R/V Heincke (HE448) Report

LightHAB
Bio-optics and biochemistry of Harmful Algal Blooms in Norwegian coastal waters and fjord systems

Cruise No. HE448

July 9th – July 31th, 2015

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1 Summary

The LightHAB cruise HE448 was focused on the detection and analysis of temporal and spatial interactions among hydrographic regimes, biogeochemical and bio-optical signatures and phytoplankton species diversity, with special attention towards potentially toxigenic species key phytoplankton general associated with Harmful Algal Blooms (HABs). A second focus was set on the relationship of bio-optical data and targeted phytoplankton species. The cruise started in Bremerhaven (Germany), transects were along the Norwegian coast, from the south tip of Norway northwards to Trondheim harbor, including incursion into the Sognefjord and the Trondheimsfjord. During the cruise, the composition of size-fractionated net plankton and Niskin bottle casts from the pelagic zone was analyzed microscopically for species identification and cell quantitation. Attempts were made to link species composition and diversity to biotic and abiotic parameters concurrent with the plankton sampling regime.

Hydrophilic paralytic shellfish poisoning (PSP) toxin were detected only in low concentrations towards the end of the expedition, especially in Trondheimsfjord. In the case of hydrophilic toxins, along the expedition transect only low concentrations were detected with exception in Sognefjord, where, concentrations of dinophysistoxins (DTX) including okadaic acid (OA) and pectenotoxins (PTX) were found known to be produced by several species of the genus *Dinophysis*. At two stations the total toxin content exceeded 3 ng per net haul.

The CTD measurements of temperature and salinity profiles essentially matched what would be expected of fjord circulation at this time of year, i.e. high stratification with low surface salinity within the fjords and a sharp pycnocline. An array of bio-optical sensors was deployed to establish relationships between optical properties and the light regime of the water masses and observed plankton population. Correlation analysis of various parameters showed good correspondence, combining CTD, FerryBox, laboratory results, and profiler data, e.g. for Chl *a*, turbidity, and backscattering. Combining optical properties and light regime at each station to observed relationship between different communities as well as abiotic and biotic parameters is still in progress.

The cruise summarizes an abundance of new knowledge on the association of plankton biodiversity in coastal and fjord systems. Further attempts are now ongoing to provide the linkage to environmental variables and potential driving forces resulting from climate-driven regime shifts in north temperate waters.

2 Participants

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3 Research Program

The ecosystem component of the HE448 cruise **LightHAB** addressed interactions and feedbacks among hydrographic regimes, biogeochemical and bio-optical signatures and phytoplankton species diversity, with a focus on potentially toxigenic species associated with Harmful Algal Blooms (HABs). In particular, we focused on key questions regarding population dynamics and associated biological activity in relation to molecular ecological and chemically defined marine provinces and potential spreading of selected toxic species along the Norwegian coast and within adjacent selected fjord systems. With respect to global change, the objective was to characterize the composition of coastal water for definable marine biodiversity, organic components and marine bio-optical properties, with linkages to the effect of coastal proximity along the coast and with the fjord systems.

These objectives were addressed by the following specific elements: (1) establishing an inventory of the phytoplankton diversity with emphasis on HAB species and of phycotoxins in various planktonic fractions; (2) on board isolation and culturing of selected HAB taxa from plankton populations and cysts for further investigations on molecular genetics, life history strategies, mixotrophy, allelochemical capacity and production of bioactive natural products including toxins; (3) determining the water chemistry in the water column. By combining data from the oceanographic, biogeochemical and bio-optical components with conceptual modeling, we contributed two major advances in understanding Norwegian coastal ecosystems with regard to the distribution and dynamics of biodiversity of targeted key HAB species and the influence of climate change scenarios.

4 Narrative of the Cruise

The *R/V Heincke* departed for the HE448 research cruise one day later than planned on 10.07.2015 at 8.00 in Bremerhaven. Under now proper weather conditions the *R/V Heincke* headed northeast towards the first station at the south tip of Norway and then north along the coast and into the adjacent fjords systems Sognefjord and Trondheimsfjord. The cruise duration was ~ 20 days over a total distance of 1375 sm, and terminated in Trondheim on 30.07.2015 at 8.00. The overarching aim of this cruise was to study putatively toxigenic dinoflagellates and address interactions among hydrographic regimes, biogeochemical, and bio-optical signatures as well as phytoplankton species diversity. This mission aspect was only partially successful. Because of late cruise timing many target species were not abundant in the water column anymore or had even completely gone, except for some stations in the Sognefjord.

After the departure from Bremerhaven all underway measurements were started. The FerryBox system continuously recorded temperature, salinity, and fluorescence in surface waters (~ approx. 6 m water depth), 5 installed radiometers at the foremast of *R/V Heincke* were used to determine remote sensing reflectance all the way until we entered the harbor of Trondheim. The first station at the south tip of Norway was reached on the 11.07.2015 at 15.00. Subsequent station work at each station included deployment of a CTD/Rosette device, with water samples from discrete depths (3 m, deep chlorophyll max., and 30 m) along the water column for chemical analysis (e.g. phytotoxin samples), and the determination of chlorophyll *a* (Chl *a*) and suspended particulate matter (SPM). Complementary vertical tows of a phytoplankton net from 30 m yielded cell concentrates of plankton of >20 µm size-fraction. Bio-optical properties were determined by a free-falling radiometric profiler combined with shipborne remote sensing, and measurements of the inherent optical properties. An overall set of 15 stations in a defined distance (~ 15 nm) were done along the Norwegian coast.

The ***Sognefjord*** area was reached at the 15.07.2015 at 12.00. An overall set up of 16 stations was done within the Sognefjord with a reduced distance of 8 nm. CTD measurements of temperature and salinity profiles matched what would be expected of fjord circulation at this time of year, i.e. high stratification with low surface salinity within the fjord and a sharp pycnocline. Along the expedition transect up to the Sognefjord only low concentrations of hydrophilic toxins were detected. But in the Sognefjord dinophysistoxins (DTX) including okadaic acid (OA) and pectenotoxins (PTX) were found related to several species of the genus *Dinophysis*. At two stations the total toxin content exceeded 3 ng per net haul (station 21 and 26). *Dinophysis* spp were found in higher abundance mainly in the Sognefjord. All three main species known from the North Sea area were present: *D. acuta*, *D. accuminata*, and *D. norvegica*.

The Sognefjord region was departed at the 19.07.2015 and we headed north to ***Runde***. In between 6 stations along the coast were done according to the standard sampling regime (CTD device, plankton net, Optical profiler). Around Runde 5 closer sampling points were chosen. This was also part of the EU funded project NeXOS with cooperation partners at the Runde Environmental Centre. The area was left at the 22.07.2015 in the early morning.

After 11 station along the Norwegian coast northwards we entered the ***Trondheimsfjord*** (25.07.2015), where an overall set of 13 stations to the end of the fjord system were done station. Compared to the Sognefjord area only low concentrations of different phytotoxins were found. In general the total toxin content did not exceed 500 pg / net. *Alexandrium* spp were almost completely restricted to the stations in the Trondheimsfjord. Now, 27 different clonal isolates of *Alexandrium* spp exist. Temperature and salinity profiles from CTD measurements showed again the expected behavior, high stratification with low surface salinity and highest temperatures at the end of the fjord system (end station ~ 20 psu, ~ 19° C in surface waters). The observed FDOM fractions indicated in the Trondheimsfjord area a strong human impact; Peak A and M were mostly observed (Coble, 2007). After the last station on the 28.07.2015 at 18.00 (station 68) in the evening we headed back towards Trondheim where the cruise terminated at the 31.07.2015 at 08.00 in the morning.

In summary, after the bad weather conditions in the North Sea and the resulting late departure from Bremerhaven the persistent proper to favorable weather and calm seas throughout the cruise HE448 resulted in no time lost or equipment failure. Microscopic observations revealed a plankton community typical for summer plankton and typical for the area. Species isolation on board was quite successful. Finally the establishment of 32 clonal cultures was possible. These cultures turned out to represent seven different species: *Azadinium spinosum*, *A. obesum*, *A. poporum*, *A. dalianense*, *A. trinitatum*, *A. polongum*, and *Amphidoma languida*. The scientists accomplished the scientific objectives and we thereby gained important comparative insights into plankton dynamics and diversity, particularly of HAB taxa, in coastal/fjord systems of Norway. Analyses are still ongoing.

5 Methodology and Instrumentation

5.1 Physical Oceanography and Bio-optics

5.1.1 Oceanographic parameters from the ship's CTD

CTD casts were performed with a Seabird 'sbe911+' CTD probe with sampling rosette (onboard device) at each station, as an initial activity at the station to determine further key discrete sampling depths, e.g. to locate chlorophyll maxima. Live data acquisition was carried out via CTD-client onboard and data post-processing with Seasoft V2. Salinity and depth were calculated from pressure values (UNESCO, 1983), and temperature was corrected to ITS-90 (Preston-Thomas, 1990). The CTD was equipped with additional sensors for turbidity, fluorescence and oxygen. All CTD data will be published via Pangaea® (www.pangaea.de). The master track of *R/V Heincke* is already published (doi:10.1594/PANGAEA.855525). CTD data are under process and will be published as soon as possible also linked to already published cruise track and to cruise identifier HE448.

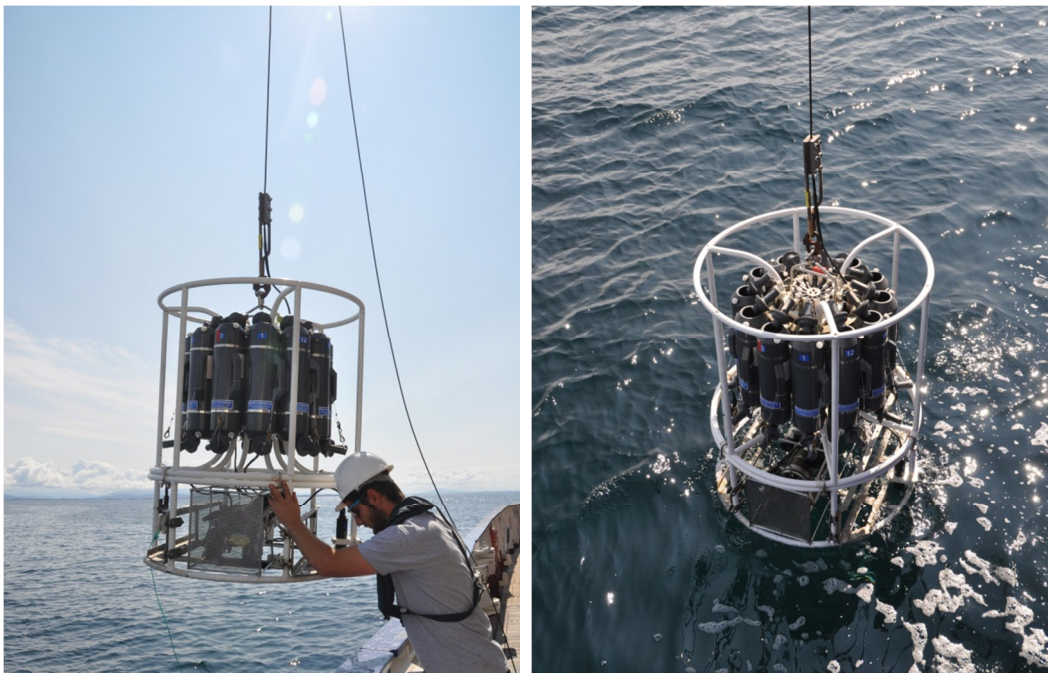

Figure 1: Onboard device Seabird 'sbe911+' CTD with sampling rosette at station work on *R/V Heincke*.

