



Progress report n°1 – EU-PRAGMA – period covered: 14/02/2006 → 20/11/2006

Grant agreement N°07.030900/2005/429172/SUB/A5 – "A pragmatic and integrated approach for the evaluation of environmental impact of oil and chemicals spilled at sea: input to European guidelines".

Main milestones for the period covered:

- March 27-29, 2006. Kick-off meeting with all project partners at the laboratory facility of IRIS in Mekjarvik (Norway)
- March/April, 2006. Web page creation of the project with address as <http://www.irisresearch.no/pragma>. The webpage is regularly updated and will be used as a platform of data exchange/information between partners.
- May 04, 2006. Presentation of EU-Pragma at the ECOBIM workshop on the use of biomarkers in environmental assessment (Brest, France)
- May 05, 2006. Meeting in Brussel between Commission representatives and the beneficiary represented by Th. Baussant
- April to June, 2006. planning and scheduling of the technical implementation of the project.
- July, 2006. Supplementary agreement N°1 to project grant agreement stating i) modification of completion date of the project (14/09/07) and ii) change of name of beneficiary institution (IRIS).
- August 22, 2006. Start of preliminary work with fish at Cedre (partner 1) together with UBO (partner 2) and with participation of UPV (partner 3) in sampling events.
- September 25, 2006. Stop preliminary study at Cedre due to technical problem and unexpected mortality of fish in exposure tanks.
- September 29, 2006. Start of experimental work with mussel and heavy oil at IRIS (Akvamiljø facility). Oil exposure ongoing. Participation of UPV (partner 3) in sampling events.
- October 13, 2006. Start of experimental work with mussel and styrene at IRIS.
- October 16, 2006. New start of experimental work with fish at Cedre. Oil exposure ongoing.
- November 15, 2006. Completion experimental work with mussel and styrene at IRIS.

A first evaluation and selection of the methods (WP1) used for the implementation of the project was initialised at the kick-off meeting in Norway. Further discussions were made between the partners. Cedre has been assigned the responsibility of the fish study while IRIS will be responsible for the mussel study. UBO and UPV participated in experimental set-up and sampling events. Planning, organisational aspects and technical implementation of the pilot studies (WP2) have been carrying on. Some technical problems have been encountered. Milestones of the technical implementation of the action are described in the next pages.

Technical implementation of the project in partner's institutions and first results

Chemical and biological methodologies are used to assess the environmental effects following spill situations of past ship accidents in EU waters. Pilot studies where marine organisms are exposed to the soluble fraction (WSF) of heavy fuel oil (FO2) and to styrene were initiated in the laboratory. The relevancy of the selected methods and their integration within an environmental index is the ultimate goal.

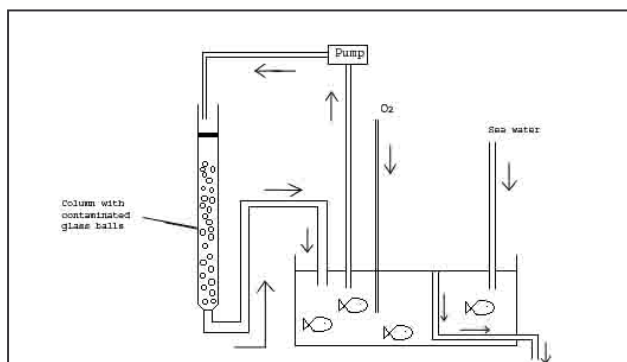


Figure 1 – Schematic description of fuel oil exposure system. Running seawater was supplied at a flow rate of $5 \text{ L}\cdot\text{min}^{-1}$ in each control and exposure tanks.

