STRATIFICATION-INDUCED MODIFICATION OF FOOD WEB DYNAMICS IN A MARINE PELAGIC ENVIRONMENT

Herwig Stibor (1), Maria Stockenreiter (1), Luna Benítez-Requena (1), Sabine Schultes (1), Claudia Alexandra Forster (1)
(1) Department of Biology II, Aquatic Ecology, Ludwig-Maximilians-Universität München, LMU Großhaderner Straße 2, 82152 Planegg-Martinsried, Germany,
E-mail: stibor@zi.biologie.uni-muenchen.de

Patrick Fink (2, 3), Maja Ilić (2),
(2) University of Cologne, Cologne Biocenter, Workgroup Aquatic Chemical Ecology, Zuelpicher Strasse 47b, 50674 Cologne, Germany
(3) Helmholtz Centre for Environmental Research, Department River Ecology and Department Aquatic Ecosystem Analysis, Brueckstrasse 3a, 39114 Magdeburg, Germany,
E-mail: patrick.fink@uni-koeln.de

Olav Vadstein (4),
(4) Department of Biotechnology and Food Science, NTNU, N-7491 Trondheim, Norway,
Email: olav.vadstein@ntnu.no

Nicolas Djeghri (5), Rudolph Corvaisier (5), Anne Donval (5) Philippe Pondaven (5)
(5) Université de Brest, UBO, Institut Universitaire Européen de la Mer, IUEM, Laboratoire des Sciences de l'Environnement Marin, LEMAR-UMR 6539, Technopôle Brest Iroise, rue Dumont d'Urville, 29280 Plouzané, France,
Email: philippe.pondaven@univ-brest.fr

ABSTRACT

Global warming is expected to increase water column stratification, thereby affecting the dynamics of plankton communities. In this context, experimental manipulations allowing mechanistic insights into the general relationship between the depth of the mixed layer and ecological dynamics are scarce and mainly limited to freshwater systems. Here, a mesocosm experiment was carried out in the Bay of Hopavågen (63°36' N, 9°33' E, Norway) on Aug-Sept 2017. Stratification was experimentally manipulated in 18 enclosures, and the responses of different trophic groups of marine plankton were determined. Preliminary analysis suggests that the total phytoplankton biomass did not change significantly between treatments during the experiment. However, disparities within and between trophic groups were recorded, and changes in the depth of the mixed layer resulted in a reorganization of food web structure and dynamics. Reasons for the observed effects are still under study; they may combine direct effects of mixing, and light exposure, and associated indirect effects through biotic interactions.

INTRODUCTION

Stratification is one of the most important physical characteristics of pelagic water columns with large effects on the suspended biota (Sommer et al. 1986; Peeters et al. 2007; Berger et al. 2010). Stratification depth will influence essential resource levels such as the daily amount of light within the upper mixed layer to which phytoplankton is exposed, or the amount of dissolved nutrients, thereby having large influence on phytoplankton growth (Diehl et al., 2002; Winder and Hunter 2008; Winder and Sommer 2012). However, strength of mixing also affects sedimentation of suspended phytoplankton – a significant aspect of phytoplankton mortality (Ptacnik et al., 2003). Besides the growth of phytoplankton, its composition might be also strongly affected by mixing depth. Large, heavy algae such as diatoms may be favored by deep mixing whereas motile phytoflagellates may benefit from lower mixing depths (Winder and Hunter 2008). Additionally, phytoplankton thin layers frequently occur at the physical discontinuities associated with stratification and create hot spots of biological activity (Durham & Stocker 2012). It is therefore not surprising that two iconic theoretical concepts of biological

oceanography are linked to stratification and mixing depth: Sverdrup's principle of critical depth is dealing with effects of mixing depth for phytoplankton development and Margalef's Mandala is describing the interaction of mixing and nutrients on phytoplankton community composition. Sverdrup's principle states that the vertical mixing of the surface layer must stay above a critical depth beyond which phytoplankton has negative growth because of light limitation (Sverdrup 1953). The concept has been re-visited in recent years taking into consideration the role of turbulence and of temporal decoupling from grazing pressure by grazers (review by Fisher et al. 2015). Margalef's Mandala is a guide to the description of phytoplankton assemblages based on combining the interactive effects of habitat mixing and nutrient conditions and basically describes the succession between major algal groups, diatoms and dinoflagellates, in water columns with different mixing depth (Margalef 1978; Margalef et al.,1979).

The above mentioned concepts clearly point towards the relevance of mixing depth for biological oceanography. Hence, as shifts in mixing depth affect phytoplankton, which is the base of pelagic food webs based on photosynthesis, it is realistic to assume that these effects will have further influence within food webs. Consequences for herbivorous zooplankton and probably even higher trophic levels such as fish or jellyfish can be expected. Beside potential positive effects of phytoflagellates on jellyfish growth via food web effects (Parsons and Lalli 2002; Colin et al. 2005) increasing stratification may additionally favor jellyfish by direct effects such as warmer temperatures in shallow mixed water layers (Richardson et al. 2009).

