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DISCOBIOL: Assessment of the Impact of Dispersant Use for Oil Spill Response in Coastal or Estuarine Areas

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ABSTRACT 300137:

Dispersants are known to be an appropriate solution for offshore spill response when sea conditions provide enough energy to disperse and then dilute oil into surface waters. In shallow coastal areas, the use of dispersant is restricted due to the potential that the dispersed oil might come into contact with sensitive resources before dilution can take place. However, after assessing the advantages and potential risks of dispersing oil in coastal areas, it may emerge after careful consideration that and in some cases the use of dispersants could provide a net environmental benefit.

The DISCOBIOL research program aimed to provide practical recommendations on dispersant use in coastal and estuarine areas by acquiring relevant (in terms of likely dispersed oil concentrations) and robust experimental information on the impact of mechanically and chemically dispersed oil on living resources. The main conclusion from these experiments was that there is no significant difference between the impacts from oil with and without dispersant use in terms of acute toxicity. However there are some observable sub-lethal effects from exposure to dispersed oil which do not persist more than a few weeks. In a natural environment, on a medium or long timescale, biota which have been exposed to oil (with and without dispersant) do exhibit some symptoms which could affect their survival rate in the field even though they do not lead to acute toxicity effects. However the DISCOBIOL project demonstrated that effects of dispersed oil were less severe than previously recorded for near shore environments.

In terms of applying these results to decision making at an oil spill, it highlights the need in coastal areas prior to the use of dispersant to complete a "Net Environmental Benefit Analysis" (NEBA) to determine whether the use of dispersant is expected to minimize the overall damage resulting from the pollution. As it is difficult to cover the number of possible spill scenarios at the contingency planning stage, instead of completing a NEBA, many countries define geographical limits where dispersion can be undertaken, based on the water depth and the distance to the shore as well as the presence of sensitive resources. The DISCOBIOL study confirmed the appropriateness of these pre-defined limits for France's

coastal waters but demonstrated that they could be less restrictive since the exposure to dispersed oil could be at least five times higher than was previously considered the safe limit.

INTRODUCTION:

Coastal waters are home to some of the world's most diverse ecosystems as well as also one of the most heavily populated areas and, hence, most affected by discharges, whether from urban, agricultural or industrial sources. Although the risk of oil pollution in coastal areas has considerably dropped since the tightening of safety regulations and maritime surveillance as a result of activities coordinated through the International Maritime Organisation, there is still a risk of oil spills in the marine environment.

Many response options are available for treating an oil spill offshore but ultimately, the choice is dependent of the environmental factors (e.g. potential of dilution, weather conditions) and the type of oil spilled. In some cases, the most relevant response may be the use of dispersant. Dispersants are designed to enhance the natural dispersion, breaking the oil slick into small droplets that are diluted in the water column. The greatest likelihood of dispersed oil having an impact on marine flora and fauna is in shallow, coastal waters as it increases their exposure to hydrocarbons (Ramachandran *et al*, 2004). Therefore, dispersant use in coastal areas, especially in the vicinity of fisheries and aquaculture areas, should be carefully considered through a net environmental benefit analysis or also called NEBA (Koyama and Kakuno, 2004). In this respect, CEDRE co-ordinated a three-year research programme called DISCOBIOL, which aimed to update and improve practical recommendations on dispersant use in coastal or estuarine areas by providing data on the impact of oil on living resources in these areas.

The DISCOBIOL program assessed the toxicity and the impact of dispersed oil (mechanically and chemically dispersed) towards the organisms living in three main eco-compartments of the coastal or estuarine environment of a temperate climate: the water column, mudflat habitats and salt marshes. The strategy was to deliver scientific and robust data to the French authorities who are in charge of defining the geographical limits where dispersion can be undertaken. To achieve this goal, the experimental work was performed in three steps:

- Phase 1: [phase 1A]- short-term acute toxicity assessment of the oil towards different species which live in the water column [pelagic fish, benthic, bivalves and crustaceans] followed by [phase 1B]- a sub-lethal effects assessment on the same species (excepted on crustaceans).
- Phase 2: long-term toxicity assessment on mudflat habitats.
- Phase 3: long-term toxicity assessment on salt marshes ecosystem through a field trial.
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MATERIALS AND METHODS:

Oil

The choice of the oil as a weathered Arabian Light Crude was determined by its worldwide use, its physical and chemical properties and information available on it. Prior the experiments, the oil was artificially weathered by bubbling air through the oil layer until approximately 10% of the petroleum mass was lost. Such a treatment of the oil simulated a 12 hour aging of a slick, released at sea in temperate climate (Nordvik, 1995). The properties

of the oil were found to be fairly similar to Arabian Light oil toped at 150°C with an API (American Petroleum Institute) gravity of 33.