5.1.2 CDOM/FDOM and Chlorophyll, SPM from water samples

Water samples were collected at each station from defined depths to measure colored dissolved organic matter (CDOM), suspended particulate matter (SPM) and chlorophyll a (chl *a*). Immediately after sampling CDOM and FDOM samples were filtered under low vacuum through 0.2 µm membrane filter (Sartorius, Germany). The filtration unit had been pre-

rinsed with Milli-Q water (Millipore, USA) to avoid contamination, followed by sample water (~100 mL). Samples were directly analysed onboard.

In a 0.01 m quartz cuvette, pre-rinsed twice with filtered seawater, both absorbance spectra and fluorescence excitation-emission matrices (EEM) for FDOM were measured with a spectrofluorometer (Aqualog®, Horiba Scientific, Germany). Measurements were performed using ultrapure water as reference. The scan ranged from 240 to 600 nm with an excitation increment of 2 nm, an emission increment of 0.8 nm, and an integration time of 2 s. From these data, the absorption coefficient $a(\lambda)$ was derived at wavelength λ according to $a(\lambda) = 2.303D\ (\lambda)/L$, where L is the path length in meters and D the absorbance measured by the instrument. The EEM data were scanned for peaks according to Coble (2007). Additionally, CDOM absorbance was measured in 0.1 m quartz cuvettes with a UV-VIS-spectrophotometer (UV-2700, Shimadzu). Samples were scanned at medium scan speed with an increment of 0.5 nm in the spectral range between 200 nm and 800 nm. Ultrapure water was used as reference. Sample and reference cells were pre-rinsed twice with sample and purified water before analysis. Absorbance values A(λ) were baseline corrected and converted to the absorption coefficient aCDOM(λ) [m-1] following $aCDOM(\lambda)=2.303\times A(\lambda)/L$, where λ is the wavelength and L is the path length of the cuvette in meters.

Figure 2 Aqualog® spectrofluorometer to determine absorbance spectra and fluorescence excitation-emission matrices (EEM) for CDOM/FDOM (left). Shimadzu UV2700 to determine CDOM absorbance spectra (right).

For SPM determination, water samples (in triplicate, up to 12 L volume) were filtered through pre-combusted and pre-weighed Whatman GF/F™ filters, pre-washed and rinsed with MilliQ water. Filters were frozen immediately at -25 °C and reweighed in the laboratory after the cruise. SPM concentrations were normalized to 1 L. To determine Chl *a*, samples (in triplicate, up to 2 L volume) were filtered through pre-combusted Whatman GF/F™ filters with nominal pore size of 0.7 µm, pre-washed and rinsed with 0.2 µm filtered seawater. Filters were stored onboard at -80 °C and analysed in laboratory afterwards via extraction with acetone followed by fluorometric examination and calculation of chlorophyll *a* concentrations according to EPA method 445 (Arar & Collins, 1997).

5.1.3 Integrating cavity measurements

Conventional PSICAM: For analyzing the discrete samples in terms of their absorption properties, a PSICAM (Röttgers et al., 2005) was used. Sample and reference measurements were performed in triplicate. Purified water (MilliQ, >18.2 MΩ) was measured as reference before each sample. Light transmission of the sample was calculated as ratio of light intensity measured when the cavity was filled with sample water and when it was filled with the reference. Subsequently, this transmission was used to calculate the total absorption of water constituents in the sample (a_{p+cdom}, with p standing for the particulate absorption and cdom for the absorption of the dissolved fraction). After each sample measurement, the cavity was rinsed with purified water. At least once a day, the reflectivity of the cavity was determined using a freshly prepared nigrosine dye with known absorption and purified water as reference. After this 'calibration'-procedure the cavity was bleached for 15 min with NaOCl solution (0.2 %, Riedel de Haën, Germany), followed by rinsing with purified water. Details regarding the theoretical background of the PSICAM, the calibration procedure, and the equations used for the different calculations can be found elsewhere (Kirk, 1995; Leathers et al., 2000; Röttgers et al., 2005). Absorption coefficients were calculated using the mean value for the reflectivity from all calibrations. Finally, data were corrected for the influence of salinity and temperature on the absorption of the water itself, using coefficients given by Pegau et al. (1997). Data for salinity were obtained from the FerryBox system, while the temperature of both sample and reference was determined immediately before the respective measurement.

The HyAbS which is under development within the EU project NeXOS is based on a flow-through PSICAM (Wollschläger et al., 2013): The conventional PSICAM cavity made from Teflon was modified with water inlet and outlets, enabling a continuous operation in flow-through mode. An outlet at the top of the cavity allows air bubbles to leave, while one at the bottom serves as an outlet for larger and heavier particles which might otherwise accumulate in the cavity. Compared to the above mentioned flow-through PSICAM, the cavity was re-designed to be more pressure-resistant and it was also equipped with larger outlet openings. Sample water and other necessary liquids were pumped into the cavity by a membrane pump (Flojet, Xylem, USA), guided by a system of tubes and solenoid valves (Bürkert Fluid Control Systems, Germany). Light was provided by a 150-W IT 3900 lamp (Illumination Technologies, USA). The light field within the cavity was measured in the wavelength range of 400 to 710 nm by a Ramses UV/VIS-spectrometer (TriOS Mess-und Datentechnik GmbH, Germany) in 2014, or an Avaspec-ULS2048XL UV/VIS-spectrometer (Avantes, The Netherlands) in 2015, respectively. Light intensity spectra were collected every five seconds and averaged in one minute intervals. To correct for fluctuations in bulk light intensity of the lamp, this intensity was also measured inside the lamp and used for data normalization.

All components of the HyAbS were controlled by custom-made, LabView-based software, allowing either a semi-automated filling and emptying of the system by switching the appropriate valves and pumps, or an automated, programmable measuring procedure. In both cases, the general procedure follows that of the manually operated PSICAM as described above. However, due to the continuous sampling principle, reference measurements could not be conducted according to each sample measurement. Instead, they were performed in larger time intervals (one to several hours in case of the semi-automated trial, every 1.5 hours in case of the complete automated trial). For the time in between, reference values were interpolated linearly. Calibration of the HyAbS was performed at least once per day (four times in case of the complete automated run) using nigrosine dye as for a conventional PSICAM.

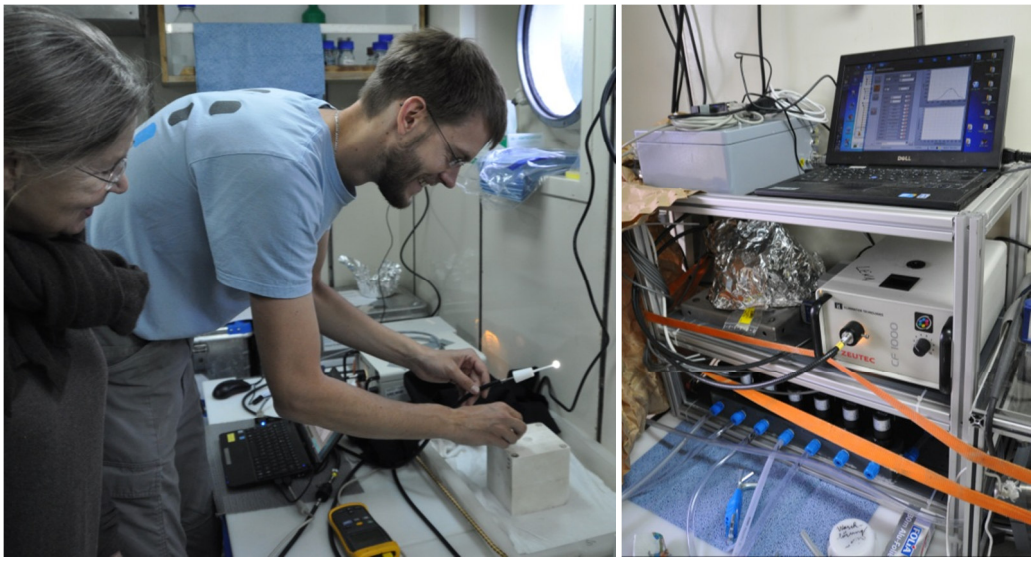

Figure 3 Conventional PSICAM (left) and flow-through version fed with sampled water from the FerryBox (right).

5.1.4 Bio-optical parameters from the profiling system

A HyperPro II profiling system (Satlantic, Halifax, Canada) was used to acquire bio-optical data for different parameters. The profiler consists of one hyperspectral irradiance and one hyperspectral radiance sensor, as well as fluorescence and backscatter sensors and an integrated CTD. A second hyperspectral irradiance sensor was mounted on the research vessel for reference measurements. On the profiler, the irradiance sensor measures downwelling and the radiance sensor upwelling light. The fluorescence sensors measure chlorophyll, CDOM, phycoerythrin and phycocyanin fluorescence signals. The backscatter sensor retrieves data at 470 nm and 700 nm.

Profiler measurements were conducted at selected stations depending on sea and weather conditions. At these stations, three casts at the back of the ship were typically performed in free-falling mode. At each cast, the profiler was lowered until the downwelling light values were of the same order of magnitude as the background noise level of the sensor.

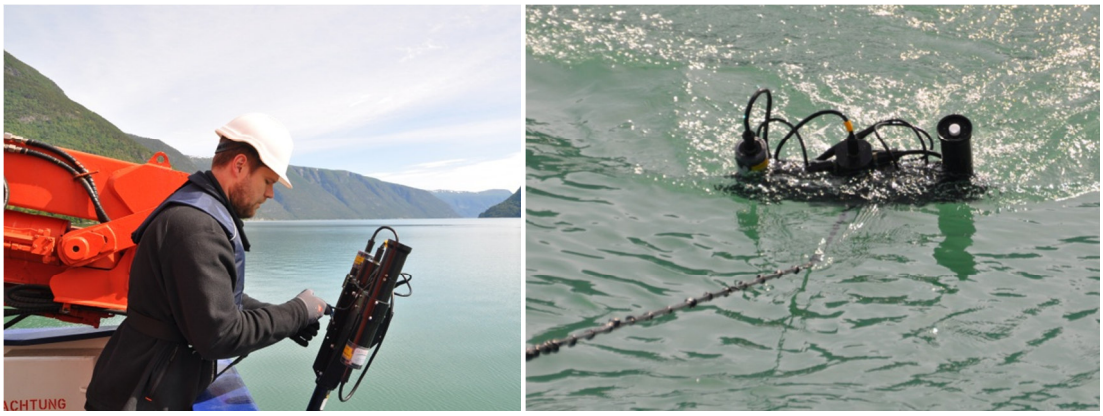

Figure 4 Hyperspectral profiling system Satlantic Inc. (Canada) to determine bio-optical data during free fall mode of *R/V Heincke*.

5.1.5 Underway data from the FerryBox

A FerryBox is a flow-through system deployed as an underway device for ship expeditions and for attendant measurements during stationary operations. The system provides basic data at high spatial and temporal resolution for various parameters, e.g. salinity, temperature (at the intake and inside the system), chlorophyll-fluorescence, CDOM-fluorescence, turbidity, and dissolved oxygen. An additional advantage is the expandable design of the system to integrate further sensors. For multi-parameter sensing, water was pumped from the moonpool of the ship. Measurements were performed at a sampling interval of 1 min. On this cruise, a second box (FerryBox AddOn) equipped with an UV- and a VIS-spectrophotometer, was used to collect absorption spectra to, e.g. improve current processing algorithms of optical nitrate detection.

Figure 5 FerryBox system (left). AddOn box equipped with an UV- and a VIS-spectrophotometer (right).