However, stratification and mixing depth of water columns are very sensitive to climate change. Increasing sea surface temperatures will decrease mixing depth and indirect consequences for biological and ecological dynamics might be larger than physiological effects of just temperature increases alone. Hence, most of the concepts dealing with mixing depth and its effects on plankton rely on theoretical considerations and purely observational data. For example, increasing stratification of water columns is discussed as one major factor that can contribute to further extending jellyfish blooms (Purcell et al. 2001; Richardson et al. 2009); however, this concept was until now never tested experimentally.

Experimental manipulations allowing more mechanistic insights into the general relationship between water column stratification and ecological dynamics are scarce and mainly limited to freshwater systems (Berger et al. 2010). Experiments are needed to contribute to a better insight into how global change might affect marine plankton communities via changes in the physical environment. To further increase such knowledge is a necessity to improve models and predictions how global change will affect European coastal waters and to foresee potential remediation measures to be taken in the future.

Here, the effects of increasing water column stratification on plankton dynamics were investigated experimentally by performing mesocosm experiments in the Bay of Hopavågen (Norway). Mixing depth of the water column was manipulated in enclosures, and consequences of the manipulations for the plankton community dynamics were followed. Three research questions related to food web dynamics and stratification were tested:

(1) Decreasing mixing depth of a water column will favor phytoflagellates over diatoms and thereby change phytoplankton communities.
(2) Decreasing mixing depth therefore decouple prevalent phytoplankton – zooplankton dynamics by changing food quantity and quality (biochemical composition) for different zooplankton groups.
(3) Decreasing mixing depth will favor jellyfish growth.

In this report, we present the experimental design and some preliminary results related to the observed food web dynamics induced by our experimental manipulations.

METHODS

The mesocosm experiment was carried out during August-September 2017 in the semi-enclosed bay of Hopavågen (63°36' N, 9°33' E), located west of Trondheim (Norway). Mixing depth was manipulated based on previous experiments performed in lakes (Diehl et al. 2015). A total of 18 enclosures were moored with a three times replicated gradient of depth of 2, 4, 6, 8, 10 and 12 m. Enclosures consisted of transparent polyethylene tubes, with a diameter of

0.9 m. They were filled on the day before the start of the experiment (Day 0) by lifting them from the depth to the surface. After filling, enclosures were daily mixed (every 12 hours; 7:00 AM and 7:00 PM) with short pulses (5 min) of compressed air; thereby ensuring a well mixed water column during the experiment.

A full sampling program was performed in all enclosures on 26/08, 31/08, 07/09 and 10/09 (end of experiment). For this purpose, the entire water column was sampled (around 7:00 AM) with an opaque integrated tube sampler (tube Ø 40 mm x 200 cm, ®KC Denmark). In addition, a lighter daily sampling program was conducted to check for short-term changes in the physical environment and plankton dynamics. This daily program included measurements of temperature, salinity, and phytoplankton biomass; temperature and salinity were determined using a CTD to verify the mixing depth and degree of stratification of the water column (inside the enclosures and inside the bay, next to the experimental raft); phytoplankton biomass was estimated from a measurement of chlorophyll *a* fluorescence using a fluorometer (see below).

Nutrient concentrations - Nutrient samples were collected in all 18 mesocosms on a weekly basis. Samples were pre-filtered (®Millex, Ø 0.2 μm) before analysis. Concentrations of soluble reactive phosphorus (SRP, PO_4^{3-}) and orthosilicic acid ($Si(OH)_4$) were measured using standard methods (Strickland and Parsons 1972). Each separate assay run was calibrated along a standard curve (range 0-1 μM-P, and 0-5 μM-Si). Standard solutions of SRP and orthosilicic acid were prepared from anhydrous potassium dihydrogen phosphate (KH_2PO_4) and sodium fluosilicate (Na_2SiF_6), respectively, diluted in deionized-water. To determine particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP), 250 mL of seawater were filtered onto precombusted and acid-washed glass-fiber filters (GF/F ®Whatman). POC and PON, as well as their isotopic signatures $^{15}\delta N$ and $^{13}\delta C$, were then measured with an elemental analyzer (CE Instruments, Milan, Italy), coupled with a mass spectrometer. POP was measured after sulfuric acid digestion followed by molybdene-blue reaction (Wetzel & Likens, 1991).

