</u>
- invertébrés macrobenthiques</td><td>- Taxons, abondances, recouvrement</td></tr>
<tr><td rowspan="4">Infralittoral</td><td rowspan="4">Sédimentaire</td><td rowspan="4">- Modérée
- Variable selon:
- Type de polluant (dispersion/adsorption, submersion/coulage)
- Processus sédimentaires (secteurs de sédimentation, bouchon vaseux, etc.)</td><td rowspan="4">Modérée</td><td rowspan="2"><u>Espèces</u>
- Endofaune sessile à cycle long (ex: bivalves)
- Macrofaune sensible (ex: amphipodes)</td><td>- Dyn. Populations (Abondances, tailles, etc.)</td></tr>
<tr><td>- Biomarqueurs : effets, exposition, génotoxicité</td></tr>
<tr><td><u>Communautés :</u>
- invertébrés macrobenthiques</td><td>- Descripteurs structure peuplements (SAB)
↳Indices?</td></tr>
<tr><td><u>Groupes</u> indicateurs</td><td>- Abondances, dominances… (ex: polychète/amphipodes)?</td></tr>
</table>

Table 1b

COMPOSANTE	Exposition (cas général)	Fluctuations naturelles	Recommandations de cibles potentielles (d'après l'expérience)	Recommandations de critères/paramètres (d'après l'expérience)
Ichtyofaune	- Elevée (démersaux) à modérée (col. d'eau) ; - Variable selon: - type de polluant - hydrologie, processus sédimentaires	Elevée*	<u>Espèces et/ou stades du développement</u>: - exposés (interface eau/sédiments) (ex: *P. platessa*, *P. flesus*) - sensibles (ex: nourriceries) (ex: *S. solea*)	- **Dyn. Populations** (abond^{ce}, biométrie, …) ↳ Analyse : Classes d'âge, indices condition, etc.
				- **Biomarqueurs :** ↳ Analyse : Exposition (EROD, métabolites HAPs) // Effets (tissus hépatiques) // Génotoxicité (adduits ADN)
			<u>Communautés</u> ichtyofauniques	- **Abondances, richesse taxonomique)** ↳ Analyse : Indices (ELFI/DCE)?
Avifaune	- Modérée - Variable selon éthologie/saison (migration/nidification/nutrition, etc.) - N.B. : effet potentiellement indirect (altération de la fonctionnalité de l'habitat)	Modérée	<u>Sites fonctionnels affectés</u> : - Vasières intertidales (limicoles, anatidés,…) - Roselières (passereaux paludicoles)	- **Recensements/comptages :** effectifs, espèces (méthodes standardisées)
				- **Descripteurs du succès reproducteur** (méthodes standardisées) // Cohérence avec critères/méthodologies établis
Flore estuarienne	- Potentiellement élevée - Variable selon: - Marées - Débordement	Modérée	<u>Associations pionnières</u>: - Prés salés atlantiques (schorres) - Roselières (scirpaies, phragmitaies, …) - Prairies subhalophiles	- **Composition spécifique** (prés^{ce}/abs^{ce} espèces) - **Abondances** (% couvert végétal) - **Distribution** (points contacts)
				- **Phénologie** - **Biométrie**
			Espèces : - <u>à statut de conservation</u> et/ou - <u>représentatives</u> des sites/habitats affectés.	- **Phénologie** - **Biométrie**

⇨ II.2) ELEMENTS FOR DETERMINING PRIORITIES AMONG OTHER PROPOSALS

In the case of a real spill, different components to those described above may be considered to be of interest and may also be included in the monitoring proposals put forward in the specifications, according to the specific spill context.
However, all the potential components cannot (or should not) necessarily be monitored, for reasons relating to:

- their relevance, in relation to the specific spill context (pollutant type, spread, fate and risks generated (exposure of various components, coincidence with biological cycles, etc.)),
- logistics and the expertise required.

The development of the content of a monitoring programme may therefore involve the integration of additional components, requiring prior assessment of their relevance.
This assessment may draw upon a prioritization approach to the proposed components, based on a certain number of criteria which should be both as objective as possible and relatively general, i.e. applicable to the specificities of the potential components and/or of the spill. The table below provides some examples (non-restrictive and non-exhaustive list)[3] of such prioritization criteria.

Criteria	**Questions** (if applicable – according to component)
Spill context/spread	• Observations: has the component been proven to have been **exposed to the pollutant**, and to what extent?
	• Risks: is the component at **risk of exposure** (e.g. proximity to the spill location, pollutant behaviour, risk of pollutant being transferred to the bottom – adsorption on silt plug, submersion, etc.)?
Importance of the component	• Is the **functional importance** of the component within the estuarine ecosystem known/established?
	• **Representative nature** of the estuary area affected: is the component (species, habitats, etc.) commonly distributed within the affected sectors?
	• **Regulatory status (conservation/protection):** does the component have a particular status (and/or is it an integral part of a regulated site) on a local, national, regional and/or international scale?
	• **Socio-economic importance:** is the component the focus of economic activities (e.g. species exploited, grown...) and/or societal/cultural activities (e.g. leisure, tourist attraction, heritage)?
Biological characteristics *Vs.* impact identification potential	• Is the component's **vulnerability (sensitivity; rehabilitation potential)** to oil known/described prior to the spill?
	• Is the **extent of natural fluctuations** (spatial, temporal) of the component known/anticipated? o Is it thought to be lower than, similar to or higher than the expected disturbance? o Does it enable a realistic monitoring strategy (frequency and number of samples taken) to be established?

[3] Selected and developed from the analysis of experience, presentations and discussions held at *Cedre* as part of the seminar: *Ecological impacts of accidental marine pollutions on benthic environment. Assessment and Prospects*" (12-14 October 2005, Cedre, Brest); from the European workshop *Pollutant monitoring and ecological impact assessment following accidental oil and other chemical spills in marine waters* (9-11 October 2007, Cedre, Brest).

	• Is the component subject to other anthropogenic disturbances at the affected sites (e.g. chronic pollution, developments, practices); if so, are they thought to be penalising in the determination of fluctuations caused by the spill?
State of knowledge	• **Availability of pre-spill data**? o Consistency with the area/sectors concerned? o Sporadic data or time series? o Old or recent data? o Data type/relevance?
Scientific practice	• Is the component the focus of **study protocols** that are: o established, commonly accepted o applicable in the given context (e.g. sampling, measurement of known variables as indicators, expertise required, data analysis, etc.)

Table 1. List (*non-restrictive, non-exhaustive*) of criteria proposed for determining priorities, in terms of the implementation of monitoring for each proposed environmental component.

III. RECOMMENDATIONS FOR DEFINING COMPONENT MONITORING PROGRAMMES

This section presents recommendations for designing monitoring for the minimum components to be considered for possible inclusion in the specifications of an impact monitoring programme (II.1). These recommendations focus on:

- Intertidal and/or subtidal soft substrate benthic endofauna (Sheet A, §A.2)
- Intertidal hard substrate benthic epifauna (Sheet A, §A.3)
- Ichthyofauna (water column + demersal fish) (Sheet B)
- Shorebirds living in estuarine mudflats and reed beds (Sheet C)
- Plant assemblages growing on banks (salt marshes, reed beds, wetlands) (Sheet D)

Where one or more of these components are included in the specifications, they provide indications and elements to be considered for the definition and assessment of the monitoring programme, in terms of constraints, types of organisms or species to be targeted in particular, potential types of approaches, strategies, limitations/advantages generated, etc.

Sheet A - RECOMMENDATIONS for the assessment of ESTUARINE BENTHOS

Benthic organisms are a component that is systematically included in oil spill impact assessment programmes in the shoreline environment. Various considerations go to explain this state of fact and, while also the case in estuary environments (e.g. potentially indicator sessile fauna, functional importance, etc.), justify the **need to implement impact assessments on estuarine benthos**.

A.1 – Selecting benthic habitats and organisms:

Various **areas** (eulittoral, sublittoral), **substrates** (soft, rocky) or **types of organisms** (species, communities, zoological or trophic groups, etc.) that make up the benthos may be monitored, targeted according to the concerns relating to the specific context of the spill (e.g. characteristics and fate of the pollutant) and the environment (e.g. areas and benthic organisms exposed to the pollutant).

- Substrates:

In terms of natural substrates, soft sediments (mud, more or less muddy sand) are generally widely present in estuaries and the endobenthic – sessile – fauna populating these beds is potentially heavily exposed to sediment contamination. It is therefore recommended that **benthic invertebrates in soft sediment be systematically monitored** in the event of estuarine pollution.

Where man-made infrastructures are present in the eulittoral zone (e.g. riprap in particular), they can be conducive to the installation of hard substrate benthic fauna. Where questions or local issues arise, and where there is a consensus among experts, the intertidal benthic fauna on rocky substrates can be monitored.

- Exposure and sensitivity:

It is recommended that **monitoring be defined** by **classifying priorities** based in particular on:

- Potential exposure of areas of the benthic compartment, generally considered as:
  - **Potentially high for the eulittoral (intertidal) zone**
  - **Moderate for the sublittoral (subtidal) zone** and decreasing with distance/depth in relation to the foreshore (except in specific circumstances)
- Knowledge of the varying sensitivity of organisms in relation to a significant oil spill. This sensitivity is recognised for a wide range of taxa, in particular crustaceans, echinoderms, as well as certain species of bivalve and gastropod molluscs, polychaete annelids, etc. These elements can, according to the populations' characteristics and local specificities (e.g. species of interest for heritage or functional reasons), contribute to the selection of biological targets.

In short: in terms of impact assessments in the benthic compartment in the estuarine environment, the previous points therefore lead us to recommend as a priority, *as a general rule*, the implementation of **benthic invertebrate monitoring**:

- **systematically** for **soft substrate endofauna in the eulittoral zone** (intertidal) (*Cf.* **§ A.2**)
- **possibly** for **soft substrate endofauna in the sublittoral zone** (subtidal) (*Cf.* **§ A.2**), where there is a risk of the pollutant being transferred to the sediment bed. This should be considered in spill scenarios liable to promote the adsorption of the dissolved fraction on matter in suspension (potential case of a light product, e.g. petrol, diesel, etc.), or the

submersion of heavy and/or weathered and/or possibly sediment-loaded products in the shallow subtidal zone (particularly in freshwater areas, due to the relatively low density of the water).

- **complementarily** for epifauna in areas of **hard substrates in the eulittoral zone** (most often infrastructures), if this issue is considered to be of interest (e.g. representativeness of the habitat in oiled areas, perceived functional importance, etc.) (*Cf.* § A.3).

A.2 - Assessment methods for soft substrate benthic endofauna

General considerations/difficulties

As a general rule, when designing the monitoring programmes proposed below (*Cf.* § A.2.1 to A.2.3), it is recommended to consider the expected extent of the **natural variability** of phenomena (parameters) to be examined in soft substrate benthic invertebrates.
This may **prove to be moderate** to **relatively high**, spatially and temporally[4], and can make it difficult to clearly assign the observed responses to the spill. This constraint can be partially reduced by:

- selecting organisms whose **ecology and biology are already known** and/or populations for which **reference data series from prior to the spill** are available,
- monitoring **"control" (unpolluted) sites** comparable to the polluted sites, where such sites exist.

N.B. **Collecting qualitative information in an emergency** (through field surveys in the first days/weeks following the spill) is recommended in as far as it can guide the monitoring choices featured in the recommendations provided below (*Cf.* § A.2.1 to A.2.3) and specify strategies. If possible, data collection should aim to:

- characterise the benthic species *visibly* affected (e.g. observations, visual surveys of phenomena such as the surfacing of sediment-dwelling bivalves, large-scale beachings, etc.)
- estimate, roughly and as an initial indication, the extent and location/extension of phenomena.

❖ A.2.1 Ecological approach, at community level

In terms of studies into soft substrate macrobenthic communities, the **perceptible advantages** are in particular (i) the acceptance – and relative standardisation – of surveillance protocols, and (ii) an established level of knowledge and experience, in terms of the analysis of community description parameters (species richness, abundance, etc.)

However, it is important to emphasise that the know-how in terms of assessment of the quality of the environment by studying macrobenthic populations **mainly focuses on marine environments: in estuarine environments, the transition system between polyhaline, mesohaline and oligohaline zones requires a few potential restrictions to be considered in terms of analysis of the data obtained according to this approach**:

- In the marine and inshore environment, except in cases of imbalance (pollution for instance), the structure of macrobenthic communities generally tends to be based on the interspecific relations (e.g. predation, competition, etc.) established between the different groups (e.g. functional, trophic, etc.) that make up the system. In estuaries, on

[4] E.g. Aggregated distribution of organisms of certain species (to a lesser extent in soft sediment than in more complex rocky habitats); seasonal to multiannual fluctuations in populations or biological parameters, etc.

the other hand, it is the increasing environmental constraints from downstream to upstream (e.g. decreasing salinity, turbidity, organic matter, etc.) which play a leading role in the composition of populations (with, for instance, maintenance in upstream areas of the species most resistant to these constraints and their fluctuations).