5.1.6 Ocean color sensing

Water transparency measurements were performed with a 50 cm Secchi disc at each station depending on sea and weather conditions to determine the penetration of light. The Forel-Ule (FU) color scale is a device that is composed of 21 colors, from 'indigo blue' to 'cola brown', and represents the range of colors that can be found in the open sea, coastal, and continental waters. Based upon a historical background, this provides an estimation of the present water constituents influencing the water color. The color of the water was determined over a Secchi disc at half the disc's depth (where the disc disappears from sight) at each day station.

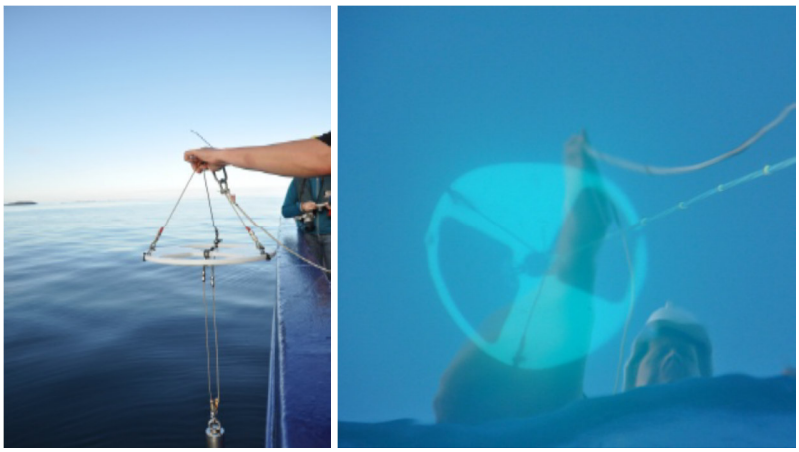

Figure 6 Secchi disc above (left) and in water (right).

When classical measurements were conducted additionally a smartphone app for FUI determination was used. Furthermore, the chl *a* content of water samples was determined in the lab by the use of a smartphone with a special designed adapter for cuvettes. Both measurements were part of the EU project Citclops (www.citclops.eu).

Above-water hyperspectral radiometric observations were conducted during the whole cruise. A radiometer setup with a RAMSES-ACC hyperspectral cosine irradiance meter to measure *ES* (λ) (downwelling solar irradiance), and two RAMSES-ARC hyperspectral radiance meters (one set-up at starboard, one at the port side of the ship) to measure *Lsfc* (θ*sfc*,Φ, λ) (upwelling water-leaving radiance) and *Lsky* (θ*sky*, Φ, λ) (sky-leaving radiance) were installed on the ships foremast (TriOS GmbH, Germany). Hyperspectral measurements were collected at 5 min intervals over a spectral range λ = 320 – 950 nm. Data processing will be done according to Garaba & Zielinski (2013). Furthermore newly developed processing algorithms will tested with the collected data set.

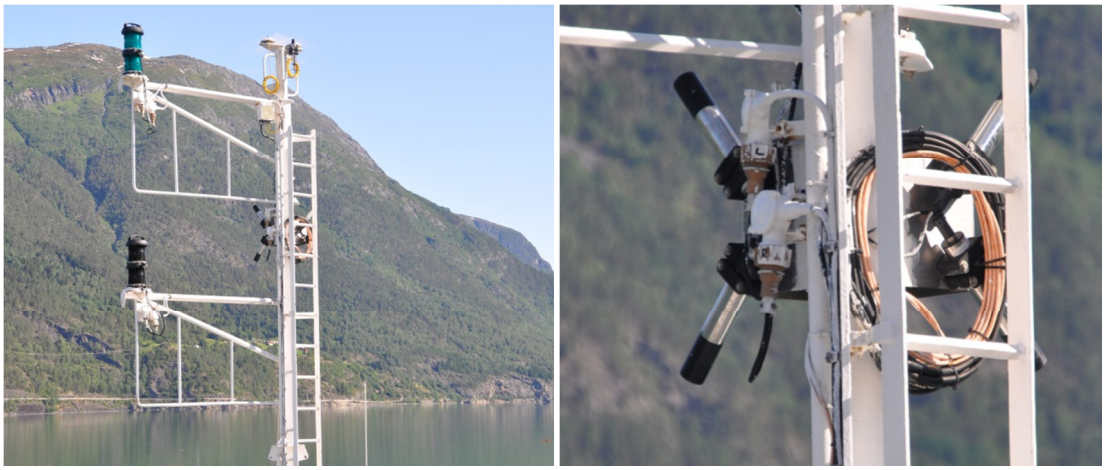

Figure 7 The radiometric setup on the foremast of *R/V Heincke*. One RAMSES-ACC hyperspectral cosine irradiance meter was installed at the top of the mast to measure total downwelling solar irradiance (yellow cable).

5.2 Toxins

5.2.1 Toxin extraction from plankton

The cell pellets from the plankton net tows were harvested by centrifugation, suspended in 500 µL methanol for lipophilic toxins or 0.03 M acetic acid for hydrophilic toxins, and subsequently extracted by homogenization, filtered and measured by LC-FLD for hydrophilic paralytic shellfish poisoning (PSP) toxins and by LC-MS/MS for all other toxin groups. Polycarbonate filters with 20 µm pre-screened samples from the rosette bottles were repeatedly rinsed with 1 mL methanol until complete decolouration of the filters. The methanolic extracts were filtered and measured by LC-MS/MS.

5.2.2 Liquid chromatography with fluorescence detection (LC-FLD)

The aqueous extracts were analyzed by reverse-phase ion-pair liquid chromatography with fluorescence detection (LC-FD) and post-column derivatization following minor modifications of previously published methods (Diener et al. 2006, Krock et al. 2007). The LC-FLD analysis was carried out on a LC1100 series liquid chromatography system equipped with a Phenomenex Luna C18 reversed-phase column (250 mm X 4.6 mm id, 5 µm pore size) precolumn. The column was coupled to a PCX 2500 post-column derivatization system. The injection volume was 20 µL and the autosampler was cooled to 4 °C. The eluate from the column was oxidized with periodic acid before entering the 50 °C reaction coil, after which it was acidified with nitric acid. The toxins were detected by dual-monochromator fluorescence (λ_{ex} 333 nm; λ_{em} 395 nm).

5.2.3 Lipophilic toxin measurement of field samples by LC-MS/MS

Mass spectral experiments were performed on a triple quadrupole mass spectrometer coupled to an Agilent model 1100 LC onboard. After injection of 5 µl of sample separation of lipophilic toxins was performed by revered phase chromatography on a C8 phase and gradient elution was performed with water and acetonitrile. The chromatographic run was divided into 3 periods: one domoic acid, one for gymnodimine and spirolides, and one for okadaic acid, dinophysistoxins, pectenotoxins, yessotoxin and azaspiracids. Selected reaction monitoring (SRM) experiments were carried out in positive ion mode (Krock et al. 2013).

Figure 8 The LC-MS/MS system installed in the dry lab onboard *R/V Heincke*.

5.3 Microplankton - and Nanoplankton

On every station plankton was sampled by vertical net tows sampling the upper 30 m column by a 20 µm net. Plankton communities were characterized by on-board microscopy of live subsamples of these net tows. Subsamples were also fixed with Paraformaldehyde. In addition, Niskin bottle samples were used to check the nanoplankton community for the presence of small and potentially toxic species e.g., of the dinophycean genus *Azadinium*. Therefore, 1 l samples were gently concentrated using 5 µm pore size polycarbonate filters and inspected using an inverted microscope. On every station, 50 ml bottle-samples were fixed with lugol (1% final concentration) for quantitative plankton analysis. Moreover, Niskin bottle samples from three sampling depth were prescreened (20 µm mesh) and combined, and 2 – 3 liter were concentrated on 5 µm pore sitze polycarbonate filters, one filter each for anlysing azaspiracids and one filter for DNA extraction and subsequent qPCR probe application. Both samples were stored at -20°C.

When species of interest were detected in the plankton, single cells were isolated by microcapilary into single wells of 96 well plates. Plates were on-board incubated at 10°C and 50 µE m^{-2} s^{-1}. After the cruise, plates were brought back to the laboratory and processed for clonal culture establishment.

6 Preliminary Results

6.1 Physical oceanography and bio-optics

6.1.1 Oceanographic parameters from the ship's CTD

In this chapter the CTD profiles for the coast of Norway and the adjacent Fjord systems (Sognefjord, Trondheimsfjord) are shown (station plot, Figure 9). For all sections, temperature, salinity, oxygen, transmission, and fluorescence profiles are plotted over the distance of each station [km].

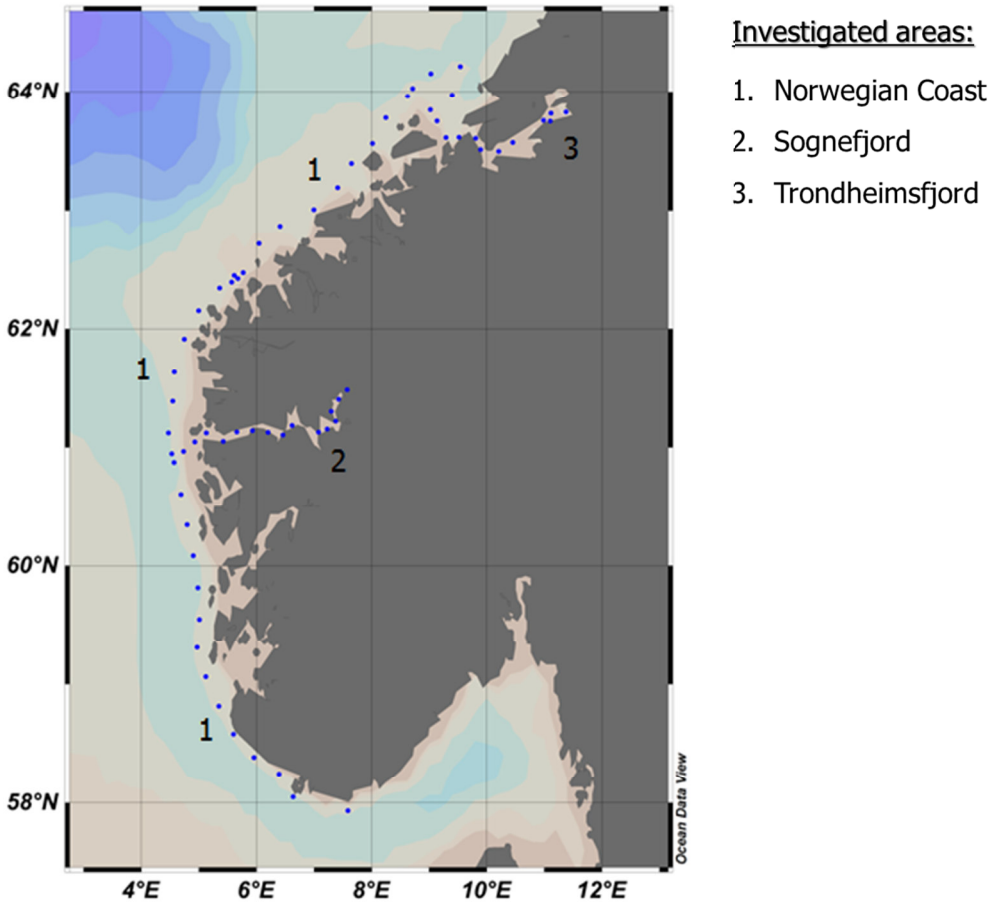

Figure 9 Map of the investigated areas during the cruise HE448 aboard *R/V Heincke*. Within the fjord systems of Sognefjord and Trondheimsfjord an overall set of 15 stations each were sampled.