Phytoplankton & microbial community composition - Phytoplankton samples were fixed with a Lugol's iodine solution, and analyzed on an inverted microscope following Utermöhl's (1958) method. Phytoplankton were identified to the species or genus level whenever possible, or merged into generic groups such as "unidentified nanophytoplankton". In addition, the composition of the phytoplankton community was characterized by the analysis of photosynthetic pigment concentrations. For this purpose, 750 mL of seawater were collected from each enclosure and filtered onto precombusted and acid-washed glass-fiber filters (GF/F ®Whatman). Pigments were extracted at -20°C in 3 mL of ethanol (95%), filtrated, and determined via high performance liquid chromatography (HPLC; ®Agilent 1200), according to Van Heukelem and Thomas (2001). External pigment standards (®DHI, Denmark) were used to calibrate the system. The software CHEMTAX (Mackey *et al*., 1996) was used to estimate the relative abundances of various algal classes from pigments concentrations ratios. Flow cytometry provided additional information on the composition of microbial communities. Samples were fixed with glutaraldehyde (0.5% final conc.) prior to analysis. Abundances of bacteria, *Synechococcus spp.*, picoeukaryotes, nanoeukaryotes, and heterotrophic nanoflagellates (HNF) were determined with a flow cytometer, according to Vadstein (2000). A multispectral Algal-Lab Analyser (®PSI Photons Systems) was also used for a daily survey of phytoplankton community composition. This fluorometry system is able to differentiate between the main "optical algal groups" (diatoms, cryptophytes, green algae and cyanobacteria). Aquapen fluorometers (®PSI Photons Systems, Brno) were used for analyses of the phytoplankton biomass (from a measure of the 'Fix Area' value: i.e. the area under the induction curve of fluorescence during a saturating light flash), and photosynthetic efficiencies of the algal communities. The net growth rates (μ; d^{-1}) of phytoplankton and other microorganism taxa were calculated as follows:

$$\mu = \ln(N_{t2}/N_{t1})/\Delta t \qquad \text{(Eq.1)}$$

where N_i is the abundance of a taxon "i", and Δt is the time step (t_2-t_1).

Zooplankton community and food web interactions- The initial composition of the zooplankton community in Hopavågen Bay was determined on day 0 using a zooplankton net (Ø 200 µm) lifted vertically from the depth to the surface, just beside the mesocosm raft. At the end of the experiment, the zooplankton community was sampled in all enclosures using the same procedure. Crustacean zooplankton samples were stored in a solution of formaldehyde (4%) before analysis. Gelatinous zooplankton (appendicularia, ctenophores, hydromedusae) were sampled using an identical net, but with a plastic bag collector to preserve the integrity of the organisms; the different taxa were then identified and counted immediately after sampling, using a microscope. Finally, lipid contents were determined by extraction of polyunsaturated fatty acids (PUFAs) with dichloromethane:methanol (2:1) and transesterified to FAME before GC-FID analysis (Fink, 2013).

Statistical Analyses- The above measurements were used to link stratification manipulations to responses in nutrient levels, light and the composition and dynamics of the different plankton communities within the mesocosms. In the present manuscript, only first preliminary analyses are presented.

PRELIMINARY RESULTS

Nutrient concentrations- Orthosilicic acid concentrations ranged from 0.12 µM-Si to 2.25 µM-Si. SRP concentrations ranged from 0.01 µM-P to 0.06 µM-P, i.e. ≤ to the precision of the method used here (Strickland and Parsons, 1972). A decrease of nutrient concentrations was recorded in all enclosures at the beginning of the experiment (from 26/08 to 31/08; Figure 1 a-b). Additionally, preliminary results suggest that changes in mixing depth had no clear influence on the dynamics of dissolved inorganic nutrient concentrations (not shown).
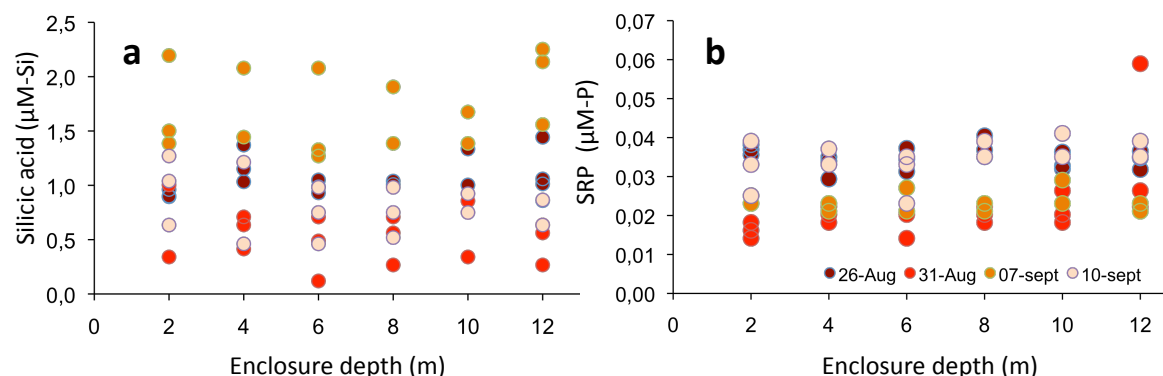

Figure 1: Concentrations of **(a)** orthosilicic acid (µM-Si) **and (b)** orthophospate (SRP, µM-P) on 26/08/17, 31/08/17, 07/09/17 and 10/09/17, in 18 enclosures moored with a three times replicated gradient of mixing depth of 2, 4, 6, 8, 10 and 12 m.