- As an outcome, the existing benthic indicators (e.g. AMBI, BQI, etc.), most often conceived and developed to analyse the structure of communities in coastal/marine environments[5], may appear to be unsuitable for estimating the level of degradation of estuarine populations, whose structure may not so much indicate a degradation of the environment as of their suitability to the typical natural constraints of an estuary (e.g. case of an abundance of species indicating organic matter enrichment, which could point to a degradation of the environment in a marine or coastal context, but be characteristic in estuarine mud).

Despite these possible difficulties, mainly arising in the least saline estuarine areas, effects can, in principle, be identified by **comparison with a certain number of basic descriptors of community structure** (or possibly indicators) between (i) contaminated sites following the spill and (ii) analogous reference data (pre-spill or from unaffected control sites).

Parameters
The assessment of the effects on community structure involves the determination of each taxon, if possible at species level, and the establishment of the following parameters: **species richness** (number of species), **abundance** and **biomass**[6]. These descriptors are the basic elements supporting subsequent analysis – entering, where relevant, into the calculation of most benthos quality indicators.

Strategy
- The data obtained (e.g. abundance, biomass, species richness) will be compared, grouped by sampling station:
    - with analogous/comparable data (i) from prior to the spill (reference data) or (ii) from "control" (unpolluted) sites, where such sites can be established, and/or
    - between stations with different levels of contamination (examination of relationships with PAH contents, for instance) and
    - between successive sampling dates, implemented <u>on a seasonal basis for at least a year</u> (inclusion of a reproductive cycle), so as to identify any medium term impacts, or the occurrence of restoration processes (e.g. data comparison at t_{+1year}).
- Comparison and monitoring can focus on various criteria, for instance:
    - the **list of the most abundant taxa** (characterising sediment)
    - the **relative abundances of distinct pollution sensitivity groups** (where this is known for the taxa/species present)[7]
    - various existing and recognised indices in terms of the ecology of benthic communities (e.g. Shannon-Wiener diversity index, AMBI, etc.) **to be selected/validated by experts according to their potential relevance with**

[5] Based, for instance, on species classification: into groups according to their sensitivity to organic pollution, trophic groups, etc.

[6] The methodologies specific to the establishment of these parameters, for instance the measurement of Ash-Free Dry Weight (AFDW) for biomass, are generally accepted and described in the scientific literature and are the focus of recommendations for macrobenthos quality monitoring as part of regulatory frameworks (Water Framework Directive) or international expert committees (ICES, for instance).

[7] E.g. unusual fluctuations in the abundances of taxa generally considered to be resistant – or even opportunistic (e.g. certain polychaetes) or, on the contrary, sensitive (e.g. amphipod crustaceans).

regard to the spill context (Cf. above remarks) and/or previous knowledge, the availability of reference data series, etc.

  - the [polychaete/amphipod] ratio has been proposed as a potential indicator of oil pollution-induced change in soft substrate communities, based on the knowledge acquired following the *Amoco Cadiz* (1978) and *Aegean Sea* (1992)[8] incidents. Refined to form the BOPA index[9], within the context of the search for indicators for the Water Framework Directive, the applicability of this index for assessing the impact of a spill in the various estuarine areas remains however to be determined.

- We should bear in mind the need to characterise and monitor, alongside these population-based parameters, **the basic sediment parameters** for each station, in particular the organic matter content and grain size.
- The acquisition and availability of relevant data on **sediment contamination** must be assured for the stations monitored – either by including this aspect in the ecological study or by drawing upon the results produced within the framework of a distinct but coordinated study that forms part of the assessment programme.
- The continuation of this type of monitoring beyond 1 year post-spill should be considered according to the results obtained at t_{+1year}, requiring possible adaptations (reduced sampling frequency, selection of a certain number of sites, etc.) to be considered on a case by case basis. A minimum frequency of 2 samples per year is recommended (early spring (March/April) and late summer (September/October)).

In the case of implementation in the sublittoral zone (identified risk of transfer of the pollution to the bottom):

- the definition of monitoring stations should, for the identification of potentially polluted sites, benefit from the expertise of scientists specialised in the dynamics of sediment transport (e.g. position and evolution of silt plug, if a risk of adsorption of the pollutant on suspended matter is identified).
- the establishment of unpolluted "control" sites can prove problematic in as far as the processes at work in the transfer of the pollution to the bottom (hydrology, settling of fine particles) probably introduce natural edaphic variability in general (highlighting the need to monitor this parameter).

Sampling protocols

Ad hoc sampling equipment (core samplers, grab samplers, etc.), the details of the sampling plan (e.g. number of replicates) and the sample processing conditions are aspects known by experts in the ecology of intertidal benthic populations, and are the focus of **recommendations** in national (e.g. ReBent), European (e.g. WFD) or other international frameworks. These recommendations should be referred to in order to ensure **compliance of the methods implemented with the accepted standards**[10] **in terms of the following considerations**, notably:

- in the eulittoral (intertidal) zone, sampling can be carried out on foot, using handheld core samplers (minimum unit surface area of 0.01 m^2, depth of 15-20 cm)
- in the sublittoral zone (or in the lower levels of the intertidal zone if necessary), sampling is conducted by boat, using grab samplers of which many models are commonly used and accepted (e.g. unit surface area of 0.1 m^2). Among those suitable for the most commonly

[8] Gesteira, J.L., Dauvin, J.C., 2000. Amphipods are good bioindicators of the impact of oil spills on soft-bottom macrobenthic communities. Marine Pollution Bulletin 40, 1017–1027.

[9] Dauvin JC, Ruellet T, 2007. Polychaete/amphipod ratio revisited. Marine Pollution Bulletin 55: 215-224.

[10] E.g. Guillaumont, B., et E. Gauthier, 2005. Recommandations pour un programme de surveillance adapté aux objectifs de la DCE - Recommandations concernant le benthos marin. Ifremer, November 2005; **Rumohr, H. 2009.** ICES Techniques in Marine Environmental Sciences/Soft-bottom macrofauna: Collection, treatment, and quality assurance of samples. 24 pp.

found sediments in estuaries (mud, muddy sand, etc. in shallow waters), we can notably refer to Van Veen grab samplers (suitable for shallow waters with muddy bottoms) and Smith-McIntyre grab samplers (which are however larger and heavier, and require a hoist).

- the number of samples/replicates per point should satisfy data representation (5 samples are generally recommended for fauna), for macrobenthos samples, but also for sediment analysis samples (organic matter and grain size), or even for contamination analysis samples if this aspect is included in the study (ensure compliance with current practices for sampling and ad hoc sample conservation with a view to chemical analysis).
- samples must be processed according to the recommendations accepted in the discipline, and referenced in the literature, from sample screening (suitable for macrofauna, preferably using a round 0.5 mm mesh[11]) to their fixing (diluted formaldehyde), not forgetting biomass calculation procedures (ash-free dry weight) or sediment analysis (e.g. grain size according to AFNOR standards in force).

Advantages and disadvantages

Over and above the general acceptance of surveillance protocols, and the knowledge acquired through past experience of significant oil spills, this approach provides an overview of the health status of the benthic environment, and the potential consequences in terms, for instance, of system diversity or functionality (recurrent concerns).

On the other hand, it has the drawback of requiring a relatively high level of expertise (e.g. taxonomy of benthic invertebrates), potentially complex logistics (mobilisation of grab samplers and vessels in the sublittoral zone), time for processing samples and considerable biological equipment (sorting, determination, counting, etc.). Data analysis and interpretation may therefore require more equipment and time than the approaches described below (§ A.2.2 and A.2.3; species targeting, or investigation of effects at individual level).

Additional remarks

- **Meiofauna**

In some cases of major oil spills (e.g. *Amoco Cadiz*, 1978), the benefit of the [nematode/copepod] ratio for impact assessment has been suggested, as well as, by various authors, for assessing imbalances due to inputs of organic matter (e.g. Raffaelli and Mason, 1981). While this potential of the meiofauna is worth mentioning, due to the results obtained following oil spills[12], this approach appears to be less of a priority than macrobenthos monitoring due to:

- the applicability/relevance in the estuaries to be assessed (question of the [nematode/copepod] relationship in normal conditions)
- protocols that appear to be less firmly established than for macrobenthos
- potentially high natural spatial and temporal variability, restrictive in terms of adequacy between the sampling strategy (e.g. high frequency to distinguish temporal variability for instance) and the interpretation of fluctuations (share that can be attributed to oil).

[11] Recommended mesh size in estuaries where the, potentially numerous, smallest specimens of macrofauna are not retained by a 1 mm mesh (more generally, double screening (1 mm and 0.5 mm) is recommended and the 2 fractions obtained should be analysed).

[12] E.g. Boucher G., 1981. Effets à long terme des hydrocarbures de l'Amoco Cadiz sur la structure des communautés de nématodes libres des sables fins sublitttoraux. In: « Amoco Cadiz » Conséquences d'une pollution accidentelle par les hydrocarbures. Actes du Colloque International, Centre Océanologique de Bretagne, Brest (France) 19-22 novembre 1979 ; C.N.E.X.O., Paris: 539-549.

❖ A.2.2 Ecological approach, at target species population level

Choice of species

Applicable to eulittoral (intertidal) and sublittoral (subtidal) zones, this approach consists in monitoring the **dynamics of populations of the target macrobenthic species** according to:

- their **sensitivity** to oil pollution, which may be:
  - known from experience (e.g. amphipod crustaceans) or
  - potentially significant due to their exposure and life cycle, in the case of **long-lived**, **sessile** and **sediment-dwelling** organisms, notably large **bivalve molluscs**. We note that specific intermediate sensitivity to oil is, in theory, more appropriate for conducting ecological monitoring, compared to very high sensitivity (e.g. major adverse effects – mortalities *and* low population restoration potential).
- their **representativeness** of the affected sediment bottoms (e.g. typical of intertidal mudflats, unlike 'accidental' species for instance on the boundaries of the geographical distribution), which also promotes the repeated collection of a satisfactory number of individuals.

In the estuaries targeted here (North-East Atlantic), a few species of macrofauna invertebrates are liable to be **potential candidates** for this type of monitoring; we can make mention for instance of:

- the bivalves:
  - *Scrobicularia plana*, a mainly deposit-feeding Semelid species, living in intertidal (eulittoral) muddy sediment (e.g. mud, muddy sand) and present in estuaries due to its tolerance to low salinities.
  - *Macoma balthica*, a deposit-feeding Tellinid species, common in freshwater estuarine environments, present in sediment in the eulittoral to sublittoral (i.e. subtidal) zone.
- the annelid *Hediste diversicolor*, the most common Polychaete (omnivorous) species in intertidal estuarine mudflats in North-West Europe – although not sensitive enough to be used in this context.
- the burrowing amphipod crustacean *Corophium volutator*, a species whose tube-dwelling specimens are also well represented in estuaries due to their tolerance to a wide range of salinities.
- …

Other macrobenthic species are known to be potentially present in tidal estuarine systems in the geographic area in question (e.g. bivalves: *Cerastoderma edule*, *Mya arenaria*, *Abra tenuis*, …), and, locally, the identification and selection of potential species for population monitoring will of course draw on **existing knowledge** (e.g. inventories, monitoring in progress...) in terms of the macrobenthic species (representativeness, biology, etc.) within the affected area.

Parameters to be measured

The measurements to be implemented within these populations are mainly the **abundance** and **size** (more easily measured in bivalves) of individuals within the target populations, so as to estimate the evolution of (i) densities, (ii) demographic structure (distribution of size/age class frequencies), and to calculate, where relevant, the survival and growth rates between the different samplings.

The **acquisition and availability of relevant data on sediment contamination must be assured** for the stations monitored – either by including this aspect in the ecological study or by drawing upon the results produced within the framework of a distinct but **coordinated**[13] study that forms part of the assessment programme.

[13] (Consistency of sampling sites and frequencies).

