

Supplementary material

related to

Population connectivity of fan-shaped sponge holobionts in the deep Cantabrian Sea

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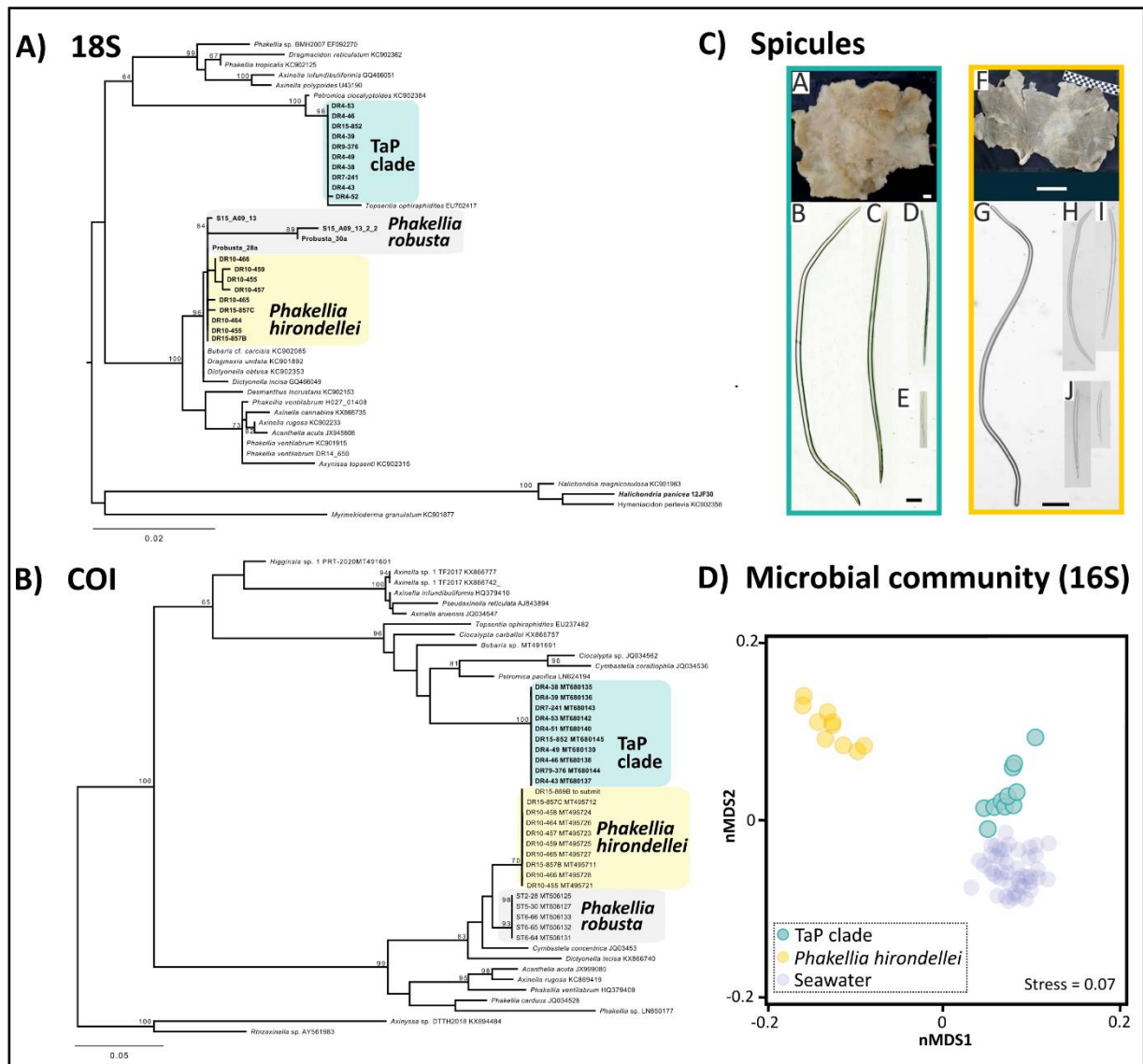
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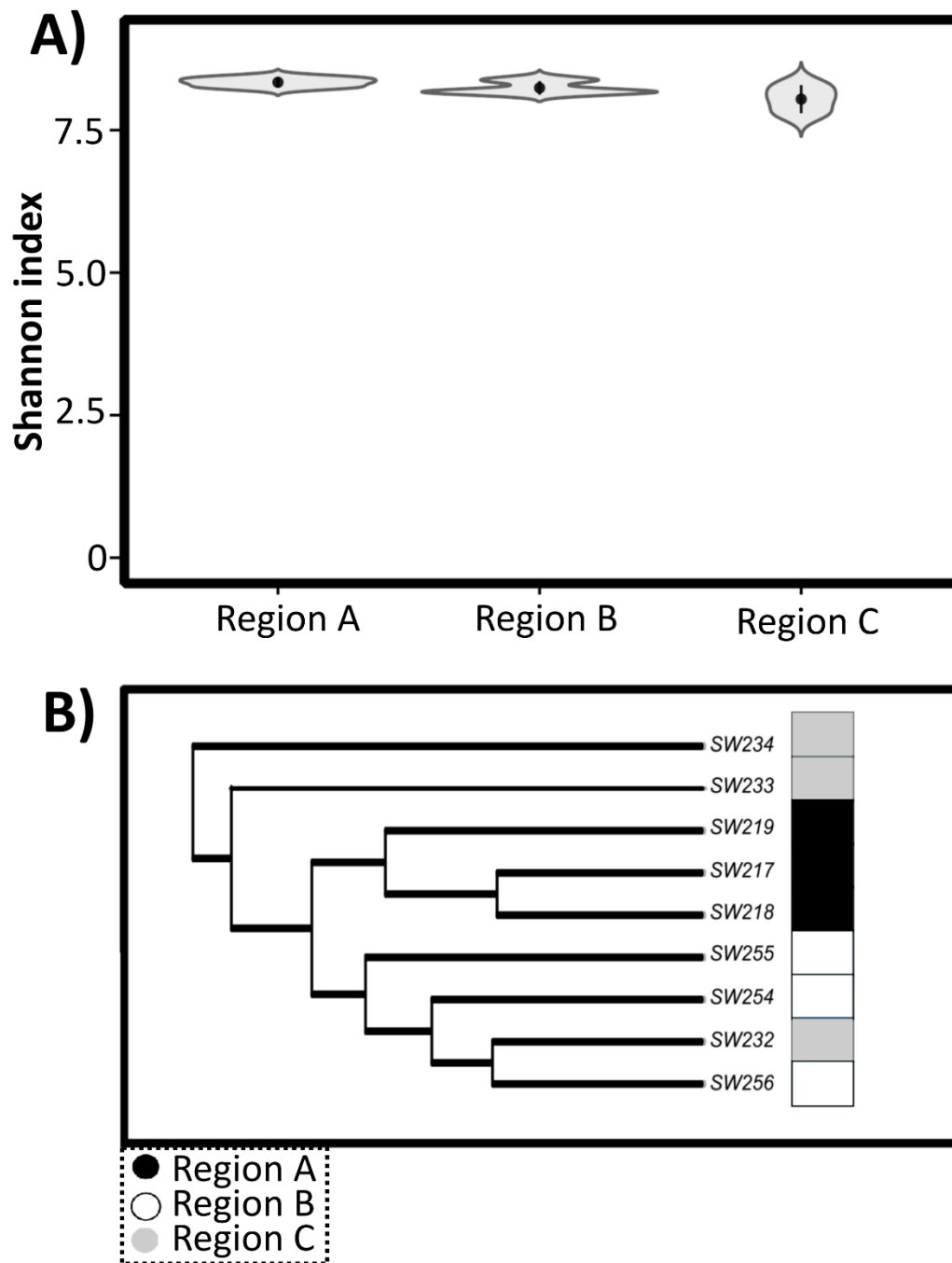
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Supplementary Table S1 Overview of spicule measurements for all 21 TaP clade and *P.hirondellei* individuals.

