On behalf of my co-authors, here is the revised version of our manuscript and the answers to the Referee #1's and Referees #2's comments. We thank them for their review that helps us to improve the quality of the manuscript. We modified deeply the ms according to their comments (new figures, introduction of nutrient data set, description of longitudinal trend, modification of the results and discussion section). Note also that one co-author was added (Mireille Pujo Pay). We hope all these modifications will correspond to the attempts or both referees.

Anonymous Referee #1
Received and published: 14 December 2010
GENERAL COMMENTS
This manuscript presents data on the abundance of different bacterial groups (as defined by flow cytometry) and bacterial production. The data were apparently collected as part of a larger project (BOUM) which is the topic of a special issue in Biogeosciences. A large number of samples were collected and processed. While I understand that a large amount of effort went into the data, I think that the manuscript needs to be revised in order to clearly present the key points and not bury the reader in repetitive correlations and dot plots. By the time I reached the end of the paper, I had completely lost track of which depth layer was important for which correlations. Also there are multiple places in the discussion where the results are just repeated rather than allowing the discussion to focus on the data that has already been presented.
We agree that the main points were diluted in a large quantity of date and correlations. We have reorganized the discussion in order to focus on the main points.

SPECIFIC COMMENTS:
I understand that studying water masses above/within/below the DCM is common, and I do not have a problem with those distinctions. However, I think that considering three layers within the water column should be sufficient. The division below the DCM is apparently arbitrary. Furthermore, the authors used two different methods to define bacterial production: one method above 200m and a second method below that point. Yet, the authors then discuss data down to 250 m as separate from samples below 250 m. Indeed, the authors begin their own discussion (page 8257, line 23-25) with a description of three water column layers
We preferred to keep the distinction between layers 'below dcm' (dcm layer down to 250 m) and 'deep' layers (250 m to the bottom) for two reasons: first, TChl a could be detected at trace levels down to 250 m depth and the layer 0-250 m is often sampled by scientists focusing on epipelagic layers in Mediterranean Sea. Second, we do not have many data available for the 'meso and bathypelagic layers and preferred to keep apart this data set in case they 'forced' the regressions between cytometric parameters and environmental variables. Thus, we feel confident in keeping this separation at 250 m depth.
The deeper depth where BP was determined with the centrifuge technique is 200 m, the shallower depth where BP was determined with the filtration technique is 250 m. In order to be less confusing in the M&M section we thus changed the sentences on the depth investigated with the filtration technique as follows (revised version, page 5 lines 24-27):
"Activities within meso- and bathy-pelagic layers were also investigated at stations A, B and C, between 250 and 910 m at st C, 250-3000 m at st B and 250-2700 m at st A. These deeper samples, where a lower activity was expected, were treated by the traditional filtration technique."

My confusion about what the important points of this manuscript are was exacerbated when the same data are repeatedly presented in tables, in the text, and in figures. I would delete all of Table 3 and only show the data in Figure 6. The text can then present the correlations most relevant to the points the authors are trying to make.
Table 3 has been modified (It is Table 2 in the revised version of the ms and it is shown at the end of the response to both referees). We removed relationships with SSC and green fluorescence, but we added relationships with nitrate+nitrite and phosphate, because this relationship with nutrients was requested by

reviewer 2. We also removed indication of n (number of data) which can be found on Table 1. The old Figure 6 (now Figure 8) only presents relations with BP, not with chlorophyll or nutrients, so it appears important to maintain this table.
In parallel, the results section was reduced in order to only focus on the most relevant relationships.

In addition, I think that table 1 can be shortened to only include the data on the different layers without the upper part of the table showing n, min, max, and so on. The boxplot in Figure 5 is a nice way to clearly show what happened at the different depths for the different variables. I think that much of the discussion in the paper could focus on this figure.
Table 1 has been shortened as suggested by the reviewer: The upper part including mean and SD has been removed but we think that it is important to keep the range of data set used to determine the correlations with environmental variables, as well as the number of data set. We also added a column giving nitrate+nitrite information.

The data in table 2 appear to only be considered briefly in the results where the authors compare their data to previous work and conclude that variability in bottom-up control as important. However this idea does not get expanded up in the discussion, and is out of context for the rest of the paper. Furthermore, table 2 should only include the Model II data. While I understand that previous work has used Model I (inappropriately as correctly noted by the authors), I do not think it is necessary to devote so much room to data incorrectly analyzed.
The results and the discussion section have been modified to include relationships with nutrients. We discussed vertical distribution of cytometric groups according to the potential limiting factors. It is thus important to point out first that BP is bottom-up limited by presenting BP-BB relationships. This was just cited in the results section (page 8, lines 9-18) but the old figure 2 and old table 2 have been removed as suggested by the referee.

