

Supplementary Material

The Mesopelagic Scattering Layer: A Hotspot for Heterotrophic Prokaryotes in the Red Sea Twilight Zone

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1 Contents of this file

The Supplementary Information includes six figures and two tables that support the discussion and conclusions of the manuscript.

Figures S1-S3 provide information on the water column structure at the time of sampling, while Figures S4-S5 and Tables S1-S2 provide information on the changes observed along the 8-days incubation experiments. All Figures and Tables are presented below with their corresponding legends.

Detailed information on the experimental set up, analysis and calculation of derived parameters is available in the Materials and Methods section of the manuscript.

2 Supplementary Figures

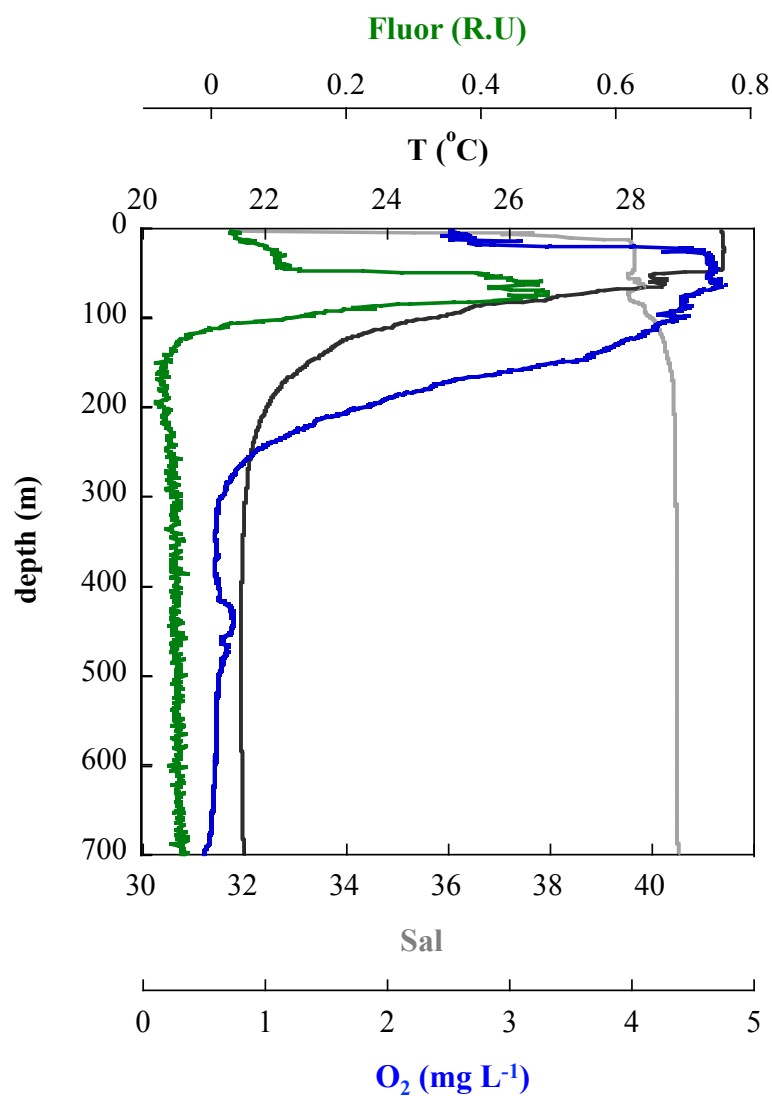


Figure S1. Vertical distribution of environmental variables. Vertical profiles displaying water column values and trends of environmental variables: Sal (grey), T (°C, black), dissolved O₂ (mg L⁻¹, blue) and fluorescence (R.U., green).