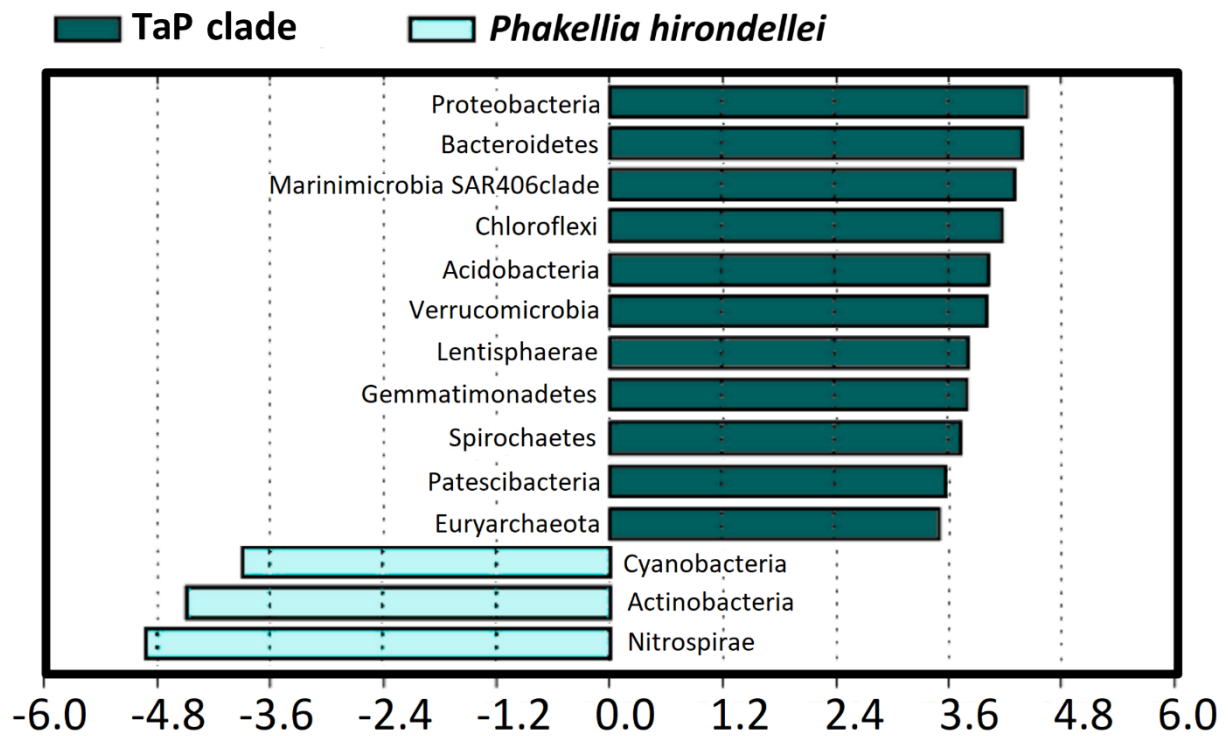
Sponge ID	Spicule types	Strongyles with gently pointed end (measurements μm)	Strongyles (measurements μm)	Oxeas I (measurements μm)	Oxeas II (measurements μm)	Styles I (measurements μm)	Styles II (measurements μm)
TaP clade (Region A, Avilés Canyon): WB-00594	strongyles and styles		1637.06 (2127.82) 2616.5 x 26.28 (30.81) 34.31 N=10			1180 N=1	395.33 (545.04) 827.79 x 12.19 (14.83) 20.59 N=11
TaP clade (Region A, Avilés Canyon): WB-00595	strongyles and styles		2232.06 x 21.93 N=1				350.86 (526.18) 752.74 x 9.02 (14.40) 17.39 N=11
TaP clade (Region A, Avilés Canyon): WB-00598	strongyles and styles	1211.6 (1854.62) 2570.43 x 16.65 (24.85) 30.47 N=10	1750 N=1			524.35 (626.73) 814.35 x 13.09 (15.68) 17.74 N=8	390.93 (426.20) 499.37 x 8.41 (11.12) 13.61 N=10
TaP clade (Region A, Avilés Canyon): WB-01102	strongyles and styles		1619.06 (1749.54) 1986.89 x 19.03 (24.02) 31.02 N=10				285.26 (515.10) 867.55 x 10.33 (15.70) 21.34 N=25
TaP clade (Region A, Avilés Canyon): WB-01105	strongyles and styles	689.92 (1446.95) 2135.7 x 14.27 (20.75) 26.92 N=8	1283.78 (1703.43) 2221.56 x 14.27 (20.92) 26.92 N=10				298.2 (561.10) 1099.11 x 9.76 (13.99) 19.83 N=18
TaP clade (Region A, Avilés Canyon): WB-01106	strongyles and styles	1204.38 (1653.59) 1943.06 x 24.84 (29.91) 33.81 N=10				658.11 (846.45) 1089.9 x 17.32 (19.60) 23.79 N=9	
TaP clade (Region A, Avilés Canyon): WB-01107	strongyles and styles	973.76 (1268.68) 1632.3 x 14.27 (18.18) 23.79 N=10	2600 N=1				428.49 (631.09) 983.28 x 10.45 (13.50) 17.39 N=10
TaP clade (Region A, Avilés Canyon): WB-01108	strongyles and styles	821.69 (1236.05) 1618.68 x 8.62 (13.33) 19.03 N=10	1750 N=1			641.34 (980.24) 1188.08 x 9.76 (16.23) 20.33 N=10	
TaP clade (Region B, Le Danois West-Bank): WB-00650	strongyles and styles	1144.43 (1542.98) 2217.23 x 26.28 (30.78) 33.81 N=10	1558.91 (1988.95) 2458.51 x 16.65 (25.62) 31.92 N=10				325 (543.44) 861.17 x 10.11 (13.61) 23.89 N=25
TaP clade (Region B, Le Danois West-Bank): WB-00673	strongyles and styles	802.29 (1049.76) 1271.44 x 13.87 (20.20) 24.49 N=10				705.68 (908.58) 1534.88 x 14.27 (18.54) 23.98 N=10	
TaP clade (Region B, Le Danois West-Bank): WB-00762	strongyles and styles	1800-2330 N=3				1160-1185 N=2	
<i>P. hirondellei</i> (Region B, Le Danois West-Bank): WB-00768	not measured						
<i>P. hirondellei</i> (Region B, Le Danois West-Bank): WB-00769	not measured						
<i>P. hirondellei</i> (Region B, Le Danois West-Bank): WB-00782	strongyles, oxeas, styles		606.68 (972.28) 1268.13 x 13.09 (16.51) 21.93 N=10	522.02 (762.85) 1166.19 x 13.12 (16.30) 19.18 N=10	132.46 (178.08) 275.96 x 3.15 (5.03) 8.32 N=10	554.39 (1015.20) 1568.1 x 11.9 (14.98) 17.32 N=20	
<i>P. hirondellei</i> (Region C, Le Danois East-Bank): WB-00699	strongyles, oxeas, styles		507.69 N=1	486.19 (600.65) 765.45 x 10.33 (13.15) 15.07 N=15	179.43 (2249.92) 341.86 x 5.03 (9.68) 12.19 N=16	208.82 (413.42) 544.72 x 11.22 (12.78) 15.47 N=13	
<i>P. hirondellei</i> (Region C, Le Danois East-Bank): WB-00701	strongyles, oxeas, styles		1103.87 (1219.25) 1364.71 x 12.13 (15.69) 17.32 N=10	647.56 (731.46) 847.3 x 12.15 (14.55) 17.98 N=7	259.04 x 9.53 N=1	302.08 (468.89) 574.13 x 11.26 (14.19) 17.46 N=10	
<i>P. hirondellei</i> (Region C, Le Danois East-Bank): WB-00702	strongyles, oxeas, styles		452.58 (534.41) 745.24 x 8.46 (10.10) 12.3 N=5	336.37 (441.65) 578.68 x 6.61 (8.96) 13.09 N=10	167.49 (232.95) 301.44 x 6.27 (8.01) 9.62 N=10	378.62 (448.45) 516 x 7.93 (10.16) 12.15 N=10	
<i>P. hirondellei</i> (Region C, Le Danois East-Bank): WB-00703	strongyles, oxeas, styles		1191.01 (1391.28) 1580 N=5	668.57 (856.74) 1285.71 N= 7	257.77 N=1	366.66 (453.56) 625.38 N=7	
<i>P. hirondellei</i> (Region C, Le Danois East-Bank): WB-01112	strongyles, oxeas, styles		622.22 (914.08) 1342.86 N=2	436.67 (643.40) 1211.11 x 6.27 (9.07) 11.37 N=9	210.33 (233.24) 265.56 x 7.54 (8.99) 10.11 N=3	256.63 (433.43) 633.56 x 7.48 (11.42) 14.15 N=15	
<i>P. hirondellei</i> (Region C, Le Danois East-Bank): WB-01113	oxeas and styles			361.76 (528.09) 644.11 x 9.35 (10.28) 11.37 N=4	212.07 (231.51) 252.73 x 6.54 (7.42) 8.41 N=2	459.52 (604.46) 798.69 N=3	
<i>P. hirondellei</i> (Region C, Le Danois East-Bank): WB-01114	oxeas and styles			746.97 x 12.15 N=1		472 N=1	