In places in the discussion, the authors go a little too far past their own data with the conclusions they reach. For example, the idea that the level of variability in green fluorescence is a direct link to adaption to response to the environment (page 8261, line 23) is a bit of a stretch. The authors go even further and bring up the switch from nutrient limitation to carbon limitation as a factor controlling SSC and green fluorescence (page 8262, line 13) – yet the manuscript has no data either carbon or nutrient limitation.
The manuscript has no data either on carbon or nutrient limitation but we added information on 2 companion papers (Talarmin et al., 2011 and Tanaka et al., 2011) which showed N and N+P limitation in surface waters during the same cruise, and we refer to another studies in the Mediterranean Sea under stratified conditions demonstrating a switch from nutrient to organic carbon limitation along the water column (Van wambeke et al., 2002; Sala et al., 2002).
We agree that the sentence page 8261 line 23 is going too far in interpretation and the whole paragraph (end page 13- up page 14) was modified as:
"In our study, depth and TChl a explained more variability in LNA cells green fluorescence within 'surface' layers whereas they explained more variability in HNA cells green fluorescence within 'below dcm' layers, suggesting that the two cytometric groups had different dynamics within distinct chlorophyll layers. Surprisingly, we found also that %HNA significantly increased with BP and TChl a only within the 'dcm' layer.
In a companion study, Talarmin et al. (2011) showed, through ^{3}H leucine labelling coupled to cell sorting that the contribution of LNA cells to bulk leucine incorporation rates was higher in surface waters whereas that of HNA cells was higher at the vicinity of the dcm at the three sites A, B and C. 'Surface' and 'below dcm' effectively constitutes 2 distinct layers if we refer to the environmental conditions that could differently influence the relationships between cytometric properties and environmental variables. Indeed, bacterial production in the 'surface layer' was limited by the availability of N or N+P in Ionian and Levantine Sea during our study (Tanaka et al., 2011) and a switch from nutrients to carbon limitation is generally associated to the dcm peak (Van Wambeke et al., 2002, Sala et al, 2002). A drastic change has

already been reported in the taxonomic composition of bacterial communities facing new limiting factors and carbon source in this layer,due to the vertical stratification of nutrients and phytoplankton populations (Dyfamed site in late summer: Ghiglione et al., 2008; Van Wambeke et al., 2009). It is thus possible that such changes might influence the vertical distribution of HNA and LNA cells as well as their cytometric properties. Finally, a potential longitudinal effect was studied through comparisons of cytometric properties within surface layers across different regions. Changes in the cytometric properties of the cells were better explained by their vertical distribution than by regional effects"

Then the discussion makes the leap to membrane physiology and SSC (page 8263, line 12) which I think is stretching their data way too far.
The discussion section has been completely changed and this part of the discussion is now removed

The authors' appear to have no killed controls for the bacterial production data. Since this is a standard part of the protocol in measuring bacterial production, the authors need to provide a strong justification for its omission and the potential impact on their results.
We apologize for the sentence missing on controls in the M&M section for BP. Yes we had controls for BP, both for the centrifuge and the filtration techniques, in all samples analyzed. The blank signal was subtracted from all incubated samples. The M&M section was modified accordingly page 5 line 10 and page 5 line 29:
"Duplicate 1.5 mL samples and one trichloracetic acid (TCA) killed control for blank correction were incubated with a mixture of [4,5-^{3}H]leucine…."
and
" Two duplicates and one formalin-killed blank were incubated in the dark at in situ temperature for 15 - 20 hours. Live samples were terminated by formalin addition (1% final concentration)…"

TECHNICAL CORRECTIONS
Page 8247, line 7: 'contrarily' is a bit awkward, how about 'contrary'?
This has been corrected

Page 8248, line 19: '. . .showed that these characteristics changed. . .' I would specify 'cytometric characteristics' because otherwise the sentence could be interpreted as referring to BP and chl a.
This has been corrected

Page 8249, line 2: '. . .using an unique procedure/instrument. . .' this study is presenting standard flow cytometry and bacterial production data, so I don't think the description of 'unique' methods is appropriate
By using this term, we wanted to point out that all the analyses made by flow cytometry were done on the same instrument by the same analyst, so that cytometric properties are comparable for the whole data set. The sentence was modified page 3 lines 14-15:
"In our study, a large number of samples were analysed to investigate flow cytometric characteristics in relation to environmental properties (temperature, total chlorophyll a, phosphate, nitrate+nitrite, bacterial production) along both vertical and horizontal gradients".
In the M&M section we specified that flow cytometric analysis were made by the same analyst (page 4 line 32)

Page 8249, line 9: 'connexions' should be 'connections'
This has been corrected

Page 8249, lines 9-11: 'were examined according to the distribution of chlorophyll by dividing vertical layers' ?? This needs to be rephrased because it is awkward and it is not clear what is mean by dividing vertical layers.
The sentence was modified (page 3, lines 20-22) as:
"The strengths of relationships were examined within layers located above, within and below the deep chlorophyll maximum, as well as longitudinally among different regions."

Page 8249, line 20: '. . .represented the majority of the area were occupied briefly. . .' not clear what is meant by majority of the area.
Page 8249, line 23: 'only one over two' ? not clear what this means.
The sentences have been modified (page 4 lines 3-6) as:
"The numbered stations were occupied briefly and sampled at different times of the day. All these numbered stations were investigated for bacterial abundance and only one half of them for bacterial production (1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 24, 25)."

Page 8252, lines 11-15: please define 'dcm' before using it to describe how the water layers were partitioned. Also, since the definition of the dcm is a key part, please specify how the depth of the dcm was actually determined.
One sentence was added in the M&M section (page 3 lines 30-32) as follows:
"Sensors on the CTD provided profiles for temperature, salinity, and fluorescence at each station. The depth of the deep chlorophyll maximum (dcm) was determined from the fluorescence profile."

Page 8253, line 3-5: Looking at figure 4, I don't see surface water temperatures down to 17degC.
Yes, you're right, this is because, due to a mistake, station 27 was not plotted on this figure, and this error is now corrected (new figure 4a, see at the end of this document).

