# <span id="page-0-0"></span>**CRUISE REPORT: P14N**

*(Updated JUN 2019)* 



# **Highlights**

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# RV SONNE SO248 Cruise Report / Fahrtbericht

Auckland, New Zealand: May 1st, 2016 Dutch Harbor, USA: June 3<sup>rd</sup>, 2016

SO248 – BacGeoPac

Functional diversity of bacterial communities and the geometabolome in the central and north Pacific

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# <span id="page-4-0"></span>**1. Cruise summary / Zusammenfassung**

# 1.1 Zusammenfassung

Der Pazifische Ozean zwischen den subpolaren Regionen der nördlichen und südlichen Hemisphäre strukturiert sich in biogeografische Provinzen, die sich hinsichtlich der Wassermassen, Hydrografie, Nährstoffe und Planktongemeinschaften unterscheiden. Sie zeigen auch große Unterschiede im Chlorophyll und der Primärproduktion, von ultraoligotrophen Bedingungen im südpazifischen Wirbel bis hin zu eutrophischen Verhältnissen in der nordpazifischen Polarfront- und der subarktischen Region und der Beringsee. Bisher ist noch unbekannt, inwiefern die Unterschiede dieser biogeografischen Provinzen und der Wassermassen zwischen der Oberfläche und dem Meeresboden sich auch in der Zusammensetzung und den funktionellen Eigenschaften der prokaryontischen Mikrobengemeinschaften und des Pools der gelösten organischen Substanz (DOM) widerspiegeln. Aus diesem Grund war das Ziel der Forschungsexpedition SO248 BacGeoPac zu untersuchen, wie die generellen planktologischen und hydrografischen biogeografischen Muster sich in der Wachstums- und Substratdynamik, Biogeografie, Zusammensetzung und funktionellen Eigenschaften der prokaryontischen Mikrobengemeinschaften und in den chemogeografischen Mustern des DOM Pools zwischen dem Südpazifischen Wirbel und der Beringsee unterscheiden. Daher haben wir einen Transekt vom westlichen Rand des südpazifischen Wirbels bei 30°S bis in die Beringsee bei 59°N entlang des 180. Längengrades untersucht. Es wurden an 19 Stationen Proben genommen, davon an 11 Stationen über die gesamte Wassersäule bis zum Meeresboden und einer Tiefe von bis zu fast 6000 m Tiefe und an 8 Stationen bis in 1000 m Tiefe. Die Probennahme an den 19 Stationen umfasste die intensive Nutzung der Niskin-CTD-Rosette, bio-optische Charakterisierungen der euphotischen Zone, horizontale und vertikale Planktonnetzzüge, Sammeln von Mikroplastikpartikeln, Wasserprobennahme mit einer in situ Pumpe nahe der Oberfläche und Sedimentbeprobung mit einem Multicorer (MUC). Der Hauptfokus war auf die epipelagische und mesopelagische Zone gerichtet. Die Untersuchungen umfassten allerdings auch die Prokaryontengemeinschaften der bathy- und abyssopelagischen Zone und des Meeresbodens und die DOM-Verteilung und wie sie mit den aphotischen Wassermassen des Pazifiks korrelieren.

Um die funktionellen Eigenschaften der prokaryontischen Mikrobengemeinschaften besser zu verstehen war ein besonderer Fokus auf prozessorientierte Studien gelegt. Deshalb setzten wir Radioisotopen- und fluoreszenzmarkierte Modellsubstrate ein und führten Experimente mit stabilen Isotopen durch. Im südpazifischen Wirbel, in der nördlichen äquatorialen Gegenströmung und in der nördlichen Polarfrontregion wurden Mesokosmenexperiments durchgeführt, um die Reaktion der Bakteriengemeinschaften auf den Zusatz von verschiedenen Substraten zu prüfen. Im Äquatorialstrom und in der nördlichen Polarfrontregion wurden 24-Stunden Zeitserien untersucht, um die Reaktion der Bakteriengemeinschaften auf tageszeitliche Unterschiede in der Lichteinstrahlung, Primärproduktion, DOM-Versorgung und der Mortalität durch Grazing und Vireninfektion zu untersuchen.

Nach den Temperaturen und dem Salzgehalt der oberflächennahen Schichten konnten wir deutlich die Wassermassen und biogeografische Provinzen unterscheiden. Die Wassertemperaturen nahmen kontinuierlich zu vom südpazifischen Subtropischen Wirbel von 23° auf 30°C und weiter nördlich wieder ab auf 22°C am nördlichen Rand des nordpazifischen Subtropischen Wirbels. Die nordpazifische Polarfrontregion war durch starke Temperaturabnahme gekennzeichnet und in der Beringsee lag die Temperatur nur noch bei 4 bis 6°C. Der Salzgehalt zeigte große Unterschiede in den verschiedenen Provinzen, insbesondere in der äquatorialen Region und der nördlichen Polarfrontzone. Durch die Messungen mit der bis ins Abyssopelagial gehenden CTD konnten wir die Wassermassen des aphotischen Pazifiks gut identifizieren.

In den permanent geschichteten Provinzen lag das tiefe Chlorophyllmaximum zwischen 60 und 120 m mit der tiefsten Position in den beiden subtropischen Wirbeln. Im äquatorialen Auftriebsgebiet war es angehoben auf 70 m. Prominente Phytoplanktonblüten existierten nur nördlich von 40°N, vor allem aber nördlich von 50°N. Nördlich von 5°N waren zwischen 200 und 1700 m Tiefe ausgeprägte Sauerstoffminimumzonen vorhanden mit Restkonzentrationen von Sauerstoff von noch etwa 15% der Oberflächenwerte.

Die bisher erhobenen mikrobiellen Parameter spiegelten die verschiedenen Wassermassen und teilweise auch die biogeografischen Provinzen gut wider. Die endgültige Interpretation der Daten wird jedoch erst möglich sein, wenn alle anderen Proben hinsichtlich der Zusammensetzung und funktionellen Eigenschaften der Prokaryontengemeinschaften mit aktuellen Methoden (next generation sequencing, Metagenomik, -transkriptomik, -proteomik) und die Zusammensetzung des DOM Pools analysiert und ausgewertet worden sind,

Erste Ergebnisse der Prokaryontenabundanz in den obersten 100 m, analysiert mittels Durchflusszytometrie an Bord, zeigten zwischen 2 und 22x10<sup>5</sup> Zellen ml<sup>-1</sup> mit kontinuierlich zunehmenden Werten von Süden nach Norden. Die bakterielle Biomassproduktion, gemessen mittels der Aufnahme von <sup>14</sup>C-markiertem Leucin, war in den obersten 100 m am höchsten in der südlichen und nördlichen äquatorialen Gegenströmung und sehr viel niedriger in den anderen Provinzen. Die Wachstumsraten der gesamten Bakteriengemeinschaft kovariierten mit der bakteriellen Biomasseproduktion mit höchsten Werten von 1 bis >2 pro Tag und viel geringeren Werten in den anderen Provinzen. Vorläufige Ergebnisse der extrazellulären hydrolytischen Enzymaktivitäten von verschiedenen Biopolymeren zeigten charakteristische Muster der verschiedenen Provinzen.

Da ein erheblicher Teil der Prokaryonten und insbesondere Archaeen in der Tiefsee chemoautotroph sind wurde die CO2-Dunkelfixierung gemessen. Höchste Raten wurden in der äquatorialen Region gemessen, aber in einigen Regionen weiter nördlich erreichten einige Werte zumindest 50% der äquatorialen Maxima. Werte in 1000 m Tiefe waren normalerweise, aber nicht immer, höher als in 2000 m.

Das Oberflächensediment entlang des Transektes zeichnete sich durch recht unterschiedliche Strukturen und Texturen aus. Die Bakterienabundanzen an der Sedimentoberfläche lagen zwischen 10<sup>8</sup> und 10<sup>9</sup> Zellen cm<sup>-3</sup> und in 20 cm unter dem Meeresboden sehr viel niedriger. Die Werte in beiden Tiefen nahmen nördlich des Äquators kontinuierlich zu.

Nach den vorläufigen Daten, die wir während der Forschungsfahrt bereits erheben konnten, sind wir sehr zuversichtlich, dass die BacGeoPac-Forschungsfahrt sehr erfolgreich war und dass wir die Ziele dieser umfangreichen Untersuchung erreichen werden.

Die meisten Untersuchungen wurden im Rahmen des durch die DFG geförderten Sonderforschungsbereichs Roseobacter (TRR51) durchgeführt.

# <span id="page-6-0"></span>1.2 Summary

The Pacific Ocean between the subpolar regions of the northern and southern hemisphere stretches over distinct biogeographic provinces which differ with respect to water masses, hydrography, nutrients and plankton communities. They also differ greatly with respect to chlorophyll *a* and primary production, from ultra-oligotrophic conditions in the south Pacific gyre to eutrophic nutrient regimes in the north Pacific polar frontal and subarctic region and the Bering Sea. So far, it is unknown how the differences in these biogeographic provinces and water masses between the surface and sea floor are reflected by the composition and functional properties of prokaryotic microbial communities and the dissolved organic matter (DOM) pool. Therefore, the aim of cruise SO248 BacGeoPac was to investigate how the general planktonand hydrography-related biogeographic patterns are reflected in the growth and substrate dynamics, biogeography, composition and functional properties of the prokaryotic microbial communities and in the chemogeography patterns of the DOM pool between the south Pacific gyre and the Bering Sea. Hence, we sampled a transect from the western edge of the south Pacific gyre at 30°S to the Bering Sea at 59°N along the 180<sup>th</sup> longitudinal degree. At 19 stations samples were collected, at 11 stations throughout the water column to the seafloor at depths of up to almost 6000 m and at 8 stations to 1000 m depth. Sampling included extensive CTD-casts, bio-optical characterization of the euphotic zone, horizontal and vertical plankton net tows, collection of microplastic particles, in situ pump deployments in near surface waters and sediment sampling with a multicorer MUC. The major focus was on the epipelagic and mesopelagic zones. However, we also included in our investigations the bathypelagic, abyssopelagic and sea floor-associated prokaryotic communities and DOM patterns and how they relate to the water masses of the dark Pacific.

To better understand the functional properties of the prokaryotic microbial communities in the different biogeographic provinces a special focus was on process studies. Therefore we applied radio- and fluorescently labelled model substrates and stable isotope tracer experiments. Further, mesocosm experiments were carried out in the south Pacific gyre, the north equatorial counter current and the north Pacific polar frontal region to examine the response of the bacterial communities to various substrate amendments. In the equatorial upwelling region and the north Pacific polar frontal region 24 hour time series were conducted to assess the response of the bacterial communities to diurnal changes in irradiance, primary production, DOM supply and mortality by grazing and virus infection.

According to temperature and salinity in the near-surface layer we could clearly identify the water masses and biogeographic provinces. Water temperatures steadily increased from the south Pacific gyre to the Pacific equatorial current from 23° to 30°C and further north decreased again to 22°C at the northern edge of the North Pacific Subtropical Gyre. The north Pacific polar frontal region was characterized by a strong decrease in temperature and in the Bering Sea temperature ranged between 4° and 6°C. Salinity exhibited strong differences in the various biogeographic provinces, in particular in the equatorial region and the north Pacific polar frontal region. From the deep CTD casts we could identify the characteristic water masses in the deep Pacific.

In the permanently stratified warm provinces the deep chlorophyll maximum was situated between 60 and 120 m with the deepest extension in both subtropical gyres. In the equatorial upwelling it was uplifted to 70 m. Prominent phytoplankton blooms were only established north of 40°N and in particular north of 50°N. North of 5°N between depths of 200 and 1700 m extensive oxygen minimum zones were established with remaining oxygen concentrations of only about 15% of surface values.

The microbial parameters assessed reflected well the different water masses and partially the biogeographic provinces. Final interpretation of the data, however, is only possible when we will have analyzed and evaluated all the samples of the composition and functional properties of the prokaryotic communities, applying state of the art analyses (next generation sequencing, metagenomics, -transcriptomics, -proteomics) and the composition of the DOM pool.

First results of the prokaryotic abundance in the upper 100 m, assessed by flow cytometry on board, ranged between 2 and 22x10<sup>5</sup> cells ml<sup>-1</sup> with continuously increasing values from south to north. Bacterial biomass production, assessed by incorporation of <sup>14</sup>C-labelled leucine, in the upper 100 m was highest in the south and north equatorial counter currents and much lower in the other provinces. Community growth rates covaried with the bacterial biomass production with highest values of 1 and >2 per day and much lower in other provinces. Preliminary results of extracellular hydrolytic enzyme activities of various biopolymers exhibited distinct patterns for the various provinces.

As quite a few prokaryotes and in particular Archaea in the deep sea are chemoautotrophic CO<sub>2</sub> dark fixation was assessed. Highest rates were measured in the equatorial region but in some regions further north values reached 50% of the equatorial maxima. Values at 1000 m were usually, but not always, higher than at 2000 m.

The surface sediment along the transect exhibited quite variable structures and textures. Bacterial abundance at the sediment surface ranged between 10<sup>8</sup> and 10<sup>9</sup> cells cm<sup>-3</sup> and much lower values at 20 cm below the seafloor. Values at both depths increased continuously north of the equator.

According to the preliminary data we were able to collect already during the cruise we are very confident that the BacGeoPac cruise was very successful and that we can reach the aims of this comprehensive study.

Most of the investigations were carried out in the frame work of the DFG-funded Collaborative Research Center *Roseobacter* (TRR51).

