

# Cruise report

## FS POSEIDON

Cruise no. 352

8.6. – 5.7. 2007

## NORCOHAB



## **Abstract**

The overall objective of the NORCOHAB cruise was to study the coastal oceanographic processes and mechanisms underlying the dynamics of key toxic bloom species and the biogeographical distribution of their toxins in the water column of different North Sea coastal waters. The cruise was endorsed by SCOR-IOC Programme GEOHAB (Global Ecology and Oceanography of Harmful Algal Blooms), under the auspices of the **Core Research Project on HABS in Fjords and Coastal Embayments**. Following the prescribed format of GEOHAB research the studies were *international*, *multidisciplinary* and *comparative* with the ultimate aim of *modeling* dynamics and behavior. We assessed and compared key genotypic and phenotypic properties of HAB species in relation to grazing and toxic and allelochemical interactions in Scottish and Norwegian/Danish (Skagerrak) coastal areas. For the first time, on board measurements of known phycotoxins collected directly from the plankton were subjected to analysis by high resolution tandem mass-spectrometry coupled with liquid chromatography (LC-MS/MS). These measurements included domoic acid and a large variety of lipophilic toxins associated with marine plankton. We also performed grazing experiments with protists and copepods on selected key toxin algal species in on-board incubations. The following main goals of the cruise programme were successfully addressed: 1) the phenotypic and genotypic characteristics of populations of selected HAB species were compared, including that of a new dinoflagellate identified as the proximal source of azaspiracid poisoning (AZP) in the North Sea; 2) the toxin profile and content of HAB species and toxin transfer and metabolism to the next trophic level were assessed and compared; and 3) insights into the relative importance of grazing by both metazoa and protists as a potential “top down” regulatory mechanism for population dynamics of HAB species was achieved.

## **1. Scientific crew:**

### **Allan Cembella<sup>1</sup> Cruise leader**

Bernd Krock<sup>1</sup>, Urban Tillmann<sup>1</sup>, Eva Käppel<sup>1</sup>, Morten Iversent<sup>1</sup>, Annegret Müller<sup>1</sup>, Sylke Wohlrab<sup>1</sup>, Paul Korb<sup>1</sup>, Uwe John<sup>1</sup> (1. leg); Tilman Alpermann<sup>1</sup> (2. leg), Ruben Neuhaus<sup>2,3\*</sup>, Timo Witte<sup>2</sup> (1. Leg), Kai Fiand<sup>2,3\*</sup> (2. leg)

<sup>1</sup>Alfred Wegener Institute for Polar and Marine Research, Section Ecological Chemistry, Am Handelshafen 12, 27570 Bremerhaven; <sup>2</sup>Optimare Sensorsysteme AG, Am Luneort 15a, 27572 Bremerhaven; <sup>3</sup>Bremerhaven Hochschule, An der Karlstadt 8, 27568 Bremerhaven;

\*under direction of Prof. O. Zielinski

## 2. Research Program

**Introduction and rationale:** Proliferation of algae in marine or brackish waters can cause massive fish kills, contaminate seafood with toxins, and alter ecosystems in ways that human perceive as harmful. These events, now generally referred to as “Harmful Algae Blooms” (HABs), a recurrent and globally increasing phenomenon causing far-reaching problems for fisheries, aquaculture, recreation and tourism, ecosystem functioning, and human health through consumption of toxic seafood. These problems are especially acute for coastal regions where many of the toxic algal events and their effects are most concentrated. A survey of affected regions and of economic losses and human poisonings throughout the world clearly demonstrates a dramatic increase in the impact of HABs over the last few decades (Hallegraeff 2003). There is a pressing need to develop effective responses to the threat of HABs through management and mitigation. This, however, requires knowledge on the ecological and oceanographic factors that control the distribution and population dynamics of HAB species. It is thus a central challenge to understand the critical features and mechanisms underlying the population dynamics and the toxicity of HAB species in a variety of oceanographic regions.

The main goals of the NORCOHAB project cruise were to assess and compare:

1. genotypic variability of populations of selected HAB species;
2. toxin profile and content of HAB species and toxin transfer and metabolism to the next trophic level
3. effect of grazing by both metazoa and protists as a potential “top down” regulatory mechanism for population dynamics of HAB species
4. potential effects of allelochemicals produced by *Alexandrium* spp. on the population dynamics and composition of the whole plankton community
5. intrinsic *in situ* growth rates of HAB field populations

Via this research cruise we conducted comparative research on toxin content, genetic variability and grazing control of known toxic plankton blooms in selected North Sea coastal waters. Although we established our key species of interest during the cruise planning phase - the dinoflagellate genus *Alexandrium*, a known producer of paralytic shellfish poisoning (PSP) and/or spirolide toxins in the North Sea and adjacent waters – we could not predict the timing of the occurrence of such blooms. We therefore made provisions for the opportunistic study of other toxic phytoplankton, including the dinoflagellate *Dinophysis*, responsible for human cases of diarrhetic shellfish poisoning (DSP) in seafood from the North Atlantic, and the toxic diatom *Pseudo-nitzschia*, which has been associated with the presence of toxins

associated with amnesic shellfish poisoning (ASP), especially from the Scottish and Danish coasts in recent years. Furthermore, our sampling programme was designed to exploit the occurrence of other potentially toxic species (e.g. *Lingulodinium polyedrum*, *Protoceratium reticulatum*, *Prorocentrum* spp., *Protoperidinium* spp., etc.) for shipboard experiments and future laboratory analysis, where they occurred in sufficient abundance in our sampling regime. The toxin composition of the plankton was surveyed first by on board tandem mass-spectrometry using a multi-toxin screening method which enabled us to refine and optimize our sampling strategy and station transect while underway.

As research areas we chose the Scottish east-coast and the southern Norwegian coast, across the Skagerrak to the nearshore of the Danish coast. These choices were based on long-term monitoring data, which show a recurrent occurrence of toxic algae, especially of *Alexandrium* and *Dinophysis*, in late spring/early summer for these coastal systems. By comparing different coastal ecosystems of the Scottish and Norwegian coasts where blooms of these various species are known to occur, but with different environmental boundary conditions and hence different biodiversity, we gained valuable insights into the factors regulating bloom dynamics. The comparative approach was adopted because it is difficult to conduct controlled experiments in large-scale ecosystems under “natural” conditions. We therefore also carried out ship-board incubations for estimating grazing rates and population growth rates for the selected toxic algal species from different coastal systems.

### **3. Narrative of the cruise**

*F/S Poseidon* departed on 8.6.2007 in the morning from Bremerhaven toward the Scottish coast. A first station was sampled on 9.6.07 on Dogger Bank in order to test all equipment and to conduct limited instrument trials. A critical desk-top centrifuge required for sample concentration for molecular genetics and toxin analysis failed to function properly and thus a replacement part was sent air freight to Aberdeen for pick-up. Serious initial problems with the temperature controlled lab-container mounted on the rear deck were fortunately resolved due to intense and creative effort of the ship’s crew. Regular transect stations were initiated on 10.6.07 off the Firth of Forth essentially followed the 100 m isobath along a transect towards the north of Scotland. On 11.6.07 a short transect was sampled perpendicular to the coast as far land-ward as Aberdeen Harbour, from where the emergency replacement part was picked up.

The primary transect sampling was continued on 2.6.07 heading further north past the Orkney Island to the Shetland Islands again following approximately the 100 m isobath. Lack of a high magnitude *Alexandrium* bloom at any station along this transect, led to the decision to reverse course and continue south along the planned secondary transect, sampling along a parallel to the primary transect but following essentially the 40 m isobath closer to the coast. Although multiple toxins were measured in the particulate fraction at almost all stations along the primary transect, the species assemblage was rather poor in number and concentration of putatively toxic plankton, leading to the assessment that the near coast may be retaining higher concentrations of the target taxa. While this proven not to be the case, based on data from these parallel transect stations (plankton composition, toxin data), a position for deployment of a surface drifter was chosen. The drifter was deployed on 18.6.08 and followed round-the-clock without any problems until 20.6.08, when it was retrieved from the surface waters. During the 48 h drift, station work was performed every 4 hours. After finishing the drift programme, the *Poseidon* entered Aberdeen Harbour. The scientific crew used the opportunity for a duty visit with colleagues at the FRS Marine Institute to discuss strategies for sampling toxin algae and analysis of their toxins along the Scottish coast. We also conferred in Aberdeen with Prof. Zielinski, who was present for the Oceans '07 conference, regarding the progress on the biooptical sampling and deployment of the COAST FLOAT™ from Optimare AG. Two scientific cruise members left the ship in Aberdeen and were replaced by colleagues from AWI and Optimare AG.

The second cruise leg started on 22.6.08 when *Poseidon* left Aberdeen Harbour. A first station of the second leg was sampled at the Scottish coast before the ship crossed the North Sea towards Stavanger on the Norwegian coast. Transect station work started on 23.6.07 at stations along the southern Norwegian coast within the Norwegian coastal current, following roughly the 100 m isobath. While oceanographic and biooptical conditions, as well as plankton composition, were dramatically different than along the Scottish coast, no high magnitude putatively toxic blooms were discovered. According, before reaching Oslofjord the ship was directed towards the Norwegian coast, retracing a parallel transect westward, following the 40 – 60 m isobath. Station work was interrupted on 24.6.07 for a two-night stay in Arendal Harbour. This scheduled stop at Arendal was used for a research visit to confer with colleagues (in particular Dr. Einar Dahl) at the Marine Station at Flødevigen regarding the historical data on Harmful Algal Blooms along the Norwegian coast and to compare sampling strategies. Routine transect station work was resumed on 26.6.08, proceeding westward along the near shore transect. In the evening of 29.6.07, the scientific crew in

consultation with the Captain and First Mate decided to leave the Norwegian coast to continue station work along the north Danish Coast, where it was expected to be more favourable weather and also to fix a initiation spot for deployment of the drifter for the second set of drift stations away from the major shipping lanes. Due to heavy wind, the first station at the Danish coast could not be started before the early morning of 29.6.07. After a couple of transect stations, the surface drifter was deployed on 30.6.07. After 60 h drift with station work at a 3 h frequency, the drifter was safely recovered on 02.7.07. On the way back to Kiel harbour, a couple of stations were sampled in the nearshore regions of the Skagerrak and Kattegat, before *Poseidon* reached Kiel on 4.7.07. Scientists and equipment left the ship on 05.7.07, effectively terminating the scientific aspects of the cruise.

We thank Captain Hansen and the entire *FS Poseidon* crew for their technical support and excellent collaboration throughout the entire cruise.

## 4. Scientific report and first results

### 4.1 Scientific Summary

The NORCOHAB P-352 cruise aboard the *FS Poseidon* (Kiel) was conducted during early summer 2007 to study the coastal oceanographic processes and mechanisms underlying the dynamics of key toxic bloom species and the biogeographical distribution of their toxins in the water column. The cruise transects were from Bremerhaven, Germany across Dogger Bank with detailed sampling initiated along the Scottish east coast from the Firth of Forth to the Shetland Islands. For comparison, sampling also conducted within the Norwegian Current on the south coast of Norway and then along the north coast of Denmark. In addition to primary and secondary transects, drift stations were sampled over several days on the Scottish and Danish coasts to obtain time series data within a given water mass. Standard physical oceanographic parameters (temperature: °C, salinity: psu,  $\sigma_t$ ) plus current velocity were supplemented with biooptical measurements with multiple profiling fluorosensors and various passive optical profilers (for turbidity and diffuse attenuation), including a hyperspectral radiometer.

Our working hypothesis was that bloom dynamics of our key toxic species in the North Sea are regulated at least as much by “top down” factors such as grazing by copepods and protists than by “bottom up” factors, including light, temperature and nutrients. One of the related major objectives was to apply liquid chromatography coupled to a highly sensitive triple-quadrupole mass spectrometer (LC-MS/MS) to identify and quantify phycotoxins at trace levels throughout the cruise trajectory. With this equipment on board we were able to respond directly to relevant findings on the composition and concentration of toxin in various size-fractions. Plankton assemblages were analyzed from various depths and size-fractions in near-real time (i.e. between stations) and from on board grazing experiments. The analytical instrumentation allowed us to characterize many toxin derivatives (of spirolides, yessotoxins, saxitoxin/gonyautoxins, domoic acid, okadaic acid/dinophysistoxins, azaspiracids) from particulate fractions from the water column at sub-picomolar concentrations. In spite of the rather low concentrations of putative causative organisms, phycotoxins were found at most stations around the perimeter of the North Sea from plankton samples. Such sensitive analytical methods proved crucial to the dynamics determination of the occurrence and fate of toxins in various components of the planktonic food web.

