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Doctoral Degree

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Title:

Hydrocarbons in the open ocean waters near the Galician Bank after the deep sea spill from the Prestige wrecks

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“Life finds a way”

Jurassic Park

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Summary

To date, most studies on the environmental consequences and fate of marine oil spills and pollution have been undertaken in coastal areas, probably as a result of the impact of spills in local economies and the vulnerability of coastal ecosystems to such events. This fact, together with analytical and technical difficulties, may explain why, in contrast, there is an apparent shortage of studies on the distribution and fate of oil hydrocarbons in open ocean waters. In fact, given the increasing exploitation of oilfields in the high seas, and the intensification of maritime traffic, in the last decades, oil pollution of the marine environment beyond the continental platforms is increasing. In addition, new sources of pollution are being recognized. Thus, in recent years it has been noted that the high number of sunken vessels scattered in the oceans sea floor are potentially a present and future source of oil spills.

In this thesis, the accident of the *Prestige* tanker in 2002 off the Galician coast is investigated as a case study of a deep sea spill in open ocean waters. Although part of the cargo carried by the tanker was released at the surface, more than 57,000 tonnes of heavy fuel oil remained in the vessel when it sank and the great majority of them were spilled from the deep ocean. In addition, the area near the Galician Bank, where the *Prestige* wrecks are located, is hydrodynamically complex. The water column is comprised by five main water masses from different origins and physico-chemical properties, which may contribute to actively spread the oil released from the Prestige in different and distinct ways.

In this context, the main aim of this study has been to determine the importance of the *Prestige* shipwrecks as a source of oil pollution in the waters near the Galician Bank several years after the accident took place. In addition, it has been investigated the role of the different water masses in the sinking area in the transport and distribution of hydrocarbons. These objectives have been tackled by a combination of laboratory and field studies. First of all, several sets of experiments were carried out under controlled temperature and salinity conditions which provided information about the potential dissolution of the *Prestige* fuel oil in seawater. In addition, two oceanographic cruises were undertaken in March and October 2006, near the wrecks location and surrounding areas, to collect seawater samples. Aliphatic and polycyclic aromatic hydrocarbons (PAHs) were determined in the suspended particulate matter (SPM) and dissolved phase (DP) in the water column of three stations. One was located above the Prestige wrecks, and the other two 73 nautical miles north and south of the Prestige incident area.

Hydrocarbons concentrations from petrogenic sources in the SPM above the wreck and surrounding areas in March 2006 were within the range of abundances previously reported for similar compounds in the North Atlantic and other marine locations, but much higher in October 2006. The concentrations of PAHs found in the DP were, both in March and October 2006, well above any expected background levels. The chemical fingerprint of the hydrocarbons indicated that in October 2006 the oil at the Prestige station originated from

the wrecks. This, together with the unusually high relative concentration of hydrocarbons in the DP at the Prestige station in March 2006, indicates that the Prestige wrecks had been releasing oil for several years after the accident. However, despite the widespread occurrence of oil hydrocarbons in the three stations and throughout their water columns, it could not be concluded that the Prestige was the main source of pollution in the area near the Galician bank, which most likely has multiple origins. In fact, different water masses contain distinct contents of hydrocarbons in the SPM and the DP, which in some instances may have remote sources. This is especially likely for the hydrocarbons in the Mediterranean water mass.

The study conducted can be viewed as an example of the potential of a sunken wreck in the deep North Atlantic as a source of pollution. Apparently a deep spill from a wreck would have initially a much localized impact, more noticeable in the dissolved hydrocarbon fractions. Eventually, the chemical signature from a deep spill cannot be easily disentangled from the background concentrations of oil pollutants after the spill is over. Although each potentially polluting shipwreck represents a singular case, knowledge obtained about the temporal and spatial distribution of hydrocarbons after the accident of the *Prestige* could be applied to deal with other deep spills in the future.

Acronyms

ΣALKs	Total n-alkanes
BDL	Below Detection Limit
CSIA	Compound Specific Isotope Analysis
DP	Dissolved Phase
EC	European Commission
ENACW	Eastern North Atlantic Central Water
EP	European Parliament
GESAMP	Joint Group of Experts on the Scientific Aspects of Marine Environment Protection
GMF	Glass Microfiber Filter
HOC	Hydrophobic Organic Contaminant
IMO	International Maritime Organization
ITOPF	International Tanker Owners Pollution Federation
LSW	Labrador Sea Water
MAE	Microwave Assisted Extraction
MW	Mediterranean Water
NADW	North Atlantic Deep Water
PAH	Polycyclic Aromatic Hydrocarbon
ΣPAHs	Total Polycyclic Aromatic Hydrocarbon
SWAF	Sea Water Accommodated Fraction
SPM	Suspended Particulate Matter
SW	Superficial Water
UCM	Unresolved Complex Mixture
UN	United Nations

CHAPTER 1

Introduction

1.1. Conceptual background of the research project

The increase of humankind’s demand for energy has led to an intensification of the extraction of fossil fuels both from the land and the sea (Fakness and Brandvik, 2008; Verma et al., 2008). The exhaustion of continental oilfields and the parallel realisation of the high potential of the oceans’ continental shelves as a petroleum source has also given rise to a remarkable increase in oil extraction from the high seas since the 1950s (UNESCO, 1998). As a consequence, the numbers of cargo ships and submerged pipelines in the ocean, that transport high amounts of oil from the extraction fields to final destinations, have increased drastically along with the number of oil spills (Papadimitrakis et al., 2006)

Oil pollution of the seas was recognized as a problem in the first half of the 20th century (IMO 2007). Several countries introduced national regulations in order to protect their coasts, but it was not until the creation of the International Law Commission of the United Nations in early fifties (UNESCO, 1998) that global policies for the regulation of the activities in the marine environment and protection of the oceans gained importance. Regulations such as the UN Convention on the Law of the Sea (UNCLOS, 1958), the International Convention for the Prevention of Pollution from Ships (MARPOL, 1973), and Convention of Safety of Life at Sea (SOLAS, 1974) delimited the sovereignty of the countries over the adjacent sea waters, established ground rules, enforced maritime safety and distributed liability for the damages generated as a consequence of the activities that take place in the marine environment.

In spite of the adopted measures, in the last decades there have been numerous large accidental oil spills in the marine environment (ITOPF, 2009), and they have become the cause of marine pollution that generates the most public concern (Anderson , 2002; Serret et al., 2003). The widespread media diffusion of some of the incidents and the subsequent social reaction has spurred policy changes in several cases (Birkland 2002), for example, the International Convention on Civil Liability for Oil Pollution Damage in 1969 (IMO, 2002), after the *Torrey Canyon* in 1967 incident when 121,000 tonnes of crude oil were spilled near English coasts (NOAA, 1992). The well-reported *Exxon Valdez* oil spill in the pristine waters of Prince William Sound, Alaska, in 1989 instigated the acceptance of the Oil Pollution Act (OPA, 1990) by the USA, one year after the accident (Birkland 2002). In Europe, the incidents of *Erika* in 1999 and *Prestige* three years later, brought out into the open the need to strengthen the existing legislation on oil shipping, and lead to the adoption of the Erika I and II legal packages by the European Union (EC, 2003), establishing a European Maritime Agency, a Committee for the Prevention of Pollution from Ships, restricting the age of oil tankers, and speeding up the removal of the single-hulled tankers (Vieites et al., 2004).

Simultaneously to the development of the legal framework, national and international organisations such as the National Oceanic and Atmospheric Organization (NOAA), the Joint Group of Experts on the Scientific Aspects of Marine Environment Protection (GESAMP), the International Maritime Organization (IMO) or the International Tanker Owners Pollution Federation (ITOPF) amongst others, made an effort to monitor each incident and its impacts in the environmental, social and economic spheres, and to offer suitable guidelines for the management plans for mitigating the adverse impacts. As far as the scientific community is concerned, distribution and fate of oil spills in the marine environment, as well as their impact in live organism, have been extensively studied. Oil spills causes short and long-term environmental and socioeconomical damages in the affected area (Frost et al., 1999;Kingston, 2002; Peterson et al., 2003). Likewise, the development of simulation models (Verma et al., 2008) and controlled oil spill field experiments (Johansen et al., 2003) have offered the possibility of predicting trajectories of the oil slicks and the dispersion of the spills, therefore becoming a powerful tool in the prevention of oil spills negative impacts (Sebastiao and Soares, 1995; Reed et al., 1999; Gonzalez et al., 2006).

Nevertheless, the spectacular technologic development of the last decades and the need to search for new oilfields in the world’s ocean floor have lead to the exploration and exploitation of areas that were beyond reach in the recent past (Faksness and Brandvick, 2008). Likewise, new problems have been realized related to the presence of abandoned sunken vessels, since they have proved to be potential sources of future spills (Michel et al., 2005). These situations represent new challenges for all the parties involved in the oil spill management and show the necessity to extend the study of pollutants.

1.2. Objectives of the thesis

The aim of this doctoral thesis is to extend the knowledge about the dynamics of the distribution of the hydrocarbons in the marine environment after a large oil spill. It has been undertaken in the framework of the FATEFUEL project *(“Biogeochemical and oceanographical implications of the dispersion in the water column of the oil spilled from the Prestige wreck”*) within the Strategic Action against Marine Pollution supported by the Ministry of Education and Science of the Spanish Government.

The present study deals with the horizontal and vertical transport of hydrocarbons, and the main processes involved in their distribution in open ocean waters, after a deep sea spill. The project uses the *Prestige* tanker accident in 2002 as a case study.

Four main objectives will be tackled in this thesis:

1. To identify the potential of the Prestige shipwreck as a source of deep sea spills.

2. To appraise the role of the Prestige wrecks as a source of contamination in the region near the Galician Bank.

3. To determine the role of the different water masses in the vicinity of the Prestige wrecks, near the Galician bank, in the distribution of petrogenic hydrocarbons in the Northeastern Atlantic.

4. To study the influence of the physico-chemical characteristics, namely temperature and salinity, of the water masses near the Galician bank in the partitioning of petroleum hydrocarbons between dissolved and particulate phases.

1.3. Oil spills in the ocean

1.3.1. Oil entering the marine environment

Spilled oil from human activities is still one of the most important sources of pollution in the ocean (Serret et al., 2003). Since the 1970s, several national and international organizations have made an effort to estimate the amount of oil entering the marine environment in order to identify the principal pollutant activities. Perhaps surprisingly to some, oil, as a natural product, has numerous natural sources of inputs to the oceans. Thus, although estimates differ according to the authors of the studies, nearly up to one half of the oil entering the sea may come from natural seeps in the seabed and from erosion processes (NRC, 2003; GESAMP, 2007). Discharges from land-based activities have also proven to be considerable (UNEP, 1995) although their contribution to the total amount is not completely clear and ranges between the 11% (National Research Council (NRC), 2003) and 37% (Australian Petroleum Production and Exploration Association (APPEA), 2002) of the total amount.

Activities related to shipping make up between the 9% (ITOPF, 2009, period 1900-1999) and the 37% (GESAMP, 2007, period 1987-1997) of the total oil contribution to the marine environment. Despite the general belief that large accidental spills are the main anthropogenic input source of oil to the ocean, the truth is that the most spills from tankers result from routine operations which usually occur in ports or oil terminals, such as loading, bunkering and discharging. These activities cause around 50 % of the spills bellow 700 tonnes and the 91 % of spills below 7 tonnes. Accidental causes, such as collisions, groundings, hull failure and fire or explosions tend to generate much larger spills, being the factors involved in the 84 % of the spills from tankers that exceed 700 tonnes. (ITOPF, 2009).

In terms of absolute amounts of oil entering the marine environment, according to GESAMP (2007), from sea-based activities it was calculated to be in average 1.25 million tonnes per year for the period between 1988-1997. This amount may fluctuate between 0.47 and 8.4 million tonnes per year when land-based activities and atmospheric sources are included, as estimated by the National Research Council (NRC,

2003) for the period 1990-1999. At least 114,000 tonnes per year correspond to accidental spills from tankers from 1990 to 1999 (ITOPF, 2009).

The majority of the oil spills from tankers are small (<7 tonnes), and their total number is uncertain, as the data on number and quantity of the spill are incomplete. However, it has been estimated that their importance in the contribution to the total amount is relatively low. On the contrary, large spills, despite being less frequent, represent a high proportion of the oil spilled. For example, in the 1990s there were 358 spills over 7 tonnes amounting to 1.138 million tonnes of oil. Nevertheless, 73 % of the total amount (830,000 tonnes) were spilled in just 10 incidents (less than 3% of the cases), which demonstrates the importance of the contribution of large accidental spills. Some of the incidents that illustrate this fact area are *Castillo de Bellver* in 1983 (252,000 tonnes) and *ABT Summer* in 1991 (260,000 tonnes), with more than the half of the total amount of the oil spilled in those years (ITOPF, 2009).

The evolution in time of the number of accidents involving large spills show a clear decreasing trend (Vieites et al., 2004; ITOPF 2009), which has been accompanied with a reduction of the total oil spilled in the ocean related to tankers (Fig. 1.1) during the last four decades (ITOPF, 2009). The total number of accidents witnessed in the 1990s was around a third of those registered in the 1970s. As consequence, the amount of oil from tanker incidents reaching the ocean has decreased in the last 40 years. Most of the large oil spills occur near the principal crude production areas, and along the major maritime transport routes (Vieites et al., 2004). Large spills also tend to occur in the Large Marine Ecosystems of the World, as defined by the NOAA, considered to be the most productive areas in the oceans and marine biodiversity hotspots (Roberts et al., 2002).

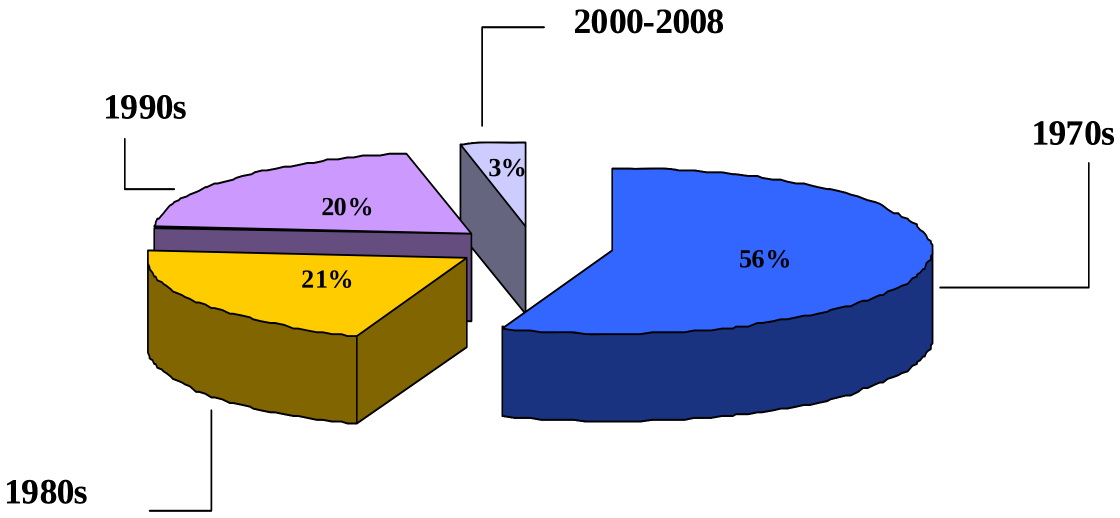


Figure 1.1. Percentage of oil spilled in the world in each decade compared to the total, for the period 1970-2008. Data source: ITOPF, 2009.

One fifth of the global oil amount (1.1 million tonnes for the period 1960-2002) spilled by maritime transport is found in the European North Atlantic, which qualifies this area as one of the most important hotspots for oil spills in the world. Within the European Atlantic, the English Channel and the Galician Coast, NW of Spain, are the most affected areas, with 526,151 and 377,765 tonnes of spilled oil respectively. This is the direct consequence of these areas being located in some of busiest maritime traffic routes of the world (Vieites et al., 2004).

1.3.2. The Prestige oil spill

The *Prestige* was a single-hulled oil tanker owned by the Liberian entity Mare Shipping, Inc. and operated by the Greek entity Universe Maritime Ltd with Bahaman flag. On 13 November 2002, during its route from Latvia to Singapore transporting 77,000 tonnes of heavy fuel oil encountered severe weather, suffered a hull failure and started leaking oil approximately 50 km off the Galician coast (Michel et al., 2005). The vessel drifted within 8 km of the coast and was denied safe haven in Spain and Portugal. The *Prestige* was then towed towards the open sea, in an effort by the Spanish political authorities to minimize the impact of a possible large oil spill in the Rias Baixas, one of the most important mussel production areas worldwide. On 19 November 2002 the *Prestige* broke in two, and it sunk at about 240 km west off the Galician coast. The stern sank at 3,565 m depth (42º10.6’N, 012º03’W approximate coordinates provided by Albaigés et al., 2003), whereas the bow sank at 3,830 m depth (42º10.8’N, 012º03.6’W approximate coordinates provided by Albaigés et al., 2003). At that time, 20,000 tonnes of fuel oil had been already spilled at the surface, and the wrecks continued spilling oil from several leaks in their structure at depth (Albaigés et al., 2003). In February 2003 the Spanish Government estimated that a total of 43,000 tonnes had been spilled, but subsequent studies raised the estimate to 63,000 tonnes. The resulting black tides affected around 1,000 km of the coasts of Spain and France.

The spilled oil was a M-100 type heavy fuel oil, the same product spilled by the *Baltic Carrier* in Denmark in 2001 (Le Cedre, 2002), with a density of 0.993 kg L^{-1} and a viscosity of 100,000 cSt (at 15 ºC). The fuel oil was composed of 22% aliphatic hydrocarbons, 50% of polycyclic aromatic hydrocarbons (PAHs) and 28 % of resins and asphaltenes (Albaigés et al., 2003). This type of fuel oil exhibits a poor capacity for evaporation and natural dispersion in the sea. It tends to generate highly viscous water in oil emulsions and patching, complicating recovery operations (Le Cedre 2002; Albaigés et al., 2003).

In the first months of 2003 a sealing operation of the leaking wrecks was undertaken with the French scientific submarine *Nautile*, and deep remotely operated vehicles (ROVs). Although all the leaks in the bow and the most of the stern were neutralized, the oil still leaked at a rate of several tonnes per day (Comité Científico Asesor (CAA) *Prestige*, 2003). It was estimated that without the removal of the remaining oil in the wrecks the *Prestige* would continue to leak until 2006. Due to the pressure of the public opinion, the Spanish government hired the Spanish oil company Repsol YPF to remove the remaining oil from the wrecks. Innovative technologies had to be developed to tackle the salvage operation in deep waters (REPSOL, 2004, Michel et al., 2005). Repsol YPF eventually claimed to have successfully removed the remaining 14,000 tonnes from the bow between May-October 2004 (Oficina Técnica de Vertidos Marinos (OTVM), 2004; Michel et al., 2005). Due to the more unstable position of the stern in the ocean bottom, the 700 tonnes of fuel oil that it was claim to contain in its tanks (European Parliament (EP), 2003) were not extracted.

The economic impact that this accidental spill had in Galicia was considerable. On November the 18th, the Spanish government banned all forms of fishing and shellfish harvesting along 96 km of the coasts of Galicia. The ban was eventually extended to 498 km and then to 554 km of coastline. The area affected by the spill is very rich in fishing and fish farming activities (oysters, mussels, turbots and many other species). The Spanish government accompanied the ban with financial aid to the 7,000 fishermen estimated to be affected (Le Cedre, 2003). Environmental damages were considerable as well. Both marine and terrestrial ecosystems were affected, although the last one also due to cleaning works (Freire et al., 2003). SEO/BirdLife estimates that the number of birds affected by the fuel was anywhere between 115,000 and 230,000, but a large proportion of corpses never arrived to the coast. The most affected species was the Guillemot (*Uria aalge)* accounting for 51% of the collected birds, more than 11,800 individuals. This has now become one of Spain’s most threatened breeding birds. Of two colonies existing in 2002, one has completely disappeared (Birdlife International, 2003). Regarding marine organisms, sedentary and sessile biotic communities, i.e. barnacles, sea urchins and several bivalves, were the most affected (Freire et al., 2003). The wreck is located near the Bank of Galicia, a seamount known by its ecologic importance and proposed as Special Area of Conservation and potential Marine Protected Area under EU Habitats Directive (Schmidt and García, 2003).

1.3.3. Deep sea spills

At the same time that the occurrence of marine causalities has decreased during the last decades, the scientific community has warned about a new problem concerning abandoned sunken vessels scattered all around the world oceans floors. The recent catastrophic large oil spills of the tankers *Erika*, *Prestige* and *Ievoli Sun*, amongst others, where the ship sank with oil in theirs tanks, has triggered the concern by the public opinion of the threat of possible future leaks, and the consequent endangerment to the environment and human activities. In consequence, governments have been pressured to act to remove the remaining pollutants from the wreckages. In parallel, the apparition of “mystery spills” which later were linked to vessels that sank decades ago, i.e. *SS Jacob Luckenbach, M/V Castillo de Salas* and *USS Mississinewa* has brought the issue of the danger of spills from relic wrecks to the fore, as well as the discussion on the convenience of removing pollutants from sunken vessels to a new level. Some authors have referred to the wrecks containing oil as “oil time bombs”, since the question is not “if” they are going to start to leak, but “when” (Girin, 2004). As an example, there is the case of the *HMS Royal Oak* wreck, which acted as a chronic source of oil pollution in the Orkney Island for over 60 years. In 2000 the wreck was estimated to be responsible for 96% of the total amount of oil released in the United Kingdom (Michel et al., 2005). In addition some of the sunken vessels contain other potentially polluting chemicals apart from oil, be it derived from their warfare or industrial transport activities. The reactive approach followed until now, based in actuation when a leak occurred has proven to be unsatisfactory for society, and there have been a growing demand for a more pro-active response, involving the removal of pollutants to avoid future threats (Basta and Kennedy, 2004).

In order to identify potentially polluting wrecks, a compilation of existing data was prepared using diverse local, national and international sources. As a result, the Environmental Research Consulting International Marine Shipwreck database was generated which covers the period between 1890-nowadays. Currently there are 8,569 potentially polluting wrecks introduced in the database and the oil remaining in them has been estimated to range between 2.5 and 20.4 million tonnes, depending on the high and low estimation models, respectively (Fig. 1.2). About 74% of wrecks correspond to incidents during the second World War (WWII), which means that after being underwater for more than 60 years, several of them may be close to reaching their oxidation state.

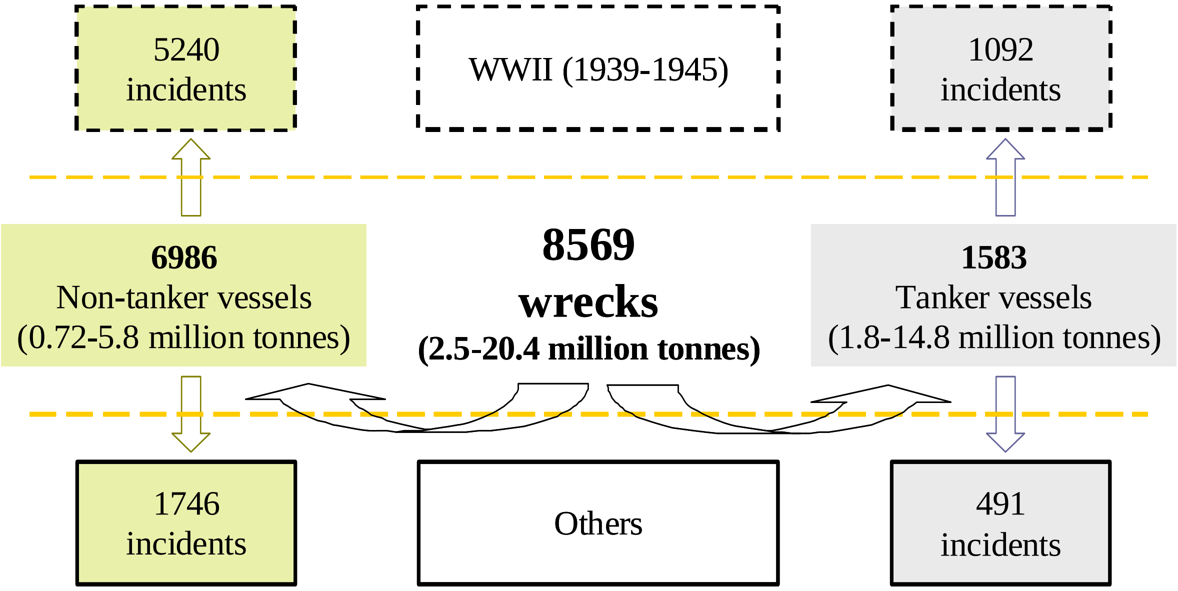


Figure 1.2. Classification of the abandoned wrecks depending on type of vessel and sinking period. Type of vessel: tanker vessels (grey) and non tanker vessels (green). Sinking period: World War II (broken line) and other periods (solid line). Data from Michel et al., 2005.

Management of potentially polluting wrecks, especially the relic ones, has proven to be rather complex. Associated difficulties are focused in three main areas: lack of a proper legal framework, unawareness of the potential environmental, economic and social risks and technological complexity for the recuperation of the remaining oil (Fig. 1.3).

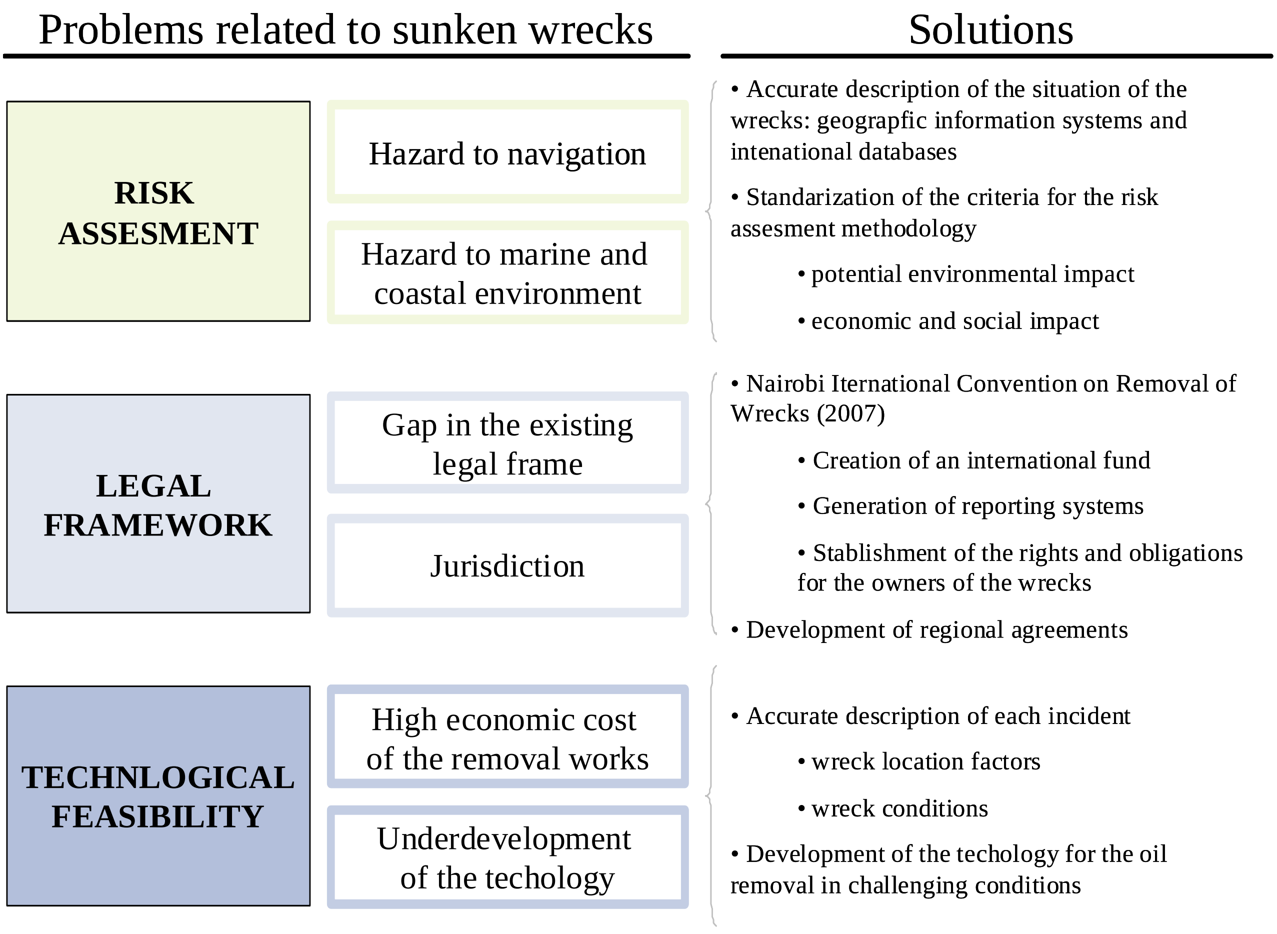


Figure 1.3 . Summary of issues that need to be addressed for the management of potentially polluting wrecks, and possible actions taken to address them.

The removal of the oil from sunken wrecks is economically expensive, time consuming and risky. Due to the large number of the potentially polluting wrecks and the insufficient human and economic resources to face future leaks, there is a need to screen the potentially most dangerous incidents, and distribute the effort in consequence. The cost-benefit analysis must take in consideration both potentially environmental impacts and socioeconomic implication of the possible spills and the remediation costs. The decision of refloating the wreck, and/or the offloading of the oil must be taken when the potential environmental or a combined environmental and socioeonomical risks outweighs the cost of the mitigation action. There have been cases when political, security or the sensibility of the public opinion has prevailed over the environmental or economic concerns. This is the case of the *USS Arizona*, which has been leaking heavy fuel oil in Pearl Harbour since 1941 and still contains around 1,700 tonnes onboard (Russel et al., 2004). Considered a National Historic Landmark, due to its status as a war grave with more than 1,000 sailors and marines, it is visited by over 1.5 million people a year. Due to the refusal of the USA authorities to remove the warship, they have designed a management strategy to asses the future risk of a catastrophic release, with the continuous monitorage of the stability of its structure, its oxidation state, oil release rates and oil degradation (Russel et al., 2004).

1.3.3.1. Risk assessment

Currently, a unified guideline for the risk assessment posed by sunken shipwreck that can be used at international level does not exist, although several organisms have developed their own for local use. Using the methodology prepared by the South Pacific Regional Environmental Programme as starting point, several authors proposed a three step methodology for the environmental risk assessment for the potentially polluting wrecks (SPREP, 2002; Nawadra and Gilbert, 200; Gilbert, 2003). First of all there would be a fist stage of "Information gathering", which implies the development of an accurate database of existing wrecks which should clearly indicate the exact map location, amount and type of cargo, vessel history and damage prior to sinking, identify the ownership and jurisdictional responsibility of each of them and mark the incidents with previous oil releases. This step would be followed by a phase of "Implication /Consequences" assessing the probable scenarios of impact in case of oil leaks, determining the natural and human resources that would be threatened and estimating the most ecologically important regions. Finally, in "Assessment of Risk Priority/Actions" stage there would be a selection of the wrecks that require regular pollution monitoring after carrying out inspections and assessment of vessel integrity in all the possible incidents, determination of the suitable contingency plans for offloading the oil cargos in the priority sites and identity the physical or ecological damages resulting from the mitigation actions.

In order to obtain the general picture of the challenges associated to sunken vessels, the risk that poses each potentially polluting wreck must be determined (Gilbert et al., 2003). The specific information gathered for an individual incident will determine in a great extent the technology that will be used for the oil recovery and clean up, since a sensitive environmental area may advise against the utilization of the most aggressive methods. Therefore modelling the possible oil release scenarios, oil fate and oil impact zones using the specific meteorological, oceanographic and physico-chemical characteristics of the area and the oil properties is essential

1.3.3.2. Legal framework

The existing legal framework has proven to be insufficient to face up the conflict presented by the potentially polluting wrecks. Although several countries pose internal financial and legal regimes, which usually involve the liability of the owners or flag state in the removal of the wrecks in their Exclusive Economic Zone, they generally constitute a weak legal tool mostly due to the lack of the necessary economic resources. Moreover, when these incidents correspond to the WWII casualties, they may be affected by national laws for the protection of the historic memory, whose objectives can be in conflict with the environmental reasons for the removal of the wreck. The particular case of the *HMS Royal Oak*, sunken in Scapa Flow, by Scottish Island of Orkney in 1939, showed the conflict of interests between the implied parties. The corrosion of the hull allowed oil release endangering both the local environment and fisheries in the area. Considered the Britain’s largest official war grave due to the nearly 500 men that died in the battleship, the Ministry of Defense was reluctant to any operation (Ministry of Defense, 2004), and it was not until Orkney authorities threatened with legal actions that the decision to offload the remaining oil was taken.

In an effort to unify the different policies and provide a legal tool for the management of the sunken wrecks and their pollution-related damages worldwide, the international community, under the guidance of the International Maritime Organization (IMO), prepared in 1998 the Draft Wreck Removal Convention, which finally resulted in the Nairobi International Convention of the Removal of Wrecks (2007). It provides a uniform set of rules for the removal of wrecks located beyond the territorial sea that can potentially affect the safety of maritime circulation or endanger the environment (IMO, 2007). The main points of the convention are the following:

- Reporting and locating the ships and wrecks
- Establishment of criteria for determining the hazard posed by wrecks

- Rights and obligations to remove the wrecks
- Financial liability
- Settlement of disputes

The most important aspect of the convention resides in making the ship-owners financially liable for the removal of the wreck, taking out insurance or other financial security and settling a determined time span to carry out the operation. It also plans to create an international fund trust to ensure the removal when the responsible partner is unable to cover it.

1.3.3.3. Technological feasibility

Technological feasibility for the recovery of a sunken wreck or for offloading the oil is a fundamental factor to take into account. Each incident is unique, and therefore the most adequate method and technology must be chosen, especially in challenging situation such as in cold waters o high depth wrecks. Most of the oil removal is carried out in the sea surface, although in especial situations, where refloating the wreck is not possible, more advanced techniques may be used to recover the oil from the vessel. Underwater oil removals are more complex and less frequent, although there have been important improvements in deep water engineering and salvage skills during the last decade, which have been proven in the incidents of *Erika*, *Ehime Maru* and *Jacob Luckenbach* amongst others. The *Prestige* possibly constituted one of the most spectacular cases of the last decade, where about allegedly 14,000 tonnes of heavy fuel oil were removed from the wreck located at more than 3,500 m depth by means of an innovative technique using ROVs and a system of shuttles (REPSOL, 2004). This incident demonstrated that oil recovering from a wreck has become technically possible at almost any depth.

Several factors can influence salvage planning and determine the application of the most suitable methodology, such as mobilization distance, sea conditions, oil type, oil viscosity, oil weathering, wreck conditions and wreck location factors. In the case of the *Royal Oak*, the attempts to install temporal stainless steel canopy or container over the hull were unsuccessful due to rough sea state and therefore the Ministry of Defense of UK was forced to offload most of the remaining oil. Sometimes, the operational requirements do not coincide with the protection of the environment objectives. In the incident of *Jacob Luckenbach*, the recovery tasks were especially difficult due to the high viscosity of the oil, strong currents and the need to maintain the stability of salvage platform used for the occasion. The removal of the oil had to occur in the summer months to maximize the chances of success, which coincided with the most biologically sensible period in the nearby Gulf of the Farallones Marine Sanctuary. In these situations, the knowledge provided by the risk assessment can be useful to design strategies which minimize the impacts in the environment.

1.4. Fate of the oil spills in the open ocean

1.4.1. Weathering processes

Once the spilled product reaches the ocean, it undergoes several physical, chemical and biological processes, also known as the oil “weathering”, which promotes its distribution in the different marine compartments, both biotic and abiotic, or its degradation (Fig. 1.4). Several factors can alter the extent of these processes being the most relevant the release conditions (the rate and total amount of oil spilled and surface release or underwater release), oil’s initial physico-chemical properties and the prevailing weather conditions (temperature, currents and sea-state amongst others). Weathering processes are differentiated into two groups depending on the time span over which they act. Short term processes, comprising evaporation, dissolution, dispersion and sedimentation, start immediately after the spill and extend for few weeks. The effect of the long term processes generally is noticed after several weeks of the spill (photooxidation), or even months (biodegradation) (Omotoso et al., 2002). Although water in oil emulsion formation is considered to belong to the latter group (Payne et al., 2003), it has been reported than many of crude oils form water in oil emulsion rapidly after being spilled (Daling et al., 2003).

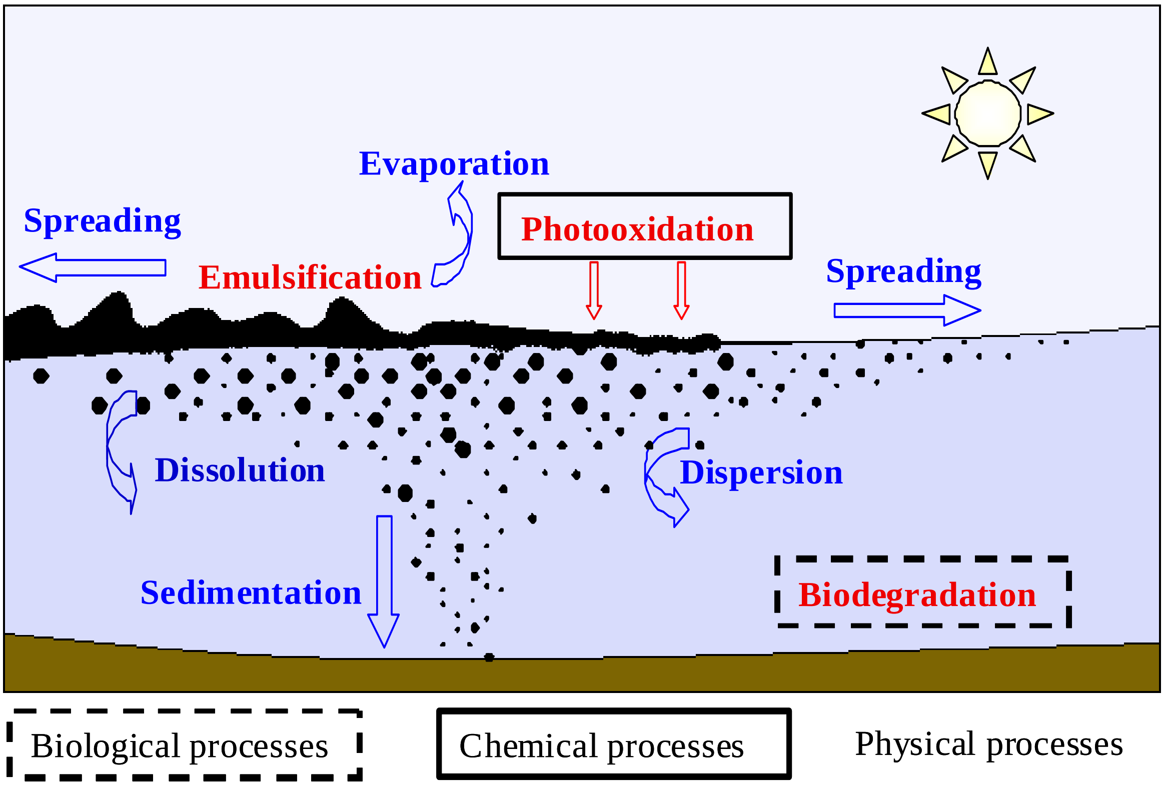


Figure 1.4. Main short-term (blue) and long-term (red) weathering processes affecting the oil in the ocean after a spill (Modified from ITOPF, 2008).

1.4.1.1. Spreading

Immediately after the spillage, oil starts to spread over the ocean surface. The spreading velocity depends on the viscosity of the product, volume spilled, water temperature and tidal stream and currents. Fluid and low viscosity oils spread more easily than the highly viscous or solid ones. They tend to form a continuous thin layer in the sea surface, and soon after start to breaking up. The most viscous oils, on the other hand, fragment instead of spreading, and form a thick layer of several centimetres. Strong tidal streams and currents usually speed up the process.

The spreading of the oil can play an important role in other weathering processes, such as emulsion formation and dispersion, where the thickness of the oil layer in the ocean surface is one of factors determining the formation of stable water-in-oil emulsions, or the natural dispersion of the oil (Daling et al., 2003).

1.4.1.2. Dispersion

In the early stages of a spill, the rate of dispersion is conditioned by the sea state and the nature of the oil. Waves and turbulence provide the required energy to break up the original oil slick in droplets of different size which will mix with the upper layer of the water column. Droplets with diameters below 50-100 µm can be considered permanently dispersed and may remain in suspension. The larger ones may rise to the surface and coalescence with other droplets or expand forming a very thin film, know as “sheen”. Dispersion can enhance other weathering processes such as dissolution, sedimentation and biodegradation, due to increasing in the surface area provided by dispersed oil.

The viscosity of the oil is also an important factor conditioning its dispersion. Low viscosity oils that remain fluid, and not largely affected by other weathering processes, can be dispersed naturally in moderate sea conditions in several days. This was the case of the *Braer* incident in the Shetlands, where most of the 84,000 tonnes of Gullfaks crude oil spilled were completely dispersed under exceptionally severe weather conditions at the time of spillage (Thébaud et al., 2003).

1.4.1.3. Emulsion formation

Some petroleum products are able to form water-in-oil emulsions, often called “chocolate –mousse” due to the brown-reddish colour that the emulsion acquires. Stable emulsions contain between 60-80 % of water, expanding the volume of the oil from two to five times. There is also a modification of the original characteristics of the spilled product, such as a noticeable increasing in the viscosity and density (Fingas et al., 2003; Fingas and Fieldhouse, 2003), and therefore, the feasibility of some countermeasure techniques, mechanical removal and chemical treatment amongst others, could be affected.

Emulsion formation “competes” to some extent with the dispersion of the oil in the sea, since factors and parameters that enhance one of the processes difficult the other and vice versa. (Daling et la., 2003). Emulsions form most readily in high viscosity fuel oils containing high abundance of stabilizing agents, such as asphtalnenes, photooxidized compounds (resins) and, in some oils, precipitated waxes (Sjöblom et al., 1992; Nour et al., 2008). Initially, formed emulsions have relatively low viscosities and are simple mixtures of water droplets and oil, which are constantly breaking down and forming back again. With time, after the more volatile and soluble components of the oil (mostly hydrocarbons) have been removed as a consequence of short time weathering processes, oil gains viscosity. There is then a precipitation of the stabilizing agents, which prevent the droplets to coalescence and drain from the emulsion stabilizing it. After an extended period of weathering and mixing due to the sea, there is water intake in the emulsion, droplets become smaller and the emulsion becomes very viscous and persistent. At this point it may remain emulsified indefinitely and it is considered “stable”. (ITOPF, 2002; Fingas and Fieldhouse, 2001).The rate of natural dispersion and also other weathering processes is retarded, which is the main reason for the persistence in the ocean of light and medium crude oils.

The viscosity of the initial product will also affect its spreading properties, the oil slick thickness, and therefore, the formation of emulsions. Thin film layers are easily disrupted in the presence of water droplets, and will split to release them. Thick layers are more capable to accommodate oil droplets, but the formation of the emulsions will also be slower, as in the case of the industrial heavy fuel oil carried by the *Erika* and the *Baltic Carrier*.

1.4.1.4. Dissolution

The dissolution of the oil in the ocean depends on its composition, salinity and temperature of the water, the turbulence of the water and degree of oil dispersion. The heaviest components of the oils (resins and asphaltenes) are practically insoluble in the water, and only a small fraction of the lightest compounds are able to dissolve (Shiu et al.,1990). Small aromatic hydrocarbons from 1 to 3 rings and sulphur and nitrogen heterocycles are the main components of the seawater soluble fraction (SWSF). Despite that this physical process is not important in the removal of oil from the marine environment, it is essential in its toxicological assessment. The SWSF is the more bioavailable fraction of the oil, and it is considered the most toxic fraction of the oil, even more than the emulsionated one (Ziolli et al., 2002).

1.4.1.5. Evaporation

The more volatile compounds of the oil evaporate to the atmosphere after the spill. At 15 ºC the fraction of oil components with a boiling point lower than 200 ºC (less than 10 carbons) could be lost in two days (Albaigés and Bayona, 2003) and therefore, the greater the proportion of the oil with low boiling point components, the bigger proportion of the loss due to evaporation. In this aspect, the refined products, such as kerosene and gasoline may evaporate completely within hours of the spill, while 80 % of the spilled diesel fuel can be loss by this process. For heavier products, such as light and heavy crudes, the loss can reach the 40% and 20% of the total, respectively. On the contrary, due to its heavy nature, only 5-10 % of the bunker C fuel oil evaporates during a spill (Verma et al., 2008). In the accident of the *Amoco Cadiz*, about the 40% of the 240,000 tonnes of oil spilled were lost due to this process and more than the half of the diesel oil cargo released form the *Jessica* in the Galapago Islands (Kingston, 2002). In contrast, only about 2-5% of the fuel oil spilled from the Prestige shipwreck evaporated, due to its heavy nature (CSIC, 2003a). The evaporated fraction consisted manily of homologous series of *n*-alkanes, with up to 10 carbon atoms, and light aromatic hydrocarbons known as BTEX (benzene, toluene, ethylbenzene and xylene). The concentration of the latter in the Prestige spill was reduced by 80% in the first two weeks after spillage (CSIC, 2003b).

The initial spreading of the oil in the sea-surface influences the evaporation rate, since the larger the oil-atmosphere contact surface, the faster the loss of the more volatile compounds. Rough sea, high temperatures and high wind speed will also enhance this process (ITOPF, 2002). In contrast, formation of emulsions drastically slows evaporation (Michel et al., 2005)

1.4.1.6. Sedimentation

Some oils have specific gravities greater than the seawater (>1.025 Kg L^{-1}) and rapidly sink to the bottom after being spilled. The vast majority of the oils, however, have lower specific gravities and float in the ocean surface, dispersing part of the load into small oil droplets. These droplets can interact with the SPM of the seawater, thus creating more dense aggregates which can sediment to the sea-floor (ITOPF, 2002). Interaction can also occur at molecular level, with compound specific adsorption of oil-sourced dissolved compounds onto SPM. Both processes contribute to the long-term transport of the oil and toxic components to the sediments (Payne et al., 2003). The nature of the released oil and the sea-state also influence oil/SPM interaction, since they both will determine the dispersion of the oil droplets from the initial slick and the dissolution of individual components. Sources of SPM include inputs from rivers, glaciers, aeolian transport, physical erosion of the shoreline sediments and resuspension of the bottom sediments. Biological particles such as phytoplankton agglomerates can also interact with oil droplets, and the ingested oil or compounds will subsequently be deposited to the sea-floor in faecal pellets (Johanson et al., 1980).

The effectiveness of this process in removing oil from the surface is conditioned by the amount of SPM, and therefore its importance in an open ocean or near shore oil spill will be very different. It has been estimated that SPM loads higher than 10 mg L^{-1} make oil/SPM interaction with subsequent transport and deposition possible. At SPM concentrations higher than 100 mg L^{-1} massive oil transport can occur (Boehm, 1987), as in the case of the *Tsesis* oil spill in the Baltic sea, where roughly 10-15 % of the 300 tonnes of the spilled oil was removed by means of oil/SPM interaction and sedimentation, which was possible due to turbulent resuspension of bottom sediments (Johansson et al., 1980). The SPM concentrations in the open ocean are low, usually less than a few mg L^{-1}, making oil/SPM interactions generally insignificant in open ocean spills (Payne et al., 2003).

In general, oil-droplets dispersion and oil/SPM interaction occur relatively soon after the spill, before weathering processes affect its viscosity. As the viscosity increases due to the loss of the lightest compounds and generation of water in oil emulsions, the concentration of oil droplets decreases and therefore oil/SPM interaction became limited (Khelifa et al., 2002; Payne et al., 2003).

1.4.1.7. Biodegradation

Seawater contains several organisms capable of metabolizing oil components and using them as a carbon source. They include bacteria, mould, yeasts, fungi, unicellular algae and protozoa. Although these organism are distributed worldwide, they concentrate in chronically polluted waters, for example in areas with heavy vessel traffic or receiving industrial discharges or sewage .The main factors affecting the extent of biodegradation are the nature of the oil, nutrients (especially nitrogen and phosphorous), oxygen and water temperature. Since these microorganisms live in the water, biodegradation only occur at the oil-water interface. Therefore, all the processes that increase the oil contact surface area, such as dispersion, will enhance biodegradation. On the contrary, once the oil is incorporated into the sediments in the sea-bed or the shoreline, this process is greatly reduced (ITOPF, 2002).

Together with photooxidation, biodegradation is one of the weathering processes that remove oil from the environment (Prince, 2002). Almost all the hydrocarbons can be degraded although each individual strain of organisms usually only degrade a limited group of compounds. The aerobic biodegradation pattern is nearly opposite to the one followed by photooxidation. Generally, *n*-alkanes are more rapidly degraded than the polycyclic aromatic hydrocarbons, and within each family, the degradation decreases with increasing size and alkylation (Prince et al., 2003).

Biodegradation is quite slow process, since it occurs in the oil-water, oil-air interphase, and it progresses on time scales of months and years (Albaigés and Bayona, 2003). In a greater or lesser extent, it depends on the fuel type, environmental conditions and the marine spill history in that location. In favourable conditions, bacterial communities can degrade between 30% to 50% of the initial product, leaving behind the heaviest residue (higher carbon number PAHs, resins and asphaltenes). In the *Prestige* wreck only 2-5% of the oil could be lost by this process , demonstrating the recalcitrant character of the spilled product (CSIC, 2003).

1.4.1.8. Photooxidation

When the oil slick is in the surface of the ocean it is exposed to sunlight ultraviolet (UV) radiation in a oxygenated environment, which promote the photooxidation of the oil and generation of some oxidized compounds, such as aliphatic and aromatic ketones, aldehydes, carboxylic acids, and fatty acids, amongst others (e.g. Nicodem et al., 1997). Generally, large aromatic hydrocarbons are more prone to photooxidation than lower carbon number ones, and the most alkylated compounds are degraded before the less substituted congeners (Prince et al., 2003). The resulting products can be more toxic than the original components (Kingston, 2002).

The seawater-soluble fraction (SWSF) increases after the photooxidation of the oil (Maki, 2001), due to, above all, the increased solubility of the resulting compounds. In some cases this leads to an increase toxicity of the SWSF for the biota (Lee, 2003). On the other hand, it has been found that photooxidized products are more available for the microbiota, not capable to degrade the original compound, and in consequence the biodegradation is strengthened (Dutta et al., 2000).