Dispersants

The two dispersants chosen were FINASOL OSR62 (from TOTAL Fluids) and INNOSPEC OD4000 (from Innospec Ltd.). Both these third generation dispersants are used in France and are produced by the partners of the DISCOBIOL project. Third generation dispersants are those effective (method NF.T.90-345, tested in CEDRE), non-toxic at the concentration recommended by the manufacturer (method NF.T.90-349, tested in CEDRE) and biodegradable in the marine environment.

Biota

Six marine species were selected to perform experiments: sea bass (*Dicentrarchus labrax,* pelagic fish), grey mullet (*Liza aurata,* coastal fish), turbot (*Scophthalmus maximus,* benthic fish), white shrimp (*Crangon crang*on), oyster (*Crassostrea gigas)* and mussel (*Mytilus edulis*). All biota were purchased from aquaculture sources. Sea bass was used in all the steps of this experimental program.

Experimental protocols

LC_{50} tests followed a standardized procedure using a modified OECD 203 guidelines. Exposure tests were performed by using the same protocols in phase 1 and phase 3 but the experiments for phase 2 were tested via the sediment, by mixing oil with the mudflat. For each of the phases, the oil concentration was monitored to determine the level of the exposure (oil concentration in the water column, phase 1A and phase 3; oil concentration in the sediment phase 2) and its effect (oil concentration in the organism tissues, bioaccumulation processes).

Animals were allocated randomly to eight experimental groups: one control group, made up using fresh seawater (C); two groups with only dispersant (Ds1 for dispersant 1 and Ds2 for dispersant 2) as internal control; two chemically dispersed oil groups (CD1 and CD2) , made by mixing 20 g of oil and 1 g of dispersant; one mechanically dispersed oil group (MD) , made by the addition of 20 g of pure oil to the seawater; one water soluble fraction of oil (WSF), consisting of an oil slick (20 g) which was contained at the sea surface by using a plastic cylinder. The fish were exposed to these different conditions for a period of 48 hours corresponding to four tidal cycles in France. This exposure regime was chosen in order to reflect realistic conditions of coastal pollution in which the dilution process is expected to reduce the dispersed oil concentration.

All the experiments at different exposures were performed in experimental systems devised to maintain the mixture of oil and dispersant as a homogeneous solution. Briefly, for phases 1A and 3, mixtures were homogenized, using a funnel (at the surface of a 300 L seawater tank), which was linked to a Johnson L450 water pump at the bottom of the tank (Milinkovitch et al., 2011). For Phase 2, oil (with/without dispersant) was added to the seawater and mudflats were contaminated during two tidal cycles before being cleaned by the use of absorbent pads (Richard et al., 2010a). During all exposure periods, several physical and chemical parameters of the sea water were measured: the pH, the dissolved oxygen concentration and the temperature.

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Oil concentration monitoring

The Total Petroleum Hydrocarbon concentrations (TPH), which is the sum of dissolved hydrocarbon concentrations plus the amount of oil droplets were measured for all exposure groups at the beginning (T = 0 h) and at the end of animals exposure (T = 48 h), using the mean of three replicated measurements for each time point. For TPHs, the seawater samples were extracted, the combined organic layers were dried and reduced under vaccum. The residues were analysed using a UV spectrophotometer (UV–vis spectrophometer, Unicam) at 390 nm, as described by Fusey and Oudot (1976).

For PAHs quantification, after sampling, the first step was a 24 hours settling phase to separate oil droplets and particulate matter from the seawater. Then, PAHs were extracted from the seawater using the stir bar sorptive extraction technique (SBSE—stir bar coated with PDMS, Gerstel), and analyzed using thermal desorption coupled to capillary gas chromatography–mass spectrometry (GC–MS). PAHs were quantified according to published procedures (Roy et al., 2005).

