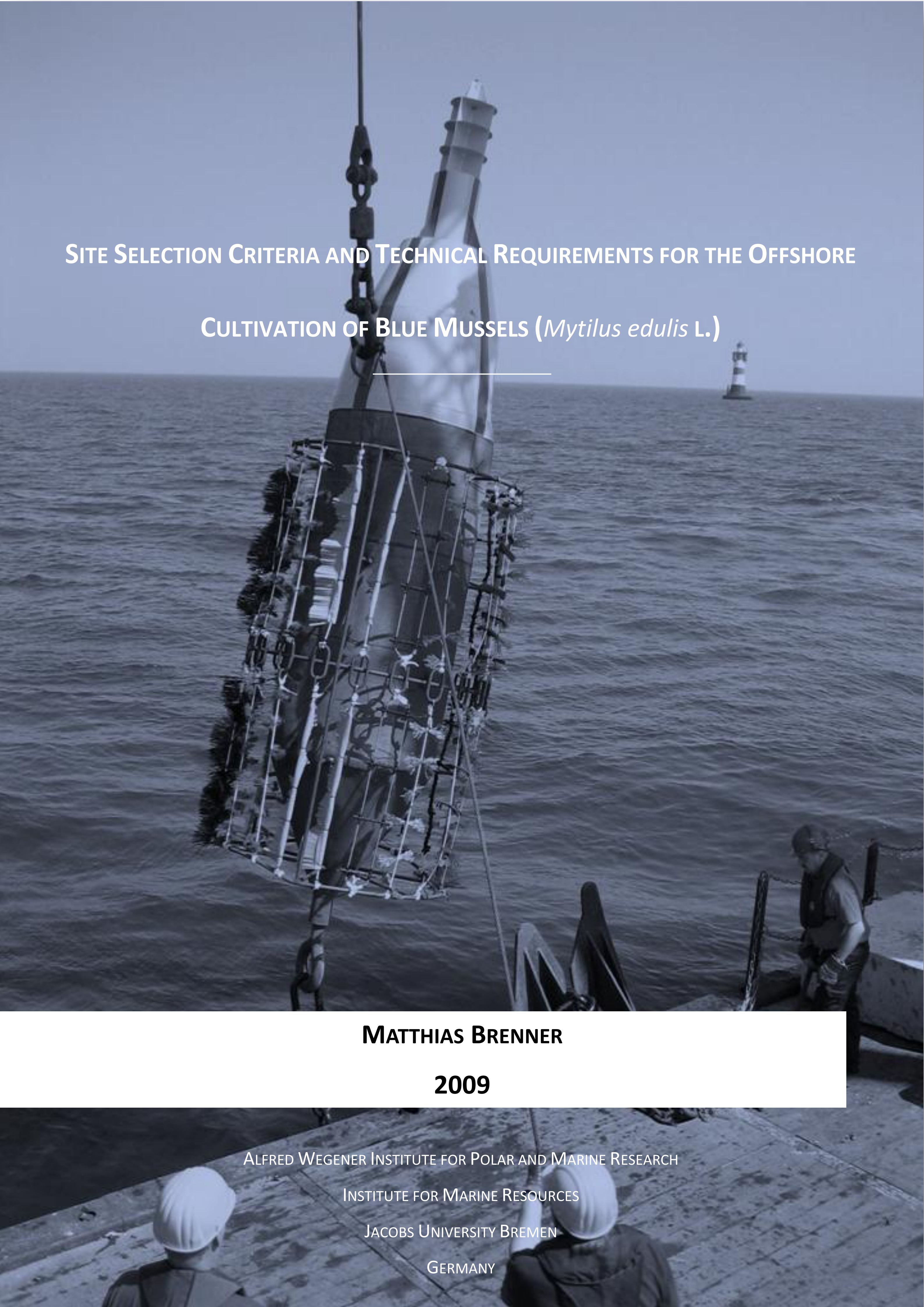

Site Selection Criteria and Technical Requirements for the Offshore Cultivation of Blue Mussels (*Mytilus edulis* L.)

Matthias Brenner

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Alfred Wegener Institute for Polar and Marine Research

Institute for Marine Resources

Jacobs University Bremen

Germany

Site selection criteria and technical requirements for the offshore cultivation of blue mussels (*Mytilus edulis* L.)

by

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A thesis submitted in partial fulfilment

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Doctor of Philosophy

in Biology

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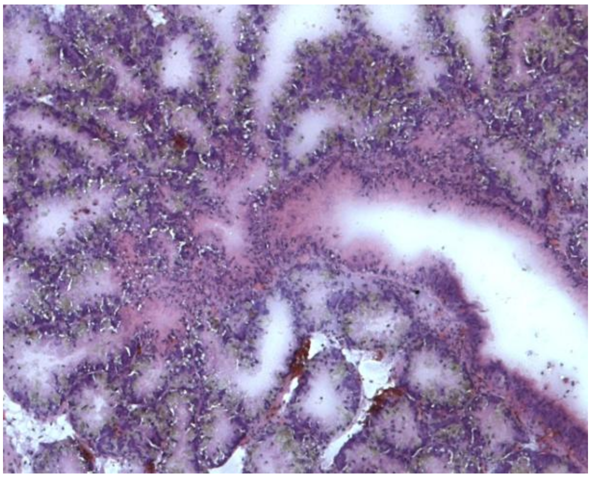

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Statement

This thesis represents original and independently conducted research that has not been submitted to any other university for the conferral of a degree.

Matthias Brenner

Bremen, Germany – August 12, 2009

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Core publications of the thesis

Below, the publications of the thesis are listed and my share thereof is outlined.

Publication I (Chapter 04)
Buck BH, Krause G, Michler-Cieluch T, **Brenner M**, Buchholz CM, Busch JA, Fisch R, Geisen M, Zielinski O (2008). Meeting the quest for spatial efficiency: Progress and prospects of extensive aquaculture within offshore wind farms. Helgoland Marine Research, 62: 269-281

The first author developed the conceptual frame, outline and design of the publication. Together with the second and third author main parts of the manuscript were written. Manuscript was discussed and improved by all the co-authors. I myself contributed the description, the preliminary results and outlook of two projects (*MytiFit* and Jade).

Publication II (Chapter 05)
Brenner M, Buck BH, Koehler A (2007). New concept combines offshore wind farms, mussel cultivation. Global Aquaculture Advocate, 10(1): 79-81

The initial idea originates from *MytiFit*-Proposal written by all 3 authors. Based on that concept I wrote this publication on my own.

Publication III (Chapter 06)
Brenner M, Buck BH (2010). Attachment properties of blue mussels (*Mytilus edulis* L.) byssus threads on culture-based artificial collector substrates. Aquacultural Engineering (accepted)

The initial concept originates from the second author who acted as my co-supervisor concerning the aquaculture aspects of my thesis. B. Buck contributed also the idea of how to measure the investigated parameters. I improved the initial concept, constructed the testing site, developed the sampling design and organized sampling and data assessment. Second author added parts of the introduction and the paragraph about “mussels experiencing hydrodynamic forces” in the discussion. I wrote the initial draft manuscript and all further versions, which resulted from discussion with second author.

Publication IV (Chapter 07)
Brenner M, Juetting E (2009). Untersuchungen zur Verzehrfähigkeit von Miesmuscheln (*Mytilus edulis* L.) aus Offshore-Windparks - Konsequenzen für die behördliche Überwachung. Journal für Verbraucherschutz und Lebensmittelsicherheit, 4: 265-272

I developed the idea for this publication, conducted the literature research and wrote the draft and all further versions of the publication, which resulted from discussion with second author. The second author provided relevant legal references and reviewed the manuscript.

Publication V (Chapter 08)
Brenner M, Ramdohr S, Effkemann S, Stede M (2009). Key parameters for the consumption suitability of offshore cultivated mussels (*Mytilus edulis*) in the German Bight. European Food Research and Technology, 230: 255-267

The concept of this publication was based on the *MytiFit*-Proposal where Mr. Stede and I were also co-authors. In addition to the initial concept the second and third author provided assessment and data about the microbial and viral contaminations and about algae toxin concentration, respectively. The last author investigated mussels for micro parasites. I adapted, improved, organised and conducted sampling for both sampling seasons, assessed data of macro parasites, condition index and length-weight-ratio of shells. Results and description of methods were added by all co-authors. I wrote the initial draft manuscript and all further versions, which resulted from discussion with second author.

Publication VI (Chapter 09)
Brenner M, Buchholz C, Buck BH, Koehler A (2009). Health and growth performance of blue mussels (*Mytilus edulis* L.) from two different hanging cultivation sites in the German Bight: a nearshore - offshore comparison (Manuscript)

The concept of this publication was based on the *MytiFit*-Proposal written by the first, third and last author. I adapted, improved, organised and conducted sampling for both sampling seasons with the help of third author and assessed data for mussels' health. Second author provided data about growth according to my concept and under my supervision. I wrote the initial draft manuscript and all further versions, which resulted from discussion with the last author.

Publication VII (Chapter 10)
Brenner M, Wilhelm C, Broeg K, Koehler A (2009). Effect of air exposure on lysosomal membrane stability of *Mytilus edulis* L. from intertidal wild banks and submerged culture ropes (Manuscript)

I developed the idea for this publication, conducted the literature research and developed, organized and conduced the sampling with the help of second author for this study. The second author assisted in the field and processed samples in the lab according to my concept and under supervision of me and the third author. Third author explained the assessment of lysosomal membrane stability and helped together with the last author with the interpretation of results. I wrote the initial draft manuscript and all further versions, which resulted from discussion with the third author and last author.

1. List of publications concerning the thesis

Additional publications, invited talks, talks, and posters presentations obtained from data of the project *MytiFit*, not presented in detail in this thesis, are listed below.

1.1. Not peer-reviewed Publications

Krone R, Brenner M (2009). Muschelzucht, künstliche Substrate & Windparks. Coquilles coquines - Harte Schale, weicher Kern. Musée National d'Histoire Naturelle, Luxembourg, 120-137

Buck BH, Krause G, Michler-Cieluch T, Brenner M, Fisch R, Zielinski O (2008). Participatory networks to meet the challenges of extensive aquaculture within offshore wind farms. Joint Proceedings of the Akademia Morska Gdynia and the University of Applied Sciences Bremerhaven, 21: 5-16

Brenner M, Buck BH, Koehler A (2008). Evaluation of a Health Management System for Offshore Cultivation of Blue Mussels (*Mytilus edulis*). Aquaculture for Human Wellbeing - The Asian Perspective. The Annual Meeting of the World Aquaculture Society, 19.-23. May 2008, Busan, Korea, 85

Buck BH, Koehler A, Brenner M, Stede M (2007). Eignung des Seegebietes am geplanten Offshore-Windpark Nordergründe für die Zucht von Miesmuscheln: Fitness, Parasitisierung und Substratwahl. Endbericht, 91 pp

Brenner M, Buck BH, Stede M, Koehler A (2007). The implementation of biodiagnostic tools helps to select appropriate sites for offshore cultivation of blue mussels (*Mytilus edulis* L.). 10th International Conference on Shellfish Restoration (ICSR) 2007, 12.-16. November 2007, Vlissingen, Netherlands, 54

Pechura A, Brenner M, Buck BH (2007). The attachment strength of offshore grown blue mussel *Mytilus edulis* L. threads according to the hard substrates' microstructure at different current velocities. 10th International Conference on Shellfish Restoration (ICSR) 2007 12.-16. November 2007, Vlissingen, Netherlands, 54

Brenner M, Ramdohr S, Stede M, Effkemann S, Bartelt E, Etzel V, Koehler A (2007). Projekt *MytiFit* - Untersuchungen zur Zucht- und Verzehrfähigkeit von Miesmuscheln (*Mytilus edulis*) aus Offshore-Windparks. 48. Arbeitstagung des Arbeitsgebietes "Lebensmittelhygiene" vom 25.- 28. September 2007 in Garmisch-Partenkirchen/ Deutsche Veterinärmedizinische Gesellschaft, 104

Brenner M, Koehler A, Buck BH (2006). Biodiagnostic techniques on blue mussels (*Mytilus edulis*) as site-selection criteria for offshore farming. 36th Annual Conference of the Ecological Society of Germany, Switzerland and Austria (GfÖ), 11.-15. September 2006, Bremen, Germany, 195

Brenner M, Buck BH, Koehler A (2006). Blue mussel culture: Does offshore cultivation lead to increasing product quality? "Linking Tradition and Technology - Highest Quality for the Consumer". The Annual Meeting of the World Aquaculture Society and the European Aquaculture Society, 9.-13. May 2006, Florence, Italy, 116

Brenner M (2006). Algen- und Muschelfarm auf hoher See. Geschäftsbericht/ Helmholtz Gemeinschaft, 1: 12-13

Michler T, Kodeih S, Brenner M, Buck BH (2006). Versuchsanlagen im Offshore-Bereich der deutschen Bucht für die Zucht von Miesmuscheln (*Mytilus edulis*). Fischerblatt, 2: 15-16

1.2. Invited talks

Brenner M, Buck BH, Koehler A (2007). Producing best quality - offshore cultivation of blue mussels. Cheju National University, 18. October 2007, Cheju, Korea

Brenner M, Buck BH, Koehler A (2007). Health management for offshore mussel (*Mytilus edulis* L.*)* farming. Korean Ocean Research and Development Institute (KORDI), 15. October 2007, Ansan, Korea

Pogoda B, Buck BH, Brenner M, Geisen M (2007). Forschungstauchen zur Weiterentwicklung Maritimer Biotechnologien: Offshore Aquakultur. 1st International Symposium for Occupational Scientific Diving (ISOSD 2007), 16. October 2007, Bremerhaven, Germany

Brenner M, Buck BH, Koehler A (2007). Health monitoring for offshore cultivation of blue mussels (*Mytilus edulis* L.). Korean-German Workshop on Polar and Marine Sciences, 25. May 2007, Alfred Wegner Institute for Polar and Marine Research, Bremerhaven, Germany

Brenner M, Buck BH, Koehler A (2007). *MytiFit* - Eignung des Seegebietes am geplanten Offshore-Windpark Nordergründe für die Zucht von Miesmuscheln. 6. Forschungsworkshop Windenergie, Thema: Perspektiven der Windenergie, 24. April 2007, Hochschule Bremerhaven, Bremerhaven, Germany

Brenner M (2006). Muscheln und Algen aus Offshore-Windparks - Projekt mit Zukunft oder Ausbeutung der Meere? Vortragsreihe Eis und Meer, 01. März 2006, Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven, Germany

Buck BH, Brenner M (2005). Offshore-Muschelzucht: Potentiale und Perspektiven, Wissenschaftsforum des Landes Bremen, Thema Ökologie, Messe HusumWind, 23. September 2005, Husum, Germany

1.3. Talks

Brenner M, Buck BH, Koehler A (2008). Evaluation of a Health Management System for Offshore Cultivation of Blue Mussels (*Mytilus edulis* L.), "Aquaculture for Human Wellbeing - The Asian Perspective". The Annual Meeting of the World Aquaculture Society, 23. May 2008, Busan, Korea

Brenner M, Buck BH, Stede M, Koehler A (2007). The implementation of biodiagnostic tools helps to select appropriate sites for offshore cultivation of blue mussels (*Mytilus edulis* L.). 10th International Conference on Shellfish Restoration (ICSR) 2007, 15. November 2007, Vlissingen, Netherlands

Pechura A, Brenner M, Buck BH (2007). The attachment strength of offshore grown blue mussel *Mytilus edulis* L. threads according to the hard substrates' nanostructure at different current velocities. 10th International Conference on Shellfish Restoration (ICSR), 15. November 2007, Vlissingen, Netherlands

Brenner M, Ramdohr S, Stede M, Effkemann S, Bartelt E, Etzel V, Koehler A (2007). Projekt *MytiFit* - Untersuchungen zur Zucht- und Verzehrfähigkeit von Miesmuscheln (*Mytilus edulis*) aus Offshore-Windparks. 48. Arbeitstagung des Arbeitsgebietes Lebensmittelhygiene der Deutschen Veterinärmedizinischen Gesellschaft (DVG), 27. September 2007, Garmisch-Partenkirchen, Germany

Brenner M, Buck BH, Koehler A (2006). Blue mussel culture: Does offshore cultivation lead to increasing product quality? "Linking Tradition and Technology - Highest Quality for the Consumer". The Annual Meeting of the World Aquaculture Society and the European Aquaculture Society, 11. May 2006, Florence, Italy

1.4. Posters

Brenner M, Koehler A, Buck BH (2007). Product quality control for offshore mussel (*Mytilus edulis*) farming - future concepts. Bremen Molecular and Marine Biology (BMMB) Meeting, Schloss Etelsen, 26.-27. January 2007, Etelsen, Germany

Brenner M, Koehler A, Buck BH (2007). Muschelzucht in Offshore-Windparks. Gemeinsame Veranstaltung der partnerschaft-umwelt-unternehmen und des Förderprogramms Angewandte Umweltforschung, 26. Februar 2007, Universität Bremen, Bremen, Germany

Brenner M, Koehler A, Buck BH (2006). Biodiagnostic techniques on blue mussels (*Mytilus edulis*) as site-selection criteria for offshore farming. 36th Annual Conference of the Ecological Society of Germany, Switzerland and Austria (GfÖ), 11.-15. September 2006, Bremen, Germany

Brenner M, Koehler A, Buck BH, Stede M, Engel M (2005). Gesunde Muscheln aus dem Windpark - Offshore-Muschelzucht: Potentiale und Perspektiven. Wissenschaftsforum des Landes Bremen, Thema Ökologie, Messe HusumWind, 20.- 24. September 2005, Husum, Germany

Buck BH, Brenner M, Rosenthal H (2005). Aus dem Windpark frisch auf den Tisch: Eine Machbarkeitsstudie über die multifunktionale Nutzung von Offshore Windparks und mariner Aquakultur. Messe InWaterTec, 31. August - 2. September 2005, Kiel, Germany

Buck BH, Brenner M, Buchholz C, Rosenthal R (2005). System-Design für Offshore-Technologien am AWI Bremerhaven. Messe InWaterTec, 31. August - 2. September 2005, Kiel, Germany

2. List of publications conducted independent of the thesis

The following publications resulted from data of the Diploma-Thesis (equivalent to Master) and from data of a student project.

2.1. Peer-reviewed Publications

Krumme U, Brenner M, Saint-Paul U (2008). Spring-neap cycle as a major driver of temporal variations in feeding of intertidal fishes: evidence from the sea catfish/ *Sciades herzbergii*/ (Ariidae) of equatorial West Atlantic mangrove creeks. Journal of Experimental Marine Biology and Ecology, 367(2): 91-99

Brenner M, Krumme U (2007). Tidal migration and patterns in feeding of the four-eyed fish *Anableps anableps* L. in a north Brazilian mangrove. Journal of Fish Biology, 70(2): 406-427

Brenner M, Buck BH, Cordes S, Dietrich L, Jacob U, Mintenbeck K, Schröder A, Brey T, Knust R, Arntz W (2001). The role of iceberg scours in niche separation within the Antarctic fish genus Trematomus. Polar Biology, 24: 502-507

List of abbreviations

ANOVA	Analysis of Variance
AQU	Aquamats (Collector)
ASP	Amnesic Shellfish Poison
ASW	Artificial Seaweed (Collector)
ATP	Adenosine Triphosphate
AZP	Azaspirazid Shellfish Poison
AWZ	Auschließliche Wirtschaftszone
BC	Before Christ
BEEP	Biological Effects of Environmental Pollution Programme
BMU	Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit – Federal Ministry for the Environment, Nature Conservation and Nuclear Safety
BS	Bordumer Sand
BSH	Bundesamt für Seeschifffahrt und Hydrographie - Federal Maritime Hydrographic Agency
CI	Condition Index
COC	Coconut Rope (Collector)
DDT	Dichlorodiphenyltrichloroethane
DIN	Deutsche Industrie Norm – German Standardisation Norm
DNA	Deoxyribonucleic Acid
DSP	Diarrhetic Shellfish Poison
EC	European Commission
EEZ	Exclusive Economic Zone
EFSA	European Food Safety Authority
EM	Electron Microscopy
EN	European Norm
EU	European Union
FAO	Food and Agriculture Organisation
FK	Forschungskutter – Research Vessel
FV	Forschungsvorhaben – Scientific Project
GAR	Galician Rope (Collector)
HAV	Hepatitis A Virus
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane (Lindane)
Hg	Mercury
HL	Helgoland
HPLC	High Performance Liquid Chromatography
HW	High Water
ICES	International Council for the Exploration of the Sea
ICZM	Integrated Coastal Zone Management
IPR	In Pond Raceways
ISO	International Organisation of Standardisation
JD	Jade estuary
LAD	Ladder (Collector)
LAVES	Landesamt für Lebensmittelsicherheit und Verbraucherschutz – State Office for Consumer Protection and Food Safety
LC-MS/MS	Liquid Chromatography Mass Spectrometry
LE	License Area Eidumstief
LEC	Leaded Christmas Tree Rope (Collector)
LMS	Lysosomal Membrane Stability
LOC	Looped Christmas Tree Rope (Collector)
LW	Low Water
MED POL	Mediterranean Pollution Monitoring Programme

mHW	mean High Water
mLW	mean Low Water
MPN	Most Probable Number
MUDAB	Marine Umweltdatenbank – Marine Environmental Data Base
MytiFit	Fitness of *Mytilus edulis* - Project
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
NFL	*Naue* Felt (Collector)
NH	Neuharlingersiel
NOX	Sum of Nitrate and Nitrite
NPorts	Niedersachsen Ports Authority
NSP	Neurotoxic Shellfish Poison
OOA	Open Ocean Aquaculture
OSPAR	Oslo-Paris Commission
Pb	Lead
PAH	Polycyclic Aromatic Hydrocarbons
PCB	Polychlorinated Biphenyls
PCR	Polymerase Chain Reaction
POC	Particulate Organic Carbon
PON	Particulate Organic Nitrogen
POP	Persistent Organic Pollutant
PSP	Paralytic Shellfish Poison
RAS	Recirculation Aquaculture Systems
REF	Reference (Collector)
RS	Roter Sand – Lighthouse Roter Sand
SEM	Scanning Electron Microscopy
SSC	Self Sinking Collector
SY	Sylt
UK	United Kingdom
VO	Verordnung – Regulation
WSA	Wasser und Schifffahrtsamt – Water and Shipping Authority

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Chapter 01

Summary

The aim of this study was to evaluate offshore areas in the German Bight (North Sea) to determine their suitability for cultivation of blue mussels (*Mytilus edulis*). A relocation of production off the coast offers a new perspective for shellfish farming, as a variety of factors including restrictions on the number of licenses, environmental protection issues and stakeholder conflicts have prevented further expansion in the intertidal and subtidal areas of the coastal sea. The development of the offshore wind farm operators, offers further the opportunity to co-use large marine areas with submerged culture systems for blue mussels. However, offshore production would entail much higher costs than the traditional on-bottom cultivation. Therefore biodiagnostic tools were used to analyse the overall health status of blue mussels grown in different areas of the North Sea. In addition to investigations on the extent of parasite infestation, bacteria, and virus and toxic algae concentrations, this information was used to determine site conditions to aid in the calculation of economic risk for potential mussel farmers. Besides the analysis of health-related parameters, fundamental technical requirements were improved and discussion on supervisional control and appropriate regulatory framework was initiated.

The study was conducted using two designated near- and offshore locations for the cultivation of blue mussels. For two consecutive sampling seasons (2006 and 2007) both testing sites and three wild banks were sampled at monthly intervals, partly with the help of research vessels and scuba divers. The mussel samples were used to assess morphometric parameters, condition indices, infestation rates of micro and macro parasites, microbial loads, viruses and the concentrations of algae toxins. Mussels attached to the substrates were used to determine dislodgement force, growth rates, and size distributions. In addition frozen tissue samples were analysed for the membrane stability of lysosomes from the digestive gland to determine the status of mussel health from these locations.

At both cultivation sites a substantial support programme was conducted to assess the biochemical parameters, nutrient concentrations and oceanographic data to describe and compare the testing areas. These data were complemented by the contaminant analysis of the water column and suspended matters at both sites provided by the Federal Marine Hydrographical Agency (BSH), Hamburg, Germany.

The data and results obtained from this complex experimental approach formed the basis for several independent scientific articles, dealing in detail with:

1) the potentials and constraints of offshore aquaculture in the German Bight and the current status of research concerning biological, technical, social and economic issues,
2) technical details identified as fundamental for the improvement of artificial mussel substrates,
3) the lack of official supervisional responsibilities and an inadequate regulatory frame for a potential offshore production of blue mussels,
4) an evaluation of parameters determining the consumption suitability of blue mussels as a healthy food,
5) the implementation of biodiagnostic tools (lysosomal membrane stability) into aquaculture research for the selection of appropriate cultivation sites, and
6) the effects of prolonged hypoxia on lysosomal membrane stability of mussels from different sites of the German Bight.

Chapter 02

1. General Introduction

1.1. Overfishing and stock depletion

Cultivation methods for freshwater fish species were applied since 2500 BC in China or in other ancient societies, e.g. on Hawaii where people practiced marine nearshore pond aquaculture. However aquatic production has always primarily been dominated by a capture based fishery. Especially the marine fishery was driven by the idea of never ending resources and a sense of sustainability has never been rooted. Hardly any effort was undertaken for centuries to restock the massive exploitation of natural marine resources. Thus leading to the situation today, where more than 50 % of the world’s fish stocks are exploited, overexploited or depleted. According to the FAO (2008) marine wild stocks contributed 80 million tons to the total world fisheries production of about 140 million tons in 2006. Together with inland fisheries and aquaculture products, 15 % of the global animal-based protein supply derives from aquatic products. This beneficial use of the natural environment will be eliminated in the near future due to overexploitation. With exploding populations (projected 6 billion) requiring this source of protein, an accelerated demand caused by improved capture technologies and by geographical expansion of fishing activity and exploitation of previously spurned species, natural stocks are being quickly depleted.

1.2. Aquaculture potentials and constraints

Following the definition of the FAO (1997) aquaculture is the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants with specified interventions in the rearing process to enhance production, by regular stocking, feeding and protection from predators.

Today, aquaculture is the fastest growing animal food-production sector world wide. With an annual growth rate of about 7 % the aquacutural production has increased since the 1950s from less than 1 million tons per year to over 50 million tons in 2006 (FAO 2008). Approximately 90 % of the global production derives from the Asia-Pacific region. This dominance is mainly due to China’s enormous production accounting for nearly 70 % of globally produced quantities (FAO 2008). Although the European Union remains the leader in global mussel production, the total contribution to the world aqauculture production does not exceed 3 %.

Today about 300 different species, ranging from fish, shellfish, crustaceans to algae are produced in aquaculture systems (FAO 2004). Most of these traditional aquaculture enterprises are located in protected in- or nearshore areas (Burbridge et al. 2001).

