# Unicellular eukaryotic community response to temperature and salinity variation in mesocosm experiments

**Natassa Stefanidou[1], Savvas Genitsaris[1,2], Juan Lopez-Bautista[3], Ulrich Sommer[4*] and Maria Moustaka-Gouni[1*]**

[1]Department of Botany, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

[2]School of Economics, Business Administration & Legal Studies, International Hellenic University, 57001 Thermi, Greece

[3]Department of Biological Sciences, The University of Alabama, P.O. Box. 870345, Tuscaloosa, AL 35484-0345, USA

[4]Geomar Helmholtz Centre for Ocean Research Kiel, 24105, Kiel, Germany

*Corresponding Authors, Email addresses: usommer@geomar.de, mmustaka@bio.auth.gr

**Keywords: coastal marine plankton, climate change, Illumina sequencing, 18S rRNA gene, cDNA, RNA/DNA ratio**

## Supplementary Tables

Supplementary Table 1: Annotated major trophic role and DNA-based and RNA-based OTUs number. Values for the Mediterranean experiment are shaded.

| Super groups | Groups | Class | Number of OTUs | | | | Trophic role | References |
|---|---|---|---|---|---|---|---|---|
| | | | DNA | RNA | DNA | RNA | | |
| Alveolates | Apicomplexa | | 2 | 2 | 3 | 3 | Parasites | Skovgaard, 2014 |
| | Ciliophora | Colpodea | 0 | 1 | 0 | 1 | Nano-grazers | Lynn, 2008 |
| | | Heterotrichea | 1 | 1 | 5 | 1 | Nano-grazers | |
| | | Litostomatea | 2 | 8 | 3 | 5 | Parasites | |
| | | Oligohymenophorea | 1 | 7 | 12 | 4 | Nano-grazers/ Parasites | |
| | | Phyllopharyngea | 8 | 23 | 4 | 5 | Nano-grazers/ Parasites | |
| | | Prostomatea | 0 | 5 | 1 | 2 | Nano-grazers | |
| | | Spirotrichea | 20 | 70 | 99 | 53 | Nano-grazers | |
| | Dinophyceae | | 125 | 50 | 280 | 103 | Micro-grazers/ Mixotrophs | Sherr and Sherr, 2007; Hansen 2011 |
| | MALVs | | 13 | 9 | 38 | 21 | Parasites | Guillou et al., 2008 |
| Amoebozoa | Lobosa | | 3 | 7 | 4 | 18 | Micro-grazers | Cavallier- Smith et al., 2006 |
| Apusozoa | Hilomonadea | | 1 | 6 | 2 | 3 | Pico-grazers | Scheckenbach et al., 2006 |
| Archaeplastida | Chlorophyta | | 81 | 46 | 59 | 67 | Autotrophs | Not et al., 2012 |
| | Rhodophyta | | 1 | 1 | 2 | 3 | Autotrophs | Yokoyama et al., 2009 |
| Excavata | Discoba | | 1 | 1 | 1 | 1 | Pico-grazers | Cavallier- Smith et al., 2006 |
| Harcobia | Centroheliozoa | | 4 | 4 | 10 | 31 | Pico-grazers | Burki et al., 2009 |
| | Cryptophyta | | 0 | 1 | 3 | 1 | Autotrophs/Mixotrophs | Not et al., 2012 |
| | Haptophyta | | 38 | 92 | 31 | 33 | Autotrophs/Mixotrophs | Not et al., 2012 |
| | Katablepharidophyta | | 2 | 4 | 1 | 0 | Pico-grazers | Okamoto et al., 2005 |
| | Picobiliphyta | | 17 | 4 | 8 | 6 | Pico-grazers | Moreira et al., 2014 |
| | Telonemia | | 7 | 53 | 6 | 7 | Pico-grazers | Boenigk et al., 2002 |
| Opisthokonta | Choanoflagellida | | | 25 | 39 | 42 | Pico-grazers | Richards et al., 2015 |
| | Fungi | Ascomycota | 39 | 55 | 42 | 130 | Decomposers | |
| | | Basidiomycota | 36 | 58 | 48 | 90 | Decomposers | |
| | | Blastocladiomycota | 0 | 1 | 1 | 0 | Parasites | |
| | | Chytridiomycota | 14 | 16 | 16 | 15 | Parasites | |
| | | Cryptomycota | 5 | 2 | 4 | 1 | Decomposers | |
| | | Entomophthoromycota | 1 | 4 | 1 | 4 | Decomposers | |
| | | Mucoromycota | 2 | 4 | 2 | 6 | Decomposers | |
| | Mesomycetozoa | | 3 | 9 | 11 | 22 | Parasites | Marshall et al., 2008 |
| Rhizaria | Cercozoa | | 71 | 56 | 40 | 41 | Parasites | Chantangsi et al., 2010 |
| Stramenopiles | Bacillariophyta | | 201 | 230 | 94 | 45 | Autotrophs | Not et al., 2012 |
| | Bicoecea | | 30 | 21 | 49 | 46 | Pico-grazers | Boenigk et al., 2002 |
| | Bolidophyceae and relatives | | 10 | 12 | 3 | 3 | Autotrophs | Brown and Sorhannus, 2010 |
| | Chrysophyceae-Synurophyceae | | 6 | 29 | 4 | 8 | Autotrophs/Mixotrophs | Brown and Sorhannus, 2010 |
| | Dictyochophyceae | | 15 | 12 | 10 | 6 | Autotrophs | Brown and |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Eustigmatophyceae | 2 | 1 | 0 | 4 | Autotrophs | Sorhannus, 2010 Brown and Sorhannus, 2010 |
| | Hyphochytriomyceta | 0 | 0 | 3 | 1 | Parasites | Kramarsky-Winter et al., 2006 |
| | Labyrinthulea | 22 | 20 | 108 | 38 | Parasites | Raghukumar, 2002 |
| | MAST | 60 | 29 | 27 | 15 | Pico-grazers | Massana et al., 2004 |
| | Oomyceta | 11 | 10 | 11 | 8 | Parasites | Park et al., 2004 |
| | Pelagophyceae | 1 | 2 | 2 | 3 | Autotrophs | Brown and Sorhannus, 2010 |
| | Phaeophyceae | 3 | 0 | 0 | 1 | Autotrophs | Brown and Sorhannus, 2010 |
| | Picophagea | 1 | 2 | 1 | 1 | Mixotrophs | Cavallier- Smith et al., 2006 |
| | Pirsonia | 3 | 4 | 4 | 14 | Parasites | Skovgaard, 2014 |
| | Raphidophyceae | 2 | 1 | 1 | 0 | Autotrophs | Brown and Sorhannus, 2010 |