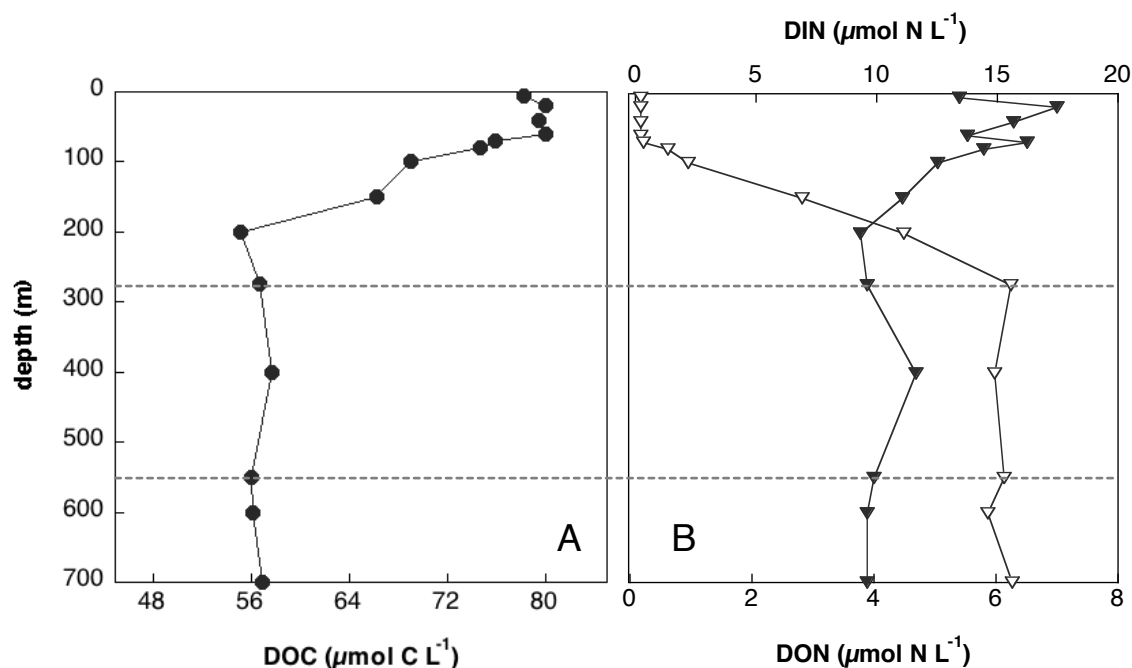


Figure S2. Vertical distribution of dissolved carbon and nitrogen. Vertical profiles displaying water column values and trends of (A) dissolved organic carbon concentration (DOC, $\mu\text{mol C L}^{-1}$, full circles), and (B) concentrations of dissolved organic nitrogen (DON, $\mu\text{mol N L}^{-1}$, full triangles) and dissolved inorganic nitrogen (DIN, $\mu\text{mol N L}^{-1}$, empty triangles). Dashed horizontal lines indicate the depths of I and D layers.