RESULTS

Due to the large number of different tests and exposures that were performed during the DISCOBIOL program (Merlin F. X. M. et al. 2012), only selected results from each phase are presented below with a focus on how these results can be used in a NEBA.

Selected results from the Phase n°1

Phase 1A

This phase of the project was designed to assess the short term acute toxicity (LC_{50} on 24 hour exposure) of the CD and MD and the Ds toward all the six species selected. The results showed that, in the test conditions (i.e. agitation), stable concentrations higher than 600 ppm were not reached for the MD while, with addition of dispersants up to 2,900 ppm stable dispersed oil concentrations were reached. In these conditions, on fish and shrimp, lethal concentration for MD were never reached while for CD, lethal concentrations ranged between 300 and 1,400 ppm. The concentration used to perform sub-lethal studies, was determined taking into account these LC_{50} results. For the bivalves, the lethal concentration could not be detected because, when placed in polluted water, the bivalves closed their shells to protect themselves due to their chemodetection abilities (Le Floch et al., 2010; Dussauze et al., 2011).

The assessment of the short term acute toxicities demonstrated that the LC_{50} concentrations were all several hundred ppm, much higher than the concentrations found during a spill (typically between 1 and 10 ppm, Lunel et al., 1996) Consequently, it was concluded that the lethal concentration measured in these tests would not be reached in real conditions. Hence, LC_{50} concentrations should not be used as a basis to quantify the toxicity of oil dispersion at an actual spill incident, though LC_{50} concentrations are still relevant for dispersant screening which is designed to ensure that only low toxicity dispersants are licenced for use in response operations.

Phase 1B

This phase assessed the sub-lethal effects resulting from exposure (48 hours, concentrations above 50 ppm) to oil with and without dispersant through immunological, physiological and behavioural bio-indicators.

For the oil monitoring in seawater (characterisation of the exposure), results obtained show, as expected, that the total petroleum hydrocarbon (TPH) concentration and the dissolved fraction of oil in the sea water increases when chemical dispersants are used. In terms of assessing the sub-lethal effects of oil on marine organisms, for example, the main results for turbot came from changes in internal regulation of temperatures and pH (homeostatis) and in blood composition (hematology). WSF induced a decrease in the number of white blood cells (leucopenia) and an alteration of hydromineral and acid-base equilibrium. Ds appeared to have only a weak effect whereas CD led to comparable effects to MD, with CD1 inducing significant changes in hematological parameters while CD2 eliciting more pronounced effects on homeostasis. To be able to discuss and interpret all results obtained from the different parameters which were monitored on turbot, a principal component analysis (PCA) was performed after the 48 hour exposure period and after the 15 days of recovery period. PCA is a statistical technique which identifies patterns in multivariate data, and compresses the data onto two axes (the 'principal components') in such a way as to highlight their similarities and differences (data points that are more similar will be plotted more closely together).

The PCA for the distribution of the individuals after 48 hours of contamination shows two distinct areas on the factorial plan (Figure 1, left graph): the first one is composed of the three "control" groups ([C], [Ds1 and Ds2], on the left) and the second one is composed of the dispersed oil groups (MD, CD1 and CD2, on the right). The fish exposed to WSF are in an intermediate position. In the same projection of the individuals onto the factorial axis after 15 days of recovery in clear water (Figure 1, right graph) it is no longer possible to differentiate these three experimental groups.