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<span id="page-10-0"></span>2.3 Scientific party / wissenschaftliche Fahrtteilnehmer



<span id="page-11-0"></span>

Figure 2.1: The scientific party of RV Sonne expedition BacGeoPac SO248

# 2.4 Crew / Mannschaft



# <span id="page-12-0"></span>**3. Narrative of the cruise / Ablauf der Forschungsfahrt**

On Sunday, May 1<sup>st</sup> around 9:00 am local time Research Vessel Sonne with the embarked 40 scientists and 30 crew members left the port of Auckland for the BacGeoPac cruise to head to the first station at 30°S, 177°E, where we arrived in the morning of May  $3^{rd}$ . Three 20 footcontainers and air fright boxes with scientific equipment had arrived in time so that everything we needed was on board. As the scientists came on board on April  $30<sup>th</sup>$  we had plenty of time to set up the labs and get ready for the first station work. This station was particularly exciting for us because we tested our brand new CTD-rosette with 24 20-Liter Niskin bottles (Fig. 3.1).

The instrument was designed and constructed in the ICBM workshop and only tested once very briefly before shipping it to Auckland. Because of the large water demand during this cruise we had decided in October 2015 to build this new instrument in order to optimize water collection and to save time. It worked perfectly right away and during the entire cruise without any malfunctioning. At station 1, a shallow station at the eastern edge of the ultra-oligotrophic South Pacific Subtropical Gyre (SPS, for the Pacific biogeographic provinces see [Fig. 4.1\)](#page-15-0) where we collected water to a depth of 1000 m, we also deployed a McLane in situ pump at 60 m for three hours to collect water for bacterial popu-



 Fig. 3.1: Scientists withdrawing water samples from the 24x20 Liter CTD rosette sampler

lation genomics studies, collected zooplankton to isolate bacteria by a vertical haul and microplastic by a horizontal tow with the Bongo net and carried out bio-optical measurements (Secchi-depth, hyperspectral und multispectral light field measurements (UV/VIS)) to characterize ocean optical properties. The next station 2 at 25°S, 179°E, still in the South Pacific Subtropical Gyre, was a deep station where, in addition to the work at station 1, we collected water all the way to the sea floor and surface sediment with a multi corer (MUC, Fig. 3.2). We used a brand new prototype-like instrument which worked perfectly during the entire cruise without

any flaw or sediment loss due to incomplete closure of the lids at the bottom of the plexiglass tubes. In addition we collected water for our first mesocosm experiments. These types of samplings became the routine at the following stations which usually alternated between shallow and deep stations along the transect. Thanks to the large volume CTD we usually needed only one shallow and one deep cast, but in quite a few instances one or two extra casts for special needs.



Fig. 3.2: Retrival of the Multi Corer

During the entire cruise we had regular meetings of the PIs of the different working groups and with the entire scientific party on board to discuss details of the planned station work and to present the planned work of each group. Later on first results of some of these groups were included in the presentations. The planned work and some first results were also presented to interested members of the crew.

The stations along the transect were selected such that we aimed at visiting at least two stations, one shallow and one deep, in each biogeographic province. This aim was achieved in all provinces except in the South and North Pacific Equatorial Counter Current (SPE, NPE) which are very narrow so that we had only one station in each of these provinces. Hence the distance between the stations was about 4 to 6° latitude. Unfortunately, we could not visit a planned station in the SPS at 20°S which is situated in the EEZ of Fiji Islands. We had applied to work in Fiji's EEZ well in time but never received any response from the authorities and thus had to skip this station.

According to temperature and salinity in the near-surface layer we could clearly identify the water masses and biogeographic provinces. Water temperatures steadily increased from the SPS to the Pacific Equatorial Current from 23° to 30°C and further north decreased again to 22° at the northern edge of the North Pacific Subtropical Gyre (NPTG). The North Pacific Polar Frontal Region (NPF) was characterized by a strong decrease in temperature such that in the Pacific Subarctic Region (PSAG) and the Bering Sea (BER) temperature ranged between 4° and 6°C [\(Fig. 7.4\).](#page-26-0) Salinity exhibited strong differences in the various biogeographic provinces, in particular in the equatorial region and the NPF [\(Fig. 7.3\).](#page-26-0) From the deep CTD casts we could identify the characteristic water masses in the deep Pacific [\(Fig. 7.6\).](#page-27-0) The water masses with the highest density at all stations was the central deep water (CDW). Overall, the tropical and subtropical water masses were clearly distinguished from those of the north Pacific.

In the permanently stratified warm provinces the DCM was situated between 60 and 120 m with the deepest extension in both subtropical gyres [\(Fig. 7.3\).](#page-26-0) In the equatorial upwelling it was uplifted to 70 m. Well pronounced phytoplankton blooms were only established north of 40°N and in particular north of 50°N.

North of 5°N between depths of 200 and 1700 m extensive oxygen minimum zones were es-tablished with remaining oxygen concentrations of only about 15% of surface values (Fig. 7.5).

The microbial parameters assessed reflected well the different water masses and partially the biogeographic provinces. Final interpretation of the data, however, is only possible when we will have analyzed all the samples for the prokaryotic community and the composition of the dissolved organic matter (DOM) pool.

Prokaryotic abundance in the upper 100 m, assessed by flow cytometry on board, ranged between 2 and 22x10<sup>5</sup> cells ml<sup>-1</sup> with continuously increasing values from south to north and highest numbers in the PSAG and BER. Bacterial biomass production, assessed by incorpora-tion of <sup>14</sup>C-labelled leucine, in the upper 100 m was highest in the SPE and NPE and much lower in the other provinces. Community growth rates in these provinces ranged between 1 and >2 per day but were much lower, usually not exceeding 0.4 per day, in the other regions. Turnover rates of dissolved free amino acids basically covaried with rates of biomass produc-tion but those of glucose and acetate exhibited different patterns with continuously increasing rates north of the equator and highest values in the PSAG and BER. Preliminary results of hydrolytic enzyme activities of various polysaccharides and peptidases exhibited distinct pat-terns for the various provinces. In particular the peptidolytic activities reached highest values in the SPE, PEQ and NPE, in line with the data of bacterial biomass production.

11 they were al-most immediate with high responses in all treatments whereas at the NPF they The mesocosm experiments at the station in the SPS, the NPE and the NPF, exhibited strik-ingly different growth responses of the ambient bacterial communities to the various substrate and vitamin B12 additions. At SPS the responses were generally slow. At NPE were interme-diate.

As quite a few prokaryotes and in particular Archaea in the deep sea are chemoautotrophic  $CO<sub>2</sub>$  dark fixation was assessed. Highest rates were measured in the equatorial region but in some regions further north values reached 50% of the equatorial maxima. Values at 1000 m were usually, but not always, higher than at 2000 m.

The surface sediment along the transect exhibited quite variable structures and textures. This was already obvious from the color of the sediment [\(Fig. 7.17\)](#page-41-0). Bacterial abundance at the sediment surface ranged between 10<sup>8</sup> and 10<sup>9</sup> cells cm<sup>-3</sup> with continuously increasing values north of the equator. At 20 cm below the seafloor cell numbers were about one order of magni-tude lower. Alkaline phosphate activities were highest in the region 6 to 20°N whereas ami-nopeptidase activities peaked between 34° and 50°N, the northern edge of the NPTG and the PSAG, regions of a pronounced oxygen minimum zone and a high sinking flux.

According to the preliminary data we were able to collect already during the cruise we are very confident that the BacGeoPac cruise was very successful and that we can reach the goals we set for this comprehensive study. However, to achieve them all the samples stored frozen in the home labs need first to be analyzed.

On May 31<sup>st</sup> we finished the work at the northernmost station. This left us enough time to finish the last incubations, pack all material and equipment before we reached the final destination, Dutch Harbor, on Unalaska one of the Aleutian Islands, Alaska. Our cruise was generally blessed with good weather even though we had to pass through two storms, one in the southeastern Trade Wind region, and one in the north Pacific polar frontal region. Despite a swell of 5 to 7 m during these storms the ship operated smoothly and we could carry out our work as usual. In fact, we did not have any interruption of our work due to weather conditions or any malfunctioning of instrumentation or ship equipment.

Dutch Harbor as final destination of this cruise was a challenge and resulted in some inconveniencies which were out of our control. It is the major fisheries harbor of the US and very much focused on services to the fisheries industries. Dutch Harbor is only reachable by small airplanes, but not during bad weather conditions, or in summer twice a month by a ship. In order to ship our frozen samples back to Germany the responsible carrier needed to charter a special airplane to Dutch Harbor. Further, the harbor can only handle 40 ft but not 20 ft containers. Therefore, our three containers needed to remain on board until RV Sonne reaches a port in Japan in mid-August or even only in September.

A post cruise meeting for shipboard and shore-based scientists to present and discuss the results will be scheduled for July 2017. This rather late meeting after the cruise is because all except two working groups are also involved in research of cruise SO254 which takes place from January  $26<sup>th</sup>$  to March 1<sup>st</sup>, 2017.



# <span id="page-15-0"></span>**4. Aims of the Cruise / Zielsetzung der Forschungsfahrt**

#### 4.1 General aims

The Pacific Ocean between the subpolar regions of the northern and southern hemisphere stretches over distinct biogeographic provinces which differ with respect to water masses, hydrography, nutrients and plankton communities (Fig. 4.1): Subantarctic, south subtropical convergence, south Pacific subtropical gyre (SPS), Pacific warm pool (WAR), Pacific equatorial current (PEQ), south and north Pacific equatorial counter current (PSE, PNE), north Pacific subtropical gyre (NPTG), north Pacific polar frontal region (NPF), Pacific subarctic region (PSAG), Bering Sea (BER). These provinces differ greatly with respect to chlorophyll *a* and primary production, from ultraoligotrophic conditions in the SPS to eutrophic nutrient regimes in the NPF, PSAG and BER (Fig. 4.2).

The overarching aim of the BacGeoPac cruise was to investigate how these general plankton- and hydrography-related biogeographic patterns are reflected in the growth and substrate dynamics, biogeography, composition and functional properties of the prokaryotic communities and in the chemogeography patterns of the DOM pool between SPS and BER. The Pacific is largely unexplored with respect to these microbial biogeography and chemodiversity patterns. The major focus was on the epipelagic and mesopelagic zones. However, we also included in our investigations the bathypelagic, abyssopelagic and sea floor-associated prokaryotic communities and DOM patterns and how they relate to the water masses of the dark ocean.

To achieve these aims we sampled a transect from the western edge of the SPS at 30°S to the BER at  $59°N$  around the 180<sup>th</sup> longitudinal degree (Fig. 4.1) and 4.2).

In order to extend this transect further south and to cover the entire Pacific from subantarctic to subarctic waters we will return to RV Sonne in late January 2017 on cruise SO254 and continue this transect from 30°S to 60°S.



Fig. 4.1: Biogeographic provinces in the Pacific visited and track of cruise SO248 (red line). For abbreviations see text.



Fig. 4.1: Chlorophyll *a* concentrations in the Pacific and track of cruise SO248 (red line).

To better understand the functional properties of the prokaryotic communities in the different biogeographic provinces a special focus was on process studies. Therefore we applied radioand fluorescently labelled model substrates and stable isotope tracer experiments and mesocosms with different substrate amendments and 24 hour time series studies.

<span id="page-16-0"></span>Besides growth activities, substrate preferences and the composition of the bacterial and archaeal communities, the phytoplankton communities, nutrient concentrations and the DOM composition were studied comprehensively for the first time on such a long transect in the Pacific.

Most of the investigations were carried out in the frame work of the DFG-funded Collaborative Research Center *Roseobacter* (TRR51).

#### 4.2 Major topics of investigations on board

#### *4.2.1 Hydrographic and biogeochemical characterization of the biogeographic provinces and water masses*

In order to characterize the biogeographic provinces and water masses we assessed the hydrographic properties of the water column from the surface to the sea floor by the sensors of the CTD rosette sampler (salinity, temperature, transparency, oxygen, fluorescence). For biogeochemical parameters such as chlorophyll, particulate organic carbon and nitrogen (POC, PON) and inorganic nutrients (nitrate, nitrite, ammonium, phosphate, silicate) samples were collected from distinct depths out of Niskin bottles mounted on the rosette sampler.

#### *4.2.2 Composition, diversity and function of the epi- and mesopelagic microbial communities*

Our aim was to comprehensively assess the composition, diversity and function of the prokaryotic communities in all biogeographic provinces in the epipelagic and mesopelagic zones. Therefore, we collected samples from the surface to 1000 m depth and prepared them for later analyses by state of the art molecular microbiological approaches such as fluorescent in situ hybridization (CARD-FISH), next generation sequencing of variable regions of the 16S rRNA gene, single cell sorting and genome sequencing, metagenomics, -transcriptomics and –proteomics.

To assess functional properties of these communities bulk rates of prokaryotic biomass production, turnover rates of amino acids, glucose and acetate were assessed by radiotracer techniques. Further, microautoradiography coupled with FISH (MAR-FISH) and using leucine as a proxy for protein synthesis and amino acids, glucose and acetate as substrates was applied. Microbial polymer hydrolysis was examined by applying fluorescently labelled polymeric substrates.