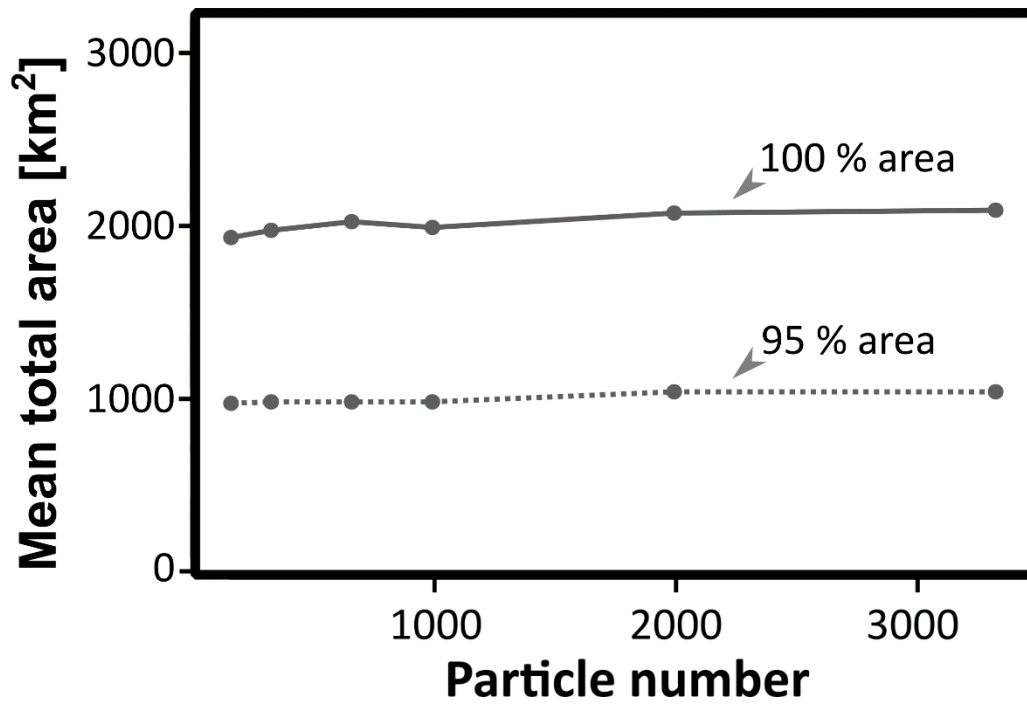
Supplementary Figure S1 Sponge taxonomy. **A-B)** Barcoding results of sponge individuals. TaP clade individuals are marked by turquoise color. *P. hirondellei* individuals are marked by yellow color. **A)** RAxML maximum likelihood phylogenetic tree based on *18S* sequences. **B)** RAxML maximum likelihood phylogenetic tree based on *COI* sequences. **C)** Sponge morphology: representative body plan and spicules of a TaP and *P. hirondellei* individual. **a-e)** TaP clade. a: habitus. b: Strongyle. c: Oxea. d: Style. e: Oxeas II. Scale bar b-e: 100 μ m. **f-j)** *P. hirondellei*. f: habitus. Scale bar: 10 cm. g: Strongyle. h: Oxea. i: Style. j: Oxeas II. Scale bar b-e: 100 μ m. **D)** Microbial profiles of the 21 TaP clade (turquoise points) and *P. hirondellei* (yellow points) individuals, presented in a non-metric multidimensional scaling plot based on weighted UniFrac distances.