The development of aquaculture has often been discussed as an alternative to conventional fishing practices of wild stocks, bridging the gap between availability and demand for marine products. However, considerable concern exists due to the large amount of protein needed to sustain carnivorous aquaculture species (Naylor et al. 2000, Roth et al. 2002). Salmon farming for example, requires a large input of fishoil or fishmeal from wild stocks for the production of feed, thus exacerbating the present problems of overexploitation. Further, common open cage systems used in fish farming have severe negative impacts on the environment, since residues of food and feaces increase euthrophycation of the surounding water bodies. In addition, an intermixing of cultured or even genetically modified individuals with wild species due to escapes can not be prevented.

Since carnivorous fish cannot be cultured extensively for economic reasons, sustainable alternative fish cultivation trend to use closed systems for the nearshore application (Fisch & Buck 2006), or towards land-based recirculation systems. These recirculation systems can be used additionally for restocking purposes. In addition to these alternatives, an increase in the cultivation of herbivorous species such as carp or filter feeders such as mussels, seems to be the most promising (Naylor et al. 2000).

1.3. Extensive aquaculture

Following the definition by Eleftheriou (1997), extensive marine aquaculture is characterized by (1) a low degree of control (i.e. environmental control, nutrition, predators, competitors and disease agents), (2) low initial costs, (3) low level technology, (4) low production efficiency as well as man power and (5) a high dependence on local climate and water quality. According to this definition the cultivation of fish is excluded, since fish cultivation requires a high degree of control and nutritional input.

Extensive aquaculture primarily comprises shellfish and algae, which are usually grown in their natural environment. The impact of this cultivation method on the surrounding environment should be low and waste volumes typically do not exceed those produced by natural populations. Additionally, the usage of indigenous species should be considered to prevent intermixing with introduced species. Buck (2002) suggests the use of blue mussels (*Mytilus edulis*), the European flat oyster (*Ostrea edulis*), and macro algae as suitable candidates for extensive cultivation in the German Bight.
In Germany only the blue mussel and the Pacific Oyster (*Crassostrea edulis*) are cultivated in the coastal waters of the German Bight. The latter only by a small enterprise situated on the island of Sylt. Mussel production in Germany has been primily based since the 1950s on the on-bottom cultivation technique. This method depends on the availability of seed mussels harvested from wild beds in the coastal sea. Seed mussels are transferred to licensed culture plots in the Wadden Sea, where favourable environmental conditions guarantee optimal growth. The on-bottom cultivated mussels reach market size of about 50-60 mm within 20 to 24 months (Van Stralen & Dijkema 1994). Due to poor recruitment over the last years, the numbers of spat has decreased dramatically, severely impacting German mussel production (Walter & Liebezeit 2003). Mussel farmers have tried to compensate for this loss with imports of seed mussels from the UK.
As an alternative to the fishing of seed mussels, basic research has been conducted to obtain mussel spat from suspended culture ropes for further use on on-bottom culture plots (Delbare 2001, Kamerman et al. 2002, Walter & Liebezeit 2001, 2003). Today, the Royal Frysk Company situated in Emmelsbüll-Horsbüll, Germany, is producing seed mussels from nearshore hanging cultures for their on-bottom license plots in North Friesland on a commercial scale (de Leeuw A. pers. comm.).
An increase in production is only possible by an intensified seeding practice since a spatial expansion of this food production sector within intertidal and subtidal areas of the German Bight is not allowed due to restrictions on the number of licences, environmental protection regulations and stakeholder conflicts (Buck et al. 2004).

1.4. The offshore alternative

Offshore aquaculture is defined as (1) being in a marine environment fully exposed to all kinds of harsh oceanographic conditions (Ryan 2005), and (2) located at least eight nautical miles off the coast (Buck 2004), avoiding stakeholder conflicts in nearer coastal areas (Dahle et al. 1991).
Along the Germany Bight, most of the protected nearshore areas are comprised of natural reserves, recreational areas and shipping routes. An expansion of marine aquaculture in suitable coastal areas is limited, since many stakeholders with vital interests compete for the same space (Buck 2004). A shifting of the mussel production off the coast would minimise spatial conflicts, but requires different culture techniques, since the water is too deep for on-bottom cultivation in most offshore areas. Technical solutions are arising with the first positive results of off-bottom cultivation experiments showing that these techniques are a potential alternative to the traditional on-bottom cultivation (e.g. Kamerman et al. 2002, Walter & Liebezeit 2003) even under harsh hydrodynamic conditions (Langan & Horton 2003). Further, using suspendend artifical substrates, spat could be obtained even in years of reduced spat fall (Walter & Liebezeit 2001).
The idea of relocating aquaculture systems offshore was provided momentum as a new stakeholder, the offshore wind farm industry entered the scene, offering a unique opportunity to co-use large marine areas (Buck 2002). A sharing of: the solid groundings of windmills to attach the culture systems (Buck 2002, Buck et al. 2006), combined service/ harvest vessels, and man-power for service needs at the wind mills and at the culture systems (Buck et al. 2004) have been proposed. However, compared to the traditional methods of mussel cultivation in Germany the offshore longline approach would be cost intensive. A realisation of this idea is perhaps desirable under the perspective of spatial efficiency and a sustainable expansion of extensive production of seafood, but it is not independent of economic restraints.
Therefore a potential scenario of a virtual offshore mussel farm was calculated and the economics drivers were determined. The two most important parameters making an offshore mussel farm profitable are the number of market sized mussels available per meter longline and the price achievable at the market (Buck & Michler-Cieluch 2009). The first parameter presents a more technical aspect of how to design the artificial substrates that mussels settle on so that they will not be

dislodged by the harsh hydrographical conditions. The second crucial point market price is highly dependent on the quality and optical appearance of the mussels. Only, healthy mussels living under the best conditions, free of growth hampering factors such as parasites, high microbial loads and pollutants with a high meat yield and good shell optics, reach the highest price on the market.

2. The multifactorial approach

Since 2000 when co-use of wind farms for off-bottom offshore cultivation (Buck 2002) in the German Bight was proposed, different studies have been conducted to elucidate the potential as well as constraints of this offshore alternative for mussel cultivation. Two pioneer studies, the project *Roter Sand* and *Offshore Aquaculture* were conducted between 2002 and 2004 by the Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, Germany, in cooperation with the Research Center Terramare, Wilhelmshaven, Germany. These two projects followed a complex approach to obtain data about suitable indigenous candidates for the offshore cultivation (Buck 2004), the technical requirements of longline systems for the cultivation of mussels or oysters (Buck 2007) and algal cultivation systems (Buck & Buchholz 2004). Insights into the feasibility of offshore seed and mussel production concerning larval, nutrient and phytoplankton concentrations (Buck 2007, Walter et al. 2009) were provided and the existing legislation and regulations concerning marine aquaculture in Germany were listed (Buck et al. 2003). In addition all stakeholders potentially involved in a multifunctional use of offshore wind farms for aquaculture were identified (Buck et al. 2004). This successful multi-pronged approach helped to disperse many concerns and doubts on the offshore idea.

Following this scheme, the outline of a project focussing on the offshore cultivation of blue mussels was developed. The project concentrated on the lack of data regarding consumption suitability of offshore-produced mussels and the necessary adaptations of the monitoring and regulation of responsible control authorities. Further, technical details about the microstructure of artificial substrates were addressed to increase production per meter longline under offshore conditions. For the first time biodiagnostic tools formerly deployed only in biomedicine and environmental monitoring were used to analyse the quality of the sites chosen for mussel cultivation.

2.1. Technical requirements for artifical substrates

Relocating cultivation systems offshore into high energy environments requires the development of suitable culture techniques able to withstand the harsh conditions and minimize risk of economic loss. Studies by Buck (2004; 2007) on offshore mussel cultivation have focussed on the longline, the “backbone” of the device. Buck suggested segmented pp-ropes suspended 3 to 5 meters in the water column to avoid breakage of longline or dislodgment of mussels due to forces generated from waves. Beside suitable mooring devices, adequate buoyancies and resistant backbone ropes, suitable collector materials being attractive for mussel larvae as artificial substrates must be available for future offshore farmers. Substrates must attract larvae, and also provide sufficient foothold to withstand storm events during wintertime if cultivation until market size is intended. Commercially used substrates have proven their suitability only in sheltered nearshore environments and should therefore be tested under offshore conditions to develop appropriate cultivation equipment.

2.2. Optimal growth – optimal health?

In nearshore intertidal areas, mussels are particularly exposed to high concentrations of pollutants, pesticides, near surface agents and estuarine runoffs etc, which can pose a threat to consumer health. Buck (2004; 2007) reported high growth rates for mussels cultivated in the German Bight. The scope of growth, i.e. the energy available for growth, is usually directly and positively correlated to a good overall health condition of the respective organism (Allen & Moore 2004). But organisms with high growth rates and a healthy appearance are no guarantee of a healthy food for human consumers. In waters, eutrophicated by urban sewage, mussels show good growth performance. The microbial status of these mussels, however, excludes them most likely from consumption, since they might carry various human pathogens. Even in developed countries with strict legislation for the treatment of wastewater, mussels can function as carriers of serious infections. Whether this is also true for offshore cultivated mussels, where the environment is cleaner due to dilution of contaminants, remain open. Data for offshore produced mussel, generated according to the analysis protocols of controlling authorities were not available for the German Bight. Therefore the State Office for Consumer Protection and Food Safety of Lower Saxony, Cuxhaven, Germany (LAVES - Institute for Fish and Fishery Products) was involved in the planning of the project from the beginning to fulfill all prerequisites for an official sampling design and assessment.

2.3. Adaptation of a regulational framework for the offshore cultivation

In most European countries monitoring systems have been established to control mussel products before they are sold on the market. Since all present aquaculture installations are situated only in nearshore areas, regulations are adjusted respectively (CEFAS 2007). Questions remained whether the existing monitoring guidelines and hygiene control regulations match the situation offshore and if parameters of analysis deployed today fit an appropriate description of consumption suitability of offshore produced mussels. In detail, doubts remained if the focus of coliform bacteria will be the crucial point for mussels originating from areas distant from anthropogenic feacal sources. Other possible hazards such as algal toxins could be underestimated. Algal blooms producing such toxins are present at many sites in the Channel, the British Isles and also in Denmark (Smaal 2002, Tillmann & Rick 2003) but appear rarely in the German Wadden Sea where the traditional on-bottom cultivation of mussels take place (Elbträchter 1996).

2.4. Parasites - a question of distance to the shore

All known micro and macro parasites of the European coastal waters are harmless to consumers, but may have negative condition effects (macro parasites) and cause higher mortalities (micro parasites) in infested hosts. Beside the potential harmful effect on a host, some macro parasites pose an aesthetic problem, since they are visible due to their bright colour (*Mytilicola intestinalis*) in raw mussels or due to their size (*Pinnotheres pisum*). Parasites living in blue mussels are numerous in intertidal and nearshore areas. Buck et al. (2005) have shown that offshore grown mussels were free of macro parasites. Infestation rates increased the closer the sites were to shore, where in particular intertidal mussels showed the highest numbers of parasites. The debate over the effects of parasites on the energy status and overall health of the host is still open, data needed to elucidate these issues are still lacking. However, with the multi-pronged approach applied in this study, the presence of parasites can be correlated to other fitness parameters, and thereby the influence of parasites on their host may be evaluated in detail.

2.5. Evaluation of health parameters - a tool for site selection?

The deployment of modified culture systems, which can withstand the high energy environment of the North Sea, will certainly mean higher investment costs. Therefore, site selection criteria should be clearly identified so that economic risks can be calculated. In this approach biodiagnostic tools (for further details see below) will be deployed for the first time to analyse the overall health status of mussels cultured in different areas. Together with data on levels of parasite infestation, bacteria, as well as virus and toxic algae concentrations, this information can be used to determine the suitability of site conditions and help calculate economic risks for potential mussel farmers.

3. First trials to apply biodiagnostic tools in aquaculture

3.1. Marine pollution and environmental monitoring

In 2000 the OSPAR Commission defined marine pollution as "the introduction by humans, directly or indirectly, of substances or energy into the maritime area which results, or is likely to result, in hazards to human health, harm to living resources and marine ecosystems, damage to amenities or interference with other legitimate uses of the sea". Marine pollution results from sources such as municipal, industrial and agricultural wastes and run-off, sewage effluents, exploitation of oil, gas and solid minerals, tourism and recreational activities and aquaculture, or from accidental events such as oil spills (GESAMP 2001).

As a response to the increase of contaminants from anthropogenic activities in the marine environment in recent years (EEA 1999), the European Commission proposed an European Parliament and Council Directive, the so called Water Framework Directive (Directive 2000/60/EC). The aim of this directive is to improve, protect and prevent further impairment of water quality across Europe. To achieve and ensure a "good quality" status of all water bodies, water quality monitoring programs have to be implemented. However, a problem of this monitoring approach is the analysis of hazardous health effects of complex mixtures of chemicals in a variable environment. The presence of toxic compounds in the environment can be detected by chemical analysis of water and sediment samples. However this approach provides only minimal information on the effects of these toxic chemicals on biological systems. Analysing the chemistry of water or sediments does not provide information on concentrations of pollutants in organisms and their tissues. Chemical analysis of biota will provide only limited indications of health effects, since interactions or combination effects of pollutants are not covered with this approach. Therefore chemical analyses alone are inapplicable as cost-effective tools to detect e.g. "hot spots" of pollution (UNEP/ STAP 2003). As a result the health of so-called sentinel organisms were assessed to describe the quality of a certain environment the investigated animals lived in.

3.2. Mussels in environmental research

Bivalves are the most commonly used sentinel organisms for the health assessment of the marine environment. The special properties of a sentinel species are that it is able to survive in a polluted habitat, and accumulates chemicals in its tissues. Due to their ability to accumulate and reflect a wide range of contaminants, mussels have been widely used in marine pollution monitoring (Goldberg 1975, Cajaraville et al. 1990, Livingstone et al. 1990, Smolders et al. 2003, Marigómez et al. 2006, ICES 2006). The blue mussel *M. edulis* occurs in all temperate marine waters of the North Pacific and the North Atlantic Ocean. In littoral to sublittoral zones down to 100 m depth the mussel is common and can be easily collected, transported and maintained in the laboratory or used for caging experiments in the field. The blue mussel predominantly inhabits shores and estuarine environments. These habitats are very complex, varying in temperature, salinity, duration of exposure to air and food supply due to tides. To cope with these factors, the blue mussel has developed a series of behavioural, physiological and metabolic adaptations. Joergensen (1990) has described the mussel as an autonomous unit, incapable of regulation of its metabolism, meaning that physiological processes of the mussel respond directly to environmental changes.

The blue mussel is an active suspension feeder, filtering mainly phytoplankton from the water column. Due to this filtering mechanism, mussels ingest, besides phytoplankton, suspended particular material, bacteria, algae toxins and all kinds of pollutants and particles from their marine environment. As sessile organisms, they directly reflect the contaminant conditions of their habitat. As a result molluscs and especially blue mussels are the bioindicator of choice in several national and international biomonitoring programs e.g. MED POL (UNEP Mediterranean Biomonitoring Programme) or BEEP (EU Biological Effects of Environmental Pollution Programme).

3.3. Established biomarkers

Biomarkers can be deployed to assess the impacts of stress at the molecular and cellular levels, thus providing the earliest warning signals of toxic chemicals on tissues and organisms (Shugart et al. 1990; 1992). On the organism level, biomarkers can be used to indicate the potential survival capacity and the reproductive performance of the investigated animals. The latter is essential when relating the measured effects of individuals to possible changes for the population. According to their level of sensitivity biomarkers are classified into three main groups: biomarkers of exposure, biomarkers of genotoxicity and biomarkers of stress (Viarengo et al. 2007).

Biomarkers of exposure are parameters whose changes can be related to the organism’s exposure to a specific class of pollutants (Viarengo et al. 2007). Widely applied biomarkers of exposure in environmental biomonitoring programs are e.g. metallothioneins, proteins with high affinity to metals over-expressed in organisms confronted with high metal concentrations. Other biomarkers of exposure are activities of enzymes inhibited by the presents of certain pesticides (Cholinesterase) or enhanced in organisms exposed to hydrocarbons (Oxygenase) (Viarengo et al. 2007). Biomarkers of genotoxicity can alter the integrity of the DNA structure, either directly or through their metabolites (Shugart 1995). Genotoxic compounds are, for example, persistent organic pollutants (POPs), present in low concentrations in the marine environment, but with the ability to accumulate in tissues where they can cause mutagenesis (Siu et al. 2004). The genotoxic effects, induced by different classes of pollutants, are detectable using special testing methods, allowing an accurate assessment of DNA fragmentation caused by genotoxic compounds even at low levels (Viarengo et al. 2007).

In this thesis only biomarkers of stress were applied, which will be described in more details below. Stress sensitive biomarkers can be used to assess the health of an ecosystem as a whole in which the organisms live in (Cajaraville et al. 1998) or for the analysis of individual organisms that live in a specific environment or at specific contaminated sites. Well established examples of biomarkers of stress are the tests for lysosome membrane stability, the lysosomal lipofuscin content and the neutral lipid accumulation in lysosomes.

3.4. Lysosome membrane stability

Lysosomes are cell organelles containing various hydrolytic enzymes necessary for different metabolic processes surrounded by a semi permeable membrane (e.g. Moore 1976, Ferreira & Dolder 2003). They are responsible for the recycling of used-up cell organelles, macro molecules and metabolic waste products, and isolate harmful substances, once they have entered the cells. Lysosomes in molluscan digestive cells accumulate metals, organic contaminants as well as nanoparticles that cannot be degraded. These substances may provoke significant alterations in the lysosomes (Moore et al. 1980a, Moore et al. 1980b, Nott et al. 1985, Viarengo et al. 1985, Sarasquete et al. 1992, Cajaraville et al. 1995, Moore et al. 2004, Koehler et al. 2008). In general, contaminants from the environment cause a significant increase in size and number of lysosomes (Marigómez et al. 1989, Regoli et al. 1998, Koehler et al 2002). When that the storage capacities of lysosomes are overloaded and cells are stressed by high concentrations of harmful substances, the lysosomal membrane becomes instable and leaky. Pollutants and hydrolytic-lysosomal enzymes can re-enter the cytoplasm with serious risk of cell death (Koehler et al. 2002). When membrane stability and the over-all health status of mussels are low, more specific tests may elucidate the type and background of the infection or pollutant. Vice versa, if membranes of the lysosomes are stable there is strong evidence that the individual mussel grew under optimal water conditions (Widdows et al. 2002, Moore et al. 2004). Impairment of lysosomal functions and, hence, of food assimilation, can result in severe alterations in the nutritional status of cells and the whole organism, and could be indicative of disturbed health. For that reason, lysosomal changes and especially lysosomal membrane destabilisation are widely accepted as general stress biomarkers (Moore et al. 2004).

3.4. Biomarkers in biomonitoring

To ensure that biomarkers are a useful tool for biomonitoring the individual power of the different markers and methods must be calculated during the planning of the programme to assure time and cost efficiency. Biomarkers, sensitive at the cellular level, able to integrate a variety of different environmental stressors; can provide clear signals of an overall stress level within a few weeks of exposure. Therefore Viarengo et al. (2007) suggested a two-tier approach beginning with an initial screening using low-coast biomarkers, like lysosomal membrane stability, with a more general sensitivity for overall health and fitness performance of the animal (Livingstone et al. 2000). Highly polluted environments are characterised by a significant increase in mortality (Viarengeo et al. 2007) accompanied with low lysosomal stability of surviving mussels.

In a second step, sites with significant alterations in lysosomal performance should be investigated using the full battery of biomarkers possible to elucidate the type and background of the potential contamination. Following this approach biomarkers are suitable tools even for large scale biomonitoring programs with hundreds of sites and thousands of individual samples.

3.5. Biodiagnostic tools in marine aquaculture

This study is the first trial to implement biodiagnostic tools for site selection and health monitoring in marine extensive aquaculture of mussels. For the sampling and assessment of the sites selected for this thesis we followed the suggested two-tier approach of Viarengo et al. (2007). Since the use of the full battery of available biomarkers for all sites over the whole sampling time would be too costly and require too much man power an initial screening was applied using the lysosomal membrane stability. Samples for lysosomal liposfuscin and neutral lipid content were taken, but not evaluated for the purpose of this thesis.

As a priority a synchronic sampling throughout one annual cycle was conducted, where parameters of interest were investigated using the same individual (lysosomal membrane stability, lipofuscin, neutral lipids) or at least mussels from the same cohort (macro and micro parasites, microbial, viral, biotoxical contaminations and growth rates) for comparison and correlation.

The initial preparation of samples (shock frosted in liquid nitrogen and stored at minus 80 °C) will allow, in a next step, additional investigation (not part of the thesis) e.g. on enzyme or NADPH-activities and the deployment of specific antibodies for certain pollutants if alterations of lysosomal stability are detected at different sites.

References

Allen JI, Moore MN (2004). Environmental prognostics: is the current use of biomarkers appropriate for environmental risk evaluation. Marine Environmental Research, 58: 227-232

Buck BH (2002). Open Ocean Aquaculture und Offshore-Windparks: Eine Machbarkeitsstudie über die multifunktionale Nutzung von Offshore-Windparks und Offshore-Marikultur im Raum Nordsee. Reports on Polar and marine research. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, 412: 252 pp

Buck BH (2004). Farming in a high energy environment: potentials and constraints of sustainable offshore aquaculture in the German Bight (North Sea). Dissertation. University of Bremen, Bremen, Germany, 258 pp

Buck BH (2007). Experimental trials on the feasibility of offshore seed production of the mussels *Mytilus edulis* in the German Bight: installation, technical requirements and environmental conditions. Helgoland Marine Research, 61: 87–101

Buck BH, Krause G, Rosenthal H, Smetacek V (2003). Aquaculture and environmental regulations: The German situation within the North Sea. In: Kirchner A (ed) International Marine Environmental Law: Institutions, Implementation and Innovation. International Marine Environmental Law and Policies Series of Kluwer Law International, The Hague, 64: 211-229

Buck BH, Buchholz CM (2004). The Offshore-Ring: A new system design for the open ocean aquaculture of macroalgae. Journal of Applied Phycology, 16: 355-368

Buck BH, Krause G, Rosenthal H (2004). Multifunctional use, environmental regulations and the prospect of offshore co-management: potential for and constraints to extensive open ocean aquaculture development within wind farms in Germany. Ocean Coastal Management, 47: 95–122

Buck BH, Thieltges DW, Walter U, Nehls G, Rosenthal H (2005). Inshore-offshore comparison of parasite infestation in *Mytilus edulis*: implications for open ocean aquaculture. Journal of Applied Ichthyology, 21(2): 107-113

Buck BH, Berg-Pollack A, Assheuer J, Zielinski O, Kassen D (2006). Technical realization of extensive aquaculture constructions in offshore wind farms: consideration of the mechanical loads. In: Proceedings of the 25th international conference on offshore mechanics and Arctic engineering, OMAE 2006: presented at the 25th International conference on offshore mechanics and Arctic engineering, 4.–9. June 2006, Hamburg, Germany/ American Society of Mechanical Engineers, 1–7

Buck BH, Koehler A, Brenner M, Stede M (2007). Eignung des Seegebietes am geplanten Offshore-Windpark Nordergründe für die Zucht von Miesmuscheln: Fitness, Parasitisierung und Substratwahl. Endbericht, 91 pp

Buck BH, Michler-Cieluch T (2009). Mussel cultivation as a co-use in offshore wind farms: potentials and economic feasibility. Aquaculture Economics and Management (submitted)

Burbridge P, Hendrick V, Roth E, Rosenthal H (2001). Social and economic policy issues relevant to marine aquaculture. Journal of Applied Ichthyology, 17: 194–206

Cajaraville MP, Díez G, Marigómez JA, Angulo E (1990). Responses of basophilic cells of the digestive gland of mussels to petroleum hydrocarbon exposure. Diseases of Aquatic Organisms, 9: 221-228

Cajaraville MP, Robledo Y, Etxeberria M, Marigómez I (1995). Cellular biomarkers as useful tools in the biological monitoring of environmental pollution: molluscan digestive lysosomes. In: Cajaraville MP (Ed). Cell biology in environmental toxicology. Bilbo, University of the Basque Country Press Service, 29-55

Cajaraville MP, Cancio I, Orbea A, Lekube X, Marigómez I (1998). Detection, control and monitoring of pollution using early warning cellular biomarkers: conventional and innovative approaches based on biotechnology. Cuad. Invest. Biol., 20: 545-548

CEFAS (2007). Microbiological monitoring of bivalve mollusc harvesting areas – Guide to good practice: Technical application. European Community Reference Laboratory for monitoring bacteriological and viral contamination of bivalve mollusc; the centre of environment, fisheries and aquaculture science (CEFAS), 3: 67 pp

Dahle LA, DePauw N, Joyce J (1991). Offshore aquaculture technology-possibilities and limitations. Aquatic Environment, 14: 83–84

Delbare D (2001). Pesca-project: Hangmosselcultuur in Belgische Kustwateren. Project Report

Directive 2000/60/EC of the European Parliament and of the Council of 23. October 2000 establishing a framework for Community action in the field of water policy

EEA (1999). European Environmental Agency. State and pressures of the marine and coastal Mediterranean environment. In: Izzo G, Moret S (Eds). Environmental issues series 5. Copenhagen, Denmark, 117 pp

Elbrächter M (1996). Toxische Algen in der Nordsee. Deutsche Hydrographische Zeitschrift, Suppl. 6: 37-44

Eleftheriou M (1997). Aqualex: a glossary of aquaculture terms. Wiley-Praxis series in aquaculture and fisheries, Aqualex Multimedia Consortium Ltd

FAO (1997). Aquaculture development. Technical guidelines for responsible fisheries. Food and Agriculture Organisation of the United Nations, Rome, 5–40

FAO (2004). The state of world fisheries and aquaculture 2004 (SOFIA). Fisheries Department. Food and Agriculture Organisation of the United Nations, Rome, Italy

FAO (2006). Fishery information, data and statistics unit. Aquaculture production: values 1984–2004. FISHSTAT Plus—universal software for fishery statistical time series. Food and Agriculture Organization of the United Nations, Rome, Italy

FAO (2008). State of World Fisheries and Aquaculture - Food and Agriculture Organization of the United Nations, Rome, Italy, 176 pp

Ferreira A, Dolder H (2003). Cytochemical study of spermiogenesis and mature spermatozoa in the lizard *Tropidurus itambere* (Reptilia, Squamata). Acta Histochemica, 105: 339-352

Fisch R, Buck BH (2006). Neues Aquakultursystem für das Meer made in Germany. Fischerblatt, 12: 13-16

GESAMP (IMO/ FAO/ UNESC OIOC/ WMO/ WHO/ IAEA/ UN/ UNEP) (2001). Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection and Advisory Committee on Protection of the Sea. A Sea of troubles. Rep. Stud. GESAMP, 70: 33 pp

Goldberg ED (1975). The Mussel Watch - a first step in global marine monitoring. Marine Pollution Bulletin, 6: 111

ICES (2006). Report of the Working Group on Biological Effects of Contaminants (WGBEC), 27. - 31. March 2006, Copenhagen, Denmark. ICES CM 2006/MHC: 04: 79 pp