A large number of samples (chlorophyll, toxins, grazing experiment cell counts) were analyzed directly on board, whereas others (plankton identification and counting, analysis of the biooptical samples) were returned to the AWI laboratory for subsequent analysis. The oceanographic data sets have been analyzed and some of the data sets on toxin profiles and molecular signatures are completed (and even published in some cases). A few research highlights to be presented in more detail later in the report are:

- Determination of the potent toxin azaspiracid in plankton samples and the subsequent isolation and culture of the causative organism of azaspiracid poisoning (AZP) in northern Europe – a truly exceptional finding
- Confirmation of the presence of the important member of the DSP toxin complex known as pectenotoxin-1 (PTX-1) in the plankton – the first definitive report of this toxin in the North Sea
- *Alexandrium margalefii* detected for the first time in the North Sea
- High grazing impact of micrograzers on *Alexandrium* populations – demonstrating that copepods may play a merely subsidiary role in bloom dynamics of this toxic dinoflagellate



## **4.2. Oceanographic and biooptical parameters (Allan Cembella, Ruben Neuhaus, Timo Witte, Kai Fiand)**

Measurements of standard physical oceanographic parameters (temperature: °C, salinity: psu,  $\sigma_t$ ) were obtained by CTD profiling instrumentation incorporated into a rosette sampling frame with Niskin sampling bottles remotely triggered for water sampling from discrete depths. A ship-mounted ADCP was used to transduce current velocity profiles along the primary and secondary transects and during the drift programme. The physical oceanographic instrumentation was supplemented with biooptical measurements with multiple profiling fluorosensors (Trios AG) and various passive optical profilers (for turbidity and diffuse attenuation) also mounted on the rosette frame. A hyperspectral profiling radiometer (Satlantic Hyperpro) was deployed at all the primary stations and during the drift programmes (day time only) for determination of the underwater light field and the associated optical parameters ( $K_d$ ,  $E_d$ ,  $L_u$ ). Filtered chlorophyll for biomass estimates and total pigment samples for HPLC analysis of photosynthetic pigments were analyzed along with discrete samples for POC, CDOM, for sea-truthing of the biooptical sensors.

A few examples of the physical and biooptical data sets are provided in the accompanying Figures 4.2.2 – 4.2.4. Among the noteworthy observations from the oceanographic sampling were the strong observed tidal flux effect and turbidity associated with the outflow plume around the Firth of Forth (not shown). Along the south coast of Norway surface turbidity was consistently higher within the Skagerrak than on the eastern North Sea coast (Frig. 4.2.2). The use of multiple fluorosensors of differing wavelengths (Chla, microFLU-chl versus micro-FLU-blue) enabled the comparison of optical signatures from fluorescence signals. Although the fluorescence profiles were somewhat similar, the micro-FLU-blue sensor indicated clear sub-surface patches attributable to cyanobacteria. Concentration of the key toxic microalgae were below detection by passive optical sensors, but the optical data provided an accurate mapping of the higher turbidity and particle loads (including phytoplankton) along the Scottish coast than on the Norwegian coast, presumably due to higher riverine run-off and tidal flux. The drift station profiles (Fig. 4.2.4) comparing the Scottish east coast with the north coast of Denmark provided time-resolved data on physical and biooptical parameters. The microstructure in the water column revealed by the optical sensors indicated much greater complexity in signature on the Scottish than on the Danish coast, likely indicating that the drifter tracked within the same water mass on the latter coast. This interpretation is consistent with the drifter track and the current velocity measurements obtained from the ADCP.

Chlorophyll a concentration remained typically low ( $<3 \mu\text{L}^{-1}$ ) at all stations throughout the cruise as was also reflected in the low number of highly pigmented plankton in the net tows and Niskin bottle casts.

