Benthic bacteria in the German Bight:
Characterising community structure and influencing environmental factors

or

‘Living along gradients’
Introduction

Methods

Results
  Spatiotemporal variations of benthic bacterial communities in the German Bight
  Impact of ocean dumping on benthic bacterial communities

Conclusions

Future perspectives
Motivation: Ocean dumping

- deposition of waste at sea
- waste includes:
  - liquid wastes (dilute acid, sludge)
  - construction waste
  - dredged material (harbours, rivers)

→ Threat for ecosystems
Motivation: Ocean dumping

Overview of the number and distribution of dumpsites within the OSPAR area
since 2005: ~ 6 mio m³
3 m high rising containing mainly sand
Motivation

• monitoring focuses on macrozoobenthic communities and flatfishes

• “Progress on investigating biological responses to the disposal of dredged material has been slow in OSPAR and more effort is needed for a wider and more systematic application of bioassays in the testing of dredged sediments.“ (OSPAR 2009b)

• bacterial community structure affected by
  – physical disturbance (Findlay et al 1990)
  – heavy metal and oil contamination (Gremion et al 2004, dos Santos et al 2011)
Motivation

- benthic bacterial communities:
  - largest variety of metabolic types
  - heterotrophic, phototrophic and lithotrophic bacteria
  - highly abundant: $10^8 - 10^{11}$ cells per ml

Are bacterial community analyses a useful supplement for monitoring programs at dumping sites?

Problem: sublittoral benthic bacterial communities uncharacterised in the German Bight
German Bight

- hydrographic regime predominantly influenced by discharges of Elbe and Weser River
- most eutrophied region in the North Sea
- shallow part of the North Sea with max. depths ~ 50 m
- Helgoland
Sediments in the German Bight

- **Grain size fractions**
  - Clay particles < 4 µm
  - Silt particles 4 - 63 µm
  - Fine sand particles 63 – 200 µm
  - Medium sand particles 200 – 630 µm
  - Coarse sand particles 630 – 2000 µm
  - Gravel particles 2000 – 6000 µm

- Proportion of each grain size fraction determines the classification into sandy or muddy sediments
Sediments in the German Bight

Introduction
Introduction

Environmental gradients

Spatial gradients

Temporal gradients
Characterising sublittoral benthic bacterial communities

I: Spatiotemporal variations influencing benthic bacterial communities in the German Bight

II: Impact of ocean dumping on benthic bacterial communities
   a) Community structure
   b) Community function
Data analysis

• Bacterial community analysis
  – ARISA fingerprinting
  – 16S pyrosequencing
  – functional gene arrays

• Environmental data (additional data provided by Prof. Karen Wiltshire and HPA)
  – CHN analysis
  – particle size analysis

• Statistical analysis
  – univariate and multivariate statistics
  – Geostatistics
Characterising sublittoral benthic bacterial communities

I: Spatiotemporal variations influencing benthic bacterial communities in the German Bight

II: Impact of ocean dumping on benthic bacterial communities
   a) Community structure
   b) Community function
I: Spatiotemporal variations influencing benthic bacterial communities

- monthly sampling from September 2010 to August 2011
- three replicates for bacterial community analysis (ARISA)
- environmental data includes:
  - temperature
  - salinity
  - chl a
  - CHN
  - particle size of the sediments
I: Spatiotemporal variations influencing benthic bacterial communities

Stable environmental conditions offshore vs variable environmental conditions nearshore
I: Spatiotemporal variations influencing benthic bacterial communities
I: Spatiotemporal variations influencing benthic bacterial communities

Fingerprinting via Automated Ribosomal Intergenic Spacer Analysis (ARISA)

Ribosomal Operon of Bacteria

Conversion of community profiles into numeric data

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## Results

### I: Spatiotemporal variations influencing benthic bacterial communities

Distance based multivariate multiple regression model (DISTLM)

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<th>P</th>
<th>Proportion of variance</th>
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<th>Pseudo-F</th>
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\[ p < 0.01 \]
I: Spatiotemporal variations influencing benthic bacterial communities

distance-based Redundancy analysis

Nearshore bacterial communities exhibit strong spatiotemporal variations
I: Spatiotemporal variations influencing benthic bacterial communities

Distribution of operational taxonomic units (OTUs)

Spatial

Temporal

Diversity of bacterial communities in nearshore habitats affected by temporal variations

OTUs defined according to ITS fragment lengths
Results

I: Spatiotemporal variations influencing benthic bacterial communities

Main findings

- amplitude of environmental gradients determines the bacterial community structure
- temporal variations affect bacterial community structure and diversity
Characterising sublittoral benthic bacterial communities

I: Spatiotemporal variations influencing benthic bacterial communities in the German Bight

II: Impact of ocean dumping on benthic bacterial communities
   a) Community structure
   b) Community function
II: Impact of ocean dumping on benthic bacterial communities
II: Impact of ocean dumping on benthic bacterial communities

Timeline sampling campaigns

08/08 - 10/08

9 months

08/09

2 months

12/09 – 02/10

2 months

04/10

4 months

08/10

△ Dumping campaigns

▲ Sampling campaigns
II: Impact of ocean dumping on benthic bacterial communities

- sampling campaigns in August 2009 and April and August 2010
- each campaign comprises 125 sampling sites
- three replicates for bacterial community analysis (ARISA)