Supplementary Table 2A: Total number of DNA-based OTUs and RNA-based reads of major taxonomic groups for each experiment. Values for the Mediterranean experiment are shaded.

| | | **Baltic** | | **Mediterranean** | |
|---|---|---|---|---|---|
| | | **DNA OTUs** | **RNA Reads** | **DNA OTUs** | **RNA Reads** |
| Alveolates | Dinophyceae | 248 | 3824 | 403 | 48784 |
| | MALVs | 31 | 123 | 60 | 1565 |
| | Other Alveolates | 90 | 64491 | 207 | 76590 |
| Archeoplastida | Chlorophytes | 139 | 2268 | 123 | 10039 |
| Harcobia | Haptophytes | 53 | 74696 | 64 | 21723 |
| | Other Harcobia | 82 | 16445 | 75 | 16593 |
| Opisthoconta | Fungi | 275 | 25128 | 239 | 182643 |
| | Other Opisthoc. | 49 | 2732 | 109 | 16073 |
| Rhizaria | Cercozoa | 117 | 6774 | 81 | 3473 |
| Stramenopiles | Bacillariophyceae | 382 | 289044 | 178 | 16905 |
| | Other Stramen. | 360 | 24775 | 426 | 83368 |
| Amoebozoa | Lobosa | 3 | 1160 | 5 | 1416 |
| Apusozoa | Hilomonadea | 5 | 1127 | 4 | 189 |
| Excavata | Discoba | 4 | 10 | 1 | 2 |

Supplementary Table 2B: Total number of DNA-based OTUs and RNA-based reads of trophic groups for each experiment. Values for the Mediterranean experiment are shaded.

