GEOMAR
Helmholtz-Zentrum für
Ozeanforschung Kiel

Date: 25.07.2017

Cruise Report

Compiled by: Dipl. Biol. Burkhard von Dewitz

R.V. ALKOR **Cruise No.: AL 493**

Dates of Cruise: 13.05. – 29.05.2017

Areas of Research: Physical, chemical, biological and fishery oceanography

Port Calls: Visby, Sweden, 19.05. – 21.05.2016

Institute: GEOMAR, FB3 (Marine Ecology, Evolutionary Ecology of Marine Fishes)

Chief Scientist: Dipl. Biol. Burkhard von Dewitz

Number of Scientists: 11

Number of Observers: 1, Regional Sea Fisheries Inspectorate in Szczecin, Poland

Projects: BONUS BIO-C3

Cruise Report

This cruise report consists of 14 pages including the cover page:

1. Scientific crew

Name	Function	Institute	Leg
Burkhard von Dewitz	Chief Scientist	GEOMAR	Entire cruise
Svend Mees	Technical Assistant	GEOMAR	Entire cruise
Henrik Gross	Technical Assistant	GEOMAR	Entire cruise
Christian Pawlitzki	Student Assistant	CAU, Kiel	Entire cruise
Caroline Utermann	Scientist	GEOMAR	Entire cruise
Annemarie Kramer	Scientist	GEOMAR	Entire cruise
Luisa Listmann	Scientist	GEOMAR	Entire cruise
Luisa Maria Berghoff	Student Assistant	GEOMAR	Entire cruise
Theresa Kuhl	Student Assistant	GEOMAR	Entire cruise
Eva Theresa Barthelmeß	Student	GEOMAR	Entire cruise
Anna Katharina Lechtenbörger	Student Assistant	GEOMAR	Entire cruise
Total	**11**		

1.1 Fisheries Observer

Name	Function	Institution	Leg
Piotr Krzysztof Rembarz	Fisheries Inspector	Regional Sea Fisheries Inspectorate in Szczecin	Entire cruise

Chief scientist:

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2. Research program

This multidisciplinary cruise extended a long-term data series on (eco-)system composition and functioning of the Baltic Sea, with a focus on the deeper basins, collected since 1986 by the GEOMAR Helmholtz Centre for Ocean Research and its predecessors IFM-GEOMAR Kiel and IFM Kiel. The key characteristic of this series is the integration of oceanographic and biological information to enhance understanding of environmental and (fish) population fluctuations, and evolutionary processes in this system, in the context of climate change and anthropogenic stressors. The resulting datasets and samples are essential for a number of ongoing projects, including the large-scale international project BONUS BIO-C3 coordinated by GEOMAR. The spatial focus lies on the Bornholm Basin (the most important spawning area of Baltic cod), but also includes the Western Baltic Sea, Arkona and Gotland Basin and Gdansk Deep (Figure 1), thus covering ICES subdivisions 22, 24, 25, 26 and 28 (Figure 2).

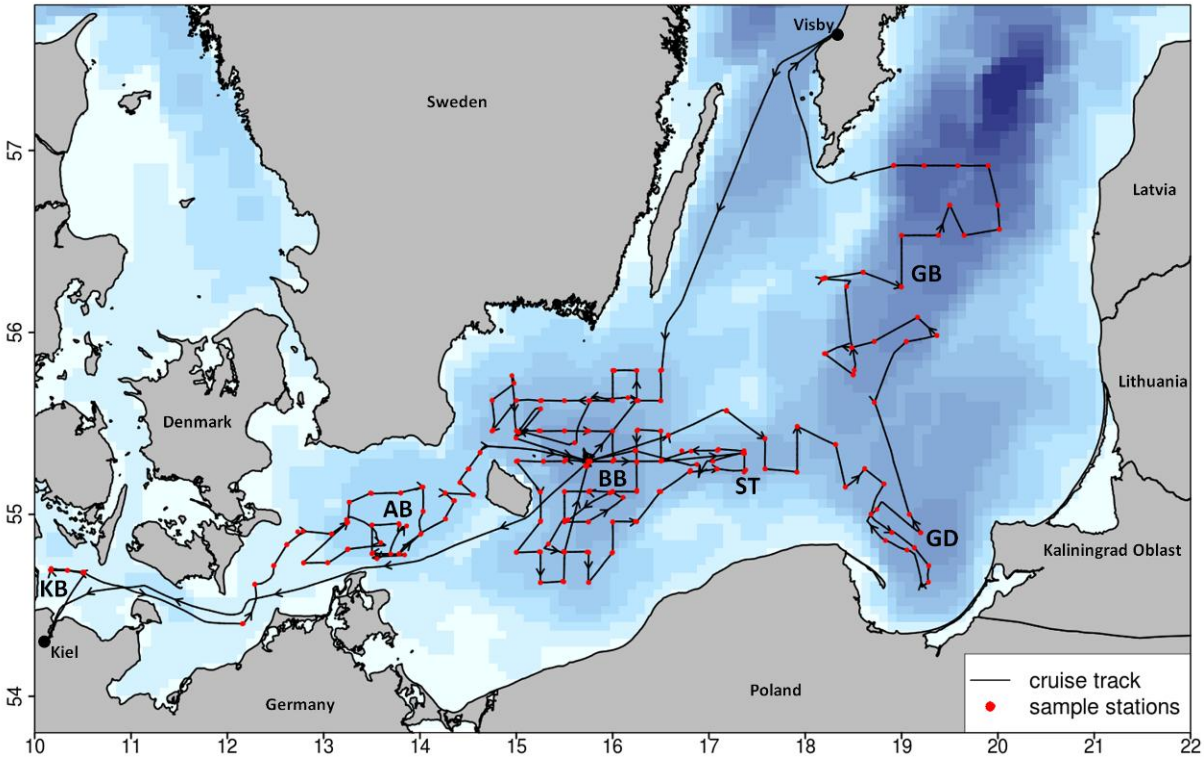

Figure 1 Cruise track of AL 493. KB = Kiel Bight, AB= Arkona Basin, BB = Bornholm Basin, ST = Stolpe Trench, GD = Gdansk Deep, GB = Gotland Basin. All realized sample stations are depicted by red dots.

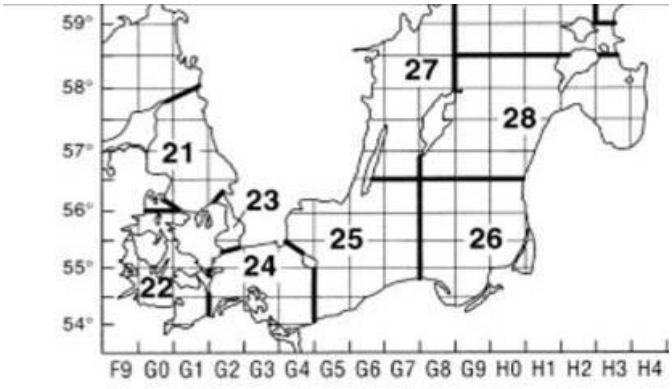

Figure 2 ICES subdivisions in the Baltic Sea area. Source: ICES

Specific investigations included a detailed hydrographic survey (oxygen, salinity, temperature, light intensity) (Figure 3), plankton surveys (zoo- and ichthyplankton, with the goal to determine the composition, abundance, vertical and horizontal distribution, and nutritional status of species, and to address questions regarding plankton phenology) (Figure 3), and pelagic fishery hauls (Figure 4). The latter served to determine size distributions, maturity status, and length – weight relationships of the three dominant fish species in the pelagic system of the Baltic, cod (*Gadus morhua*), herring (*Clupea harengus*) and sprat (*Sprattus sprattus*), as well as the benthic flatfish flounder (*Plathichthys flesus*). Secondly, various different samples were obtained for more detailed analyses, including gonad samples of cod, stomachs of cod, herring and sprat for dietary analyses, otoliths of cod for aging, and tissue samples of cod, flounder, whiting, plaice and others for genetic analyses. In addition, along the cruise track, hydroacoustic (echosounder) data were collected continuously for later analysis of fish abundance and distribution.