Phytoplankton community composition- Phytoplankton biomass, shown as chlorophyll *a*, ranged from 1.5 to 3.5 µg L^{-1}. Transient dynamics were recorded in all enclosures with a decrease at the beginning of the experiment (from 26/08 to 31/08; Figure 2 a), followed by a net increase from 26/08 to 10/09. At the end of the experiment (day 16), the phytoplankton biomass was close to that observed at the beginning (day 0; figure 2a).
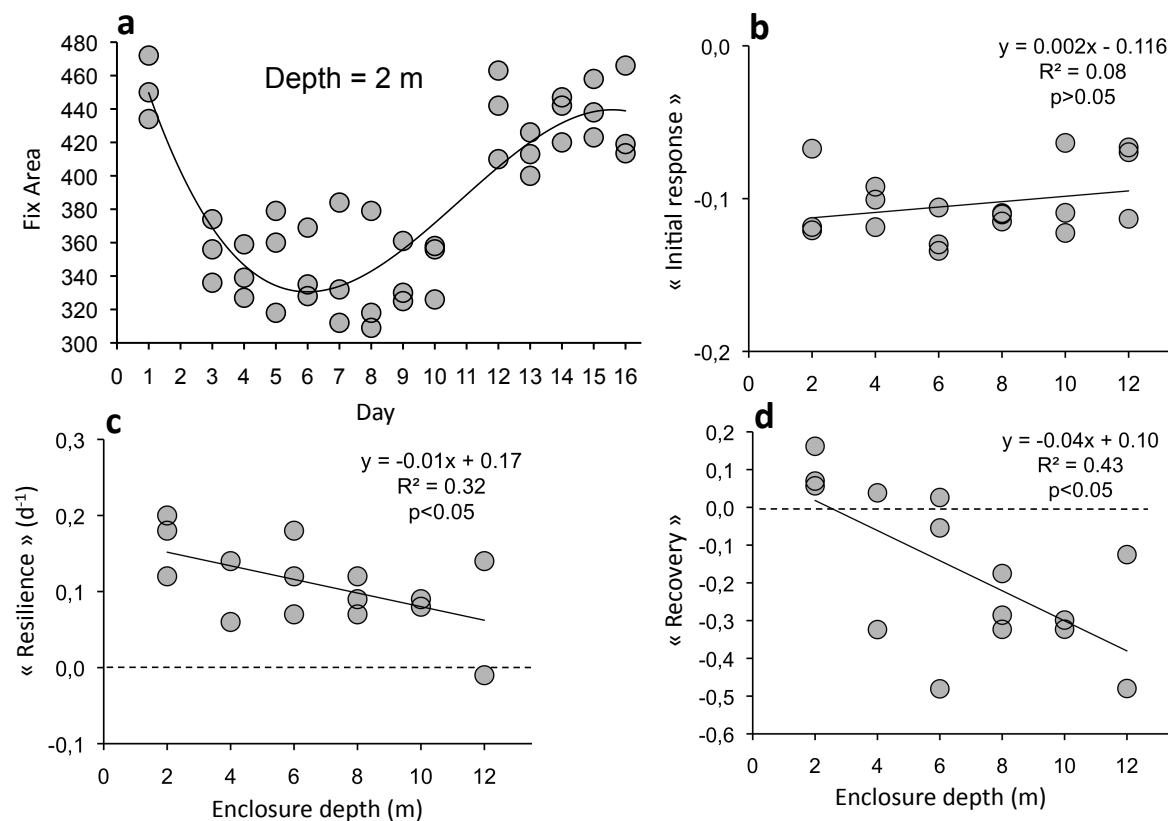
Figure 2: (a) Example of the evolution over time of the "Fix Area" (proxy of the chlorophyll *a* content) in three enclosures (depth = 2 m); (b) Initial response of the phytoplankton community, calculated using the formula $\ln(N_{t5}/N_{t1})$, where N is the biomass of chlorophyll *a* at time t_1 (26/08) or t_5 (31/08); (c) Index of "resilience": phytoplankton Net Growth Rate (NGR, d^{-1}) from t_5=31/08 to t_{16}=10/09; (d) Index of "recovery", calculated as $\ln(N_{t16}/N_{t1})$; for details on (b), (c) and (d) see Hillebrand et al. (2018).