Joergensen CB (1990). Bivalve filter feeding: Hydrodynamics, bioenergetics, physiology and ecology. Olsen & Olsen, Fredensborg, Denmark

Kamermans P, Brummelhuis E , Smaal A (2002). Use of spat collectors to enhance supply of seed for bottom culture of blue mussels (*Mytilus edulis*) in the Netherlands. World Aquaculture, 33(3): 12-15

Koehler A, Wahl E, Soeffker K (2002). Functional and morphological changes of lysosomes as prognostic biomarkers of toxic liver injury in a marine flatfish (*Platichthys flesus* L.). Environmental Toxicology and Chemistry, 21(11): 2434–2444

Koehler A, Marx U, Broeg K, Bahns S, Bressling J (2008). Effects of Nanoparticles in *Mytilus edulis* Gills and Hepatopancreas - a New Threat to Marine Life? Marine Environmental Research, 66: 12–14

Langan R, Horton F (2003). Design, operation and economics of submerged longline mussel culture in the open ocean. Bulletin of the Aquaculture Association of Canada, 103: 11–20

Livingstone DR, García-Martínez P, Michel X, Narbonne JF, O'Hara S, Ribera D, Winston GW (1990). Oxyradical production as a pollutionmediated mechanism of toxicity in the common mussel, *Mytilus edulis* L., and other molluscs. Functional Ecolology, 4: 415-424

Marigómez JA, Vega MM, Carajaville MP, Angulo E (1989). Quantitative response of the digestive-lysosomal system of winkles to sublethal concentrations of cadmium. Cellular and Molecular Biology, 35: 555-562

Marigómez I, Soto M, Cancio I, Orbea A, Garmendia L, Cajaraville MP (2006). Cell and tissue biomarkers in mussel, and histopathology in hake and anchovy from Bay of Biscay after the Prestige oil spill (Monitoring Campaign 2003). Marine Pollution Bulletin, 53: 287-304

Moore MN (1976). Cytochemical demonstration of latency of lysosomal hydrolases in the digestive cells of the common mussel, *Mytilus edulis*, and changes induced by thermal stress. Cell and Tissue Research, 175: 279-287

Moore MN, Bubel A, Lowe DM (1980a). Cytology and cytochemistry of the pericardial gland cells of *Mytilus edulis* and their lysosomal response to injected horseradish peroxidise and anthracene. Journal of the Marine Biological Association of the United Kingdom, 60: 135-149

Moore MN, Koehn RK, Bayne BL (1980b). Leucine aminopeptidase (aminopeptidase-I), N-acetyl-hexosaminidase and lysosomes in the mussel, *Mytilus edulis* L., in response to salinity changes. Journal of Experimental Zoology, 214: 239-249

Moore MN, Lowe DM, Koehler A (2004). Biological effects of contaminants: Measurements of lysosomal membrane stability. ICES Techniques in Marine Environmental Sciences (TIMES), vol. 36. ICES, Copenhagen

Naylor RL, Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000). Effect of aquaculture on world fish supplies. Nature, 405: 1017–1024

Nott JA, Moore MN, Mavin LJ, Ryan KP (1985). The fine structure of lysosomal membranes and endoplasmatic reticulum in the digestive cells of *Mytilus edulis* exposed to anthracene and phenanthrene. Marine Environmental Research, 34: 226-229

Regoli F, Nigro M, Orlando E (1998). Lysosomal and antioxidant response to metals in the Antarctic scallop *Adamussium colbecki*. Aquatic Toxicology, 40: 375-392

Roth E, Ackefors H, Asche F, Balnath C, Black E, Black K, Boghen A, Browdy C, Burbridge P, Castell JD, Chamberlain G, Dabrowski K, Davies I, Dosdat A, Eleftheriou A, Ervik A, Gordin H, Heinig CS, Hilge V, Karakassis I, Kuhlmann H, Landry T, von Lukowicz M, McGlade J, Price A, Rhenault RB, Rosenthal H, Saint-Paul U, Sandifer PA, Saroglia M, Silvert W, Steffens W, Soto D, Varadi L, Verreth J, Verdegem M, Waller U (2002). An intellectual injustice to aquaculture development: a response to the review article on “Effect of aquaculture on world fish supplies”. Report of the ICES working group on environmental interactions of mariculture, F:04 REF ACME, Annex 4: 83–89

Ryan J (2005). Offshore aquaculture - Do we need it, and why is it taking so long? International Salmon Farmers Association (Ireland). Expert workshop on sustainable aquaculture, DG JRC European Commission, Institute for Prospective Technological Studies, 17.–18. January 2005, Seville, Spain

Saraquete MC, Gonzales de Canales ML, Gimeno S (1992). Comparative histopathological alterations in the digestive gland of marine bivalves exposed to Cu and Cd. European Journal of Histochemistry, 36: 223-232

Shugart LR, McCarthy JF, D’Surney SJ, Greeley MS, Hull CG (1990). Molecular and cellular markers of toxicity in the Japanease medaka (*Oryzias latipes*). Report No. CONF-9008165-1

Shugart LR, McCarthy JF, Halbrook RS (1992). Biological markers of environmental and ecological contamination: An overview. Risk Analysis, 12: 353-360

Shugart LR (1995) Environmental genotoxicology. In: Rand GM (Ed). Fundamentals of Aquatic Toxicology: Effects, Environmental Fate, and Risk Assessment. Taylor & Francis, Bristol, PA 405-420

Siu WHL, Cao J, Jack RW, Wu RSS, Richardson BJ, Xu L, Lam PKS (2004) Application of the comet and micronucleus assays to the detection of B[a]P genotoxicity in haemocytes of the green-lipped mussel (*Perna viridis*). Aquatic Toxicology, 66: 381-392

Smaal AC (2002). European mussel cultivation along the Atlantic coast: production status, problems and perspectives. Hydrobiologia, 484: 89–98

Smolders R, Bervoets L, Wepener V, Blust R (2003). A conceptual framework for using mussels as biomonitors in whole effluent toxicity. Human and Ecology Risk Assessment, 9: 741-760

Tillmann U, Rick H-J (2003). North Sea phytoplankton: a review. Senckenbergiana maritime, 33: 1-69

UNEP/ STAP (2003). Report of the STAP/ GEF Workshop on the use of Bioindicators, Biomarkers and Analytical Methods for the Analysis of POPs in Developing Countries. Tsukuba, Japan 10.-12. December 2003, 34 pp van Stralen MR, Dijkema RD (1994). Mussel culture in a changing environment: the effects of a coastal engineering project on mussel culture (*Mytilus edulis* L.) in the Oosterschelde estuary (SW Netherlands). Hydrobiologia, 282/283: 359-379

Viarengo A, Moore MN, Mancinelli G, Mazzucotelli A, Pipe RK (1985). Significance of metallothioneins and lysosomes in cadmium toxicity and homeostasis in the digestive gland cells of mussels exposed to the metal in presence or absence of phenanthrene. Marine Environmental Research, 17: 184-187

Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007). The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comparative Biochemistry Physiology, 146C: 281-300

Walter U, Liebezeit G (2001). Nachhaltige Miesmuschel-Anzucht im niedersächsischen Wattenmeer durch die Besiedlung natürlicher und künstlicher Substrate. Abschlußbericht des ersten Projektjahres, 98 pp

Walter U, Liebezeit G (2003). Efficiency of blue mussel (*Mytilus edulis*) spat collectors in highly dynamic tidal environments of the Lower Saxonian coast (southern North Sea). Biomolecular Engineering, 20: 407-411

Walter U, Buck BH, Liebezeit G (2009). Larval occurrence and settlement in the German Bight – a trial to estimate potentials for *Mytilus edulis* culture in offshore areas. Aquaculture International (submitted)

Widdows J, Donkin P, Staff FJ, Matthiessen P, Law RJ, Allen YT, Thain JE, Allchin CR, Jones BR (2002). Measurement of stress effects (scope of growth) and contaminant levels in mussels (*Mytilus edulis*) collected from the Irish Sea. Marine Environmental Research, 53: 327-356

Chapter 03

Scope of the thesis

A project proposal, creating a framework, in which remaining questions about technical details, health and monitoring aspects could be answered, was written in the summer of the year 2005. The premises of the project idea were to produce the most optimal quality of shellfish for human consumption with indigenous species using extensive cultivation methods. By deploying biodiagnostic tools the individual health of cultivated mussels in a given environment was evaluated. At the same time the cultivated organism is used in reverse for the selection of proper sites. All results of the project and of this thesis should be directly applicable for potential farmers.
The proposal was granted in late summer 2005 and was begun in November of the same year (Buck et al. 2007). Some of data obtained from this project, called *MytiFit* (Fitness of *Mytilus edulis*), was used in this thesis and form the basis of **Chapters 04** through **10**. A detailed review of the status of marine aquaculture along the German North Sea coast, focussing on all completed and ongoing projects conducted in the last year is given in **Chapter 04**.

The aim of the thesis was to answer the following questions:

Technical details:

- Is commercially deployed nearshore spat collecting equipment suitable for offshore conditions?
- How should a collector for exposed conditions look?
- What are the requirements of spat collectors used for production of seed versus market sized mussels?

Consumption suitability:

- Will the quality of offshore produced mussels re: microbes, viruses and algal toxins fulfill legal requirements?
- Does the aesthetic appearance of the mussel, for example extent of parasites, shell commensals etc depend on location of site or particular farming method used?

Legal issues:

- Are existing regulations and the official monitoring programme adequate for the offshore production of mussels?
- Which public body is responsible for mussels produced in the Exclusive Economic Zone (EEZ)?
- Are the obligatory analysis parameters for nearshore on-bottom cultivation areas suitable for offshore production sites?

Assessment of health for potentially farmed shellfish:

- Are methods developed for environmental monitoring suitable for the selection of culture sites?
- Is an optimal growth performance equal to an optimal health of the organism?
- Can the deployed methods be modified for suitable daily use in aquaculture?

The conceptual framework of the project addressing the questions listed above was introduced to the broader public during the Annual Meeting of the World Aquaculture Society (Brenner et al. 2006) and with a publication in a journal focusing on aquaculture related themes (**Chapter 05**).

There are mainly two possibilities for offshore mussel cultivation: (i) the production of seed mussel for an intensified on-bottom grow out and (ii) farmers could profit from high growth rates (Buck 2007, Brenner unpublished data) and bring mussels to market size within the second season. The first possibility would help to reduce the pressure on wild mussel populations, since removal of wild seed mussels would not be necessary. Further, offshore seed mussel production would provide the opportunity to intensify the production on on-bottom cultivation areas. However, if mussels are transferred to nearshore on-bottom areas, they lose their predicted microbial purity. On the other hand the economic risk of the seed mussel production is low, since they are harvested in autumn of the first year and loss of both -mussels and culture systems- during winter storm events is likely to be low.

Suitable artificial offshore substrates are necessary for both approaches which collect enough larvae from the water column in spring and offer optimal foothold for mussels during all growing stages until harvest. Preconditions for offshore deployed substrates and data on the performance of previously applied substrate from nearshore cultivation areas as well as self constructed substrates are discussed in **Chapter 06**.

In Germany, all products on the market sold as edible must undergo a complex monitoring procedure controlled by the state authorities responsible for edible hygiene and consumer safety (EC 853 and 854/2004). A well established monitoring system with defined responsibilities for traditional on-bottom cultivation areas exists (Sassen et al. 2005). Most of the offshore production areas will, however, be established beyond the 12 nautical miles zone and therefore are not within the scope of responsibility for the counties which are organising the sampling and shipment to the accredited analysis laboratories. These issues need to be clarified and organisational structures must be established. **Chapter 07** provides an overview of the current situation and the changes which need to be made when relocating the mussel production offshore.

Besides the more technical issues such as responsibilities and shipment procedures there exists detailed regulations set by the EU, as well as Germany and its states, on the limits of microbiological contamination and how to maintain and monitor the water quality in areas of mussel cultivation. Our data and a critical review of existing literature show (see **Chapter 08**), however, that the scope and intentions of the EU-standards (CEFAS 2007) need fundamental change since (i) focussing on geo- and hydrographic influences are adapted to nearshore requirements and should be completed for offshore situations and (ii) insisting on limits only for *E. coli* and Salmonella as sole criteria for marketability of mussels does not consider different production areas and their peculiarities (e.g. Formica-Cruz et al. 2002, Romalde et al. 2002, Croci et al. 2002). Hygiene control should further consider regional consumer taste, for example preference for raw or cooked shellfish (oysters/ mussels.) For raw products analysis for the presence of viruses, human pathogenic germs and algal toxins should be added to the monitoring routine, whereas in mussels, especially offshore cultivated ones, the monitoring of algal toxin should be required (see **Chapter 07** and **08**).

Optimal growth rates and an aesthetic appearance of mussel products are the focus of a mussel farmer, but hygienic production conditions are far from guaranteed as mussels are able to survive in highly euthrophicated waters, where microbes and viruses are commonly present in high concentrations. Further, punctual sources of pollutants, so-called hot-spots may also occur in offshore areas. It is known that in the aftermath of World War II many tons of munitions and weapons were dumped in the North Sea. A continuous mapping has not been implemented and many sites have slipped from memory over the decades. Today, the gradual release of pollutants may result in a contamination of edible products cultured in the vicinity of these sites. Other hazards may originate from offshore platforms for oil and gas, shipping routes or pipelines. All these potentially negative influences must be assessed and evaluated to achieve the most optimal quality of shellfish.

In a new approach biodiagnostic tools were deployed to analyse the overall health status of mussels cultured in different off- and nearshore areas of the North Sea (see **Chapter 09**). With these methods the tissues of the mussel’s digestive system responsible for food uptake, storage of reserve substances and detoxification can be investigated providing a clear signal on the health status of the mussel. The combined analysis which encompasses macro and micro parasites, the extent of bacterial and viral infections, and accumulation of algal toxins, will allow better determination of how to achieve the most rapid growth and best product quality for human consumption (see **Chapters 08** and **09**).

To obtain further insights into the methodology of deployed biodignostic tools a specific experiment was conducted to elucidate influence of hypoxia on the lysosomal membrane stability, as a central tool of the biodiagnostic approach, in intertidal and hanging cultivated mussels. The results are presented in **Chapter 10**.

A discussion of the methodical approach of this thesis, the results and the impact for further investigations is presented in **Chapter 11**. The overall summary is found in **Chapter 01**.

References

Brenner M, Buck BH, Koehler A (2006). Blue mussel culture: Does offshore cultivation lead to increasing product quality? Linking Tradition and Technology Highest Quality for the Consumer. The Annual Meeting of the World Aquaculture Society and the European Aquaculture Society, 9.-13. May 2006, Florence, Italy 116

Buck BH (2007). Experimental trials on the feasibility of offshore seed production of the mussels *Mytilus edulis* in the German Bight: Installation, technical requirements and environmental conditions. Helgoland Marine Research, 61: 87-101

Buck BH, Koehler A, Brenner M, Stede M, Engel M (2007). Projekt *MytiFit*: Eignung des Seegebietes am geplanten Offshore-Windpark *Nordergründe* für die Zucht von Miesmuscheln: Fitness, Parsitisierung und Substratwahl. Endbericht, 91 pp

CEFAS (2007). Microbiological monitoring of bivalve mollusc harvesting areas – Guide to good practice: Technical application. European Community Reference Laboratory for monitoring bacteriological and viral contamination of bivalve mollusc. The centre of environment, fisheries and aquaculture science, 3: 67 pp

Croci L, De Medici D, Ciccozzi M, Di Pasquale S, Suffredini E, Toti L (2003). Contamination of mussels by hepatitis A virus: a public-health problem in southern Italy. Food control, 14: 559-563

EC 853/2004. Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin, 61 pp

EC 854/2004. Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption, 139 pp

Formica-Cruz M, Tofino-Quesada G, Bofill-Mas S, Lees DN, Henshilwood K, Allard AK, Conden-Hansson A–C, Hernroth BE, Vantarakis A, Tsibouxi A, Papapetropoulou M, Furones MD, Girones R (2002). Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden and the United Kingdom. Applied and Environmental Microbiology, 68: 5990-5998

Romalde JL, Area E, Sánchez G, Ribao C, Torrado I, Abad X, Pintó RM, Barja JL, Bosch A (2002). Prevalence of enterovirus and hepatitis A virus in bivalve molluscs from Galicia (NW Spain): inadequacy of the EU standards of microbiological quality. International Journal of Food Microbiology, 74: 119-130

Sassen K, Velleuer R, Feldhusen F, Stede M, Effkemann S, Graf K, Zander HD, Pohlenz F, Heyken F, Hagena W, Hanslik M (2005). Niedersächsische Ausführungshinweise für die Überwachungsbehörden zur Durchführung der Muschelhygieneüberwachung. Niedersächsisches Ministerium für den ländlichen Raum, Ernährung, Landwirtschaft und Verbraucherschutz, Hannover, Germany, 18 pp

Chapter 04

MEETING THE QUEST FOR SPATIAL EFFICIENCY: PROGRESS AND PROSPECTS OF EXTENSIVE AQUACULTURE WITHIN OFFSHORE WIND FARMS

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Abstract

Along the German North Sea coast the observed high spatial competition of stakeholders has encouraged the idea of integrating open ocean aquaculture in conjunction with offshore wind farms beyond the 12 miles zone. The article provides an overview on the current state of transdisciplinary research on a potential implementation of such a multifunctional use concept on a showcase basis, covering biological, technical, economic and social/ policy aspects, as well as private-public partnerships and the relevant institutional bodies. We show that the cultivation of seaweeds and blue mussels is biologically and technically feasible in a high-energy environment using modified cultivation strategies. The point of departure of our multi-use concept was that the solid groundings of wind turbines could serve as attachment points for the aquaculture installations and become the key to the successful commercial cultivation of any offshore aquatic organism. However, spaces in between the turbines are also attractive for farming projects, since public access is restricted and thus the cultivation site protected from outside influences. An economic analysis of different operation scenarios indicates that the market price and the annual settlement success of juvenile mussels are the main factors that determine the breakeven point. Social and policy science research reveals that the integration of relevant actors into the development of a multi-use concept for a wind farm - mariculture interaction is a complex and controversial issue. Combining knowledge and experience of wind farm planners as well as mussel fishermen and mariculturists within the framework of national and EU policies is probably the most important component for designing and developing an effective offshore co-management regime to limit the consumption of ocean space.

Keywords: offshore aquaculture, offshore wind farms, co-management, ICZM, mussel cultivation, seaweed cultivation

1. Introduction

The political recognition on national as well as on EU level that the implementation of integrated coastal zone management (ICZM) is still fragmentary acted as incentive to investigate in more detail how this could be overcome (BMU 2006). In Germany, it generated a call of the Federal Ministry of Education and Research to the various Federal States to develop projects that address ICZM on a

regional level. In 2004, the programme *Coastal Futures* (Kannen 2004) tying up various administrative and scientific bodies and the public along the west coast of the State of Schleswig-Holstein was granted funding. This programme focussed on two issues: (1) to develop the future of the coast as a living, working and recreational space for the local population, and (2) to consider the potential contribution of coastal resources to the sustainable development on the national and EU/ global level, i.e. by providing regenerative energy through wind power. In order to sustain sufficient open space for future development, the idea of combining offshore wind power generation with other uses, such as aquaculture operations, emerged (Buck 2002). Marine aquaculture is a growing enterprise in Germany as well as in the whole of Europe, strongly motivated by the decline of fisheries production and the search for alternative income options for rural peripheral coastal regions.
In order to stimulate multifunctional use of marine space it was decided to develop a project on a showcase basis which deals not only with different scientific fields, but also with private-public partnerships and the relevant institutional bodies. In the following, we provide an overview on the current state of research undertaken within this focus.

1.2. Background

“Fisheries have rarely been sustainable”. This statement by Pauly et al. (2002) was driven by the lack in sustainability induced through a serial depletion of wild stocks worldwide because of improved technology, geographical expansion, and exploitation of previously spurned species lower in the food web. In exchange, aquaculture was often discussed to bridge the gap between supply and demand or, in contrast, even to exacerbate this scenario.
Since the 1970s aquaculture production has grown quite rapidly and is by now one of the fastest growing aquatic food production sectors in the world (FAO 2004). Besides the rapid development of this sector the wide-ranging decline in fisheries yields has been enhanced by an increase in public demand for aquatic products. With an annual share of more than 15% of total animal protein supplies, the production of capture fisheries and aquaculture plays a significant role for the global food security (FAO 2004). In 2004, approximately 160 million tonnes of aquatic organisms were produced worldwide (Fig. 1). From that amount, global aquaculture accounts for almost 37.7 % of total edible production totalling about 60 million tonnes of aquatic organisms (FAO 2006).

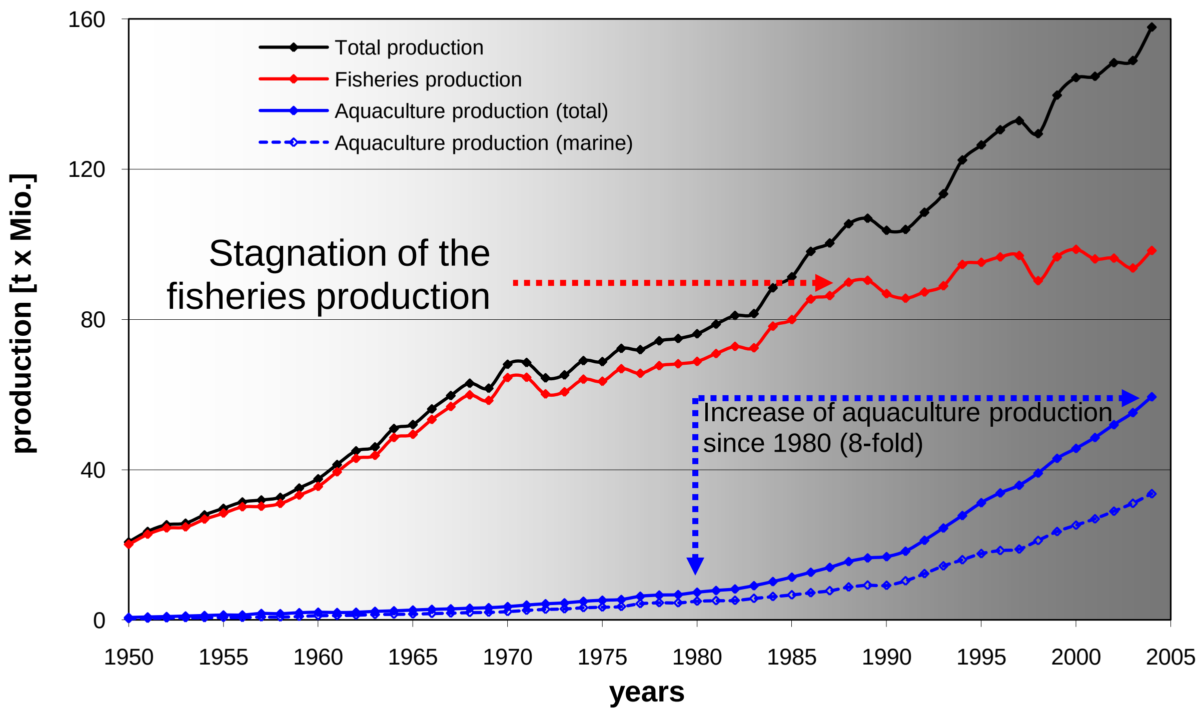

Fig. 1: Global production of aquatic organisms originating from fisheries and aquaculture within the last 55 years (data source FAO 2006, modified after Buck 2007b)

Today, a wide range of aquatic species is raised in various systems onshore as well as in the ocean. According to the FAO (2004), approximately 300 different species, ranging from fish to shellfish, crustaceans, and algae are produced in aquaculture systems. Most of these traditionally founded aquaculture enterprises are concentrated in well-protected and therefore favourable inshore water areas (Burbridge et al. 2001). Even if over-reporting its aquaculture production (Rawski & Xiao 2001) it was the People's Republic of China contributing approx. 70 % to world aquaculture production in 2004. It is nevertheless debatable, whether this production can compensate for the global deficiency in aquatic food. In addition, the intensive traditional aquaculture of carnivorous species does not automatically relieve pressure on ocean fisheries (Naylor et al. 2000). Salmon farming, e.g., requires large inputs of wild fish as fish oil and fish meal for the production of feed. On the other hand the major increase in aquaculture production originates from herbivorous species and the production is anyway heavily skewed towards herbivores (Roth et al. 2002). Hence, the farming of non-carnivorous species that is not dependent on fishmeal-based feeds is considered a sustainable way of producing food.

On top of this debate, nowadays, an increasing limitation of favourable coastal sites for the development of modern aquaculture is evident in countries such as Germany (Buck 2004). This spatial limitation is mainly caused by the lack of protected nearshore areas and by the fact that regulatory frameworks that assign specific areas for aquaculture operations in Germany and other countries bordering the North Sea are diverse and still emerging. This situation is in contrast to production progress in developing countries, in which the installation of aquaculture systems benefits from the often weak enforcement of integrated coastal management schemes, which regulate the equal access to the coastal resources (Davis & Bailey 1996, Adger & Luttrell 2000). Thus, the rise of aquaculture production has specifically taken place in developing countries, especially in Asia, which hold approx. 91.4 % of the global production share (Rana 1997; Lee & Turk 1998; FAO 2006). In addition, the intensive and in various cases overlapping use of coastal habitats adds to the increasing pollution of coastal waters and gives rise to spatial conflicts, thus leaving little room for the expansion of modern coastal aquaculture systems. This problem has triggered the movement to offshore areas, where little spatial regulations have been established so far and clean water can be expected (Krause et al. 2003). There is an enormous economic potential for *extensive marine aquaculture* in offshore areas.

1.3. What is extensive marine aquaculture?

In contrast to intensive aquaculture operations *extensive aquaculture* covers a line of production in a sustainable manner. Following the definition of the FAO (1989; 1997) *aquaculture* is the farming of aquatic organisms including fish, molluscs, crustaceans, and aquatic plants with some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding, and protection from predators. Specifically, *marine aquaculture* – also called *mariculture* – concentrates on aquatic organisms cultivated in brackish or marine environments. If aquaculture operations are characterised by (1) a low degree of control (i.e. environmental control, nutrition, predators, competitors, and disease agents), (2) low initial costs, (3) low level technology, (4) low production efficiency, and (5) high dependence on local climate and water quality (natural water bodies such as bays, ponds, embayments) (Eleftheriou 1997) it is defined as *extensive marine aquaculture*. Finally, *offshore aquaculture* or *open ocean aquaculture* is defined as (1) being in a marine environment fully exposed to all kinds of oceanographic conditions (Ryan 2005), and (2) located at least eight nautical miles off the coast (Buck 2004) avoiding tremendous stakeholder conflicts in nearer coastal areas (Dahle et al. 1991). The procedures and applied techniques for the cultivation of organisms mainly depend on the species, their life cycle determines the phase of cultivation, and the location for the grow-out, where market size is reached.

