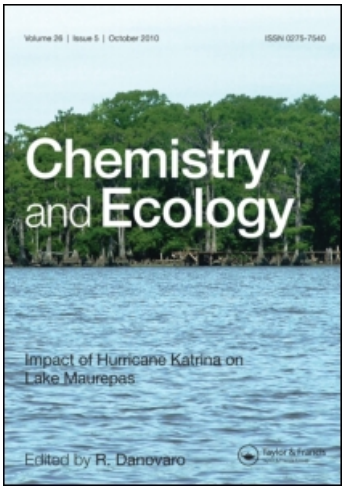

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Luminous bacteria in the deep-sea waters near the ANTARES underwater neutrino telescope (Mediterranean Sea)

Badr Al Ali[a]; Marc Garel[a]; Philippe Cuny[a]; Juan-Carlos Miquel[b]; Tarik Toubal[b]; Anne Robert[a]; Christian Tamburini[a]
[a] Université de la Méditerranée, Centre d'Océanologie de Marseille, LMGEM UMR 6117 CNRS - INSU, Marseille, Cedex 9, France [b] IAEA Marine Environment Laboratories, Monaco

Online publication date: 09 February 2010

To cite this Article Al Ali, Badr , Garel, Marc , Cuny, Philippe , Miquel, Juan-Carlos , Toubal, Tarik , Robert, Anne and Tamburini, Christian(2010) 'Luminous bacteria in the deep-sea waters near the ANTARES underwater neutrino telescope (Mediterranean Sea)', Chemistry and Ecology, 26: 1, 57 — 72
To link to this Article: DOI: 10.1080/02757540903513766
URL: http://dx.doi.org/10.1080/02757540903513766

Chemistry and Ecology
Vol. 26, No. 1, February 2010, 57–72

Taylor & Francis
Taylor & Francis Group

Luminous bacteria in the deep-sea waters near the ANTARES underwater neutrino telescope (Mediterranean Sea)

Badr Al Ali[a], Marc Garel[a], Philippe Cuny[a], Juan-Carlos Miquel[b], Tarik Toubal[b], Anne Robert[a] and Christian Tamburini[a]*

[a] *Université de la Méditerranée, Centre d'Océanologie de Marseille, LMGEM UMR 6117 CNRS – INSU, Campus de Luminy, Case 901, 13288 Marseille, Cedex 9, France;* [b] *IAEA Marine Environment Laboratories, 4 quai Antoine 1er, MC98000, Monaco*

(*Received 3 November 2009; final version received 26 November 2009*)

Marine luminous deep-sea bacteria may represent a potential source of signal perturbation for the ANTARES neutrino telescope installed between 2000 and 2475 m depth in the Mediterranean Sea. Using the CARD-FISH method, we have estimated the relative abundances of total prokaryotes of Bacteria, γ-proteobacteria and *Vibrinoceae* (domain, class and family affiliation of marine luminous bacteria, respectively) through the water column close to the ANTARES site. At 2200 m depth, *Vibrionaceae* appeared to be far from negligible, representing 40% of γ-proteobacteria, 25% of Bacteria and 9% of the total DAPI-stained cells, while Bacteria and Archaea represented 35% each. Furthermore, during a high luminous background period detected by the neutrino telescope, we isolated, from a 2200 m depth sample, a piezophilic luminous bacterium, phylogenetically determined as *Photobacterium phosphoreum* strain ANT-2200. We have used this strain to investigate the effect of hydrostatic pressure on bioluminescence by developing a new high-pressure apparatus. First assays showed that the bioluminescence intensity of *Photobacterium phosphoreum* strain ANT-2200 was 5 times higher at 22 MPa than at 0.1 MPa (atmospheric pressure).

Keywords: deep-sea microbiology; hydrostatic pressure; CARD-FISH; luminous bacteria; high-pressure device; prokaryotic diversity; marine microbial ecology; Mediterranean Sea; ANTARES neutrino telescope

1. Introduction

This study is related to the ANTARES programme dedicated to the installation of an underwater telescope immersed at 2475 m depth and 20 nautical miles (NM) off the French Mediterranean coast (42°50′N/06°10′E). It comprises a large array of optical light sensors connected to a permanent deep-sea station. The telescope covers a 0.1 km^2 lines array comprising 12 lines with 900 photomultiplier tubes in 12 vertical strings with an active height of 300 m. The project was completed in May 2008 and was thus the completion of the largest undersea neutrino telescope to date (for more information see http://antares.in2p3.fr/). Indeed, although designed to detect light produced by high energy muons induced by neutrino interactions with matter, the detector

*Corresponding author. Email: christian.tamburini@univmed.fr

ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757540903513766
http://www.informaworld.com

is also sensitive to light emission from the radioactive decay of dissolved potassium-40 (^{40}K) in seawater and from luminescence of marine organisms [1,2]. ^{40}K and bioluminescence contribute mainly one photoelectron hit distributed randomly in time over the entire array, as also observed during the DUMAND and AMANDA experiences [3].

Bioluminescence is the process by which living micro- or macro-organisms emit light. Marine luminous bacteria are ecologically versatile and enjoy more than one lifestyle, occurring as free living forms, epiphytes, saprophytes, parasites, symbionts in the light organs of fish and squid and commensals in the gut of various marine organisms [4–6]. Luminescent bacteria are Gram negative γ-proteobacteria, rod-shaped cells, chemoorganotrophic, nonsporlating, and motile by flagella [7]. The ecological role and physiological process of symbiotic luminous bacteria associated with marine macroorganisms are relatively well understood, while little is known about planktonic luminous bacteria. For example, the presence of symbiotic bacteria in fish is of mutual benefit: the bacteria are supplied with nutrients and a protected environment, whereas the fish are supplied with light [7]. In contrast, the reason why marine planktonic bacteria emit light is less obvious, and several hypotheses have been proposed. For instance, luminous bacteria attached to sinking faecal pellets ejected by plankton might produce sufficient light to attract organisms to feed on the pellets and therefore can recover favourable conditions (e.g. the gut of animals such as fish or zooplankton), thus enhancing the propagation of the bacteria [8]. Another hypothesis is that bioluminescence plays a continuous antioxidative role [9–11].

In this study, we focused on planktonic luminous bacteria living in the water column close to the ANTARES site. The first objective was to quantify and then identify and isolate luminous bacteria close to the ANTARES site during a high background activity period detected by the ANTARES telescope. The second objective was to verify the ability of isolated strains to grow and emit light under high-pressure conditions. For this purpose we have developed a new hyperbaric system able to measure both luminescence and growth of bacteria under high-pressure conditions. Complementary environmental data of the study site during the observation period is also reported.

2. Material and methods

2.1. *Sampling area and sampling protocol*

Sampling was performed in April and in September 2005 during the NOCTILUC cruises on board the R/V TETHYS II in the North-Western Mediterranean Sea, close to the ANTARES site. This sampling station was located 20 NM off La Seyne-sur-Mer on the French Mediterranean coast (42°54′N/06°06′ E) in the vicinity of the ANTARES telescope (Figure 1). Several depths were sampled using Niskin bottles: 25, 50, 200, 500, 800, 1000, 1500, 1800, 1900, 2000 and 2200 m in depth.

2.2. *Time-series particle and carbon flux*

A mooring with a Technicap PPS5 sediment trap (conical, baffled, 1 m^2 collecting surface) was deployed on 31 March 2005 for 159 days. Sampling took place from 5 April 2005 (day of year, DOY, 94) to 4 September 2005 (DOY 243) at 2325 m depth, 150 m above the sea-floor of ∼2475 m depth. Each cup integrated the flux during a period of 6 days and 15 hours, and samples were preserved with 2% buffered formaldehyde. Back in the laboratory, swimmers were carefully removed by sieving and hand-picking under a dissecting microscope. Prior to mass and carbon content determination samples were freeze-dried. Concentrations of total and organic

