

1 Lipid biomarkers and bacterial lipase activities as indicators of organic matter and bacterial
2 dynamics in contrasting regimes at the Dyfamed site, NW Mediterranean

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1 **Abstract**

2 This study investigated relationships between dissolved organic matter (DOM)
3 composition and bacterial dynamics on short time scales during spring mesotrophic (March
4 2003) and summer oligotrophic (June 2003) regimes, in a 0-500m deep water column with
5 little advection, at the DYFAMED site, NW Mediterranean. DOM was characterized by
6 analyzing dissolved organic carbon (DOC), colored dissolved organic matter (CDOM) and
7 lipid class biotracers. Bacterial dynamics were assessed through the measurement of *in situ*
8 bacterial lipase activity, abundance, production and bacterial community structure. We made
9 the assumption that by coupling the ambient concentration of hydrolysable acyl-lipids with
10 the measurement of their *in situ* bacterial hydrolysis rates (i.e. the free fatty acid release rate)
11 would provide new insights about bacterial response to changes in environmental conditions.
12 The seasonal transition from spring to summer was accompanied by a significant
13 accumulation of excess DOC (+ 5 μM) in the upper layer (0-50m). In this layer, the free fatty
14 acid release rate to the bacterial carbon demand (BCD) ratio increased from 0.6 ± 0.3 in March
15 to 1.3 ± 1.0 in June showing that more uncoupling between the hydrolysis of the acyl-lipids
16 and the BCD occurred during evolution of the season, and that free fatty acids contributed to
17 the excess DOC. The increase of lipolysis index and CDOM absorbance (from 0.24 ± 0.17 to
18 0.39 ± 0.13 and from 0.076 ± 0.039 to 0.144 ± 0.068 , respectively), and the higher contribution of
19 triglycerides, wax esters and phospholipids (from <5% to 12-31%) to the lipid pool reflected
20 the change in DOM quality. In addition to a strong increase of bacterial lipase activity per
21 cell (51.4 ± 29.4 to 418.3 ± 290.6 $\text{Ag C cell}^{-1} \text{h}^{-1}$), a significant percentage of ribotypes (39%)
22 was different between spring and summer in the DCM layer in particular, suggesting a shift in
23 bacterial community structure due to the different trophic conditions. In both seasons, diel
24 variations of DOM and bacterial parameters in the chlorophyll layers reflected a greater
25 bioavailability and/or DOM utilization by bacteria at night (the ratio of free fatty acid release

1 rate to bacterial carbon demand decreased), most likely related to zooplankton trophic
2 behaviour. In mesotrophic conditions, such a day/night pattern was driving changes in the
3 bacterial community structure. In the more oligotrophic period, diel variations in bacterial
4 community structure were depth-dependent in relation to the strong summer stratification.

5

6 **Keywords: Lipids, Lipase activity, DOM, Bacteria, Community structure**

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8 Running title: Lipid biomarkers and bacterial lipase activities in the NW Mediterranean

1 **1. Introduction**

2 Dissolved organic matter (DOM) produced through photosynthesis (algal excretion, cell
3 lysis) or zooplankton metabolism (egestion of fecal material, excretion by metazoan
4 zooplankton or ‘sloppy feeding’) is a key factor in the regulation of bacterial activities and
5 growth (Azam et al. 1983; Bochlansky et al., 1995; Richardot et al., 2001; Kragh and
6 Søndergaard, 2004). Short- and long-term variations of the quality and quantity of carbon
7 resources force bacteria to adapt their metabolic functions like production (Zohary and
8 Robarts, 1992; Gasol et al., 1998; Kirchman et al. 2004) and ectoenzymatic activities
9 (Middelboe and Søndergaard, 1993; Bochlansky et al., 1995). Pronounced changes in
10 bacterial abundance, production and ectoenzyme activities were observed during changes in
11 organic matter concentration and composition (Palumbo et al., 1984; Rath et al., 1993; Smith
12 et al., 1995). For example, mesocosm experiments underline the increase of bacterial
13 numbers, growth rates and enzyme activities following peak phytoplankton blooms (Smith et
14 al., 1995; Van Wambeke, 1995; Fajon et al., 1999). In turn, bacterial processes are
15 responsible for qualitative and/or quantitative transformations of the organic matter pool.

16 Bacteria are the only organisms capable of using low concentrations of dissolved
17 organic molecules. Because a large part of organic matter is in the form of large size
18 macromolecules (Amon and Benner, 1994) but only small molecules (<600 Da) are directly
19 assimilated by bacteria (Weiss et al., 1991), bacterial ectoenzymes play a key function in the
20 transformation of biopolymer into small monomers (Chróst, 1991). They are thus responsible
21 for the persistence or collapse of biological production during their transport to depth (Azam,
22 1998). However, *in situ* biopolymer hydrolysis and monomer assimilation rates by bacteria
23 remain poorly documented (Nagata and Kirchman, 1997). Research on DOM cycling through
24 bacterial processes suffers several weaknesses, among which methodologies for measuring *in*
25 *situ* bacterial activity are still controversial. Several studies have attempted to identify

1 innovative substrates aimed at providing realistic data regarding organic matter hydrolysis
2 (Arnosti, 1996; Pantoja et al., 1997; Bourguet et al., 2003) and production rates (Van Mooy et
3 al., 2006) in the marine environment. Thus, while it is now clear that bacteria are important
4 for determining the fate and loss of organic carbon exported to the mesopelagic layer and the
5 deep ocean (Legendre and Rassoulzadegan, 1996), the rates at which bacterial processes
6 occur in the marine environment, how they vary in response to small or larger spatio-temporal
7 scale events, how they relate to bacterial community structure, and how they impact
8 biogeochemical cycles, are still under question.

9 The connections between phylogenetic diversity and ecological function of marine
10 bacteria in natural environments are poorly documented. Throughout a phytoplankton bloom
11 in a Danish coastal area, only a few differences in microbial community composition were
12 observed, while changes in bacterial abundance and production were pronounced (Riemann
13 and Middelboe, 2002). This result suggests that environmental changes could occur without
14 major shifts in the bacterial community composition (Hollibaugh and Azam, 1983). However,
15 a succession of bacterial communities is often associated with the formation and senescence
16 of phytoplankton blooms, possibly reflecting the proliferation of bacterial phylotypes
17 specialized in particle colonization and degradation (Van Hannen et al., 1999; Riemann et al.,
18 2000; Fandino et al., 2001; Larsen et al., 2001; Schäfer et al., 2001). Moreover, different
19 isolates of marine bacteria grown under the same conditions were shown to display different
20 patterns of hydrolytic enzyme activities, suggesting that expression of ectoenzyme activities
21 in oceans could result from species selection and population dynamics (Martinez et al., 1996).
22 Actually, whether the observed bacteria-related changes in carbon fluxes are due to variations
23 in bacterial community structure is still unclear.

24 Lipids are energetic compounds for cell metabolism. They are helpful markers to
25 determine sources and fate of organic matter in marine systems (Wakeham, 1995). They

1 generally represent 3-20% of biogenic carbon (Rullkötter, 2000) and can be easily
2 concentrated and analyzed from suspended particles and/or the dissolved fraction.
3 Biogeochemical studies using lipid biomarkers, either intact compounds (Parrish, 1988;
4 Goutx et al., 1990; Derieux et al., 1998; Rütters et al., 2002) and/or molecular moieties
5 (Grimalt et al., 1992; Bouloubassi and Saliot, 1993; Volkman et al., 1998; Wakeham et al.,
6 2002; Goutx et al., 2005) are numerous. An improved Iatroscan TLC-FID analysis of intact
7 acyl-lipids and their degradation products (Parrish et al., 1992; Striby et al., 1999) was
8 proposed as a tool for studying organic matter dynamics in relation to bacterial degradation
9 processes (Goutx et al., 2003). Such analysis allows discrimination of lipid polymers
10 (requiring hydrolysis before uptake) from directly assimilable monomers.

11 Using this approach, observations at the Dyfamed site (NW Mediterranean sea, Cruise
12 Dynaproc I, 1995) revealed the presence of chemical gradients of lipid monomers that
13 accumulated during transition from spring mesotrophic to summer oligotrophic regimes,
14 suggesting that peculiar conditions for uncoupling between bacterial hydrolysis and
15 assimilation prevailed in surface waters at this time of the year (Goutx et al., 2000; Van
16 Wambeke et al., 2001). However, amino peptidase, not lipase activity, was measured (Van
17 Wambeke et al., 2001). In addition, as the enzyme activity was measured with an analog
18 fluorochrome substrate, it may not be representative of naturally occurring rates (Bourguet et
19 al., 2003). The ³H-triolein lipase assay developed in our laboratory by Bourguet et al. (2003)
20 may provide a better estimate of lipid polymer *in situ* hydrolysis rates.

21 With the hope of better resolving the response of bacteria to change in environmental
22 conditions, we studied the effect of contrasting spring mesotrophic and summer oligotrophic
23 regimes of production on DOM composition, bacterial activity and community structure at the
24 DYFAMED time-series station, NW Mediterranean, along the vertical dimension. We made
25 the assumption that coupling the ambient concentration of hydrolysable acyl-lipids with the

1 measurement of their *in situ* bacterial hydrolysis rates (i.e. the free fatty acid release rate)
2 would provide new insight about bacterial response to change in environmental conditions.
3 We coupled the analysis of lipid classes used as both biomarker of source and transformation
4 of DOM, and substrate for bacterial hydrolysis, to the measurement of *in situ* bacterial lipase
5 activity, bacterial abundance, production and community structure together with
6 environmental parameters such as nutrients, Chl a, primary production, zooplankton biomass,
7 DOC, CDOM, along a 0-500 m water column. The aim of the study was to determine the
8 extent to which changes in environmental conditions were reflected in the DOM composition,
9 the bacterial metabolism and the structure of the community.

1 **2. Material and methods**

2

3 2.1. CTD casts and sample collection

4 Sampling was conducted on board the 25-m long R/V Tethys II during two short
5 surveys in 2003 March (24-31) and June (19-24) at the French JGOFS Dyfamed time series
6 station (43°25'N, 07°52'E), NW Mediterranean (Figure 1). Water samples were collected
7 along the water column from 3 to 500m, at 8-10 depths between surface and 100m and 2-4
8 depths between 100 and 500m, using a Seabird SBE911 CTD-Rosette sampler equipped with
9 a Chelsea AIII fluorescence auxiliary sensor and 12 l Niskin bottles. During the two surveys,
10 CTD profiles (20) were done at regular time intervals to characterize the hydrological
11 conditions at the study site. Seawater samples were collected from two profiles done at
12 around noon and midnight (local time, GMT + 2) on March 26 and June 21. Samples for
13 chemical and microbiological analyses were immediately treated on board, and microbial
14 processes measured without delay on board. Seawater samples for nutrients, phytoplankton
15 biomass and primary production (PP) were collected from the late night CTD/rosette cast
16 immediately before coming back to the laboratory (usually between 1 and 2 am). Zooplankton
17 biomass was collected in vertical tows (0-200m and 0-500m) with a triple WP11 (200 µm
18 mesh size) deployed twice (day and night) during the May and June surveys (March 26-30,
19 June 19-22).

20

21 2.2. Nutrients, chlorophyll biomass and primary production

22 Samples for nutrients (0-250m in March and 0-100m in June) were collected in
23 polyethylene flasks and immediately poisoned with HgCl₂ (10 µg l⁻¹). Analyses were
24 conducted in the laboratory using a Technicon AutoAnalyzer II according to Tréguer and le
25 Corre (1975). At 3, 10, 20, 30, 40 and 60m depths, primary production was assayed using the

1 dual labeling $^{13}\text{C}/^{15}\text{N}$ procedure (Fernandez et al., 2005) and a 12h incubation time from
2 sunset to sunrise. Precision was $\pm 0.05 \mu\text{mole}$ for nitrates, ammonium and silicate data, ± 0.03
3 μmole for nitrites, $\pm 0.02 \mu\text{mole}$ for phosphates, $\pm 0.03 \mu\text{g L}^{-1}$ for chlorophyll and ± 0.5
4 μmole for primary production.