The fact that the total phytoplankton biomass did not change substantially during the experiment is probably due to the low concentrations of dissolved nutrients at the beginning of the experiment, regardless of the depth of the enclosure. However, this apparent lack of response masks disparities within and between trophic groups.

We first explored how the response of phytoplankton communities to changes in mixing depth could be described using variables such as "resistance", "resilience" or "recovery" (see Hillebrand et al, 2018). Using these concepts in the case of the Hopavågen experiment, it was found that resilience and recovery of the phytoplankton community were significantly lower in deeper enclosures (Fig. 2 c-d).

In addition, a more detailed analysis of the structure of phytoplankton communities suggests that, although the total biomass varied little between the beginning and end of the experiment (Figure 2), a proliferation of prymnesophytes (probably calcifying coccolithophores) occurred in shallow mesocosms (Figure 3).
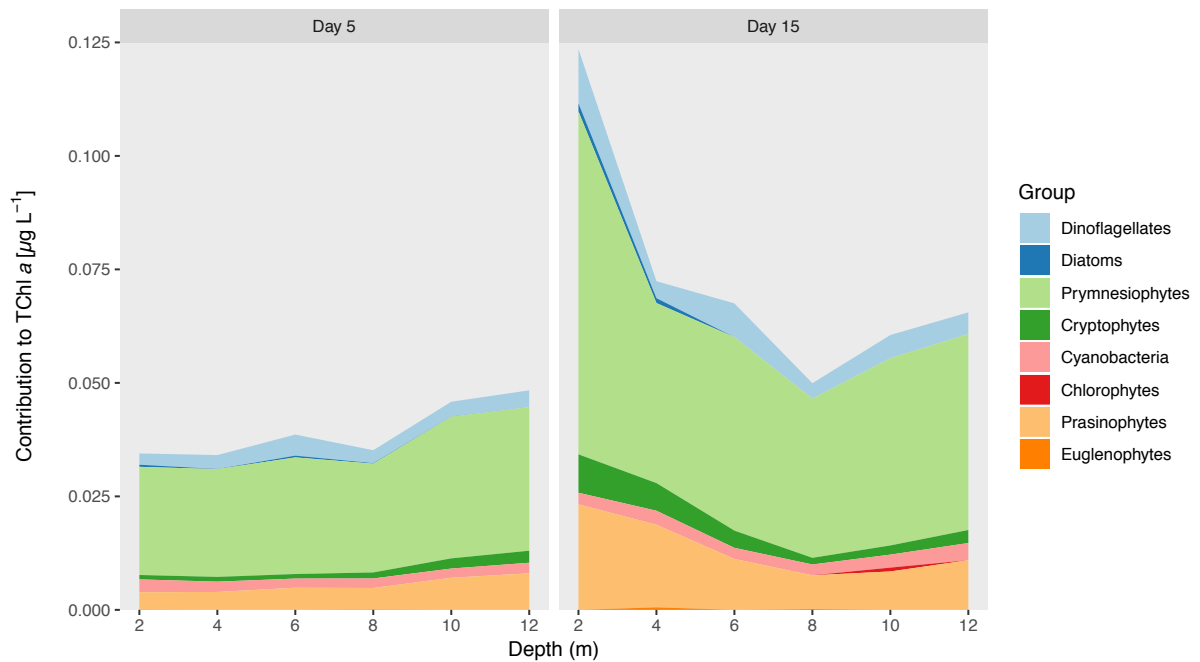
Figure 3: Evolution over time (day 5 and day 15) of the abundance of different algal groups (contribution to total Chlorophyll *a* estimated from pigments analysis and CHEMTAX; see text), along a gradient of mixing depth of 2, 4, 6, 8, 10 and 12 m.