1.4.2. Vertical processes

The oceans play an important role in the transport and fate of the hydrophobic organic contaminants (HOCs) at regional and global scales (Dachs et al., 2002; Lohmann et al., 2007). The importance of the water column in the organic matter cycle in the marine environment (Lipiatou et al., 1997) and as consequence in the fate the contaminants associated to it has been widely studied. The key processes that take part in the distribution of the HOCs in the water column can be summarized in five groups (Fig. 1.5):

- Mechanical transport by ocean currents
- Air-water exchange
- Phase partitioning
- Degradation of compounds
- Water-sediment exchange

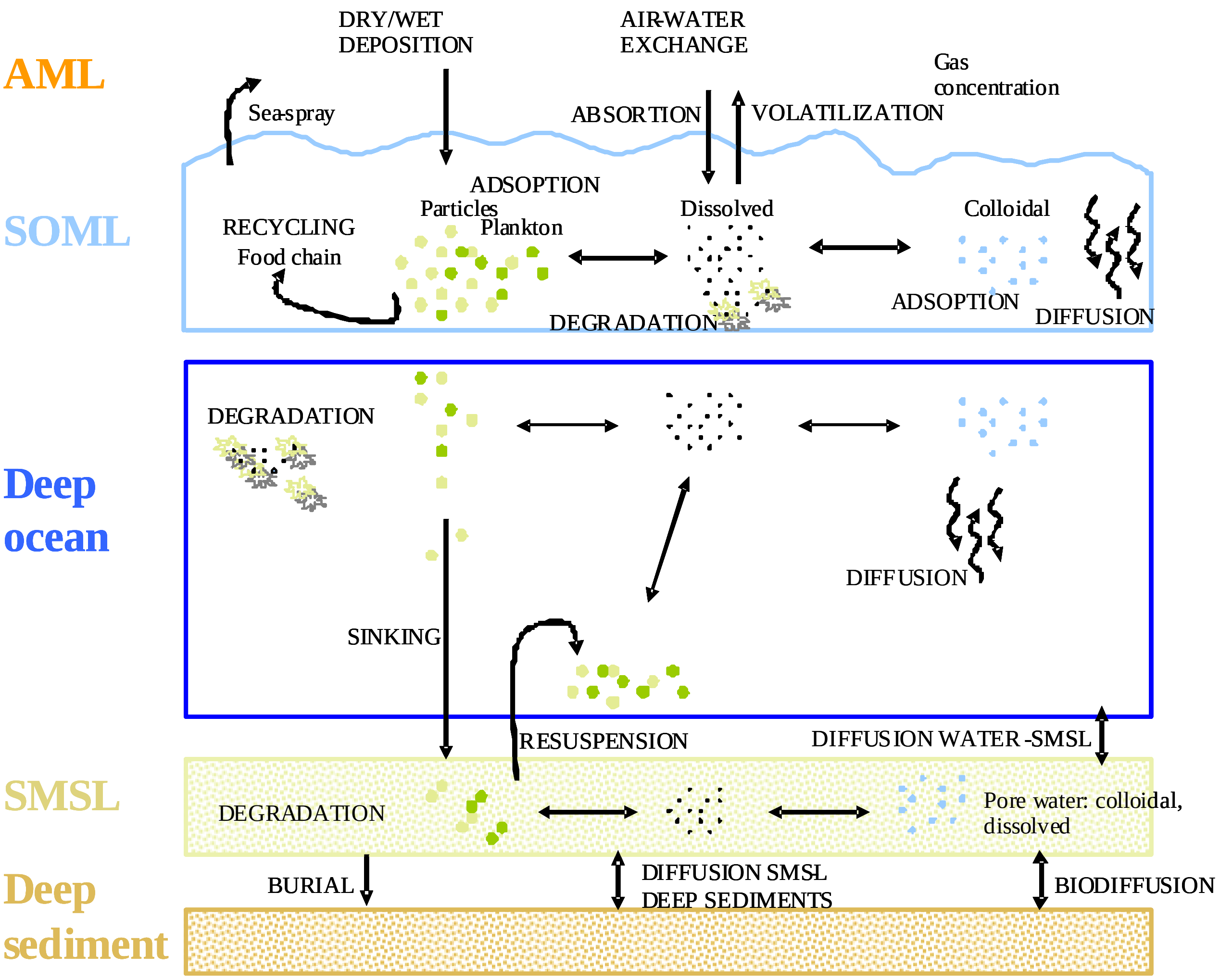


Figure 1.5. Principal water column processes in the ocean for the HOCs.AML: Atmospheric mixed layer; SOML; Surface ocean mixed layer; SMSL: Surface mixed sediment layer. Adapted from Jaward et al., 2004 and Jurado et al., 2007.

Mechanical transport of HOCs is one of the simplest ways for the distribution of pollutants in the marine environment and include advective and difussive movements. Advection is governed by current velocity (Ilyina et al., 2006), and implies the displacements of the contaminants with the moving water mass. Several authors consider migratory displacements of the polluted living organisms, such as salmons, part of this advective process (Ewald et al., 1998; Wania 1998). In the case of HOCs diffusion, the turbulence of the water plays an important role in the mixing, intensifying the process (Jurado et al., 2007).

Air-water exchange in the mixed surface layer is one of the most important pathways for the entry and loss of HOCs in the water column (Iawata et al., 1993; Wania et al., 1998). In remote locations atmospheric transport is the principal input of particulate and vapour contaminants in the marine environment, in fact, the distribution of PAHs and polychlorinated biphenyls (PCBs) in the suspended particulate matter (SPM) and the dissolved phase (DP) in the atmosphere and the seawater have been observed often to be similar (Achman et al., 1993; Jeremiason et al., 1999). The entry of contaminants to the ocean from the atmosphere occurs by means of wet and dry deposition processes. While the fist implies intense periodic inputs during rain episodes the later means a slower dynamic process in dry weather (Cotham and Bidleman, 1991; Brorström-Lundén, 1996). Several studies suggest that this air-water exchange and HOCs uptake by phytoplankton are coupled (Dachs et al., 1999). Pollutants uptake by phytoplankton and subsequent sedimentation decreases HOCs concentrations in the DP, which in turn enhances the flux from atmosphere to ocean (Millard et al., 1993; Dachs et al., 1999; Jeremiason et al., 1999). In general, all the processes that promote the removal of the HOCs from the superficial waters, such as vertical advection of water masses, sedimentation of the SPM and mixing of waters by turbulent diffusion reduce their loss by evaporation (Wania et al., 1998). In addition, the formation of marine aerosols can also transfer HOCs from seawater to the atmosphere (Allen et al., 1996). Both the bursting of the bubbles created during the breaking of waves and wind induced sea-spray are the primary processes that generate atmospheric particles from seawater, although bubbles are also formed from methane releases from sediment due to anaerobic decomposition of the organic matter and supersaturation of the superficial waters as a consequence of marine algae photosynthesis (Wania et al., 1998). Other processes, such as raindrop splashing during severe rainstorms can also enhance the formation of atmospheric particles (Offenberg and Baker, 1997).

In the marine environment, HOCs can be found in their dissolved form or associated to particles. While the DP follows the direction of the water masses, the SPM tends to settle and remain in the sediments (Ilyina et al., 2006). Amongst the transport and transformation processes governing the fate of pollutants in the water column, the vertical flux of HOCs associated to settling particles is assumed to be the most significant (Dachs et al., 2002; Wania and Daly, 2002). The most important characteristic of the HOCs that govern their distribution in the different compartments is their hydrophobicity (Froescheis et al., 2000). It is translated in poor solubility and high affinity to lipids, which promote preferential association to SPM in the ocean (Jurado et al., 2007). Particles possess surfaces and phases similar to the lipids, hence the sorption to them is rather favourable in the aqueous environment (Zhou et al., 1995; Luthy et al., 1997; Zhow and Roelan 1997). This partition will influence the final fate of the HOCs in the ocean and their addition to the food chains, since only the dissolved phase is small enough to go through the biological membrane and therefore the toxicity of pollutants will depend to great extent of the phase they are concentrated in. In open ocean waters a large portion of particles are formed by living phytoplankton, which grow and sediment to the ocean bottom providing one of the most effective process for the HOCs secuestration from the surface waters (Baker et al., 1991; Dachs et al., 1996; Gustafsson et al., 1997).

Benthic organism communities are greatly affected by the HOCs partition in the SPM, since biota in the abysm depends on the input of organic carbon from the ocean surface and therefore HOCs that are not degraded in the upper layers are to be transported by living or dead biota, or adsorbed to detritus in marine snow (Froescheis et al., 2000). High phytoplankton growth intensify vertical fluxes and therefore the removal of HOCs from the superficial water, which in turn means the increase of pollutant concentration in the benthic organisms (Millard et al., 1993; Gunnarsson et al., 1996; Jeremiason et al., 1999).

HOCs in general are transformed very slowly in the marine environment, which puts in evidence their recalcitrant nature (Michaud et al., 2007) . Abiotic (photooxidation and hydrolysis) and biotic degradation of the compounds in the water column depend to a large extent of the nature of the compounds and their phase partitioning. Compounds associated to particles show longer lifetimes than the ones freely dissolved in the aqueous phase, which are more readily attacked by living organisms (Ohkouchi et al., 1999). Pollutants such as PCDD/Fs and PCBs have half life reported values in sediments of 10-300 years and 3-40 years respectively (Sinkkonen and Paarsivita 2000). On the contrary, reported half lives in sediments for three-ring PAHs range from 16 to 126 days while for five ring PAHs may rise up to from 229 days to more than 1,400 days (Shuttleworth and Cerniglia, 1995). Opposite to other HOCs, hydrocarbons are readily metabolized by different marine vertebrate and invertebrate species (Selck et al., 2003; Jonsson et al., 2004; Nfon et al., 2008) and microorganisms that use hydrocarbons as a sole of carbon and energy source are widely distributed in nature (Atlas, 1981; Kasai et al., 2002; Van Hamme et al., 2003). The preferential degradation of the low molecular weight PAHs and their higher bioavalability compared to the heavier PAHs (Kanaly and Harayama, 2000; Arulazhagan and Vasidevan, 2009) translates in compositional changes in PAHs in the course of the sinking in the water column (Maldonado et al., 1999; Ohkouchi et al., 1999). Therefore, higher proportion PAHs with more than four rings in the sediments compared to the SPM of the surface waters can be found. The biodegradation process of the HOCs continues in the sediments after particles have settled (De Lange et al., 2009).

Interactions between the seawater and sediments also play an important role in the distribution of pollutants in the ocean. Historically, polluted sediments can act as a secondary contamination source of HOCs for overlying water column, thus prolonging the exposure to the benthic biota even after the emission has stopped (Larsson et al., 1990). The two key processes governing the transport of HOCs between the seawater-sediment interphase are sedimentation/resuspension of the sediments and diffusion movement of the HOCs and HOCs attached to the organic matter. In environments with oxygen, biodiffusion by the benthic organism such as bivalves, molluscs and worms enhances the mixing of the particles with the pollutants, which translates in the increase of the transfer of HOCs to the seawater (Gilek et al., 1997; Schaffner et al., 1997)

1.5. References

Achman, D., Hornbuckle, K.C., Eisenreich, S.J., 1993. Volatilization of polychlorinated biphenyls from Green bay, Lake Michigan. Environ. Sci. Technol. 27, 75-86.

Albaigés, J., B. Morales-Nin, Vilas, F., 2006. The Prestige oil spill: A scientific response. Mar. Pollut. Bull., 53, 205-207.

Albaigés, J., Bayona, J..M., 2003. La “huella” del fuel : Ensayos sobre el *Prestige*. Fundación Santiago Rey Fernández-Latorre, A Coruña, pp. 80-103.

Allen, J.O., Dookeran, N.M., Smith, K.A., Sarofim, A.F., Taghizadeh, K., Lafleur, A.L., 1996. Measurements of polycyclic aromatic hydrocarbons associated with size-segregated atmospheric aerosols in Massachusets. Environ. Sci. Technol. 30, 1023-1031.

Anderson, A.G., 2002. The media politics of oil spills. Spill. Sci. Tecnol. Bull 7, 7-15.

APPEA, 2002. Discovery: Explore the world of oil and gas: Oceans and oil spills. Available from: http://www.appea.com.au/Publications/docs/oceans.html

Arulazhagan, P., Vasidevan, N., 2009. Role of a moderately halophilic bacterial consortium in the biodegradation of polyaromatic hydrocarbons. Mar. Pollut. Bull. 58, 256-262.

Atlas, R.M., 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. Microbiol. Rev. 45, 180-209.

Baker, J.E., Eisenreich, S.J., Eadie, B.J., 1991. Sediment trap fluxes and benthic recycling of carbon, polycyclic aromatic hydrocarbons and polychlorobiphenyl congeners in Lake Superior. Environ. Sci. Technol. 25, 500-509.

Basta D.J., Kennedy, D.M., 2004. The need for a proactive approach to underwater threats. Mar. Technol. Soc. J. 38. 9-11.

Birkland, T. A., Lawrence, R.G., 2002. The Social and Political Meaning of the Exxon Valdez Oil Spill. Spill Sci. & Technol. B. 7, 17-22.

Brorström-Lundén, E., 1996. Atmospheric deposition of persistent organic compounds to the sea surface. J. Sea Res. 35, 81-90.

CAA, 2003. Hundimiento del buque *Prestige*. Informe nº7. Available from: http://otvm.uvigo.es/investigacion/informes/informcca.html.

Cotham, W.E., BidlemanT.F., 1991. Estimating the atmospheric deposition of organochlorine contaminants to the Arctic. Chemosphere 22, 165-188.

CSIC, 2003a. Informe técnico CSIC *Prestige* nº1: Caracterización del vertido y evolución preliminar en el medio. Available from: http://csicprestige.iim.csic.es/desarro/informcsic/1/.

CSIC, 2003b. Informe técnico CSIC *Prestige* nº10: Contenido de componentes volátiles en le fuel-oil vertido por el *Prestige*. Available from: http://csicprestige.iim.csic.es/desarro/informcsic/10/.

Dachs, J., Bayona, J.M., Fowler, S.W., Miquel, J.C., Albaigés, J., 1996. Vertical fluxes of polycyclic aromatic hydrocarbons and organochlorine compounds in the western Alboran Sea (southwestern Mediterranean). J. Mar. Chem. 52, 75-86.

Dachs, J., Bayona, J.M., Fillaux, J., Saliot, A., Albaigés, J., 1999. Evaluation of anthropogenic and biogenic inputs into the western Mediterranean using molecular markers. Mar. Chem. 65: 195-210.

Dachs, J., Lohmann, R., Ockenden, W.A., Méjanelle, L., Eisenreich, S.J., Jones, K.C., 2002. Oceanic biogeochemical controls on global dynamics of persistent organic pollutants. Environ. Sci. Technol. 36, 4229-4237.

Daling, P. S., Moldestad, Ø.M., Johansen, Ø.,Lewis, A., Rødal, J., 2003. Norwegian testing of emulsion properties at sea-the importance of oil type and release conditions. Spill Sci. Technol. Bull. 8, 123-136.

De Lange, H.J., Peeters, E.T.H.M., Maas, H., De Jonge, J., 2009. Seasonal variation of total and biochemically available concentrations of PAHs in a foodplain lake sediment has no effect on the benthic invertebrate community. Chemosphere, article in press.

Dutta, T.K., Harayama, S., 2000. Fate of crude oil by the combination of photooxidation and biodegradation. Environ. Sci. Technol. 34, 1500-1505.

EC, 2003. Prestige accident. Available from: http://ec.europa.eu/transport/maritime/safety/doc/prestige/2003_01_08_memo_en.pdf

EP, 2003. Caso studio informe MA 2003: Recuperación del fuel del buque petrolero *Prestige*. Available from: http://www.europarl.europa.eu/comparl/tempcom/mare/pdf/031201_hernan_es.pdf

Ewald, G., Larsson, P., Linge, H., Okla, L., Szarzi, S., 1998. Biotransport of organic pollutants to an inland Alaska lake by migrating sockeye salmon (*Oncorhynchus nerca*). Arctic 51, 40-47.

Faksness, L., Brandvick, P.J., 2008. Distribution of water soluble components from Arctic marine oil spills- A combined laboratory and field study. Cold Reg. Sci. Technol. 54, 97-105.

Fingas, M., Fieldhouse, B., 2001. What causes the formation of water-in-oil emulsions?. International Oil Spill Conference 2001, Wahington D.C. Available from: http://www.iosc.org/papers/02366.pdf#search="water-in-oil"

Fingas, M., Fieldhouse, B., 2003. Studies of the formation process of water in oil emulsions. Mar. Pollut. Bull. 47, 369-396.

Fingas, M., Fieldhouse, B., Wang, Z., 2003. The long term weathering of water-in-oil-emulsions. Spill Sci. Technol. Bull. 8, 137-143.

Freire, J., Fernández, L, González-Gurriarán, E., Muiño, R., 2003. Impacto ambiental de la marea negra del *Prestige* : efectos sobre los ecosistemas marinos y recursos pesqueros. Papel de la comunidad científica en la respuesta a la crisis. Available from: http://otvm.uvigo.es/investigacion/informes/documentos/archivos/Impacto_ambiental_Prestige_jFreire.pdf

Froescheis, O., Looser, R., Cailliet, G., Jarman, W.M., Ballschmiter, K., 2000. The deep-sea as a final global sink of semivolatile persistent organic pollutants? Part I: PCBs in surface and deep-sea dwelling fish of the North and South Atlantic and the Monterey Bay Canyon (California). Chemosphere 40: 651-660.

Frost, K.L., Lowry, L.F., Ver-Hoef, J.M., 1999. Monitoring the trend of harbour seals in Prince William Sound, Alaska, after the Exxon Valdez oil spill. Mar. Mammal Sci. 15, 494-506.

GESAMP, 2007. Reports and studies 75: Estimates of oil entering the marine environment from sea-based activities. International Maritime Organization, London, pp.83.

Gilbert, T.D., Nawadra, S., Tafileichig, A., Yinug, L., 2003. Response to an oil spill from a sunken WWII oil tanker in Yap State. Proceedings of the 2003 Intrenational Oil Spill Conference. American Petroleum Institute, Washington, D.C., pp.1-8.

Gilek , M., Björk, M., Broman, D., kautsy, N., Näf, C., 1997. The role of the blue mussel, *Mytilus edulis*, in the cycling of the hydrophobic organic contaminants in the Baltic proper. Ambio 26, 202-209.

Girin, M., 2004. European experience in response to potentially polluting shipwrecks. Mar. Technol. Soc. J. 38, 21-25.

Gonzalez, M., Uriarte, A., Pozo, R., Collins, M., 2006. The Prestige crisis: Operational oceanography applied to oil recovery, by the Basque fishing fleet. Mar. Pollut. Bull. 53, 369-374.

Gunnarsson, J., Schaanning, M.T., Hylland, K., Sköld, M., Eriksen, D.Ø, Berge, J.A., Skei, J.,1996. Interaction between eutrophication and contaminants. III. Mobilization and bioaccumulation of benzo(a)pyrene from marine sediments. Mar. Pollut. Bull., 33, 80-89.

Gustafsson, Ö, Gschwend, P.M., Buesseler, K.O., 1997. Settling removal rates of PCBs into the northwestern Atlantic derived from ^{238}U-^{234}Th disequilibria. Environ. Sci. Technol. 31, 3544-3550.

Iawata, H., Tanabe, S., Sakal, N., Tatsukawa, R., 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of the ocean on their global transport and fate. Environ. Sci. Technol. 27, 1080-1098.

Ilyina, T., Pohlmann, T., Lammel, G., Sündermann, J., 2006. A fate and transport ocean model for persistent organic pollutants and its application to the North Sea. J. Mar. Sys. 63, 1-19.

IMO, 2007. Conventions. Available from: http://www.imo.org/

ITOPF, 2002. fate of marine oil spills. Available from: http://www.itopf.com/marine%2Dspills/fate/weathering%2Dprocess/documents/tip2.pdf

ITOPF, 2009. Oil tanker spill statistics. Available from: http://www.itopf.com/information%2Dservices/data%2Dand%2Dstatistics/statistics/documents/Statpack2008_001.pdf

Jeremiason, J.D., Eisenreich S.J., Paterson, M.J., Beaty, K.G., Hecky, R., Elser, J.J., 1999. Biogeochemical cycling of PCBs in lakes of variable trophic status: a paired lake experiment. Limnol. Oceanogr. 44, 889-902.

Johansen, O., Rye, H., Cooper, C., 2003. Deepspill.Field study of a simulated oil and gas blowout in deep waters. Spill. Sci. Tecnol. Bull. 8, 433-443.

Johanson, S.U., Larsson, U., Boehm, P.D., 1980. The *Tsesis* oil spill : impact on the pelagic ecosystem. Mar. Pollut. Bull. 11, 284-293.

Jonsson, G., Bechmann, R.K., Bamber, S.D., Baussant, T., 2004. Bioconcentration, biotransformation and elimination of polycyclic aromatic hydrocarbons in sheepshead minnows (*Cyprinodon variegates*) exposed to contaminated seawater. Environ. Toxicol. Chem. 23, 1538-1548.

Jurado, E., Zaldívar, J.M., Marinov, D., Dachs, J., 2007. Fate of persistent organic pollutants in the water column. Does turbulent mixing matter?. Mar. Pollut. Bull. 54: 441-451.

Kanaly, R.A., Harayama, S., 2000. Biodegradation of high-molecular-weight polycyclic aromatic hydrocarbons by bacteria. J. Bacteriol. 182, 2059-2067.

Kasai, Y., Kishira, H., Harayama, S., 2002. Bacteria belonging to the genus *Cycloclasticus* play a primary role in the degradation of aromatic hydrocarbons released in marine environment. Appl. Environ. Microb. 68, 5625-5633.

Khelifa, A., Stoffyn-Egli, P., Hill P.S., Lee, K., 2002. Characteristics of oil droplets stabilized by mineral particles: effects of oil type and temperature. Spill Sci. Technol. Bull. 8, 19-30.

Kingston, p., 2002. Long-term environmental impact of oil spills. Spill Sci. Technol. Bull. 7, 53-61.

Larsson, P., Okla, L., Ryding, S.O., Westöö, B., 1990. Contaminated sediments as a source of PCBs in a river system. Can. J. Fish. Aquat. Sci. 47, 746-754.

Le Cedre 2002. Informes sobre el Prestige : Intento de salvamento. Available from: http://www.cedre.fr/es/accidentes/prestige/sauvetage.htm

Lee, K., 2003. Oil-particle interactions in aquatic environments: influence on the transport, fate, effect and remediation of oil spills. Spill Sci. Technol. Bull. 8, 3-8.

Lipiatou, E., Tolosa, I., Simó, R., Bouloubassi, I., Dachs, J., Marti, S., Sicre, M.A., Bayona, J.M., Grimalt, J.O., Saliot, A., Albaiges, J., 1997. Mass budget and dynamics of polycyclic aromatic hydrocarbons in the Mediterranean Sea. Deep-Sea Res, Pt. II 44: 881-905.

Lohmann, R., Breivik, K., Dachs, J., Muir, D., 2007. Global fate of POPs: current and future research directions. Environ. Pollut. 150, 150-165.

Maki, H., Sasaki, T., Harayama, S., 2001. Photo-oxidation of biodegraded crude oil and toxicity of the photo-oxidized products. Chemosphere 44, 1145-1151.

Maldonado, C., J. M. Bayona, J.M., Bodineau, L., 1999. Sources, distribution, and water column processes of aliphatic and polycyclic aromatic hydrocarbons in the northwestern Black Sea water. Environ. Sci. Technol. 33: 2693-2702.

Michaud, L., Di Marco, G., Bruni, V., Lo Giudice, A., 2007. Biodegradative potencial and characterization of psychrotolerant polychlorinated biphenyl degrading bacteria isolated from a coastal station in the Terra Nova Bay (Ross Sea, Antarctica). Mar. Pollut. Bull. 54, 1754-1761.

Michel, J., Gilbert, T., Waldron., J., Blocksidge, C.T., Schmidt, S.F., Etkin, D., Urban, R., 2005. Potentially polluting wrecks in marine waters. In: Proceedings of the international oil spill conference (IOSC) , Miami beach, USA.

Millard, E.S., Halfon, E., Minns, C., Charlton, C.C., 1993. Effect of primary productivity and vertical mixing on PCB dynamics in planktonic model ecosystems. Environ. Toxicol. Chem. 12, 931-946.

Ministry of Defense, 2004. Available from: http://news.mod.uk/news/.

Nawadra, S., Gilbert, T.D., 2002. Risk of marine spills in the Pacific Island Region and its evolving response arrangements. Proceedings of the International Spill Conference, SpillCon, Sidney, Australia. Available from: http://spillcon.com/2002/2002papers.htm.

NRC, 2003. Oil in the sea: inputs, fates and effects. Available from: http://books.nap.edu/openbook.php?record_id=10388&page=63

Nfon, E., Cousins, I.T., Broman, D., 2008. Biomagnification of organic pollutants in benthic and pelagic marine food chains from the Baltic Sea. Sci. Total. Environ. 397, 190-204.

Nicodem, D.E., Guedes, C.L.B., Correa, R.J., Fernández, M.C.Z., 1997. Photochemical processes and the environmental impact of petroleum spills. Biogeochemistry 39, 121-138.

NOAA, 1992. Report nº HMRAD93-11. Oil spill case histories. Available from: http://response.restoration.noaa.gov/book_shelf/26_spilldb.pdf

Nour, A.H., Suliman, A., Hadow, M.M., 2008. Stabilization mechanisms of water-in-crude-oil emulsion. J. Appl. Sci. 8, 1571-1575.

Offenberg, J.H., Baker, J.E., 1997. Polychlorinated biphenyls in Chicago: enhanced wet deposition to near-shore Lake Michigan. Environ. Sci. Technol. 31, 1534-1538.

Ohkouchi, N., Kawamura, K., Kawahata, H., 1999. Distribution of three-to seven-ring polynuclear aromatic hydrocarbons on the deep sea floor in the central Pacific. Environ. Sci. Technol. 33, 3086-3090.

Omotoso, O.E., Munoz, V.A., Mikula, R.J., 2002. Mechanisms of crude oil-mineral interactions. Spill Sci. Technol. Bull 8, 45-54.

OTVM, 2004. Noticias de prensa: Repsol finaliza la extracción del fuel del Prestige en menos de un mes. Available from: http://otvm.uvigo.es/prensa/documentos/voz120804.pdf

Papadimitrakis, J.M., Psaltaki, M., Christolis, M., Markatos, N.C., 2006. Simulating the fate o fan oil spill near coastal zones: the case of a spill (from a power plant) at the Greek Island of Lesvos. Environ. Modell. Softw. 21, 170-177.

Payne, J.R., Clayton, J.R., Kirstein, B.E., 2003. Oil/suspended particulate material interactions and sedimentation. Spill Sci. Technol. Bull. 8, 201-221.

Peterson, C.H., Rice, S.D., Short, J.W., Esler, D., Bodkin, J.L., Ballachey, B.E., Irons, D.B., 2003. Long-term ecosystem response to the Exxon Valdez oil spill. Science 302, 2082-2086.

Prince, R.C., Garrett, R.M., Bare, R.E., Grossman, M.J., Townsend, T., Suflita, J.M., Lee, K., Owens, E.H., Sergy, G.A., Braddock, J.F., Lindstrom, J.E., Lessard, R.R., 2003. The roles of photooxidation and biodegradation in long-term weathering of crude and heavy fuel oils. Spill Sci. Technol. Bull. 8, 145-156.

Prince, R.C., Owens, E.H., Sergy, G.A., 2002. Weathering of an Arctic oil spill over 20 years: the BIOS experiment revisited. Mar. Pollut. Bull. 44, 1236, 1242.

Reed, M., Johansen, O., Brandvik, P.J., Daling, P., Lewis, A., Fiocco, R., MacKay, D., Prentki, R., 1999. Oil spill modelling towards the close of the 20th century: overview of the state of the art. Spill Sci. Technol. Bull. 5, 3-16.

REPSOL, 2004. Recuperación del fuel del buque petrolero del *Prestige*,. Available from:http://www.repsol.com/es_es/todo_sobre_repsol_ypf/informacion_para_accionistas_e_inversores/inf_economicofinanciera/informes_financieros/HTML/Social/04/default.aspx?Pagina=13

Roberts, C.M., McClean, C.J., Veron, J.E.N., Hawkins, J.P., Allen, G.R., McAllister, D.E., Mittermeier, C.G., Schueler, F.W., Spalding, M., Wells, F., Vynne, C., Werner, T.B., 2002. Marine biodiversity hotspots and conservation priorities for tropical reefs. Science 295, 1280-1284.

Russel, M.A., Murphy,, L.E., Johnson, D.L., Foecke, T.J., Morris, P.J., Mitchel, R., 2004. Science for stewardships. Multidisciplinary research on *USS Arizona*. Mar. Technol. Soc. J. 38, 54-63.

Schaffner, L.C., Dickhut, RM., Mitra, S., Lay, P.W., Brower.Riel, C., 1997. Effects of physical chemistry and bioturbation by estuarine macrofauna on the transport of the hydrophobic organic contaminants in the benthos. Environ. Sci. Technol. 31, 3120-3125.

Schmidt, S.F., García, R., 2003. The Galicia Bank: a potential MPA. WWF North-East Atlantic Programme. Available from: http://www.ngo.grida.no/wwfneap/overview/overfset.htm.

Sebastiao, P., Soares, C.G., 1995. Modelling the fate of oil spills at sea. Spill Sci. Technol. Bull. 2, 121-131.

Selck, H., Palmqvist, A, Forbes, B., 2003. Biotransformation of dissolved and sediment-bound fluoranthene in the polychaete, *Capitella* sp. I. Environ. Toxicol. Chem. 22, 2373-2379.

Serret, P., Álvarez-salgado, X.A., Bode, A., 2003. Spain’s Earth scientist and the oil spill. Science 299, 511.

Shiu, W. Y., Bobra, M., Bobra, A.M., Maijanen, A., Suntio, L., Mackay, D., 1990. The water solubility of crude oils and petroleum products. Oil Chem. Pollut. 7,57-84.

Shuttleworth, K.L., Cerniglia, C.E., 1995., Environmental aspects of PAH biodegradation. Apll. Biochem. Biotechnol. 54, 291-302.

Sinkkonen, S., Paasivirta, J, 2002. Degradation half-life times of PCDDs, PCDFs and PCBs for environmental fate modelling. Chemosphere 40, 943-949.

Sjöblom, J., Mingyuan, L., Christy, A.A., Gu, T., 1992. Water-in-oil emulsions from the Norwegian continental shelf. 7. Interfacial pressure and emulsification stability. Colloid. Surface. 66, 55-62.

SPREP, 2002. Regional Strategy to Address Marine Pollution from World War II Shipwrecks, Thirteenth SPREP Meeting of Officials (Item 7.2.2.1) Majuro, Marshall Islands 21-25 July, 2002. (Pub. SPREP). Available from: http://www.sprep.org.ws.

Thébaud, O., Bailly, D., Hay, J., Pérez, J. 2003. The cost of oil pollution at sea: an analysis of the process of damage valuation and compensation following oil spills. Libro-ponencias seminario Prestige “Efectos económicos, sociales y ambientales de la marea negra del Prestige”. Asociación Hispano-Portuguesa de Economía de los Recursos Naturales y Ambientales (AERNA). Available from: http://otvm.uvigo.es/investigacion/informes/documentos/archivos/Prestige_Hayetal.pdf

UNEP, 1995. Global Programme of Action for the Protection of the Marine Environment from Land-Based Activities. Available from: http://www.gpa.unep.org/documents/full_text_of_the_english.pdf

UNESCO, 1998. Leyes para el océano. Available from: http://www.unesco.org/courier/1998_08/sp/dossier/txt31.htm#e2

Van Hamme, J.D., Singh, A., Ward, O.P., 2003. Recent advances in petroleum microbiology. Microbiol. Mol. Biol. R. 67, 503-549.

Verma, P., Water, S.R., Devotta, S., 2008. Simulation of impact of oil spill in the ocean-a case study of Arabian Gulf. Environ. Monit. Assess. 146, 191-201.

Vieites, D.R., Nieto-Román, S., Palanca, A., Ferrer, X., Vences, M., 2004. European Atlantic: the hottest oil spill hotspot wordwide. Naturwissenschaften 941, 535-538.

Wania, F., 1998. the significance of long range transport of persistent organic pollutants by migratory animals. WECC report 3/98. Available from: http://www.scar.utoronto.ca/~wania/reports/WECC3-1998.pdf

Wania, F., Axelman, J., Broman, D., 1998. A review of processes involved in the exchange of persistent organic pollutants across the air-sea interface. Environ. Pollut. 12, 3-23.

Wania, F., Daly, G.L., 2002. Estimating the contribution of degradation in air and deposition to the deep sea to the global loss of PCBs. Atmos. Environ. 36, 5581-5593.

Ziolli, R.L., Jardim, W.F., 2002. Operational problems related to the preparation of the seawater soluble fraction of crude oil. J. Environ. Monitor. 4, 138-141.

CHAPTER 2

Material and methods

2.1. Study area and approach

2.1.1. Bottom topography

The wrecks of the Prestige are located 240 km off northwestern Spain. The bow lays in its natural position practically horizontal and SW orientation at coordinates 42º 10.8’N, 12º 03.6’W, on a moderate slope area (10%) at 3830m depth. The stern is also in its natural position and lays in a more pronounced slope (30%) in at coordenation 42º 10.6’N, 12º 03’W at 3565 m depth (Albaigés et al., 2003; Comité Científico Asesor, (CAA), 2003). Both are located on the SW slope of the Galician Bank (Fig. 2.1), (42.7º N, 11.6º W), a seamount located about 200 km offshore, which presents and average depth of 700 m at the shallowest parts and a minimum of 500 m. To the west is found the Iberian Basin, which reaches depths of more than 5000 m, while to the east, the corridor formed between the coast and the bank do not exceed the 3000 m (Peliz et al., 2005; Ruiz-Villarreal et al., 2006). The Basin is connected with the Bay of Biscay (43 ºN) by a passage between the Galician Bank and the NW Iberian margin of approximately 2600 m depth (Peliz et al., 2005). The Galician Bank has an inherent ecological importance (Schmidt and García, 2003). It is also known to impact in the circulation of the Eastern North Atlantic (Maze et al., 1997; Coelho et al., 2002) through the amplification of tidal currents, internal tides, enhanced mixing, turbulence and internal waves, eddy generation and retention of materials.

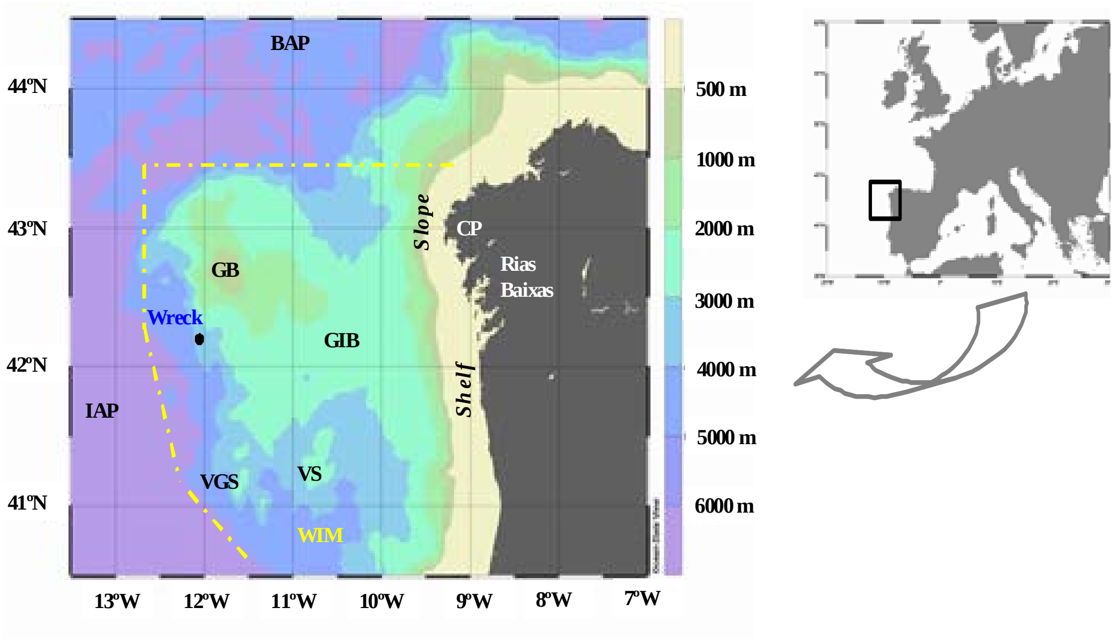


Figure 2.1. Bathymetry and main topographic features near the wreck. BAP: Biscay Abyssal Plain; GB: Galician Bank; GIB: Galicia Interior Basin IAP: Iberian Abyssal Plain; VGS: Vasco da Gama seamount; VG: Vigo seamount; WIM: Western Iberian Margin. The *Prestige* shipwreck is marked in blue and the limits of the WIM in bright yellow.

2.1.2. Hydrography

At surface, the study area is part of the North Atlantic Eastern Boundary Current (EBC) system, which transports cool water in a general southerly direction. Near the coast, this current system is called the Portugal Coastal Current (PCC), and flows southward only during spring-summer. The weakening of the Azores High in the North Atlantic during autumn-winter causes a change in the wind direction giving rise to a countercurrent called the Iberian poleward Current (IPC) or Portugal Coastal Contra Current (PCCC), which transports warm and saline water northwards (Alvarez-Salgado et al., 2003; García-Soto et al., 2002). The IPC eastward extension enters the Cantabrian Sea usually around Christmas, which is why is also known as the “Navidad” Flow (Pingree and Le Cann, 1992).

The hydrographic vertical profile in the area is characterised by the variability of the physico-chemical and hydrodynamic properties of the principal water masses in the water column (Fig. 2.2). The upper water mass is termed the Superficial Water (SW) layer. It comprises the seasonal thermocline and upper ocean mixed layer, and it is highly influenced by seasonal variations in sea-air heat (winter cooling and summer heating) and water fluxes (evaporation/precipitations regimes), and wind-driven mixing. As a consequence its thickness has a large variability so that the mixed layer can reach depths of 700 m in winter, while its extents no deeper than 200 m in summer (Fiúza, 1998).

Below the SW water mass is found the so-called Eastern North Atlantic Central Water (ENACW). In the study area is basically a mixture of water from two different origins. An upper layer has a subtropical origin (ENACW subtropical or ENACWst) formed at the Azores front, around 35º N, as a result of subduction of surface waters. It is characterised by temperature (Tº) and salinity values (S) of Tº=13.13-18.50 ºC and S=35.80-36.75 psu (Fiúza, 1998). The general circulation of this upper layer of the Central Water in the East North Atlantic is a weak eastward flow toward the coast of the Iberian Peninsula. Near the coast, the circulation of the ENACWst couples with that of the SW. During winter the ENACW flows poleward, forming the IPC, while in summer follows the PCC, upwelling waters to the Galician shelf. Underlying the ENACWst, there is a less saline and colder branch of subpolar origin (ENACW subpolar or ENACWsp) which fluctuates between Tº=10.00-12.20 ºC and S=35.40-35.66 psu (Fiúza, 1998). It is formed in the eastern North Atlantic, north of 46º N by winter cooling and deep convection and flows slowly southwards through the region off the Iberian Peninsula as far as 30º-35º N (Fiúza, 1998). Average dissolved oxygen content in the ENACW ranges between [O_2]= 6.43-8.57 mg L^{-1} (CSIC, 2003b).The SW and ENACW flow at average 10 cm s^{-1} with maximum peaks of 50 cm s^{-1} near the surface. The speed and variability decreases with depth, and while speeds of 13.9±7.38 cm s^{-1} are found at 20 m, the values decrease to 6±3.7 cm s^{-1} at 400 m (Villarreal et al., 2006).

The lower boundary edge of the ENACW is marked by a minimum in salinity, at about an average depth of 600 m. Immediately below is located the Mediterranean Water (MW), which carries saline and relatively warm water northward at an average speed of 18 cm s^{-1}(Ruiz-Villarreal et al., 2006). It enters the Atlantic through the Gilbraltar Strait, mixing with fresher and cooler Atlantic waters at different stages. Two branches can be differentiated depending on the salinity and temperature conditions: the upper core centred around 800 m, has a temperature maximum and characteristic values of Tº= 11.5-11.9 ºC, S=36.08-36.13 psu and lower core, centred at 1200 m, showing a salinity maximum and values of Tº=11.00-11.5 ºC, S=36.10-36.25 psu (Varela et al., 2005). Dissolved oxygen concentration range between $[O_2]$= 5.14-5.71 ng L^{-1} (CSIC, 2003b). The average speed of the this water mass is about 20 cm s^{-1} although peaks of 40 cm s^{-1} have been observed (Ruiz-Villarreal et al., 2006)

The SW, ENACW and MW compose the entire water column in the shelf. In the study area, the Galician Bank has an important influence in the MW (Daniault et al., 1994; Maze et al., 1997; Iorga and Lozier 1999), generating a purer core flowing northward close to the slope and another core west to the bank with more mixed MW, especially with Labrador Sea Water, due to intrusion phenomena and formation of eddies (with maximum speeds of 30 cm s^{-1}).

Below 1600 m, two deep water masses can be found off the shelf. The Labrador Sea Water (LSW), with a salinity minimum and a maximum in dissolved oxygen concentration of the water column, defined by values of Tº=3.5 ºC and S=34.89 psu (Talley and McCarney, 1982). The deepest water body is the North Atlantic Deep Water (NADW), which has a higher salinity and lower temperature (T=2.5 ºC, S=34.943 psu) than the LSW (Saunders, 1986), and its dissolved oxygen contents range between 8.04±0.028 mg L^{-1} (CSIC, 2003a), quite above the concentrations reported in other oceans, generally < 0.143 mg L^{-1} (Olson et al., 1993). At this latitude, NADW receives contributions from Iceland-Scotland, the,Denmark Strait Overflow Waters, and the Antarctic Bottom Water (ABW) (Varela et al., 2005). Both of them move slowly (3±2.5 cm s^{-1}) with maximum peaks of 15 cm s^{-1} (Ruiz-Villarreal et al., 2006). The general direction of the circulation of the deep water masses in the study area is not completely clear. Some author suggest a cyclonic recirculation of the water at the Galician Bank, and therefore these water bodies would move northward (Van Aken 2000)

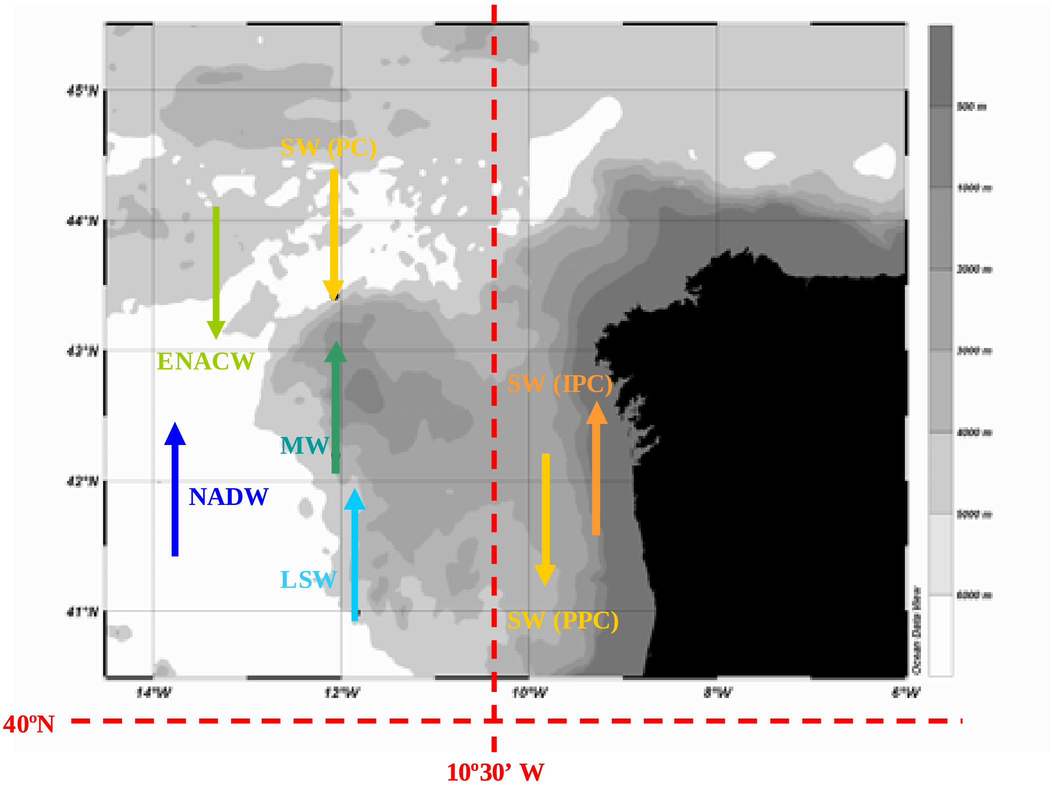


Figure 2.2. Diagram of the general circulation of the main water masses near the Galician Bank. Abbreviations for the water masses as indicated in the text.

One of the most relevant phenomenon affecting the western Galician coast is the wind-driven annual upwelling/downwelling pulse. The seasonality of this event is conditioned mainly by two atmospheric systems: the Azores High and the Iceland Low (Varela et al., 2005). In the spring-summer season (from March-April to September-October), the Azores High dominates over North Atlantic, while Iceland Low migrates northward, producing shelf winds with northerly component. As a result, considerable volumes of water are transported off the shelf, creating filaments that penetrate far offshore (as far as 200 km). The upwelled water is replaced by cold, less salty and nutrient rich water of the ENACW. During the autumn/winter months (from September-October to March-April), the Azores High adopts its southern location and the Iceland Low is reinforced, generating a considerable increase in wave height, and producing downwelling favourable southwesterly winds, the driving force of the IPC (Varela et al., 2005; Herrera et al., 2008). The continental run-off is maximum during this period, and the cold and fresh waters carried by Douro River, Minho River and local rivers draining into the Rias Baixas cause a strong coastal thermohaline front with the warm and salty waters carried by the IPC. These low saline water lenses extend over the shelf and constitute the Western Iberia Buoyant Plume (WIBP). Although it is present all the year round, during winter is more marked while in summer, WIBP is advected southward and off shore with the upwelling jet (Varela et al., 2005; Álvarez-Salgado et al., 2006). Nevertheless, this well differentiated two season regime is often altered by upwelling pulses during downwelling season and vice versa (Alvarez-Salgado et al., 2003). The high variability of the wind causes a complex small time variability of the hydrographic and circulation structure, especially in winter, when variations of more than 2 days can be a 25% higher than during the summer season. Intermediate periods, known as spin-up (preparation) and spin-down (relaxation) events that occur in the established upwelling/downwelling regime tend to create changes in the dynamic, geochemical and hydrographical characteristics (Varela et al., 2005).

2.2. Cruises and sampling methodology in the open ocean

2.2.1. Cruises and sampling strategy

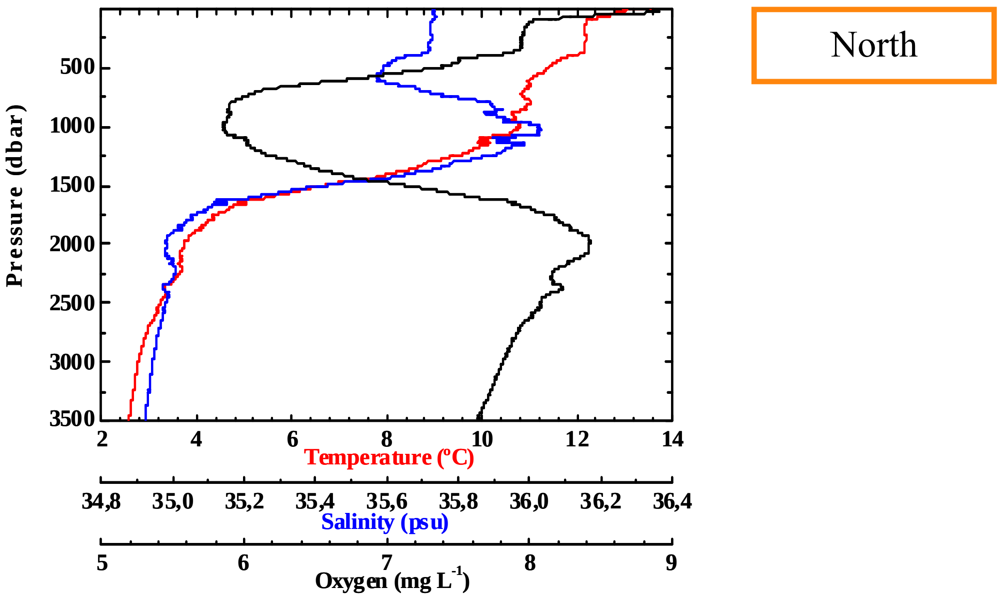
Field work was performed on board the research vessel *Cornide de Saavedra,* in two oceanographic cruises undertaken in spring and autumn 2006. The spring cruise was held from March 27th to April 3rd and three sampling locations (Table. 2.1) were selected, corresponding to the of the *Prestige* sinking site (42º11.8' N, 12º03.3' W), a station 73.3 nautical miles northwards (43º25.1' N, 12º04.16' W) and another one 73.2 nautical miles southwards (40º59.23' N, 11º50.52' W) to determine the role of the main currents in the area in the transport and distribution of contaminants. The water column of each location was sampled at five different depths, corresponding to the different water masses present in the area: 5 m (SW), 400 m (ENACW), 1,000 m (MW), 2,000 (LSW) and bottom (NADW). Physico-chemical characteristics of the water masses at the sampling depths and their temperature, salinity and dissolved oxygen profiles in the water column are listed in table 2.1 and figure 2.3. Looking at the temperatures it can be deducted the prevailing direction of the water masses. Thus the NADW increases its temperature from south to North, whereas its source is colder either from the GIN seas (Greenland, Icelandic and Norwegian seas) or ABW which suggests that it flows northwards. The opposite is the case for the LSW, flowing southwards from the gradients in T and S. The MW cools as it flows northwards and becomes less saline, whereas the 5 m water mass cools as it flows northwards, becoming al fresher and more oxygenated as the water cools due to the increase in gas solubility associated to cooler waters. It also seems that the Prestige location water mass is different than the north and southern location at 400 m and 1,000 m from the values in T and S. At 1,000m seems to have a northerly source with more oxygen and cooler T. In the three deepest water masses the Prestige station registers higher O_2 values decoupled from T changes, probably related to mixing with a younger water source from higher latitudes than in the north and source stations.

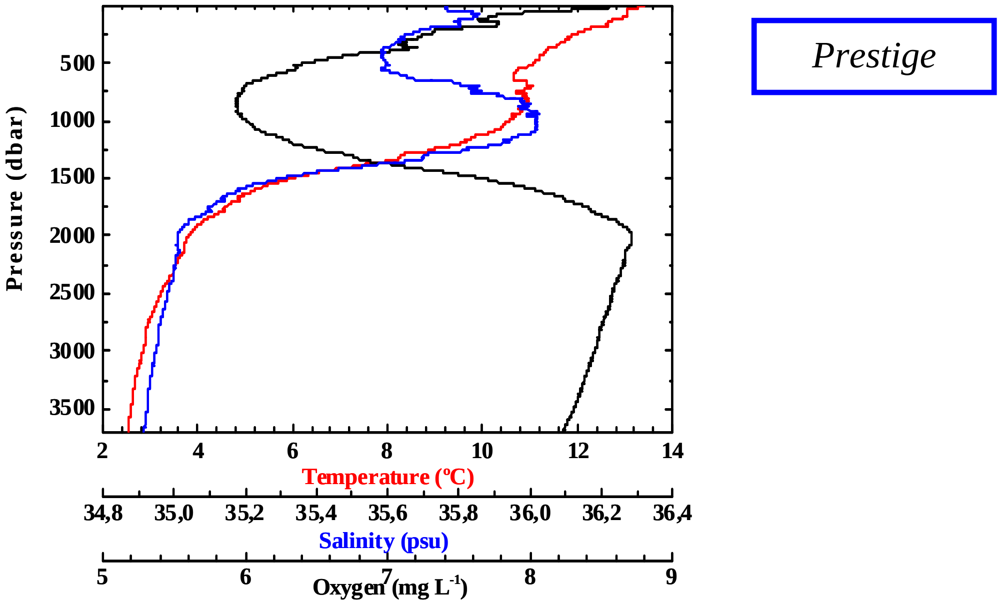
Sample depth	**Station**	**T (ºC)**	**S (psu)**	**O_2 (mgL^{-1})**
5 m	North	13.06	35.74	8.8
	Prestige	13.4	35.76	8.53
	South	13.83	35.91	8.13
400 m	North	11.91	35.67	7.70
	Prestige	11.31	35.58	7.00
	South	11.99	35.67	6.84
1,000 m	North	10.8	36.03	5.85
	Prestige	10.56	36.02	6.00
	South	11.11	36.14	5.82
2,000 m	North	3.75	34.98	8.41
	Prestige	3.81	35.01	8.71
	South	4.19	35.10	8.08
3,500 m	North	2.57	34.92	7.63
3,700 m	*Prestige*	2.53	34.92	8.22
4,000 m	South	2.49	34.91	7.54

Table 2.1. Salinity (psu), temperature (ºC) and oxygen (mg L^{-1}) data registered by CTD at the selected sampling depths for the samples collected in the spring cruise, March 2006.

The second oceanographic cruise, under the coordination of Instituto Español de Oceanografía (IEO) was carried out between October 29th and November 1st 2006, with the objective of the exhaustive sampling of the Prestige tanker wreck area (42º 12,487' N, 12º 03,121' W) and determine the origin of the iridescent slicks observed in March in the same area. Seawater was collected at 5 m, 500 m, 1,000 m, 2,000 m and 3,706 m to reproduce the sampling strategy carried out in the spring cruise.