In the North Sea, only indigenous species can be considered for aquaculture to avoid the disruption of local flora and fauna in ecologically highly sensitive areas of the Wadden Sea. This limits economic opportunities of aquaculture enterprises, since only a few indigenous candidates are regarded as high-value species (BLE 2003). Following a feasibility study by Buck (2002) only culture species with modest service needs can be considered as favourable candidates. In this study, the most suitable candidates identified for offshore extensive aquaculture were the sugar kelp (*Laminaria saccharina*), dulse (*Palmaria palmata*), the blue mussel *Mytilus edulis*, and two oyster species, the Pacific oyster (*Crassostera gigas*) and the European flat oyster (*Ostrea edulis*), respectively.

Mussels and seaweed are cultured mainly in extensive systems throughout the world (Hickman 1992, Critchley et al. 2006, Buck 2007a), the latter for historical and traditional reasons being mostly found in Asian countries. Worldwide, several techniques exist to cultivate mussels and seaweed, either in co-culture or in single culture. Basically, both species are cultured in a suspended manner in the water column, floating or submerged. Today the use of the rafts, longlines, and ring methods dominate (Fig. 2). The latter two were the main cultivation techniques used throughout our research studies in offshore areas of the German Bight (North Sea) (Hickman 1992, Buck & Buchholz 2004, Buck 2007a).

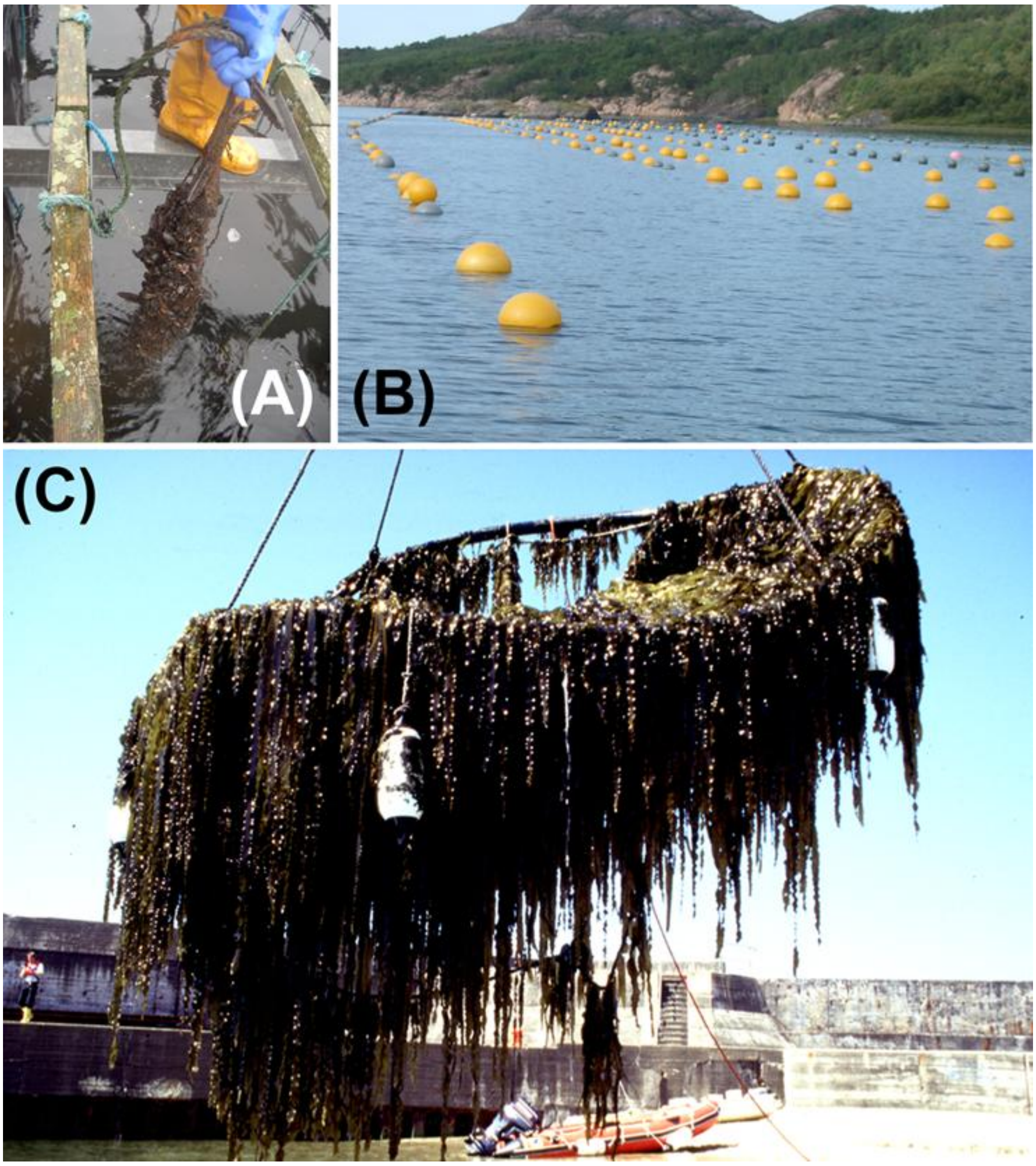

Fig. 2: Methods to cultivate mussels and seaweed in a suspended or floating design. (a) Shows a raft system where mussel collectors are attached vertically from a wooden frame. (b) A horizontally installed longline floating on the surface. The mussel collectors are attached below the water surface vertically to the longline. (c) A floating ring system for the cultivation of seaweed at harvest time (Buck and Buchholz 2004).

2. Offshore wind farms as a newcomer

High and reasonably steady wind speeds occur regularly in offshore areas, making such areas prime candidates for renewable energy production by wind energy farms. In Germany a major political incentive[1] exists currently to install large offshore wind farms (Tiedemann 2003; BMU/ Stiftung Offshore Windenergie 2007). The promotion of wind power is mainly driven by the policy to reduce the dependence on conventional fossil energy resources as well as the need to reduce the environmentally harmful CO_2 loads. Thus, the emerging branch of offshore wind farms appears as a new stakeholder on the list of users (Gierloff-Emden 2002, Dahlke 2002, Tiedemann 2003).
At present 47 project applications for wind farms in the Economic Exclusive Zone (EEZ) of the German North Sea and in the Baltic Sea are in the planning process (BSH 2008) with a total number of wind turbines per farm ranging between 80 and 500 (Buck 2002). In November 2001, the Federal Maritime and Hydrographic Agency (BSH) granted the first approval for the installation of a pilot offshore wind farm: The *Prokon Nord Company* received the permit to install 12 wind turbines in the German EEZ of the North Sea, approximately 45 km north of the island of *Borkum* with the option to expand the wind farm up to a total of 208 wind turbines by the year 2010. Since then a total of 20 wind farm development projects have been approved in German waters most of them planned seaward of the 12 nautical miles zone: 17 in the EEZ of the North Sea and three in the EEZ of the Baltic Sea (BSH 2008). Currently, only a test field of 12 wind turbines at the site of the licensed wind farm “Borkum West” is being constructed. Experience gained in this project should give developers practical knowledge in the construction and operation of offshore wind farms at depths and at distances from the shore that are not comparable to anywhere in the world (BMU/ Stiftung Offshore Windenergie 2007, Dena 2007).
In contrast to neighbouring European states, the prospect to move wind energy developments offshore stagnates in Germany mainly due to a very complex licensing procedure and the high environmental restraints (BMU/ Stiftung Offshore Windenergie 2007, BWE 2007). A further constraint lies in the spatial competition of offshore wind farms with other utilisation of the marine waters in the German Bight. Such conflicts result from shipping (trade or private), recreational activities, extraction or disposal of gravel, military missions, fisheries, aquaculture, cable and pipelines, nature reserves areas, and other marine and coastal protected areas (Wirtz et al. 2003, Buck et al. 2004, BSH 2007). However, despite the number of competing users within offshore regions being lower compared to coastal areas (Jentoft 2000), the quest for spatial efficiency remains to be a key incentive also for offshore developments in the future.

2.1. Moving offshore: the multi-use idea

The plans for the massive expansion of wind farms in offshore areas of the North Sea triggered the idea of a combination of wind turbines with installations of extensive shellfish and macroalgae aquaculture (Buck 2002; 2004). Offshore wind farms provide an appropriately sized area free of shipping traffic. At the same time the infrastructure for regular service support is readily available and hence such sites provide an ideal opportunity for devising and implementing a multiple-use concept (Buck et al. 2004, Michler-Cieluch unpublished data). However, in contrast to coastal inshore areas where beaches and their adjacent nearshore zones act as buffers to absorb wave energy, offshore regions are high-energy environments fully exposed to waves, weather, and currents. Numerous studies have demonstrated that waves can reach remarkable heights in offshore areas (e.g. Führböter

[1] Wind energy continues to be the world’s most dynamically growing energy source. The first initiative towards an economy based on renewable energy resources in Germany was set by the governmental decision to gradually reduce the use of nuclear energy and to respond to the gradually diminishing fossil and nuclear energy reserves. Simultaneously the output of CO_2 to the atmosphere is reduced (Kyoto protocol). So far, this development has been successful to such an extent that around 7.2 % of the energy needs in Germany are covered by this technology. At the end of 2007, Germany had an installed capacity of 22,247 MW, which contributed to 19,460 operating wind turbines (BWE 2008). Within Europe as the leading market for wind energy with over 57 GW, Germany thus accounted for 39 % in terms of installed capacity and still remains the world’s leader. However, with the North American market currently experiencing a strong growth it is expected that the US will overtake Germany by the end of 2009 (GWEC 2007).

& Dette 1983, Becker et al. 1992). In this context, the solid foundation structure of wind turbines provides support for anchoring cultivation devices that can withstand the harsh weather conditions (Buck et al. 2006). Furthermore, offshore structures are well known for their artificial reef function, thus supporting biodiversity in ecosystems. In comparison to inshore areas, water quality – a major element in aquaculture operations – is regarded as very good (Takayanagi 1998, BSH 2006). Finally, the multifunctional use of offshore areas reduces conflicts between stakeholders if activities are concentrated and conjointly managed within so-called multiple-use marine areas. This, in turn, increases the amount of open ocean territory free of utilisation by man. The above issues are considered as key incentives to move offshore with aquaculture operations.
Since an overall interest exists to move aquaculture activities to offshore locations, different suggestions for technical structures were proposed (see proceedings of various OOA-Conferences: e.g. Stickney 1998, Bridger & Costa-Pierce 2003). Major difficulties in the development of suitable techniques for open ocean (offshore) aquaculture are the harsh environmental conditions, which place an enormous stress on the materials used. It would be advantageous for Germany's offshore aquaculture development to plan for a combination of uses. While windmills use the wind above the surface to produce energy, their fixed pylons, commonly concrete fundaments (gravity foundation), metal jackets or tripods, offer a possibility to connect systems used in aquaculture. The combination of these two industries has to cope with the forces generated by the high energy environment. Hence in designing such structures the forces must be taken into consideration.

2.2. Scope of offshore aquaculture research activities in the German North Sea
Only few scientific studies dealing with the prospects of offshore aquaculture were available prior to the beginning of our studies presented below, and little was known about the biotechnological requirements, the economic potential, or the socio-economic influence to the general feasibility of offshore aquaculture. Very few long-term experiments under harsh hydrodynamic conditions exist (e.g. Langan & Horton 2003 for offshore mussel cultivation, Neushul & Harger 1985, Neushul et al. 1992 for offshore seaweed cultivation) but data on system and species performance are urgently needed to derive methodologies for the assessment of its environmental and economic viability. Therefore, the assessment of the potentials and constraints for sustainable aquaculture development in all marine habitats requires input from various scientific disciplines in order to direct this development towards a successful aquaculture undertaking. In particular, this holds true for offshore aquaculture, where little practical experience is available to date, although research in this area is evolving rapidly (e.g. Turner 2001, Pérez et al. 2003, Bridger & Costa-Pearce 2003, Dalton 2004, Naylor & Burke 2005).
The *Coastal Futures Programme* initiated an integrated assessment of theoretical and practical challenges of aquaculture operations in the North Sea in combination with offshore wind farms. Several studies were carried out, all of which contribute to specific aspects of such a combined utilisation of offshore space.
In the following we group the completed and ongoing projects (see Tab. 1) into the following key areas of research:

(a) **Biological studies**, in which the focus is placed on cultivation and subsequent performance characteristics of indigenous bivalve and seaweed species exposed to extensive offshore aquaculture farming conditions. Further, health status and infestation rates of parasites, bacteria and viruses of candidates are determined to gain reliable predictions where highest growth rates and best product quality for consumers can be achieved. To evaluate the significance and comparability of the employed parameters, the area of investigation was extended along the Atlantic coast from southern Portugal to northern Denmark. Further on, the closely related blue mussel *Mytilus galloprovincialis* was included in the analysis to test the effectiveness of all parameters in different species.
(b) **Physical and technical studies**, where the effects of the prevailing hydrodynamics at specific offshore sites within the national boundaries of the German North Sea on candidates and culture constructions are investigated. At the same time the technical requirements needed for farming structures in high energy environments and their possible combination with offshore wind farms are assessed. New system designs for offshore farming will be developed and prototypes (e.g.

offshore ring, offshore collector) are in the test stage. In addition to offshore seaweed and mussel cultivation, a new technology for finfish mariculture of turbot and cod will be tested in the project *AquaInno* (Fisch & Buck 2006). A floating plant (pond-in-pond systems), combining “In pond raceways” (IPR) and “Recirculation aquaculture systems” (RAS), will be designed for nearshore employment and be developed further for the use in more exposed habitats.

(c) **Management and institutional studies** which focus on the analysis of potential management approaches to implement a multi-use concept of offshore areas, integrating various stakeholders, and their respective views and knowledge systems. This endorses the examination of the prevailing case laws and regulative and management framework conditions as well as a suggestion of decisive offshore co-management strategies to support such activities.

(d) **Economic studies**, in which economic evaluation of such multi-use concepts in offshore locations is conducted taking into consideration market conditions as well as investment and operating costs.

The conceptual approach relied on the results of a theoretical feasibility study (Project No. 1 in Tab. 1), which was carried out prior to the practical research in the field. The sequence of, and relations between the different projects are displayed in Figure 3.

All of the results contributed to the Coastal Futures programme and support the quest to find innovative new approaches for sustainable use and alternative livelihoods of coastal populations.

Table 1: List and description of offshore aquaculture projects including funding agencies ([1]completed, [2]ongoing, [3]interdisciplinary between all projects).

No.	Project	Funding	Description
1	*Feasibility Study* (1)	Alfred Wegener Institute (AWI)	**Proof of concept** for **aquaculture** operations in offshore **wind farms**: • **Review** of worldwide offshore aquaculture experiences (biology, techniques, multi-use ideas), • feasibility study for a **combination of aquaculture in offshore wind farms** in the North Sea, • preliminary **market analysis**
2	*Roter Sand* (1)	AWI	Development of **Offshore-Technology** and **System-Design**: • **Technical** aspects and **biological** feasibility at the offshore test site *Roter Sand*, • Development of **submersible longline systems** and **floating ring systems**.
3	*Offshore-Aquaculture* (1)	Senate for Construction, Environment & Transport (SBUV); AWI	**Potential** of **blue mussel** and **sugar kelp culture** at all planned offshore wind farm sites in the North Sea: • **Settlement success** of mussel larvae, • **growth rates** of seaweed and mussels, • biological and physical **site-selection-criteria**.
4	*Coastal Futures* (2, 3)	Federal Ministry for Education and Research (BMBF); AWI	Assessment of **multi-use issues** of a wind farm – mariculture interaction in offshore areas within an **Integrated Coastal Zone Management (ICZM)** approach: • aspects of **co- management** and **cooperation** between involved actor groups such as wind farmers, fishery groups, and public authorities, • potential schemes for **governance** and **management arrangements**.
5	*MytiMoney* (2)	BMBF; AWI	Assessment of **economic potentials** for offshore mussel cultivation: • Calculation of **the economic valuation** of **joint wind farm – mariculture use** in offshore locations by taking into consideration **market conditions**, **investment and operating costs**, • development of a **model** in order to assess **different scenarios**.
6	*MytiFit* (2)	SBUV; AWI, Bremerhaven	**Fitness**, **settlement success** and **overall health** of offshore cultivated mussels • Test of different **substrates** for the offshore collection of mussel spat, • analysis of mussel infestations with **macro-** and **microparasites**, **bacteria** and **viruses** • assessment of **health** and **energy situation** of mussels using biodiagnostic techniques, • analysis of mussel **growth** and **shell stability**.
7	*AquaLast* (2)	SBUV; AWI, Bremerhaven	Use of **offshore wind turbines** as anchor points for **open ocean longline aquaculture**: • **Modelling** and **calculation** of possible loads by currents and wave action, • evaluation of the **hydrological conditions** and **mechanical loads** onto the foundation in a **field experiment** off Sylt, • development of a **computer program** to correctly predict **loads for large scale offshore longlines**.
8	*GIS German Bight* (2)	AWI	**GIS-based decision support tool** for offshore aquaculture development: • **Mapping** of all German **mariculture sites**, • **determination** of **potential open ocean aquaculture installations** in the German Bight.
9	*River Jade* (2)	SBUV; AWI	**Settlement, fitness** and **health** of nearshore cultivated mussel: • **Nearshore reference site** (settlement, growth, **mussel health** and shell stability) for the *MytiFit* project, • part of student **education** programme.

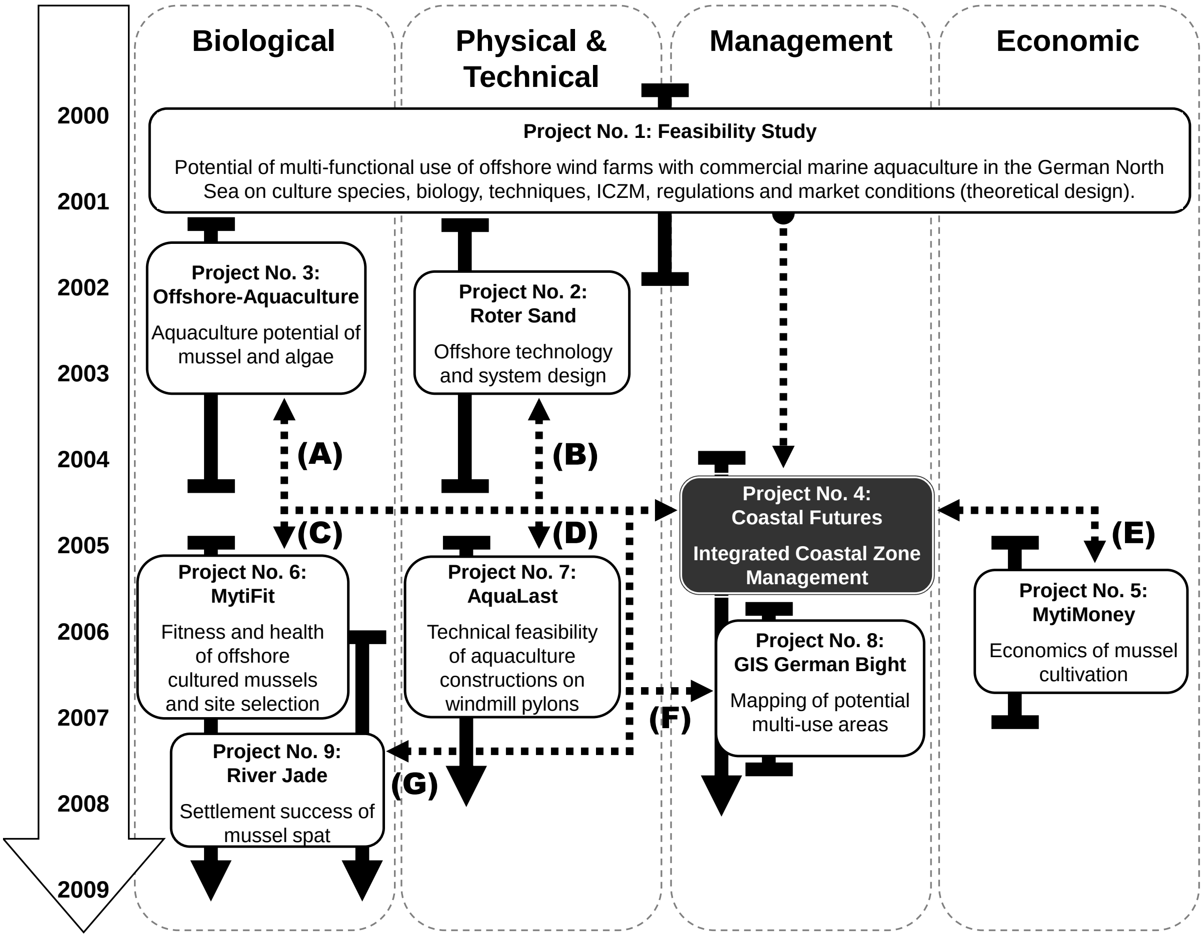

Fig. 3: Chronological order of conducted and ongoing research projects dealing with the combination of offshore wind farming and open ocean aquaculture. Project No. 1, the feasibility study, constituted the basis for all subsequent research. The *Coastal Futures Project* acts as a key node project to which the other projects either have contributed or by which they have been stimulated because of its transdisciplinary approach. It is visible that: (*A*) calls the wind farm developers' attention to offshore aquaculture; (*B*) and (*C*) include authorities and fishermen into the planning process for site selection criteria of appropriate aquaculture sites; (*D*) involves offshore engineers and wind farm developers/ operators into the technical part of an offshore aquaculture enterprise; (*E*) introduces (mussel) fishermen to the co-management idea and appraises the economics of mussel cultivation; (*F*) supplies authorities with maps and tools to limit regional stakeholder conflicts, and (*G*) establishing an inshore reference station to support the data collected offshore.

3. Detailed results of the projects

Over the last decades, substantial insight has been gained on the terms and conditions active in the offshore environment. However, these data are only partly useful for the selection of offshore aquaculture sites, because they have been gathered primarily for other user needs and thus lack the essential specificity to

address the biological and cultivable potential of these sites. Prior to a multifunctional development comprising mariculture activities it is therefore necessary to determine the appropriate biological,

technological, and management requirements as well as the performance characteristics that would allow the employment of favourable and cost-effective methodologies. To meet this end, special focus was placed on the combination of extensive offshore shellfish and seaweed farming at exposed sites within the proposed offshore wind farm boundaries.
Due to the wide spectrum, which is covered in the nine projects specified above, the outcomes are manifold. In the following, we present the various project results according to their contributions towards the main research topics involved.

3.1. Biological studies

The theoretical *Feasibility Study Project* (No. 1) was aimed to ascertain the biological, technical, and economic feasibility of an offshore marine aquaculture structure with respect to the cultivation of marine organisms within wind farm sites in the German North Sea. One result was that to date in terms of commercial marine aquaculture Germany had little knowledge and background on offshore aquaculture compared to many other coastal countries throughout the world. Nevertheless, a synthesis from a selection of parameters (e.g. geo-physical and biological parameters) allowed the identification of suitable candidates for commercial offshore aquaculture. These candidates include blue mussels (*Mytilus edulis*) and oysters (*Ostrea edulis*, *Crassostrea gigas*), which could be maintained extensively in the offshore region. Moreover, labour requirement for these candidates as well as for seaweeds, such as the sugar kelp (*Laminaria saccharina*) and dulse (*Palmaria palmata*), is supposed to be low.
In the *Offshore Aquaculture Project* (No. 3) the biological feasibility of cultivating mussels, oysters, and kelp within offshore wind farm sites was investigated. Growth of these species was excellent in offshore environments but different depending on exposure sites, system designs, installation mode, and season. Settlement of young mussels on artificial collector substrates decreased with increasing distance from shore (Walter et al. in press). However, this does not limit the economic potential, if the thinning procedure is omitted following a “One-Step-Cultivation” concept (Buck 2004). Further, mussels were free of parasites at offshore locations due to dilution effects and the interrupted reproduction cycles of some macroparasites (Buck et al. 2005). Hydrodynamic forces could support length increase of seaweed blades when transferring young sporophytes to sea. These algae will adapt to the occurring loads and develop strong holdfasts which prevent detachment of the entire plant (Buck & Buchholz 2005).
After the principal feasibility of offshore cultivation of algae and mussels was proven by the outcome of the *Offshore Aquaculture Project* two further ongoing projects, *MytiFit* and *River Jade* (No. 6 and No. 9), were started in 2005 to elucidate more details regarding cultivation techniques. Additionally, the overall health status of mussels cultured under different conditions and the impact on economic aspects was investigated (Brenner et al. 2007). Specific aims of the projects were the development of suitable offshore spat collecting techniques, detailed knowledge about parasites (macro and micro), bacteria, and virus infestations at different sites, implementation of biodiagnostic techniques for the health analysis of cultured mussels, and collection of all relevant data (e.g. shell stability and attachment strength of mussels) for the further processing of mussels as a product for human consumption. Modified and improved techniques for offshore farming withstand the high energy environment of the North Sea, but will certainly cause higher investment costs. Therefore, site selecting criteria for a culture area should be clearly identified to assess economic risks. Important for the cultivation success is the water quality. The analysis of the cultured organisms with biodiagnostic tools provides detailed insights in the water conditions the animals live in. By this approach, reliable predictions are possible at which locations highest growth rates and best product quality for consumers can be achieved. Preliminary results attest offshore areas satisfying settlement success and excellent growth rates (Manefeld 2006), low infestations of macro-parasites (Voss 2006), micro-parasites, bacteria, and toxins (Brenner et al. 2009).
First results in the Euro-Tour Project (No. 11) show that mussels originating from offshore habitats have a better health status regarding the infestation of macro and micro parasites (Buck & Brenner, unpublished data). Further, macro parasites were found in mussels from nearshore areas in the

Wadden Sea (the Netherlands, Germany, Denmark) whereas micro parasites were absent in all samples from the German Bight.

3.2. Physical and technical studies

The first technically oriented studies were conducted in the project *Roter Sand* (No. 2). The results of this study allowed the identification of two offshore aquaculture systems that were best suited for offshore operations from a biological point of view. Depending on the impacting hydrodynamic properties, different technical setups were regarded as favourable. The first one is a floating and submergible ring system for the cultivation of seaweed. It withstands rough weather conditions and allows easy handling (Buck & Buchholz 2004). The second system is a submerged longline design for blue mussel culture (Buck 2007a). The longline should be installed 5 m below the water surface and be connected to foundations of offshore windmills. For the longline, polypropylene proved to be an appropriate material. The system design is made of various connected segments allowing an easy harvest and replacement of parts of the construction. However, more technical engineering research is required to find the most cost-effective mode of construction and best choice of materials (e.g. corrosion, longevity in spite of mechanical stress), so that easy handling can be guaranteed under relatively harsh weather conditions (e.g. construction, deployment, retrieval, service, repairs).

The technical realisation and the implications of aquaculture technical requirements on design and construction of the grounding construction of offshore wind turbines were considered in the *AquaLast Project* (No. 7, Buck et al. 2006).

