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Foraminifera as potential bio-indicators of the "*Erika*" oil spill in the Bay of Bourgneuf: Field and experimental studies

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Abstract – Benthic foraminifera are used as potential bio-indicators of pollution due to the *"Erika"* oil spill. The foraminiferal assemblages from a site situated on the tidal mudflat of the southern Bay of Bourgneuf (Vendée, France) have been sampled 19 times on a monthly/bimonthly scale. The field study reveals uncommon low densities and poor faunas in the first 21 months of the survey. In order to understand the effect of the *"Erika"* fuel, foraminiferal cultures with 0 to 72.0 mg per 100 ml of *"Erika"* oil were maintained in controlled conditions in the laboratory. In the laboratory, an experiment with 5.5 mg per 100 ml of oil shows morphological abnormalities, cellular modifications and a low rate of reproduction. These first results confirm the potential toxicity of the fuel No. 2 from *"Erika"* and the sensitivity of foraminifera to this pollutant.

Key words: Foraminifera / Bio-indicators / Cultures / Oil spill / North Atlantic coast

Résumé – Les foraminifères comme bio-indicateurs potentiels de la marée noire de l'« *Erika* » en Baie de Bourgneuf : études expérimentale et de terrain. Les foraminifères benthiques sont utilisés comme bio-indicateurs potentiels de pollution due à la marée noire de l'« *Erika* ». Les assemblages de foraminifères d'un site de la slikke du sud de la Baie de Bourgneuf (Vendée, France) ont été échantillonnés 19 fois sur une base mensuelle à bimestrielle. L'étude de terrain révèle de faibles densités et une faune pauvre dans les premiers 21 mois du suivi. Afin de comprendre l'effet du fioul de l'« *Erika* », des cultures de foraminifères ont été maintenues en conditions contrôlées en laboratoire avec de 0 à 72,0 mg de pétrole de l'« *Erika* » pour 100 ml d'eau de mer. En laboratoire, une expérience avec 5,5 mg de pétrole pour 100 ml montre des anomalies morphologiques, des modifications cellulaires et un faible taux de reproduction. Ces premiers résultats confirment la toxicité potentielle du fioul No. 2 et la sensibilité des foraminifères à ce polluant.

1 Introduction

In the Bay of Bourgneuf (Vendée, Atlantic coast of France), the *Erika* oil spill of December 1999 strongly affected intertidal areas (mudflats and saltmarshes). Benthic foraminifera from these environments have been monitored for 40 months and are used as bio-indicators to evaluate the impact of oil pollution and the recovery of the study area.

A number of studies on living foraminifera have used these organisms as bio-indicators of intertidal environments (e.g. Murray 1971; de Rijk 1995; Redois and Debenay 1996; Redois and Debenay 1999; Debenay et al. 2000; Debenay and Guillou 2002). Previous studies concluded that the composition of foraminiferal faunas and the presence of morphological abnormalities in foraminiferal tests can be used as indicators of pollution (e.g. Alve 1991; Yanko et al. 1994, 1999; Alve 1995; Yanko 1997; Samir 2000; Samir and El-Din 2001). The few studies dealing with the effects of hydrocarbon pollution on foraminifera have contradictory results: Locklin and Maddocks (1982) noted the absence of effects on foraminiferal faunas while Vénec-Peyré (1981) and Yanko and Flexer (1992) observed a depletion in foraminiferal abundance as well as morphological abnormalities. The aims of this study are to observe eventual effects of *Erika* oil on the density, composition and morphology of the assemblages observed in the field and to determine the influence of various amounts of oil on a foraminiferal species grown in cultures.

2 Material and methods

2.1 In situ study

One exposed site (A2, La Noue Fromagette, Beauvoir-sur-Mer; Fig. 1) in the intertidal zone of the southern part of the Bay of Bourgneuf has been monitored from the oil spill in January 2000, to April 2003, by collecting the uppermost layer (0−1 cm) of a sediment surface of 50 dm^2 on a