Strategy

- The results obtained at the polluted stations will be compared:
  - with analogous/comparable data (i) from prior to the spill (reference data) or (ii) from "control" (unpolluted) sites, where such sites can be established, and/or
  - between points (stations) with different levels of contamination (examination of relationships with PAH contents, for instance) and
  - between successive sampling dates, implemented **every two to three months for at least a year** (inclusion of a reproductive cycle), so as to identify any medium term impacts or the occurrence of restoration processes (e.g. data comparison at t_{+1year}).
- We should bear in mind the need to characterise and monitor, alongside these population-based parameters, **the basic sediment parameters** (organic matter content and grain size) for each station.
- In the case of implementation in the sublittoral zone (expected risk of transfer of the pollution to the bottom):
  - the definition of monitoring stations should, for the identification of potentially polluted sites, benefit from the expertise of scientists specialised in sediment transport dynamics (e.g. position and evolution of silt plug, if a risk of adsorption of the pollutant on suspended matter is identified).
  - the establishment of unpolluted "control" sites in the subtidal zone can prove problematic in as far as the processes at work in the transfer of the pollution to the bottom (hydrology, settling of fine particles) probably introduce natural edaphic variability in general.
- The continuation of this type of monitoring beyond 1 year post-spill should be considered according to the results obtained, requiring possible adaptations (reduced sampling frequency, selection of a certain number of sites, etc.) to be considered on a case by case basis.

Sampling protocols

- The choice of tools (hand-held core samplers, manual sampling within quadrats, etc.) and of the sampling plan (unit surface area, satisfactory number of stations and replicates for the collection of a significant number of individuals and statistical testing, mesh size for sediment screening, etc.), should be **specified on a case by case basis in close connection with the characteristics of the selected populations**. The consideration of these elements (e.g. dimension, distribution, abundance of individuals)[14] in **the definition of sampling procedures** is an aspect that is well known to experts in benthic ecology.
- If an analytical aspect (i.e. sediment contamination) is included in the study, standard practices for sampling and ad hoc sample conservation must therefore be followed.

Advantages

This approach has the advantage of being **relatively easy to implement** (limited logistics – in particular in the intertidal zone), and does not require complex data measurements or analysis, nor too high a level of expertise (sorting and determination of a set of species for instance). It can therefore provide relatively medium-term results (a few months after monitoring begins).

[14] (E.g. several mm, approx. 1 cm, etc.; from a few specimens to several dozen – or even hundreds – per m^2; regular or aggregated distribution, etc.)

❖ A.2.3 Ecotoxicological approach, at target species level

Within the framework of post-spill impact assessments on the benthic compartment, approaches can be implemented aiming to identify the appearance of sublethal effects in the individuals of a given species: these effects can be expressed on different scales (e.g. organic to subcellular, or even molecular) and vary in their specificity to the pollutant in question.

There are therefore many varied techniques that can potentially be used (e.g. genotoxic effect biomarkers, enzymatic biomarkers, histopathology, etc.) and their relevance can vary according to the specificities of the pollution (spill characteristics, oil composition and fate, etc.).
The recommendations provided in this paragraph therefore:

- focus on **the main features of a general minimum approach**,
- are partly based on currently identified experience in terms of oil pollution; they are therefore **neither exhaustive nor restrictive**.

Choice of species

Over and above the general recommendations to be followed (Cf. above **General considerations/difficulties**), the organism selection criteria are similar to those mentioned in the previous section (Cf. **A.2.1**), i.e.:

- their known **sensitivity** to oil pollution (probable induction of a response),
- the **potential exposure** of the species to the pollutant (e.g. sediment-dwelling, filter-feeding bivalve),
- their **representativeness** of the affected area,
- possibly their **status** as a consumed/commercial and/or heritage species.

In the sediments of estuaries in the North-East Atlantic, a few species of invertebrates of the macrofauna present these characteristics and are therefore liable to be **potential candidates** for ecotoxicological monitoring, for instance:

- the long-lived sediment-dwelling bivalves *Scrobicularia plana* (deposit feeder) and *Macoma balthica* (suspension feeder) common in fine estuarine sediment,
- the annelid *Hediste diversicolor*, a very common omnivorous Polychaete in intertidal estuarine mud,
- the amphipod crustacean *Corophium volutator*, a species which is also well represented in estuaries,
- …

Evidently, other "candidate" species[15] can be selected based on **existing knowledge** (e.g. inventories, monitoring in progress...) in terms of the macrobenthos (representativeness, biology, etc.) within the affected area.
Where present, the monitoring of **sentinel species** may be recommended, in particular, among benthic invertebrates, the filter-feeding bivalve *Mytilus* sp., a species often present in estuaries (tolerance to salinities of 20 to 40) although on hard substrates (where present, on infrastructures made of metal, concrete, etc.).

Parameters to be measured

Firstly, if **data series** collected as part of routine monitoring of the estuary environment quality, on a specific biomarker and species, are available in the affected area, it is recommended that the possible appearance of **anomalies in the basic fluctuations** of this biomarker be examined soon after the spill, even if this biomarker is not thought to be specific to oil pollution.

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[15] Species of sediment-dwelling benthic macrofauna, common in fine sediment and tolerant to brackish water, for instance (among bivalves): the soft-shell clam *Mya arenaria*, the cockle *Cerastoderma edule*, possibly the carpet shells *Venerupis decussata* and *V. philippinarum*, etc.

The assessment of the biological effect of a pollutant on sentinel bivalve molluscs (mussels, *Mytilus* spp.) or other potentially candidate species (mentioned above) may be implemented through **a crossover study of a set of biomarkers**, whether specific or not to the impact caused by PAHs.

Wherever possible, according to the spill context (e.g. applicability to species present), these biomarkers will be preferentially selected on the basis of **recommendations** made by international expert groups (e.g. the Joint Assessment and Monitoring Programme, of the OSPAR Convention; the International Council for the Exploration of the Sea (ICES), etc.).

In this regard, we suggest the application as a priority of a set of biomarkers (listed in the table below)[16]:

- of **exposure**, recognised as controls of contact with the major contaminants of aquatic systems, including hydrocarbons,
- of **genotoxicity**,
- of **effects** (not specific, however, to a type of contaminant),
- and associating the investigation of **short term** effects (hours, days) and **medium/long terms** effects (weeks, months).

[16] Although more specifically described for mussels, the application of these biomarkers can reasonably be considered for the potentially candidate species mentioned in this paragraph (due to expectedly similar biological responses).

	Biomarkers	Biological function	Recommendations (JAMP or ICES)	Application to taxonomic groups		
				Bivalves	Crustaceans	Polychaetes
Exposure	Benzo(a)Pyrene Hydroxylase (short term response)	PAH detoxification enzyme activity (phase 1 of metabolisation activities)		X	X	X
	Acetylcholinesterase (short term response)	Neurotoxic effect: general stress marker indicating the organism's physiological status	Yes	X	X	X
Genotoxicity	Micronucleus (long term response)	Formation following the breaking of DNA strands by exposure to clastogenic and aneugenic substances	Yes	X	X[17]	X
	Comet assay (long term response)	DNA strand breakage	Yes (additional method)	X	X[18]	X
Effects	Lysosomal stability (short and long term response)	Subcellular damage. Good pathology predictor: indicates a relationship between exposure and pathological effects	Yes	X	X[19]	X
	Antioxidant enzyme activities (CAT, SOD, GPx, GST, …) (short term response)	Cellular response to oxidant stress		X	X	X
	Malondialdehyde (long term response)	Lipid breakdown substance (lipid peroxidation: cellular response to oxidant stress)		X	X	X
	Histopathology (long term response)	General control response to a pathological effect (e.g. digestive glands, gonads)	Yes	X	X[20]	?

Overview of recommendations of the main biomarkers for benthic invertebrates (N.B. indications established based on experience, suggesting their suitability for the current issue. This list is not restrictive but rather provides an <u>indication</u>).

[17] Applicable in theory, performed on crabs (Nudi et al, 2010), although little hindsight for this taxon.
[18] Applicable in theory, performed on Daphnia (Den Besten and Tuk, 2000) and Gammarus (Lacaze, 2011), although little hindsight for this taxon.
[19] Applicable in theory, performed on Daphnia (Den Besten and Tuk, 2000), terrestrial isopods (Nolde et al, 2006) and shrimps (Bechmann et al, 2010), although little hindsight for these taxa.
[20] Applicable in theory, performed on *Carcinus maenas* (Moralles-Caselles, 2008).

Strategy

- The biological responses obtained will be compared:
    - o with analogous/comparable data: (i) from prior to the spill (reference data i.e. base level of indicators chosen where they exist) or (ii) from "control" (unpolluted) sites, where such sites can be established, and/or
    - o between stations with different levels of contamination (examination of relationships with PAH contents, for instance) and
    - o between successive sampling dates, implemented at a suitable frequency on the time scale of the biological process examined (i.e. high for initial short term response biomarkers, lower for long term responses)
- As previously mentioned, it is important to ensure the acquisition and availability of relevant data on **contamination of the sediment, water mass** and **biota** for the stations monitored – either by including this aspect in the ecotoxicological study or by drawing upon the results produced within the framework of a distinct but coordinated study that forms part of the assessment programme.
- Similarly, it is also beneficial to combine the biomarker responses with the environmental data acquired at the stations monitored (temperature, salinity, dissolved oxygen...).
- The continuation of ecotoxicological monitoring beyond an initial annual cycle should be considered according to the results (e.g. effective and persistent response), requiring possible adaptations (reduced sampling frequency, monitoring of certain 'chronic' biomarkers, selection of a certain number of sites, etc.).

Additional remarks

- **Bioassays**

In post-spill follow-up, bioassays may be conducted in the laboratory in order to rapidly provide elements on the (i) assessment of the risk generated by the substance spilt/matrix on the affected environment, or (ii) interpretation of the results obtained by impact studies[21].

Where relevant (and in addition to *in situ* research into impacts on organisms, populations or communities), we recommend implementing a series of bioassays:

- to initially **screen** the toxic potential of the substance or the matrix.
- whose protocols, commonly accepted and described, are the focus of **recommendations** and/or draw upon **standardised protocols** (e.g. ISO standard).

We also note that there are six trophic levels recommended for risk assessment – in the marine environment, but potentially recommended in the estuarine environment –, which are: bacteria, phytoplankton, crustaceans, molluscs, urchins and fish.

The table below gives an overview of bioassays of potential interest in the event of an oil spill.

[21] It is important to remember that the aim of bioassays is not to extrapolate the results in terms of effects induced *in situ*, but rather to determine the potential toxicity of the environments tested.

Recommended method	Matrix tested	Organisms	Biological function	References
DR-Calux (*in vitro* bioassay)	Water, WAF[22], sediment elutriate, biota extracts	Rat liver cell line	Measurement of exposure to planar compounds (PAHs, PCBs...)	Klamer *et al.* (2005)
Microtox assay (*in vitro* bioassay)	Liquid matrices: Water, WAF, sediment elutriate	Bioluminescent marine bacteria *Vibrio fischeri*	Measurement of acute toxicity	ISO standard 11348 [23]
Embryo-larval development (test *in vivo* – whole organisms)	Water, WAF, aqueous sediment extracts	Urchins, oysters	Teratology from the blockage of embryonic divisions to embryo-larval development anomalies	Beiras *et al.* (2012)[24], or Quiniou[25] *et al.* (2005)
Sediment bioassays (*in vivo* test)	Sediment, aqueous sediment extracts	Polychaete amphipods, urchins, bivalves	Mortality and capacity to re-burrow after exposure.	ISO standard 16712[26] and/or Thain & Bifield (2001)[27]
Scope for growth (*in vivo* or mesocosm test)	Liquid matrices: Water, WAF, sediment elutriate. Applicable at the spill site.	Used on mussels, transposable to other bivalves	Sublethal measurement of the energy available for growth	Widdows & Staff (2006)[28]

Overview of recommendations of the main bioassays for benthic invertebrates.

[22] Water accommodated fraction: aqueous fraction containing the dissolved and/or suspended and/or emulsified component of a multi-component substance such as oil.
[23] International Organization for Standardization. 2007. Water quality -- Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) -- Part 3: Method using freeze-dried bacteria. EN ISO 11348-3. It is worth noting however that the sensitivity of this test varies with salinity.
[24] Beiras, R., Durán, I., Bellas, J., and Sánchez-Marín, P. 2012. Biological effects of contaminants: *Paracentrotus lividus* sea urchin embryo test with marine sediment elutriates. ICES Techniques in Marine Environmental Sciences No. 51. 13 pp. Available at: http://www.ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20%28TIMES%29/times51/TIMES%2051%20Final%20120522.pdf
[25] Quiniou F., His É. Delesmont R., Caisey X., 2005. Bio-indicateur de la toxicité potentielle de milieux aqueux : bio-essai « Développement embryo-larvaire de bivalve ». Éd. Ifremer, Méthodes d'analyse en milieu marin, 24p.
[26] International Organization for Standardization. 2005. Water quality - Determination of acute toxicity of marine or estuarine sediment to amphipods. EN ISO 16712
[27] Thain, J. & Bifield, S. 2001. Biological effects of contaminants: Sediment bioassay using the polychaete *Arenicola marina*. ICES Techniques in Marine Environmental Sciences No.29. 16 pp. Available at: http://www.ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20%28TIMES%29/times29/TIMES29.pdf
[28] Widdows, J. & Staff, F. 2006. Biological effects of contaminants: measurement of scope for growth in mussels. ICES Techniques in Marine Environmental Sciences No.40. 34 pp. Available at: http://www.ices.dk/sites/pub/Publication%20Reports/Techniques%20in%20Marine%20Environmental%20Sciences%20%28TIMES%29/times40/TIMES40.pdf

A.3 - Assessment methods for hard substrate intertidal benthic epifauna

General considerations/difficulties

As a general rule, we note that the extent of the **natural variability** of macrobenthic populations can **prove relatively high in rocky environments**, temporally but also spatially in connection with the variability in the habitat's structural complexity, and can also make it difficult to clearly attribute the response observed to the spill. This constraint can be partially reduced by:

- selecting organisms whose **ecology and biology are already known** and/or populations for which **reference data series prior to the spill** are available,
- monitoring **"control" (unpolluted) sites** comparable to the polluted sites , where such sites exist.

