Supplement

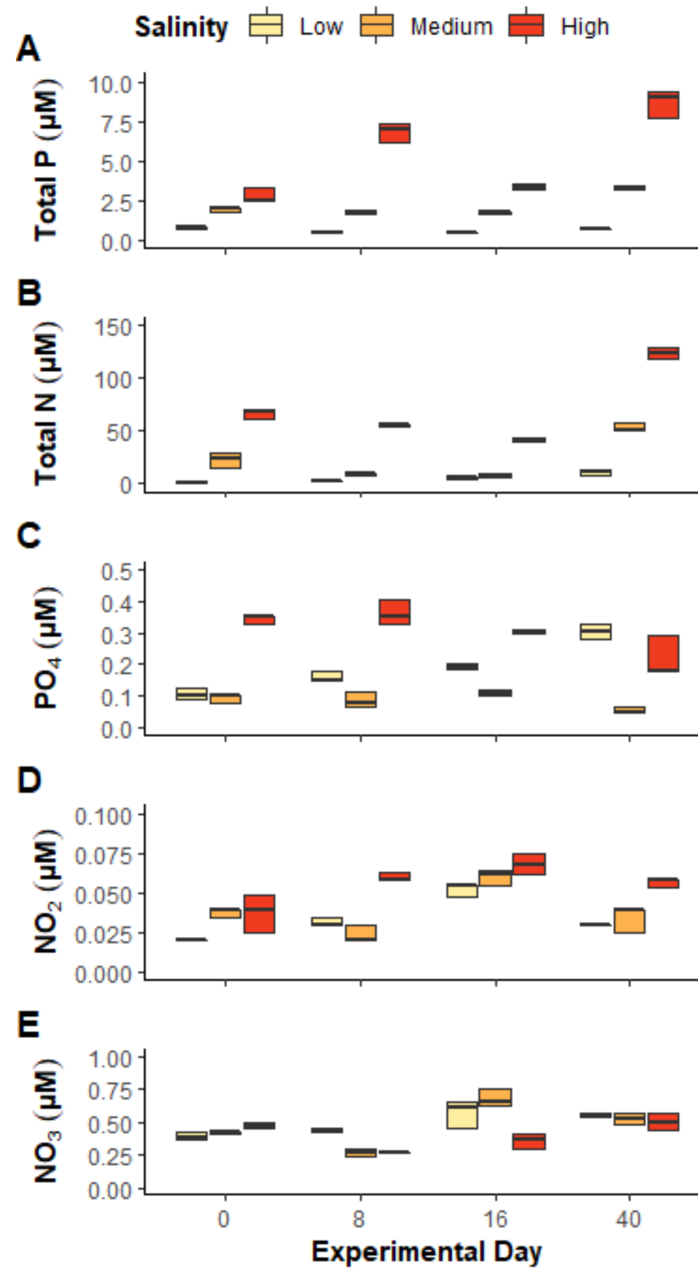

Figure S1. Nutrient dynamics recorded in three replicate control samples within each pond and around the mesocosm enclosure (1m distance) at selected sampling days along the course of the experiment from day 0 to 40.

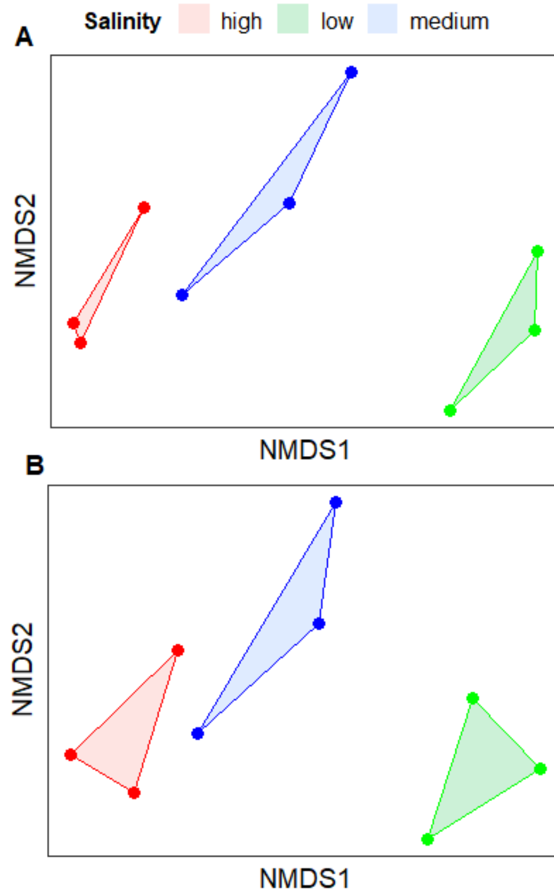

Figure S2. Compositional differences between the replicate mesocosms of three salinity levels (40, 46, and 61 psu) at day 0 before the connectivity between different salinity assemblages was initiated. Assemblage differences were calculated with the Bray-Curtis similarity index using species presence absence data (panel A) and non-transformed, species abundance data (panel B).