In both cruises, between16 to 20 L of seawater were collected by means of a rosette array of Niskin bottles at specific depths, with the exception of the surface samples, where Go Flo bottles were used. Temperature, salinity, oxygen and fluorescence were measured in all the stations with a CDT (SBE 9 plus, Sea-Bird electronics, Inc., USA). The seawater was stored in high density polyethylene containers, previously extracted with hexane, and immediately carried to the ship laboratory for its analysis.





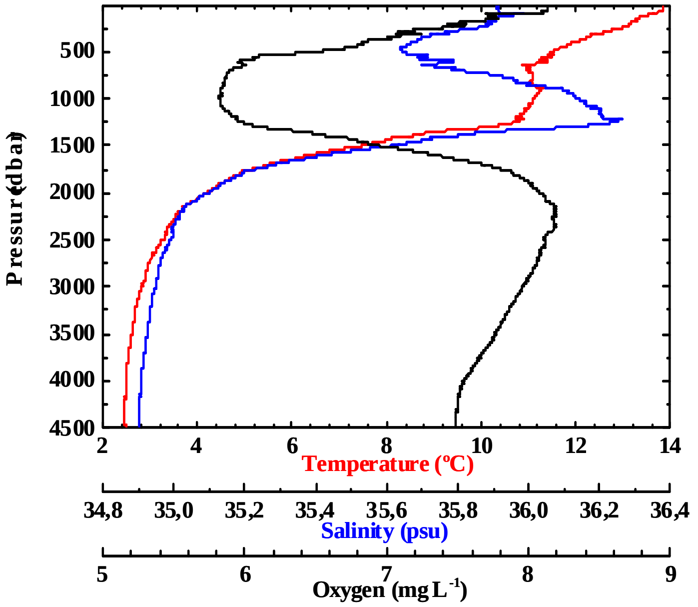
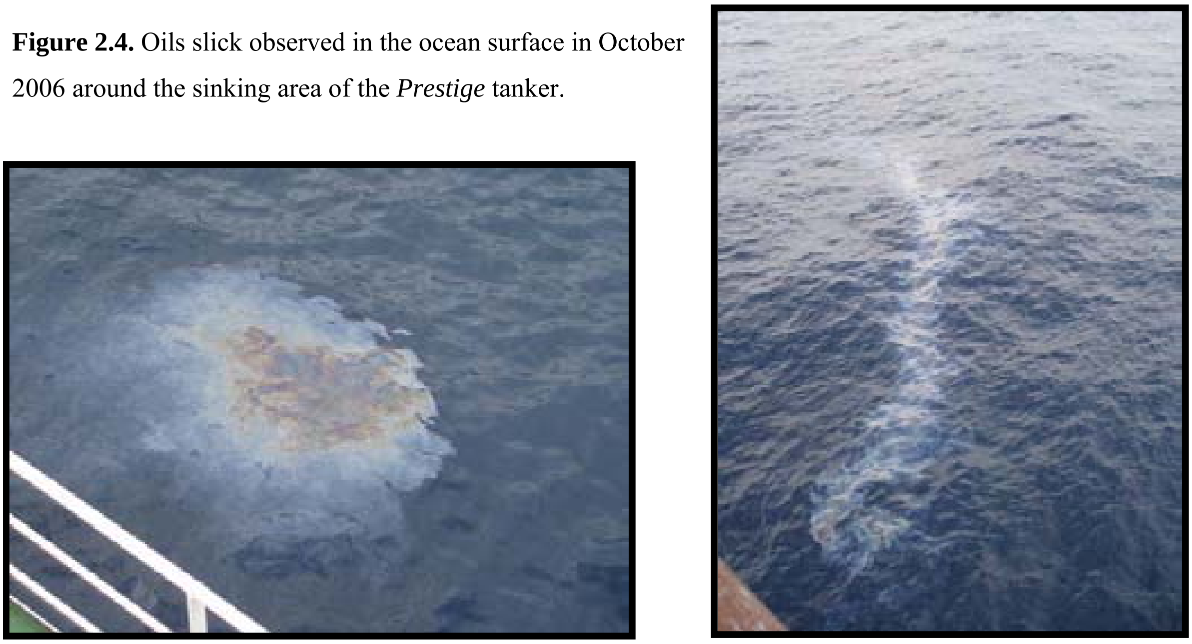


Figure 2.3. Temperature (ºC), salinity (psu) and .dissolved oxygen (mg L^{-1}) profiles registered by a CTD in the different sampling stations in March 2006.

2.2.2. Sampling of the oil slicks

The favourable weather during the autumn cruise permitted the utilization of a dinghy to have better access to the oil slicks floating in the ocean surface (Fig. 2.4). Oil was collected by means of aluminium webs from the bow of the dinghy and it was stored in glass flasks and kept in a freezer as the GMF filters.

Figure 2.4. Oils slick observed in the ocean surface in October 2006 around the sinking area of the *Prestige* tanker.



2.3. Laboratory methodology

2.3.1. Sampling the SPM and DP of the seawater

The filtration of the SPM and extraction of the DP was carried out consecutively; by placing a glass microfiber filters (GMF) (0.7 µm, Ø 47 mm, APFF type, Millipore, Ireland) on top of a C_{18} bonded silica (ENVI-Disk) extraction disk (Ø 47 mm, Supelco, Sigma-Aldrich Bellefonte, USA) in a filtration holder (Fig. 2.5). Glass matrix SPE disk were chosen instead of PTFE ones since they give higher flow rates and shortened the filtration times (Urbe and Ruana, 1997). Two tree-place PVC manifolds (Millipore), with 47 mm diameter filter holders were connected to two vacuum pumps (KNF

Neuberger, Inc., Trenton, USA). In each support a 47 mm diameter C_{18} disk, at the bottom, and a prefired GMF on top of were placed. This system allowed us to filter six different samples at the same time reducing the seawater storage time and thus avoiding the redistribution of contaminants between the dissolved phase and SPM (Wolska et al., 1999). Each 10 L of seawater, both the GMF and the C_{18} disk were replaced to avoid analyte breakthrough, except when the clogging of the GMF filter due to high SPM required more frequent changes.

Figure 2.5. Filtration system used to sample the seawater onboard Cornide de Saavedra, March 2006.

The conditioning of the C_{18} disks was carried out by soaking eluting through them while in the filter holder 10 ml of hexane for 3 min, and then connecting the vacuum. Before all the solvent was eluted the vacuum was stopped, and 10 ml of methanol were added, repeating the elution process. Before eluting all the methanol through the disk, seawater was added. After the water was filtered the vacuum was left on for a minute to remove the remaining water. The GMF and C_{18} disk were wrapped in aluminium foil separately and maintained at -20 ºC until they were analysed.

2.3.2. Determination of hydrocarbons in the seawater

PAHs are found at pg-ng L^{-1} levels in marine waters and for this reason suitable preconcentration techniques are needed to reach typical detection limits (Dachs and Bayona, 1997, Wolska et al., 1999). GMFs of a nominal pore diameter of 0,7 µm are routinely used for the sampling of the suspended particulate matter (SPM) of the seawater (Mitra and Bianchi, 2003, Ko and Baker, 1995, Countway et al., 2003). On the contrary, there is a lack of standard procedures for the analysis of dissolved PAHs in the open ocean waters, principally due to their low abundance (Filipkowska et al., 2005). Moreover, several problems can arise when PAHs are not sampled immediately after seawater is collected. Storage stability studies have shown that the distribution of hydrophobic compounds between the particulate and dissolved phase of the seawater change while the samples are carried to the laboratory or storing them several days before their analysis. The decrease in the PAH recovery can even reach 20-70 % (Wolska eta al., 1999, Michor et al., 1996) which shows the need to find suitable methods which allow sample both phases in the ship. Solid phase extraction (SPE) methods, such as amberlite resins, polyurethane foams and C_{18} modified silica cartridges has been largely used for preconcentration on hydrophobic compounds from sea, fresh and produced water (Font et al., 1996, Cao et al., 2005). It provides several advantages upon liquid-liquid extraction, such as the simplicity of sampling, avoid the transport of large water volumes and less need of solvents for the compound analysis. Moreover, the launch into the market of the disks type adsorbents, adapted to analyse volumes between 1-10 L, had reduced the clogging, improve the blanks, eliminate the channelling and increase the flow rates (Filipkowska et al., 2005; McMillin and Means, 1996). These characteristics, together with the easy of transport make them suitable for the field sampling.

2.3.2.1. Spiking experiments

Spiking experiments were carried out in the laboratory to determine the suitability and efficiency of C_{18} extraction disks to preconcentrate PAHs from the water. Some 8 L of milli-Q water were poured in glass flasks and spiked with with a mixture of 17 PAHs in acetonitrile containing acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]flouranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, dibenzo[*a,h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, naphthalene, perylene, phenanthrene and pyrene, (Dr. Ehrenstorfer, Germany), with a concentration of 100 ng L^{-1} each compound. The bottles were covered and left 48 hours at room temperature. After this time the water was extracted as described in the previous section. Thiphenylamine (Sigma-Aldrich) was used as an injection standard and response factors were calculated relative to this compound. The samples were analysed by Trace-Ultra (Thermo-Finnigan) GC-FID in a DB-5 (Agilent) fused silica capillary column (30 m x 0.25 mm I.D. x 0.25 µm film thickness).

Extraction efficiency of individual PAHs obtained by the C_{18} disks were determined (Fig. 2.6). Recuperation of individual PAHs ranged from 58 % to 90 %, decreasing according to the molecular weight of the compound, which has been previously observed in experiments of similar characteristics (Michor et al., 1996). The reason for this pattern resides in the microporous surface of the silica (60 Å), that allows better interactions with the lightest compounds than the largest ones, which do not fit as easily in the microporus (Dachs and Bayona, 1997; Michor et al., 1996). It was decided that C_{18} extraction disks offered a suitable alternative for the PAHs concentration from the seawater and easy to manipulate onboad for the field sampling.

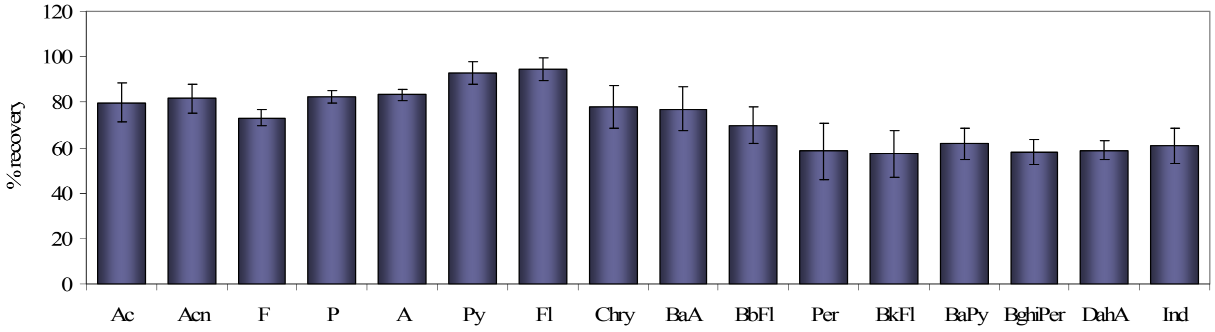


Figure 2.6. Extraction efficiency of individual PAHs spiked in Milli Q water. Quantification was carried out per triplicate and standard deviation is indicated above each bar. Compound identification as follows:Ac: Acenaphthylene; Acn: Acenaphthene; F: Fluorene; P: Phenanthrene; A: Anthracene; Py: Pyrene; Fl: Fluoranthene; Chry: Chrysene; BaA: Benzo[*a*]anthracene; BbF: Benzo[*b*]fluoranthene; Per: Perylene; BkFl: Benzo[*k*]fluoranthene; BaPy: Benzo[*a*]pyrene; BghiPer: Benzo[*g,h,i*]perylene; DahA: Dibenzo[*a,h*] anthracene; Ind: Indeno[1,2,3,*c*-*d*]pyrene.

Sampling of the hydrocarbons in the SPM from the seawater has more uniform methodology and the vast majority of authors agree in the use of the filters GMF of 0.7 µm Ø nominal pore (Dachs and Bayona, 1997; Schulz-Bull et al., 1998; Mitra and Bianchi, 2003).The suitability of the microwave assisted extraction (MAE) for the analysis of hydrocarbons in the SPM was determined by spiking previously fired GMF, similar to the ones used in the cruise, with a mixture of 17 PAHs and 5 n-alkanes and extracted and fractionated as the field samples. Recuperations for the different compounds ranged from 71 to 101 % and 82 to 95 % for PAHs and n-alkanes respectively (table 2.2).

Compound	Rec. SPM (%)	SD
N	71	11.3
Ac	85	9.0
Acn	87	6.8
F	78	3.9
P	88	2.9
A	89	2.8
Py	99	5.2
Fl	101	5.3
Chry	83	10.0
BaA	82	10.3
BbFl	74	8.5
Per	81	13.0
BkFl	77	8.3
BaPy	78	15.8
BghiPer	69	16.3
DahA	73	9.7
Ind	76	11.8
C_{16}	88	1.0
C_{20}	82	1.5
C_{28}	94	17.4
C_{32}	95	11.2
C_{36}	87	16.3

Table 2.2. Average recuperations obtained in the spiking experiments about the hydrocarbon extraction efficiency of the proposed method for the SPM. Abbreviations of the compounds as in the text.

2.3.2.2. Extraction and fractionation of analytes in the SPM and DP samples

The extraction of hydrocarbons from the filters was performed in a microwave (CEM MARSx, Matthews, NC, USA). The followed methodology used the optimization of MAE conditions and solvent mixtures reported for PAH extraction from sediments SPM samples (Piñeiro-Iglesias et al., 2000; Shu et al., 2000). Freeze dried filters were inserted uncut in the Teflon vessels and a mixture of anthracene-d_{10} and pyrene-d_{10} was added as recovery standards. When there were more than one filters corresponding to the same sample, they were extracted together. Some 15 ml of hexane:acetone 1:1 (v/v) and a magnetic agitation bar were added to each vessel which was subsequently sealed. The extraction was carried out at a power of 1200 W and temperature of 115 ºC. After irradiation the vessels were left to cool to below 35 ºC before they were opened.

Organic extracts were fractionated by column chromatography using 1 g silica (bottom) and 1 g alumina (middle), and 1 g sodium sulphate, the first two previously activated at 110 ºC and deactivated with 5 % of milli-Q water (w/w). First fraction (aliphatic hydrocarbons) was collected eluting 2.5 ml of hexane, and the second fraction (PAHs) eluting 10 ml of hexane:dichloromethane 2:1 (v/v). Extracts were concentrated to 1 ml with a rotary evaporator and then with a gentle stream of N_2, never reaching complete dryness, especially in the case of the PAHs fraction.

C_{18} disks were extracted in a 47 mm of diameter glass filter holder. They were soaked with 10 ml of methanol for 3 min, before connecting then vacuum to elute and collect the solvent. The same process was repeated with 10 ml of dichloromethane and finally with 10 ml of hexane. These last two fractions were collected together. The methanol fraction was transferred into a separatory funnel and liquid-liquid extracted three times with 2 ml of hexane. The three hexane fractions were combined with the dichloromethane/hexane fraction. Recovery standards were added to the apolar extracts before fractionate them by flash chromatography with silica as described above for the GMF filters extracts.

2.3.2.3. Instrumental analysis

The quantification of hydrocarbons was carried out in a Konik HRGC 4000B gas chromatograph (GC) coupled to a Konik MS Q12 mass spectrometer (MS) (Konik, Sant Cugat del Vallès, Spain). The GC was fitted with a fused silica capillary column (30 m x 0.25 mm I.D. x 0.25 µm film thickness) DB5 MS (Agilent, Santa Clara, USA). The initial column temperature was held for 1 minute at 70 ºC, then programmed to 320 ºC at a rate of 6 ºC min^{-1} and kept at this temperature for 10 minutes, for the PAHs, while the program was slightly modified for the aliphatic hydrocarbons, being the temperature ramp of 15ºCmin^{-1} from 70 to 150 ºC and 6ºcmin^{-1} from 150ºC to 320 ºC. Helium was used as carrier gas at a constant flow of 1.5 mL min^{-1}. 2 µL were injected in the splitless mode (splitless time: 1 min), keeping the injector temperature at 300 ºC. Data were acquired in the selective ion monitoring (SIM) mode at a 70 eV and processed by the Konikrom Data Reduction software. Quantification was performed calculating the response factors for each compound at different concentrations, correcting the values with the internal standards. A solution of 17 PAHs containing acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]flouranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, dibenzo[*a,h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, naphthalene, perylene, phenanthrene and pyrene (Dr. Ehrenstorfer,Germany) were used to calculate the response factors for PAHs, and a mixture of C_{16}, C_{20}, C_{28}, C_{32} and C_{36} n-alkanes (Sigma-Aldrich) for aliphatic hydrocarbons.

2.3.1.5. Quality assurance/quality control (QA/QC)

Laboratory and field blanks were carried out as follows. Milli-Q water was sterilized in the laboratory and stored in glass containers. During the cruises it was transferred to the polyethylene containers and analysed as if the collected seawater. Unused prefired GMF were also analysed in order to monitor the contamination during transport and storage. Laboratory blanks were carried out in each microwave extraction batch, adding in one of the extraction vessel the same solvent and standard mixture as in the other, without adding a GMF. Average recoveries were 55-94% and 77-83% for the GMF and the C_{18} disks and reported values are recovery corrected.

The detection limit was calculated from the blanks with the formula $DL=Y_B+3SD$ (Eurachem, 1998), where YB and SD are the average concentration and standard deviation respectively. Detection limit varied for the different compounds analysed and ranged from 4 to 14 pg $L^{-1}$and 8 to 140 pg L^{-1} for PAHs and n-alkanes respectively..

2.3.3. Oil identification

2.3.2.1. Oil fractionation

Samples of the original oil carried in the Prestige tanker and the one found in the oceanographic cruise floating in the ocean surface was fractionated using a glass column (30 cm x 1 cm) packed with 6 g of silica (bottom) (SiO_2, 40-60 mesh, Acros Organics, Belgium), 6 g of aluminium oxide (middle) (Al_2O_3, 70-230 mesh, Merck, Germany) and 2 g of sodium sulphate (top), in hexane, as described in Alzaga et al. (2003) . Between 10-20 mg of the oil sample were dissolved in hexane, spiked with a solution of anthracene-d_{10} (Acros Organics, Belgium) and pyrene-d_{10} (Sigma-Aldrich, USA) in isooctane and added at the top of the column. The aliphatic hydrocarbons were eluted in the first fraction with 17 mL of hexane (Suprasolv, Merck), and the PAHs with 20 mL of hexane:dichloromethane (2:1, v/v). The recovered fractions were concentrated in a rotary evaporator, followed by a gentle stream of nitrogen until near dryness, redissolved with isooctane and spiked with a solution of thiphenylamine (Sigma-Aldrich) before further analysis by GC/MS.

2.3.2.2. Compound specific isotope analysis (CSCIA) of $^{13}C/^{12}C$

This technique was used as a complementary tool to confirm the origin of the oil found at the sea surface in the sinking area of the Prestige shipwreck. All the analyses were carried out in the Marine Environmental Studies Laboratory of the International Atomic Energy Agency (MESL-IAEA). Measurements were performed using a Hewlett-Packard HP5890 Series II gas chromatograph coupled to a Finnigan MAT Delta C IRMS via a combustion furnace heated at 940 ºC. 2 µl of FI oil the compared oils in isooctane were injected on column onto a HP5 column (60 m x 0.32 mm i.d.x 0.25 mm film thickness). The GC oven temperature was programmed as follows: initial temperature 60 ºC was held for 2 min and raised to 100 ºC at a rate of 10ºCmin^{-1}, then to 310 ºC at 4ºcmin^{-1} and held at this temperature for 43 min. Samples were injected three times each and the average values and standard deviation was calculated. A chromatogram of an analysed samples is shown in Fig. 2.7.

The reported carbon isotopic data were reported in $\delta^{13}C$ traditional notation of per mil (‰) deviation of the isotope ratio from a standard. Before and after each analysis pulses of reference CO_2 were bled into the source in order to calibrate it relative to Pee Dee Belemnite. Between samples standard mixture composed of three n-alkanes with known isotope composition were injected in order to control the performance of the instrument.

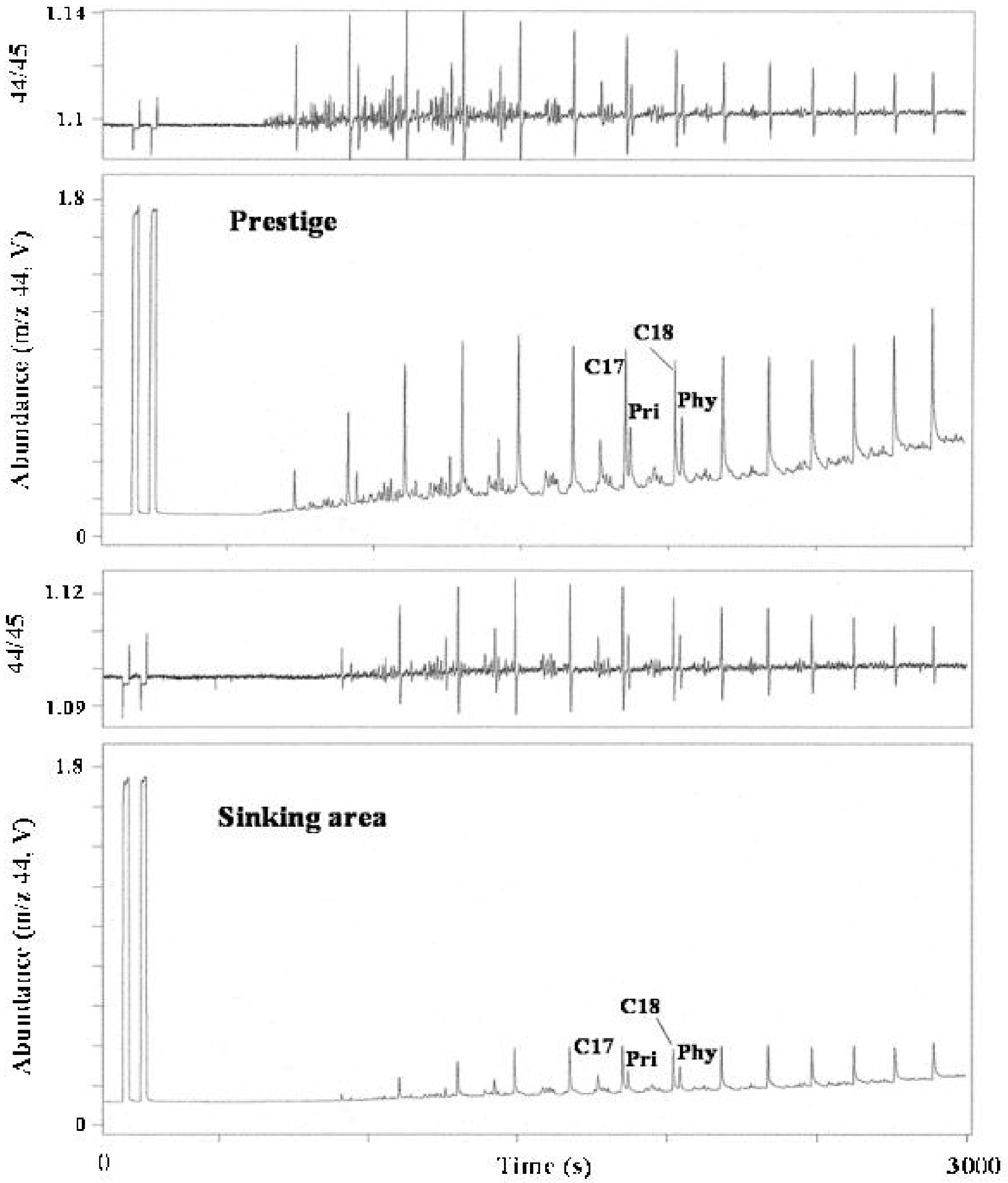


Figure 2.7. Chromatograms of the $\delta^{13}C$ analysis for *n*-alkanes (C_{14} to C_{24}) of the compared fuels. Analyses carried out in the MESL-IAEA of Monaco in 2007.

2.4. References

Albaigés, J., Bayona, J..M., 2003. La “huella” del fuel : Ensayos sobre el *Prestige*. Fundación Santiago Rey Fernández-Latorre, A Coruña, pp. 80-103.

Álvarez-Salgado, X.A., Figueiras, F.G., Pérez, F.F., Groom, S., Nogueira, E., Borges, A.V., Chou, L., Castro, C.G., Moncoiffe, G., Ríos, A.F., Miller, A.E.F., Frankignoulle, M., Savidge, G., Wollast, R., 2003. The Portugal coastal counter current off NW Spain: new insights on its biogeochemical variability. Prog. Oceanogr. 56, 281-321.

Álvarez-Salgado,X.A., Herrera, J.L., Gago, J., Otero, P., Soriano, J.A., Pola, C.G., García-Soto, C., 2006. Influence of the oceanographic conditions during spring 2003 on the transport of the Prestige tanker fuel oil to the Galician coast. Mar. Pollut. Bull. 53,239-249.

Alzaga, R., Montuori, P., Ortiz, L., Bayona, J.M., Albaigés, J., 2003. Fast-solid phase extraction-gas chromatography-mass sectrometry procedure for oil fingerprinting. Application to the Prestige oil spill. J. Chromatogr. A. 1025, 133-138.

Cao, Z. H., Wang,Y.Q., Ma, Y., Xu, Z., Shi, G., Zhuang, Y., Zhu, T., 2005. Occurrence and distribution of polycyclic aromatic hydrocarbons in reclaimed water and surface water of Tianjin, China. J. Hazard. Mater. 122, 51-59.

Coelho, H.S., Neves, R.J.J., White, M., Leitao, P.C., Santos, A.J., 2002. A model for ocean circulation on the Iberian coast. J. Mar. Sys. 32, 153-179.

CAA, 2003. Comité Científico Asesor sobre el hundimiento del *Prestige*: Informe sobre la neutralización del pecio. Available from: http://otvm.uvigo.es/investigacion/informes/documentos/CSIC/pecio/Informe8.pdf

Countway, R. E., Dickhut, R.M., Canuel, E.A., 2003. Polycyclic aromatic hydrocarbon (PAH) distributions and associations with organic matter in surface waters of the York River, VA Estuary. Org. Geochem. 34, 209-224.

CSIC, Informe técnico Prestige nº3, 2003a. Datos de oxígeno e hidrográficos en las proximidades del Prestige. Available from: http://csicprestige.iim.csic.es/desarro/informcsic/3/.

CSIC, Informe técnico nº 5, 2003b. Escenario oceanográfico en la zona de hundimiento del Prestige. Available from: http://csicprestige.iim.csic.es/desarro/informcsic/5/.

Dachs, J., Bayona, J.M., 1997. Large volume preconcentration of dissolved hydrocarbons and polychlorinated biphenyls from the seawater. Intercomparison between C_{18} disks and XAD-2 column. Chemosphere 35, 1669-1679.

Daniault, J.P., Maze, J.P., Arhan, M., 1994. Circulation and mixing of Mediterranean Water west of the Iberian Peninsula. Deep-Sea Res. Pt. I 41, 1685-1714.

Eurachem, 1998. The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics. In: Eurachem Guide (first edition), Teddington, Middlesex. (www.eurachem.org).

Filipkowska, A., Lubecki, L., Kowalewska, G., 2005. Polycyclic aromatic hydrocarbon analysis in different matrices of the marine environment. Anal. Chim. Acta 547, 243-254.

Fiuza, A., F.G., Hamann, M., Ambar., Díaz del Río, G., González, N., Cabanas, J.M., 1998. Water masses and their circulation off Iberia during May 1993. Deep-Sea Res. Pt.I 45, 1127-1160.

Font, G., Mañes, J., Moltó, J.C., Picó, Y., 1996. Current developments in the analysis of water pollution by polychlorinated biphenyls. J. Chromatogr. A 733, 449-471.

García-Soto, C., Pingree, R.D., Valdés, L., 2002. Navidad development in the southern Bay of Biscay: climate change and swoddy structure from remote sensing and in situ measurements. J. Geophys. Res. 107.

Herrera. J.L., Rosón, G., Varela, R.A., Piedracoba, S., 2008. Variability of the western Galician upwelling system (NW Spain) during an intensively sampled annual cycle. An EOF analysis approach. J. Mar. Sys. 72, 200-217.

Iorga, M.C., Lozier, M.S., 1999. Signatures of the Mediterranean outflow from North Atlantic climatology 1. Salinity and density fields. J. Geophys. Res. 104, 25985-26009.

Ko, F.C., Baker, J.E., 1995. Partitioning of hydrophobic organic contaminants to resuspended sediments and plankton in the mesohaline Chesapeake Bay. Mar. Chem. 49, 171-188.

Maze, J.P., Arhan, M., Mercier, H., 1997. Volume Budget of the Eastern Boundary layer off the Iberian Peninsula. Deep-Sea Res. Pt. I 44, 1543-1574.

McMillin, D., Means, J.C., 1996. Spatial and temporal trends of pesticide residues in water and particulates in the Mississippi River plume and the northwestern Gulf of Mexico. J. Chromatogr. A 754, 169-185.

Michor, G., Carron, J., Bruce, S., Cancilla, D.A., 1996. Analysis of 23 polynuclear aromatic hydrocarbons from natural water at the sub-ng/l level using solid-phase disk extraction and mass-selective detection. J. Chromatogr. A 732, 85-99.

Mitra, S., Bianchi, T.S., 2003. A preliminary assessment of polycyclic aromatic hydrocarbon distributions in the lower Mississippi River and Gulf of Mexico. Mar. Chem. 82, 273-288.

Olson, D.B., Hitchcok, G.L., Fine, R.A., Warren, A.B., 1993. Maintenance of the low oxygen in the central Arabian sea. Deep-Sea Res. 48, 1905-1921.

Peliz, Á., Dubert, J., Santos, A.M.P., Oliveira, P.B., Le Cann, B., 2005. Winter upper ocean circulation in the Western Iberian Basin-Fronts, Eddies and Poleward Flows: an overview. Deep-Sea Res. Pt I 52, 621-646.

Pingree, R.D., LeCann, B., 1992. Three anticyclonic slope water oceanic eddies (swoddies) in the southern Bay of Biscay in 1990. Deep-Sea Res. 39, 1147-1175.

Piñeiro-Iglesias, M., López-Mahía, P., Vazquez-Blanco, E., Muniategui-Lorenzo, S., Prada-Rodríguez, D., Fernández-Fernández, E., 2000. Microwave assisted extraction of polycyclic aromatic hydrocarbons from atmospheric particulate samples. Fresenius J. Anal. Chem. 367, 29-34.

Ruiz-Villarreal, M., C. González-Pola, Diaz del Rio, G., Lavin, A., Otero, P., Piedracoba, S.,Cabanas, J.M., 2006. Oceanographic conditions in North and Northwest Iberia and their influence on the Prestige oil spill. Mar. Pollut. Bull. 53, 220-238.

Saunders, P.M., 1986. The accuracy of measurements of salinity, oxygen and temperature in the deep ocean. J. Phys. Oceanogr. 16, 1274-1285.

Schmidt, S.F., García, R., 2003. The Galicia Bank: a potential MPA. WWF North-East Atlantic Programme. Available from: http://www.ngo.grida.no/wwfneap/overview/overfset.htm.

Schulz-Bull, D. E., Petrick, G., Bruhn, R., Duinker, J.C., 1998. Chlorobiphenyls (PCB) and PAHs in water masses of the northern North Atlantic. Mar. Chem. 61, 101-114.

Shu, Y.Y., Lao, R.C., Chiu, C.H., Turle, R., 2000. Analysis of polycyclic aromatic hydrocarbons in sediment reference material by microwave-assisted extraction. Chemosphere 41, 1709-1716.

Talley, L.D., McCarney; M.S., 1982. Distribution and circulation of Labrador Sea water. J. Phys. Oceanogr. 12, 1189-1205.

Urbe, I., Ruana, J., 1997. Application of solid-phase extraction discs with a glass fiber matrix to fast determination of polycyclic aromatic hydrocarbons in J. Chromatogr. A 778, 337-345.

Van Aken, H.M., 2000. The hydrography of the mid-latitude northeast Atlantic Ocean I: The deep water masses. Deep-Sea Res. Pt. I 47, 757-788.

Varela, R. A., Rosón, G., Herrera, J.L., Torres-López, S., Fernández-Romero, A., 2005. A general view of the hydrographic and dynamical patterns of the Rías Baixas adjacent sea area. J. Mar. Sys. 54, 97-113.

Wolska, L., Galer, K., Górecki, T., Namiesnik, J., 1999. Surface water preparation procedure for chromatographic determination of polycyclic aromatic hydrocarbons and polychlorinated biphenyls. Talanta 50, 985-991.

CHAPTER 3

Distribution of hydrocarbons in the water column after a deep sea spill: the Prestige shipwreck

3.1. Introduction

Accidental marine pollution can have a major impact on the health of the marine environment, affecting the inhabiting organisms such as birds (Oropesa et al., 2007), fish (Pérez del Olmo et al., 2009), sea mammals (Garrott et al., 1993) and marine invertebrates (Laffon et al., 2006). Severe accidental pollution is usually associated with oil tanker accidental spills. Although statistics indicate that the number of spills and amounts of oil involved have declined since the mid 1980s, one accident can create considerable media interest and great public controversy (Birkland and Lawrence, 2002; Serret et al., 2003). The wide coverage of these accidents by the media usually reflects society's fear of catastrophic degradation of the marine environment. This fear is a reality that policy and decision makers must consider when developing response and regulatory strategies (Vieites et al., 2004).

In recent years the scientific community has alerted of the potential pollution coming from wrecks in marine waters (Girin, 2004). At present there are thousands of sunken ships worldwide and the oil remaining in them has been estimated to range between 2.5 and 20.4 million tonnes (Michel et al., 2005), which represents between 180 and 1500 times the fuel spilled annually at surface (calculated from ITOPF 2009., considering and annual rate of 14.000 Tn/year, for the period 2000-2007). Almost two thirds of the potentially polluting wrecks are concentrated in the South-Asian Pacific (35%), and the North Atlantic Ocean (27%) (Michel et al., 2005). Most of the casualties correspond to vessels sunk decades ago, mainly during World War II. Some of them have begun to release oil to the environment, for example SS *Jacob Luckenbach, M/V Castillo de Salas and USS Mississinewa* (Michel et al., 2005).

In order to avoid future threats, the removal of the oil before the emergency occurs has been encouraged from different sectors (Basta and Kennedy, 2004), which leads to the need of evaluating the individual risk posed by each sunken vessel. Several factors must be taken into account, such as the location of the wreck, behaviour and weathering characteristics, possible oil release scenarios derived from the seasonal oceanographic and meteorological data and the sensibility of the regional environment likely to be impacted by the spill, amongst others (Gilbert et al., 2003). To date little is known of the fate of a deep sea-oil spill, particularly beyond the oceans' continental platforms, where many of the currents wrecks are found.

Hydrocarbons are one of the most widespread organic pollutants entering the marine environment (Schulz-Bull et al., 1998, Cincinelli et al., 2008) and they are susceptible to long range transport (Jaward et al., 2004). It is unknown to which extent sunken vessels may already provide an important input of hydrocarbons at present or in the future, given that the wrecks are bound to release their cargo when they reach a certain oxidation state. Besides the obvious difficulty of monitoring a deep sea wreck for leaks, the physico-chemical transformation of the oil in its dissolved and dispersed phases, in the pelagic water column, and its interaction with biological and sedimentological processes has not been studied.

There is no reason to expect that dispersion models of hydrocarbon in coastal environments, where the main processes relate to advection and mixing, are applicable to spills that have been occurring over time scales of years in the deep sea (e.g. Turrell, 1994). No studies have modelled spills below 1000 m depth, and most address leaks of oil or gas at depths shallower than 200 m, recognizing shortcomings in the understanding of physical processes that control the dispersion of the oil mixture under water. Simulations do not take into account the processes of dissolution and diffusion that will fractionate the original liquid and where the environmental conditions, such as temperature, salinity and depth are key (Yapa and Zheng, 1997). Potentially, some of the oil may remain at great depth, as part of the plume could be trapped in the water column below the thermocline (Daling et al., 2003). To shed some light into this issue we have investigated the deep spill from the wreck of the *Prestige* oil tanker.

In March 2006, an oceanographic expedition was undertaken on board the IEO (Insituto Español de Oceanografia) vessel *Cornide de Saavedra* to the area of the sinking of the *Prestige* with the objective of establishing the distribution of polycyclic aromatic hydrocarbons (PAHs) in the water column and evaluate if any trace of the spill could still be found four years after the incident. Near the sinking area, iridescent slicks were observed, but the severe weather during the cruise did not allow proper sampling of the oil slicks. In July 2006, IEO scientists in the area of the incident reported the presence of oil slicks and managed to collect a small sample from the oil attached to the frame of a rosette array of Niskin bottles. The oil sample was shown to be chemically indistinguishable from the original Prestige fuel oil (Albaigés et al, personal communication). Due to this fact, another cruise in the same vessel was planned for October 2006 to the sinking area. In this occasion, facilitated by excellent weather conditions, the occurrence of oil slicks of different thickness and size, and small tar balls were also observed and sampled accordingly, while also undertaking a detailed sampling of the water column near the wreck.

In this chapter we discuss the origin of the oil slicks found in the ocean surface of the *Prestige* shipwreck, and describe the distribution of the hydrocarbons in the water column of the sinking area in March and October 2006. In order to achieve the established objectives, the distributions of PAHs and *n*-alkanes were analysed in the SPM and in the oil collected in October 2006 in the *Prestige* sinking area, and compared to the chemical signatures of the original oil carried by the tanker.

3.2. Results and discussion

3.2.1. Origin of the oil slicks

The presence of oil slicks above the Prestige wrecks does not constitute proof *per se* that they are the origin of any spillage. As argued in the introduction, the ocean floor, and particularly in the North Atlantic, is littered with ship wrecks and many of them could potentially be the source of deep sea spills (Michel et al., 2005). Moreover, the water column above the wrecks is hydrographically complex, with different water masses (Ruiz-Villarreal et al., 2006) that can potentially transport oil away from its source. It is thus only with chemical fingerprinting techniques that the origin of the oil can be demonstrated. Several complementary approaches were used to verify that the oil collected in the sea surface in October 2006 in fact originated from the Prestige wreck. The relative abundance profile of the distribution of aliphatic hydrocarbons displayed by the suspected oil matched well the hydrocarbon distribution from the original Prestige fuel oil (Fig. 3.1), showing the bimodal distribution previously reported for this oil, as the result of mixing a heavy oil fraction with a lighter one to reduce the viscosity of the former (CSIC, 2003).

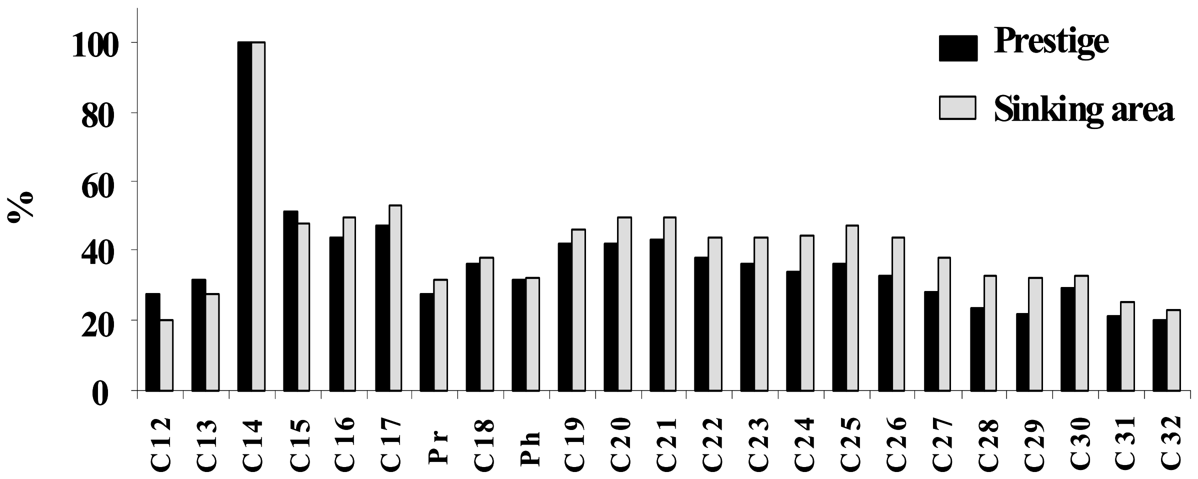


Figure 3.1. C_{12}-C_{32} *n*-alkane distribution in the Prestige fuel oil and oil collected in the sinking area in October 2006.

In Table 3.1 we show that the hydrocarbon composition of some of these samples is practically identical to that of the original fuel oil. The oil does not show signs of biodegradation despite the time past since the incident.

	A	B	C	*Prestige*
n-C_{17}/Pristane	-	1.7	1.6	1.5
n-C_{18}/Phytane	-	1.6	1.5	1.4
Pristane/Phytane	0.8	0.9	0.9	0.9
%27Ts[a]	24	22	24	23
%29αβ[a]	46	46	46	44
%32 αβ S[a]	57	58	57	59
%27d[a]	33	35	33	35
%29ααS[a]	52	50	54	49
%29ββ (R+S)[a]	52	47	49	48
%27ββ[a]	35	34	35	35
%28ββ[a]	25	24	25	25
%29ββ[a]	40	41	40	40

Table 3.1. Biomarker indices measured in three samples (A, B and C) from oil slicks collected on 30/10/2006 above the location of the *Prestige* wrecks. "*Prestige* values" drawn from mean values of 200 samples from the *Prestige* fuel oil collected during 2003 (unpublished data from Albaigés et al.). [a]: Indices defined in Diez et al. (2005). Coordinates of samples: A: 42º12,14´ N, 12º05,0´ W, B: 42º12,49´ N, 12º03,12´ W, C: 42º12,49´ N, 12º03,12´ W.

The isotopic composition of the individual *n*-alkanes was also used to confirm the identity of the oil found in the sinking area since the discriminative nature of this technique has been largely used to unmistakably determine the origin of the oils from different locations (Mansuy et al., 1997, Philp et al., 2002). The results of the Compound Specific Isotope Analysis (CSIA) of $^{13}C/^{12}C$ of the two oils are showed in Fig.3.2. The values provided are the average of three analyses and the standard deviation is better than 0.3 ‰, comparable to the 0.5 ‰ usually reported by the instrument manufacturer. The compared oils exhibited very similar isotopic composition profiles, and the $\delta^{13}C$ values of both Prestige and suspected oils ranged between -30.9 ‰ and -32.4 ‰, which agreed with the carbon isotope ratios previously reported for n-alkanes of similar petroleum products (Bjorøy et al., 1991, Mazeas and Budzinski, 2002). We are thus able to ascertain that the oil from the slicks is that carried originally by the *Prestige*, and that its presence in the ocean obeys most likely to a deep sea spill from the wrecks. The possibility of remobilization of the oil sunken in the bottom being the origin of the slicks found in the sea surface was ruled out. In weathering processes *n*-alkanes <20 carbons gradually decrease as a consequence to evaporation, as well as *n*-C_{17}/Pr and *n*-C_{18}/Ph ratios do in biodegradation. Since the collected oil did not show major sign of transformation due to exposition to the environment, it was supposed that the spill may be quite recent, that is, coming directly from the wrecks.

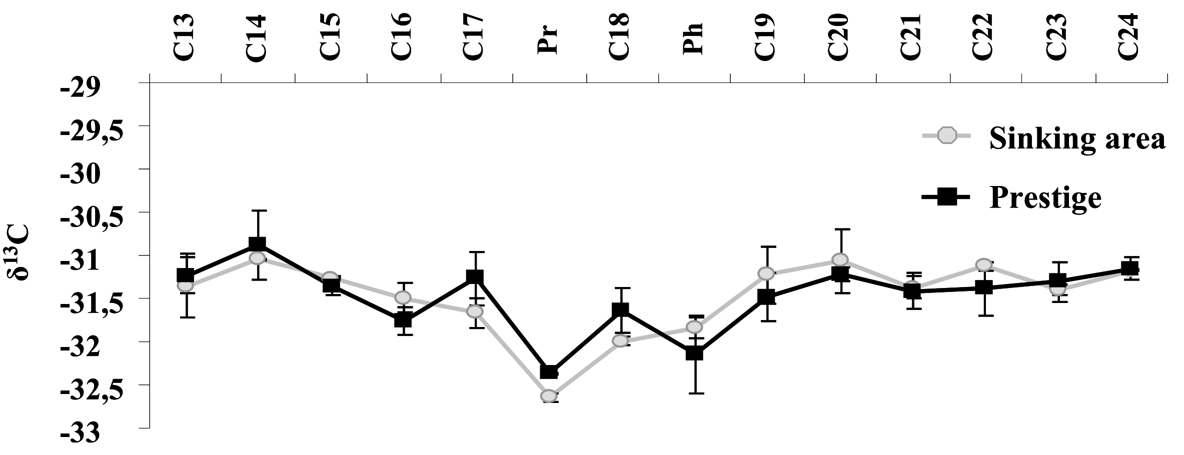


Figure 3.2. Isotopic composition of the n-alkanes (C_{13} to C_{24}) of the fuel oil carried by the Prestige, and from the oil slicks at the sea surface above the location of the Prestige wrecks in October 2006-

3.2.2. PAHs in the SPM

Noticeable differences in the hydrocarbon concentrations were observed in the water column of the Prestige sinking area between the two sampling periods of March and October 2006 (Fig.3.3). The ΣPAHs (sum of infividual PAHs concentrations represented in Fig.3.4) in March ranged between 0.3 to 2.1 ng L^{-1}. These values are not far from the background reference concentration established by the OSPAR in 2004 for the area of the Eastern North Atlantic (0.7-1.6 ng L^{-1},sum of 15 parent PAHs), although they were closer to the 1.05 ng L^{-1} (sum of 10 parent PAHs) and 0.5-12 ng L^{-1} (sum of 15 parent PAHs) found in supposedly more polluted areas such as the Mediterranean (Lipiatou et al., 1997) and Baltic Seas (Witt, 2002), respectively. In contrast, ΣPAHs levels found in October of the same year were well above the background levels, and were three orders of magnitude higher than the concentrations measured in March 2006. ΣPAHs fluctuated between 308 ng L^{-1} and 1218 ng L^{-1}, similar to the levels determined in December 2002 off Costa da Morte (0.29-5.8 µg L^{-1}, sum of 25 PAHs), in the most affected coastal areas by the Prestige oil spill (González et al., 2006).

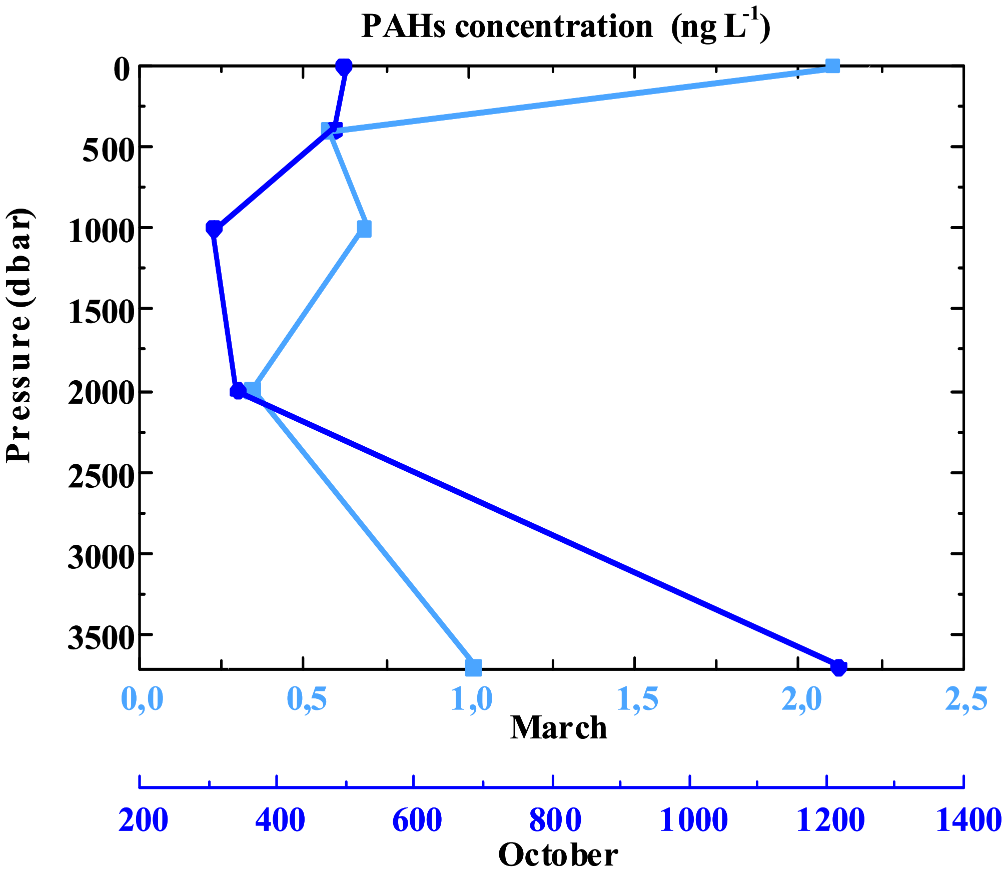


Figure 3.3. Total PAHs concentrations in the SPM of water column at the Prestige shipwreck area, in March (light blue square) and October (dark blue dot) 2006. Note that the ranges of concentration in the x-axis are different.

In both sampling periods the vertical profile of ΣPAHs was characterized by the highest hydrocarbon concentration in the most superficial (5 m) and the deepest (3,706 m) samples, and a decrease in the abundance of the mid-depth layers. The high concentration of contaminants at surface is not surprising given the presence of oil slicks in the sea surface. Near the floor, we attribute the high concentrations to various possible factors. One option is the secondary contamination of the water body due to sediment resuspension (Nemirovskaya, 2007). The proximity to the release point is also one important factor to take into account when the distribution and fate of hydrocarbons are studied since background levels can been reached at relatively short distance from the source (Utvik and Johnsen, 1999, Neff et al., 2006). Thus, high concentration in the NADW water mass probably reflects that this is the water mass in contact with the spill source, likely to be the Prestige wrecks. The main difference exhibited by the two periods was that in March, the maximum abundance was found at 5 m depth and doubled the concentration of the bottom layer, while in October, the concentration at 3,706 m triplicated the one near the surface, which effectively confirms the increasing of the oil input in the water column due to a major deep sea spill coming from the Prestige wreck between the two sampling periods. Another option is that samples were not retrieved exactly from the same locations and water depths during the two periods. Thus the research vessel Cornide de Saavedra has not got a dynamical positioning system, and the ship drifted at different speeds in each occasion. Consequently, the highest concentrations of hydrocarbons in October in comparison to March at depth could be related to the proximity of the sampling device to the spill source.

The relative distribution of the individual PAHs in the water column also exhibited divergences during the two sampling periods (Fig. 3.4). In March, the mixture was dominated by phenanthrene and methylphenanthrenes, which suggest a chronical petrogenic pollution source (Maldonado et al., 1999), followed by naphthalene and its alkyl derivatives. In fact, the relative importance of the latter group was higher at 5 m and 3,700 m depth, in accordance with the maximum concentration of the ΣPAHs. The presence of the family of naphthalene derivatives indicates a fresh petrogenic input, which could be a contribution of the oil from Prestige wreck, suggesting that the wreck was already leaking at that time. Compounds higher than 5 rings were below the detection limit in all the samples. In October, the PAH mixture is clearly dominated by 2-3 ring hydrocarbons, a fact that was also observed after the main black tides in the affected areas by the Prestige (González et al., 2006), and that agrees with the fresh character of the oil found in the sea surface, suggesting that the spilled product was recently leaked from the wreck and had not undergone significant weathering. In general, the relative distribution of the PAHs was quite uniform in the water column, which has been previously observed after the oil spill of the Amoco Cadiz (Marchand, 1980), and naphthalene and its alkyl derivatives represented 95-99 % of the total mixture.