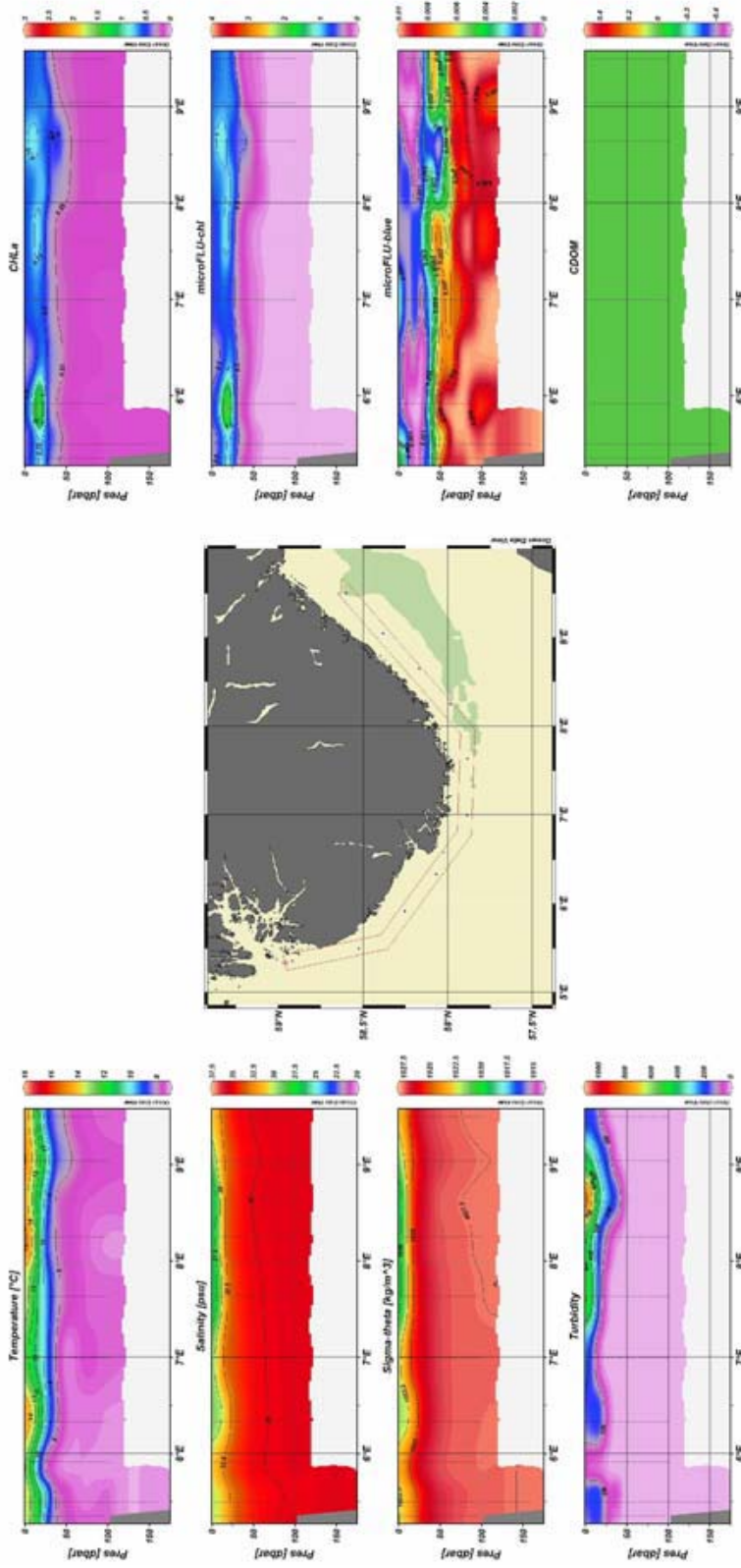


Fig. 4.2.2 CTD data set for the transect Norwegian coast

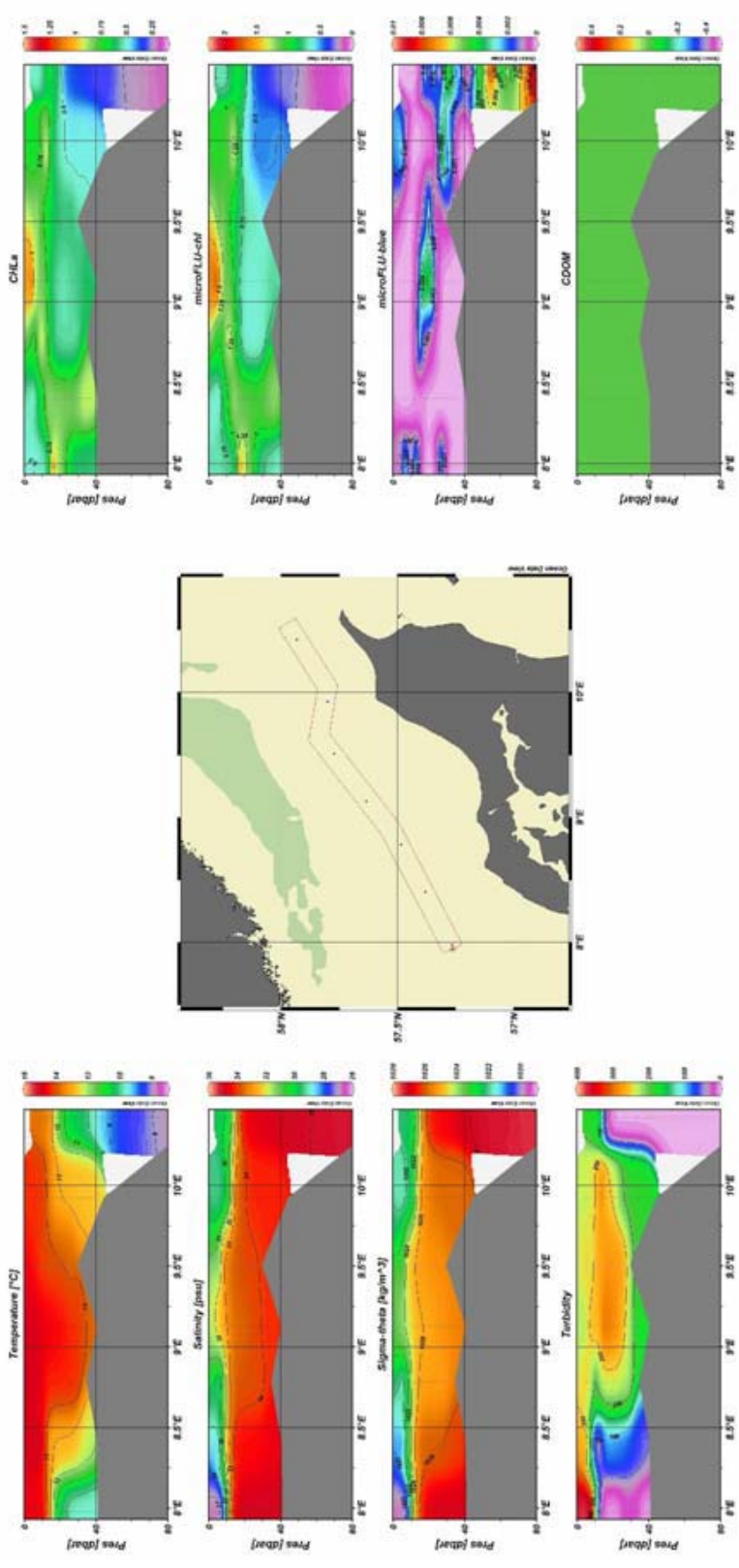


Fig. 4.2.3 CTD data set for the transect Danish coast

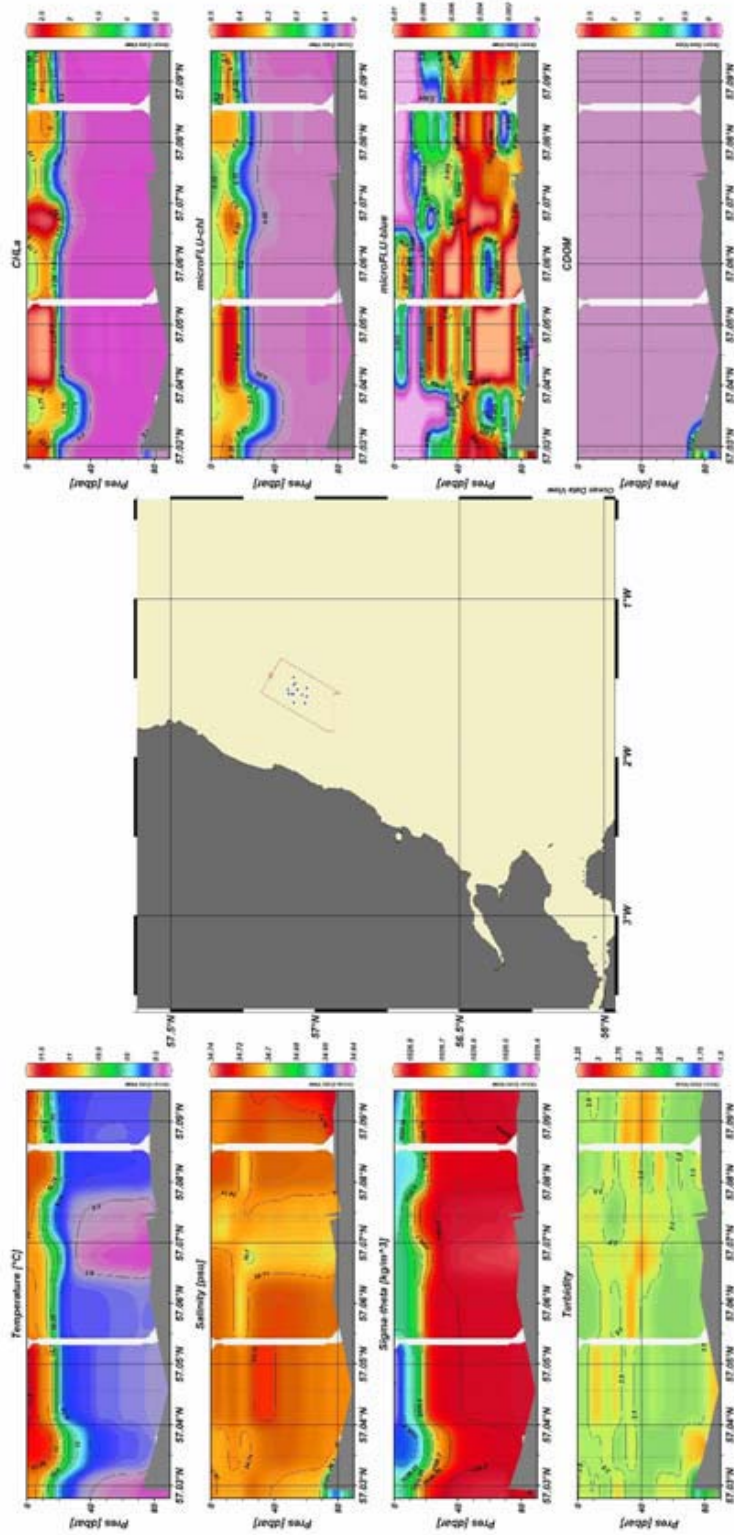


Fig. 4.2.4 CTD data set for the Drift station Scotland

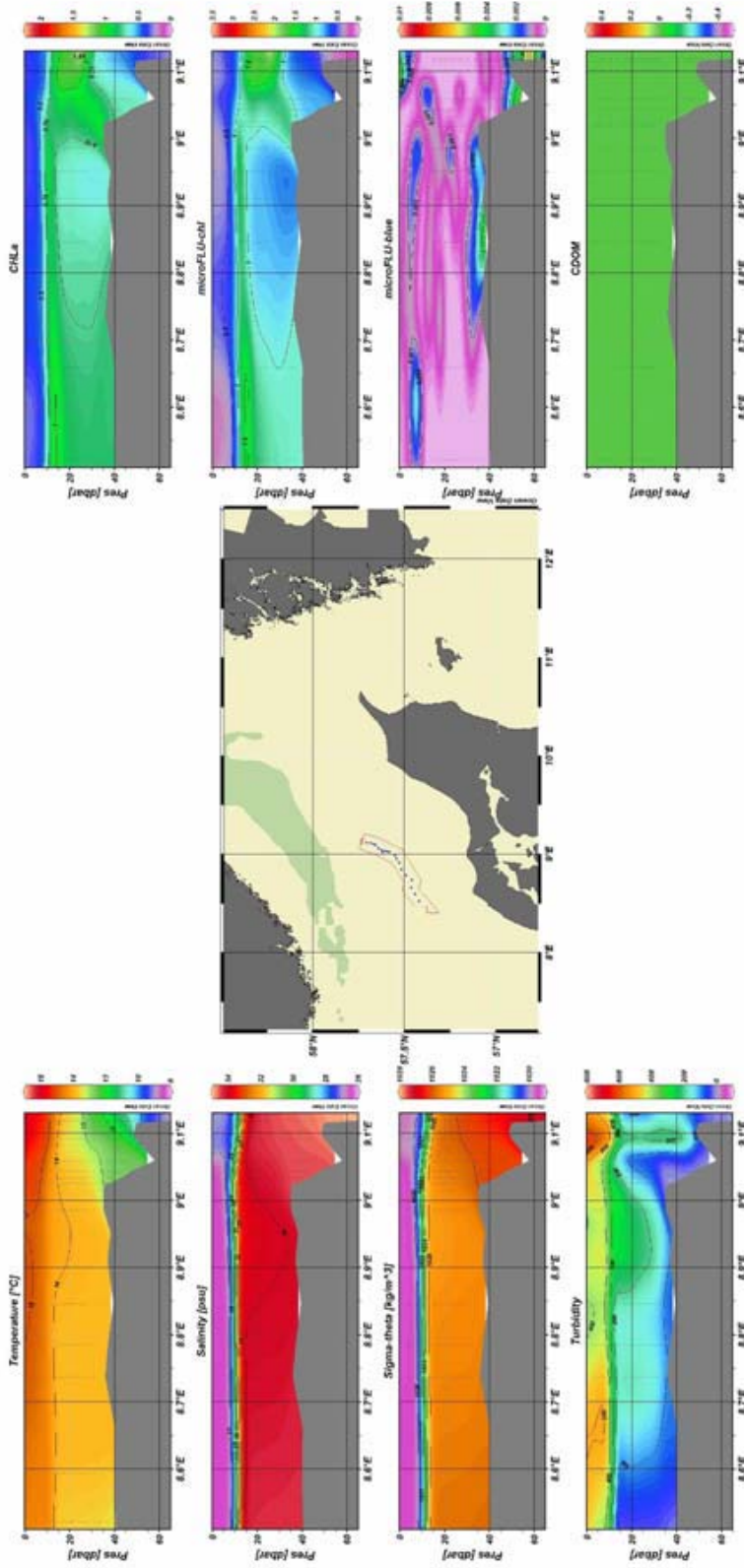


Fig. 4.2.4 CTD data set for the Drift stations Denmark

### 4.3. Plankton and Grazing Interactions (Urban Tillmann, Morten Iversen, Eva Käppel, Tilman Alpermann)

An unusually warm winter-spring transition in 2007 appears to have accelerated the typical bloom succession and we were left with mainly grazers and detritivores accompanied by relatively few pigmented dinoflagellates and some rather unhealthy diatom chains at most stations around the North Sea perimeter from early June to early July. During the whole cruise, net tows indicate that plankton densities were rather low. Generally, plankton communities were dominated by dinoflagellates with *Dinophysis* and *Ceratium* as the dominant genera. In addition a high number of heterotrophs (i.e. large ciliates, heterotrophic dinoflagellates, rotifers, copepod nauplii, etc.) were present indicating that mainly “post-bloom” situations have been sampled.

Abundance of *Alexandrium* spp. as estimated from net tows was low (<100 cells L<sup>-1</sup>). Nevertheless, a number of cultures were established from isolated single *Alexandrium* cells. Interestingly, a number of these clonal cultures turned out to be *Alexandrium margalefii*, a species previously unknown from the North Sea. Species determination has been verified by both molecular diagnostic methods as well as by SEM analysis of thecal plates (Fig. 4.3.1)

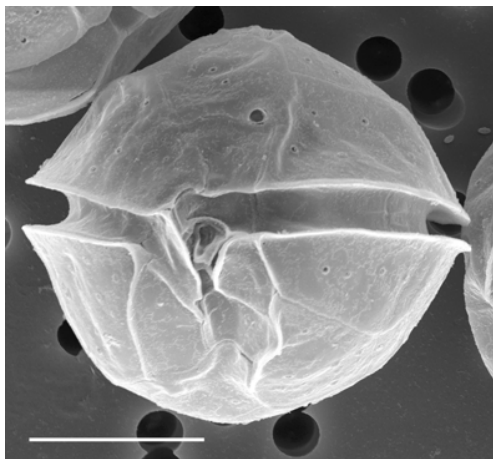


Fig. 4.3.1: SEM micrograph of *Alexandrium margalefii* isolated from the North Sea. Scale bar represents 10  $\mu\text{m}$ .



## **Estimating microzooplankton grazing on *Alexandrium tamarense***

**Introduction:** Estimation of microzooplankton grazing on bloom forming microalgae is an important component in describing plankton dynamics. In this study, fluorescently labelled algae (FLA) were used as a tracer for estimating grazing rates and the effect on microalgae in field experiments. On the *Poseidon\_352* expedition, experiments were undertaken to specifically estimate the grazing impact of microzooplankton on the bloom-forming dinoflagellate *Alexandrium tamarense*.

**Methods:** The following Standard protocol was used for onboard experiments. The general setup on-board was derived from results of preliminary laboratory studies. *Alexandrium tamarense* cells were brought from the laboratory and cultured under standard conditions in a temperature-controlled incubation chamber installed on the ship. *A. tamarense* cells were stained at a concentration of 5,000 ml<sup>-1</sup> with 0.5 µM CMAC (7-amino-4-chloromethylcoumarin) for 4 h. A defined volume of the incubated samples were pre-filtered through a plastic filter apparatus on 20 µm Nitex gauze. The gauze was resuspended in 50 ml Whatman GF/F-filtered seawater. The suspension was filtered through the glass filter apparatus on 8 µm membrane filters. The membrane filters were prepared on an objective glass with immersion oil, counted by epifluorescence microscopy and grazing impact was calculated for the differences in the cell counts. For all experiments on board, incubation condition were standardized on a plankton wheel operating at 1 rpm under dim light on a light:dark photocycle of 16:8 h at 12 °C for 24 h.

**Station-specific experiments:** At ten stations (374, 376, 401, 404, 407, 410, 415, 427, 437, 445) during the cruise, grazing experiments were conducted using the standard protocol outlined above. Fluorescently labelled non-lytic *A. tamarense* cells were added to sampled seawater to estimate the grazing impact on the natural plankton population. Seawater from 3 and 10 m depth was sampled with the rosette sampler during day-time. Feeding experiments were conducted with unfractionated seawater and also with seawater pre-filtered through 200 µm Nitex gauze. The fraction <200 µm was chosen to determine the grazing impact of the microzooplankton (range: 20 to 200 µm) in comparison to the entire grazing effect. Seawater pre-filtered on Whatman GF/F filters was used as control. Stained *A. tamarense* cells were added to achieve a starting concentration of about 1 cell ml<sup>-1</sup>. The samples were incubated in triplicate in 1 l bottles under standard conditions for 24 h. Triplicate bottles (one for each



fraction) were used to measure the actual start concentration of labelled *A. tamarensis* cells. After 24 h incubation, a defined volume of each bottle was filtered on Nitex gauze, resuspended in Whatman GF/F-filtered seawater and filtered on nitrocellulose membrane filters. All *A. tamarensis* cells per filter were counted. Because cells were counted on membrane filters, identification below the generic level was not possible for most organisms, except for very common and distinct species. Cells of the tintinnid grazer *Favella* sp. present in the unfractionated sample were counted for one filter per station.

**Results:** The application of fluorescent label to *A. tamarensis* cells allowed the identification of potential grazer species from natural plankton communities. Figure 4.3.2 shows examples of grazers on *A. tamarensis* discovered by fluorescent labelling of cells which were then retained in their guts and/or in food vacuoles. Grazers detected included copepods (plus nauplii), *Favella* spp., the dinoflagellate *Polykrikos kofoidii*, an unknown rotifer and an unknown micrograzer.