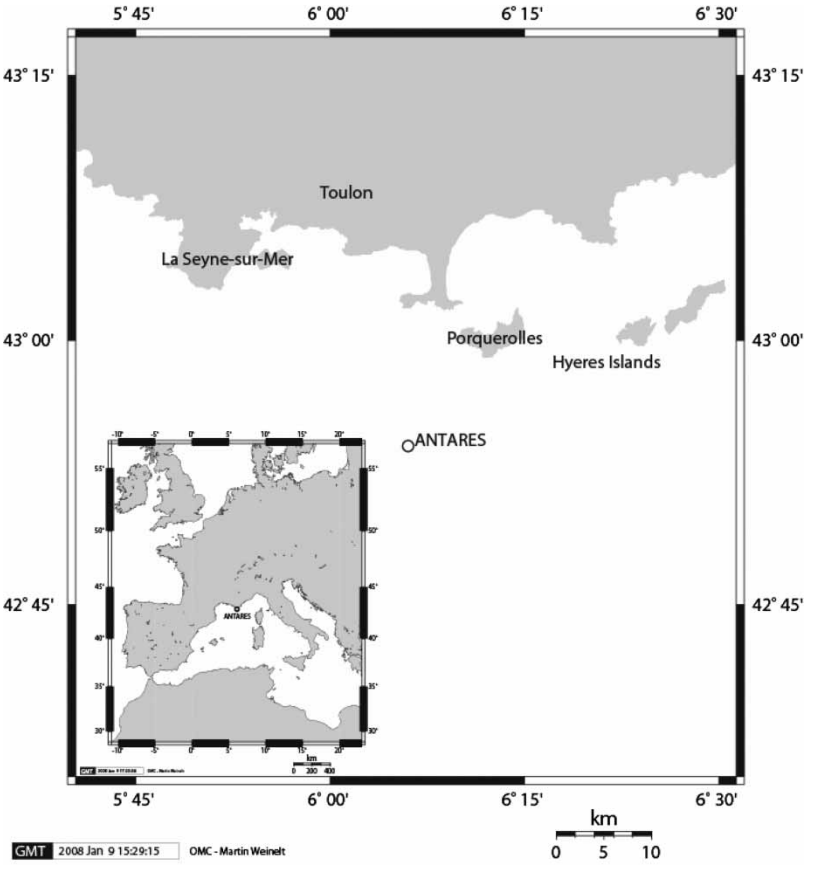

Figure 1. Map of the sampling area: ANTARES site. Map constructed using Only Map Creation (OMP) program (Geomar, Kiel, Germany; http://www.aquarius.geomar.de).

(POC) carbon were measured using a Vario-El CHN elemental microanalyser on aliquots of the dessicated samples [12,13].

2.3. *Possible colonisation by luminous bacteria of the optical modules*

The optical module (OM), the basic optical unit of ANTARES, consists of a photomultiplier tube (PMT) housed in a water-pressure resistant glass sphere [14]. In order to verify the ability of luminous bacteria to attach to these OMs, we have incubated glass-slides mounted on a frame (Figure 2) carried on a OMs frame test. The slides-frame was composed of a plastic plate (300 × 300 mm) with two compartments of six glass slides (750 × 260 mm). This slides-frame was fixed on a photomultiplier frame (oriented toward the bottom at 45 °C as ANTARES OMs) and deposited at 2000 m depth between April 2005 and September 2005. After six months of *in situ* incubation, the slides-frame was recovered and the glass slides were washed with sterilised filtered sea water to wash bacteria not fixated on the glass slides (e.g. during the recovery), deposited in liquid SeaWater Complete (SWC) medium amended with glycerol, and incubated at room temperature in the dark for 48 h. The emission of light of the tubes containing glass slides incubated into liquid ZoBell medium was checked visually in a dark room.

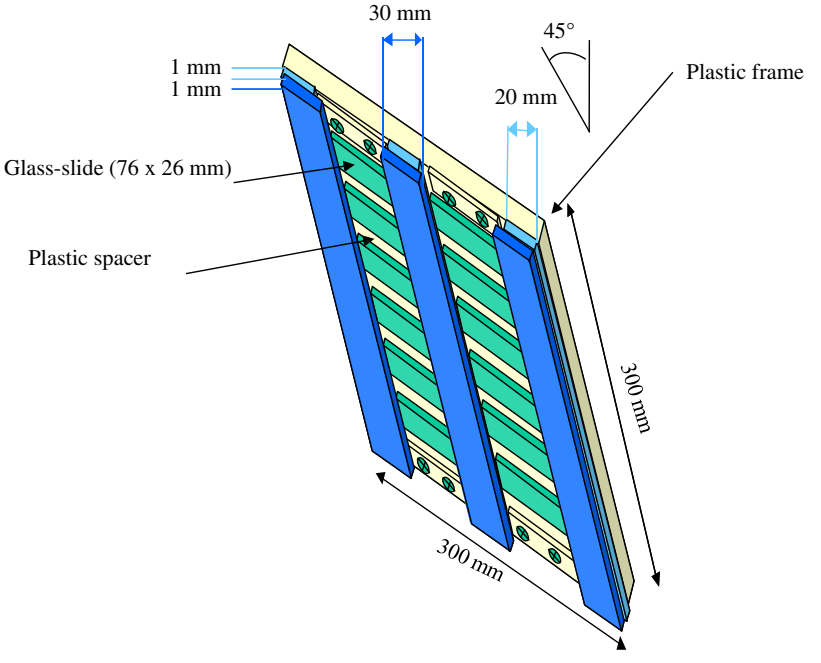

Figure 2. Schematic diagram of the glass-slides frame that was placed at the ANTARES site for 6 months. The frame was oriented downwards at 45° as the optical modules photodiodes of the ANTARES neutrino telescope.

2.4. *Isolation and culture medium*

Seawater samples (500 mL) were filtered through 3.0 μm and then through 0.2 μm sterilised polycarbonate filters (diameter of 47 mm). Filters were then placed with the cells side up on SWC agar medium amended with glycerol (0.3% glycerol, 0.3% yeast extract, 0.5% bio-peptone, 25% distilled water, 75% old sea water and 1.5% agar; pH 7.5; according to Nealson and Hastings [8] and incubated at room temperature in the dark for 48 h. The presence of attached-to-particles (retained by 3.0 μm-pore-size polycarbonate filter) and free-living (passed throughout 3.0 and retained by 0.2 μm-pore-size-filter) luminous colonies was checked visually in a dark room. Among the isolated colonies, one (called hereafter strain ANT-2200) was chosen as a model for this study. It was isolated from samples recovered at 2200 m depth.

2.5. *Catalysed reporter deposition coupled with fluorescence* in situ *hybridisation (CARD-FISH) analysis*

Sub-samples were fixed with 0.2 μm filtered formaldehyde solution (final concentration, 2% [vol/vol]), kept at room temperature for 15 min and subsequently stored at 4 °C for around 12 h. Then subsamples were filtered onto 0.2-μm-pore-size white polycarbonate membrane filters using a gentle vacuum and cellulose nitrate support filters (pore size, 0.45 μm). The volume of seawater filtered onto 0.2-μm-pore-size was 20 mL at 25 and 100 m, 40 mL at 500 m and 120 mL below 1000 m depth. Filters were subsequently washed twice with 5 mL of 0.2-μm filtered MilliQ water, air-dried and stored frozen in plastic tubes at −20 °C until analysis. Pieces of filters were analysed as previously described in Tamburini et al. [15]. Probes used are listed in Table 1. At the end of the CARD-FISH procedure, the pieces of filters were mounted on slides, with a mixture of Citifluor, Vectashild, and PBS (5.5/0.5/0.5 in volume, respectively) containing 1 μg mL^{-1} of 4′, 6′-diamidino-2-phenylindole (DAPI). The slides were stored at −20 °C until counting with an

Table 1. 16S rRNA-targeted oligonucleotide probes used in this study.

Probe	Sequence (5′ to 3′) of probe	Target organisms	% Formamide	References
Eub338	GCTGCCTCCCGTAGGAGT	Domain of Bacteria	55	[60]
Gam42a*	GCCTTCCCACATCGTTT	γ-subclass of *Proteobacteria*	55	[61]
Vibrio	AGGCCACAACCTCCAAGTAG	Specific for the group of *Vibrionaceae*	55	[30]
Arch915	GTGCTCCCCCGCCAATTCCT	Domain of Archaea	20	[62]
NegControl	TAGTGACGCGCTCGA	For non-specific probe binding	55	[41]

Note: *Including an unlabelled competitor probe BET42a (5′-GCCTTCCCACTTCGTTT-3′), see Manz et al. [61] for details.

Olympus BX61 microscope. The fraction of CARD-FISH-stained cells in at least 1000 DAPI-stained cells per sample was quantified. Negative control counts averaged 1% and were always below 5% of DAPI-stained cells.

2.6. *PCR amplification of the 16S rRNA gene*

DNA was extracted using the DNA/RNA Mini Kit Qiagen® according to the manufacturer's protocol. The 16S rRNA gene of the strain was amplified by PCR with universal primers for eubacteria – EU3 and EU5 – as previously described in Cuny et al. [16]. The nucleotide sequence of the PCR product was determined by the Plateforme Génomique Fonctionnelle de l'Université Victor Ségalen Bordeaux 2 (Bordeaux, France).