These results show that the dispersion (either mechanical or chemical) enhances the sub-lethal effects of an exposure to the weathered crude oil initially in the first 48 hour exposure period. However, after a 15 day recovery period it is not possible to detect these sub-lethal effects in a comparison between the exposed and control groups. This would imply that the initial sub-lethal effects do not have long-term consequences.
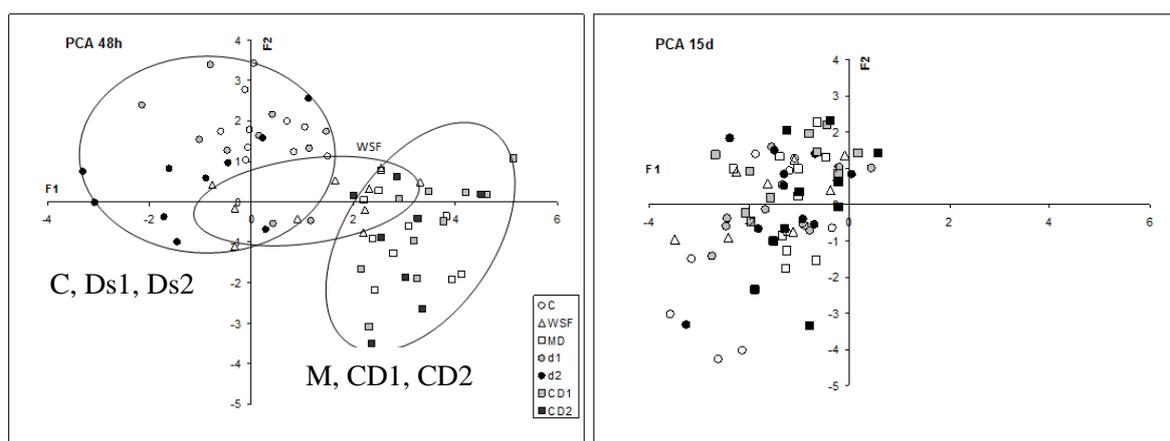

Figure 1- Principal component analysis of the distribution of the individuals in the factorial plan (F1*F2). **Left**: After 48 hours of contamination. **Right**: After 15 days of recovery.

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This study demonstrated that hydrocarbon contamination at concentrations closer to those experienced in real spills induces a large number of biological changes, essentially due to respiratory issues, which impact the immune system, but also swimming, and growth rate changes. Mechanical and chemical dispersion induces a further increase of these effects (Figure 1, left graph), though the effects are still reversible (Figure 1, right graph). However, it is important to note that these results cannot be extrapolated to field conditions without a degree of caution for the reason that they have been obtained in optimal stabilisation conditions moving the biota into completely clean water.

Selected results from the Phase n°2

Experiments of the phase 2-A were carried out at the experimental station at Lauzieres (Charentes-Maritimes, France). The objective was to test the influence of chemically dispersed and non-dispersed oil spill on the oil penetration into the sediment (mudflat). The concentration of hydrocarbon [HC] contained in two core sections (surface to 0.5 cm depth and 0.5 to 1cm) of the mudflat was measured for two different contamination level: (+) for 65 ppm and (++) for 260 ppm, which is a higher concentration than one finds in reality (Figure 2). The left side of the graph show that the [HC] in the core 0-0.5cm was elevated for the mudflat treated with MD or CD. However, the right side of the graph shows that there is not a statistical difference between the control and the MD and CD groups in both concentrations tested. Therefore, the oil contaminated the sediment up to the first ½ cm of sediment but it is not influenced by the presence of dispersant or the concentration of oil.
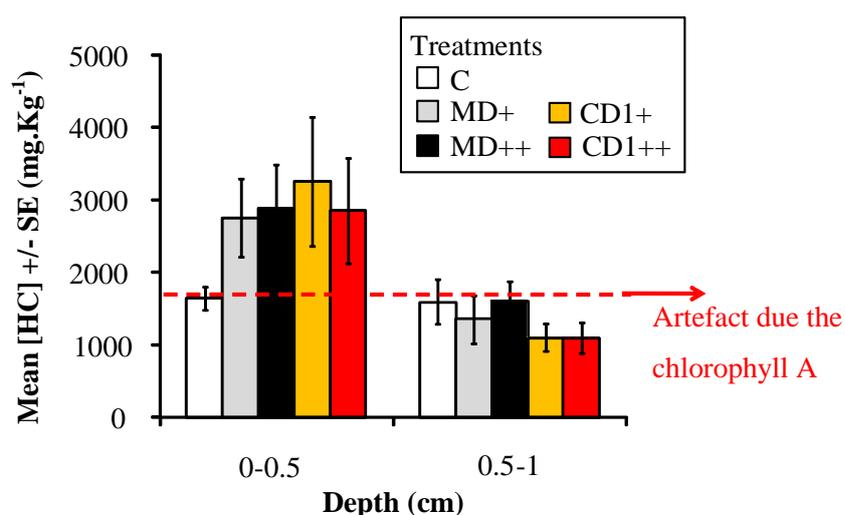

Figure 2 - Oil concentration [HC] found in sediment cores between two depths (0-0.5 and 0.5-1 cm) including the standard deviation (SE). The white bar represents the control, the grey and black the mudflat with MD and the yellow and red represent the mudflat with CD. (+) corresponds to 65 ppm of contamination and (++) corresponds to 260 ppm.