Although, as far as toxicity is concerned, more attention is generally paid to high molecular weight PAHs, 2-3 ring PAHs contribute notably to the toxicity of the mixture and therefore they should be taken into account as a toxicological assessment in a deep sea spill (Neff et al., 2006). PAHs of 4-5 rings were found throughout the water column but only constituted less than 1 % of the total, due to their poor solubility in saline waters (Schwarzenbach et al., 1993) and low concentration in the fuel of the Prestige (CSIC, 2003). Pyrene abundance dominated over fluoranthene, a trait of petroleum product spills (Tronczyski et al., 2004) and the fluoranthene/pyrene ratio was <1 in the sinking area for all the sampled depths. In environments where the main PAH inputs are of pyrogenic nature, Fl dominates over Py, and Fl/Py is usually>1 and therefore this ratio is used as origin indicator (Maldonado et al., 1999). The high P/A ratio, always above 10, also confirms the attribution of PAH to petrogenic pollution (De Luca et al., 2005).

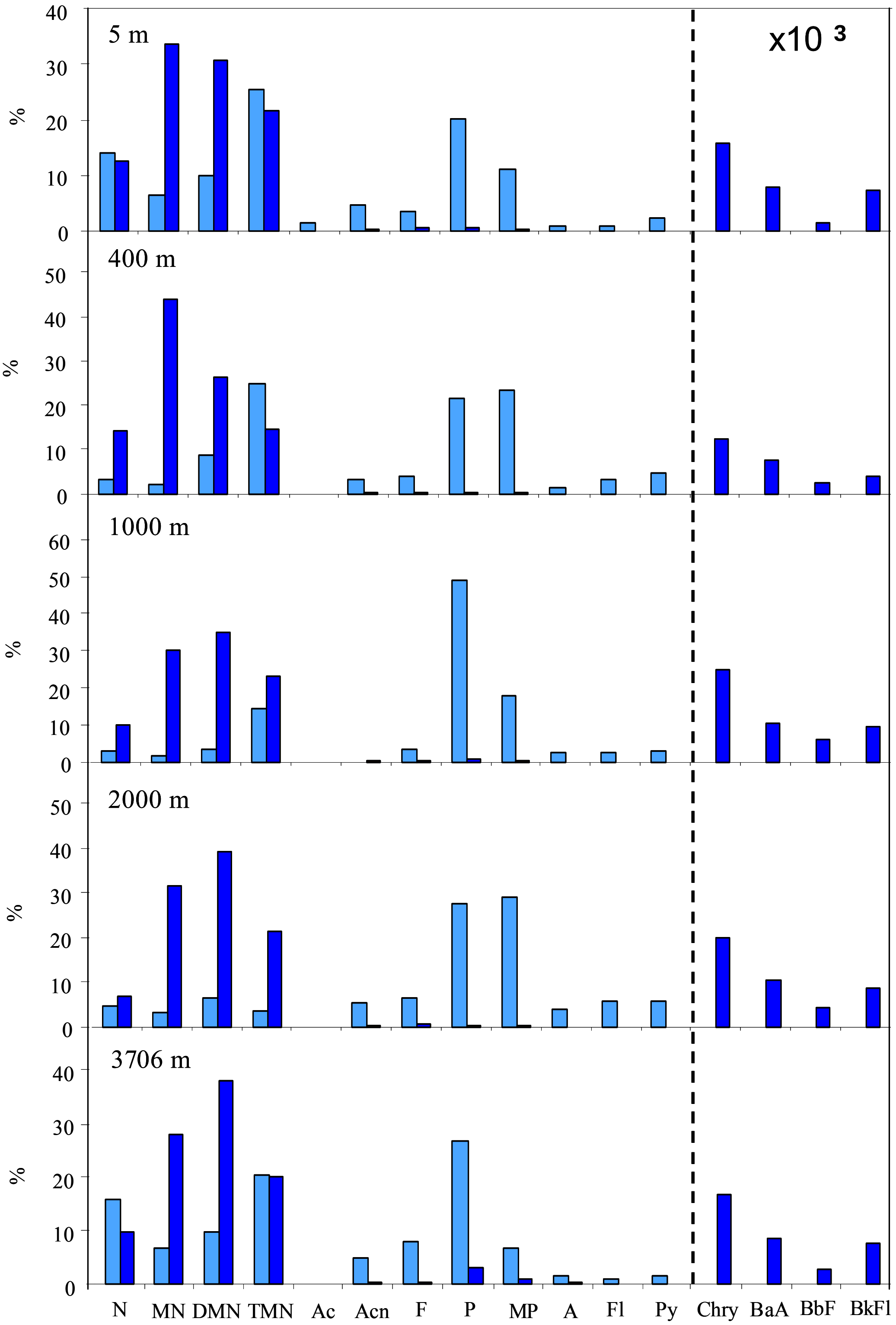


Figure 3.4. Relative distribution of PAHs in the SPM of the water column at the Prestige shipwreck sinking area in March (light blue) and October (dark blue) 2006. N: Naphthalene, MN: Methylnaphthalenes, DMN: Dimethylnaphthalenes, TMN: Trimethylnaphthalenes, Ac: Acenaphthylene, Acn: Acenaphthene, F: Fluorene, P: Phenanthrene, MP: Methylphenanthrenes, A: Anthracene, Fl: Fluoranthene, Py: Pyrene, Chry: Chrysene, BaA: Benzo[*a*]anthracene, BbF: Benzo[*b*]fluorene, BkFl: Benzo[*k*]fluoranthene.

3.2.3. Aliphatic hydrocarbons in the SPM

Alkanes from C_{14} to C_{35}, together with pristane (Pr) and phytane (Ph) were also determined. Despite that these compounds are not usually taken into account in the toxicological assessments of a spill, they are very useful to identify the source and the fate of spills in the environment (Kaplan et al., 1997, Wang et al., 2003). In March, the total concentration of aliphatic hydrocarbons in the water column ranged from 4.8 to 40.5 ng L^{-1}. These concentrations agreed with the 6-34 ng L^{-1} reported in the Western Mediterranean open surface waters (Dachs et al., 1999), 0.8-6.8 ng L^{-1} of deep sea waters (1700 m) in the Alboran Sea (Martí et al., 2001) and 3-101 ng L^{-1} in the water column (maximum of 200 m) of the open ocean stations in the Black Sea (Maldonado et al., 1999).

In March, the *n*-alkane abundance decreased with depth probably as consequence of the reduction of the SPM (Fig. 3.5). In contrast, the samples collected in October showed a vertical profile much similar to the one found for the PAHs in the same period, with maximum concentration in the superficial waters, a depletion in the mid depth water and enrichments near the bottom. As for the PAHs, this trend could be attributed to the contribution of the oil coming out from the Prestige wrecks. Higher concentration in the total aliphatic hydrocarbons in October seems to confirm the occurrence of a deep sea spill, although the increment was less spectacular than in the aromatic fraction, only about one order of magnitude. Total aliphatic hydrocarbon concentrations ranged from 65.7 to 413.7 ng L^{-1}, values that are well over the concentration reported in the open ocean.

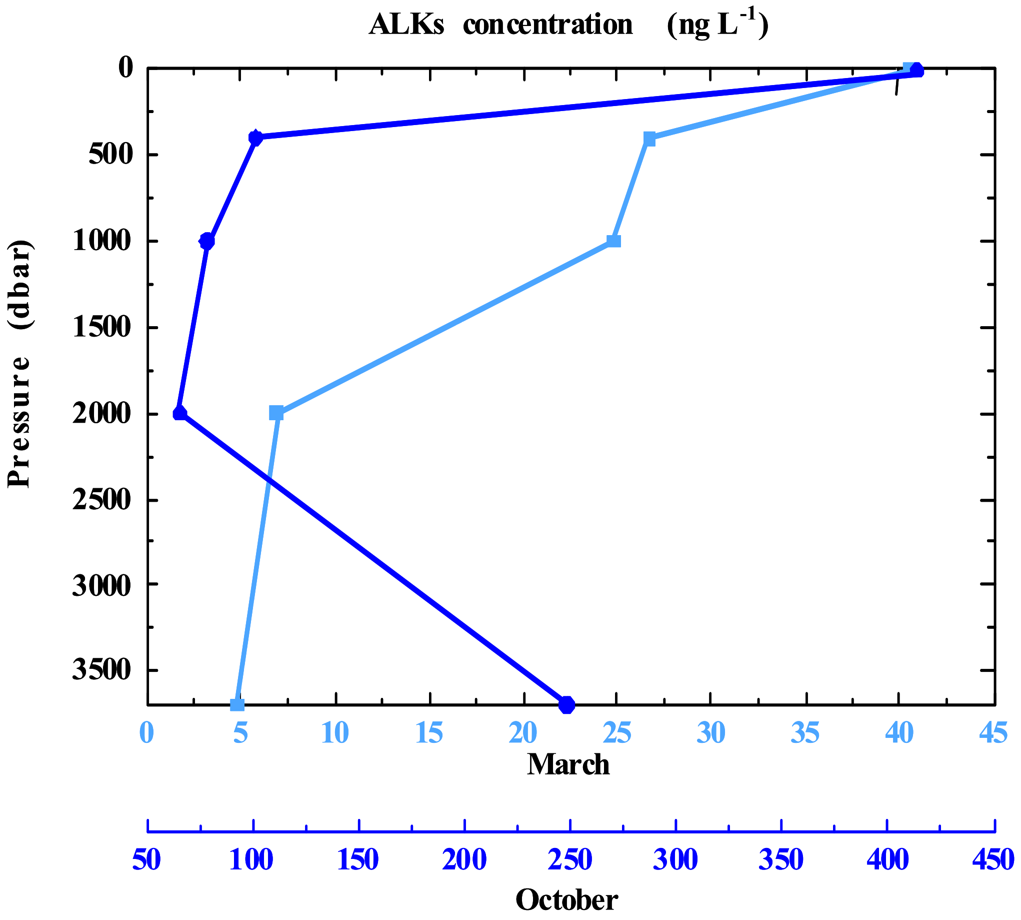


Figure 3.5. Total n-alkane (ΣALKs) concentrations in the SPM of water column at the Prestige shipwreck area, in March (light blue square) and October (dark blue dot) 2006. Note that the ranges of concentration in the x-axis are different.

Similarly to the distribution of the PAHs, the relative distribution of individual n-alkanes in the mixture changed noticeably between the samples collected in the two cruises (Fig.3.6 and 3.7). In March, the most important feature of the mixture was the dominance of compounds between C_{25}-C_{30} in all the depths. The odd-to-even carbon number ratio showed values of unity in the superficial waters and values of <1 (from 0.82 to 0.91) in the remaining depths. These elevated values were more elevated than the ones found in the Ross Sea (Antarctica) (from 0.4 to 0.8), with dominance of hydrocarbons coming from pelagic species (Bubba et al., 2004). This suggests a mixture of petrogenic and biogenic hydrocarbons. One of the most important features of the distribution of n-alkanes in October was the relative uniformity in the n-alkane profile in the water column, although the profiles displayed by the most superficial and deepest samples fitted best the distribution pattern of the Prestige oil. In the mid-depth samples C_{16}, C_{17} and n-alkanes between C_{27}-C_{35} were generally present at higher proportion than the other components. Although n-alkanes from C_{15} to C_{17} are characteristics of lipids of phytoplankton origin (Avigan and Blumer, 1968), odd to even carbon number ratio showed values around the unity for all the depths, which it is evidence for a major contribution of petrogenic origin hydrocarbons to the aliphatic fraction (Martí et al., 2001).

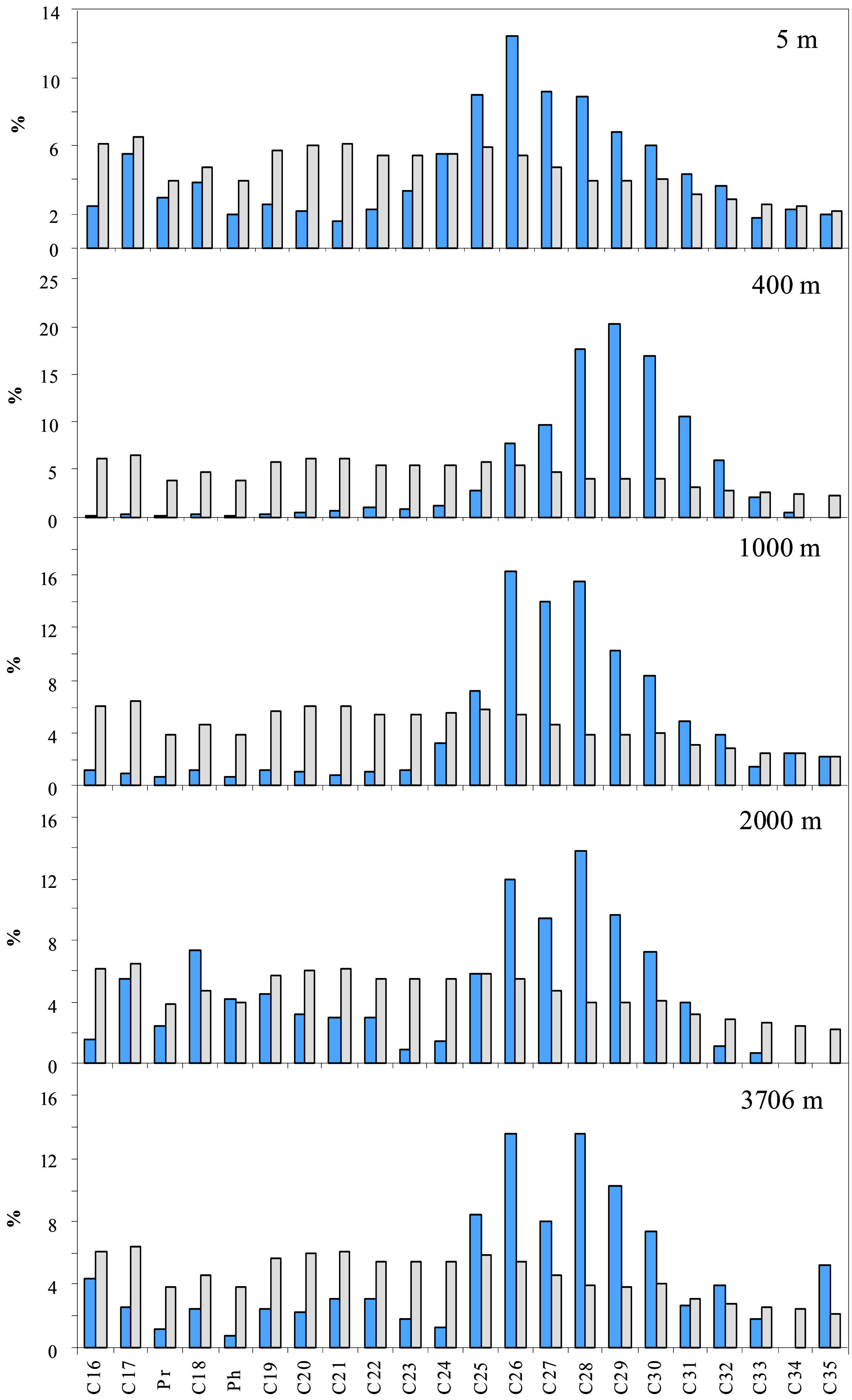


Figure 3.6. Distribution of individual *n*-alkanes, including pristane (Pr) and phytane (Ph) in the SPM in March 2006 (light blue) and in the oil slick found in the sinking area in October 2006 (grey).

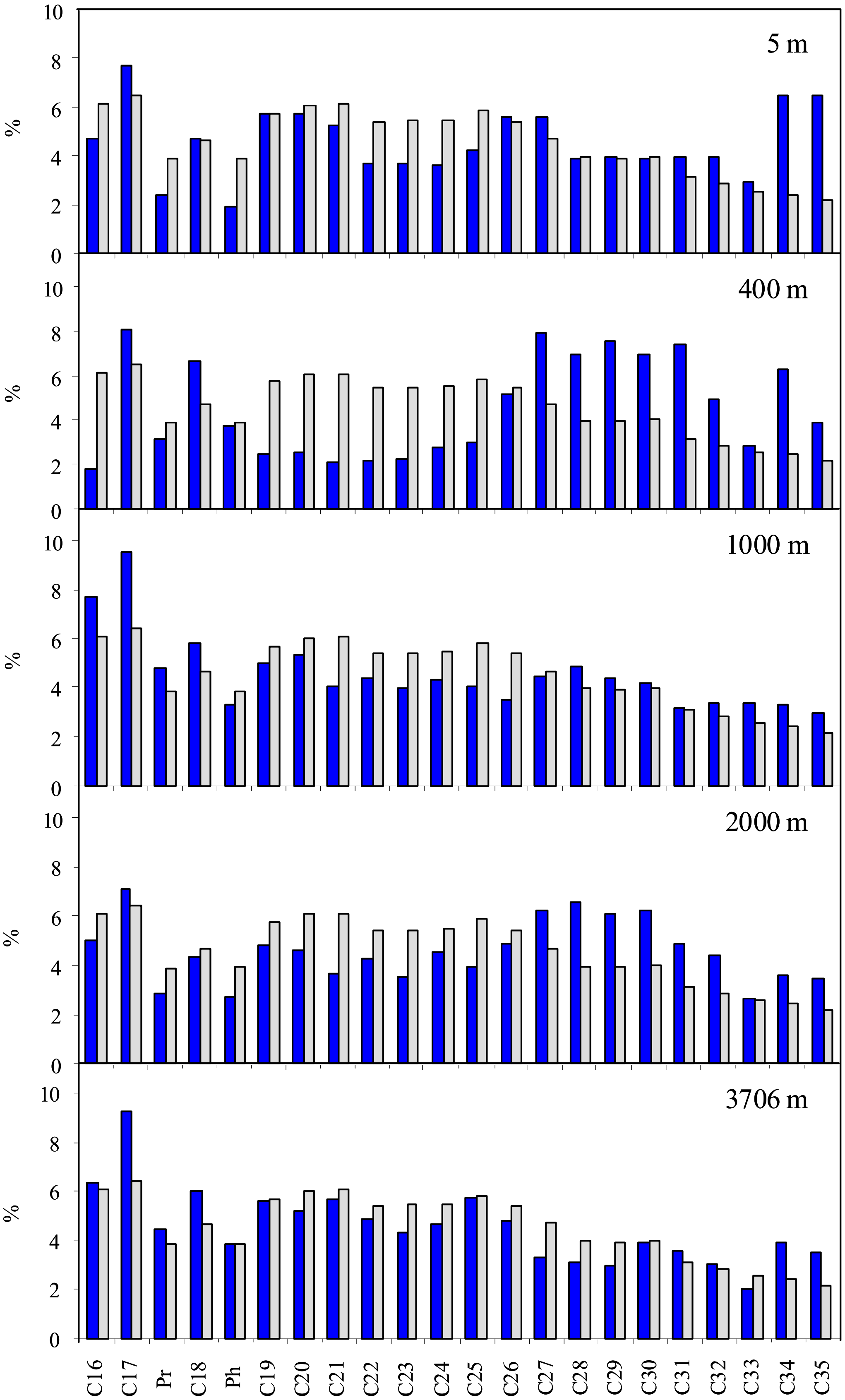


Figure 3.7. Distribution of individual *n*-alkanes, including pristane (Pr) and phytane (Ph) in the SPM (dark blue) and in the oil slick (grey) found in the sinking area in October 2006.

3.3. Conclusions

The chemical signature of the oil slicks detected in the *Prestige* sinking area in October 2006 is equivalent to that of the fuel oil carried by the Prestige, and do not reflect a weathered oil signature. Given that the Prestige accident took place more than three years since the oil slicks were found, we propose that their most likely origin is from a recent deep sea spill from the shipwreck. The surface oil signature was also found in the entire water column above the wrecks. The hydrocarbon concentration levels in the different water masses of the area are different, and overall much higher than the concentrations reported previously in the area, and were close to the ones observed near the Galician coast on the most affected areas soon after the spill in 2002. Likewise, the maximum hydrocarbon abundances were located in the deepest water mass, as a consequence of its proximity to the wreck, and superficial waters, mostly due to the floating oil, which acted as a secondary source. Distribution profiles of individual hydrocarbons in the SPM also showed a clear modification between the two sampling periods. In October they significantly resembled the Prestige fuel profiles, overpowering the signature of biogenic and other possible anthropogenic sources that could be observed in March.

Clearly, the deep sea-spill from the wrecks introduced large amounts of hydrocarbons in the water column, and could be pointed at as the most relevant hydrocarbon source in the area. Although each potentially polluting shipwreck represents a unique situation, knowledge of the influence of a deep sea spill in the water column and the hydrocarbons distribution in the different water masses acquired from the case study of the *Prestige*, could be applied to deal with other deep spills in the future.

3.4. References

Avigan, J., Blumer, M.,1968. On the origin of pristane in marine organisms. J. Lipid Res. 9, 350–352.

Basta D.J., Kennedy, D.M., 2004. The need for a proactive approach to underwater threats. Mar. Technol. Soc. J. 38. 9-11.

Birkland, T. A., Lawrence, R.G., 2002. The Social and Political Meaning of the Exxon Valdez Oil Spill. Spill Sci. & Technol. B. 7, 17-22.

Bjorøy, M., Hall, K., Gyllion, P., Jumeau, J., 1991. Carbon isotope variations in n-alkanes and isoprenoids of whole oils. Chem. Geol. 93, 13-20.

Bubba, M., Cincinelli, A., Checchini, L., Lepri, L., Desideri, P., 2004. Horizontal and vertical distributions of biogenic and anthropogenic organic compounds in the Ross Sea (Antarctica). Intern. J. Environ. Anal. Chem. 84, 441–456.

Cincinelli, A., T. Martellini, T., Bittoni,L., Russo, A., Gambaro, A., Lepri, L., 2008. Natural and anthropogenic hydrocarbons in the water column of the Ross Sea (Antarctica). J. Mar. Sys. 73, 208-220.

CSIC, 2003. Informe técnico CSIC Prestige nº1: Caracterización del vertido y evolución preliminar en el medio. Available from: http://csicprestige.iim.csic.es/desarro/informcsic/1/.

Dachs, J., Bayona, J.M., Fillaux, J., Saliot, A., Albaigés, J., 1999. Evaluation of anthropogenic and biogenic inputs into the western Mediterranean using molecular markers. Mar. Chem. 65, 195-210.

Daling, P. S., Moldestad, Ø.M., Johansen, Ø.,Lewis, A., Rødal, J., 2003. Norwegian Testing of Emulsion Properties at Sea--The Importance of Oil Type and Release Conditions. Spill Sci. Technol. Bull. 8, 123-136.

De Luca, G., Furesi, A., Micera, G., Panzanelli, A., Piu, P.C., Pilo, M.I., Spano, N., Sanna, G., 2005. Nature, distribution and origin of polycyclic aromatic hydrocarbons (PAHs) in the sediments of Olbia harbor (Northern Sardinia, Italy). Mar. Pollut. Bull. 50. 1223-1232.

Diez, S., Sabate, J., Viñas, L., Bayona, J.M., Solanas, A.M., Albaigés, J., 2005. The *Prestige* oil spill. I. Biodegradation of a heavy fuel oil under simulated conditions. Environ. Toxicol. Chem. 24, 2203-2217.

Diez, S., E. Jover, J.M. Bayona, J. Albaigés. Prestige Oil Spill., 2007. III. Fate of a heavy oil in the marine environment. Environ. Sci. Technol. 41, 3075-3082.

Garrott, R. A., Eberhardt, L.L.,D. M. Burn, D.M., 1993. Mortality of Sea Otters in Prince William Sound Following the *Exxon Valdez* Oil Spill. Mar. Mammal Sci. 9, 343-359.

Gilbert T.D., Nawadra, S., Tafileichig, A., Yinug, L., 2003. Response to an oil spill from a sunken WWII oil tanker in Yap State, Micronesia. Proceedings of the 2003 International Oil Spill Conference. American Petroleum Institute, Washington, D.C. pp.1-8.

Girin, M., 2004. European experience in response to potentially polluting shipwrecks. Maine Technology Society Journal 38, 21-25.

González, J. J., Viñas, L., Franco, M.A., Fumega, J., Soriano, J.A., Grueiro, G., Muniategui, S., López-Mahía, P., Prada, D., Bayona, J.M., Alzaga, R., Albaigés, J., 2006. Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the Prestige oil spill. Mar. Pollut. Bull. 53, 250-259.

ITOPF, 2009. Oil tanker spill statistics. Available from: http://www.itopf.com/information%2Dservices/data%2Dand%2Dstatistics/statistics/documents/Statpack2008_001.pdf

Jaward, F. M., Barber, J.L., Booij, K., Jones, K.C., 2004. Spatial distribution of atmospheric PAHs and PCNs along a north-south Atlantic transect. Environ. Pollut. 132 , 173-181.

Kaplan, I. R., Galperin, Y., Lu, S.T., Lee, R.P., 1997. Forensic environmental geochemistry: differentiation of fuel-types, their sources and release time. Org. Geochem. 27, 289-299.

Laffon, B., T. Rábade, T., Pásaro, E., Méndez, J., 2006. Monitoring of the impact of Prestige oil spill on Mytilus galloprovincialis from Galician coast. Environ. Int. 32, 342-348.

Lipiatou, E., Tolosa, I., Simó, R., Bouloubassi, I., Dachs, J., Marti, S., Sicre, M.A., Bayona, J.M., Grimalt, J.O., Saliot, A., Albaiges, J., 1997. Mass budget and dynamics of polycyclic aromatic hydrocarbons in the Mediterranean Sea. Deep-Sea Res. Pt. II 44, 881-905

Maldonado, C., J. M. Bayona, J.M., Bodineau, L., 1999. Sources, distribution, and water column processes of aliphatic and polycyclic aromatic hydrocarbons in the northwestern Black Sea water. Environ. Sci. Technol. 33, 2693-2702.

Mansuy, L., Philp, R.P., Allen, J., 1997. Source Identification of Oil Spills Based on the Isotopic Composition of Individual Components in Weathered Oil Samples. Environ. Sci. Technol. 31, 3417-3425.

Marchand, M. H., 1980. The Amoco Cadiz oil spill. Distribution and evolution of hydrocarbon concentrations in seawater and marine sediments. Environ. Int. 4, 421-429.

Marti, S., Bayona, J.M, Albaigés, J., 2001. Potential Source of Organic Pollutants into the Northeastern Atlantic: the outflow of the Mediterranean deep-lying waters through the Gibraltar Strait. Environ. Sci. Technol. 35, 2682-2689.

Mazeas, L., Budzinski, H., 2002. Molecular and stable carbon isotopic source identification of oil residues and oiled bird feathers sampled along the Atlantic coast of France after the Erika oil spill. Environ. Sci. Technol. 36, 130-137.

Michel, J., Gilbert, T., Waldron., J., Blocksidge, C.T., Schmidt Etkin, D., Urban, R., 2005. Potentially polluting wrecks in marine waters. In: Proceedings of the international oil spill conference (IOSC) , Miami beach, USA.

Neff, J. M., Johnsen, S., Frost, T.K., Utvik, T.I., Durell, G.S., 2006. Oil well produced water discharges to the North Sea. Part II: Comparison of deployed mussels (Mytilus edulis) and the DREAM model to predict ecological risk. Mar. Environ. Res. 62, 224-246.

Nemirovskaya, I.A., 2007. Hydrocarbons in the water and bottom sediments of a region with continuous petroleum contamination. Geochem. Int+ 45, 638-351.

Oropesa, A.L., Pérez-López, M., Hernández, D., García, J.P., Fidalgo, L.E., López-Beceiro, A., Soler, F., 2007. Acetylcholinesterase activity in seabirds affected by the Prestige oil spill on the Galician coast (NW Spain). Sci. Total Environ. 372, 532-538.

OSPAR, 2004. OSPAR/ICES Workshop on the evaluation and update of the background reference concentrations (BCRs) and ecotoxicological assessment criteria (EACs). Available from: www.ospar.org.

Pérez-del-Olmo, A., Montero, F.E, Raga, J.A., Fernández, M., Kostadinova, A., 2009. Follow-up trends of parasite community alteration in a marine fish after the Prestige oil-spill: Shifting baselines?. Environ. Pollut. 157, 221-228.

Philp, P., Allen, R.J., Kuder, T., 2002. The use of the isotopic composition of individual compounds for correlating spilled oils and refined products in the environment with suspected sources. Environ. Forensics 3, 341-348.

Ruiz-Villarreal, M., C. González-Pola, Diaz del Rio, G., Lavin, A., Otero, P., Piedracoba, S.,.Cabanas, J.M., 2006. Oceanographic conditions in North and Northwest Iberia and their influence on the Prestige oil spill. Mar. Pollut. Bull. 53, 220-238.

Schulz-Bull, D. E., Petrick, G., Bruhn, R., Duinker, J.C., 1998. Chlorobiphenyls (PCB) and PAHs in water masses of the northern North Atlantic. Mar. Chem. 61, 101-114.

Schwarzenbach, R.P., Gschwend, P.M.,Imboden, D.M., 1993. Environmental Organic Chemistry, New York, John Wiley and Sons, Inc.

Serret, P., Álvarez-Salgado, X.A., Bode, A., 2003. Spain's earth scientists and the oil spill. Science 299,511.

Tronczyski, J., Munschy, C., Héas-Moisan, K., Guiot, N., Truquet, I., Olivier, N., Men,S., Furaut, A., 2004. Contamination of the Bay of Biscay by polycyclic aromatic hydrocarbons (PAHs) following the T/V "*Erika*" oil spill. Aquat. Living Resour. 17, 243-259

Turrell, W. R. (1994) Modelling the Braer oil spill:A retrospective view. Mar. Pollut. Bull._28, 211-218.

Utvik, T. I.R., Johnsen, S., 1999. Bioavailability of polycyclic aromatic hydrocarbons in the North Sea. Environ. Sci. Technol. 33, 1963-1969.

Vieites, D.R., Nieto-Román, S., Palanca, A., Ferrer, X., Vences, M., 2004. European Atlantic: the hottest oil spill hotspot Worldwide. Naturwissenschaften 91, 535-538.

Wang, Z. and M. F. Fingas, M.F., 2003. Development of oil hydrocarbon fingerprinting and identification techniques. Mar. Pollut. Bull. 47, 423-452.

Witt, G., 2002. Occurrence and transport of polycyclic aromatic hydrocarbons in the water bodies of the Baltic Sea. Mar. Chem. 79, 49-66.

Yapa, P. D. , Zheng, L., 1997. Modelling oil and gas releases from deep water: A review. Spill Sci. Technol. Bull. 4, 189-198.

CHAPTER 4

Hydrocarbons in the particulate matter of the open-ocean water masses near the Galician Bank

4.1. Introduction

In November 2002 the *Prestige* broke in two, 240 km offshore the western Iberian Peninsula coast, and sunk at more than 3,500 m depth with around 58,000 tonnes of heavy fuel oil in its tanks (Albaigés et al., 2006). Despite salvage and sealing operations, still in 2006 fresh oil slicks of fuel carried by the vessel could be found in the sinking area (see chapter 3). This suggests the possibility that the wrecks had been releasing oil from the depth for a number of years, and consequently contributed to a chronic hydrocarbon contamination in the area. Distribution and spreading of the fuel oil near the coast was thoroughly studied after the *Prestige* incident, (González et al., 2006; Serrano et al., 2006; Varela et al., 2006) mainly due to the importance of the local fisheries off NW Spain, natural interest and tourisms activities in the affected areas (Garzas-Gil, et al., 2006). Studies of the distribution of the oil in the open ocean beyond the continental platform, however, have been limited in scope, and the majority of them were related to the effect of the spill in the pelagic/benthic organisms, such as plankton and fish (Sánchez et al., 2006; Varela et al., 2006). In fact, studies of the distribution of organic contaminants in open-ocean waters in the North Atlantic are in general scarce, and information on the occurrence of organic pollutants in deep waters is even more occasional (Lipiatou et al., 1997; Schulz-Bull et al., 1998; Martí et al., 2001). The sinking of the *Prestige* tanker represented, thus, a unique scientific opportunity as a case study to investigate the presence of organic petrogenic pollutants in the deep sea, and to assess the role of open-ocean currents in the distribution of hydrocarbons from a deep spill in the open ocean.

Several factors are involved in the distribution of the hydrocarbons between different water masses. These influence physico-chemical properties of the water bodies, such as temperature, salinity, pH and amount of particulate and organic carbon and pressure amongst others (Xie et al., 1997; Maldonado et al., 1999; Jurado et al., 2007; Elordui-Zapatarietxe et al., 2008) (see a detailed discussion in chapter 1). In addition, advective transport also plays a decisive role in the long-range worldwide distribution of hydrophobic organic pollutants (Lohmann et al., 2007), where the origin, formation process, circulation direction and mixing of water masses must be taken into account (Martí et al., 2001; Lohmann et al., 2006). Given that the hydrodynamic area where the

Prestige sank is complex, comprising five water masses with different origins and distinct physicochemical characteristics (see chapter 2), their hydrocarbon composition can be expected to be relatively variable (Elordui-Zapatarietxe et al., 2008).

The study presented in this chapter is part of a study aimed at determining the distribution of hydrocarbons in open ocean waters off the Galician coast, and assess the role of the different water masses in their transport. The samples were collected in March 2006, as described in chapter 2, section 2.2. As an initial hypothesis, we assumed that the *Prestige* shipwreck would be the major source of hydrocarbon pollution in the vicinity of the incident area. The sampling stations selected were far enough from the coast not to be influenced in principle by inputs from land-based and coastal activities, and the study area is not in the path of major shipping lanes. We were also assuming that other shipwrecks in the area were not contributing significantly to background levels of contaminants in the Prestige wreck station. Thus, from accidental discharges of produced waters rich in PAHs in the North Sea waters, it has been described that concentrations of hydrocarbons reach background levels a short distance from the discharge point (Utvik et al., 1999). In consequence, in our study it was initially assumed that both north and south stations were far enough from the wreck to consider them in principle not affected by the *Prestige* spill.

To tackle this study we pursued two main goals: i) to determine the origin of the hydrocarbons (PAHs and *n*-alkanes) in the SPM in the water column at three different stations, corresponding to the sinking area of the *Prestige* tanker wreck, and in one station located to the north and another one to the south of the sinking region, and ii) to determine the relative distribution of hydrocarbons in the various water masses (see chapter 2) off the Galician coast. The expectation was that the study should provide some insights of the potential spread of the *Prestige* deep sea spill since the vessel sank.

Detailed description of the study area and methodology are provided in chapter 2.

4.2. Results and discussion

4.2.1. Hydrocarbon distribution

4.2.1.1. PAHs

The total concentration of 21 PAHs analysed (ΣPAHs) in the SPM of the water column of the three stations are shown in Fig. 4.1. Several resemblances were observed in the vertical distribution of concentration profiles among stations. Overall, an increase in the ΣPAHs concentration could be observed in the deepest water mass, NADW, compared to the overlaying water body, the LSW. In the northern station, the NADW constituted the water mass with the highest PAH abundance while in the *Prestige* site displayed the second highest ΣPAHs values after the SW. On the contrary, in the south station this tendency was not as clear as in the other sites, and the profile was characterized by a relative maximum concentration peak at 1000 m depth, corresponding to the MW. This slight increase in the ΣPAHs abundance was less evident in the *Prestige* station and non-existent in the north.

Another common trait related to the vertical distribution profiles was the south-north increasing gradient in the ΣPAHs abundance in the two deepest water masses. This tendency seemed to be also followed in the SW and ENACW for the north and south sites, being concentrations lower in the later. On the other hand, the highest ΣPAHs concentrations for these water masses were found in the Prestige station.

Finally, opposite to the general trend followed by the two most superficial and two deepest water masses, the MW showed a south-north decreasing gradient, being the concentrations in the north the lowest of the three stations.

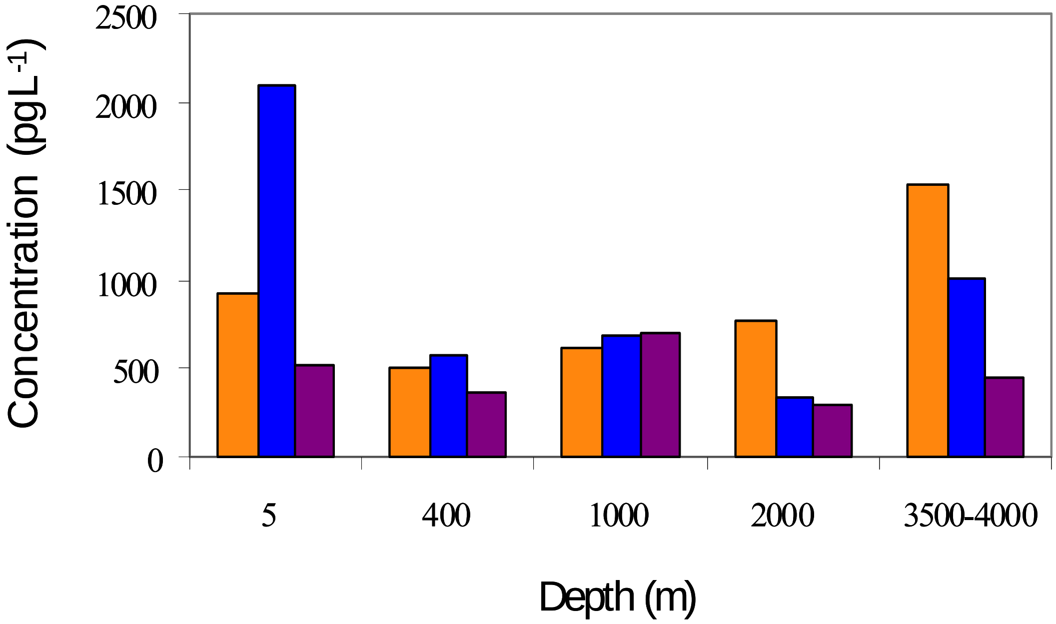


Figure 4.1. Concentration of the sum of 21 PAHs (ΣPAHs) in the SPM of selected stations at different depths in March 2006. Sampling locations: north station (orange), *Prestige* station (blue) and south station (purple). ΣPAHs: Naphthalene (N); Methylnaphthalenes (MN); Dimethylnaphthalenes (DMN); Trimethylnaphthalenes (TMN); Acenaphthylene (Ac); Acenaphthene (Acn) ; Fluorene (F); Phenanthrene (P); Methylphenanthrenes (MP); Anthracene (A), Fluoranthene (Fl); Pyrene (Py); Chrysene (Chry); Benzo[*a*]anthracene (BaA), Benzo[*b*]fluorene (BbF), Perylene (Per); Benzo[*k*]fluoranthene (BkFl); ; Benzo[*a*]pyrene (BaPy); Benzo[*g,h,i*]perylene (BghiPer); Dibenzo[*a,h*] anthracene (DahA); Indeno[1,2,3,*c-d*]pyrene (Ind).

ΣPAH levels in the SPM of the water column at the studies station were quite similar. The ΣPAHs concentrations in the *Prestige* station ranged between 0.3 to 2.1 ng L^{-1}, from 0.5 to 1.5 ng L^{-1} in the north, and 0.3 to 0.7 ng L^{-1} in the south. For most depth intervals the reported PAH abundances were in the range of the background reference concentrations (BRC) established by the OSPAR in 2004 for the Eastern North Atlantic (0.7-1.6 ng L^{-1} for 15 unsubstituted PAHs). However, previous studies on the PAH distributions in the North Atlantic have reported very varied concentrations, due to a large extent to the divergence in the target compounds and analytical methodology. Even so, when only PAHs common in all the studies are compared, the levels described in the literature regarding to the open ocean water do not seem to agree. Concentration from the 5 pg L^{-1} (P+A+Fl+Py) described by Lipiatou et al. (1997), 21-139 pg L^{-1} (P+Fl+Py) determined by Schulz-Bull et al. (1998) to 4.5 -54 pg L^{-1} (P+Fl+Py, <200

m) and 1.4-21 pg L^{-1} (P+Fl+Py, >1000 m) found by Martí et al. (2001) have been reported. For this group of compounds, the PAHs levels found in this study ranged between 77-188 pg L^{-1}in the north, 133-488 ng L^{-1} in the Prestige site and 92-180 pg L^{-1} in the south, which with exception of some water masses, were not far from some of the values reported in the previously mentioned studies. Anyway, from the studied stations, the nearer to the wreck seemed to present the highest concentration.

The distribution of PAHs in the mixture (Fig.s 4.2, 4.3 and 4.4) was dominated by light hydrocarbons from two to four rings, being phenanthrene, naphthalene and its alkylated derivatives the most abundant compounds. The contribution of alkylated compounds to the ΣPAHs was quite elevated and ranged from 42 to 67 % (mean = 52,5, s.d.= 8,3). These families of compounds were also abundant in the water column near the Galician and French coast immediately after the *Prestige* and *Erika* oil spills respectively (Tronczynski et al., 2004; Gonzalez et al., 2006). In the *Prestige* station, SW and the NADW displayed higher contribution of the naphthalene family to the ΣPAHs than in the middle depth layers, and their distribution resembles that of the seawater samples collected the months following the *Prestige* accident in the water column off Galicia (Gonzalez et al., 2006). This distinction was not observed in the south and north stations, where the proportions were more similar in throughout the water column. Nevertheless, the family of naphthalene compounds was also the most abundant in the north, while phenanthrene and methylphenanthrenes were dominant in the south. PAHs higher than 5 rings were below the detection limit in all samples.

Nevertheless, individual PAHs distributions in the water column (appendix 2) did not always follow the general profiles of ΣPAHs established in the Fig. 4.1. The lightest compounds of the mixture tended to follow more accurately the general patterns in the respective sampling station, while other PAHs, such as anthracene, fluoranthene and pyrene showed more different behaviours. Several authors has observed compositional changes in the PAH composition in the SPM with depth (Maldonado et al., 1999; Martí et al., 2001), a phenomenon that has been attributed to the easier degradation and adsorption of the lightest petrogenic compound that enter the superficial waters in the upper water bodies. This phenomenon translated in an increasing presence of the pyrogenic PAHs in the deepest water masses. Nevertheless, such tendency has not been observed in this study.

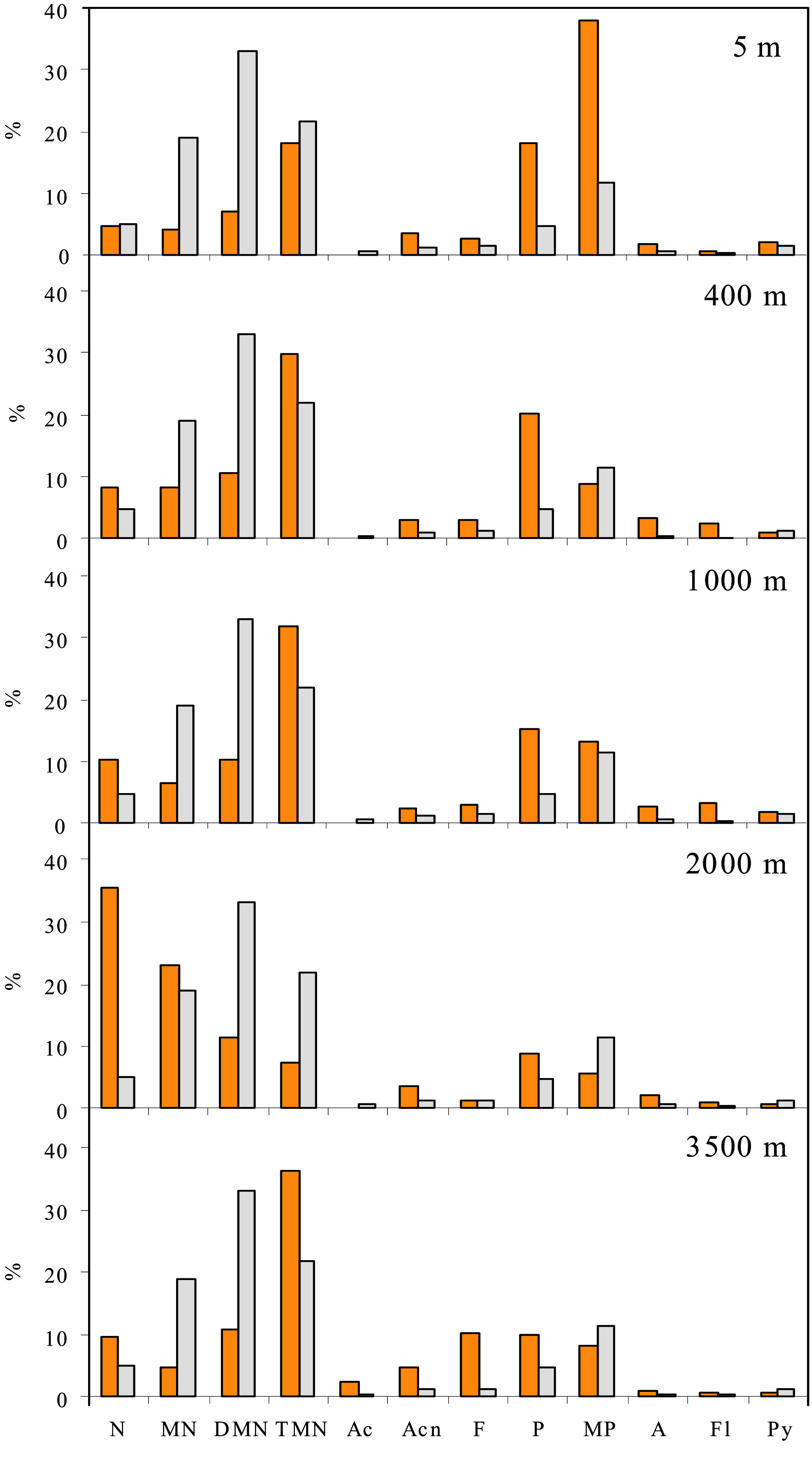


Figure 4.2. Relative abundance of PAHs in the SPM at the north station (orange) in March 2006 and the fuel oil from the *Prestige* (grey). Sampling depths correspond to the water masses in the area (detailed description in chapter 2). PAH abbreviation as in fig. 4.1.

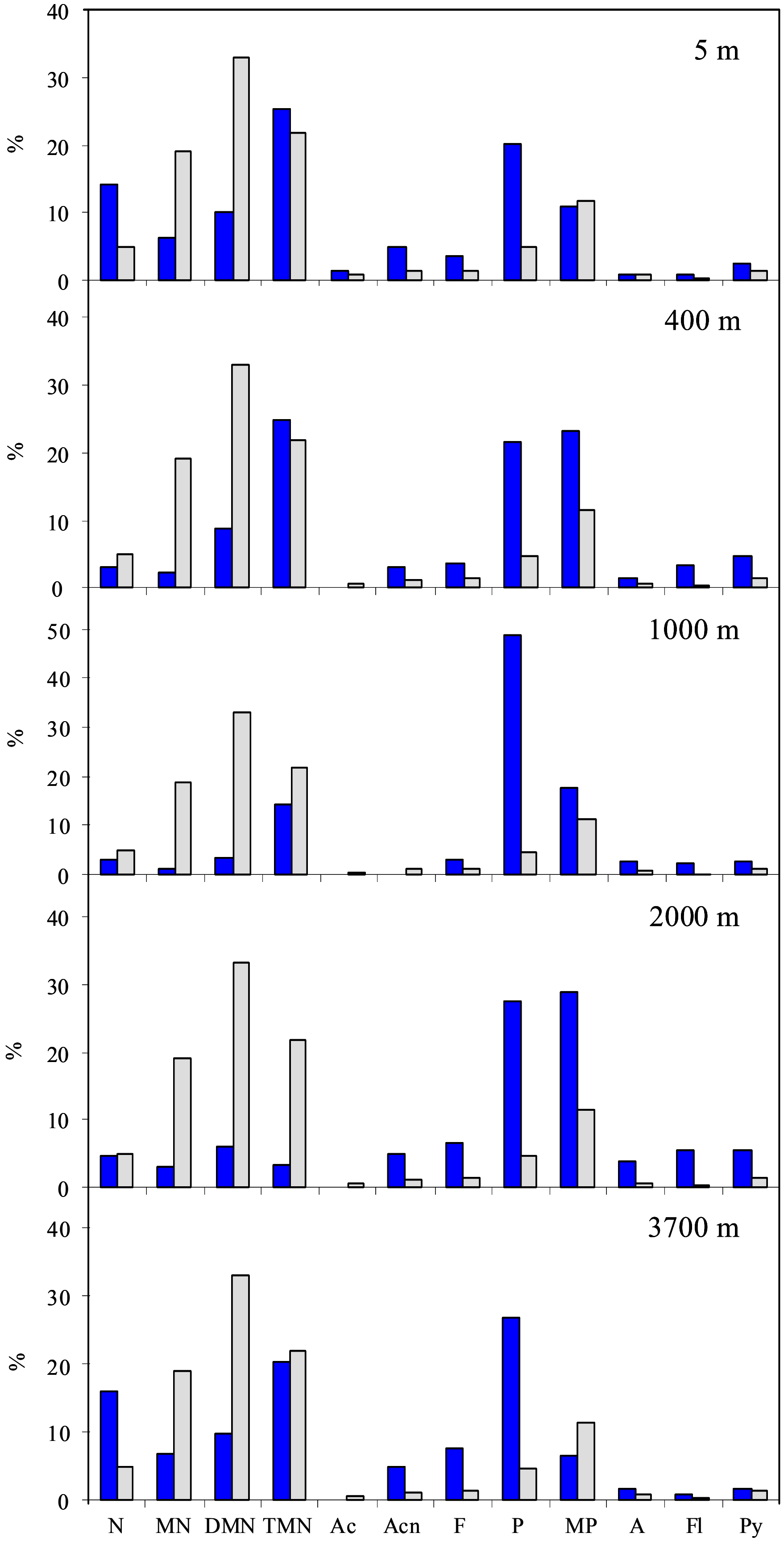


Figure 4.3. Relative abundance of PAHs in the SPM at the *Prestige* station (blue) in March 2006 and the fuel oil from the *Prestige* (grey). Sampling depths correspond to the water masses in the area (detailed description in chapter 2). PAH abbreviation as in fig. 4.1.

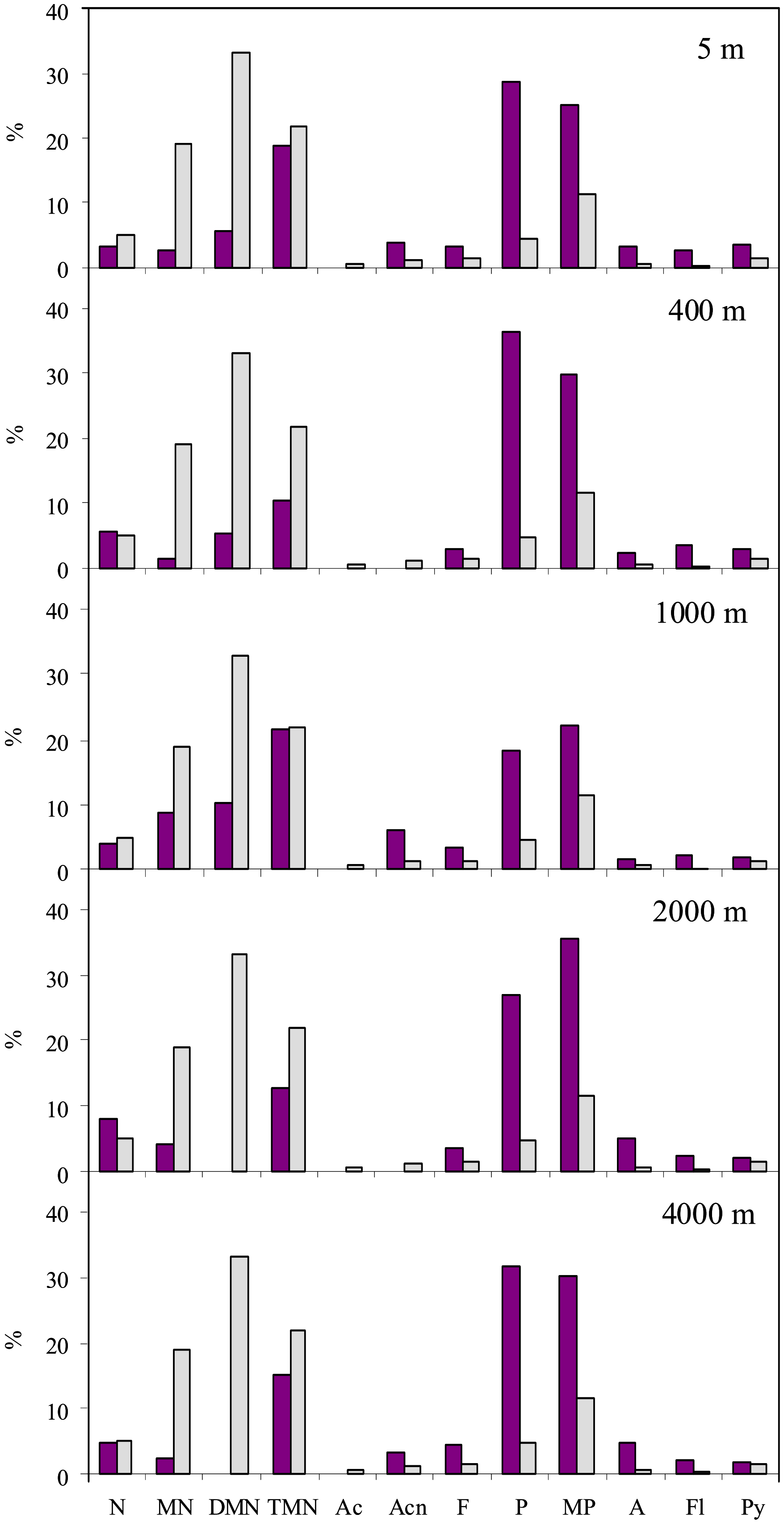


Figure 4.4. Relative abundance of PAHs in the SPM at the south station (purple) in March 2006 and the fuel oil from the *Prestige* (grey). Sampling depths correspond to the water masses in the area (detailed description in chapter 2). PAH abbreviation as in fig. 4.1.