N.B. **Collecting qualitative information in an emergency** (through field surveys in the first days/weeks following the spill) is recommended in as far as it can guide the monitoring choices featured in the recommendations provided below (*Cf*. § A.3.1 to A.3.3) and specify strategies. If possible, data collection should aim to:

- characterise the benthic species *visibly* affected,
- estimate, roughly and as an initial indication, the extent and location/extension of phenomena.

A.3.1 Ecological approach, at target species population level

Choice of species:

This approach consists in monitoring the **dynamics of populations of the target macrobenthic species** according to:

- their **sensitivity** to oil pollution, which may be:
  - known from experience (e.g. amphipod crustaceans) or
  - potentially significant due to their exposure and life cycle, in the case of **long-lived**, **sessile** and **epigeal** organisms, notably large **gasteropod molluscs** (e.g. limpets in rocky environments).
  - We note that **intermediate sensitivity** to oil is, in theory, more appropriate for conducting ecological monitoring, compared to tolerance (no effect on populations) or very high sensitivity (extreme case of a temporary and localised near-disappearance of the population).
- their **representativeness** of potentially affected eulittoral hard substrates (e.g. boulders, riprap, etc.), which also promotes the repeated collection of a satisfactory number of individuals.

In the estuaries targeted here (North-East Atlantic), a few common species of macrofauna invertebrates are liable to be **potential candidates** for this type of monitoring; we can make mention for instance of the following commonly found and easily identifiable species:

- the gastropod *Patella vulgata*, possibly *Littorina littorea* (more mobile), grazing species, sensitive to oil (acute toxicity) and potentially present on a wide range of hard eulittoral substrates (natural or artificial), although probably restricted to polyhaline areas,
- the bivalves *Mytilus edulis*, *M. galloprovincialis* and *Crassostrea gigas*, suspension-feeding, sessile species, potentially present on a wide range of hard eulittoral substrates (natural or artificial), although probably restricted to polyhaline areas despite a certain tolerance to decreasing salinity).

Locally, the identification and selection of potential species for population monitoring will of course draw on **existing knowledge** (e.g. inventories, monitoring in progress...) in terms of species of hard substrate benthic epifauna (representativeness, abundance/distribution, biology, etc.) within the affected area.

<u>Parameters to be measured:</u>

Like soft sediment population monitoring, the measurements to be implemented within these populations are mainly the **abundance** (individual counts, or percentage cover for sessile macrobenthic species[29]), which enables the identification of fluctuations in densities and, where relevant, their rehabilitation (restoration process). In the case of limpets, the measurement of the **size** of individuals has also been used (e.g. cases of the *Sea Empress* and the *Braer*) to identify the occurrence of effects through the evolution of the demographic structure (distribution of size/age class frequencies, growth rate between the various sampling events, recruitment phenomena).

It is important to ensure the **acquisition and availability of relevant data on the level of oiling** of the hard substrates monitored or the **contamination** of their immediate surroundings – either by including this aspect in the ecological study or by drawing upon the results produced within the framework of a distinct but **coordinated**[30] study that forms part of the assessment programme.

<u>Strategy:</u>

- The parameters measured at the polluted stations will be compared:
  - with analogous/comparable data (i) from prior to the spill (reference data) or (ii) from "control" (unpolluted) sites, where such sites can be established (see below), or
  - between stations with different levels of contamination (examination of relationships with PAH contents, for instance) and
  - between successive sampling dates, implemented **every 2 to 3 months for at least 1 year** (inclusion of a reproductive cycle), so as to identify any medium term impacts, or the occurrence of restoration processes (e.g. data comparison at t_{+1year}).
- It is important to remember to **minimise biases related to spatial variability**, naturally high in rocky areas, by preferentially sampling at stations that are as similar as possible in terms of environmental factors: slope, exposure[31]/orientation, structural complexity of the substrate (crevices, boulders, etc.).
- The continuation of this type of monitoring beyond 1 year post-spill should be considered according to the results obtained, requiring possible adaptations (reduced sampling frequency, selection of a certain number of sites, etc.) to be considered on a case by case basis.

<u>Sampling protocols:</u>

- It is recommended, wherever possible, that the methodologies proposed as part of **existing monitoring programmes in this field** be adopted, for instance the methodology proposed as part of REBENT (monitoring of fauna on rocky foreshores)[32].
- Classically, the quantitative/semi-quantitative estimation of abundances (counts, percentage cover) of epibenthic macrofauna on rocky substrates is implemented within quadrats with a unit surface area of 0.1 m^2, ideally with around ten replicates per point, within which the above-mentioned parameters are measured.

[29] E.g. Mussels, oysters, barnacles
[30] (Consistency of sampling sites and frequencies).
[31] Mainly sheltered estuarine areas.
[32] ftp://ftp.ifremer.fr/ifremer/delao/.snapshot/nightly.1/gt_benthos_dce/FT05-2003-01.pdf

- To assess abundances, it is recommended here that non-destructive sampling be implemented within permanent quadrats defined on substrates that are as similar as possible, in order to minimise the introduction of biases related to the habitat's spatial variability. In the case of the measurement of the size of individuals within a population, a destructive sample may be taken within random quadrats, whereby the number of replicates should be assessed and chosen appropriately (Cf. following point).
- The choice of tools and of the sampling plan (satisfactory number of stations and replicates for the collection of a significant number of individuals and statistical testing, etc.) remains to be **chosen/specified on a case by case basis in close connection with the characteristics of the selected populations**. The consideration of these elements (e.g. dimension, distribution, abundance of individuals)[33] in **the definition of sampling procedures** is an aspect that is well known to experts in benthic ecology.
- Where relevant and if necessary, sampling (*n* quadrats) can be implemented, at each station, along a hypsometric radial, in particular in sampling the upper and medium eulittoral levels (isolines at the *Pelvetia canaliculata* and *Fucus vesiculosus/Ascophyllum nodosum* belts respectively).

Advantages:
Where this approach targets relatively abundant, large species with limited mobility (e.g. limpets), it has the advantage of being **relatively easy to implement** (limited logistics), and does not require complex data measurements or analysis, nor a prohibitively high level of expertise (taxonomy) (sorting and determination of a set of species for instance). It can therefore provide relatively short-term results (a few months after monitoring begins).

❖ A.3.2 Ecotoxicological approach, at target species level

Approaches similar to those used for soft substrate macrofauna invertebrates can be implemented (Cf. A.2.3), aiming to identify the appearance of sublethal effects in the individuals of a given hard substrate epibenthic species. These effects can be expressed on different scales (e.g. organic to subcellular, or even molecular) and vary in their specificity to the pollutant in question.

There are therefore many varied techniques (e.g. genotoxic effect biomarkers, enzymatic biomarkers, histopathology, etc.) that can potentially be used and their relevance can vary according to the specificities of the pollution (spill characteristics, oil composition and fate, etc.).
The recommendations provided in this paragraph therefore:
- focus on **the main features of a general minimum approach**,
- are partly based on currently identified experience in terms of oil pollution; they are therefore **neither exhaustive nor restrictive**.

Choice of species:
Over and above the general recommendations to be followed (Cf. above **General considerations/difficulties**), the organism selection criteria are similar to those mentioned in the previous section (Cf. A.2.2), i.e.:
- their known **sensitivity** to oil pollution (probable induction of a response),
- the **potential exposure** of the species to the pollutant,
- their **representativeness** of the affected area,
- possibly their **status** as a consumed/commercial and/or heritage species.

[33] (E.g. several mm, approx. 1 cm, etc.; from a few specimens to several dozen – or even hundreds – per m^2; regular or aggregated distribution, etc.)

On the hard substrates of estuaries in the North-East Atlantic, a few species of invertebrates of the macrofauna present these characteristics and are therefore liable to be **potential candidates** for ecotoxicological monitoring, for instance:

- the bivalves *Mytilus edulis*, *M. galloprovincialis* and *Crassostrea gigas* common on hard estuarine substrates, commonly present in estuaries, at least in polyhaline areas.
- the gastropods *Patella vulgata*, *Littorina littorea* and *L. saxatilis*, at least in polyhaline areas.

Evidently, other "candidate" species can be selected based on **existing knowledge** (e.g. inventories, monitoring in progress...) in terms of the macrobenthos (representativeness, biology, etc.) within the affected area.
In the case of hard substrates, the monitoring of **sentinel species** may be recommended, in particular, among benthic invertebrates, the filter-feeding bivalve *Mytilus* sp., or **exploited/consumed species** (*C. gigas*) also often targeted as part of health monitoring programmes (potentially providing contamination data).

<u>Parameters to be measured:</u>
Firstly, if **data series** collected as part of routine monitoring of the estuary environment quality, on a specific biomarker and species, are available in the affected area, it is recommended that the possible appearance of **anomalies in the basic fluctuations** of this biomarker be examined soon after the spill, even if this biomarker is not thought to be specific to oil pollution.

The assessment of the biological effect of a pollutant on sentinel bivalve molluscs (mussels, *Mytilus* sp.) or other potentially candidate species (mentioned above) may be implemented through **a crossover study of a set of biomarkers**, whether specific or not to the impact caused by PAHs.

If possible, according to the spill context (e.g. applicability to species present), these biomarkers will be preferentially selected on the basis of **recommendations** made by international expert groups (e.g. the Joint Assessment and Monitoring Programme, of the OSPAR Convention; the International Council for the Exploration of the Sea (ICES), etc.).

In this regard, we suggest the application as a priority of a set of biomarkers (listed in the table below):

- of **exposure**, recognised as controls of contact with the major contaminants of aquatic systems, including hydrocarbons,
- of **genotoxicity**,
- of **effects** (not specific, however, to a type of contaminant),
- and associating the investigation of **short term** effects (hours, days) and **medium/long terms** effects (weeks, months).

	Biomarkers	Biological function	Recommendations (JAMP or ICES)	Application to taxonomic groups		
				Bivalves	Gastropods	Crustaceans
Exposure	Acetylcholinesterase (short term response)	Neurotoxic effect: general stress marker indicating the organism's physiological status	Yes	X	X	X
	Metallothionein (short term response)	Metal detoxification and aromatic organic compounds, inductors of cellular oxidative stress	Yes (additional method)	X	X	X
Genotoxicity	Micronucleus (long term response)	Formation following the breaking of DNA strands by exposure to clastogenic and aneugenic substances	Yes	X	X	X
	Comet assay (long term response)	DNA strand breakage	Yes (additional method)	X	X	X
Effects	Lysosomal stability (short and long term response)	Subcellular damage. Good pathology predictor: indicates a relationship between exposure and pathological effects	Yes	X	X	X
	Histopathology (long term response)	General control response to pathological effect (e.g. digestive glands, gonads)	Yes	X	X	X

Overview of recommendations of the main biomarkers for benthic invertebrates (N.B. indications established based on experience, suggesting their suitability for the current issue. This list is not restrictive but rather provides an indication).