2.7. *Phylogenetic analysis and alignment*

The 16S rRNA gene sequences were aligned with the same region of the closest relatives. Sequences were obtained with BLAST Version 2.2.13 (http://www.ncbi.nlm.nih.gov/BLAST) [17]. The sequences were aligned using ClustalX Version 1.83 [18]. A phylogenetic tree was constructed using the neighbour-joining method [19] and a bootstrap analysis with 1,000 replicates was carried out to check the robustness of the tree. Finally, the tree was plotted using MEGA Version 4 [20]. The tree was rooted using *Escherichia coli* ATCC 11775 (GenBank accession no. X80725) as an outgroup. The sequence determined in this study have been submitted to the GenBank database and assigned accession no. bankitEU881910.

2.8. *Description of the high-pressure bioluminescent tank*

Figure 3 presents the high-pressure bioluminescent tank designed in collaboration with the company Métro-Mesures (Mennecy, France). This high-pressure bioluminescent tank is constituted by a hyperbaric tank and a culture chamber. The hyperbaric tank is able to support 40 MPa of hydrostatic pressure. It is made up of a main core in APX4 stainless steel cylinders (215 mm OD, 150 mm ID and 200 mm total length), a APX4 stainless steel end-cap (205 mm D, 69.7 mm height) fitted with one O-ring and a screw-top in bronze (160 mm OD, 98 mm height). The end-cap is fitted with two filling ports, a safety valve (920.86.00 H, Top Industrie S.A., France) and a manometer. Within the main core filled with sterilised MilliQ water, the culture chamber is connected by a 1/8″ PEEK (polyetheretherketone) tube. The culture chamber, in ertalyte (chemically and biologically inert material, white in colour to reflect light), consists of a main core (80 mm ID, 100 mm height) and a floating piston fitted with one O-ring and with a housing for a stir bar. To measure the bioluminescence within the culture chamber, a plexiglass cone (13.3 mm down-diameter, 8.8 mm up-diameter, 21.4 mm height) is positioned in an APX4 stainless support fitted with one O-ring and inserted into the ertalyte culture chamber. The whole system

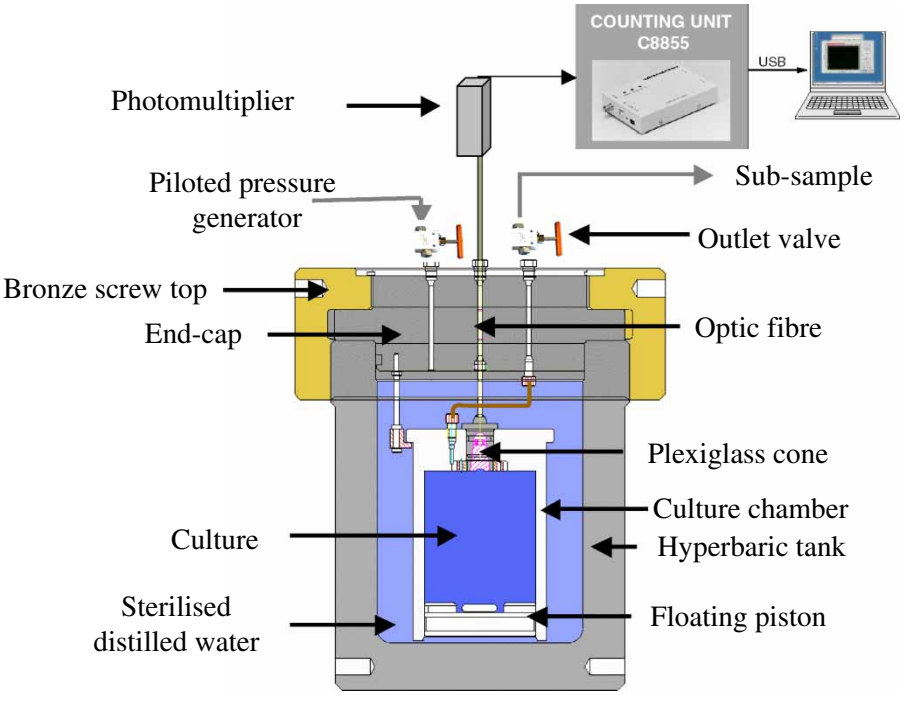

Figure 3. Schematic diagram of the high-pressure bioluminescence tank.

is autoclavable to ensure sterilised conditions of the culture. The plexiglass cone transmits the photons emitted by luminous bacteria growing and glowing into the culture chamber via an optical fibre connected to a photomultiplier (H7155, Hammamatsu®) linked to a counting unit (C8855, Hammamatsu®). Using the sample software for controlling the counting unit (C8855) according to the manufacturer's protocol, photon counting was obtained by integrated signals on 10 sec. The baseline obtained with sterilised MilliQ water is in average equal to 2.8 ± 0.6 photons sec^{-1}. The maximum volume of the culture chamber is 500 mL. The temperature is regulated using an external housing with a coil of tubing connected to a temperature cooler. The hydrostatic pressure is induced using a piloted pressure generator [21] connected to the hyperbaric tank and transmitted to the culture chamber via the floating piston. Sub-sampling is performed using the piloted pressure generator.

2.9. *Culture of luminous bacteria using the high-pressure bioluminescent tank*

The strain isolated at 2200 m depth at the ANTARES site was diluted on a streak plate and maintained at room temperature in the dark for 24 h. A single colony was selected and used to prepare a pre-culture by inoculating it with 30 mL of liquid medium. The pre-culture was incubated at room temperature in the dark for 18 h (before the end of the log phase). After 18 h, part of the preculture was transferred to fresh medium in order to obtain an optical density of 0.05. This culture was transferred, into a pre-filled sterile high-pressure bottle, after that it was transferred into the culture chamber within the high-pressure tank without air bubbles. The culture was performed under two different pressure conditions, 0.1 MPa (atmospheric pressure conditions) and 22 MPa (corresponding to 2200 m depth). Previous experiments demonstrated the necessity of oxygen to ensure the growth of the strain. Ju et al. [22] proposed use of perfluorocarbons (fluorinert, FC72) in enhancing oxygen transfer in bioreactors; the oxygen, being not bound to

perfluorocarbons is easily released to the cells. This procedure was also used with success in high pressure cultivation [23–25]. Hence, in order to obtain optimal growth of both cultures (at 0.1 and 22 MPa), we added fluorinert (FC-72; 3M), saturated beforehand with oxygen (25% total volume).

Bacterial growth was estimated by measuring the optical density at the wavelength of 600 nm (OD_{600}) using a spectrophotometer (Perkin Elmer, Lamda EZ201 UV/Vis spectrophotometer). Direct counting was also performed using epifluorescence microscopy using DAPI staining to obtain the relation between total cell counts (DAPI counts) and the optical density (OD_{600}) according to Equation (1):

$$\text{Number of DAPI-stained cells, mL}^{-1} = 5 \times 10^8 \times \text{OD}_{600} (\text{R}^2 = 0.95, n = 40). \quad (1)$$

3. Results and discussion

3.1. *Field experiments*

The ANTARES site is located at the south edge of the Mediterranean North Current (NC), which has a variable influence on its hydrographic parameters. The most important hydrographic characteristics of the water column at the ANTARES site are summarised on the potential temperature (θ) versus salinity (S) diagram of Figure 4. The surface layer (up to 100 m depth) is dominated by the Modified Atlantic Water (MAW) entered at the Gibraltar Strait following a classical cyclonic pattern [26,27]. MAW salinity and temperature values evolve throughout the year upon seasonal variability. The Winter Intermediate Water (WIW) is formed during cold and dry winter conditions [28] in the Ligurian Sea, usually centred at 200 m depth. Only old WIW from the previous winter is noticeable. The Levantine Intermediate Water (LIW), originated from the Levantine Basin, located between 300 and 600 m depth, is characterised by high temperature and high salinity ($T > 13.3\,^{\circ}C$, $S > 38.5$). The Western Mediterranean Deep Water (WMDW) is deeper than 1000 m depth and is characterised by temperature of 12.8 °C and salinity ranging from 38.42 to 38.45.

Mass flux was very variable in 2005, with high flux at the beginning of the experiment (1321 mg m^{-2} d^{-1} from 5–11 April) and then a sharp decrease in flux during the rest of the sampling period (Figure 5). During the whole time-series period, the mass flux at 2325 m depth averaged 77.9 mg m^{-2} d^{-1}, whereas the mean organic carbon flux was 1.6 mg m^{-2} d^{-1}, representing 5.5% of the mass flux. However, these mean fluxes average an initial period with particularly high flux and the remaining period with low flux. The high mass and POC flux (and low OC %, 1.5%) in April 2005 could result from sediment resuspension associated with deep water formation or from large transport of sediment during cascading events [29]. We consider that the very high flux measured in April could not be due to the mooring line deployment because the opening of the first cup collecting material was 6.5 days after the deployment, and the trap was sampling at 150 m above the sea-floor. If the first two periods associated to this resuspension phenomenon are not considered then the average mass and OC flux were 9.4 and 0.34 mg m^{-2} d^{-1}, respectively, and the OC content 5.9%. A less intense but very significant increase of mass and OC fluxes occurred between 16 June and 6 July 2005 (between DOY 165 and 185), with mass flux reaching 73.4 mg m^{-2} d^{-1} and OC flux reaching 1.95 mg m^{-2} d^{-1}; this late spring peak in flux is the result of the transfer to the deep of organic matter produced in surface waters during the spring bloom.