Page 8254, lines 13-15: 'Box plot distributions of HNA and LNA cell abundances relating to layer ("surface", "dcm", "below dcm", "deep") were similar (Fig. 5a, b)' . . . this sentence is too vague, so I can't figure out what the authors think is similar in subplots 5a and 5b. I think the sentence can be removed since the authors go into detail in the following paragraphs.
The sentence has been removed

Page 8258-8259: if the %HNA with depth is the most striking feature in the dataset, this point should not be buried in the results section.
We removed many descriptions of irrelevant relationships in the results section, trying to keep the more interesting for discussion.

Also, the statement about LNA cells decreasing with depth faster cannot be seen in the way the data in figure 4 are currently plotted.
We added a plot of %HNA versus depth on the figure 4 (Fig 4 g, h) and abundances of HNA and LNA cells are on separated plots (Figure 4 e, f, g, h).

Furthermore, while the authors do not need to repeat their results in the discussion section, they should check as to why the discussion has n = 55 but the results have n = 53 for what appears to be the same conclusion.
Sorry for this mistake, it was n=55, 53 was the number of degrees of freedom.

Page 8259, lines 9-28: The extended discussion on factors other than size which affect SSC can be shortened because the authors' data really did not cover those points.
The paragraph on distribution of cytometric properties in meso and bathy pelagic layer has been shortened dealing on assumptions on effects of hydrostatic pressure and modification of membrane properties, but as

requested by referee 2, we added new sentences on potential top-down effects (lysis and grazing) on % HNA at depth (end page 14, up page 15) as:
"SSC of LNA cells also increased but the slope was 6 times lower than that of HNA cells. SSC values of both HNA and LNA cells were not related to BP in the deep layers, nor to nutrient (PO4, NO3+NO2) concentrations. Since SSC variations are sometimes related to the biovolume (Bouvier et al., 2001; Felip et al., 2007) therefore, an increase in SSC values could be interpreted as an increase in cell biovolume. However, this should be considered with caution because it is not congruent with other reports or observations through epifluorescence microscopy (Tamburini, pers com). La Ferla et al. (2004) estimated biovolume and the lipopolysaccharide content of bacterioplankton down to 4000 m in the Ionian Sea and the mean cell volume varied in a similar range in both the euphotic and aphotic zone. It is important to consider that SSC is much more complex than only cell biovolume and can be affected by both the cell structure and the chemical composition of the outer membranes. Presumably hydrostatic pressure and dominance of Archaea (Tamburini et al., 2009) might influence SSC properties among the deep-sea prokaryotes. It is also possible that selective grazing of HNA reduces the contribution of HNA to total bacteria within upper layers where bacterial production is higher but not within deeper layers (Corzo et al., 2005), or that different virus-host interactions may exist in bathypelagic layers when compared to epipelagic layers (Winter et al., 2009). There is clear evidence than more work should be carried out on this topic in the future."

Page 8260, line 28: '. . .along the different sub-groups of chlorophyll categories. . .' not clear what this means – perhaps the different layers of the water column being considered?
Yes. This part of the discussion has been shortened

Page 8261, line 3: '. . .the correlation was significant. . .' not clear which correlation is being discussed, HNA cells? LNA cells?
Both. But this part of the discussion has been removed

Also: 'The slope of the regression of abundance versus chlorophyll was slightly higher for HNA than for LNA cells within the "dcm" layer, suggesting that the HNA cells were very responsive to changes in phytoplankton stocks in the "dcm" layer. . ..since the reader doesn't know the slopes for the data only within the dcm layer, this statement is hard to evaluate. Also, there are statistical tests to compare slopes and they should be done if the data are that important (see Zar, Biostatistical Analysis for one book with an excellent description of how to determine if two slopes are in fact significantly different)
The slopes were 0.44 ± 0.10 for LNA abundance, 0.61 ± 0.14 for HNA abundance (model II regressions on log-log transformed data) but this part of the ms has been removed.

Page 8261, line 21: 'hypothesize' not 'hypothesise'
This has been corrected.

There appear to be references which are not cited in the text, but do appear in the references section (Schlitzer for example. I did not look for others, but that one stood out).
Schlitzer is the reference for the use of ODV for plotting figure 1 and 2 and is now cited in the legend of figure 1. We checked the entire reference list as some were removed in the new version because they are not anymore cited in the text. At the opposite, we added new information and some new references are added.

Table 1: in the column for temperature, does the 'pot' indicate that potential temperature was used? And if so, why?
Because we also used deep data this is best if we remove the effect of compressibility. This is why potential temperature was used.

Also, I am not clear about what '. . .for the data set used for comparison of abundance and cytometric characteristics of HNA & LNA cells' means – the statement seems to imply there are other data not being presented in the manuscript.
Yes, especially for temperature and salinity. Some other CTD casts and/or some other depths were sampled without measurements of bacterial abundances. We refer only to the physical/biogeochemical data (CTDs and depths) where flow cytometric analyses were made. More data on nutrients, BP or chlorophyll data can exist in the data base of the BOUM cruise but not necessarily with an accompanying value of bacterial abundance. For this reason, Table 1 only presents range and means of environmental variables used in regressions with cytometric properties.

Table 2: please define 'ns' in the legend.
This has been done: 'not significant"

Figure 1: please indicate what the colors in the map are. I would imagine they are depth, but that should be indicated.
Yes they are depth. The scale bar showing colour code for depths has been added on this figure.