5

6 2.3. Zooplankton biomass and gut content

7 Material from two of the cod ends was collected onto a filter, dried (60°C for 48h) and
8 weighed with a Sartorius balance. Material from the third cod end was preserved in buffered
9 formalin (4%) for species abundance determination. As zooplankton are known to be active
10 mainly at night, gut contents were determined on dominant species sampled with a surface
11 bongo net or a multinet (BIONESS) around midnight. Organisms were put in vials with 10 ml
12 of 90% acetone, then crushed. Samples were extracted at 4°C for ca. 12 h. Chlorophyll and
13 pheopigments were determined by a standard fluorimetric procedure, and the gut content was
14 estimated according to Bamstedt et al. (2000).

1

2 2.4. Organic matter

3 2.4.1. Dissolved organic carbon

4 Samples were filtered through 2 precombusted (24 h, 450°C) glass fiber filters
5 (Whatman GF/F, 25mm), placed in precombusted glass tubes (closed with a screw cap and a
6 Teflon liner), poisoned with orthophosphoric acid (H₃PO₄) and stored at room temperature
7 until analysis. Dissolved organic carbon (DOC) was analyzed on a Shimadzu TOC-V using
8 high- temperature catalytic oxidation (HTCO) (Sugimura and Suzuki, 1988; Cauwet, 1994).
9 Analytical precision of measurements was close to 2 μM. Deep Sargasso Sea reference water
10 (47 mol L⁻¹ C, ± 0.5 SE, <http://www.rsmas.miami.edu/groups/biogeochem/CRM.html>) was
11 injected every 10-12 samples to insure stable operating conditions.

12

13 2.4.2. Dissolved lipids

14 Seawater was filtered onto precombusted glass fiber filters (Whatman GF/F, 47 mm).
15 Dissolved lipids were extracted from the filtrate (2 liters) by liquid-liquid extraction, using
16 dichloromethane (two extractions at sea water natural pH, followed by two extractions at pH
17 2). Each lipid extract was separated into classes of compounds, according to polarity, on
18 chromarods SIII (0.9mm diameter, 150 mm length, 75 μm silica thick) and quantified using
19 thin layer chromatography-flame ionization detection (TLC-FID) Iatroscan TH10 apparatus
20 model MK-IV (Iatron, Japan; hydrogen flow, 160 ml min⁻¹; air flow, 2000 ml min⁻¹). The
21 elution scheme has been described previously (Striby et al., 1999). The procedure allows
22 reliable separation and quantification of twelve classes of acyl-lipids and free lipids, including
23 storage lipids (wax esters and triacylglycerols), chloroplast lipids (pigments and glycolipids),
24 cellular membrane lipids (phospholipids that include phosphoglycerides,
25 phosphatidylethanolamines and phosphatidylcholines), and their hydrolysis metabolites

1 (alcohols, free fatty acids, monoglycerides and 1,2- and 1,3-diglycerides). The ratio of
2 hydrolysis metabolites to the sum of reserve and membrane acyl-lipids (i.e. the lipolysis
3 index, LI) was used to characterize the degradation status of organic matter (Goutx et al.,
4 2003). Metabolites and their source acyl-lipids were considered as lipid monomers and
5 polymers, respectively. Total lipids refer to the sum of all lipid classes.

6

7 2.4.3. Colored dissolved organic matter (CDOM)

8 CDOM was analyzed according to Momzikoff et al. (1994). Seawater samples (500 ml)
9 were filtered through precombusted glass fiber filters (Whatman GF/F, 25mm). CDOM
10 substances were extracted from the filtrate using HLB Oasis cartridges (Waters) after HCl
11 acidification to pH 2.2. They were sequentially eluted with 4 ml of methanol and 4 ml of 0.1
12 M NaOH. Only the MeOH eluates were considered here. Optical density (o.d.) of eluate was
13 measured at 412 nm in a 1cm path length quartz cell in a conventional spectrophotometer,
14 then converted to absorbance coefficient (A) through the relationship $A = 2.3 \text{ o.d. } L^{-1}$ where L
15 = 0.01m. CDOM initially present in seawater before extraction and originating from the
16 MeOH eluate was quantified using the 125 enrichment factor. Data used here do not take into
17 account losses due to extraction. The recovery yield of the CDOM was measured to be ca
18 70% by comparing the absorption coefficient at 412 nm of the combined MeOH and NaOH
19 eluates with that of the corresponding seawater before CDOM extraction of several samples.

20

21 2.5. Bacterial parameters

22 2.5.1. Bacterial abundance and activities

23 For bacterial abundance (BA) measurements, seawater samples (10 ml) were poisoned
24 with 2% buffered formalin that was previously filtered through a 0.2 μ m pore-size filter. BA
25 was measured using the acridine orange direct count (AODC) method (Hobbie et al., 1977).

1 Samples were stained and filtered onto black 0.2 μm pore-size filters (Isopore, 25mm) and
2 observed using an Olympus microscope (magnification x 1250). Twenty fields containing 80
3 to 100 bacteria each were counted for each filter (CV inter-field, 2 to 14 %), and the samples
4 were analyzed in duplicate (CV inter-filter, 1 to 7 %).

5 Bacterial production (BP) was estimated from ^3H -leucine (specific activity = 161 Ci mmol^{-1})
6 incorporation according to Kirchman et al. (1993). Three replicates (10 to 30
7 ml depending on sampling depth) and one 2 % formalin control received 20 nM leucine
8 mix (2 nM ^3H -leucine and 18 nM cold leucine). This concentration was experimentally
9 determined to be saturating at different depths. Because we had only one incubation
10 chamber set up at 13°C on board, activities measured at 3 and 10 m depth in June, when
11 the sea water temperature was 26 and 20°C , respectively, were corrected for temperature
12 effect by using a $Q_{10}=2.3$ according to Li and Dickie (1987). Samples were incubated in the
13 dark at $13 \pm 1^\circ\text{C}$ for 2 to 8 h. Previous experiments showed that leucine incorporation was
14 linear over these time periods (data not shown). The reaction was terminated with formalin
15 (final concentration 2%), and all samples were filtered onto nitrocellulose 0.2 μm -pore size
16 filters (25mm, Millipore). Samples were then extracted with 5% trichloroacetic acid (TCA)
17 for 10 min followed by three 3 ml rinses with 5% TCA. Filters were placed in scintillation
18 vials containing 5 ml scintillation cocktail (Amersham), and radioactivity was counted on a
19 Tri-CARB 1500 Packard liquid scintillation counter. Quenching was corrected by internal
20 standard, and control counts were subtracted. The mean coefficient of variation of the
21 triplicate measurements was 11.5%. BP rates were then calculated from leucine incorporation
22 rates using the conversion factor of $1.5 \text{ kg C mol}^{-1}$ leucine, assuming a dilution factor of 1
23 (Van Wambeke et al. 2002).

24 Bacterial lipase activity was measured using the ^3H -triolein lipase assay described by
25 Bourguet et al. (2003), slightly modified as follows: Water samples (20 ml) were amended

1 with ^3H -triolein (specific activity = 53 Ci mmol^{-1}) (0.13 to 0.26 nM final concentration).
2 Subsamples (1.5 ml) were distributed into 15 ml centrifuge tubes. At $t=0$, 1h, 2h and 3h,
3 incubations ($13 \pm 1^\circ\text{C}$, in the dark) were terminated by the addition of 1 ml TRIS solution
4 (1M, pH 11) and stored at -20°C until analysis. Samples were extracted with 6.5 ml of the
5 solvent mixture (methanol:chloroform:heptane, 38.6:34:27.4) and centrifuged (3 min at 5500
6 rpm) to allow separation of the phases. A portion (2 ml) of the upper aqueous phase,
7 containing the released [^3H]-oleic acids, was transferred into scintillation vials containing 10
8 ml of Hionic-Fluor liquid scintillation cocktail (Packard Bioscience) for radioactivity
9 counting. Any $^3\text{H}_2\text{O}$ formed by mineralization of the [^3H]oleic acid would end up in the
10 aqueous phase as well (as would other transformation products). However, previous tests
11 (Bourguet et al., 2003) suggested that negligible fatty acid mineralization occurs during this
12 short incubation time. The counts of [^3H]-oleic acids were corrected according to a $\sim 50\%$ pre-
13 determined oleic acid partition coefficient between solvent and aqueous phases. Hydrolysis
14 rates ($\% \text{ h}^{-1}$) (HR) were calculated from the appearance rate of the labeled free fatty acids
15 [^3H]-oleic acid (r^2 of the regression slope ranged from 0.915 to 0.992, $P < 0.05$, $n=8$)
16 compared to the initial [^3H]-triolein concentration (considered as “esterified fatty acids”). As
17 for BP, activities measured at 3 and 10 m depth in June were corrected for temperature by
18 using a $Q_{10}=2.3$.

19 Bacterial carbon demand (BCD) was calculated as the ratio of bacterial production to
20 bacterial growth efficiency (BGE). BGE values at the DYFAMED site were taken from
21 Lemée et al. (2002) (0.19 and 0.15 in March and June, respectively). Free fatty acid (FFA)
22 release rates quantify the flux of FFA released through ectoenzymatic hydrolysis of acyl-
23 lipids by bacteria. Rates were calculated assuming that ^3H -triolein hydrolysis rates (^3H -
24 triolein HR) were representative of the hydrolysis rates of one fatty acid ester bond per mole
25 of wax esters, glyco- and phospholipids and two ester bonds per mole of triglyceride lipids in

1 the lipid polymer pool according to Bourguet et al. (2003), Arrese et al. (2006) and Aoki et al.
2 (2007). FFA release rates were calculated as the product of HR and the concentration of
3 hydrolyzable carbon lipid.

4

5 2.5.2. Bacterial community structure.

6 Samples (2 liters) were filtered under low vacuum pressure (<100 mbar) using a
7 peristaltic pump flushing water through polycarbonate 0.22 μm -pore-size filters (Nuclepore,
8 47mm). Filters were put into sterile 2 ml Eppendorf tubes and stored at -20°C until analysis.
9 The procedure used for extraction of total DNA and PCR amplification of the variable V3
10 region of the 16S rDNA has been described elsewhere (Ghiglione et al., 2005). A mix of
11 fluorescently labelled TET-PCR products and a ROX-internal size standard (Genescan-400
12 ROX, Applied Biosystems) were heat denatured at 94°C for 5 min and immediately placed on
13 ice for at least 10 min. CE-SSCP electrophoresis was carried out as described previously
14 (Ghiglione et al., 2005) using the ABI 310 Genetic Analyzer equipped with a capillary tube
15 (Applied Biosystems). Data were collected with ABI Prism 310 collection software (Applied
16 Biosystems). The size standard being marked with a different fluorophore (ROX) permitted a
17 reliable comparison of patterns from each sample after computing alignment (Genescan
18 analysis software, Applied Biosystems). A binary matrix based on the presence or absence of
19 peaks of the compiled ATTP and 0.22 μm -pore-size Nuclepore-filtered 16S rDNA PCR-CE-
20 SSCP profiles was used to construct a similarity matrix with Jaccard's dichotomy coefficient.
21 Distance matrix was then calculated and dendrograms were generated using the unweighted
22 pair group method with arithmetic averages (UPGMA, SYSTAT 5.2.1.).