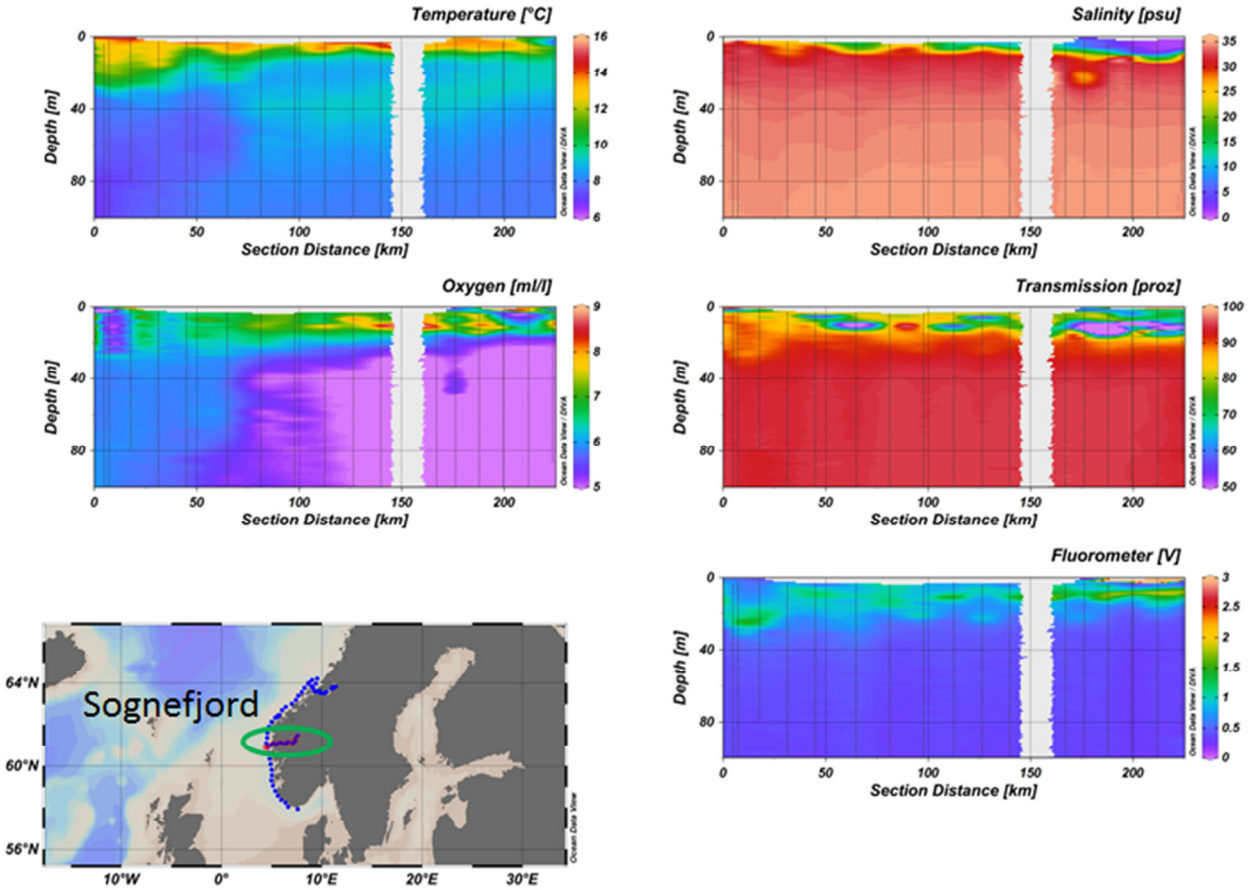

Figure 10 Map of the investigated Sognefjord area and associated CTD profiles for 100 m depth. Plots are shown over the section distance [km] from outside to inside stations (marked with a black line) for temperature [ITS-90, °C], salinity, oxygen [mL/L], transmission [%] and fluorescence [V].

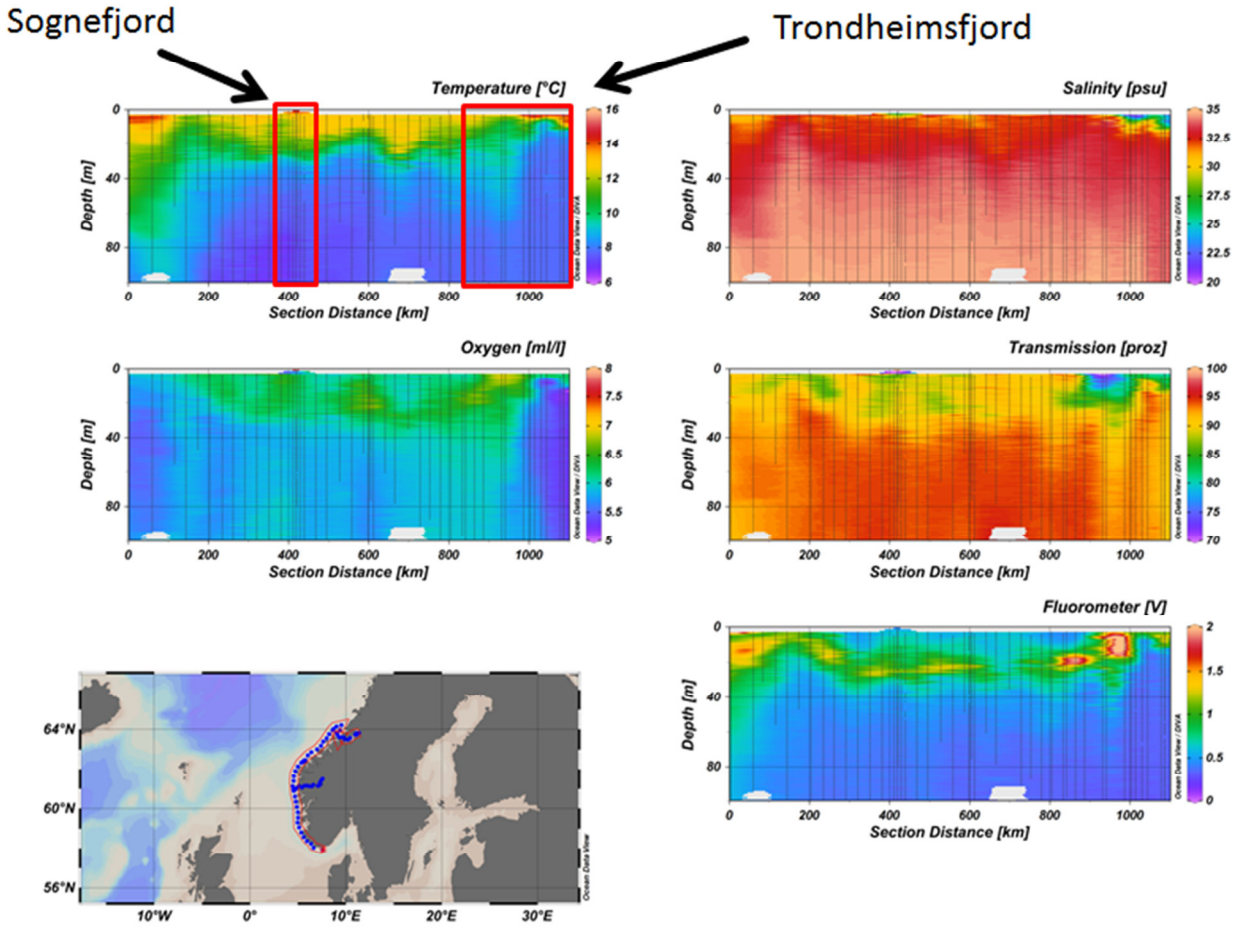

Figure 11 Map of the investigated areas along the Norwegian coast, the Sognefjord, and the Trondheimsfjord as well as their associated CTD profiles for 100 m depth. Plots are shown over the section distance [km] from the lowest station at the south tip of Norway to the latest inside station of Trondheimsfjord (marked with a black line) for temperature [ITS-90, °C], salinity, oxygen [mL/L], transmission [%] and fluorescence [V].

6.1.2 CDOM/FDOM from water samples

Absorption coefficients of defined wavelengths and Coble peaks over the whole spectral range were determined from absorption and fluorescence data (excitation emission matrices, EEM compare Figure 12). FDOM Peaks were analyzed for water samples from 3 m depth for main peak components (Figure 13). A comparison between FDOM peaks as well as CDOM absorption coefficients and DOC concentrations will be done in further analysis.

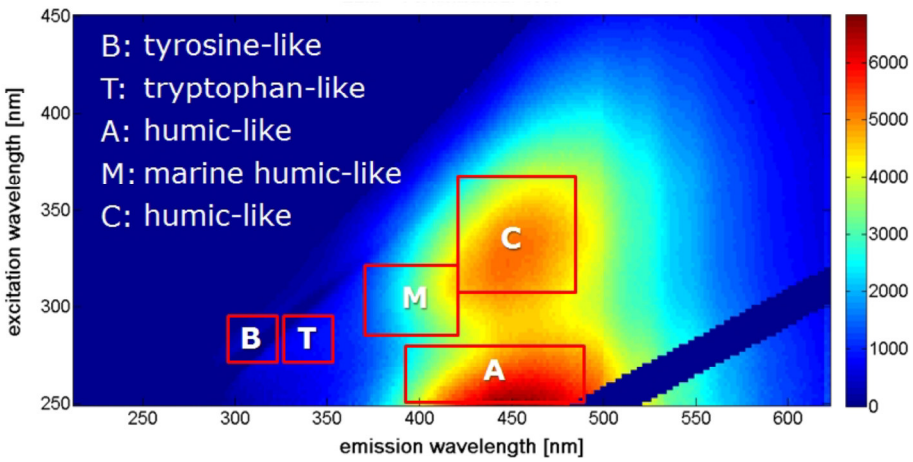

Figure 12 Example of an EEM indicating the important peak areas according to Coble (2007).

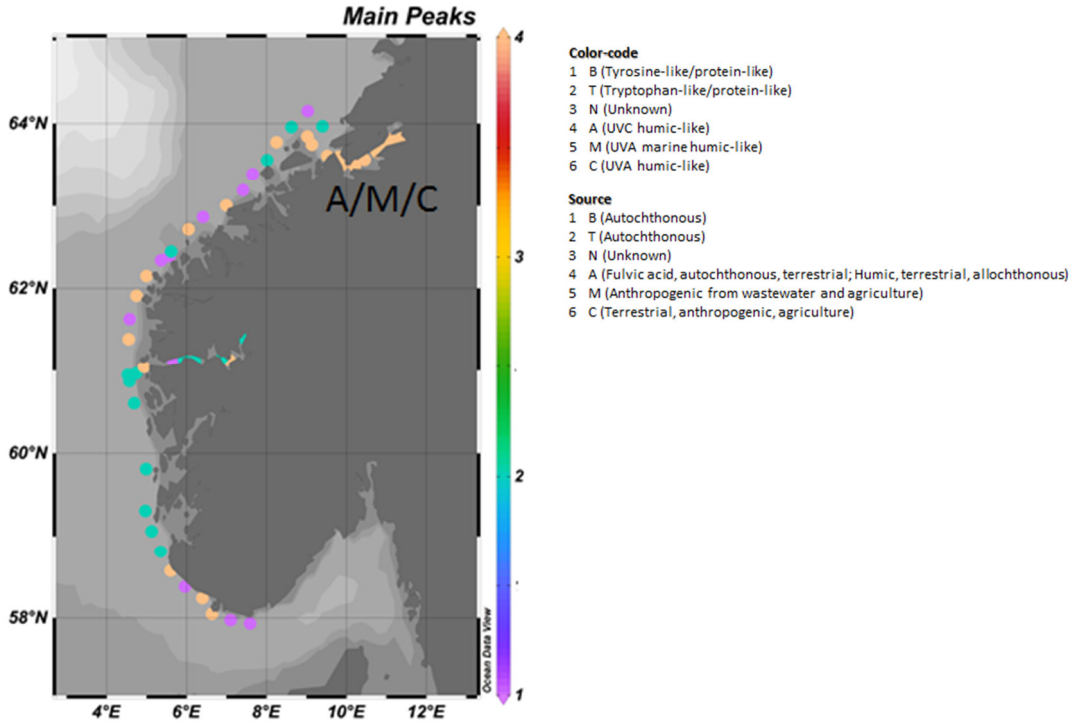

Figure 13 FDOM main peaks in water samples from 3 m depth. Especially tyrosine- and tryptophan-like components (1/2, peak B and T) were mainly found along the Norwegian coast and in the Sognefjord, whereas humic-like components (4, peak M) were dominant in the Trondheimsfjord.