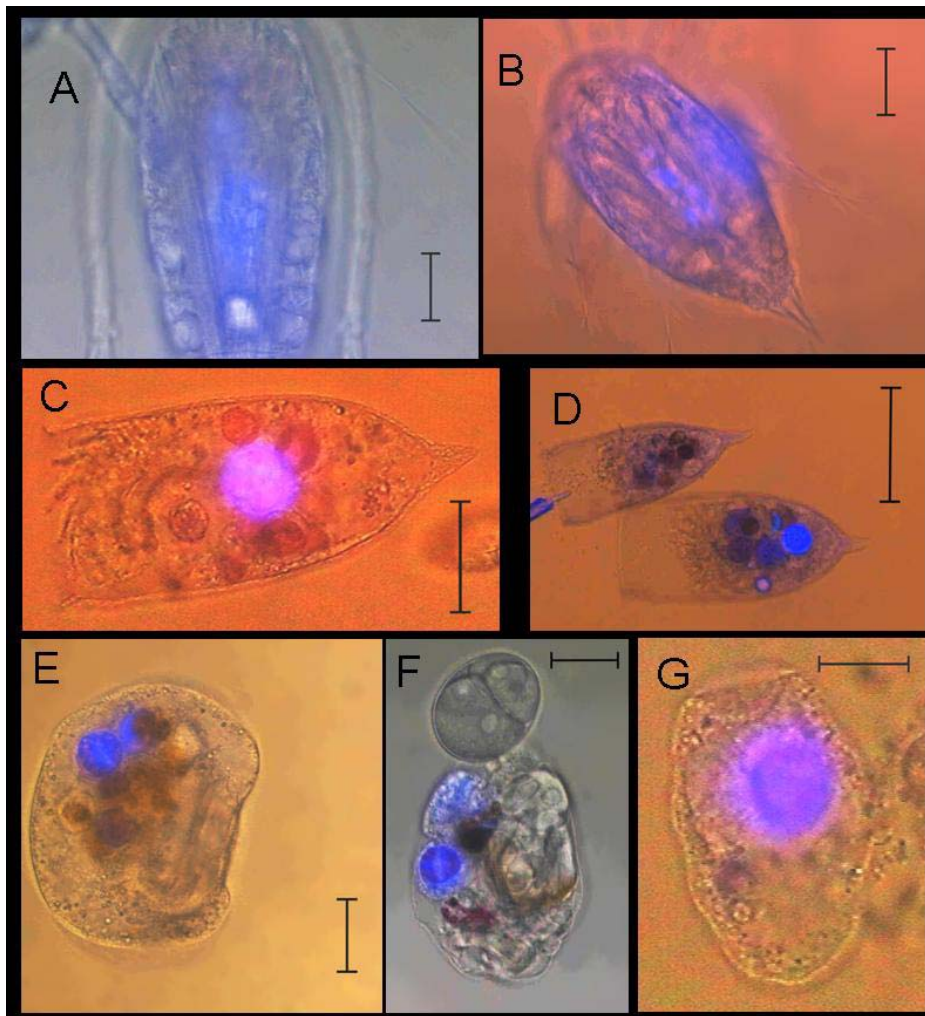


Fig. 4.3.2: Various planktonic grazers upon *A. tamarensis* as indicated by blue fluorescence inside the grazers. (A) copepod *Acartia clausii*; (B) copepod nauplii, (C, D) the tintinnid *Favella ehrenbergii*, (E) an unknown grazer (F) rotifer *Rotatoria* (G) dinoflagellate *Polykrikos kofoidii*. Scale bar represents 150  $\mu\text{m}$  for (A), 70  $\mu\text{m}$  for (B), 50  $\mu\text{m}$  for (C, E, and F), 80  $\mu\text{m}$  for (D) and 30  $\mu\text{m}$  for (G), respectively.

Grazing coefficients calculated for the whole grazer community were close to or only slightly higher than the grazing rate of microzooplankton (Fig. 4.3.3). Highest grazing rates on *A. tamarensis* of around  $2 \text{ d}^{-1}$  were observed at St 374 and 407. At the last four stations, grazing coefficients were rather low. On average the microzooplankton grazing at the Scottish coast (St 374 to 410) was  $73\% \pm 17$  of total grazing. At the Norwegian and Danish coast (St 415 to 445), the average microzooplankton grazing was  $75\% \pm 125$ . The high standard deviation was due to the wide ranging values at St 415 and 445.

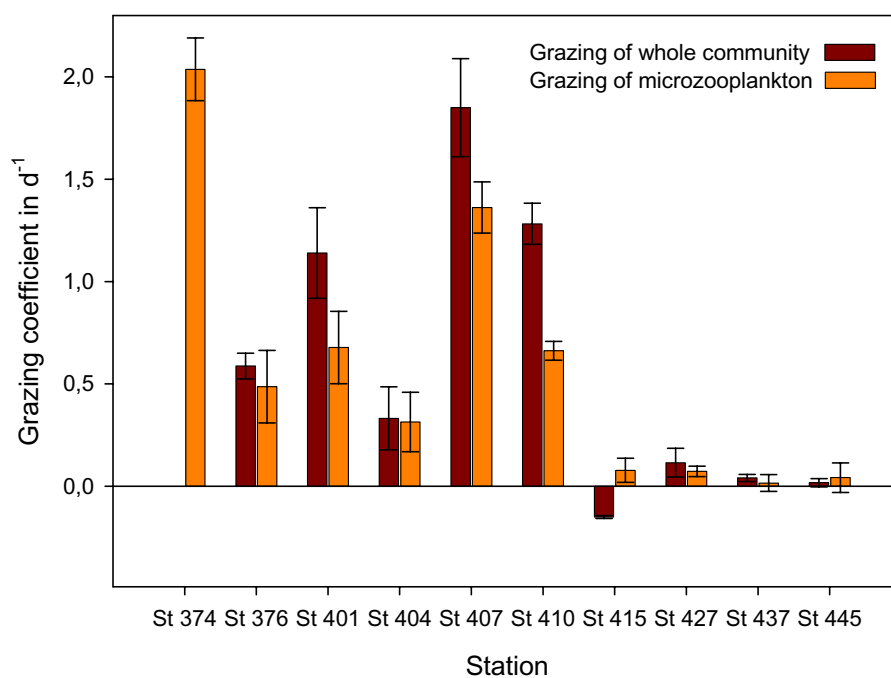


Figure 4.3.3 : Grazing coefficients ( $d^{-1}$ ) of the whole grazer community versus only microzooplankton

Table 4.3.1. Comparison of grazing coefficients of the total community and of only microzooplankton

Station	Grazing coefficient of total community in $d^{-1}$	Grazing coefficient of microzooplankton in $d^{-1}$	% of total grazing
374	-	2.04	-
376	0.59	0.49	82.8
401	1.14	0.68	59.5
404	0.33	0.31	94.8
407	1.85	1.36	73.6
410	1.28	0.66	51.6
415	-0.15	0.08	-52.0
427	0.12	0.07	63.3
437	0.04	0.02	39.9
445	0.02	0.04	246.7

Grazing on *A. tamarensis* was significantly correlated to the abundance of a large tintinnid *Favella ehrenbergii*, indicating the importance of this micrograzer. (Fig. 4.3.4).

**Summary and Conclusion:** At the Scottish coast, maximum grazing rates of microzooplankton on *A. tamarensis* of 2 d<sup>-1</sup> were measured. Grazing rates at the Norwegian and Danish coasts were extremely low (<0.1 d<sup>-1</sup>). In laboratory experiments, the growth rate of *A.*

*tamarensis* was measured as 0.3 d<sup>-1</sup>. Thus microzooplankton grazing rates were shown to be higher than the growth rate of *A. tamarensis*. Microzooplankton grazing may therefore contribute in a major way to a bloom cessation, as suggested by previous studies. Furthermore, field observations indicated that copepods may support dinoflagellate bloom-formation by grazing on tintinnids, i.e. by reducing the concentration of the direct microzooplankton predator upon the dinoflagellate.

The FLA method is easy to handle and yields only minor disruption of the natural assemblage thereby allowing the clear identification of the major grazers. In conclusion, utilization of fluorescently labelled algae proved to be a valuable complement to methods for estimating microzooplankton grazing impacts on bloom-forming microalgae.

#### 4.4. Analysis of phycotoxins in plankton fractions (Bernd Krock, Annegret Müller)

##### Application of LC-MS/MS onboard ship

The development and application of advanced methods in liquid chromatography coupled to mass spectrometry (LC-MS) has become essential for monitoring of phycotoxins, particularly in plankton and shellfish. Yet most such chemical analyses are conducted in land-based laboratories on stored samples, and thus much information on the near real-time

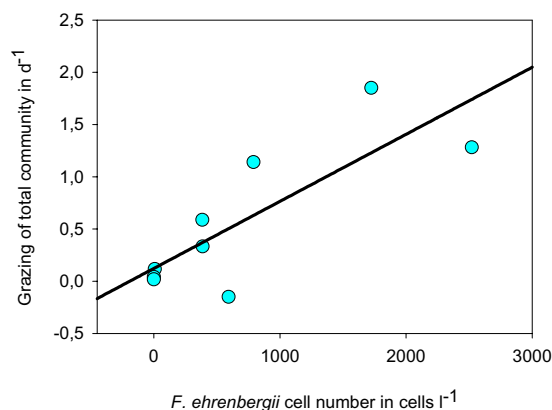


Fig. 4.3.4. Correlation of *Favella* sp. cell numbers vs. grazing of total community;  $n = 8$ ;  $r^2 = 0.80$

biogeographical distribution and dynamics of phycotoxins in the plankton is unavailable. One of the major objectives on our cruise was to apply liquid chromatography coupled to a highly sensitive triple-quadrupole linear ion-trap hybrid mass spectrometer (LC-MS/MS) to identify and quantify phycotoxins at trace levels throughout the cruise trajectory. With this equipment on board we were able to respond directly to relevant findings on the composition and concentration of toxin in various size-fractions and plankton assemblages from various depths and size-fractions in near-real time (i.e. between stations). The analytical instrumentation provided a wealth of information, allowing us to characterize many toxin derivatives (of spirolides, yessotoxins, saxitoxin/gonyautoxins, domoic acid, okadaic acid/dinophysistoxins, azaspiracids [AZA]) from particulate fractions from the water column at sub-picomolar concentrations.

Amazingly, in spite of the rather low concentrations of putative causative organisms, phycotoxins were found at most stations around the perimeter of the North Sea from plankton samples. Such sensitive analytical methods proved crucial to the dynamics determination of the occurrence and fate of toxins in various components of the planktonic food web.

Marine toxins of putative microalgal origin were analyzed directly from size-fractionated plankton net tows (20 µm mesh size) and Niskin bottle samples from discrete depths after rapid methanolic extraction but without any further clean-up. Almost all expected phycotoxins were detected in North Sea plankton samples, with domoic acid and 20-methyl spirolide G being most abundant. Hereby we have proven that even sophisticated mass spectrometers can be operated in ship laboratories without any limitation caused by vibrations of the ship engine or by wave movement during heavy seas at wind forces up to nine Beaufort. On board LC-MS/MS is a valuable method for near real-time analysis of phycotoxins in the plankton for studies on bloom dynamics and fate of toxins in the food web, as well as for characterization and isolation of putatively toxigenic organisms.