Figure 2: please specify what the vertical bar is around 1000 km. Also, even though you do not have bacterial production data for all of the stations, please make the plots with the same x-axis to allow for easier comparison between the two variables.
The bar at St 17 in the Sicily strait shows the bottom depth (116 m). This was added in the legend. The two plots have been re-drawn with the same x-axis (old figure 2 is now new figure 3).

Figure 3: the points would be easier to distinguish if only one circle were used, and the other circles were replaced with non-circle and non-square shapes.
Old Figure 3 has been removed. We modified colour codes in old figure 6 (new fig 8) to be the same as in figure 7 (new figure 9).

Figure 4: The LNA and HNA abundances overlap enough that I would separate them into two figures or add some color so that the reader can see any possible patterns between LNA and HNA abundances. Also, the figure legend indicates that the scales are different for 4b and 4f; however, there also appears to be a difference scale for bacterial production (0-50 in the upper plot and 0-5 in the lower plot).
We draw HNA and LNA abundances on separated figures. We add also vertical distribution of % HNA
The text of the legend is modified to point out changes in some scales:
"Fig. 4. Vertical distributions of a,b: temperature; c,d total Chlorophyll a TChl a) and bacterial production (BP); e,f: LNA cells abundances, g,h HNA cells abundance and %HNA; i,j: side scatter signal (SSC); k,l: green fluorescence signal. Note different variable scales for the deeper layers (bottom figures), except for SSC, green fluorescence and %HNA (same scale)."

Figure 5: please make the letters for each of the subplots the same in order to prevent confusion between the letters indicating statistical significance and the letters for each of the subplots.
Letters for the subplots are now in capital and always drawn at the same place on the subplot for both old Figure 5 (new figure 5) and new figure 6.

Anonymous Referee #2

Received and published: 7 January 2011

This manuscript presents large amounts of data on distribution patterns of prokaryote cells and their single cell characteristics in the Mediterranean Sea. One anonymous reviewer has already provided critical comments on this manuscript, pointing out the need of revisions. After careful examination of the manuscript and the review, I reached the conclusion that I mostly share the opinion with the other referee. In order to minimize repetition, the following review will focus on the points that were not mentioned or not adequately stressed by the other referee.

1. Testable hypotheses (research questions) should be formulated in order to contribute to the progress of science. Introduction states two aims. One is to explore the factors that determine HNA-LNA cell variability in the Mediterranean Sea. This sort of "aim" would have been justifiable in 80-90's when the flow cytometric technique in marine science was relatively new. However, given that extensive amounts of data on single-cell characteristics of marine prokaryotes have been published during the past decade, this naïve formulation of research question cannot be acceptable.

The end of the introduction was reformulated to point out that although we know a lot on the distribution of LNA and HNA in some environments, to our best knowledge the distribution of HNA and LNA cells was never investigated in the Eastern basin, nor in deep layers. Moreover, variations in these parameters have not yet been understood and are generally only related to the trophic level of the waters. We extended the factors not only to depth, BP and chlorophyll, but also nutrients and this was also written at the end of the introduction.

The second stated aim (to explore HNL-LNA connections) appears to be more specific, yet I could not figure out what "HNA-LNA connections" means at all on the basis of the information provided in Introduction. It was unfortunate that what this question (HNALNA connections) means was unclear even after reading the entire text including the relevant section in Discussion (Section 4.4).

My suggestion is that the manuscript be reorganized to address specific (and meaningful) hypotheses regarding single cell properties (biochemistry and ecology, not just "cytometric characteristics") of marine prokaryotes. What was the question? What was the answer? What was the news?

This feeling may be due to the important number of results and sometimes to the "dilution" of the informations and results. The discussion section was shortened to better focus on relevant results.

The main point was that the vertical distribution better explain the distribution of cytometric properties than longitudinal distribution, and notably that phosphate and nitrate + nitrite were not particularly factors explaining the variability of cytometric properties.

The second one is the surprising increase in the SSC values of the HNA group and the increase in %HNA in meso and bathy pelagic layers.

2. Need more examination regarding prokaryote-environment relationships. It was rather disappointing that the authors did not explore potentially interesting questions regarding prokaryote-environment relationships in the Mediterranean Sea. In fact, the environmental factor that was dealt with by this study was only chlorophyll. I suspect that there were gradients in nutrient concentrations and stoichiometry along the transect and over depth. Mediterranean ecosystem in general is known to be severely limited by P, but the extent of P-limitation may vary depending on regions and depths. Also, there is a vertical gradient in temperature but Mediterranean is unique with relatively warm waters at great depths. How these unique environmental settings of the system examined by this study may affect prokaryote single cell properties?

We investigated relationships between cytometric parameters and nutrients (nitrate + nitrite and phosphate). Ranges of nitrate + nitrite were added Table 1, and results of correlations with nutrients Table 2 (new Table 2 is old Table 3).

Two news figures were added (Figure 6, Figure 7, see at the end of this document) to examine longitudinal trends.
The discussion has been modified to include prokaryote - nutrients relationships and regional variability.

3. SSC-FL diagrams should be presented. Definitions of HNA and LNA differ among studies using different instrument and/or conditions. Since SSC and FL variations are the focus of this study, it is important to show representative diagrams.
A figure has been added to show four representative diagrams (new Figure 2, see at the end of this document).

Specific comments
Section 3.2 As pointed out by the other referee, this section and related tables and figures can be substantially reduced or eliminated
Old Table 2 and old Figure 3 have been removed.

