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Supplement of

Ecological divergence of a mesocosm in an eastern boundary upwelling system assessed with multi-marker environmental DNA metabarcoding

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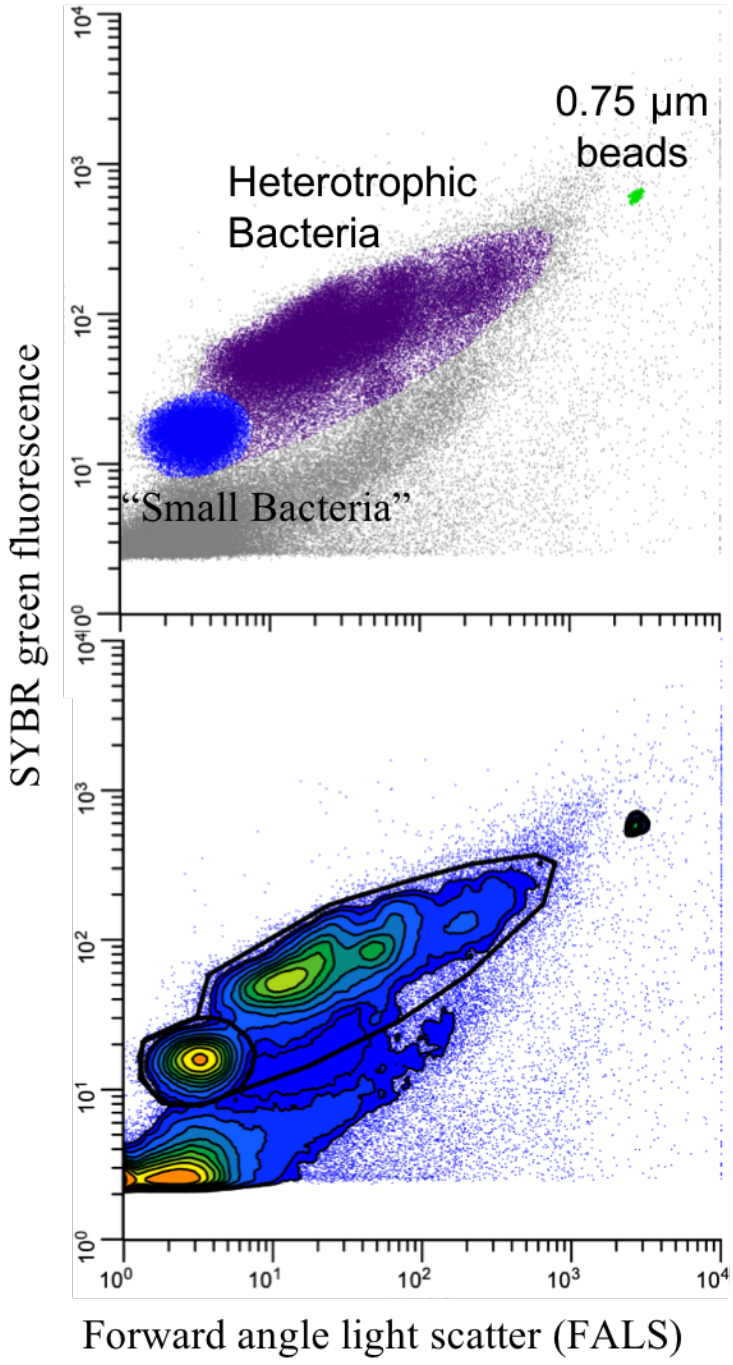

Figure S1: Representative flow cytogram from Mesocosm 1, day 36 indicating the population of "small bacteria" that became highly prevalent later in the experiment in both Mesocosm 1 and Pacific (Figure 2j). As shown, gating of this population was based on lower forward angle light scatter and SYBR green fluorescence than the larger population of heterotrophic bacteria.