| | **Baltic** | | **Mediterranean** | |
|---|---|---|---|---|
| | **DNA** | **RNA** | **DNA** | **RNA** |
| Autotrophs | 374 | 304766 | 129 | 76153 |
| Mixotrophs | 40 | 74443 | 298 | 21268 |
| Decomposers | 97 | 25128 | 114 | 180643 |
| Parasites | 124 | 9077 | 212 | 43156 |
| Micrograzers | 67 | 3486 | 70 | 7413 |
| Picograzers | 166 | 95264 | 270 | 149458 |

Supplementary Table 3: Ratios of auto- and mixo- trophic OTUs to heterotrophic OTUs ($S_{auto}/S_{het}$ ratio) and auto- and mixo- trophic OTUs to consumers ($S_{auto}/S_{grazers+parasites}$ ratio) for each experiment. Values for the Mediterranean experiment are shaded.

| **Baltic** | **Mediterranean** |
|---|---|

| | $S_{auto}/S_{grazers+parasites}$ | $S_{auto}/S_{het}$ | $S_{auto}/S_{grazers+parasites}$ | $S_{auto}/S_{het}$ |
|---|---|---|---|---|
| $A_i$ | 1.48 | 1.13 | 1.43 | 1.10 |
| $A_f$ | 1.23 | 0.92 | 0.97 | 0.74 |
| $A_{f+}$ | 1.17 | 0.91 | 1.49 | 1.21 |
| $A_{f-}$ | 1.41 | 0.95 | 1.13 | 0.70 |
| $H_i$ | 1.34 | 0.89 | 0.52 | 0.41 |
| $H_f$ | 0.62 | 0.46 | 0.58 | 0.50 |
| $H_{f+}$ | 1.13 | 0.81 | 0.67 | 0.55 |
| $H_{f-}$ | 1.99 | 1.01 | 0.62 | 0.49 |

Supplementary Table 4: Percentages of DNA-based and RNA-based abundant and rare OTUs for each experiment. Values for the Mediterranean experiment are shaded.

| | **DNA** | | | | **RNA** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Abundant OTUs %** | | **Rare OTUs %** | | **Abundant OTUs %** | | **Rare OTUs %** | |
| | **Baltic** | **Mediterranean** | **Baltic** | **Mediterranean** | **Baltic** | **Mediterranean** | **Baltic** | **Mediterranean** |
| $A_i$ | 3.7 | 4.6 | 87.6 | 82.7 | 3.3 | 4.9 | 92.0 | 82.3 |
| $A_f$ | 5.3 | 10.4 | 88.4 | 85.4 | 3.2 | 6.5 | 93.0 | 82.1 |
| $A_{f+}$ | 10.2 | 10.9 | 84.2 | 77.4 | 2.3 | 5.3 | 94.5 | 82.6 |
| $A_{f-}$ | 8.0 | 8.9 | 84.5 | 81.7 | 2.8 | 6.3 | 91.5 | 86.2 |
| $H_i$ | 7.6 | 3.3 | 83.9 | 91.7 | 3.3 | 3.5 | 88.0 | 88.8 |
| $H_f$ | 4.1 | 5.2 | 86.8 | 87.4 | 1.5 | 6.5 | 88.7 | 81.3 |
| $H_{f+}$ | 5.9 | 6.9 | 85.2 | 85.6 | 4.6 | 7.7 | 90.8 | 79.5 |
| $H_{f-}$ | 3.8 | 5.9 | 86.7 | 87.5 | 1.9 | 8.3 | 90.3 | 79.4 |

## References


Boenigk, J., Arndt, H. (2002). Bacterivory by heterotrophic flagellates: community structure and feeding strategies. Anton van Lee 81, 465-480.

Brown, J.W., Sorhannus, U. (2010). A molecular genetic timescale for the diversification of autotrophic Stramenopiles (Ochrophyta): substantive underestimation of putative fossil ages. PLoS ONE 5 (9), e12759.

Burki, F., Inagaki, Y., Bråte, J., Archibald, J.M., Keeling, P. J., Cavalier-Smith, T. et al. (2009). Large-scale phylogenetic analyses reveal that two enigmatic protist lineages, Telonemia and Centroheliozoa, are related to photosynthetic Chromalveolates. Genome Biol Evol 1, 231-238.

Cavallier- Smith, T., Chao, E.Y.E. (2006). Phylogeny and Megasystematics of Phagotrophic Heterokonts (Kingdom Chromista). J Mol Evol 62, 388-420.