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Table 4.4.1. Limit of detection (LOD, S/N=3), minimal and maximal amounts of phycotoxins found in North Sea plankton samples (DTX = dinophysistoxin; PTX = pectenotoxin; YTX = yessotoxin; AZA = azaspiracid)

Toxin	LOD [ng/NT]	Min [ng/NT]	Max [ng/NT]
Domoic acid	0.3	0.5	12,800
13-desmethyl spirolide C	0.015	n.d.	3.670
20-methyl spirolide G	0.07	n.d.	83

Okadaic acid	0.085	0.085	41.7
DTX-1	0.15	n.d.	24.4
DTX-2	0.15	n.d.	60.9
PTX-2	0.25	n.d.	207
PTX-2 seco acid	0.07	n.d.	69.8
PTX-11	0.13	n.d.	11
YTX	0.05	n.d.	56
AZA-1	0.015	n.d.	6.7

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### **Proof of PTX-1 occurrence in North Sea plankton**

Lipophilic phycotoxins in size-fractionated plankton net tows (20  $\mu\text{m}$  mesh-size) were measured on board by LC-MS/MS. For pectenotoxins (PTXs) the following ion masses ( $[\text{M}+\text{H}]^+$ ) were monitored:  $m/z$  876 for PTX-2,  $m/z$  892 for PTX-11 and PTX-13, and  $m/z$  874 for PTX-12 and PTX-14. The PTX levels in net plankton were highest along the Danish north coast, but levels over 50 ng per net tow were also detected on the southern Scottish east coast and in the northern Skagerrak. Abundance of PTXs was highly correlated with the occurrence of the marine dinoflagellate *Dinophysis* spp. Whereas in the eastern North Sea PTX-2 was the most abundant PTX, in the western North Sea PTX-1 was the major component, but it was also present in lower proportions in the Norwegian and Danish waters than in the western North Sea. Isobaric PTX-11 was absent or only detected at trace levels throughout the entire cruise, and PTX-13 and PTX-14 were not detected at all. The identity of PTX-1 was confirmed by comparison of retention time and mass spectrum of the North Sea phytoplankton sample to PTX-1 previously isolated from shellfish. Statistical analysis showed the best correlation between the occurrence of PTX-1 and *D. acuminata* cell concentration. Nevertheless, we could not rule out the possibility of metabolic transformations of PTXs by organisms that have grazed upon *Dinophysis*. Such biotransformations could conceivably occur in heterotrophic dinoflagellates or ciliates, or even via oxidation in copepod fecal pellets. In any case, this study confirmed the presence of PTX-1 in the plankton and is the first definitive report of this toxin in the North Sea.

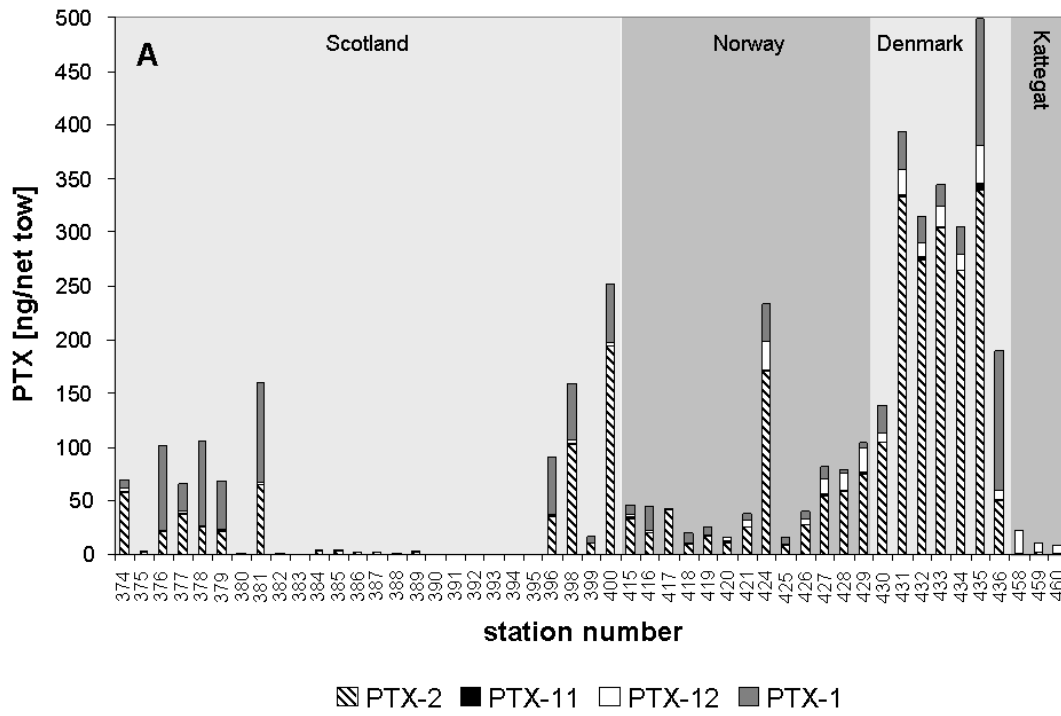


Fig. 4.4.1. Semi-quantitative geographical distribution of PTXs in the North Sea

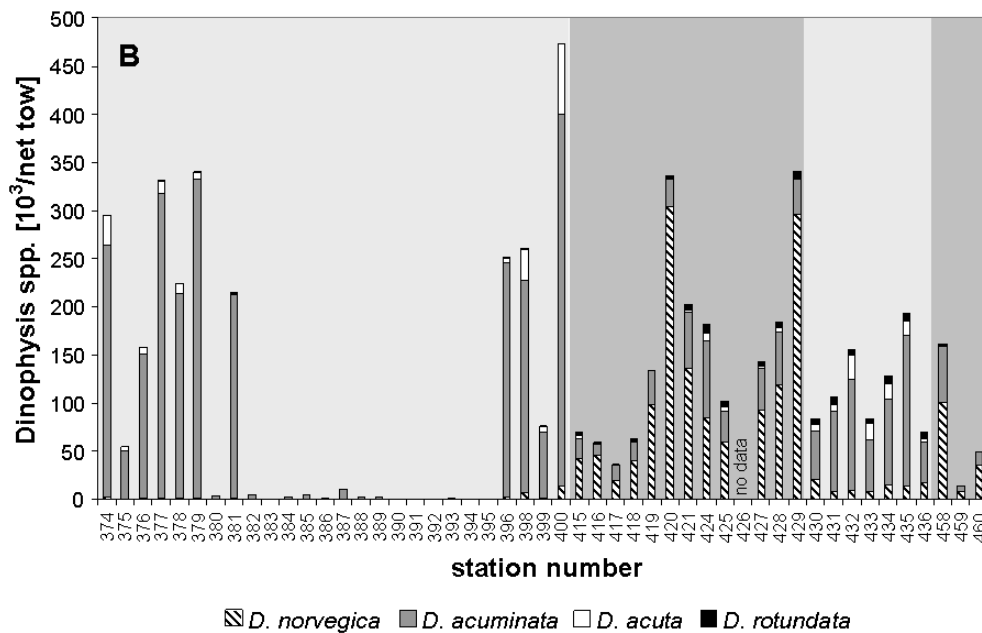


Fig. 4.4.2. Semi-quantitative geographical distribution and cell abundance of *Dinophysis* spp.

Table 4.4.2. Matrix of Pearson product-moment correlation coefficients (upper right,  $n = 48$ ) and significance level (lower left),  $*= < 0.05$ ;  $**= < 0.01$ ;  $***= < .001$  comparing the distribution of toxins with the putative producing organisms.

	PTX-2	PTX-11	PTX-12	PTX-1	Total PTX	<i>D. norvegica</i>	<i>D. acuminata</i>	<i>D. acuta</i>	<i>D. rotundata</i>	Total <i>Dinophysis</i>
PTX-2		0.72	0.74	0.43	0.97	-0.03	0.30	0.52	0.66	0.30
PTX-11	***		0.86	0.54	0.79	0.24	0.16	0.19	0.65	0.30
PTX-12	***	***		0.28	0.74	0.34	0.06	0.16	0.74	0.27
PTX-1	**	***	*		0.64	-0.17	0.55	0.34	0.30	0.41
Total PTX	***	***	***	***		-0.04	0.39	0.51	0.66	0.37
<i>D. norvegica</i>			*				-0.14	-0.13	0.38	0.43
<i>D. acuminata</i>	*			***	**			0.71	-0.02	0.83
<i>D. acuta</i>	***			*	***		***		0.10	0.63
<i>D. rotundata</i>	***	***	***	*	***	**	*			0.23
Total <i>Dinophysis</i>	*	*		**	**	**	***	***		



### Isolation of the AZA-producing organism

Azaspiracids (AZAs) are a group of lipophilic polyether toxins implicated in incidents of shellfish poisoning in humans, particularly in northern Europe. One highlight of the *Poseidon* cruise was the on board determination of azaspiracids in plankton samples and the subsequent isolation and culture of the causative organism of azaspiracid poisoning (AZP). The occurrence and abundance of AZA analogues was measured by LC-MS/MS in size-fractionated plankton samples collected by net tows (20  $\mu\text{m}$  mesh size), by pumping from discrete depths and from Niskin entrapment bottle casts to fixed depths. Although AZA was the least abundant of the many phycotoxin groups measured, the high sensitivity of the LC-MS/MS allowed a detailed quantification. The highest amounts of AZA-1 were present in the southern Skagerrak in the 3-20  $\mu\text{m}$  size-fraction. AZA-1 was consistently the major azaspiracid component.

In eastern Scottish coastal waters, the highest amounts of AZA-1 in net tow samples were in the 50-200  $\mu\text{m}$  fractions, with lesser amounts detected in the  $>200$   $\mu\text{m}$  and 50-20  $\mu\text{m}$  fractions. At these stations, the 50-200  $\mu\text{m}$  fractions were rich in the ciliate *Favella ehrenbergii*. Cells of *F. ehrenbergii* isolated by microcapillary indeed contained AZA-1, but isolated cells grown and fed the non-toxic dinoflagellate *Scrippsiella trochoidea* for one week failed to contain any detectable AZA-1 – evidence that *F. ehrenbergii* is merely a vector for AZA.

Detailed analysis of plankton from Niskin bottle samples from around the North Sea typically showed highest amounts of AZA in the 3-20  $\mu\text{m}$  fraction. From this fraction, a large number of crude cultures were established and subsequently screened for the presence of AZAs. A small photosynthetic thecate dinoflagellate, provisionally designated as strain 3D9 was isolated by microcapillary and brought into pure culture. This isolated dinoflagellate strain 3D9 was found to produce AZA-1, AZA-2 and an isomer of AZA-2, and was detected in field samples rich in AZA by sequence comparisons using molecular genetic techniques. This discovery of a novel causative dinoflagellate for AZA toxicity essentially explains the lack of correlation of AZA with the abundance and distribution of the previously postulated culprit species *Protoperidinium crassipes*. We propose instead that such large phagotrophic dinoflagellates can act as an AZA vector following grazing upon the proximal source.

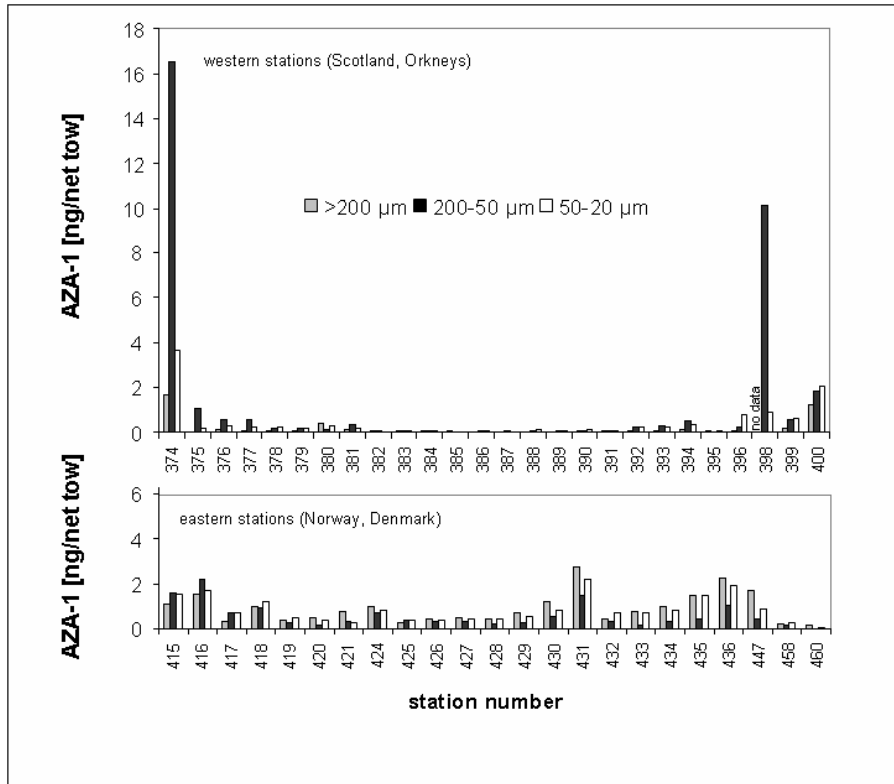


Fig. 4.4.3. Amount of AZA-1 [pg per net tow] in size fractions: > 200 μm (grey bars), 50-200 μm (black bars) and 20-50 μm (white bars)