While these analyses and samples mainly stood in the context of the continuation of the long-term data series, also sediment and invertebrate samples were taken for marine natural products research in a pilot study initiated, prepared and conducted during the cruise by M.Sc. Caroline Utermann from the department of Marine Natural Product Chemestry at GEOMAR supervised by Prof. Dr. Deniz Tasdemir (see section 5 in this report for details).

3. Narrative of cruise with technical details

The previous cruise of RV ALKOR AL491 was part of the same time series and was performed with most of the heavy gear needed also for this cruise AL493. Because ALKOR completed AL491 on April 28th 2017 on the West shore institute pier with a small detected leakage she was hauled into the dry dock for repairs. Therefore all heavy gear needed also for cruise AL493 remained on board during the time in the dry dock.

On May 12th 2017 repairs were done and back at the West shore institute pier remaining scientific gear was loaded on RV ALKOR, including heavy gear needed for microbial sampling and laboratory work. First preparations of gear and laboratories already started then by the technicians, the microbial sampling team and the chief scientist.

On May 13th 2017 at 7:00 (all times board times) all scientific personal embarked RV Alkor and preparations of laboratories and sampling gear continued while RV Alkor was hauled to Sartori Pier inside the Kiel Fjord to take fuel and provisions. At 10:15 all supplies were on board and RV Alkor departed from Sartori Pier heading to the first research area in the Kiel Bight.

Over the duration of the cruise, hydroacoustic data obtained with four different echosounder frequencies (38, 70, 120 and 200 kHz) were continuously recorded. All planned scientific activities were accomplished during the cruise with some minor changes in the amount of fisheries hauls performed due to unsuitable bottom topography or a lack of fish in the area. Here now follows a detailed description and narrative of the cruise with detailed time designations.

In the Kiel Bight (SD22) the first working area of the cruise 3 Stations were covered during the first day (May 13th) with one cast of each of our standard zooplankton and hydrology sampling gear the Bongo net and the CTD probe. All 3 Stations were placed in the deeper ditch in the central Bight running from Maasholm eastward to the Fehmarn Belt (Figure 3). This area is frequently used as fishing ground from surrounding ports and is therefore suitable for sampling of fishery related projects and of high investigational interest for scientific questions regarding east and west comparisons of for example cod populations or Ichthyoplankton compositions. Additionally 2 Fishery hauls were performed on the western and eastern end of the trench for adult fish sampling (Figure 4). During the evening

RV Alkor steamed to the next operational area the Arkona Basin.

The next Day May 14th 2017 at 01:18 station work continued in the Arkona Basin (SD 24) and together with the following day all planned scientific work for the Arkona Basin could be accomplished until the 15th of May at 23:16. 27 Stations of the previously set Station grid in this Area were covered with CTD and Bongo net hauls starting in the west with station H31 and finishing with H10 in the east (Figure 3). The Zooplankton and hydrological sampling was interrupted on May 14th for 2 pelagic fishery trawls performed between 8:00 and 11:00 followed by efforts for microbial community sampling on station H24. To this end the big rosette water sampler was deployed first followed by 3 van Veen grab sampler and one mini MUC (small multi corer for sediment) until the station was completed with mini MUC on deck at 14:18. Standard Plankton sampling stations were paused again the next day the 15th of May from 5:17 until 17:56 to perform 4 more pelagic fishery hauls in the area and process the caught fish. The fishery stations were chosen to obtain a suitable sample size of Cod individuals in this area and therefore were oriented on the current commercial fishery activities in the area and the previously recorded echosounder information (Figure 4).

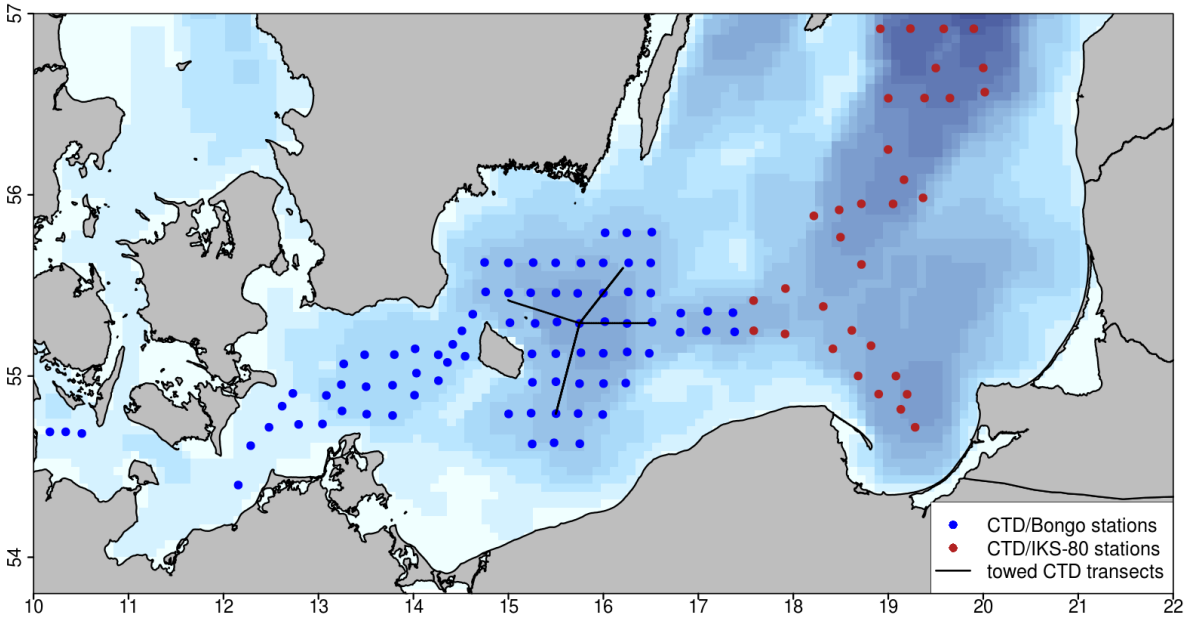

Figure 3 Stations on AL493 with vertical CTD profiles and Bongo-net hauls (blue dots) or CTD profiles and IKS-80 net hauls (red dots), respectively. Transects of towed CTD recordings are indicated by black lines.

Following the completed station work in the Arkona Basin RV ALKOR steamed to the central station of the main working area in the Bornholm Basin (SD 25) BB23. This station acts as standard position for sampling efforts regarding the time series of zooplankton composition, phytoplankton spring bloom observation and micro-/nanoplankton composition realized in cooperation with Dr. Jörg Dutz at IOW, Warnemünde. During the cruise this station was covered as early as possible and at the end of the cruise, respectively, with the rosette water sampler and 3 replicates of each of the vertical deployed plankton nets WP2 (100µm mesh size) and Apstein (50µm mesh size). On May 16th 2017 at 8:52 the first set of deployments were concluded and were followed by two stations within the Bornholm Basin continuing the sample efforts of the sediment microbial community. Stations were chosen on the eastern brink of the basin in the direction towards the next planned working area the Stolpe Trench and in accordance to available information on bottom sediment distribution.