1 3. Results

2

3 3.1. Hydrology, phytoplankton and zooplankton

4 The 0-1000m CTD casts done at regular intervals showed little advection and a great
5 stability of the water masses at the DYFAMED site during the day and night surveys (Fig. 2),
6 according to Andersen and Prieur (2000). In March, a temperature instability marked by a
7 12.9°C temperature minimum was observed between 100 and 200 m depth (Fig. 2a), but we
8 did not sample at these depths. In the euphotic layer, the temperature was homogenous (13.2
9 ± 0.1 °C) (Fig. 3a), and a large fluorescence layer (Fig.3b) was observed with a maximum
10 between 10 and 20m similar to the Chl *a* concentrations ($2.9 \mu\text{g l}^{-1}$, 15m) (Fig.4). Nitrates
11 ranged from $<0.1 \mu\text{M}$ in the first 10 m up to approximately $5 \mu\text{M}$ between 60 m and 100m
12 and were $>7 \mu\text{M}$ at 250m (Fig. 4). Phosphate concentrations were from undetectable (<0.02
13 μM) in the first 30m up to approximately $0.30\mu\text{M}$ deeper than 120m. Ammonium
14 concentration was $0.13\mu\text{M}$ in the first 20m, peaked at $0.6\mu\text{M}$ between 30 and 40m and was
15 $<0.03\mu\text{M}$ between 60 and 250m. Primary production ranged from 28.7 to a maximum of 64.3
16 $\text{mg m}^{-3}/12 \text{ h}$ at the depth of the fluorescence peak. Zooplankton biomass amounted to 4 to
17 8mg DW m^{-2} (Fig. 5), the organisms being mainly found in the 0-200m layer, during day and
18 night. In spring, the gut content of some species of zooplankton revealed that zooplankton
19 were actively feeding at night in the upper layer, contrasting with the summer period when the
20 gut content was much lower than in the spring (Table 1).

21 In June, data showed a strong thermal stratification, with a temperature gradient ranging
22 from 26°C at the extreme surface to 14°C at 40m depth and with a fairly constant temperature
23 below (13.3 ± 0.1 °C) and no advection (Figure 2b and c). Below the thermocline, a thick
24 fluorescence peak was observed between 40 and 60m (Fig. 3c and d) with a deep chlorophyll
25 maximum (DCM) at 50m ($2.5 \mu\text{g l}^{-1}$ Chl *a*) (Fig. 4). Nitrate was undetectable ($<0.04\mu\text{M}$) in

1 the first 40m, increased drastically to $6.17\mu\text{M}$ at 80m and then more slightly up to $7.9\mu\text{M}$ at
2 250m (Fig. 4). Phosphate was also undetectable ($<0.02\mu\text{M}$) in the first 60m, then increased
3 rapidly to $0.23\mu\text{M}$ at 80m and finally more slightly to $0.35\mu\text{M}$ at 250m. Ammonium ranged
4 between 0.08 and $0.17\mu\text{M}$ in the first 40m with two peaks at 10 and 30m, decreased down to
5 undetectable between 60 and 150m and then increased again to approximately $0.5\mu\text{M}$
6 between 200 and 250m. Primary production in June (2.48 to $8.26\text{ mg m}^{-3}/12\text{ h}$) was 8 to 10
7 times lower than in March, indicating a transition from a period of highly productive bloom to
8 the beginning of a thermal stratification period (Fig. 4). Zooplankton biomass was on average
9 4 time lower than in March (on average $< 1\text{ mg DW m}^{-2}$) and exhibited a strong variability
10 likely due to capture of large organisms (euphausiids sampled in the net) as shown by the high
11 integrated dry weight values during the first night (Fig. 5).

12

13 3.2. DOC distribution

14 DOC concentration profiles followed similar trends during the sampling period:
15 maximum values in surface waters (0-30m), a steep gradient from 15-30m to 50-60m, then a
16 moderate gradient down to 250m, and fairly homogeneous values in deeper waters (Fig.6).
17 DOC concentrations ranged from $61.2\pm 1.5\mu\text{M}$ to $39.7\pm 1.8\mu\text{M}$ in March and from
18 $67.3\pm 1.8\mu\text{M}$ to $43.5\pm 2.9\mu\text{M}$ in June. A significant increase ($+5\mu\text{M}$) (ANOVA, $p<0.05$, $n=8$)
19 was noticeable at the surface above 50m depth between March and June (Fig. 64). Below
20 100m, no such variability was noticeable. There was no apparent difference between day and
21 night concentrations.

22

23 3.3. Dissolved lipids

24 Concentrations varied from 12.4 ± 0.4 to $39.6\pm 2.7\text{ }\mu\text{g C l}^{-1}$ and from 11.8 ± 0.3 to 78.4 ± 0.8
25 $\mu\text{g C l}^{-1}$ in March and June, respectively (Table 2) with a higher contribution to DOC in June

1 than in March (3.4 ± 0.6 - 6.7 ± 1.9 and 2.9 ± 1.7 - 4.2 ± 1.0 % of DOC, respectively). At both
2 seasons, chloroplast lipid biomarkers (CL) (which include pigments and glycolipids)
3 dominated the lipid pool ($54.8\pm 18.2\%$ of TL) indicating a phytodetritus source for DOM. In
4 spring CL phytoplankton lipids made up 45 to 87% of the total lipid pool, whereas
5 triglycerides (TG), wax esters (WE) and phospholipids (PL), and varied from below the
6 detection limit of analysis to 5% of total lipids (except at 10m during the day, PL~ 10% TL)
7 (Table 2). From spring to summer, CL decreased from 76 ± 9 to $43\pm 13\%$ of TL, whereas the
8 proportions of TG, WE and PL reached higher values (up to 31%, 12% and 13%,
9 respectively) indicating inputs of organic matter from heterotrophs. In the surface layer (0-
10 50m), the lipolysis index (LI) increased significantly (0.24 ± 0.17 to 0.39 ± 0.13) (ANOVA,
11 $p<0.07$, $n=8$) related to the increase of monomers. The pattern of day/night variation was
12 demonstrated by a significant increase of LI (X3) at night in March, which reflected lipid
13 hydrolysis. The major increase was at the base of the chlorophyll layer (30m) where total
14 dissolved lipids also accumulated. In June at night, both lipid polymers and monomers
15 increased, indicating inputs of biogenic matter to DOM. In the DCM, total dissolved lipid
16 concentrations were *circa* 3 times higher at night than during the day (Table 2) with a larger
17 increase of monomers compared to polymers resulting in an enhanced LI (0.41 to 0.70). LI
18 increases at night were also noticeable down to 150 m depth.

19

20 3.4. CDOM

21 In March, the average absorption coefficient was 0.076 ± 0.041 m^{-1} in the surface (0-50
22 m) water column. The absorption coefficient increased from a surface minimum (0.025 m^{-1} ,
23 3m) to the base of the chlorophyll layer (0.074 m^{-1} , 30m) then decreased (Fig. 7). In June, the
24 values were higher than in March in the surface waters (0.134 ± 0.067 m^{-1}) ($p<0.05$, $n=8$), with
25 a deeper maxima than in March located at the depth of the DCM (50m). In both March and

1 June in the 0-100 m layer, the absorption coefficients were higher during the day than the
2 night (Fig. 7).

3

4 3.5. Bacterial abundance (BA), production (BP), bacterial production specific activity (BP_{SA})
5 and bacterial carbon demand (BCD)

6 In March, a pronounced vertical gradient of BA (Table 3) was observed with a
7 maximum between 15 and 30 m, and no significant changes between day and night. BP
8 exhibited a stronger vertical gradient and increased at night up to 2.8 times at the depth of the
9 chlorophyll peak (10-15m). BP per cell (BP_{SA}) (1.90 ± 0.30 to 80.90 ± 1.60 Ag C cell⁻¹h⁻¹)
10 strongly decreased with depth (less than 5 Ag C cell⁻¹h⁻¹ below 50 m), with 1.4 to 2.4 times
11 more activity at night than during the day (ANOVA, $p < 0.05$, $n = 8$). In June, BA was lower
12 than in March, maximal in the surface layer and decreasing with depth (12.73 ± 0.28 to
13 $0.14 \pm 0.01 \times 10^8$ cells l⁻¹) (Table 3) with no influence of the chlorophyll maxima. BA was
14 about 3 times lower from day to night at the surface (12.73 ± 0.28 to $4.32 \pm 0.16 \times 10^8$ cells l⁻¹,
15 respectively) and in deeper layers (Table 23). BP was significantly lower than in March
16 (55.88 ± 0.02 to 0.11 ± 0.02 - μ g C l⁻¹ d⁻¹), with no significant change between day and night,
17 except at the depths of the chlorophyll maximum (50-60 m). BP_{SA} activity per cell followed
18 the same vertical distribution as BA and BP, decreasing from surface to depth (121.67 ± 4.50
19 to 1.25 ± 0.20 Ag C cell⁻¹h⁻¹). Bacterial cells were more active at night than during the days
20 above (3-30 m) and below (250-500 m) the chlorophyll layer (3 and 6 times, respectively). At
21 both seasons, BP was correlated to primary production ($R^2 = 0.76-0.77$, $p < 0.05$, $n = 5$). As for
22 BP, BCD values decreased from the surface to 500 m (Table 23). In March, BCD exhibited a
23 significantly higher maximum than in June (474.79 ± 21.84 and 372.53 ± 0.11 ng C l⁻¹ h⁻¹,
24 respectively) and a significant increase between day and night (ANOVA, $p < 0.05$, $n = 8$). In
25 June, diel variations were not significant.

1

2 3.6. ^3H -triolein hydrolysis rates (HR), free fatty acids (FFA) release rates and specific
3 hydrolysis activity (HR_{SA})

4 ^3H -triolein hydrolysis rates were higher in March than in June (0 to 2.72 ± 0.02 and 0 to
5 $1.20 \pm 0.01 \text{ \% h}^{-1}$, respectively) in the 0-50 m surface layer, while a reverse trend was observed
6 in the 150-500 m deeper layer (Table 2). The FFA release rates were in the same range at both
7 seasons due to the increase of the hydrolyzable lipid pool in June. In March, HR_{SA} (on average
8 $51.4 \pm 29.4 \text{ Ag C cell}^{-1}\text{h}^{-1}$) decreased from surface to 30 m depth and then increased again at
9 250 m. It was significantly higher during the day than during the night (ANOVA, $p < 0.05$,
10 $n=5$). HR_{SA} (on average $418.3 \pm 290.6 \text{ Ag C cell}^{-1}\text{h}^{-1}$) was much higher (X7) in June than in
11 March and strongly increased from day to night above (3-30 m) and below (250-500 m) the
12 chlorophyll layer (*circa* 8 and 4 times, respectively).

13

14 3.7. Free fatty acid release rates to bacterial carbon demand ratio (FFA release rates/BCD)

15 In the 0-50 m surface layer, FFA release rate to BCD ratios were lower in March than
16 in June (0.6 ± 0.3 and 1.3 ± 1.0 , respectively) (ANOVA, $p < 0.05$, $n=8$). The highest values were
17 found below this layer (~ 3 to 11), in June in particular. In March, the ratios decreased at night
18 except at 30 m depth. In June, the day/night pattern followed a depth-dependant distribution;
19 the ratios increased in the surface (0-50 m) and in deeper waters (500 m), whereas it
20 decreased between 50-250 m (Table 2).

21

22 3.8. Bacterial community structure

23 In March, the number of CE-SSCP peaks per sample varied from 23 to 30, with a
24 maximum in the chlorophyll layer (Table 3). Only slight changes in the number of ribotypes
25 per sample were observed from day to night. Samples from the first 50 m exhibited more

1 ribotypes per sample during the day (~29, range 27 to 30) than at night (~27, range 25 to 28),
2 while a reverse trend was observed at 250 m (23 at day and 26 at night). Cluster analysis
3 indicated clear differences between day and night bacterial community structures, especially
4 for the upper productive layer (Fig. 8). During the day, samples from 3 to 50 m clustered
5 together with slight changes in the community structure for the 30 m sample (bottom of the
6 Chl *a* peak). During the night, samples from 3 to 50 m clustered together with distinguishable
7 profiles at 15 m (chlorophyll maxima). Bacterial community structure of 250 m samples at
8 day and night presented the lowest similarity values (from 77 to 82 %) with both day and
9 night clusters of samples from the upper productive layer (3 m to 50 m). Thirty-nine % of
10 ribotypes were different between March and June, when considering the total number of
11 ribotypes encountered for both periods (57 peaks). In June, the number of peaks per sample
12 was also maximum in the DCM layer, varying from 25 to 30 peaks in the upper layer (3 m to
13 30 m), from 34 to 39 peaks in the DCM layer (50 m to 100 m) and from 33 to 37 peaks in
14 deeper layers (250 m to 500 m) (Table 3). When submitted to cluster analysis, samples from 3
15 to 100 m in June were organized in a different cluster with a relatively low similarity
16 percentage (72%) compared to the samples from March (Fig. 8). Three different layers could
17 be distinguished according to the distribution of the ribotypes. A clear difference was
18 observed between the upper and the DCM layers, with a low similarity value (72.4%). The
19 influence of the DCM level appeared to be restricted at 50 m during the day and extended to
20 50 and 60 m depth during the night. The deeper layer (250 to 500 m) clustered with only 70%
21 of similarity with the other groups. Samples from day and night followed the same depth-
22 dependant distribution, but a day/night influence could be clearly observed in the surface (0-
23 30 m) and in deeper (250-500 m) waters.