The increase in %HNA with depth has been ascribed to the alleviation of P-limitation (Nishimura et al. 2005 AEM) and this notion has been theoretically considered under the framework of temperature-resource acquisition trade offs (Hall et al. 2008 ISME J. 2:471-). I would recommend that the authors mention this.
This has been done -page 12 lines 21-27:
"Nishimura et al. (2005) hypothesized that P limitation exerts more severe constraints on the growth of bacterial groups with higher nucleic acid content. Furthermore, a modelling demonstration by Hall et al. (2008) would suggest that warm adapted species had lower minimal P cell quotas than cold-adapted species, which gives advantage to small species (like LNA-type cells) in warm-resource limited environment. Temperature range in 'surface' layers during our study could reach 11°C (26°C down to 15°C) in 'surface' layers, where %HNA did not seems to be affected by temperature. Rather, resource limitation is a more explanatory factor to explain vertical distribution of LNA and HNA cells."

The first paper that described %HNA-chl relationship in the ocean is Li et al. (1995 L&O). I would recommend that this paper be cited.
This reference has been added in the introduction section (page 2 line 14)

Although the authors considered only bottom up forces as determinants of single-cell properties of prokaryotes, there is ample evidence in support of the notion that selective grazing and viral infection may also affect size and surface properties of cells.
This is an interesting question. Yes, there is literature data on size - selection of preys by phagotrophic protozans. Scharek & Latasa (2007) compared growth and grazing of HNA and LNA cells in surface and dcm layers in Mediterranean sea waters, and growth and grazing of both cytometric groups are equilibrated.
Dealing on the increase of %HNA and SSC of HNA in deep layers, we added a sentence suggesting that top-down controls could influence also %HNA (page 15 lines 3-7):
"It is also possible that the selective grazing on HNA cells due to their larger size reduces their contribution to total bacteria within upper layers where bacterial production is higher but not within deeper layers (Corzo et al., 2005). In addition, different virus-host interactions in bathypelagic layers exist compared to epipelagic layers (Winter et al., 2009)."
We also added additional hypothesis (page 15 lines 1-3):
"Although we are not aware of any investigations on marine microorganisms, SSC characteristics may also be affected by changes in the zeta potential of the cells when infected by viruses or when producing polysaccarides (Wilson et al 2001)".

Wilson, W. W., Wade, M. M., Holman, S. C., and Champlin, F. R.: Status of methods for assessing bacterial cell surface charge properties based on zeta potential measurements. Journal of Microbiological Methods, 43**,** 153-164, 2001.