Unfortunately the sediment encountered on both stations BB13 at 11:55 and BBCU at 14:31, was not suitable for obtaining sediment samples by neither grabbing nor coring.

From 16:30 on the 16th of May station work continued in the adjacent ditch the Stolpe Trench (SD25) toward the east. Here the 4 eastern stations of the scheduled grid were covered with CTD casts and IKS-80 casts, respectively. Stations on longitudes east of 17.5° E are as a rule covered with IKS-80 plankton nets instead of Bongo net hauls to be comparable with a historic time series of samples taken by colleagues in Latvia in the years before 1985. Therefore all standard plankton stations performed before the station grid of the Bornholm Basin was covered again with Bongo net hauls on the second leg of the cruise, were sampled with the IKS-80 plankton net. 8 more plankton stations in the Gdansk Deep (SD26) could be executed until 7:30 on the 17th of May 2017 and before the central and eastern part of the Gdansk Deep was covered by 4 pelagic fishery hauls (Figure 4) between 08:18 and 15:00.

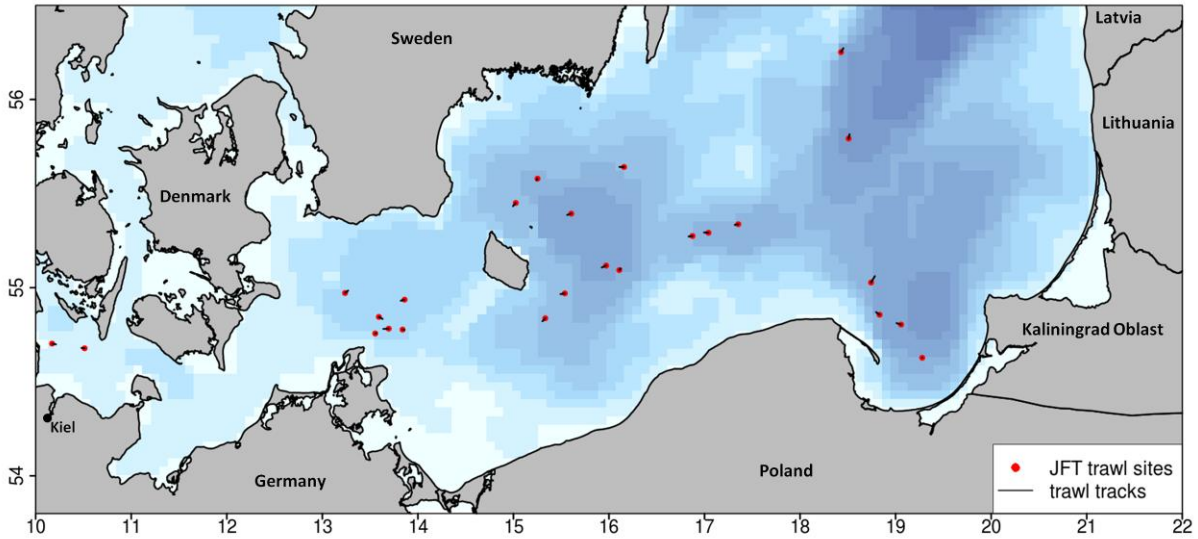

Figure 4 Fishery stations covered during AL493 with pelagic trawls.

During the evening and night of the 17th of May station work was continued towards the north in the southern Gotland Basin (SD26) with 10 plankton stations followed by 2 fishery hauls during the next day the 18th of May from 8:58 to 12:38 (Figure 4). Due to the small amount of obtained samples of the main target species cod in the area fishery efforts were cut and the afternoon was used to cover two more sediment sampling stations in the Gotland Basin area. The shallower station attempted first turned out not suitable for sediment sampling as well but the next station chosen in deeper waters was sampled successfully (for details see 5.4). After completion of sediment sampling efforts at 17:06 standard plankton and hydrology sampling (CTD and IKS-80) continued till the 19th of May at 09:53 on 11 stations of the planned station grid towards the north within the Gotland Basin (SD26 and SD28; Figure 3). The first leg of AL493 was then completed with a scheduled visit to Visby on Gotland, Sweden (May 19th 17:00 – May 21st 9:00). A disagreement regarding legal issues touching security protocols of the harbor and special security permits for the vessel almost prevented the visit but relentless efforts by the Captain Jan Peter Lass solved finally the issue making the much needed break possible.

On the second leg of the cruise RV ALKOR steamed at 9:00 on May 21st from Visby harbor towards the remaining working area the Bornholm Basin and station work could be continued at 19:28. Weather conditions during the transit were something harsher than the previous part of the cruise with stronger winds and less good working conditions. But it remained the only short period of bad weather conditions during the entire cruise. The following 60.5 hours until 8:13 on May 24th 2017 the regular spaced grid of 45 stations

covering the complete Bornholm Basin area deeper than 60m and the remaining 6 stations within the Stolpe Trench were covered with standard plankton and hydrological sampling (CTD and Bongo-net casts). The sequence of 51 stations started at the north eastern corner of the basin on station BB11 and ended in the middle of the southern half of the basin on BB35.Additionally on the 22nd of May between 09:18 and 10:15 two more attempts for microbial sediment sampling at a shallow depth were realized in the north western corner of the basin. Time consumed by these efforts were kept at a minimum by equipping an CTD probe with a portable camera and scouting the sediment surface before deploying any sediment sampling gear. Both stations showed dens coverage of bigger rocks at the sediment surface and were therefore not sampled.

On May 24th between 09:49 and 17:00 4 fishery hauls were performed in the southern half of the basin followed by an overnight towed CTD and hydro acoustic transect towards the north eastern corner and through the centre of the basin (Figure 3) until 05:38 on May 25th. These towed CTD and hydro acoustic transects serve as detailed data sampling of the vertical distribution of pelagic fish species and the accompanying hydrological conditions for habitat analytical studies and detailed assessments of the hydrological inflow situation below the permanent halocline in the basin. The complementing east west transect was performed after 4 more fishery hauls in the northern part of the basin on May 25th (08:11 to 16:49; Figure 4) from 18:30 until 05:00 on May 26th. On May 26th fishery efforts for the cruise were completed with 3 more pelagic hauls in the area of the Stolpe Trench (Figure 4).

On the transit route from the last Stolpe Trench fishery station towards the central station of again the Bornholm Basin station BB23 the last sampling of the sediment microbial community was performed. This time a deeper station of 75m depth was chosen. Here bottom sediment characteristics were suitable for sediment sampling gear and the rosette water sampler, 3 van Veen grab sampler and 1 mini MUC were deployed and the planned samples could be obtained.

As mentioned before, to add a second time point to the time series of zooplankton composition, phytoplankton spring bloom observation and micro-/nanoplankton composition of the central Bornholm Basin, same sampling efforts as performed already on May 16th were repeated from 08:00 to 09:49 on May 27th. These included CTD casts, zoo- and phytoplankton sampling with Apstein and WP-2 nets, oxygen measurements and micro-/nanoplankton sampling with the rosette water sampler.

The same central station of the Bornholm Basin also served as location for efforts dedicated to the intensive vertically and temporally resolved sampling of plankton communities by four towed Multinet MAXI and four vertical Multinet MIDI double hauls over a 22 hour period, covering the water column in 5 m and 10 m depth layers, respectively. Sampling was started at 12:27 on May 27th with the towed Multinet MAXI and ended with the last vertical haul of the Multinet Midi at 09:19 on May 28th.