1

2 **4. Discussion**

3

4 4.1. General conditions

5 To achieve our goals, it was essential to explore a simple and stable biological system
6 in a field where physical forcings were low. The Dyfamed site fulfilled this requirement. The
7 site is known to have a predictable succession of species and trophic regimes throughout the
8 year. In addition, its location sheltered from the coast by the Ligurian current confers to this
9 site a remarkable hydrological stability with little advection. Thanks to the data accumulated
10 at this site by the Service d'Observation for about 20 years (Andersen and Prieur, 2000;
11 Stemmann et al., 2000; Lemée et al., 2002; Marty and Chiaverini, 2002), we were able to
12 anticipate and successfully sample contrasting production regimes. Indeed, during both
13 sampling periods, the hydrological and meteorological conditions were stable (cf figure 2),
14 and the biological characteristics of the system were typical of the area. In spring (March
15 2003), phytoplankton were abundant in the surface layer (PP = 64-28 mg m⁻³ d⁻¹, Chl /a/max=
16 2.9 µg l⁻¹) and consumed extensively nitrates and phosphates, which started to be depleted.
17 An abundant zooplankton community, dominated by copepods, was actively feeding in the
18 upper layer during the night, with a high potential for OM export and excretion (Banse, 1990).
19 NH₄⁺ regeneration was 159-237 nM 12h⁻¹ (data not shown) and led to ammonium
20 accumulation down to 50m. In the summer period (June 2003), thermal stratification was
21 established and the 0-50m surface layer was depleted in nitrate and phosphate. Primary
22 production was low and limited to a sub surface maximum above a deep chlorophyll
23 maximum (PP = 2-8 mg m⁻³ d⁻¹, Chla/max = 1.6 µg l⁻¹). Zooplankton were less abundant and
24 active than in spring. In spring, the system was net autotrophic, and a high particle flux was
25 recorded in sediment traps moored by the MedFlux team during the same period (Flux_{mass}=

1 800 and 700 mg m⁻² d⁻¹ & Flux_{POC} = 76 and 42 mg m⁻² d⁻¹, at 200 m and 800 m,
2 respectively). In summer, the system was net heterotrophic, characterized by a weak export at
3 200 m (Flux_{mass} = 100 mg m⁻² d⁻¹ and Flux_{POC} = 14 mg m⁻² d⁻¹) (Peterson et al., 2005; Lee
4 et al. this issue).

5

6 4.2. Dynamics of dissolved organic matter from meso- to oligotrophic regimes

7 The general trends of DOC dynamics in the water column was consistent with those
8 found in various oceanic regions and compared well with profiles already reported by Avril
9 (2002) for the Northwestern Mediterranean. The DOC surface maximum and the marked
10 depth gradient, showed the net source of DOC that exists in surface euphotic waters, and
11 DOC consumption or removal in deeper waters. The major change observed from spring to
12 summer periods was an excess DOC, during oligotrophic conditions, which was consistent
13 with the seasonal accumulations of DOM already reported by several authors in the NW
14 Mediterranean zone when oligotrophic conditions prevailed (Copin-Montégut and Avril,
15 1993; Avril, 2002; Lemée et al., 2002; Pujo-Pay and Conan, 2003).

16 Chromophoric dissolved organic matter (CDOM) is an important fraction of the DOM
17 composed of molecules absorbing light in the UV and the visible portions of the solar
18 spectrum (Nelson and Siegel, 2002). The biological situation (high phytoplankton production
19 and zooplankton grazing in March) during the sampling periods and the DOM sources that we
20 identified using lipid biotracers, were consistent with CDOM formation mechanisms in
21 offshore waters reported by several authors: CDOM is thought to be formed from
22 intramolecular reaction of organic biomolecules released by decaying phytoplankton (Yentsch
23 and Reichert, 1961; Sieburth and Jensen, 1969) and zooplankton grazing (Momzikoff et al.,
24 1983; Momzikoff et al., 1994). Bacterial processes have been implicated in the formation of
25 CDOM as well (Chen and Bada, 1992). In surface waters, sunlight plays a crucial role in the

1 fate of CDOM, breaking down large organic molecules into low molecular weight gases and
2 smaller molecules, some of which are removed by the microbial community (Nelson et al.,
3 2004; Biers et al., 2007). Both UV condensation effects and ammonium releases by
4 zooplankton might increase CDOM production (Kieber et al., 1997). As the regime changed,
5 the nearly 2 fold increase of CDOM absorbance observed in the 0-50m surface layer was
6 consistent with the evolution of the CDOM followed on a yearly scale in surface layers
7 (Dujmov et al., 1992; Kahru and Mitchell, 2001) and indicated the predominance of formation
8 processes over removal. The day/night patterns observed at surface and depth, which were
9 similar at both seasons, suggested that both biotic and abiotic light-dependant factors
10 (phytoplankton exudation, zooplankton grazing, UV irradiance) were responsible for diel
11 CDOM variations.

12 Change in the quality of DOC was also noticeable from the contribution of the lipid
13 pool to total DOC. It increased significantly from around 3.5 to 6.7 % as the system changed
14 from meso- to oligotrophy. These ratios were in the range of values reported by Goutx et al.
15 (2000) and Van Wambeke et al. (2001) at the same site in May 1995, when the system was
16 changing towards oligotrophy. Such changes reflected the metabolic pathways of plankton
17 organisms, favoring the synthesis of storage lipids in response to nutrient depletion in summer
18 (Parrish and Wangersky, 1987). They might also be due to the decrease of protein
19 contribution to the organic carbon pool since heterotrophs might have used proteins faster
20 than carbohydrates or lipids.

21 Lipid biomarkers indicated a major phytoplankton source for DOC at both seasons. In
22 summer, the increase of phospholipids and neutral lipids (wax esters and triglycerides) in
23 DOC was consistent with the shift in phytoplankton carbon allocation from glycolipids in
24 diatoms-dominated regime to neutral lipids and phospholipids in flagellates-dominated
25 regime reported by Smith et al. (1997). Bactivorious flagellates are rich in triglyceride storage

1 lipids (Méjanelle et al. 2002; 2005). We did not quantify the flagellate biomass during this
2 study. However, flagellates usually represent a larger amount of the biomass during the
3 May/June period than at any other time of the year (Tanaka and Rassoulzadegan; 2002).
4 Because the shift was noticeable in the dissolved lipid pool, this suggests a rapid transfer of
5 synthesis products from living cells or detritus to DOM through viral lysis, sloppy feeding,
6 dissolution and/or bacterial enzymes attack. However, it also may be related to the filtration
7 protocol as some of the DOM passing through GF/F filters may contain small particles like
8 colloids or small flagellates (Lee and Henrichs, 1993). The qualitative distribution of these
9 neutral lipids (WE and TG) was strikingly noticeable down to 500m. A scenario, including
10 emission of slowly settling particles from the 0-50m surface layer ecosystem and subsequent
11 release of DOM through the mesopelagic layer is consistent with lipid biomarker distributions
12 in the dissolved pool with depth. The enhanced concentration of slowly-sinking particles
13 bearing a strong signature of bacterial degradation in the oligotrophic summer sediment traps
14 deployed during the MedFlux experiment (Wakeham et al. 2008) also supported this scenario.

15

16 4.3. Relationships between bacterial activities and DOM

17 Besides nutrients, bacteria draw their resources for growth from the readily available
18 pool of dissolved organic matter. This pool is composed of small easily assimilable monomers
19 mainly released in the medium through phytoplankton exudation, in addition to large
20 biopolymers originating from plankton detritus that constitute the major part of the bio
21 reactive bulk of organic carbon (Amon and Benner, 1994). When small phytoplankton-
22 derived molecules start to be depleted, bacteria activate the enzymatic systems that cleave
23 biopolymers into monomers (Hoppe, 1993). Chrost (1991) suggests that hydrolysis is a major
24 limiting process for carbon assimilation by bacteria. Polypeptide and polysaccharide
25 hydrolysis have been the topic of numerous studies (Mulholland et al. 2002; Murray et al.

1 2007 and reference herein). On the contrary, marine bacterial lipases are less studied and rate
2 measurements in marine ecosystems are scarce. Lipases (triacylglycerol acylhydrolases; EC
3 3.1.1.3) are defined as the enzymes that catalyze the hydrolysis of triacylglycerols, releasing
4 long-chain fatty acids (Bourguet et al. 2003). These ubiquitous enzymes are found in animals,
5 plants and microorganisms, including fungi and bacteria and are mainly sought for their
6 biotechnological potential. In the marine environment, they have been found in tidal flat
7 sediments (Lee et al. 2006), in the intestinal content of marine fish (Yazawa et al. 1988), in
8 sponges (Kiran et al. 2007) and bacteria (Ando et al. 1991; Martinez et al. 1996). In the
9 present study, we hypothesized that coupling the measurement of ambient concentrations of
10 hydrolysable acyl-lipids biomarkers with the measurement of their *in situ* bacterial hydrolysis
11 rates (i.e. the free fatty acid release rate) would be a proxy to study bacterial response to
12 change in environmental conditions.

13 During the period investigated, production processes (photosynthesis and secondary
14 production) were much more active in spring than in summer. Bacterial abundance and
15 production were in the range of values reported by Tanaka and Rassoulzadegan (2004) at the
16 DYFAMED site. Bacterial production was tightly related to the peaks of primary production,
17 where the highest quantity of phytoplankton-derived organic matter was available to support
18 heterotrophic production. In these conditions, a major feature of DOM dynamics in the
19 surface waters was the accumulation of excess DOC observed in summer, the origin of which
20 must be sought in the imbalance between inputs of biogenic material in the euphotic layer and
21 a slowed-down OM utilization by bacteria. The lipid hydrolysis rates that we measured at the
22 DYFAMED site (<0.1-2.7%, 10^7 - 10^9 cells l^{-1}) were in the lower range of values reported in
23 previous studies. Bourguet et al. (2003) reported hydrolysis rates in a New-Caledonia lagoon
24 in the range 0.9-7.12 % \cdot h $^{-1}$ using the same substrate (0.57 to 1.12 nM of [3 H]-triolein) as we
25 used. Marine microbiologists commonly assay lipase activities by using synthetic fluorescent

1 analogs 4-methylumbelliferyl (MUF) fatty acyl esters that may be non-specifically
2 hydrolyzed by carboxyl-esterase. Using saturating concentrations of a MUF-oleate analog
3 substrate (20 μ M) in samples from pure culture of bacterial strains and from the open field,
4 both containing $\sim 10^9$ - 10^{10} bacterial cells l^{-1} , Martinez et al. (1996) reported lipase activities in
5 the range <0.1-14 %. The lipase activities measured in estuarine environments by Taylor et al.
6 (2003) were in the same range of values, although these authors were expecting higher values
7 knowing that all activities were elevated in these estuarine systems.

8 However, in these previous studies, absolute hydrolysis rates were not calculated
9 because ambient substrate concentrations were unknown. Here, we present for the first time
10 the simultaneous analysis of ambient lipid concentrations associated with the measurement of
11 lipase activity, giving access to the flux of free fatty acid release rates by bacterial hydrolytic
12 enzymes. We did not estimate the fraction of lipid carbon supporting the bacterial carbon
13 demand because the protocol for measuring assimilation of radiolabelled free fatty acids
14 “monomers” by bacteria was not set up at the time of the cruise, but we propose the ratio of
15 free fatty acid release rate to BCD as a proxy for evaluating the relative importance of
16 biopolymer hydrolysis versus monomer assimilation by the bacterial community. A ratio >1
17 would reflect no balance between hydrolysis and assimilation (hence, free fatty acid
18 accumulation), whereas a ratio<1 would reflect a better coupling between hydrolysis and
19 assimilation.