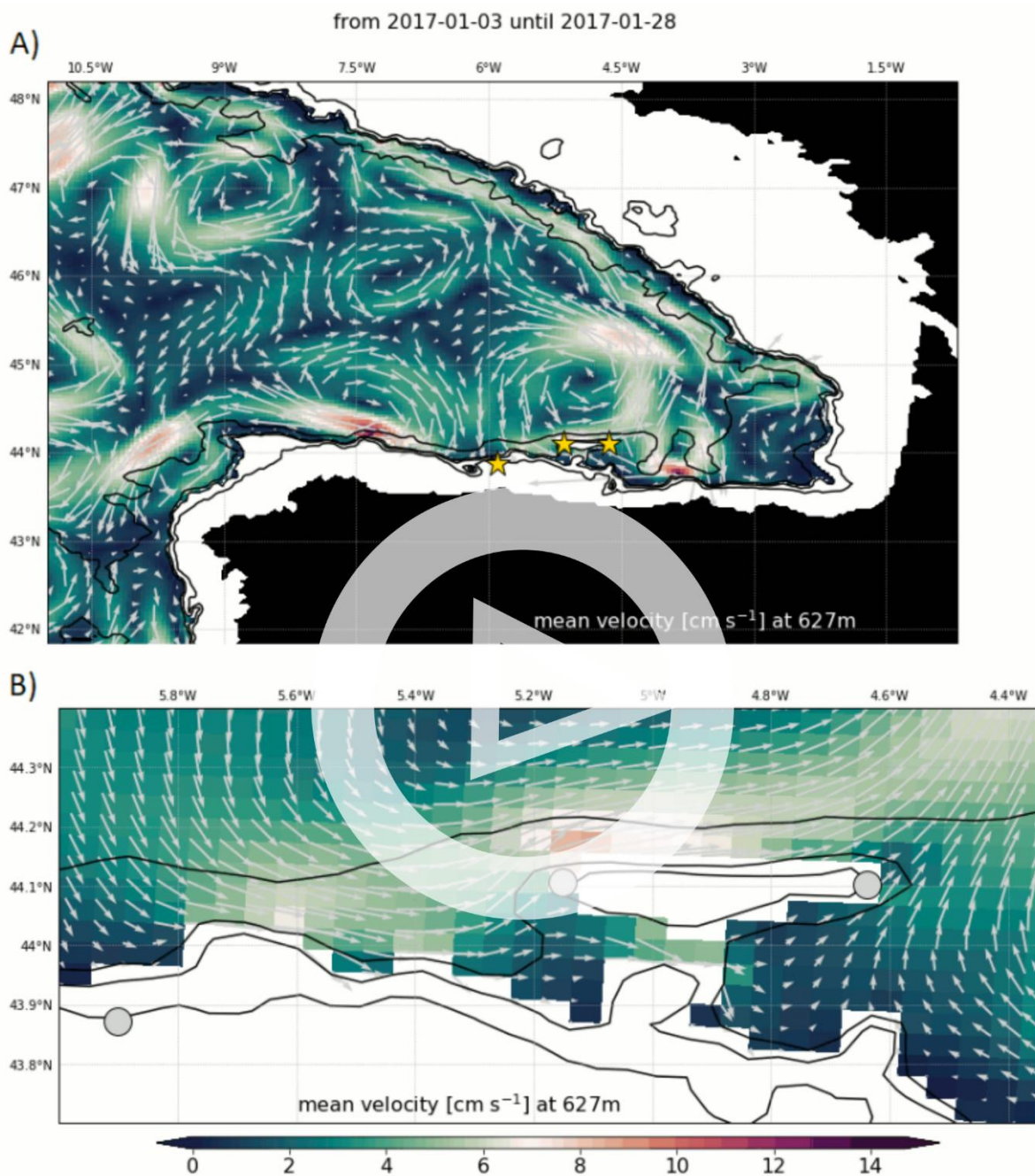
Supplementary Figure S2 Seawater microbial reference communities. **A)** Alpha diversity metrics (Shannon index) are presented for all three regions. Dots show means and whiskers represent standard deviations. **B)** Clustering dendrogram of seawater samples, based on weighted UniFrac distances. Upgma-clustering was performed and grey-shaded boxes next to the nodes of the dendrogram indicate respective sampling stations.



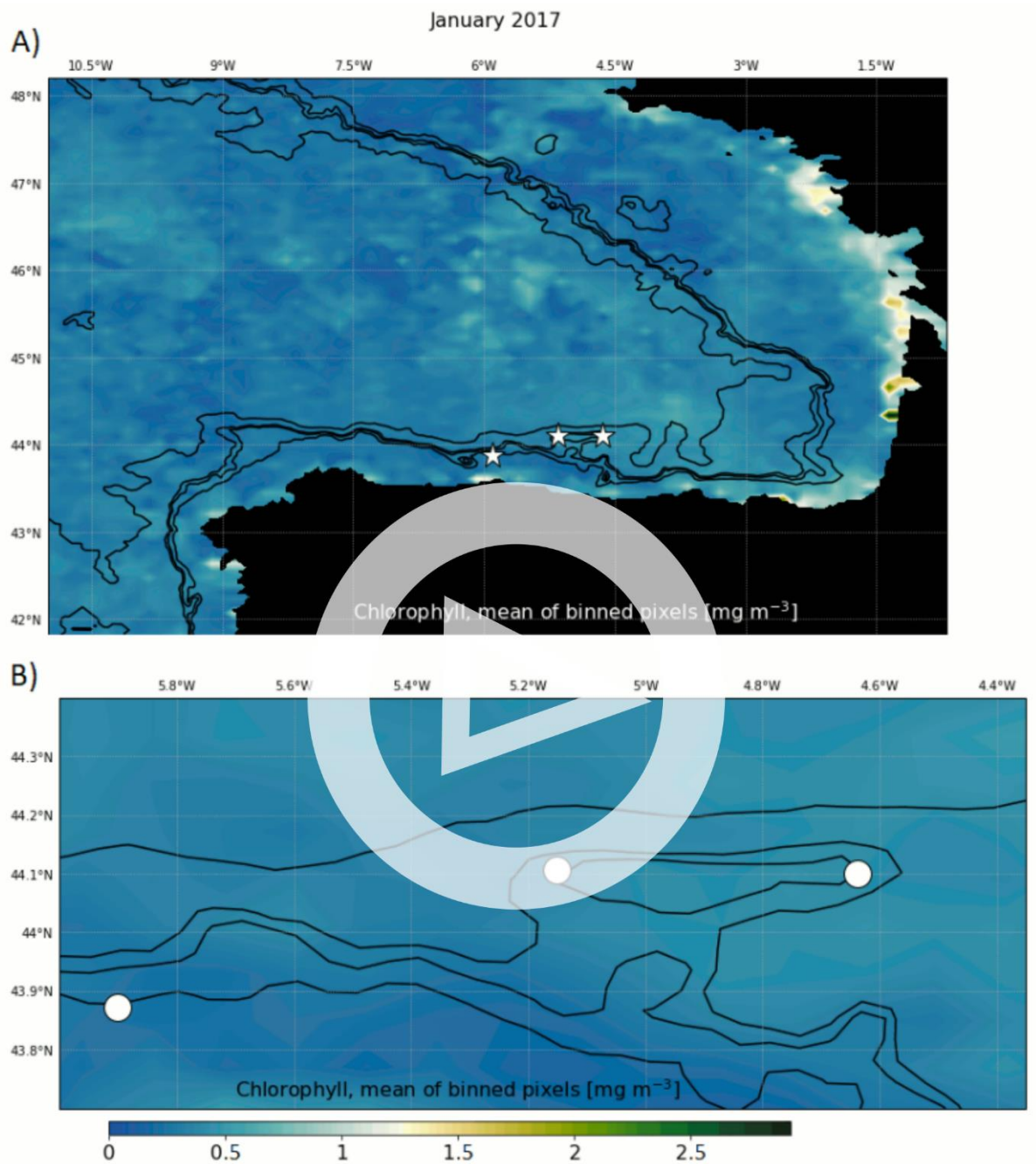
Supplementary Figure S3 Statistically significant enriched phyla visualised with Linear discriminant analysis Effect Size (LEfSe) plots. Analysis was performed on the microbial phylum level and the rank in the plot is given according to effect size. The two sponge clades (TaP clade and *P. hironellei*) are contrasted.