Text S1. Osmotic stress test

Salinity could be a strong stressor, instantly affecting phytoplankton cells through osmotic pressure, causing cell rupture, when working at a high salinity gradient. To test for potential osmotic stress, a laboratory experiment was performed. Samples from the different salinity levels meant to be used in the mesocosm experiment were transported in the lab. Their exact salinity was measured, and artificial seawater of the corresponding salinities was made. Each phytoplankton assemblage was inoculated in different salinity levels, and its growth was monitored for two days (Fig S3A). The use of artificial seawater ensured salinity to be the only factor affecting phytoplankton cells. Since the goal was to test for immediate reaction to salinity, and not for growth potential, two days were enough to monitor for that. Osmotic stress did not occur, enabling us to rule it out as a confounding factor in any potential observations. The phytoplankton assemblages were grouped based on the salinity they came from and not based on the salinity that was imposed on them (FigS3B).

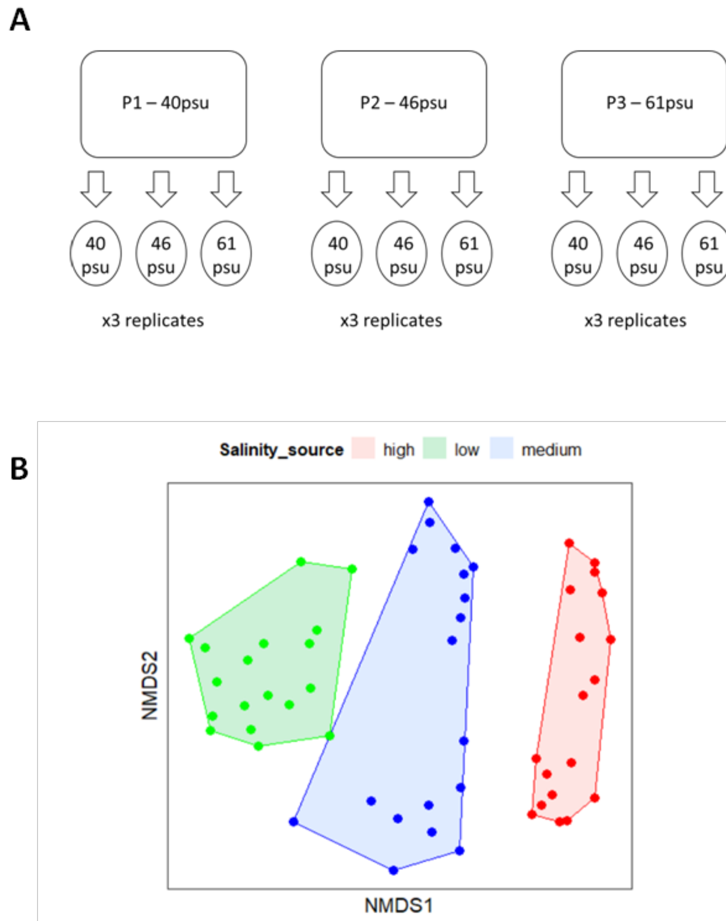

Figure S3. Experimental setup of the lab experiment on osmotic stress (Panel A) and Multidimensional scaling analysis of the phytoplankton assemblages for the two days of the osmotic stress experiment, where phytoplankton assemblages are grouped based on the salinity level they originated (Panel B)