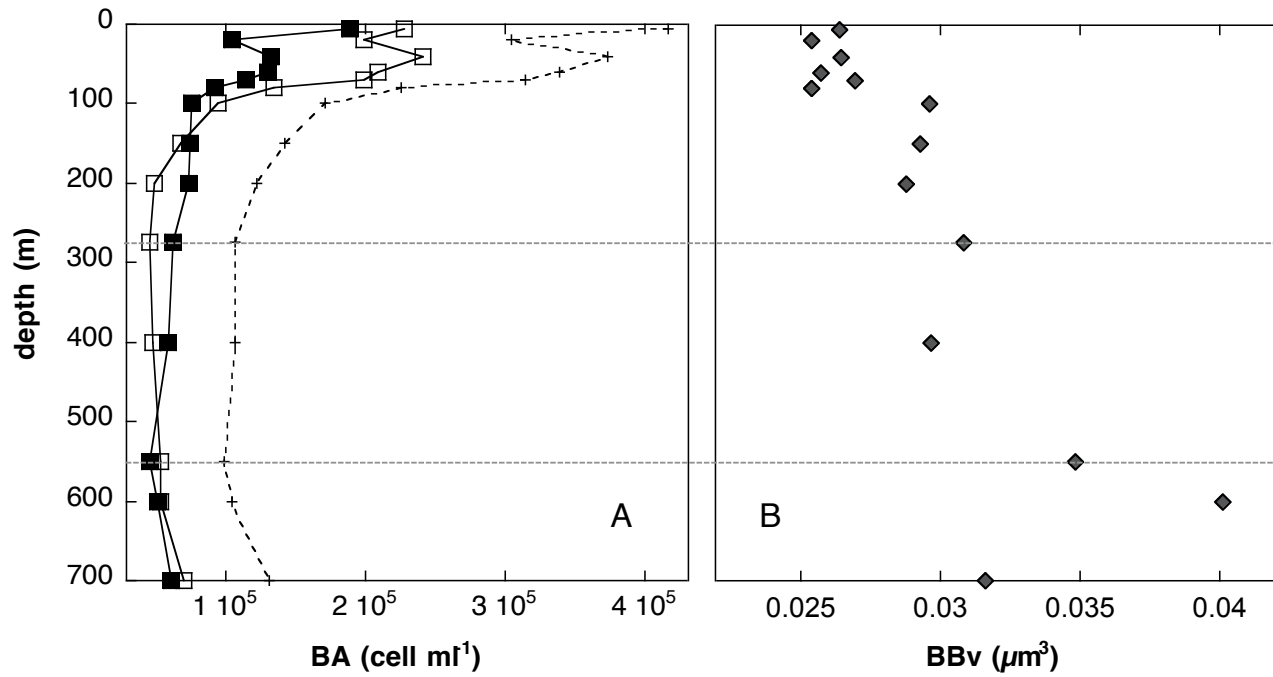


Figure S3. Vertical distribution of prokaryotes' abundance and cell size. Vertical profiles displaying water column values and trends of (A) bacterial abundance (BA, cells ml^{-1}) of high nucleic acid (HNA, full squares), low nucleic acid (LNA, empty squares) and total (dashed profile with crosses) cells, and (B) bacterial cell size or bio-volume (BBv, μm^3 , grey diamonds). Dashed horizontal lines indicate the depths of I and D layers.

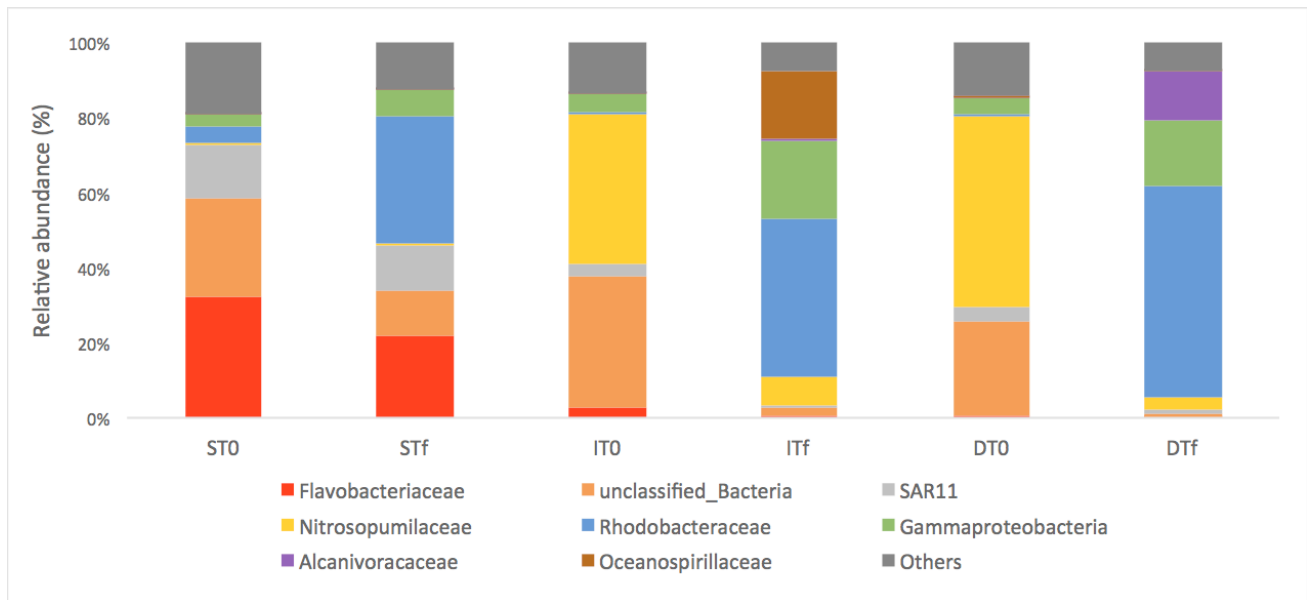


Figure S4. Prokaryotic community structure. Bacterial community structure at the family level in the initial (T0) and final (Tf) water samples of the different water layers studied: surface (S), intermediate (I) and DSL (D).

