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ScienceDirect
Marine Micropaleontology 61 (2006) 76–93
MARINE MICROPALEONTOLOGY
www.elsevier.com/locate/marmicro

Benthic foraminiferal response to experimentally induced *Erika* oil pollution

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Received 14 July 2005; accepted 23 May 2006

Abstract

Benthic foraminifera from an intertidal mudflat (Bay of Bourgneuf, France) have been exposed to different types of oil-polluted seawater in an experimental laboratory setting. The aim of this experiment was to study the response of foraminiferal faunas from the intertidal zone to oil spills, as observed after the wreckage of the *Erika* oil tanker in December 1999 on the French Atlantic coast. In the course of the experiment a saturated seawater mix (SSW) and a water accommodated fraction of the oil (WAF) were on several occasions added to a part of the mesocosms, and the response of the foraminiferal assemblages was monitored during 3 months after the start of oil treatments. Several potential effects of oil-pollution were studied: 1) foraminiferal standing stocks, 2) anomalous growth patterns resulting in deformed or abnormal foraminiferal tests, and 3) changes in the taxonomical composition of the assemblage.

The foraminiferal assemblages at the start of the experiment were dominantly composed of *Haynesina germanica*. The experimental results show a strong decline in density in all mesocosms (including the control mesocosms) over time. During the first 2 weeks of the experiment, this decline was stronger in the oil-treated mesocosms than in the control mesocosms. After the first 2 weeks, the replicates of the oil-treatments showed an extreme variability, whereas the control mesocosms showed less variability with densities steadily decreasing over time. In some of the oil-treated mesocosms, we observed strongly increased densities, indicative of reproductive events. In all other oil-treated mesocosms foraminiferal densities decreased more severely than in the control mesocosms. The different types of oil–seawater mixtures did not cause a significantly different response. After 3 months, some taxa that had not been encountered previously in the field samples or in the mesocosms (e.g., *Textularia earlandi*) appeared in the 63–125 μm fractions of the oil-treated mesocosms. We conclude that there is a dual response to oil-induced pollution: foraminiferal faunas may respond by a strongly increased mortality, and/or by an accelerated reproduction of some of the taxa.

Keywords: benthic foraminifera; mesocosm experiment; oil pollution; *Erika* wreckage; France

1. Introduction

Worldwide accidents and wreckages of oil tankers are a cause of great public concern, especially because of the clear visual ecological impact of such incidents. However, these accidents are not the major causes of oil

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doi:10.1016/j.marmicro.2006.05.005

dispersal in the marine environment. Of all oil released in the marine environment about 33% is generated by the transport of oil; only one quarter of this quantity (8% of all oil) is caused by accidents and major spillages (Kennish, 1992). Despite the fact that oil tanker accidents are not the major contributors to oil released in the natural environment, they have raised a wide public concern by showing that oil pollution has profound effects on the sea and its inhabitants. For example, accidents of the Amoco Cadiz, the Exxon Valdez, the *Erika* and the recent wreckages of the Prestige and the Jessica have had great impact on the awareness of the hazards involved in oil transports (e.g., Lee and Page, 1997; Baars, 2002; Marshall and Edgar, 2003; Zenetos et al., 2004). The more invisible release of oil and its toxic compounds in nature is probably of even greater concern to us, especially since about one third of all oil released is ultimately accumulating in estuaries (Kennish, 1992), that contain a wide range of very fragile environments.

Several studies have shown the impact of oil pollution on various benthic organisms, such as bivalves, gastropods, copepods, nematodes and sea urchins (Temara et al., 1999; Hamoutene et al., 2002; Lee et al., 2002; Le Hir and Hily, 2002; Suderman and Thistle, 2003; Martinéz-Jerónimo et al., 2005). Effects range from lethal, carcogenic and/or mutagenic effects. Different oils have specific compositions and the proportions of toxic components vary strongly (Singer et al., 2000; Tsvetnenko and Evans, 2002). Crude oils are composed of many thousands complex gaseous, liquid and solid organic compounds of which hydrocarbons are the most abundant (Kennish, 1992). Import constituents are the alkanes (parrafins), cycloalkanes (cycloparrafins, naphtalenes), alkenes, alkynes and the aromatic hydrocarbons including polynuclear or polycyclic hydrocarbons (PAHs). This last group forms the most toxic part of the oil and generally the toxicity increases from alkanes, cycloalkanes, and alkenes to the aromatics. Already in the first hours following oil spills the composition of the released oil changes significantly. A number of processes are responsible for this compositional change: spreading, evaporation, photochemical oxidation, dissolution, emulsification, sedimentation, adsorption and microbial degradation (Kennish, 1992; Kingston, 2002). After being released in the marine environment, it mostly takes at least a few hours to transport the oil into the coastal zone. During this time interval, the aforementioned processes will strongly affect the composition and toxicity of the oil.

In December 1999 the oil tanker *Erika* wrecked 80 miles off the coast of Brittany, France. This accident caused 5.8 million gallons of heavy fuel oil to be released in the marine environment. The transported oil was lowly volatile, had a poor solubility and a low dispersal potential (for a specific analysis of the *Erika* oil see IFP, 2003). In the weeks/months following the accident, the oil was washed ashore along a 400 km stretch of the French coastline.

Live foraminifera are increasingly used and studied for their value as bioindicators in a wide range of marine environments (e.g., Alve, 1991, 1995; Yanko et al., 1994, 1999; Debenay et al., 2001; Samir, 2000; Samir and El-Din, 2001; Armynot du Châtelet et al., 2004; Saraswat et al., 2004). Foraminifera are valuable since they respond fast to environmental changes, occur in high densities and produce a fossil record giving access to the pre-pollution background faunas. Since the degree of bioavailability of toxic compounds cannot be derived directly from analyses of sediments or waters, the study of foraminiferal faunas that give an integrated account of the effects of pollution is important. However, very few studies dealing with the effects of natural or anthropogenic oil pollution on benthic foraminifera have been performed until today (e.g., Vénec-Peyré, 1981; Yanko et al., 1994; Alve, 1995; Bernhard et al., 2001). Since January 2000 a field survey has been carried out in tidal mudflats and marshes in the Bay of Bourgneuf (French Atlantic coast), that were heavily affected by the *Erika* oil spill (see Baars, 2002), with the aim to study the effect of the *Erika* oil pollution on the benthic foraminiferal faunas (Morvan et al., 2004). Further, a series of monospecific cultures and in vitro experiments with specimens of *Ammonia tepida* were carried out under laboratory conditions (Le Cadre, 2003; Morvan et al., 2004). These experiments were designed to test the impact of different quantities of *Erika* oil mixed in seawater on populations of this taxon. The results of both field and laboratory studies urged the need for a series of complementary mesocosm experiments, in which an oil spill was simulated under controlled environmental conditions and in which foraminifera were kept as far as possible in natural conditions, i.e., in original sediments. For this purpose, 26 mesocosms were incubated and two different methods of introducing oil in the experimental environment were tested over a period of 4 months. We did not particularly intend to study a mono-specific assemblage as was performed in previous experiments with *Ammonia beccarii* (Le Cadre, 2003). However, these intertidal environments require special capacities from organisms experiencing regularly extreme environmental variability. In these environments only few foraminiferal taxa thrive and often the assemblages are

strongly dominated by a single taxon, with seasonally or temporally changing dominance (e.g., Murray and Alve, 2000). Since the experimental period may be too short to monitor the recovery of the foraminiferal assemblages or to observe a potential ecological succession of the assemblage, the present study can be considered as complimentary to more long-term field studies (Ellis, 2003; Morvan et al., 2004). The focus of this study is to look especially at the initial and short-term (<4 months) response of foraminiferal assemblages to oil pollution.

2. Materials and methods

2.1. Collection site and incubation

Sediment was collected at the Port du Collet in the Bay of Bourgneuf (Fig. 1). The collection site is located on an intertidal mudflat falling dry at low tide, with salt marshes in the near vicinity.

During low tide (February 4, 2003), a total of about 15 l of superficial (the upper 5 to 10 mm) sediment was collected on the mudflat. Surficial material was collected in the field since most foraminifera live in the surficial 5 to 10 mm of the sediment in these environments (e.g., Richter, 1964, 1965). The foraminiferal taxa in these intertidal environments are known to be capable of rapid migration (Richter, 1965; Lee et al., 1969) after burial and we expected that incubating 7 cm of superficial sediment would result in high densities of living foraminifera near the sediment–water interface. Further, a sediment column is necessary to keep open the possibility that foraminifera migrated or survived deeper in the sediment as an escape for the introduced oil. Foraminifera in these environments are found alive up to depths of 6 cm (Richter, 1964; Langer et al., 1989). Finally, the incubation of 7 cm of sediment allowed the development of the various redox zones in the experimental situation.