Selected results from the Phase n°3

The primary objective of this phase was to examine and compare the effects of exposure between untreated oil, chemically dispersed oil and dispersant alone, upon a fish's capability to adapt to environmental shocks. To reach that goal, we implemented high-throughput, non-lethal challenge tests to estimate the resistance to the reduction of oxygen levels (hypoxia) and tolerance to variation of temperature as surrogate measures of their capacity to face natural contingencies (Claireaux et al. 2013). These tests were carried out

two weeks before the exposure to oil (for 48 hours), then a week and a month after exposure. Fish were divided into 4 groups: control, MD, CD and Ds groups.

Results obtained in the challenge tests, before the transfer to the semi natural tidal ponds, showed that responses to both environmental challenges displayed a noticeable variability between individuals (Figure 3). Typically, for both challenge tests a difference of 2-3 h was found in the time it took to the least tolerant and the most tolerant individuals to lose their ability to maintain equilibrium. It must be noted, however, that the coefficient of variation (CV) of individual performances was significantly higher for the hypoxia challenge test (HCT) than for the temperature challenge test (TCT). On the other hand, for a given challenge test, no significant difference among trials (i.e. before exposure, two days after exposure and one months after) was observed.).
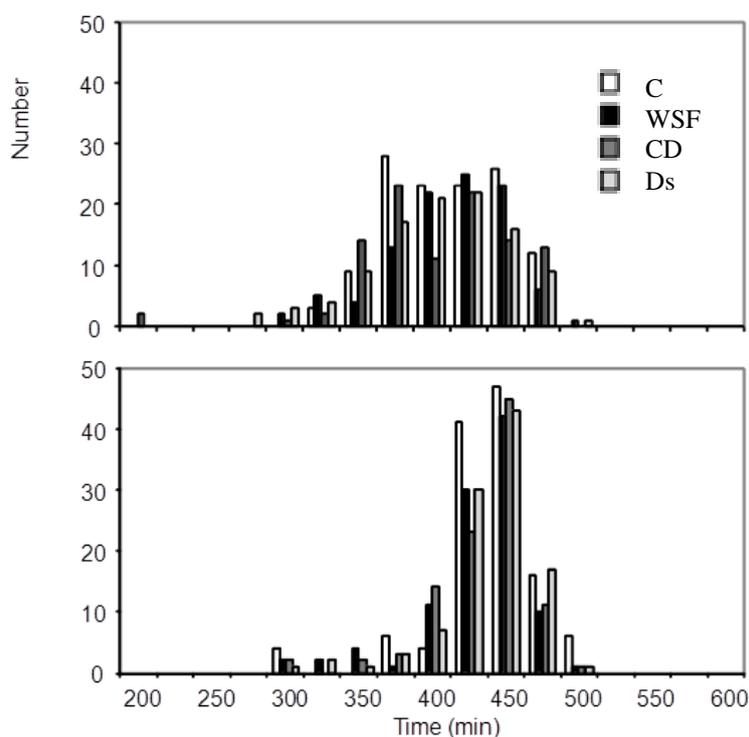

Figure 3- Example of variability between individual test animals in the time to lose equilibrium during a hypoxia challenge test (top panel) and temperature challenge test (bottom panel). White bars: C; black bars: WSF; dark grey bars: CD; light grey bars: Ds.