6.1.3 Integrating cavity measurements

Differences were found in the absorption spectra of the different size classes indicating probably different types of phytoplankton. The red Chl *a* peak was used as a proxy for Chl *a*. A station example for the absorption characteristic of the different size classes of phytoplankton and the "all constituents" spectrum is shown in Figure 14.

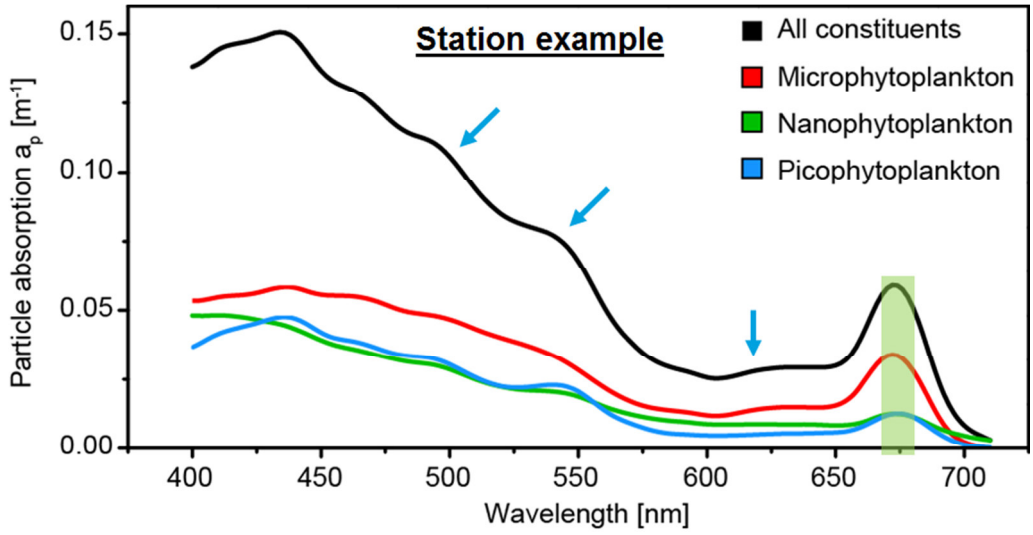

Figure 14 Example of absorption spectra of a selected station during HE448 showing the differences in shape characteristics between the three size classes (Microphytoplankton, red) > 20 µm, (Nanophytoplankton, green) 2-20 µm, and (Picophytoplankton, blue) > 2 µm. A sum up of all constituents is shown by the black line.

The correlation between both optical proxies (PSICAM Chl *a*, FerryBox Chl *a*, Figure 15) showed a solid linear correlation, the R^2 increases to 0.6 when the outlier is omitted. Comparable only low Chl *a* concentrations were observed. The fluorescence signal might be variable due to light acclimatization processes of the cells. Furthermore, the water mass measured by both systems might not be completely identical.

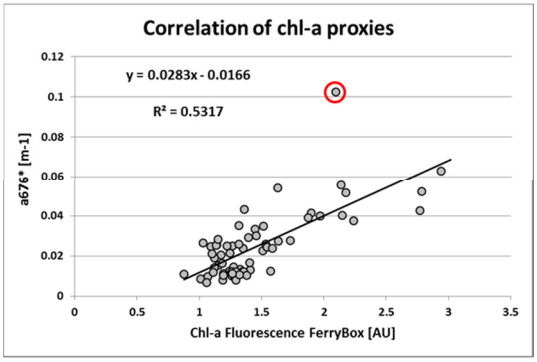

Figure 15 Correlation between a676 [m^{-1}] from PSICAM measurement and Chl-a fluorescence from FerryBox system.

The Chl *a* distribution calculated from manually operated PSICAM measurements at the discrete depth of the "Deep Chlorophyll Maximum (DCM)" is shown for all sampled stations in Figure 16. The proportion [%] of each size class contributing to the total Chl *a* concentration is shown in Figure 17.

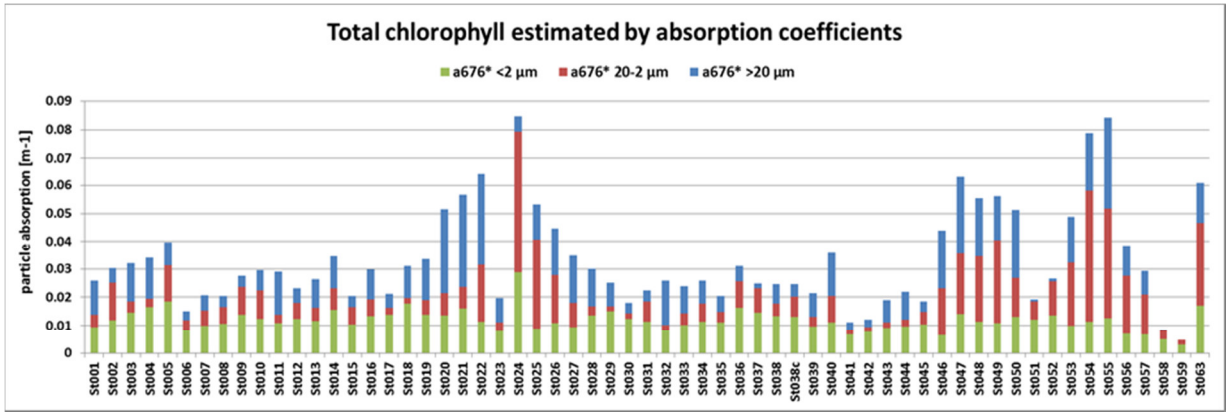

Figure 16 Total Chl *a* estimation by the absorption coefficient a676 for the three different size classes picophytoplankton a676 < 2 µm (green), nanophytoplankton a676 20-2 µm (red), and microphytoplankton a676 > 20 µm (blue).

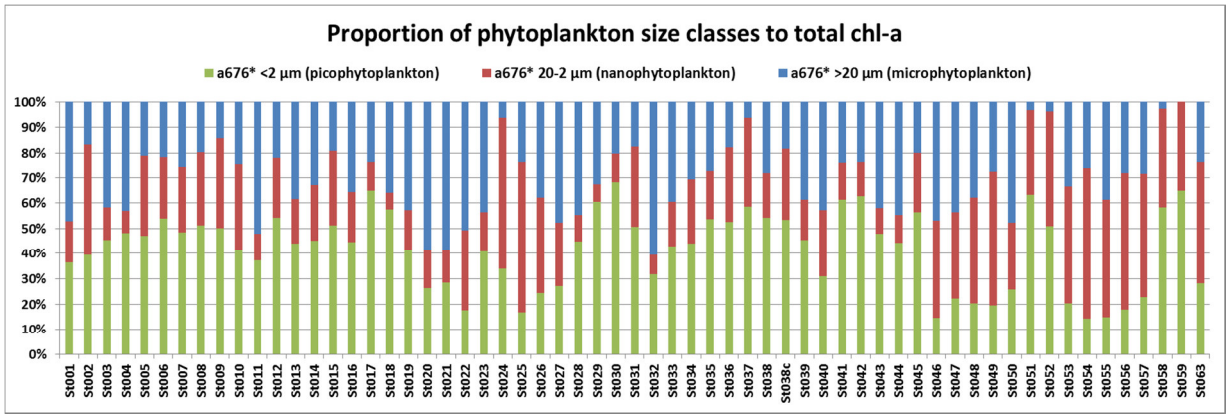

Figure 17 Proportion [%] of phytoplankton size classes to the total Chl *a* estimation with picophytoplankton a676 < 2 µm (green), nanophytoplankton a676 20-2 µm (red), and microphytoplankton a676 > 20 µm (blue).

The amount of chromophoric dissolved organic matter (CDOM) appears to influence the size distribution. The proportion of microphytoplankton to total Chl a is not affected, whereas the proportion of nanophytoplankton on total Chl *a* increases and the proportion of picophytoplankton to total Chl *a* decreases (Figure 18).

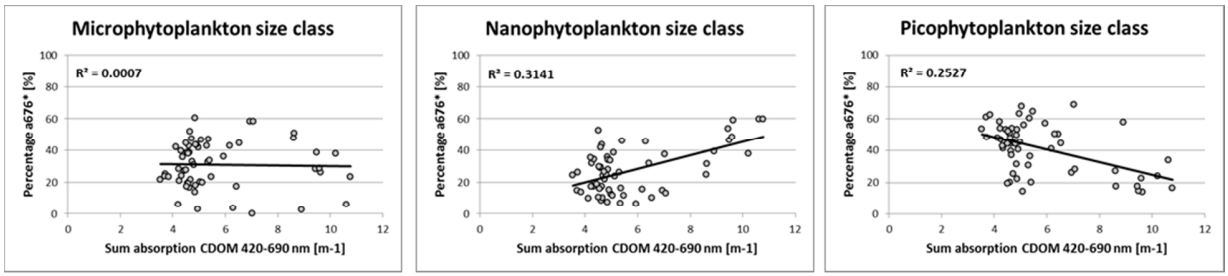

Figure 18 Correlation plots for the different size classes of phytoplankton. Microphytoplankton vs. the sum absorption of CDOM (left), nanophytoplankton vs. the sum absorption of CDOM (middle), and picophytoplankton vs. the sum absorption of CDOM (right).

The automation of the PSCICAM (HyAbs, ftPSCIAM - flow-through version) worked very well. Measurements of a whole spectrum were done every 5 seconds. Before further processing was done, an automated quality check of each spectrum was conducted; with (1) minimum between a400 and a700 >0 and (2) a690 > a700. In Figure 19 the measurements of the continuous operated ftPSCIAM over the whole cruise track is shown. Further shown is the signal of fluorescence sensor of the FerryBox system [AU]. As reference the point measurements of the PSICAM are plotted. The overall trend for PSICAM and FerryBox is very similar. Further investigations and corrections are in process.

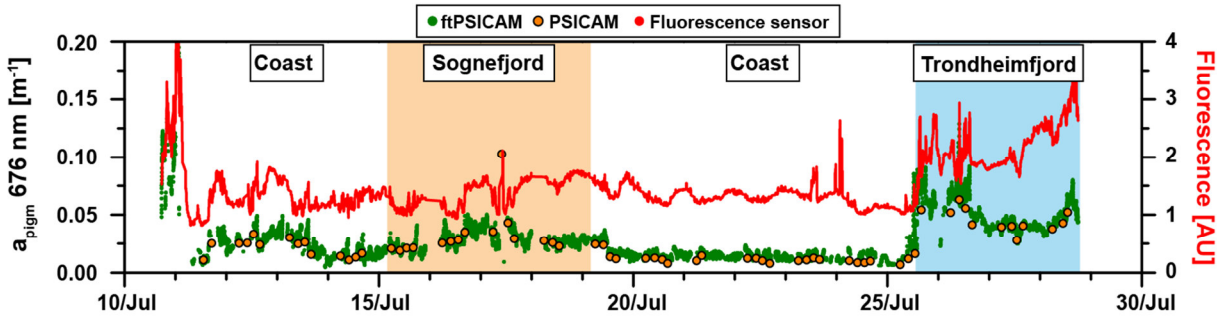

Figure 19 Measurements of the continuous operated ftPSCIAM [a676] over the whole cruise track compared to the signal of fluorescence sensor of the FerryBox system [AU]. Additionally, as a reference the point measurements of the manually operated PSICAM are plotted in orange.