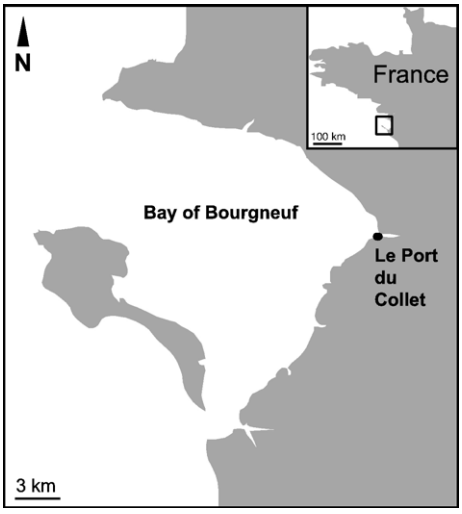

Fig. 1. Map of the Bay of Bourgneuf area with the location of the collection site.

The sediment was stored in 2 cooling boxes and covered by ambient seawater. Upon arrival in the laboratory at the same day, a sample was taken from each cooling box (Fig. 2; $T=0$, sub-sample boxes 1 and 2). The next day (February 5, 2003) the sediment from both cooling boxes was mixed and gently sieved over a 0.5 mm mesh-size screen in order to get rid of large metazoans, shell and plant debris that would strongly disturb the mesocosms. After sieving the sediment was randomly divided over two large containers that were left to settle for 2 days. A 9×6 grid (54 squares of 36 cm^2) was constructed and pushed into the sediment in both containers in order to obtain equal volumes of sediment to be incubated in the mesocosms. As mesocosms, we used Plexiglas tubes (∅=6 cm) closed at the bottom with PVC-stoppers. Each mesocosm was filled with 4 randomly chosen portions, two of the middle and two of the outer part of the containers. Afterwards, the mesocosms were filled with natural microfiltered (0.2 μm) seawater and left for 1 day in order to have all sediment settled, allowing us to introduce an air supply into each individual mesocosms. Two air pumps (Rena air 400) were used to construct a series of tubes with air stones in order to introduce air into the water column of the mesocosms. In order to avoid excessive evaporation, each mesocosm was covered with parafilm. The mesocosms were placed in an incubator (Snijders ECD10E) with UV-lights creating a day/night cycle of 10/14 h with temperatures of 18/16 °C.

2.2. Preparation of the oil–water mixture

In order to introduce the oil into the mesocosms two different methods were used. In both cases we used a sample of *Erika* oil supplied by CEDRE (Centre de Documentation de Recherche et d'Expérimentations sur les pollutions accidentelles des eaux), Brest. Although several suggestions and propositions are made (Singer et al., 2000), a widely accepted standard method for introducing oil in water, and testing its ecotoxicity, is still lacking. In this paper, we have followed two methods:

1) The first method consisted of the preparation of a saturated mixture of soluble oil compounds in seawater (IFP, 2003). This oil-saturated seawater (SSW) was

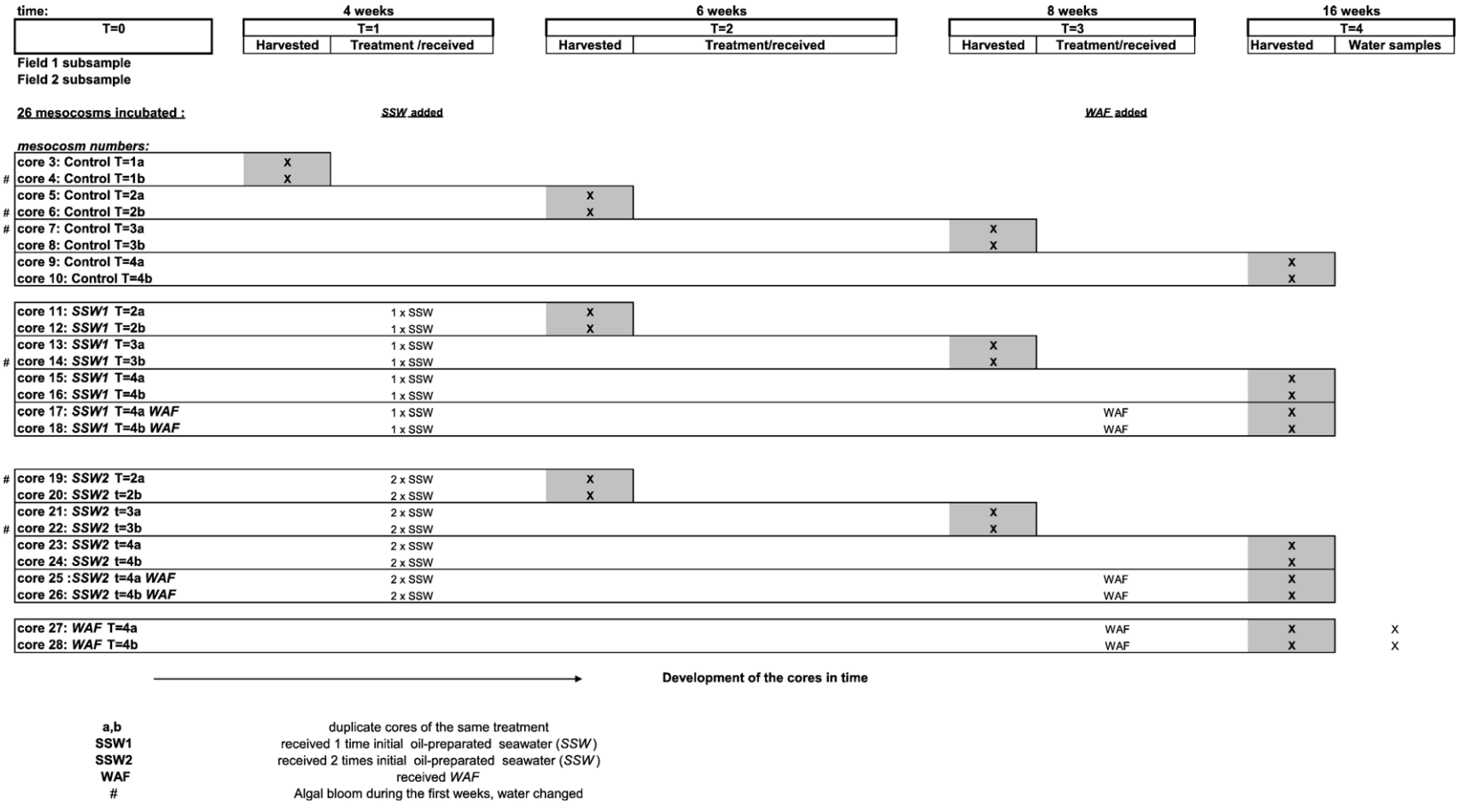

Fig. 2. Schematic representation of the experiment. See text for explanation.

prepared by mixing rigorously 25 ml of *Erika* oil with 5 l of 0.2 μm-filtered seawater, and this mixture was left for 5 days in a closed jerry can. After 5 days, large oil droplets floating at the surface were removed, and this oil–seawater mixture was introduced in equal volumes (0.5 l) into the mesocosms.

2) The second method consisted of the preparation of a water accommodated oil fraction (WAF) as described by Singer et al. (2000). This method involves mechanically mixing of water and oil under dark conditions to artificially increase the load of soluble oil compounds in (sea) water. A volume of 5 ml of oil was placed on the surface of a volume of 4.5 l of seawater in a 5 l glass flask. A magnetic stirring device was used to mix for 18 h at a speed of about 200 rpm, without creating a visible vortex at the water surface. Afterwards, the remaining oil-droplets floating at the surface were removed with a filter paper, and the mixture was introduced in equal volumes (0.5 l) into the mesocosms. After preparation, a sample of the WAF-mixture was taken and stored in a Nalgene® jar in order to be analyzed for its oil constituents.

2.3. Experimental design

All treatments were performed twice in order to have, to a certain degree, control on the variability between replicates of the same treatments. No sub-samples were taken out of the mesocosms; pseudo-replication was avoided by sampling entire mesocosms. A schematic overview of the experimental setup, including all the mesocosms, the received treatments, and the times of harvesting is reported in Fig. 2.

After the initial treatments of collection and sieving at the start of the experiment, 26 mesocosms were incubated ($T=0$). In total, 8 mesocosms (cores 3–10) were left unaffected to be used as controls, and at each sampling date two of these control mesocosms were harvested.

After 3 weeks, in 6 mesocosms (Fig. 2; cores 4, 6, 7, 14, 19 and 22) an algal bloom developed suddenly. In the incubator, these 6 mesocosms were located closest to the UV-lights. The water in these 6 mesocosms was replaced with fresh 0.2 μm filtered seawater, and they were marked (see Fig. 2) so that we were able to notice any potential effects of this algal bloom. After replacing the seawater, the UV-lights were covered from the sides in order to reduce their direct impact, and to have a more equal light distribution to all mesocosms, coming from above.