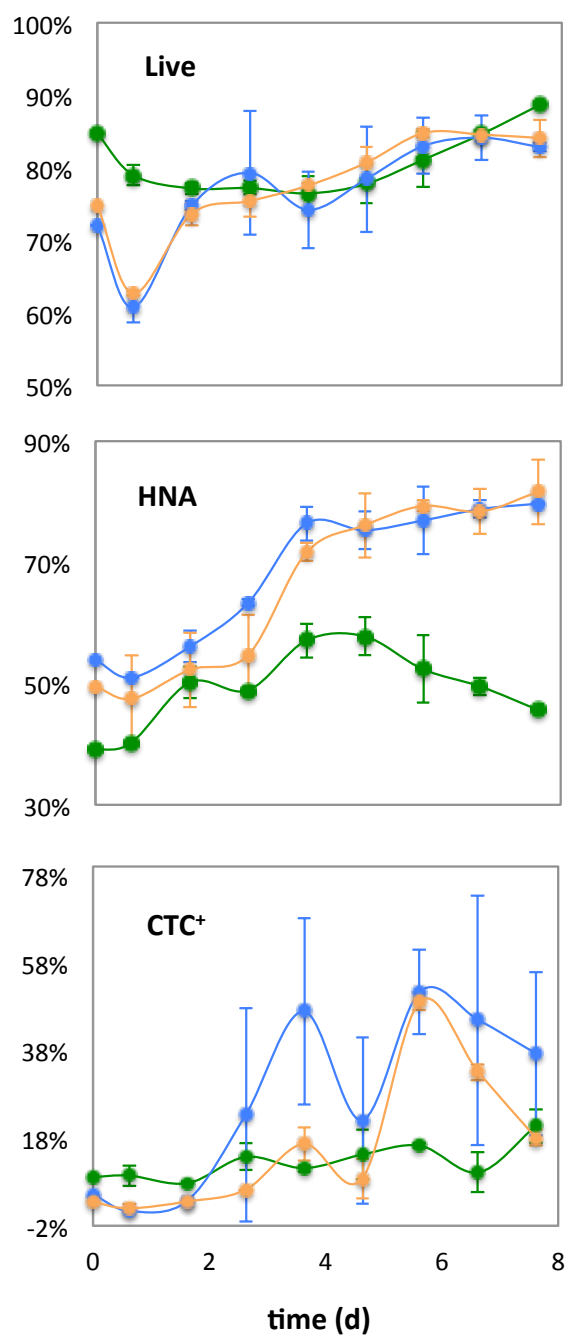


Figure S5. Bacteria physiological state along the incubations. Evolution of the percentage of live (% Live, A), high nucleic acid content (% HNA, made up by the taxa more responsive to nutrient inputs, B), and actively respiring (% CTC⁺, C) bacteria along the incubations, from the different water layers studied: surface (S, green), intermediate (I, blue) and deep scattering layer (D, orange).

3 Supplementary Tables

	$\mu_{\text{H+L}}$	μ_{Live}	$\mu_{\text{CTC+}}$
Surface	0.08 ± 0.01	0.18 ± 0.04	0.23 ± 0.04
Intermediate	0.26 ± 0.10	0.17 ± 0.05	1.35 ± 0.41
DSL	0.24 ± 0.05	0.24 ± 0.00	0.90 ± 0.20

Table S1. Bacteria specific growth rates. Specific growth rates (μ , d^{-1}) of the total bacteria cells (HNA plus LNA cells, $\mu_{\text{H+L}}$), the live cells (μ_{Live}), and the actively respiring bacteria or CTC+ cells ($\mu_{\text{CTC+}}$) and its associated SD for the incubations of the three examined layers.

	utilized DOC (%)
Surface	2.1 ± 0.1
Intermediate	6.2 ± 0.0
DSL	4.3 ± 1.0

Table S2. Consumed DOC. Percentage of initial DOC utilized (% DOC) and its associated SD for the incubations of the three examined layers.