#### *4.2.3 Mesocosm experiments to assess functional responses to substrate amendmens*

In order to examine how the ambient bacterial communities respond to substrate amendments mesocosm experiments in triplicate 20 L carboys and controls were conducted in the SPS, NPE and NPF. An exudate of the diatom *Thalassiosira rotula*, alginate and vitamin B12 and a precursor were added to separate sets of mesocosms and incubated for six days at ambient temperature. Subsamples for bacterial abundance, biomass production, substrate turnover, bacterial community composition and DOM analysis by ultrahigh resolution mass spectrometry (see below) were withdrawn periodically.

#### *4.2.4 Diversity and function of the microbial communities in the dark ocean*

The bacterial and archaeal communities in the dark ocean (>1000 m depth) were investigated with respect to community composition and key functional processes, e.g. heterotrophic biomass production and  $CO<sub>2</sub>$  dark fixation. The specific aim was to examine whether the different water masses in the deep ocean, the subantarctic and subarctic intermediate waters and the deep water harbor distinct microbial communities with different rates of biomass production and  $CO<sub>2</sub>$ -dark fixation. Similar methods as in the upper water column were applied.

#### *4.2.5 Diversity and function of surface-sediment-associated microbial communities*

In order to examine how the greatly varying trophic state and sinking flux of the different biogeographic processes is reflected in the sea floor-associated microbial communities their composition and polymer degradation potential was investigated in the upper 20 cm of the sediment. Similar methods as in the upper water column were applied.

#### *4.2.6 Dissolved Organic Matter (DOM)*

The composition of the DOM pool was assessed by ultrahigh resolution mass spectrometry, FT-ICR-MS, (Fourier transform ion cyclotron resonance mass spectrometry, Dittmar and Stubbins 2014) in the entire water column to characterize the biogeographic provinces and water masses also by these geochemical features. They are mainly a result of the processing of organic matter by the resident microbes. Concentrations of dissolved amino acids and carbohydrates and of dissolved organic carbon were measured as well.

# <span id="page-18-0"></span>**5. Agenda of the cruise / Programm der Forschungsfahrt**

#### *5.1 Cruise track*

A south – north transect across the Pacific was investigated from 30°S to 59°N at or close to the 180<sup>th</sup> latitudinal degree to comprehensively cover the major biogeographic provinces of this ocean (Fig. 5.1). In contrast to the impression o[f Fig. 4.1](#page-15-0) and as verified by our hydrographic measurements this transect did not go through the Pacific Warm Pool but a little east and covered the various equatorial provinces with their complex currents, counter currents (PSE, PEQ, PNE) and upwelling regions. Sampling included extensive CTD-casts throughout the water column, bio-optical characterization of the euphotic zone, horizontal and vertical plankton net tows, collection of microplastic particles, in situ pump deployments in near surface waters and sediment sampling with a MUC at all deep stations. In the SPS, the PNE and NPF mesocosm experiments were carried out to examine the response of the bacterial



cruise SO248 across the Pacific.

communities to various substrate amendments. In the PEQ and NPF 24 hour time series were conducted to assess the response of the bacterial communities to diurnal changes in irradiance, primary production, DOM supply and mortality by grazing and virus infection.

#### *5.2 Station work*

The investigation included 19 stations, 11 "deep" stations from the surface to the sea floor, including the surface sediment, and 8 "shallow" stations from the surface to 1000 m depth (Fig. 5.1). The locations of the stations were planned and executed such that we had usually at least two stations in each biogeographic province. This resulted in distances of  $\sim$ 3-6° latitude between two stations. Only for the narrow PSE, PEQ and PNE this scheme did not work such that we had only one deep station in each of these provinces.

<span id="page-19-0"></span>The operations of the deep stations usually started with either a MUC or a shallow CTD down to 1000 m, depending on the time of the day (Fig. 5.2). The aim was to have the CTD on deck in the morning around 8:00 hours local time whenever possible so that we had a morning situation for all analyses. We had a fixed number of defined depths between 20 and 1000 m depth for collecting samples (20, 40, 60, 100, 200, 300, 500, 1000 m) and only the depth of the deep chlorophyll maximum (DCM) was adjusted according the fluorescence reading of the CTD downcast. Sample collection with the Niskin bottles was always done during the CTD



Fig. 5.2: Retrival of the 24x20 Liter CTD, sediment cores by the MUC and the McLane in situ pump.

upcast. When the MUC was operated prior to the CTD, between 2:00 and ~6:00 hours local time we relocated the ship for the CTD cast to a new site at a distance of 2 nm. The deep CTD, covering the water column below 1000 m and to 10 m above sea floor with sampling collection roughly every 1000 m, followed thereafter and, if needed, another CTD for special water requirements. Samples for various parameters were withdrawn from the Niskin bottles (see below). The McLane in situ pump was usually deployed at 20 or 60 m for 3 hours shortly after the start of the shallow CTD from the stern on the starboard side (Fig. 5.2). This deployment scheme saved time and still allowed operating the CTD smoothly.

Parameter	$0-200$ m	200-1000 m	1000 m-seafloor
Particulate organic carbon / nitrogen	+	+	
Chlorophyll			
Inorganic nutrients		+	
(nitrate, nitrite, ammonium, phosphate, silikate)			
Dissolved amino acids / carbohydrates	٠	+	
<b>DOC</b>			
DOM (FT-ICR-MS)			
<b>CARD-FISH</b>			
Bacterial biomass production			
Turnover rates of amino acids, glucose, acetate	$\ddot{}$		
<b>MAR-FISH</b>			
Microbial polymer hydrolysis		+	
Metagenomics, -transcriptomics, -proteomics			
Prokaryotic community composition		٠	

Table 5.1: Measured parameters in samples withdrawn from the Niskin bottles:

<span id="page-20-0"></span>Optical characterization of the upper 200 m followed the CTD operations. This included Secchi depth reading, Forel Ule estimates of ocean color and UV and optical profilers. The profilers were set out from the stern portside to 200 m in free falling mode as the ship gently moved forward.

Thereafter a vertical or horizontal tow of the Bongo net with a mesh size of 200 um followed. The vertical tow for collecting zooplankton covered the upper 200 m of the water column. With the horizontal tow at the surface microplastic was collected which lasted 30 min at a ship speed of 2-3 kn.

In case the MUC was not operated prior to the CTD casts the station work was terminated with sediment collection by the MUC. From the sediment slices from the surface and 20 cm below seafloor were prepared for analysis of pore water, phosphatase and leucine aminopeptidase activities, prokaryotic cell numbers and community analysis.

Shallow stations followed basically the same sequence of operations except the deep CTD and the MUC casts.

During the two 24 hour time series stations CTD casts down to 500 m were run every 3 hours in addition to the operations of a regular deep station.

#### *5.3 Underway measurements*

In addition to ship-based underway data (weather conditions, incident light, ADCP) additional parameters were assessed by a FerryBox for a better characterization of the extension of the biogeographic provinces. This device is a flow through system connected to the ship's continuous pump system to monitor continuously temperature, salinity, chlorophyll fluorescence, turbidity and dissolved oxygen.

Discrete Aerosol Optical Thickness (AOT) measurements were made whenever clear and cloudless sky allowed. Readings were done with a handheld MICROTOPS II instrument kindly provided by the NASA/Goddard Space Flight Center in conjunction with the AERONET Maritime Aerosol Network program.

# <span id="page-21-0"></span>*6. Settings of the working area / Beschreibung des Arbeitsgebiets*

The Pacific Ocean, the largest water mass globally, exhibits distinct hydrographic patterns and biogeographic provinces between the tropics and subpolar regions [\(Figs. 4.1,](#page-15-0) [4.2,](#page-15-0) Fig. 6.1, 6.2, Longhurst 2006). The largest areas are covered by the ultraoligotrophic South Pacific and the North Pacific Subtropical Gyres, permanently stratified and nutrientdepleted areas. The tropics are characterized by the equatorial currents and southern and northern counter currents with pronounced upwelling features, leading to substantially higher rates of primary production and phytoplankton biomass than the subtropical gyres locked out of nutrient input from nutrient-enriched deep water. The polar frontal and the subarctic region are affected by the westerly and polar winds and pronounced seasonal hy-



Fig. 6.1: Surface currents in the Pacific (Tomczak and Godfrey 1994).

drographic and plankton dynamics. High concentrations of inorganic nutrients, including silicate, lead to pronounced spring and summer phytoplankton blooms and a high sinking flux and sediments enriched in organic matter.

Quite a few studies have been carried out on the composition and growth dynamics of bacterioplankton communities in various regions of the Pacific Ocean including the equatorial upwelling, the subtropical gyres and the colder regions, mainly on the northern hemisphere (Shi et al. 2011, Sowell et al. 2011, Tada et al. 2011, Ottesen et al. 2013). However, only scarce information and none applying state of the art community analysis exists on basin-wide patterns of the composition of bacterioplankton communities and microbial polymer hydrolysis in the Pacific (Baldwin et al. 2005, Arnosti et al. 2011). In fact no study is available on the composition and biodiversity of bacterioplankton communities and their active players in relation to the major biogeographic provinces in the Pacific. Further, no information is available on the DOM composition in the biogeographic provinces of this ocean.



Fig. 6.2: Vertical structure of the water masses in the Pacific at 160°W between the surface and seafloor (Dietrich et al. 1992).

The deep Pacific ocean with the oldest oceanic water masses (Hansell 2013), pronounced northern and southern intermediate waters and a large central deep water mass [\(Fig. 6.2,](#page-21-0) Die-trich et al. 1992) is also rather unexplored with respect to assessing structure and function of the prokaryotic microbial communities. Only a few studies from the North Pacific Subtropical Gyre are available (DeLong et al. 2006, Swan et al. 2011, Ottesen et al. 2014). The only large scale studies including the dark ocean carried out so far investigated bulk growth properties of the microbial communities between the Bering Sea and tropical regions but did not address microbial community composition (Nagata et al. 2000, Yokokawa et al. 2013).

# <span id="page-23-0"></span>**7. Work details and first results / Beschreibung der Arbeiten im Detail einschließlich erster Ergebnisse**

Investigations described in chapters 7.3, 7.4, 7.6, 7.7, 7.8, 7.9 and 7.10 were carried out as projects and key work packages of the Transregional Collaborative Research Center Ecology, Physiology and Molecular Biology of the *Roseobacter* clade: Towards a Systems Biology Understanding of a Globally Important Clade of Marine Bacteria (TRR 51, [www.roseobacter.de\)](http://www.roseobacter.de/).

# *7.1 Oceanographic Measurements - New Deepwater-CTD Rosette*  (TH Badewien, H Winkler, KL Arndt, O Zielinski)

The oceanographic measurements were carried out with the help of a CTD- (Conductivity, Temperature, Depth) probe attached to a rosette water sampler. The major goal was to identify the thermohaline structure of the water column. In addition, different water masses of the Pacific had to be identified.

To adequately analyze the genomic, proteomic and biochemical characteristics of bacteria in oligotrophic oceanic regions, high volumes of water from different depths had to be obtained. In addition, several mesocosm experiments were conducted onboard using ambient seawater. Thus, to serve the high demand for seawater, we designed and constructed a new frame sized in such a way that it could accommodate 24 water-sampling bottles with a volume of 20 L each as well as the standard Seabird probes.

# *Methods and instrument details*

We tested the newly designed CTD-rosette for the first time under oceanographic conditions during this cruise. The system worked well even under harsh weather conditions and the high pressure present at the deepest sampling stations (up to about 5900 m depth).

We used a Sea-Bird Electronics Inc. CTD SBE 911plus probe, SN 09-1266, attached to a SBE 32 Carousel Water Sampler SN 32-1119 containing 24 20-liter Ocean Test Equipment Inc. bottles. The CTD system is equipped with double temperature and conductivity sensors, an oxygen sensor, a pressure sensors, an altimeter, and a combined chlorophyll fluorometer and turbidity sensor.

Before each measurement, the CTD was adapted to the ambient water temperature at 10 m water depth for 3 minutes. We obtained temperature, conductivity, oxygen, fluorescence, turbidity profile from the surface down to 1000 m for "shallow" casts and down to 10 m above the sea floor for "deep" casts. Temperature was calculated according to the ITS-90 temperature standard (potential T in °C). Absolute salinity (g/kg) was calculated using the TEOS-10 standard (IOC, SCOR and IAPSO, 2010; Millero et al. 2008, McDougall et al. 2012). Within the upper 500 m, the CTD was lowered at 0.5 m/s due to constraints by the winch system. At depths higher than 500 m, the speed could be increased up to 1 m/s.

All data were recorded and stored using the standard software Seasave V 7.23.2. The data were processed by means of ManageCTD, loops deleted, and data were added such as the CTD header, and ship position, based on the aboard data system DSHIP. We checked the data for unusual spikes using the despike-routine of the ManageCTD. Finally, the data were converted into different formats for subsequent analyses and publication. Other data from the DSHIP data set (salinometer, Ferrybox, weather recordings, ADCP, GPS) were extracted for further processing and as a supplement to the CTD data.

Table 7.1: Specifications and dates of calibration of the CTD sensors.



All sensors attached to the frame were pre-calibrated by the manufacturers (Table 7.1). We compared the data of both temperature and conductivity sensors using data from 56 and 57 casts, respectively. The temperature sensors had a very high accuracy, with a mean difference of 0.0007 °C (Fig. 7.1). The conductivity sensors had an accuracy of 0.0071 ms/cm (Fig. 7.1). There was a small constant offset between both conductivity sensors. We collected about 30 seawater samples for further analyzes using a lab-based salinometer.



Figure 7.1: Accuracy check of the temperature and conductivity double sensors based on nearly all CTD-casts during cruise SO248.