4.3.1.2. *n*-alkanes

Concentration and vertical and horizontal distributions of *n*-.alkanes from C_{14} to C_{35} together with pristane and phytane were determined for the north, *Prestige* and south sites. Total alkane concentrations(ΣALKs) did not show substantial differences between the compared stations and ranged between 5.1-27 ng L^{-1}, 4.8-40.6 ng L^{-1} and 7.3-32.3 ng L^{-1} in the north, *Prestige* and south stations respectively. They agreed with the values described for water column in the Black Sea (3-1500 m), were ΣALK $_{C14-C36}$ values of 3-10 ng L^{-1} were reached (Maldonado et al., 1999), or in North Atlantic waters off the Gibraltar Strait (800-1700 m) with average values of 20.4±12.9 ng L^{-1} in small particles (Martí et al., 2001).

Vertical profiles of ΣALKs (Fig. 4.5) showed a decreasing abundance with depth in all the stations which follows the typical decrease in the SPM abundance in the water column with depth (Martí et al., 2001; Bubba et al., 2004). Maximum concentrations were found at the SW of the Prestige sinking area, which coincided with the trend described for the ΣPAHs in this water body. Otherwise, the distribution of ΣALKs did not follow the profiles described for the PAHs and there has not been found neither an enrichment in abundance in the NADW nor a relative maximum peak in the MW. In fact, with the exception of the SW in the Prestige station, it could not be established a relationship between maximum concentration of ΣPAHs and ΣALKs.

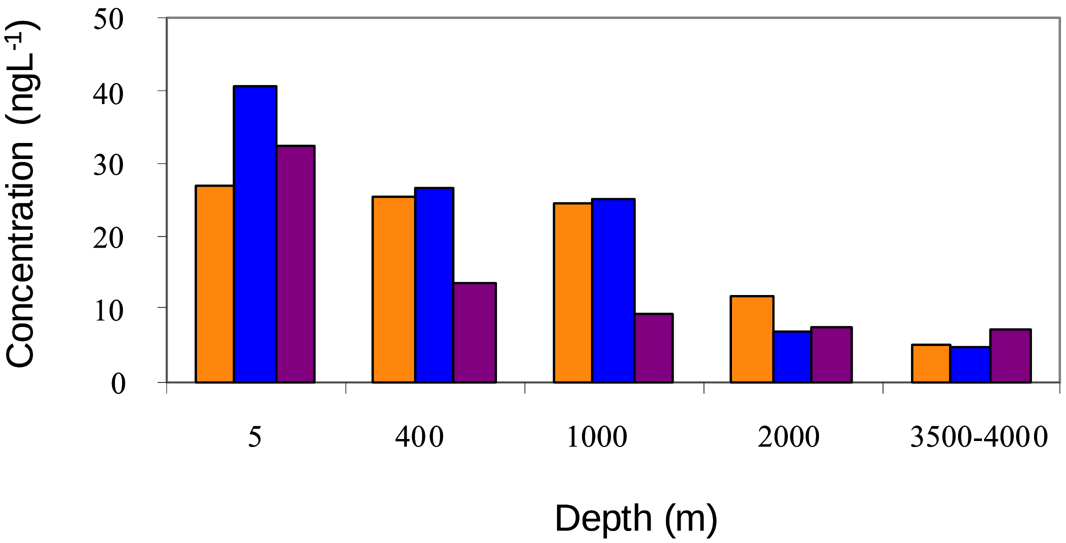


Figure 4.5. Comparison of the concentration of the ΣALKs in the SPM of selected stations in March 2006 . Sampling locations: north station (orange), Prestige station (blue) and south station (purple). ΣALKs: sum of n-alkanes between C_{14} to C_{35} together with Ph and Pr.

Differences in the concentrations were also noted when the sampling stations were compared between them. First of all, ΣALKs abundance was higher in the SW and NADW and lower in the MW in the south station than in the north, opposite to the trend described for the ΣPAHs. In the ENACW and the LSW, on the contrary, the increasing concentration gradient northward described for the PAHs was observed for ΣALKs as well. The Prestige station still displayed the highest abundance of the three, agreeing with that described for PAHs.

Composition of the *n*-alkane mixture also varied with depth and between stations (Fig.s 4.6, 4.7, and 4.8). The most remarkable feature observed was the compositional differences of the mixture between the most superficial and deepest water masses and the mid-depth water masses. In general, the three intermediate water masses (ENACW, MW and LSW) showed more similar individual compound distribution, where the heaviest *n*-alkanes (C_{25}-C_{35}) were predominant. For these water bodies, in the north station, C_{28} and C_{29} were the most abundant alkanes, closely followed by C_{30}. In the Prestige and south stations, the presence of C_{26} was also very noticeable, especially at MW and LSW. This station also showed an exception of the general trend and the *n*-alkane distribution found in the LSW appeared to be closer to the one in the NADW and the SW than the profiles displayed by the ENACW and the MW.

The most superficial and deepest water masses showed a less homogeneous *n*-alkane distribution and clearly differentiated from the previously described water bodies. In the SW, compounds between C_{15}-C_{20} were dominant in the mixture, being the most relevant fraction in the north station. In general, C_{15} and C_{17} dominated the light *n*-alkanes, which are characteristics of phytoplankton lipids (Green et al. 1992). The lightest fraction seemed to gain importance at the deepest samples as well, being C_{16}, C_{17} and C_{18} the most abundant in the north and south sites and C_{14} and C_{16} in the *Prestige* station.

The unresolved complex mixture (UCM) concentrations of aliphatic hydrocarbons in the SPM of the studied locations are showed in table 4.2. The presence of UCM in the SW represented a common trait in the three stations with concentrations between 36.5 ng L^{-1} and 144.6 ng L^{-1}, decreasing from south to north. For the remaining water masses, the profile of the UCM distribution in the *Prestige* station was completely opposite to the one in the north and south. The later two locations were characterized by a lack of UCM in the water column except in the MW. Surprisingly, this water mass was the only one in which no UCM could be detected in the *Prestige* station. In the water column near the wreck UCM concentrations ranged between 12.7 and 47.8 ng L^{-1} and decreased with depth until the NADW, where an icreasing in the abundance was observed. The described levels were lower than the 12.1-742 ng L^{-1} described in the open water of the Black sea (Maldonado et al., 1999), but were within the range of the 48-131 ng L^{-1} found in the small particles in Western Mediterranean (Dachs et al., 1997).

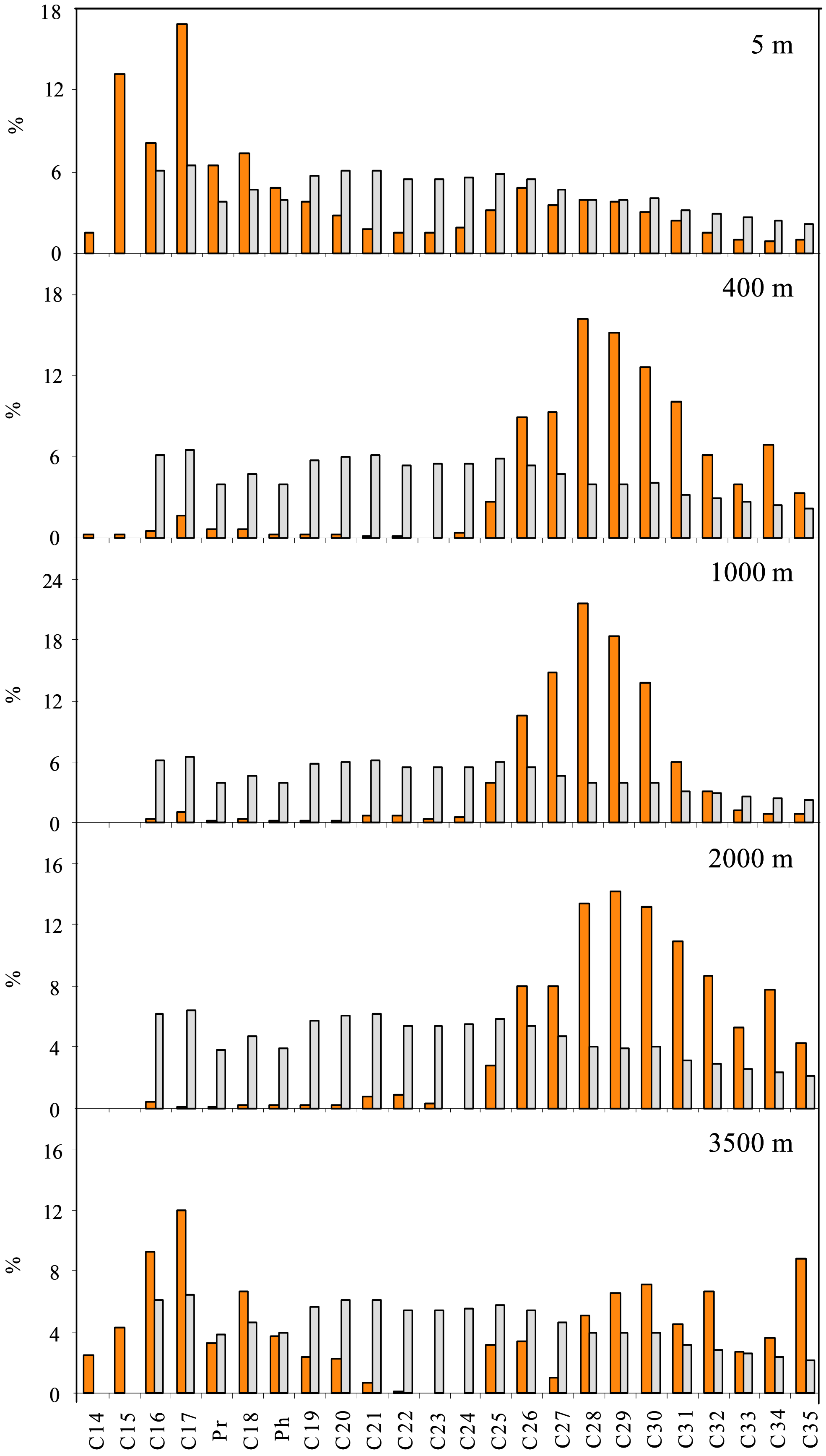


Figure 4.6. Relative abundance of the *n*-alkanes , together with pristane (Pr) and phytane (Ph) in the SPM at the north station (orange) in March 2006 and the fuel oil from the *Prestige* (grey). Sampling depths correspond to the water masses in the area (detailed description in chapter 2).

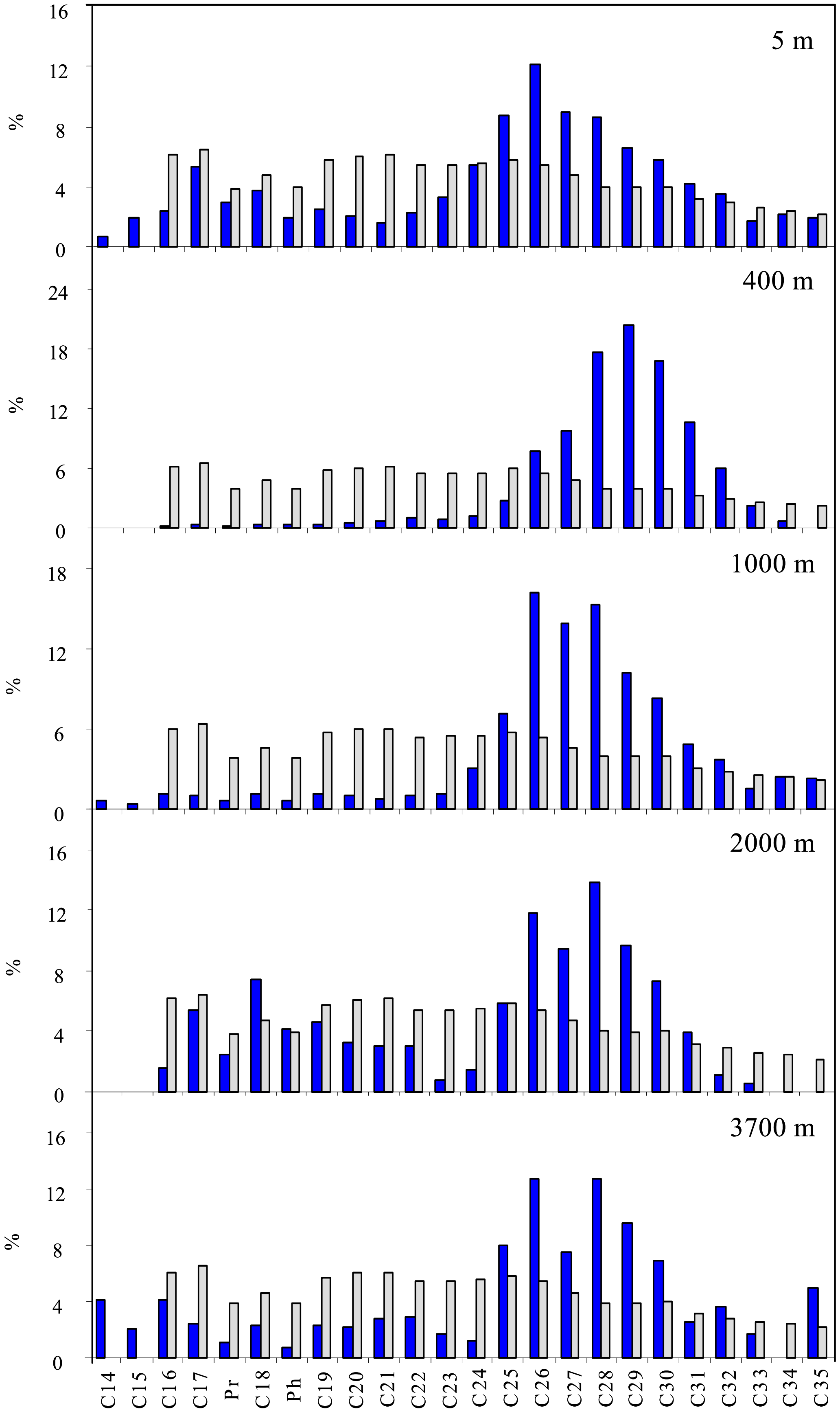


Figure 4.7. Relative abundance of the *n*-alkanes, together with pristane (Pr) and phytane (Ph) in the SPM at the *Prestige* station (blue) in March 2006 and the fuel oil from the *Prestige* (grey). Sampling depths correspond to the water masses in the area (detailed description in chapter 2).

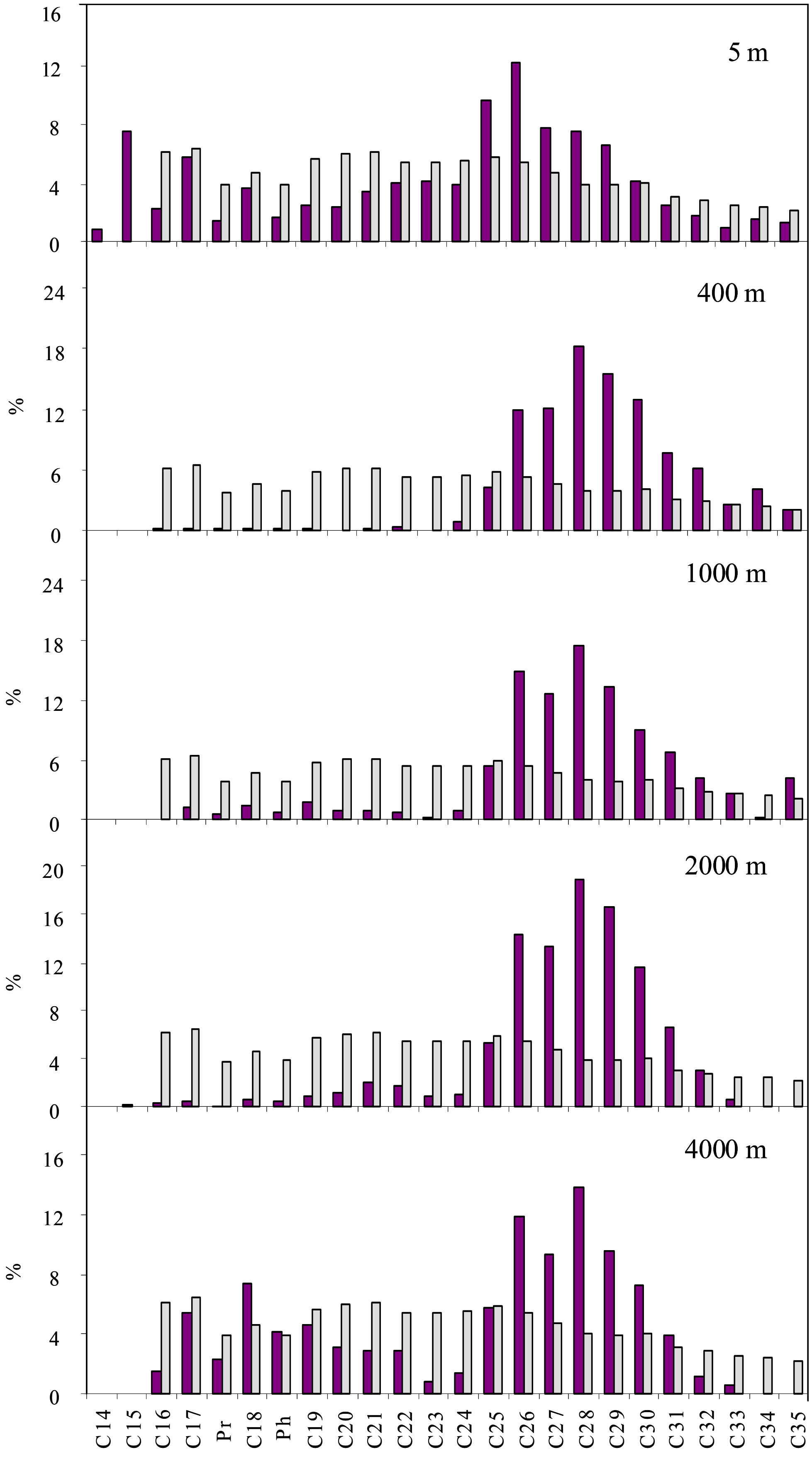


Figure 4.8. Relative abundance of the *n*-alkanes, together with pristane (Pr) and phytane (Ph) in the SPM at the south station (purple) in March 2006 and the fuel oil from the *Prestige* (grey). Sampling depths correspond to the water masses in the area (detailed description in chapter 2).

4.2.2. Origin of the hydrocarbons

One of the most evident facts of the results is the widespread occurrence of PAH and non-biogenic hydrocarbons in relatively high concentration throughout the water column of the three stations. The hydrocarbons levels and individual compound distribution in each sampling site was the result of both local vertical processes and longer range transport of hydrocarbons due to the circulation of water masses with its own pollutant loads. In this aspect, the SW and NADW clearly differed from the mid-depth water masses as can be deduced from *n*-alkane distributions and ΣPAHs concentrations.

Likewise, in the intermediate water masses, the MW carried the highest PAHs load and proportion of particle-bound compounds compared to the other water masses in the remaining water column. This is possibly an inherent feature of this concrete water mass. Thus, the potential of the MW as a source of organic pollutants into the north-eastern Atlantic had been previously described in studies carried out in Atlantic waters off the Gibraltar Strait and Alboran Sea (Martí et al., 2001), were water lenses of Mediterranean origin showed higher average concentration (12.1±9.5 ng L^{-1}) of ΣPAHs in small size particles at mid-depth waters than the North Atlantic waters (6.5±6.7 ng L^{-1}). The unusual increases of ΣPAHs found at the present study can be a reflection of the same phenomenon. The decreasing gradient in concentration northward may be due to the mixing of the MW with relatively less polluted adjacent water masses, which has also been observed previously for nutrient concentration and other parameters (Van Aken, 2000). This fact was confirmed by the decreasing in the salinity northward of the MW in the studied stations (chapter 2, fig. 2.3).

The heterogeneity of hydrocarbon distributions in the water column suggested multiple sources of the pollutants in the area studied. In the open ocean waters one of the most important pathways for the entrance of contaminants is dry atmospheric deposition in the water surface, which generally is characterized by the high contribution of pyrogenic PAHs of 4 or more rings (Wania et al., 1998; Dahle et al., 2003). The dominance of the 2-3 ring compounds in the PAH mixture of the three sampling stations suggested sources derived from petroleum as the most probable anthropogenic input of hydrocarbons in the area, which seemed to be confirmed by the high percentage of alkylated compounds, very abundant in the waters with predominant petrogenic contamination (Lipiatou and Saliot, 1991; Maldonado et al., 1999; Zakaria et al., 2002; Neff et al., 2006). It is known by the time the cruise was undertaken that some leaks had reappeared in the wrecks and some of the remaining oil was being released in the deep ocean. However, due to the lack of dominance of the hydrocarbon concentration in the station nearer to the *Prestige* over the other sites in the SPM, the wrecks are not likely to be the only point source of hydrocarbons at depth. Although there is no evidence to reject the option of the *Prestige* being one of the possible contributors, PAHs contamination in the area can also be consequence of a chronic petrogenic pollution from multiple local or relatively remote sources, or deepspills from other wrecks.

Origin indicators using ratios of PAHs were also calculated to obtain additional information about the origin of the hydrocarbons found in the studied area (table 4.1). Phenanthrene and anthracene are two structural isomers but due to their distinct physico-chemical properties they behave differently in the environment. Phenanthrene is thermodynamically more stable than anthracene, so that high P/A ratios can be related to petrogenic pollution, but relatively low ratios are linked to pyrogenic sources, since combustion processes help the formation of anthracene (Soclo et al., 2000; Culotta et al., 2006). Hence P/A >10 is taken as evidence of the occurrence of petrogenic contamination (De Luca et al., 2005), although this can only be taken as indicative. Thus, the P/A value of the ratio in the Prestige fuel oil is 7.6. Another frequently used source indicator ratio is based on the concentrations of fluoranthene and pyrene. These are considered typical combustion products generated as a consequence of condensation of low molecular weight compounds (Soclo et al., 2000). Similarly, fluoranthene is thermodinamically less stable than pyrene hence in pyrolytical processes the predominance of fluoranthene over pyrene yields Fl/Py values >1 (Qiao et al., 2005). In petroleum derived PAHs pyrene is more abundant and therefore petrogenic pollution is characterized by Fl/Py values <1 (Guinan et al., 2001). The mentioned ratios have been widely used by several author in the assessment of the origin of PAHs from different environments (Budzinski et al., 1997; Baumard et al., 1998; Doong and Lin, 2004).

From the P/A and Fl/Py ratios in the three locations (table 4.1) it could be argued that the petrogenic signature was more evident throughout the water column in the *Prestige* station than in the other two northern and southern sites, and in the surface water mass in the three stations. Conversely, the LSW water mass contains in the three stations a predominant pyrolytic signal rather than petrogenic. One of the possible explanations for this could be the fresher nature of petroleum derived pollution near the wrecks, which would act as a recent source and hydrocarbons would be less affected by dispersive processes than in the other areas. The superficial waters of the other stations also exhibited signs of petrogenic PAHs which would be expected since this water body will be affected by frequent surface oil spills from ships.

Data provided by P/A and Fn/Py ratios did not always completely coincide between the different location for some depths, as in ENACW and MW in the south station and NADW in the north. In this cases the ratios showed in-between values, not petrogenic but either entirely pyrogenic, which reinforced the supposition of the multiple sources of hydrocarbons.

PAH ratios	**P/A**			**Fl/Py**		
Stations	*N*	*P*	*S*	*N*	*P*	*S*
SW (5 m)	11	26	9	0.2	0.3	0.7
ENACW (400 m)	6	15	17	2.2	0.7	1.1
MW (1000 m)	5	19	11	1.8	0.9	1.1
LSW (2000 m)	4	7	6	1.4	1	1.2
NADW (bottom)	10	17	7	1.	0.5	1.1
Prestige fuel oil		*7.6*			*0.22*	

Table 4.1. Selected PAH ratios in the SPM at the different stations and water masses, where P: Phenanthrene , A: Anthracene, Fl: Fluoranthene and Py: Pyrene. Locations: N: north station; P: Prestige station; S: south station. Sampling depths correspond to the water masses in the area (detailed description in chapter 2). In petrogenic sources P/A >10 and Fl/Py values <1.

The distribution of *n*-alkanes showed more clearly the mixed origin of the hydrocarbons found in the three stations. Even though the n-alkane profiles in the superficial and deep water seemed to resemble more closely the profile of the fuel oil carried by the *Prestige*, the distribution found at intermediate depth were completely different. It leads to think that the *Prestige* shipwreck was not the main contributor of hydrocarbons in the area, even in the Prestige station, especially when the signature of *Prestige* could be clearly noted for all the water masses in October 2006 (see chapter 3). Besides, *n*-alkanes of sea algae origin (C_{16} and C_{18}) (Green et al., 1992) were found to be very abundant in the hydrocarbons mixture of the SW and NADW, and the low correlation between alkanes and UCM observed for the study area (R^2= 0.29) has been previously associated to the biogenic character of the *n*-alkanes in other studies (Maldonado et al., 1999).

The odd-to-even carbon number ratio (table 4.2) was analysed to obtain additional information about the origin of the *n*-alkanes. The values around 1 of this ratio indicate petrogenic contamination, while results different from 1 indicate biogenic origin, such as plants or marine algae (odd to even ratio >1) and pelagic species (odd to even ratio <1) (Bubba et al., 2004). With exception of the superficial waters of the north and south stations, field sampled displayed an odd to even ratio <1, which would point open sea organisms as origin of the hydrocarbons (Green et al., 1992). Values between 1.2-1.4 for this ratio have been previously found in Antarctic marine waters 300 km offshore and long-range transport of aerosols containing terrestrial high plan hydrocarbons have been suggested as a possible source. Seeing as the samples which exhibited values >1 were the most superficial, aeolian deposition is a plausible explanation in the SW (Bubba et al., 2004).

	UCM (ng L^{-1})			**Odd-to-even**		
Stations	N	P	S	N	P	S
SW (5 m)	36.5	47.8	144.6	1.4	1	1.2
ENACW (400 m)	BDL	34.2	BDL	0.9	0.9	0.8
MW (1000 m)	43.1	BDL	35.4	0.9	0.8	1
LSW (2000 m)	BDL	12.7	BDL	0.9	0.8	0.9
NADW (bottom)	BDL	29.5	BDL	1	0.9	0.8
Prestige fuel oil					1	

Table 4.2. Selected ratio/parameters corresponding to n-alkanes determined in the SPM the different stations .Locations: N: north station; P: Prestige station; S: south station. Sampling depths correspond to the water masses in the area (detailed description in chapter 2). UCM: Unresolved complex mixture. BDL: Below detection limit.

On the other hand, the Pr/Ph ratio, also used to determine the origin of the hydrocarbon contamination, exhibited a relatively low value in all of the sampling sites and depths (<2.29), which would suggest the presence of petrogenic origin hydrocarbons (Venkatesan et al., 1980; Shaw et al., 1985). Similarly to the observations made for the PAHs, the general distribution of the *n*-alkanes in the different water masses and origin diagnostic ratios seemed to suggest mixed, both petrogenic and biogenic, origin of hydrocarbons.

4.3. Conclusions

Concentration and distribution of hydrocarbon in the water column of the *Prestige* sinking area and two other locations, one in the north and the other in the south were studied in order to ascertain if the Prestige wreck was the main source of hydrocarbons in the area and determine the role of the different water masses in the dispersion of pollutants.

The studied stations showed a wide contamination by hydrocarbons that extended to the whole water column, although the distribution profiles and diagnostic ratios showed both biogenic and anthropogenic origins. The petrogenic input was determined to be the most important anthropogenic hydrocarbon source of the area. However, there was not sufficient evidence to consider the *Prestige* wrecks the main source of petrogenic pollution in March 2006. Alternatively, the area seems to be affected by a chronic petrogenic contamination from multiple sources.

The water masses in the area have been shown to actively transport contamination far from the sources and be able to carry signatures of the contamination from their source region. Anyway, the high hydrodynamic variability of the open ocean waters near the Galician Bank which translates in the seasonal change of the direction of water masses and local turbulent phenomenon, makes it difficult to determine the influence of the circulation of the water masses in the area in the hydrocarbon distribution described in this study.

4.4. References

Albaigés, J., B. Morales-Nin, Vilas, F., 2006. The Prestige oil spill: A scientific response. Mar. Pollut. Bull. 53, 205-207.

Baumard, P., Budzinski, H., Garrigues, P., Sorbe, J.C., Burgeot, T., Belloq, J., 1998. Concentrations of PAHs (polycyclic aromatic hydrocarbons) in various marine organisms in relation of those in sediments and to trophic level. Mar. Pollut. Bull. 36, 951-960.

Bubba, M., Cincinelli, A., Checchini, L., Lepri, L., Desideri, P., 2004. Horizontal and vertical distributions of biogenic and anthropogenic organic compounds in the Ross Sea (Antarctica). Intern. J. Environ. Anal. Chem. 84, 441–456.

Budzinski, H., Jones, I., Belloq, J., Pierard, C., Garrigues, P., 1997. Evaluation of sediments contamination by polycyclic aromatic hydrocarbons in the Gironde stuary. Mar. Chem. 58, 85-97.

Culotta, L., De Stefano, C., Gianguzza, A., Mannino, M.R., Oreccgio, S., 2006. The PAH composition of surface sediments from Stagnone coastal lagoon, Marsala (Italy). Mar. Chem. 99, 117-127.

Dachs, J., Bayona, J.M.. , Raoux, C., Albaigés, J., 1997. Spatial, vertical distribution and budget of polycyclic aromaric hydrocarbons in the western Mediterranean seawater. Environ. Sci. Technol. 31, 682-688.

Dahle, S., Savinov, V.M., Matishov, M.M., Evenset, A., Naes, K., 2003. Polycyclic aromatic hydrocarbons (PAHs) in the bottom sediments of the Kara Sea shelf, Gulf of Ob and Yenisey Bay. Sci. Total Environ. 306, 57-71.

De Luca, G., Furesi, A., Micera, G., Panzanelli, A., Piu, P.C., Pilo, M.I., Spano, N., Sanna, G., 2005. Nature, distribution and origin of polycyclic aromatic hydrocarbons

(PAHs) in the sediments of Olbia harbor (Northern Sardinia, Italy). Mar. Pollut. Bull. 50. 1223-1232.

Doong, R, Lin, Y., 2004. Characterization and distribution of polycyclic aromatic hydrocarbon contaminations in surface sediment and water from Gao-ping River, Taiwan. Water Res. 38, 1733-1744.

Elordui-Zapatarietxe, S., Albaigés, J., Rosell-Melé, A., 2008. Fast preparation of the seawater accomodated fraction of the heavy fuel oil by sonication. Chemosphere 73, 1811-1816.

Garzas-Gil, M. D., Prada-Blanco, A., Vázquez-Rodríguez, M.X., 2006. Estimating the short-term economic damages from the Prestige oil spill in the Galician fisheries and tourism. Ecol. Econ. 58, 842-849.

González, J. J., Viñas, L., Franco, M.A., Fumega, J., Soriano, J.A., Grueiro, G., Muniategui, S., López-Mahía, P., Prada, D., Bayona, J.M., Alzaga, R., Albaigés, J., 2006. Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the Prestige oil spill. Mar. Pollut. Bull. 53, 250-259.

Green, G., Skerratt, J.H., Leeming, R., Nichols, P.D., 1992. Hydrocarbons and coprostanol levels in seawater, sea-ice algae, and sediments near Davis Station in eastern Antarctica: A regional survey and preliminary results for a field fuel spill experiment. Mar. Pollut. Bull. 25, 293-302.

Guinan, J., Charlesworth, M., Service, M, oliver, T., 2001. Sources and geochemical constraints of polycyclic aromatic hydrocarbons (PAHs) in sediments and mussels of two Northern Irish-Sea loughs. Mar. Pollut. Bull. 42, 1073-1081.

Jurado, E., Zaldívar, J.M., Marinov, D., Dachs, J., 2007. Fate of persistent organic pollutants in the water column. Does turbulent mixing matter?. Mar. Pollut. Bull. 54, 441-451.

Lipiatou , E., Saliot, A., 1991. Fluxes and transport of anthropogenic and natural polycyclic aromatic hydrocarbons in the western Maditerranean sea. Mar. Chem, 32, 51-71.

Lipiatou, E., Tolosa, I., Simó, R., Bouloubassi, I., Dachs, J., Marti, S., Sicre, M.A., Bayona, J.M., Grimalt, J.O., Saliot, A., Albaiges, J., 1997. Mass budget and dynamics of polycyclic aromatic hydrocarbons in the Mediterranean Sea. Deep-Sea Res, Pt. II 44, 881-905.

Lohmann, R., Jurado, E., Pilson, M.E., Dachs, J., 2006. Oceanic deep water formation as a sink of persisten organic pollutants. Geophys. Res. Lett. 33, L12607.

Lohmann, R., Breivik, K., Dachs, J., Muir, D., 2007. Fate of POPs: Current and future research directions. Environ. Pollut. 150, 150-165.

Maldonado, C., J. M. Bayona, J.M., Bodineau, L., 1999. Sources, distribution, and water column processes of aliphatic and polycyclic aromatic hydrocarbons in the northwestern Black Sea water. Environ. Sci. Technol. 33, 2693-2702.

Martí, S., Bayona, J.M, Albaigés, J., 2001. A potential source of organic pollutants into the Northeastern Atlantic: the outflow of the Mediterranean deep-lying waters through the Gibraltar Strait. Environ.Sci.Techol. 35, 2682-2689.

Neff, J. M., Johnsen, S., Frost, T.K., Utvik, T.I., Durell, G.S., 2006. Oil well produced water discharges to the North Sea. Part II: Comparison of deployed mussels (Mytilus edulis) and the DREAM model to predict ecological risk. Mar. Environ. Res. 62, 224-246.

OSPAR, 2004. OSPAR/ICES Workshop on the evaluation and update of the background reference concentrations (BCRs) and ecotoxicological assessment criteria (EACs). Available from: www.ospar.org.

Qiao, M., Wang, C., Huang, S., Wang, D., Wang, Z., 2005. Composition, sources and potential toxicological significance of PAHs in the surface sediments of the Meiliang Bay, Taihu Lake, China. Environ.l Int. 32, 28-33.

Sánchez, F., Velasco, F., Cartes, J.E., Olaso, I., Preciado, I., Fanelli, E., Serrano, A., Gutierrez-Zabala, J.L., 2006. Monitoring the Prestige oil spill impacts on some key species of the Northern Iberian shelf. Mar, Pollut. Bull. 53, 332-349.

Schulz-Bull, D. E., Petrick, G., Bruhn, R., Duinker, J.C., 1998. Chlorobiphenyls (PCB) and PAHs in water masses of the northern North Atlantic. Mar. Chem. 61, 101-114.

Serrano, A., Sánchez, F., Preciado, I., Parra, S., Frutos, I., 2006. Spatial and temporal changes in benthic communities of the Galician continental shelf after the Prestige oil spill. Mar. Pollut. Bull. 53, 315-331.

Shaw, D.G., Hogan, T.E., McIntosh, D.J., 1985. Hydrocarbons ins the sediments of Port Valdez, Alaska: Consequences of five years' permitted discharge.. Estuarine Coastal Shelf Sci. 21, 131-144.

Soclo, H.H., Garrigues, P.H., Ewald, M., 2000. Origin of polycyclic aromatic hydrocarbons (PAHs) in coastal marine sediments: case studies in Cotonou (Benin) and Aquitaine (France) areas. Mar. Pollut. Bull. 40, 387-396.

Tronczyski, J., Munschy, C., Héas-Moisan, K., Guiot, N., Truquet, I., Olivier,N., Men,S., Furaut, A., 2004. Contamination of the Bay of Biscay by polycyclic aromatic hydrocarbons (PAHs) following the T/V "*Erika*" oil spill. Aquat. Living Resour. 17, 243-259

Utvik, R.T. I., Durell, G.S., Johnsen, S., 1999. Determining produced water originating polycyclic aromatic hydrocarbons in North Sea waters: Comparison of sampling techniques. Mar. Pollut. Bull. 38, 977-989.

Van Aken, H.M., 2000. The hydrography of the mid-latitude northeast Atlantic Ocean I: The deep water masses. Deep-Sea Res. Pt. I 47, 757-788.

Varela, R. A., Rosón, G., Herrera, J.L., Torres-López, S., Fernández-Romero, A., 2005. A general view of the hydrographic and dynamical patterns of the Rías Baixas adjacent sea area. J. Mar. Sys. 54, 97-113.

Venkatesan, M.I., Brenner, S., Ruth, E., Bonilla, J., Kaplan, I.R., 1980. Hydrocarbons in age-dated sediment cores for two basins in the Southern California Bight. Geochim. Cosmochim. Acta 44, 789-802.

Wania, F., Axelman, J., Broman, D., 1998. A review of processes involved in the exchange of persistent organic pollutants across the air-sea interface. Environ. Pollut. 12, 3-23.

Xie, W., Shiu, W., McCay, D., 1997. A review of the effect of salts on the solubility of organic compounds in seawater. Mar. Envir. Res. 44, 429-444.

Zakaria, MP., Takada, H., Tsutsumi, S., Ohno, K., Yamada, J., Kouno, E., Kumata, H., 2002. Distribution of polycyclic aromatic hydrocarbons (PAHs) in rivers and estuaries in Malaysia: a widespread input of petrogenic PAHs. Environ. Sci. Technol. 36, 1907-1918.

CHAPTER 5

Fast preparation of the seawater accommodated fraction of heavy fuel oil by sonication

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5.1. Introduction

The seawater accommodated fraction (SWAF) of a crude oil is a mixture mainly composed by light polycyclic aromatic hydrocarbons (PAHs), phenols and heterocyclic compounds containing nitrogen and sulphur (Saeed and Al-Mutairi, 2000). Several of these PAHs are known to be neurotoxic, mutagenic and carcinogenic (Khan et al. 1995; Fernandez et al., 2006). Since the SWAF is the fraction which is more readily bioavailable soon after an oil spill, it has been widely used for the assessment of the toxicity of the oils in different living organisms, such as crustaceans (Maki et al., 2001; Martinez-Jeronimo et al., 2005), fish (Akaishi et al., 2004) and microbiota (Ohwada et al., 2003). The SWAF can also produce long term effects in areas that are not directly affected by the spill (Navas et al., 2006).

The preparation in the laboratory of the SWAF is usually carried out by gently stirring the oil and seawater by means of a low energy mixing system to avoid the formation of oil in water emulsions (Ali et al., 1995; Rayburn et al, 1996; Ziolli and Jardim, 2002). Consequently, the procedure is slow, taking several days for the concentration of the SWAF to reach a steady state (Hokstad et al., 1999; Page et al., 2000). Moreover, the preparation of replicates of the SWAF is tedious and time consuming. On the other hand, in the assessment studies of the toxicological effects on biota, it is convenient to prepare the SWAF rapidly, as it is not possible to add a biocide to the water to avoid the onset of bacterial activity after 24 h (Singer et al., 2000).

The final composition of the SWAF depends chiefly on parameters such as oil-water ratio, stirring and settling time, salinity and temperature (Ziolli and Jardim, 2002; Martínez-Jerónimo et al., 2005). Given that there is not a common procedure for its preparation the results from different authors are difficult to compare (Singer et al., 2000). Therefore, it is not easy to assess, for instance, how oceanic water masses properties may affect the formation and composition of the SWAF in different spill conditions. For example, in the incident of the *Prestige* tanker tens of thousands of tonnes of heavy fuel oil were released from the wreck at more than 3,500 m water depth (Albaigés et al., 2006). On its way towards the surface, the oil had to cross up to five water masses with different temperature and salinity conditions (Ruiz-Villarreal et al., 2006). Consequently, the concentration and composition of the SWAF in each water mass was likely to be different.

In this chapter we propose a simple, fast and reproducible method for the preparation of SWAF. We apply a high energy mixing system, using an ultrasonic bath, but avoiding the formation of oil-water emulsions. The method is appraised by studying the changes in the concentration of PAHs in the SWAF of a heavy fuel oil in some of the salinity and temperature conditions commonly found in the North Atlantic Ocean, in the area of the incident of the *Prestige* tanker.

5.2. Materials and methods

5.2.1. Fuel oil and seawater

The fuel oil employed was a marine fuel oil IFO 380, with a density of 0.981 kg L^{-1} at 15 ºC, provided by the Coordination Technical Bureau from the Scientific Intervention Program against Accidental Marine Spills (Vigo, Spain) in April 2005. It was similar in its physicochemical properties to that carried by the *Prestige* tanker.

Natural seawater was obtained from the Gulf of Biscay (33.3 psu; Cantabrian Sea) and from the Mediterranean Sea (37.7 psu). The salinity was measured using a YSI FT Model 556 conductimeter (YSI, Ohio, USA). The seawater was sterilized by adding $HgCl_2$ and filtrated before use through a precleaned glass fibre filter (0.7 µm, Ø 47 mm, APFF type, Millipore, Ireland) to remove suspended particulate material. To determine background levels of hydrocarbons in the natural SWAF, three aliquots of 400 mL from each water type were extracted with the same procedure used to analyze the SWAF, as described in the next section. The background PAH concentrations were subtracted from those found in the SWAF samples prepared in the laboratory.

The effect of temperature in the dissolution of fuel oil was appraised at two temperatures, i.e. 20 ºC (i.e. coded high temperature or HT in the text and figures) and 3 ºC (i.e. low temperature or LT). These temperatures were chosen as representative of the values of the surface and bottom water masses in the sinking area of the *Prestige* tanker, in the North Eastern Atlantic, 150 nautical miles offshore from the Spanish coast (Ruiz-Villarreal et al., 2006).

The effect of salinity was studied using natural seawater from the Gulf of Biscay (low salinity or LS), and from the Mediterranean Sea (high salinity or HS). The salinity of both of them was measured directly in the storing tanks, before and after the experiments to monitor any changes due to evaporation.

5.2.2. Preparation of SWAF

The dissolution apparatus (Fig. 5.1) was adapted from Ali et al. (1995). Seawater (1L) was poured into a 1.5 L volume glass flask (94 mm x 200 mmL). Two PTFE tubs (0.7 mm I.D. x 500 mmL) were inserted in the cap, one of them kept over the surface of the seawater to blow nitrogen, and the other used for the collection of water samples inserted deep into the seawater. Fuel was added in a 1:500 (v/v) oil to water ratio, close to the surface of the seawater by means of a stainless steel spatula. The surface area to volume ratio was 0.03 and the headspace represented the 33% of the volume. The cap on the flask was sealed with PTFE film first, and then with plastic film. All the apparatus was placed carefully in an ultrasonic bath, sonicated for 30 min with an energy of 360 W and left to settle down at a constant temperature.

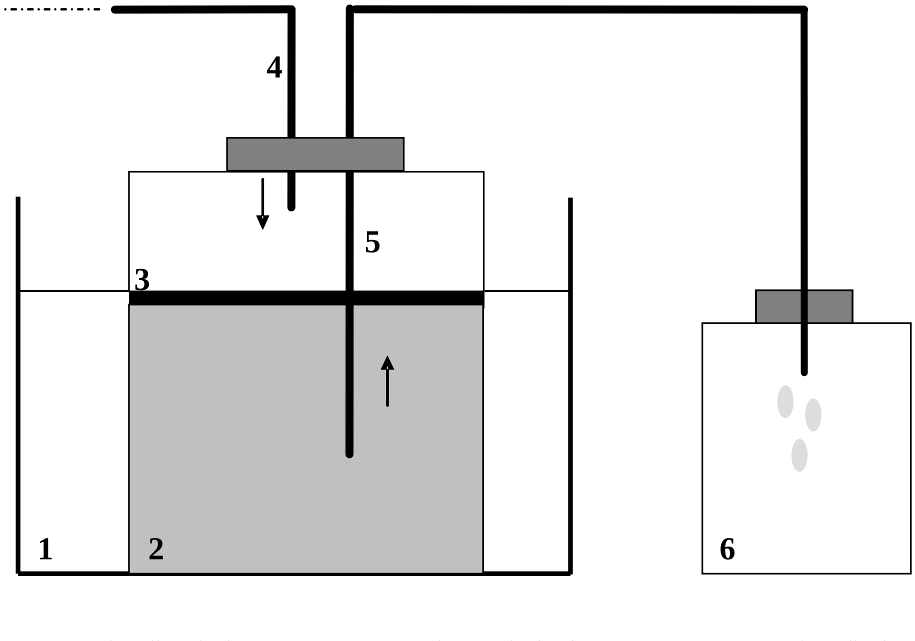

Figure 5.1. The dissolution apparatus. 1: Ultrasonic bath; 2: SWAF preparation flask; 3: Oil slick; 4: tube for N_2 application; 5: Tube for sample retrieval; 6: Sample recovery jar.

The apparatus was covered with aluminium foil to minimize the photodegradation of the fuel oil during the experiment. To maintain the sonication conditions reproducible, the location of the flask and the water level in the ultrasonic bath were exactly the same in all the experiments. Emulsions did not form as long as the flasks did not vibrate substantially in the bath. Cork plates were used to avoid direct contact between the flasks and the bath walls.

The retrieval of the water samples was carried out by applying a gentle stream of nitrogen through the tube over the seawater surface, while SWAF aliquots were collected in a clean glass flask through the tube inserted in the bottom. Special attention was paid not to disturb the water surface during this process to avoid dispersion of the oil.

The temperature was controlled mainly at two different stages of the SWAF preparation. First of all, natural seawater in the flasks, and distilled water filling the ultrasonic bath was added at the corresponding temperature at the beginning of each preparation experiment. Some of the seawater was simply stored in closed tanks in the laboratory at room temperature, maintained at 20 ± 2 ºC, while water at 3 ºC was obtained using a refrigeration system. This parameter was also controlled during the equilibration time. Half of the replicas were left to equilibrate at 3 ºC, and the other half at 20 ºC. The temperature of the water in the ultrasonic bath was measured before and after stirring and a maximum increment of 2 ºC was observed.

Four experiments (at two different temperatures and salinities) were carried out in triplicate. In each type of experiment two identical sets were prepared for different purposes. In the first one, between 1 and 3 mL aliquots of water were collected at 0, 24, 48, 72, 96 and 120 h to monitor the progress of the oil dissolution by fluorescence analysis. In the second, 400 mL of water were collected after 24 h for the identification and quantification of individual PAHs.

The significance of the different experimental effects were confirmed using a one way ANOVA ($p<0.05$ for all the preparation parameters) and several post-hoc tests (Tuckey HSD and Bonferroni), to make pairwise comparison of the average concentration and appraise which factor had the strongest influence in the dissolution of fuel oil in the seawater.

In parallel, another experiment was prepared where the fuel was added to the water without sonication, in order to assess the differences in the solubility of the fuel caused by the present method. The experiment was performed in triplicate under HT and HS conditions. Besides the oil-water mixing, the rest of the process was followed exactly as in the sonication experiments.

5.2.3. Characterization of PAHs in sea water and fuel oil

Sea water (400 mL) was filtered through a Durapore membrane (0.22 µm and Ø 47 mm, Millipore) in order to eliminate the particulate bulk oil material generated when using high energy stirring systems (Singer et al., 2000). The filtrated sea water was poured into a separatory glass funnel, spiked with a solution of anthracene-d_{10}, and extracted three times with 50 mL of dichloromethane (Suprasolv, Merck, Germany). The combined extracts were passed through a glass column filled with cotton wool and 7 g of dried Na_2SO_4 (>99%, Merck) to eliminate residual seawater, and concentrated in a rotary evaporator to 1mL, followed by a gentle stream of nitrogen, avoiding complete removal of the solvent.

The PAH fraction from the fuel oil was isolated using a glass column (30 cm x 1 cm) packed with 6 g of silica (bottom) (SiO_2, 40-60 mesh, Acros Organics, Belgium), 6 g of aluminium oxide (middle) (Al_2O_3, 70-230 mesh, Merck, Germany) and 2 g of sodium sulphate (top), in hexane, as described in Alzaga et al. (2003). Between 10 and 20 mg of the oil sample was dissolved in hexane, spiked with a solution of anthracene-d_{10} (Acros Organics, Belgium) and pyrene (Sigma-Aldrich, USA) in isooctane and added at the top of the column. The aliphatic hydrocarbons were eluted in the first fraction with 17 mL of hexane (Suprasolv, Merck), and the PAHs with 20 mL of hexane:dichloromethane (2:1, v/v). The recovered fractions were concentrated in a rotary evaporator, followed by a gentle stream of nitrogen until near dryness, redissolved with isooctane and spiked with a solution of thiphenylamine (Sigma-Aldrich) before further analysis by gas chromatography-mass spectrometry (GC/MS).

Quantification of the PAHs was carried out in a Konik HRGC 4000B gas chromatograph coupled to a Konik MS Q12 mass spectrometer (Konik, Sant Cugat del Vallès, Spain). The GC was fitted with a fused silica capillary column (30 m x 0.25 mm I.D. x 0.25 µm film thickness) DB5 MS (Agilent, Santa Clara, USA). The initial column temperature was held for 1 min at 70 ºC, then programmed to 320 ºC at a rate of 6 ºC min^{-1} and kept at this temperature for 10 min. Helium was used as carrier gas at a constant flow of 1.5 mL min^{-1}. The injection was made in the split/splitless mode (splitless time: 1 min), keeping the injector temperature at 300 ºC. Data were acquired in the selective ion monitoring (SIM) mode at a 70 eV and processed by the Konikrom Data Reduction software. Quantification was performed calculating the response factors for each compound at different concentrations, correcting the values with the internal standards. A solution of 17 PAHs containing acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]flouranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, dibenzo[*a,h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, naphthalene, perylene, phenanthrene and pyrene were used for response factors calculation (Dr. Ehrenstorfer,Germany).

5.2.4. Spectrofluorimetric analysis of the SWAF

The spectrofluorimetric analysis is a very sensitive technique largely used for the measurement of oil in water (Ali et al., 1995; Gonzalez et al., 2006). Even though the results are dependent of the calibration and the oil composition, it provides a useful tool for rapid monitoring of total aromatic hydrocarbons in water. Therefore, the progress of the dissolution experiment was followed by measuring the fluorescence directly in the water phase using a Surveyor Thermo-Finnigan (Waltham, USA) high performance liquid chromatograph (HPLC), coupled to a SpectraSystem FL3000 fluorescence detector. The system was operated in the off-column mode, by-passing the chromatographic column. Milli-Q water was used as mobile phase at a flow rate of 1 mL min^{-1}. Any dilutions of the sea water were made with deionised water (Milli-Q, Millipore) to keep the detector signal within the linear range of the instrument. Excitation and emission wavelengths were at 254 and 320 nm, respectively, as they coincide closely with the excitation/emission profiles of the naphthalene derivatives (Groner et al., 2001).