### Contextual data

#### Grain size fractions

- < 20µm
- 20-63µm
- 63-100µm
- 100-200µm
- 200-630µm
- 630-1000µm
- 1000-2000µm

#### S, N, P, C

- TOC (C)
- nitrogen (N)
- sulphur (S)
- phosphor (P)

#### Hydrocarbons

**Sum Polycyclic Aromatic Hydrocarbons (PAH)**

- naphthaline
- fluorene
- phenanthrene
- anthracene
- fluoranthene
- pyrene
- benzo[a]anthracene
- chrysene
- benzo[b]fluoranthene
- benzo[k]fluoranthene
- benzo[a]pyrene
- dibenzo[a]anthracene
- benzo(ghi)perylene
- indeno(1.2.3cd)pyrene

**Sum Chlorinated Biphenyls (PCB)**

- PCB28
- PCB52
- PCB101
- PCB118
- PCB138
- PCB153
- PCB180

**Sum Hexachlorocyclohexane (HCH)**

- alphaHCH
- betaHCH
- gammaHCH
- deltaHCH

**Sum Dichlorodiphenyldichloroethane (DDT) and metabolites**

- ppDDE
- opDDD
- ppDDD
- opDDT
- ppDDT

**Sum Organotin Compounds**

- monobutyltin (MBT)
- dibutyltin (DBT)
- tributyltin (TBT)
- tetrabutyltin

**Heavy Metals**

- arsenic
- lead
- cadmium
- chrome
- copper
- nickel
- mercury
- zinc
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<td>chrysene</td>
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- fluoranthene
- pyrene
- benz(a)anthracene
- chrysene
- benzo(b)fluoranthene
- benzo(k)fluoranthene
- benzo(a)pyrene
- dibenz(ah)anthracene
- benzo(ghi)perylene
- indeno(1.2.3cd)pyrene

**Heavy Metals**

- arsenic
- lead
- cadmium
- chrome
- copper
- nickel
- mercury
- zinc

**Sum Chlorinated Diphenyls (PCB)**

- PCB28
- PCB52
- PCB101
- PCB118
- PCB138
- PCB153
- PCB180
II: Impact of ocean dumping on benthic bacterial communities

Biplot Redundancy analysis August 2009

Bacterial communities at the dumping site significantly affected by fine sand (100 – 200 µm) and organic pollutants
II: Impact of ocean dumping on benthic bacterial communities

- based on significant differences (analysis of similarities) in the community structure nine representative samples were selected
- via pyrosequencing the V1-V5 region of the 16S rDNA was sequenced
II: Impact of ocean dumping on benthic bacterial communities

Distribution of operational taxonomic units (OTUs)

lowest diversity was observed in Elbe and at dumping centre

OTUs = sequence similarity > 97 %
Singletons (n=1) were removed prior to the analysis
II: Impact of ocean dumping on benthic bacterial communities

considerable higher abundance of *Flavobacteriaceae* at the dumping centre

indication for organic pollution?

singletons (n=1) were removed prior to the analysis
II: Impact of ocean dumping on benthic bacterial communities

**Betaproteobacteria**

![Bar chart showing distribution of bacterial groups](chart.png)

- **Typical freshwater groups** still detectable nine months after dumping activity

- Singletons (n=1) were removed prior to the analysis
II: Impact of ocean dumping on benthic bacterial communities

*Deltaproteobacteria*

considerable higher numbers of *Desulfuromonadaceae* and lower numbers of *Desulfurobacteraceae* indication for organic pollution?

singletons (n=1) were removed prior to the analysis
Characterising sublittoral benthic bacterial communities

I: Spatiotemporal variations influencing benthic bacterial communities in the German Bight

II: Impact of ocean dumping on benthic bacterial communities
   a) Community structure
   b) Community function
II: Impact of ocean dumping on benthic bacterial communities

Results
II: Impact of ocean dumping on benthic bacterial communities

GeoChip analysis

- GeoChip 4.2 (functional gene array) (He et al 2007, Lu et al 2012)

- contains 103 666 probes encoding for functional genes involved in biogeochemical key processes

- genes are categorised according to these processes e.g. sulphur cycling, heavy metal resistance, organic remediation
II: Impact of ocean dumping on benthic bacterial communities

Distribution of functional genes of the gene category „organic remediation“

significantly lower functional diversity at the dumping centre as compared with the reference sites
II: Impact of ocean dumping on benthic bacterial communities

Hierarchical clustering based on Euclidean distance for the gene category „organic remediation“
Main findings

• similar results for all gene categories
• no accumulation of genes involved in pollution related processes at the dumping centre detectable
• significant lower functional diversity at the dumping centre
• differences among detected gene groups based on phylogenetic background
Main conclusions

• most pronounced environmental gradients affect the bacterial community structure significantly

• bacterial community structure at the dumping site was significantly influenced by the dumping activity
  – lower alpha and functional diversity
  – mix-community containing fresh water and adapted marine bacteria

• bacterial community analysis represent a useful supplement for monitoring programs

• But: further elaboration is needed!!
• deepening the knowledge about benthic bacterial communities in the German Bight
  – identifying community composition and function „Metagenomic approaches“
  – simultaneous investigation of pelagic and benthic bacteria „Benthopelagic coupling“

• adaptation of monitoring conditions and experimental set up for the inclusion of bacterial community analysis
  – controlled experiments focusing on the impact of relevant pollutants on the bacterial communities
  – identifying indicator organisms
  – inclusion of physicochemical parameters such as pH, oxygen penetration, bioavailability of pollutants
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