So far, modelling and experimental validation of a submerged 50 m longline aquaculture construction mounted between two steel piles 17 nautical miles off the coast, showed significant forces of up to 90 kN (equivalent to 9 tons) induced by waves of up to 1.8 m significant wave height and tidal currents of up to 1.0 m/s (Zielinski et al. 2006). Given the high energy environment in the North Sea and the non-linear relationship between water movement and its resulting forces, even higher mechanical loads are to be expected within the life cycle of such an arrangement.

Future work will (a) continue to monitor the experimental setup under different environmental conditions, (b) improve model parameters, such as drag coefficients by means of laboratory experiments, and (c) scale up the 50 m longline experiment as part of a simulation to full length using realistic foundations, such as monopiles and tripods. The resulting mechanical loads will then be considered as boundary conditions for the construction of wind farm foundations and additional demands in material, risk calculations, and safety measures will be identified and economically quantified. The latter aspect will be a substantial factor for the successful integration of wind farmers in an offshore co-management scheme.

Furthermore, commercially produced nearshore collectors and self designed collectors made of PP-fleece, PE or natural strand like coconut were tested under harsh offshore conditions. First results show that postlarvae and settled juvenile mussels prefer different materials and different material surfaces. With these results a new collector for the use in high energy environments can be developed.

Finally, the new Pond-in-Pond system (No. 10, Fisch & Buck 2006) with an internal waste water treatment for the use in sheltered and nearshore areas has the potential to lower environmental impact of fish culture facilities, such as net pens or cages, where pollution from faeces, medicine, and food pellets is mainly “solved” by dilution. It is able to reduce the nutrient load not only from the fish holding unit but also from the surrounding water column in which the entire system is floating. The system will be improved to fulfil new guidelines set by the EU.

3.3. Management and institutional studies

In the *Coastal Futures Project* (No. 4) research on stakeholders’ perspectives towards a potential wind farm–mariculture interaction reveals that integrating the perceptions and demands of the various resource users into the development of a multiple-use concept is a complex and controversial issue (Michler-Cieluch & Krause 2008). Different values need to be harmonized. So far, disagreements on the distribution of entitlements to benefits and profits between the different stakeholders can be observed (Table 2).

Table 2: Pros and cons expressed by representatives of different stakeholder groups towards suggested "wind farm–mariculture integration" (modified after Michler & Buck 2007, Michler-Cieluch & Kodeih (2008).

PROS	CONS
• Combination would increase acceptance of offshore wind farms among population (administration)	• Uncertain profitability due to monumental technical requirements for operating mussel and algae cultures in the open sea (administration)
• Additional societal benefit (policy)	• Economic feasibility very questionable (fishery)
• Good innovative idea even though many open questions have to be answered before (wind energy)	• Offshore wind farms are a source of danger for shipping; danger of oil spills. (tourism)
• Multi-use of ocean territory reduces claiming of additional areas (tourism)	• Lack of in-depth information on impacts; not imaginable at present (policy)
• Additional jobs in the rural region likely (economy)	• Lack of "neutral" information (fishery)
• Opens up potentials for research and economic/financial benefit; possibility for international image (research)	• Exaggerated synergetic effects should not be interpreted; cultivation of oysters is not part of a wind farm (economy)
• Initiation of a co-management scheme for the EEZ (research)	• The mariculture concept needs to see for itself. Otherwise it does not convince in combination with offshore wind farms (administration).
• Interesting with regard to local gastronomy (tourism)	• Loss of traditional fishery activities, e.g. trawl fishery (fishery)

Our ongoing multidisciplinary social science research implies that the effective input of wind farmers and future mariculture operators such as mussel harvesters into a negotiation and bargaining process is the most essential component for the formation of an effective wind farm–mariculture co-management regime. Both acting groups know best what kind of tasks and activities have to be performed offshore for their purposes and what kind of rules would have to be applied for jointly using an ocean territory. Top-down induced management schemes by e.g. the National government hold a high potential of failure. Moreover, involving the relevant actors improves the social acceptability of new innovating concepts, and their applicability (Heinelt 2002). Consequently, we argue that for developing and implementing a wind farm–mariculture multiple-use concept, co-management such as described by Carlsson and Berkes (2005) should ideally "be carried out with the participation of different actors that typically try to find ways to learn from their actions and adapt the behaviour to the consequences of their own, and other's actions" (p. 67). This must be supported by the relevant authorities on all levels and find its way into the legislative framework on EU and national level.

If we consider co-management to be a network activity between private actors, such as wind farmers or mariculture operators/fishermen, and public authorities, one of its basic characteristics is the fact that a third party can coordinate the activities of formally separated parties (Carlsson & Berkes 2005). Since ways and means have to be developed that balance the respective interests of dominant and politically supported wind farming participants and small-scale entrepreneurial harvesters this model can also be suggested for a prospected wind farm–mariculture interrelation. Therefore, the key question has to be resolved on how institutional arrangements could act as "boundary organizations" (see Cash & Moser 2000) in the prospect of an offshore co-management process between wind farmers, future mariculture operators, and governmental agencies. However, in order to define the functional structure of such a co-management regime in detail, reliable outcomes on economic and technical integration prospects of a joint wind farm–mariculture venture have to be produced. The latter is a key research demand, which was voiced by most of the interview partners so far (Michler-Cieluch & Krause 2008).

The *GIS German Bight Project* (No. 8) demonstrates that there are sites in the German Bight appropriate for open ocean aquaculture installations despite plenty of stakeholder conflicts. All test areas are situated near planned or approved offshore wind farms at places with adequate water depth, still not too far from the coast. Co-management arrangements comprising offshore wind farms and aquaculture allow for installing the latter within or in close vicinity to wind farm territory. This is due to the fact that the use of designated wind farm areas is restricted for other stakeholders such as fisheries. In addition, the planned wind farms offer enough room for large aquaculture installations (Koch 2006).

3.4. Economic studies

The *Feasibility Study Project* (No. 1) gave a general overview on market prices, market demands, classification of candidate species as high value products, and the cost of some infrastructure. The study looked into the possible market value of offshore aquaculture products in comparison to the performance of existing conventionally operated farms in coastal waters. Main focus was placed on existing experience within the European community. A result was that a strong market exists for the suggested mussels and oysters as well as for brown and red algae. This market is likely to expand in the near future. Red algae can be directly sold to the consumer as a healthy “green and clean” bio-food or used in industry for various purposes (e.g. emulsifiers, ingredients for food, medicine). The feasibility study rounds up with a final evaluation of all factors, which are critical for the development of a commercial offshore aquaculture in combination with the planned wind farms in the North Sea region.

However, the *MytiMoney Project* (No. 5) principally highlights specific economic criteria needed for mussel farming at a site within an offshore wind farm in the German Bight. The economic analysis of the combined wind farm–mariculture use, focusing on seed mussel production, demonstrates that the service life of infrastructure as well as the mussel yield obtained per meter longline are crucial factors determining profitability (Fig. 4). In contrast, shipping costs and the idea to develop an offshore servicing vessel that can jointly be used for wind farm and mariculture operations and maintenance (Buck et al. 2004), does not seem to have a strong influence on general cost reduction patterns. Finally, a full economic analysis of different operation scenarios (best case – worst case) indicated that the market price and the annual settlement success of juvenile mussels were the main factors that determine the breakeven point.

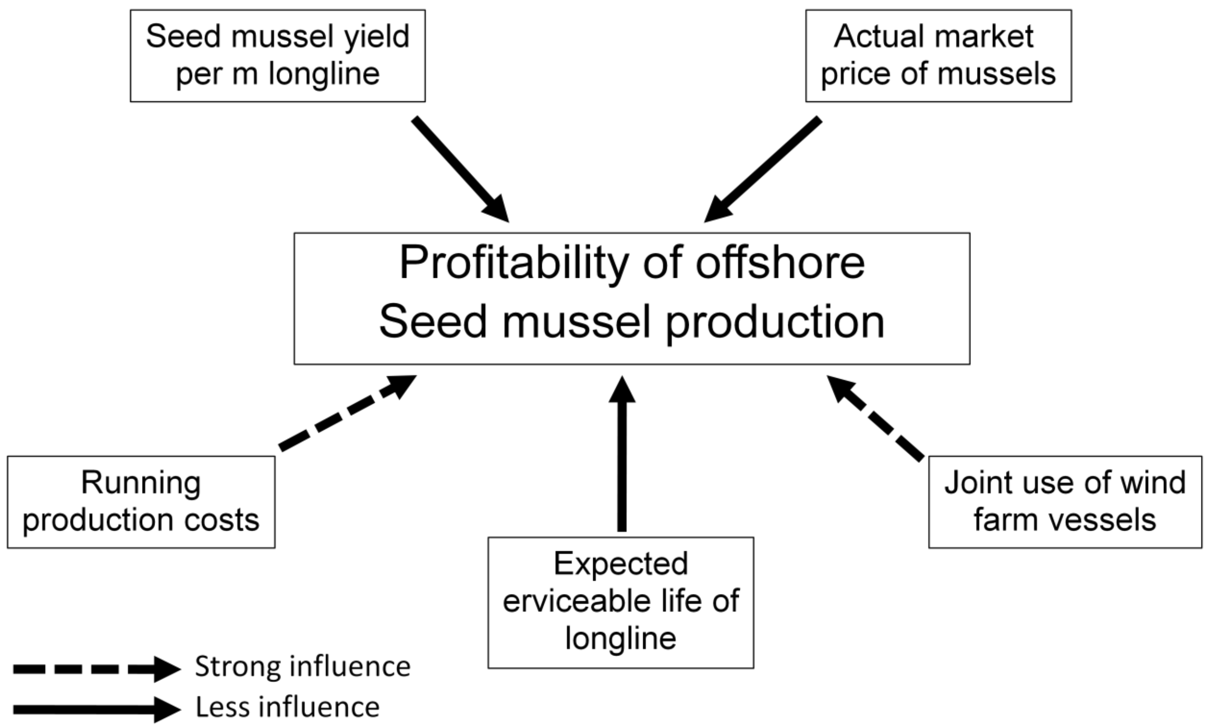

Fig. 4: Factors influencing cost effectiveness of offshore seed mussel production within a wind farm territory in the North Sea.

Despite of these studies the economics of a joint wind farm–mariculture utilization scheme still remain to be evaluated in detail. First results suggest concentrating on how to reduce investment costs for longlines used per culturing plot at offshore locations. This includes a more detailed analysis of the general start-up costs. Moreover, the prospect of cultivating mussels for direct consumption has to be considered in the future. The idea of having two production cycles in a single year is not possible due to the main spat fall event of blue mussels being between April and May (Pulfrich 1997).

4. Conclusion and Outlook

By setting higher value on an inclusion of stakeholder knowledge and opinion, the initiation of the *Coastal Futures Project* (No. 4) resulted in a stronger focus on the practicability of multifunctional use of offshore areas. It can be shown that such innovative new concepts are highly complex and interdependent. First results indicate that secured technical and economic feasibility appears to be a basic prerequisite to assure that both, offshore wind farm operators and aquaculturists, will support the multi-use concept, especially as far as the management of joint activities is concerned.

This suggests that as soon as technical and economic aspects are evaluated in more detail it is important to initialize a comprehensive communication program to provide information to the key public and private actor groups (stakeholders). This in turn will increase their knowledge on the overall feasibility of combining different offshore uses and will contribute to adding a joint wind farm–mariculture venture to their future portfolio.

Detailed data are needed to calculate economic potentials and risks of a co-used wind farm area for the production of seafood. Apart from the principal feasibility of an area as an aquaculture site, growth rates and product quality must be predictable. First results on blue mussels from test areas offshore show that highest product quality can be expected from testing areas offshore. A proven product quality ensures higher market prizes, should compensate for higher investment costs for the culture systems, and helps to install a functioning offshore aquaculture system in the German Bight.

References

Adger WN, Luttrell C (2000). Property rights and the utilisation of wetlands. Ecological Economics, 35: 75-89

Becker G, Dick S, Dippner J (1992). Hydrography of the German Bight. Marine Ecology Progress Series, 91: 9–18

BLE (2003). Der Markt für Fischereierzeugnisse in der Bundesrepublik Deutschland im Jahre 2003. Bericht über die Versorgung der Bundesrepublik Deutschland mit Fischereiprodukten aus Eigenproduktion und Importen sowie die Exportsituation. Bundesanstalt für Landwirtschaft und Ernährung, Referat 521/ Fischwirtschaft, 7-10

BMU (2006). Integriertes Küstenzonenmanagement in Deutschland (IKZM). Nationale Strategie mit Bestandsaufnahme nach der EU-Empfehlung 2002/413/EG. Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit (Federal Ministry for the Environment, Nature Conservation and Nuclear Safety)

BMU/ Stiftung Offshore Windenergie (2007). Offshore wind power deployment in Germany. Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (eds)

Brenner M, Buck BH, Koehler A (2007). New concept combines offshore wind farms, mussel cultivation. Global Aquaculture Advocate, 10: 79-81

Brenner M, Ramdohr S, Effkemann S, Stede M (2009). Key parameters for the consumption suitability of offshore cultivated blue mussels (*Mytilus edulis* L.) in the German Bight. European Food Research and Technology, 230: 255-267

Bridger CJ, Costa-Pierce BA (2003). Open Ocean Aquaculture: From Research to Commercial Reality. The World Aquaculture Society, Baton Rouge, Louisiana USA

BSH (2006). MURSYS - Marine Environment Reporting System Information from the North Sea and Baltic Sea. Bundesamt für Seeschifffahrt und Hydrographie (BSH), Hamburg, Germany

BSH (2007). Bundesamt für Seeschifffahrt und Hydrographie – BSH (Federal Maritime and Hydrographic Agency), CONTIS-Geodata. Hamburg/ Rostock, Germany

BSH (2008). Wind farms. Bundesamt für Seeschifffahrt und Hydrographie (Federal Maritime and Hydrographic Agency). Hamburg/ Rostock, Germany

Buck BH (2002). Open Ocean Aquaculture und Offshore-Windparks: Eine Machbarkeitsstudie über die multifunktionale Nutzung von Offshore-Windparks und Offshore-Marikultur im Raum Nordsee. Reports on Polar and Marine Research, Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, 412: 252 pp

Buck BH (2004). Farming in a High Energy Environment: Potentials and Constraints of Sustainable Offshore Aquaculture in the German Bight (North Sea). Dissertation. University of Bremen, Bremen, Germany

Buck BH (2007a). Experimental trials on the feasibility of offshore seed production of the mussels Mytilus edulis in the German Bight: Installation, technical requirements and environmental conditions. Helgoland Marine Research, 61:87-101

Buck BH (2007b). Marikultur als Co-Nutzung in Offshore-Windparks: Status Quo, Probleme und Perspektiven, Meeresumwelt-Symposium 2006 : 16. Symposium, 13. bis 14. Juni 2006, CCH - Congress Center Hamburg / Bundesamt für Seeschifffahrt und Hydrographie in Zusammenarbeit mit dem Bundesumweltamt im Auftrag des Bundesministeriums für Umwelt, Naturschutz und Reaktorsicherheit, 167-179.

Buck BH, Berg-Pollack A, Assheuer J, Zielinski O, Kassen D (2006). Technical Realization of Extensive Aquaculture Constructions in Offshore Wind Farms: Consideration of the Mechanical Loads. Proceedings of the 25th International Conference on Offshore Mechanics and Arctic Engineering, OMAE 2006: presented at the 25th International Conference on Offshore Mechanics and Arctic Engineering, 4-9 June 2006, Hamburg, Germany / American Society of Mechanical Engineers, 1-7

Buck BH, Thieltges D, Walter U, Nehls G, Rosenthal H (2005). Inshore-offshore comparison of parasite infestation in Mytilus edulis: Implications for open ocean aquaculture. Journal of Applied Ichthyology, 21:107-113

Buck BH, Buchholz CM (2005). Response of offshore cultivated *Laminaria saccharina* to hydrodynamic forcing in the North Sea. Aquaculture, 250: 674-691

Buck BH, Krause G, Rosenthal H (2004). Multifunctional Use, Environmental Regulations and the Prospect of Offshore Co-Management: Potential for and Constraints to Extensive Open Ocean Aquaculture Development within Wind Farms in Germany. Ocean Coastal Management, 47: 95-122

Buck BH, Buchholz CM (2004). The Offshore-Ring: A new system design for the open ocean aquaculture of macroalgae. Journal of Applied Phycology, 16: 355-368

Burbridge P, Hendrick V, Roth E, Rosenthal H (2001). Social and economic policy issues relevant to marine aquaculture. Journal of Applied Ichthyology, 17: 194-206

BWE (2006). Datenblatt Windenergie 2005. Bundesverband WindEnergie, Assessed December 2006 at http://www.wind-energie.de/de/statistiken/datenblatt-2005

BWE (2007). Offshore Windenergie. BWE Hintergrundpapiere. Bundesverband WindEnergie, Assessed February 2007 at http://www.wind-energie.de/no_cache/de/presse-service/hintergrundpapiere

BWE (2008). Datenblatt 2007: Datenblatt Windenergie 2007. Bundesverband WindEnergie. Assessed March 2008 at http://www.wind-energie.de/de/statistiken/datenblatt-2005

Carlsson L, Berkes F (2005). Co-Management: concepts and methodological implications. Journal of Environmental Management, 75: 65-76

Cash DW, Moser SC (2000). Linking global and local scales: designing dynamic assessment and management processes. Global Environmental Change, 10:109-120

Critchley AT, Ohno M, Largo DB (2006). World Seaweed Resources. An authoritative reference system. ETI BioInformatics. DVD-ROM

Dahle LA, DePauw N, Joyce J (1991). Offshore aquaculture technology - possibilities and limitations. Aquaculture and the Environment, 14: 83-84

Dahlke V (2002). Genehmigungsverfahren von Offshore-Windenergieanlagen nach der Seeanlagenverordnung. Natur und Recht, 24: 472-479

Dalton R (2004). Fishing for Trouble. Nature, 431: 502-504

Davis A, Bailey C (1996). Common in Custom, Uncommon in Adantage: Common Property, Local Elites, and Alternative Approaches to Fisheries Management. Society and Natural Resources, 9: 251-265

Dena (2007). Renewable Energies: Germany's first test field for offshore wind energy. Dena fact sheet. German Energy Agency, Berlin

Eleftheriou M (1997). Aqualex: A Glossary of Aquaculture Terms. Wiley-Praxis Series in Aquaculture and Fisheries, Aqualex Multimedia Consortium Ltd

FAO (1997). Aquaculture development. Technical Guidelines for Responsible Fisheries, Food and Agriculture Organisation of the United Nations, Rome, Italy, 5-40

FAO (2004). The State of World Fisheries and Aquaculture - 2004 (SOFIA). Fisheries Department. Food and Agriculture Organisation of the United Nations, Rome, Italy

FAO (2006). Fishery Information, Data and Statistics Unit. Aquaculture production: values 1984-2004. FISHSTAT Plus - Universal software for fishery statistical time series. Food and Agriculture Organization of the United Nations, Rome, Italy

FAO/FIDI (1989). Aquaculture production (1984-1986). Food and Agriculture Organisation of the United Nations, Rome Fisheries Circular, 815 pp

Fisch R, Buck BH (2006). Neues Aquakultursystem für das Meer made in Germany. Fischerblatt, 12: 13-16

Führböter A, Dette HH (1983). Wasserstände, Wind und Seegang im Seegebiet um Helgoland in den Jahren von 1969 bis 1983. Leichtweiß-Institut für Wasserbau der TU Braunschweig, Bericht Nr. 577

Gierloff-Emden, HGR (2002). Wandel der Umwelt der See- und Küstenlandschaft der Nordsee durch Nutzung von Windenergie. Mitteilungen der Österreichischen Geographischen Gesellschaft, 144: 219-226

GWEC (2007). Continuing boom in wind energy – 20 GW of new capacity in 2007. Press release 15. February 2000, Global Wind Energy Council, http://www.gwec.net , assessed March 2008

Heinelt H (2002). Achieving Sustainable and Innovative Policies through Participatory Governance in a Multi-level Context. In: Heinelt H, Getimis P, Kafkalas G, Smith R, Swyngedouw E, Opladen (eds). Particpatory Governance in Multi-Level Context. Leske + Budrich, 17-32

Hickman RW (1992). Mussel Cultivation. In: Gosling E (Ed). The mussel Mytilus: Ecology, Physiology, Genetics and Culture. Development in Aquaculture and Fisheries Science No. 25. Elsevier, Amsterdam, 465-510

Jentoft S (2000). Co-managing the coastal zone: is the task too complex? Ocean Coastal Management, 43: 527-535

Kannen A (2004). Holistic Systems Analysis for ICZM: The Coastal Futures Approach. In: Schernewski G and Dolch T (eds). Geographie der Meere und Küsten. Coastline Reports, 1: 171-181

Koch A (2006). Marine Aquakulturen im Nordseeraum. Status, Probleme und Potentiale unter besonderer Berücksichtigung der Deutschen Bucht. Diploma Thesis, University of Hannover, Germany

Krause G, Buck BH, Rosenthal H (2003). Multifunctional Use and Environmental Regulations: Potentials in the Offshore Aquaculture Development in Germany. Proceedings of the Multidisciplinary Scientific Conference on Sustainable Coastal Zone Management “Rights and Duties in the Coastal Zone”, 12.-14. June 2003, Stockholm, Sweden

Langan R, Horton F (2003). Design, operation and economics of submerged longline mussel culture in the open ocean. Bulletin of the Aquaculture Association of Canada, 103: 11–20

Lee PG, Turk PE (1998). Overview of a Modern, Shore-Based Hatchery for Offshore Mariculture Support. In: Stickney RR (Ed). Joining Forces with Industry - Open Ocean Aquaculture, Proceedings of the Third Annual International Conference, 10.-15. May, Corpus Christi, Texas. Corpus Christi, Texas Sea Grant College Programme, 87-102

Manefeld T (2006). Ansiedlungspotential von Miesmuschellarven (*Mytilus edulis*) an verschiedenen Substraten freihängender Brutkollektoren. Thesis. University of Applied Sciences Bremerhaven, Bremerhaven, Germany

Michler T, Buck BH (2007). Multi-purpose marine areas: Integrating mariculture in offshore wind farms. Proceedings of the 2nd International Conference on Coastal Conservation and Management in the Atlantic and Mediterranean (ICCCM ’07), 22.-26. March 2007, Hammamet, Tunisia, 107-110

Michler-Cieluch T, Kodeih S (2008). Mussel and seaweed cultivation in offshore wind farms: An opinion survey. Coastal Management (in press)

Michler-Cieluch T, Krause G (2008). Perceived concerns and possible management strategies for governing ‘wind farm – mariculture integration’. Marine Policy (in press)

Naylor R, Burke M (2005). Aquaculture and Ocean Resources: Raising Tigers of the Sea. Annual Review of Environment and Resources, 30: 185-218

Naylor RL Goldburg RJ, Primavera JH, Kautsky N, Beveridge MCM, Clay J, Folke C, Lubchenco J, Mooney H, Troell M (2000). Effect of aquaculture on world fish supplies. Nature, 405: 1017-1024

Neushul M, Benson J, Harger BWW, Charters, AC (1992). Macroalgal farming in the sea: Water motion and nitrate uptake. Journal of Applied Phycology, 4: 255–265

Neushul, M, Harger BWW (1985). Studies of biomass yield from a near-shore macroalgal test farm. Journal of Solar Energy Engineering, 107: 93–96

Pauly D, Christensen V, Guénette S, Pitcher TJ, Sumaila RU, Walters CJ, Watson R, Zeller D (2002). Towards sustainability in world fisheries. Nature, 418: 689-695

Pérez OM, Telfer TC, Ross LG (2003). On the calculation of wave climate for offshore cage culture site selection: a case study in Tenerife (Canary Islands). Aquaculture Engineering, 29: 1-21

Pulfrich A (1997). Seasonal variation in the occurrence of planktic bivalve larvae in the Schleswig-Holstein Wadden Sea. Helgoland Wissenschaftliche Meeresuntersuchungen, 51: 23-39

Rana KJ (1997). Aquatic Environments and Use of Species Subgroups. In: Review of the State of World Aquaculture. FAO Fisheries Circular FIRI/C886 (Rev. 1). Rome, Italy, Food and Agriculture Organisation (FAO), 7-16

Rawski TG, Xiao W (2001). Roundtable on Chinese economic statistics: introduction. China Economic Review, 12: 298-302

Roth E, Ackefors H, Asche F, Balnath C, Black E, Black K, Boghen A, Browdy C, Burbridge P, Castell JD, Chamberlain G, Dabrowski K, Davies I, Dosdat A, Eleftheriou A, Ervik A, Gordin H, Heinig CS, Hilge V, Karakassis I, Kuhlmann H, Landry T, von Lukowicz M, McGlade J, Price A, Rhenault RB, Rosenthal H, Saint-Paul U, Sandifer PA, Saroglia M, Silvert W, Steffens W, Soto D, Varadi L, Verreth J, Verdegem M, Waller U (2002). An intellectual injustice to aquaculture development: a response to the review article on "Effect of aquaculture on world fish supplies", Report of the ICES Working Group on Environmental Interactions of Mariculture, F:04 REF ACME, Annex 4, 83-89

Ryan J (2005). Offshore Aquaculture - Do we need it, and why is it taking so long? International Salmon Farmers Association (Ireland). Expert workshop on “Sustainable Aquaculture”, DG JRC European Commission, Institute for Prospective Technological Studies, 17.-18. January 2005, Seville, Spain

Stickney RR (1998). Joining Forces With Industry - Open Ocean Aquaculture. Proceedings of the Third Annual International Conference, 10.-15. May, Corpus Christi, Texas. TAMU-SG-99-103, Corpus Christi, Texas Sea Grant College Programme

Takayanagi K (1998). Water quality guidelines for aquaculture: an example in Japan. In: Howell WH, Keller BJ, Park PK, McVey JP, Takayanagi K, Uekita Y (eds) Nutrition and Technical Development of Aquaculture, Proceedings of the Twenty-Sixth U.S.-Japan Aquaculture Symposium, Durham/ New Hampshire/ USA, 16.-18. September 1997. UJNR Technical Report No. 26, Durham, University of New Hampshire Sea Grant Programme, 247-254

Tiedemann A (2003). Windenergieparke im Meer - Perspektiven für den umweltverträglichen Einstieg in eine neue Großtechnologie. In: Lozán J, Rachor E, Reise K, Sündermann J, Westernhagen H v (eds) Warnsignale aus Nordsee & Wattenmeer: Eine aktuelle Umweltbilanz. Wissenschaftliche Auswertungen, Hamburg, Germany, 142–148

Turner R (2001). Offshore mariculture: Site evaluation. In: Muir J, Basurco B (eds) Options méditerranéennes - Mediterranean offshore mariculture. Etudes et recherches, Serie B, Numéro 30, Zaragoza, CIHEAM, INO Reproducciones, 141-157

Voss D (2006). Parasitierungsgrad der Miesmuschel (*Mytilus edulis*) in Abhängigkeit zur Tiefenzonierung und Entfernung zur Küste. Bachelor-Thesis. University of Applied Sciences Bremerhaven, Bremerhaven, Germany

Walter U, Buck BH, Liebezeit G (2009). Larval occurrence and settlement in the German Bight – a trial to estimate potentials for *Mytilus edulis* culture in offshore areas. Aquaculture International (in press)

Wirtz KW, Tol RSJ, Hooss KG (2003). Mythos “Offene See”: Nutzungskonflikte im Meeresraum. In: Lozan L et al. (eds) Warnsignale aus Nordsee & Wattenmeer. Eine aktuelle Umweltbilanz. Wissenschaftliche Auswertungen, Hamburg, Germany, 157-160

Zielinski O, Assheuer J, Berg-Pollack A, Buck BH, Geisen M, Henkel R, Kassen D (2006). Assessment of Mechanical Loads and Environmental Conditions for Extensive Aquaculture Constructions within Offshore Wind Farms: First Results from the AquaLast Study Site. Proceedings of DEWEK 2006: presented at the DEWEK 2006, 22.-23. November 2006, Bremen, Germany, 1-4

Chapter 05

NEW CONCEPT COMBINES OFFSHORE WIND FARMS AND MUSSEL CULTIVATION

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Abstract

Thanks to ongoing research, expansion of blue mussel culture to offshore locations may be possible in Germany. Initial trials showed that basic biotic conditions in the German North Sea support the cultivation of mussels under harsh conditions. Studies are now examining line culture options, installation configurations, and substrates.