1. A preliminary experiment with fuel oil and fish has been initiated at Cedre (Partner 1) in August 2006. An exposure system has been set up, as illustrated in figure 1. It was built after a model used at IRIS (see picture of system under paragraph 2). The system

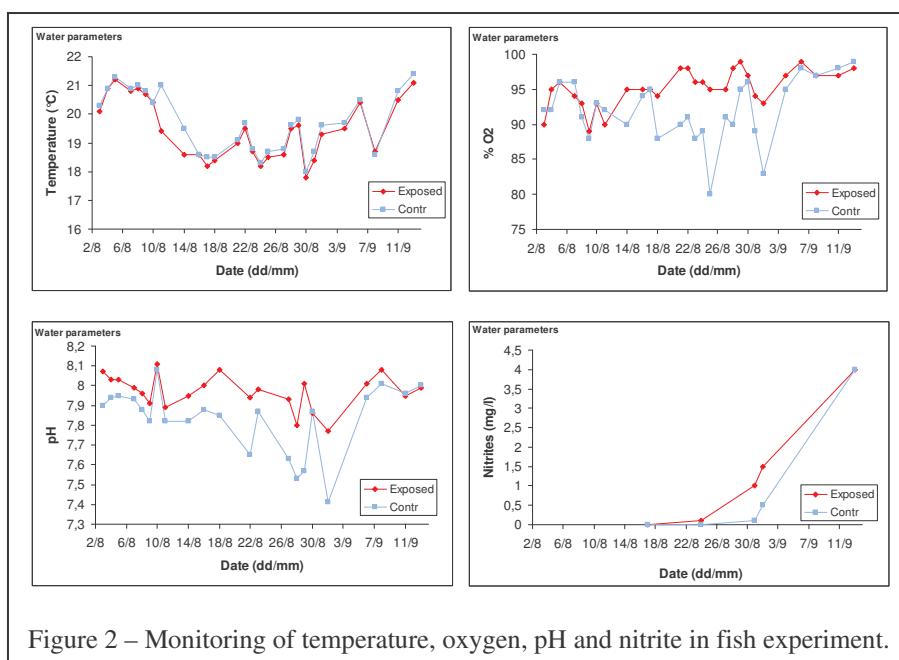


Figure 2 – Monitoring of temperature, oxygen, pH and nitrite in fish experiment.

can generate water-soluble fraction (WSF) of oil. It is made of a column of 2 m high filled with glass beads (25 kg) beforehand soaked with oil: the flow through the column was $0.5 \text{ L}\cdot\text{min}^{-1}$ and total flow in the tank is $5 \text{ L}\cdot\text{min}^{-1}$. Hence FO2 is

supplied at a dilution rate of 1:10. During all the experimental period, the seawater parameters in fish tanks ($\% \text{O}_2$, temperature, salinity, pH) were quite stable except the nitrite concentration (strongly increased in September, see figure 2) which demonstrated a critical accumulation over time.

Preliminary results - Measurements made shortly (within first week) after the start of the exposure to WSF of FO2 showed that

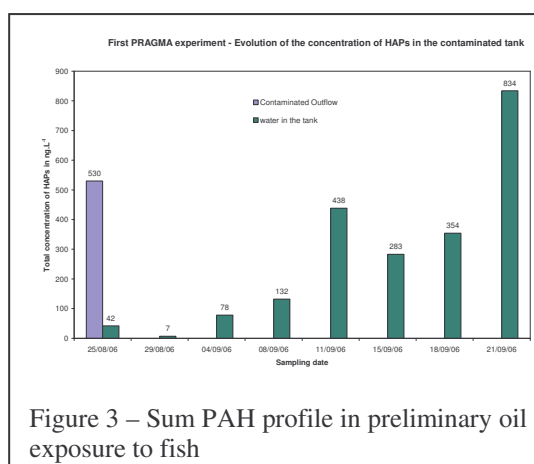


Figure 3 – Sum PAH profile in preliminary oil exposure to fish

exposure of the fish initiated an efficient decontamination system as measured by fluorescence analyses of fish bile samples. Nevertheless, no major changes in physiological parameters in fish were detected within a short period. Later, from the 11th to 25th/09/06, a high mortality was observed: 90 exposed fish died. Yet, this mortality was apparently due to a technical failure in the experimental system leading to high fuel oil concentrations in the exposure tanks (see figure 3; increase in polycyclic aromatic compounds i.e. PAH in water).