The vertical distribution of total DAPI-stained cells at the ANTARES site is in concordance with profiles described from the DYFAMED site [30–32] which is located to the east of the ANTARES site in the Ligurian Sea. In April 2005, prokaryotic cell abundance (as DAPI-stained cells) decreased from the surface to the deep-sea waters, from a maximum of 100.2×10^4 to a minimum of 3.7×10^4 cells mL^{-1} at 25 m and 2200 m, respectively (Figure 6). We observed that the number of luminous CFUs per 100 mL of seawater samples varied between 0 and 7

for samples filtered on the 3-μm-pore-size filters (attached bacteria), while no luminous CFU (free-living bacteria) was observed on the 0.2-μm filters after filtration on 3-μm-pore-size filters. That suggests that luminous bacteria are more attached-to-particles than free-living, as already suggested by Herren et al. [33].

Amram et al. [14] found that the number of total bacteria (counted by epifluoresence microscopy) can reach on glass-slides (surface facing downwards at 45° as the ANTARES OMs are oriented) is 10^5 cells cm^{-2} after 3 months of incubation at 2000 m depth at the ANTARES site. They also visualised that some bacteria were embedded in exopolymers, so fouling by bacteria is possible at 2000 m depth. These authors have also shown that this biofouling has particular importance for vertical glass slides but minor importance for surfaces facing downwards, where the light transmission is negligible. However, we found that when using glass-slides incubated for 6 months at the ANTARES site (surface facing downwards at 45°) which were then incubated for 48 h in liquid SWC medium, half of the cultures were luminescent. These results suggest a potential bioluminescent ability of the bacteria attached to optical modules that may contribute (according the natural conditions) to the background of the neutrino telescope baseline.

Marine luminous planktonic bacteria are widely distributed. They have been isolated throughout the world from shallow coastal environments to open ocean waters and from the surface to the deep ocean. Their concentration estimated by means of the culture method is generally low. For instance, in the Puerto Rico Trench, the number of luminous bacteria ranged from 0.04 CFUs per 100 mL at 1000 m depth and 30 CFUs per 100 mL in surface seawater [4]. In the Mediterranean Sea, few values have been reported in the literature. De Domenico et al. [34] estimated a number of CFU varying between 0 and 12 CFU per 100 mL, with a maximum often found in the deep-waters (between 900–1200 m depth) of the Ionian Sea (Capo Passero). However, as underlined by Gentile et al. [35], luminous bacteria represent between 0.4 and 0.8% of all prokaryotic cells (DAPI counts) in the Tyrrhenian Sea. So, in order to obtain a quantitative estimation of luminous bacteria in the deep-sea waters near the ANTARES station, we have attempted to use the CARD-FISH

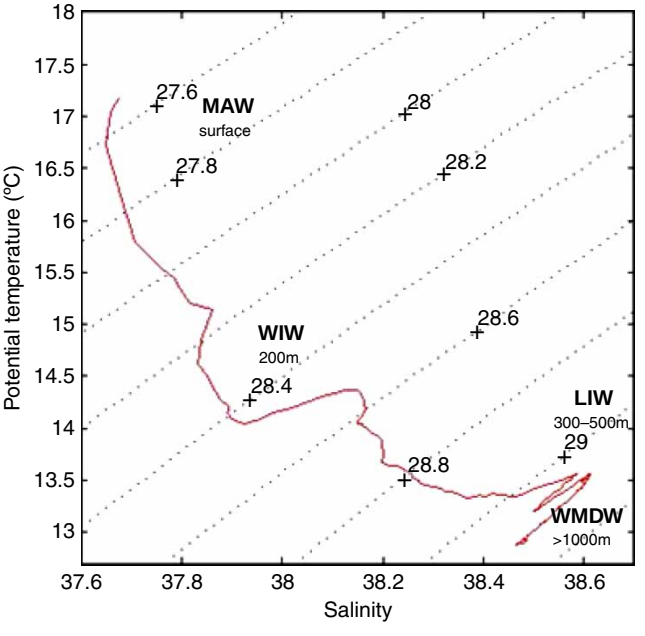

Figure 4. Theta-S diagram at ANTARES site in May 2007. MAW: Modified Atlantic Water; WIW: Winter Intermediate Water; LIW: Levantine Intermediate Water; WMDW: Western Mediterranean Deep Water.

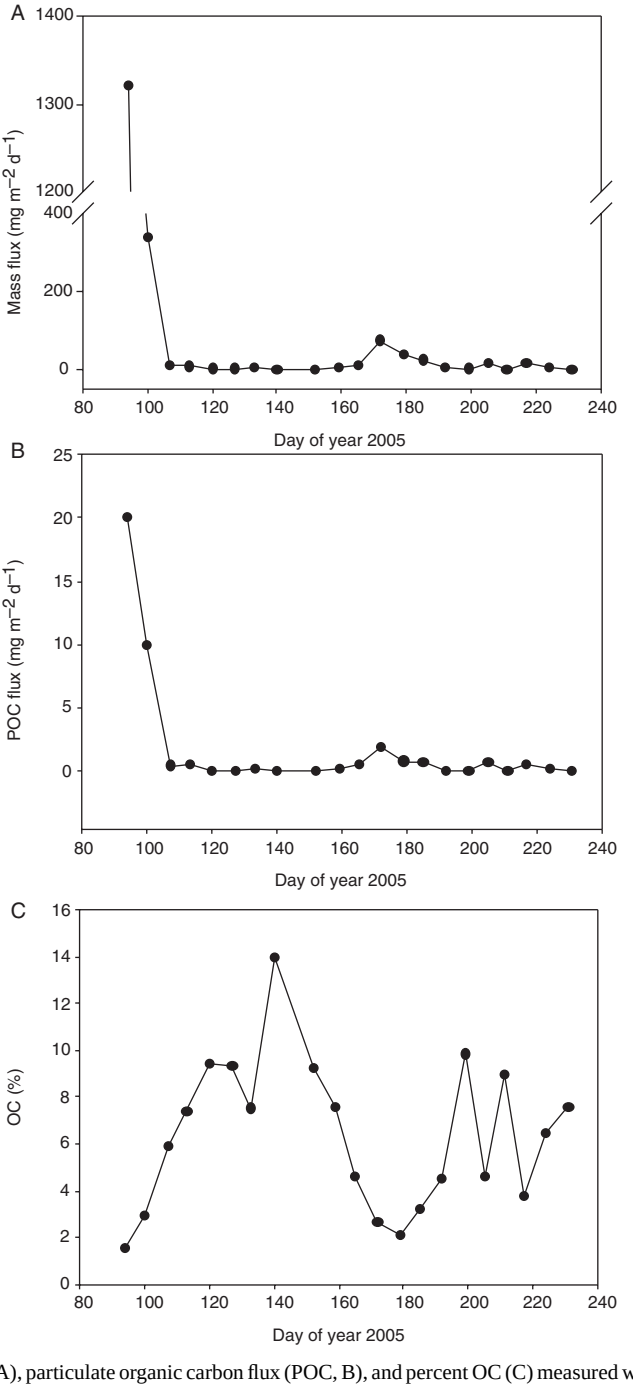

Figure 5. Mass flux (A), particulate organic carbon flux (POC, B), and percent OC (C) measured with the PPS5 sediment trap at 2325 m depth.

technique. Unfortunately, our attempt to develop a specific probe for mRNA luminous bacteria failed, probably owing to the low copy number of mRNAs and their instability [36]. However, using rRNAs CARD-FISH technique, we have estimated the relative abundance to total prokaryotes of Bacteria, γ-proteobacteria and *Vibrinoceae* (domain, subclass and group affiliation of marine luminous bacteria) and Archaea through the water column. At the ANTARES site, the bacterial contribution to total prokaryotic abundance ranged from 73% at 25 m to 35% at 2200 m depth (Figure 6). The percentage of Archaea to total prokaryotic abundance was checked to complete our understanding of their importance in terms of distribution, and because pelagic Archaea were observed to be nearly as abundant as Bacteria in deep-sea waters of different parts of the ocean [37–41] and in the Tyrrhenian Sea [15]. At the ANTARES site, we found that Archaea increased from 9% at 25 m to 35% of DAPI-stained cells between 2000 and 2200 m depth, where they reach the same proportions as those observed for the Bacteria at 2000 m depth. Among the Bacteria, the γ-proteobacteria relative abundance increased from 10% of DAPI-stained cells at 25 m depth, to 47% at 500 m depth, then declined to 22% in the deepest samples (Figure 6). In addition, *Vibrionaceae* relative abundance varied from 3% to 15% of DAPI-stained cells, with two peaks of 15% and 9% at 500 m and at 2200 m depth, respectively. These results are in good agreement with those obtained by Giuliano et al. [30], who have shown that the *Vibrio* group presented a maximum in the superficial layer and in the deep water layers of the Mediterranean (DYFAMED study site). Relative abundance of *Vibrionaceae* ranged from 24–40% of γ-proteobacteria detected by CARD-FISH. All *Vibrionaceae* are not exclusively luminous bacteria. However, the numbers of luminous CFUs (data not shown) were more abundant at the depths where the numbers of *Vibrinonaceae* were at a maximum.