After 4 weeks ($T=1$) the experimental treatment started. Two control mesocosms (Fig. 2; cores 3 and 4) were harvested. At $T=1$, in two series of mesocosms (SSW1 and SSW2, cores 11–26) the seawater was replaced with 0.5 l of SSW. After another 2 days in half of these mesocosms (the SSW2 series, cores 19–26) the oil–seawater mixture was replaced again with fresh SSW. We decided to renew the water in these 8 cores, in order to increase again the bioavailability of oil compounds in case the oil compounds present in the first volume of SSW had been adsorbed to organic matter or on the glass surface of the mesocosm.

After two more weeks ($T=2$, 6 weeks) the first series of oil-treated (cores 11–12, 19–20) and another couple of control mesocosms (cores 5–6) were harvested, a procedure that was repeated after another 2 weeks ($T=3$, 8 weeks, harvesting of treated cores 13–14, 21–22 and control cores 7–8).

At $T=3$ (8 weeks), in 2 remaining mesocosms of each of the three previous types (i.e., control, SSW1 and SSW2, cores 27–28, 17–18 and 25–26) 0.5 l of WAF (water accommodated fraction) was introduced. Finally, after another 8 weeks (16 weeks in total, $T=4$) all 12 remaining mesocosms were harvested.

2.4. Sampling protocol and analytical analyses

Sampling of the experimental mesocosms involved a number of steps, which were followed during all sampling exercises in the course of the experiment. Firstly, salinity levels (using the Practical Salinity Scale) and pore-water oxygen profiles (ml O_2/l) were measured in the mesocosms. Salinity levels were also measured in the mesocosms every week with a refractometer (ATAGO 10, precision 1‰). Oxygen concentration was measured in the overlying water column and in the pore-water of the sediment by using an electrode (Unisense OX500) and picoamperemeter (Unisense PA2000). The electrode was deployed by using a micromanipulator (Unisense MM33) allowing measuring increments of 0.25 mm. Seawater collected from the mesocosms was aerated in a small chamber for half an hour and afterwards measured, and served as calibration value for seawater fully saturated in oxygen. In order to calibrate the zero-oxygen level a measurement in the

Table 1
Measured oil constituents in the WAF of this experiment and the measurements of the IFP on a saturated oil–seawater (based on IFP, 2003)

	WAF	SSA (IFP)
Fluoranthene	0.020	0.6
Benzo(a)anthracene	0.330	<0.02
Benzo(b)fluoranthene	0.500	<0.010
Pyrene	0.065	0.6
Benzo(a)pyrene	0.250	<0.14

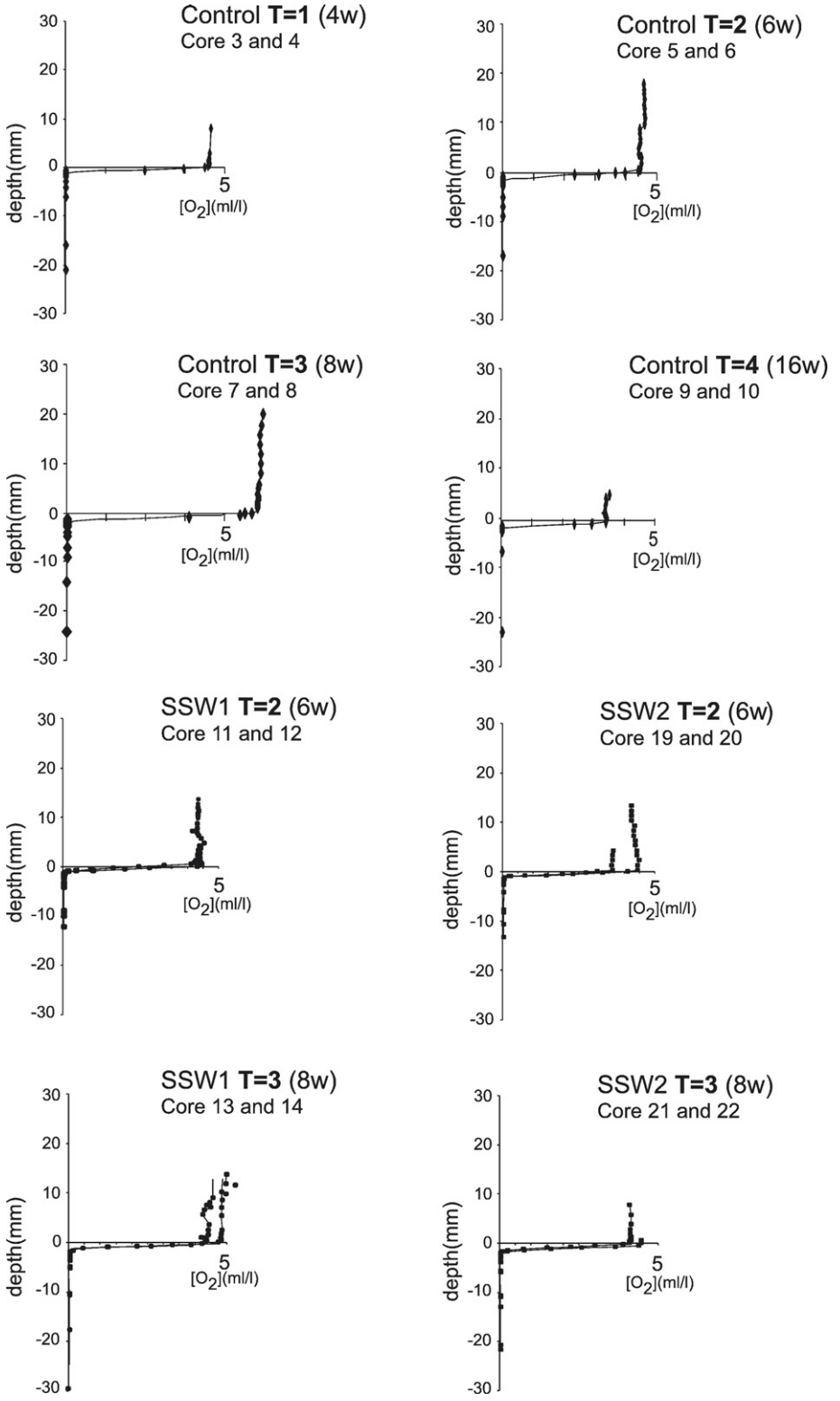

Fig. 3. Oxygen profiles in the sediment of the control ($T=1$–$T=4$) and the treatments with oil-saturated seawater (SSW, $T=2$ and $T=3$).

anoxic sediments at 5 to 6 cm depth in the mesocosms was taken. Levels of the pH in the individual mesocosms were measured by using PANPEHA special papers, pH range 0–14. The mesocosms were photographed and any special observations were noted. Water samples were taken and stored in Nalgene© bottles and finally the core was pushed upwards using a stopper at the bottom and the sediment was sliced in 0.5 or 1 cm thick intervals by using rings of fixed thickness. The sediment was stored in jars containing 96% ethanol with Rose Bengal (1 g/l). Samples were sieved over 150, 63 and 38 μm mesh-size screens. After being dried, the foraminiferal tests were separated from the mineral fraction by a flotation technique using the high-density liquid trichlorethylene (Cl_3C_2H, d=1.46). The well-stained tests have been picked dry in the size-fractions 63–150 and >150 μm. In case of high foraminiferal densities, an Otto microsplitter was used to make a split of the sample containing at least 300 foraminifera. Foraminiferal abundances are standardized to a volume of 50 cc for all analyses.