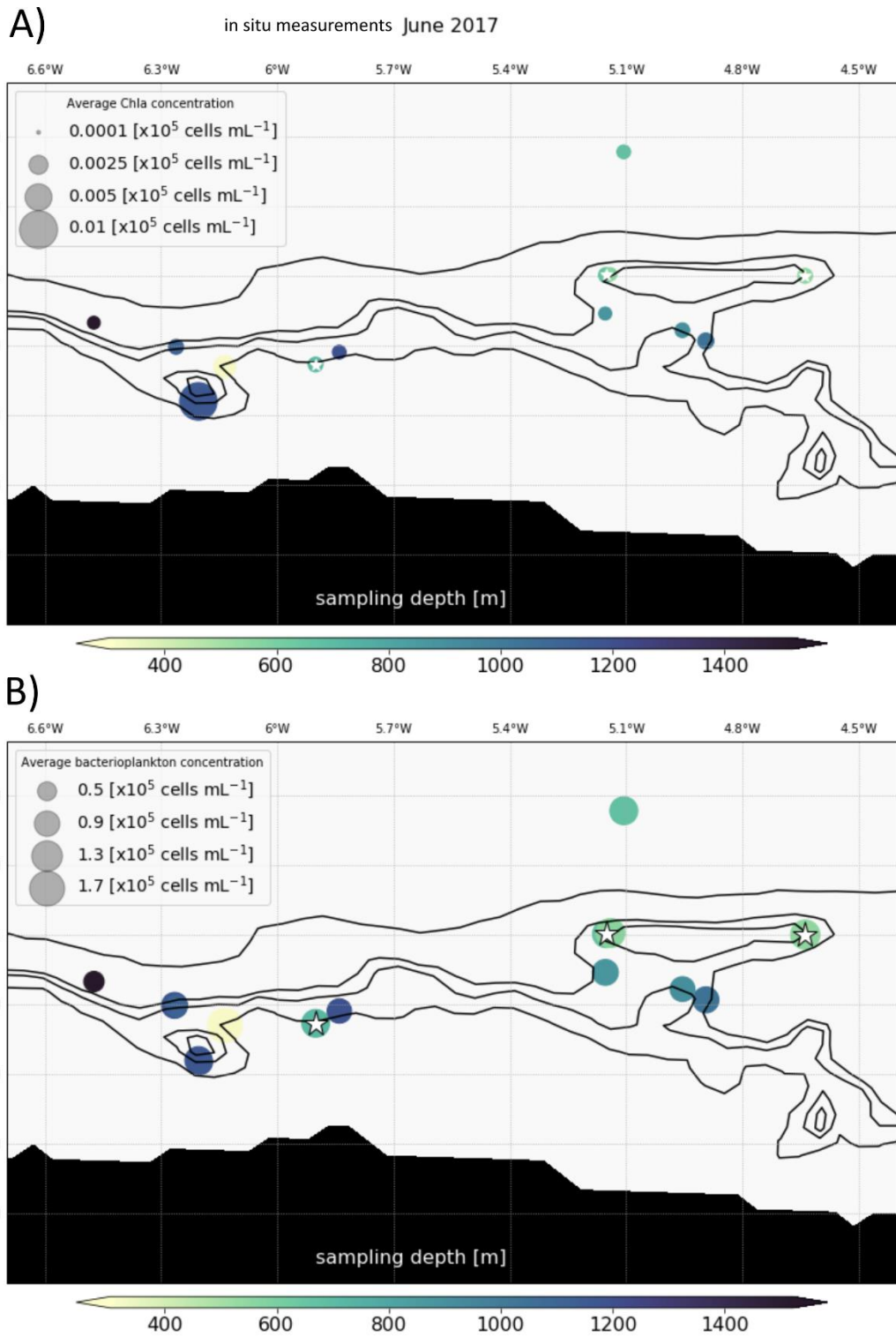
Supplementary Figure S4 Particle saturation curves, computed to determine optimal number of particles for model simulations. ‘Number of particles’ on the x-axis refers to particles per grid box and day. The particles were advected for 43 days (30 day release time, 14 days pelagic duration time, minus 1 day). ‘Mean total area’ on the y-axis refers to the average area (across all three stations) covered by larval drift. The solid line represents the 100 % area, while the dotted line refers to the area which is given by the 95 % probability of the particles binned onto a geographic grid (determined according to Seville *et al.*, 2018).



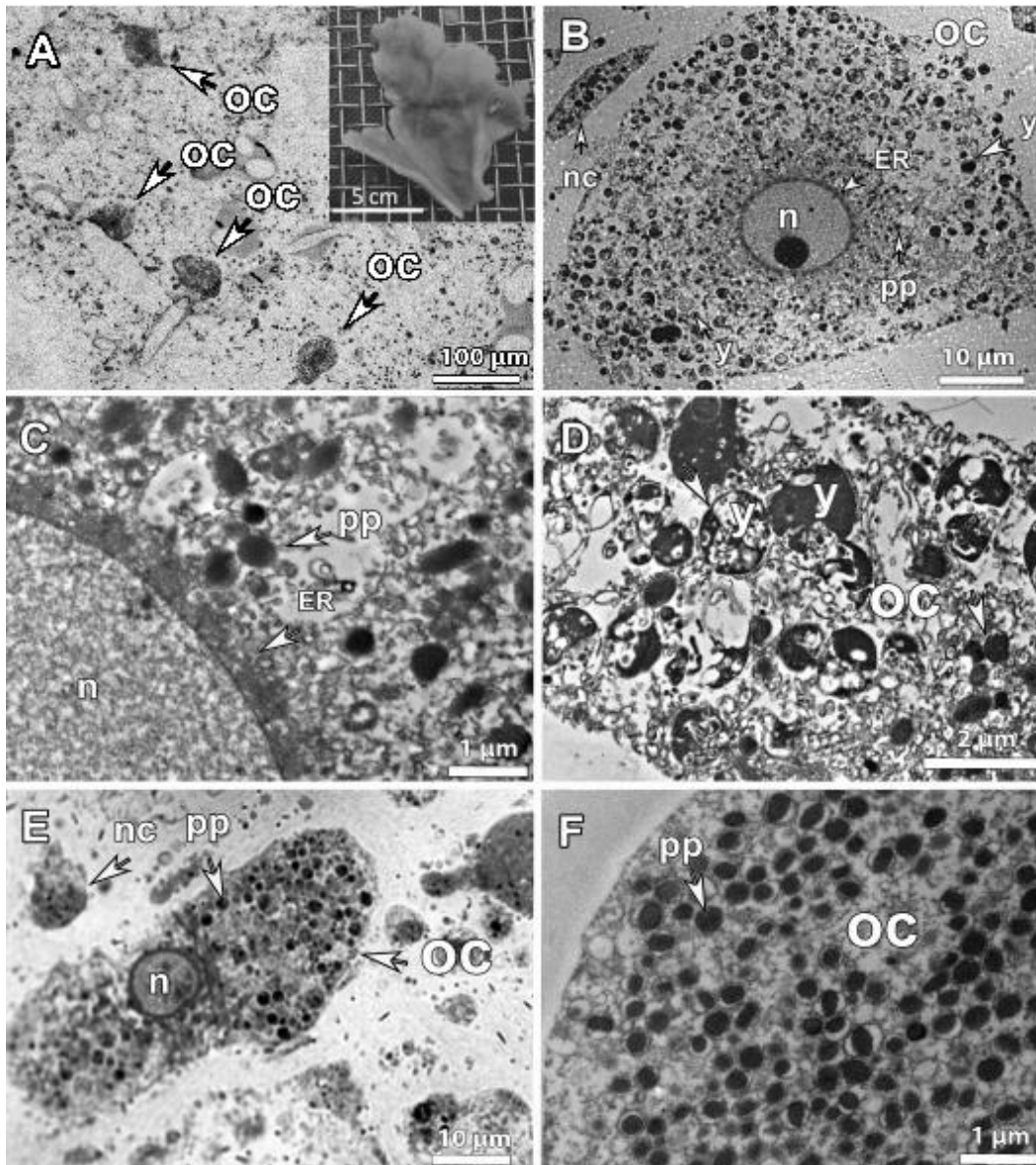
Supplementary Figure S5 Intra-annual variability of current velocities and current directions. Animation of monthly averaged current velocities for 2017 at the 627 m depth layer extracted from VIKING20X. Bathymetry contours are indicated by black lines. A) Overview map of the larger study area. Yellow stars indicate sampling region (A: ‘Canyon’, B: ‘West-Bank’, C: ‘East-Bank’), the coastline is indicated by the black filled area. B) Zooming into the study region. Grey dots indicate the sampling regions (A: ‘Canyon’, B: ‘West-Bank’, C: ‘East-Bank’).