# <span id="page-25-0"></span>*Oxygen Calibration*

For calibration of the Aanderaa oxygen sensor, we used the standard Winkler titration technique to determine the concentration of dissolved oxygen in seawater (Grasshoff et al. 1999). At almost each station we took two to four replicate water samples from two to three different depths. In total, we used 98 water samples for the calibration of the oxygen sensor. The results obtained from the oxygen sensor and the titration measurements compare well ( $R^2$  = 0.96, see Fig. 7.2). The correction function has an offset of 7.75  $\mu$ mol L<sup>-1</sup>; the slope is 1.13.



Fig. 7.2: Validation of the Aanderaa-type oxygen optode by using Winkler titration of samples obtained at 14 stations during cruise SO248.

#### *Preliminary results*

Altogether, we collected samples at 19 stations with 59 CTD-casts. About 30,000 L of seawater were used for analyses and experiments.

Contour plots of salinity and temperature down to 1000 m water depth over the entire transect from the southernmost to the northernmost station in the Bering Sea along 180° E/W are shown in [Figs. 7.3 and 7.4.](#page-26-0) Black lines indicate isopycnals. The white dashed line indicates the depth of the chlorophyll maximum, as determined by the fluorescence sensor.

We can clearly identify the water masses in the tropical and subtropical regions with highest temperatures and salinity south of the equator. Another prominent feature is the strong salinity gradient with the frontal systems in the northern Pacific around 40-45 °N and the subarctic and Bering Sea regions with salinities below 33 and surface temperatures as low as 4 °C.

Fig. 7.5 shows the oxygen distribution down to a depth of 3000 m along the transect. The socalled oxygen minimum zone extends from about 200 m down to almost 2000 m in the northern Pacific and complies with previous studies (Dietrich et al. 1992). The maximum extension [of the oxygen mini](#page-27-0)mum zone was between 50° and 60° N.

Temperature-Salinity diagrams obtained from the measurements at the "deep" stations are shown in Fig. 7.6. The water masses with the highest density at all stations were identified as the central deep water (CDW) of the Pacific Ocean. Overall, we can clearly distinguish the tropical and subtropical water masses (stations 2-12) from those of the North Pacific (stations 14-19).

<span id="page-26-0"></span>

Figure 7.3: Contour plot of the absolute salinity distribution along the meridional transect of cruise SO248 and biogeographic provinces (top). Black lines indicate the isopycnals; the white dashed line the depth of the chlorophyll maximum and the dashed blue lines the positions of the stations.



Figure 7.4: Contour plot of the potential temperature distribution along the meridional transect of cruise SO248 and biogeographic provinces (top). Black lines indicate the isopycnals; the white dashed line the depth of the chlorophyll maximum and the dashed blue lines the positions of the stations.

<span id="page-27-0"></span>

Figure 7.5: Contour plot of the oxygen concentration along the meridional transect of cruise SO248 and biogeographic provinces (top). Black lines indicate the isopycnals; the white dashed line the position of the chlorophyll maximum and the dashed blue lines the positions of the stations.



Figure 7.6: T-S-Diagram (potential temperature versus absolute salinity) of all deep stations of cruise SO248.

Data Management:

The data are available at PANGAEA:<https://doi.pangaea.de/10.1594/PANGAEA.864673> Badewien TH, Winkler H, Arndt KL, Simon M (2016) Physical oceanography during SONNE cruise SO248 (BacGeoPac). Institute for Chemistry and Biology of the Marine Environment, Carl-von-Ossietzky University of Oldenburg, Germany*, Dataset #864673,*  (doi:*10.1594/PANGAEA.864673).* 

#### <span id="page-28-0"></span>*7.2 Bio-optics*

(D Voss, D Meier, R Henkel, O Zielinski)

The main objective of the bio-optics part of the cruise was to determine and correlate the underwater light field, combined with transparency measurements and ship-based ocean color sensing to biogeochemical properties and the composition of plankton and bacterioplankton communities and the biogeographic provnices. As light availability controls phytoplankton growth and community composition and only few investigations were performed within this oceanic region covering distinct hydrographic patterns and biogeographic provinces, observations play an important role to elucidate causative links. To obtain a better insight into the biodiversity patterns of (bacterio-) plankton communities and their biogeochemical significance and role in DOM turnover the assessment of DOM was complemented by measuring chromophoric (CDOM) and fluorescence properties (FDOM). These measurements allow a highly sensitive DOM analysis (Coble 2007, Moore et al. 2009, Baszanowska et al 2011) and the correlation of DOM signatures to water masses and their specific microbial biogeochemical processes. A further objective of the cruise was to investigate the improvement of optical processing methods. Here we focused on the validation and calculation of nitrate in oligotrophic surface waters with an UV spectrophotometer continuously running over the whole cruise transect.

#### *Methods*

#### *Hyperspectral und multispectral light field measurements (UV/VIS)*

A HyperPro II profiling system (Satlantic, Halifax, Canada) was used to acquire bio-optical data for different parameters. The profiler consists of one hyperspectral irradiance and one hyperspectral radiance sensor as well as fluorescence and backscatter sensors and an integrated CTD. A second hyperspectral irradiance sensor was mounted on the research vessel for reference measurements. On the profiler, the irradiance sensor measures downwelling and the radiance sensor upwelling light. The fluorescence sensors measure chlorophyll, CDOM, phycoerythrin and phycocyanin fluorescence signals. The backscatter sensor retrieves data at 470 nm and 700 nm. Profiler measurements were conducted at almost all stations depending on sea, weather and light conditions. At these stations, three casts at the back of the ship were typically performed in free-falling mode (1x full depth, 2 x 50 m). At each cast, the profiler was lowered until the downwelling light values were of the same order of magnitude as the background noise level of the sensor. Besides the VIS version a second UV profiling system (Satlantic, Halifax, Canada) was used at the stations to determine the penetration of UV light within the water column. On the profiler, two irradiance sensors (selected wavelengths) measure the downwelling light. A second hyperspectral irradiance sensor was mounted on the research vessel for reference measurements of the full light availability. Three casts at the back of the ship were typically performed in free-falling mode (1x full depth, 2 x 50 m). Data processing was done onboard; further modelling will be performed afterwards.



Fig. 7.7: HyperPro II profiler to determine the underwater light field.

As a reference for the underwater light field measurements spectral absorption coefficients of particles and pigments were determined in discrete water samples afterwards in the lab. Therefore, particles were concentrated on filters for subsequent absorption analysis. One to 2 L of seawater from selected light field depths were filtered under low vacuum trough precombusted Whatman GF/F filters (47mm). Filters were immediately frozen at -80°C. Further analysis will be done in the home lab afterwards.

#### *Ocean Color Sensing*

Water transparency measurements were performed with a 0.9 m diameter Secchi disk at almost each station depending on sea and weather conditions. The Forel-Ule (FU) color scale is a device that is composed of 21 colors, from 'indigo blue' to 'cola brown', and represents the range of colors that can be found in the open sea, coastal, and continental waters. Based upon a historical background, this provides an estimate of the present water constituents influencing the water color. The color of the water was determined over a Secchi disk at half the disk's depth at each day station. When classical measurements were conducted at some stations additionally a smartphone app for FUI



Fig. 7.8: Forel-Ule scale for observations during cruise SO248.

determination was used. Measurements were part of the EU project Citclops [\(www.citclops.eu,](http://www.citclops.eu/) [eyeonwater.org.](../../../../../Users/Dani/Desktop/eyeonwater.org)

Above-water hyperspectral radiometric observations were conducted during the whole cruise. A radiometer setup with a RAMSES-ACC hyperspectral cosine irradiance meter to measure *ES* (λ) (downwelling solar irradiance), and two RAMSES-ARC hyperspectral radiance meters to measure *Lsfc* (θ*sfc*,Φ, λ) (upwelling water-leaving radiance) and *Lsky* (θ*sky*, Φ, λ) (skyleaving radiance) were installed on the ships foremast (Fig. 7.9, TriOS GmbH, Germany). Hyperspectral measurements were collected at 5 min intervals over a spectral range of  $λ = 320$  – 950 nm. Data processing will be done according to Garaba & Zielinski (2014). Furthermore newly developed processing algorithms will be tested with the collected data set. Measurements were combined with a security camera, taking pictures every 10 minutes for a later validation of spectra.



Fig. 7.9 Radiometric setup at the foremast of RV Sonne.

# *FDOM / CDOM measurements*

Water samples were collected at each station from defined depths to measure CDOM and FDOM on shipboard fluoro- and spectrophotometrically, respectively, in 0.2 µm prefiltered samples.

#### *Preliminary Results*

#### *Hyperspectral und multispectral light field measurements (UV/VIS)*

Underwater light field measurements were performed at almost every station and showed pronounced differences in the different biogeographic provinces (Fig. 7.10). The vertical profile of the photosynthetic active radiation (PAR) decreased from the PNE (station 7) to the NPF (station 12) and BER (Station 17). stations were selected to show the changes in the. Analysis and modeling are in progress for all stations along the cruise transect.



Figure 7.10: Vertical profile of absolute PAR (left) and percent (right) at 3 stations of cruise SO248 in the Pacific. For location of the stations see Fig. 5.1.

#### *FDOM measurements*

For each station and distinct depths water samples were taken for FDOM measurements. Data of station 3 in SPS show the typical differences of oligotrophic waters in the profile between the surface and 1000 m depth with increased fluorescence in the mesopelagic zone [\(Fig. 7.11\)](#page-31-0).

<span id="page-31-0"></span>

Figure 7.11: FDOM characteristics of station 3 in SPS between 20 and 1000 m depth. DCM: deep chlorophyll maximum.

#### *Ocean Color Sensing*

Water transparency and Forel-Ule (FU) color scale measurements were performed at almost every station. As shown on Figure 7.12 Secchi depth in the oligotrophic biogeographic provinces in the equatorial regions exceeded 40 m and decreased in the more eutrophic ones further north to <20 m. The Forel-Ule index exhibited inverse patterns.



Figure 7.12: Station numbers (left), Secchi depth (central) and Forel-Ule index (right) of stations of cruise SO248. Due to bad weather or light conditions no observations were possible at station 11, and 13 to 15.

#### *Data Management*

All data will be transferred to the PANGAEA database as soon as they are available and quality checked. Depending on data type and progress of sample analysis, this will be done within 2-3 years. All datasets will be submitted to PANGAEA, allocated by the cruise identifier SO248.

<span id="page-33-0"></span>*7.3 Bacterioplankton cell numbers, biomass production and turnover rates of labile substrates.* 

(M Simon, I Bakenhus, S Billerbeck, N Bergen, F Mielke, L Wolter, M Wolterink, B Kuerzel, HA Giebel)

We aimed at a comprehensive assessment of the bacterioplankton community in the Pacific and its biogeographic provinces with a special emphasis on the Roseobacter clade and its major bacterioplankton subclusters. The work includes investigations of the biogeography, growth and population dynamics and the bacterioplankton community composition.

A list of investigated parameters is given i[n Table 5.1.](#page-19-0) 

#### *Methods*

Our main work on shipboard was the collection and processing of water samples from depths between 20 and 1000 m. Samples were withdrawn from the Niskin bottles mounted on the CTD rosette from the mixed layer and the mesopelagic zones (for details on the CTD see chapter 7.1). Our sampling scheme included fixed depths between 20 and 200 m, the chlorophyll maximum and at "deep" stations also 500 and 1000 m. Samples for bacterial abundance, production and turnover of dissolved free amino acids and glucose were analyzed on shipboard. Bacterial abundance was assessed by flow cytometry and bacterial production and substrate turnover by radiotracer techniques and applying  $14C$ -leucine,  $3H$ -leucine, -glucose, –amino acids and -acetate. For details on the methods see Simon and Azam (1989) and Simon and Rosenstock (2007). In addition, samples for CARD-FISH and MAR-FISH analyses of the bacterial communities applying the same radiolabelled substrates were taken and processed for the upper 200 m. Further, samples for the analysis of dissolved free and combined amino acids and neutral sugars were collected, prefiltered through 0.2 µm polysulfon membranes (Gelman Acrodisc) and stored frozen until later analysis in the home lab by HPLC.

# *Preliminary Results*

Bacterial cell numbers in the near surface layer ranged between 2 and  $22x10^5$  cells ml<sup>-1</sup> with a trend of increasing numbers from south to north, in particular at 20 and 60 m depth (Fig. 7.13). Bacterial biomass production and bulk growth rates were extremely low in the SPS and exhibited highest rates in the PSE and PNE at 20 m depth exceeding 2 per day (Fig. 7.13). In other tropical and subtropical regions bulk growth rates ranged between 0.3 and 1 per day with a trend of decreasing values further north in the colder water masses and biogeographic provinces. The extremely low rates of bacterial biomass production in the SPS and the high values in the PSE and PNE and generally decreasing values further north in the NPTG, NPF, PSAG and BER are well reflecte[d in the integrated rates of bacterial biomass production of the upper](#page-34-0) 300 m of the water column (Fig. 7.14).

Turnover rates of dissolved free amino acids in the upper 100 m ranged from below 0.005 per day in the SPS to 0.8 per day in the equatorial region. Values in the colder regions further north were between 0.05 and 0.3 per day without a clear latitudinal gradient. In contrast, turnover rates or glucose and acetate exhibited a clear latitudinal gradient with increasing rates towards the PSAG and BER from below 0.05 to 0.2 per day.