2.5. Analyses of the WAF mixture

The analyses of the PAHs content (Table 1) in the WAF mixes were carried out by two different methods, firstly by GC-MS (Saturn 2 Varian, column CP SIL 5 CB, ion-trap technology) after extraction from sea water by hexane, and secondly, for the samples with lowest PAHs contents, by Laser-induced fluorescence (system LYOPO: Nd:Yag laser

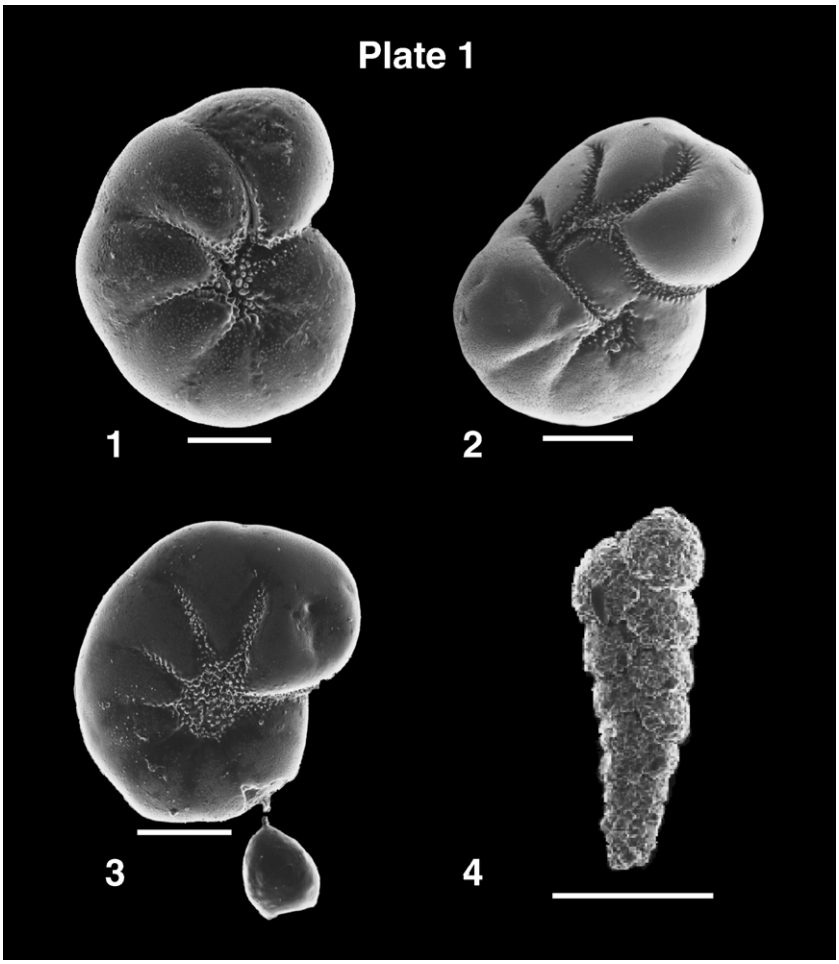

Plate 1. All scalebars represent 100 μm.

1. *Haynesina germanica* (Ehrenberg).
2. Deformed test of *H. germanica*.
3. *H. germanica* with turbelarian cocoon.
4. *Textularia earlandi* (Parker).

associated with an Optical Parametric Oscillator and a ICCD camera allowing temporal resolution. The latter technique makes it possible to reach a detection limit (3 sigma) in seawater of 0.3 ng/l in the case of benzo-*a*-pyrene. Values below this limit will be indicated as zero.

3. Results

3.1. O_2-measurements, salinity, and pH

The oxygen profiles of the control mesocosms show no significant variability between the treatments and in time during the experimental period from $T=1$ to $T=4$ (Fig. 3). Very similar oxygen profiles are observed in the control cores and in the treatments with oil-saturated seawater (SSW) at $T=2$ and $T=3$ (Fig. 3). The average oxygen penetration depth is at most 2 mm in the measured treatments. This lies well within the sample resolution of 5 mm used for the foraminiferal faunas. In the course of the experiment the salinity was checked every week and kept between 30 and 32 psu. In case of increased salinity levels droplets of distilled water were added to decrease the salinity. The pH-levels did not vary in the mesocosms during the experimental period.

3.2. Oil compounds

The measurements of oil compounds in the oil-saturated waters SSW1 and SSW2 were strongly hampered by the presence of large amounts of phtalates due to storage in a jerry can. In the second phase of the experiment, when a WAF was used, all contact with plastics was avoided, and concentrations of the various oil compounds in the initial WAF could be determined. The five measured oil components (Table 1) in the WAF (prior to addition to the mesocosms) include the most toxic PAHs found in oils. In the table the values of these PAHs are compared with the concentrations encountered in a saturated mixture of *Erika* oil and seawater, prepared and analyzed by the IFP (IFP, 2003). We decided to compare the composition of our WAF with the measurements of IFP on their saturated oil–seawater mixture, because the preparation methods for the two mixtures are rather similar. This comparison shows that the values of benzo(*a*)anthracene, benzo(*b*)fluoranthene and benzo(*a*)pyrene (considered as the most toxic component), have higher concentrations in the WAF mixture prepared in our experiment than in the WAF prepared by IFP.

At $T=4$, 8 weeks after the addition of the oil–water mixture, no more detectable oil compounds (>0.3 ng/l) were found in the mesocosms.

3.3. Composition of the foraminiferal assemblage

The foraminiferal assemblages consisted mainly of *Haynesina germanica* (Plate 1, Fig. 1), often comprising almost 100% of the total standing stock of living (Rose Bengal stained) foraminifera. Other Rose Bengal stained taxa encountered in the samples, although infrequent and mostly in low densities, are *A. tepida* and *A. beccarii*

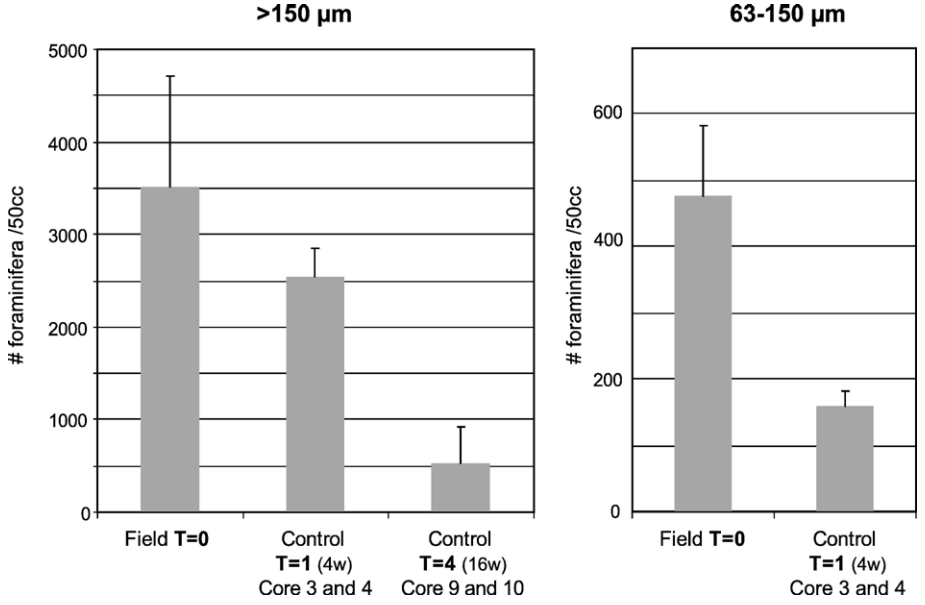

Fig. 4. Average standardized standing stock of the field samples (superficial 1 cm) and the control treatments at $T=1$ and $T=4$ (2σ, $n=2$). All data have been standardized for 50 cc.