Luminous bacteria produce specific substances, auto-inducers (AI), which accumulate during bacterial growth and multiplication. If the quantity of auto-inducers exceeds a critical threshold, the enzyme luciferase is induced, thus generating the luminous emission [42]. This phenomenon is called auto-induction [43–45], and is also known as quorum sensing. It was first observed with the bacterium *Vibrio fischeri*, and there exists a threshold concentration of AI (10^7 cells mL^{-1}) that activates the synthesis of luciferase and other enzymes implied in luminescence [46]. *V. fischeri* achieves high cell densities (10^{10}–10^{11} cells mL^{-1}) as a symbiont in light organs, whereas in seawater the density of this bacterium does not exceed 10^2 cells mL^{-1}. Therefore

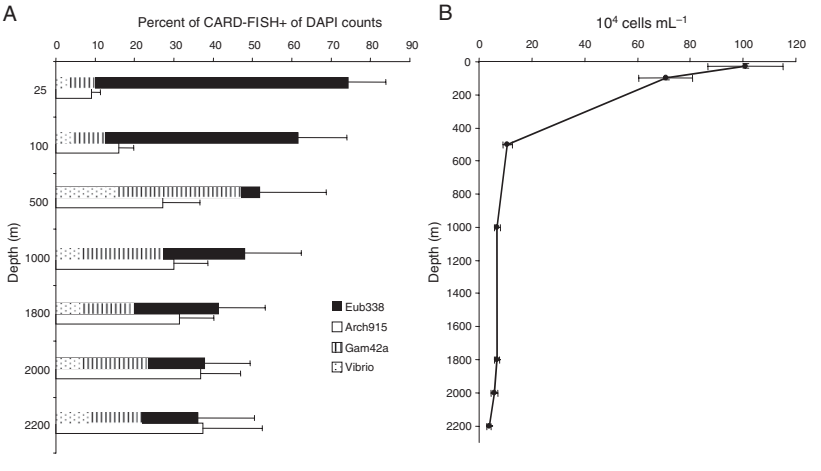

Figure 6. (A) Relative bacterial and archaeal abundance (expressed as percent of total prokaryotes, DAPI stained cells) at the ANTARES site in April 2005 detected by catalysed reporter deposition coupled with fluorescence *in situ* hybridisation (CARD-FISH). Eub338: Bacteria; Arch915: Archaea; Gam42a: γ-Proteobacteria; Vibrio: Group of *Vibrionaceae*. See Table 1 for details. (B) Depth profiles for total prokaryotic cells (DAPI-stained cells) at the ANTARES site in April 2005.

V. fischeri appears luminescent as a symbiont but not as a planktonic bacterium [4,8,47]. Thus, in natural ecosystems, and in particular in the seawater ecosystem, bacteria will appear luminous only if a sufficient quantity of auto-inducers accumulates; this would occur when natural conditions favour the development of a high density bacterial population [48]. So, we hypothesise that these conditions may occur in particles, marine snow, gel particles and biofilm attached to ANTARES' optical modules.

3.2. *Laboratory experiments*

Among the 11 luminous CFUs isolated from 500 mL filtered water at 2200 m depth, we isolated two strains according to morphotype. One strain was sequenced in order to achieve its 16S phylogenetic affiliation. The 16S rRNA gene sequence of ANT-2200 obtained in this study consisted of 1510 nt. In terms of 16S rRNA gene sequence similarity, the closest relatives of strain ANT-2200 were *Photobacterium phosphoreum* (100%) strain IFO 13896, *P. leiognathi* strain RM1 (99 %), *P. 'kishitanii clade'* strain piapo.8.1 (99%) and *P. iliopiscarium* strain NCIMB 13481 (99%). Lower sequence similarities (<96%) were found with all other *Photobacterium* species with valid published names (Figure 7). On the basis of phylogenetic data, the isolate identified is then called *Photobacterium phosphoreum* strain ANT-2200. Previous observations have shown that the *Photobacterium* genus seems to constitute the majority of numerically important luminous bacteria in deep-sea waters [7,35,49,50].

Our knowledge on pressure effects on marine microbes has been improved by both laboratory experiments using pure cultures of microbes and field experiments using special pressure apparatus to recover undecompressed samples from the deep sea. Laboratory experiments have elucidated microbial adaptations to high hydrostatic pressure in respect to growth [51], membrane lipids [52], membrane proteins [53], enzymes [54], respiratory chain [55] and DNA replication and translation [56]. Hence, we have logically developed a special apparatus (high-pressure bioluminescent tank) to check the effect of pressure on bioluminescence and growth of the *Photobacterium phosphoreum* strain ANT-2200. Thereafter, we present two preliminary experiments carried out successively on the same bacterial colony using the high-pressure bioluminescent tank. The first experiment was carried out at 0.1 MPa, followed by the second experiment at 22 MPa. Figure 8 shows that *P. phosphoreum* ANT-2200 reached the stationary phase after 7 h with an optical density equal to 0.316 (corresponding to 1.6×10^8 cell mL^{-1}) at 0.1 MPa, while at 22 MPa the stationary phase was reached after 8 h with an OD_{600nm} equal to 0.421 (corresponding to

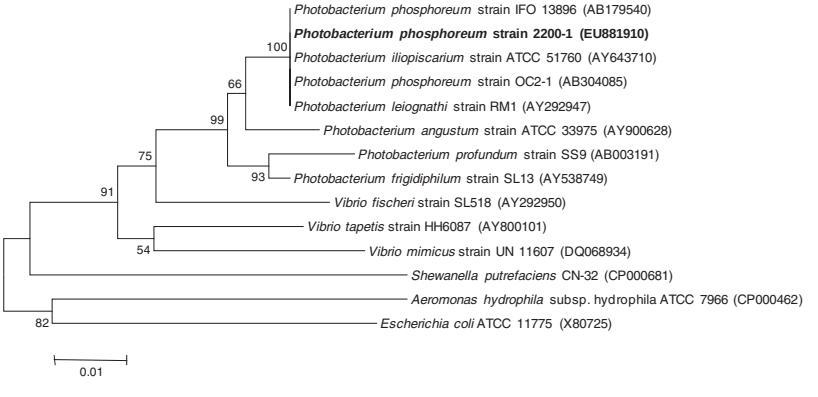

Figure 7. Neighbour-joining tree showing the phylogenetic positions of strain ANT-2200 and related taxa based on 16S rRNA gene sequences. Numbers at nodes are levels of bootstrap support (%) based on neighbour-joining analyses of 1,000 resampled datasets; only values above 70% are given. Bar, 0.01 substitutions per nucleotide position.

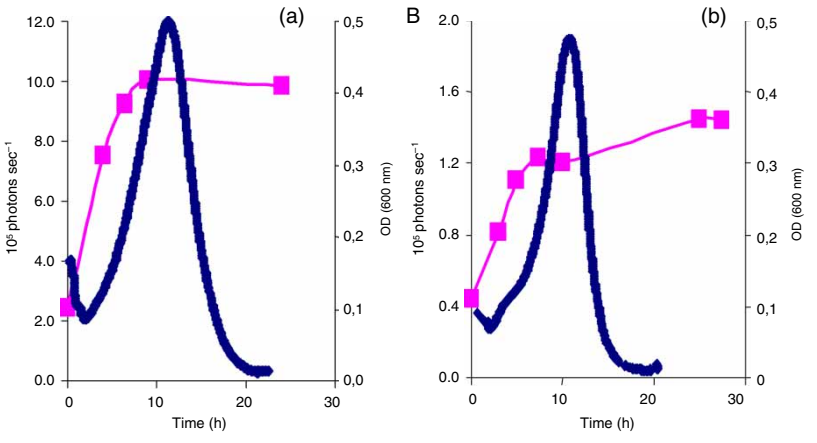

Figure 8. Bioluminescence (photons sec^{-1}) (♦) and curve of growth (–■–) of *Photobacterium phosphoreum* strain ANT-2200 during incubation at 22 MPa (a) and at atmospheric pressure (0.1 MPa) (b).