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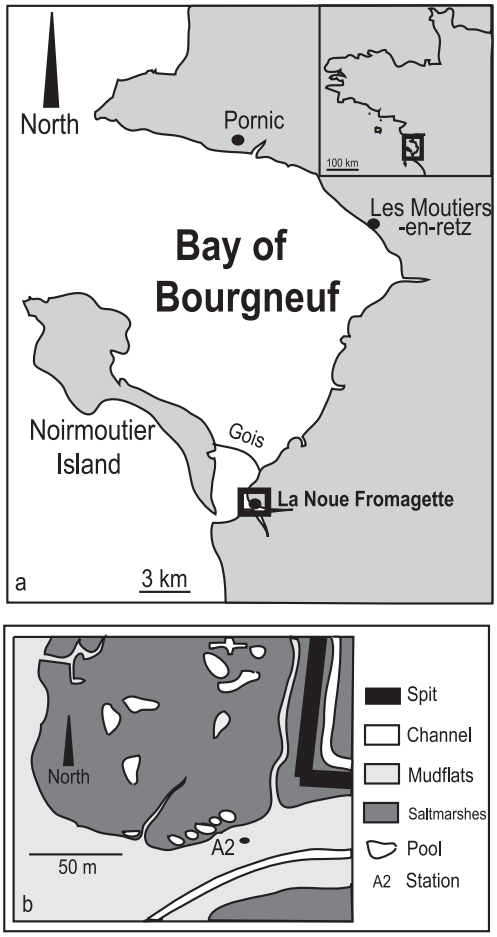

Fig. 1. Location area; (a) La Noue Fromagette in the Bay of Bourgneuf , (b) A2 station.

monthly/bimonthly basis. Sediments were collected manually at low tide in the intertidal mudflats of the Sallertaine channel and were stored in 96% ethanol containing 1 g l^{-1} Rose Bengal stain (Walton 1952). After at least 3 days, a constant volume of 50 cm^3 of sediment was sieved through 500 and 63 μm mesh sieves. The meiofauna from the 63–500 μm fraction was separated by flotation on trichlorethylene and, when available, about 300 living (stained by Rose Bengal) foraminifera were counted. When more individuals were present, the sample was splitted and the results were extrapolated for the whole sample.

2.2 Culture studies

In the laboratory, living specimens of *Ammonia tepida* were grown in culture. This species was chosen because it can be maintained quite easily and reproduces in culture. Moreover, this species was used successfully in previous studies for evaluating the impact of either natural (pH, salinity) (e.g. Stouff 1998; Stouff et al. 1999a,b; Geslin 1999, Le Cadre et al. 2003) or anthropogenic stress (e.g. Alve 1991; Sharifi et al. 1991; Yanko et al. 1994; Yanko et al. 1998; Samir 2000). Adult specimens were collected manually in October 2001 together with seawater in an area not affected by the oil spill (Auray river, Golfe du Morbihan, southern Brittany). After

Table 1. Description of foraminiferal (Ammonia tepida) cultures.

Experiment	Amount of *Erika* oil (mg per 100 ml^{-1})	Number of specimens	Length of the experiment (month)
Control	-	9	12
Exp. 1	72	3	4
Exp. 2	30	4	4
Exp. 3	5.5	7	8
Exp. 4	3.0	9	2
Exp. 5	1.5	8	2

selection of adult specimens, a small number of foraminifera were introduced in 70 mm diameter Petri dishes with 100 ml of (0.2 μm membrane filter) in order to monitor them individually (Table 1). The control culture in microfiltered natural seawater is composed of 3 replicates with 3 foraminifera each. Amounts of *Erika* fuel provided by the CEDRE (Centre de Documentation de Recherche et d'Expérimentations sur les pollutions accidentelles des eaux) were added to the cultures: 1.5, 3.0, 5.5, 30.0 and 72.0 mg. Cultures were placed in an incubator (LMS Bioblock scientific) with UV-lights creating a day/night cycle of 12/12 hours with a temperature of 20 °C. Salinity = 35 and pH = 7.8 were checked daily. One drop of a diatom culture with *Phaeodactylum triconurtum* (colony 1052/14) et *Amphiprora* sp. (colony 1003/5) was added weekly. The behaviour of the foraminifera in the microcosms was followed weekly for about a year, and at the end of each experiment, live specimens were fixed (full details in Le Cadre et al. 2003). Morphological and cytological observations were made under a scanning electron microscope, SEM (Jeol 6301F) and a transmission electron microscope, TEM (Jeol), respectively, at the SCME (Service Commun de Microscopie Electronique) of the University of Angers (France).