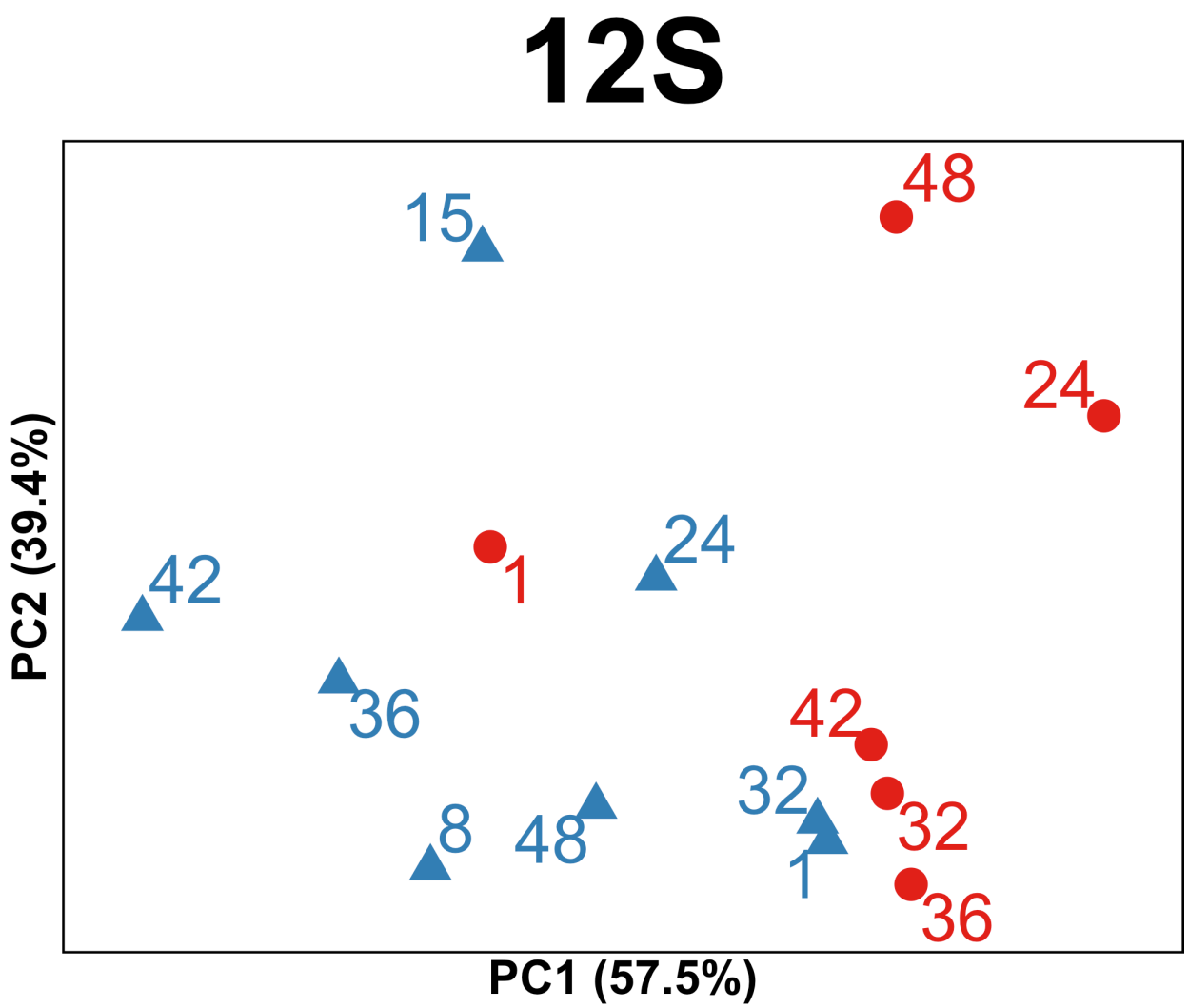

Figure S2. Beta diversity analyses using DEICODE robust Aitchison PCA for 12S rRNA ASVs, subset for only chordate sequences. M1 samples are shown as red circles and Pacific samples are shown as blue triangles. M1 days 8 and 15 were excluded from the analysis because of their low read counts.