Keywords: *Mytilus edulis*, blue mussel, offshore cultivation, wind farms, macro parasites, biomarker

1. Introduction

Wind energy is the world’s most dynamically growing energy source. The first initiative toward an economy based on renewable energy resources in Germany was set by the governmental decision to gradually reduce the use of nuclear energy. Plans were promoted to implement large wind farms far out in the German North Sea and large areas within the coastal sea (Tiedemann 2003). As wind energy operations only take place above water surfaces, the idea was born to co-use these areas for offshore aquaculture (Buck 2002).

1.1. Offshore Opportunity

Because no expansion of traditional bottom mussel culture is possible in the intertidal zone of the Wadden Sea due to licensing restrictions, environmental protections, and stakeholder conflicts, offshore aquaculture was considered a possibility. However, several questions remained. Are longline cultivation techniques adaptable to high-energy environments and other new culture candidates? Is it feasible to attach the culture constructions to the groundings of the wind turbines, while maintaining the strong hydrodynamic conditions (Buck et al. 2006) (Fig. 1)? These were the main questions pursued by the authors and other scientists at the Alfred Wegener Institute for Polar and Marine Research when starting the first offshore aquaculture projects in 2000.

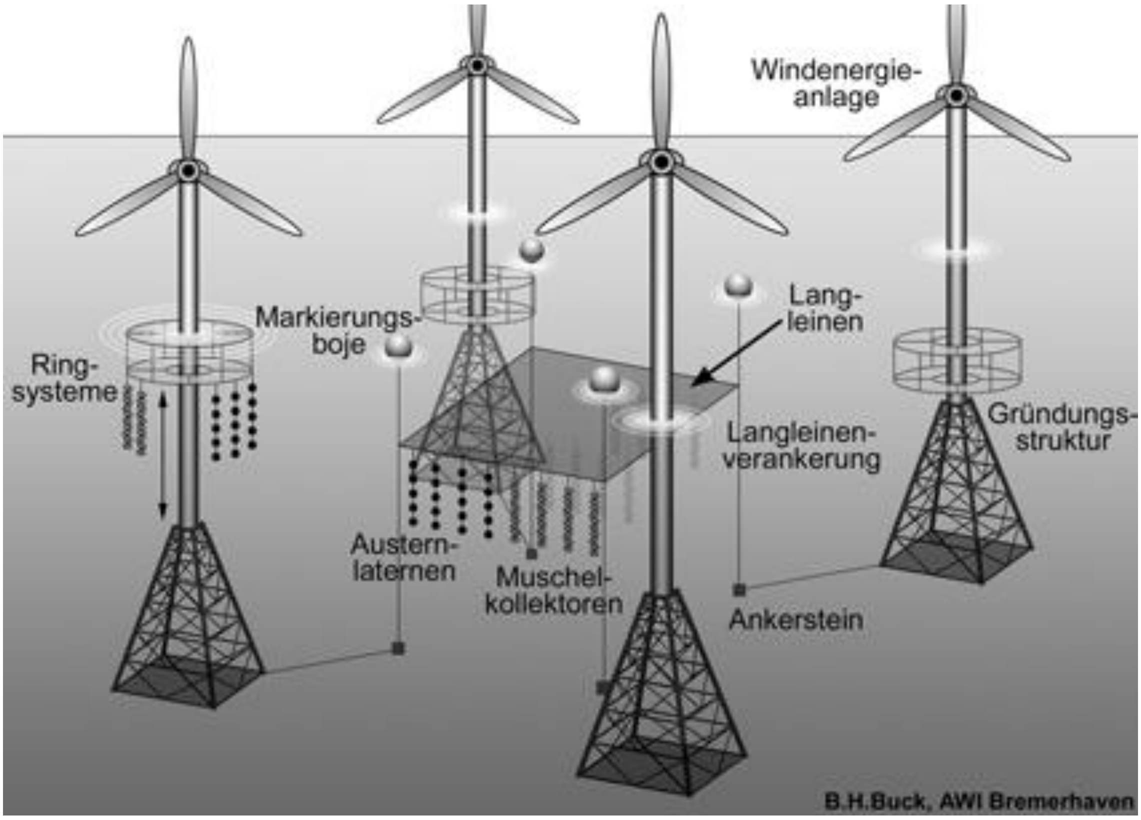

Fig. 1: Ongoing work on mussel culture at wind farms is studying construction configurations that can handle the high-energy offshore environment (Graphic: Buck et al. 2004).

2. First Results

Initial trial results showed that the basic biotic conditions in many parts of the German North Sea support the cultivation of mussels under harsh conditions. Food availability and quality, and larval supply are sufficient. The blue mussel, *Mytilus edulis,* showed excellent growth rates and reached market size within a season and a half (Buck 2007, Walter et al. 2010) (Fig. 2). Furthermore, modified and improved offshore culture techniques can now withstand the environmental forces of the North Sea. However, these new constructions could certainly raise investments costs. All such relevant site selection criteria of potential culture plots should be well known to calculate economic risks.

Fig. 2: In trials, blue mussels reached market size within a season and a half.

2.1. Parasitic Infestations

Research also showed that macro parasites would be an important consideration. Although all known macro parasite species of blue mussels are considered harmless to human consumers, obviously infested mussels lose their economic value. For example, the parasitic copepod *Mytilicola intestinales* is bright red in color, and at up to 10 mm in length, easily visible (Fig. 3). It is simply an aesthetic problem. Interestingly, mussels grown offshore are free of macro parasites, in contrast to bottom-grown mussels in nearshore areas (Buck et al. 2005). The complex life cycle of parasitic trematodes is only completed near shore, where mussels, periwinkles, and sea birds live in close communities. Other parasitic copepods need the long-living natural beds of blue mussels to develop and establish their populations.

Infestations with micro parasites and viruses may follow the same pattern, but in contrast to their larger counterparts, infestations with viruses can provide serious risks to the health of cultured candidates. Although the life cycles of common micro parasites like *Bonamia* or *Marteilia* species are still unknown (OiE 2003), their abundance might also be connected with the natural mussel beds nearshore.

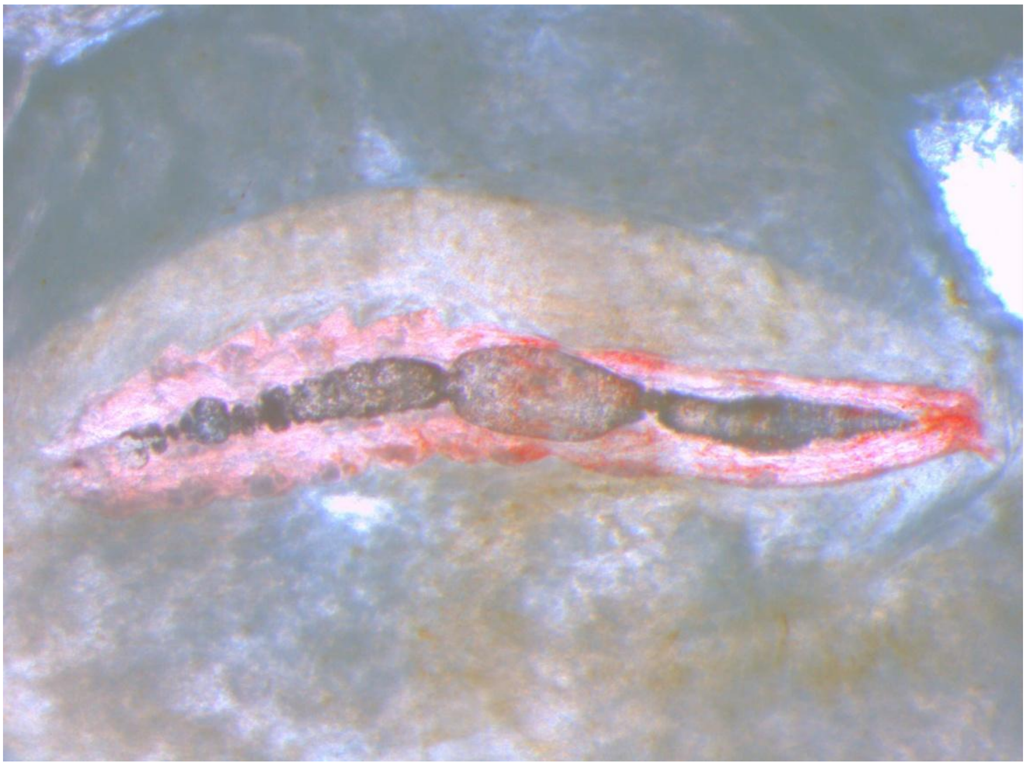

Fig. 3: Although harmless to humans, the brightly colored *Mytilicola intestinales* copepod that affects mussels is easily visible.

2.2. Water Quality

One can assume that offshore areas have lower concentrations of pollutants, pesticides, and near-surface agents due to dilution of urban sewage and estuarine runoffs. In fact, offshore areas provide a maximum supply of clean water with good dissolved-oxygen conditions. Mussels grown under such conditions accumulate fewer toxins and would likely have less stressed immune systems and better overall health than mussels grown in near- and inshore areas. As shown by various European research institutes, these factors result in higher growth rates and yield a qualitatively better product for human consumption.

2.3. Biodiagnostic Tools

In a new approach, the authors are using modern biodiagnostic tools to quickly and inexpensively analyze site conditions and the overall health of mussels cultured in different offshore areas (Cajaraville et al. 1998). With these methods, the tissue of the mussels' digestive systems responsible for food uptake, storage of reserve substances, and detoxification provides a clear signal on the health status of the mussels. For example, the condition of the surrounding membranes of lysosomes is tested. Lysosomes are usually responsible for the recycling of other spent cell organelles and metabolic products, as well as harmful substances that enter the cells. When the storage capacities of lysosomes are overloaded, and cells are stressed by high concentrations of harmful substances, the lysosomal membranes can become unstable and pollutants leak back into the cytoplasm with serious risk for health (Koehler et al. 2002). If membrane stability and the overall health status of mussels are low, more specific tests can elucidate the types and backgrounds of the causal pollution. In contrast, if lysosome membranes are stable, there is strong evidence that individual mussels grow under good water conditions (Brenner et al. 2006).

2.4. Targeted Sites

The implementation of biodiagnostic methodologies to identify potential culture areas within the zones proposed for offshore wind farms is one focus of the new *MytiFit* project financed by the Ministry for Construction, Environment, and Transport in Bremen, Germany, and the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. Other aspects include the quantification of micro and macroparasites, shell stability, and the attachment strength of mussels using different artificial substrates. In a test field located in the vicinity of a planned offshore wind farm about 31 km off the coast at Bremerhaven, three test moorings with large buoyancy will be deployed to test the described parameters in different water depths (Fig. 4). The aim of the project is to accumulate relevant information for the selection of appropriate cultivation sites for mussels in the planned wind farm areas. The prediction of rapid growth and high product quality should compensate for the higher investment costs of the culture systems and help install a functioning offshore aquaculture system in the German Bight (for more details refer to Buck et al. 2007).

Fig. 4: One element of the *MytiFit* project involves an evaluation of blue mussel attachment to varied artificial substrates that could be used for culture at offshore wind farms.

References

Brenner M, Buck BH, Koehler A (2006). Blue mussel culture: Does offshore cultivation lead to increasing product quality? "Linking Tradition and Technology - Highest Quality for the Consumer". The Annual Meeting of the World Aquaculture Society and the European Aquaculture Society, 9.-13. May 2006, Florence, Italy, 116

Buck BH (2002). Open Ocean Aquakultur und Offshore-Windparks. Eine Machbarkeitsstudie über die multifunktionale Nutzung von Offshore-Windparks und Offshore-Marikultur im Raum Nordsee. Berichte zur Polar- und Meeresforschung, 412: 252 pp

Buck BH, Krause G, Rosenthal H (2004). Multifunctional use, environmental regulations and the prospect of offshore co-management: potential for and constraints to extensive open ocean aquaculture development within wind farms in Germany. Ocean Coastal Management, 47: 95–122

Buck BH, Thieltges DW, Walter U, Nehls G, Rosenthal H (2005). Inshore offshore comparison of parasite infestation in *Mytilus edulis*: implications for open ocean aquaculture. Journal of Applied Ichthyology, 21: 107-113

Buck BH, Berg-Pollack A, Assheuer J, Zielinski O, Kassen D (2006). Technical realization of extensive aquaculture constructions in offshore wind farms: consideration of the mechanical loads. In: Proceedings of the 25th international conference on offshore mechanics and Arctic engineering, OMAE 2006: presented at the 25th International conference on offshore mechanics and Arctic engineering, 4.–9. June 2006, Hamburg, Germany/ American Society of Mechanical Engineers, 1–7

Buck BH (2007). Experimental trials on the feasibility of offshore seed production of the mussel Mytilus edulis in the German Bight: installation, technical requirements and environmental conditions. Helgoland Marine Research, 61: 87-101

Buck BH, Koehler A, Brenner M, Stede M (2008). Eignung des Seegebietes am geplanten Offshore-Windpark Nordergründe für die Zucht von Miesmuscheln: Fitness, Parasitisierung und Substratwahl. Endbericht, 91 pp

Cajaraville MP, Cancio I, Orbea A, Lekube X, Marigómez I (1998). Detection, control and monitoring of pollution using early warning cellular biomarkers: conventional and innovative approaches based on biotechnology. Cuad. Invest. Biol., 20: 545-548

Koehler A, Wahl E, Soeffker K (2002). Functional and morphological changes of lysosomes as prognostic biomarkers of toxic liver injury in a marine flatfish (*Platichthys flesus* L.). Environmental Toxicology and Chemistry, 21(11): 2434–2444

OiE (2003). Manual of diagnostic tests for aquatic animals. World Organisation for Animal Health. http://www.oie.int/eng/normes/fmanual/A_index.htm. , assessed January 2009, 7 pp

Tiedemann A (2003). Windenergieparke im Meer - Perspektiven für den umweltverträglichen Einstieg in eine neue Großtechnologie. In: Lozán J, Rachor E, Reise K, Sündermann J, Westernhagen H v (eds). Warnsignale aus Nordsee & Wattenmeer: Eine aktuelle Umweltbilanz. Wissenschaftliche Auswertungen, Hamburg, 142–148

Walter U, Buck BH, Liebezeit G (2010). Larval occurrence and settlement in the German Bight – a trial to estimate potentials for *Mytilus edulis* culture in offshore areas. Aquaculture International (submitted)

Chapter 06

ATTACHMENT PROPERTIES OF BLUE MUSSEL (*MYTILUS EDULIS* L.) BYSSUS THREADS ON CULTURE-BASED ARTIFICIAL COLLECTOR SUBSTRATES

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Abstract

The attachment strength of blue mussels (*Mytilus edulis*) growing under exposed conditions on ten different artificial substrates was measured while assessing microstructure of the applied substrate materials. Fleece-like micro structure attracted especially mussel larvae, however, most settled individuals lost attachment on this type of microstructure with increasing size during the time of experiment. Substrates with thick filaments and long and fixed appendices were less attractive to larvae but provided a better foothold for juvenile mussels as shown by the results of the dislodgement trials. In addition these appendices of substrates could interweave with the mussels, building up a resistant mussel/ substrate conglomerate. Our results show that a mussel byssus apparatus can withstand harsh conditions, if suitable substrates are deployed. Depending on cultivation aims (seed or market sized mussels) and cultivation method (one or two step cultivation), different collector types for larval attraction, good foothold and interweaving abilities or collectors combining these properties should be developed and applied. The study suggests that substrates need to be very precisely tailored according to the major environmental conditions. Furthermore, the results imply that a "one size fits all substrate" may not be the most culture-effective approach, but, in the contrary, substrates need to be changed and modified according to the size of the mussel as they go through their different life cycle stages.

Keywords: spat collectors, offshore aquaculture, offshore wind farms, *Mytilus edulis*, dislodgement

1. Introduction

Commercial fisheries and aquaculture in Europe have long been engaged in the farming of blue mussels (*Mytilus edulis*), a stable food source in this part of the world for many centuries (Smaal 2002). The exploitation of natural mussel beds along the German North Sea coast has a long tradition (Kleinsteuber & Will 1988). Since the 1950s a combined fishery-culture system has developed (Korringa 1976), using the techniques described by Seaman & Ruth (1993). Juveniles are collected with dredges from natural intertidal mussel beds and transferred to licensed culture plots, where environmental conditions are suitable for growth and fattening. However, due to poor recruitment over the last several years in the Wadden Sea (Germany, Denmark and the Netherlands), research commenced in the year 2000 to investigate whether or not suspended culture techniques could be used to obtain seed mussels in an inshore area of the Jade estuary (Walter & Liebezeit 2001; 2003) as well as in offshore sites of the coastal sea and the economic exclusive zone within the German Bight (Buck 2007, Walter et al. 2010).

The development of European mussel farming has taken place nearly exclusively in protected nearshore waters and estuarine habitats. In Germany, however, sheltered locations are rare and because of tremendous stakeholder conflicts the advancement of coastal mussel culture has been rather stagnant (CWSS 2008). As a consequence, aquaculture activities have tended to move to more exposed offshore sites (Ryan 2005). It is widely believed that the commercialization of mariculture in the open ocean has enormous future economic potential (Stickney 1998, Bridger & Costa-Pierce 2003, Buck et al. 2004). In 1998, the University of New Hampshire initiated the Open Ocean Aquaculture Demonstration Project to investigate the commercial potential of environmentally responsible seafood production, employment opportunities, engineering solutions and operational methodologies of offshore aquaculture (Bucklin & Howell 1998). As a subproject Langan and Horton (2003) tested the biological and commercial feasibility of *M. edulis* cultivation with 120 m long submerged test facilities 10 km off the coast of Portsmouth (New Hampshire).

Recently, a new stakeholder is expanding far off the coast – the offshore wind farm industry. Within this new development the concept of combining renewable energy production with the cultivation of different organisms was born and a synergistic resource use in the form of aquaculture and offshore wind farming has been intensively investigated (for review see Buck et al. 2008). This requires a suspended culture technique where mussels settle on artificial substrates, commonly called collectors, attached to a horizontal floating or submerged longline and hanging vertically in the water column (Hickman 1992). Unfortunately, offshore sites in the German Bight are exposed to high waves and strong currents (Becker et al. 1992), requiring techniques and a system design that can withstand these conditions (Langan & Horton 2003, Buck 2007) while at the same time support settling and good growth of cultivated mussels as well as preventing detachment and subsequent loss. Settlement of mussels on substrates is defined by Connell (1985) as being the point when larvae first take up a durable residence and, if necessary during inhospitable conditions, allowing a detachment and a subsequent second residence on another substrate (Alfaro et al. 2006). Following the compilation by Filgueira et al. (2007) (Tab. 1) the attachment behaviour of mussel threads on a substratum is quite manifold and is affected by (1) the composition of material, (2) surface condition, (3) exposure to stress conditions and (4) type of spat collectors used for aquaculture. Furthermore, fouling organisms such as barnacles, bryozoans and seaweeds may increase mussel mortality by increasing the mussel’s weight as well as its drag (Witman & Suchanek 1984), which results in an increased risk of detachment by wave action and tidal currents.

Parameters which increase the hydrodynamic forces exerted on mussels will be important factors when considering mussel cultivation in offshore environments, since they will alter the probability that mussels will be dislodged. Thus, the survival of mussels is to a large extent dependent on their ability to form a strong attachment in order to withstand large forces imparted by wave shock and surge (Witman & Suchanek 1984) as well as current velocity (Price 1982, Bell & Gosline 1997).

Table 1: Compilation of parameters investigated having an effect on larval settlement and post-mortality (modified after Filgueira et al. 2007).

Characteristics	parameter	data resources
composition	surface free energy	Nishida et al. 2003
	wettability	Alfred et al. 2005
	polarity	Hansen & Waite 1991
	associated fouling	Alfaro et al. 2006
structural properties	surface area	Walter & Liebezeit 2003
	thickness of filaments	Alfaro and Jeffs 2002 Lekang et al. 2003
post-mortality	hydrodynamic disturbance	Eckman 1987 McShane & Nylor 1995
	competition for space and food	Guiñez & Castilla 1999 Guiñez 2005
	predation	Schiel 2004 Morrisey et al. 2006
	intraspecific competition	Filgueira et al. 2007
characteristics of spat collector ropes	strength of seed attachment (visual judgement only)	Lekang et al. 2003
	structural complexity to reduce predation	Moreno 1995 Filgueira et al. 2007 Walters & Wethey 1996 Frandsen & Dolmer 2002
	high density in ropes with filamentous loops	Filgueira et al. 2007
	high yield in ropes with non-filamentous loops	Filgueira et al. 2007
	combination of spat collecting devices until harvesting	Bomo et al. 1998

With the exception of studies by Walter and Liebezeit (2003) and Lekang et al. (2003) on aspects such as settlement success on different substrates, growth performance and shaking resistance (visually), the attachment and detachment strength of mussel threads on spat collectors in high energy environments has remained unexplored. The aim of this work is to gain insight into the attachment strength of mussels on various materials enabling them to withstand strong environmental conditions, for optimal cultivation at exposed sites. Therefore we examine the effectivity of spat collectors under exposed conditions by investigating the following: (i) the attachment of mussel thread plaques on different materials, (ii) the number of threads mussels use to attach to a certain substrate, and (iii) the force necessary to dislodge the mussels from a given artificial substrate.

2. Material and Methods

2.1. Experimental configuration

The experiments were conducted with cultivated blue mussels (*M. edulis*) settled on artificial spat collectors. These spat collectors originated from a testing area located in a high energy environment at the outer Jade estuary in the German Bight (53° 35’ 05.12’’ N, 08° 09’ 14.57’’ E, North Sea, Fig. 1).

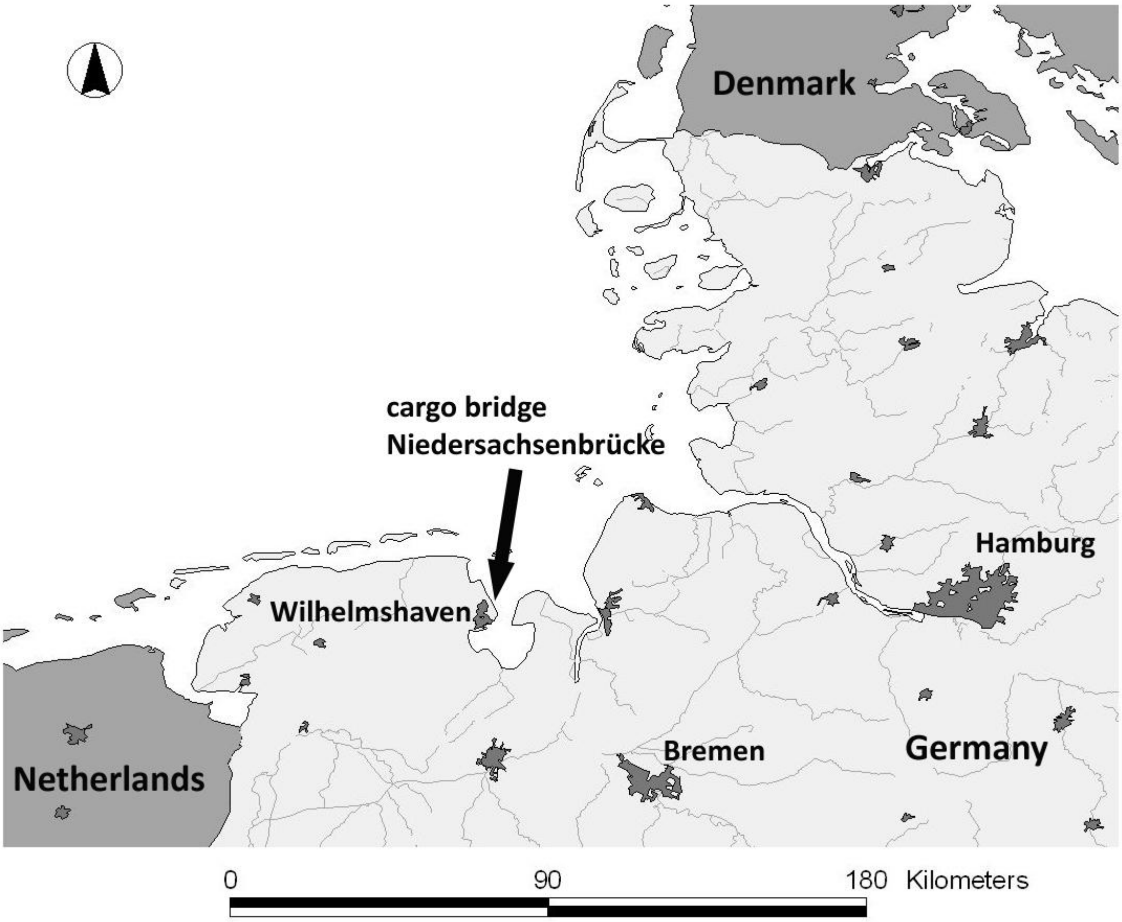


Fig. 1: Map of the German Bight showing the test site at the cargo bridge *Niedersachsenbrücke* near the city of Wilhelmshaven.

The test collector harnesses were suspended loose-hanging from a cargo bridge (Coal Cargo Bridge *Niedersachsenbrücke* of the Niedersachsen Ports GmbH & Co. KG) approx. 1,300 m off the coast in the vicinity of a waterway (Fig. 2a). A total of 10 harnesses were hung below the jetty allowing a distance of 20 m between harnesses (Fig. 2b). Each harness consisted of a 20 mm polypropylene rope with an iron plate (5 kg) at its distal end, weighing down the substrates into the water column even at strong current velocities (Fig. 2c). The upper part of the harness was attached to a steel beam between the pillars of the bridge. The polypropylene (pp) rope was strengthened with two swirls and shackles to prevent entanglement. The lower swirl was placed one meter above the mean high water (mHW) level to prevent corrosion and fouling. To insure that the cultivated mussels were submerged during the whole of the experiment, samples of artificial test collectors were attached to the pp-rope from one to approximately three meters below mean low water (mLW) level using sea water- and uv-resistant plastic binders. The deployed substrate samples were about 15 cm in length and fixed every 20 cm to the rope. All harnesses equipped with the collector samples were hung from the bridge before the spawning time of mussels in mid April to early May 2007 (Pulfrich 1997), and remained there for 16 months until the mussels were harvested in August 2008.