20 The significant increase of the FFA release rate to BCD ratio that we measured in the
21 0-50m surface layer, suggested that the dissolved lipid metabolites (i.e. free fatty acids)
22 contributed to the excess DOC when the season changed from spring to summer. However,
23 the signal (on average) was weak and more related to the base of the surface layer (30m,
24 50m). Other factors such as the nutrient limitation that prevailed at the surface in summer
25 (Van Wambeke et al. 2002), may have slowed down the assimilation of monomers and

1 enhanced the lipid metabolite accumulation. Van Wambeke et al. (2001), using the potential
2 aminopeptidase hydrolysis rate to BCD ratio, showed that proteins contributed a large part of
3 the excess DOC at this site (5m depth) during the meso- to oligotrophic transition period. As
4 in marine temperate areas, the surface DOM accumulation in summer could also result from a
5 competition between phytoplankton and bacteria for nutritive resources as nitrogen or
6 phosphorus (Thingstad, 1997; Pujol-Pay and Conan, 2003). Because both BA and BP
7 decreased to a similar extent between spring and summer, and thus BCD was in the same
8 range at both seasons, the increase of the ratio was attributable to the increase of FFA release
9 rate and was related to a much higher bacterial cell specific lipase activity (x 3-4 fold). The
10 more active bacterial metabolism per cell and the hydrolysis of a greater quantity of acyl-
11 lipids while lipid monomers were not fully assimilated, was a striking feature of the bacterial
12 community in the 0-50m surface layer in summer (cf Table 3). In the DCM layer, peculiar
13 conditions (low primary production, accumulation of phytodetritus and CDOM) appeared to
14 promote enhanced cell specific lipase activities. The DCM was located at the depth of the
15 nitracline and the phosphocline, where traces of nutrients were available to bacteria. Intense
16 lipase activity per cell may provide additional energy necessary for keeping intracellular
17 equilibrium and enzyme activation in addition to carbon resource. Finally, the shift in species
18 distribution suggested by the increase in the number of ribotypes at the depth of the DCM (see
19 discussion below), may account for the observed enhanced lipase activity per cell.

20 Below the 0-50m layer at both seasons, BA and BP drastically decreased, whereas the
21 decrease of lipid hydrolysis activity was less pronounced. As the readily utilizable compounds
22 became scarce below the productive layer, the transfer of biopolymers from POC to DOC
23 provided the major carbon resources for bacteria (Tanaka and Rassoulzadegan, 2002). Most
24 probably this transfer was occurring through the action of enzymes, including lipases. In
25 spring, depth distribution was difficult to interpret as there were only a few samples available

1 below the 0-50m layer. In summer oligotrophy, the increase of the free fatty acid release rate
2 to BCD ratios with depth suggested an activation of the lipase enzymes compared to the
3 bacterial carbon demand. The hydrolysis of acyl-lipids per cell was very high at these depths
4 and may constitute a strategy developed by the bacterial community for its survival in this
5 carbon limited environment (Van Wambeke et al. 2002). Additional experimental work is
6 needed to test this hypothesis further. Finally, biases due to the choice of carbon equivalence,
7 calibration protocols, varying bacterial volume, inherent to the acquisition of the different
8 variables (BP rate, HR rate, BA) may have overestimated this ratio. However, relative
9 changes would similarly reflect the *in situ* dynamics of acyl-lipid hydrolysis.

10

11 4.4. Bacterial community structure

12 Changes in bacterial community structure could be associated with the biotic and
13 abiotic changes that occurred from meso- to oligotrophic conditions. Of a total of 57
14 ribotypes, 39% of them were different from spring to summer. At both seasons, the maximum
15 number of ribotypes per samples were found at the chlorophyll *a* maximum, suggesting that
16 the organic matter released by phytoplankton could be used directly or indirectly by a large
17 number of phylotypes at the DCM level. In general, the number of ribotypes per samples
18 decreased with depth from the DCM to deeper levels. These results are in accordance with the
19 abundant information in the literature supporting the thesis that prokaryotic diversity in the
20 open ocean varies with depth (Field et al., 1997; Wright et al., 1997; Acinas et al., 1999;
21 Carlson et al., 2004; Ghiglione et al., 2005).

22 The number of ribotypes per sample increased with the change of the system toward
23 oligotrophy, but the evolution was not significant enough to suggest that selection or
24 competition mechanisms probably occurred at the different spatial and temporal scales
25 studied. However, the cluster analysis of the structure of the bacterial community revealed

1 significant seasonal changes in the organization of the different ribotypes throughout the
2 water column (Fig. 68). These changes probably resulted from a complex succession of
3 bacterial strains occurring between the two seasons. In coastal environments, different studies
4 have emphasized the importance of time for the evolution of bacterial community structure
5 from days or weeks (Acinas et al., 1997; Fandino et al., 1998; 2001) to months, seasons or
6 annual scales (Murray et al., 1998; Riemann et al., 1999; Schauer et al., 2003; Ghiglione et
7 al., 2005).

8 Interestingly, the distribution of the samples in the two different seasonal clusters
9 appeared to be different and can be related to the trophic status of each period. The
10 organization of samples from the spring cluster was clearly influenced by diel physico-
11 chemical variations, resulting in rapid changes in bacterial functionality (see below). Bacterial
12 community structure of the summer cluster was organized with depth, according to the strong
13 summer stratification. Such seasonal changes in the ribotype identity and organization in the
14 water column from meso- to oligotrophy resulted in an increase of specific activity at the cell
15 level but a decrease in total bacterial abundance and production that played a determinant role
16 in the accumulation of DOM in summer. These results suggest the adaptation capacities of the
17 bacterial community structure to environmental changes and its influence in the functional
18 role of bacteria in the ecosystem functioning. These observations emphasize the need for
19 further studies at the single cell level for identifying whether the whole community or a
20 fraction of the community realizes the acyl-lipid cleavage during summer oligotrophy.

21

22 4.4. Diel variations

23 In both meso- and oligotrophic conditions, DOC concentrations in the productive layer did
24 not change between day and night, but its composition varied substantially. CDOM
25 absorbance increased during the day while accumulations of biogenic dissolved lipids were

1 observed at night, suggesting an opposite diel periodicity between input processes of fresh
2 biogenic matter and formation of CDOM matter. The day/night pattern of CDOM absorbance
3 was similar in spring and summer. At both seasons, CDOM absorbance increased during the
4 day in the 15-30m upper layer suggesting that diel biotic/abiotic interactions occurred at these
5 depths, for example the effect of UV light on the labile molecules released by the functioning
6 of the biological system. Dissolved lipid accumulations were related to the depths of the
7 chlorophyll maxima. At these depths, lipid class biomarkers (i.e. the high lipolysis indices
8 (LI)) reflected acyl-lipid hydrolysis at night, a biomarker pattern that has already been
9 observed in areas undergoing either high zooplankton grazing pressure (Week et al.1993) or
10 bacterial acyl-lipid hydrolysis (Goutx et al. 2003; Caradec et al. 2004). Most probably both
11 processes coexisted.

12 In spring, the presence of abundant zooplankton in the upper layer with high feeding
13 activity at night (cf Table 1) may release readily available labile DOM (Richardot et al., 2001;
14 Kragh and Søndergaard, 2004) that supports the enhanced bacterial activities (cf Table 3). As
15 bacterial abundance remained fairly constant, the enhanced activities were not the result of a
16 bacterial bloom during the night, but rather may result from a shift in bacterial community
17 structure as shown by CE-SSCP fingerprinting (cf Fig. 8). Such rapid changes in structure of
18 the community on daily to weekly time scales have already been observed in several
19 mesocosm experiments (Van Hannen et al., 1999; Schäfer et al., 2001).

20 Changes in bacterial community were related to the observed increase of cell-specific
21 activity from day to night, increasing drastically the total bacterial production at night. On the
22 contrary, the ratio of the free fatty acid release rate to bacterial carbon demand (while
23 indicating an overall balanced lipid monomer consumption over production in the upper
24 layers in March) decreased at night. This suggests that small lipid monomers were being
25 consumed less at night than during the day in the 0-15m surface layer, or at least only a

1 fraction were being consumed, indicating a preferential utilization of compounds other than
2 lipid monomers by bacteria under the surface water conditions prevailing at night in March.
3 At 30m depth, contrary to what was observed in the surface waters, the high production rate
4 of FFA in relation to the BCD at night, may likely be due to the peculiar conditions of
5 lowering phytoplankton resources and nutrient regeneration (ammonium in particular) at this
6 depth.

7 In summer, cluster analysis of CE-SSCP pattern based on UPGMA showed that
8 bacterial community structure at the different depths was more influenced by thermal
9 stratification than by diel changes, even if a slight day/night influence could be observed in
10 the upper (0-30 m) and deeper layers (250-500 m). This pattern was similar to the FFA
11 release rate to BCD ratio pattern that was related to changes in the nutrients and carbon
12 resources from surface to depth characterizing the DYFAMED site in summer oligotrophic
13 conditions (see Van Wambeke et al. 2002) (cf &4.3.), while no clear day/ night pattern was
14 observed. In the deeper layers, most bacterial parameters (abundance, production and
15 hydrolysis) decreased from day to night in association with a notable shift in bacterial
16 community structure. However, it was not possible from our data to relate these changes to
17 any significant pattern of organic matter quality or quantity. These observations outline the
18 need for more research on short term variations in the deep water column, which should be
19 investigated over a longer time.

20

21

22 Conclusion

23 In this study, we present for the first time the simultaneous analysis of ambient acyl-lipid
24 concentrations associated with the measurement of lipase activity, allowing estimation of the
25 free fatty acid release rates by bacterial hydrolytic enzymes. At the DYFAMED site, the

1 variations of dissolved lipid biomarkers and bacterial lipase activities during the seasonal
2 transition toward oligotrophy support the idea that the excess DOC was, at least partly, related
3 to the uncoupling between fresh biogenic lipid inputs and its consumption by heterotrophs,
4 leading to accumulation of the under-utilized free fatty acids. Major changes in the lipase
5 activity per cell occurred between day and night in spring and were depth-related in summer,
6 in relation with the trophic conditions. In summer, within the 0-100m euphotic layer, the
7 highest cell specific lipase activities were measured at the depths of the DCM where higher
8 numbers of ribotype were found. In the aphotic layer, high lipase activity per cell but low
9 bacterial production would reflect the community-level response to ambient trophic
10 conditions. This study illustrates the importance of the diversity and functions of bacterial
11 communities to the carbon dynamics at the Dyfamed site and, more generally, to marine
12 biogeochemical cycles.

13

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2

3

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Figure legends

Figure 1: Location of the DYFAMED time series station

Figure 2: Temperature profiles during the March (a) and June (b) surveys, and zoom of the June profile (c). For each survey, filled symbols are used for data from the first period (March 24-26, June 19-21) and open symbols for data from the second period (March 29-31, June 22-24).

Figure 3: Temperature and fluorescence within the 0-100m layer during March and June 2003 surveys. Dots represent CTD casts.

Figure 4: Nutrients, chlorophyll a and primary production during March and June 2003 surveys.

Figure 5: Zooplankton biomass expressed as dry weight in mg m^{-3} obtained by vertical tows (0-200 m and 0-500 m) at day and night during March and June 2003 surveys.

Figure 6: DOC concentrations at day and night during March (filled symbols) and June (open symbols) 2003 surveys.

Figure 7: CDOM absorbance coefficient (m^{-1}) at day and night during March and June 2003 surveys.

- 1 Figure 8: UPGMA similarity dendrogram generated from 16S rDNA PCR-CE-SSCP profiles
- 2 at day (D) and night (N) during the March and June 2003 surveys.