Chantangsi, C., Leander, B.S. (2010). An SSU rDNA barcoding approach to the diversity of marine interstitial cercozoans, including descriptions of four novel genera and nine novel species. Int J Syst Evol Microbiol 60, 1962-1977.

Guillou, L., Viprey, M., Chambouvet, A., Welsh, R.M., Kirkham, A.R., Massana, R., et al. (2008). Widespread occurrence and genetic diversity of marine parasitoids belonging to Syndiniales (Alveolata). Environ Microbiol 10, 3349-3365.

Hansen, P.J. (2011). The Role of Photosynthesis and Food Uptake for the Growth of Marine Mixotrophic Dinoflagellates. Eukaryot Microbiol 58, 203-214.

Kramarsky-Winter, E., Harel, M., Siboni, N., Ben Dov, E. Brickner, I., Loya, Y. et al. (2006). Identification of a protist-coral association and its possible ecological role. MEPS 317: 67-73.

Lynn, D. (2008). The ciliated protozoa: characterization, classification, and guide to the literature. New York: Springer.

Marshall, W.L., Celio, G., McLaughlin, D.J., Berbee, M.L. (2008). Multiple isolations of a culturable, motile Ichthyosporean (Mesomycetozoa, Opisthokonta), Creolimax fragrantissima n. gen., n. sp., from marine invertabrate digestive tracts. Protist 159, 415-433.

Massana, R., Castresana, J., Balagué, V., Guillou, L., Romari, K., Groisillier, A. et al. (2004). Phylogenetic and Ecological Analysis of Novel Marine Stramenopiles. Appl Environ Microbiol 70 (6), 3528-3534.

Moreira, D. and López-García, P. (2014). The rise and fall of Picobiliphytes: How assumed autotrophs turned out to be heterotrophs. BioEssays 36, 468–474.

Not, F., Siano, R., Kooistra, W.H.C.F., Simon, N., Vaulot, D., Probert, I. (2012). Diversity and ecology of eukaryotic marine phytoplankton. In: Genomic insights into the biology of algae. Piganeau, ed. USA: Elsevier Chapter One, pp. 1-54.

Okamoto, N., Inouyen, I. (2005). The Katablepharids are a Distant Sister Group of the Cryptophyta: A Proposal for Katablepharidophyta Divisio Nova/Kathablepharida Phylum Novum Based on SSU rDNA and Beta-Tubulin Phylogeny. Protist 156, 163-179.

Park, M.G., Yih, W., Coats, D.W. (2004). Parasites and phytoplankton, with special emphasis on Dinoflagellate infections. *J Eukaryot Microbiol* 51, 45-155.

Raghukumar, S. (2002). Ecology of the marine protists, the Labyrinthulomycetes (Thraustochytrids and Labyrinthulids*)*. Eur J Protistol 38, 127-145.

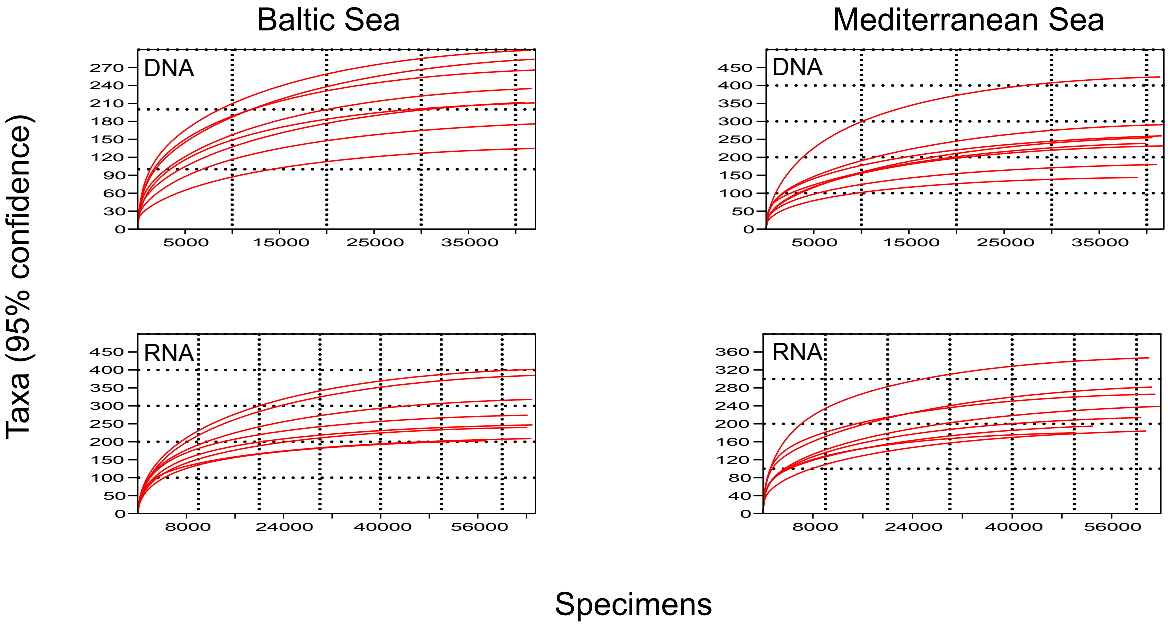
Richards, T.A., Leonard, G., Mahé, F., del Campo, J., Romac, S., Jones, M.D.M. et al. (2015). Molecular diversity and distribution of marine fungi across 130 European environmental samples. Proc R Soc B 282, 2015-2243.