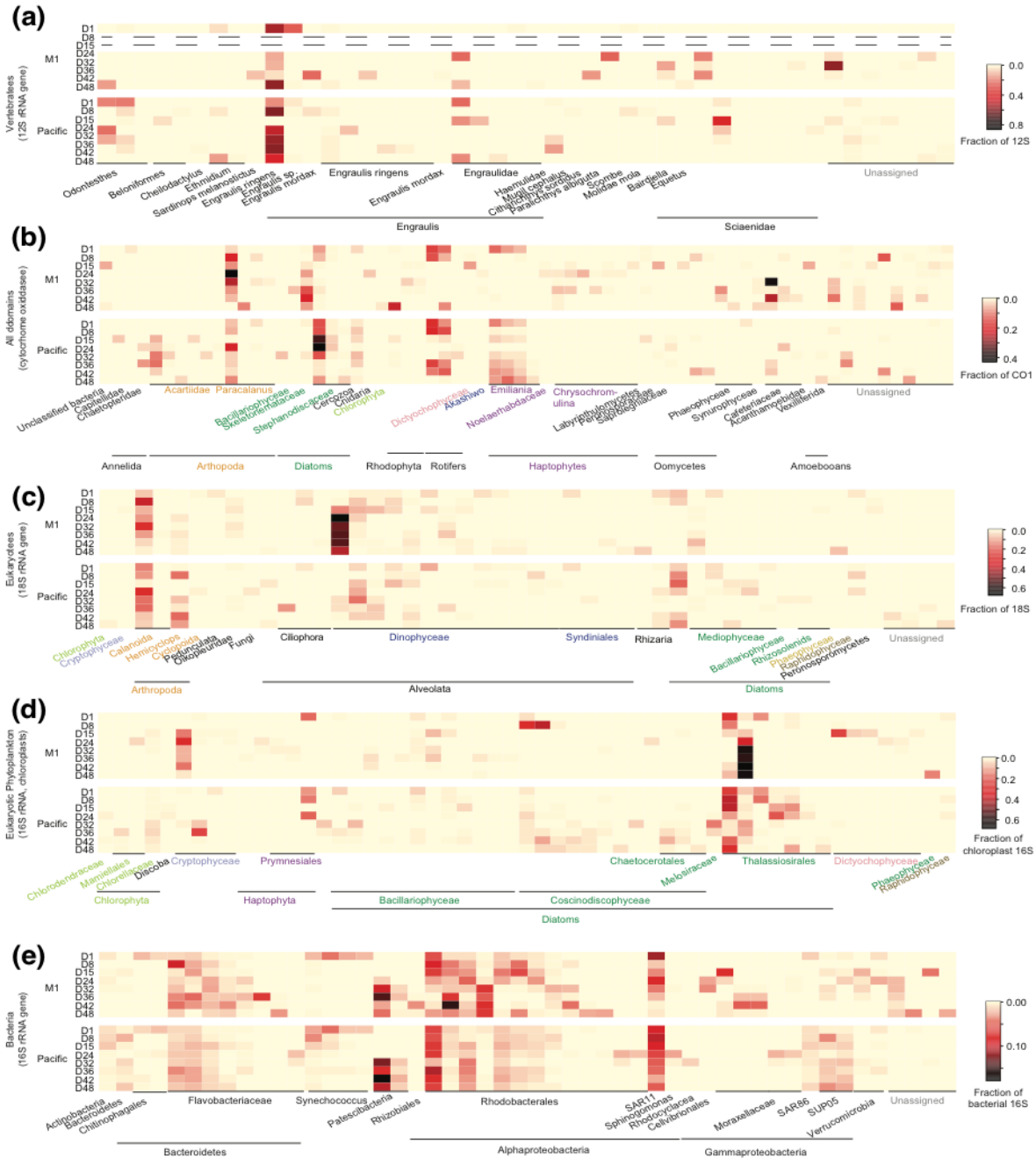

Figure S3. Relative abundance (percent of reads per sample) of ASVs that were among the top 10 most abundant ASVs in any individual sample and that were ever more than 1% of the reads in any sample for (a) 12S rRNA, (b) COI, (c) 18S rRNA, (d) 16S rRNA plastidial ASVs, and (e) 16S rRNA bacterial ASVs. Here, the QIIME 2 classify-consensus-blast plugin using the SILVA 132 database and the 7-level majority taxonomy was used for 18S rRNA sequence classification. The taxonomy assigned to 16S rRNA ASVs corresponds to that of the SILVA taxonomy.