Strategy

- The biological responses obtained will be compared:
  - with analogous/comparable data: (i) from prior to the spill (reference data i.e. base level of indicators chosen where they exist) or (ii) from "control" (unpolluted) sites, where such sites can be established, and/or
  - between stations with different levels of contamination (examination of relationships with PAH contents, for instance) and
  - between successive sampling dates, implemented at a suitable frequency on the time scale of the biological process examined (i.e. high for initial short term response biomarkers, lower for long term responses)
- As previously mentioned, it is important to ensure the acquisition and availability of relevant data on **contamination of adjacent sediment, water mass** and **biota** for the stations monitored – either by including this aspect in the ecotoxicological study or by drawing upon the results produced within the framework of a distinct but coordinated study that forms part of the assessment programme. For hard substrate invertebrates, we note the existence of the coastal chemical contamination observation network

ROCCH (formerly RNO) coordinated by Ifremer, which provides contamination data on the three matrices (water, sediment, biota) in transitional and coastal waters.
- Similarly, it is also beneficial to combine the biomarker responses with the environmental data acquired at the stations monitored (temperature, salinity, dissolved oxygen...).
- The continuation of ecotoxicological monitoring beyond an initial annual cycle should be considered according to the results (e.g. effective and persistent response), requiring possible adaptations (reduced sampling frequency, monitoring of certain 'chronic' biomarkers, selection of a certain number of sites, etc.).

Additional remarks:

- **Bioassays**

As highlighted previously (Cf. **A.2.3**), bioassays can rapidly provide elements for risk assessment as well as elements for interpreting the results of impact studies[34].
This additional method to that of in situ impacts on organisms, populations or communities is recommended by implementing a series of bioassays comparable to those proposed previously.
Reference can thus be made to the suggested list in the previous section (Cf. **A.2.3**), with adaptation to the present issue if necessary.

❖ A.3.3 Ecological approach, at community level

Like for soft substrate studies, the monitoring of a certain number of basic rocky substrate macrobenthic community description parameters (species richness, abundance, etc.) can assess evolutions in terms of the quality of the benthic environment. However, experience in this field mainly **relates to the marine environment, and the approach described below is in principle a restricted application limited to polyhaline, or possibly mesohaline, areas.**

Sampling protocols

Wherever possible, methodological consistency should be sought with the protocols adopted as part of existing monitoring programmes on a national scale, in France ReBent in particular[35] - or even on a European scale (e.g. WFD).
As for intertidal rocky substrates, the following guidelines are recommended:

- at each sampling station, sampling should be carried out, where possible, in the upper and middle **eulittoral** area (isolines at the *Pelvetia canaliculata* and *Fucus vesiculosus/Ascophyllum nodosum* belts respectively).
- **non-destructive** sampling should be carried out (*i.e.* without removal), at each level, from 3 to 10 **permanent quadrats** (carefully marked and easily locatable – for instance georeferenced by GPS) with a unit surface area of 0.1 m^2 and defined on surfaces that are as **homogeneous** as possible (exposure, crevices, overhangs, etc.), in order to **minimise biases related to the habitat's spatial variability**.
- for the monitoring of small, possibly abundant species (e.g. barnacles, small species of gastropods), random subsampling may be performed within sub-quadrats defined by a quadrat mesh of 0.1 m^2.

[34] It is important to remember once again that the aim of bioassays is not to extrapolate the results in terms of effects induced *in situ*, but rather to determine the potential toxicity of the environments tested.
[35] Hily C., Grall J., 2006. Suivi stationnel des estrans rocheux (faune). FT-05-2006-01. http://www.rebent.org//medias/documents/www/contenu/documents/FT05_Hily_Rebent_Rocheux_2006.pdf

Parameters

There are relatively few reliable, established indicators in rocky benthic environments, and experience of post-oil spill monitoring studies has shown how difficult it can be to distinguish natural variation from that due to an effect of the spill – particularly in the absence of reference data.

However, the assessment of the effects of a spill on the composition of the benthic epifauna can be attempted by monitoring fluctuations, within each sample/quadrat, of:

- the **list/number of taxa present**, identified if possible at species level, in particular those **characteristic** (most frequent/abundant) of the sites studies,
- the **abundances** of the species present, established:
  - by evaluating the **percentage cover** of species of attached flora and fauna (semi-quantitative index), consistent with pre-existing programmes where they exist (e.g. Rebent; Cf. table below),
  - by **counting** individuals of attached or mobile species, within 0.1 m^2 quadrats for large fauna (e.g. limpets), or within sub-quadrats for small fauna.

Cover index	Percentage cover
0	0 (absence)
1	0-5 %
2	5-25 %
3	25-50 %
4	50-75 %
5	75-100 %

Correspondences between cover percentages and indices, as defined for Rebent
(*Source: Hily C., Grall J., 2006. Suivi stationnel des estrans rocheux (faune). FT-05-2006-01*.
http://www.rebent.org//medias/documents/www/contenu/documents/FT05_Hily_Rebent_Rocheux_2006.pdf)

Strategy

- The data obtained will be compared, grouped by sampling station:
  - with analogous/comparable data (i) from prior to the spill (reference data) or (ii) from "control" (unpolluted) sites, where such sites can be established, and/or
  - between stations with different levels of contamination and
  - between successive sampling dates, implemented on a seasonal basis for at least 1 year (inclusion of a reproductive cycle), so as to identify any medium term impacts, or the occurrence of restoration processes (e.g. data comparison at t_{+1year}).
- comparison and monitoring can focus on various criteria, in particular:
  - the list of the most abundant taxa (characterising sediment),
  - the relative abundances of the main species (limpets, barnacles, winkles),
- The acquisition and availability of data on the level of oiling of the hard substrates monitored or the contamination of their immediate surroundings must be ensured – either by including this aspect in the ecological study or by drawing upon the results produced within the framework of a distinct but coordinated study that forms part of the assessment programme.
- The continuation of this type of monitoring beyond 1 year post-spill should be considered according to the results obtained at t_{+1year}, requiring possible adaptations (reduced sampling frequency, selection of a certain number of sites, etc.) to be considered on a case by case basis. A minimum frequency of 2 samples per year is recommended (early spring (March/April) and late summer (September/October)).

Advantages and disadvantages

While the approach aims to produce an overview of the state of intertidal hard substrate epibenthic communities, it has the disadvantage of possibly requiring a high level of expertise (e.g. taxonomy) and demands a certain amount of time to process the results (determination, counts, etc.). It is also important to remember the above-mentioned reservations (Cf. **General considerations/difficulties** at the beginning of § A.3) in terms of the difficulty in clearly identifying impacts, in relation to the high natural variability within this compartment.

Sheet B - RECOMMENDATIONS for assessing impacts on the ICHTHYOFAUNA

Fish are a component that is **almost systematically included in oil spill impact assessment programmes** in the shoreline environment. The reasons for this are outlined above (Cf. literature review on experience in this field) and, while mainly related to marine and shoreline pollution, remain relevant in estuary environments. The accomplishment of certain stages of the biological cycle, for many species of both freshwater and marine ichthyofauna, is closely linked to the maintenance of the ecological function of the estuary: nursery for juveniles, breeding grounds for adults or migration corridor for amphihaline species, etc. Due to this sensitivity and potential exposure, the assessment of the effects of major estuarine pollution on the ichthyofauna is therefore recommended with regard to the functional, economic, heritage etc. importance of this component.

B.1 - Selection of organisms within the "fish" component

The sensitivity of fish to oil is known, and the risk of exposure to the pollutant is generally higher in shallow coastal areas, as well as being related to the rate of renewal of the water mass (case of semi-confined environments compared to open sea for instance) – this typology corresponds to **muddy estuarine habitats** for which it is worthwhile implementing ichthyofauna monitoring efforts.

Furthermore, the risks of impacts on the ichthyofauna are related to the **stages of development** present in the affected area; larvae and juveniles – possibly abundant at coastal and estuarine sites (e.g. nurseries in outer estuary areas) – are more sensitive and vulnerable to spilt contaminants than adults. Thus when a **spill coincides with a critical phase of the biological cycle** (e.g. egg-laying, hatching, migration...) this may provide one of the priority selection criteria for one or more species.

In response to logistical constraints (especially the extent of the monitoring programme) or the relevance of monitoring efforts (selection of species liable to determine whether or not the spill has had effects), it may be desirable, if not necessary, to determine monitoring priorities, which may depend on:

- **The potential exposure of species**. In relatively sheltered, soft sediment, shallow coastal areas as is the case of estuaries, demersal fish, in particular flat fish living at the water/sediment interface, are in principle more exposed to pollutant than pelagic fish[36] (particularly when the pollution is transferred to the bottom). In real cases of spills, the ethology of flat fish has caused them to be a priority monitoring target, due to their supposed integrative nature and their role as an indicator of change in the benthic environment (e.g. study of individual biological responses).
- **Prior knowledge of biological cycles.** Such knowledge can be a determining factor of the study's potential to determine any impacts, in particular when the stages of development (e.g. larvae, juveniles) and/or descriptors (e.g. abundance, enzyme activity, etc.) investigated are known to fluctuate under the influence of factors external to the incident which can therefore be confusing.

As concerns the second point, it is important to select – where possible and relevant – species which are the focus of monitoring programmes liable to provide long term data series, as part of **conservation** measures (e.g. reintroduction measures/maintenance of amphihaline populations) and/or **resource management** (e.g. fished species).

[36] As the water column is a more open habitat, whose contamination is in theory relatively short-lived *following a spill*, in connection with the extent of physical processes promoting the natural dispersion of the pollutant and preventing the persistence of noxious concentrations.

The targeting of **consumed** species is doubly useful, as it may be complementary to risk assessments for human health, generally conducted in the event of significant pollution (assessment of the need for bans on fishing, sales, etc.), and can be a source of additional data (in particular fish meat contamination).

In short: In terms of the prioritisation of the assessment of impacts on the ichthyofauna in estuary environments, the above-mentioned points therefore lead us to recommend, *as a general rule*:

- **priority** monitoring of populations of **demersal fish**, in particular benthic fish, on **sites/habitats** which:
  - are **potentially exposed** (e.g. potential areas where the pollutant may settle) and/or
  - have a specific **functional importance** within the biological cycles (e.g. nursery area)
- **possible** monitoring of pelagic fish populations, on a case by case basis if this issue is felt to be of interest.

Preferentially, the generally high natural variability of fish populations advocates the **selection of species (or groups of species) for which reference data series are available** (most often species of commercial or heritage value).

B.2: Assessment methods for the fish compartment (bentho-demersal and pelagic)

General considerations/difficulties

When designing monitoring programmes based on the recommendations provided below, it is important to bear in mind the difficulty raised by the **natural variability** of the processes studied in fish populations, which can prove **relatively high** at various levels of biological organisation (e.g. recruitment variability, under the influence of climate variations or fishing pressure on exploited species; variability at sub-individual level in connection with biological cycles or the, possibly chronic, presence of xenobiotics other than the oil spilt...). This constraint, which is common in fishery resources monitoring, can, in the context of a study aiming to attribute phenomena observed to a spill, be partially reduced by:

- Selecting organisms whose ecology and biology are already known, and/or
- Obtaining pre-spill, and if possible relatively recent, reference data series and/or
- Monitoring, if possible, "control" sites (unpolluted) identified as comparable to the polluted sites.

Different types of approaches can be implemented to identify the impacts generated on the ichthyofauna: the ecological approach (at population and/or community level) and the biological approach (at target species (sub)individual level), detailed below.

Note: In an emergency (first days/weeks after the incident) and if possible, the collection of qualitative information (through field surveys) aiming to (i) characterise the species *visibly* affected (e.g. distress phenomena, mortality, etc.) and (ii) estimate, roughly and as an initial indication, the extent and location/extension of such phenomena, is recommended in as far as this information can guide the decision-making and definition processes for the monitoring programme(s) to be implemented and proposed below.

❖ B.2.1 Ecological approach, at target species population level

Choice of species:

Applicable to different haline areas of the estuary environment, this approach consists in monitoring the **dynamics of populations** of the target species according to:

- their **sensitivity** to oil pollution, which may be:
    - known from experience or
    - potentially significant due to their exposure and life cycle, in the case of species which (i) develop completely or partially (case of catadromous/anadromous species) in estuaries and/or (ii) are ethologically linked to shallow turbulent water habitats, or even living at the water/sediment interface, notably **benthic flat fish**.
- their **commercial** and/or **recreational importance**.

In the estuaries targeted here (North-East Atlantic), a few species of benthic fish are liable to be **potential candidates** for this type of monitoring; we can make mention for instance of:

- righteye flounders:
    - the European plaice *Pleuronectes platessa*, a flat fish present in estuaries up to the salt intrusion limit,
    - the European flounder *Platichthys flesus*, a flat fish that is tolerant to low salinities and relatively common in estuaries, where individuals stay during the first years of their development,
- the sole *Solea solea*, a species present in downstream estuary areas and of particular commercial importance,
- …

Other pelagic species are known to be potentially present in tidal estuarine systems in the geographical area considered (e.g. sea bass, pout, stickleback...) and, locally, the identification and selection of candidate species for population monitoring should draw upon **existing knowledge** (e.g. WFD inventories, fishing statistics, monitoring programmes in progress...) of the species (representativeness, biology, etc.) within the affected area.