The research program of cruise AL493 was completed at 09:25 on May 28th at the central station of the Bornholm Basin with no personal accidents and no severe technical failures. RV ALKOR then steamed for Kiel harbor and reached port on May 29th at 08:00. After unloading, the cruise ended at 12:00. Additional detail on the cruise timeline, first scientific results including a report of the sediment sampling efforts, the station list (Appendix E1) and an overview of gear deployments (Table 1) are provided below.

Table 1 Overview of gear deployment. Mesh sizes are given in brackets.

Gear	Deployments (n)
ADM-CTD vertical	118
ADM-CTD towed	2
Hydroacoustic transect (continuous)	1
Watersampler + CTD	7
Bongo, Babybongo (150µ, 335µ, 500µ)	81
IKS-80 (500µ)	33
WP-2 (100µ)	6
Apstein (50µ)	6
Multinet MAXI horizontal (335µ)	8
Multinet MIDI vertical (50µ)	8
pelagic trawl (Jungfischtrawl)	25
van Veen grab sampler	19
mini MUC	6

4. Detailed cruise timeline (all times board time):

Friday 12/05/2017 loading equipment preparing gear.

Saturday 13/05/2017 1015 leaving Sartori pier, steaming to Kiel Bight.
1258 start of station work in Kiel Bight with pelagic fishery
1758 continued station work Kiel Bight CTD, Bongo.
1927 steaming for Arkona Basin station H31

Sunday 14/05/2017 0118 station work Arkona Basin CTD, Bongo
0813 pelagic fishery Arkona Basin
1336 microbial sediment community sampling Arkona Basin
1433 continued station work Arkona Basin CTD, Bongo

Monday 15/05/2017 0035 continued station work Arkona Basin CTD, Bongo
0757 pelagic fishery Arkona Basin
1756 continued station work Arkona Basin CTD, Bongo
2316 steaming for central Bornholm Basin BB23

Tuesday 16/05/2017 0630 water sampler, WP2, Apstein on central Bornholm Basin BB23
1155 microbial sediment community sampling BB13
1431 microbial sediment community sampling BBCU
1630 station work Stolpe Trench and Gdansk Deep CTD, IKS-80

Wednesday 17/05/2017 0007 continued station work Gdansk Deep CTD, IKS-80
0818 pelagic fishery Gdansk Deep
1640 continued station work Gdansk Deep, Gotland Basin CTD, IKS-80

Thursday 18/05/2017 0000 continued station work Gotland Basin CTD, IKS-80
0858 pelagic fishery Gotland Basin
1406 microbial sediment community sampling GBCU
1620 microbial sediment community sampling GBCU2
1831 continued station work Gotland Basin CTD, IKS-80

Friday 19/05/2017 0043 continued station work Gotland Basin CTD, IKS-80
0953 steaming for Visby harbor
1700 reaching port

Saturday 20/05/2017 harbor day Visby, Sweden

Sunday 21/05/2017 0900 departure from Visby steaming to Bornholm Basin BB11
1928 continued station work Bornholm Basin CTD, Bongo

Monday 22/05/2017 0001 continued station work Bornholm Basin CTD, Bongo
0918 microbial sediment community sampling BBCU2
1149 continued station work Bornholm Basin CTD, Bongo

Tuesday 23/05/2017 0028 continued station work Bornholm Basin CTD, Bongo

Wednesday 24/05/2017 0001 continued station work Bornholm Basin CTD, Bongo
0949 pelagic fishery Bornholm Basin
1757 towed CTD and hydro acoustics transect Bornholm Basin S-N

Thursday 25/05/2017 0000 continued CTD and hydro acoustic transect Bornholm Basin S-N
0811 pelagic fishery Bornholm Basin
1830 towed CTD and hydro acoustic transect Bornholm Basin W-E

Friday 26/05/2017 0000 continued CTD and hydro acoustic transect Bornholm Basin W-E
0803 pelagic fishery Bornholm Basin
1455 microbial sediment community sampling BBCU3

Saturday 27/05/2017 0800 sampler, WP2, Apstein on central Bornholm Basin BB23
1227 Start of 24h sampling with towed multinet MAXI, vertical multinet MIDI

Sunday 28/05/2017 0000 continued 24h sampling
0925 steaming for Kiel harbour

Monday 29/05/2017 0800 arrival Kiel GEOMAR east shore pier, unloading
0930 relocation to GEOMAR west shore pier, unloading
1200 end of cruise

5. Scientific report and first results

5.1 Ichthyo– and zooplankton sampling

Bongo- and Babybongo hauls covered Kiel Bight (3 hauls), Arkona Basin (27 hauls), and Bornholm Basin including the western part of the Stolpe Trench (51 hauls) (Figure 3). Larvae of cod (*Gadus morhua*; n = 1 in total), sprat (*Sprattus sprattus*; n = 1140), flounder (*Plathichthys flesus*; n = 525), common seasnail (*Liparis liparis*; n = 20), sand eels (*Hyperoplus lanceolatus*; n = 29) and other species (n = 16) were picked from the 500 µm bongo-samples and conserved at -80 °C for subsequent RNA/DNA analysis. All the 500 µm Bongo samples were also checked for the presence of gelatinous zooplankton. The jellyfish species *Aurelia aurita*, *Cyanea capillata* and *Obelia spp.* were present regularly. Of the invasive combjelly *Mnemiopsis leidyi* only 1 specimen was found. Following these initial on board steps, all Bongo samples were conserved in formol, and will be used for the determination of species composition and abundance of zooplankton and ichthyoplankton.

Stations in the eastern part of Stolpe trench and the Gdansk Deep and Southern Gotland Basin were covered with IKS-80 instead of Bongo hauls (Figure 3) to ensure compatibility of data with a long-term IKS-80 sampling series maintained by the Latvian Fish Resources Agency (LATFRA; Andrei Makarcuks).

Repeated Multinet MAXI (335µ, towed, sampling of the water column in 5 m layers) and MIDI (50µ, vertical, sampling of the water column in 10 m layers) (HYDROBIOS, Kiel) casts were done over a 24 hour period on May 27/ May 28 on the central deep Bornholm Basin station BB23 to reveal the vertical distribution of ichthyo- and zooplankton. In addition, WP-2 (100 µm) and Apstein (55 µm) nets and the rosette water sampler were deployed to obtain additional samples, including nano/micro phytoplankton samples in the context of plankton phenology work within the BONUS BIO-C3 project (Dr. Jörg Dutz, IOW) and the determination of vertical oxygen profiles using Winkler titration.

5.2 Fishery

Pelagic fishery was conducted in the Kiel Bight (2 hauls), Arkona Basin (6 hauls), Bornholm Basin (8 hauls), Stolpe Trench (3 hauls), Gotland Basin (2 hauls) and Gdansk Deep (4 hauls) (Figure 4). In parallel to the fishery hauls, hydroacoustic measurements of fish distribution patterns were recorded continuously. Catches were dominated by sprat (*Sprattus sprattus*, n = 273955) and herring (*Clupea hargenus*, n = 3815) followed by cod (*Gadus morhua*, n = 1331), whiting (*Merlangius merlangus*, n = 580) and flatfishes. The latter were comprised of flounder (*Platichthys flesus*, n = 17) and plaice (*Pleuronectes platessa*, n = 5); and in western parts by common dab (*Limanda limanda*, n = 136) and one long rough dab (*Hippoglossides platessoides*). One four-bearded rockling (*Enchelyopus cimbrius*), 171 three-spined sticklebacks (*Gasterosteus aculeatus*), one European eelpout (*Zoarces viviparus*), one hooknose (*Agonus cataphractus*), one short spined sea scorpion (*Myoxocephalus scorpius*) and fife sand eels (*Hyperoplus lanceolatus*) completed the catches.