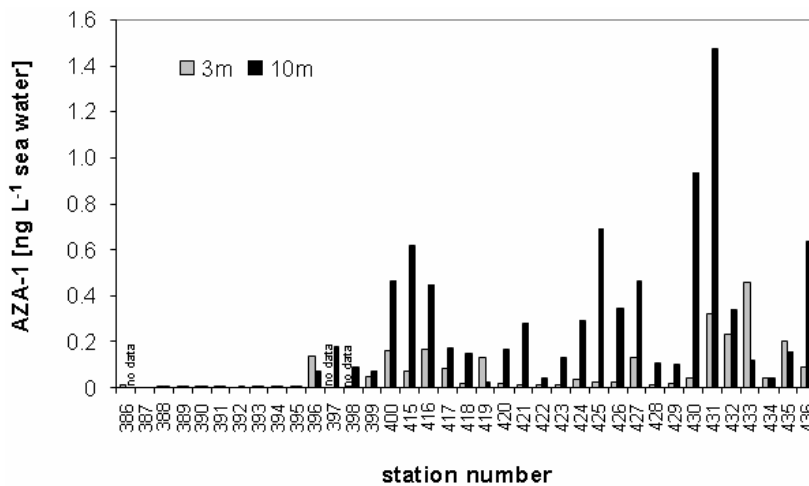


Fig. 4.4.4. AZA-1 concentrations [pg L<sup>-1</sup> seawater] in plankton size fractions 3-20 μm at 3 m (grey bars) and 10 m water depth (black bars) collected by Niskin bottles

#### **4.5 Molecular Genetics and Ecology (Uwe John, Sylke Wohlrab, Paul Korb, Tilman Alpermann)**

In addition to the oceanographic, biooptical and phycotoxin data, we also attempted to examine biodiversity at different stations. Therefore we sampled for taxonomic analysis and microscopic quantification of key species as well as for the determination of the molecular biodiversity via amplification of molecular markers.

Paraformaldehyde-fixed plankton samples for microscopic examination and for DNA-analysis were taken at 51 stations. For each station, plankton net samples were fractionated (200µm, 50µm and 20µm) and then divided for fixation (taxonomic examination) and DNA extraction (molecular diversity). All DNA samples consisted of two fractionated subsamples from one plankton net tow.

For a total of 24 drift-stations, 60L of seawater were pumped with a diaphragm pump on board and fractionated as described above. Seawater was pumped from different depth (20, 10 and 3m) for the first drift stations series. For the second drift-station series, seawater was additionally sampled from the depth of the chlorophyll maximum as determined with a profiling fluorosensor mounted on the CTD package.

##### **Molecular characterization of the source organism of azaspiracid**

From the isolate of the AZA-producing dinoflagellate, provisionally dubbed strain D39, four genes (18s rDNA, 28s rDNA [D1'/D2 region], ITS and cox1) were sequenced and compared phylogenetically with sequences in the genetic databases. Phylogenetic analysis by different molecular markers permitted construction of phylogenetic trees which placed this novel dinoflagellate as a representative of a sister group closely related to the Peridinales but did not show affinities to any known genus or species of dinoflagellate. With molecular probes we also demonstrated that this organism (or at least sequences consistent with its genotype) was consistently present wherever AZA toxins were detected in field plankton samples.

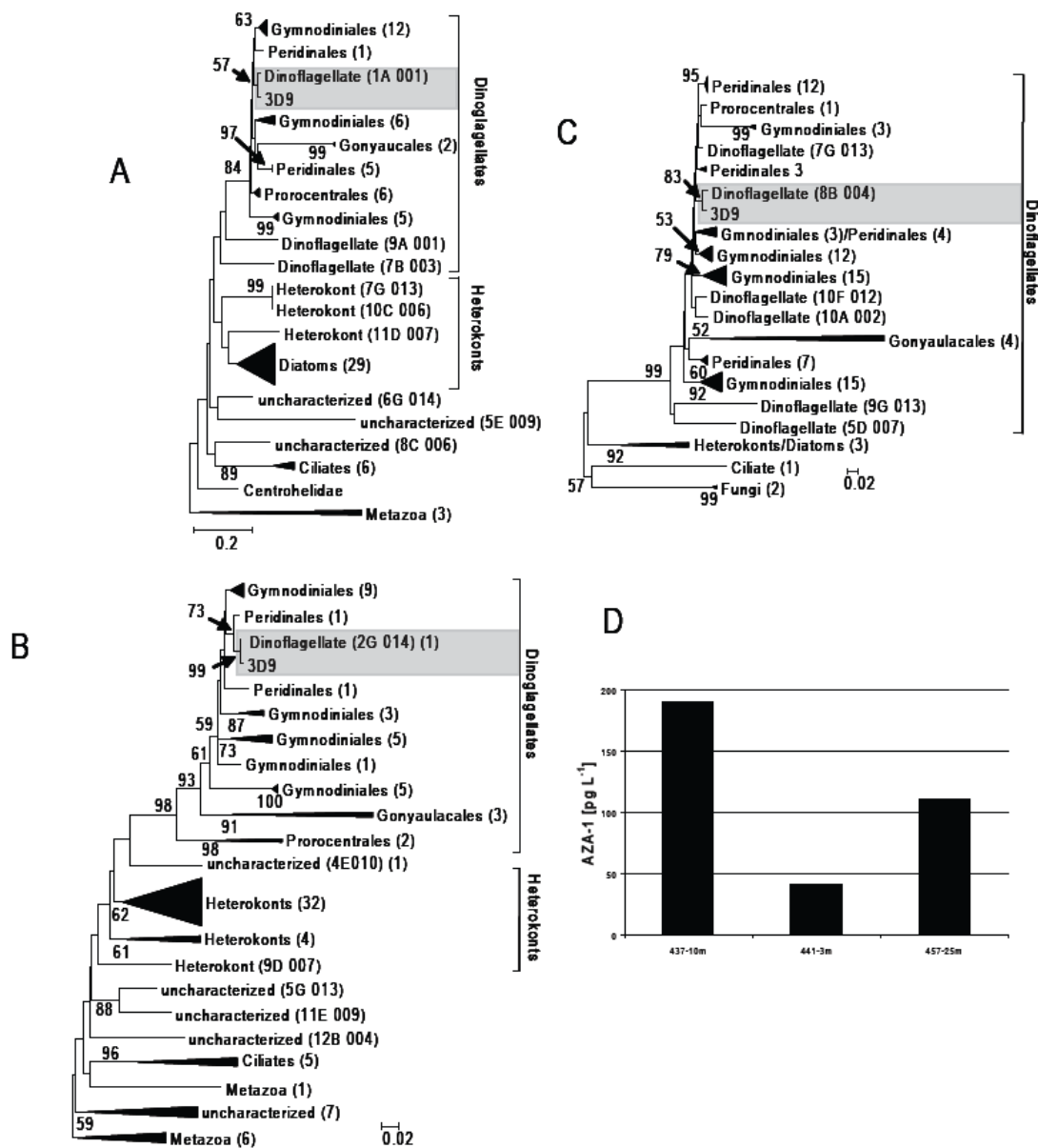


Fig.4.5.1. Neighbor Joining dendrogram of partial sequence of LSU genes from three field plankton samples: A) St 437 (10 m at 8:00h); B) St 441 (3 m at 20:00h); C) St 457 (25 m at 02:00h) and d) AZA-1 concentration in plankton samples from these three sampling stations. The sequence for strain 3D9 was added as reference for the AZA-producing organism; corresponding clade is marked by grey shading. Numbers at nodes are bootstrap percentage of 1000 replicates. Numbers in parentheses after the names indicate the number of sequences in that clade; clone code numbers are given if no reliable characterization was possible

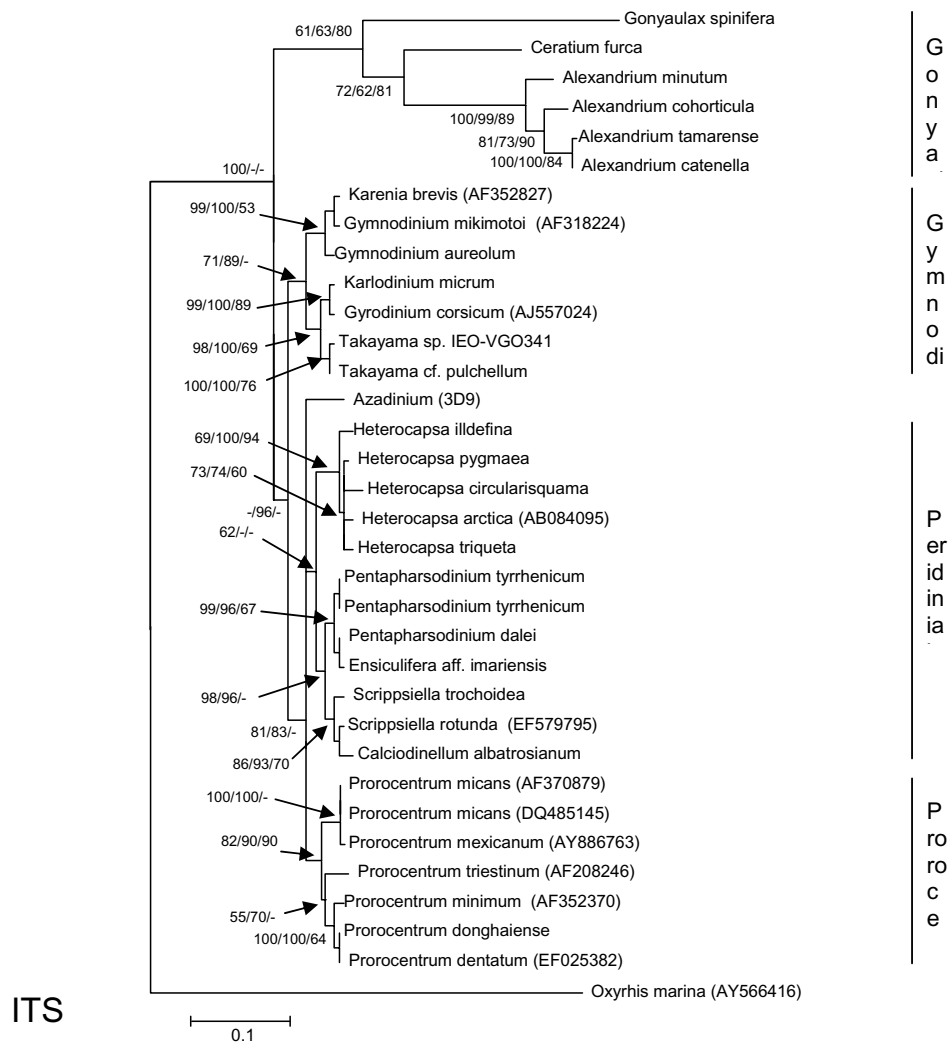


Fig. 4.5.2. Maximum likelihood phylogenetic tree of dinoflagellates inferred from the internal transcript spacer (ITS) from the rDNA operon. The dinoflagellate *Oxyrrhis marina* was used as outgroup. Bootstrap values are given at the nodes in the following order: Neighbor joining, Maximum Parsimony, and Maximum likelihood.

### Effects of copepod grazing and their waterborne cues on *Alexandrium tamarense*

As copepods are one of the major grazers in coastal ecosystems where HABs occur, we attempted to determine the nature of any chemical ecological interactions between copepods and potential prey species. We set up an experiment on board with cultured *Alexandrium tamarense* cells exposed to freshly collected copepods to examine the effect of direct grazing on *Alexandrium* cells and any possible effects from waterborne cues from the copepods on

*Alexandrium* responses. For this purpose, *Alexandrium* cells were either incubated directly with copepods or in seawater containing potential cues from starved copepods.

Copepods with different foraging strategies were chosen for the experiments and collected with a zooplankton net. Adult females of *Calanus helgolandicus* (filter-feeder) and *Oithona similis* (ambush-feeder) were sorted after collection in the North Sea off the west coast of Scotland. Females of *Acartia clausii* (mixed ambush-/filter-feeder) were collected in the North Sea off the northwest coast of Jydland (Denmark).