Diesel oil solutions (between 1.2 and 4.5 µg L^{-1} equivalents diesel oil) were tested for the calibration of the detector, as reported elsewhere (Ali et al., 1995), because it contains low molecular weigh aromatic hydrocarbons similar to the ones in the SWAF of the fuel oil. A stock solution was prepared in acetone and subsequent dilutions in milli-Q water were made until the desired concentration was reached. The detection limit (DL) was calculated with the formula $DL=Y_B+3SD$ (Eurachem, 1998), where Y_B and SD where the mean signal and standard deviation of the blank, respectively. It was 0.3 µg L^{-1} of diesel equivalents.

5.3. Results and discussion

5.3.1. Solubility of the total aromatic hydrocarbons

A summary of the results for all the fuel oil-water accommodation experiments is shown in Fig. 5.2 (as µg L^{-1} diesel equivalents of dissolved hydrocarbons). As it can be seen in all the experimental set ups, after sonication of the water/fuel oil mixture is completed, the concentration of the soluble fraction increases markedly during the first 24h of settling time. From then onwards the concentrations of total aromatic hydrocarbons only show a slight relative increase so that they can be considered, in practice, constant in their average value.

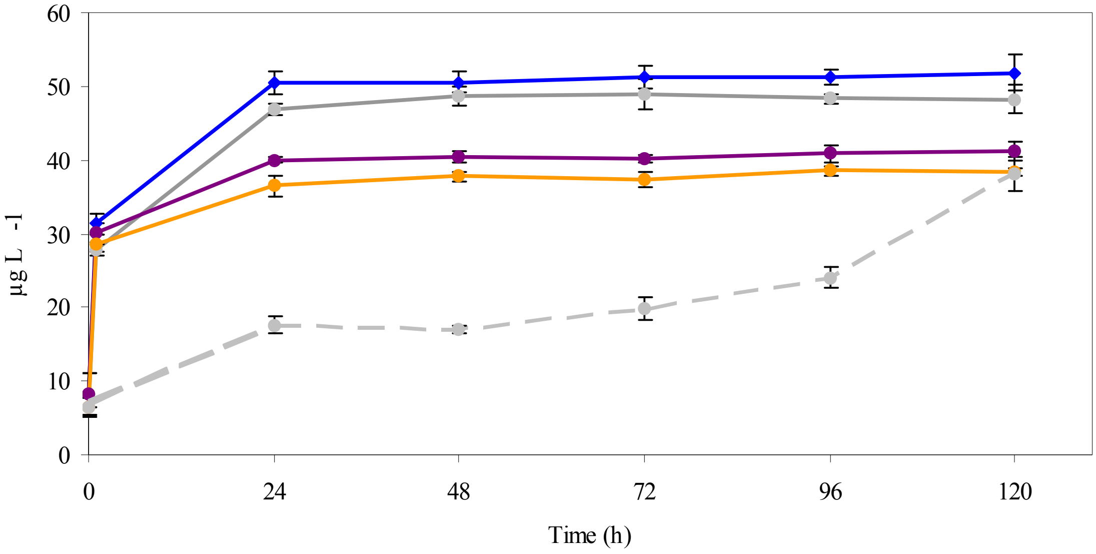

Figure 5.2. Average concentration in µg L^{-1} diesel equivalents (n=3) of the total aromatic hydrocarbons of IFO380 fuel oil in seawater with settling time, prepared at different experimental conditions: a) with sonication, HTLS (solid blue), HTHS (solid grey), LTLS (solid purple), LTHS (solid orange), and without sonication HTHS (dotted grey). * means that significantly differ at $p<0.05$.

In fact, some of the changes in the concentration after 24h can be attributed to the fact that all aliquots were taken from the same preparation flask, producing a small change in the fuel oil to water ratio and a subsequent slight increase of the concentration of total aromatic hydrocarbons in the SWAF. Thus, we conducted an additional set of six experiments under the same experimental conditions for sampling each preparation flask at a different settling time. In this case, no change in the fuel oil to water ratio occurs, and thus no rise in the concentration of SWAF was observed after 96 and 120 h (Fig. 5.3). Three independent replicas were performed for each set of experimental conditions, to check the reproducibility of the proposed sonication method. The RSD of all the series ranged from 1% to 5% (n=3), which indicates that ultrasonic mixing is a reproducible method for SWAF preparation.

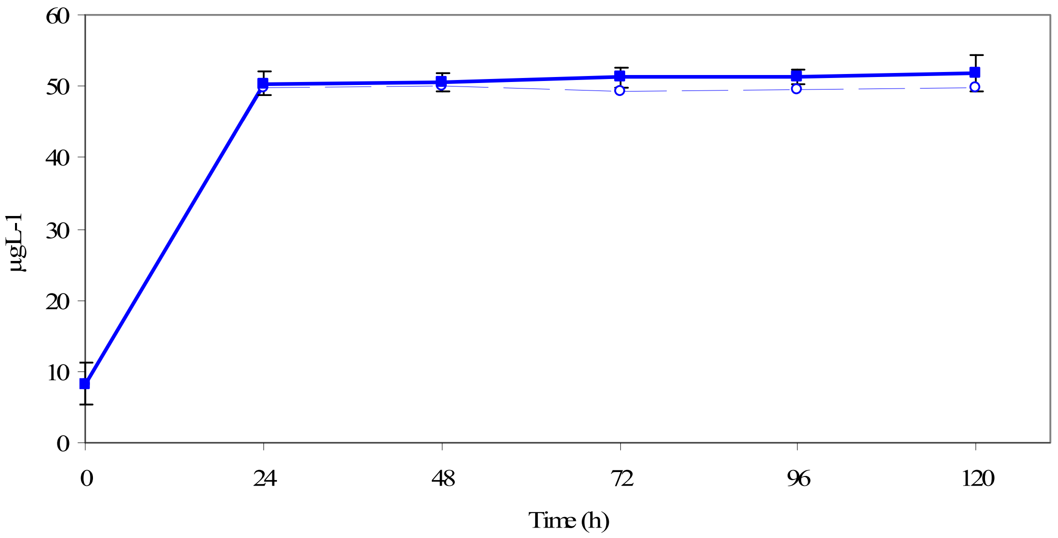

Figure 5.3. Average concentration in µg L^{-1} diesel equivalents (n=3) of the total aromatic hydrocarbons of IFO380 fuel oil in seawater with settling time, prepared with sonication (HTLS) and collection of aliquots from the same flask (solid line) or independent flasks (dotted line)

The improvement in the speed of the process provided by the proposed method can be observed when the results are compared with the ones obtained in the experiment without sonication (Fig. 5.2, grey). The total aromatic hydrocarbons in the water after 24 h reaches to 62% of the assumed equilibrium concentration (48.3 µg mL^{-1} equivalents of diesel) that was not attained until after 96 h.

From Fig. 2 it is apparent that the maximum average concentration of the total aromatic hydrocarbons in the SWAF is influenced by both the temperature and salinity of the seawater. Differences were shown to be statistically significant for all the treatments by ANOVA test ($p<0.01$) and confirmed by HSD-Tukey and Bonferroni tests ($p<0.05$ for all cases). However, the time required to reach the maximum concentration seems to be independent of these conditions, and was found to be 24h after sonication was concluded. Therefore, the whole SWAF preparation process using an ultrasonic bath can be completed in little more than 24 h, so that it is suitable for both, chemical analysis and toxicological studies of the SWAF. This system also offers some technical advantages compared to magnetic or vortex mixing when the preparation of several replicas is required. The classical stirring methods require the availability of a number of stirring devices whereas with the same ultrasonic bath as many as wanted replicas can be prepared continuously.

The present experiments have shown that the solubility of the aromatic hydrocarbons increases as the temperature increases and salinity decreases (May and Miller., 1981; Schwazenbach et al, 2003). Thus, the higher concentrations in the SWAF are obtained at the highest temperature and lowest salinity, and vice versa. In the range of conditions used, the temperature of the seawater has a larger effect on the solubility of the aromatic hydrocarbons than salinity. While the concentration in the SWAF increases an average of 27% over a temperature range of 3- 20 ºC, only an 8% difference was observed from the more to the less saline seawater. These results are consistent with those found previously in the laboratory and the field (Whitehouse, 1984), where two to five fold increase was observed in the solubility of PAHs when the temperature was risen from 5 to 30 ºC. The effect of salinity is even lower, at most by a factor of two when the salinity changed from 36 to 0 psu (May and Miller., 1981; Readman et al., 1982).

5.3.2. Solubility of individual PAHs

The SWAF of the fuel used in the experiments showed a compound distribution consistent with this type of product (e.g. Barron et al., 1999; Saeed and Al-Mutairi, 2000) (Fig. 5.4). The most abundant components were two and three ring PAHs and nitrogen heterocycles, which represented 94% of the total concentration of hydrocarbons in the SWAF. Alkanes and PAHs of four or more rings were only found at trace levels (Ali et al., 1995; Saeed and Al-Mutairi, 2000).

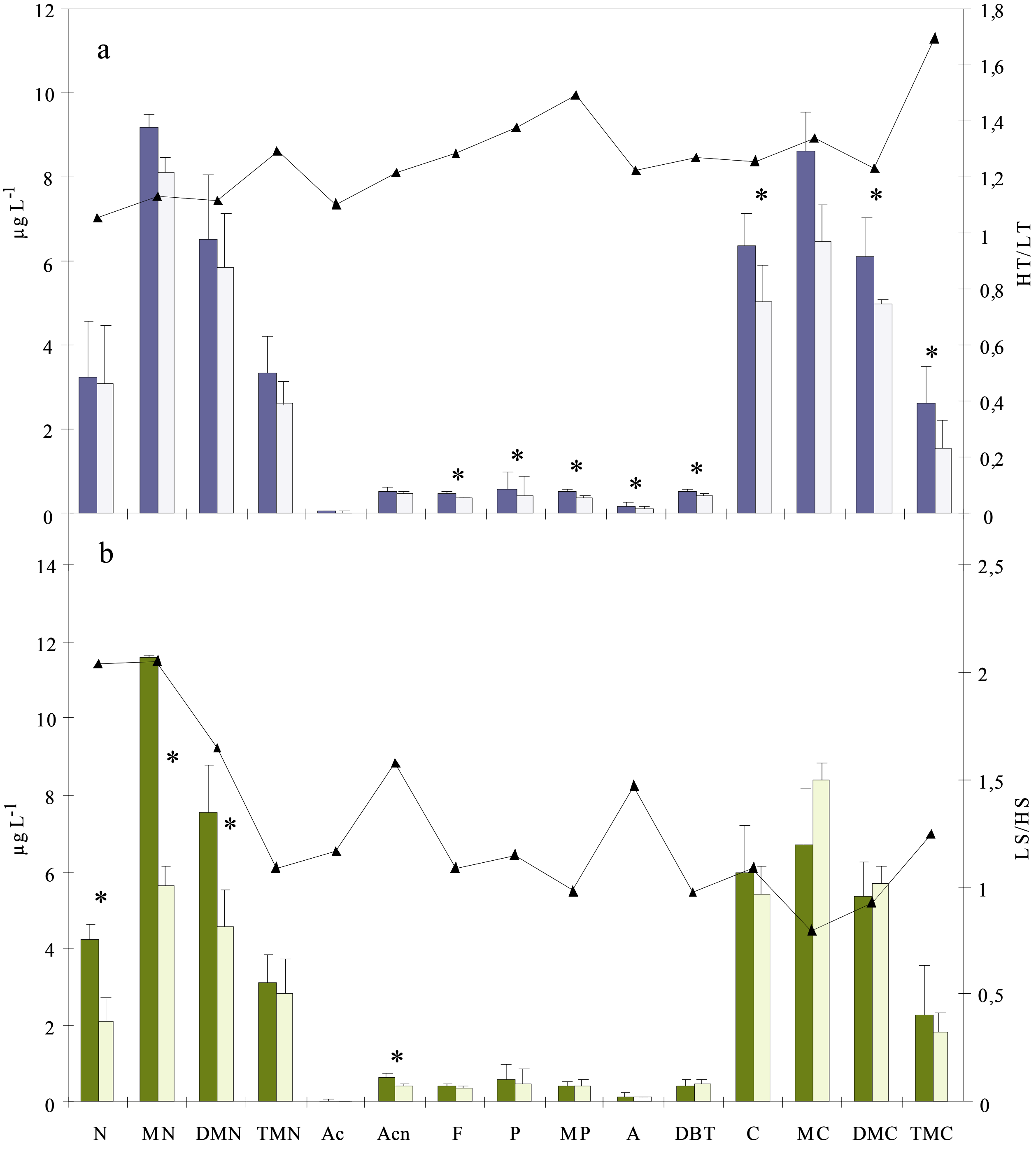

Figure 5.4. Individual compound concentrations (bars) and relative difference (lines) under different SWAF preparation conditions: a) temperature (HT in dark blue and LT in light blue), b) salinity (LS dark green and HS light green). N: Naphthalene; MN: Methylnaphthalenes; DMN: Dimethylnaphthalenes; TMN: Trimethylnaphthalenes; Ac: Acenaphthylene; Acn: Acenaphthene; F: Fluorene; P: Phenanthrene; MP: Methylphenanthrenes; A: Anthracene; DBT: Dibenzothiophene; C: Carbazole; MC: Methylcarbazole; DMC: Dimethylcarbazole; TMC: Trimethylcarbazole. * values significantly different at $p<0.05$.

Naphthalene and its alkyl derivatives represent between 86 and 90 % of the total concentration of PAHs, which agrees with the proportion (89%) reported in tests carried out with similar fuel oils (González et al., 2006, Saeed and Al-Mutairi, 2000). Carbazole and its alkyl derivatives are as abundant as the family of naphthalenes despite the fact that they are relatively much less abundant in the fuel oil studied. This is the result of their higher water solubility (Kraak et al, 1997). As shown in Table 1, the concentration of carbazole in the SWAF is between 71.1 to 89.4% relative to their concentration in the original fuel oil. On the other hand, the solubility decreases with increasing alkylation (Dimitriou et al., 2003), since alkyl groups contribute to the hydrophobicity of the molecule (Schwarzenbach et al., 2003). A similar pattern is observed by the group of naphthalenes and carbazoles, but more evident in the case of the latter.

	HT	**LT**	**HS**	**LS**
Naphthalene	1.5	1.4	0.9	1.9
Methylnaphthalene	0.9	0.8	0.6	1.16
Dimethylnaphthalene	0.4	0.3	0.3	0.4
Trimethylnaphthalene	0.3	0.2	0.2	0.3
Carbazole	89.4	71.1	76.5	83.8
Methylcarbazole	12.3	9.2	12.0	9.6
Dimethylcarbazole	10.3	8.3	9.6	8.9
Trimethylcarbazole	3.3	1.9	2.3	2.9

Table 5.1. Percentage of individual compounds of the fuel oil dissolved in the seawater after ultrasonic stirring, relative to their original concentration in the oil, at different preparation conditions. See Fig. 5.3 for abbreviations.

Both naphthalene and carbazole families are the focus of toxicological concern in marine oil spills and produced waters, not just for their direct action but due to their potential to generate carcinogenic and toxic metabolites in marine organisms (Wilson et al., 1997; Wiegman et al., 1999).

The concentrations of individual compounds in the SWAF varied as a function of the experimental conditions (Fig.5.4, Table 5.1), following over time the patterns observed for the total aromatic hydrocarbons. Generally, the dissolution of individual PAHs in seawater increases as temperature increased and salinity decreased (Schlautman et al., 2004; Tremblay et al., 2005; Viamajala et al., 2007), but the solubility varies for each compound (Fig. 5.4).

Temperature and salinity show uneven influence in the dissolution of the individual PAHs in the seawater at the experimental conditions of the study.
As it can be observed in Fig. 5.4, even though all the compounds follow the general trend of more abundance at higher temperature, this effect is more pronounced for the heaviest compounds of the SWAF, such as fluorene, phenanthrene, methylphenanthrene, anthracene and dibenzothiophene, exhibiting statistically significant differences (ANOVA test, $p<0.05$). On the contrary, the influence of salinity is more noticeable for the lighter compounds, such as naphthalene and its alkylated derivatives, which decreased even 2- fold when the seawater salinity raised from 33 to 37 psu. In contrast, 3-ring PAHs show little change in solubility at the two different salinities investigated, a trend observed previously in solubility experiments involving phenanthene and fluorene, where a very slight decrease in their dissolution was observed when the water salinity increased from 0 to 33 psu (Whitehouse, 1984). This can be explained by the "salting out" effect (Schwarzenbach et al., 2003).

This effect can be also observed comparing the variation of total PAHs and *N*-heterocycles abundance according to the preparation conditions. The total PAHs concentration in the aqueous phase reached 25 µg L^{-1} at 20 ºC and 22 µg L^{-1} at 3 ºC. These concentrations are lower than some found in previous laboratory experiments (67-174 µg L^{-1}) with different fresh fuel oils at several loadings (Hokstad et al., 1999). However, a large range in the PAHs concentrations (ΣPAHs=171-2176 µg L^{-1}) and distributions of the SWAF of different types of a Kuwaiti crude oil has been reported, demonstrating that a great variability exists even within the same type of product (Saeed and Al-Mutairi, 2000).

The preceding experiments indicate that a fractionation of the fuel oil may occur in the ocean, depending on the salinity and temperature characteristics of the water masses in contact with the product. Based on the results obtained in this study, higher concentrations of PAHs and *N*-heterocycles should be found in the warmest and less saline waters of the water column. Nevertheless, the factors controlling the abundance of these compounds in the dissolved phase of the water masses are not limited to salinity and temperature. There exists important factor such as the quantity of dissolved organic matter, the type and quantity of suspended particulate matter, and the type of ions dissolved in the water that also need to be assessed (Xie et al., 1997; Tremblay et al., 2005).

5.4. Conclusions

We have evaluated a simple system, using a sonication bath, to speed-up the process of producing oil sea water accommodated fractions (SWAF) in the laboratory. We have shown that these can be reproducibly obtained in 24 h, regardless of the experimental conditions (water temperature and salinity), as long as some basic precautions for avoiding the formation of emulsions are adopted, namely excessive vibration of the flask in the sonication bath.

The tests conducted in a heavy fuel oil similar to that carried by the *Prestige* tanker have shown that naphthalene and its alkyl derivatives and *N*-heterocycles of the family of carbazoles were the most abundant hydrocarbons present in the SWAF. Both temperature and salinity affect to some extent the dissolution of the fuel oil in the seawater. The concentration of the total aromatic hydrocarbons in the SWAF increases with the increase of water temperature and the decrease of salinity. Individual PAHs follow the same pattern. Changes in temperature usually found in the open ocean are bound to have a much larger impact in the concentration of PAHs in the SWAF than the corresponding values of sea water salinity. In summary, our laboratory results show that after a spill the highest SWAF should be expected in the warmest and less saline waters of the water column.

5.5. References

Akaishi, F. M., H. C. S. de Assis, Jakobi, S.G.C., Eiras-Stofella, D.R., St-Jean, D., Courtenay, S.C., Lima, E.F., Wagener, A.L.R., Scofield, A.L.S., Oliveira-Ribero, C.A., 2004. Morphological and neurotoxicological findings in tropical freshwater fish (*Astyanax* sp.) after waterborne and acute exposure to water soluble fraction (WSF) of crude oil. Arch. Environ. Con. Tox. 46, 244-253.

Albaiges, J., Morales-Nin, B., Vilas F., 2006. The *Prestige* oil spill: A scientific response. Mar. Pollut. Bull. 53, 205-207.

Ali, L. N., Mantoura, R. F. C., Rowland, S., 1995. The dissolution and photodegradation of Kuwaiti crude-oil in seawater.1. Quantitative dissolution and analysis of the seawater-soluble fraction. Mar. Environ. Res. 40, 1-17.

Alzaga, R., Montuori, P., Ortiz, L., Bayona, J.M., Albaiges, J., 2003. Fast-solid phase extraction-gas chromatography-mass sectrometry procedure for oil fingerprinting. Application to the *Prestige* oil spill. J. Chromatogr. A 1025, 133-138.

Barron, M. G., Podrabsky, T., Ogle, S., Ricker, R.W., 1999. Are aromatic hydrocarbons the primary determinant of petroleum toxicity to aquatic organisms?. Aquat. Toxicol. 46, 253-268.

Eurachem, 1998. The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics. In: Eurachem Guide (first edition), Teddington, Middlesex. (www.eurachem.org).

Fernandez, N., Cesar, A., Salamanca, M.J., Del Valls, T.A., 2006. Toxicological characterisation of the aqueous soluble phase of the *Prestige* fuel-oil using the sea-urchin embryo bioassay. Ecotoxicology 15, 593-599.

Gonzalez, J. J., Vinas, L., Franco, M.A., Fumega, J., Soriano, J.A., Grueiro, G., Muniategui, S., Lopez-Mahia, P., Prada. D., Bayona, J.M., Alzaga, R., Albaiges, J.,

2006. Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the *Prestige* oil spill. Mar. Pollut. Bull. 53, 250-259.

Groner, M., Muroski, A.R., Myrick, M.L., 2001. Identification of major water-soluble fluorescent components of some petrochemicals. Mar. Pollut. Bull. 42, 935-941.

Hokstad, J. N., Daling, P.S., Buffagni, M., Johnsen, S., 1999. Chemical and ecotoxicological characterisation of oil-water systems. Spill Sci. Technol. B. 5, 75-80.

Khan, M. A. Q., Alghais, S.M., Al-Marri, S., 1995. Petroleum-hydrocarbons in fish from the Arabian Gulf. Arch. Environ. Con. Tox. 29, 517-522.

Kraak, M. H. S., Ainscough, C., Fernandez, A., van Vlaardingen., P.L.A., Voogt, P., Admiraal, W.A., 1997. Short-term and chronic exposure of the zebra mussel (*Dreissena polymorpha*) to acridine: Effects and metabolism. Aquat. Toxicol. 37, 9-20.

Maki, H., Sasaki, T., Harayama, S., 2001. Photo-oxidation of biodegraded crude oil and toxicity of the photo-oxidized products. Chemosphere 44, 1145-1151.

Martinez-Jeronimo, F., Villasenor, R., Rios, G., Espinosa-Chavez, F., 2005. Toxicity of the crude oil water-soluble fraction and kaolin-adsorbed crude oil on *Daphnia magna* (Crustacea: *Anomopoda*). Arch. Environ. Con. Tox. 48, 444-449.

May, W. E., Miller, M.M., 1981. High-performance liquid-chromatographic methods for determining aqueous solubilities, octanol-water partition-coefficients and ambient-temperature vapor-pressures of hydrophobic compounds. Abstr. Pap. Am. Chem. S. 182(AUG), 35-PEST.

Navas, J. M., Babin, M., Casado, S., Fernanadez, C., Tarazona, J.V., 2006. The *Prestige* oil spill: A laboratory study about the toxicity of the water-soluble fraction of the fuel oil. Mar. Environ. Res. 62, S352-S355.

Ohwada, K., Nishimura, M., Nomura, H., Shibata, A., Okamoto, K., Toyoda, K., Yhoshida, A., Takada, H., Yamada, M., 2003. Study of the effect of water-soluble fractions of heavy-oil on coastal marine organisms using enclosed ecosystems, mesocosms. Mar. Pollut. Bull. 47, 78-84.

Page, C. A., Bonner, J.S., Sumner, P.L., Autenrieth, R., 2000. Solubility of petroleum hydrocarbons in oil/water systems. Mar. Chem. 70, 79-87.

Rayburn, J. R., Glas, P.S., Foss, S., Fisher, W.S., 1996. Characterization of grass shrimp (*Palaemonetes pugio*) embryo toxicity tests using the water soluble fraction of number 2 fuel oil. Mar. Pollut. Bull. 32, 860-866.

Readman, J. W., Mantoura, R.F.C., Rhead, M.M., Brown, L., 1982. Aquatic distribution and heterotrophic degradation of polycyclic aromatic-hydrocarbons (Pah) in the Tamar Estuary. Estuar. Coast. Shelf S. 14, 369-389.

Ruiz-Villarreal, M., Gonzalez-Pola, C., Diaz del Rio, G., Lavin, A., Otero, P., Piedracoba, S., cabanas, J.M., 2006. Oceanographic conditions in North and Northwest Iberia and their influence on the *Prestige* oil spill. Mar. Pollut. Bull. 53, 220-238.

Saeed, T. and Al-Mutairi, M., 2000. Comparative composition of volatile organic compounds in the water-soluble fraction of different crude oils produced in Kuwait. Water Air Soil Poll. 120, 107-119.

Schlautman, M. A., Yim, S.B., Carraway, E.R., Lee, J.L., Herbert, B.E., 2004. Testing a surface tension-based model to predict the salting out of polycyclic aromatic hydrocarbons in model environmental solutions. Water Res. 38, 3331-3339.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., Dieter, M., 2003. Environmental organic chemistry (second edition). John Wiley & Sons, inc., New Jersey.

Singer, M. M., Aurand, D., Bragin, G.E., Clark, J.R., Coelho, G.M., Sowby, M.L., Tjeerderma, R.S., 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. Mar. Pollut. Bull. 40, 1007-1016.

Tremblay, L., Kohl, S.D., Rice, J.A., Gagne, J.P., 2005. Effects of temperature, salinity, and dissolved humic substances on the sorption of polycyclic aromatic hydrocarbons to estuarine particles. Mar. Chem. 96, 21-34.

Viamajala, S., Peyton, B.M., Richards, L.A., Petersen, J.N., 2007. Solubilization, solution equilibria, and biodegradation of PAH's under thermophilic conditions. Chemosphere 66, 1094-1106.

Whitehouse, B. G., 1984. The effects of temperature and salinity on the aqueous solubility of polynuclear aromatic-hydrocarbons. Mar. Chem. 14, 319-332.

Wiegman, S., Van Vlaardingen, P.L.A., Peijnenburg, W.J.G.M., van Beusekom, S.A.M., Kraak , M.H.S., Admiraal, W., 1999. Photokinetics of azaarenes and toxicity of phototransformation products to the marine diatom *Phaeodactylum tricornutum*. Environ. Sci. Technol. 33, 4256-4262.

Wilson, A. S., Davis, C.D., Williams, D.P., Buckpitt, A.R., Pirmohamed, M., Park, B.K., 1997. Characterisation of the toxic metabolite(s) of naphthalene . Toxicology 120, 75-75.

Xie, W. H., Shiu, W.Y., Mackay, D., 1997. A review of the effect of salts on the solubility of organic compounds in seawater. Mar. Environ. Res. 44, 429-444.

Ziolli, R. L., Jardim, W.F., 2002. Operational problems related to the preparation of the seawater soluble fraction of crude oil. J. Environ. Monitor. 4, 138-141.

CHAPTER 6

Phase distribution of hydrocarbons in the water column above the Prestige wrecks and surrounding areas

6.1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are one of the most widespread organic pollutants entering the marine environment (Schulz-Bull et al., 1998; Cincinelli et al., 2008). They are susceptible to long range transport (Jaward et al., 2004) and therefore they are widespread in a global scale (Cao et al., 2005). Since oceans are likely to be the ultimate sink for several organic contaminants (Froescheis et al., 2000), hydrocarbons amongst them, it is essential to understand the processes involved in their distribution in the different biotic and abiotic compartments (Jurado et al., 2007). Even though, unlike most of the persistent organic pollutants (POPs), they have no apparent capacity for bioaccumulation in the food chain (Nfon et al., 2008), several of them are known to be potentially toxic, mutagenic and carcinogenic for human health and damaging for the environment (McCarty et al., 1992; Clemons et al., 1998; Di Toro et al., 2000).

The water column plays a significant role in the organic matter cycle in the marine environment (Lipiatou et al., 1997), and therefore in the fate of any associated contaminants. In aqueous environments, hydrophobicity is the main physico-chemical property governing the distribution of apolar organic pollutants, such as aliphatic and non functionalized aromatic hydrocarbons, between the free dissolved phase (DP) and all types of suspended solid particles (SPM) (Jurado et al., 2007). Their low solubility in water makes them to be preferentially associated to biotic or abiotic particulate matter (Koelmans et al., 1996; Razzaque and Grathwohl, 2008). The impact of the PAHs in the benthic and pelagic organisms and incorporation to the food webs in open ocean waters will be conditioned to a large extent by this phase partitioning (González-Doncel et al., 2008), since only the DP is small enough to pass biological membranes. Conversely, their adsorption to particles decreases their bioavailability (Ohkouchi et al., 1999).

In general, PAHs partition between the SPM and the DP of seawater is known to be influenced by several characteristics of the water masses and the nature of the solid particles. The main factors driving phase partitioning are particle size (Zhou et al., 1999), soot percentage (Buchelli and Gustafsson, 2000), organic carbon amount (Koelmans et al.,1996), dissolved organic matter (Gustafsson and Gschwend, 1997), temperature (Viamajala et al., 2007) and salinity (Xie et al., 1997), amongst others. The resulting partition between phases will determine to a large extent the fate and transport of the hydrocarbons, since they are influenced by environmental processes such as sedimentation, degradation and volatilization (Broman et al., 1991; Leppard et al., 1998; Jaward et al., 2004). Living organisms also play an important role in the modification of the PAHs distribution in the water, since the uptake of these pollutant is not only governed by equilibrium partitioning, but also by particular biological mechanisms, unlike in the case of the more persistent organic contaminants. As a consequence, some planktonic species can alter the expected PAHs levels in the DP and SPM, from physicochemical parameters, (Cailleaud et al., 2007) due to their metabolization (Lotufo 1998; Nfon et al., 2008).

Accidental oil spills constitute an important input of hydrocarbons in the marine environment. The pollution generated can extend throughout the water column and change temporally the background vertical and horizontal distribution of these compounds (Tronczyski et al., 2004). Most of the studies about the distribution and spreading of the oil in the sea are focused in relatively shallow waters or areas near the coast, mostly due to the economic and recreational interest of the affected areas (González et al., 2006; Garzas-Gil, et al., 2006; Serrano et al., 2006). The studies about the distribution of the oil in the open ocean waters, however, are limited, and the vast majority of them focussed to study the effect of a spill in the pelagic/benthic organisms such as plankton and fish (Johansson et al., 1980; Sánchez et al., 2006; Varela et al., 2006). In the accident of the Prestige tanker in 2002, a large part of the cargo, about 42.000 tonnes of M-100 type heavy fuel oil, were spilled after the vessel broke in two and sunk at more than 3,500 m depth, 240 km offshore Galician coast, NW of Spain (Michel et al., 2005; Albaigés et al., 2006).

In previous chapters it has been reported how the wrecks of Prestige have been leaking oil probably for several years after the accident happened (chapter 3). However, in the wider area north and south of the Prestige incident, in the SPM throughout the water column, levels of PAH hydrocarbons from diverse petrogenic and pyrolitic sources reach concentrations within similar orders of magnitude as in the water column above the wrecks (chapter 4).

The general aim of this chapter is to report the distribution of the PAHs in the DP of the seawater for the different water masses present in the sinking area of the Prestige in March and October 2006. The purpose of the study was (i) to determine the distribution of the PAHs between the different water phases during a deep sea spill and compare it to the distributions reported in the literature and in open ocean waters not affected by the deep spill and (ii) to appraise the main parameters affecting the distribution of PAHs in the water column between SPM and DP.

Detailed description of the study area and the methodology are given in chapter 2.

6.2. Results and discussion

6.2.1. Dissolved PAHs in March 2006.

During the oceanographic expedition in March 2006 water column samples were retrieved above the location of the Prestige wrecks, and also in two locations 73 nautical miles north and south from the Prestige sinking location. The SPM data from the three sites was discussed in chapter 4. In this section it is discussed the data of 21 PAHs determined in the DP, namely naphthalene (N), methylnaphthalene (MN), dimethylnaphthalene (DMN), trimethylnaphthalene (TMN), acenaphthylene (Ac), acenaphthene (Acn), fluorene (F), phenanthrene (P); methylphenanthrene (MP), anthracene (A), fluoranthene (Fl), pyrene (Py), chrysene (Chry), benzo[*a*]anthracene (BaA), benzo[*b*]fluorene (BbF), perylene (Per), benzo[*k*]fluoranthene (BkFl), benzo[*a*]pyrene (BaPy), benzo[*g,h,i*]perylene (BghiPer), dibenzo[*a,h*] anthracene (DahA), indeno[*1,2,3,c-d*]pyrene (Ind). Some of them were found bellow detection limit in all the samples (individual concentrations are listed in appendix 1) and therefore were not represented in the figures. The northern and southern stations, as argued in the previous chapter, should provide some insights into the background levels of PAHs in the area near the Galician bank sites, in principle not affected by Prestige oil. Unfortunately, due to operational constrains, the DP phase was not sampled in the LSW of the northern and southern stations.

Vertical distribution profiles of the total concentration of PAHs (ΣPAHs) in the DP in the main water masses of the three sampling stations in March are shown in Fig. 6.1. As observed for the SPM, concentration profiles show overall a common general trend where the maximum concentration of PAHs are found in the surface waters, decreasing at mid-depth and relatively increasing in abundance near the bottom. However, unlike the concentration profiles of ΣPAHs in the SPM, the DP ΣPAHs do not increase in the MW in the southern and Prestige station. Opposite behaviour to the gradients described in the SPM were found when ΣPAHs abundances in the north and south stations were compared, being the concentration in the SW and ENACW higher in the south while in the case of MW ΣPAHs were more abundant in the north. The deepest water mass, the NADW, did not exhibit large differences in ΣPAHs levels between these two locations.

The most striking result is that the concentration of the DP ΣPAHs in the Prestige station was an order of magnitude higher than in the two other sites throughout the water column. This is quite unlike the results of ΣPAHs in the SPM, where all the locations displayed similar ΣPAHs concentrations (chapter 4, Fig. 4.1). ΣPAHs abundance in the DP of the Prestige station ranged from 31.3 to 187.8 ng L^{-1}. These ΣPAHs levels are well above the ones described in previous studies where usually concentrations lower than 1 ng L^{-1} have been reported for the open ocean waters (Lipiatou et al., 1997; Dachs et al., 1997; Schulz-Bull et al., 1998; Maldonado et al., 1999). In fact, the values found at the Prestige station in March 2006 are more similar to the levels found in the DP near the coast after the Erika oil spill (Troncynsky et al., 2004), of around 20.9-139 ng L^{-1}.

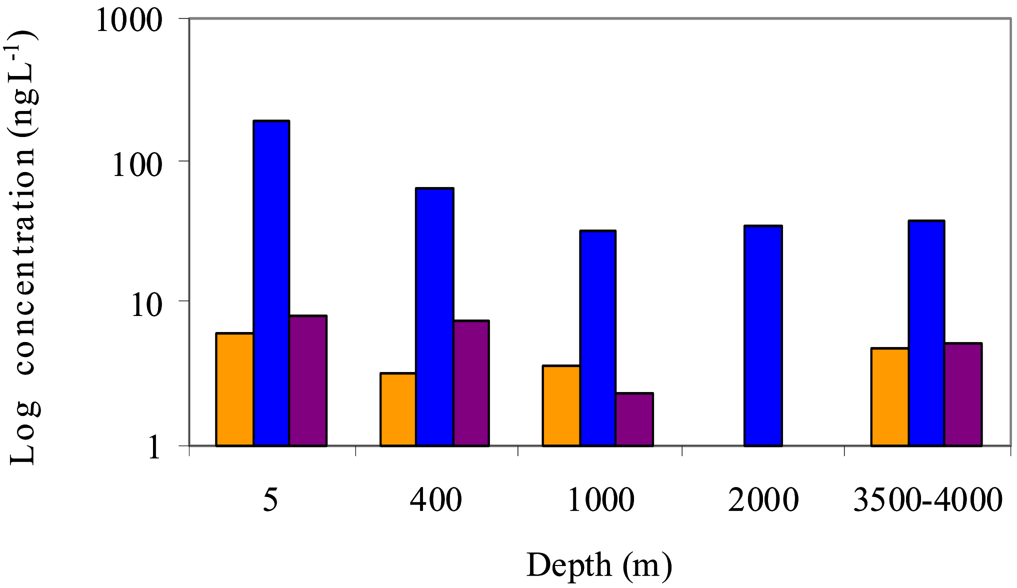

Figure 6.1. Log concentration (ng L^{-1}) of the sum of 21 PAHs (ΣPAHs) in the DP of selected stations at different depths in March 2006. Sampling locations: north station (orange), *Prestige* station (blue) and south station (purple). ΣPAHs: Naphthalene (N); Methylnaphthalenes (MN); Dimethylnaphthalenes (DMN); Trimethylnaphthalenes (TMN); Acenaphthylene (Ac); Acenaphthene (Acn) ; Fluorene (F); Phenanthrene (P); Methylphenanthrenes (MP); Anthracene (A), Fluoranthene (Fl); Pyrene (Py); Chrysene (Chry); Benzo[*a*]anthracene (BaA), Benzo[*b*]fluorene (BbF), Perylene (Per); Benzo[*k*]fluoranthene (BkFl); ; Benzo[*a*]pyrene (BaPy); Benzo[*g,h,i*]perylene (BghiPer); Dibenzo[*a,h*] anthracene (DahA); Indeno[1,2,3,*c-d*]pyrene (Ind). Individual concentrations are listed in appendix 1.

In the north and south stations, ΣPAHs concentrations ranged between 3.1-6.2 and 2.3-8.1 ng L^{-1} respectively. Despite that these values are not as high as those above the wreck they are higher than the concentrations described in the literature for the North Atlantic (5-65 pg L^{-1}) (Schulz Bull et al., 1998), and are close to the DP values reported for polluted waters, such as the 1.8 ng L^{-1} of ΣPAHs found in the DP of ocean sea waters near Barcelona (Dachs et al., 1997), 3-8 ng L^{-1} of the Seine estuary and 3-43 ng L^{-1} of the Chesapeak estuary (Maldonado et al., 1999). Anyway, it must be taken into account that since it is in the sampling step were the most uncertainties are generated, the used sampling method in each study may influence the final results. Whereas the glass microfiber filters are widely accepted to sample the SPM (Font et al., 1996; Michor et al., 1996; Filipkowska et al., 2005), there is no consensus on which is the most reliable methodology to sample DP PAHs from the seawater, and consequently the use of different pre-concentration techniques may lead to some discrepancies in the reported absolute concentration values . Thus, the previously mentioned studies isolated the DP fraction by pumping water using in-situ pumps through a XAD-2 resin. This is an adsorbent which is more efficient in the retention of high molecular PAHs, generally of pyrolitic origin, than the C_{18} disks. In return, lower molecular weight PAHs which are associated with petrogenic sorces, are more efficiently concentrated from seawater with the C_{18} disks compared to the resins, mainly to the smaller diameter of the microporous surface of the silica (Dachs and Bayona, 1997).

Similarly to the SPM, light PAHs between 2 to 4 rings were the most abundant PAHs in the DP mixture, even though overall, the distribution of individual compounds proved to be rather different between the phases and the stations (Fig.s 6.2, 6.3 and 6.4). In general, the most soluble compounds, such as naphthalene and its family, constituted the bulk of the ΣPAHs in the DP, but their importance varied greatly depending on the location. While in the north and Prestige stations this group of compounds represented between 81-97 % of the ΣPAHs, the percentages dropped to 47-78 % in the south. At this last station fluoranthene and pyrene were also very abundant in the PAH mixture, a feature that was not observed in the north and Prestige stations. These two PAHs have been widely found in high concentration in different marine samples (Lipiatou and Saliot, 1991;Schulz-Bull et al., 1998). Likewise, phenanthrene and its alkylated family presented a higher proportion in the PAH mixture for all the depths in the south (13-38% of the total) compared to the north (1.3-15%) and Prestige (2.2-10%) stations.

The abundance of low molecular weight PAHs in the DP, together with the results obtained in the SPM, suggest a dominant petrogenic contamination in the studied locations. In any case, from the data it cannot be concluded that the Prestige was the main hydrocarbon source in the area. Chronic contamination of PAHs, especially naphthalene and its family, has been reported in several areas, such as off the France coast (Tronczynski et al., 2004) and eastern Antarctica (Green et al., 1992). In fact, naphthalene, phenanthrene, fluoranthene, fluorene and pyrene are the most abundant unsubstituted PAHs in the mixture of the background reference concentrations (BCRs) established for the area (OSPAR, 2004), although the reported BCR levels for this compounds were lower than the ones found in this study.

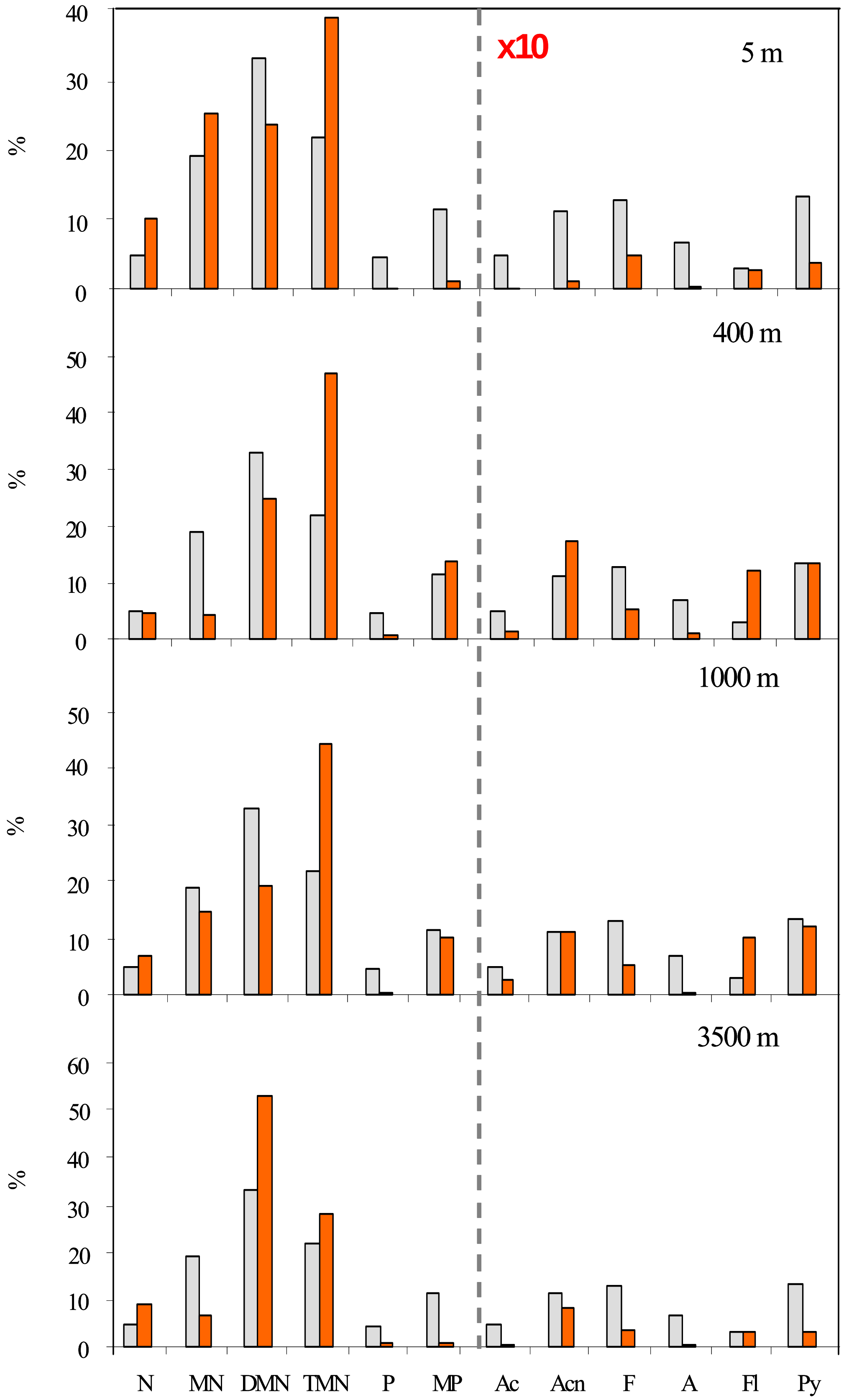

Figure 6.2. Relative abundances of the PAHs in the DP at the north station (orange) in March 2006. and in the fuel oil from the *Prestige* (grey) . Sampling depths correspond to the water masses in the area (detailed description in chapter 2). PAH abbreviations are given in the text.

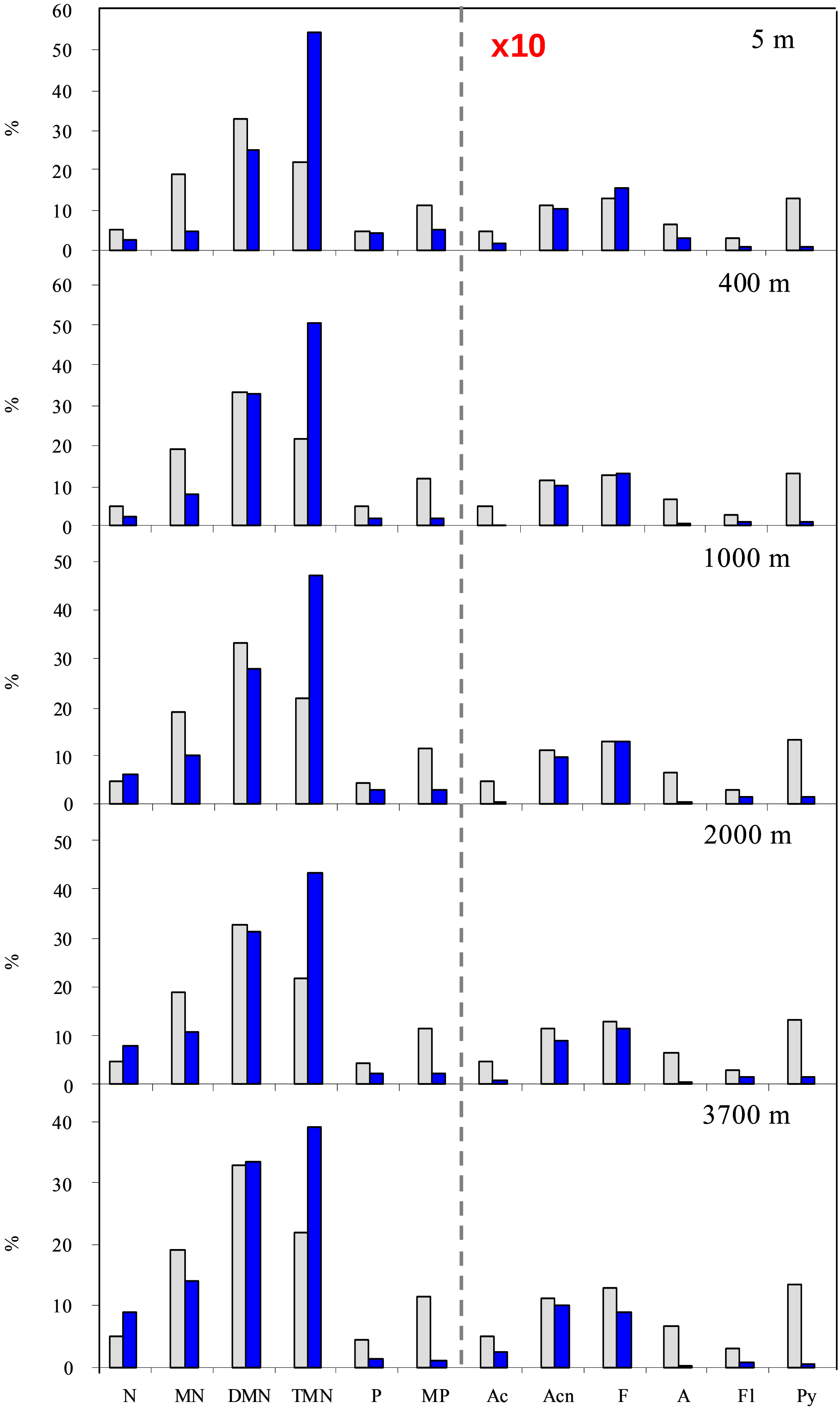

Figure 6.3. Relative abundances of the PAHs in the DP at the *Prestige* station (blue), in March 2006. and in the fuel oil from the *Prestige* (grey) . Sampling depths correspond to the water masses in the area (detailed description in chapter 2). PAH abbreviations are given in the text.

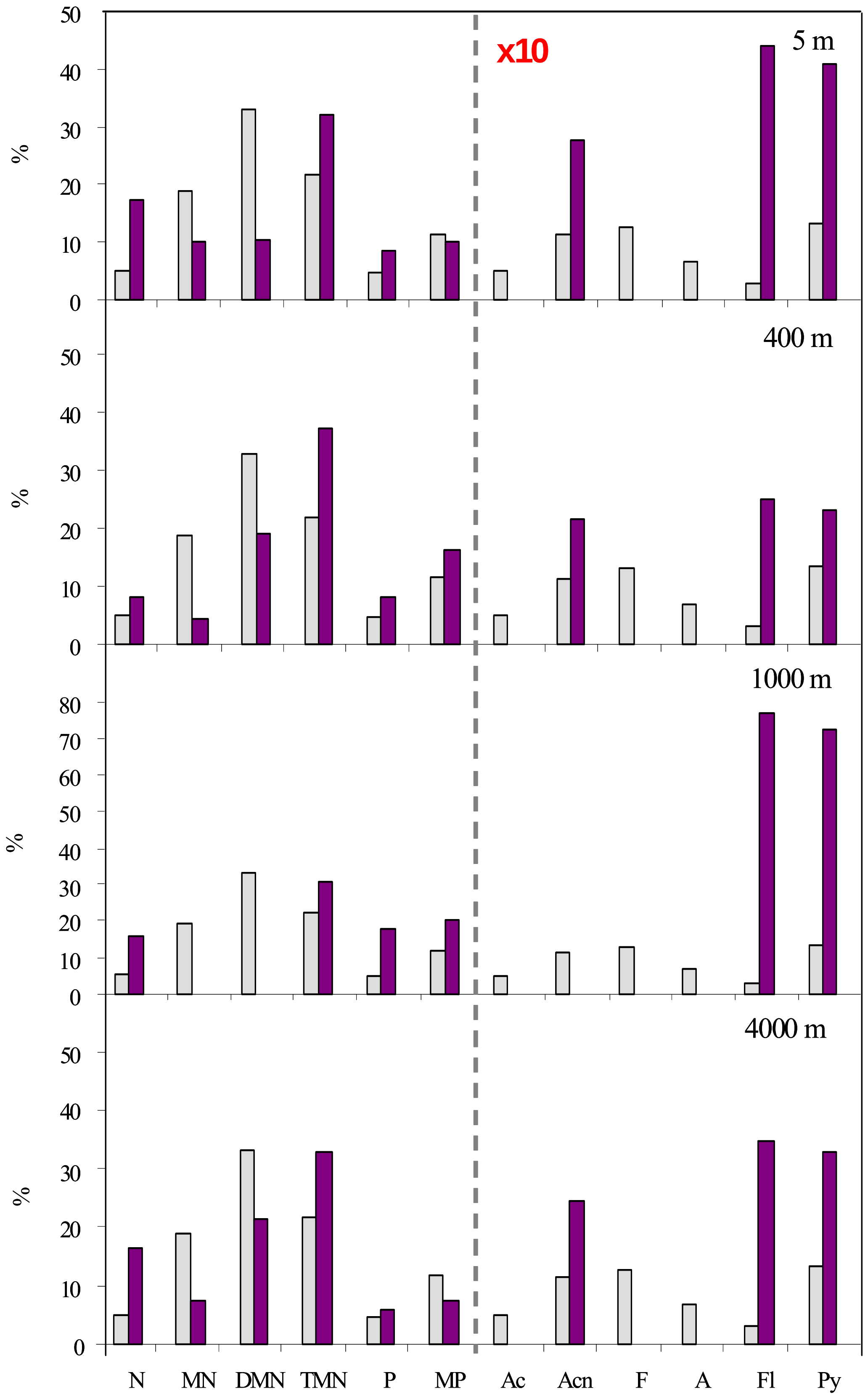

Figure 6.4. Relative abundances of the PAHs in the DP at the south station (purple), in March 2006. and in the fuel oil from the *Prestige* (grey) . Sampling depths correspond to the water masses in the area (detailed description in chapter 2). PAH abbreviations are given in the text.

6.2.2. Dissolved PAHs in October 2006

Vertical distribution profile of the ΣPAHs in the DP above the Prestige tanker wrecks is showed in Fig.6.5. As in March, compounds bellow detection limit are not represented in the figures and individual PAH concentrations are listed in appendix 3. With the exception of the SW, the water mass concentration pattern was very similar to the one observed in the SPM for the same sampling period, which was characterized by a maximum concentration in the deepest water mass, the NADW, and a relative minimum in the MW. On the contrary, the major difference between the two phases resided in the SW, since in the SPM represented the water mass with higher concentration after the NADW, while in the DP displayed the lowest abundance.