After the challenge tests and just over a month after the exposure period, the animals were transferred into semi natural tidal ponds (saltmarshes ecosystem, Figure 4) where the individuals' fitness (growth and survival) were measured at 4 dates (P1, P2, P3 and P4) over a period of 6 months. Results show that fish growth rates varied significantly over the duration of the experiment (ANOVA, $p < 0.0001$; Figure 5 top panel). ANOVA or Analysis of Variance is used to compare differences of means among more than two groups, by looking at variation in the data and where that variation is found. A significant probability value (p-value) (usually taken as $p<0.05$) suggests that at least one group is significantly different from the others. While fish gained nearly 20 % body weight (BW) during the first monitoring period (P1; May - June), they lost approximately 2, 6 and 5 % BW during periods P2 (June - July), P3 (July - August) and P4 (August - October) respectively. Moreover, it was observed that the CD fish group experienced significantly lower growth than the other 3

experimental groups (C, WSF and Ds; ANOVA, $p < 0.01$). Fish survival significantly varied with both the monitoring period and the initial contaminant exposure conditions (ANOVA, $p < 0.005$; Figure 5 bottom panel).

Figure 4- Semi-natural tidal ponds where fish were placed during 6 months

Post hoc Tukey-test showed that survival during the second monitoring period was significantly higher than during periods 1 and 3. Survival during P4 was the even lower than during P2 and P3. Moreover, fish from the CD group had significantly lower survival than fish from C and Ds groups. However, survival in WSF and CD groups was not statistically different.

Growth and survival calculated over the entire experimental period on those fish that were still alive at the end of the survey are also noted on Figure 5 (hatched bars). Fish overall growth was 14.17 ±1.58 %, 9.45 ±3.15 %, 3.07 ±2.30 % and 12.40 ±2.48 % BW for group C, WSF, CD and Ds respectively. Fish overall survival was 22.98 %, 17.16 %, 15.77 % and 23.63 % for group C, WSF, CD and Ds respectively.
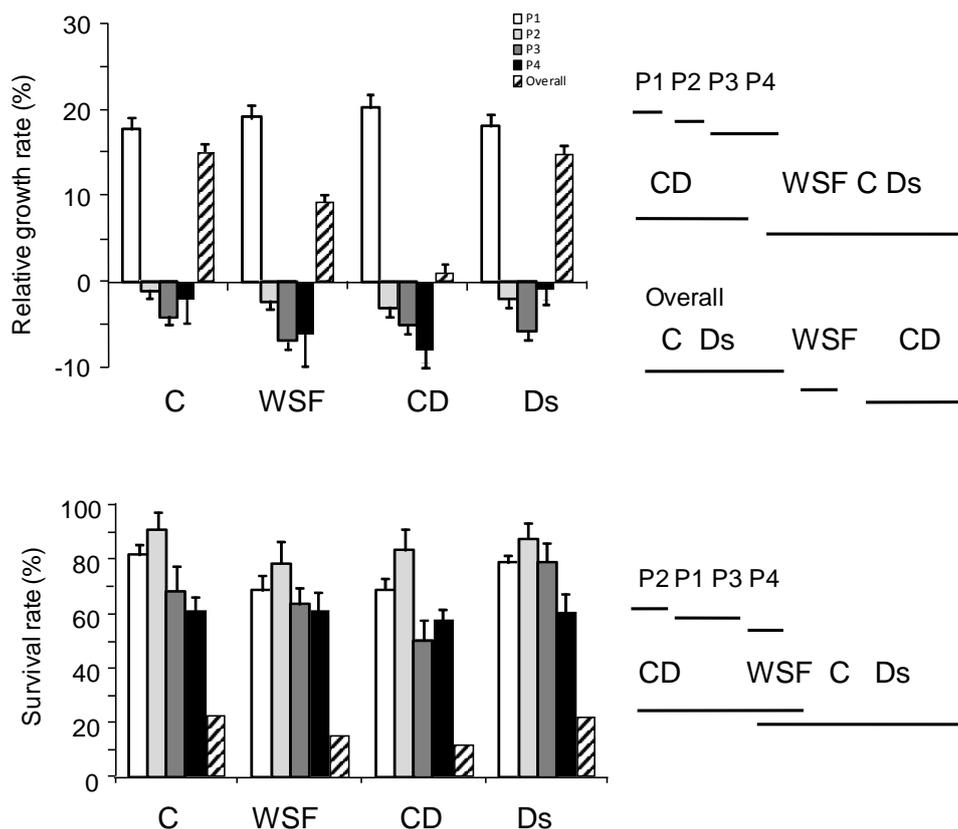
Figure 5- Relative growth rate (top panel) and survival (bottom panel) as a function of time and initial contamination exposure. White bars: P1 (May 16th to June 17th); light grey bars: P2 (June 17th to July 30th); dark grey bars: P3 (July 30th to August 31st); black bars: P4 (August 31st to October 14th); hatched bars overall experimental period (May 16th to October 14th). The absence of standard error above the hatched bars for survival (bottom panel) results from the fact that fish having been regularly reallocated among ponds during the experiment, ponds are no true replicate of survival. Results from *post hoc* tests are also presented on the side of the figure. A line spanning two groups or periods indicates that they are not significantly different.