Scheckenbach, F., Wylezich, C., Mylnikov, P.A., Weitere, M., Arndt, H. (2006). Molecular Comparisons of Freshwater and Marine Isolates of the Same Morphospecies of Heterotrophic Flagellates. Appl Environ Microbiol 72 (10), 6638-6643.

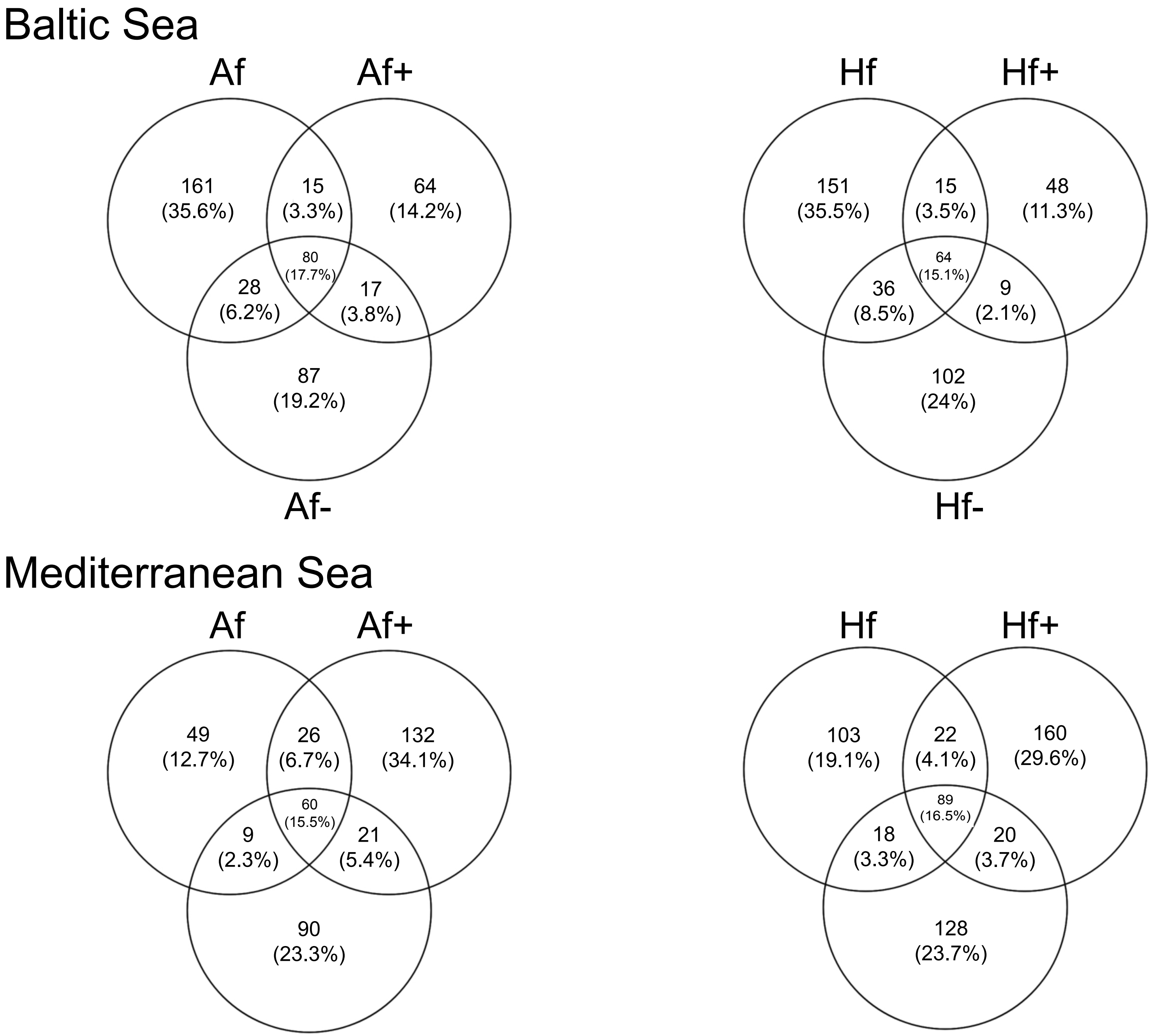
Sherr, B.F., Sherr, E.B., Caron, D.A., Vaulot, D., Worden, A.Z. (2007). Oceanic protists. Oceanography 20, 130-134.