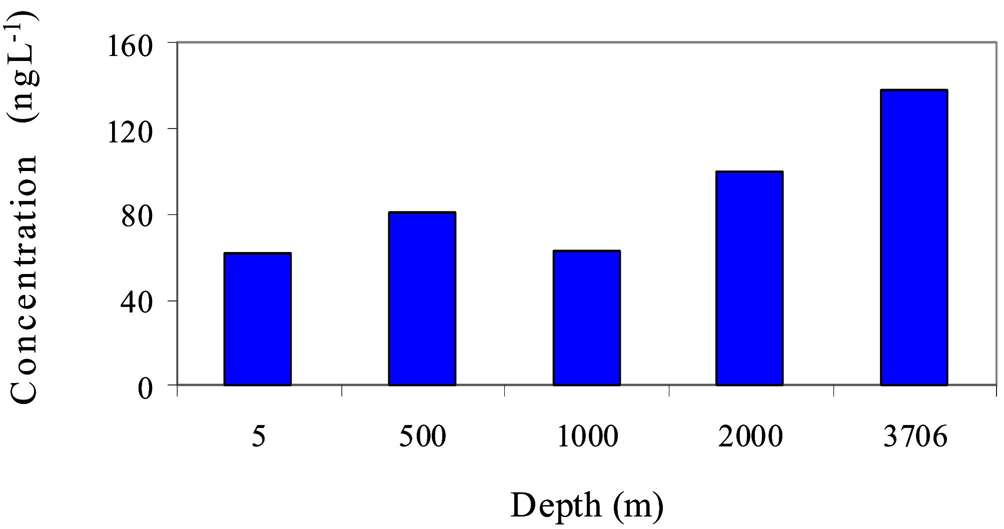

Figure 6.5. Concentration of the sum of 21 PAHs (ΣPAHs) in the DP in the *Prestige* station in October 2006 at the different sampling depths. The list of measured compounds is in fig. 6.1. Individual concentrations are listed in appendix 3

The differences observed in the vertical ΣPAHs concentration patterns in the DP between the two sampling periods were more pronounced. The distribution of the ΣPAHs in the water column in October was nearly opposite to the one found in March, which showed a decreasing tendency with depth after a maximum concentration in the SW. However, the concentration of the ΣPAHs in March and October 2006 were in a similar order of magnitude. In March the ΣPAHs concentration in the DP of the Prestige station ranged from 31.3 to 187.8 ng L^{-1} and in October ranged from 61 to 137 ng L^{-1}. As already mentioned, these values are in concordance with the concentrations reported for the same phase in 2000, in the most heavily impacted shore by the Erika oil spill in France (Tronczynsky et al., 2004). In fact, the abundances of PAH in the DP were overall higher in October than in March for all the water masses except at the surface in the SW. In this water mass the DP PAH values were higher in March than in October.. Nevertheless, the increase in the ΣPAHs concentrations in the water column between March and October for the DP is far from being as large as measured in the SPM (chapter 3, appendix3).

Distribution of the individual PAHs in the water column in October is plotted in Fig. 6.6. As in March, the compounds that mostly contributed to the PAH mixture in the DP of the seawater were naphthalene and its alkyl derivatives. Nonetheless their relative distribution changed compared to that of the SPM in October (chapter 3). The surface, SW and ENACW, and deepest water masses, NADW, showed similar distribution of light PAHS where the concentrations were in the order TMN>DMN>N>MN, whereas the intermediate water masses, MW and LSW, displayed a profile of concentrations where TMN>DMN>MN> N. These differences could not be observed in the PAH distribution in the DP in March, where the distributions of PAHs in the water column were more uniform for all the water masses. The relative importance of the phenanthrenes and methylphenanthrenes was also slightly higher in March, although their presence is still considerable in October.

Unlike in the SPM, most of the 4-5 ring PAHs could not be detected in the DP, with the exception of pyrene and fluoranthene, and in the SW also chrysene and benzo[*a*]anthracene.. Their concentration in the SPM was higher in October than in March, which seems to be reflected at least in the DP of the SW despite their low solunility in saline waters (Schwarzenbach et al., 1993).

Taking into account the petrogenic nature of the PAHs in the SPM in October, together with the confirmation of a deep sea spill at that time, it is reasonable to suppose that the source of hydrocarbons in the DP also have their origin in the Prestige wreck. Thus maximum level observed in the NADW would be consequence of the proximity of the water mass to the source.

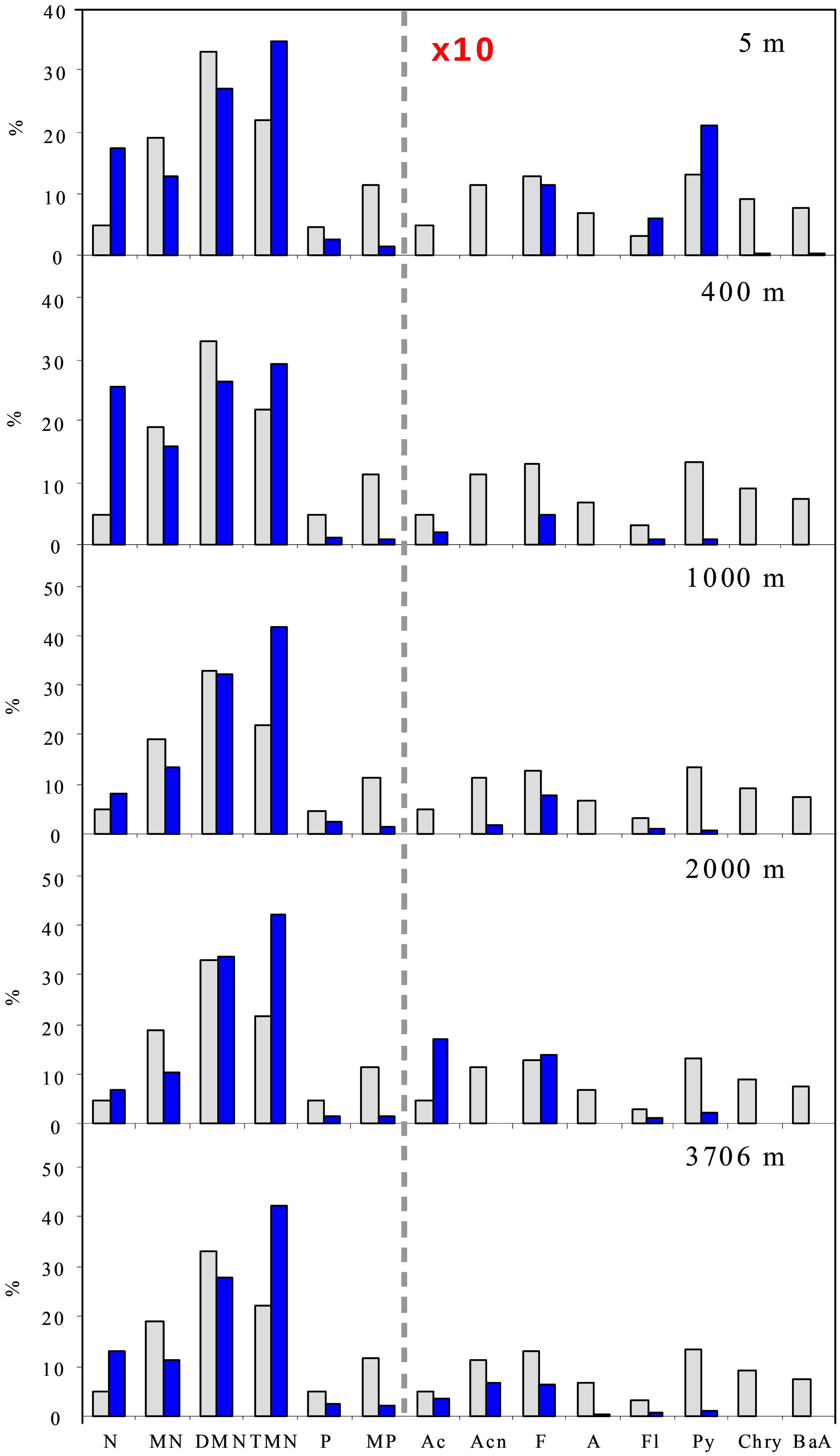

Figure 6.6. Relative abundances of the PAHs in the DP at the *Prestige* station (blue) , in October 2006. and in the fuel oil from the *Prestige* (grey) . Sampling depths correspond to the water masses in the area (detailed description in chapter 2). PAH abbreviations are given in the text.

6.2.3. Distribution between the DP and SPM of the seawater

There are many factors that affect the distribution of the hydrophobic organic compounds in the water column, such as salinity, temperature, and quantity and quality of SPM amongst others, (Zhou et al., 1999) and also transport processes such as diffusion and advection (Gustafsson et al., 1998; Tremblay et al., 2005). The importance of the organic matter in the partition equilibrium is also well accepted (Shi et al., 2007; Razzaque and Gratwohl, 2008), but the influence of the salinity of the seawater is still controversial. While some authors find that the petrogenic PAHs are influenced by salinity (Maldonado et al., 1999), other studies about the effect of this parameter in the estuarine particles suggest that sorption equilibrium is rather insensitive to salinity (Brunk et al., 1997). In theory, the increase in the water salinity is accompanied by a decrease in the solubility of hydrophobic organic contaminants due to salting-out effect. Therefore, when the salinity of the water increases, adsoption of hydrocarbons to SPM and organic phases is enhanced (Schwarzenbach et al., 1993). The laboratory experiments described in chapter 5 to determine the effect of temperature and salinity in the dissolution of the heavy fuel oil in seawater suggested that individual PAHs were affected differently by each parameter (Elordui-Zapatarietxe et al., 2008). The lightest hydrocarbons, especially naphthalene and its alkyl derivatives were more influenced by salinity than temperature. Since light PAHs dominate the mixture found in October 2006, the association to SPM would be expected to be enhanced in the MW, i.e. relatively lower concentration of PAH in the DP to the SPM, the most saline of the body waters. Nonetheless, this tendency in the PAHs phase distribution due to salinity was not found in October. On the contrary a clear change in the DP/SPM distribution is observed in the water column of the south station in March relative to the MW. The increment of the PAHs in the SPM in this water mass compared to the SW, ENACW and NADW could suggest an enhanced adhesion to particles due to salinity. Anyway, this phenomenon is not observed in the Prestige and north stations for the same period, which could mean that either there is a mixing of the MW with the fresher ENACW and LSW when it moves northward, or that salinity is not the principal factor influencing this concrete distribution of PAHs in the south.

Temperature also may not play an important role in the PAHs adsorption to the SPM, since the increment in the concentration observed in the deepest water mass might rather be caused by proximity to the wreck than effect of temperature. It must be taken into account that observations made in the laboratory experiments correspond to equilibrium condition between the oil and the aqueous phase. Thus, is expected that conclusions obtained in the laboratory did not necessarily coincide with the distributions in the field samples, where particles of different origin are present and non-equilibrium conditions may prevail if the spill was relatively recent.

The importance of each phase as a PAH pool differs from site to site in marine waters (Fig. 6.7), even though there is a general agreement that the PAH associated to the particles increases with proximity to the coast, mostly due to the particle input from riverine run-off and land (Gustafsson et al.,1998; Luo et al., 2006). Far from the continent, in open ocean waters, this trend seems to reverse and most of the dissolved/dispersed PAHs apparently tend to be in their dissolved form.

Proportion of PAHs in the different phases also varies between the different oceans and seas, and the PAHs in the DP have been found to represent from the 71% to the vast majority of 98% in theoretically *unpolluted* areas (Fig. 6.7). Nevertheless, there are exceptions to this general pattern, and higher abundance of PAHs in the SPM has also been reported for the North Atlantic (Schulz-Bull et al., 1998).

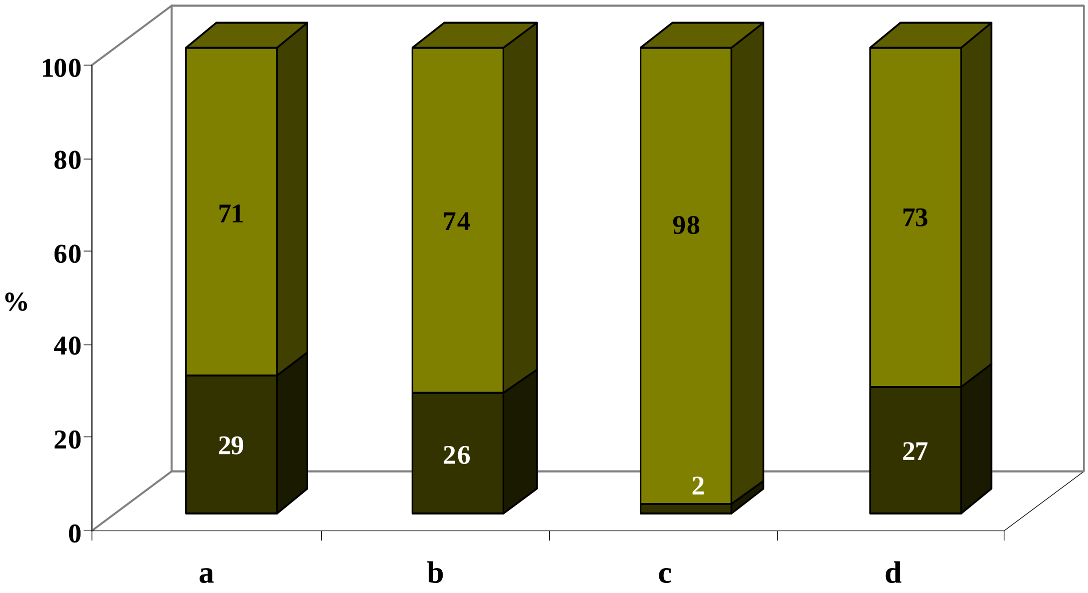

Figure 6.7. PAHs percentage associated to the SPM (dark green) and DP (light green) in marine waters based in data described in the literature. a: Black Sea (Maldonado et al., 1999); b: Mediterranean Sea (Lipiatou et al., 1997); c: North Atlantic (Lipiatou et al., 1997); d: Baltic Sea (Broman et al., 1996). Percentages have been calculated from the data of ΣPAHs described for each phase in the studies.

In order to gather further information about the role of the different water masses in the transport of pollutants, the proportion of PAHs in the DP and SPM was calculated in the water column of the studies sites for the two sampling periods. Overall, there existed a dominance of dissolved PAHs over the particle-bound PAHs in all the stations in March (Fig. 6.8), although the importance of each phase varied depending on the sampling site and water depth. This partition agreed with previous studies of the distribution of PAHs in the open ocean waters, where higher abundances were found in the DP (Lipiatou et al., 1997; Maldonado et al., 1999) mostly due to the input of solid particles on the high seas is more limited (Payne et al., 2003; Luo et al., 2006).

The Prestige station showed the largest fraction of the ΣPAHs concentration in the DP (97-99%) over SPM of the three sites and the distribution was maintained fairly uniform throughout the water column. Distributions in the south were quite similar to the ones in the Prestige station, being the PAH percentages in the DP for the SW, ENACW and

NADW only 4-5% lower. On the contrary, the proportion of this phase in the MW was the lowest of three locations (77%). Otherwise, the size of the DP fraction was overall the lowest in the northern station (76-87%) in comparison to the other two stations. As in the other two stations, the phase distribution was quite constant for the upper three water masses and it was not observed the compositional change in the MW described previously in the south. In contrast the proportion of the PAHs in the DP of the NADW was noticeably lower than in the other water masses of the same station, and also 16 and 21% lower than in the two most southern stations.

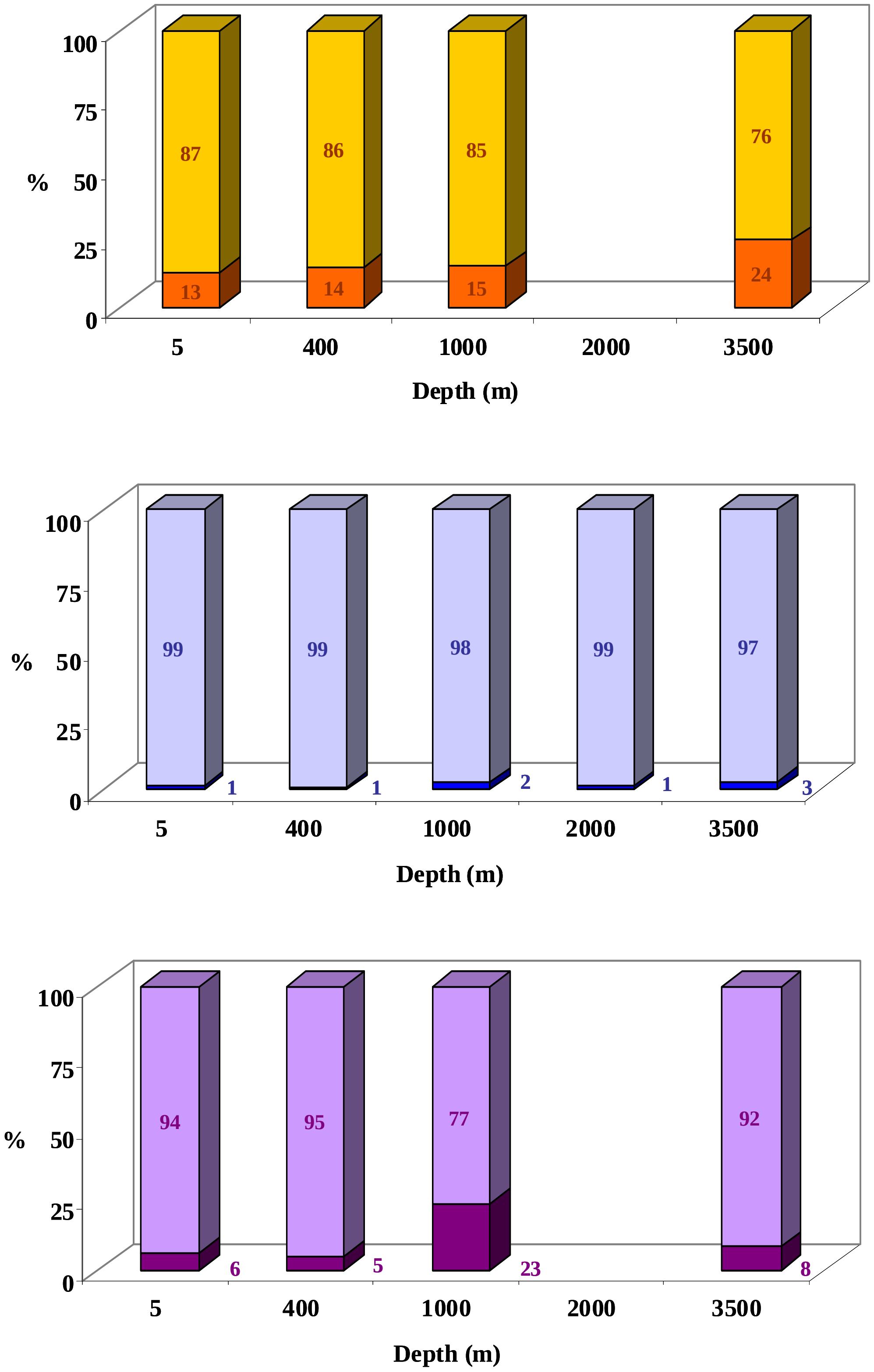

Figure 6.8. ΣPAHs percentage associated to the SPM (dark shade) and DP (light shade) in the water column at the north (orange), Prestige (blue) and south (purple) stations in March 2006. Percentages have been calculated from the ΣPAHs values described for each depth and station in chapter 3 and this chapter.

The phase distribution found in October 2006 in the Prestige tanker wreck (Fig. 6.9) did not agree with the ones observed for open ocean waters in the literature and DP/SPM proportions described for the same station in March. The ΣPAHs in the DP represented between the 10 to 23 % of the mixture, being the importance of this phase the lowest in the most superficial and deepest water masses, coinciding with the water bodies with most contact with the oil. Since the plankton and free-living bacteria are potential particles, the higher PAH proportion associated to SPM can be explained with their higher abundance in the upper zone. Several hypothesis were considered in order to interpret the observed results. One of the possible explanations of the dominance of the particulate PAHs and apparent distribution of PAHs could be the recent character of the spill. Equilibrium concentration between DP and SPM are not immediately reached after the spill, in fact it can take from days to weeks (Broman et al., 1996). Other of alternative explanations not mutually exclusive is the presence of the colloidal oil in the water column as a result of the deep sea spill, which could have also caused the resemblance of the individual PAH distributions to the profiles of the Prestige oil (chapter 3, fig. 3.4). The formation of colloids is usually preceded by turbulence overall provided by physical factors such as wind, waves and currents in the ocean surface (Sterling et al., 2003). Anyway, the local turbulent processes above the wreck are unknown and therefore the possibility of the fuel oil colloids formation can neither be confirmed nor rejected. Data of SPM amount and a study of the local turbulent processes near the Galician Bank would provide valuable information about the explanation of the phase distribution in October.

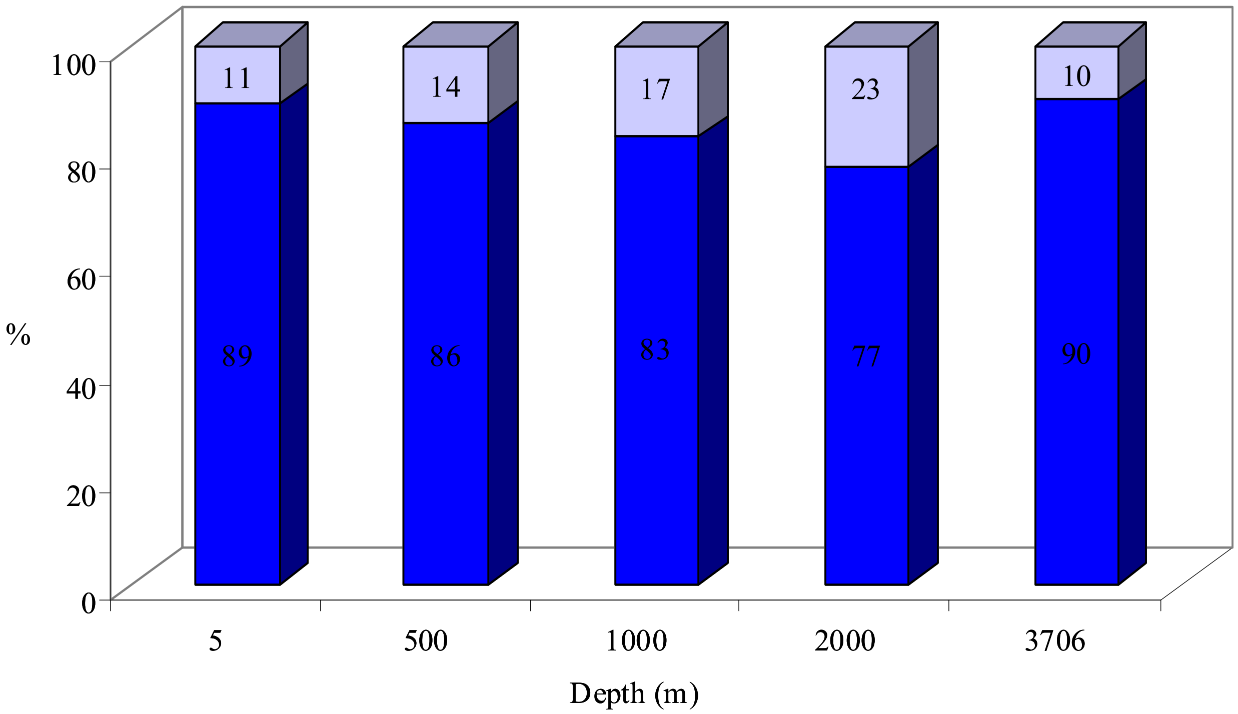

Figure 6.9. ΣPAHs percentage associated to the SPM (dark blue) and DP (light blue) in the water column at the Prestige station in October 2006. Percentages have been calculated from the ΣPAHs values described for each depth in chapter 3 and this chapter.

6.3. Conclusions

Distributions of the PAHs in the DP were studied in the north, Prestige and south stations in March 2006. The water column in the sinking area was also analysed in October of the same year. In March, the PAH mixture of the three stations is dominated by light hydrocarbons which, together with the distributions observed in the SPM, suggest an important input of petrogenic hydrocarbons in the area. Even so, the ΣPAHs concentrations found in the north and south stations were in the range of levels reported in the literature for the North Atlantic and similar areas. However, the concentrations determined in the Prestige site exceeded in more than one order of magnitude the levels found in the south and north, which lead to think that the Prestige location could have been affected by an oil spill from the wreck, even so not as recent as in October.

There was a clear quantitative and qualitative change in the PAH distribution in the water column above the wreck between the two sampling periods. While the maximum concentrations in the DP in March were observed in the SW, in October were found in the deepest water mass, which seemed to confirm the deep spill coming from the wreck. Moreover, the increase of PAH concentrations in the DP from March to October also agreed with this supposition.

Overall, the PAH distribution between the DP and SPM in March agreed with the ones reported in the literature for the open ocean waters, where the PAHs in the DP dominated the ones associated to the particles. The proportions between the phases changed drastically in the Prestige station in October 2006, being the SPM the dominant. Although the processes behind this partition were not fully clarified, the deep spill from the wrecks detected at that time was pointed out to be the main cause of the observed PAHs distribution between the DP/SPM.

6.4. References

Albaigés, J., B. Morales-Nin, Vilas, F., 2006. The Prestige oil spill: A scientific response. Mar. Pollut. Bull., 53: 205-207.

Broman, D., Näf, C., Rolff, C., Zebür, Y., 1991. Occurrence and dynamics of polychlorinated dibenzo-p-dioxins and dibenzofurans and polycyclic aromatic hydrocarbons in the mixed surface layer of remote coastal and offshore waters of the Baltic. Environ. Sci. Technol. 25:1850-1864.

Broman, D., Näf, C., Axelman, J., Bandh, C., Pettersen, H., Johnstone, J., Wallberg, P., 1996. Significance of bacteria in marine waters for the distribution of the hydrophobic organic contaminants. Environ. Sci. Technol. 30:1238-1241.

Brunk, B. K., Jirka,G.H., Lion, L.W., 1997. Effects of salinity changes and the formation of dissolved organic matter coatings on the sorption of phenanthrene: Implications for pollutant trapping in estuaries. Environ. Sci. Technol. 31: 119-125.

Bucheli, T.D. and Gustafsson,O., 2000. Quantification of the soot-water distribution coefficient of PAH provides mechanistic basis for enhanced sorption observations. Environ. Sci. Technol. 34: 5144-5151.

Cailleaud, K., Forget-Leray, J., Souissi, S., Hilde, D., LeMenach, K., Budzinski, H., 2007. Seasonal variations of hydrophobic organic contaminant concentrations in the water-column of the Seine Estuary and their transfer to a planktonic species Eurytemora affinis (Calanoïda, copepoda). Part 1: PCBs and PAHs. Chemosphere 70: 270-280.

Cao, Z., Wang, Y., Ma, Y., Xu, Z., Shi, G., Zhuang, Y., Zhu, T., 2005. Occurrence and distribution of polycyclic aromatic hydrocarbons in reclaimed water and surface water of Tianjin, China. J. Hazard. Mat. A 122: 51-59.

Cincinelli, A., T. Martellini, T., Bittoni,L., Russo, A., Gambaro, A., Lepri, L., 2008. Natural and anthropogenic hydrocarbons in the water column of the Ross Sea (Antarctica). Journal of Marine Systems 73: 208-220.

Clemons, J. H., Allan, L.M., Marvin, C.H., Wu, Z., McCarry, B.E., Bryant, D.W., Zacharewski, T.R., 1998. Evidence of estrogen- and TCDD-like activities in crude and fractionated extracts of PM10 air particulate material using in vitro gene expression assays. Environ. Sci. Technol. 32: 1853-1860.

Dachs, J., Bayona, J.M., 1997. Large volume preconcentration of the dissolved hydrocarbons and polychlorinated biphenyls from seawater. Intercomparison between C_{18} disks and XAD-2 column. Chemosphere 35, 1669-1679.

Dachs, J., Bayona, J.M.. , Raoux, C., Albaigés, J., 1997. Spatial, vertical distribution and budget of polycyclic aromaric hydrocarbons in the western Mediterranean seawater. Environ. Sci. Technol. 31, 682-688.

Di Toro, D. M., McGrath, J.A., Hansen, D.J., 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. Environ. Toxicol. Chem. 19: 1951-1970.

Elordui-Zapatarietxe, S., Albaigés, J., Rosell-Melé, A., 2008. Fast preparation of the seawater accomodated fraction of the heavy fuel oil by sonication. Chemosphere 73: 1811-1816.

Filipkowska, A., Lubecki, L., Kowalewska, G., 2005. Polycyclic aromatic hydrocarbon analysis in different matrices of the marine environment. Anal. Chim. Acta 547, 243-254.

Font, G., Mañes, J., Moltó, J.C., Picó, Y., 1996. Current developments in the analysis of water pollution by polychlorinated biphenyls. J. Chromatogr. A 733, 449-471.

Froescheis, O., Looser, R., Cailliet, G., Jarman, W.M., Ballschmiter, K., 2000. The deep-sea as a final global sink of semivolatile persistent organic pollutants? Part I:

PCBs in surface and deep-sea dwelling fish of the North and South Atlantic and the Monterey Bay Canyon (California). Chemosphere 40: 651-660.

Garza-Gil, M. D., Prada-Blanco, A., Vázquez-Rodríguez, M.X., 2006. Estimating the short-term economic damages from the Prestige oil spill in the Galician fisheries and tourism. Ecol. Econ. 58: 842-849.

González, J. J., Viñas, L., Franco, M.A., Fumega, J., Soriano, J.A., Grueiro, G., Muniategui, S., López-Mahía, P., Prada, D., Bayona, J.M., Alzaga, R., Albaigés, J., 2006. Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the Prestige oil spill. Mar. Pollut. Bull. 53: 250-259.

González-Doncel., M., González, L., Fernández-Torija, C., Navas, J.M., Tarazona, J.V., 2008. Toxic effects of an oil spill on fish early life stages may not be exclusively associated to PAHs: Studies with the Prestige oil and medaka (*Oryzias latipes*). Aquat. toxicol. 87: 280-288.

Green, G., Skerratt, J.H., Leeming, R., Nichols, P.D., 1992. Hydrocarbons and coprostanol levels in seawater, sea-ice algae, and sediments near Davis Satation in eastern Antarctica: A regional survey and preliminary results for a field fuel spill experiment. Mar. Pollut. Bull. 25, 293-302.

Gustafsson, Ö, Gschwend, P.M., 1997. Aquatic colloids: concepts, definitions and current challenges. Limnol. Oceanogr. 42: 519-528.

Gustafsson, Ö, Buesseler, K.O., Geyer, W.R., Moran, S.B., Gschwend, P.M., 1998. An assessment of the relative importance of the horizontal and vertical transport of particle-reactive chemicals in the coastal ocean. Cont. Shelf Res. 18; 805-829.

Jaward, F. M.: Barber, J.L., Booij, K., Jones, K.C., 2004. Spatial distribution of atmospheric PAHs and PCNs along a north-south Atlantic transect. Environ. Pollut. 132 : 173-181.

Johansson, S., Larsson, U., Boehm, P., 1980. The Tsesis oil spill impact on the pelagic ecosystem. Mar. Pollut. Bull. 11: 284-293.

Jurado, E., Zaldívar, J.M., Marinov, D., Dachs, J., 2007. Fate of persistent organic pollutants in the water columna. Does turbulent mixing matter?. Mar. Pollut. Bull. 54: 441-451.

Koelmans, A.A., Gillisen, F. Makatita, W., Van Den Verg, M., 1996. Organic carbon normalisation of PCB, PAH and pesticide concentrations in suspended solids. Wat.Res. 31: 461-470.

Leppard, G.G., Flannigan, D.T., Mavrocordatos, D., Marvin, C.H., Bryant, D.W., McCarry, B.E., 1998. Bindinh of polycyclic aromatic hydrocarbons by size classes of particulate in Hamilton Harbor water. Environ. Sci. Technol. 32 : 3633-3639.

Lipiatou , E., Saliot, A., 1991. Fluxes and transport of anthropogenic and antural polycyclia aromatic hydrocarbons in the western Maditerranean sea. Mar. Chem,., 32, 51-71.

Lipiatou, E., Tolosa, I., Simó, R., Bouloubassi, I., Dachs, J., Marti, S., Sicre, M.A., Bayona, J.M., Grimalt, J.O., Saliot, A., Albaiges, J., 1997. Mass budget and dynamics of polycyclic aromatic hydrocarbons in the Mediterranean Sea. Deep-Sea Res, Pt. II 44: 881-905.

Lotufo, G.R., 1998. Bioaccumulation of sediment-associated fluoranthene in bethic copepods: uptake, elimination and biotransformation. Aquat. Toxicol. 44:1-15.

Luo, X., Chen, S., Mai, B., Yang, Q., Sheng, G., Fu, J., 2006. Polycyclic aromatic hydrocarbons in the suspended particulate matter and sediments from the Pearl River Estuary and adjacent coastal areas, China. Environ. Pollut. 139: 9-20.

Maldonado, C., J. M. Bayona, J.M., Bodineau, L., 1999. Sources, distribution, and water column processes of aliphatic and polycyclic aromatic hydrocarbons in the northwestern Black Sea water. Environ. Sci. Technol. 33: 2693-2702.

McCarty L.S., Mackay, D., Smith, A.D., Ozburn, G.W., Dixon, D.G., 1992. Residue-based interpretation of toxicity and bioconcentration QSARs from aquatic bioassays: neutral narcotic organics. Environ Toxicol Chem 11:917-930.

Michel, J., Gilbert, T., Waldron., J., Blocksidge, C.T., Schmidt Etkin, D., Urban, R., 2005. Potentially polluting wrecks in marine waters. In: Proceedings of the international oil spill conference (IOSC) , Miami beach, USA.

Michor, G., Carron, J., Bruce, S., Cancilla, D.A., 1996. Analysis of 23 polynuclear aromatic hydrocarbons from natural water at the sub-ng/l level using solid-phase disk extraction and mass-selective detection. J. Chromatogr. A 732, 85-99.

Nfon, E., Cousins, I.T., Broman, D., 2008. Biomagnification of organic pollutants in benthic and pelagic marine food chains from the Baltic Sea. Sci. Total Environ. 397: 190-204.

Ohkouchi, N., Kawamura, K., Kawahata, H., 1999. Distribution of three-to seven-ring polynuclear aromatic hydrocarbons on the deep sea floor in the central Pacific. Environ. Sci. Technol. 33, 3086-3090.

Payne, J.R., Clayton, J.R., Kirstein, B.E., 2003. Oil/suspended particulate material interactions and sedimentation. Spill Sci. Technol. Bull. 8, 201-221.
8, 201-221.

Razzaque, M. M., Grathwohl, P., 2008. Predicting organic carbon-water partitioning of hydrophobic organic chemicals in soils and sediments based on water solubility. Water Res. 42: 3775-3780.

Sánchez, F., Velasco, F., Cartes, J.E., Olaso, I., Preciado, I., Fanelli, E., Serrano, A., Gutierrez-Zabala, J.L., 2006. Monitoring the Prestige oil spill impacts on some key species of the Northern Iberian shelf. Mar, Pollut. Bull. 53: 332-349.

Schulz-Bull, D. E., Petrick, G., Bruhn, R., Duinker, J.C., 1998. Chlorobiphenyls (PCB) and PAHs in water masses of the northern North Atlantic. Mar. Chem. 61: 101-114.

Schwarzenbach, R.P., Gschwend, P.M.,Imboden, D.M. 1993 Environmental Organic Chemistry, New York: John Wiley and Sons, Inc.

Serrano, A., Sánchez, F., Preciado, I., Parra, S., Frutos, I., 2006. Spatial and temporal changes in benthic communities of the Galician continental shelf after the Prestige oil spill. Mar. Pollut. Bull. 53: 315-331.

Shi, Z., Tao, S., Pan, B., Liu, W.X., Dhen, W.R., 2007. Partitioning and source diagnostics of polycyclic aromatic hydrocarbons in rivers in Tianjin, China. Environ. Pollut. 146: 492-500.

Sterling, M.C., Bonner, J.S., Page, C.A., Fuller, C.B., Ernest, A.N.S., Autenrieth, R.L., 2003. Partitioning of polycyclic aromatic hydrocarbons in aquatic systems. Environ. Sci. Technol. 37, 4429-4434.

Tremblay, L., Kohl, S.D., Rice, J.A., Gagne, J.P., 2005. Effects of temperature, salinity, and dissolved humic substances on the sorption of polycyclic aromatic hydrocarbons to estuarine particles. Mar. Chem. 96, 21-34.

Tronczyski, J., Munschy, C., Héas-Moisan, K., Guiot, N., Truquet, I., Olivier,N., Men,S., Furaut, A., 2004. Contamination of the Bay of Biscay by polycyclic aromatic hydrocarbons (PAHs) following the T/V "*Erika*" oil spill. Aquat. Living Resour. 17, 243-259

Varela, M., Bode, A., Lorenzo, J., Álvarez-Ossorio, M.T., Miranda, A., Patrocinio, T., Anadón, R., Viesca, L., Rodríguez, N., Valdés, L., Cabal, J., Urrutia, A., García –Soto, C., Rodríguez, M.,Álvarez-Salgado, X.A., Groom, S., 2006. The effect of the "Prestige" oil spill on the plankton of the N-NW Spanish coast. Mar. Pollut. Bull. 53: 272-286.

Viamajala, S., Peyton, B.M., Richards, L.A., Petersen, J.N., 2007. Solubilization, solution equilibria, and biodegradation of PAH's under thermophilic conditions. Chemosphere 66: 1094-1106.

Xie, W., Shiu, W., McCay, D., 1997. A review of the effect of salts on the solubility of organic compounds in seawater. Mar. Envir. Res. 44: 429-444.

Zhou, J. L., Fileman, T.W., Evans, S., Donkin, P., Readman, J.W., Mantoura, R.F.C., Rowland, S., 1999. The partition of fluoranthene and pyrene between suspended particles and dissolved phase in the Humber Estuary: a study of the controlling factors. Sci. Total Environ. 243-244: 305-321.

CHAPTER 7

General conclusions

The general conclusions obtained from the present study are the following:

Deep sea spill

i. The oil detected near the Prestige sinking area in October 2006 had a chemical signature equivalent to that of the oil originally carried by the tanker. This suggests the occurrence of a deep spill from the Prestige wrecks at the time the cruise was undertaken. The lack of major signs of transformation due to exposition to the environment confirmed that the spill was recent, that is, coming directly from the wrecks.

ii. Large amounts of hydrocarbons were introduced to the water column as a consequence of the deep sea oil spill and total concentrations detected in October near the wreck were similar to the ones reported in the most affected coastal areas by the black tides in 2002 and 2003.

iii. As a consequence of the deep oil spill, there was a variation in the distribution of individual hydrocarbons in the SPM from March to October, where the signature of the Prestige oil overpowered hydrocarbons from other origins.

iv. The change in the phase distribution of the ΣPAHs in the sinking area between March and October, where the SPM dominated over the DP, showed that the hydrocarbons were in a non-equilibrium state, thus reflecting the recent nature of the oil spill.

v. The proximity to the source proved to be a key factor determining the abundance of the total dissolved and particle-bound hydrocarbons and therefore the water mass in contact with the wrecks, the NADW, exhibited the highest hydrocarbon levels. Oil slicks floating on the ocean surface acted as a secondary hydrocarbons source and concentrations in the superficial waters were higher than expected with relation to the distance from the wreck. Similarly, mid-depth water masses presented the lowest values in general due to being the more distant from the two main focuses.

Distribution of hydrocarbons near the Galician Bank

vi. In March 2006, the open ocean waters near the Galician Bank showed a wide contamination by hydrocarbons that extended to the whole water column.

vii. The petrogenic input was determined to be the most important anthropogenic hydrocarbon source of the area. However, there was not enough evidence to consider the *Prestige* wrecks the main source of hydrocarbon pollution in March 2006. Alternatively, the area seems to be affected by a chronic petrogenic contamination from multiple sources.

viii. Hydrocarbon concentration in the SPM in March were in the range of the values reported in the literature for similar areas, even so, individual PAH profiles resembled distributions found in waters with oil pollution.

ix. The sampling station located above the shipwreck showed signs of having being affected by a deep oil spill, which was reflected in the exceedingly high concentration of ΣPAHs found in DP compared to the other two locations. However, since the abundance of PAHs in the SPM and the DP/SPM proportions in the Prestige site were in concordance with the ones found in the south and north, it was deduced that the spill probably was not as recent as the one observed in October 2006.

x. The water masses in the area have been shown to actively transport contamination far from the sources and be able to carry signatures of the contamination from their source region. In this respect, both superficial and deep water masses seemed to carry different hydrocarbon loads from the mid-depth water masses. The MW also displayed higher PAH levels in the SPM than the adjoining water masses, which showed its origin from an more polluted area, such as the Mediterranean Sea.

xi. Although DP/SPM proportions varied between the different stations and water masses in March, overall ΣPAHs were far more abundant in the DP than in the SPM, which agreed with the proportions reported in the literature for the North Atlantic waters and similar areas.

Laboratory work

xii. Ultrasonication of the oil-water mixture is as fast, reliable and reproducible method for the preparation of the seawater accommodated fraction in the laboratory.

xiii. The concentration of the total aromatic hydrocarbons in the SWAF increases with the decrease of salinity and the increase of water temperature. Individual PAHs follow the same pattern.

xiv. Changes in temperature usually found in the open ocean are bound to have a much larger impact in the concentration of PAHs in the SWAF than the corresponding values of sea water salinity. Taking into account obtained results, after a spill the highest SWAF should be expected in the warmest and less saline waters of the water column.

Implications of the study and future work

xv. Sunken vessels with oil in them can potentially generate future spills and therefore act as a chronic hydrocarbon contamination sources if the remaining oil is not removed, as the wreck of *Prestige* tanker has demonstrated.

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Figure 6.1. Log concentration (ng L^{-1}) of the sum of 21 PAHs (ΣPAHs) in the DP of selected stations at different depths in March 2006. Sampling locations: north station (orange), *Prestige* station (blue) and south station (purple). ΣPAHs: Naphthalene (N); Methylnaphthalenes (MN); Dimethylnaphthalenes (DMN); Trimethylnaphthalenes (TMN); Acenaphthylene (Ac); Acenaphthene (Acn) ; Fluorene (F); Phenanthrene (P); Methylphenanthrenes (MP); Anthracene (A), Fluoranthene (Fl); Pyrene (Py); Chrysene (Chry); Benzo[*a*]anthracene (BaA), Benzo[*b*]fluorene (BbF), Perylene (Per); Benzo[*k*]fluoranthene (BkFl); ; Benzo[*a*]pyrene (BaPy); Benzo[*g,h,i*]perylene (BghiPer); Dibenzo[*a,h*] anthracene (DahA); Indeno[1,2,3,*c-d*]pyrene (Ind). Individual concentrations are listed in annex 1.

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APPENDIX

APPENDIX 1

Individual concentrations of hydrocarbons in the SPM and DP in March 2006

	North					Prestige					South				
	5 m	**400 m**	**1000 m**	**2000 m**	**3500 m**	**5 m**	**400 m**	**1000 m**	**2000 m**	**3700 m**	**5 m**	**400 m**	**1000 m**	**2000 m**	**4000 m**
N	43	42	63	272	152	297	17	21	16	161	17	21	28	23	20
MN	36	42	41	176	73	132	12	10	11	67	13	5	62	12	11
DMN	63	55	63	88	166	211	50	24	21	99	29	19	72	BDL	BDL
TMN	168	153	195	56	555	532	143	99	12	205	98	37	150	37	66
Ac	BDL	BDL	BDL	BDL	36	28	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Acn	33	16	16	26	74	99	18	BDL	17	48	19	BDL	42	BDL	14
F	24	16	18	11	158	70	22	22	22	78	17	11	22	10	19
P	166	104	93	67	153	424	124	333	95	271	149	133	128	79	139
MP	349	46	80	43	128	231	134	121	99	67	131	109	153	104	132
A	15	18	17	16	16	16	8	18	13	16	17	8	11	14	21
Fl	4	13	20	7	13	16	19	17	19	7	13	12	14	7	9
Py	18	6	11	5	13	48	27	19	19	15	18	11	13	6	8
Total	919	511	617	767	1537	2102	574	683	344	1013	521	366	696	292	439

Concentratioin (pg L^{-1}) of the individual PAHs determined in the SPM samples in March 2006. BDL: Below detection limit; na: not analysed.