Conversely, analysis of flow cytometry data revealed that the abundance of small prokaryotes belonging to the genus *Synechococcus spp.* was higher in deeper enclosures ($p < 0.05$; Figure 4a).
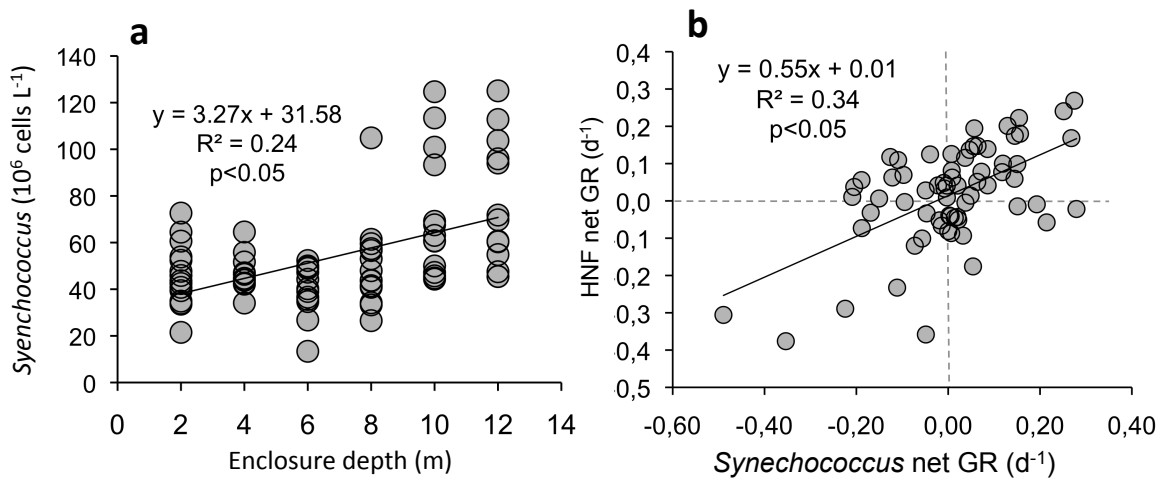

Figure 4: Preliminary results from flow cytometry; **(a)** Abundance of *Synechococcus* spp along a gradient of mixing depth of 2, 4, 6, 8, 10 and 12 m; **(b)** net Growth Rate (GR, d^{-1}) of heterotrophic nanoflagellates (HNF) *versus* net GR of *Synechococcus* in all 18 enclosures.

A preliminary conclusion that can be drawn from Figures 3 and 4a is that, all other things being equal, changes in the depth of the mixing layer may have resulted in a reorganization of the structure of the phytoplankton community. Ongoing analyses of stored phytoplankton samples will verify this pattern.

6

Heterotrophic nanoflagellates (HNF), micro- and mesozooplankton- A stratification-induced change in the dynamics of the zooplankton community was also recorded during the experiment (Figure 5).
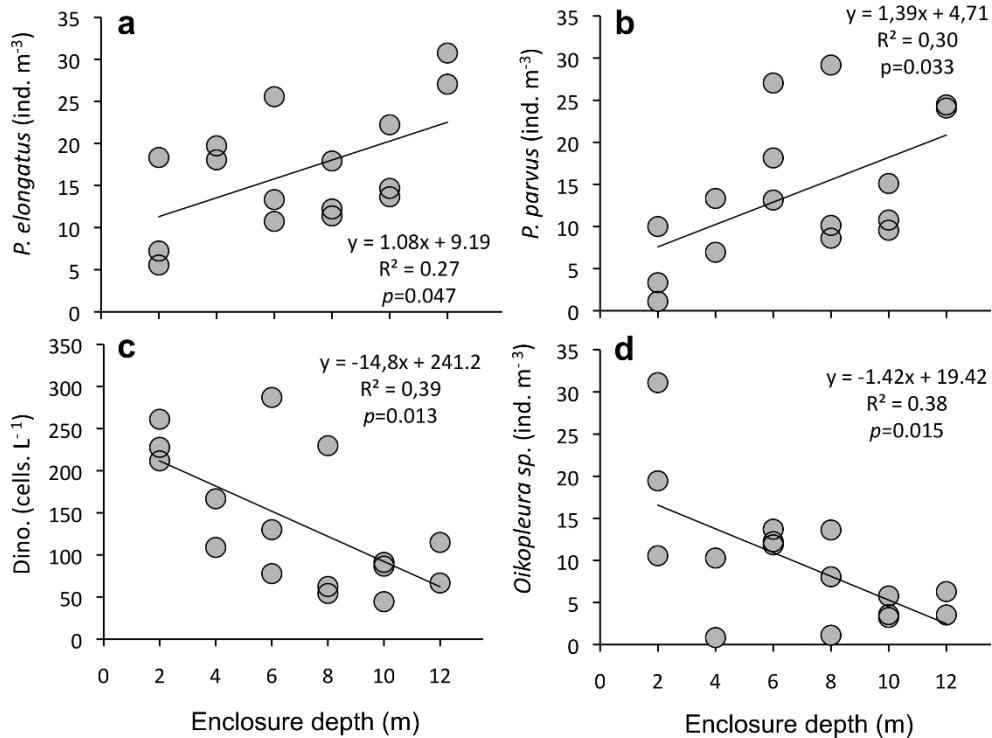

Figure 5: Abundances of **(a)** *Pseudocalanus elongatus,* **(b)** *Paracalanus parvus,,* **(c)** *large dinoflagellates*, and **(d)** *Oikopleura sp* along a gradient of mixing depth.