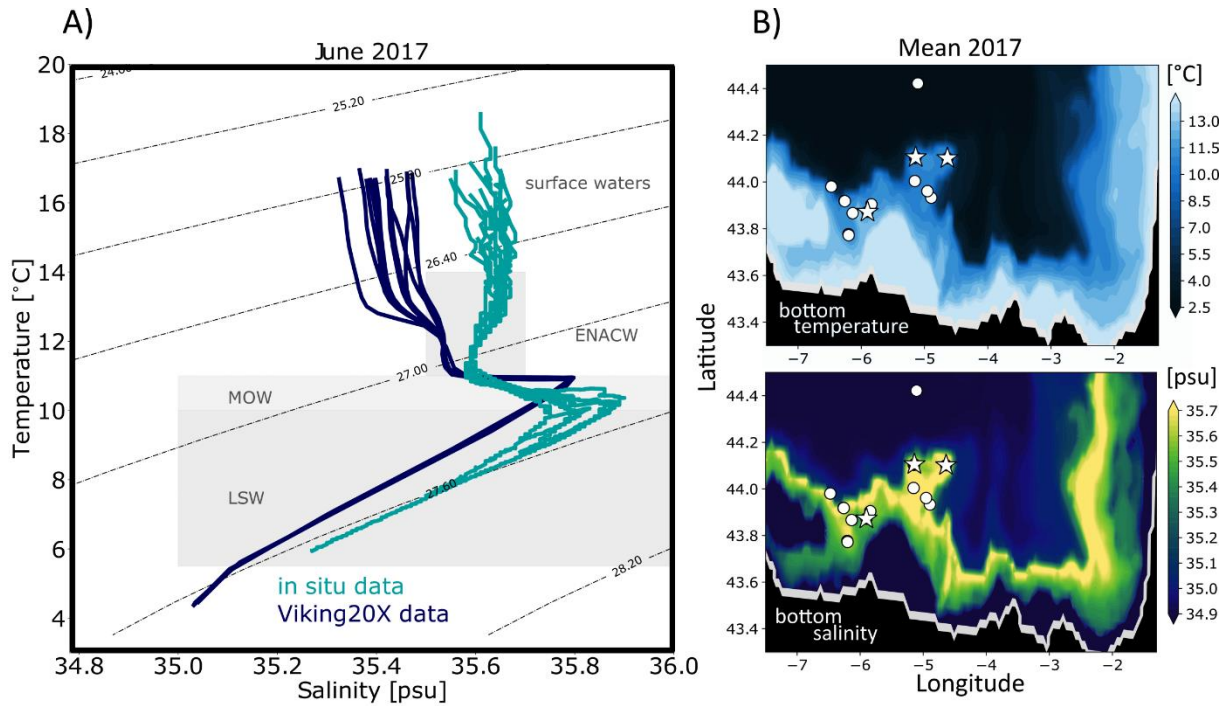
Supplementary Figure S6 Intra-annual variability of primary productivity. Animation showing seasonality of chlorophyll a concentrations for 2017 at the surface, extracted from satellite remote sensing data (provided by ESA). Bottom bathymetry contours are indicated by black lines. A) Overview map of the larger study area. White stars indicate sampling regions (A: 'Canyon', B: 'West-Bank', C: 'East-Bank'), the coastline is indicated by black filled area. B) Zoom into the study region. White dots indicate sampling regions (A: 'Canyon', B: 'West-Bank', C: 'East-Bank').



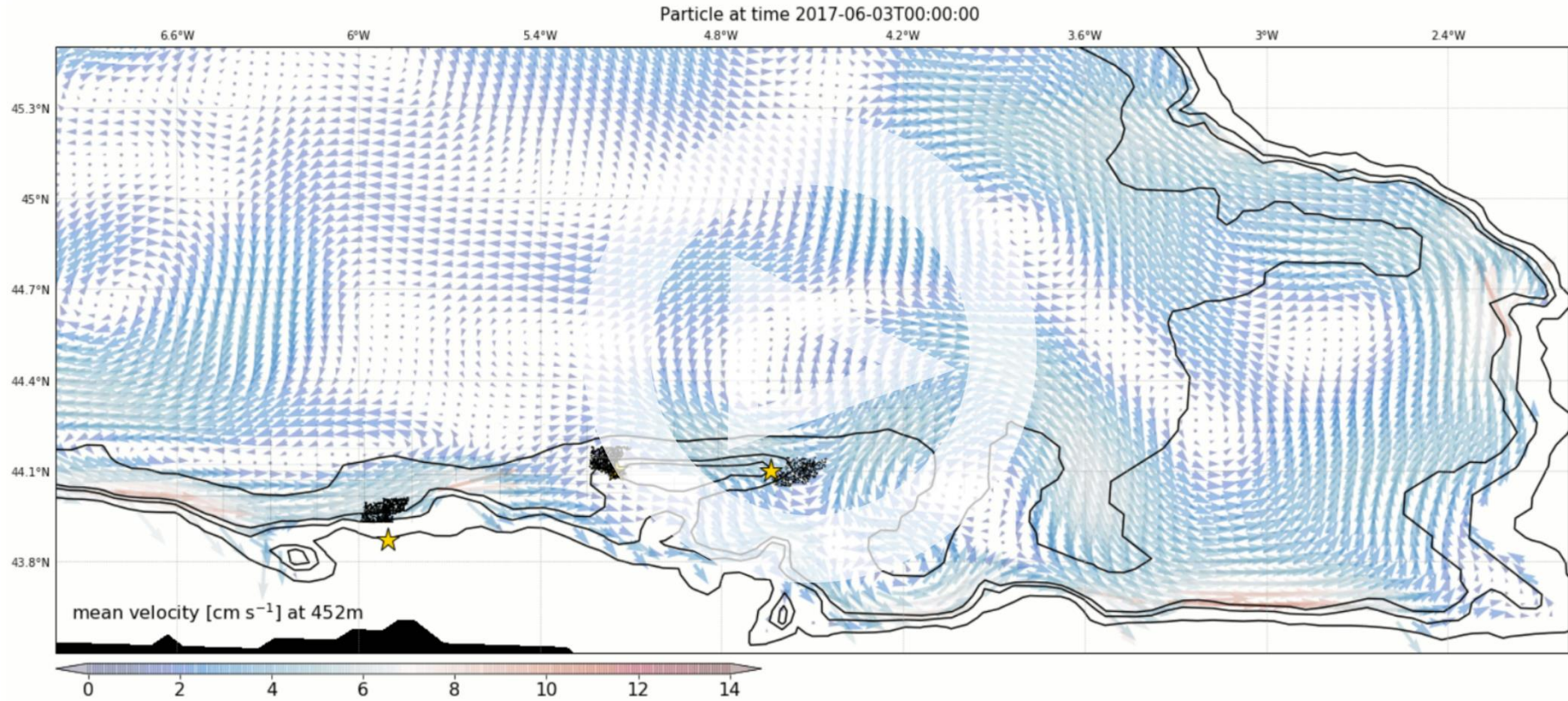
Supplementary Figure S7 Flow cytometry data from in situ measurements in June 2017. Average values derived from three biological replicates per CTD site are shown. The focus regions (A: ‘Canyon’, B: ‘West-Bank’, C: ‘East-Bank’) are indicated by white stars. Colors depict sampling depths and sizes of bubbles parameter concentrations. A) Presents chlorophyll a concentrations. B) Shows bacterioplankton concentrations.