Parameters to be measured:

The basic measurements to be implemented within these populations mainly aim to determine whether or not the pollution has affected the **abundance of individuals** or the **population's demographic structure**; they are biometry measurements (**length**, **weight**, etc., possibly sclerochronology) and should enable the monitoring of factors such as:

- abundance or biomass (related to the fishing effort – commonly expressed by the CPUE[37]),
- size frequency distribution, for the identification of the possibly variable effects according to age classes (e.g. case of disturbances in the year's recruitment),
- synthetic descriptors of the physiological state of individuals (e.g. condition index),
- mortality rate, growth rate, etc.

It is important to ensure **(i) the acquisition – or availability – of analogous, relevant** (e.g. recent for pre-spill data) **reference data** in order to minimise ambiguities due to natural fluctuations, and **(ii)** the possibility of examining the relationships between the phenomena observed and the **oil contamination** (e.g. sediment, benthic invertebrates) – either by including this aspect in the ecological study or by drawing upon the results produced within the framework of a distinct but **coordinated**[38] study that forms part of the assessment programme.

[37]Catch Per Unit Effort
[38](Consistency of sampling sites and frequencies).

Strategy:

- The results obtained at the polluted stations will be compared:
  - with analogous/comparable data (i) preferably from prior to the spill (reference data) or (ii) if not, from "control" (unpolluted) sites, where such sites can be established, even although the comparison of polluted sites and unpolluted sites is a delicate task given the high natural variability of fish populations and/or
  - between points (stations) with different levels of contamination (examination of relationships with PAH contents, for instance) and
  - between successive sampling dates, implemented **on a seasonal basis for at least 1 year** (inclusion of a reproductive cycle), so as to identify any medium term impacts, or the occurrence of restoration processes (e.g. data comparison at t_{+1year}). The sampling plan may be reviewed (scaled up or down) according to financial and field constraints, while taking account of local specificities.
- It is also useful to monitor **the basic parameters** of water masses (temperature, salinity, conductivity, dissolved oxygen, etc.) for each station.
- The continuation of this type of monitoring beyond 1 year post-spill should be considered according to the results obtained, requiring possible adaptations (reduced sampling frequency, selection of a certain number of sites, etc.) to be considered on a case by case basis.

Sampling protocols:

There is currently a **standardised sampling protocol** defined and validated by IRSTEA[39] for fish inventories in transitional waters, and which should be drawn upon for sampling in relation to the ichthyofauna.

Broadly speaking[40], we note that:

- the sampling tool required for this task is the beam trawl, whose size should be chosen according to the size of the estuary (e.g. 3 m is the recommended size for large estuaries (Seine, Loire, Gironde), while 1.5 m is recommended for moderate to small estuaries)
- trawling should be carried out against the current, at an absolute speed of between 1.5 and 3 knots according to the dimensions of the gear, for 15 minutes during tidal coefficients of less than 90, at various depths.
- 6 to 8 hauls are recommended per haline area concerned by the pollution.

It is important to note that these sampling techniques were defined, tested and validated during fish inventory campaigns for the WFD and therefore data on ichthyological communities has been acquired using this methodology for a certain number of transitional water masses.

Advantages and disadvantages:

While this approach has disadvantages in terms of logistics and its relatively complex implementation (campaigns at sea), it can nevertheless be coupled in terms of its organisation with other studies at individual level (ecotoxicology) or community level. Furthermore, this approach does not require complex data measurements or analytical procedures, except for the sclerochronological analysis of growth rates (preparation and interpretation phases in particular).

[39] French National Research Institute of Science and Technology for Environment and Agriculture (formerly CEMAGREF).

[40] Cf. AFNOR. Qualité de l'eau - Échantillonnage au chalut à perche des communautés de poissons dans les estuaires. XP T90-701. Paris: AFNOR, 2011, 15 p.

❖ B.2.2 Ecotoxicological approach, at target species level

Within the framework of post-spill impact assessments on the "fish" compartment, like for benthic endofauna, approaches can be implemented aiming to identify the appearance of sublethal effects in the individuals of a given species: these effects, although tricky to characterise, can be expressed on different scales (e.g. organic to subcellular, or even molecular) and vary in their specificity to the pollutant in question.

There are many varied techniques (e.g. biomarkers of exposure, effect, histopathology, etc.) that can potentially be applied and their relevance can vary according to the specificities of the pollution (spill characteristics, oil composition and fate, etc.).
The recommendations provided in this paragraph therefore:

- focus on **the main features of a general minimum approach**,
- are partly based on currently identified experience in terms of oil pollution; they are therefore **neither exhaustive nor restrictive**.

Choice of species:

Over and above the general recommendations to be followed (Cf. above **§B.2 General considerations/difficulties**), the organism selection criteria are similar to those mentioned in the previous section (Cf. **B.2.1**), i.e.:

- their known **sensitivity** to oil pollution (probable induction of a response),
- the **potential exposure** of the species to the pollutant (e.g. benthic fish),
- possibly their **status** as a consumed/commercial and/or heritage species.

In the estuaries of the North-East Atlantic, a few species of fish present these characteristics and are therefore liable to be **potential candidates** for ecotoxicological monitoring. The following can be chosen for instance:

- preferably, flat fish such as the European flounder (*Platichthys flesus*), the European plaice (*Pleuronectes platessa*) or the sole (*Solea solea*),
- secondly and if necessary, pelagic fish such as sea bass (*Dicentrachus labrax*) or pouting (*Trisopterus luscus*),
- …

If they are present, the monitoring of **sentinel species** is recommended, in particular among benthic fish such as flounders, a species abundantly present in estuaries and widely studied in ecotoxicology, or sole.

Parameters to be measured:

Firstly, if **data series** collected as part of routine monitoring of the estuary environment quality, on a specific biomarker and species, are available in the affected area, it is recommended that the possible appearance of **anomalies in the basic fluctuations** of this biomarker be examined soon after the spill, even if this biomarker is not thought to be specific to oil pollution. Certain data obtained through estuary research programmes can also help to establish the pre-spill status.

The assessment of the biological effect of a pollutant on sentinel fish (flounder, *Platichthys flesus*) or other potentially candidate species (mentioned above) may be implemented through **a crossover study of a set of biomarkers**, whether specific or not to the impact caused by PAHs.

Wherever possible, according to the spill context (e.g. applicability to species present), these biomarkers will be preferentially selected on the basis of **recommendations** made by international expert groups (e.g. the Joint Assessment and Monitoring Programme (JAMP), of the OSPAR Convention; the International Council for the Exploration of the Sea (ICES), etc.).

For the fish compartment, reference should be made to the JAMP document recommending the monitoring of PAH-specific biological effects[41], based however on a context of chronic contamination, but also to the document by Martinez-Gomez et al (2010)[42] published by ICES relating to accidental pollution. These two documents are summarised in the tables below.

In this regard, we suggest the application as a priority of a set of biomarkers (listed in the table below):

- of **exposure**, recognised as controls of contact with the major contaminants of aquatic systems, including hydrocarbons,
- of **genotoxicity**,
- of **effects** (not specific, however, to a type of contaminant),
- and associating the investigation of **short term** effects (hours, days) and **medium/long terms** effects (weeks, months).

[41] JAMP (Joint assessment monitoring programme) 2009. Guidelines to contaminant-specific biological effects (OSPAR agreement 2008-2009). Technical annex 2: PAH-specific biological effects monitoring. OSPAR convention for the protection of the marine environment of the North-East Atlantic. Technical annexes of monitoring guidelines. Ref No:2008-9. 48pp.

[42] Martinez-Gomez C., Vethaak A.D., Hylland K., Burgeot T., Köhler A., Lyons B.P., Thain J., Gubbins M.J. et Davies I.M. 2010. A guide to toxicity assessment and monitoring effects at lower levels of biological organization following marine oil spills in European waters. ICES Journal of Marine Science, 67:1105-1118.

	Biomarkers	Biological function	Recommendations	
			JAMP	ICES
Exposure	Cytochrome P450A1/EROD	PAH detoxification enzyme activity (activation phase 1 of metabolisation activities)	X	X
	PAH metabolites	Exposure to PAHs and metabolisation	X	X
Genotoxicity	DNA adducts (long term response)	Formation of bonds to DNA following exposure to DNA-reactive components	X	X
	Comet assay (long term response)	DNA strand breakage		X
Effects	Lysosomal stability (short and long term response)	Subcellular damage. Good pathology predictor: indicates a relationship between exposure and pathological effects		X
	Antioxidant enzyme activities (CAT, SOD, GPx…) (short term response)	Cellular response to oxidant stress		X
	Malondialdehyde (long term response)	Lipid breakdown substance (lipid peroxidation: cellular response to oxidant stress)		X
	Liver histopathology (long term response) including macroscopic hepatic neoplasms	General responses indicating a pathological effect	X	X
	External signs of disease (including fin lesions)	General responses indicating a pathological effect	X	

Overview of recommendations of the main biomarkers for benthic and pelagic fish (N.B. indications established based on experience, suggesting their suitability for the current issue. This list is not restrictive but rather provides an <u>indication</u>).

Strategy

- The biological responses obtained will be compared:
    - with analogous/comparable data: (i) from prior to the spill (reference data) or (ii) from "control" (unpolluted) sites, where such sites can be established, and/or
    - between stations with different levels of contamination (examination of relationships with PAH contents, for instance) and
    - between successive sampling dates, implemented <u>at a suitable frequency on the time scale of the biological process</u> examined (i.e. high for initial short term response biomarkers, lower for long term responses)
- As previously mentioned, it is important to ensure the acquisition and availability of relevant data on **contamination of the sediment, water mass** and **biota** for the stations monitored – either by including this aspect in the ecotoxicological study or by drawing upon the results produced within the framework of a distinct but coordinated study that forms part of the assessment programme.
- Similarly, it is also beneficial to combine the biomarker responses with the environmental data acquired at the stations monitored (temperature, salinity, dissolved oxygen...).
- The continuation of ecotoxicological monitoring beyond an initial annual cycle should be considered according to the results (e.g. effective and persistent response), requiring possible adaptations (reduced sampling frequency, monitoring of certain 'chronic'

biomarkers, selection of a certain number of sites, implementation of the 'caging' technique etc.).

Additional remarks:

- **Bioassays**

As highlighted previously (Cf. soft substrate endofauna sheet **A.2.3**), bioassays can rapidly provide elements for risk assessment as well as elements for interpreting the results of impact studies.
This additional method to that of in situ impacts on organisms, populations or communities is recommended by implementing a series of bioassays comparable to those proposed previously.
Reference can thus be made to the suggested list in the previous section (Cf. **A.2.3**), with adaptation to the present issue if necessary.

❖ B.2.3 Ecological approach, at community level

The search for alterations to the structure of fish communities, considered as an indicator of the estuary environment quality, is a potential approach in order to evaluate the impact of a spill.
This method consists in monitoring the evolution of descriptors/metrics of the structure of assemblages, or else of synthetic indices incorporating the descriptors – offering a more integrative and functional approach.

Parameters:
Certain basic community structure parameters such as **biomass**, **abundance** or even **species richness** (number of species) can descriptively characterise various aspects of fish populations. Other metrics draw upon notions of **ecological**, **trophic or position guilds**, and provide a vision of the functional diversity of communities. These descriptors are generally aggregated for the calculation of indicators/indices.
In this respect, there are different types of indices to characterise fish communities, associating different numbers of metrics (monometric or multimetric). Among the best known indices we can make mention of the Biological Health Index (**BHI**), the Index of Biotic Integrity specific to fish communities (**IBI for fish**) and the French Estuarine and Lagoon Fish Index (**ELFI**[43]) developed through the WFD and based on a pressure-impact approach. Note that, to the best of our knowledge, these indices have never yet been applied for an oil spill impact assessment. Their use remains worthy of consideration, as they are in principle relevant where reference series exist for a comparative approach (identification of variations in relation to a baseline).
The acquisition of metrics should therefore be taken into account according to the calculation of the index and the issue it is intended to address.

Sampling protocol:
Wherever possible, the standardised sampling protocol developed by IRSTEA for estuarine fish should be followed, as outlined above (Cf. **§ B.2.1**), and may be used jointly for ecological (populations and/or communities) and ecotoxicological approaches, in order to pool resources and reduce costs.
During sampling, all catches will be identified down to the species, always in an equivalent way.

[43] Delpech, C., Courrat, A., Pasquaud, S., Lobry, J., Le Pape, O., Nicolas, D., Boët, P., Girardin, M., Lepage, M., 2010. Development of a fish-based index to assess the ecological quality of transitional waters: The case of French estuaries. Marine Pollution Bulletin 60, 908–918.