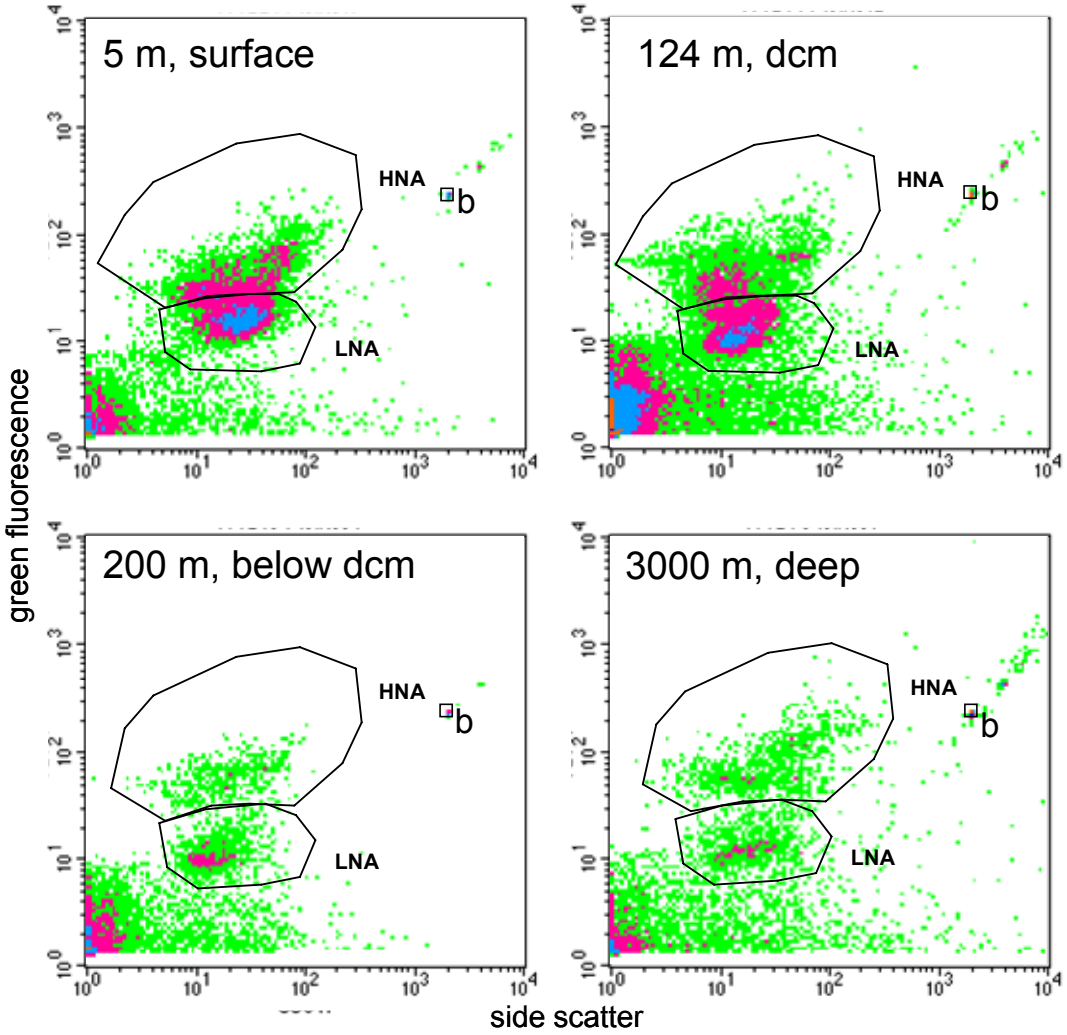
New Table 1

Ranges of salinity, temperature, total chlorophyll a (TChl a), phosphate (PO4), nitrate + nitrite (NO3+NO2), total bacterial abundance (total ab), and bacterial production (BP) for the data set used for comparison of abundance and cytometric characteristics of HNA and LNA cells. Means, coefficient of variation and number of data for each group of water column partition: layers above the deep chlorophyll maximum (surface), layers at the deep chlorophyll maximum ('dcm'), layers below the dcm but above or equal to 250m ('below dcm'), and layers below 250m 'deep' . bdl: below detection limits.

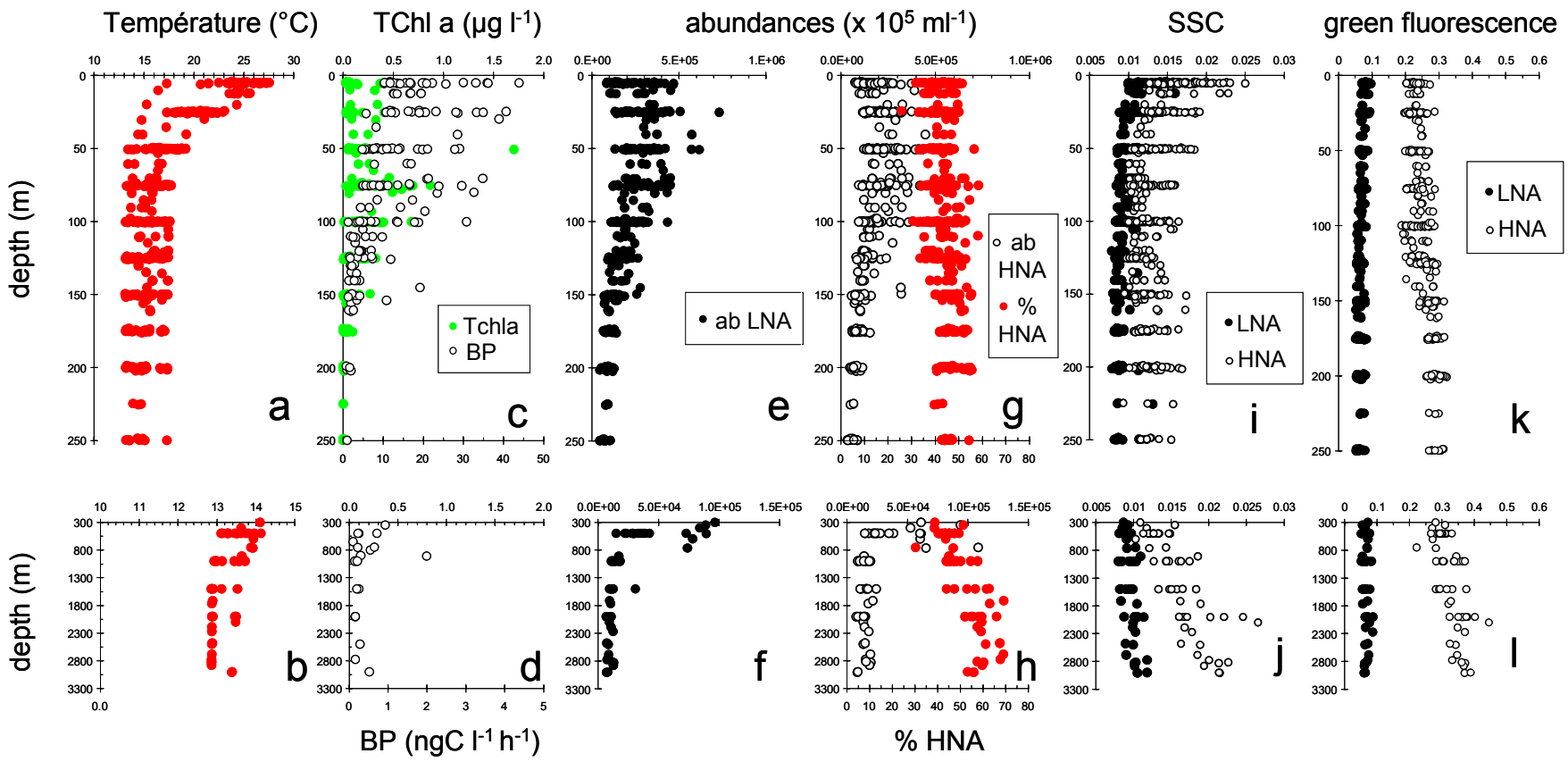
	Salinity PSU	temperature °C	TChl a µg l^{-1}	PO4 µM	NO3+NO2 µM	total ab x 10^5 ml^{-1}	BP ng C l^{-1} h^{-1}
N	493	493	296	417	417	493	198
min	37.24	12.9	0.0004	bdl	bdl	0.15	0.15
max	39.65	27.5	1.7	0.393	9.81	13.40	43.9
surface	38.33 (1.8%, n=192)	19.7 (18%, n=192)	0.098 (74%, n=111)	0.008 (155%, n=148)	0.040 (392%, n=148)	4.62 (33%, n=192)	15.0 (60%, n=110)
dcm	38.42 (1.6%, n=45)	15.8 (7%, n=45)	0.43 (67%, n=30)	0.015 (112%, n=38)	0.46 (130%, n=38)	5.18 (43%, n=45)	15.2 (61%, n=24)
below dcm	38.60 (1.2%, n=201)	14.9 (8%, n=201)	0.059 (137%; n=154)	0.101 (98%, n=175)	3.46 (79%, n=175)	2.36 (49%, n=201)	3.62 (96%, n=54)
deep	38.63 (0.4%, n=55)	13.4 (5%, n=55)		0.277 (35%, n=55)	7.50 (28%, n=55)	0.53 (110%, n=55)	0.48 (123%, n=10)