For each haul and the entire catch, catch weight and length frequencies of all species (illustrated in Figure 5 for cod) were taken. Stomach samples were taken from sprat (10 per 1 cm length class) and herring (10 per 2 cm length class). For cod, single fish data (length, weight, sex and maturity stage) and samples (otoliths, fin clips for genetic analysis, stomachs and gonads) were obtained for 852 individuals (see Figure 6 for illustration), whereas only length and weight were measured for the remaining 479 individuals. All length and weight data together suggests that the caught individuals are representing foremost the lower third of

the possible size spectrum in the Baltic Sea (Figure 7). Fishing pressure of fish longer than 35 cm is most likely too high to find these individuals in significant numbers.

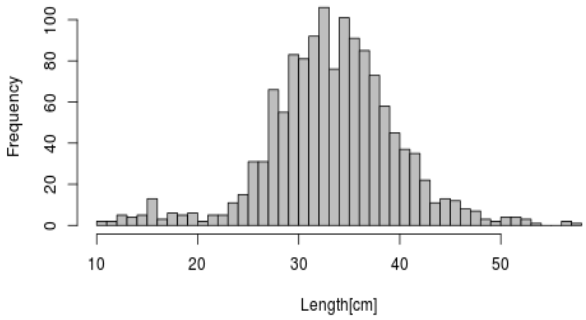

Figure 5 Relative length frequency distribution of individual sampled cod during AL 493 (n = 1331).

Figure 6 Samples (otoliths, fin clips, stomach contents, gonads) and measures (total length, weight, gutted weight, liver and gonad weight) taken from 852 out of 1331 cod individuals during the cruise (illustrated here for a 38 cm female, maturity stage IV, with full stomach, from Bornholm Basin). Photo: Nickel

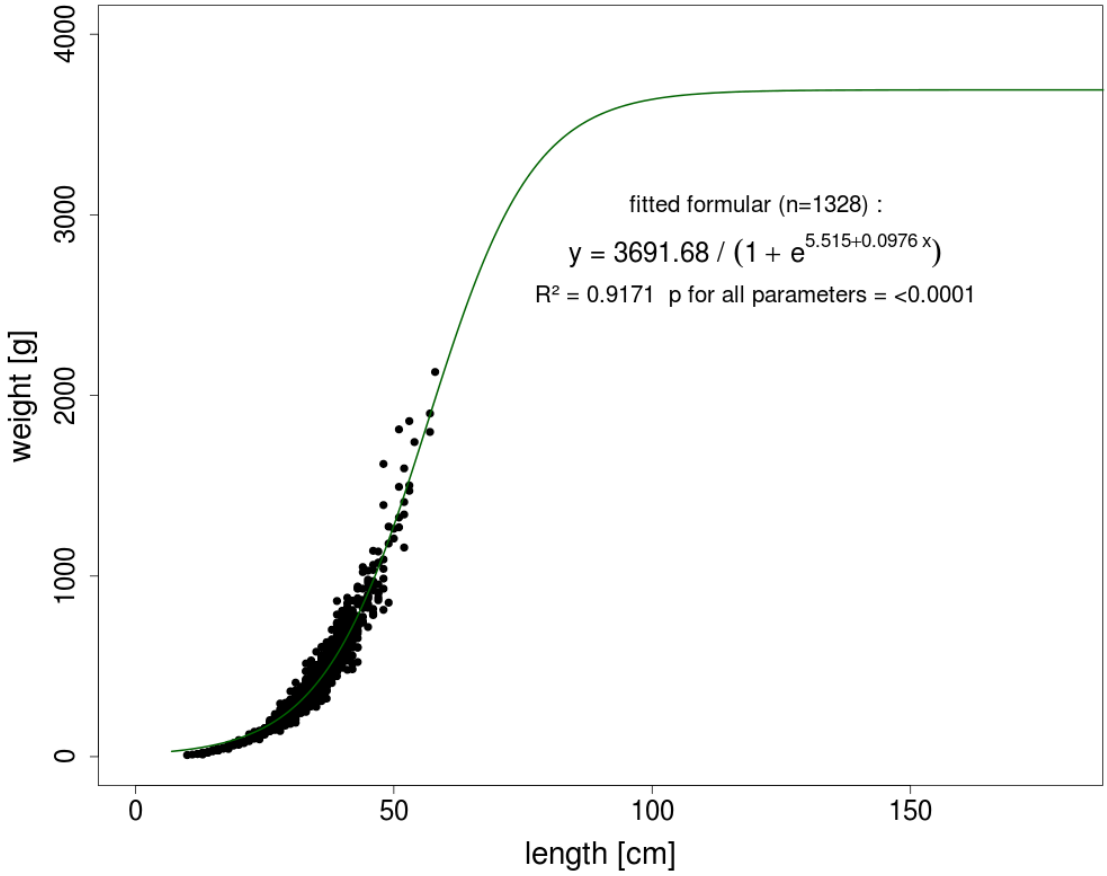

Figure 7: Length weight relationship of all caught cod individuals on AL493. Dots indicate each individual and solid green line represents best fit for a limited growth formula with the upper limit unknown (parameters and fit criteria are given in the plot).

5.3 Hydrography

CTD profiles from 120 stations were obtained with the ADM-CTD and the HYDROBIOS water sampler with attached CTD. In the Bornholm Basin two transects were covered with a towed ADM-CTD for a higher resolution cross section of the hydrographic situation (for locations see Figure 3). Conditions varied depending on the basin and location of the Baltic sampled, and will be analyzed in depth in context of the long-term data series on hydrographic conditions.

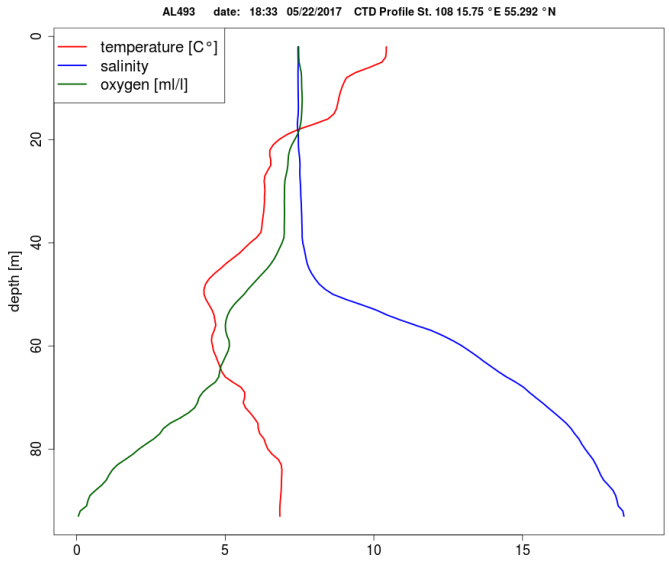

Figure 7 CTD profile of station BB23 in the central Bornholm Basin in May 2017. Temperature red line, salinity blue line and oxygen green line.

Oxygen concentrations at the bottom in the Bornholm Basin were found to be somewhat reduced to the concentration measured 2016 in spring. This year, 2017, hypoxic to anoxic conditions were found at the bottom of all sampled stations with water depths >81m (see Figure 7). But from water depths of 81m upward the oxygen concentrations was measured as above 2 ml/l. Within this water body below the halocline but above 81m the diluted oxygen did decrease slightly compared to 2016. The position of the halocline was found to be about 3 m lower in the water column.