3 Results

3.1 In situ study

In the first 21 months after oil deposition, very low density living assemblages were recorded (0 in June 2001 to about 50 foraminifera per 50 cm^3 of sediment in September 2001; Fig. 2). The species richness was also very low (1 in June 2001 to 11 species in December 2000). In October 2001, density and species richness strongly increased until a maximum of about 1050 living individuals per 50 cm^3, belonging to 19 species in December 2001. During the first part of the year 2002, densities ranged from 30 to 110 individuals per 50 cm^3 and species richness from 7 to 15. After a small peak in foraminiferal abundance in November 2002 (about 300 individuals per 50 cm^3, belonging to 25 species), the first months of 2003 showed again a low number of living specimens (35–105 per 50 cm^3) increasing again in April 2003 which contains 515 individuals per 50 cm^3. Six taxa dominated the assemblages. The most frequent and abundant was *Haynesina germanica*, present in all samples, except in June 2001 when only one living foraminifer was encountered; its density showed a strong increase in autumn 2001 and 2002 as well as in April 2003. In autumn 2000,

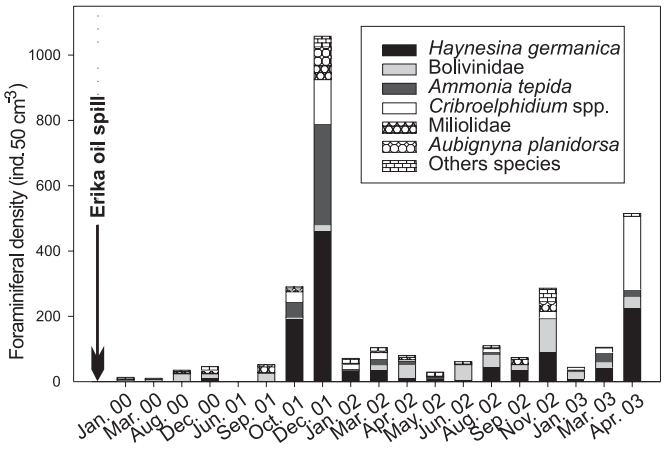

Fig. 2. Standing crop of foraminiferal fauna during the oil spill monitoring from January 2000 to April 2003.

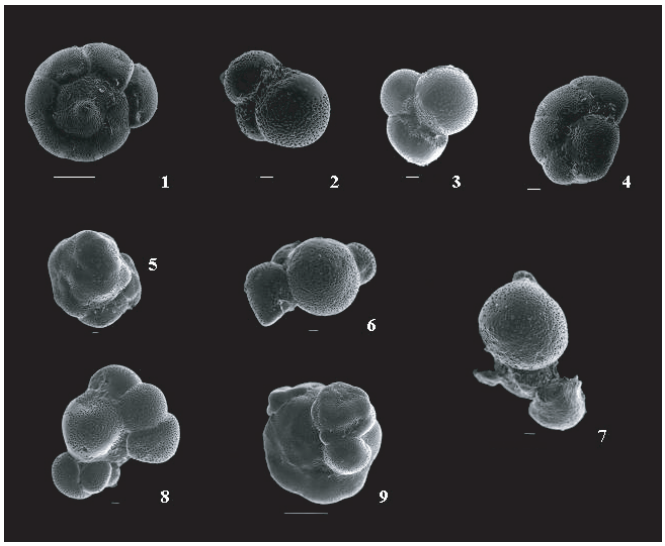

Plate 1. 1-4 Specimens from the control culture. **1** *Ammonia tepida* introduced in the control culture (scale bar = 100 μm). **2-3** Juveniles with three chambers born in the control culture (scale bar = 10 μm). **4** Juveniles with six chambers born in the control culture (scale bar = 10 μm). **5-9** Deformed juveniles born in contaminated culture with 5.5 mg of hydrocarbons. **5** Complex form (scale bar = 10 μm). **6** Triple test (scale bar = 10 μm). **7** Wrong coiling of the first chambers (scale bar = 10 μm). **8** Double test issue of the formation of two spires (scale bar = 10 μm). **9** Complex form (scale bar = 100 μm).

on the contrary, such a density increase has not been observed. Bolivinids were also present in all samples and in relative high abundance. Only a few specimens of *Ammonia tepida* were found during the first 21 months of monitoring, but this species represented about 30% of the assemblage during the abundance peak observed in December 2001. In 2002 and 2003, its relative abundance was low in all samples. *Cribroelphidium* spp. were encountered alive since October 2001, and were especially frequent when the overall foraminiferal abundance was high (December 2001, April 2003). Miliolids, that were present in almost all samples and *Aubignyna planidorsa*, found in a few samples, had always a low relative abundance.