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Table 1. Gut content (ng Chla eq. mg⁻¹ DW) of zooplankton species at the two seasons. standard deviation into brackets, non available (na), Total length (TL),

	March	June
Zooplankton species	Gut content (ng Chla eq. mg ⁻¹ DW)	
<i>Diacria trispinosa</i>	81.2 (na)	38.9 (na)
<i>Pyrosoma atlanticum</i> (< 2 cm TL)	38.6 (na)	23.3 (0.2)
<i>Pyrosoma atlanticum</i> (> 3cm TL)	38 (14.6)	4.8 (2.1)
<i>Meganictyphanes norvegica</i>	1.21 (0.12)	0.48 (na)

Table 2. Dissolved lipid (Ld) concentrations and lipid class composition in samples collected during days and night in March and June 2003. Not analyzed (Nd); Below detection (bd), Phospholipids (PL), Chloroplast lipids (CL), Wax esters (WE), Triglycerides (TG), Sterols (ST), Lipolysis index (LI), standard deviation (sd).

	Depth m	Ld		Lipid classes					Acyl-lipids		Metabolites		Hydrolyzable		LI -	
		$\mu\text{g C l}^{-1}$	<i>sd</i>	PL	CL	WE %	TG	ST	Polymers $\mu\text{g C l}^{-1}$	<i>sd</i>	Monomers $\mu\text{g C l}^{-1}$	<i>sd</i>	lipid C $\mu\text{g C l}^{-1}$	<i>sd</i>		
March	Day	3	21.6	3.5	0.00	86.12	bd	0.00	4.27	18.6	3.0	2.1	0.3	6.2	1.0	0.11
		10	21.2	0.9	9.97	80.92	bd	2.56	0.00	19.9	0.8	1.4	0.1	6.8	0.3	0.07
		15	27.8	3.0	5.09	78.73	bd	1.78	0.00	23.8	2.6	4.0	0.4	8.1	0.9	0.17
		30	25.8	0.5	3.79	74.90	bd	2.33	2.20	20.9	0.4	4.3	0.1	7.2	0.1	0.21
		50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		150	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		250	19.4	2.3	4.82	87.89	bd	0.00	3.76	17.9	2.1	0.7	0.1	6.0	0.7	0.04
	500	26.0	1.7	3.46	44.56	bd	2.92	1.17	13.2	0.9	12.4	0.8	4.7	0.3	0.94	
	Night	3	12.4	0.4	0.00	86.94	bd	0.00	5.47	10.8	0.3	0.9	0.0	3.6	0.1	0.09
		10	14.7	0.6	1.56	73.78	bd	0.00	2.64	11.1	0.5	3.2	0.1	3.7	0.2	0.29
		15	16.7	0.1	2.19	66.85	bd	0.00	2.39	11.5	0.1	4.8	0.0	3.8	0.0	0.41
		30	39.6	2.7	2.28	59.19	bd	0.00	3.02	24.3	1.7	14.1	1.0	8.1	0.6	0.58
		50	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		150	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
250		16.3	0.4	2.71	51.05	bd	0.00	1.31	8.8	0.2	7.3	0.2	2.9	0.1	0.84	
500	24.6	1.3	2.42	46.14	bd	1.07	0.94	12.2	0.6	12.1	0.6	4.2	0.2	1.00		
June	Day	3	20.6	0.3	7.66	62.72	1.95	4.97	1.57	15.9	0.2	4.4	0.1	5.7	0.1	0.27
		10	35.2	0.5	5.93	41.71	3.29	16.76	3.02	23.8	0.3	10.3	0.1	9.9	0.1	0.43
		30	19.7	0.5	11.31	56.35	1.62	8.74	0.37	15.4	0.4	4.3	0.1	5.7	0.1	0.28
		50	27.6	0.4	6.41	52.78	0.00	11.02	0.84	19.3	0.3	8.0	0.1	7.5	0.1	0.41
		60	33.7	0.5	7.24	42.64	4.06	13.19	2.90	22.6	0.3	10.1	0.1	9.0	0.1	0.45
		100	30.2	0.5	7.49	39.37	9.64	10.70	1.61	20.3	0.3	9.4	0.2	7.8	0.1	0.46
		150	11.8	0.3	12.95	61.89	0.00	2.61	0.94	9.1	0.2	2.5	0.1	3.1	0.1	0.28
		250	24.3	0.4	8.44	30.25	12.53	19.72	2.34	17.2	0.3	6.5	0.1	7.3	0.1	0.38
		500	25.8	0.4	17.45	36.68	1.40	14.44	1.41	18.1	0.3	7.4	0.1	7.3	0.1	0.41
	Night	3	51.1	0.3	9.56	43.00	3.82	19.24	0.00	38.7	0.2	12.5	0.1	16.2	0.1	0.32
		10	53.6	0.6	8.78	32.41	5.56	25.42	2.00	38.7	0.4	13.9	0.1	19.1	0.1	0.36
		30	56.2	0.4	8.0	21.8	7.3	31.6	3.90	38.6	0.4	15.3	0.1	18.8	0.1	0.40
		50	78.4	0.8	12.99	38.63	1.53	1.76	6.56	43.1	0.4	30.2	0.3	14.8	0.1	0.70
		60	30.1	0.6	8.88	47.37	1.27	3.24	6.22	18.3	0.4	9.9	0.2	6.4	0.1	0.54
		100	36.6	0.7	10.54	39.64	2.17	4.79	6.39	20.9	0.4	13.3	0.3	7.6	0.1	0.64
		150	31.6	0.3	10.36	41.99	0.00	8.29	3.09	19.1	0.2	11.4	0.1	7.3	0.1	0.60
		250	15.9	0.2	10.10	60.52	6.36	4.08	1.12	12.9	0.2	2.8	0.0	4.5	0.1	0.22
		500	20.0	0.2	10.63	55.73	3.48	11.31	1.11	16.2	0.2	3.5	0.0	6.2	0.1	0.22

Table 3. Bacterial numbers, production, hydrolysis activities and ribotypes, and calculated fluxes in samples collected during days and night in March and June 2003. Not analyzed (Nd); Below detection (bd), 3H-triolein Hydrolysis Rate (3H-triolein HR), Free Fatty Acids release rate (FFA release rate), Bacterial abundance (BA), Bacterial production (BP), Bacterial carbon demand (BCD).

	Depth m	3H-triolein HR		FFA release rate		BA		BP		BP cell ⁻¹		3H-triolein HR cell ⁻¹		BCD		FFA release rate		Ribotypes	
		% h ⁻¹	<i>sd</i>	ng C l ⁻¹ h ⁻¹	<i>sd</i>	x10 ⁸ cells l ⁻¹	<i>sd</i>	ngC l ⁻¹ h ⁻¹	<i>sd</i>	AgC cells ⁻¹ h ⁻¹	<i>sd</i>	AgC cells ⁻¹ h ⁻¹	<i>sd</i>	ngC l ⁻¹ h ⁻¹	<i>sd</i>	/BCD	<i>sd</i>		
March	Day	3	1.70	0.06	105.5	17.5	11.9	0.2	47.0	0.2	39.4	0.6	88.5	3.1	247.4	0.9	0.43	0.01	30
		10	1.80	0.10	122.6	8.5	13.6	0.6	40.8	0.5	30.1	1.3	90.5	3.7	214.9	2.4	0.57	0.01	29
		15	1.23	0.14	99.8	15.5	20.9	0.4	26.2	0.5	12.5	0.3	47.7	1.4	138.1	2.7	0.72	0.02	30
		30	1.73	0.23	124.4	16.7	22.6	0.2	28.3	0.3	12.5	0.2	55.0	0.9	149.1	1.5	0.83	0.01	27
		50	0.73	0.49	nd	0.0	14.5	0.6	6.3	0.3	4.3	0.3	nd	nd	33.2	1.4	nd	nd	30
		150	0.41	0.06	nd	0.0	3.2	0.4	1.7	0.1	5.2	0.7	nd	nd	8.7	0.6	nd	nd	nd
		250	0.18	0.07	11.0	4.4	1.4	0.1	0.3	0.0	2.3	0.2	77.7	4.9	1.7	0.2	6.31	0.63	23
		500	bd	bd	bd	bd	1.1	0.1	0.2	0.0	1.9	0.3	bd	bd	1.1	0.2	bd	bd	nd
	Night	3	2.22	0.03	80.0	2.8	11.0	0.2	88.9	0.6	80.9	1.6	72.9	1.4	467.6	2.9	0.17	0.00	27
		10	2.72	0.02	100.2	4.2	19.6	0.4	90.2	4.2	46.0	2.3	51.1	1.0	474.8	21.8	0.21	0.01	28
		15	2.72	0.12	104.5	4.7	24.2	0.4	74.3	2.3	30.7	1.1	43.1	0.7	391.1	12.2	0.27	0.01	28
		30	2.43	0.17	197.3	19.3	16.7	0.4	29.1	0.5	17.4	0.5	11.8	3.3	152.9	2.7	1.29	0.03	26
		50	0.91	0.06	nd	0.0	14.5	1.1	8.0	0.3	5.5	0.5	nd	nd	41.9	1.6	nd	nd	25
		150	0.17	0.01	nd	0.0	5.3	0.5	1.2	0.0	2.2	0.2	nd	nd	6.1	0.1	nd	nd	nd
		250	0.12	0.03	3.6	0.9	1.8	0.1	0.5	0.0	3.0	0.2	20.7	1.7	2.8	0.1	1.30	0.04	26
		500	0.01	0.04	0.4	1.7	0.6	0.1	0.2	0.0	3.4	0.6	6.9	2.8	1.1	0.2	0.38	0.16	nd
June	Day	3	2.40	0.09	135.7	5.1	12.7	0.3	55.9	0.0	43.9	1.0	106.6	1.0	372.5	0.1	0.36	0.00	30
		10	1.38	0.11	136.7	10.9	10.5	0.3	31.1	0.2	29.7	1.0	130.4	2.8	207.6	0.8	0.66	0.01	28
		30	0.62	0.06	35.6	3.5	4.3	0.2	13.3	1.3	30.7	3.5	82.5	4.7	88.3	6.9	0.40	0.03	30
		50	0.78	0.06	58.1	4.5	1.6	0.2	2.5	0.0	15.8	1.5	360.8	33.7	16.9	0.2	3.43	0.05	38
		60	0.57	0.04	51.7	3.7	0.9	0.1	0.7	0.1	7.9	0.9	562.3	36.9	4.9	0.4	10.63	0.81	39
		100	0.67	0.10	52.4	7.9	0.8	0.1	0.9	0.1	10.7	1.1	646.8	56.7	5.8	0.3	9.03	0.43	38
		150	0.66	0.17	20.6	5.4	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		250	0.25	0.18	18.1	13.2	1.2	0.1	0.3	0.0	2.8	0.4	153.1	20.2	2.2	0.2	8.21	0.84	37
	500	0.10	0.05	7.1	3.6	1.6	0.3	0.2	0.0	1.3	0.2	44.3	7.2	1.4	0.1	5.09	0.46	33	
	Night	3	1.76	0.12	284.4	19.4	4.3	0.2	52.5	0.3	121.6	4.5	658.5	9.9	350.2	1.4	0.81	0.01	25
		10	0.88	0.06	168.7	11.5	3.7	0.2	32.9	1.1	88.6	5.2	455.0	14.2	219.2	5.6	0.77	0.01	30
		30	1.20	0.01	225.2	2.5	1.9	0.1	17.6	0.0	90.6	5.1	1160.6	65.8	117.2	0.2	1.92	0.00	27
		50	0.52	0.19	77.7	28.2	1.9	0.1	7.0	0.0	36.9	2.1	406.6	27.7	46.9	0.2	1.65	0.06	36
		60	0.65	0.16	41.7	10.3	1.2	0.1	2.1	0.0	16.9	1.5	339.2	31.5	13.9	0.2	3.01	0.08	34
		100	0.52	0.06	39.6	4.6	0.6	0.1	1.4	0.1	21.8	3.0	639.3	62.3	9.0	0.7	4.40	0.34	37
		150	0.17	0.05	12.3	3.6	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
250		0.18	0.04	7.9	1.8	0.1	0.0	0.2	0.0	15.0	2.4	564.3	42.3	1.4	0.2	5.64	0.65	33	
500	0.13	0.04	8.0	2.5	0.2	0.0	0.1	0.0	5.2	1.2	383.1	56.0	0.7	0.1	10.97	1.61	34		