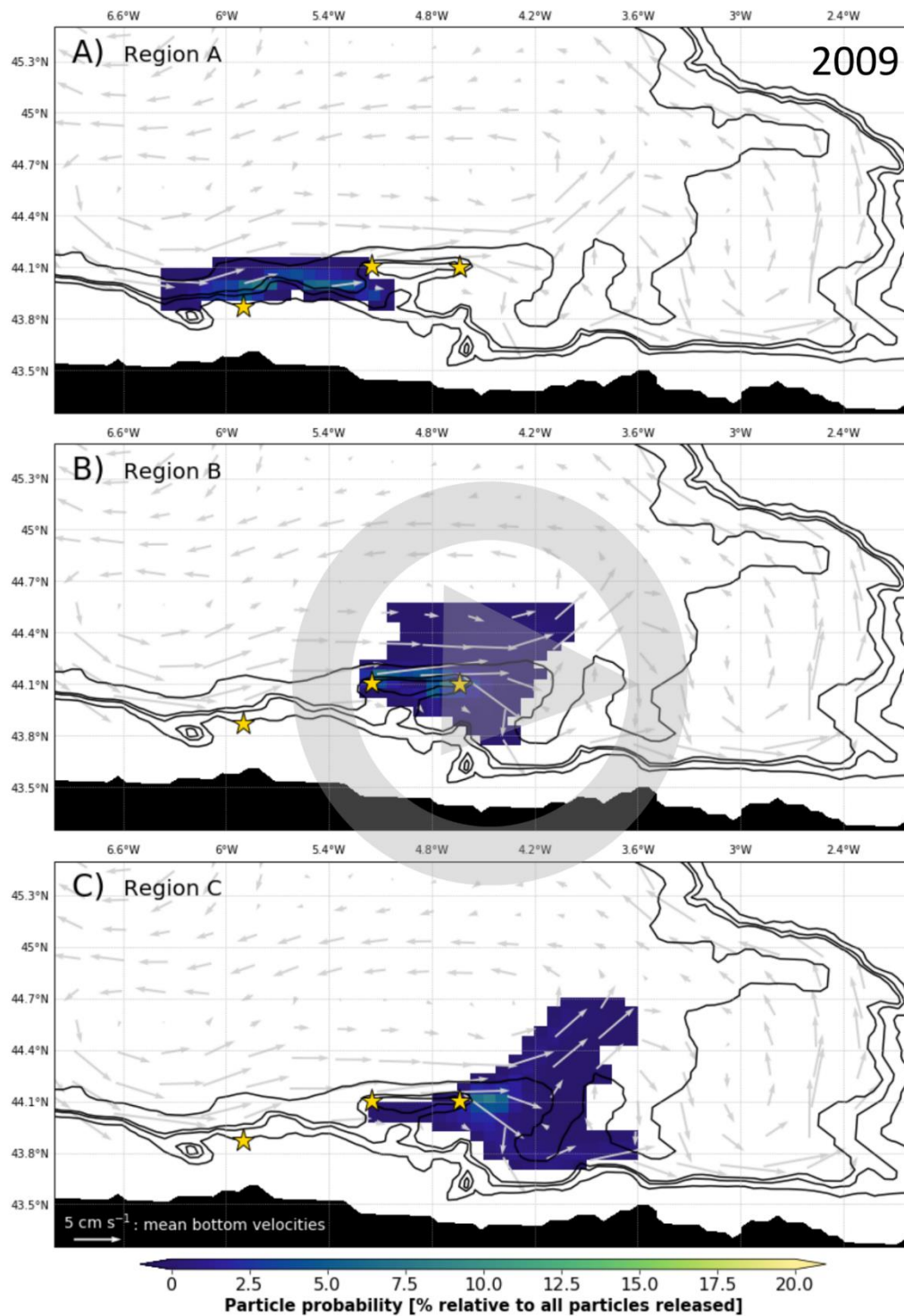
Supplementary Figure S8 Sponge histology for validation of reproductive status of TaP clade and *P. hirondellei* individuals collected in June 2017. A) Overview of TaP clade tissue, showing oocytes (oc) close to the water channel. Small inset micrograph shows habitus of a TaP clade individual. B) Close-up of TaP clade oocyte (oc) and nurse cell (nc). The nucleus (n), endoplasmic reticulum (ER), protein platelets (pp) and yolk (y) are indicated. C) Zooming-in further into the TaP clade oocyte. Close-up of the nucleus (n), endoplasmic reticulum (ER) and protein platelets (pp). D) Close-up of a TaP clade oocyte (oc). Big droplets of heterogenous yolk (y) are marked. E) Close-up of a *P. hirondellei* oocyte (oc) and nurse cell (nc). The nucleus (n) and protein platelets (pp) are indicated. F) Zooming-in further into the *P. hirondellei* oocyte. Close-up of protein platelets (pp).