We assume, that the last two inflow events in the winter 2014/15 and 2015/16 increased the salinity and oxygen conditions below the halocline in the Bornholm Basin substantially but were not followed by a third one this winter 2016/2017 and therefore the impact by them is ceasing. Intermediate water depths are not yet depleted of oxygen but less severe inflow events would layer within this depth range because of the still relatively high salinity in the deep basin.

The Stolpe Trench connecting the Bornholm Basin with the Gdansk Deep was found oxygenated down to the bottom (~3ml/l). The Gdansk Deep showed oxygen conditions below the halocline in the central basin around the hypoxic mark. This is a slight increase to the conditions found last year in May. A likely explanation is that the large amounts of oxygenated water entering the Baltic Sea system during the last two major inflow events reached the Gdansk Deep with at least 6 month delay and the remains are still visible in the observed profiles.

Further into the southern Gotland Basin the effect of increasing oxygen concentrations is still detectable. Below an oxygen minimum of 1.5 ml/l at 80m depth the oxygen concentration is increasing in deeper water layers to over 2.2 ml/l at 110m. The oxygen situations in deeper stations of the Gotland Basin where water depths are exceeding 120m

were not systematically assessed, but are also less important for biological processes acting on fish populations in the Baltic Sea.

In biological terms the critical hypoxic border of 2 ml/l is still not dominating in the system and with that the overall state of oxygen distribution within the basins of the Baltic Proper was found to be still in a poor condition. This stresses the fact, that also big inflow events like the one observed for the winter 2014/15 are solitary not able to change the course of spreading hypoxia and anoxia in the Baltic Sea and that other factors like eutrophication and changes in ecosystem functioning processes are contributing to this development.

5.4 Sediment sampling on the deep basins of the Baltic Sea

The additional line of work carried out in parallel to the above cruise program successfully completed the planned sampling. A report compiled by Caroline Utermann who planned and executed this project containing details of the project and describing the work realized on board is given below.

5.4.1 Summary

The research cruise AL493 (13.05.2017-29.05.2017) was attended to obtain sediment and invertebrate samples for marine natural products research from the deep basins of the Baltic Sea. For this purpose, a Van Veen Grab Sampler, a sediment multicorer equipped with 4 tubes (Mini Muc) and a CTD/water sampler were employed at several sampling stations. Sediment samples, including water reference samples, were taken in the Arkona, Bornholm and Gotland Basins (stations H24, BBCU3, GBCU2). Moreover, small bivalves were sampled along with reference water samples in the Bornholm Basin (station BB13). First morphological identification assigned them to *Mytilus* sp. and *Macoma balthica*. Inoculation of sediment, bivalves and water on four different growth media yielded several hundred microbial strains. The isolation process is still ongoing and the identification of all strains via Sanger sequencing (bacteria: 16S rRNA gene; fungi: 18S rRNA gene, ITS1) as well as cryo-preservation of the strains will follow soon. As a future perspective, the most promising bacterial and fungal strains could be re-cultivated later in order to test their bioactivity potential (*e.g.* environmental panel, cancer) and elucidate their metabolic potential via UPLC-QTOF-MS measurements.

5.4.2 Station schedule & sampling

All sampling stations of AL493 are depicted in Figure 8 and the stations for microbiological sampling are highlighted in red. During the cruise, seven different stations were selected for sediment sampling and their chronology as wells as key parameters are presented below (Table 1). Among the seven stations, only three were suitable for sediment sampling by the multicorer (H24, BBCU3, GBCU2; Table 2). These three stations are characterized as comparably deep stations (44-95 meters) and the sediment type can be described as muddy. Sediment samples from these three stations had in common that they had a strong hydrogen sulfide (H_2S) smell. The four remaining stations were inaccessible for sediment sampling by coring or grabbing because the sandy sediment was densely covered with stones and rocks although the available information (sediment distribution maps) indicated a purely sandy sediment composition on those stations.

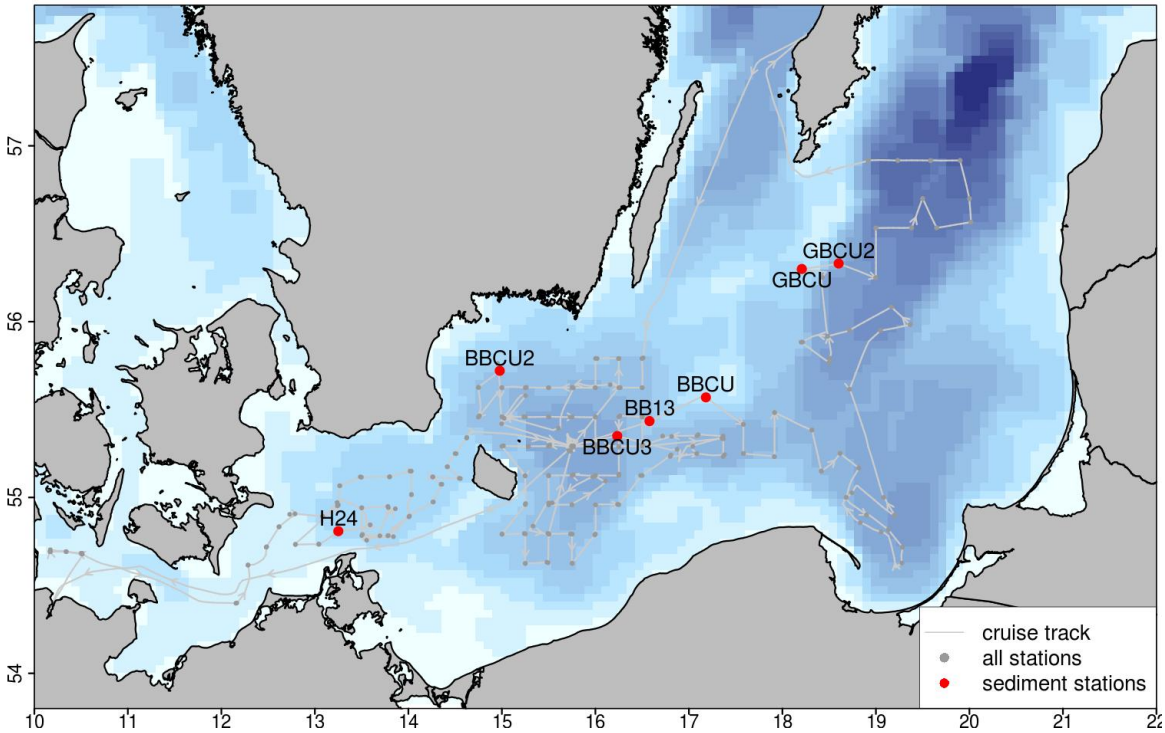

Figure 8: Map of AL493 cruise track. Microbiological sampling stations are highlighted in red and include seven stations in three deep basins of the Baltic Sea. Stations are assigned to the basins as follows: Arkona Basin: H24; Bornholm Basin: BB13, BBCU, BBCU2, BBCU3; Gotland Basin: GBCU, GBCU2. The map was generated by Burkhard von Dewitz with the statistical software R.

Initially, the sampling sequence at each station followed the sequence depicted in Figure 9 (water, grabbing, coring) and is described below.