# *Data management*

All finally processed data will be stored on a server at ICBM, of TRR 51 and will be available on request if not otherwise mentioned. Most of the data will be published in international peerreviewed journals.

<span id="page-34-0"></span>

Fig. 7.13: Bacterial cell numbers (upper panel), biomass production (central panel) and bulk growth rates (lower panel) at 20, 60 and 100 m depth in the Pacific during cruise SO248.

Fig. 7.14: Bacterial biomass production integrated from 0 to 300 m depth in the Pacific during cruise SO248.

#### <span id="page-35-0"></span>*7.4 Mesocosm experiments*

#### (M Wietz, G Wienhausen, M Simon, M Dogs, M Wolterink, HA Giebel)

Mesocosm experiments were carried out to examine the response of the ambient bacterial communities in the SPS, the PNE and NPF to amendments of an exudate of the diatom *Thalassiosira rotula*, alginate and of vitamin B12 and a precursor.

#### *Methods*

Twenty-liter Nalgene carboys were filled with water from 20 m depth at station 2 (SPS) and station 14 (NPF) and from 40 m at station 7 (PNE). In one experiment two sets of triplicates were amended with the diatom exudates and incubated for 6 days, one set of triplicates and controls without amendment in the dark and the other set in a 12:12 light-dark cycle. In the second experiment, another set of triplicates was amended with alginate as a model polysaccharide and incubated in the dark for six days. In a third experiment vitamin B12 or a precursor and inorganic nutrients (nitrate, phosphate, silicate) was added to triplicates and incubated together with controls without any addition for six days in the dark. All incubations were at in situ temperature and subsampled periodically for bacterial abundance, bacterial biomass production and the vitamin experiment also for turnover rates of amino acids, phytoplankton and the alginate experiment also for turnover rates of glucose. After six days a large part of the remaining volume was filtered onto 0.2 µm filters for metagenomic and transcriptomic analyses of the bacterial communities.

#### *Preliminary Results*

Bacterial abundance in all experiments increased only little over time. However, bacterial biomass production increased over time in all experiments indicating that the bacterial communities did respond to the different amendments. However, the responses differed in the various biogeographic provinces. The response in the SPS was low and a short lag time, that in the PNE very rapid exhibiting highest rates and that in the NPF slow but steady over the entire incubation time.

The later analyses of the bacterial community composition will show which bacterial lineages responded and elucidate differences in the response patterns to the various amendments and incubations conditions, i.e. dark versus light:dark.
# *7.5 Extracellular Peptidase and polysaccharide activities*

### (C Arnosti, JP Balmonte)

The objective was to measure heterotrophic activities of microbial communities in the different biogeographic provinces and at different depths in order to determine the extent to which biogeographic and depth-related differences in microbial community composition and genetic con-tent are reflected in activities of the extracellular enzymes that initiate the remineralization of organic matter.

### *Methods*

We measured peptidase and polysaccharide hydrolytic activities at 8 stations (1, 2, 4, 6, 7, 10, 14, 16) and 5 different depths along the latitudinal transect. Peptidase activities at the surface and the DCM were also measured at the stations 3, 5, 8, 9, 11, 12, 13, 15 and 17. Further, hydrolytic activities of particle-associated (>3 um) bacteria were compared to wholewater ac-tivities at the 8 stations and 3 depths (surface, DCM, bottom water). Peptidase and polysac-charide hydrolytic activities were also measured in the mesocosms that were amended with a diatom exudate and alginate so that these activities can be related to the growth dynamics and composition of the bacterial communities responding to the added substrates.

Glucosidase and peptidase activities were measured using MUF and MCA-tagged small sub-strates (glucose; leucine; short peptides to measure trypsin and chymotrypsin activities) after the method of Hoppe (1983) and Obayashi & Suzuki (2008), although our measurements were made using a plate reader. Activities of polysaccharide hydrolyzing enzymes were measured with fluorescently-labeled polysaccharides, using the method of Arnosti (1995; 2003). Gravity-filtration measurements of enzyme activities were made by gravity-filtering water through a 3.0 um pore-sized filter, then cutting the filter into 12 equal portions for measurements of enzyme activity in duplicate, after D'Ambrosio et al (2014).

#### *Preliminary results*

We have compiled preliminary results for peptidase and  $\beta$ -glucosidase activities from the sur-face and DCM between stations 1 and 11 (30 S to 28 N). Rates and patterns of activities var-ied by location; across all substrates, activities were lower at St 1-3 and 11 than at St 4-10. For some substrates and sites (L-MCA at St 5, 7, 9 and 10; AAPF-chym at St 4, 5, and 7), activi-ties in the DCM exceeded those in surface water. Trypsin/chymotrypsin activities showed greater range and spatial variability than did leucine aminopeptidase activity. Trypsin activities in surface waters were notably high at St 3 and 4, whereas chymotrypsin activities were high at St 6 and 10. Ranges of these activities were a factor of 3 greater than for leucine aminopepti-das[e \(Fig. 7.15\).](#page-37-0) 

The raw data for the rest of the peptidase samples has been collected, but still need to be processed. All of the samples for polysaccharide hydrolase activities remain to be run (in excess of 10,000 samples); we have the capacity to analyze a maximum of 50 samples per day, and we have a backlog of samples from other cruises that are still in the pipeline. We estimate that we will complete running the samples on our HPLC/GPC system during the spring of 2017. Data analysis will commence at/after that time.

#### *Data management*

34 All of the raw data and processed data will be posted and stored on our lab's password-protected Sakai website, which is backed up and maintained by the University of North Caroli-na. After publication, data can be made publicly available also through public databases.

<span id="page-37-0"></span>

Fig. 7.15: Hydrolysis rates of  $\alpha$ - and  $\beta$ -glucosidases, leucine-amino-peptidase, chymotrypsin and trypsin at 20 m depth (surface) and the deep chlorophyll maximum (DCM) at stations 1 to 11 of cruise SO248 in the Pacific.

# *7.6 Bacterioplankton biogeography and bacterial algal interactions*

#### (I Wagner-Döbler, J Tomasch)

#### *Algae-Bacterial Interactions*

A topic still controversially debated in microbial ecology is the extend to which the microorganisms colonizing eukaryotes like corals and microalgae are stable symbionts selected for specific functions or the result of random attachment (Hester et al. 2016). We will use chlorophyll auto-fluorescence-activated cell sorting to isolate single phototrophic microalgal cells together with their attached microbial communities (the holobiont) from water samples of the photic zone. Multiple-displacement amplification (SDA) will be used to obtain DNA from the single holobionts. 16S rRNA gene amplification and next generation sequencing (tag-sequencing, amplicon sequencing) will be used to characterize the microbial communities from approx. 200 single microalgae holobionts. Statistical methods will be employed to determine the stable and variable part of the community (Hester et al. 2016). Based on these results, the metagenomes of algal cells with highly similar as well as divergent microbiomes will be analyzed in order to reveal how community divergence influences functional diversity or conservation.

#### *Bacterioplankton biogeography*

Deep sequencing of the 16S rRNA gene of bacteria and chloroplasts from microalgae will be used to determine the composition of microbial communities in the epipelagic zone of the latitudinal gradient crossing the biogeographic provinces. Water samples will be fractionated into three size classes by sequential filtration to determine the influence of particle size on biogeographic patterns and to compare bacterioplankton communities associated with microalgae to free-living ones. The same approach has previously been used for the Atlantic Ocean (Milici et al. 2016a, b, c). The data will serve as a background for interpreting the results of single holobiont sequencing.

#### *Diel changes in gene expression*

Bacteria in the photic zone of the oceans adapt their transcriptional activity to the diel regime (Ottesen et al. 2013, 2014). We will use a data set of the two 24 h time-series with sampling intervals of three hours to analyze the transcriptional activity patterns of the bacterioplankton using Illumina RNA sequencing.

# *Methods*

Unfiltered CTD water samples for single cell sorting, subsequent 16S rRNA gene sequencing and metagenomics were obtained at 20 m and 60 m at each station along the cruise. Samples were cryo-conserved using three different protocols (glycine-betaine, glycine-betaine and TE, glycerol).

At each station the water column was sampled at 20, 40, 60, 100, 200, 500, and 1000 m depth. The DCM was additionally sampled if it differed from the standard depths by more than 5 m. Samples (10 L) were sequentially filtered from 8 µm to 3 µm to 0.22 µm to separate the plankton into three size fractions (large particle associated, small particle associated and free living bacteria). For RNA extraction, 20 L water samples were taken at 60 m depth and the DCM.

At two stations (0.00 N and 50.00 N) 20 L water samples were taken over a 24 h period every three hours at two depths (20 m and DCM) and filtered as above.

All samples were stored at -80°C until further analysis in the home lab.

# *Data management*

Raw data will be sequenced by the Genome Analysis group of the HZI. Raw and processed data will be stored on HZI servers. Analyses will be stored on the servers of the group Microbial Communication and published in international peer reviewed journals. Raw and processed data will be made publicly available on the European Nucleotide Archive [\(http://www.ebi.ac.uk/ena\)](http://www.ebi.ac.uk/ena) upon publication.

# *7.7 Population structure and divergence in the Roseobacter group*

# (HM Freese, A Methner, C Lepleux)

Bacteria of the *Roseobacter* group are abundant and widely distributed in marine systems as well as physiologically and phylogenetically highly diverged which suggests that adaptation and selection have been the major drivers of their evolution. Exclusively surface-associated *Phaeobacter* species from the *Roseobacter* group showed clade-specific preferences in association with a host or environmental resource patches. Their population structure and evolutionary mechanisms differed to so far investigated pelagic *Prochlorococcus* and *Pelagibacter* but also to vibrios which switch between free-living and associated lifestyles. This leads to the question if this population structure and these evolutionary mechanisms are representative for the *Roseobacter* group even at different hierarchical levels or for a specific lifestyle. Therefore, the relevance of these evolutionary mechanisms and the ecological niches of the intraspecific genome based cluster will be elucidated for closely related genera, like *Pseudophaeobacter*, which occur associated and in sediments as well as the distantly related genome streamlined pelagic *Roseobacter* clade affiliated (RCA) cluster.

# *Methods*

At all stations >30°N 9 water samples from 20 m depth were amended with glycine betaine and frozen at -80°C for later cultivation independent population genomic analysis of the RCA cluster *via* single cell genomics. In parallel, bacteria from large volumes [\(Fig. 7.16\)](#page-39-0)  were size

<span id="page-39-0"></span>fractionated and concentrated onto membrane filter (0.2  $\mu$ m, 3  $\mu$ m, 10  $\mu$ m) with a McLane WTS-LV Sampler in situ at 20 m depth and frozen at -80°C for later DNA and RNA extraction.

To elucidate physiological potential and niches of subpopulations of various species of the *Roseobacter* group water samples from  $20$  m, the DCM or 100 m and 500 m were incubated with 14 different substrates in combination with 5-Ethynyl-2'-deoxyuridine (Click-iT<sup>®</sup>) in microcosm experiments at 11 stations. After 6 h incubation, cells were harvested, fixed, washed and permeabilized on board and frozen for further processing at home. At three stations surface samples were additionally incubated with 3 different CNS substrates in mesocosms, filtered, fixed and frozen for later RNA extraction.



Fig. 7.16: Volumes of surface water filtered with the McLane in situ pump within 3 h at stations  $>30^{\circ}N$ 

To enrich strains closely related to Phaeobacter, samples of the surface sediment, zooplankton concentrated by a 100 um or 300 um Bongo net were incubated in replicates of serial dilutions using a general and a specific medium over the transect. After at least 10 days of continuously incubation at 15°C, 96 well plates with grown wells were subject to a specific PCR and screened by gel electrophoresis. Positive single wells were streaked on agar plates, transferred into fresh medium and cryo-preserved which will be continued at home.

### *Preliminary Results*

Overall, 328 enrichments in 96 deep well plates and 40 agar plates of exemplary stations were inoculated. Bacteria were readily enriched from plankton samples over the whole transect but only exceptional growth was observed in sediment enrichments so far. However, further growth of the sediment enrichments is expected in the next weeks. Phaeobacter related bacteria did not dominate the plankton enrichments but 11% of the so far 131 screened 96 well plates contained at least one strain. From one larger crab, already 5 different bacteria from the *Roseobacter* group with a 16S rDNA similarity of 97 – 93% to *Phaeobacter* were isolated which may point to an organism dependent association of *Roseobacter* bacteria. However, this has to be verified when screening, isolation and molecular analysis of all enrichments are completed. Depending on the isolation success, selected bacterial strains will be genome sequenced and their population genomics analyzed.

The result of the cultivation independent population genomic analysis and of the substrate dependent physiological potential of bacterial subpopulations in 460 assays will become available after processing of the samples (single cell genomics, metagenomics and transcriptomics as well as counting, sorting and sequencing of active (i.e. fluorescing cells) in the laboratories at home after several months.

#### Data management

The results will be published in international peer-reviewed journals and molecular data will be submitted to the respective data base (e.g. NCBI).

### *7.8 Microbial abundance, diversity and activity in Pacific deep sea sediments*

#### (M Pohlner, J Degenhardt, B Engelen)

For the analysis of seafloor sediments along the SO248 transect, we followed three main objectives: First, we wanted to determine if the site-specific conditions of the biogeographic provinces in the water column are reflected by the microbial abundance and diversity at the seafloor. Second objective was to compare enzymatic activities of benthic communities along the transect. To identify the role of benthic viruses in shaping microbial community structures and supporting microbial turnover by the viral shunt was the third objective.