	North					Prestige					South				
	5 m	**400 m**	**1000 m**	**2000 m**	**3500 m**	**5 m**	**400 m**	**1000 m**	**2000 m**	**3700 m**	**5 m**	**400 m**	**1000 m**	**2000 m**	**4000 m**
N	0.62	0.14	0.25	na	0.43	4.80	1.35	1.92	2.67	3.37	1.41	0.59	0.37	na	0.82
MN	1.55	0.13	0.52	na	0.32	8.98	5.01	3.11	3.69	5.29	0.81	0.33	BDL	na	0.38
DMN	1.45	0.77	0.69	na	2.54	47.30	20.35	8.78	10.75	12.62	0.86	1.43	BDL	na	1.07
TMN	2.39	1.47	1.61	na	1.34	102.60	31.59	14.75	14.85	14.62	2.59	2.75	0.72	na	1.68
Ac	BDL	BDL	0.01	na	BDL	0.32	0.03	0.01	0.03	0.09	BDL	BDL	BDL	na	BDL
Acn	0.01	0.05	0.04	na	0.04	1.94	0.63	0.30	0.31	0.38	0.23	0.16	BDL	na	0.12
F	0.03	0.02	0.02	na	0.02	2.97	0.82	0.41	0.39	0.33	BDL	BDL	BDL	na	0.00
P	0.01	0.02	0.02	na	0.03	8.39	1.24	0.92	0.67	0.43	0.69	0.62	0.41	na	0.29
MP	0.07	0.43	0.37	na	0.03	9.42	1.15	0.97	0.69	0.38	0.81	1.21	0.46	na	0.37
A	BDL	BDL	BDL	na	BDL	0.57	0.04	0.01	0.01	0.01	BDL	BDL	BDL	na	BDL
Fl	0.02	0.04	0.04	na	0.01	0.23	0.06	0.05	0.05	0.03	0.36	0.19	0.18	na	0.18
Py	0.02	0.04	0.04	na	0.01	0.23	0.07	0.05	0.05	0.02	0.33	0.17	0.17	na	0.17
Total	6.176	3.112	3.603	na	4.791	187.74	62.33	31.28	34.16	37.55	8.09	7.46	2.30		5.08

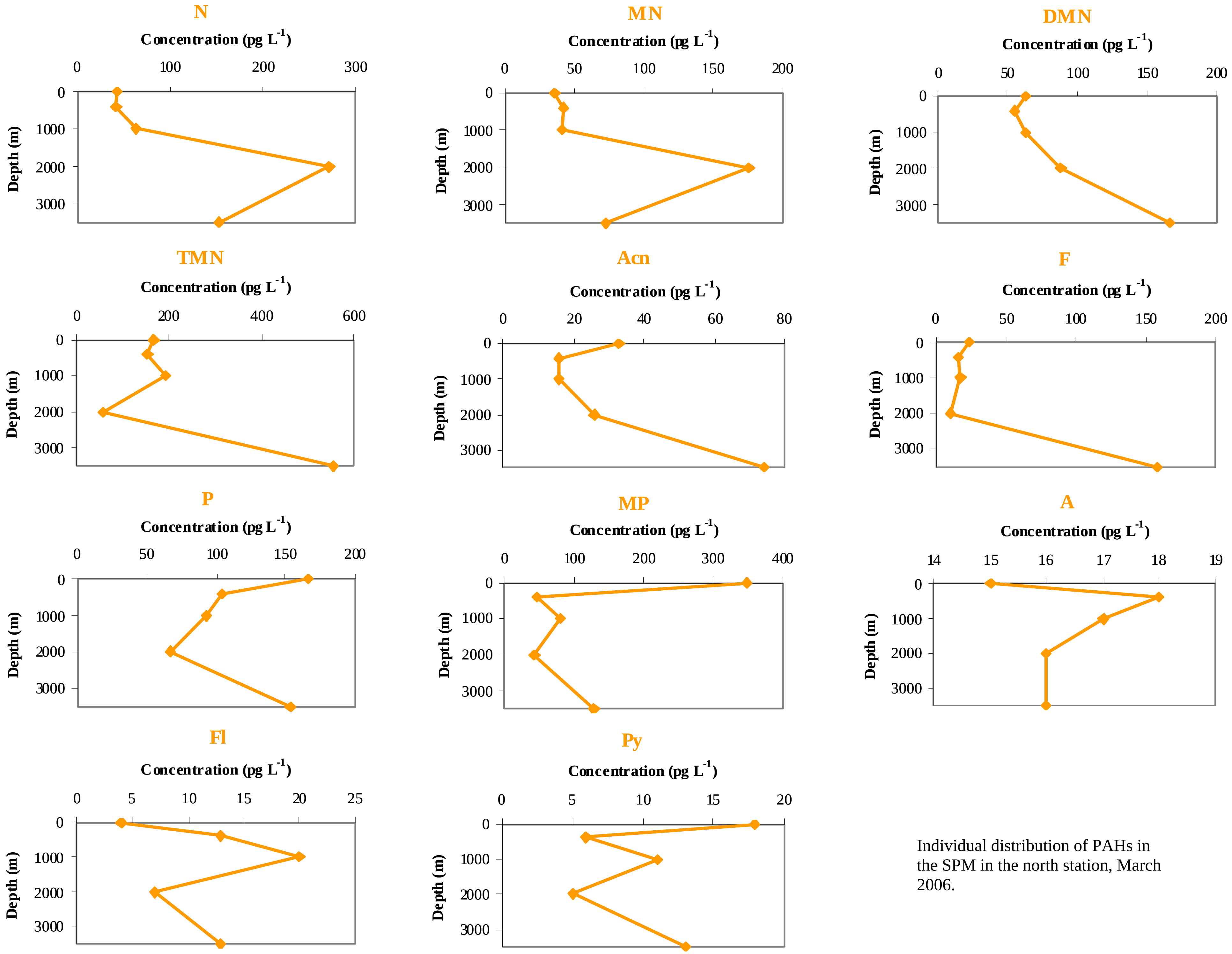
Concentration (ng L^{-1}) of the individual PAHs determined in the DP samples in March 2006. BDL: Below detection limit; na: not analysed

	North					Prestige					South				
	5 m	**400 m**	**1000 m**	**2000 m**	**3500 m**	**5 m**	**400 m**	**1000 m**	**2000 m**	**3700 m**	**5 m**	**400 m**	**1000 m**	**2000 m**	**4000 m**
C_{14}	0.37	0.05	BDL	BDL	0.13	0.26	BDL	0.16	BDL	0.20	0.28	BDL	BDL	BDL	BDL
C_{15}	3.58	0.08	BDL	BDL	0.22	0.79	0.01	0.10	BDL	0.10	2.44	BDL	BDL	0.01	BDL
C_{16}	2.18	0.11	0.08	0.05	0.47	0.95	0.04	0.29	0.10	0.20	0.75	0.03	BDL	0.02	0.11
C_{17}	4.54	0.41	0.22	0.01	0.61	2.17	0.09	0.24	0.38	0.12	1.86	0.03	0.11	0.03	0.40
Pr	1.77	0.15	0.07	0.01	0.17	1.17	0.04	0.15	0.17	0.05	0.48	0.02	0.04	BDL	0.17
C_{18}	1.98	0.13	0.10	0.03	0.34	1.51	0.09	0.30	0.51	0.11	1.18	0.03	0.13	0.05	0.54
Ph	1.28	0.06	0.06	0.02	0.19	0.78	0.05	0.16	0.29	0.03	0.52	0.02	0.07	0.03	0.30
C_{19}	1.01	0.06	0.06	0.02	0.12	1.00	0.10	0.29	0.31	0.11	0.84	0.01	0.15	0.06	0.33
C_{20}	0.75	0.05	0.07	0.03	0.12	0.83	0.14	0.24	0.22	0.10	0.78	0.01	0.08	0.09	0.23
C_{21}	0.45	0.03	0.15	0.09	0.03	0.61	0.19	0.21	0.20	0.14	1.12	0.02	0.09	0.14	0.21
C_{22}	0.41	0.02	0.17	0.10	0.01	0.89	0.29	0.27	0.20	0.14	1.31	0.04	0.07	0.13	0.21
C_{23}	0.39	BDL	0.09	0.04	BDL	1.31	0.22	0.29	0.06	0.08	1.36	BDL	0.01	0.07	0.06
C_{24}	0.50	0.09	0.12	BDL	BDL	2.21	0.33	0.78	0.10	0.06	1.28	0.12	0.09	0.08	0.10
C_{25}	0.84	0.68	0.92	0.34	0.16	3.53	0.72	1.78	0.40	0.38	3.15	0.60	0.49	0.39	0.42
C_{26}	1.29	2.24	2.58	0.96	0.17	4.93	2.06	4.03	0.82	0.61	3.93	1.65	1.36	1.06	0.86
C_{27}	0.97	2.36	3.59	0.97	0.05	3.61	2.59	3.44	0.65	0.36	2.50	1.68	1.16	0.98	0.68
C_{28}	1.06	4.10	5.28	1.61	0.26	3.50	4.72	3.82	0.95	0.61	2.43	2.52	1.60	1.39	1.00
C_{29}	1.01	3.84	4.48	1.69	0.33	2.69	5.41	2.54	0.66	0.46	2.12	2.13	1.22	1.22	0.70
C_{30}	0.81	3.17	3.37	1.58	0.36	2.36	4.49	2.07	0.50	0.33	1.35	1.80	0.83	0.85	0.53
C_{31}	0.62	2.54	1.44	1.31	0.23	1.70	2.81	1.20	0.27	0.12	0.79	1.05	0.62	0.48	0.28
C_{32}	0.42	1.53	0.77	1.03	0.34	1.42	1.58	0.94	0.08	0.18	0.59	0.84	0.37	0.23	0.08
C_{33}	0.27	1.00	0.30	0.63	0.14	0.68	0.56	0.37	0.04	0.08	0.31	0.36	0.24	0.04	0.04
C_{34}	0.24	1.75	0.21	0.93	0.18	0.87	0.15	0.62	BDL	BDL	0.50	0.55	0.02	BDL	BDL
C_{35}	0.25	0.82	0.20	0.51	0.45	0.79	BDL	0.57	BDL	0.24	0.44	0.28	0.39	BDL	BDL
Total	26.99	25.27	24.33	11.95	5.11	40.56	26.69	24.86	6.91	4.81	32.32	13.79	9.14	7.35	7.27

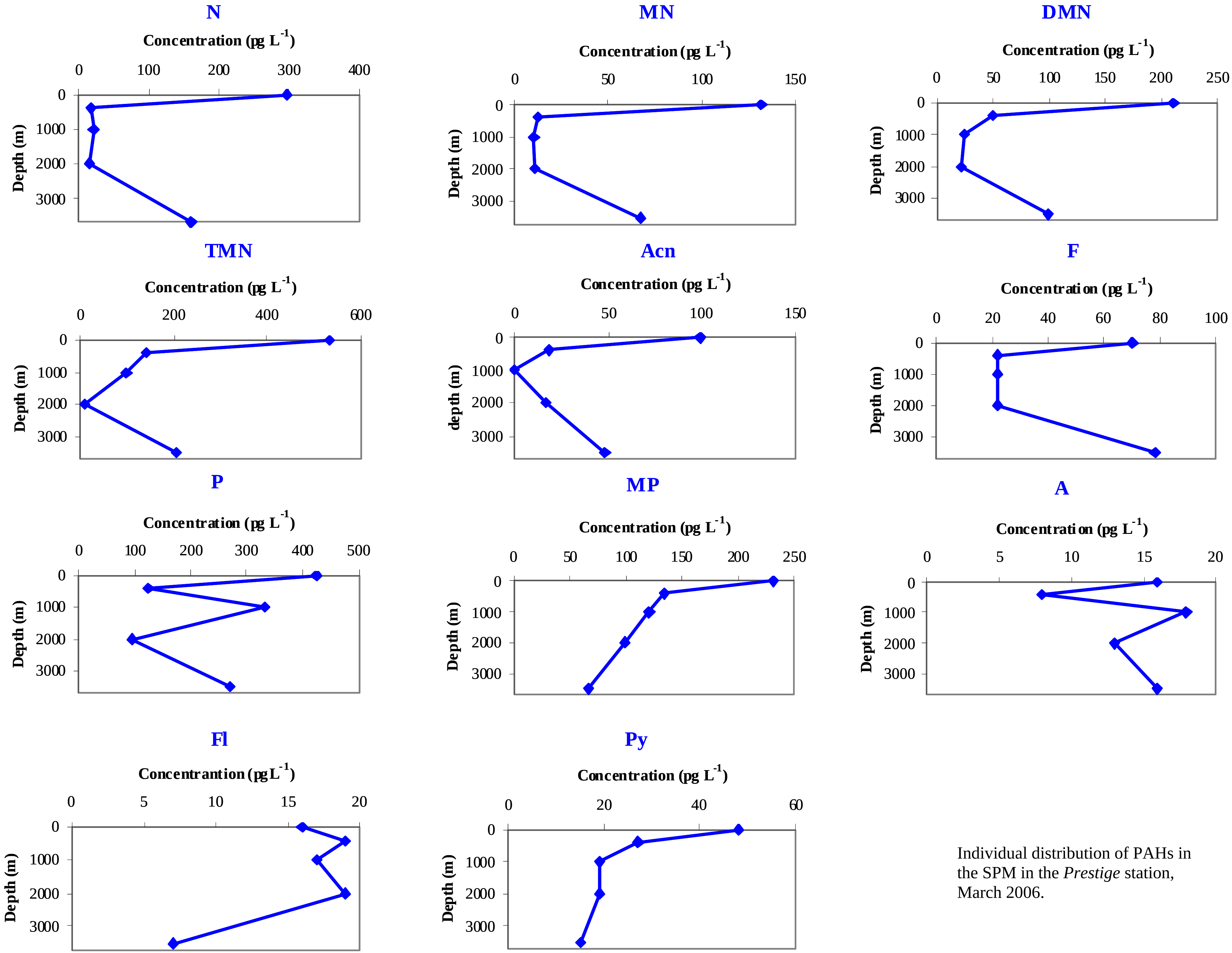
Concentration (ng L^{-1}) of the individual n-alkanes determined in the SPM in March 2006. BDL: Below detection limit; na: not analysed

APPENDIX 2

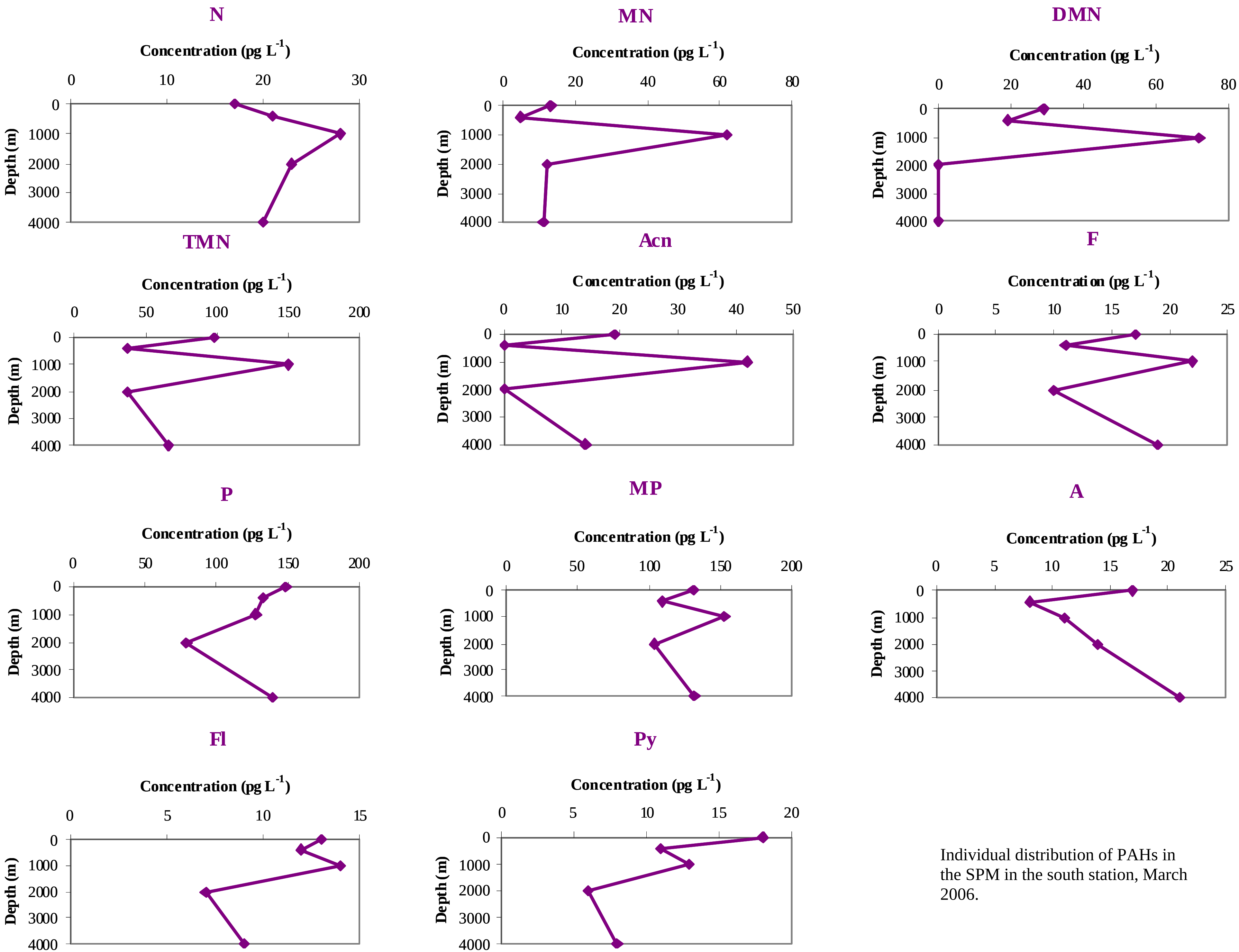
Distribution profiles of individual PAHs in the SPM of the water column in March 2006



Individual distribution of PAHs in the SPM in the north station, March 2006.



Individual distribution of PAHs in the SPM in the *Prestige* station, March 2006.



Individual distribution of PAHs in the SPM in the south station, March 2006.

APPENDIX 3

Individual concentrations of hydrocarbons in the SPM and DP in October 2006

	SPM					**DP**				
	5 m	**500 m**	**1000 m**	**2000 m**	**3706 m**	**5 m**	**500 m**	**1000 m**	**2000 m**	**3706 m**
N	62.87	68.40	30.18	23.38	117.43	10.71	20.79	5.12	6.92	17.89
MN	166.81	212.61	93.10	108.39	337.94	7.79	12.95	8.50	10.35	15.27
DMN	153.48	127.43	108.04	134.51	463.85	16.62	21.40	20.33	33.59	38.12
TMN	107.92	70.97	70.96	73.23	242.48	21.34	23.70	26.47	42.18	58.24
Ac	BDL	BDL	BDL	BDL	BDL	BDL	0.16	BDL	1.70	0.43
Acn	1.27	1.11	0.73	0.90	3.59	BDL	BDL	0.11	BDL	0.90
F	1.56	1.63	1.18	1.68	3.93	0.70	0.38	0.48	1.39	0.86
P	2.25	1.18	2.17	0.89	37.49	1.58	0.94	1.41	1.67	3.07
MP	0.87	0.64	0.98	0.43	8.75	0.90	0.64	0.89	1.64	2.99
A	0.13	0.05	0.15	0.04	1.60	BDL	BDL	BDL	BDL	0.04
Fl	0.05	bdl	0.01	bdl	0.08	0.28	BDL	BDL	0.03	0.02
Py	0.25	0.08	0.07	0.04	0.16	1.20	BDL	BDL	0.15	0.05
Chry	0.08	0.06	0.08	0.07	0.20	0.01	BDL	BDL	BDL	BDL
BaA	0.04	0.04	0.03	0.04	0.10	0.01	BDL	BDL	BDL	BDL
BbF	0.01	0.01	0.02	0.01	0.03	BDL	BDL	BDL	BDL	BDL
BkFl	0.04	0.02	0.03	0.03	0.09	BDL	BDL	BDL	BDL	BDL
Total	497.602	484.234	307.742	343.632	1217.724	61.139	80.942	63.306	99.627	137.867

Individual PAH concentrations (ng L^{-1}) in the SPM and DP of the water column. October 2006. BDL: Below detection limit.

	SPM					**DP**				
	5 m	**500 m**	**1000 m**	**2000 m**	**3500 m**	**5 m**	**500 m**	**1000 m**	**2000 m**	**3700 m**
C_{16}	19.6	1.8	6.0	3.3	15.7	6.8	BDL	BDL	BDL	8.1
C_{17}	31.8	8.2	7.5	4.7	23.0	6.3	BDL	BDL	4.3	9.6
Pri	10.1	3.2	3.8	1.9	11.0	1.5	0.2	BDL	1.6	1.9
C_{18}	19.4	6.7	4.6	2.8	15.0	4.6	BDL	BDL	1.9	6.3
Phy	7.9	3.8	2.6	1.8	9.6	0.4	BDL	BDL	0.4	1.5
C_{19}	23.8	2.5	3.9	3.1	13.9	2.9	0.3	BDL	1.4	8.0
C_{20}	23.6	2.5	4.2	3.0	12.8	1.2	BDL	BDL	0.0	2.7
C_{21}	21.7	2.1	3.2	2.4	14.1	0.5	0.1	BDL	0.3	1.4
C_{22}	15.2	2.2	3.4	2.8	12.1	0.5	BDL	0.2	0.2	0.6
C_{23}	15.2	2.3	3.1	2.3	10.7	0.2	BDL	0.1	0.1	0.4
C_{24}	15.1	2.7	3.4	2.9	11.5	BDL	1.5	0.3	0.1	0.1
C_{25}	17.4	3.0	3.2	2.6	14.2	0.3	BDL	0.5	0.2	0.2
C_{26}	23.0	5.2	2.7	3.2	11.9	0.5	0.6	1.0	0.4	0.6
C_{27}	23.0	8.0	3.5	4.1	8.2	0.7	0.3	0.7	0.5	0.5
C_{28}	16.2	7.0	3.8	4.3	7.8	1.3	1.9	1.8	1.0	1.5
C_{29}	16.2	7.6	3.5	4.0	7.4	1.8	1.4	1.4	1.0	1.7
C_{30}	16.2	7.0	3.3	4.1	9.7	2.3	2.9	2.7	2.0	2.7
C_{31}	16.2	7.5	2.5	3.2	8.9	2.2	2.0	2.1	1.6	2.6
C_{32}	16.3	5.0	2.7	2.9	7.5	2.2	2.6	2.3	2.0	3.2
C_{33}	12.2	2.9	2.7	1.7	4.9	1.4	2.0	1.9	1.4	2.4
C_{34}	26.7	6.4	2.6	2.3	9.7	4.2	3.6	1.2	3.0	4.7
C_{35}	26.9	4.0	2.3	2.2	8.7	2.7	4.5	2.0	2.3	4.4
Total	413.8	101.5	78.5	65.7	248.4	44.4	23.9	18.2	25.9	65.2

Individual aliphatic hydrocarbon concentrations (ng L^{-1}) in the SPM and DP in the water column, October 2006. BDL: Below detection limit.

APPENDIX 4

Paper related to chapter 5

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Fast preparation of the seawater accommodated fraction of heavy fuel oil by sonication

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ABSTRACT

The seawater accommodated fraction (SWAF) of oil is widely used for the assessment of its toxicity. However, its preparation in the laboratory is time consuming, and results from different authors are difficult to compare as preparation methods vary. Here we describe a simple and fast set up, using sonication, to produce reproducible SWAF in the laboratory. The system was tested on heavy fuel oil placed on seawater at different salinity and temperature conditions. Maximum dissolution of the oil was achieved after 24 h, independently of both seawater salinity and temperature. Our findings are discussed in relation to the fate of the oil from the deep spill of the *Prestige* tanker. Changes in temperature in the open ocean are bound to have larger impact in the concentration of the SWAF than the corresponding values of sea water salinity. We anticipate that in this type of incident the highest SWAF, as the oil reaches the sea surface, should be expected in the warmest and less saline waters of the water column.

1. Introduction

The seawater accommodated fraction (SWAF) of a crude oil is a mixture mainly composed by light polycyclic aromatic hydrocarbons (PAHs), phenols and heterocyclic compounds containing nitrogen and sulphur (Saeed and Al-Mutairi, 2000). Several of these PAHs are known to be neurotoxic, mutagenic and carcinogenic (Khan et al., 1995; Fernandez et al., 2006). Since the SWAF is the fraction which is more readily bioavailable soon after an oil spill, it has been widely used for the assessment of the toxicity of the oils in different living organisms, such as crustaceans (Maki et al., 2001; Martinez-Jeronimo et al., 2005), fish (Akaishi et al., 2004) and microbiota (Ohwada et al., 2003). The SWAF can also produce long term effects in areas that are not directly affected by the spill (Navas et al., 2006).

The preparation in the laboratory of the SWAF is usually carried out by gently stirring the oil and seawater by means of a low energy mixing system to avoid the formation of oil in water emulsions (Ali et al., 1995; Rayburn et al, 1996; Ziolli and Jardim, 2002). Consequently, the procedure is slow, taking several days for the concentration of the SWAF to reach a steady state (Hokstad et al., 1999; Page et al., 2000). Moreover, the preparation of replicates of the SWAF is tedious and time consuming. On the other hand, in the assessment studies of the toxicological effects on biota, it is convenient to prepare the SWAF rapidly, as it is not possible to add a biocide to the water to avoid the onset of bacterial activity after 24 h (Singer et al., 2000).

The final composition of the SWAF depends chiefly on parameters such as oil–water ratio, stirring and settling time, salinity and temperature (Ziolli and Jardim, 2002; Martinez-Jeronimo et al., 2005). Given that there is not a common procedure for its preparation the results from different authors are difficult to compare (Singer et al., 2000). Therefore, it is not easy to assess, for instance, how oceanic water masses properties may affect the formation and composition of the SWAF in different spill conditions. For example, in the incident of the *Prestige* tanker tens of thousands of tonnes of heavy fuel oil were released from the wreck at more than 3500 m water depth (Albaigés et al., 2006). On its way towards the surface, the oil had to cross up to five water masses with different temperature and salinity conditions (Ruiz-Villarreal et al., 2006). Consequently, the concentration and composition of the SWAF in each water mass was likely to be different.

In this paper we propose a simple, fast and reproducible method for the preparation of SWAF. We apply a high energy mixing system, using an ultrasonic bath, but avoiding the formation of oil–water emulsions. The method is appraised by studying the changes in the concentration of PAHs in the SWAF of a heavy fuel oil in some of the salinity and temperature conditions commonly found in the North Atlantic Ocean, in the area of the incident of the *Prestige* tanker.

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2. Materials and methods

2.1. Fuel oil and seawater

The fuel oil employed was a marine fuel oil IFO 380, with a density of 0.981 kg L^{-1} at 15 °C, provided by the Coordination Technical Bureau from the Scientific Intervention Program Against Accidental Marine Spills (Vigo, Spain) in April 2005. It was similar in its physicochemical properties to that carried by the *Prestige* tanker.

Natural seawater was obtained from the Gulf of Biscay (33.3 psu; Cantabrian Sea) and from the Mediterranean Sea (37.7 psu). The salinity was measured using a YSI FT Model 556 conductimeter (YSI, Ohio, USA). The seawater was sterilized by adding $HgCl_2$ and filtrated before use through a precleaned glass fibre filter (0.7 μm, Ø 47 mm, APFF type, Millipore, Ireland) to remove suspended particulate material. To determine background levels of hydrocarbons in the natural SWAF, three aliquots of 400 mL from each water type were extracted with the same procedure used to analyze the SWAF, as described in the next section. The background PAH concentrations were subtracted from those found in the SWAF samples prepared in the laboratory.

The effect of temperature in the dissolution of fuel oil was appraised at two temperatures, i.e. 20 °C (i.e. coded high temperature or HT in the text and figures) and 3 °C (i.e. low temperature or LT). These temperatures were chosen as representative of the values of the surface and bottom water masses in the sinking area of the *Prestige* tanker, in the North Eastern Atlantic, 150 nautical miles offshore from the Spanish coast (Ruiz-Villarreal et al., 2006).

The effect of salinity was studied using natural seawater from the Gulf of Biscay (low salinity or LS), and from the Mediterranean Sea (high salinity or HS). The salinity of both of them was measured directly in the storing tanks, before and after the experiments to monitor any changes due to evaporation.

2.2. Preparation of SWAF

The dissolution apparatus (Fig. 1) was adapted from Ali et al. (1995). Seawater (1 L) was poured into a 1.5 L volume glass flask (94 mm I.D. × 200 mm height). Two PTFE tubs (0.7 mm I.D. × 500 mm length) were inserted in the cap, one of them kept over the surface of the seawater to blow nitrogen, and the other used for the collection of water samples inserted deep into the seawater. Fuel was added in a 1:500 (v/v) oil to water ratio, close to the surface of the seawater by means of a stainless steel spatula. The surface area to volume ratio was 0.03 and the headspace represented the 33% of the flask volume. The cap on the flask was sealed with PTFE film first, and then with plastic film. All the apparatus was placed carefully in an ultrasonic bath, sonicated for 30 min with an energy of 360 W and left to settle down at a constant temperature.

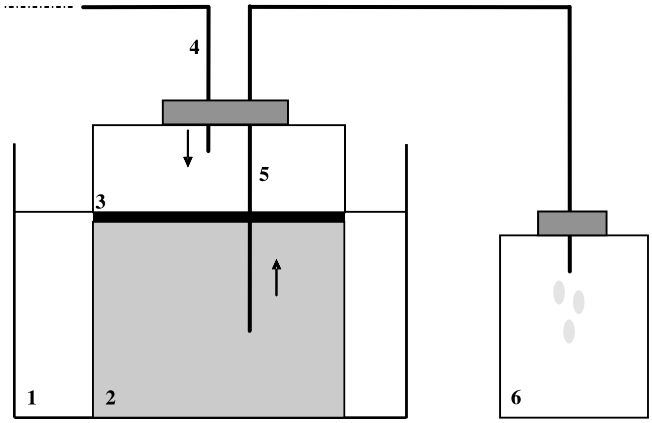

Fig. 1. The dissolution apparatus. (1): Ultrasonic bath; (2): SWAF preparation flask; (3): oil slick; (4): tube for N_2 application; (5): tube for sample retrieval; (6): sample recovery jar.

The apparatus was covered with aluminium foil to minimize the photodegradation of the fuel oil during the experiment. To maintain the sonication conditions reproducible, the location of the flask and the water level in the ultrasonic bath were exactly the same in all the experiments. Emulsions did not form as long as the flasks did not vibrate substantially in the bath. Cork plates were used to avoid direct contact between the flasks and the bath walls.

The retrieval of the water samples was carried out by applying a gentle stream of nitrogen through the tube over the seawater surface, while SWAF aliquots were collected in a clean glass flask through the tube inserted in the bottom. Special attention was paid not to disturb the water surface during this process to avoid dispersion of the oil.

The temperature was controlled mainly at two different stages of the SWAF preparation. First of all, natural seawater in the flasks, and distilled water filling the ultrasonic bath was added at the corresponding temperature at the beginning of each preparation experiment. Some of the seawater was simply stored in closed tanks in the laboratory at room temperature, maintained at 20 ± 2 °C, while water at 3 °C was obtained using a refrigeration system. This parameter was also controlled during the equilibration time. Half of the replicas were left to equilibrate at 3 °C, and the other half at 20 °C. The temperature of the water in the ultrasonic bath was measured before and after stirring and a maximum increment of 2 °C was observed.

Four experiments (at two different temperatures and salinities) were carried out in triplicate. In each type of experiment two identical sets were prepared for different purposes. In the first one, between 1 and 3 mL aliquots of water were collected at 0, 24, 48, 72, 96 and 120 h to monitor the progress of the oil dissolution by fluorescence analysis. In the second, 400 mL of water were collected after 24 h for the identification and quantification of individual PAHs.

The significance of the different experimental effects were confirmed using a one way ANOVA ($p < 0.05$ for all the preparation parameters) and several post-hoc tests (Tuckey HSD and Bonferroni), to make pairwise comparison of the average concentration and appraise which factor had the strongest influence in the dissolution of fuel oil in the seawater.

In parallel, another experiment was prepared where the fuel-water mixture was not sonicated to assess the differences in the solubility of the fuel in comparison to the proposed method with sonication. The experiment without sonication performed in triplicate under HT and HS conditions. Besides the oil–water mixing, the rest of the process was followed exactly as in the sonication experiments.

2.3. Characterization of PAHs in sea water and fuel oil

Sea water (400 mL) was filtered through a Durapore membrane (0.22 μm and Ø 47 mm, Millipore) in order to eliminate the particulate bulk oil material generated when using high energy stirring systems (Singer et al., 2000). The filtrated sea water was poured into a separatory glass funnel, spiked with a solution of anthracene-d_{10}, and extracted three times with 50 mL of dichloromethane (Suprasolv, Merck, Germany). The combined extracts were passed through a glass column filled with cotton wool and 7 g of dried Na_2SO_4 (>99%, Merck) to eliminate residual seawater, and concentrated in a rotary evaporator to 1 mL, followed by a gentle stream of nitrogen, avoiding complete removal of the solvent.

The PAH fraction from the fuel oil was isolated using a glass column (30 cm × 1 cm) packed with 6 g of silica (bottom) (SiO_2, 40–60 mesh, Acros Organics, Belgium), 6 g of aluminium oxide (middle) (Al_2O_3, 70–230 mesh, Merck, Germany) and 2 g of sodium sulphate (top), in hexane, as described in Alzaga et al. (2003). Between 10 and 20 mg of the oil sample was dissolved in hexane, spiked with a solution of anthracene-d_{10} (Acros Organics, Belgium) and pyrene (Sigma–Aldrich, USA) in isooctane and added at the top of the column. The aliphatic hydrocarbons were eluted in the first fraction with 17 mL of hexane (Suprasolv, Merck), and the PAHs with 20 mL of hexane:dichloromethane (2:1, v/v). The recovered fractions were concentrated in a rotary evaporator, followed by a gentle stream of nitrogen until near dryness, redissolved with isooctane and spiked with a solution of thiphenylamine (Sigma–Aldrich) before further analysis by gas chromatography-mass spectrometry (GC/MS).

Quantification of the PAHs was carried out in a Konik HRGC 4000B gas chromatograph coupled to a Konik MS Q12 mass spectrometer (Konik, Sant Cugat del Vallès, Spain). The GC was fitted with a fused silica capillary column (30 m × 0.25 mm I.D. × 0.25 μm film thickness) DB5 MS (Agilent, Santa Clara, USA). The initial column temperature was held for 1 min at 70 °C, then programmed to 320 °C at a rate of 6 °C min^{-1} and kept at this temperature for 10 min. Helium was used as carrier gas at a constant flow of 1.5 mL min^{-1}. The injection was made in the split/splitless mode (splitless time 1 min), keeping the injector temperature at 300 °C. Data were acquired in the selective ion monitoring (SIM) mode at a 70 eV and processed by the Konikrom Data Reduction software. Quantification was performed calculating the response factors for each compound at different concentrations, correcting the values with the internal standards. A solution of 17 PAHs containing acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]flouranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, dibenzo[*a,h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, naphthalene, perylene, phenanthrene and pyrene were used for response factors calculation (Dr. Ehrenstorfer, Germany).

2.4. Spectrofluorimetric analysis of the SWAF

The spectrofluorimetric analysis is a very sensitive technique largely used for the measurement of oil in water (Ali et al., 1995; Gonzalez et al., 2006). Even though the results are dependent of the calibrant and the oil composition, it is a useful method for the rapid monitoring of total aromatic hydrocarbons concentration in water. Therefore, the progress of the dissolution experiment was followed by measuring the fluorescence directly in the water phase using a Surveyor Thermo-Finnigan (Waltham, USA) high performance liquid chromatograph (HPLC), coupled to a SpectraSystem FL3000 fluorescence detector. The system was operated in the off-column mode, by-passing the chromatographic column. Milli-Q water was used as mobile phase at a flow rate of 1 mL min^{-1}. Any dilutions of the sea water were made with deionised water (Milli-Q, Millipore) to keep the detector signal within the linear range of the instrument. Excitation and emission wavelengths were at 254 and 320 nm, respectively, as they coincide closely with the excitation/emission profiles of the naphthalene derivatives (Groner et al., 2001).

Diesel oil solutions (between 1.2 and 4.5 μg L^{-1} equivalents diesel oil) were tested for the calibration of the detector, as reported elsewhere (Ali et al., 1995), because it contains low molecular weigh aromatic hydrocarbons similar to the ones in the SWAF of the fuel oil. A stock solution was prepared in acetone and subsequent dilutions in milli-Q water were made until the desired concentration was reached. The detection limit (DL) was calculated with the formula $DL = Y_B + 3SD$ (Eurachem, 1998), where Y_B and SD where the mean signal and standard deviation of the blank, respectively. The DL was 0.3 μg L^{-1} of diesel equivalents.

3. Results and discussion

3.1. Solubility of the total aromatic hydrocarbons

A summary of the results for all the fuel oil–water accommodation experiments is shown in Fig. 2a (as μg L^{-1} diesel equivalents of dissolved hydrocarbons). As it can be seen in all the experimental set ups, after sonication of the water/fuel oil mixture is completed, the concentration of the soluble fraction increases markedly during the first 24 h of settling time. From then onwards the concentrations of total aromatic hydrocarbons only show a slight relative increase so that they can be considered, in practice, constant in their average value. In fact, some of the changes in the concentration after 24 h can be attributed to the fact that all aliquots were taken from the same preparation flask, producing a small change in the fuel oil to water ratio and a subsequent slight increase of the concentration of total aromatic hydrocarbons in the SWAF. Thus, we conducted an additional set of six experiments under the same experimental conditions, sampling each preparation flask at a different settling time. In this case, no change in the fuel oil to water ratio occurs, and thus no rise in the concentration of SWAF was observed after 96 and 120 h (Fig. 2b). Three independent replicas were performed for each set of experimental conditions, to check the reproducibility of the proposed sonication method. The RSD of all the series ranged from 1% to 5% ($n = 3$), which indicates that ultrasonic mixing is a reproducible method for SWAF preparation.

The improvement in the speed of the process provided by the proposed method can be observed when the results are compared with the ones obtained in the experiment without sonication (Fig. 2a, (5)). The total aromatic hydrocarbons in the water after 24 h reaches 62% of the assumed equilibrium concentration (48.3 μg mL^{-1} equivalents of diesel) that was not attained until after 96 h.

From Fig. 2 it is apparent that the maximum average concentration of the total aromatic hydrocarbons in the SWAF is influenced by both the temperature and salinity of the seawater. Differences were shown to be statistically significant for all the treatments by ANOVA test ($p < 0.01$) and confirmed by HSD-Tukey and Bonferroni tests ($p < 0.05$ for all cases). However, the time required to reach the maximum concentration seems to be independent of these conditions, and was found to be 24 h after sonication was concluded. Therefore, the whole SWAF preparation process using an ultrasonic bath can be completed in little more than 24 h, so that it is suitable for both, chemical analysis and toxicological studies of the SWAF. This system also offers some technical advantages compared to magnetic or vortex mixing when the preparation of several replicas is required. The classical stirring methods require the availability of a number of stirring devices whereas with the same ultrasonic bath as many as wanted replicas can be prepared continuously.

The present experiments have shown that the solubility of the aromatic hydrocarbons increases as the temperature increases and salinity decreases (May and Miller, 1981; Schwarzenbach et al., 2003). Thus, the higher concentrations in the SWAF are obtained at the highest temperature and lowest salinity, and vice versa. In the range of conditions used, the temperature of the seawater has a larger effect on the solubility of the aromatic hydrocarbons than salinity. While the concentration in the SWAF increases an average of 27% over a temperature range of 3–20 °C, only an 8% difference was observed from the more to the less saline seawater. These results are consistent with those found previously

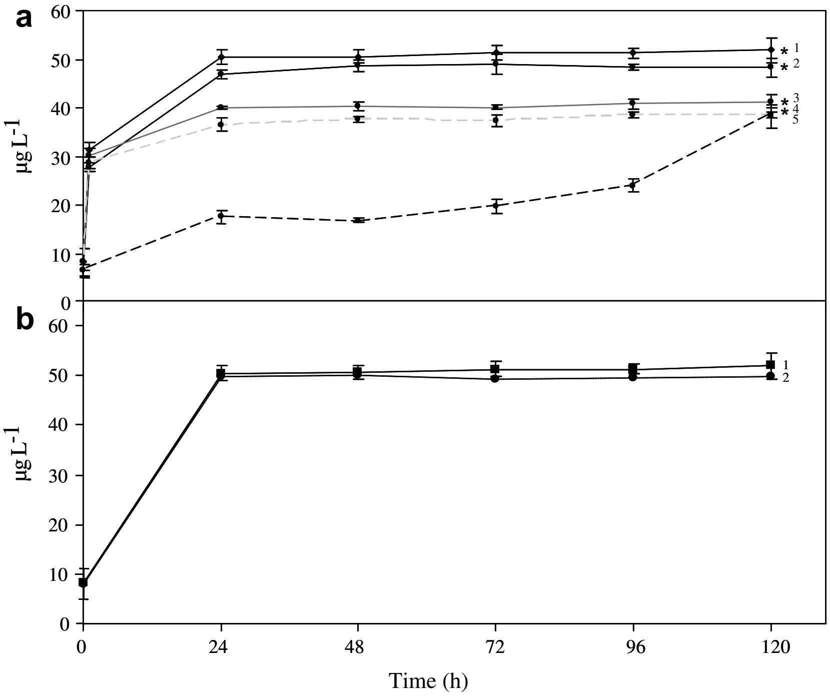

Fig. 2. Average concentration in μg L^{-1} diesel equivalents ($n = 3$) of the total aromatic hydrocarbons of IFO380 fuel oil in seawater with settling time, prepared at different experimental conditions: (a) with sonication, HTLS (1), HTHS (2), LTLS (3), LTHS (4), and without sonication HTHS (5), (b) with sonication (HTLS) and collection of aliquots from the same flask (1) or independent flasks (2). * Means that significantly differ at $p < 0.05$.

in the laboratory and the field (Whitehouse, 1984), where two to five fold increase was observed in the solubility of PAHs when the temperature was risen from 5 to 30 °C. The effect of salinity is even lower, at most by a factor of two when the salinity changed from 36 to 0 psu (May and Miller, 1981; Readman et al., 1982).

3.2. Solubility of individual PAHs

The SWAF of the fuel used in the experiments showed a compound distribution consistent with this type of product (e.g. Barron et al., 1999; Saeed and Al-Mutairi, 2000) (Fig. 3). The most abundant components were two and three ring PAHs and nitrogen heterocycles, that represented 94% of the total concentration of hydrocarbons in the SWAF. Alkanes and PAHs of four or more rings were only found at trace levels (Ali et al., 1995; Saeed and Al-Mutairi, 2000).

Naphthalene and its alkyl derivatives represent between 86% and 90% of the total concentration of PAHs, which agrees with the proportion (89%) reported in tests carried out with similar fuel oils (Gonzalez et al., 2006; Saeed and Al-Mutairi, 2000). Carbazole and its alkyl derivatives are as abundant as the family of naphthalenes despite the fact that they are relatively much less abundant in the fuel oil studied, but this is the result of their higher water solubility (Kraak et al, 1997). As shown in Table 1, the concentration of carbazole in the SWAF is between 71.1% and 89.4% relative to their concentration in the original fuel oil. On the other hand, the solubility decreases with increasing alkylation (Dimitriou et al., 2003), since alkyl groups contribute to the hydrophobicity of the molecule (Schwarzenbach et al., 2003). A similar pattern is observed by the group of naphthalenes and carbazoles, but more evident in the case of the latter.

Both naphthalene and carbazole families are the focus of toxicological concern in marine oil spills and produced waters, not just for their direct action but due to their potential to generate carcinogenic and toxic metabolites in marine organisms (Wilson et al., 1997; Wiegman et al., 1999).

The concentrations of individual compounds in the SWAF varied as a function of the experimental conditions (Fig. 3, Table 1), following over time the patterns observed for the total aromatic hydrocarbons. Generally, the dissolution of individual PAHs in seawater increases as temperature increased and salinity decreased (Schlautman et al., 2004; Tremblay et al., 2005; Viamajala et al., 2007), but the solubility varies for each compound (Fig. 3).

Temperature and salinity show uneven influence in the dissolution of the individual PAHs in the seawater at the experimental conditions of the study. As it can be observed in Fig. 3, even though all the compounds follow the general trend of more abundance at higher temperature, this effect is more pronounced for the heaviest compounds of the SWAF, such as fluorene, phenanthrene, methylphenanthrene, anthracene and dibenzothiophene, exhibiting statistically significant differences (ANOVA test, $p < 0.05$). On the contrary, the influence of salinity is more noticeable for the lighter compounds, such as naphthalene and its alkylated derivatives, which decreased even 2-fold when the seawater salinity raised from 33 to 37 psu. In contrast, 3-ring PAHs show little change in solubility at the two different salinities investigated, a trend observed previously in solubility experiments involving phenanthene and fluorene, where a very slight decrease in their dissolution was observed when the water salinity increased from 0 to 33 psu (Whitehouse, 1984). This can be explained by the “salting out” effect (Schwarzenbach et al., 2003).

This effect can be also observed comparing the variation of total PAHs and *N*-heterocycles abundance according to the preparation conditions. The total PAHs concentration in the aqueous phase reached 25 μg L^{-1} at 20 °C and 22 μg L^{-1} at 3 °C. These concentrations are lower than some found in previous laboratory experiments (67–174 μg L^{-1}) with different fresh fuel oils at several loadings (Hokstad et al., 1999). However, a large range in the PAHs concentrations (ΣPAHs = 171–2176 μg L^{-1}) and distributions of the SWAF of different types of a Kuwaiti crude oil has been re-

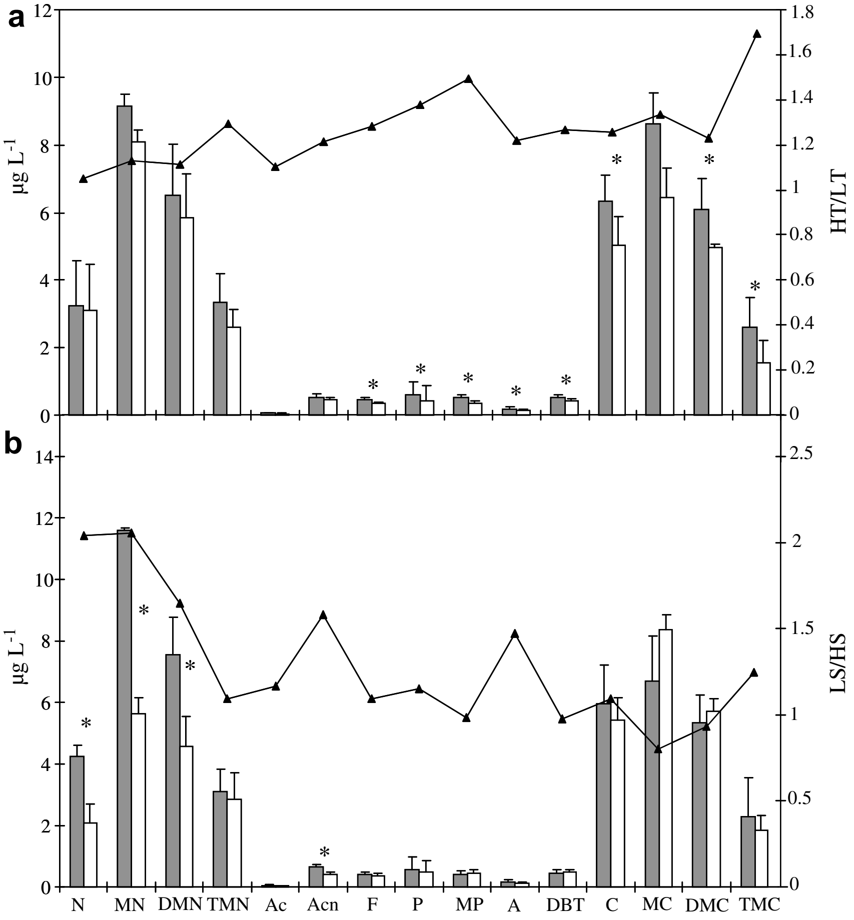

Fig. 3. Individual compound concentrations (bars) and relative difference (lines) under different SWAF preparation conditions: (a) temperature (HT in grey and LT in white), (b) salinity (LS in grey and HS in white). N: naphthalene; MN: methylnaphthalene; DMN: dimethylnaphthalene; TMN: trimethylnaphthalene; Ac: acenaphthylene; Acn: acenaphthene; F: fluorene; P: phenanthrene; A: anthracene; DBT: dibenzothiophene; C: carbazole; MC: methylcarbazole; DMC: dimethylcarbazole; TMC: trimethylcarbazole. * Values significantly different at $p < 0.05$.

Table 1
Percentage of individual compounds of the fuel oil dissolved in the seawater after ultrasonic stirring, relative to their original concentration in the oil, at different preparation conditions

	HT	LT	HS	LS
Naphthalene	1.5	1.4	0.9	1.9
Methylnaphthalene	0.9	0.8	0.6	1.16
Dimethylnaphthalene	0.4	0.3	0.3	0.4
Trimethylnaphthalene	0.3	0.2	0.2	0.3
Carbazole	89.4	71.1	76.5	83.8
Methylcarbazole	12.3	9.2	12.0	9.6
Dimethylcarbazole	10.3	8.3	9.6	8.9
Trimethylcarbazole	3.3	1.9	2.3	2.9

See Fig. 3 for abbreviations.

ported, demonstrating that a great variability exists even within the same type of product (Saeed and Al-Mutairi, 2000).

The preceding experiments indicate that a fractionation of the fuel oil may occur in the ocean, depending on the salinity and temperature characteristics of the water masses in contact with the product. Based on the results obtained in this study, higher concentrations of PAHs and *N*-heterocycles should be found in the warmest and less saline waters of the water column. Nevertheless, the factors controlling the abundance of these compounds in the dissolved phase of the water masses are not limited to salinity and temperature. There exists important factor such as the quantity of dissolved organic matter, the type and quantity of suspended particulate matter, and the type of ions dissolved in the water that also need to be assessed (Xie et al., 1997; Tremblay et al., 2005).

4. Conclusions

We have evaluated a simple system, using a sonication bath, to speed-up the process of producing oil sea water accommodated fractions (SWAF) in the laboratory. We have shown that these can be reproducibly obtained in 24 h, regardless of the experimental conditions (water temperature and salinity), as long as some basic precautions for avoiding the formation of emulsions are adopted, namely excessive vibration of the flask in the sonication bath.

The tests conducted in a heavy fuel oil similar to that carried by the *Prestige* tanker have shown that naphthalene and its alkyl derivatives and *N*-heterocycles of the family of carbazoles were the most abundant hydrocarbons present in the SWAF. Both temperature and salinity affect to some extent the dissolution of the fuel oil in the seawater. The concentration of the total aromatic hydrocarbons in the SWAF increases with the increase of water temperature and the decrease of salinity. Individual PAHs follow the same pattern. Changes in temperature usually found in the open ocean are bound to have a much larger impact in the concentration of PAHs in the SWAF than the corresponding values of sea water salinity. In summary, our laboratory results show that after a spill the highest SWAF should be expected in the warmest and less saline waters of the water column.

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References

Akaishi, F.M., de Assis, H.C.S., Jakobi, S.G.C., Eiras-Stofella, D.R., St-Jean, D., Courtenay, S.C., Lima, E.F., Wagener, A.L.R., Scofield, A.L.S., Oliveira-Ribero, C.A., 2004. Morphological and neurotoxicological findings in tropical freshwater fish (*Astyanax* sp.) after waterborne and acute exposure to water soluble fraction (WSF) of crude oil. Arch. Environ. Con. Tox. 46, 244–253.

Albaiges, J., Morales-Nin, B., Vilas, F., 2006. The Prestige oil spill: a scientific response. Mar. Pollut. Bull. 53, 205–207.

Ali, L.N., Mantoura, R.F.C., Rowland, S., 1995. The dissolution and photodegradation of Kuwaiti crude-oil in seawater1. Quantitative dissolution and analysis of the seawater-soluble fraction. Mar. Environ. Res. 40, 1–17.

Alzaga, R., Montuori, P., Ortiz, L., Bayona, J.M., Albaiges, J., 2003. Fast-solid phase extraction-gas chromatography-mass spectrometry procedure for oil fingerprinting Application to the Prestige oil spill. J. Chromatogr. A 1025, 133–138.

Barron, M.G., Podrabsky, T., Ogle, S., Ricker, R.W., 1999. Are aromatic hydrocarbons the primary determinant of petroleum toxicity to aquatic organisms? Aquat. Toxicol. 46, 253–268.

Dimitriou-Christidis, P., Harris, B.C., McDonald, T.J., Reese, E., Autenrieth, R.L., 2003. Estimation of selected physicochemical properties for methylated naphthalene compounds. Chemosphere 52, 869–881.

Eurachem, 1998. The fitness for purpose of analytical methods. A laboratory guide to method validation and related topics. In: Eurachem Guide (first edition), Teddington, Middlesex. (http://www.eurachem.org).

Fernandez, N., Cesar, A., Salamanca, M.J., Del Valls, T.A., 2006. Toxicological characterisation of the aqueous soluble phase of the Prestige fuel-oil using the sea-urchin embryo bioassay. Ecotoxicology 15, 593–599.

Gonzalez, J.J., Vinas, L., Franco, M.A., Fumega, J., Soriano, J.A., Grueiro, G., Muniategui, S., Lopez-Mahia, P., Prada, D., Bayona, J.M., Alzaga, R., Albaiges, J., 2006. Spatial and temporal distribution of dissolved/dispersed aromatic hydrocarbons in seawater in the area affected by the Prestige oil spill. Mar. Pollut. Bull. 53, 250–259.

Groner, M., Muroski, A.R., Myrick, M.L., 2001. Identification of major water-soluble fluorescent components of some petrochemicals. Mar. Pollut. Bull. 42, 935–941.

Hokstad, J.N., Daling, P.S., Buffagni, M., Johnsen, S., 1999. Chemical and ecotoxicological characterisation of oil–water systems. Spill Sci. Technol. B 5, 75–80.

Khan, M.A.Q., Alghais, S.M., Al-Marri, S., 1995. Petroleum-hydrocarbons in fish from the Arabian Gulf. Arch. Environ. Con. Tox. 29, 517–522.

Kraak, M.H.S., Ainscough, C., Fernandez, A., vanVlaardingen, P.L.A., Voogt, P., Admiraal, W.A., 1997. Short-term and chronic exposure of the zebra mussel (*Dreissena polymorpha*) to acridine: effects and metabolism. Aquat. Toxicol. 37, 9–20.

Maki, H., Sasaki, T., Harayama, S., 2001. Photo-oxidation of biodegraded crude oil and toxicity of the photo-oxidized products. Chemosphere 44, 1145–1151.

Martinez-Jeronimo, F., Villasenor, R., Rios, G., Espinosa-Chavez, F., 2005. Toxicity of the crude oil water-soluble fraction and kaolin-adsorbed crude oil on Daphnia magna (Crustacea: *Anomopoda*). Arch. Environ. Con. Tox. 48, 444–449.

May, W.E., Miller, M.M., 1981. High-performance liquid-chromatographic methods for determining aqueous solubilities octanol–water partition-coefficients and ambient-temperature vapor-pressures of hydrophobic compounds. Abstr. Pap. Am. Chem. Soc. 182 (Aug), 35 (PEST).

Navas, J.M., Babin, M., Casado, S., Fernanadez, C., Tarazona, J.V., 2006. The Prestige oil spill: a laboratory study about the toxicity of the water-soluble fraction of the fuel oil. Mar. Environ. Res. 62, S352–S355.

Ohwada, K., Nishimura, M., Nomura, H., Shibata, A., Okamoto, K., Toyoda, K., Yhoshida, A., Takada, H., Yamada, M., 2003. Study of the effect of water-soluble fractions of heavy-oil on coastal marine organisms using enclosed ecosystems, mesocosmos. Mar. Pollut. Bull. 47, 78–84.

Page, C.A., Bonner, J.S., Sumner, P.L., Autenrieth, R., 2000. Solubility of petroleum hydrocarbons in oil/water systems. Mar. Chem. 70, 79–87.

Rayburn, J.R., Glas, P.S., Foss, S., Fisher, W.S., 1996. Characterization of grass shrimp (*Palaemonetes pugio*) embryo toxicity tests using the water soluble fraction of number 2 fuel oil. Mar. Pollut. Bull. 32, 860–866.

Readman, J.W., Mantoura, R.F.C., Rhead, M.M., Brown, L., 1982. Aquatic distribution and heterotrophic degradation of polycyclic aromatic-hydrocarbons (Pah) in the Tamar Estuary. Estuar. Coast. Shelf Sci. 14, 369–389.

Ruiz-Villarreal, M., Gonzalez-Pola, C., Diaz del Rio, G., Lavin, A., Otero, P., Piedracoba, S., Cabanas, J.M., 2006. Oceanographic conditions in North and Northwest Iberia and their influence on the Prestige oil spill. Mar. Pollut. Bull. 53, 220–238.

Saeed, T., Al-Mutairi, M., 2000. Comparative composition of volatile organic compounds in the water-soluble fraction of different crude oils produced in Kuwait. Water Air Soil Pollut. 120, 107–119.

Schlautman, M.A., Yim, S.B., Carraway, E.R., Lee, J.L., Herbert, B.E., 2004. Testing a surface tension-based model to predict the salting out of polycyclic aromatic hydrocarbons in model environmental solutions. Water Res. 38, 3331–3339.

Schwarzenbach, R.P., Gschwend, P.M., Imboden, D.M., Dieter, M., 2003. Environmental organic chemistry, second ed. John Wiley & Sons, Inc., New Jersey.

Singer, M.M., Aurand, D., Bragin, G.E., Clark, J.R., Coelho, G.M., Sowby, M.L., Tjeerderma, R.S., 2000. Standardization of the preparation and quantitation of water-accommodated fractions of petroleum for toxicity testing. Mar. Pollut. Bull. 40, 1007–1016.

Tremblay, L., Kohl, S.D., Rice, J.A., Gagne, J.P., 2005. Effects of temperature salinity and dissolved humic substances on the sorption of polycyclic aromatic hydrocarbons to estuarine particles. Mar. Chem. 96, 21–34.

Viamajala, S., Peyton, B.M., Richards, L.A., Petersen, J.N., 2007. Solubilization, solution equilibria, and biodegradation of PAH's under thermophilic conditions. Chemosphere 66, 1094–1106.

Whitehouse, B.G., 1984. The effects of temperature and salinity on the aqueous solubility of polynuclear aromatic-hydrocarbons. Mar. Chem. 14, 319–332.

Wiegman, S., Van Vlaardingen, P.L.A., Peijnenburg, W.J.G.M., van Beusekom, S.A.M., Kraak, M.H.S., Admiraal, W., 1999. Photokinetics of azaarenes and toxicity of phototransformation products to the marine diatom *Phaeodactylum tricornutum*. Environ. Sci. Technol. 33, 4256–4262.

Wilson, A.S., Davis, C.D., Williams, D.P., Buckpitt, A.R., Pirmohamed, M., Park, B.K., 1997. Characterisation of the toxic metabolite(s) of naphthalene. Toxicology 120, 75.

Xie, W.H., Shiu, W.Y., Mackay, D., 1997. A review of the effect of salts on the solubility of organic compounds in seawater. Mar. Environ. Res. 44, 429–444.

Ziolli, R.L., Jardim, W.F., 2002. Operational problems related to the preparation of the seawater soluble fraction of crude oil. J. Environ. Monit. 4, 138–141.