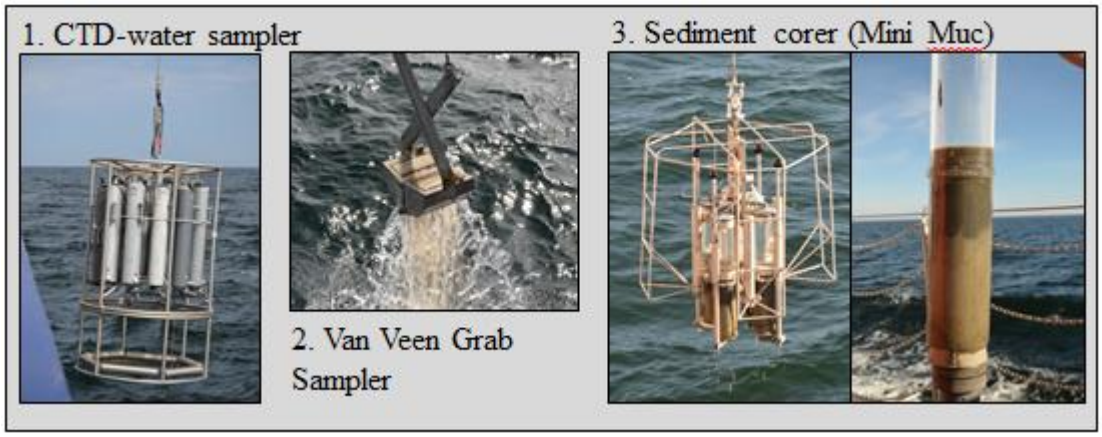

Figure 9: Sequence of all sampling devices employed at each station selected for microbiological sampling of sediment and water.

Water samples were collected with a CTD/water sampler by closing the attached Niskin bottles directly above the sediment. Three Niskin bottles were sampled by filling water in sterile 50 mL Falcon tubes. Subsequently, sediment samples were first collected with a grab sampler to evaluate the sediment composition and presence of invertebrates. Invertebrates were only detected at station BB13 in the Bornholm Basin. Small bivalves were attached to stones that the grab sampler brought up and placed into Kautex bottles before further processing in lab (Figure 10).

Figure 10: Bivalves sampled from stones collected in the Bornholm Basin (station BB13).

Afterwards, undisturbed surface sediment samples were collected with the multicorer. Three of the four sediment cores were processed by removing the first centimeter aseptically into sterile glass petri dishes before further processing. At the second and third station (BB13, BBCU) the expected sandy sediment was interspersed with stones, why no sediment coring could be conducted. In order to protect corer tubes from breaking and to save time during sampling, a CTD was equipped with an underwater camera (GoPro; CTD-GP) and two bicycle lamps. This device was successfully applied to record videos of the sediment surface until 40 m depth. Thus, at all stations with a suitable depth (GBCU, BBCU2) the sampling sequence was changed as shown in Figure 11. However, at these stations no sediment samples could be obtained because stones were observed with the CTD-GP (GBCU, BBCU2-1) or the sediment did not remain in the corer tubes due to its sandy nature (BBCU-2).

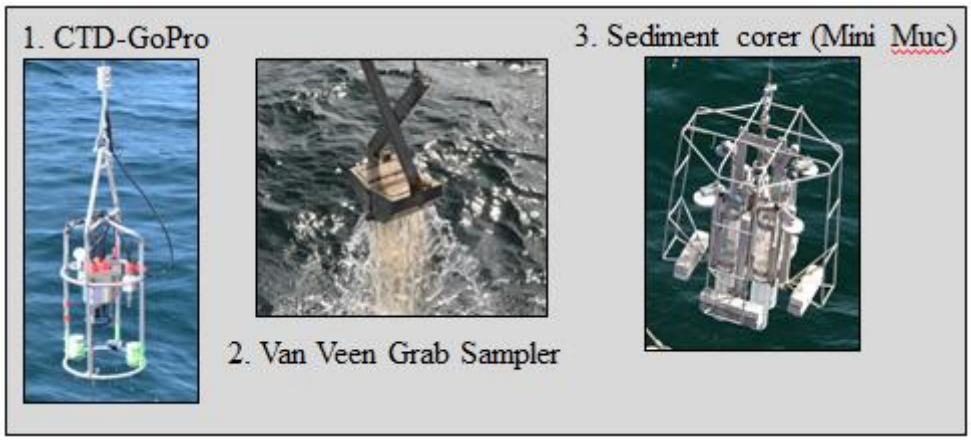

Figure 11: Altered sampling sequence for sediment observation via a CTD equipped with an underwater camera (GoPro).

At subsequent stations with a depth below 40 meters, the regular sampling sequence (Figure 9) was altered in a way that the grab sampler was done at first in order to test if the expected sediment type is present. This procedure verified that the sediment at the stations GBCU2 and BBCU3 is muddy and no stones are present. The successfully sampled station in the Gotland Basin (GBCU2) differed from the respective stations in the Arkona and Bornholm Basins because the water column above the sediment showed very low oxygen conditions. The sediment surface at those stations was found to be colonized by the filamentous sulfur bacterium *Beggiatoa* sp., which is a typical observation in the hypoxic regions of the Baltic Sea (Figure 12; e.g. Rosenberg & Diaz 1993 or at

http://www.geomar.de/index.php?id=4&no_cache=1&tx_ttnews%5btt_news%5d=3985&tx_ttnews%5bbackPid%5d=185).

Figure 12: Sediment core obtained in the Gotland Basin at 95 meters depth (station GBCU2). A mat of the filamentous sulfur bacterium *Beggiatoa* sp. is clearly visible.

Table 2: Detailed sampling schedule of all microbiological sampling stations of the research cruise AL493. Each station is indicated with a cruise specific name (*e.g.* H24) and a consecutive station number (*e.g.* 14).

Basin	Station	Date	Time (start)	Depth (m)	Sediment type	Device	No. of Hols	Hol-number(s)	Coordinates
Arkona	H24 / 14	14.05.2017	13:36	44	Mud	CTD-WS	1	1	N 54°48´51 E 13°15´05
						GS	3	1-3	
						MM	1	1	
Bornholm	BB13 / 40	16.05.2017	11:55	46	Sand & stones	CTD-WS	1	3	N 55°26´07 E 16°34´72
						GS	3	4-6	
						Station skipped (stones in GS)			
Bornholm	BBCU / 41	16.05.2017	14:31	27	Sand & stones	CTD-WS	1	4	N 55°34´17 E 17°10´92
						GS	3	7-9	
						MM	1	2	
						No sediment cores (stones in GS)			
Gotland	GBCU / 71	18.05.2017	14:06	31	Sand & stones	CTD-GP	1	53	N 56°17´65 E 18°10´71
						GS	1	10	
						Station skipped (stones detected by CTD-GP)			
					Sand	CTD-GP	1	54	N 56°17´98 E 18°12´47
						GS	3	11-13	
						MM	2	3, 4	
						Station skipped (sand did not remain in cores)			
Gotland	GBCU2 / 72-74	18.05.2017	16:20	95	Mud	GS	3	14-16	N 56°19´84 E 18°36´09
						MM	1	5	N 56°19´84 E 18°36´08
						CTD-WS	1	5	N 56°19´86 E 18°36´08
Bornholm	BBCU2 / 99	22.05.2017	09:18	32-33	Sand & stones	CTD-GP	1	78	N 55°43´24 E 14°58´63
						Station skipped (stones detected by CTD-GP)			
						CTD-GP	1	79	N 55°45´74 E 14°57´26

						Station skipped (stones detected by CTD-GP)			
Bornholm	BBCU3 / 154	26.05.2017	14:55	75	Clay/mud	GS	3	17-19	N 55°20´99 E 16°13´98 to N 55°21´00 E 16°14´02
						MM	1	6	N 55°21´01 E 16°14´01
						CTD-WS	1	6	N 55°21´01 E 16°14´02

CTD-GP = CTD coupled with GoPro, CTD-WS = CTD-water sampler, GS = Van Veen Grab Sampler, MM = Mini Muc (sediment corer).