For the field part of the study, survival data clearly confirmed that conditions in the ponds impacted fish performance (high salinity and temperature, poor oxygen content due to the summer period). During the first period of the field survey (P1), feeding conditions were optimal as illustrated by the nearly 20 % weight gain observed in all experimental groups (Figure 5). During that period, we observed that fish which performed well during HCT and TCT had significantly better survival then fish that did not. During the subsequent periods (P2 to P4), on the other hand, the culling of hypoxia and heat sensitive individuals, combined with the progressive deterioration of feeding conditions, changed the circumstances of natural of selection, survival being then mostly determined by starvation tolerance. In a recent publication, is has been shown that individual variation in growth rate in sea bass reflects a trade-off against tolerance of food deprivation (Dupont-Prinet et al., 2010). These authors demonstrated that fast growing individuals have physiological or behavioral attributes which lead to larger weight loss when food is not available. Present data clearly support this view by showing that those individuals that experienced fast growth during P1 were also those that suffered lower survival when trophic conditions deteriorated (P2 to P4). Fish groups exposed to oil or to chemically dispersed oil suffered poorer growth compared to control or

dispersant-exposed groups. Moreover, the OIL+DISP population also endured significantly lower survival rate.

In conclusion, functional physiological bases exist to support the claim that OIL and OIL+DISP fish groups suffered impaired physiological performance and lessened ability to face environmental challenges under natural conditions. Although not observed shortly after exposure to contaminants, these impairments were revealed over time, as fish were exposed to increasingly selective feeding and physico-chemical conditions. Challenge tests did not discriminate experimental groups shortly after contaminant exposure, but they turned to be decisive in deciphering the constraints that occurred under field conditions and their consequences upon determinants of individual fitness such as growth and survival. Experiments are currently being conducted to examine the long-term consequences (<6 months post-exposure) upon fish responses during HCT and TCT. As already demonstrated in rainbow trout (Oncorhynchus mykiss) by Ramachandrana et al., (2004), chemical treatment of oil did not directly affect fish ability for environmental adaptation. However, it contributed to increase exposure to hydrocarbons and therefore increased their detrimental influence upon fish physiology. From the fact that proxies of Darwinian fitness such as survival and growth were affected following exposure to oil and to chemically-treated oil, it would be tempting to speculate that these conditions may interfere with genetic adaptation of sea bass populations. However, trans-generational experiments, including an assessment of population genetic changes, need to be conducted before this point is ascertained.

RECOMMENDATIONS ON THE USE OF DISPERSANTS IN COASTAL AREAS:

In coastal areas prior to the use of dispersant it is necessary to complete a "Net Environmental Benefit Analysis" (NEBA) to check if the use of dispersant is predicted to minimize the overall damage resulting from the pollution. Specifically, without dispersion, oil will remain at the sea surface, and then, reach the coast and most likely result in cleaning operations that can be very damaging the shoreline. By contrast dispersion will transfer the oil into the water column and thus prevent that slicks reach the coast under the action of wind may increase the exposure in the near-shore waters if there is not sufficient dilution capacity. NEBA looks to assess the relative environmental and economic impacts of each of these two options "chemical dispersion versus no chemical dispersion". To carry out a full NEBA assessment in advance of a spill is difficult as it is not possible to adequately cover the infinite number of possible spill scenarios. In fact, depending on specific scenarios chosen (amount of the spill, nature of the shoreline, biodiversity…), conclusions of different NEBA analyses can be contradictory at the planning stage and the responsible authority will need to make a decision at the planning stage on "most-likely" scenarios.