Skovgaard, A. (2014). Dirty tricks in the plankton: diversity and role of marine parasitic protists. Acta Protozool 53, 51-62.

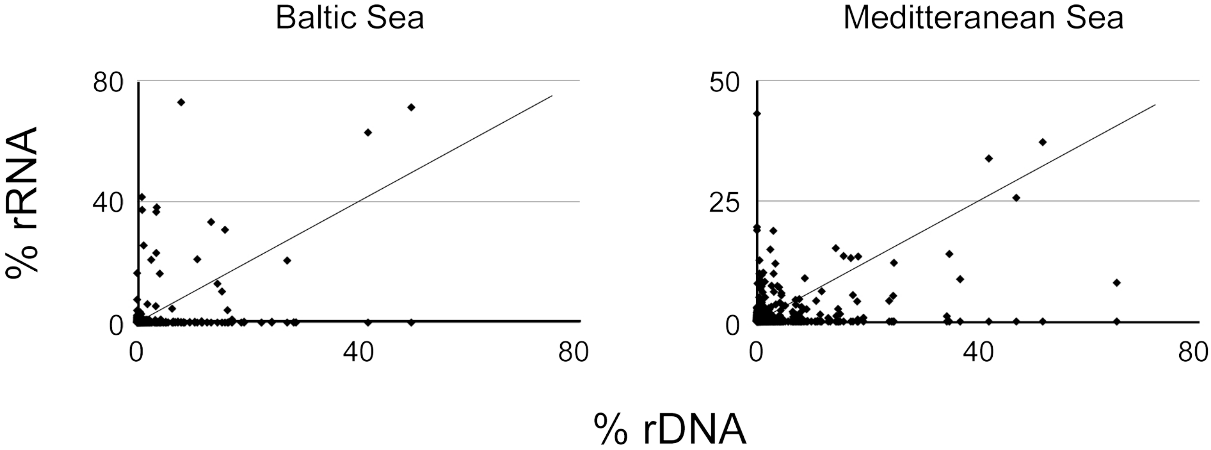
Yokoyama, A., Scott, J.L., Zuccarello, G.C., Kajikawa, M., Hara, Y., West, J.A. (2009). Corynoplastis japonica gen. et sp. nov. and Dixoniellales ord. nov. (Rhodellophyceae, Rhodophyta) based on morphological and molecular evidence. Phycol Res 57, 278-289.