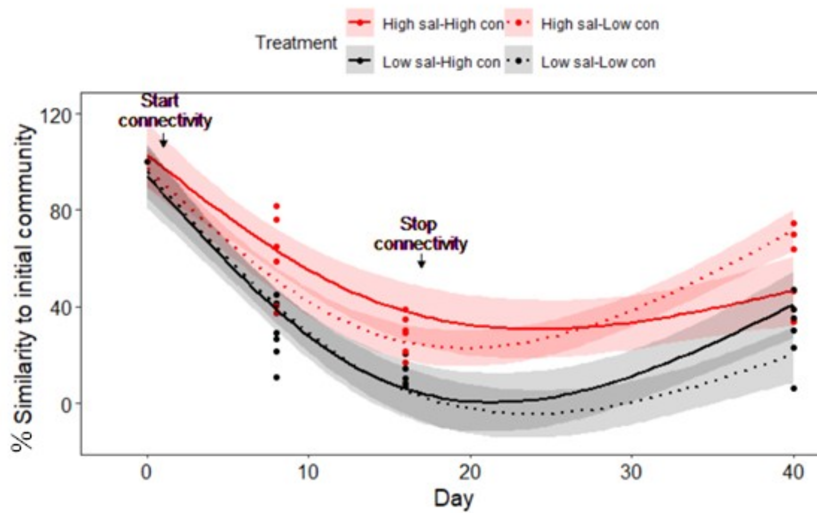

Figure S4. Resilience of the assemblages in low and high salinity and low and high connectivity. Y-axis is the similarity from day 0 presented as percentage, x-axis is the day of the experiment. Resistance was measured as assemblage similarity of day 8 from day 0 and recovery was measured as similarity of day 40 from day 0 noting that connectivity had ceased in day 16 of the experiment. Similarity was calculated with the Bray-Curtis similarity index using **non-transformed, species abundance data**.

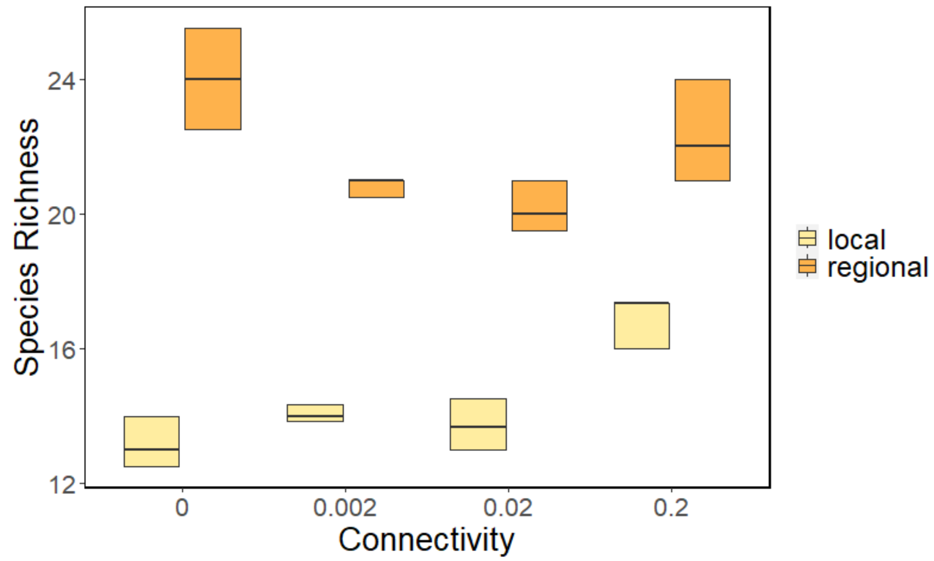

Figure S5. Species richness at local and regional scales at different connectivity levels, at day 16.

Text S2. DNA extraction and sequencing, 18S rRNA gene amplicon analysis

DNA extraction from filters was performed with the MoBio Power Soil kit (MoBio Inc. Carlsbad, CA, USA) following its standard protocol with minor modifications for filters processing. Sequencing of the V2-V3 region of the 18S rRNA gene was performed upon amplification using the primer pair 18S-82F (5′-GAAACTGCGAATGGCTC-3′) (López-García et al. 2003) and Euk-516r (5′-ACCAGACTTGCCCTCC-3′) for Eukaryotes (Amann et al. 1990). Construction of libraries was performed by 'Genes Diffusion' company (Lille, France) and amplicons were finally sequenced with Illumina MiSeq PE 2x300 (CNRS-UMR8199, Lille). Processing of the resulting sequences, i.e. sequence assembly and quality control, was performed with the MOTHUR software (v 1.35) (Schloss et al. 2009). Only sequences with ≥480 bp, no ambiguous bases and homopolymers shorter than 8 bp were considered for further analysis. These sequences were aligned using the SILVA SSU database (release 119) (Pruesse et al. 2007). Chimeras were removed using the Uchime Software (Edgar et al. 2011). All sequences were binned into Operational Taxonomic Units (OTUs) and were clustered (average neighbour algorithm) at 97% sequence similarity. Single singletons, that appeared only once in the whole dataset, were removed using MOTHUR (v 1.35). Coverage values were calculated with MOTHUR (v 1.35) as well as diversity indices. Sequences from this study (mesocosms) and sequences used from the coastal area study (Spatharis et al. 2019) have been submitted in NCBI Short Read Archive under accession code PRJNA952408 and PRJNA515026 respectively. Taxonomic classification was assigned using BLAST (Altschul et al. 1990) on the Protist Ribosomal Reference (PR4.14.0) curated Database (built on Genbank; June 2021), containing 197,602 sequences (Guillou et al. 2013).

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