Morphological abnormalities were encountered in *H. germanica*, *A. tepida* and Miliolids. The abnormalities corresponded to deformations and distortions of the test (wrong direction of coiling). Their proportion never exceeded 3% of the population.

3.2 Culture studies

After an incubation of 2 to 4 weeks in unpolluted conditions, *A. tepida* reproduced with an average of 15 juveniles for each reproduction event (every 2 weeks). Unpolluted microcosms were maintained for one year. Most foraminifera born in culture (a total of 684 juveniles) presented a normal morphology; only 12 abnormal tests were observed (1.75%) (Plate 1, see 1-4).

Eight adults were introduced in the microcosm with 1.5 mg of *Erika* oil per 100 ml of seawater. A first reproduction event occurred after 8 days and a second one after 40 days. Among the 43 juveniles born in the culture, only one showed morphological abnormalities.

In the culture contaminated with 3.0 mg of oil (9 adults introduced), 20 juveniles were born from 2 adults after 15 days and all of them were normal.

After 9 days in a culture subjected to 5.5 mg of oil (7 adults), 7 juveniles were observed. Two new reproductions took place after 30 days with emission of 7 juveniles each time. Among the 21 newborn juveniles, 9 showed strong morphological abnormalities (wrong direction of coiling, double or triple forms and complex forms). Abnormalities were also observed on an adult specimen during the formation of new chambers. After 7 months, new reproductions took place, producing 29 juveniles, 13 of them (45%) being deformed (Plate 1, see 5 to 9). One *A. tepida* with morphological abnormalities was removed from this culture (with 5.5 mg of oil) and placed in an unpolluted microcosm with only microfiltered seawater. After 20 days, 20 juveniles were emitted, all without morphological abnormalities. In order to confirm this result, another experiment was performed removing 19 normal and abnormal individuals from a culture subjected to 5.5 mg of oil, and introducing them into an unpolluted microcosm. After 20 days, a first reproduction occurred and after 2 months, 280 newborn juveniles were observed in this culture. Only five juveniles were abnormal (1.8%).

In microcosms with 30.0 mg of *Erika* oil per 100 ml of seawater (4 adults introduced), specimens stayed alive during 6 weeks. However, no growth or reproduction was observed. After 2 months, no more life activity was detected. The death of these organisms was confirmed by the degradation of the cell material. The same observations were made for the culture with 72.0 mg of oil (3 individuals). All foraminifera were dead after 2 months.

3.3 Cytological study

Transmission electron microscope observations of ultrathin sections of the contaminated and deformed specimens raised in 5.5 mg of *Erika* fuel showed a general aspect different from that of the control cultures. In the specimens from control cultures, the inner organic layer (IOL) was compact and