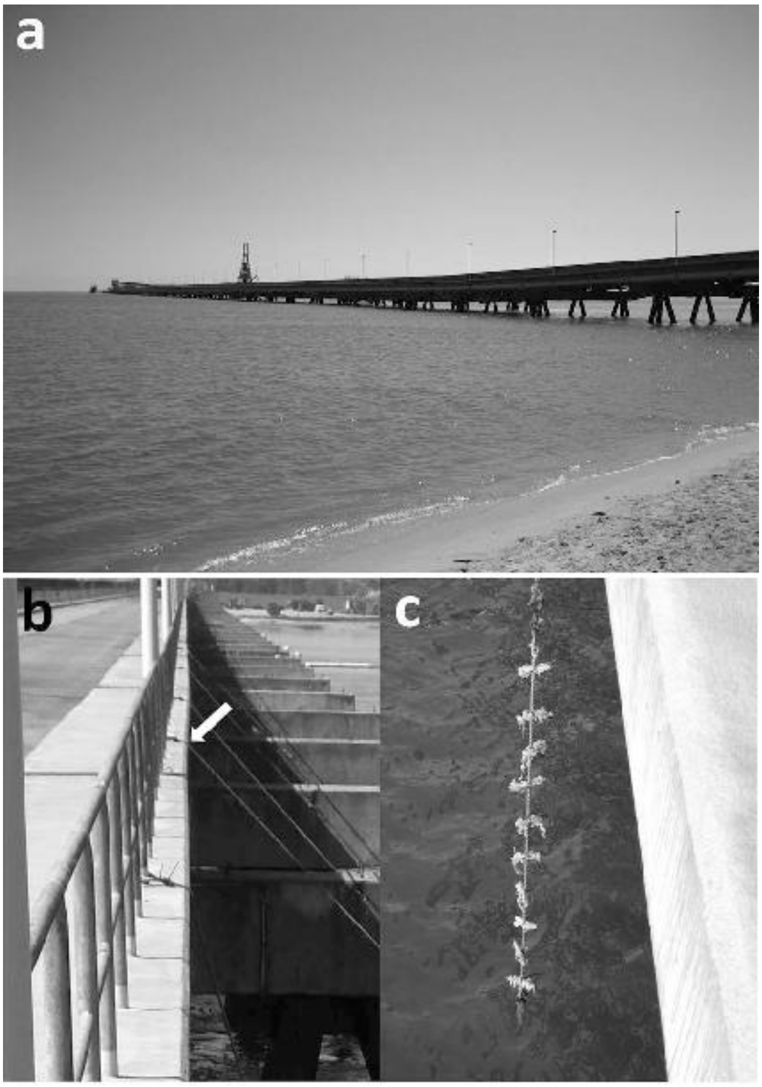


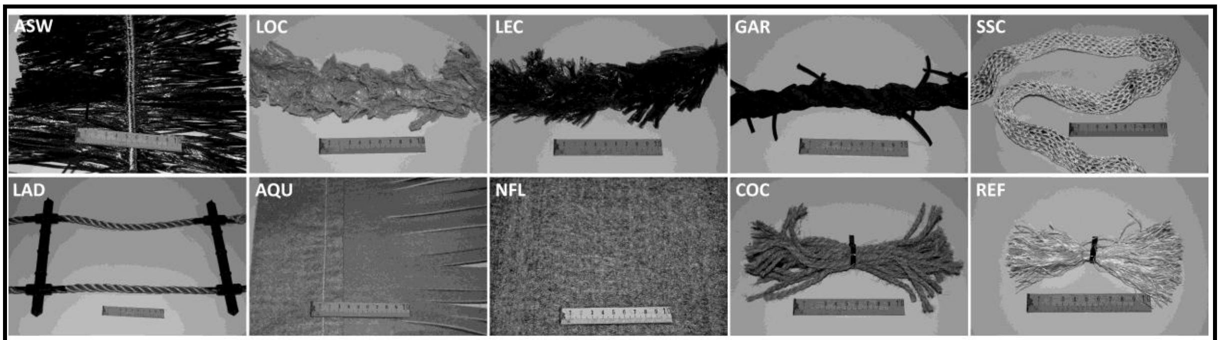
Fig. 2a-c: (a) The 1,300 m long cargo bridge at the Jade estuary, (b) harnesses attached to a steel beam, hanging between the pillars of the bridge (see white arrow), each of them consisting of (c) a 20 mm rope carrying substrate samples and a weight at the distal end.

Samples were retrieved one day prior to the start of the testing by lifting the harnesses, including the mussel collectors, by hand to the pavement of the bridge. Collector samples were separated from the rope by cutting the binders and stored dry for 1.5 hours in cool boxes for transportation. Back at the laboratory collector samples were transferred to a seawater recirculation system of about 15 °C where they stayed until the testing started the following day.

2.2. Collector materials used

10 different spat collector materials, both synthetic and natural, were used in this experiment (Tab. 2).

Table 2: Spat collector types used for this experiment.



No.	shortcut	name	characteristics	origin
(1)	ASW	*Artificial Seaweed*	10 mm nylon rope as back bone with 10 cm long pp-leaves attached at both sites	Japan
(2)	LOC	*Leaded Christmas Tree*	extruded polypropylene with a straight trim, strands of lead in the center help sinking	New Zealand
(3)	LEC	*Looped Christmas Tree*	extruded polypropylene with a looped trim, strands of lead in the center help sinking	New Zealand
(4)	GAR	*Galician Rope*	rough surfaced nylon-pe ropes with strands	Spain
(5)	SSC	*Self-Sinking Collector*	polyester net formed as a tube, small stones help sinking	Norway
(6)	LAD	*Ladder Collector*	16 mm parallel running pp-ropes connected every 35 cm by a plastic bar	Norway
(7)	AQU	*Aquamats®*	strands of pp fleece material with ballast sleeve	USA
(8)	NFL	*Naue® Fleece*	pp fleece, cost-saving alternative to AQU,	Germany
(9)	COC	*Coconut Rope*	24 mm rope of coconut fibres	India
(10)	REF	*Reference Collector*	bushy tufts of a unravelled 10 mm pp-rope	Germany

Five of these collectors are patent-registered and are used at commercial mussel cultivation sites in nearshore environments: (1) The *Artificial Seaweed* (ASW) is a Japanese patent. It consists of a 10 mm nylon rope as its back bone to which 10 cm long polypropylene (pp)-leaves are attached perpendicularly at both sites. Each centimetre of back bone holds 20 pp-leaves to enlarge the substrate's surface. (2, 3) The *Christmas Tree Ropes* are registered patents in New Zealand. These collectors are used on commercial farms in the Netherlands as well as in New Zealand. The ropes are made from black extruded polypropylene with straight (**le**aded) or **lo**oped appendices of ca. 5 cm length (LEC, LOC). Both collector types have three strands of lead in their center to support sinking. (4) The *Galician Rope* (GAR) is patented and produced in Spain. It consists of three nylon-polyethylene (pe) strands added with transversal filaments of the same material and plastic pegs fixed to the center rope every 5 and 25 cm, respectively. (5) The *Self-Sinking Collector* (SSC) is a Norwegian patent made of polyester net designed as a tube of approx. 3 cm in diameter. Inside the tube are small stones to ensure sinking. (6) The *Ladder* (LAD) collector is also a Norwegian patent displaying two 16 mm thick parallel running pp-ropes, connected to each other every 35 cm by a plastic bar. With the

exception of the GAR which is designed to be used as a single dropper from Galician raft cultures, all other described collector types can be deployed vertically from longline constructions as droppers or looped form end-to-end of the longline as a continuous rope. Additionally, all collector types are known to withstand the mechanical harvesting of either seed or adult mussels and can be reused several times. Two further spat collectors were tested, both consisted of fleece material: (7) *Aquamats*® (AQU) consists of pp-fleece strands of ca. 30 cm length ending at a ballast sleeve of the same material. The patented *Aquamats*® (USA) was originally used for shrimp cultivation. Weights in the ballast sleeve guarantee its use on the bottom of a shrimp tank or pond thereby separating the growing area into smaller compounds. Spatial separation and places to hide for juvenile animals help to decrease stress and adult predation. *Aquamats*® are a favoured substrate for fouling organisms, a reason for the use of this collector material in this experiment. (8) Another pp-fleece, the *Naue*® Fleece (NFL), is used to produce e.g. shore protection bags. These bags are known to be fully covered by different fouling organisms, especially by mussels after a few months (*Naue*®, pers. comm.). The structure of this material is similar to *Aquamats*® but not as costly. Representative of a natural material (9), a coconut rope (COC) was tested as well. Coconut ropes are used in France for spat collection in the traditional pole (“bouchot”) method. Finally, a (10) reference collector (REF) was also suspended in the water. It consists of bushy tufts of an unravelled 10 mm pp-rope. This collector type has been used in several studies to investigate settlement success of *Mytilus* larvae (e.g. Alfaro & Jeffs 2003, Buck 2007, Walter et al. 2010) and can help to compare the results of this study to other investigations.
The AQU and NFL substrates are fabricated as mats. For the experiments pieces of 15 cm x 10 cm and 15 cm x 5 cm were used, respectively. To test the LAD substrate a piece of rope between two pegs was cut out and fixed to the harness. The self sinking substrate (SSC) was cut in pieces and the two endings of the nylon tube were sealed before fixing the sample to the harness. Like this the woven fabric of SSC did not unravel and stones (helping the substrate to sink) were kept inside the tube. All other substrates were just cut in 15 cm pieces before they were deployed on the harness ropes. Samples of all substrates were fixed crosswise to the vertically hanging harness ropes. Like this chafing between sample and harness could be reduced and the whole surface of the substrate sample was accessible by mussel larvae.

2.3. Measurement of current velocity

Current velocity was measured by using a RCM 7 current meter (*Aandera*® Instruments), which was deployed in the vicinity of the collectors suspended in the water column. The tidal regime was bi-directional (NNW-SSE) and highest current speeds were measured three hours after slack time, independently from seasonal influence. Data were logged for a short time (eight weeks) from 30th August to 25th October 2007.

2.4. Measurement of dislodgement force on mussel byssus threads

To measure the dislodgement force a digital force gauge (FH 10, *Sauter*®, Germany) with a sensory range from 0 – 11 N was deployed, hanging vertically from the top of a wooden frame (1 m in height, Fig. 3a). The force gauge was equipped with a clamp to pull a single mussel (Fig. 3b) from the respective collector, which was mounted at the bottom of the wooden frame on an acrylic glass plate.

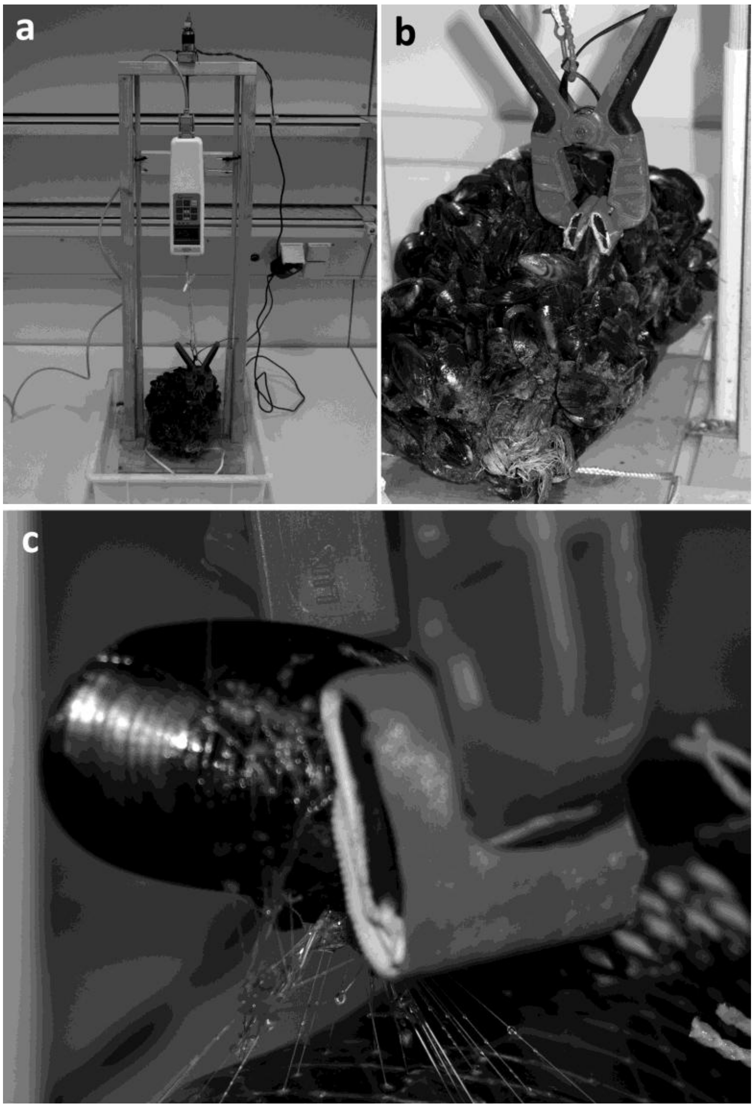


Fig. 3a-c: (a) Dislodgement device with force gauge, (b) sample and clamp to pick up a single mussel showing (c) the number of byssus threads by lifting up the clamp.

Data on dislodgement force were recorded by lifting the gauge at a constant and slow velocity using an electric motor until byssus threads or plaques were ruptured. Data were recorded at intervals of 0.1 seconds over the whole dislodgement process. The maximum load of the gauge was limited to 10.5 N (11 N minus 0.5 N weight of the clamp). If the maximum value was achieved while dislodgement process was conducted the dislodgement force was denoted as 10.5 N. Once a mussel was fixed to the clamp of the gauge, the dislodgement procedure was started by gently lifting the clamp until single byssus threads were visible (Fig. 3c). The numbers of threads were counted thereafter and the location of the byssus plaque attachment was determined. Byssus attachments were classified into three categories: (1) all byssus attached to the collector surface, (2) byssus threads attached to the shell of other mussels and to the collector substrate and (3) all byssus threads attached to the surface of other mussels. Subsequently, the dislodgement was conducted and the final force measured and recorded. For all three categories approx. 30 mussels were lifted to break the threads.

2.5. Assessment of substrate microstructure

To evaluate the appropriateness of the collector surfaces for suitable attachment of byssus plaques, the microstructures of all substrates were investigated using a Scanning Electron Microscope (SEM). First, photos of all collector materials were taken prior to the experiment. All substrates were then exposed for 1-3 weeks below the jetty during the spawning event. Once a week samples of each substrate were taken, dried and examined for post larval settlement using a binocular microscope (magnification 50-fold) before SEM photos were taken. Finally, the substrates were dried (24 hours at 60 °C) after the dislodgement experiment and SEM photos were taken of the remaining byssus plagues. For comparability all photos were taken under 100-fold magnification.

2.6. Statistical analysis of data

In this study, data on dislodgement forces were compared between the ten collector types by Kruskal-Wallis one-way ANOVA followed by Dunn's Multiple Comparisons test using the *Prism*® 4 software. Arithmetic mean values and standard deviations were calculated for data on shell morphology (length, width, and height) and byssal numbers as well as for hydrodynamic parameters (current velocity) using *MS Excel*® 2007.

3. Results

3.1 General observations on larval settlement during grow-out

During the grow-out phase from April 2006 to August 2008 larval settlement was observed and artificial collectors inspected for e.g. entanglements and abrasions at least once a month. A detailed examination of the substrate-mussel sample was conducted before samples were placed in the dislodgement device. Preliminary observations at this stage were that both fleece-like substrates, AQU and NFL, were fully covered with well attached juvenile mussels (≤ 5 mm) in late June of the first season, whereas all other substrates (ASW, LEC, LOC, GAR, COC, LAD, REF and SSC) had fewer individuals attached to the surface. Filamentous fringes of the substrates ASW, LEC, LOC, GAR, COC, and REF were partly free of larvae, observed visually. Regarding the rope-like collectors (ASW, LEC, LOC, GAR, COC, and REF), all post larvae settled on or in the vicinity of the central part of the collector, but were equally distributed on fleece (NFL) and on tubular substrates (SSC), however, in the latter case in lower numbers. AQU showed intermediate results due to its slit-like character. Later in the year and especially after the first storm events in autumn, the number of juvenile mussels on fleece and tubular substrates (AQU, NFL and SSC) decreased tremendously, but seemed to stabilize on the filamentous substrates (ASW, LEC, LOC, GAR, and REF). The COC-collector lost most of its filaments during the experiment due to an improper fixation by the plastic binders, resulting in an inaccurate interpretation of the development of post larval numbers.

In spring 2008 mussels on the filamentous substrates were heavily entangled with the collectors' fringes and formed conglomerated compact units (ASW and GAR). Conglomerates were also built up within the substrate-mussels units on LOC, LEC and REF, but only to a minor extent, due to the short fringes. NFL, LAD and SSC lost most of the mussels and the remaining mussels seemed to migrate to the backbone rope of the harness. However, the loss of mussels was not as strong as on the AQU where the bands of the substrate helped to interweave mussels and substrate.

3.2. Hydrodynamic conditions

The current conditions at the cargo bridge in the Jade estuary varied in a diurnal cycle during periods of measurement due to the local tidal regime. The mussels encountered rapid tidal currents flowing in one direction for some hours (S to SE), slowing and stopping at slack tide, and then flowing rapidly in the opposite direction (N-NW). Thus, the cultivated mussels experienced a wide range of current velocities ranging from 0 to 1.42 $m \cdot s^{-1}$ (Fig. 4). The mean significant wave height was not measured during the experiment, but should not have exceeded 1.2 m in height (GKSS 2006).

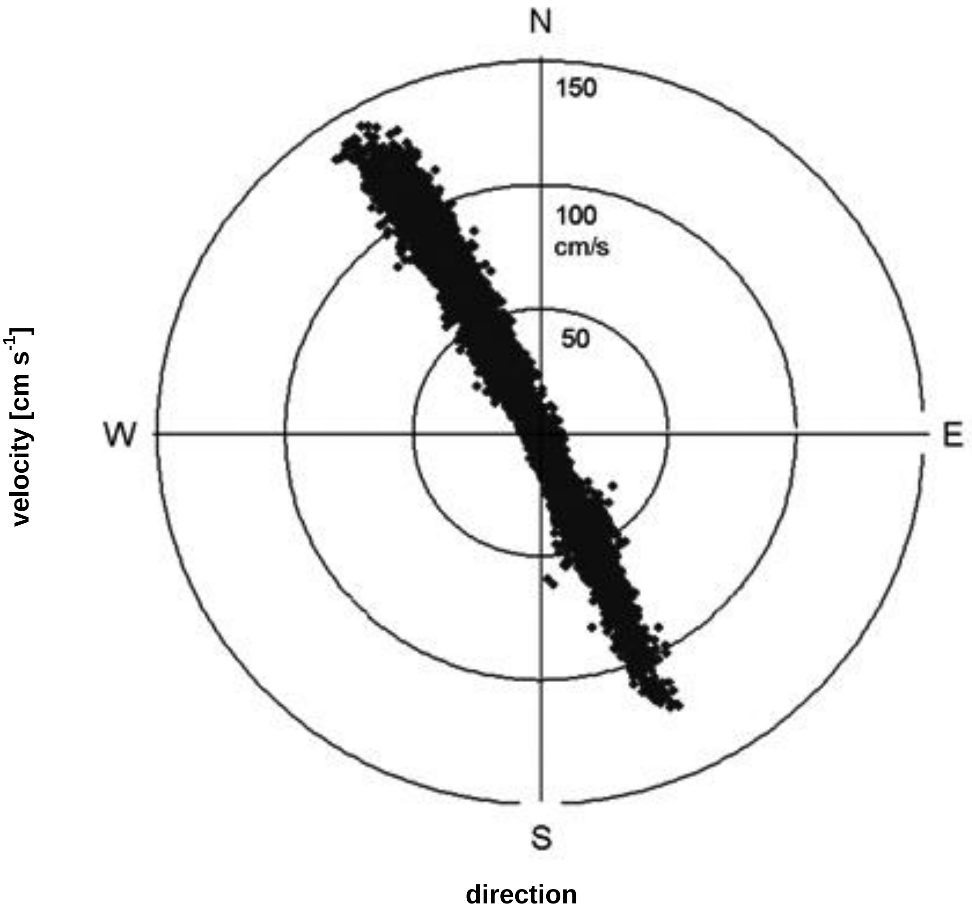


Fig. 4: Direction and velocity of tidal currents in the Jade estuary measured with *Aandera* RCM7 during 8 weeks from August to October 2007 at the cargo bridge.

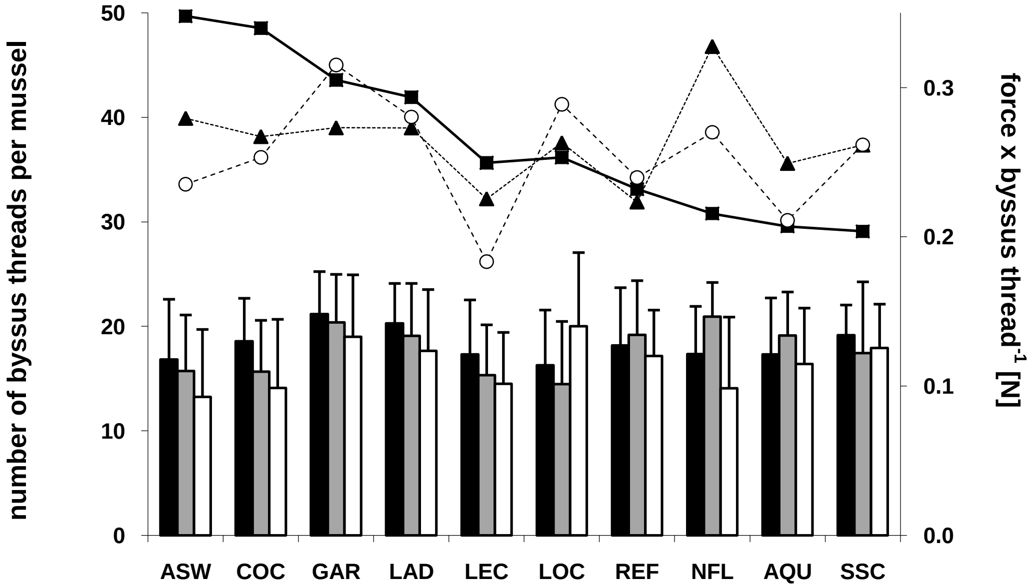
3.3. Morphometric and byssal analysis of *M. edulis* settling on the collectors

Mussels harvested at the end of the experiment prior to the drag experiment reached a size of 4.61 ± 0.28 cm (ML ± SD) in length ranging from 4.12 to 5.08 cm, 2.22 ± 0.15 cm (MW ± SD) in width ranging from 1.93 to 2.56 cm, and 1.67 ± 0.13 cm (MH ± SD) in height ranging from 1.42 to 1.93 cm (n = 35). The number of byssus threads per mussel varied between 13.24 ± 6.48 SD and 21.17 ± 4.09 SD. The bars in Figure 5 show the mean numbers of byssus threads of a single mussel fixed only to the substrate (zone 1), to neighbouring mussels (zone 3) or to both (zone 2), respectively.

3.4. Dislodgement force

The graphs showing dislodgement forces (Fig. 5a) indicate the mean maximum dislodgement-force [N] per byssus thread and substrate of mussels from zone 1 to 3. The dislodgement force of mussels from zones 2 and 3 vary around 0.25 N per thread, while the force per thread of mussels from zone 1 decrease continuously from filamentous substrates with long fringes (ASW = 0.35 N; GAR = 0.31 N), over filamentous substrates with shorter fringes (LEC = 0.25 N; LOC = 0.25 N), to fleece-like substrates (NFL = 0.22 N; AQU = 0.21 N) and shows the lowest force for the tubular substrate (SSC = 0.20 N).

a



b

	ASW	COC	GAR	LAD	LEC	LOC	REF	NFL	AQU	SSC
zone 1										
mean force per thread [N]	0.35	0.34	0.31	0.29	0.25	0.25	0.23	0.22	0.21	0.20
standard deviation	0.12	0.13	0.09	0.13	0.06	0.09	0.08	0.07	0.08	0.07
zone 2										
mean force per thread [N]	0.28	0.27	0.27	0.27	0.23	0.26	0.22	0.33	0.25	0.26
standard deviation	0.08	0.10	0.10	0.11	0.06	0.09	0.08	0.10	0.12	0.12
zone 3										
mean force per thread [N]	0.24	0.25	0.32	0.28	0.18	0.29	0.24	0.27	0.21	0.26
standard deviation	0.11	0.15	0.08	0.12	0.04	0.08	0.09	0.12	0.07	0.08

Fig. 5a/b: (a) Mean number of byssus threads per mussel and substrate (shown as bars) divided in three zones. Zone 1 (black): all byssus threads were attached to the collector's substrate; zone 2 (grey): byssus threads are attached both to the collector substrate and to the surface of mussels; zone 3 (white): all byssus threads are attached to mussels. Graphs: Mean maximum dislodgement-force [N] per byssus thread and substrate of mussel from zone 1 (square); 2 (triangle) and 3 (circle). To calculate mean number of byssus threads and mean maximum dislodgement force per byssus thread of each substrate and zone, numbers of investigated individuals were as follows (zone 1/zone 2/zone 3): ASW: 25/23/25; COC: 14/24/26; GAR: 30/32/7; LAD: 24/22/12; LEC: 30/25/4; LOC: 26/26/2; REF: 27/23/17; NFL: 14/16/12; AQU: 29/23/18; SSC: 12/18/14. Differences between GAR and REF and GAR and AQU for zone 1 were significant ($p < 0.05$ and 0.01, respectively). For zone 2 differences between LEC, LOC and NFL were significant ($p < 0.01$ and 0.05, respectively). (b) Table shows the mean maximum dislodgement forces [N] per byssus thread and the respective standard deviations for zone 1, 2 and 3.