Supplementary Results 1. Taxon Distributions in M1 and Pacific (Heatmap)

The COI, 18S rRNA and 16S rRNA communities showed differentiation between M1 and the Pacific and over time; for 12S rRNA, because of the deliberate exclusion of nekton from M1, this differentiation is also present but is due to differences in initial conditions rather than change due to experimental manipulation. In the 12S rRNA dataset, the exclusion of vertebrates in M1 is evidenced by lack of data for days 8 and 15, due to the decay of the vertebrate DNA initially trapped in the mesocosm. Throughout the experiment, the Pacific 12S rRNA community is dominated by Peruvian anchoveta, *Engraulis ringens*, which also comes to dominate M1 in the latter half of the experiment due to the impact of resting seabirds defecating into the mesocosm (Fig. S3a). For COI, the dominant zooplankton sequences were the calanoid copepod *Paracalanus* (abundant in both M1 and the Pacific), the family Acartiidae (abundant only in the Pacific), and rotifers of the family Synchaetidae (abundant in both M1 and the Pacific at the start of the experiment, abundant only the Pacific from day 15 on) (Fig. S3b). The phytoplankton community as detected by COI was dominated by the haptophyte *Emiliania huxleyi* (abundant in both M1 and the Pacific initially, only abundant in the Pacific in subsequent samples) and diatoms (more abundant in the Pacific, with the exception of *Skeletonema*, which was more abundant in M1) (Fig. S3b). Additionally, the heterotrophic protist family Caferiaceae dominated for much of the latter dates in M1. In the 18S rRNA dataset, the primary taxa differentiating M1 and the Pacific is the mixotrophic dinoflagellate *Akashiwo sanguinea*, which dominated 18S rRNA reads in M1 beginning on day 24. The phytoplankton community as detected via chloroplast sequences were most dominated by diatoms, in particular those of the groups Skeletonemataceae (especially M1), Thalassiosirales (Pacific), and cryptophytes (mostly M1) (Fig. S3d). The bacterial communities were relatively more "even" and dominated by fewer taxa, but among those most relatively abundant in both M1 and Pacific were Alphaproteobacteria (SAR11 and Rhodobacterales), Bacteroidetes, and Saccharimonadales (Fig. S3e).

Six cyanobacterial ASVs were represented with 500+ reads in at least one sample. Five ASVs had at most one difference over the 275 nucleotides of the V1-V2 16S rRNA amplicon to a cultured representative of *Synechococcus* clade I, II, IV (Rocap et al., 2002) and WPC2 (Choi and Noh, 2009), and *Prochlorococcus* LLIV (Rocap et al., 2002). Surprisingly, the last ASV is identical over its entire length to the V1-V2 region of Cyanobium sp. Suigetsu-CR5, isolated from a Japanese saline lake (Ohki et al., 2012). The same ASV has two mismatches to a *Synechococcus* clade III representative, which is a marine strain and thus the more likely relative of this ASV, but we do not have the additional phylogenetic information (i.e. full length 16S sequence) to resolve this. ASVs most similar to Cyanobium sp. Suigetsu-CR5 have been identified in the Northeastern Pacific before (Sudek et al., 2015). The relative abundances of all six ASVs decreased dramatically over the course of the experiment, both in Pacific and mesocosm. This is in agreement with our flow cytometry data (Fig. 2f) which for *Synechococcus* shows a precipitous decline in the mesocosm from ~340,000 cells ml^{-1} on day 2 to less than 10,000 cells ml^{-1} from day 8 onward. The Pacific control sample shows the same trend though less drastic, starting from only 123,000 cells ml^{-1} on day 2 and dropping to 13,000 cells ml^{-1} or less from day 22 onward (Fig. 2f).

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