Strategy:

- The data obtained (e.g. abundance, biomass, species richness) will be compared, grouped by sampling station:
    - with analogous/comparable data (i) from prior to the spill (reference data). In the case of an ichthyological community approach, the comparison of "control" (unpolluted) sites with polluted sites is advised against, unless a sufficient and substantial number of control sites are available to determine the levels of natural variability.
    - between stations with different levels of contamination (examination of relationships with PAH contents, for instance) and
    - between successive sampling dates, implemented on a seasonal basis for at least 1 year (inclusion of a reproductive cycle), so as to identify any medium term impacts, or the occurrence of restoration processes (e.g. data comparison at t_{+1year}).
    - comparison and monitoring can focus on various existing indices as mentioned above (e.g. ELFI, etc.) **to be selected/validated by experts according to their potential relevance with regard to the spill context** (Cf. above remarks) and/or previous knowledge, the availability of reference data series, etc.

Advantages and disadvantages:

While this approach can prove complex to implement (campaigns at sea, arduous sampling), it aims, in principle, to obtain an overall diagnosis of the health status of ichthyological communities and their rehabilitation. Although undeniably worthwhile, it is nevertheless important to bear in mind:

(i) the potential pitfalls, inherent to the typically high variability of fish populations (Cf. **§ B.2 General considerations/difficulties**) and therefore also dependent on the level of *pre*-spill knowledge, and

(ii) the lack of concrete experience – to the best of our knowledge and at the present time – of the response, in the case of an oil spill, of "fish indicators" based on the community structure.

SHEET C - RECOMMENDATIONS for the assessment of AVIFAUNA

Bird populations are a component that is almost systematically included in oil spill impact assessment programmes in both shoreline and estuarine (case of the pollution of the Loire estuary in 2008) environments. The rationale behind these monitoring programmes depends, firstly, on the exposure and vulnerability of certain species to oil. Although in principle less exposed than many marine species[44], estuarine avifauna may be the focus of impact assessments due to (i) their position in the food chain (liable, according to the ethology of the species, to suffer indirect contamination by the ingestion of contaminated prey) and/or (ii) the presence of species with a special status in terms of conservation.

Finally, the integration of avifauna in an estuary monitoring programme also, and most often, responds to the issue of indirect impacts, in particular in the case of **a temporary alteration of habitats** following clean-up operations. This remark applies more specifically to marsh-dwelling passerines (e.g. the scything of oiled reeds can sometimes be necessary).

C.1 – Selection of types of habitats and avifauna in estuaries:

The selection of natural habitats that may require avifauna to be monitored as a priority can draw upon all or some of these criteria:

- their **functional importance** (feeding areas, breeding areas, etc.) in relation to bird populations,
- their **representativeness**/extent within the areas affected by the pollution,
- their **observed exposure** to the pollution (oiling),
- their **possible disturbance due to the clean-up sites** required,
- the **coincidence between the spill and the presence of birds in the habitat** (in connection with a particular stage in the biological cycle).

Given these *a priori* criteria, certain estuarine bird habitats potentially appear as priorities, for which the need to implement impact studies should be specified **according to the specific context of each incident** (e.g. correspondence between the time of the spill and the biological cycle/presence of species, etc.). Particular consideration should be given to:

- **intertidal mudflats**: potentially exposed to contamination (pollutant deposited or infiltrated), they provide the basis of the diet of migrating water bird populations: Anatidae, Rallidae and especially waders (of which they are probably home to the most abundant and diversified populations on the French Channel and Atlantic coasts). These birds feed at low tide on the benthic invertebrates (polychaetes, gastropods, bivalves, etc.) that populate these coastal or estuarine (mainly downstream part) mudflats during part of their annual cycle. These species are mainly northern and Arctic and, in the targeted estuaries, their populations generally peak during the non-breeding season, i.e. between **early autumn and early winter** according to species. Also, the intertidal mudflats of the large estuaries of the Channel and Atlantic are sites of national or

[44] Exposure of marine species living at the water/air interface (e.g. divers such as auks) to drifting slicks.

even international importance for temperate species, including the Common Shelduck and the avocet.

- **reed beds** (on the upper foreshore, wetlands or marshes bordering polyhaline waters): submersible and therefore potentially exposed to pollutant deposits, they are home to large populations of marsh-dwelling passerines (nutritional function and shelter from predators), for all or part of the year according to biological cycles, with particular sensitivity during the **spring breeding period**, generally between April and June for the estuaries targeted. Reed beds also have a functional importance as a migratory stopover, in particular in the **autumn**, thus another potentially sensitive period.

The need to monitor one and/or other of these habitats should be assessed based on surveys, to first check that:

- the pollution has spread to the habitats mentioned (reed beds and/or mudflats),
- the sites are visited/used by bird populations (nesting, hibernation, etc.) at the time of the incident,
- individuals are exposed (reports of dead birds, oiling) or are at reasonable risk of exposure.

In addition to these checks, **the consideration of changes to the habitat in connection with possible operations required for clean-up** (for instance scything of oiled reed beds to remove pollutant)[45], may reinforce the decision to implement monitoring for a given habitat/site.

C.2 - Assessment methods for avifauna in mudflats or reed beds

General considerations

Compared to seabirds, various considerations suggest that it may be less difficult to identify fluctuations in shoreline populations that can be attributed to a spill. Particular mention can be made of:

- the generally lower natural variability than in marine species,
- the higher probability of having exploitable reference data, due to the existence of surveillance networks as part of various frameworks (e.g. international networks, management measures for sites of special interest – nature reserves, bird sanctuaries, etc.) promoting the collection of up-to-date data,
- in direct correlation with the previous point, the availability of standardised protocols suitable for the annual monitoring of hibernating or nesting populations.

Ideally, it is recommended[46] that monitoring be implemented where it is possible to draw upon:

- the availability of relevant (up-to-date), pre-spill biological or population reference data,
- the inclusion of "control" (unpolluted) sites comparable to the polluted sites[47],
- the consideration of habitat changes that can be attributed to variables other than the pollution (e.g. extension/reduction of mudflats or reed beds due to variability in practices and uses).

[45] Example of the pollution of the Loire estuary in 2008, in particular.

[46] Recommendations supported by the experience of the monitoring of marsh-dwelling bird life in the Loire estuary following the 2008 spill.

[47] Such a "spatialised approach" is not however relevant to waterbirds, as the available data covers the total number of birds of each species frequenting an estuary (functional site), rather than the use of different mudflats within an estuary.

The **collection of quantitative information in an emergency** (through field surveys in the first days/weeks after the spill) is recommended in as far as it can usefully guide monitoring choices according to the recommendations provided below. Data collection should aim to:

- characterise the species *visibly* affected (e.g. waders, marsh-dwelling birds, etc.),
- estimate, roughly and as an initial indication, the extent (e.g. oiling, mortalities, disturbance, etc.) and geographical spread of these phenomena.

❖ C.2.1 Ecological approach, at target species population or community level

Choice of species:

The risks of direct exposure of coastal bird life are, generally, minimised by avoidance behaviour (and/or by the disturbance caused by clean-up operations), even if contact with the pollutant can in principle be caused by oiled materials (e.g. plant debris, tide mark, etc.) or by the ingestion of contaminated prey (e.g. contaminated benthos on mudflats). Furthermore, the oiling of birds' feathers is, in principle, less penalising for the survival of shorebirds than for marine species (loss of buoyancy, thermal insulation, etc.).
In waterbirds like in marsh-dwelling passerines in estuaries, the risks of impact are in theory less directly linked to their specific vulnerability to oil than indirectly to the **alteration of the functionality of the polluted habitats** with which they are associated (e.g. feeding, resting, shelter, etc.).

For these reasons:

- the targeting of bird monitoring relies less on the selection of bird species and more on the selection of **affected habitats** – a risk source for avifauna (Cf. selection criteria in § C.1),
- we recommend, according to the specific context of each spill:
  - a monitoring programme covering all wading or marsh-dwelling species present in the assessed habitats (mudflats or reed beds),
  - where relevant, a detailed analysis can focus on the species with one or more of the following characteristics:
    - most abundant populations, **representative** of the habitats monitored,
    - of **heritage value**, due to a special conservation status on a regional, or even European or global, scale,
    - considered as **quality indicators of the affected environment** (e.g. Avocet, related to the benthic environment quality in the Loire indicators),
    - in connection with the previous points, that are the focus of **pre-existing monitoring programmes** (long term surveillance) liable to provide reference data series in relation to their biology.

We recommend calling upon the expertise of local ornithologists (knowledge obtained from inventories, monitoring in progress, etc. in terms of the populations present within the affected area) to assess, according to the specific context of the spill, the relevance of implementing a monitoring programme targeting one or more candidate species.

Parameters to be measured:

The measurements to be taken within shorebird populations are mainly based on censuses of the **number of species** and **number of individuals** visiting the selected sites. The ultimate aim is to estimate the spatial and temporal variabilities in the use of the site that can be attributed to the pollution (e.g. decrease in total species richness, fluctuation in site use/population redistribution phenomena to unaffected areas, etc.).

In order to interpret possible fluctuations in numbers, these censuses can also include the collection of data on:

- the **number of oiled individuals** (or even the extent of oiling) by species. This approach is nevertheless limited by the visual detectability firstly of birds and secondly of traces of oil (colour of feathers, size of individuals, distance, etc.). It mainly aims to indicate the possibility of contact between individuals and the pollutant, and the persistence of the phenomenon over time,
- where relevant (drastic case), the **number of dead individuals** per species. In this case, the link between the pollution and the dead birds should be examined (in particular through autopsies),
- more generally, **observations of any ringed individuals**, to help to interpret changes in abundance (movements or mortalities),
- the **initial oiling of the sites monitored** (degree affected, form, etc.),
- any **clean-up operations** that may have been implemented (type and chronology),
- any changes to habitats related to factors external to the pollution (e.g. developments, agricultural practices, etc.).

If possible, these types of observations are worth extending to sites (mudflats, reed beds) at varying distances from the affected estuary, in order to identify a possible bird dispersion phenomenon towards other functional habitats[48].

Strategy:

- The results obtained at the polluted sites will be compared:
    - with analogous/comparable data (i) from prior to the spill (reference data) and/or (ii) from similar "control" (unpolluted) sites, where such sites can be established,
    - between successive sampling dates*, subject to the availability of analogous pre-spill data series*[49]:
        - at high frequency (to be appropriately chosen, e.g. weekly, monthly), during the habitat occupation period (hibernation or nesting) following the spill and,
        - at low frequency (annual, during the habitat occupation period) to check, where relevant, for the persistence of long term effects on the number of nesting and/or hibernating birds visiting the site.

Sampling protocols:

The generally recognised importance of wetland birds and the presence of these birds in the targeted estuaries mean that **considerable data exists locally**.

This data is updated at varying frequencies according to its focus, for instance in the case of:

- surveillance programmes coordinated by international organisations (for instance Wetlands International, monitoring in relation to Ramsar[50] sites, etc.),
- community initiatives involving the inventory, upon the initiative of state services (DREAL in France), of the species listed in Annex 1 of the Birds Directive for Special Protection Areas (SPAs)[51] within the network of *Natura 2000* sites (present in the 3 target estuaries),

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[48] Following an oil spill in the Loire estuary, due to a collision between 2 vessels in January 2006, oiled avocets were observed in the Gulf of Morbihan, around 60 km to the north-west, a few days after the incident (Gélinaud G./Bretagne Vivante, personal communication)

[49] Where such data is not available, the attribution of fluctuations in the parameter measured (numbers of individuals, etc.) to the spill is highly likely to prove problematic, if not impossible.

[50] *The Ramsar Convention on Wetlands of International Importance especially as Waterfowl Habitat*.

- national inventories, for instance the French national inventory of natural heritage (*INPN*, of the French national museum of natural history) or nature reserve inventories.

Just as the availability and relevance of such local data (Cf. table of potential "Avifauna" data concerning the target estuaries) should be checked, it is preferable to comply with the methodologies underlying its acquisition.

When surveying bird populations, **standardised methodologies** should be complied with:

- for **waterbirds** (Anatidae, Rallidae, waders) living in mudflats, monitoring should comply with the methods adopted notably within the framework of:
    - the French contribution to the annual International Waterbird Census (IWC) (organised by Wetlands International)[52] – contribution coordinated by LPO and the French national hunting and wildlife agency.
    - the *Observatoire des limicoles côtiers* (shorebird observatory), run since 2000 by *Réserves Naturelles de France* and consisting of monthly counts (conducted based on a standardised methodology)[53].
- for **reed bed passerines**, the semi-quantitative study of "Point Abundance Indices" (PAI) is recommended, measured using the Simple Point Sampling (SPS) technique at "listening posts". Note that the sites should be surveyed by trained observers familiar with the specific procedures of the protocol[54]. This well established[55] protocol, commonly used in ornithology, enables the standardised assessment of spatial and temporal variations in the abundance of nesting populations of common birds "contacted". It is, for instance, recommended by the French research centre on bird population biology (CRBPO) as part of the STOC programme on the temporal monitoring of common birds by the French national museum of natural history.