6.1.4 Bio-optical parameters from the profiling system

At all daytime station free-falling profiler measurements were conducted to determine light penetration in the water column. A comparison of two stations in the Sognefjord is shown in Figure 20. Station 15 (blue) represents an outer fjord station whereas station 22 (red) is at the end of the Sognefjord. Correlation plots of various parameters concerning the free-falling profiler are shown in Figure 21.

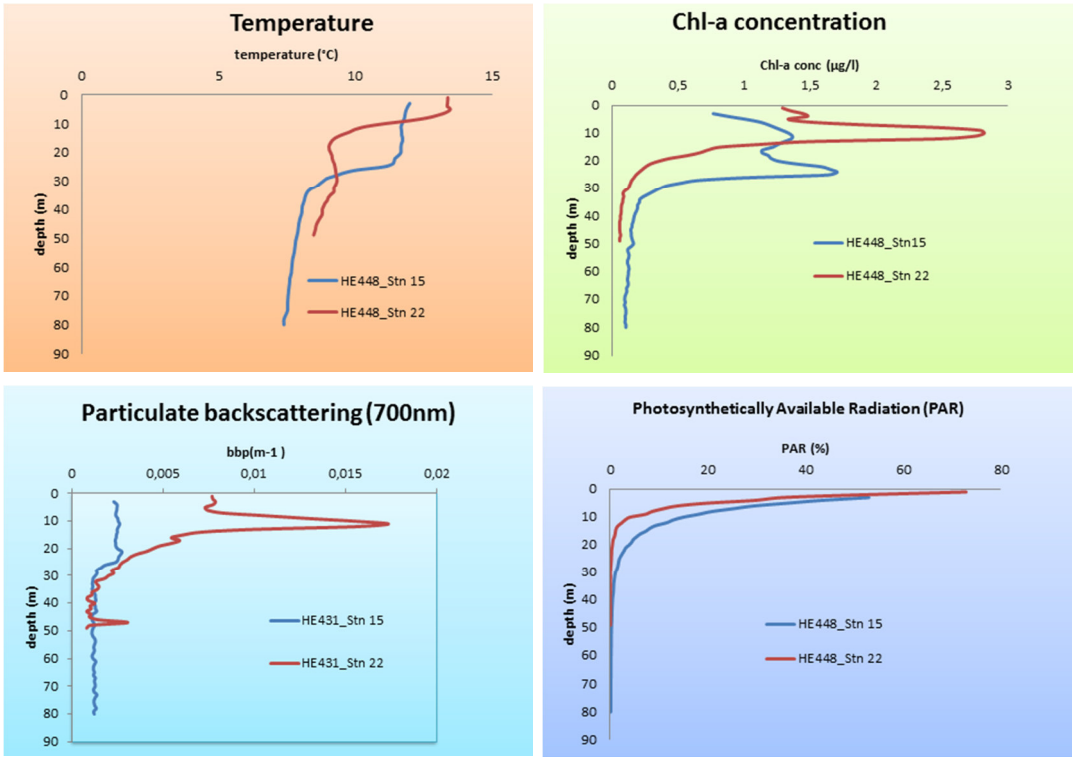

Figure 20 Comparison of two selected profiler stations in the Sognefjord. Station 22 was more stratified with higher backscattering due to particles in the water column and higher chlorophyll content. The 1% PAR depth for station 15 was 30m, but at station 22 this occurred at 16m.

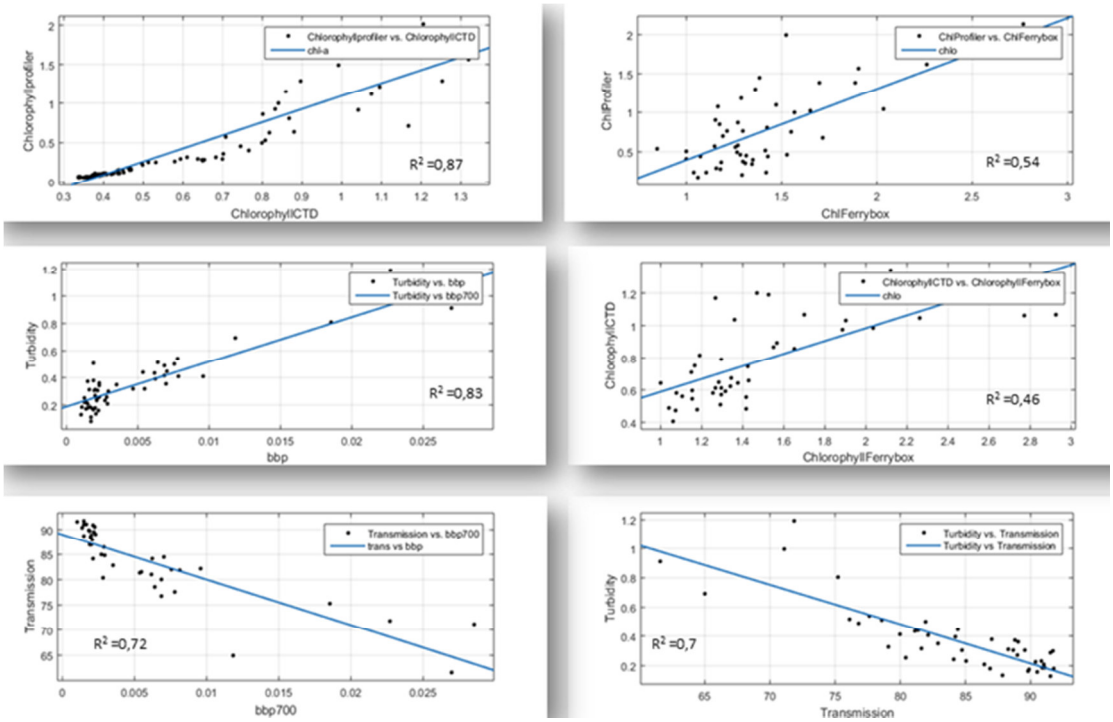

Figure 21 Correlation plots of various parameters showing good correspondence of results by combining CTD, FerryBox, laboratory, and profiler data. Chl *a* (profiler data) versus Chl *a* (CTD data; upper panel, left); Chl *a* (profiler data) versus FerryBox Chl *a* (upper panel, right); turbidity (FerryBox data) versus backscattering bbp700 (profiler data, middle panel, left); Chl *a* (CTD data) versus FerryBox Chl *a* (middle panel, right); transmission (CTD data) versus backscattering bbp700 (profiler data, lower panel, left), and turbidity (FerryBox data) versus transmission (CTD data, lower panel, right).

6.1.5 Underway data from the FerryBox

Continuous flow-through measurements for various parameters worked very well along the whole cruise track. Selected results for surface waters for temperature at the intake point, salinity, and fluorescence measurements are shown in Figure 22.

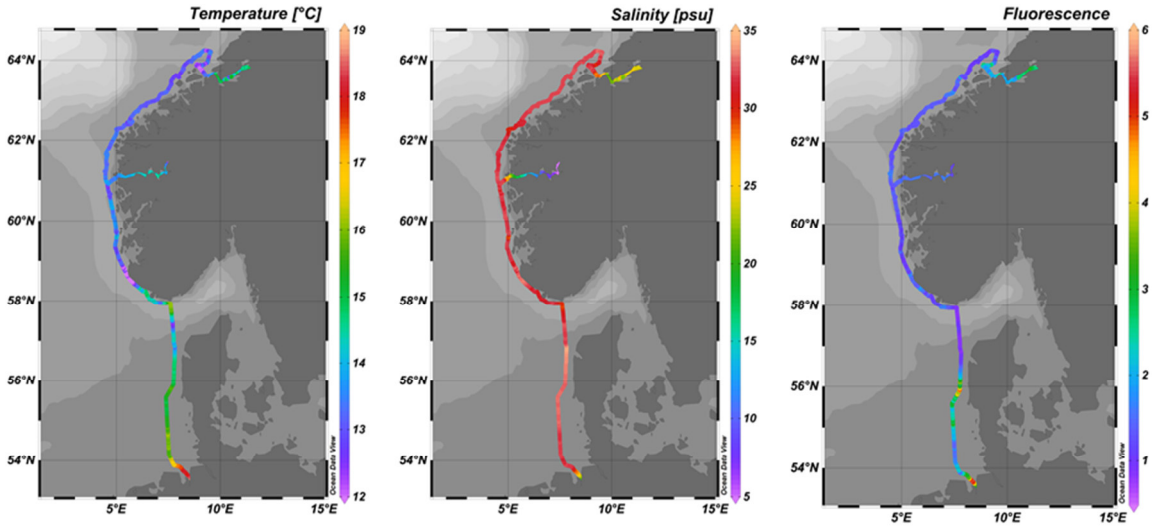

Figure 22 Temperature (left), salinity (middle), and fluorescence (right) measurements from FerryBox investigations in surface waters over the whole cruise track of HE448.

6.1.6 Ocean color sensing

At all stations, depending on weather conditions, daytime Secchi disc measurements and FUI observation were performed. The results can be seen in Figure 23 (left). Highest penetration depths were recorded for the upper Norwegian stations (~63°N) and close to the Trondheimsfjord, with values up to 18 m at the outer Trondheimsfjord station.

Forel-Ule indices (FUI) are shown in Figure 23 (right), representing open sea and coastal regions. High penetration depths correspond well with lower FUI values, clearly seen in the Trondheimsfjord. With further processing, more conclusions about existing components in the water (e.g., as an index of phytoplankton biomass in clearest waters) can also be drawn. Both data sets are needed as a reference for hyperspectral radiometric observations, as one goal is the determination of ocean color by radiometric measurements (compare Garaba et al, 2014). Analysis is still in progress.

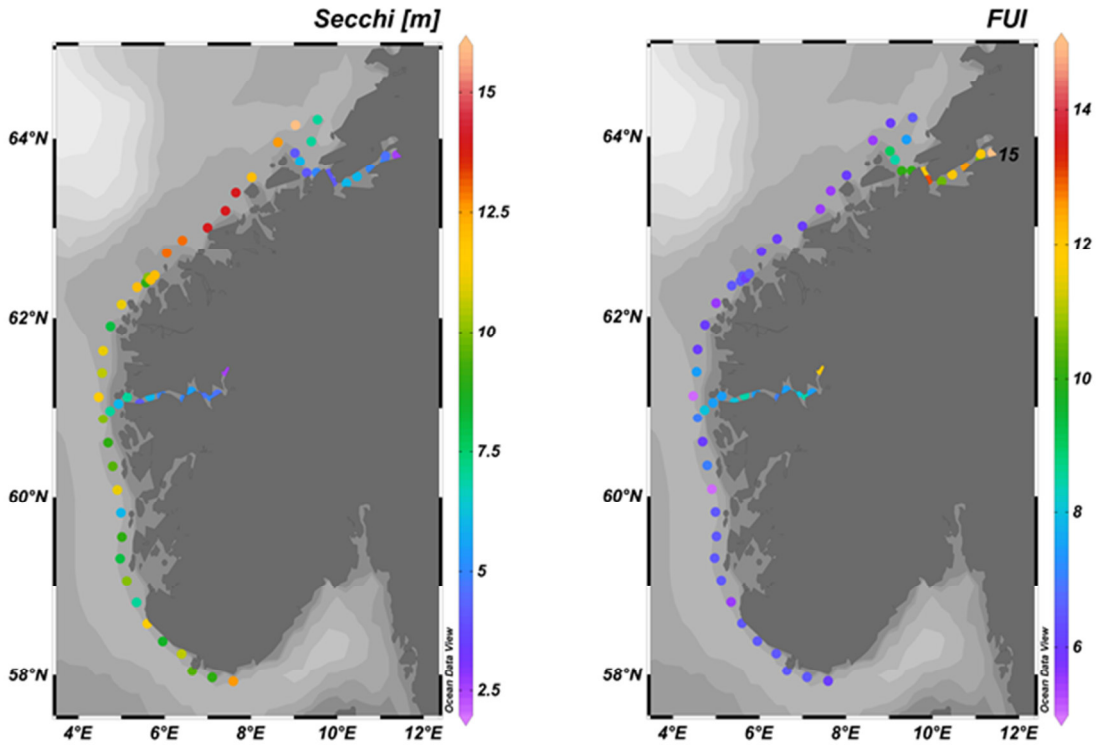

Figure 23 Measured Secchi depths (left) at daytime and Forel-Ule indices (FUI, right) determined for all stations along the Norwegian coast as well as the areas of Sognefjord and Trondheimsfjord.