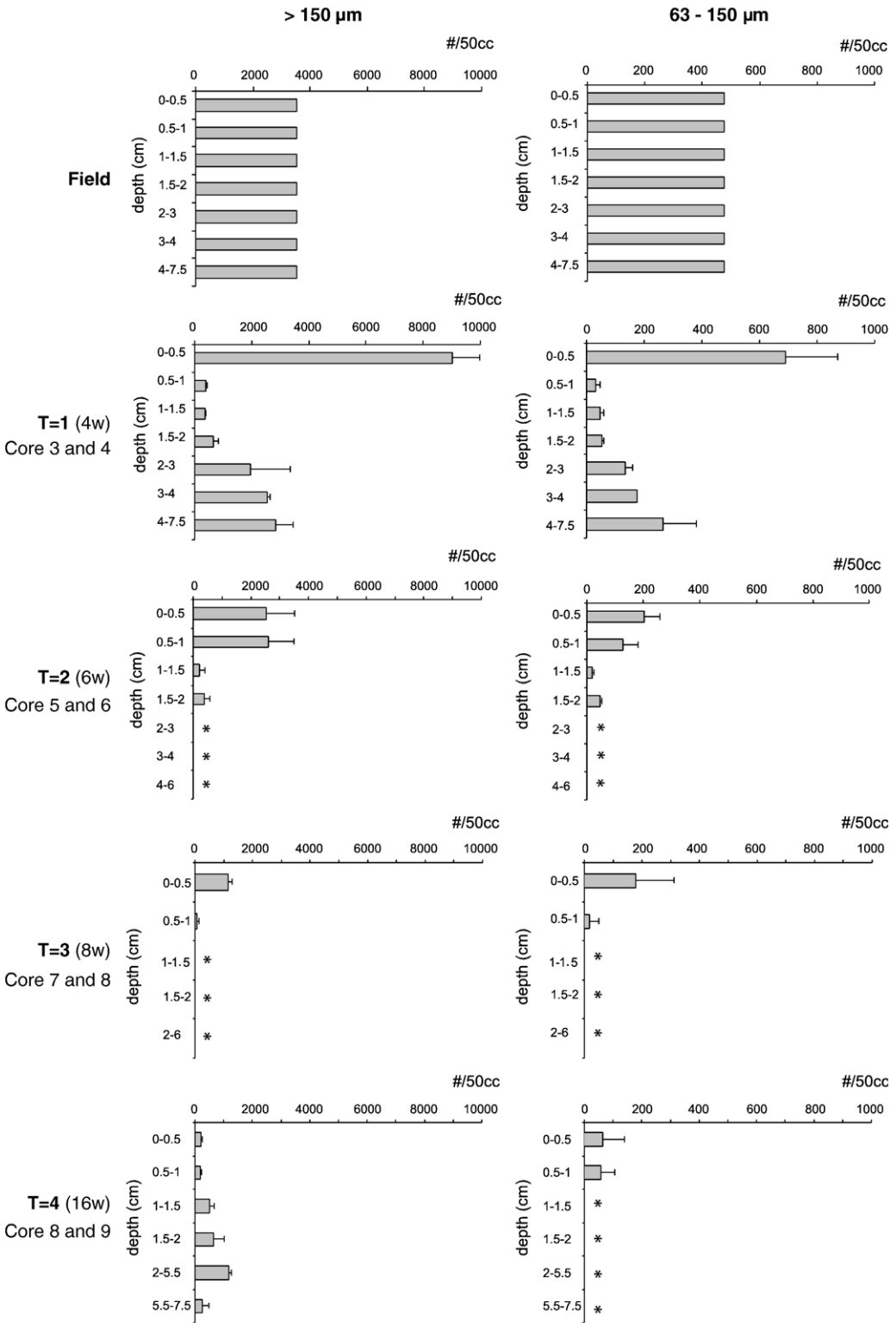

Fig. 5. Vertical distribution of the control treatments in the >150 µm fraction and in part of the mesocosms in the 63–150 µm fraction (2σ, $n=2$) during the experiment. All data have been standardized for 50 cc (∗ no data available).

(grouped in *Ammonia* spp.), *Bolivina variabilis*, *Quinqueloculina seminula* and *Textularia earlandi* (Plate 1, Fig. 4), *Cribroelphidium gunteri*, *Cribroelphidium excavatum*, *Aubignyna planidorsa*, *Stainforthia fusiformis*, *Buliminella elegantissima* and *Reophax* spp.

3.4. Control mesocosms vs. field

The total faunal densities (>150 μm and 63–150 μm fractions) of the superficial sediment (0–1 cm) sampled in the field are compared (Fig. 4) to the foraminiferal densities observed in the control treatment mesocosms at $T=1$ (cores 3 and 4) and $T=4$ (cores 9 and 10). In order to calculate the standardized foraminiferal density (per 50 cc) in the 7,5 cm sediment columns sampled at $T=1$ and $T=4$, that were originally entirely filled with superficial (upper ~1 cm) sediment, the total numbers of individuals in all levels were added, divided by the total sediment volume in the core, and multiplied by 50. The standing stock (>150 μm) in the field samples in the superficial 1 cm of the sediment shows an average density of 3500 foraminifera per 50 cc (Fig. 4). After 4 weeks, densities in the control treatments ($T=1$, >150 μm) were lower (averagely 2538 specimens) and after 16 weeks ($T=4$) the total standing stock decreased to an average of 532 foraminifera per 50 cc (Fig. 4). For the 63–150 μm fraction there is a strong decrease between the field density ($T=0$, ~470 specimens per 50 cc) and the control situation at $T=1$ (core 3 and 4, ~160 specimens per 50 cc).

The mixing of the superficial sediment at the start of the experiment ($T=0$) will cause an initial homogeneous depth distribution of the total fauna, theoretically without any microhabitat partitioning (Fig. 5). In the course of the experiment the vertical distribution of both size fractions shows some perspicuous changes. At $T=1$ both the >150 μm and the 63–150 μm fractions show a skewed distribution with a strong maximum in the first half cm, and minimum values between 0.5 and 2 cm. However, significant proportions of stained specimens stained were also found at deeper levels in the sediment (Fig. 5, 2–7.5 cm). This suggests that in the 4 weeks after incubation, an important part of the specimens (but not all) of the 0.5–2 cm interval have migrated towards the shallowest sediment layer. Apparently, specimens of still deeper sediment intervals (> 2 cm) were not able to do so. At $T=2$, when no data are available for the sediment layers below 2 cm, most specimens are found in the upper 1 cm. At $T=3$, when only data for the upper 1 cm are available, a peak can be noticed in the upper 0.5 cm. At $T=4$ the density of stained specimens is highest in intermediate depth levels (2–5.5 cm). The standing stock in the shallow surface layer has strongly decreased now. In all cases, the data for the smaller size fraction (63–150 μm) display very similar patterns as those observed in the larger size fraction (Fig. 5).

3.5. Oil-treated mesocosms

The resulting total standing stocks of the upper 1 cm of each (replicate) mesocosm of the control and oil (replicate) treatments are for both size fractions summarized in Fig. 6a,b. Starting point of all treatments is the situation at $T=1$, showing a high total standing stocks for both size-fractions. At $T=2$, the total standing stocks in the >150 μm as well as 63–150 μm fraction are lower in the oil-treated mesocosms (cores 11, 12, 19 and 20) than in the control treatments (cores 5 and 6). Only in oil-treated core 12, the values are of the same order of magnitude as those observed in the control cores. Values in the SSW2 treatments (cores 19 and 20, treated twice with SSW) are in both size fractions lower than those in the SSW1 treatments (cores 11 and 12, only one treatment with SSW). At $T=3$, for two oil-treated cores (14 and 22), values in both size fractions are much lower in the treated cores than in the reference cores (7 and 8). In core 21, values are about comparable (although the density is very low in the 63–150 μm fraction), but in core 13, the values in the oil-treated core are about 10 times higher than those of the reference cores, in the >150 μm as well as in the 63–150 μm fraction. At $T=4$, next to two control cores (9–10), 4 cores treated only with SSW (15–16, 23–24), 2 cores treated only at $T=3$ with WAF (25–26) and 4 cores treated both with SSW and at $T=3$ with WAF (17–18, 27–28) have been harvested. Both fractions give a picture with extremely variable results, essentially of two types. In 5 of the 10 cores that were subjected to oil treatments (cores 15, 24, 27, 18 and 26), in both size fractions the faunas are much poorer than in the control mesocosms (cores 9 and 10); they are almost devoid of living foraminifera. In 4 other cores (numbers 16, 23, 17 and 25), faunal densities were in both size fractions much higher (up to 20 times more foraminifera) than in the control cores. In a last core (n° 28), the >150 μm fraction contained very few foraminifera, whereas in the 63–150 μm fraction, about 850 living specimens were observed. In a large majority of the mesocosms, *H. germanica* is strongly dominating (>95%) both size-fractions. At $T=4$ however, also some other species are observed in fair numbers in some of the oil-treated mesocosms. A very high quantity of *T. earlandi* is observed in the 63–150 μm fraction of the WAF-treated cores no. 24 (an almost monospecific assemblage of this species) and