Status: A new experiment is planned and currently conducted at Cedre, starting October 2006 (see experimental calendar below). The styrene exposure of fish will be performed at the beginning of 2007.

02-16/10/06 : Acclimatization period of fish (90 + 90 in two tanks)
16/10/06 : First sampling (t = 0) and beginning of fuel oil exposure.
23/10/06 : Sampling n° 2 (t = 1 week)
20/11/06 : Sampling n° 3 (t = 1 month) and recontamination.
08/01/06 : Sampling n° 4 (t = 5 weeks)
05/02/07 : Sampling n°5 (t = 5 months)
02/04/07 : Sampling n°6 (t = 7 months)

2. A similar experiment with bivalve molluscs (mussel) exposed to WSF of FO2 has been initiated in September 2006 at IRIS&Akvamiljø. Moreover, the experiment with styrene was started shortly after, in October 2006. A picture of the two experimental systems is shown in figure 4. Sampling for chemical data and biological effects has occurred following the plan described in table 1.

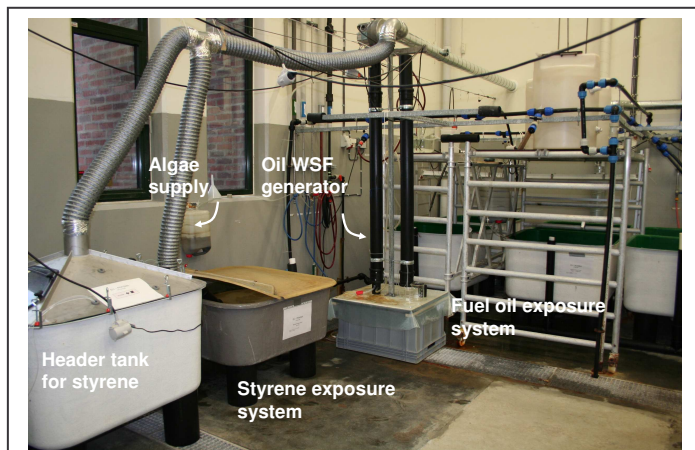
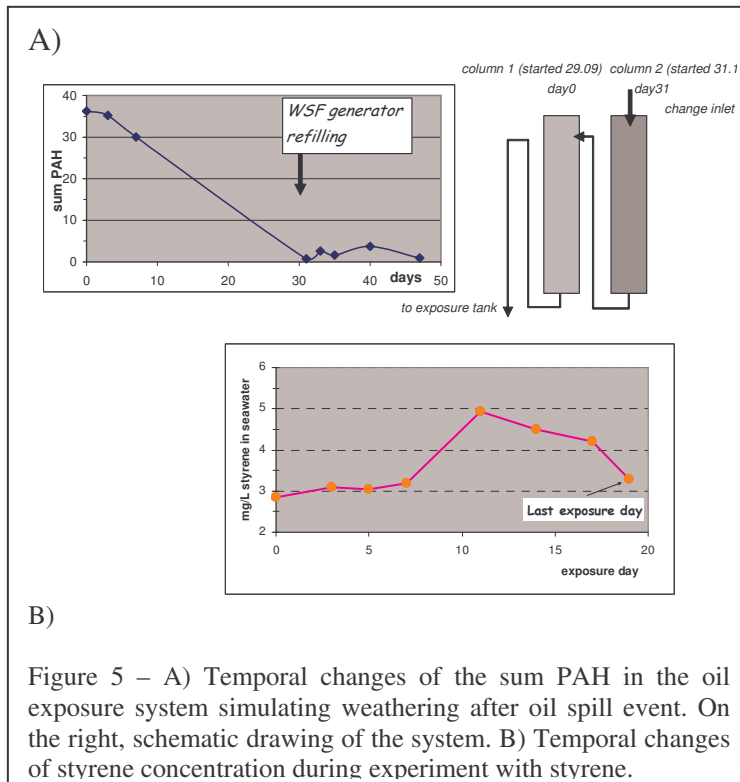


Figure 4 – Picture of the exposure systems for oil and styrene used at IRIS&Akvamiljø. The oil exposure system is using the same principle as described in figure 1.