2.11×10^8 cell mL^{-1}). Bacterial growth rates were equal to 0.098 h^{-1} and 0.147 h^{-1} at 0.1 MPa and 22 MPa, respectively (Figure 8). During the first hour after inoculation, the light emission declined to reach a minimum. After this lag phase relative to the growth rate, the quantity of photons produced per second increased up to a maximum when the maximum OD_{600nm} was reached (Figure 8). This agrees with previous observations found in the literature [44,57]. The conditions in both experiments were the same except for the pressure (0.1 and 22 MPa), making it possible to compare them. The light emission curves showed a peak at 1.82×10^5 and 12×10^5 photons sec^{-1} at 0.1 MPa and 22 MPa, respectively. While the OD_{600nm} at 0.1 and 22 MPa were equal to 0.316 and 0.421, respectively (Figure 8). We can observe the luminescent peak during the early stationary growth phase. The failure of these bacteria to emit light during the first stages of growth is probably due to an insufficient quantity of auto-inducer to induce the enzyme luciferase.

It is possible to calculate the number of photons emitted per second per cell using the relationship between OD_{600nm} and DAPI counts (Equation (1)). The specific intensity of luminescence reached 0.58 and 2.85 photon sec^{-1} $cell^{-1}$ at 0.1 and 22 MPa, respectively. Then, *P. phosphoreum* ANT-2200 emitted 5 times more photons sec^{-1} $cell^{-1}$ at 22 MPa than at 0.1 MPa. These results should be treated with caution but are consistent with additional experiments done at different pressure and temperature (Al Ali, in preparation). Still, the present study demonstrates our ability to measure both bioluminescence and growth of luminous bacteria using our high-pressure bioluminescent tank.

Our next goal is to measure the bioluminescence of microorganisms under *in situ* conditions, using this new apparatus with natural samples taken using our High-Pressure Serial Sampler [58,59]. Bioluminescence due to macroorganisms has been estimated at the ANTARES site using an autonomous free-fall lander vehicle [2] to study simulated bioluminescence (bioluminescence due to mechanical stimulation), but this apparatus is unable to estimate the diffuse bioluminescence due to free-living or attached-to-particles microorganisms. Priede et al. [2] estimated that the mean density of luminous macroorganisms at depths >1500 m in the Ligurian Sea was 0.622 ind m^{-3}. At these densities the authors predicted that the effect of natural background bioluminescence due to macroorganisms (by opposition to microorganisms studied in the present work) would be negligible for the telescope. Thus, if macroorganisms are negligible, the light background sometimes occurring close to the ANTARES deep underwater telescope might be due to the important contribution of luminous microorganisms, mainly attached bacteria to OMs and/or attached-to-particles falling through the water column.

To the best of our knowledge, this is the first time that both growth and luminescence of bacteria have been measured under high pressure conditions. These preliminary results must be confirmed, but we have found that the luminous *P. phosphoreum* strain ANT-2200 isolated at 2200 m depth at the ANTARES site exhibit higher bioluminescence activity at high pressure (22 MPa) than at atmospheric pressure (0.1 MPa), suggesting its ability to produce light in a natural environment and its potential impact in terms of light perturbation of the ANTARES deep underwater neutrino telescope. However, a better estimation of the seasonal variability of luminous bacteria in the deep sea and direct measurements using our high pressure bioluminescence tank are required in order to better understand this phenomenon. With this aim, the apparatus we have designed is a promising tool to achieve better understanding of luminous deep-sea bacteria and their role in the biogeochemical cycle of the oceans.

Acknowledgements

This work was funded by the ANR-POTES (see http://www.com.univ-mrs.fr/LMGEM/potes) program (ANR-05-BLAN-0161-01) supported by the Agence Nationale de la Recherche (France), by the INSU and IN2P3 with the ANTARES-Bioluminescence project and by the AAMIS project (Université de la Méditerranée). BAA was supported by a fellowship funded by the Syria Ministry of Higher Education. Authors thank S. Escoffier, M. Billault, J.P. Schuller, the captains and crews of R.V. Tethys II for cruise assistance and the ANTARES collaboration. We also thank Michael Paul for improving the English of the manuscript and France Van Wambeke and Dominique Lefèvre for their critical comments. The International Atomic Energy Agency is grateful for the support provided to its Marine Environment Laboratories by the Government of the Principality of Monaco.

References

[1] J.A. Aguilar, A. Albert, F. Ameli, M. Anghinolfi, G. Anton, S. Anvar, E. Aslanides, J.J. Aubert, E. Barbarito, S. Basa, M. Battaglieri, Y. Becherini, R. Bellotti, J. Beltramelli, V. Bertin, A. Bigi, M. Billault, R. Blaes, N. de Botton, M.C. Bouwhuis, S.M. Bradbury, R. Bruijn, J. Brunner, G.F. Burgio, J. Busto, F. Cafagna, L. Caillat, A. Calzas, A. Capone, L. Caponetto, E. Carmona, J. Carr, S.L. Cartwright, D. Castel, E. Castorina, V. Cavasinni, S. Cecchini, A. Ceres, P. Charvis, P. Chauchot, T. Chiarusi, M. Circella, C. Colnard, C. Compère, R. Coniglione, N. Cottini, P. Coyle, S. Cuneo, A.-S. Cussatlegras, G. Damy, R. van Dantzig, C. De Marzo, I. Dekeyser, E. Delagnes, D. Denans, A. Deschamps, F. Dessages-Ardellier, J.-J. Destelle, B. Dinkespieler, C. Distefano, C. Donzaud, J.-F. Drogou, F. Druillole, D. Durand, J.-P. Ernenwein, S. Escoffier, E. Falchini, S. Favard, F. Feinstein, S. Ferry, D. Festy, C. Fiorello, V. Flaminio, S. Galeotti, J.-M. Gallone, G. Giacomelli, N. Girard, C. Gojak, P. Goret, K. Graf, G. Hallewell, M.N. Harakeh, B. Hartmann, A. Heijboer, E. Heine, Y. Hello, J.J. Hernández-Rey, J. Hößl, C. Hoffman, J. Hogenbirk, J.R. Hubbard, M. Jaquet, M. Jaspers, M. de Jong, F. Jouvenot, N. Kalantar-Nayestanaki, A. Kappes, T. Karg, S. Karkar, U. Katz, P. Keller, H. Kok, P. Kooijman, C. Kopper, E.V. Korolkova, A. Kouchner, W. Kretschmer, A. Kruijer, S. Kuch, V.A. Kudryavstev, D.L. Lachartre, H.P. Lagier, R. Lahmann, G. Lamanna, P. Lamare, J.C. Languillat, H. Laschinsky, Y. Le Guen, H. Le Provost, A. Le Van Suu, T. Legou, G. Lim, L. Lo Nigro, D. Lo Presti, H. Loehner, S. Loucatos, F. Louis, F. Lucarelli, V. Lyashuk, M. MarcelinM, A. Margiotta, R. Masullo, F. Mazéas, A.J. Mazure, E. McMillan, R. Megna , M. Melissas , E. Migneco, A. Milovanovic, M. Mongelli, T. Montaruli, M. Morganti, L. Moscoso, M. Musumeci, C. Naumann, M. Naumann-Godo, V. Niess, C. Olivetto, R. Ostasch, N. Palanque-Delabrouille, P. Payre, H. Peek, C. Petta, P. Piattelli, J.-P. Pineau, J. Poinsignon, V. Popa, T. Pradier, C. Racca, N. Randazzo, J. van Randwijk, D. Real, B. van Rens, F. Réthoré, P. Rewiersma, G. Riccobene, V. Rigaud, M. Ripani, V. Roca, C. Roda, J.F. Rolin, M. Romita, H.J. Rose, A. Rostovtsev, J. Roux, M. Ruppi, G.V. Russo, F. Salesa, K. Salomon, P. Sapienza, F. Schmitt, J.-P. Schuller, R. Shanidze, I. Sokalski, T. Spona, M. Spurio, G. van der Steenhoven, T. Stolarczyk, K. Streeb, D. Stubert, L. Sulak, M. Taiuti, C. Tamburini, C. Tao, G. Terreni, L.F. Thompson, P. Valdy, V. Valente, B. Vallage, G. Venekamp, B. Verlaat, P. Vernin, R. de Vita, G. de Vries, R. van Wijk, P. de Witt Huberts, G. Wobbe, E. de Wolf, A.-F. Yao, D. Zaborov, H. Zaccone, J.D. Zornoza, and J. Zúñiga, *First results of the instrumentation line for the deep-sea ANTARES neutrino telescope*, Astropar. Phys. 26 (2006), pp. 314–324.