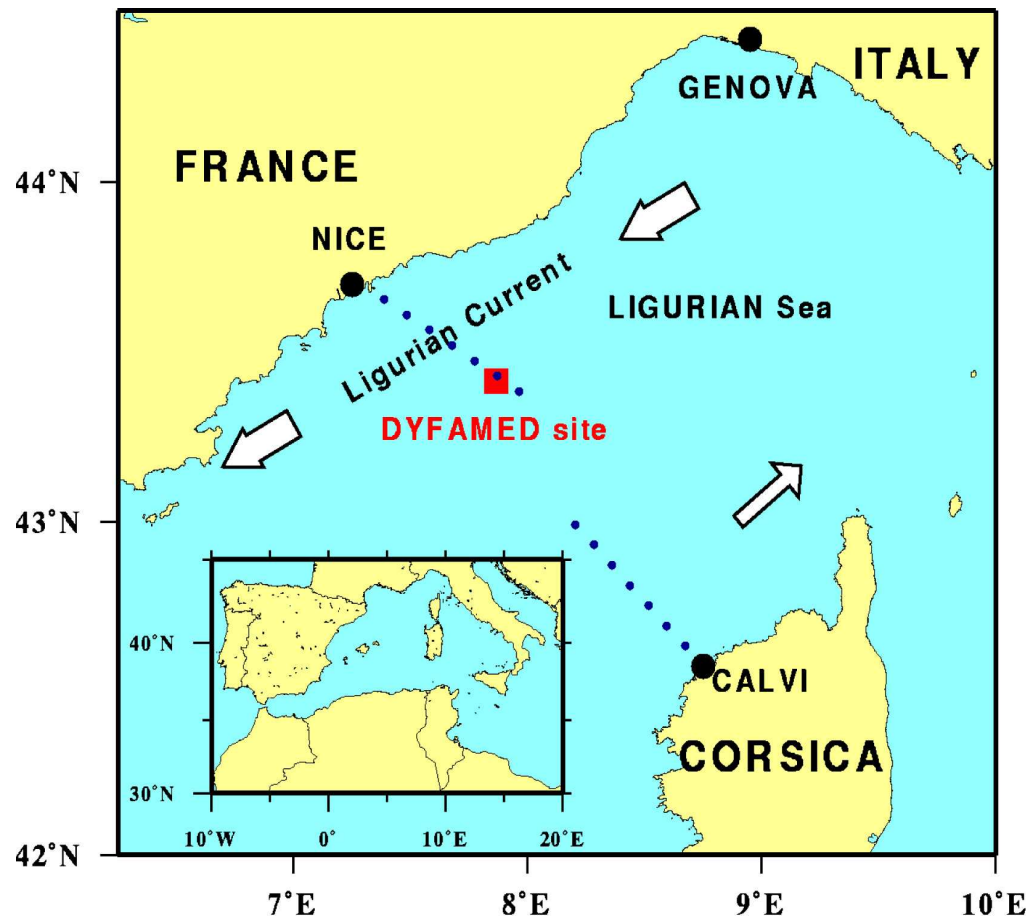


Fig 1

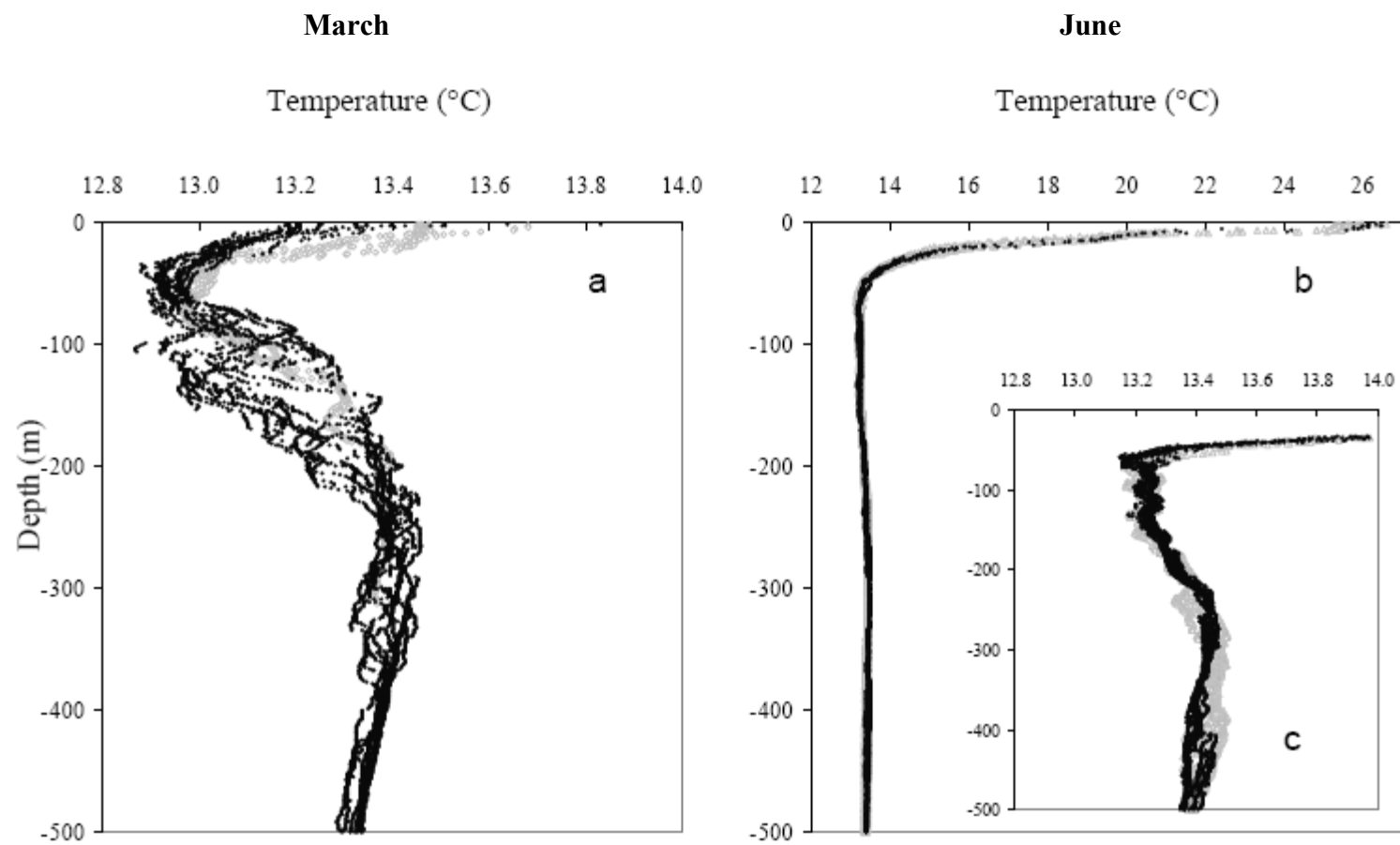


Fig 2

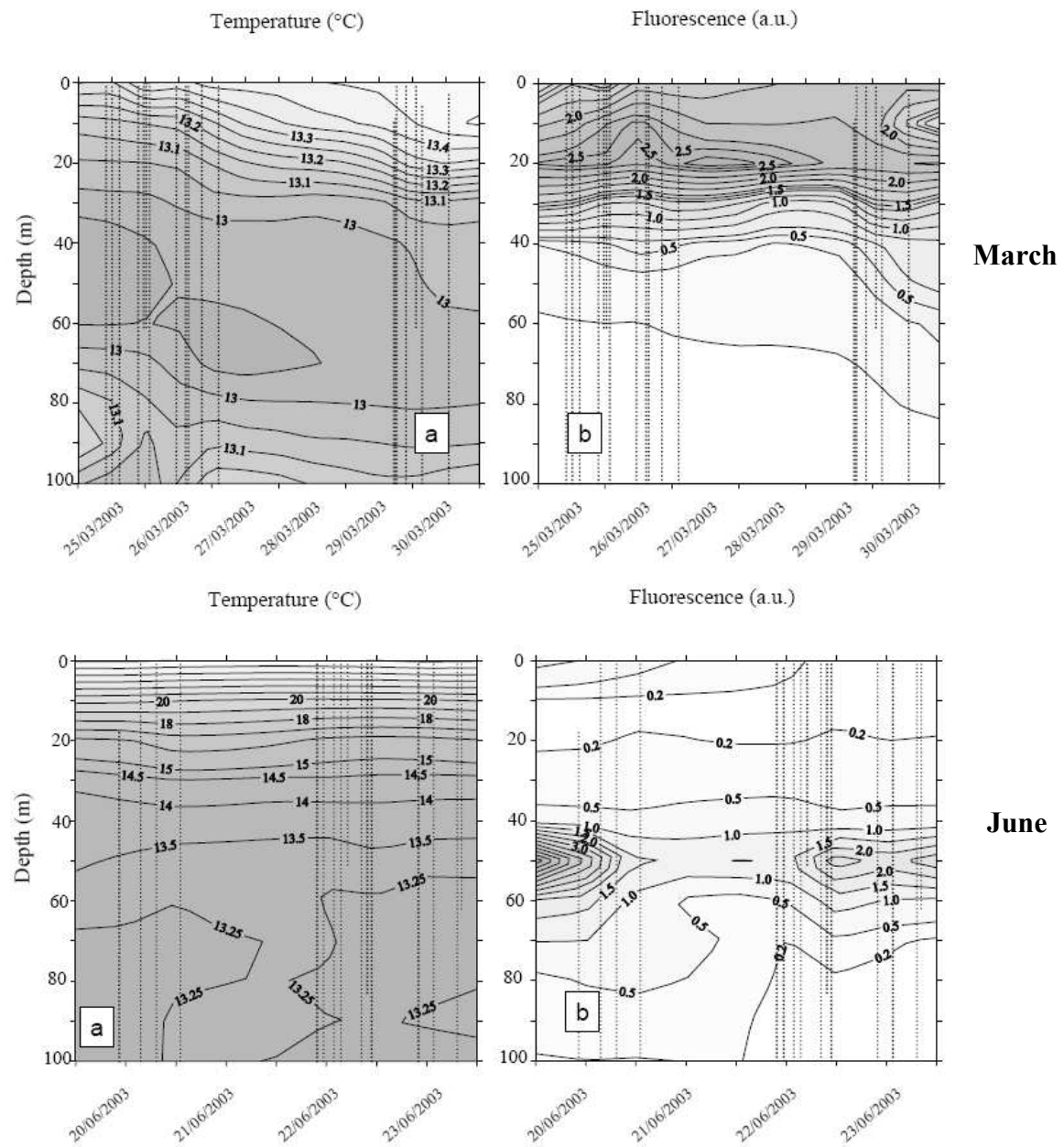


Figure 3

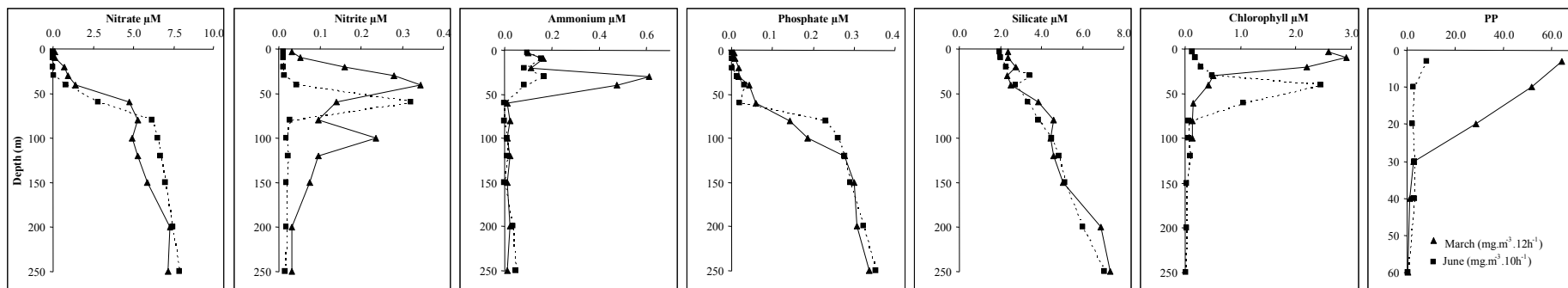


Figure 4

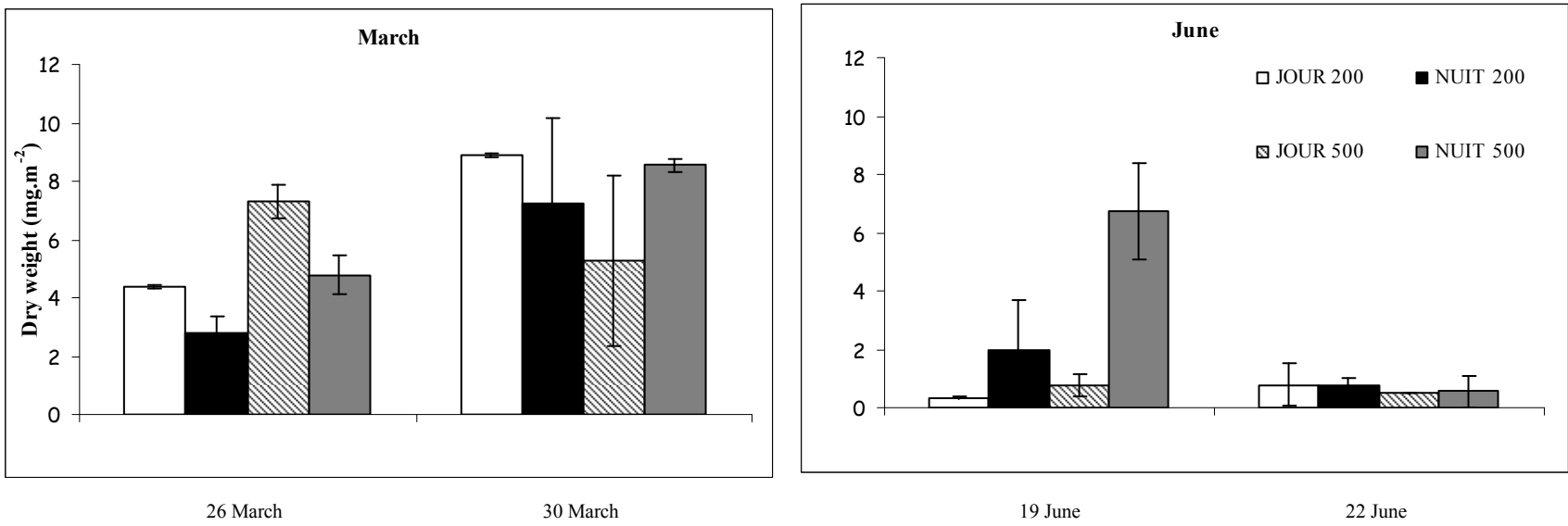