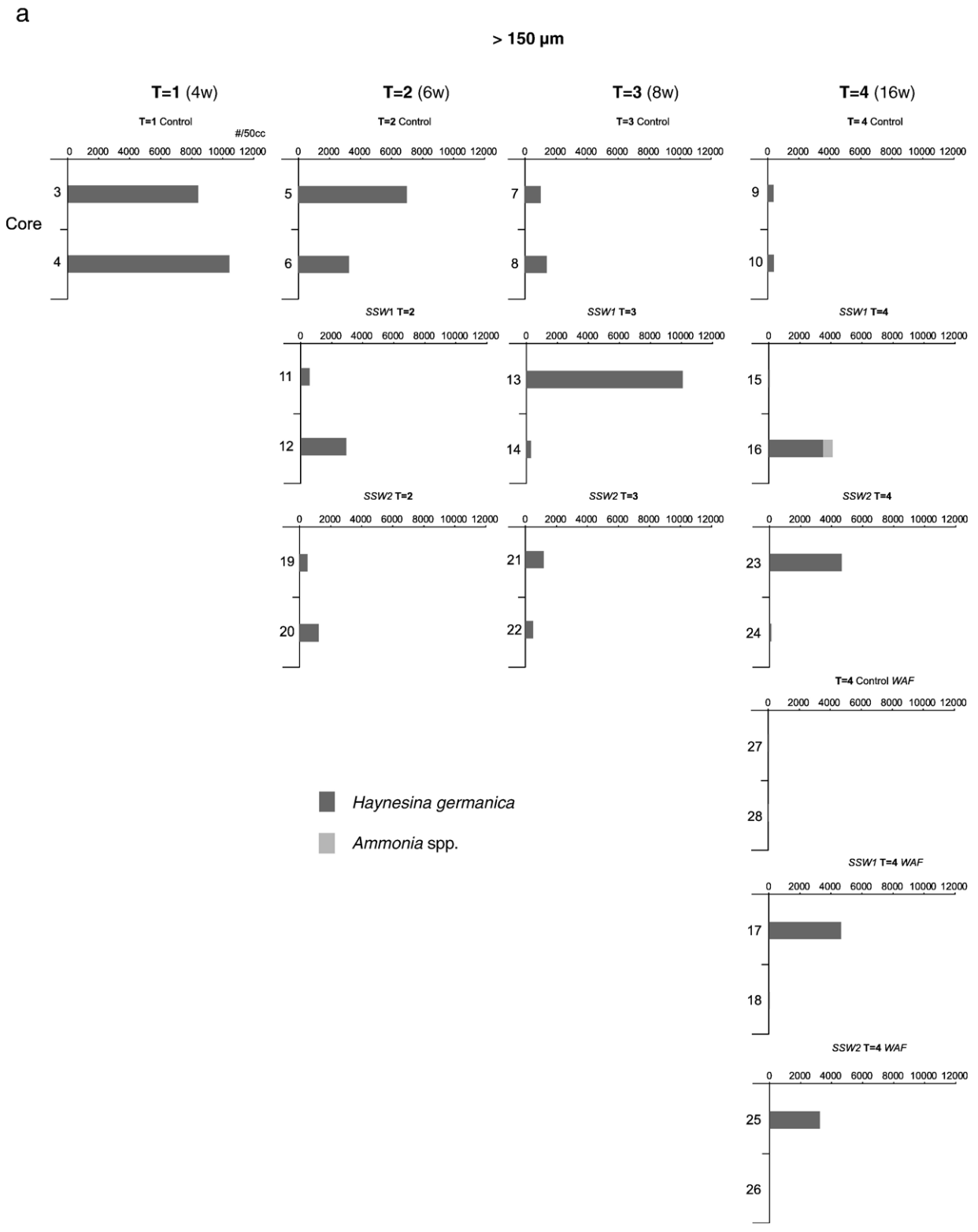

Fig. 6. Total standing stocks and species composition in the first 1 cm of *all* treatments in the a) >150 µm fraction, and b) 63–150 µm fraction. Replicates of each experimental treatment are presented separately (see core numbers).

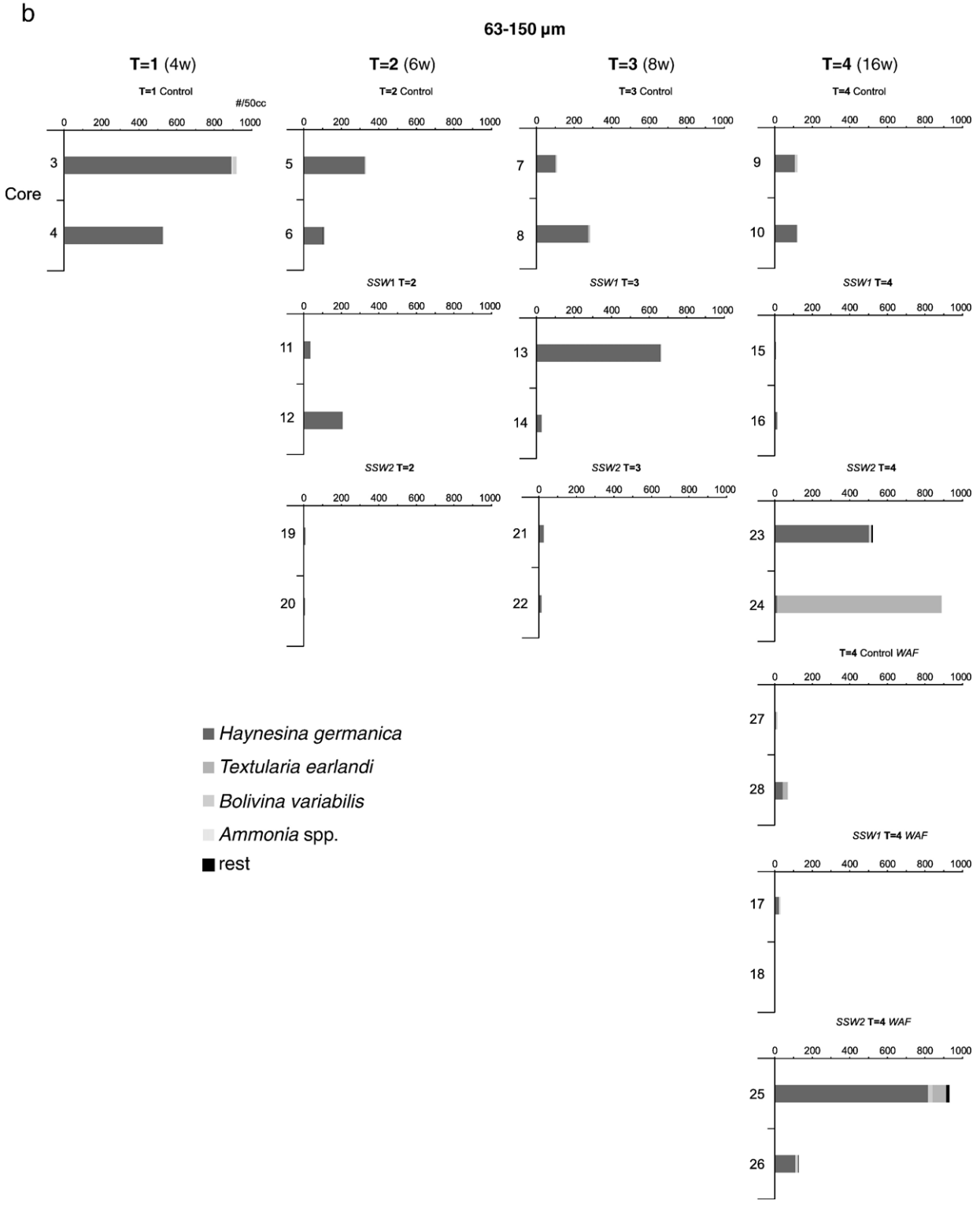

Fig. 6 (*continued*).

Table 2
Relative frequency in both size fractions of the anomalous tests of *Haynesina germanica* encountered in the upper 0.5 cm in the replicates of all treatments

63–150 μm														
		Treatment												
		Control				SSW 1			SSW 2			WAF $T=4$		
	Replicate	$T=1$	$T=2$	$T=3$	$T=4$	$T=2$	$T=3$	$T=4$	$T=2$	$T=3$	$T=4$	Control	SSW1	SSW2
Deformed test	a	–	–	–	–	6.3	0.3	–	–	–	–	–	–	–
	b	–	2.2	–	–	–	–	–	–	–	–	–	–	–
Egg pockets	a	–	–	–	–	–	–	–	–	–	–	–	–	–
	b	–	–	–	–	–	–	–	–	–	–	–	–	–
>150 μm														
Deformed test	a	–	0.1	–	–	–	–	–	–	–	–	–	–	–
	b	0.3	–	–	–	0.1	–	–	–	–	–	–	–	–
Egg pockets	a	–	–	–	4.5	–	0.5	–	–	–	–	–	–	–
	b	–	1.0	–	–	–	–	0.6	–	–	–	–	–	–

no. 26 (about 100 specimens, about 10% of a fauna otherwise dominated by *H. germanica*). *Ammonia* spp. is encountered in both size fractions of one of the other WAF-treated mesocosms (core 17).

3.6. Anomalous tests

Among the stained tests of the dominant taxon, *H. germanica,* two types of anomalous tests were encountered during the experiment. Firstly, strongly deformed tests are found that display twinning, strongly deformed chambers, strongly in- or deflated chambers and changes in coiling direction (Plate 1, Fig. 2). Such strongly deformed tests of *H. germanica* were rarely encountered (max. 6.3%, Table 2), and only in the upper 5 mm of the sediment. They are not restricted to the oil-treated mesocosms but are also found in the control treatments (Table 2).

Secondly, tests are found with epibiontic worm (turbelarian) cocoons attached to them (Plate 1, Fig. 3). One individual specimen had four of these pockets attached to its test. The presence of these cocoons is restricted to tests in the >150 μm size fraction and the highest densities are observed in the control treatments (Table 2).