New Table 2. Relationships between abundance of HNA cells, abundance of LNA cells and %HNA versus TChl a, versus BP, versus soluble reactive phosphorus (PO4) and versus nitrate+nitrite (NO3+NO2) concentrations. Data were transformed before fitting with linear regressions (see methods). n: number of data, r2: determination coefficient, nd: not determined, ns: not significant (Significant threshold set at p=0.05). For clarity, sign of slopes for weak correlations (r2 < 0.1, in italics) were not indicated. The number of data used in the regressions for each type of environmental variable and for each layer are indicated in Table 1.

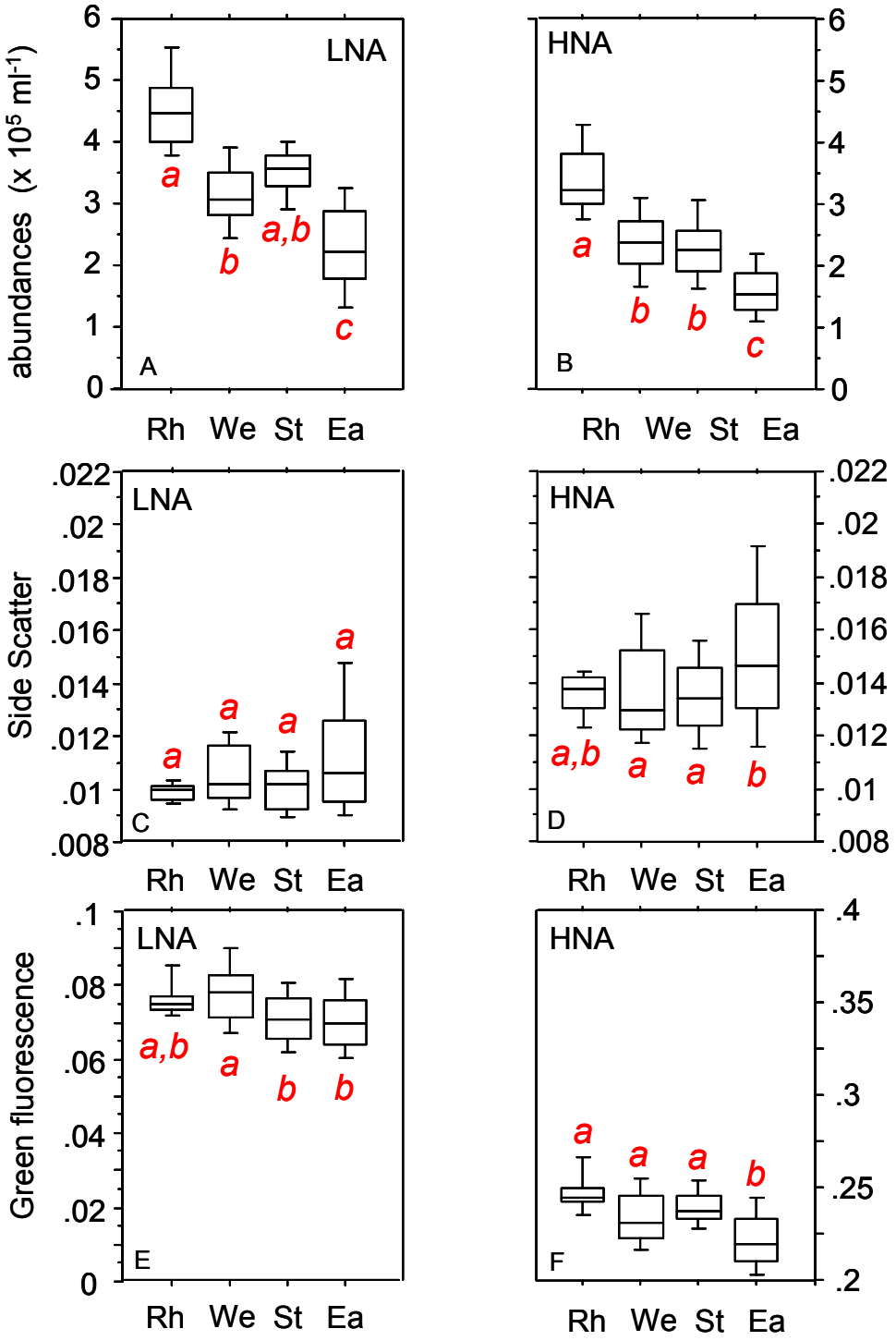
		relation with Tchla		relation with BP		relation with PO4		realtion with NO3+NO2	
		Sign of slope	r2	sign of slope	r2	sign of slope	r2	sign of slope	r2
HNA abundance	all data	+	0.59	+	0.65	-	0.46	-	0.41
	surface	+	0.32	+	0.25		ns		ns
	dcm	+	0.41	+	0.52		ns		ns
	below dcm	+	0.55	+	0.42		*0.08*	-	0.19
	deep		nd		ns		ns		ns
LNA abundance	all data	+	0.6	+	0.70	-	0.49	-	0.46
	surface	+	0.26	+	0.21		ns		ns
	dcm	+	0.4	+	0.49		ns		ns
	below dcm	+	0.64	+	0.46		*0.07*	-	0.16
	deep		nd		ns		ns		ns
%HNA	all data		*0.07*	-	0.19	+	0.21	+	0.24
	surface		ns		ns		ns		ns
	dcm	+	0.12	+	0.21		ns		ns
	below dcm		*0.02*		ns		ns		ns
	deep		nd		ns		ns		ns