Figure 5

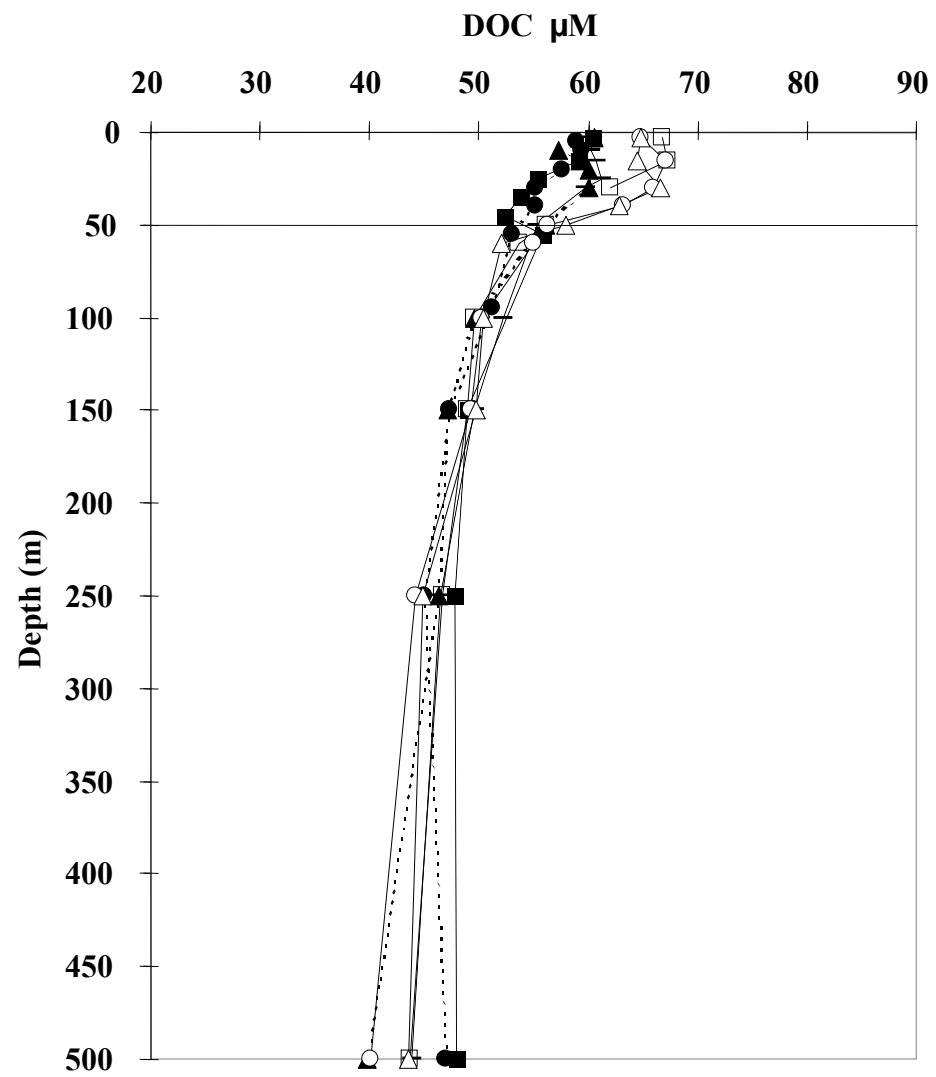


Figure 6

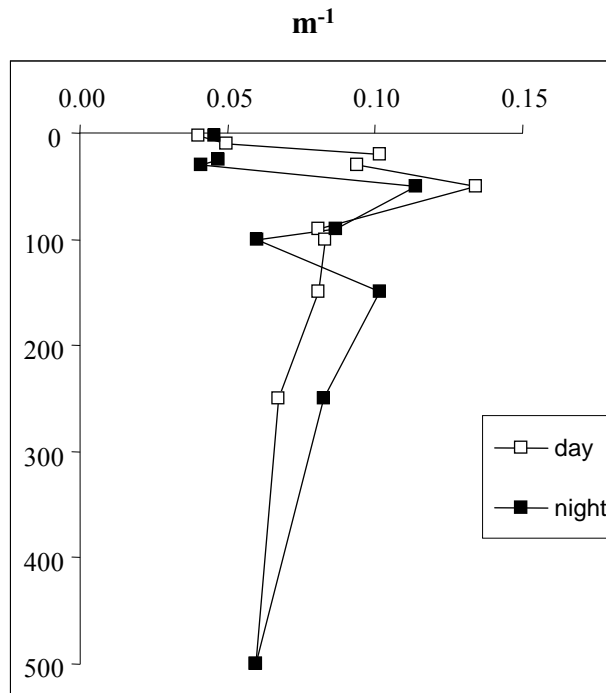
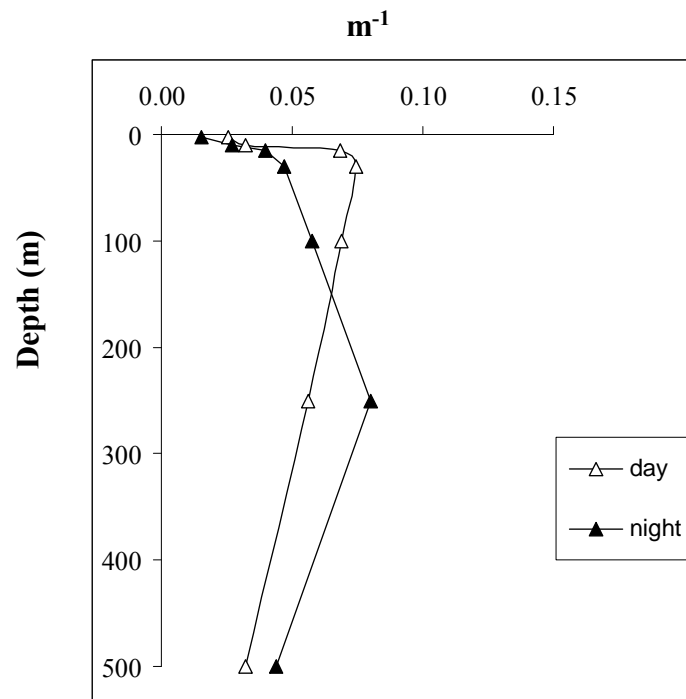


Figure 7

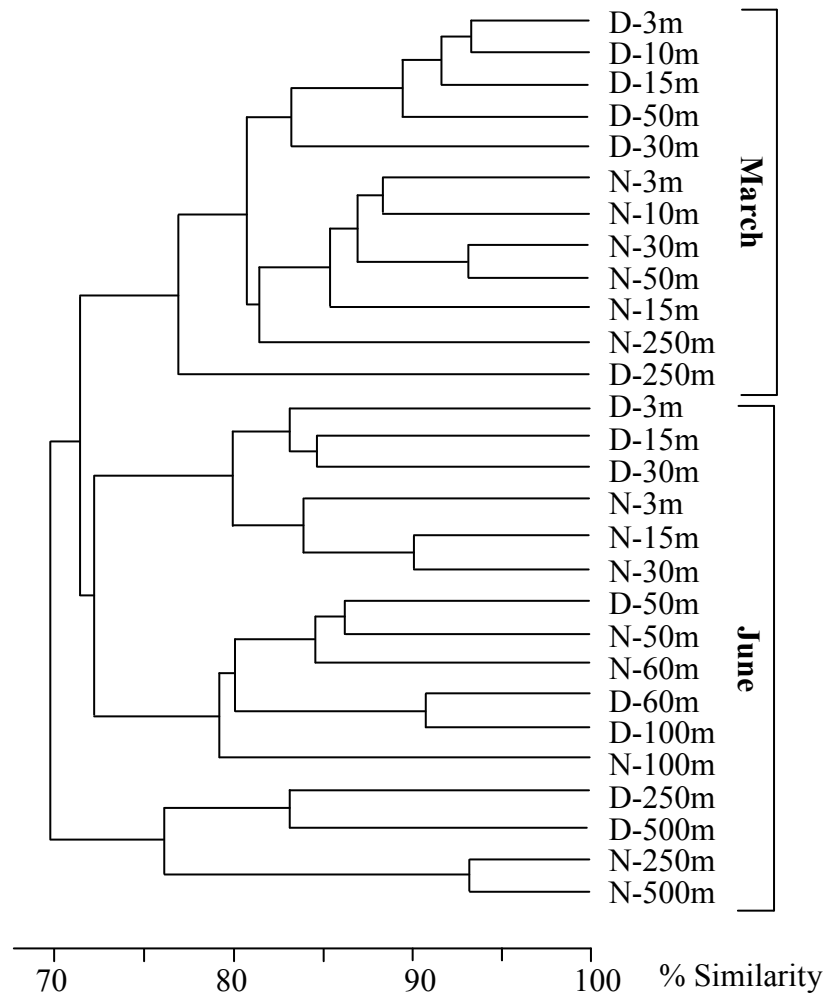


Figure 8