The following questions will be addressed:

- Are there regional microbial distribution patterns and is there an overlap in diversity between sediments and the overlying waters?
- Which factors specifically trigger the distribution, abundance and diversity of the *Roseobacter* group in marine sediments?
- Are the environmental settings at the seafloor reflected by exo-enzyme activities of phosphatase and amino peptidase?
- Which members of the benthic communities are affected by viral lysis and which part takes advantage of the viral shunt?

#### *Methods*

To answer the above-mentioned questions, samples from seafloor sediments were taken by a MUC at 10 stations along the transect (Table 7.1). The water depth of the sites ranged between 3258 and 5909 meters below sea level (mbsl). At all sampling sites, sufficient amounts of sediments were recovered. However, the surface layer of site 18 was lost as the MUC penetrated too deep into the seafloor. Due to the tight time schedule of the cruise, no further attempts were made to sample this station.

<b>Station</b>	Date	Latitude	Longitude	Depth (mbsl)	
2	04.05.16	26° 59,6' S	178° 13,8' E	4185	
$\overline{\mathbf{4}}$	08.05.16	$10^{\circ}$ 20,0' S	176° 28,5' W	4130	
6	11.05.16	$0^{\circ}$ $1,9'$ S	179° 59,4' W	5285	
8	15.05.16	$10^{\circ}$ 58,0' N	$179^{\circ}$ 0.1' E	5402	
10	18.05.16	21° 58,0' N	178° 19,0' E	3258	
12	21.05.16	33° 58.4' N	177° 20,5' E	3486	
14	24.05.16	45° 0,0' N	178° 45,0' E	5909	
16	26.05.16	49° 60.0' N	179° 33,0' E	5621	
18	29.05.16	56° 60,0' N	179° 35,0' E	3811	
19	30.05.16	58° 54,5' N	178° 55,8' W	3300	

Tab. 7.1: Position and depth of sediment sampling sites of cruise SO248.

At all stations, subsamples were regularly taken from the seafloor and 20 cm below seafloor (cmbsf). Aliquots were processed for total cell counting and phosphatase and amino peptidase activity measurements by applying fluorescently labelled model substrates, both performed directly onboard. For later analyses, subsamples were taken for virus counting, CARD-FISH quantification, DNA/RNA-extraction, inorganic sediment composition, sediment density and porosity. Porewater was collected separately form the respective sediment layers to analyse the concentrations of phosphate, DOC, amino acids, mono- and polysaccharides as well as high-resolution DOM composition. To investigate the viral shunt in seafloor sediments, phageinduction experiments were performed with samples from sites  $6$  and 10. A third experiment was set-up with samples from site 19, but was started two weeks after return to the homelab. The experiments will be evaluated in the frame of a Master thesis.

#### *Preliminary results*

The sediment cores showed a large variety in texture and colour indicating different elemental sediment compositions (Fig. 7.17). A detailed geochemical description will be performed at ICBM in the frame of a Bachelor thesis.



Fig. 7.17: Representative sediment cores from the SO248 transect. Numbers correspond to the stations. At station 18, the surface layer was lost due to overfilling the core-liner.

Total cell counts at the seafloor were in the range of 10<sup>8</sup>-10<sup>9</sup> cells cm<sup>-3</sup> (Fig. 7.18). Covarying with the productivity in the overlying water column, they increased from station 8 to 19 with highest numbers in the highly productive Bering Sea. Cell counts at 20 cmbsf decreased by one to two orders of magnitude at all stations.

The results of the direct counting will be confirmed and extended within the frame of a Master thesis by specific quantification of *Bacteria* and the *Roseobacter* group using CARD-FISH and quantitative PCR targeting the 16S rRNA gene. Accordingly, all samples will be subjected to next generation sequencing to determine the microbial diversity with special focus on the *Roseobacter* group. Data from this diversity analysis will be compared to metagenomic and metatranscriptomic studies (see chapter 7.9).



Fig. 7.18. Total cell counts along the SO248 transect. Cells were counted onboard by epifluorescence microscopy using SybrGreen as fluorescent dye.



Fig. 7.19. Phosphatase activities along the SO248 transect at the stations indicated (sampling site). A: Total activities; B: Background-corrected activities. Left Y-axis: Surface samples; Right Y-axis: samples from 20 cm bsf. Note the different scales of the y-axes.

While the total phosphatase activities show a trend of increasing values from south to north (Fig. 7.19a), the assay was biased by a relatively high background-activity of the heatinactivated control. This phenomenon was also found previously for deep-subsurface sediments of the South Pacific Gyre (unpublished data) and will further be investigated. The background-corrected activities (Fig. 7.19b) were roughly tenfold lower than total activities.

Aminopeptidase activities reflect the ability of benthic microbial communities to degrade proteins and to potentially use them as substrates. The aminopeptidase activities were not influenced by unspecific protein degradation as visualized in Fig. 7.20a and b. Generally, the benthic communities also showed increasing activities from south to north with higher activities at the seafloor in comparison to the deeper layer. The results will be correlated to the other hydrolytic activities of other biopolymers obtained by C Arnosti (see chapter 7.5).



Fig. 7.20. Aminopeptidase activities along the SO248 transect. A: Total activities; B: Backgroundcorrected activities. Left Y-axis: Surface samples; Right Y-axis: samples from 20 cmbsf. Note the different scales of the v-axes.

#### Data management

The results will be transferred to a database which will be available for the other cruise participants and finally published in international peer-reviewed journals.

- *7.9 Metagenomic, metatranscriptomic and metaproteomic analysis of bacterial communities in the water column and surface sediment*
- (B Wemheuer, A von Hoyningen-Huene, L Wöhlbrand)

The aim was to investigate the diversity and function of the *Roseobacter* clade and other marine microbes along the longitudinal transect from the SPS at 30°S to BER at 59°N. Community structure and diversity are assessed by community barcoding using universal primers for bacteria and archaea. Furthermore, the potential and functions of the microbial communities will be assessed using comparative metagenomic, metatranscriptomic and metaproteomic approaches.

### *Methods*

Water samples were taken at ever station from 20 m depth, the DCM and 300 m depth from the Niskin bottles. A total of 40 L per depth were pre-filtered through a 20 µm nylon net in order to remove large particles and micro- and macroplankton. For DNA and RNA analysis, bacterioplankton was harvested by serial filtration of 20 L of prefiltered sea water using a 2.7 µm glass fibre filter (Whatman GF/D, GE Healthcare, Freiburg, Germany) followed by a filter sandwich consisting of a 0.7 µm glass fibre filter (Whatman GF/F, GE Healthcare) and a 0.2 µm polycarbonate filter (Whatman Nuclepore, GE Healthcare). Particle-associated bacterioplankton was caught in the 2.7 µm filter while the free-living community was caught in the filter sandwich. For proteome analysis, the remaining 20 L of seawater were prefiltered using the 2.7 µm glass fibre filter. The free-living bacterioplankton was subsequently harvested on four 0.2 µm (47mm diameter) polyether sulfone filters (Satorius, Göttingen, Germany). After filtration, all filter samples were stored at -80°C until further analysis.

At the two 24 h time series stations, samples were collected in a 3 hours' routine in a similar way as described for proteomic analysis. In case of these samples, six 0.2 um polyether sulfone filters were used for filtration of approx. 40 L of sea water. Four filters will be used for proteome analysis and two filters for DNA extraction to allow for the construction of a stationspecific metagenome-based protein database for protein identification.

Moreover, a total of 6.5 L of oxygen-depleted water of detected oxygen minimum zones (OMZ) was collected at stations 6, 7, 8 and 17. In addition, 24 bottles containing 80 ml anoxic seawater medium were inoculated on board with 4 ml collected from the OMZ. Samples of anoxic or oxygen depleted sediments were collected at stations 18 and 19 and covered with anoxic seawater medium. These samples are designated for starting enrichment cultures in the home laboratory.

In addition to water samples, sediment samples were collected at the sediment surface as well as at 20 cm depth for metagenomic and metaproteomic analysis. For each sampling station and sediment depth, around 5 g sediment were collected in quadruple and stored at -80°C.

# *Functional Community Fingerprints*

To obtain a functional fingerprint of the microbial community inhabiting ocean surface waters, approximately 400 µl of the 2.7 µm filtered seawater were incubated for three days at 15°C in 48 deep well plates containing 50 µl of different nutrient solutions, sugars, trace elements or vitamins in triplicates, with artificial seawater as a control. After incubation, DMSO was added and the plates were frozen at -80°C until further use. Changes in community structure will be assessed by community barcoding.

### *Preliminary results*

In the course of the cruise, a total of 57 and 114 filter samples were obtained for metagenomic and metatranscriptomic analysis of the particle-associated and free-living bacterioplankton communities. DNA and RNA will be extracted and purified. For metagenomic analysis, isolated DNA will be directly sequenced using a HiSeq 4000 (Illumina, Madison, USA). In addition, the DNA will be used as a template in PCRs targeting bacterial and archaeal 16S rRNA genes. Obtained PCR products will be sequenced using a MiSeq sequencer (Illumina, Madison). For metatranscriptomics, ribosomal RNA will be depleted in total envrionmental DNA. Obtained RNA will be converted to cDNA and sequenced using a HiSeq 4000 (Illumina, Madison, USA). In addition, the structure of the putatively active bacterioplankton community will be assessed by sequencing of 16S rRNA transcripts generated from total environmental RNA.

A total of 324 filters were collected for meta-proteomic analysis of the bacterioplankton: 228 at the regular stations and additional 96 filters at the 24 h sampling campaigns. In addition, 72 sediment samples were collected at nine distinct sampling stations. Filter and sediments samples will be subjected to cellular lysis, protein extraction, generation of peptides per sample and analysis by mass spectrometry. Final protein identification will be based on the metagenome/-transcriptome-based protein sequence database generated from the same samples.

### *Data management*

Sequence data generated by community barcoding and sequencing of environmental DNA and RNA will be made publically available by submission to the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI). Subsequent to protein identification and publication of respective data, the obtained proteomic data will be made publicly available via appropriate proteomic databases (e.g. proteome exchange) or own server structures if necessary.

# *7.10 Dissolved Organic Matter*

# (J Niggemann, B Noriega-Ortega, M Hinrichs, T Dittmar)

It is unknown whether and if so how the composition of the DOM pool reflects the different biogeographic provinces in general and in particular in the Pacific Ocean. Further, the DOM pool in the water masses of the dark Pacific, the oldest water oceanic masses (Hansell 2013) is till vey poorly characterized. Therefore the objective was to characterize the DOM pool in these waters. Together with other physical and biological parameters, i.e. characterizing the microbial community growth dynamics and composition, investigated on this cruise we will be able to shed light on controls of the DOM patterns.

#### *Methods*

For the bulk DOM characterization, water samples (4 L) from most depths of all stations covering the entire water column were withdrawn from the Niskin bottles, passed through GF/F glass fiber filters (precombusted 400°C, 4 h, Whatman, Maidstone, UK) and acidified to pH 2. Duplicate subsamples for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) will be analyzed as non-purgeable organic carbon by high temperature catalytic combustion using a Shimadzu TOC-VCPH/CPN instrument equipped with a TNM-1 module in the home lab. In order to desalt and concentrate the marine DOM for the analysis of the molecular composition, the remaining sample was solid-phase extracted using commercially available modified styrene divinyl benzene polymer columns after Dittmar et al. 2008 (PPL, Agilent, Santa Clara, CA, USA). The obtained DOM extracts will be analyzed upon arrival in the home lab on a 15 tesla FT-ICR-MS (Bruker Daltonics, Billerica, MA, USA) equipped with an electrospray ionization source (Bruker Apollo II).

Selected samples from the main stations were filtered, frozen, and shipped to Aron Stubbins and Leanne Powers at Skidaway Institute of Oceanography (Savannah, Georgia, USA). Apparent quantum yield (AQY) spectra, defined as moles DBC lost per moles photons absorbed by CDOM will be obtained. These describe the efficiency of DBC loss and can be used in ocean color based models to estimate its loss on regional/global scales. Coupling the photochemical model with a physical model will allow for a better assessment of the supply (upwelling of deep DBC rich waters) and subsequent photochemical loss in the sunlit waters.

Preliminary results are not yet available.

### *7.11 Dark ocean microbial biogeography*

(T Reinthaler, C Amano, B Bayer , RH Hansman, E Sintes, M Pinto, C Baranyi, GJ Herndl)

About 75% of the ocean is deeper than 200 m, however, most concepts on the interaction between the physical and chemical environment and the biota are derived from the relatively thin ocean surface layer. Moreover, the link between prokaryotic activity and biogeochemistry in the dark ocean is not firmly established despite recent studies that highlight the role of Bacteria and Archaea in the cycling of organic and inorganic matter in the dark ocean (Baltar et al. 2009, Herndl et al. 2005, Reinthaler et al. 2006). Among others, the observation that the most important source of substrate for prokaryotes, i.e., DOC, is not depleted (Barber 1986) led to the longstanding view that microbes in the deep are dormant or even dead (Jannasch and Wirsen 1973). This paradigm is challenged, however, by recent evidence suggesting that prokaryotes in the dark ocean are as active (or even more active) on a per-cell level as compared to the sunlit surface (Kirchman et al. 2007, Reinthaler et al. 2006, Varela et al. 2008).