Advantages:

As mentioned previously, wetlands and the bird populations they are home to are the focus of widely recognised interest and therefore of relatively frequent pre-existing inventories (generally unlike marine species). This has a number of advantages:

- higher potential availability of reference data than for marine species,
- existence of commonly accepted standard methodologies, possibly requiring adaptation to the scale of the affected sites,
- possibility of mobilising local ornithological expertise, with knowledge of the affected sectors and trained in common protocols in terms of bird population censuses (public establishments, local branches of nature protection associations, scientific laboratories, etc.).

❖ C.2.2 Biological approach, at target species level

Where field observations suggest notable impact on a **nesting species** (e.g. mortalities, severe oiling of feathers), and subject to good knowledge of nesting sites, experience in shoreline pollution has shown the potential of certain monitoring programmes to identify a **sublethal effect** on reproduction of the species. These monitoring programmes are usually based on counts of **reproductive**

[51] or European Important Bird Areas (IBAs), destined to become SPAs.

[52] Cf. also Wetlands International, 2010. Guidance on waterbird monitoring methodology: Field Protocol for waterbird counting; http://www.wetlands.org/LinkClick.aspx?fileticket=SzPEwscxuXs%3D&tabid=2791&mid=11794

[53] http://www.aires-marines.fr/Media/Agence/Images/Affiches/Poster-Observatoire-Limicoles

[54] E.g. 2 minutes silence and immobility after arrival at the point, no searching for distant individuals using binoculars, standardised duration of observation, compliance with times and climate conditions, etc.

[55] Blondel, Ferry, Frochot; 1970. Méthode des Indices Ponctuels d'Abondance (IPA) ou des relevés d'avifaune par stations d'écoute. Alauda, vol 38 pp. 55-70.

adults/nesting couples, and/or the measurement of other **descriptors of reproductive success** such as the number of eggs per nest, hatch rate, number of fledged chicks, etc.
However, with regard to the natural variability of reproductive success, the probability of identifying an impact that can be attributed to the spill is dependent on the availability of reference data obtained from pre-existing monitoring programmes, a prerequisite recommended for implementing such an approach. Consequently, and if such a post-spill assessment is felt worthwhile, it is recommended that the methodology used should comply with that adopted by existing monitoring programmes.

Sheet D- RECOMMENDATIONS for assessing impacts on COASTAL FLORA

Coastal plant communities are a component that is often included in oil spill impact assessment programmes, wherever their exposure to oil is reported – for instance due to oil being splashed or sprayed onto supralittoral plant communities (e.g. *Braer* and *Erika* spills) or due to the submersion of eulittoral communities, influenced by tides, in particular marshes (e.g. *Amoco Cadiz* and *Sea Empress* spills, or estuarine formations such as in the Loire following the Donges incident in 2008). Vegetation is considered to be an essential component due, on the one hand, to its **functional** status (primary production, habitat function and bank maintenance) but also, on the other hand, its potentially **integrative** function of fluctuations affecting the environment of which they help to describe the integrity. Finally, certain plant species or groups are of heritage value and may constitute habitats of interest, for instance under the European Habitats Directive. The monitoring of affected flora can therefore also be justified for conservation reasons, if the spill affects an area containing protected habitats and/or species.

These reasons are applicable in estuarine contexts, and justify the **relevance of implementing impact assessments on plant communities** in the event of a spill.

D.1 – Selecting habitats and species:

Estuaries contain varied plant associations, distributed according to the salinity gradient, the type of substrate and the tidal range. Among these habitats, particular mention can be made of pioneer vegetation in sandy or muddy areas, **Atlantic salt marshes**, **reed beds** (phragmites, sea clubrushes or saltmarsh bulrushes), and **subhalophilic meadows**.

Meanwhile, post-spill monitoring of a plant community as a whole can provide answers to the question of **the possible impact of clean-up operations in polluted habitats**. Such monitoring efforts can focus on oiled communities which are potentially sensitive to clean-up techniques, or to unoiled areas that could be disturbed by the set-up of clean-up sites (trampling, vehicle traffic, equipment storage, etc.).

These monitoring efforts can, where relevant, focus on one or more selected species due to their **vulnerability and/or their rarity** (e.g. *Angelica heterocarpa* during the pollution of the Loire estuary) or, on the other hand, their **representativeness** within habitats affected by the oil.

We also note that the monitoring of ***consumed species*** *can be considered to be of interest where a risk for human and/or animal health has been identified. In estuaries, the collection of species destined for human consumption (e.g. marsh samphire) is unlikely due to chronic contamination of the environment, but the question of the transfer of oil compounds (PAHs) to herbivores is highly likely to be raised in the case of grazing on contaminated wetlands or salt marshes) (plant PAH content). This however is less a form of environmental impact monitoring and more a health risk monitoring measure, as part of a separate monitoring programme.*

<u>In short</u>: In terms of the prioritisation of the assessment of impacts on the flora in estuary environments, the above-mentioned points therefore lead us to recommend, as a general rule:

- **priority monitoring** of **plant communities representative of the estuary environment affected** by the spill (and possibly clean-up operations),
- **complementary monitoring**, if necessary, **of a particular species** in response to **conservation concerns in particular.**

D.2 - Plant community assessment methods

General considerations/difficulties

Plant monitoring is based on recognised and commonly accepted protocols, applied in various post-spill contexts, following major coastal or estuarine spills.

Although the methodology of such programmes is relatively common, it is important to remember the importance, when designing monitoring programmes based on the recommendations provided below, of having **reference data series** from prior to the spill, and if possible relatively recent, but also of monitoring **control sites** (unpolluted) – where such sites can be identified – that are comparable to the polluted sites.

Note: In an emergency (first days/weeks after the incident), and to guide the decision-making and definition processes for the monitoring programmes to be implemented, information should be collected (through field surveys) on:

(i) visible signs of alteration, in order to characterise the species *visibly* affected (e.g. defoliation, necrosis, discolouration, etc.)

(ii) to estimate, roughly and as an initial indication, the extent and location/extension of the habitats affected.

(iii) clean-up operations implemented (type and chronology).

❖ D.2.1 Ecological approach, at target species population or community level

Following a spill, the monitoring of plant community status descriptors (e.g. species composition, etc.), liable to highlight any changes induced in the habitat as a whole, can identify the occurrence of impact.

Parameters

Simple descriptors of the plant communities and plant species (taxa) present at the site at the time of monitoring can be used to characterise any post-spill variations. By establishing the following parameters such as **species composition** (species present), the evolving trends in the vegetation can be monitored.

Finer characterisation of plant units can also be implemented through sigmatistic[56] **phytosociological surveys** according to the Braun-Blanquet abundance-dominance coefficients method. This method enables **floristic surveys** to be conducted by identifying all the plant species present (taxa) within a defined area (permanent plots – indicated using a permanent marker – and mapped) and by estimating the **total coverage** of the vegetation then the **coverage of each species** expressed using **abundance-dominance coefficients**. Alongside phytosociological monitoring, **georeferenced photographic** surveys of the monitored areas should be taken for archiving and to contribute to the analysis of the spatial and temporal evolution of the vegetation.

[56] **De Foucault B., 1986.** Petit manuel d'initiation à la phytosociologie sigmatiste. Mémoire n°1 *Soc.Linn.du Nord de la France*, Amiens, 25 pp.
Lahondère, Ch., 1997. Initiation à la Phytosociologie sigmatiste*, Bulletin de la Société Botanique du Centre-Ouest*, Nouvelle série, Numéro spécial 16.

Phenology, i.e. the study of stages of plant development and quality (e.g. plant development, flowering, fruit production, seed production), is also a factor used to check the plant development cycle.

A description of certain **biometric parameters** can also be provided alongside phytosociological surveys: **plant size** (maximum, minimum and average height), which, although subject to variations related to climate and soil conditions, is an indicator of development difficulties, as are **stem diameter** and **density. The existence of unpolluted controls is necessary in order to establish comparisons.**

Where direct or indirect impact results in a significant drop in plant cover, the phytosociological survey may be completed by establishing **contact points** on the monitored plots. This method involves noting the different plant species with which a metal spike comes into contact when inserted into the vegetation at regular intervals along a survey line. This monitoring method is used to determine the **spatial progression** and to identify the vitality and the evolution of the different species present on the plots.

A **map of the vegetation units** monitored could be used to characterise the spread of the pollution of the vegetation and habitats concerned, if human resources and equipment allow, in order to efficiently complete the parameters studied for vegetation monitoring. The CORINE[57] typology as well as a suitable scale for the site (1:25,000 for instance) could be used to describe the vegetation.

In addition to vegetation monitoring, it may be worthwhile characterising the soil in terms of its nature (grain size, chemical composition) or contamination (content of metal elements – nickel and vanadium; PAHs, etc.). These analyses may be carried out where impact indices are reported (e.g. chlorosis, mortalities) and/or to help to explain the possible differences observed in plant descriptors.

Strategy

- The results obtained at the polluted stations will be compared:
  - with analogous/comparable data (i) from prior to the spill (reference data) or (ii) from "control" (unpolluted) sites, where such sites can be established, and/or
  - between stations (plots) with different levels of contamination (choice of sites with moderate to high levels of contamination for instance) and
  - between sites on which different clean-up techniques have been used (self-cleaning, manual clean-up, mechanical clean-up)
  - between successive sampling dates, implemented **at least annually, generally at the beginning of the summer season for phytosociological surveys** (inclusion of flowering for annual plants) and regularly during the vegetation season for the determination of certain species (e.g. early autumn for marsh samphire, spring for early species) or for the inventory of other parameters, so as to identify any medium term impacts, or the occurrence of restoration processes (e.g. data comparison at t_{+1year}).
- In order to study the relationship between the phenomena observed and the spill, it is possible to couple the monitoring with **soil samples** to characterise the type (e.g. edaphic

[57] The CORINE Biotopes typology is a hierarchical classification system for European habitats, developed through the CORINE programme (Coordination of Information on the Environment). The French edition was published in 1997: Bissardon M. & Guibal L., 1997. *Corine biotopes. Version originale. Types d'habitats français*. ENGREF, Nancy, 217 p.

nature) as well as the possible contamination, and in certain cases, with **analyses of plant oil contents**.

- The continuation of this type of monitoring beyond 1 year post-spill should be considered according to the results obtained (the minimum duration taken is generally two plant cycles, including to confirm an absence of impact observed during the first year). Thereafter, possible adaptations (reduced sampling frequency, selection of a certain number of sites, etc.) can be considered on a case by case basis.

Sampling protocols:

The choice of sampling plan (surface area of quadrats, satisfactory number of permanent plots and replicates for the collection of a significant number of locations and statistical testing, etc.) remains to be **chosen/specified on a case by case basis in close connection with the characteristics of the selected communities**. The consideration of these elements (e.g. survey dimension, homogeneous plant cover in terms of floristic structure and composition for phytosociology) in the **definition of sampling procedures** is an aspect that is well known to botanical experts.

Advantages:

This approach has the advantage of being relatively easy to implement (limited logistics), enabling it to be launched earlier and implemented more quickly in the field, and does not require complex data measurements or analysis. Botanical expertise for species identification is however necessary, as well as good field knowledge in order to determine the plant communities or species of heritage value to be monitored. The botanical approach provides relatively medium-term (a few months after its launch) or long-term (> 1 year of monitoring) results.

❖ D.2.2 Biological approach, at target species level

In certain cases and in addition to ecological monitoring of plant communities, monitoring programmes can target species of heritage value or species representative of estuarine habitats if field observations suggest obvious impact.

In addition to monitoring of **phenology** and **biometric criteria** (e.g. size, diameter, densities; Cf. D.2.1), a few rare ecophysiology studies aiming to qualify the health status of target species and to assess the lethal and sublethal effects generated by the oil have been conducted following past spills. Demographic parameters (**growth**, **fertility, survival)** can thus be determining criteria to assess the impact of the oil *in situ.* Meanwhile, laboratory **bioassays** on plants can also be conducted and combined with a series of plant physiology tests (transplantation of roots into contaminated substrate, tests involving spraying with a water/oil mixture, etc.).

The relevance of such an approach should however be **assessed on a case by case basis**, and the strategy will consist in comparing the results with (i) pre-spill reference data or (ii) similar "control" (unpolluted) sites or control groups in the case of laboratory exposure.