The effect on the *Alexandrium* cells was determined by measuring the PSP-toxin content in the cells after the treatment compared to a control treatment. The samples were also screened by microarray hybridizations with the *Alexandrium* RNA on an array containing probes from expressed sequence tag (EST) libraries of *Alexandrium* spp. The microarray approach allows for the analysis of the transcriptomic response of *Alexandrium* towards: a) direct grazing of copepods including potential wounding effect, and/or b) the potential chemical signals excreted resulting by copepods into the surrounding seawater.

Results from the PSP-toxin measurement showed a higher PSP-toxin content in *Alexandrium* cells exposed to adult individuals and waterborne cues from *C. helgolandicus* and also a possible but less distinct shift-up in *Acartia clausii* (see Fig. 4.5.3).

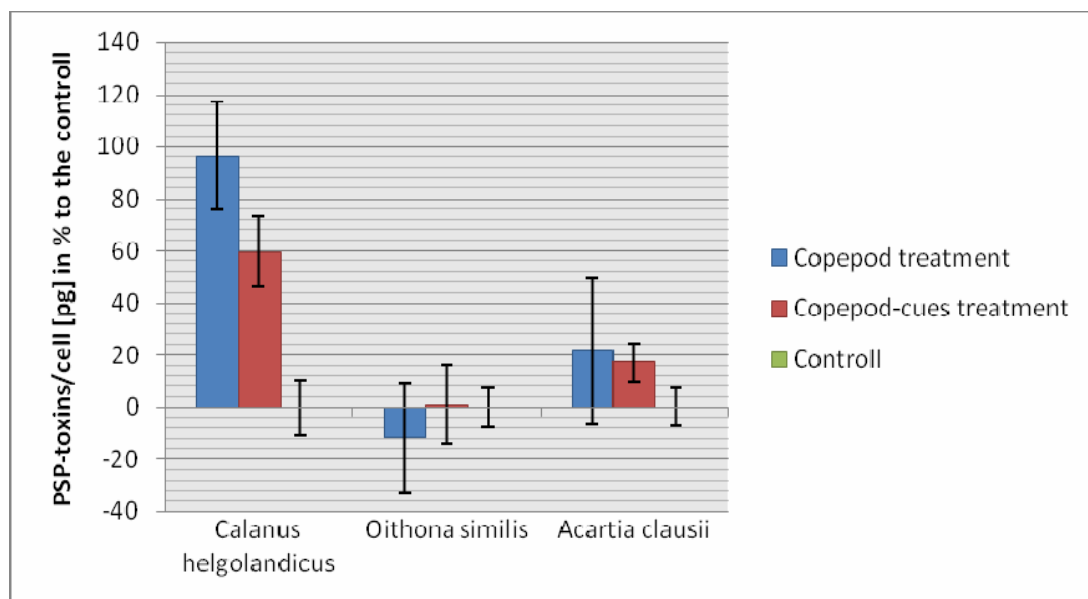


Fig. 4.5.3. PSP-toxin content of *Alexandrium tamarense* cells after incubation with copepods or filtrate of starved copepods containing possible copepod cues

Results from the microarray hybridization with *Alexandrium* RNA showed a response to all treatments compared to the control treatment at the level of gene regulation. Furthermore, there seems to be a common set of genes that is regulated in response to all tested copepods or their waterborne cues (Fig. 4.5.4).

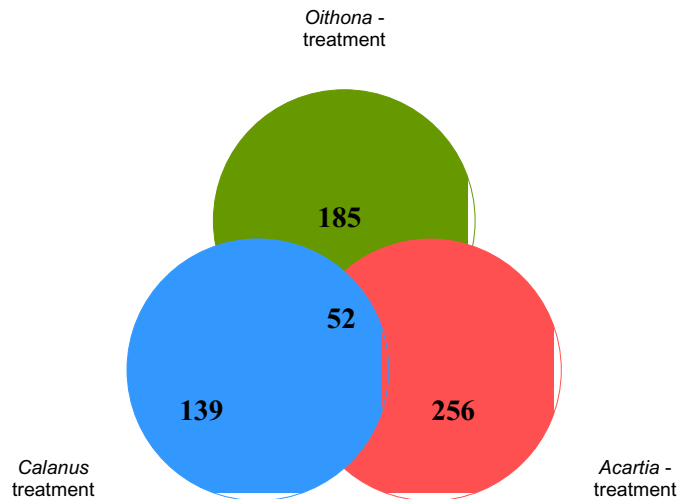


Fig. 4.5.4. Gene regulation response pattern; numbers indicate genes that are regulated in each treatment and in response to all copepods or cues treatments.

In summary, results from these experiments show that waterborne cues are part of this interspecific interaction and thus may influence top-down control of HAB growth and toxicity. The results from the microarray hybridizations give further evidence for the ability of *Alexandrium tamarense* to detect copepods through waterborne cues. The response measured as global gene regulation pattern even showed a similar response on the genetic level in the treatments where no higher PSP- toxin content could be detected.

#### 4.6 Future Perspectives

Further NORCOHAB expeditions in the North Sea are planned under the comparative GEOHAB framework. Following the proof of concept for integrated operation of chemical analytical instrumentation and biooptical profilers for detection of HABs and their toxins in near-real time, we will continue work on the biogeographical distribution of toxigenic organisms and their toxins in relation to food web transfer and chemical ecology of key species in coastal waters.

## 4.7 Dissemination of results

### Presentations, thesis, reports, publications etc.:

Cembella, A.D., Krock, B., Tillmann, U., John, U. (2008): The *Poseidon* adventure redux – NORCOHAB cruise in North Sea. *Harmful Algae News*, 36: 8-10

Cembella, A., Krock, B., Tillmann, U., John, U. (2008): The *Poseidon* expedition – Hunt for the source of azaspiracids. Report Of The Working Group on Harmful Algal Bloom Dynamics (WGHABD), 10 – 13 March 2008, Galway, Ireland, ICES, pp. 23-24.

Fiand, K-F. (2007): Ozeanographische Messungen in der Nordsee – Darstellung und Auswertung von Messdaten einer Forschungsfahrt mit Schwerpunkt einer Chlorophyll a-Analyse. Bachelorarbeit Maritime Technologien, University of Applied Sciences Bremerhaven, Germany. 55 pp

Käppel, E.C. (2007): Estimation of microzooplankton grazing effects on bloom forming dinoflagellates by use of fluorescently labelled algae (FLA). Diploma Thesis, CAU University of Kiel, Germany. 74 pp.

Krock, B., Tillmann, U., Selwood, A.I., Cembella, A.D. (2008, submitted): Unambiguous identification of pectenotoxin-1 and distribution of pectenotoxins in plankton from the North Sea. Submitted to *Toxicon*

Krock, B., Tillmann, U., John, U., Cembella, A.D. (2008): Characterization of azaspiracids in plankton size-fractions and isolation of an azaspiracid-producing dinoflagellate from the North Sea. *Harmful Algae*, in press

Krock, B., Tillmann, U., John, U., Cembella, A.D. (2008): LC-MS/MS on board - isolation of an unknown toxin producer from the North Sea. Poster, presented at the 41st annual meeting of the Deutsche Gesellschaft für Massenspektrometrie (DGMS) in Gießen, 2nd – 5th March 2008

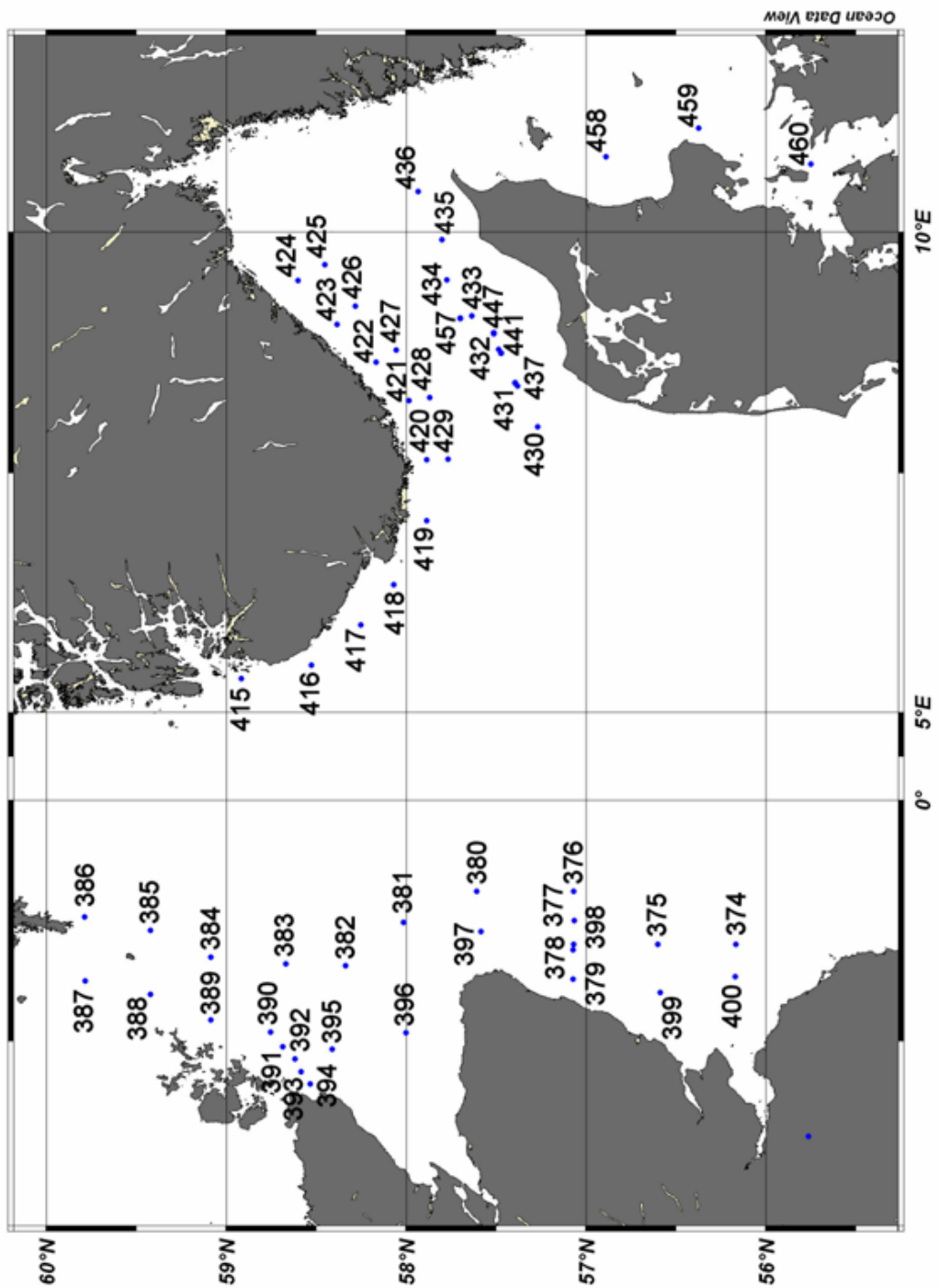
Krock, B., Tillmann, U., John, U., Cembella, A.D. (2008, in press): LC-MS/MS on board ship - tandem mass spectrometry in the search for phycotoxins and novel toxicogenic plankton from the North Sea. *Analytical and Bioanalytical Chemistry*, 2008, in press

Tillmann, U., Elbrächter, M., Krock, B., John, U., Cembella, A.D. (2008) Characterization of a novel azaspiracid-producing dinoflagellate from the North Sea. Accepted presentation for the XIII HAB Conference in Hong Kong China, November 2008