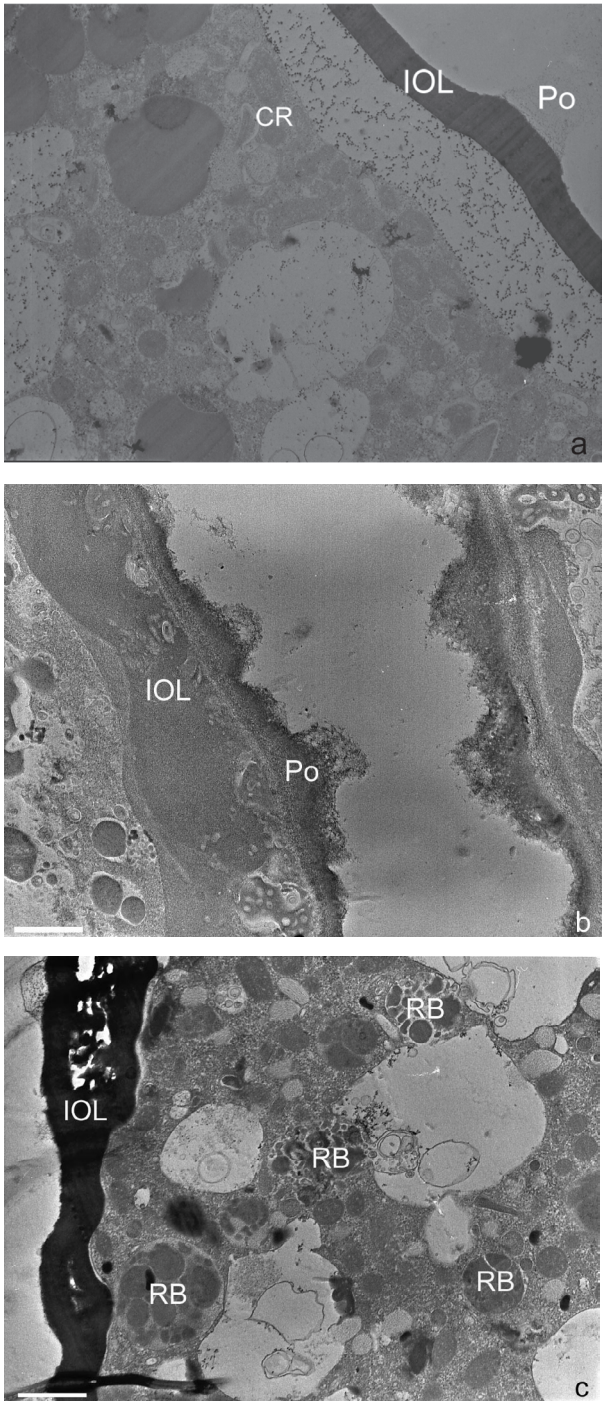

Fig. 3. (a) Compact and homogeneous inner organic lining (IOL) in specimens raised in a control culture (Po: pore; RB: residual body); (b) Thickening of the IOL; (c) Proliferation of residual bodies (RB) in foraminifera raised in a microcosm with 5.5 mg of *Erika* oil per 100 ml seawater.

homogeneous (Fig. 3a) whereas in contaminated specimens, it was thickened (Fig. 3b). This thickening corresponded to an enlargement of the organic lining with an infill of fibrous material. Proliferation of residual bodies was also observed in these contaminated specimens (Fig. 3c).

4 Discussion

Taking into account the seasonal cycles observed over more than three years, the density and the species richness were uncommonly low during the 21-months period following the oil spill. Such depletions of faunas were observed in one of the most polluted estuarine area (Huelva, Spain) by Ruiz et al. (2004). The low values, recorded in the Bay of Bourgneuf, suggest that foraminiferal populations were impacted, either by the oil spill itself or by the strong disturbance of the environment resulting from cleaning activities. However, our temporal record is still too short to fully know the potential degree of interannual variability; moreover, at present it is still impossible to firmly recognise oil pollution or cleaning activities as the main causes of this low density. It is absolutely necessary to extend the field record in time in order to obtain a better knowledge of foraminiferal seasonal cycles and interannual variability. Small and medium scales patchiness are another factors that may interfere with temporal variability. A comparison of the data of station A2 presented in this paper, with the temporal succession found at 5 others impacted sites nearby shows very similar patterns for all stations, indicating that those patterns actually represent temporal faunal variability and not small and medium scales spatial patchiness. The rates of morphological abnormalities observed in the field never exceed those recorded in unpolluted environments (Geslin et al. 2000). Therefore, at the present stage of our study, we can not firmly ascribe the observed population changes to an impact of the *Erika* oil spill on the benthic foraminiferal faunas in the Bay of Bourgneuf. However, the absence of a reproductive event in December 2000 and the very perspicuous population increase in autumn 2001 are comparable to the recovery of a rocky shore fauna described for the same area by Le Hir and Hily (2002). These authors showed that after low abundances of grazer species recorded in crevices for 10 months, new and less sensitive species took advantage of newly available space.