[2] I.G. Priede, A. Jamieson, A. Heger, C. Jessica, and A.F. Zuur, *The potential influence of bioluminescence from marine animals on a deep-sea underwater neutrino telescope array in the Mediterranean Sea*, Deep Sea Res. I 55 (2008), pp. 1474–1483.

[3] J. Wilkes, *DUMAND and AMANDA: High energy neutrino astrophysics*, Proceedings of the SLAC Summer Institute on Particle Physics, Standford, CA, 8–19 August, 1994.

[4] E.G. Ruby, E.P. Greenberg, and J.W. Hastings, *Planktonic marine luminous bacteria: Species distribution in the water column*, Appl. Envir. Microbiol. 39 (1980), pp. 302–306.

[5] J.W. Hastings, *Chemistry and control of luminescence in marine organisms*, Bull. Mar. Sci. 33 (1983), pp. 818–828.

[6] P.J. Herring, *Systematic distribution of bioluminescence in living organisms*, Biolumines. Chemilumines. 1 (1987), pp. 147–163.

[7] P. Dunlap and K. Kita-Tsukamoto, *Luminous bacteria*, Prokaryotes 2 (2006), pp. 863–892.
[8] K. Nealson and J.W. Hastings, *Bacterial bioluminescence: Its control and ecological significance*, Microbiol. Rev. 43 (1979), pp. 496–518.
[9] E.G. Ruby, *Lessons from a cooperative, bacterial-animal association: The Vibrio fischeri*; Euprymna scolopes *light organ symbiosis*, Ann. Rev. Microbiol. 50 (1996), pp. 591–624.
[10] H. Szpilewska, A. Czyz, and G. Wegrzyn, *Experimental evidence for the physiological role of bacterial luciferase in the protection of cells against oxidative stress*, Curr. Microbiol. 47 (2003), pp. 379–382.
[11] G.S. Timmins, S.K. Jackson, and H.M. Swartz, *The evolution of bioluminescent oxygen consumption as an ancient oxygen detoxification mechanism*, J. Mol. Evol. 52 (2001), pp. 321–332.
[12] J.C. Miquel, S.W. Fowler, J. La Rosa, and P. Buard-Menard, *Dynamics of the downward flux of particles and carbon in the northwestern Mediterranean Sea*, Deep-Sea Res. I 41 (1994), pp. 243–261.
[13] J.C. Miquel and J. La Rosa, *Suivi à long terme des flux particulaires au site DYFAMED (Mer Ligure, Méditerranée Occidentale)*, Océanis (1999), pp. 303–318.
[14] P. Amram, M. Anghinolfi, S. Anvar, F.E. Ardellier-Desages, E. Aslanides, J.J. Aubert, R. Azoulay, D. Bailey, S. Basa, M. Battaglieri, R. Belloti, J. Beltramelli, Y. Benhammou, R. Berthier, V. Bertin, M. Billault, R. Blaes, R.W. Blaes, F. Blondeau, N. de Botton, J. Boulesteix, C.B. Brooks, J. Brunner, F. Cafagna, A. Calzas, A. Capone, L. Caponetto, C. Cârloganu, E. Carmona, J. Carr, S.L. Cartwright, S. Cecchini, F. Ciacio, M. Circella, C. Compère, S. Cooper, P. Coyle, S. Cuneo, M. Danilov, R. van Dantzig, C. De Marzo, J.J. Destelle, R. De Vita, G. Dispau, F. Druillole, J. Engelen, F. Feinstein, C. Ferdi, D. Festy, J. Fopma, J.M. Gallone, G. Giacomelli, P. Goret, J.F. Gournay, G. Hallewell, A. Heijboer, J.J. Hernández-Rey, J.R. Hubbard, M. Jaquet, M. de Jong, M. Karolak, P. Keller, P. Kooijman, A. Kouchnern, V.A. Kudryavtsev, H. Lafoux, P. Lagier, P. Lamare, J.C. Languillat, L. Laubier, J.P. Laugier, B. Leilde, H. Le Provost, A. Le Van SuU, L. Lo Nigro, D. Lo Presti, S. LoucatoS, F. LouiS, V. Lyashuk, P. Magnier, M. Marcelin, A. Margiotta, R. Masullo, F. Mazéaso, B. Mazeas, A. Mazur, J.E. McMillan, E. Migneco, C. Millot, P. Mols, F. Montanet, T. Montaruli, L. Moscoso, M. Musumeci, E. Nezri, G.J. Nooren, J.E.J. Oberski, C. Olivetto, A. Oppelt-Pohl, N. Palanque-Delabrouille, R. Papaleo, P. Payre, P. Perrin, M. Petrucceti, C. Petta, P. Piattelli, J. Poinsignon, R. Potheau, Y. Queinec, C. Racca, G. Raia, N. Randazzo, F. Rethore, G. Riccobene, J.S. Ricol, M. Ripani, V. Roca-Blay, A. Romeyer, A. Rostovstev, G.V. Russo, Y. Sacquin, E. Salusti, J.P. Schuller, W. Schuster, J.P. Soirat, O. Souvorova, N.J.C. Spooner, M. Spurio, T. Stolarczyk, D. Stubert, M. Taiuti, C. Tao, L.F. Thompson, S. Tilav, R. Triay, A. Usik, P. Valdy, V. Valente, I. Varlamov, G. Vaudaine, P. Vernin, E. Vladimirsky, M. Vorobiev, P. de Witt Huber, E. de Wolf, V. Zakharov, S. Zavatarelli, J.d.D. Zornoza, J. Zúñiga, J.-C. Aloïsi, P. Kerhervé, and A. Monaco, *Sedimentation and fouling of optical surfaces at the ANTARES site*, Astropart. Phys. 19 (2003), pp. 253–267.
[15] C. Tamburini, M. Garel, B. Al Ali, B. Mérigot, P. Kriwy, B. Charrière, and G. Budillon, *Distribution and activity of Bacteria and Archaea in the different water masses of the Tyrrhenian Sea*, Deep Sea Res. II 56 (2009), pp. 700–712.
[16] P. Cuny, J. Faucet, M. Acquaviva, J.C. Bertrand, and M. Gilewicz, *Enhanced biodegradation of phenanthrene by a marine bacterium in presence of a synthetic surfactant*, Lett. Appl. Microbiol. 29 (1999), pp. 242–245.
[17] S.F. Altschul, T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, and D.J. Lipman, *Gapped BLAST and PSI-BLAST: A new generation of protein database search programs*, Nucleic Acids Res. 25 (1997), pp. 3389–3402.
[18] J.D. Thompson, T.J. Gibson, F. Plewniak, F. Jeanmougin, and D.G. Higgins, *The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools*, Nucleic Acids Res. 25 (1997), pp. 4876–4882.
[19] N.N.M. Saitou, *The neighbor-joining method: A new method for reconstructing phylogenetic trees*, Mol. Biol. Evol. 4 (1987), pp. 406–425.
[20] K. Tamura, J. Dudley, M. Nei, and S. Kumar, *MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software Version 4.0*, Mol. Biol. Evol. 24 (2007), pp. 1596–1599.
[21] C. Tamburini, M. Goutx, C. Guigue, M. Garel, D. Lefèvre, B. Charrière, R. Sempéré, S. Pepa, M.L. Peterson, S. Wakeham, and C. Lee, *Effects of hydrostatic pressure on microbial alteration of sinking fecal pellets*, Deep Sea Res. II 56 (2009), pp. 1533–1546.
[22] L.-K. Ju, J.F. Lee, and W.B. Armiger, *Enhancing oxygen transfer in bioreactors by perfluorocarbon emulsions*, Biotechnol. Progr. 7 (1991), pp. 323–329.
[23] C. Kato, S. Suzuki, S. Hata, T. Ito, and K. Horikoshi, *The properties of a protease activated by high pressure from* Sporosarcina *sp. strainDSK25 isolated from deep-sea sediment*, Jamstecr (Japan Marine Science and Technology Center) 32 (1995), pp. 7–13.
[24] C. Kato, T. Sato, M. Smorawinska, and K. Horikoshi, *High pressure conditions stimulate expression of chloramphenicol acetyltransferase regulated by the lac promoter in* Escherichia coli, FEMS Microbiol. Lett. 122 (1994), pp. 91–96.
[25] M. Yanagibayashi, Y. Nogi, L. Li, and C. Kato, *Changes in the microbial community in Japan Trench sediment from a depth of 6292 m during cultivation without decompression*, FEMS Microbiol. Lett. 170 (1999), pp. 271–279.
[26] M. Astraldi, S. Balopoulos, J. Candela, J. Font, M. Gacic, G.P. Gasparini, M. Manca, A. Theocharis, and J. Tintore, *The role of straits and channels in understanding the characteristics of Mediterranean circulation*, Progr. Oceanogr. 44 (1999), pp. 65–108.
[27] C. Millot and I. Taupier-Lepage, *Circulation in the Mediterranean Sea*, in *The Handbook of Environmental Chemistry*, A. Saliot (ed.), Springer-Verlag, Heidelberg, 2005, pp. 29–66.