4. Discussion

4.1. Experimental design

Carrying out ecological experiments in laboratory settings with (benthic) foraminifera involves a number of practical issues to be dealt with. It is evident that the sampling of the foraminiferal faunas and the subsequent transport to the laboratory will cause a disturbance to the faunas, of which the effects are very difficult to evaluate. However, a number of experimental studies show that a wide range of taxa can bear these manipulations and adapt fairly well to the partially artificial environments offered in the laboratory (e.g., Weinberg, 1990; Hemleben and Kitazato, 1995; Ernst et al., 2002; Heinz et al., 2002; Duijnstee et al., 2003). The taxa studied here are characteristic of intertidal environments, and experience highly variable wave, wind and tidal regimes in their natural environment, as well as very strong daily changes in temperature and salinity. Therefore, the initial disturbance caused by collection and the subsequent manipulations (e.g., sieving) will probably not have a lethal impact.

The vertical distribution in the sediment at $T=1$ suggests that in the 4 weeks following incubation, many specimens placed in deeper sediment layers, were able to migrate to the surface layer (Fig. 5). Apparently, a large percentage of the foraminifera artificially introduced into the 0.5–2 cm depth level appear to have migrated to the superficial 0.5 cm, whereas individuals that were introduced in even deeper sediment levels (below 2 cm) were less capable to do so.

One of the factors that could have negatively influenced the foraminiferal faunas is the total lack of variability of most of the parameters that are extremely variable in nature, such as temperature and salinity. However, since the aim of this study was to study the impact of oil compounds on these taxa, no efforts were made to create conditions closer to the natural ones. Since in the laboratory, all environmental conditions were kept as constant as possible, one may expect that an important part of faunal differences between treated and control mesocosms may be due to the addition of SSW and WAF mixtures of the *Erika* oil.

4.1.1. Rose Bengal staining and selection of sample depth

Using Rose Bengal stain for identifying living foraminifera can result in erroneous identification of living individuals (Bernhard, 1988). However, in studies with high foraminiferal densities the Rose Bengal method appears to be the only accurate and relatively reliable method (Lutze and Altenbach, 1991; Murray and Bowser, 2000). The exact nature (intensity) of the staining appears to be an important criterion, since many stained tests found deeper in the sediment are less intensively stained than their counterparts living in the more superficial sediments. These weakly stained specimens found deeper in the sediment most likely are dead, but because of the anoxic conditions the protoplasm has only degrades rather slowly, explaining the weaker staining. Consequently, we did not include these weakly stained specimens in our counts of living specimens. New staining techniques are presently being developed, in order to have a more objective method to recognise deep infaunal living individuals in anoxic or strongly dysoxic sediments (Bernhard et al., 2004). The shallow habitat for the dominant taxon, *H. germanica*, is in agreement with observations from other field (Haake, 1962; Richter, 1967) and experimental studies (Langezaal et al., 2003). It appears therefore reasonable to focus our study on the first cm interval. Another argument to concentrate our study on this interval is that the strongest impact of oil pollution for these benthic organisms is expected to take place near or just below the sediment–water interface.

4.1.2. Light conditions in the incubator

During the experiment UV-light (10.000 lx) in the incubator provoked algal blooms in the mesocosms located closest to these lights. Replacement of the seawater in the mesocosms, the lowering of the light levels to half of the initial intensity (5.000 lx) and changing the direction of the light source solved this algal bloom problem since no blooms occurred afterwards. A comparison of the affected and non-affected mesocosms (by these algal blooms) does not show a clear effect on the foraminiferal community. Theoretically, there could also be an impact from light penetrating deeper sediment layers through the transparent walls of the core. At present, it is not clear to what extent this phenomenon has affected the foraminiferal distribution in our mesocosms. In future experiments mesocosms with opaque walls should be used.

4.1.3. Preparation of oil–seawater mixtures

The use of two different methods for introducing the *Erika* oil in the experiments is symptomatic for the difficulties encountered for this procedure. The studies in literature on the ecotoxicological effects of different types of oil on different organisms show a lack of consistency with respect to the followed experimental procedures (e.g., Singer et al., 2000). The methods of introducing oil in environments or laboratory settings (ranging from spraying oil on surfaces, using oil slicks, mixing oil with sediments, preparing WAFs, etc.), and the quantification of the oil constituents is often not clearly explained. Also the use of degraded or fresh oil is one of the important issues for which no clear protocol appears to exist. The geographical distribution of oil spills suggests that the release of oil in the environment never results in a homogeneous distribution of the oil in the coastal zone (Kennish, 1992). Certain areas are affected more strongly and by different forms of the evolved oil (e.g., oil slick, droplets, water fraction, etc.). However, in order to work with a repeatable standard method, we have finally decided to use the water-accommodated fractions of the *Erika* oil in our experiments (Singer et al., 2000). According to Singer et al. (2000) this procedure should increase the comparability of tests of studies on oil ecotoxicology. We used fresh *Erika* oil, which is different from the field situation where rather degraded oil arrived on the coast. In nature, oil slicks covering the sediment cause anoxic conditions below the slick. Even without considering the toxic effects of the oil on the organisms, the anoxic conditions below the oil slicks will probably kill most organisms, including foraminifera. Because the aim of our study was to investigate the foraminiferal response to the toxicity of the oil compounds, we decided to use a WAF, and not to introduce oils slicks into the mesocosms, that would have caused very localized anoxia.

4.2. Oil toxicity

One of the main questions with respect to oils spills is what the exact ecotoxicological effects are of the various oil compounds. The analyses performed on the WAF show the presence of polycyclic or polynuclear aromatic hydrocarbons (PAHs). We remain uncertain about the evolution of these WAFs after the introduction in the mesocosms and about the fate of the various oil compounds in general. The bioavailability of various oil compounds could be significant; for example, PAHs are relatively insoluble and tend to concentrate on the seafloor, where they are adsorbed to clay particles and to particulate organic matter or accumulate in organisms (Mekenyan et al., 1994; Bennet et al., 1999). However, these oil compounds may still be available to foraminifera since they feed on various kinds of organic material (Lipps, 1983). Although UV-degradation of oil components could have taken place and the air supply

could have dispersed the evaporated components, the introduction of SSW and later of WAF should have made significant amounts oft oil compounds available to the organisms in the mesocosms.

Among the most carcinogenic, mutagenic and toxic oil compounds found in estuaries are the PAHs (Kennish, 1992; Watts, 1998). The general fate of PAHs in estuaries is dispersal in the water column, concentration in biota, incorporation in bottom sediments, chemical oxidation and biodegradation. Although not all PAHs are dangerous, the low molecular weight PAHs are often highly toxic and have adverse effects on organisms, without being carcinogenic. High molecular PAHs, on the contrary, are carcinogenic, mutagenic or teratogenic. So, anthracenes, fluorenes, napthalenes and phenantrenes are directly toxic, whereas benzo(*c*)phenanthrene, benzo(*a*)pyrene and dibenzo (*a*,*I*)pyrene are carcinogenic. Further, oil toxicity could strongly increase by the action of photo-toxicity and photo-oxidation (e.g., Mekenyan et al., 1994; Barron and Ka'aihue, 2001; Lee, 2003).

Many aquatic organisms rapidly metabolize PAHs, increasing the carcinogenic and/or mutagenic effects of these compounds. PAHs are activated in organisms that dispose of an enzymatic activity, called MFO (mixed function oxygenase), in the liver, kidney and/or gills (Kennish, 1992). Invertebrates show a greatly reduced ability to metabolize PAHs, and MFO-activity in invertebrates appears limited to Annelida and Arthropoda. However, biota lacking MFO-activity, like foraminifera, may accumulate great quantities of PAHs in their cytoplasm, especially since most foraminifera feed on various types of food (e.g., particulate organic matter, detritus) that is easily affected by oil pollution. Most benthic foraminifera occupy shallow to deep infaunal habitats and in these often anoxic environments the PAHs degrade very slowly and may persist almost indefinitely (Kennish, 1992). So, the effects of PAHs could strongly affect foraminifera and they might monitor oil pollution in such environments, but detailed cytoplasm measurements are needed in the future.

4.3. Development of the control mesocosms

The evolution of the control mesocosms over time clearly shows two different phenomena. First of all there is an ongoing decrease of the foraminiferal standing stocks over time, from about 3500 live foraminifera per 50 cc at the beginning of the experiment, down to about 900 foraminifera per 50 cc after 16 weeks ($T=4$). Next, after the even distribution over the top 7.5 cm imposed at the beginning of the experiment, a part of the foraminifera inhabiting deeper sediment levels (below the first 0.5 cm) appear to migrate to the topmost half cm. This migration behavior mainly concerns the foraminifera in the 0.5–2 cm depth level. The standing stocks in the deeper depth levels (2–7.5 cm) show a continuous presence of stained foraminifera over time. The strongly decreasing foraminiferal numbers in the topmost level as well as in deeper sediment levels (below 2 cm) after the initial increase at $T=1$ suggest a strong mortality. The fact that at $T=4$, infaunal densities are much higher than epifaunal densities may be an artifact, that can perhaps be explained by persistent staining of dead foraminifera, due to very slow protoplasm degradation in anoxic conditions.