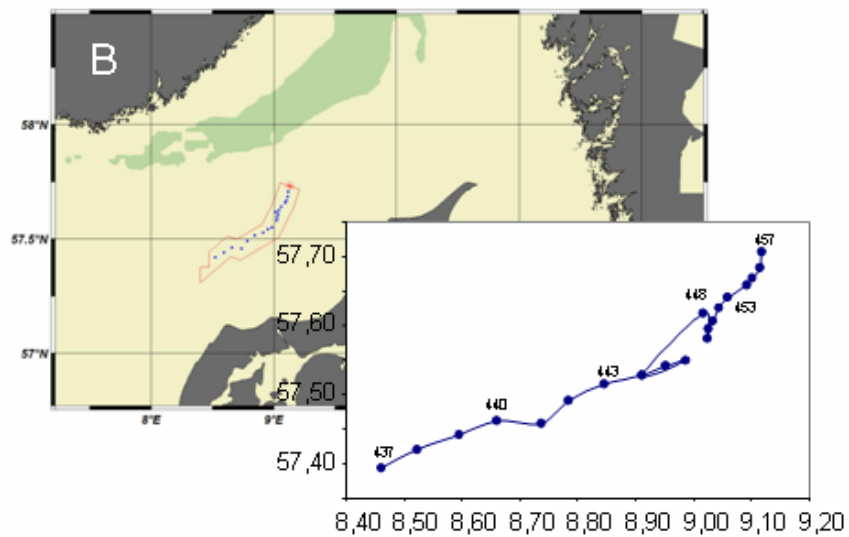
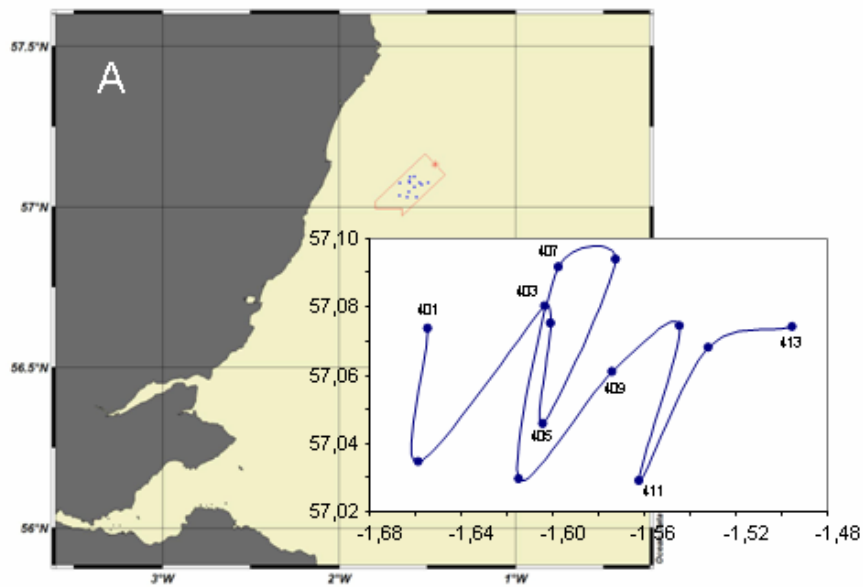


## 5. Appendix

### 5.1 Poseidon 352, map of stations



Poseidon 352, Driftstations. A: 48 h drift at the Scottish coast. B: 60 h drift at the Danish Coast. Numbers in the insert plot are station numbers



## 5.2. Station list

day.mon.year	hh:mm	Station	Lon [degrees_east]	Lat [degrees_north]	Bot. Depth [m]	Equipment
09.06.2007	12:24:00	373	-3,4991	55,7610	50	CTD, bottle sampler, secci, Plankton net, waterpump
10.06.2007	07:02:00	374	-1,5001	56,1656	55	CTD, bottle sampler, Secci, Profiler, Plankton net
10.06.2007	11:24:00	375	-1,4999	56,5988	55	CTD, bottle sampler, Secci, Profiler, Plankton net
11.06.2007	06:23:00	376	-0,9502	57,0655	68	CTD, bottle sampler, Secci, Profiler, Plankton net
11.06.2007	08:30:00	377	-1,2497	57,0618	56	CTD, bottle sampler, Secci, Profiler, Plankton net
11.06.2007	10:25:00	378	-1,5596	57,0686	73	CTD, bottle sampler, Secci, Profiler, Plankton net
11.06.2007	12:40:00	379	-1,8588	57,0687	80	CTD, bottle sampler, Secci, Profiler, Plankton net
12.06.2007	13:53:00	380	-0,9514	57,6060	100	CTD, bottle sampler, Secci, Profiler, Plankton net
12.06.2007	17:31:00	381	-1,2691	58,0135	99	CTD, bottle sampler, Profiler, Plankton net
13.06.2007	06:15:00	382	-1,7240	58,3335	99	CTD, bottle sampler, Secci, Profiler, Plankton net
13.06.2007	09:28:00	383	-1,7008	58,6661	102	CTD, bottle sampler, Secci, Profiler, Plankton net
13.06.2007	13:13:00	384	-1,6322	59,0824	103	CTD, bottle sampler, Secci, Profiler, Plankton net
13.06.2007	16:31:00	385	-1,3563	59,4180	98	CTD, bottle sampler, Secci, Profiler, Plankton net
14.06.2007	06:03:00	386	-1,2133	59,7853	100	CTD, bottle sampler, Secci, Profiler, Plankton net
14.06.2007	11:47:00	387	-1,8831	59,7819	103	CTD, bottle sampler, Plankton net
14.06.2007	14:38:00	388	-2,0200	59,4206	75	CTD, bottle sampler, Plankton net
14.06.2007	17:44:00	389	-2,2899	59,0847	80	CTD, bottle sampler, Plankton net
15.06.2007	06:03:00	390	-2,4152	58,7504	73	CTD, bottle sampler, Plankton net
15.06.2007	07:16:00	391	-2,5644	58,6834	67	CTD, bottle sampler, Plankton net
15.06.2007	08:21:00	392	-2,6939	58,6157	64	CTD, bottle sampler, Plankton net
15.06.2007	09:24:00	393	-2,8264	58,5808	66	CTD, bottle sampler, Plankton net
15.06.2007	10:22:00	394	-2,9503	58,5316	64	CTD, bottle sampler, Plankton net
15.06.2007	12:20:00	395	-2,5940	58,4076	52	CTD, bottle sampler, Plankton net
16.06.2007	06:03:00	396	-2,4177	58,0000	65	CTD, bottle sampler, Plankton net
16.06.2007	11:28:00	397	-1,3673	57,5829	86	CTD, bottle sampler
16.06.2007	14:56:00	398	-1,5000	57,0656	90	CTD, bottle sampler, Plankton net
17.06.2007	06:03:00	399	-2,0001	56,5851	60	CTD, bottle sampler, Plankton net
17.06.2007	10:25:00	400	-1,8369	56,1691	45	CTD, bottle sampler, Plankton net
18.06.2007	10:19:00	401	-1,4952	57,0743	82	CTD, bottle sampler, Waterpump
18.06.2007	14:13:00	402	-1,5318	57,0680	79	CTD, bottle sampler, Waterpump
18.06.2007	18:15:00	403	-1,5622	57,0292	89	CTD, bottle sampler, Waterpump
18.06.2007	22:13:00	404	-1,5442	57,0745	71	CTD, bottle sampler, Waterpump
19.06.2007	02:21:00	405	-1,5737	57,0611	77	CTD, bottle sampler, Waterpump
19.06.2007	06:17:00	406	-1,6147	57,0295	72	CTD, bottle sampler, Waterpump
19.06.2007	10:15:00	407	-1,5972	57,0917	77	CTD, bottle sampler, Waterpump
19.06.2007	14:53:00	408	-1,5723	57,0938	79	CTD, bottle sampler, Plankton net, Waterpump
19.06.2007	18:14:00	409	-1,6040	57,0457	89	CTD, bottle sampler, Waterpump
19.06.2007	22:14:00	410	-1,6005	57,0751	82	CTD, bottle sampler, Waterpump
20.06.2007	02:18:00	411	-1,6034	57,0802	78	CTD, bottle sampler, Waterpump
20.06.2007	06:19:00	412	-1,6583	57,0347	79	CTD, bottle sampler, Waterpump
20.06.2007	10:19:00	413	-1,6543	57,0736	75	CTD, bottle sampler, Waterpump
20.06.2007	11:51:00	414	-1,3660	57,5840	84	Plankton net, Waterpump
23.06.2007	09:32:00	415	5,3554	58,9159	103	CTD, bottle sampler, Secci, Profiler, Plankton net
23.06.2007	12:41:00	416	5,4994	58,5243	274	CTD, bottle sampler, Secci, Profiler, Plankton net
23.06.2007	15:31:00	417	5,9187	58,2516	316	CTD, bottle sampler, Secci, Profiler, Plankton net
23.06.2007	17:57:00	418	6,3329	58,0661	346	CTD, bottle sampler, Secci, Profiler, Plankton net
24.06.2007	06:02:00	419	6,9989	57,8839	426	CTD, bottle sampler, Secci, Profiler, Plankton net
24.06.2007	08:42:00	420	7,6348	57,8828	481	CTD, bottle sampler, Secci, Profiler, Plankton net
24.06.2007	11:34:00	421	8,2499	57,9823	531	CTD, bottle sampler, Profiler, Plankton net

24.06.2007	14:23:00	422	8,6476	58,1666	333	CTD, bottle sampler
26.06.2007	08:51:00	423	9,0449	58,3811	372	CTD, bottle sampler
26.06.2007	11:38:00	424	9,4986	58,5993	501	CTD, bottle sampler, Plankton net
26.06.2007	13:25:00	425	9,6663	58,4507	684	CTD, bottle sampler, Plankton net
26.06.2007	15:44:00	426	9,2320	58,2807	426	CTD, bottle sampler, Plankton net
26.06.2007	18:13:00	427	8,7788	58,0538	584	CTD, bottle sampler, Plankton net
27.06.2007	06:05:00	428	8,2834	57,8659	525	CTD, bottle sampler, Plankton net
27.06.2007	09:14:00	429	7,6414	57,7664	460	CTD, bottle sampler, Plankton net
29.06.2007	06:06:00	430	7,9798	57,2660	41	CTD, bottle sampler, Plankton net
29.06.2007	08:09:00	431	8,4048	57,3796	41	CTD, bottle sampler, Plankton net, Waterpump
29.06.2007	10:12:00	432	8,7841	57,4845	35	CTD, bottle sampler, Plankton net
29.06.2007	12:05:00	433	9,1308	57,6337	41	CTD, bottle sampler, Plankton net
29.06.2007	13:56:00	434	9,5091	57,7717	30	CTD, bottle sampler, Plankton net
29.06.2007	15:44:00	435	9,9261	57,7992	45	CTD, bottle sampler, Plankton net
29.06.2007	17:47:00	436	10,4239	57,9308	100	CTD, bottle sampler, Plankton net
30.06.2007	06:02:00	437	8,4608	57,3932	38	CTD, bottle sampler, Waterpump
30.06.2007	09:02:00	438	8,5219	57,4196	41	CTD, bottle sampler
30.06.2007	12:00:00	439	8,5944	57,4415	40	CTD, bottle sampler, Plankton net, Waterpump
30.06.2007	15:03:00	440	8,6598	57,4614	40	CTD, bottle sampler
30.06.2007	18:03:00	441	8,7377	57,4574	36	CTD, bottle sampler, Waterpump
30.06.2007	20:58:00	442	8,7848	57,4905	37	CTD, bottle sampler
30.06.2007	23:59:00	443	8,8465	57,5147	40	CTD, bottle sampler, Waterpump
01.07.2007	03:03:00	444	8,9116	57,5277	37	CTD, bottle sampler
01.07.2007	06:00:00	445	8,9520	57,5421	38	CTD, bottle sampler, Waterpump
01.07.2007	09:00:00	446	8,9863	57,5498	35	CTD, bottle sampler
01.07.2007	12:00:00	447	8,9116	57,5277	37	CTD, bottle sampler, Waterpump
01.07.2007	15:03:00	448	9,0177	57,6176	35	CTD, bottle sampler
01.07.2007	18:01:00	449	9,0243	57,5814	36	CTD, bottle sampler, Waterpump
01.07.2007	20:55:00	450	9,0264	57,5953	39	CTD, bottle sampler
01.07.2007	23:55:00	451	9,0334	57,6071	43	CTD, bottle sampler, Waterpump
02.07.2007	03:01:00	452	9,0436	57,6260	48	CTD, bottle sampler
02.07.2007	05:59:00	453	9,0591	57,6405	58	CTD, bottle sampler, Waterpump
02.07.2007	09:01:00	454	9,0922	57,6583	50	CTD, bottle sampler
02.07.2007	11:56:00	455	9,1020	57,6681	49	CTD, bottle sampler, Waterpump
02.07.2007	15:00:00	456	9,1155	57,6843	50	CTD, bottle sampler
02.07.2007	18:00:00	457	9,1186	57,7067	68	CTD, bottle sampler, Waterpump
03.07.2007	06:50:00	458	10,788	56,887	9	Plankton net
03.07.2007	11:00:00	459	11,083	56,371	17	Plankton net
03.07.2007	15:45:00	460	10,711	55,75	18	Plankton net