6.2 Toxins

Hydrophilic paralytic shellfish poisoning (PSP) toxin were detected only in low concentrations towards the end of the expedition and especially in Trondheimsfjord (Figure 24). Among the PSP toxins detected in planktonic field samples only saxitoxin (STX) and gonyautoxins-2 and -3 were detected, whereas N-sulfocarbamoyl B- and C-toxins, which are the main variants produced by North Sea *Alexandrium tamarense* (Tillmann et al. 2009), were completely absent. Other toxins produced by the genus *Alexandrium* are spirolides (SPX) (Cembella et al. 2000) and as found recently also gymnodimines (GYM) (Van Wagoner et al. 2011, Harju et al. 2016). Whereas GYM were hardly detected in samples along the expedition transect, the occurrence of SPX generally coincided with the occurrence of PSP. The general coincidence of PSP and SPX in planktonic samples and the unusual PSP profile indicate that *Alexandrium ostenfeldii* could be the producer of both toxin classes.

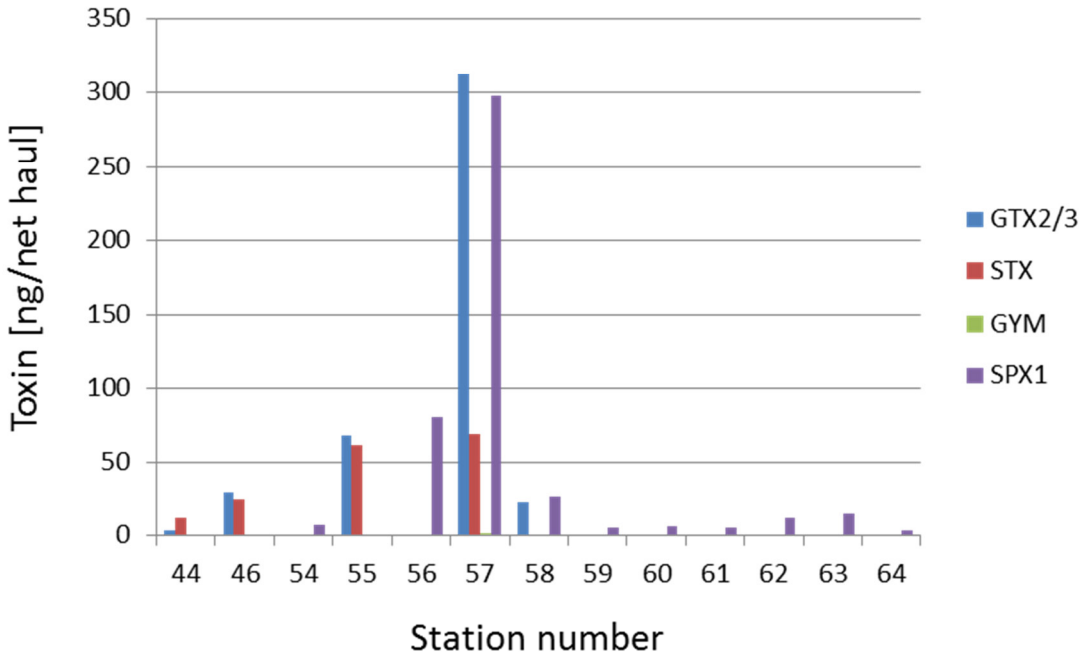

Figure 24 Quantities of the putative *Alexandrium* toxins PSP (STX and GTX2/3), SPX-1 and GYM found on the expedition transect.

As in the case of the hydrophilic toxins, along the expedition transect only low concentrations of phycotoxins were detected (Figure 25). The only exception consisted in Sognefjord where elevated concentrations of dinophysistoxins (DTX) including okadaic acid (OA) and pectenotoxins (PTX) were found. At two stations total toxin content exceeded 3 ng per net haul. These toxins are known to be produced by several species of the genus *Dinophysis*, which has been known to occur in Sognefjord (Wenche Eikrem, personal communication).

Noteworthy is the persistence of the characteristic toxin profile which includes the otherwise rarely found 36-*S*-PTX-12 and its enantiomer 36-*R*-PTX-12 that has been described for the first time by Miles et al. (2004).in the northern part of the expedition.

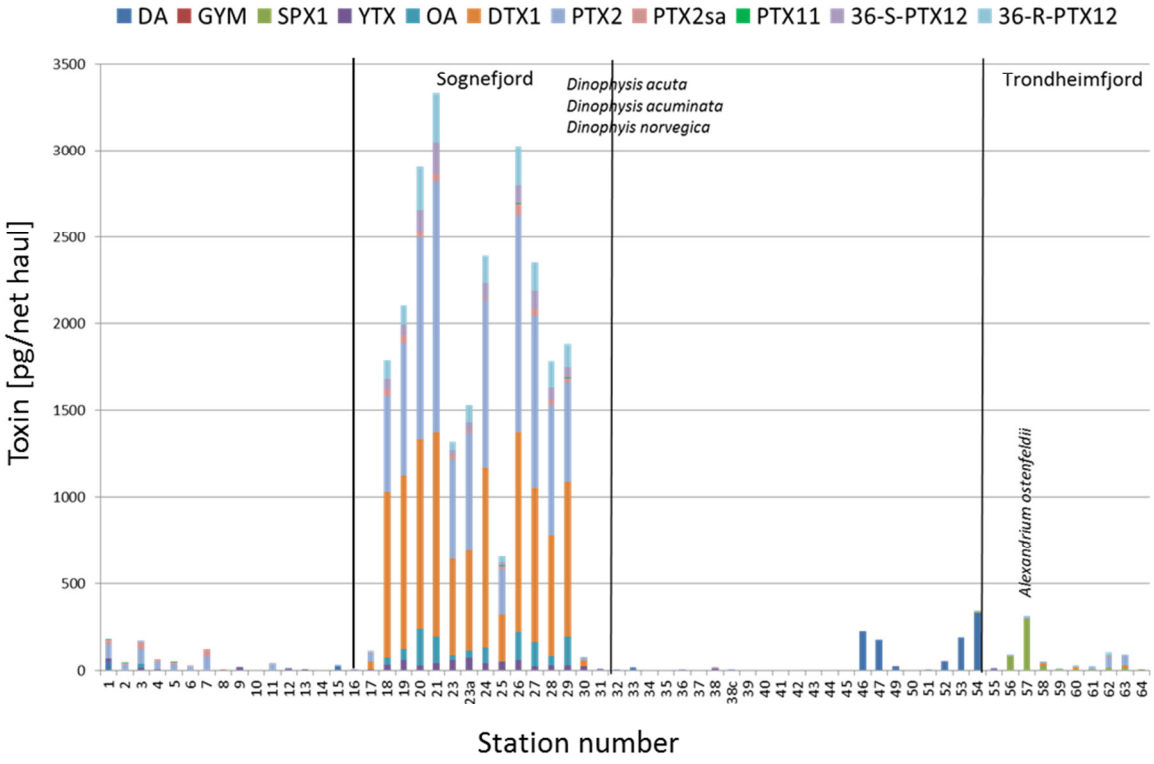

Figure 25 Lipophilic phycotoxins detected on the expedition transect: Domoic acid (DA), gymnodimine A (GYM), 13-desmethyl spirolide C (SPX-1), okadaic acid (OA), dinophysitiontoxin-1 (DTX-1), pectenotoxins-2 (PTX-2), pectenotoxins-2 seco acid (PTX-2sa), pectentoxin-11 (PTX-11), 36-*S*-pectenotoxins-12 (36-*S*-PTX-12) and 36-*R*-pectenotoxin-12 (36-*R*-PTX-12).

6.3 Microplankton - and Nanoplankton

Generally, micro- and nanoplankton, as inferred from live on-board microscopical observations, were composed of plankton species typical for summer plankton and typical for the area. Diatoms were dominating just a few stations with species of the genus *Pseudo-Nitzschia* as the most important species. Net plankton was dominated by dinophyceae, with large species of *Ceratium* (*C. tripos*, *C. furca*, *C. fusus*, *C. macroceros*) and a diverse community of *Protoperidnium* spp. being most abundant. Niskin bottle samples revealed a high abundance and high diversity of Nanoplankton with *Emiliania huxleyi* and various other coccolithophores in high abundances. The targeted on-bord microscopical search for species of the genus *Azadinium* and *Amphidoma* revealed the presence of species at almost all stations (Figure 26), although at very low abundances. This reflects the fact that toxin analyses for the corresponding azaspiracid (AZA) compounds were negative for all stations of the cruise. Nevertheless microscopical analysis was confirmed by application of a genus specific molecular probe, which likewise revealed the presence of *Azadinium* spp., albeit at generally low abundance, at all stations. Species isolation on board was quite successful and finally leads to establishing 32 clonal cultures. These cultures turned out to represent seven different species: *Azadinium spinosum*, *A. obesum*, *A. poporum*, *A. dalianense*, *A. trinitatum*, *A. polongum*, and *Amphidoma languida*. For most of these species this is the first record for the Norwegian coast. All cultures are currently characterized in terms of molecular sequence information and in terms of presence of AZA compounds.

Other toxic species encountered were *Dinophysis* spp., which were found in higher abundance mainly in the Sognefjord. All three main species known from the North Sea area were present: *D. acuta*, *D. accuminata*, and *D. norvegica*. In contrast, species of the genus *Alexandrium* were almost completely restricted to the stations in the Trondheimsfjord. Culture establishment again was quite successful and now 27 different clonal isolates of *Alexandrium* spp. of that area are available. All isolates are currently investigated in detail for a morphological species determination, for their molecular signatures, and for the toxin profile. PSP toxins were detected in seven of the isolates with a toxin profile almost identical to *Alexandrium* isolates obtained from the West coast of Greenland. At least three isolated were identified as *A. ostenfeldii*. All these were found to produce spirolides but lacked PSP toxins.