Date (expected)	Uke	Exposure		Water		NRRT	Comet	Immuno (cell count & phago-)	other Cl, lipid, BB, SoS, Clearance	UPV
		day	day	oil	styrene					
29.09.2006	39		0							
02.10.2006	40		3			X (n=18)			X	X (n=40 UPV)
06.10.2006	40		7			X (n=18)			X	X (n=20 UPV)
13.10.2006	41		0							
16.10.2006	42		3			X (n=18)	X (n=18)		X	X (n=40 UPV)
20.10.2006	42		7							X (n=20 UPV)
30.10.2006	44					X (n=18)	X (n=18)		X	X (n=40 UPV)
31.10.2006		Refilling oil generator								
01.11.2006	44	day1	19			X (n=18)	X (n=18)	X (n=18)	X	X (n=40 UPV)
02.11.2006										
03.11.2006		day3								
08.11.2006	45	1week				X (n=18)	X (n=18)		X	X (n=40 UPV)
15.11.2006	46	2week				X (n=18)		X (n=18)	X	X (n=40 UPV)
22.11.2006	47	3week								
20.12.2006										
2007										
26.02.2007	9		150			X (n=18)	X (n=18)	X (n=18)	X	X (n=40 UPV)
26.03.2007	13		150+30			X (n=18)	X (n=18)	X (n=18)	X	X (n=40 UPV)

Table 1 – Sampling Schedule for fuel oil and styrene exposure of blue mussels. N= number of samples analysed at each sampling time

2a) Oil exposure - Figure 5A shows the temporal changes of the sum of PAHs in the oil exposure system. The decrease of the sum PAHs in the exposure water simulates the weathering of oil that naturally follows a spill event. After 30 days of exposure, the WSF system was refilled with FO2 to simulate an oil re-suspension (pulse) event in the water. However this operation will be repeated later in December since the exposure level in seawater did not rise as high as expected. In addition to the biological sampling, a mussel valve gape sensor system (figure 6) has been



placed in oil exposure tanks after one month of exposure to assess the soundness of this warning technique for spill events and for biomonitoring in the field. The principle is based on the voltage readings that result from changes in distance between two tiny magnets placed each on one side of the shell. At closure, this voltage is minimum and it increases as the shell opens. The system will be operated during pulse events and thereafter to test the sensitivity.

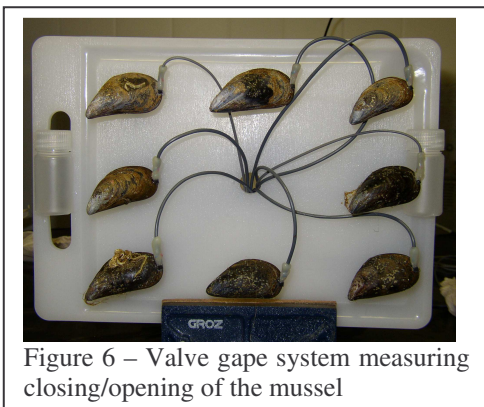


Figure 6 – Valve gape system measuring closing/opening of the mussel

Further plan: The oil exposure is planned to go on until spawning season of the mussels is reached i.e. in the beginning of spring 2007.

2b) Styrene exposure Figure 5B illustrates the temporal changes of styrene during 20 days of exposure. The mean concentration level in the exposure system was 3.6 ppm but concentration was higher after exposure day 10. Following exposure, mussels were placed for 2 weeks in clean seawater to assess recovery.

Status: The styrene experiment is currently completed. Data will be analysed in 2007.

All chemical, biological- and sensing-based methodologies tested for the exposure scenarios described above are currently under evaluation. A first assessment of the results should be available in the next progress report.