This is why, instead completing a full NEBA at the planning stage, many countries define geographical limits where dispersion can be undertaken when far enough from the shore. So for these scenarios the dilution is expected to be great enough in all feasible scenarios for the NEBA analysis to provide a net environmental benefit in favour of dispersing the oil into the water column if this is possible for the given oil type and weather conditions. Then if at the time of an actual incident the spill is closer to the shore than these pre-defined limits, the authority can go through a NEBA process on the scenario which is much more tightly defined to determine whether there is a net environmental benefit even in these shallower waters.

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These pre-defined geographical limits are generally based on the water depth and the distance to the shore as well as the presence of sensitive resources. France defined 3 geographical limits along its coast line considering 3 spill scenarios, 10, 100 and 1 000 t spills based on a threshold of not exceeding "10 ppm initial dispersed oil concentration" (Figure 6). However, both UK and the USA for example have a different system. In the United Kingdom, the 20 metres bathymetric contour line and a distance of 1 nautical mile from the coastline are taken as the baseline. The USA also works on threshold distances from shore and minimum depths. Many States in the USA have pre-approved the use of dispersants outside three nautical miles from shore and/or in depths greater than 10 metres.

Results and observations from DISCOBIOL study validate in principle the need for limitations to the use of dispersant along the coast but confirm that current limitations are too conservative and can be eased.
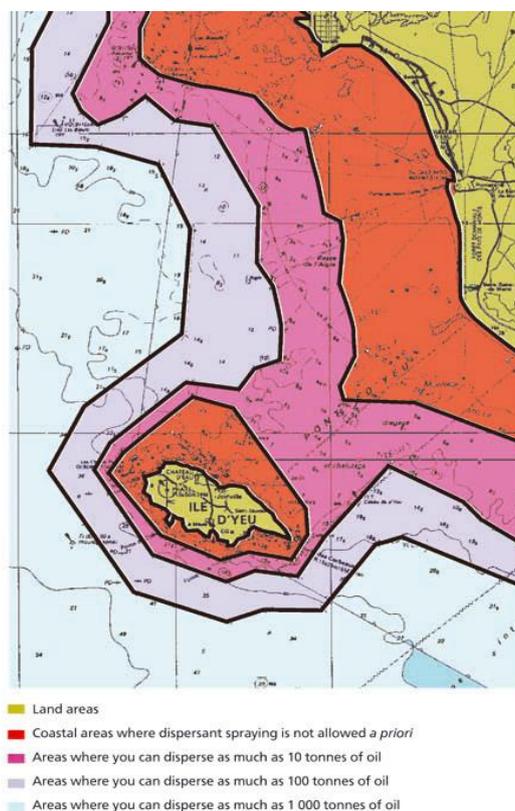

Legend:
- Land areas
- Coastal areas where dispersant spraying is not allowed *a priori*
- Areas where you can disperse as much as 10 tonnes of oil
- Areas where you can disperse as much as 100 tonnes of oil
- Areas where you can disperse as much as 1 000 tonnes of oil

Figure 6 - The three geographical limits for France where dispersion is authorized depending the depth and the volume of oil at the sea surface.

Considering that exposure conditions (i.e. concentration over a determined period) used in the study (60 to 140 ppm over two days should not be exceeded, it has been concluded in France that it is possible to increase by five times the "10 ppm initial dispersed oil concentration" would lead to 50 ppm over a two days exposure. Thus the quantity of oil which can be dispersed in the French pre-defined zones (10, 100, 1 000 t) could be multiplied by 5 leading to 50, 500 and 5 000 t limits. On the basis of the "precautionary principle", it has been decided in France that for seas with low tides and low mixing energy (i.e. Mediterranean sea), where the dilution of the dispersed plume can be at a rather slower rate it would be advisable to keep the limits on the volume of oil that can be dispersed in these predefined zones to half these quantities (25, 250 and 2 500 t). Other countries may wish to

consider relaxing their criteria for pre-defined zones for dispersant use on the basis of this data from the DISCOBIOL project.

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