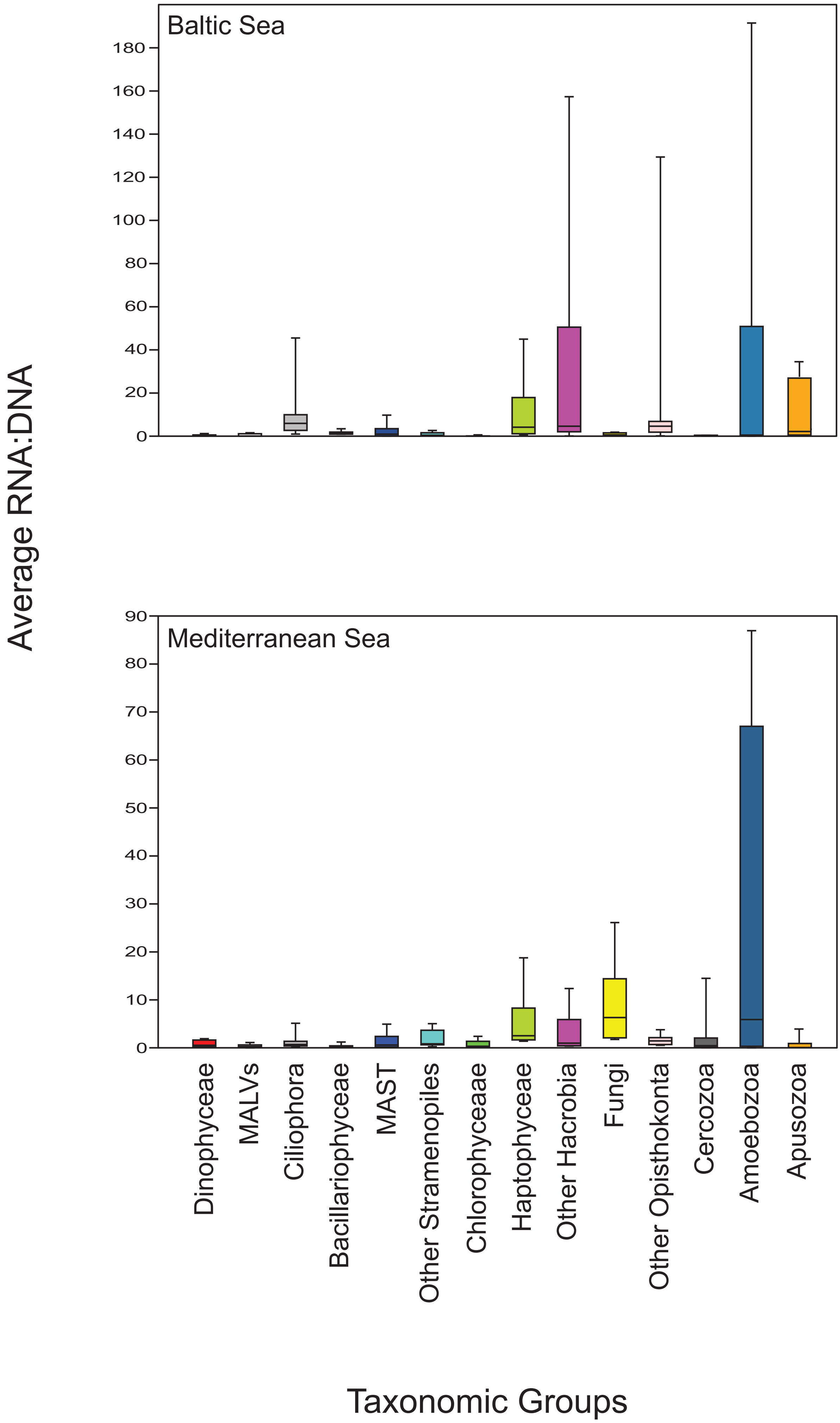

**Supplementary Figure 1**. Rarefaction curves of DNA and RNA samples in the Baltic experiment and in the Mediterranean experiment.




**Supplementary Figure 2.** Venn diagram showing the number of unique and shared DNA OTUs across the different salinities of A and H communities in the Baltic experiment and in the Mediterranean experiment. Af: ambient temperature and salinity, Af+: ambient temperature and high salinity (+5psu), Af-: ambient temperature and low salinity (-5psu), Hf: heat shock (+6oC) and ambient salinity Hf+: heat shock (+6oC) and high salinity (+5psu), Hf-: heat shock (+6oC) and low salinity (-5psu).




**Supplementary Figure 3.** Relative read abundance of RNA (y axis) and DNA (x axis) reads in the Baltic experiment and in the Mediterranean experiment. Data points represent the common OTUs in RNA and DNA datasets.




**Supplementary Figure 4.** Range of average RNA:DNA ratios for each taxonomic group in the Baltic experiment and in the Mediterranean experiment. Boxplots represent variation among the average RNA:DNA ratios in each treatment and whiskers denote minimum and maximum values.