Despite the major insights gained from studies on microbial activity in the surface ocean, knowledge on the microbial processing of organic matter and nutrients in the dark ocean is still in its infancy due to the lack of data. For this reason, the IPCC called attention to the fact that it is not possible to parameterize prokaryotic activity for an enhanced understanding of the global ocean carbon cycle (Intergovernmental Panel on Climate Change 2001) and an interdisciplinary workshop of experts on integrating biogeochemistry and ecosystems in a changing ocean emphasized to study the interactions of the physics, chemistry and biology on an interdisciplinary basis (IMBER IMBIZO [http://www.imber.info/IMBIZO1.html\).](http://www.imber.info/IMBIZO1.html)

Thus, the objectives here are to establish a biogeography of microbial diversity and activity in the meso- and bathypelagic waters of the Pacific which is a particularly under-sampled ocean.

#### *Methods*

# *Prokaryotic heterotrophic production*

Samples for prokaryotic heterotrophic production were taken from Niskin bottles at all "deep" stations at depths ranging from 200 m to 10 m above sea floor (see [Table A3](#page-103-0) in appendix). Occasionally, a sample was also taken from the supernatant of the MUC. Triplicate life and 2 control samples were incubated in glass vials. Microbial heterotrophic production was measured by incubating 20–40 mL of seawater with 5 nM  ${}^{3}$ H-leucine (final concentration, specific activity 120 Ci mmol<sup>-1</sup>, ARC) in the dark at in situ temperature ( $\pm$ 1°C) for 5 to 24 h. Incubation times and volumes were chosen according to the sampling depth and based on previous experience. Duplicate formaldehyde-killed blanks were treated in the same way as the samples. Incubations were terminated by adding formaldehyde (2% final concentration) to the samples. Samples and blanks were filtered through 0.2-µm polycarbonate filters (Whatman Nuclepore, 25 mm filter diameter) supported by cellulose acetate filters (Millipore HA, 0.45-µm pore size). Subsequently, the filters were rinsed with 5% ice-cold trichloroacetic acid and with Milli-Q. Fil-ters were dried, 8 ml of scintillation cocktail (FilterCount, Canberra-Packard) added, and after about 18 h measured on board by liquid scintillation counting (Perkin Elmer Tricarb).

# *MAR-FISH*

Samples for fluorescence in situ hybridization combined with microautoradiography were incubated in plastic tubes (Greiner Bio-One). To volumes between 20 mL and 80 mL (depending on the depth)  $3H$ -leucine was added (5 nM final concentration), similar to the activity measurements. After 5 to 24 h the samples were fixed with 2% filtered formaldehyde and incubated for up to 18 h at 4ºC to fix the cells. Subsequently the samples were filtered onto 0.2-µm polycar-45

bonate filters (25 mm diameter, Millipore GTTP) and rinsed with Milli-Q. The filters were placed into 2 mL microfuge tubes and dried. Finally, the tubes with the filters were frozen at -80ºC until analysis in the home laboratory.

# *Abundance of active bacteria in the dark ocean (Click-it*

To evaluate the active prokaryotic community in the dark ocean seawater samples were withdrawn at all "deep" stations from the Niskin bottles at most depths at 100 m and below into acid-washed polycarbonate bottles. Duplicate 15 mL water samples were transferred into 15 mL sterile tube (Greiner Bio-One) and incubated with either homopropargylglycine (HPG) or 5 ethynyl-2'-deoxyuridine (EdU) (20 and 40 nM final concentration, respectively) at in situ tem-perature (±2ºC) for 10-24 h (see [Table A3,](#page-103-0) appendix). Duplicate 1 mL subsamples were col-lected at the beginning and end of the incubation in 1.5 mL tubes, fixed with glutaraldehyde (0.5% final concentration) and stored at -80ºC. These samples will be used to evaluate the active prokaryotic abundance by flow cytometry. The remaining volume was stored at -80°C after adding 0.2-um filtered Glycerol TE buffer (10% final concentration) for single-cell ge-nomics. Further analysis will be conducted in the home lab.

#### *DIC fixation*

DIC fixation by deep ocean microbes was measured through the incorporation of  ${}^{14}C$ bicarbonate. Samples were collected between 200 m to either 1000 m or 2000 m depth de-pending on the station [\(Table A3, a](#page-103-0)ppendix). Seawater was collected from Niskin bottles and split into triplicate 40 ml samples and duplicate blanks in 50-ml plastic screw-top tubes (Greiner Bio-One). 50 µCi of  $^{14}$ C-sodium bicarbonate in 50 µl pH 9 Milli-Q water was added to each tube. Blanks were killed by adding 2.5 ml formaldehyde (2% final concentration) prior to substrate addition. All samples and blanks were incubated at in situ temperature for 48 to 72 h. Samples were terminated by adding formaldehyde (2% final concentration) filtered along with the blanks through 25 mm 0.2-µm polycarbonate filters (Whatman Nuclepore) supported by 0.45-µm cellulose acetate filters (Millipore HA). Sample tubes and filters were rinsed twice with 10 ml 0.2-µm filtered seawater. Filters were then exposed to fuming HCl for 20-24 h, before being placed in 20-ml scintillation vials with 8 ml scintillation cocktail (FilterCount, Canberra-Packard). After approximately 18 h, samples were counted on board in a liquid scintillation counter (Perkin Elmer Tricarb).

Additional experiments on bicarbonate uptake by the autotrophic prokaryotic community were also conducted at several stations and depths. One of these types of experiments was to determine the contribution of ammonia oxidizing Archaea to bulk DIC fixation by inhibiting ammonia oxidation through nitric oxide scavenging with 2-phenyl-4,4,5,5,-tetramethylimidazoline-1 oxyl-3-oxide (PTIO, Sigma; Martens-Habbena et al. 2015). PTIO was added to selected sea-water samples [\(Table A3, appendix\) at 100 µM final concentratio](#page-103-0)n for at least 30 min prior to substrate addition, and incubations then proceeded as described above. In separate experiments, the effect of temperature on bicarbonate uptake was determined by incubating deep seawater samples with bicarbonate additions at 20°C (Table A3) rather than at in situ temperature.

MAR-FISH samples for bicarbonate uptake were collected as detailed above at stations and depths indicated i[n Table A3.](#page-103-0) One hundred  $\mu$ Ci of  $^{14}$ C-sodium bicarbonate was added to 80 ml seawater for 48-72 h.

# *Amino acids*

Samples were collected for dissolved amino acids concentrations throughout the water column at deep stations (Table S2, appendix). Duplicate samples of 2 ml seawater were filtered through 0.2-µm syringe filters (Acrodisc, Pall) into pre-combusted 4-ml amber glass vials and then frozen at -20˚C for analysis in the home lab.

# *Prokaryotic Abundance*

Water samples were collected at every deep station from the surface to the bottom layers (Table S2, appendix). Duplicate 1.8 mL samples were fixed with glutaraldehyde (final concentration 0.5%), frozen in liquid nitrogen, and stored at -80°C. The abundance of prokaryotes will be measured in the home lab by flow cytometry after nucleic acid staining with SYBRGreen.

### *CARD-FISH*

Samples for Catalyzed reporter depositions fluorescence in situ hybridization (CARD-FISH) were fixed with 2% filtered formaldehyde in plastic tubes (Greiner Bio-One) in volumes between 20 mL and 80 mL (depending on the depth) and incubated for 12-18h. Subsequently, samples were filtered onto 0.2-µm polycarbonate filters (25 mm diameter, Millipore GTTP) and rinsed with Milli-Q. Filters were placed into 2 mL microfuge tubes and dried. Finally, the tubes with the filters were frozen at -80ºC until analysis in the home lab.

### *DNA samples*

Samples for DNA and RNA analysis were taken at all deep stations between 200 m depth and 10 m above sea floor (Table S2 appendix). Depending on the depth, between 1 and 5 L were filtered onto 0.2-µm polycarbonate filters (47 mm diameter, Millipore GTTP) in duplicate, using a peristaltic pump. After filtration, filters were folded and placed into microfuge tubes, flash frozen in liquid nitrogen and stored at -80ºC. In the home lab, DNA and RNA will be extracted for biodiversity analysis.

#### *Genome analysis of single cells*

Samples for single cell DNA analyses were taken at depths ranging from 100 m to 5000 m depth. One mL was cryo-preserved with 100 µl GlyTE Buffer for 5 min and subsequently frozen at -80°C. Samples will be shipped to the Single Cell Genomics Center at the Bigelow Laboratory for Ocean Sciences for sequencing.

# *High volume sampling for proteomics and exoproteomics*

At 4 stations high volume samples were taken for later proteomics and exo-proteomics analysis. For 500 and 2000 m depth 250 or 480 L were collected in Niskin bottles and transferred to several 80 L barrels. The raw seawater was pre-filtered over a 3.0 um (147 mm diameter, Millipore) and a 1.0 µm (293 mm diameter, GE Water Tech) polycarbonate filter using positive pressure diaphragm pumps (Verderair Cont-EX). The pre-filtered seawater was filtered onto a 0.2-µm Durapore membrane (293 mm diameter, Millipore). Initially, the permeate was concentrated to ~1 L using an ultrafiltration system with a pore size of 5000 Da and 0.5 m<sup>2</sup> filtration area (Millipore Pellicon Ultrafiltration System). This volume was further concentrated to ~30 mL using a small scale 5000 Da ultrafiltration cassette (Vivaflow Sartorius). The final concentrate was flash frozen in liquid nitrogen and stored at -80ºC. In the home lab the proteins from these samples will be extracted and analyzed using an nanoLC-MS/MS approach.

#### *Preliminary results*

Leucine incorporation in the dark ocean decreased by one to two orders of magnitude relative to the epipelagic zone (Fig. 7.21). This trend was obvious for all stations irrespective of the biogeographic province and water mass. In near-bottom samples, leucine incorporation deviated occasionally substantially from this exponentially decreasing trend with depth.



DIC fixation varied greatly vertically and along the transect (Fig. 7.22). Highest values occurred in the SPS and NPTG at 200 m and lowest values in the deep water in the southern Pacific and north of 45°N (Fig. 7.22B, C, D). Enhanced values were also measured at 700 and 1000 m in the equatorial upwelling region (Fig. 7.22D).



**Fig. 7.22:** DIC fixation rates in the Pacific during cruise SO248. A: Transect and stations visited; B; contour plot of DIC fixation rates; C and D: DIC fixation rates at depths of 200, 300, 700, 1000 and 2000 m depth along the transect.

### *Data management*

The data will be archived on the server of University of Vienna as a safe backup. Until publication, the data are available to members of PacGeoBac and on request from the authors. After publication the data will be submitted to the data information system Pangaea and will be available to everyone.

# *7.12 Prokaryotic leucine incorporation measurement under in situ pressure conditions with an in situ microbial incubator*

### (C Amano, T Reinthaler, GJ Herndl)

Prokaryotic activity and community composition is highly depth-stratified in the oceanic water column reflecting the increasing recalcitrance of dissolved organic matter and decreasing temperature with depth (Delong et al. 2006). While the abundance and microbial activity of dark ocean microbes have been investigated over the past decade (Aristegui et al. 2009, Reinthaler et al. 2010, Yokokawa et al. 2013), their metabolic rates have been largely determined under atmospheric pressure conditions. Moreover, the role of increasing hydrostatic pressure in controlling deep ocean microbial activity is less studied. To characterize marine prokaryotic activities at in situ pressure condition, an in situ microbial incubator (ISMI) was deployed during this cruise. The ISMI can collect and incubate seawater at a chosen depth and is also able to fix a certain volume of the incubated samples at specific time intervals. Here we tested the operation of the in situ incubator and measured prokaryotic leucine incorporation both at in situ and at atmospheric pressure conditions.

#### *Methods*

# *Leucine incorporation under in situ vs. atmospheric pressure incubations*

In situ incubations with the ISMI were done at 1000 and 2000 m depth at stations 10 and 16. The HCl washed polycarbonate bottles (500 mL) and all silicon tubings were filled with ultrapure water or filtered seawater to prevent rupture of the materials. Formaldehyde was added into the fixation cylinders (2% final concentration), and 380  $\mu$ L of <sup>3</sup>H-leucine was injected into triplicate incubation bottles (final concentration 5 nM leucine, 120 Ci mmol<sup>-1</sup>) with luer-lock syringes. Prior to deployment, the sampling schedule was programed with a custom made software (N-Com communicator ver. 3.01, Nichiyu Giken Co Ltd) and the ISMI was mounted on the CTD frame. After reaching the target depth, seawater was pumped into the incubation bottles and thereby, mixing the water with the added  ${}^{3}$ H-leucine.. Sub-samples were collected in the fixing cylinders and terminated by formaldehyde in situ at the initial time (T=0) and at the end of the incubation (T=8h). After recovering the ISMI and collecting the fixed water samples, the samples were filtered onto 0.2-µm pore size polycarbonate filters (25 mm diameter, Millipore GTTP) on board. The filters were used for leucine incorporation rate measurements using the on board scintillation counter and will be further used for MAR-FISH analysis as described above. To compare the in situ rates to the leucine incorporation at atmospheric pressure, water samples were also taken from decompressed Niskin bottles and incubated in the incubation ISMI bottles at atmospheric pressure conditions.