Table 3: List of all samples collected for microbiological work during the research cruise AL493.

Basin	Station	Depth (m)	Sediment type	Samples taken	Replicates
Arkona	H24 / 14	44	Mud	Sediment	3
				Water	3
Bornholm	BB13 / 40	46	Sand & stones	*Mytilus* sp.	1
				Macoma balthica	1
				Water	3
Bornholm	BBCU / 41	27	Sand & stones	-	-
Gotland	GBCU / 71	31	Sand & stones	-	-
Gotland	GBCU2 / 72-74	95	Mud	Sediment	3
				Water	3
Bornholm	BBCU2 / 99	32-33	Sand & stones	-	-
Bornholm	BBCU3 / 154	75	Clay/mud	Sediment	3
				Water	3

5.4.3 Microbiological work

Collected sediment and water samples were inoculated onto four different growth media. Their composition is shown in Table 3. The pH value of PDA and WSP30 medium was adjusted before adding agar to 5.6 and 7.3.

Sample preparation was similar for all three sediment sampling stations (H24, GBCU2, BBCU3). Sediment samples were diluted 10- and 100-fold with sterile sodium chloride (0.8%) and 100 µL of each dilution were plated onto the four different growth media. Sediment samples were conserved with both 20% Glycerol and 20% DMSO. Approximately 6 mL of the respective solution were transferred to a sterile 15 mL Falcon tube and a sediment subsample (approximately 2-3 mL) was added. Tubes were frozen at -80°C. At the first station (H24) 100µL of each water sample and a 10-fold dilution were inoculated on the four different growth media. First observations showed that the colonization of the agar plates was very low why inoculation of water samples was altered slightly at the next two stations (GBCU2, BBCU3) by plating 100 µL and 500 µL but not a 10-fold dilution. Collected invertebrates (BB13) were morphologically identified as two different bivalve species, *Mytilus* sp. (I2) and *Macoma balthica* (I3). Of each species, one individual was selected for microbial cultivation. The shell was sterilized with 70% ethanol and removed aseptically with a scalpel. The mussel flesh was transferred to a sterile reaction tube (1,5 mL) and homogenized with a sterile single use mortar. A 1- and 10-fold dilution prepared with sterile 0.8% sodium chloride was plated on the same media like sediment and water samples. A few individuals of each bivalve species were placed in a sterile 1.5 mL reaction tube that contained 96% ethanol and frozen at -80°C. Remaining animals were placed into a plastic bag

and frozen as well. Isolation of microorganisms was done during the first two weeks of growth and will be repeated after approximately one month to include also slow growing microorganisms. It is attempted to isolate all different morphotypes from sediment (invertebrates) and water samples separately at all stations. Since isolation is still ongoing, a complete list of all isolated microorganisms will be submitted later. More than 200 bacteria and fungi have been already isolated. Their identification will be done by Sanger sequencing whenever the isolation of all microorganisms is finished. Finally, it was has to be noted that at station BB13 a small invertebrate identified as *Saduria* sp. (I1) was collected as well. This sample is not mentioned any further because no microorganisms could be grown from the prepared homogenate. It is assumed that the ethanol used for surface sterilization diffused into the animal as well and killed all associated microorganisms.

Table 4: The composition of growth media used for microorganisms obtained during water and sediment sampling on the research cruise AL493. Abbreviations of the respective media are given in brackets.

Component	**Amount (per litre)**
Marine Broth (MA)	
Ready-to-use mixture Difco™ Marine Broth 2216	37.4 g
Bacto agar	18.0 g
Potato-Dextrose-Agar (PDA)	
Potato extract (Fluka)	4.0 g
Glucose monohydrate	20.0 g
Bacto agar	18.0 g
Tryptic Soy Broth medium 3+10 (TSB3+10)	
Bacto tryptic soy broth	3.0 g
Sodium chloride	10.0 g
Bacto agar	18.0 g
Wickerham medium (WSP30)	
Glucose monohydrate	10.0 g
Peptone from soymeal	5.0 g
Malt extract	3.0 g
Yeast extract (Merck, Darmstadt, Germany)	3.0 g
Sea salt (Instant Ocean, Virginia, USA)	30.0 g
Bacto agar	18.0 g

5.4.4 Acknowledgements

I want to thank all responsible and involved scientists and technicians from the research unit Evolutionary Ecology of Marine Fishes as well as the whole crew of AL493 for making this research cruise such a success. Many thanks go to Burkhard von Dewitz for tremendous support and enthusiasm during planning, preparations and station selection. Special credits go to Svend Mees for his technical support during water sampling and the implementation of underwater movie observations. Theresa Bartelmess, Theresa Kuhl, Luisa Listmann and Christian Pawlitzki are thanked for their support during sampling and microbiological laboratory work. No less appreciation goes to Dr. Annemarie Kramer for scientific advice and for contributing to most pictures presented in this written report. The captain, the 1st and 2nd officer are acknowledged for their help during station selection and the bosun for his essential guidance during sediment coring with the Mini Muc. Coming to my own department, the Marine Natural Products Chemistry, I would like to thank Prof. Dr. Deniz Tasdemir for giving me the opportunity to join this research cruise, her trust and helpful advice during

setting up this research project. Finally, I am grateful to Dr. Martina Blümel and Arlette Wenzel-Storjohann for their assistance in formalities and cruise preparations as well as scientific discussions.

5.4.5 References

Rosenberg, R., & Diaz, R. J. (1993). Sulfur bacteria (*Beggiatoa* spp.) mats indicate hypoxic conditions in the inner Stockholm Archipelago. Ambio, 32-36.

6. Scientific equipment: instruments and gear

Hydrography:
- ADM-CTD with additional O2 sensor
- Hydrobios Water Sampler with CTD and O2 sensor

Zooplankton:
- Baby Bongo-Net (150 µm)
- Bongo-Net (335 µm)
- Bongo Net (500 µm)
- WP-2 (100 µm)
- Apstein net (55 µm)

Ichthyoplankton:
- Bongo-Net (335 µm and 500 µm)
- Hydrobios Multinet MAXI (335 µm horizontal tows)
- Hydrobios Multinet MIDI (50 µm vertical hauls)
- IKS-80 (500 µm)

Fish:
- Jungfisch Trawl (pelagic trawls) (0.5 cm)

Hydroacoustic:
- 38, 70, 120 and 200 kHz-echosounder EK60

Sediment sampling:
- Van Veen grab sampler ca. 15l volume
- Mini MUC, sediment multicorer equipped with 4 Tubes

7. Acknowledgements

Thanks to Captain Jan Peter Lass and the entire crew of RV ALKOR for their outstanding support during the cruise and to Svend Mees for his support in preparing the technical gear for the cruise and maintaining it during the cruise! Their extended experience and flexibility was crucial for the successful execution of the research program. Many thanks also goes to Dr. Jan Dierking chief scientist on AL491 with whom I jointly prepared all paper work including diplomatic formalities and scientific preparation of the cruise. I would also like to thank all scientific personal and student assistants on AL493 for their enthusiastic and relentless work on board. In particular I want to thank Caroline Utermann for making the cooperation in microbial community sampling successful and always very enjoyable.

The cruise received funding from BONUS (BIO-C3 project), the joint Baltic Sea research and development programme (Art 185), funded jointly from the European Union's Seventh Programme for research, technological development and demonstration (Forschungszentrum Jülich Beteiligungsgesellschaft mbH (Germany) and BMBF (Grant No. 03F0682A).

8. Appendix E1: Station list of AL 493

Supplied with the report in electronic form as Excel table, "Appendix E1 " – AL493_station_list.xlsx