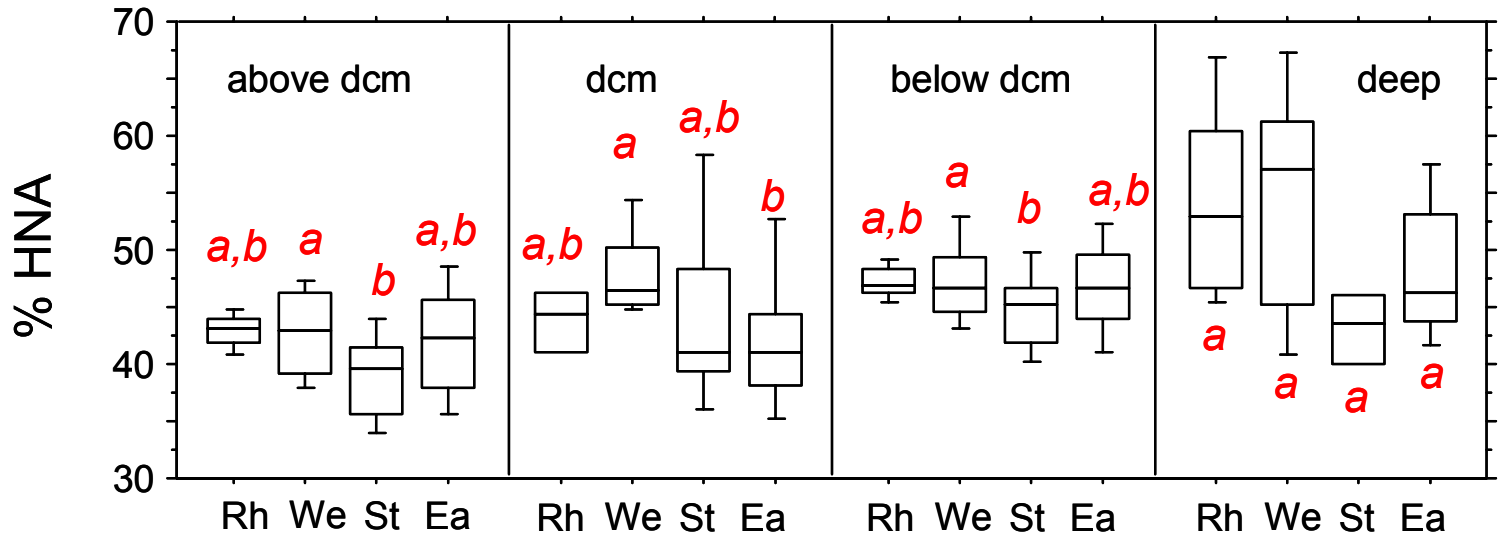
New Fig. 2. Examples of four cytograms obtained after SyBR green I staining. The data comes from Station B (Ionian Sea): surface (5 m), at the dcm (125 m), below dcm (200 m) and in deep layers (3000 m). Time of analysis was set to 1 minute for the 3 first layers, and 3 minutes for the 3000 m layer. Windows for HNA and LNA cells are drawn and were adjusted from one sample to another. b: 1 µm beads.



New figure 4. Vertical distributions of a,b: temperature; c,d total Chlorophyll a TChl a) and bacterial production (BP); e,f: LNA cells abundances, g,h HNA cells abundance and %HNA; i,j: side scatter signal (SSC); k,l: green fluorescence signal. Note different variable scales for the deeper layers (bottom figures), except for SSC, green fluorescence and %HNA (same scale).



New Fig. 6. Box plots showing distribution of abundance (A, B), side scatter (C, D) and green fluorescence (E, F) of subgroups LNA and HNA in the 'surface' layers according a regional distribution: 'Rh' (stations 27, 26, 25), 'We' (stations 24, 23, A, 22, 21, 20, 19), 'St' (Stations 18, 17, 16, 15, 14) and 'Ea' (stations 13, 12, B, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, C). Groups connected by the same letter (in italics & in red) are not significantly different at the 0.01 probability level.



New Fig. 7. Box plots distributions of %HNA in the different regions and in the different layers. Within a common layer, groups connected by the same letter (in italics & in red) are not significantly different at the 0.01 probability level.