#### *Comparison of ISMI bottles and commercially available material*

To test whether there is a difference between ISMI bottles and other commercially available bottles, <sup>3</sup>H-leucine incorporation rates were determined in 50 mL tubes (Greiner Bio-One), polycarbonate bottles, and the custom made polycarbonate bottles of the ISMI. Seawater was collected from 2000 m depth at St. 14 and incubated with 5 nM  ${}^{3}$ H-leucine at 4 ${}^{0}$ C under atmospheric pressure. Triplicate live samples and duplicate formaldehyde-killed blank were incubated in the three types of bottles. The leucine incorporation rate was measured as described above.

### Checking the instrumental bias with full setup of the system

To check for potential biases in rate measurements due to instrument tubing and rinse water, seawater was collected from 500 m depth at St. 17 and incubated to determine  ${}^{3}$ H-leucine incorporation. The incubation tests were performed at 4ºC under atmospheric pressure using the full setup of the ISMI and in parallel the ISMI bottles without the system.

### *Preliminary Results*

Although the leucine incorporation rate obtained in the ISMI bottle was ca. 20% lower than that obtained with the other materials, no difference was found between ISMI bottles and the full setup of the ISMI. At both stations, leucine incorporation obtained under in situ pressure conditions was lower than under atmospheric pressure conditions (Fig. 7.23).



Fig. 7.23: Leucine incorporation rates at in situ and atmospheric pressure conditions. The error bars indicate the mean  $\pm$  |mean-replicate| of duplicate bottles or mean  $\pm$  SD.

#### **Data management**

The data from the single measurements will be combined in an excel spreadsheet. A version of this file will be archived on the server of the University of Vienna as a safe backup. After publication the data will be submitted to Pangaea and available to everyone.

# *7.13 The role of Archaea in the oxygenated water column*

### (B Bayer, GJ Herndl)

Planktonic prokaryotes are phylogenetically and metabolically diverse (DeLong et al 2006, Giovannoni and Stingl 2005) and play central roles in mediating a variety of different biogeochemical cycles (Azam and Malfatti 2007). It has now been firmly established that Archaea appear to be ubiquitous in the oxygenated oceanic water column (Karner et al 2001, Varela et al 2008). We aim at investigating the metabolic potential of deep-sea prokaryotes during cruise SO248, specifically focusing on Archaea.

Like Bacteria, Archaea have evolved a variety of different metabolisms that utilize organic or inorganic electron donors and acceptors, with many of them being able to assimilate inorganic carbon  $(CO_2, HCO_3)$  (Offre et al 2013).

Marine Group I Thaumarchaeota (formerly known as Marine Group I Crenarchaeota) have been found to be consistently abundant throughout the water column, comprising up to 40% of the total prokaryotic community in deep waters (Karner et al 2001, Teira et al 2006). Previous studies point towards the presence of additional metabolic capacities of Marine Group I Thaumarchaeota (Luo et al 2014, Swan et al 2014). One objective is to investigate if urea can serve as an alternative substrate for Thaumarchaeota in the deep Pacific, using Stable Isotope Probing (SIP) to detect substrate incorporation.

In addition to Thaumarchaeota, typically two euryarchaeal groups can be detected in considerable abundances in the marine water column: Marine Group II and III Euryarchaeota (Fuhrman and Davis 1997). Metagenomic analyses indicated that Group II Euryarchaota in surface waters may be capable of proteorhodopsin-dependent photoheterotrophy (Iverson et al 2012), however, metabolic capacities of deep sea Euryarchaeota remains enigmatic. Recent analyses of Single-Amplified-Genomes indicated that deep sea Marine Group II and III Euryarchaeota are heterotrophs able to utilize proteins and amino acids (Bayer et al., unpublished). The objective is to investigate the response of Euryarchaeota to amino acid additions in order to test this hypothesis.

# *Methods*

Seawater from 3 different depths (500 m, 1000 m, bottom) was sampled at stations 4 and 12 and incubated in 2-10 L carboys amended with either L-Alanine, D-Alanine, Arginine (final concentration: 10 µM) or no substrate. Prokaryotic cell abundance was measured on board via Flow Cytometry (Accuri, BD) over the time course of the experiments. Incubations were terminated when exponential growth was reached, or after 14 days. Additionally, samples for DNA, proteomics, CARD-FISH and amino acid analyses were taken at the beginning and at the end of the incubations. DNA and proteins will be extracted in the home lab and community composition will be analyzed.

### *High volume mesocosms amended with <sup>13</sup>C-labelled urea*

Seawater at stations 4 and 12 was taken from 500, 1000 and 4000 m depth and incubated in high volume mesocosms (200 L for 1000 m, 100 L for 500 m depth) amended with  ${}^{12}$ C- or  ${}^{13}$ C Urea (final concentration: 10 µM). After 6-14 days, the total volume was filtered onto 0.2 µm Durapore membranes (293 mm diameter, Millipore) and stored at -80°C. Stable isotope probing (SIP) will be carried out in the home lab.

# *Preliminary results*

Increases of cell abundances towards the end of the experiment at station 4 show that the bacterial community responded to the amino acid additions (Fig. 7.24).



500m, Station 4

Fig. 7.24: Prokaryotic abundance during incubation of samples amended with the amino acids shown in the legend from the station and depth indicated.

# **Data management**

Sequencing data will be archived on the server of the University of Vienna• as a safe backup. Until publication, the data is available to members of PacGeoBac and on request from the authors. After publication the sequencing data will be submitted to NCBI and will be available to everyone.

# *7.14 Plastic and microbial communities*

### (M Pinto, GJ Herndl)

The aim was to study the microbial community composition and metabolism of the biofilm of plastics in different biogeographic provinces during this cruise and to determine how they differ from the microbial communities in the surrounding seawater.

# *Methods*

Plastics were collected with horizontal tows at the surface with a Bongo net composed of two nets of 1 m diameter and 3 m length, which was held 15 to 20 m away from the ship. The net was launched at 13 stations, and was towed at a speed of 1-2 knots for 30-45 min. The mesh size of the net was 300 um.

To divide the collected material into 4 different size classes, the material from the cod end of the net was passed through a sieve tower, composed of four sieves with mesh sizes of 9.5 mm, 4.75 mm, 1.4 mm, 300 µm. Afterwards, plastics were counted and separated either by naked eye or, in the case of smaller pieces, under the dissection microscope and separated for different analysis: Scanning electron microscopy (SEM), fluorescence in situ hybridization (FISH), Raman spectroscopy and molecular analysis. Additionally, to monitor the biofilm establishment on plastics, plastic squares of 1 cm<sup>2</sup> were incubated in glass bottles with 1 L of seawater for 10 days. A bottle without any plastic incubated and another with plastic incubated in 0.2-µm filtered seawater were kept as controls. Every other day, both plastic and water were sampled for molecular analysis, SEM, cell abundance and FISH. At the end of the experiment, the heterotrophic activity on the plastics was measured with radiolabelled leucine and the remaining seawater was filtered onto 0.2 µm polycarbonate filters for metagenomics.

#### *Preliminary results*

Plastics were found at 4 stations between 11°N and 40°N, with the highest plastic abundance at 33°N with 178 collected plastics (around 63 pieces per km<sup>2</sup> of water surface) (Fig. 7.25). All samples were frozen and transported to Vienna, and are currently stored at -80°C until further processing.



# **Data management**

seawater.

All data obtained from this cruise will be available after publication in a peer-reviewed journal. Additionally, information on the amount of plastic collected at different latitudes will be shared with organizations interested in plastic pollution research.

### *7.15 Under way measurements by FerryBox*

# *Continuous FerryBox flow-through measurements of surface waters*

A FerryBox is a flow-through system deployed as an underway device for ship expeditions and for attendant measurements during stationary operations (Fig. 7.26). The system provides basic data at high spatial and temporal resolution for various parameters, e.g. salinity, temperature (at the intake and inside the system), Chl *a* -fluorescence, turbidity, and dissolved oxygen. For multi-parameter sensing and validation of the ships flow-through system (equipped with thermosalinograph and bbe FluoroProbe sensor), the FerryBox was fed by water from the ship system via a bypass. Measurements were performed at a sampling interval of 1 min. Once a day, a salinity reference sample was taken (glass bottle, 250 ml) to validate the salinity sensor of the FerryBox as well as the salinity probe of the thermosalinograph of the ships system. Analysis of the salinity samples is ongoing.

On this cruise, a second box (FerryBox AddOn) equipped with an UV- and a VISspectrophotometer, was used to collect absorption spectra to, e.g. improve current processing algorithms of optical nitrate detection for oligotrophic waters. Measurements were performed at a sampling interval of 10 min. Processing will be performed according to Zielinski et al (2011) and Frank et al (2014). Validation and improvement of processing algorithms to calculate optical nitrate and transmission are still under progress.

Further investigations in this section include the sampling for harmful algae toxins along the whole cruise transect. Therefore a special absorber pad was installed in the outflow of the flow-through boxes. Within a fixed time interval of 5 days water was running through these absorber pads with respect to the investigated area. Pads were stored at 4°C until further processing, wash out of toxins and analysis via LC-MS/MS.



Figure 7.26 FerryBox system (right) for hydrographic parameters in surface waters and AddOn Box (left) for the collection of UV/VIS spectra for calculation of transmission and optical nitrate.

### *7.16 Meteorology data on aerosol and water vapor*

(H Winkler, TH Badewien, KL Arndt, S Kinne, A Smirnov)

Meteorological data during the SO248 cruise were continuously obtained via built-in systems *(weather-station, thermosalinograph / TSG,* so-called "*underway-data"*). We used handheld instruments for measuring aerosol and water vapor.

#### *Meteorological Underway Data*

Weather data such as air temperature, wind speed, wind direction and global radiation were extracted from the underway data, which are logged and controlled by the DSHIP-Software. Hydrographic data from the sea surface were taken via a thermosalinograph connected to a through-flow-system in the front of the ship.

#### *Microtops Aerosol and Water Vapor Survey*

During cruise SO248 we measured direct solar attenuation by means of a handheld Microtops instrument during cloud-free conditions at daytimes. The system provides information on atmospheric aerosol amount, aerosol size and atmospheric water vapor. The data will be included in the *Aeronet Maritime Aerososol Network* (http://aeronet.gsfc.nasa.gov). Data were delivered to Alexander Smirnov (Science Systems and Applications, Inc., NASA/Goddard Space Flight Center), who processed them. First results can be seen at [http://aeronet.gsfc.nasa.gov/new\\_web/cruises\\_new/Sonne\\_16\\_0.html.](http://aeronet.gsfc.nasa.gov/new_web/cruises_new/Sonne_16_0.html)

In collaboration with NASA-GSFC, Microtops measurements are conducted worldwide on an opportunity basis aboard (research) vessels in order to complement continental aerosol monitoring at AERONET sites.



**Figure 7.27** Microtops and GPS unit provided by the Marine Aerosol Network of AERONET at NASA-GSFC.



Figure 7.28 Overview of measuring points of aerosol and water vapor during cruise SO248 *[\(http://aeronet.gsfc.nasa.gov/new\\_web/cruises\\_new/Sonne\\_16\\_0.html,](http://aeronet.gsfc.nasa.gov/new_web/cruises_new/Sonne_16_0.html) as processed until July, 06 2016).*

# **8. Acknowledgements**

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# **10. Abbreviations / Abkürzungen**



# **11. Appendices**

# **A) Participating Institutions / Liste der teilnehmenden Institutionen**

### **ICBM**

Institut für Chemie und Biologie des Meeres Universität Oldenburg Carl von Ossietzky Str. 9-11 D-26129 Oldenburg, **Germany [www.icbm.de](http://www.icbm.de/)**

### **HZI**

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### **DSMZ**

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# **UVI**

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# **UNC**

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# **B) Station list / Stationsliste**

Table A1: Station number, start and end of station work, latitude, longitude and water depth of stations. For a detailed list of station work at each station see Table A2.



**Table A2: Detailed overview on the equipment used and number of tasks, station number, date, time, device used and action, latitude, longitude and water depth.** 





**lost Equipment:** none

 $\sum$  142

**Station Date / Time UTC Device Action Comment (Action) Latitude Longitude Depth (m)** SO248\_1-1 02.05.2016 18:03:17 CTD station start 29° 59,969' S 176° 59,890' E 4280,1 SO248 1-1 02.05.2016 18:10:03 CTD in the water  $\vert$  so 200 0,056' S 176° 59,968' E 4267,8 SO248 1-1 | 02.05.2016 18:51:55 | CTD max depth/<br>on ground maxSL: 1000m | 30° 0,049' S | 176° 59,958' E | 4268,2 SO248\_1-1 | 02.05.2016 19:01:45 | CTD | hoisting | Beg. Hieven | 30° 0,050' S | 176° 59,959' E | 4268,9 SO248\_1-1 02.05.2016 19:34:42 CTD on deck 30° 0,044' S 176° 59,970' E 4265,2 SO248\_1-1 02.05.2016 20:10:15 CTD station end 30° 0,030' S 176° 59,971' E 4270,2










































































Table A3: List of the main parameters sampled by the dark ocean team of the Department of Limnology and Bio-Oceanography, University of Vienna during SO248.





## **Abbreviations:**

Stn: Station

 $3$ H-Leu: incorporation of  $3$ H labelled leucine

MAR: Microautoradiography combined with Fluorescence in situ Hybridization

 $DI<sup>14</sup>C$ : incorporation of  $14^{\circ}C$  labeled bicarbonate

PTIO: inhibitor experiments

PA: prokaryotic abundance

SAGs: Single Cell Genomes

CARD-FISH: Catalyzed Reporter Deposition Fluorescence in situ Hybridization OMICS: includes metagenomics and exo-proteomics

AA: Amino Acids