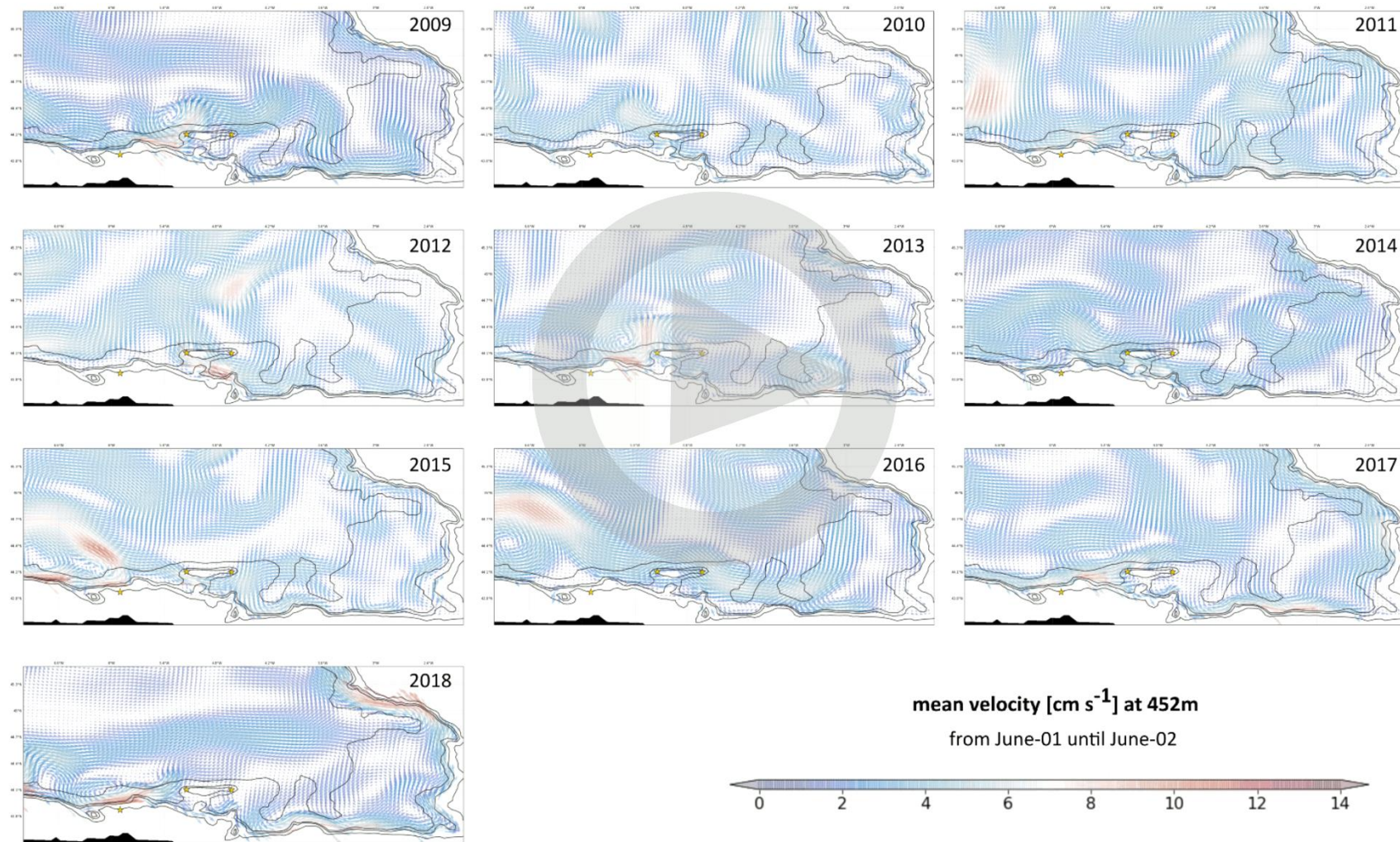
Supplementary Figure S9 Validation of VIKING20X temperature and salinity data with in situ measurements conducted in June 2017. A) T-S-diagram of vertical sections (from 20 m water depth down to the seafloor) from 15 CTD casts performed during the SponGES0617 campaign (turquoise color) and the VIKING20X data extracted for the same timeframe and locations (dark blue color). Watermasses are indicated after Lavin *et al.* (2006) by grey boxes and the following abbreviations: ENACW (Eastern North Atlantic Central Water), MOW (Mediterranean Outflow Water), LSW (Labrador Sea Water). B) Maps of temperature and salinity averages for the year 2017, extracted from VIKING20X for bottom depths (i.e. no single depth layer, but varying depths). The locations of the 15 CTD casts are indicated by white dots, with white stars highlighting the three focus regions (A: ‘Canyon’, B: ‘West-Bank’, C: ‘East-Bank’). The coastline is represented by the black filled area.



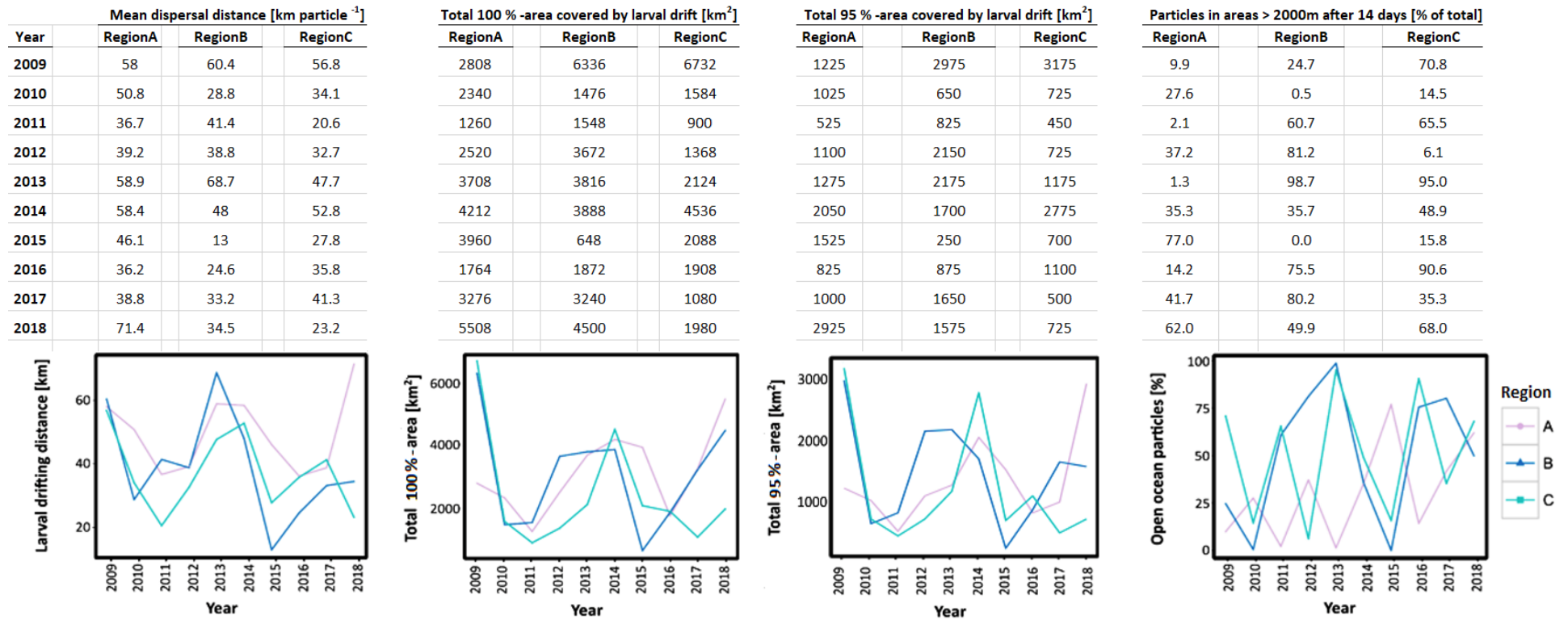
Supplementary Figure S10 Animation of virtual particles, advected with Parcels. Particle trajectories as well as daily current velocities fields are indicated for June 2017 (until mid of July 2017). Particle positions were recorded every 6 hours. Larval duration time was set to 14 days, with settled larvae vanishing from the particle set. The three sampling regions (A: 'Canyon', B: 'West-Bank', C: 'East-Bank') are indicated by yellow stars. Bathymetry contours are shown by black lines and a small edge of the coastline is indicated by black area.



Supplementary Figure S11 Animation spanning ten years (2009-2018) and showing particle probabilities in space (in % relative to all particles released) after a 14 days drifting time and a daily release of larvae over a period of 30 days in June every year for the three regions (A: 'Canyon', B: 'West-Bank', C: 'East-Bank'). Bathymetry contours are shown by black lines and the Spanish coastline is indicated by the black area. Mean monthly current velocities for each year are indicated by grey arrows.



Supplementary Figure S12 Animation of daily current velocities fields in June until mid of July for the ten analysed years (2009-2018).



Supplementary Figure S13 Inter-annual variability of oceanographic connectivity between 2009 to 2018. (i) Mean dispersal distance, (ii) total 100 %-areas covered by larval drift (i.e. total number of grid boxes covered by larval drift multiplied by size of one gridbox), as well as (iii) total 95 %-areas covered by larval drift (i.e. the area which is given by the 95 % probability of the particles binned onto a geographic grid) (iv) percentage of particles drifting in areas with a seafloor depth below 2000 m on drifting day 14, are indicted per sampling region and simulated year.