In unpolluted environments, usually only a low proportion of morphological abnormalities is encountered. The deformations occurring in non polluted environments may result from a regeneration of the cell after reproduction, when a small quantity of cytoplasm with a nucleus remains in the test (Stouff et al. 1999a,b). Only a rate superior of 3% was considered as indicators of stressed (Geslin 1999; Le Cadre 2003). However, this rate may rise in stressed environments, with stress resulting either from natural or from anthropogenic parameters. (Alve 1991). Carr et al. (1996) showed that sediments surrounding oil spill areas within 150 m offshore platform were toxic. Polycyclic aromatic hydrocarbon (PAH), a major toxic component of oil, can be adsorbed on particles in suspension or on organic matter, lowering their bio-availability (Maskaoui et al. 2002; Geffard et al. 2003; Zhou and Maskaoui 2003). Vénec-Peyré (1981), who studied the consequences of the Amoco-Cadiz oil spill in the Northern Brittany coast in March 1978, observed high rates of morphological abnormalities in foraminiferal tests. She attributed these morphological abnormalities to the toxicity of the product, but she could not exclude that they were an indirect consequence of the oil spill, for instance of a rapid decrease of pH (Locklin and Maddocks 1982; Le Cadre et al. 2003). In our study, an amount of 5.5 mg of oil per 100 ml of seawater provoked

numerous morphological abnormalities in juveniles. This elevated rate of malformation confirmed the potential toxicity of the fuel No. 2 from *Erika* tanker. The number of juveniles emitted during one reproduction was lower in contaminated cultures (7 juveniles) than in control cultures (15 juveniles). The same phenomenon was already observed in cultures contaminated with heavy metals (Le Cadre 2003). This low rate may be due to a low reproduction or to the death more important of the juveniles (proloculus state) in the reproduction cyst. The low densities often encountered in environments contaminated by hydrocarbons (Mayer 1980 in Geslin 1999; Yanko and Flexer 1992; Bonetti et al. 1999) may be explained by this phenomenon. Yet, the delay before the first reproduction event is not noticeably influenced by the presence of hydrocarbons.

The cytological study of specimens presenting morphological abnormalities showed the same cellular modifications as the ones observed in individuals contaminated by heavy metals (Le Cadre 2003). The thickening of the IOL can be related to a defence mechanism against penetration of xenobiotic components in the cell. The proliferation of residual bodies can be the result of a metabolism perturbation (Amiard et al. 1995; Cajaraville et al. 2000; Domouhtsidou and Dimitriadis 2001).

After reintroduction of deformed individuals into unpolluted microcosm, juveniles with a normal morphology were produced. Therefore, it seems that the modifications are due to metabolism disturbance, but do not affect the genome.

5 Conclusion

The laboratory experiments clearly showed the potential impact of the *Erika* fuel on the foraminiferal test shape, cytology and on reproduction processes. In the field, the proportion of deformed tests did not indicate a significant impact of the oil spill. Even if our knowledge on seasonal variability of foraminiferal assemblages is still poor, the uncommon low densities and species richness during the 21 months following the oil spill strongly suggest a negative impact of the oil spill or of the cleaning activities on the foraminiferal assemblages.

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Appendix 1. Benthic foraminifera: Absolute abundance of major species, other species and species richness.

Taxons	*Haynesina germanica*	Bolivinidae	*Ammonia tepida*	*Cribroelphidium* spp.	Miliolidae	*Aubignyna planidorsa*	Other species	Species richness
12.01.00	1	4	0	1	2	0	5	6
10.03.00	1	5	1	3	0	0	0	5
10.08.00	3	21	1	1	4	1	4	9
28.12.00	11	13	0	1	10	1	10	11
26.06.01	0	0	1	0	0	0	0	1
4.09.01	2	24	1	0	18	3	4	9
31.10.01	194	4	46	31	6	6	3	11
5.12.01	462	19	309	135	44	57	32	19
5.01.02	32	2	5	14	1	2	15	15
4.03.02	35	17	18	19	3	2	10	13
25.04.02	11	42	10	4	7	1	5	14
29.05.02	10	0	4	2	2	0	11	10
27.06.02	5	47	1	0	1	1	6	9
13.08.02	45	39	8	9	3	1	5	13
27.09.02	36	16	0	0	17	0	5	7
9.11.02	90	103	3	19	21	9	41	25
22.01.03	7	24	3	0	0	0	1	5
4.03.03	43	18	29	13	0	0	2	6
22.04.03	226	36	20	225	0	0	8	6