For example, the net growth rate of HNF was significantly related to that of *Synechococcus spp.* (Figure 4b), suggesting direct or indirect trophic relationships between these organisms (e. g. Sundt-Hansen et al., 2006). With respect to micro- and mesozooplankton abundance, changes in the depth of the mixing depth had also contrasting impacts across taxa (Figure 5a-d). On the one hand, the abundance of some species of small sized calanoid copepods, such as *Pseudocalanus elongatus* and *Paracalanus parvus*, was significantly lower in shallow mesocosms compared to deep mesocosms at the end of the experiment (Figure 5a-b). On the other hand, the abundance of large dinoflagellates and *Oikopleura sp.* was higher in shallow mesocosms than in deep mesocosms at the end of the experiment (Figure 5c-d). The higher abundance of *Synechococcus* in deeper mesocosms (Figure 4a) could then be partly explained by a decrease in mortality due to a lower abundance of their potential consumers, heterotrophic dinoflagellates or *Oikopleura* sp. (e. g. Sundt-Hansen et al., 2006). This is at least suggested in Figure 6, where *Synechococus* abundance is negatively correlated with that of large dinoflagellates and Oikopleura sp. at the end of the experiment.

The causal links that may explain the observed stratification induced change in the dynamics of the zooplankton community probably combine bottom-up and top-down effects (Diehl et al., 2002; Stibor et al., 2004; Stibor et al., 2006; Sundt-Hansen et al., 2006; Diehl et al., 2015). For instance, differences among zooplankton taxa regarding starvation tolerance, metabolic efficiency to use the available food, or feeding mode may play a role. Further analyses, such as zooplankton isotopic signatures or changes in fatty acids contents, may help to disentangle the relative importance of these effects.
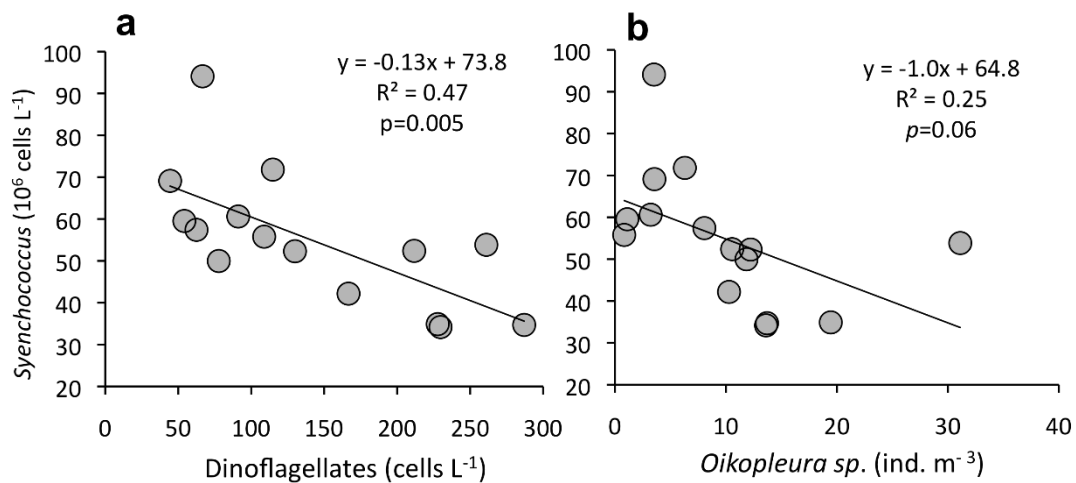
Figure 6: Abundances of **(a)** *Synechococcus versus* Dinoflagellates and **(b)** *Synechococcus versus Oikopleura sp.* at the end of the experiment, along a gradient of mixing depth of 2, 4, 6, 8, 10 and 12 m.

PRELIMINARY CONCLUSIONS AND PERSPECTIVES

In this study, water column stratification was experimentally manipulated in a mesocosm experiment carried out in the Bay of Hopavågen (63°36' N, 9°33' E, Norway). A total of 18 enclosures were moored, with a three times replicated gradient of mixing depth of 2, 4, 6, 8, 10 and 12 m. Responses from different trophic groups of marine plankton were determined over a 16-day period. Preliminary results suggest that the total phytoplankton biomass did not change significantly, possibly due to the low dissolved nutrient concentrations at this time of year (August-September 2017). However, disparities within and between trophic groups were recorded, and it was observed that changes in the depth of the mixed layer lead to a reorganization of the structure and dynamics of the food web. The mechanisms underlying these reactions are still under study and ongoing analyses (such as phytoplankton taxonomy) should provide further insights on how changes in the stratification of the upper surface layers can influence food web dynamics.

ACKNOWLEDGEMENTS

REFERENCES

Berger, S. A., Diehl, S., Stibor, H., Trommer, G., and Ruhenstroth, M. (2010). Water temperature and stratification depth independently shift cardinal events during plankton spring succession. *Global Change Biology*, *16*, 1954-1965.