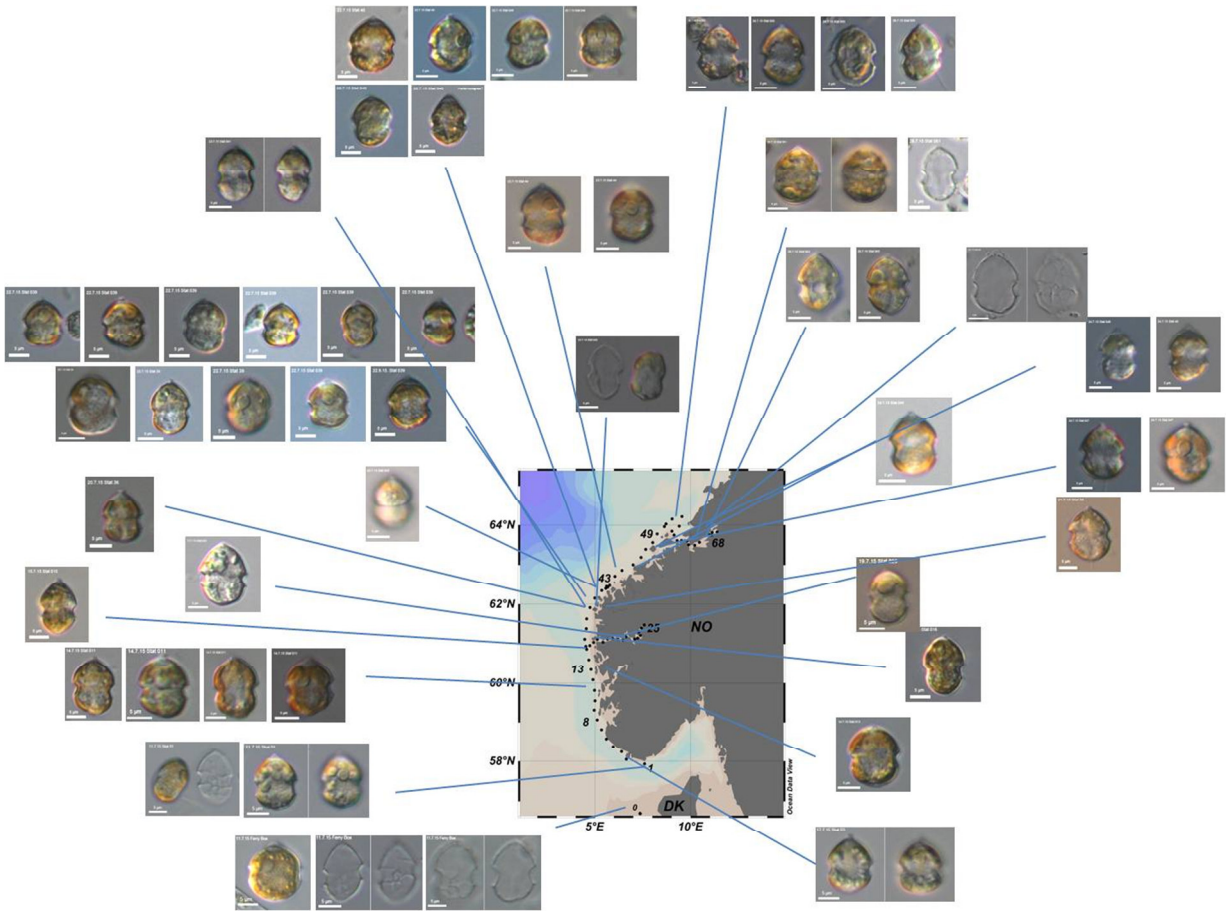

Figure 26 On-board live plankton documentation of the presence *of Azadinium*/*Amphidoma* spp. along the Norwegian coast.

7 Station List

ID	Station No	Date	Time	Latitude	Longitude
1	HE448/0001	11.07.2015	13:19:00	57° 56,12' N	007° 36,13' E
2	HE448/0002	11.07.2015	16:43:00	57° 56,72' N	007° 05,91' E
3	HE448/0003	12.07.2015	06:02:00	58° 03,03' N	006° 38,18' E
4	HE448/0004	12.07.2015	10:03:00	58° 14,66' N	006° 23,54' E
5	HE448/0005	12.07.2015	13:01:00	58° 22,95' N	005° 57,45' E
6	HE448/0006	12.07.2015	15:59:00	58° 34,60' N	005° 35,85' E
7	HE448/0007	13.07.2015	06:01:00	58° 48,84' N	005° 20,82' E
8	HE448/0008	13.07.2015	10:00:00	59° 03,78' N	005° 07,22' E
9	HE448/0009	13.07.2015	13:12:00	59° 18,56' N	004° 57,93' E
10	HE448/0010	13.07.2015	16:01:00	59° 32,90' N	005° 00,48' E
11	HE448/0011	14.07.2015	06:01:00	59° 48,86' N	004° 59,05' E
12	HE448/0012	14.07.2015	10:02:00	60° 04,91' N	004° 53,98' E
13	HE448/0013	14.07.2015	13:08:00	60° 20,67' N	004° 47,71' E
14	HE448/0014	14.07.2015	16:03:00	60° 36,19' N	004° 41,16' E
15	HE448/0015	15.07.2015	05:59:00	60° 52,24' N	004° 34,15' E
16	HE448/0016	15.07.2015	10:01:00	60° 57,85' N	004° 44,01' E
17	HE448/0017	15.07.2015	13:15:00	61° 07,24' N	005° 07,70' E
18	HE448/0018	15.07.2015	16:18:00	61° 07,73' N	005° 39,50' E
19	HE448/0019	16.07.2015	06:01:00	61° 07,33' N	006° 12,10' E
20	HE448/0020	16.07.2015	10:03:00	61° 11,07' N	006° 36,98' E
21	HE448/0021	16.07.2015	13:28:00	61° 07,61' N	007° 04,54' E
22	HE448/0022	16.07.2015	15:40:00	61° 08,33' N	007° 20,44' E
23	HE448/0023	16.07.2015	16:39:00	61° 13,16' N	007° 22,55' E
24	HE448/0024	17.07.2015	06:02:00	61° 24,12' N	007° 25,76' E
25	HE448/0025	17.07.2015	10:01:00	61° 28,86' N	007° 34,61' E
26	HE448/0026	17.07.2015	13:02:00	61° 18,04' N	007° 17,86' E
27	HE448/0027	17.07.2015	15:58:00	61° 09,11' N	007° 13,77' E
28	HE448/0028	18.07.2015	06:01:00	61° 09,27' N	006° 52,17' E
29	HE448/0029	18.07.2015	10:02:00	61° 06,18' N	006° 27,50' E
30	HE448/0030	18.07.2015	13:16:00	61° 08,35' N	005° 55,61' E
31	HE448/0031	19.07.2015	06:14:00	61° 03,09' N	005° 24,97' E
32	HE448/0032	19.07.2015	09:58:00	61° 02,68' N	004° 55,82' E
33	HE448/0033	19.07.2015	13:09:00	60° 56,73' N	004° 31,75' E
34	HE448/0034	19.07.2015	15:59:00	61° 07,27' N	004° 28,20' E
35	HE448/0035	20.07.2015	05:59:00	61° 23,18' N	004° 32,84' E
36	HE448/0036	20.07.2015	10:10:00	61° 37,78' N	004° 34,36' E
37	HE448/0037	20.07.2015	13:41:00	61° 54,78' N	004° 44,67' E
38	HE448/0038	20.07.2015	16:25:00	62° 09,06' N	004° 59,90' E
39	HE448/0039	21.07.2015	05:59:00	62° 20,46' N	005° 21,55' E

ID	Station No	Date	Time	Latitude	Longitude
40	HE448/0040	21.07.2015	08:41:59	62° 23,48' N	005° 33,79' E
41	HE448/0041	22.07.2015	06:02:00	62° 26,80' N	005° 36,74' E
42	HE448/0042	22.07.2015	10:00:00	62° 25,26' N	005° 40,54' E
43	HE448/0043	22.07.2015	13:01:00	62° 28,25' N	005° 46,18' E
44	HE448/0044	22.07.2015	16:36:00	62° 42,96' N	006° 02,74' E
45	HE448/0045	23.07.2015	06:01:00	62° 51,95' N	006° 24,78' E
46	HE448/0046	23.07.2015	09:59:00	63° 00,28' N	006° 59,77' E
47	HE448/0047	23.07.2015	13:09:00	63° 11,49' N	007° 24,57' E
48	HE448/0048	23.07.2015	15:59:00	63° 23,55' N	007° 39,01' E
49	HE448/0049	24.07.2015	06:01:00	63° 33,57' N	008° 01,38' E
50	HE448/0050	24.07.2015	09:56:00	63° 46,61' N	008° 14,91' E
51	HE448/0051	24.07.2015	13:13:00	63° 57,71' N	008° 37,49' E
52	HE448/0052	24.07.2015	16:00:00	64° 01,58' N	008° 42,90' E
53	HE448/0053	25.07.2015	06:00:00	64° 09,11' N	009° 01,68' E
54	HE448/0054	25.07.2015	09:59:00	64° 12,67' N	009° 32,32' E
55	HE448/0055	25.07.2015	13:09:00	63° 58,28' N	009° 23,88' E
56	HE448/0056	25.07.2015	16:05:00	63° 50,71' N	009° 01,14' E
57	HE448/0057	26.07.2015	05:55:00	63° 44,88' N	009° 08,02' E
58	HE448/0058	26.07.2015	09:56:00	63° 36,56' N	009° 17,62' E
59	HE448/0059	26.07.2015	12:59:00	63° 36,75' N	009° 31,02' E
60	HE448/0060	26.07.2015	16:00:00	63° 36,25' N	009° 48,47' E
61	HE448/0061	27.07.2015	05:58:00	63° 30,53' N	009° 53,61' E
62	HE448/0062	27.07.2015	10:05:00	63° 29,75' N	010° 12,64' E
63	HE448/0063	27.07.2015	13:00:00	63° 34,19' N	010° 27,34' E
64	HE448/0064	27.07.2015	16:00:00	63° 39,61' N	010° 45,84' E
65	HE448/0065	28.07.2015	05:59:00	63° 44,88' N	011° 06,13' E
66	HE448/0066	28.07.2015	10:00:00	63° 49,35' N	011° 22,35' E
67	HE448/0067	28.07.2015	13:01:00	63° 48,79' N	011° 06,90' E
68	HE448/0068	28.07.2015	15:58:00	63° 45,33' N	010° 59,32' E

8 Data and Sample Storage and Availability

All data will be transferred to the PANGAEA database as soon as they are available and quality checked. Depending on data type and progress of sample analysis, this will be done within 2-3 years. All datasets will be submitted to PANGAEA, allocated by the cruise identifier HE448. The overall cruise track of the HE448 is already available doi:10.1594/PANGAEA.855525.

The following compilation names the scientists who are responsible for access to the different data and sample sets.

CTD and bio-optics data are held at the ICBM (Oldenburg) and were analyzed by the group of Prof. Dr. O. Zielinski. CTD data and accompanying information were already submitted to PANGAEA.

Underway data (FerryBox) are held by the HZG (Geesthacht) and the results will be uploaded to PANGAEA. PSICAM measurements are also held by the HZG (Geesthacht). The processing and validation is ongoing.

Toxin analyses (Dr. B. Krock) were performed by AWI (Bremerhaven) and the results will be uploaded to PANGAEA.

9 Acknowledgements

The scientific team is very grateful to Master Werner Riederer and crew of the *R/V Heincke* for cooperative and efficient assistance with operation and deployment of scientific equipment, and for the friendly and positive working and social atmosphere on board. The HAB research was conducted as part of the IOC/SCOR program on Global Ecology and Oceanography of Harmful Algal Blooms (GEOHAB) within the Core Research Project on HABs in Fjords and Coastal Embayments. The AWI contribution was provided by the HGF Program Earth and Environment under PACES Theme 2 (Coast) work package 3. The cruise is associated to the EU-funded projects NeXOS (Next Ocean Sensors) and Citclops (Citizens observatory for coastal and ocean optical monitoring).

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