An important question is why the faunal densities in the control cores show a considerable decrease over time. Faunal density in our microcosms is controlled by the initial faunal concentration (about 3500 individuals per 50 cc), additions by reproduction and/or growth (transition of individuals from the fine to the coarse size fraction) and losses by mortality. It is evident that in our mesocosm control setup the last parameter (i.e., mortality) was predominant. As a first interpretation, it could be advanced that, in spite of our concern to reproduce the natural conditions as good as possible, the artificial conditions created in the laboratory were not favorable for the development of the foraminiferal faunas.

A comparison with the temporal fluctuations of faunal densities in the Bay of Bourgneuf (Morvan et al., 2004) learns that also in the natural environment the faunas show very important fluctuations of their densities, with prolonged periods with decreasing densities (mortality predominant), interrupted by short periods of massive reproduction and growth, during periods when environmental conditions are particularly favorable. For example, a 75% decrease of faunal density was observed in the Bay of Bourgneuf between autumn 2001 and winter 2002. These observations in the natural environment, with prolonged periods of strongly decreasing population densities, suggest that we cannot conclude that the conditions developed in the laboratory were unfavourable for the foraminiferal fauna. We should rather conclude that favorable conditions, necessary for the massive reproduction and rapid growth of foraminifera, did not develop, and the population dynamics was mainly determined by mortality.

4.4. Foraminiferal response to addition of oil compounds

The experiment carried out was designed to study the (short-term) effects of oil pollution on the almost

monospecific assemblages of *H. germanica*. Several potential effects were envisaged in response to the introduced SSW1/2 and WAF mixtures: 1) increased mortality, 2) compositional changes in the foraminiferal faunas due to different degrees of tolerance of the various species, and 3) deformations of test morphology due to deregulation of the calcification process.

Firstly, field and experimental data provide evidence for acute toxicity of foraminifera after contact with oil. Morvan et al. (2004) describe experiments in petri-dishes in which all foraminifera die when exposed to quantities of >30 mg oil in 100 ml of seawater, whereas a doses of 5.5 mg oil in 100 ml of seawater provoked serious morphological abnormalities in a significant number of tests. In our experiments, however, foraminifera are maintained in ambient sediments, and have been subjected to much smaller quantities of fuel.

The oil-treatments in this experiment appear to provoke two contrasting faunal responses: firstly, a strong decrease in foraminiferal standing stocks, already in the first few weeks after addition of the oil. The decrease of faunal density in the oil-treated mesocosms appears to be more important than that observed in the control mesocosms. Furthermore, low values in cores 19 and 20 suggest that increased oil availability (SSW2) could provoke a stronger mortality over short periods of time ($T=2$). At $T=3$ (8 weeks) and especially at $T=4$ (16 weeks), a majority of the mesocosms contains only very poor foraminiferal faunas.

However, in some other oil-treated mesocosms, a return to higher foraminiferal densities can be observed in the course of the experiment. For instance, at $T=3$, in one of the mesocosms treated with SSW (core 13, SSW1) a sharp increase in the smaller and larger size-fraction can only be explained by a reproductive event. Similar reproductive events appear to have taken place at $T=4$ in cores 16, 23 and 24, all treated with SSW. Also in cores 17 and 25, treated by WAF, a reproductive response to oil pollution seems evident. In comparison to the control mesocosms at $T=4$, in all oil-treatments except cores 27 and 28, of the replicates has far higher standing stocks in both size fractions, suggesting reproduction. An interesting observation is the appearance, especially in the 63–150 μm size fraction at $T=4$, of other taxa than *H. germanica*, that is strongly dominant in all control cores. For instance, in one of the SSW treated mesocosms (core 24) very high numbers of *T. earlandi* are observed. Other taxa (e.g., *C. gunteri*, *C. excavatum*, *A. planidorsa*, *S. fusiformis*, *B. elegantissima* and *Reophax* sp.) appear but in less pronounced densities (see Appendix A, core 16, 23, 25, 26). The reproduction events and the appearance of other taxa could be explained by the stress experienced in the mesocosms. The newly appearing taxa could have been present as propagules or as resting stages profiting from the strongly modified environmental conditions (Alve and Goldstein, 2002). The absence of these species in the field data (Morvan et al., 2004) and appearance in the mesocosms appears an interesting phenomenon that needs further attention.

Summarizing, the comparison of faunal density and composition between control and oil-treated mesocosms shows a dual response to oil treatments; the faunas appear to respond either by increased mortality, or by a reproductive event. This dual response suggests that the addition of oil components has had a profound effect on the faunas. However, no significant relation could be established between the preparation method of the oil–water mixture, the quantity of the oil mixture and the foraminiferal response.

Previous laboratory studies with *A. tepida* (Le Cadre, 2003) showed that in petri-dish cultures quantities above 5.5 mg *Erika* oil per 100 ml seawater caused serious deformations of the test in many specimens. Some of these deformed specimens that were placed in oil-free seawater reproduced, and the newly formed juvenile individuals had all normal tests. This suggests that no mutagenetic effects were caused by the petrol in the mother specimens suggesting a lack of MFO activity in foraminifera. In our experiment, no significant effects of oil on the morphology of the foraminiferal tests were observed (Table 2). The number of deformed tests was always very low, as was the proportion of tests with turbelarian cocoons. After the Amoco Cadiz oil spill Vénec-Peyré (1981) described increased numbers of foraminiferal tests with these cocoons, which she interpreted as a potential result of the oil pollution. The results of our experiment and of the field survey of the oil-spill in the Bay of Bourgneuf (Morvan et al., 2004), where no significant increase of tests with cocoons was found, give no evidence to support this hypothesis.

5. Conclusions

The foraminiferal assemblage at the start of the experiment was dominantly composed of *H. germanica*. A comparison of control mesocosms and oil-treated mesocosms shows that the addition of oil-polluted seawater may provoke two different responses of the foraminiferal faunas:

1) The oil-treated mesocosms show a sharp decline in density (including the control) over time. The fact that this decline was stronger in the oil-treated mesocosms than in the control mesocosms, suggests that the toxicity of the oil causes an increase of foraminiferal mortality, eventually accompanied by a decrease of foraminiferal reproduction.

2) After 4 weeks, in some of the oil-treated mesocosms strongly increased densities were observed, suggesting reproduction events. In other oil-treated mesocosms, densities continued to decrease. After 4 months, certain taxa (e.g., *T. earlandi, Ammonia* spp.), that were not encountered, previously, or were only observed in very small quantities, appeared massively in the 63–150 μm fraction of some of the oil-treated mesocosms.

Replicates of the oil-treatments showed an extreme variability, with foraminiferal densities being either very low, or suddenly becoming very high. In the meantime, the control mesocosms were much less variable, and showed steadily decreasing densities over time in both replicates. These data suggest that foraminiferal faunas can respond to oil pollution either by increased mortality, or by rather unpredictable reproductive events of some of the taxa. The different types of oil–seawater mixtures used did not cause a significantly different response.

6. Taxonomic remarks

Aubignyna planidorsa Atkinson 1969
Ammonia beccarii (Linné) *Nautilus beccarii* Linné 1758
Ammonia tepida (Cushman) *Rotalia beccarii* (Linné) var. *tepida* Cushman 1926
Buliminella elegantissima (d'Orbigny) *Bulimina elegantissima* d'Orbigny 1839
Cribroelphidium gunteri (Cole) *Elphidium gunteri* Cole 1931
Cribroelphidium excavatum (Terquem) *Polystomella excavata* Terquem 1875
Haynesina germanica (Ehrenberg) *Nonionina germanica* Ehrenberg 1840
Quinqueloculina seminula (Linné) *Serpula seminulum* Linné 1758
Stainforthia fusiformis (Williamson) *Bulimina pupoides* d'Orbigny *var. fusiformis* Williamson 1858
Textularia earlandi (Parker) *Prolixoplecta earlandi* Parker 1952

Acknowledgements

Julie Vincendeau, Julie Vandeville and Anne-Lise Libeaut are thanked for processing part of the samples. Thanks are due to Mélissa Gaultier and Didier Martinez for their technical assistance. The constructive reviews of R. Serandrei-Barbero and S.E. Ishman considerably improved the manuscript. The SEM photographs were taken at the Service Commun de Microscopie électronique (SCME) of Angers University. This research was supported by the Conseil Général de La Vendée and INERIS.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.marmicro.2006.05.005.

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