[28] G.P. Gasparini, G. Zodiatis, M. Astraldi, C. Galli, and S. Sparnocchia, *Winter intermediate water lenses in the Ligurian Sea*, J. Mar. Syst. 20 (1999), pp. 319–332.
[29] M. Canals, P. Puig, X.D. de Madron, S. Heussner, A. Palanques, and J. Fabres, *Flushing submarine canyons*, Nature 444 (2006), pp. 354–357.
[30] L. Giuliano, M. De Domenico, E. De Domenico, M.G. Hofle, and M.M. Yakimov, *Identification of culturable oligotrophic bacteria within naturally occurring bacterioplankton communities of the Ligurian Sea by 16S rRNA sequencing and probing*, Microbial Ecol. 37 (1999), pp. 77–85.
[31] C. Tamburini, J. Garcin, M. Ragot, and A. Bianchi, *Biopolymer hydrolysis and bacterial production under ambient hydrostatic pressure through a 2000 m water column in the NW Mediterranean*, Deep Sea Res. II 49 (2002), pp. 2109–2123.
[32] T. Tanaka and F. Rassoulzadegan, *Full-depth profile (0–2000 m) of bacteria, heterotrophic nanoflagellates and ciliates in the NW Mediterranean Sea: Vertical partitioning of microbial trophic structures*, Deep Sea Res. II 49 (2002), pp. 2093–2107.
[33] C.M. Herren, A.L. Alldredge, and J.F. Case, *Coastal bioluminescent marine snow: Fine structure of bioluminescence distribution*, Continental Shelf Res. 24 (2004), pp. 413–429.
[34] M. De Domenico, S. Scarfì, M. Leonardi, F. Raffa, M. De Luca, and E. De Domenico, *Microbial communities temporal variations in a pelaic site offshore Cape Passero (Southern Ionian Sea)*, Biol. Mar. Medit. (2003), pp. 994–997.
[35] G. Gentile, M. De Luca, R. Denaro, F. Smedile, E. De Domenico, M. De Domenico, M.M. Yakimov, and S. Scarfì, *PCR based detection of bioluminescent microbial populations in marine environment*, Deep Sea Res. II 56 (2009), pp. 763–767.
[36] R. Amann and B.M. Fuchs, *Single-cell identification in microbial communities by improved fluorescence* in situ *hybridization techniques*, Nature Rev. Microbiol. (2008), pp. 339–348.
[37] M.B. Karner, E.F. DeLong, and D.M. Karl, *Archaeal dominance in the mesopelagic zone of the Pacific Ocean*, Nature 409 (2001), pp. 507–510.
[38] M.J. Church, E.F. Delong, H.W. Ducklow, M.B. Karner, C.M. Preston, and D.M. Karl, *Abundance and distribution of planktonic Archaea and Bacteria in the waters west of the Antarctic Peninsula*, Limnol. Oceanogr. 48 (2003), pp. 1893–1902.
[39] G.J. Herndl, T. Reinthaler, E. Teira, H. van Aken, C. Veth, A. Pernthaler, and J. Pernthaler, *Contribution of Archaea to total prokaryotic production in the deep Atlantic Ocean*, Appl. Envir. Microbiol. 71 (2005), pp. 2303–2309.
[40] D.L. Kirchman, H. Elifantz, A.I. Dittel, R.R. Malmstrom, and M.T. Cottrell, *Standing stocks and activity of Archaea and Bacteria in the western Arctic Ocean*, Limnol. Oceanogr. 52 (2007), pp. 495–507.
[41] M. Karner and J. Fuhrman, *Determination of active marine bacterioplankton: A comparison of universal 16S rRNA probes, autoradiography, and nucleoid staining*, Appl. Envir. Microbiol. 63 (1997), pp. 1208–1213.
[42] N. Ramaiah, J. Chun, J. Ravel, W.L. Straube, R.T. Hill, and R.R. Colwell, *Detection of luciferase gene sequences in nonluminescent bacteria from the Chesapeake Bay*, FEMS Microbiol. Ecol. 33 (2000), pp. 27–34.
[43] A. Eberhard, *Inhibition and activation of bacterial luciferase synthesis*, J. Bacteriol. 109 (1972), pp. 1101–1105.
[44] E.G. Ruby and K.H. Nealson, *Pyruvate production and excretion by the luminous marine bacteria*, Appl. Envir. Microbiol. 34 (1977), pp. 164–169.
[45] J.W. Hastings and E.P. Greenberg, *Quorum sensing: The explanation of a curious phenomenon reveals a common characteristic of bacteria*, J. Bacteriol. 181 (1999), pp. 2667–2668.
[46] V.D.S. Nunes-Halldorson and N.L. Duran, *Bioluminescent bacteria: Lux genes as environmental biosensors*, Brazil. J. Microbiol. 34 (2003), pp. 91–96.
[47] E.G. Ruby and K.H. Nealson, *Seasonal changes in the species composition of luminous bacteria in nearshore seawater*, Limnol. Oceanogr. 23 (1978), pp. 530–533.
[48] P. Perego, L. Fanara, M. Zilli, and M. Del Borghi, *Applications of luminous bacteria in environmental monitoring*, Chem. Biochem. Eng. Q. 16 (2002), pp. 87–92.
[49] C.F. Wimpee, T.L. Nadeau, and K.H. Nealson, *Development of species-specific hybridization probes for marine luminous bacteria by using in vitro DNA amplification*, Appl. Envir. Microbiol. 57 (1991), pp. 1319–1324.
[50] J.C. Ast and P.V. Dunlap, *Phylogenetic resolution and habitat specificity of members of the* Photobacterium phosphoreum *species group*, Envir. Microbiol. 7 (2005), pp. 1641–1654.
[51] F. Abe, C. Kato, and K. Horikoshi, *Pressure-regulated metabolism in microorganisms*, Extremophiles Deep-Sea Envir. 7 (1999), pp. 447–453.
[52] A.A. Yayanos, *Microbiology to 10,500 meters in the deep-sea*, Ann. Rev. Microbiol. 49 (1995), pp. 777–805.
[53] D.H. Bartlett, M. Wright, A.A. Yayanos, and M. Silverman, *Isolation of a gene regulated by hydrostatic pressure in a deep-sea bacterium*, Nature 342 (1989), pp. 572–574.
[54] C. Kato, T. Sato, F. Abe, E. Ohmae, H. Tamegai, K. Nakasone, K.S. Siddiqui, and T. Thomas, *Protein adaptation to high-pressure environments*, in *Protein Adaptation in Extremophiles. Molecular Anatomy and Physiology of Proteins Series*, T. Thomas and K. S. Siddiqui (eds.), Nova Science Publisher, New York, 2008, pp. 168–197.
[55] M. Yamada, K. Nakasone, H. Tamegai, C. Kato, R. Usami, and K. Horikoshi, *Pressure regulation of soluble cytochromes c in a deep-sea piezophilic bacterium*, Shewanella violacea, J. Bacteriol. 182 (2000), pp. 2945–2952.
[56] D.H. Bartlett, C. Kato, and K. Horikoshi, *High pressure influences on gene and protein expression*, Res. Microbiol. 146 (1995), pp. 697–706.
[57] E.A. Meighen, *Molecular biology of bacterial bioluminescence*, Microbiol. Rev. 55 (1991), pp. 123–142.
[58] A. Bianchi, J. Garcin, and O. Tholosan, *A high-pressure serial sampler to measure microbial activity in the deep sea*, Deep-Sea Res. I 322 (1999), pp. 2129–2142.

[59] C. Tamburini, J. Garcin, and A. Bianchi, *Role of deep-sea bacteria in organic matter mineralization and adaptation to hydrostatic pressure conditions in the NW Mediterranean Sea*, Aquat. Microbial Ecol. 32 (2003), pp. 209–218.
[60] R.I. Amann, B.J. Binder, R.J. Olson, S.W. Chisholm, R. Devereux, and D.A. Stahl, *Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations*, Appl. Envir. Microbiol. 56 (1990), pp. 1919–1925.
[61] W. Manz, R. Amann, W. Ludwig, M. Wagner, and K.-H. Scheiler, *Phylogenetic oligodeoxynucleotide probes for the major subclasses of proteobacteria: Problems and solution*, Systemat. Appl. Microbiol. 15 (1992), pp. 593–600.
[62] D.A. Stahl and R. Amann, *Development and application of nucleic acid probes*, in *Nucleic acid techniques in bacterial systematics*, E. Stackebrandt and M. Goodfellow (eds.), John Wiley & Sons Ltd., Chichester, UK, 1991, pp. 205–248.