Colin, S. P., Costello, J. H., Graham, W. M., and Higgins, J. (2005). Omnivory by the small cosmopolitan hydromedusa *Aglaura hemistoma*. *Limnology and Oceanography*, *50*, 1264-1268.

Diehl, S., Berger, S., Ptacnik, R., and Wild, A. (2002). Phytoplankton, light, and nutrients in a gradient of mixing depths: field experiments. *Ecology*, *83*, 399-411.

Diehl, S., S.A. Berger, Q. Soisson, D. Giling, and Stibor, H. (2015). An experimental demonstration of the critical depth principle. *ICES Journal of Marine Science,* 72, 2051–2060.

Durham, W.M., and Stocker R. (2012). Thin Phytoplankton Layers: Characteristics, Mechanisms, and Consequences. *Annual Review of Marine Science*, 4, 177-207.

Fink, P. (2013). Invasion of quality: high amounts of essential fatty acids in the invasive Ponto-Caspian mysid *Limnomysis benedeni. Journal of plankton research*, 35, 907-913.

Hillebrand, H., Langenheder, S., Lebret, K., Lindström, E., Östman, Ö., Striebel, M. (2018). Decomposing multiple dimensions of stability in global change experiments. Ecology letters, 21, 21-30.

Margalef, R. (1978). Life-forms of phytoplankton as survival alternatives in an unstable environment. *Oceanologica Acta, 1*, 493-509

Margalef, R., Estrada, M., and Blasco, D. (1979). Fuctional morphology of organisms involved in red tides, as adapted to decaving turbulence. In Tylor, D. and Seliger, H (eds). Toxic dinoflagellate blooms. Elsevier New York, 89-94.

Parsons, T.R., and Lalli, C.M. (2002). Jellyfish population expolsions: revisting a hypothesis of possible causes. *La mer, 40,* 111-121.

Peeters, F., Straile, D., Lorke, A., and Livingstone, D. M. (2007). Earlier onset of the spring phytoplankton bloom in lakes of the temperate zone in a warmer climate. *Global Change Biology*, *13*, 1898-1909.

Ptacnik, R., Diehl, S., and Berger, S. (2003). Performance of sinking and nonsinking phytoplankton taxa in a gradient of mixing depths. *Limnology and Oceanography*, *48*, 1903-1912.

Purcell, J. E., and Arai, M. N. (2001). Interactions of pelagic cnidarians and ctenophores with fish: a review. *Hydrobiologia*, *451*, 27-44.

Richardson, A. J., Bakun, A., Hays, G. C., and Gibbons, M. J. (2009). The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Trends in Ecology Evolution*, *24*, 312-322.

Sommer, U., Gliwicz, Z.M., Lampert, W., and Duncan, A. (1986). The PEG-Model of seasonal succession of planktonic events in fresh waters. *Archiv für Hydrobiologie, 106,* 433–471.

Stibor, H., Vadstein, O., Diehl, S., Gelzleichter, A., Hansen, T., Katechakis, A., Lippert, B., Løseth, K., Peters, C., Roederer, W., Sandow, M., Sundt-Hansen, and L. Olsen, Y. (2004). Copepods act as a switch between alternative trophic cascades in marine pelagic food webs. *Ecology Letters,* 7, 321-328.

Stibor, H., Gelzleichter, A., Hantzsche, F., Sommer, U., Striebel, M., Vadstein, O., and Olsen, Y. (2006). Combining dialysis and dilution techniques to estimate gross growth rate of phytoplankton and grazing by micro-and mesozooplankton *in situ*. *Archiv für Hydrobiologie,* 167, 403-419.

Strickland, J. D. H. and Parsons, T. R. (1972). A practical handbook of seawater analysis. Second Edition, Bulletin 167. Fisheries Research Board of Canada, Ottawa.

Sundt-Hansen, L. E., Olsen, Y., Stibor, H., Heldal, M., Vadstein, O. (2006). Trophic cascades mediated by copepods, not nutrient supply rate, determine the development of picocyanobacteria. *Aquatic microbial ecology*, 45, 207-218.

Sverdrup, H.U. (1953). On conditions for the vernal blooming of phytoplankton. *Journal du Conseil International pour l'Exploration de la Mer, 18,* 287-295.

Vadstein, O. (2000). Heterotrophic, planktonic bacteria and cycling of phosphorus. In Advances in microbial ecology (pp. 115-167). Springer, Boston, MA.

Wetzel, R. G., and Likens, G. E. (1991). Limnological analyses, 2nd ed. Springer Verlag.

Winder, M., and Hunter, D. A. (2008). Temporal organization of phytoplankton communities linked to physical forcing. *Oecologia*, *156*, 179-192.

Winder, M., and Sommer, U. (2012). Phytoplankton response to a changing climate. In *Phytoplankton responses to human impacts at different scales* (pp. 5-16). Springer Netherlands.