It can be demonstrated that the substrates with the higher dislodgement values in zone 1 (ASW, COC, GAR, LAD and LEC) exceed their particular value of zone 2 and 3 (except GAR) while at the same time the five substrates with the lower dislodgement value in zone 1 (LOC, REF, NFL, AQU and SSC) are consequently below the respective forces determined for zone 2 and 3 (Fig. 5a). Only a small number of mussels were solely attached to other mussels (zone 3) for GAR/ LEC/ LOC (n = 7/4/2, respectively). The standard deviations of each mean force per byssus thread (calculated as maximum forces measured divided by the respective number of threads) is displayed in the table of Figure 5 b.Significant differences were detected for zone 1 between GAR and REF ($p < 0.01$) and between GAR and AQU ($p < 0.05$). For zone 2 LEC and LOC differed significantly from NFL ($p < 0.01$ and 0.05, respectively). Zone 3 was not tested for differences.
The materials used to fabric the different substrates are displayed in Tab. 2. The highest dislodgement forces were achieved from substrates made of a mixture of nylon and pp-fibres (ASW), natural fibres (COC) or a mixture of nylon and pe-fibres. These three substrates are followed by the group of collectors fabricated of pp-fibres (LAD, LEC, LOC, REF, NFL, and AQU). Lowest dislodgement forces were obtained using polyester fibres (SSC).

3.5. Microstructure of Substrates
The EM-photos of ASW display a plane surface of the substrate with elongated fissures within the pp leaves (Fig. 6.1a). The mussel post larva is attached to the substrate's surface without being entangled (Fig. 6.1b). The plague of the byssus thread has full contact to the substrate (Fig. 6.1c). Figures 6.2a-c show the slightly rough filaments of the coconut fibre (COC), the attached post larva and byssus plagues with full contact to the substrate. The plaited filament of GAR is shown as an unused substrate in Figure 6.3a. Figures 6.3b&c show the deeply rooted post larva and the byssus plague with full contact on the relatively rough material profile. Filaments of LAD are displayed in Figure 6.4a. The attached post larva of the LAD substrate is shown in Figure 6.4b and the byssus plague with full contact around a single filament is shown in Figure 6.4c. Figures 6.5a-c show the split filaments of the unused LEC-substrate, the attached post larva and the byssus plague bridging the split for full contact to the substrate.
Similarly to the leaded rope, LOC filaments are split lengthwise (Fig. 7.1a) and larvae's byssus plague have to bridge the splits for full contact to the substrate (Fig. 7.1c). The REF-substrate is displayed in Figure 7.2a, with an attached post larva (Fig. 7.2b) and the byssus plague having full contact around a single filament (Fig. 7.2c). Figures 7.3a-c show the very thin and loosely interwoven filaments of NFL as unused substrate (Fig. 7.3a), the deep rooted post larva (Fig. 7.3b), and three byssus plagues (Fig. 7.3c) with a relatively small contact area to the substrate, due to the felt structure. Likewise NFL, AQU (Fig. 7.4a-c) show the same felt-like structure of thin and loosely interwoven filaments (Fig. 7.4a), a deeply rooted post larva (Fig. 7.4b), and byssus plagues not having full contact to the substrate (Fig. 7.4c). The twisted nylon filaments of SSC are shown as unused substrate (Fig. 7.5a), with an attached post larva (Fig. 7.5b) and the byssus plague on the substrate with full contact (Fig. 7.5c).

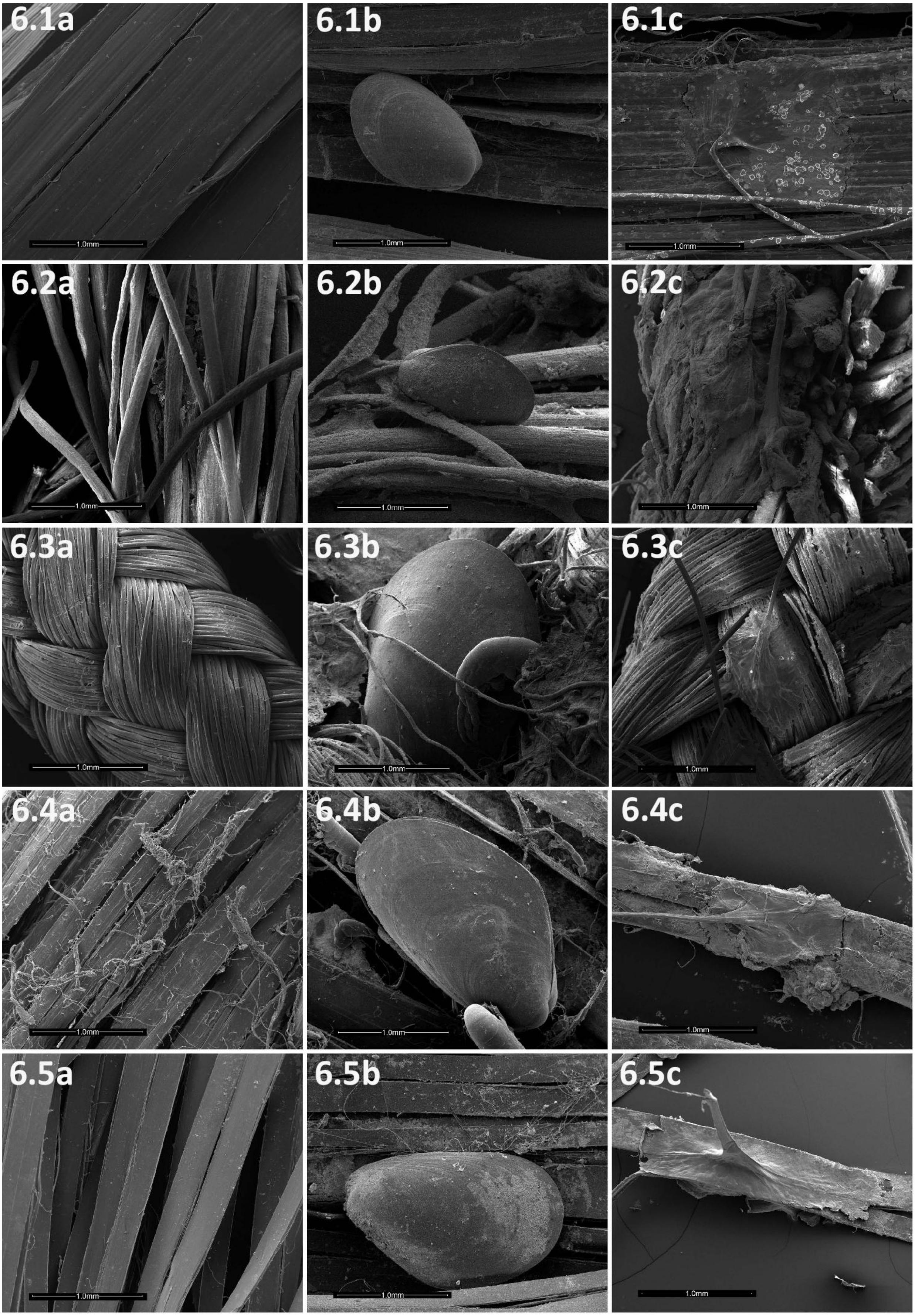


Fig. 6.1-6.5a-c: EM-Photos of (1) ASW, (2) COC, (2) GAR, (3) LAD, and (4) LEC as substrate (a), with attached post larvae (b) and remaining byssus plagues (c) using 100 fold magnification.

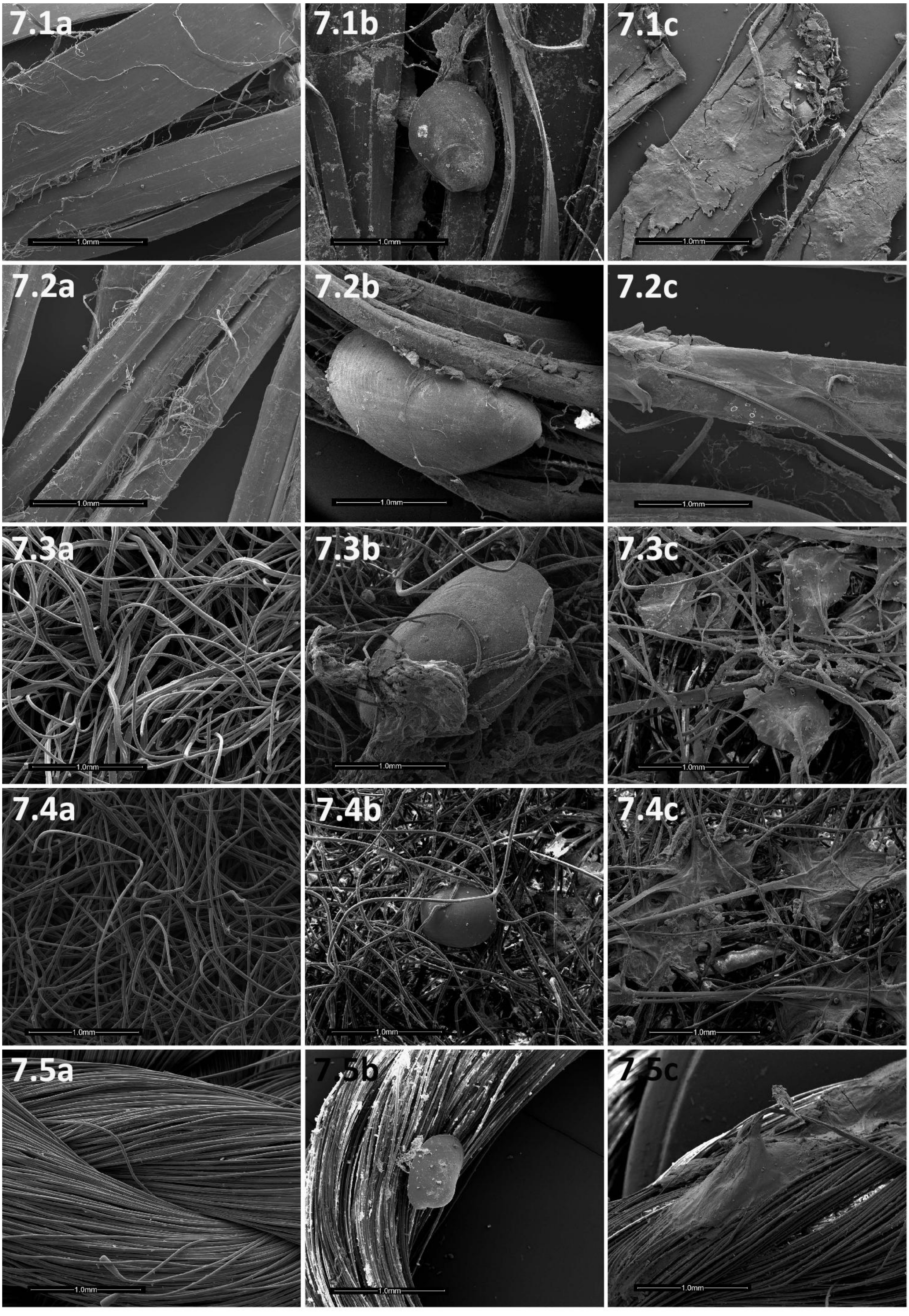


Fig. 7.1-7.5a-c: EM-Photos of (1) LOC, (2) REF, (3) AQU, (4) NFL, and (5) SSC as substrate (a), with attached post larvae (b) and remaining byssus plagues (c) using 100 fold magnification.

With the exception of zone 1 where mussels have only contact to the artificial substrates, mussels build conglomerates by gluing their byssus plagues on the shells of other mussels in their vicinity. Exemplarily, figure 8 shows the slightly corrugated surface of a mussel shell with byssus plague attached to it with full contact.

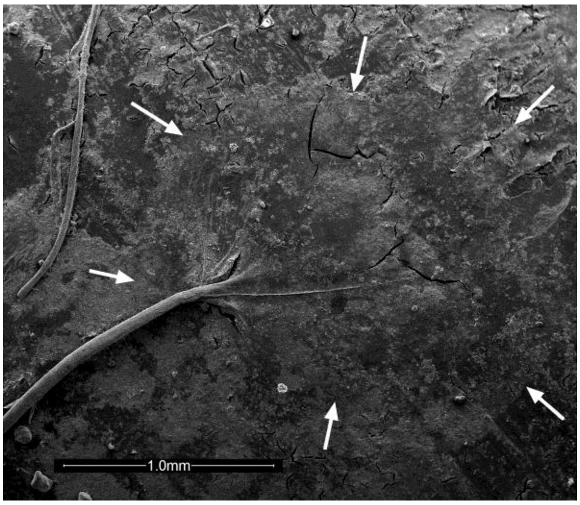


Fig. 8: EM-Photo of a byssus plague attached to a mussel shell using 100 fold magnification.

4. Discussion

The selection of a suitable site is one of the most important steps toward making a mussel cultivation enterprise profitable, since it is directly linked to the success or failure of a mussel culture business. While site selection depends upon a wide range of criteria, one important parameter is the prevailing hydrodynamic conditions of the local environment. Forces originating from currents, waves, and swell stress the culture candidate as well as the system design and therefore have to be taken into account as an initial consideration. This is especially valid when considering cultivation in high energy environments.

In this study the mechanical forces necessary to dislodge or break byssus threads were investigated. Such research has been thus far concentrated on culture systems situated in protected bays or rias (Lekang et al. 2003, Filgueira et al. 2007) or the applied method produced no comparable data, since attachment strength was tested visually by shacking the collector ropes (Lekang et al. 2003).

The results of our experiment showed that there were at least two main factors affecting the suitability of artificial substrates for the commercially successful cultivation of blue mussels under exposed conditions. First, there were significant differences among the tested collector types regarding the forces necessary to dislodge a single mussel from the substrate. Second, there was a difference in the resulting spatial structure of the substrates-mussel conglomerates, according to number, form and length of the substrates' filaments. The latter factor is especially important when mussels grow in various layers around a certain substrate. The longer and more numerous the filaments or appendices of a substrate are, the better the interweaving of mussels with the substrate is even at the outer regions. In the following, we discuss the implications of our experiment in detail and offer advice for substrate development and application for potential farmers.

4.1. Attachment to the Substrates

Commercially applied filamentous substrates and natural coconut fibres followed by fleece- or felt-like substrates, provided the best adhesive conditions for mussel attachment to substrates. The tube-like structure of the self sinking collector provided the weakest adhesive condition. Concerning the materials used to fabric the substrates best adhesive conditions were obtained by nylon-pe, nylon-pp fabrics or by collectors made of natural fibres. Second best results were achieved by the group of substrates made off pp-fibres. The weakest attachment was measured using polyester as substrate material.

Interestingly, the dislodgement forces for the best four substrates were even higher than for mussel shells, which represent the natural substrate of *M. edulis*. The SEM photos (Fig. 6 & 7) of byssus plagues show full contact on or around the more or less plane surfaces of the filamentous substrates. In contrast byssus plagues on fleece substrates had only partial contact to the thin filaments. Most of the potential contact area is needed to bridge gaps between the filaments. Further, fleece filaments are only interwoven, not welded or glued together. It was observed that the impinging forces on mussels and their plagues dissociate the respective filaments from the fleece fabric, thus decreasing the foothold of the mussel. Dislodged mussels from fleece substrates were observed having remains of fleece filaments on the plagues of the byssus threads. All filamentous substrates still had numerous adult mussels after 16 months of exposure (Brenner, unpublished data), whereas the mat-like fleece (NFL) and the tubular SSC lost most of their mussel load. These findings have important implications for cultivation management, since different substrates should be used according to the desired product (seed or market sized mussels), or depending on the type of cultivation approach (one or two step). The filamentous substrates build strongly interwoven conglomerates with the mussels. Depending on the length of appendices and their number per cm collector length, even mussels from the outer region of the conglomerate had contact to the substrate in the centre. In contrast spatially unstructured substrates provide fixation only for mussels close to the inner part of the artificial substrate. This can be explained by the fact that mussel conglomerates on collectors are multi-layered, leading to a different attachment degree of mussels. While mussels in the central region of the collector are all attached to the substratum, individuals in the periphery are only attached to their neighbouring mussels or only partly attached to the substrate. The same results were observed in the central and peripheral regions in mussel beds by Dayton (1971) and Witman and Suchanek (1984). These studies showed that mussels attached to conspecifics were more easily dislodged than those attached directly to the substratum. On the other hand, mussels in the outer regions are more exposed to high energy conditions in the currents, waves and swell, which should stimulate the build up of a higher number and more stable threads. However, our experiment show in concordance with other scholars, that the shells of the mussels in the periphery were more overgrown by epiphytes (e.g. barnacles, polychaetes, bryozoans) and therefore experienced more forces than their neighbouring mussels in the central part of the conglomerates (Witman & Suchanek 1984).

Interestingly, the settlement of post larvae on the substrate differed from the settlement of juvenile or adult mussels. In early summer fleece-like substrates were covered with small larvae (≥5 mm) (Brenner, unpublished data). In the beginning of the settlement process, drag force on larvae should be low, since they were deeply rooted into the fleece-substrates. These results correspond only with the first part of the primary/ secondary settlement model of Bayne (1964), postulating a preference of mussel larvae for filamentous substrates. The loss of juvenile mussels later in the year due to a second larval phase seems, however, not likely since the loss of mussels occurred in August to September, where mussels are already too big in size for a second larval phase. Once they grow in length and weight, drag increases (Harger 1970). By autumn fleece substrates had already lost most of their mussels. On filamentous substrates fewer post larvae were found in the beginning of settlement process, and were oriented towards the core of the substrate. While growing over time the mussels grip themselves by interweaving with the long appendices of the substratum. By this behaviour the loss of mussels appeared to be diminished and numbers of settled mussels seem to be rather stable.

With the experimental approach deployed in this study the influence of the different materials used to fabric the substrates cannot fully be clarified. However, it is noticeable that best adhesive conditions were provided using nylon and natural fibres for the fabrication of the substrates. Second best conditions were provided using pp-fibres. Whether polyester is generally unsuitable for mussels as substrate material, since lowest dislodgement values were measured using this material, should be clarified in further studies.

4.2. Mussels experiencing hydrodynamic forces

In this study we have shown which forces are necessary to artificially detach mussels from the substrates surface. In doing so byssus threats attached to either the collector or to other mussels or a combination of both were investigated. However, at exposed sites more factors generating forces act on the body of blue mussels as well as on their shape and their attachment. These forces include current velocities, waves, and swell as well as forces resulting from bio-pumping and their own growing size and overgrowing fouling organisms, leading to an enlarged surface area and thus increasing the forces acting on the individual mussel. While mussels have the ability to adapt their attachment strategy according to these changes Witman and Suchanek (1984) measured a 15 times higher attachment strength of mussels living in exposed vs. protected habitats. Several studies have suggested that mussels go “with the flow”, producing more byssal threads that increase attachment strength when higher water velocities are encountered (Price 1982, Bell & Gosline 1997, Carrington 2002). However, Moeser et al. (2006) relativised this general conclusion and stated a hindered thread production at water velocities above 18 cm/s. In our experiment the measured current velocities were approx. 7 times higher (up to 1.4 m/s) and mussels were not dislodged and continued producing threads.

Taking into account that in this study no single mussel but in fact mussel conglomerates experience forces, we suggest that forces on the clusters of organisms will be smaller than the total force on all solitary individuals in sum (Johnson 2001, Vogel 1984). Forces will decrease with increasing cluster density. In our experimental cultures collectors floating below the cargo bridge created an artificial vertical mussel bed that certainly further reduced flow and therefore the impact of drag at least on the more centrally growing mussels. The low resistance against dislodgement or breaking forces of mussels growing in aggregations versus those growing individually is probably balanced by a neighbourhood protection against the full impact of currents (Buck and Buchholz 2005).

To get an insight about forces affecting mussel conglomerates it is important to know that these forces also act on the entire collector which is going with the flow too. It would be necessary to examine drag and lift as well as the acceleration reaction for mussels, mussel conglomerates and for full collectors also. As these data could not be obtained in this study we cannot go in more detail with the forces impacting on mussels and the collector.

4.3. Implications for mussel farming at exposed sites

Artificial substrates for spat collection in exposed or offshore sites must combine different properties. Due to the distance to other adjacent wild populations larval concentration in the water column is probably low in offshore areas (Walter et al. 2010). Therefore, deployed substrates must be highly attractive for remaining mussel larvae. Furthermore, substrates must hinder dislodgement of growing juvenile mussels by strong currents and waves. Considering options such as whether to produce either seed or market sized mussels or taking a thinning step during the grow out if numbers of settled post larvae are high, different scenarios of deployed substrates are possible. If the production of seed mussels is intended, substrates with a high fleece percentage and only short but numerous appendices are required. The harvesting of seed mussels should be organized before mussels achieve a growth bigger than approx. 1-2 cm and before the storm season begins. In contrast, if market sized mussels are required, fleece percentage should be reduced around the core of a collector rope and appendices should be long and numerous allowing the possibility to build up strong interwoven mussel-substrate conglomerates, able to survive winter storm events without bigger losses of mussels. A combination of both collector types might be suitable if settlement success is high and a thinning step during grow out is possible or necessary. In this case the collector of choice for the first step would be a fleece-like and after the thinning a filamentous substrate with long appendices. This scenario would also be suitable for a combined production of seed and market sized mussels.

5. Conclusions and Outlook

In this study evidences were achieved that the surface properties relevant for the adhesive conditions are influenced by the materials used for the fabrication of the substrate. More important, however, seems to be the emerging spatial structure of a substrate with its appendices building up conglomerates together with the settled mussels. Substrates designed for the exposed and offshore application should have a strong core, coated with a fleece-like material for larval attraction, combined with numerous well-fixed appendices which secure even big juvenile mussels by building a strong conglomerate of substrate and mussels. Depending on the type of mussel cultivation desired, such as production of either seed or market sized mussels, percentages of fleece and number and length of appendices can vary.

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References

Alfaro, A.C., Jeffs, A.G. 2003. Variability in mussel settlement on suspended ropes placed at Ahipara Bay, Northland, New Zealand. Aquaculture 216: 115-126

Alfaro, A.C., Copp, B.R., Appleton, D.R., Kelly, S., Jeffs, A.G. 2006. Chemical cues promote settlement in larvae of the green-lipped mussel *Perna canaliculus*. Aquaculture International, 14: 405-412

Alfred, N., Ista, L.K., Callow, M.E., Callow, J.A., Lopez, G.P., Clare, A.S. 2005. Mussel (*Mytilus edulis*) byssus deposition in response to variations in surface wettabillity. Journal of the Royal Society Interface, 3(6): 37-43

Bayne, B.L. 1964. Primary and secondary settlement in *Mytilus edulis* L. (Mollusca). Journal of Animal Ecology, 33: 513-523

Becker, G., Dick, S., Dippner, J. 1992. Hydrography of the German Bight. Marine Ecology Progress Series, 91: 9–18

Bell, E.C., Gosline, J.M. 1997. Strategies for a life in flow: tenacity, morphometry, and probability of dislodgement of two *Mytilus* species. Marine Biology Progress Series, 159: 197-208

Bomo, A.M., Stevik, T.K., Myklebust, H. 1998. Development of technology for Norwegian shellfish production. ITF-report 96

Bridger, C.J., Costa-Pierce, B.A. 2003. Open ocean aquaculture: from research to commercial reality. The World Aquaculture Society, Baton Rouge

Buck, B.H. 2007. Experimental trials on the feasibility of offshore seed production of the mussel *Mytilus edulis* in the German Bight: installation, technical requirements and environmental conditions. Helgoland Marine Research, 61: 87–101

Buck, B.H., Krause, G., Rosenthal, H. 2004. Multifunctional use, environmental regulations and the prospect of offshore co-management: potential for and constraints to extensive open ocean aquaculture development within wind farms in Germany. Ocean Coastal Management, 47: 95–122

Buck, B.H., Buchholz, C.M. 2005. Response of offshore cultivated *Laminaria saccharina* to hydrodynamic forcing in the North Sea. Aquaculture 250, 674–691

Buck, B.H., Krause G., Michler-Cieluch, T., Brenner, M., Buchholz, C.M., Busch, J.A., Fisch, R., Geisen, M., Zielinski, O. 2008. Meeting the quest for spatial efficiency: progress and prospects of extensive aquaculture within offshore wind farms. Helgoland Marine Research, 62: 269-281

Bucklin, A., Howell, W.H. 1998. Progress and prospects from the UNH Open Ocean Aquaculture Demonstration Project. In: Stickney RR (Ed.). Joining Forces with Industry. Proceedings of the Third International Conference of Open Ocean Aquaculture, Corpus Christi, TX, US, 10.-15. May 1998, 7 – 30

Carrington, E. 2002. Seasonal variation in the attachment strength of blue mussels: causes and consequences. Limnology and Oceanography, 47: 1723-1733

Connell, J.H. 1985. The consequences of variation in initial settlement vs. post settlement mortality in rocky intertidal communities. Journal of Experimental Marine Biology and Ecology, 93: 11-45

CWSS, 2008. Nomination of the Dutch-German Wadden Sea as World Heritage Site. Volume One. Common Wadden Sea Secretariat, Wilhelmshaven, Germany

Dayton, P.K. 1973. Competition, disturbance, and community organisation: the provision and subsequent utilisation of space in a rocky intertidal community. Ecol. Monogr. 41(4), 351-389.

Eckman, J.E. 1987. The role of hydrodynamics in recruitment, growth, and survival of *Argopecten irradiands* (L.) and *Anomia simplex* (D'Orbigny) within eelgrass meadows. Journal of Experimental Marine Biology and Ecology, 106: 165-191

Filgueira, R., Peteiro, L.G., Labarta, U., Fernandez-Reiriz, M.J. 2007. Assessment of spat collector ropes in Galician mussel farming. Aquacultural Engineering, 37: 195-201

Frandsen, R.P., Dolmer, P. 2002. Effects of substrate type on growth and mortality if blue mussels (*Mytilus edulis*) exposed to the predator *Carcinus meanas*. Marine Biology, 141: 253-262

GKSS, 2006. GKSS Research Centre Geesthacht, CoastDat database, http://www.coastdat.de/data/index.html.de , assessed in May 2009

Guiñez, R. 2005. A review on self-thinning in mussels. Revista Biologia Marina y Oceanografia, 40: 1-6

Guiñez, R., Castilla, J.C. 1999. A tridimensional self-thinning model for multilayered intertidal mussel. American Naturalist, 153: 341-357

Hansen, D.C., Waite, J.H. 1991. Marine metal chelating proteins. College of Marine Studies. University of Delaware. American Chemical Society, Lewes

Harger, J.R.E. 1970. The effect of wave impact on some aspects of the biology of mussels. Veliger 12, 401-414

Hickman, R.W. 1992. Mussel Cultivation, in: Gosling, E. (Ed.), The mussel *Mytilus*: ecology, physiology, genetics and culture. Development in aquaculture and fisheries science no. 25. Elsevier, Amsterdam, pp. 465–510

Johnson, A.S. 2001. Drag, drafting, and mechanical interactions in canopies of the red alga *Chondrus crispus*. Biol. Bull. Mar. Biol. Lab. 201(2), 126–135

Kleinsteuber, H., Will, R. 1988. Populationsdynamik der Miesmuschel und Entwicklung der Miesmuschel in den Wattgebieten der Nordsee unter besonderer Berücksichtigung der niedersächsischen Küste. Landesfischereiverband Weser-Ems e.V., Oldenburg, Germany

Korringa, P. 1976. Farming marine organisms low in the food chain: a multidisciplinary approach to edible seaweed, mussel and clam production Developments in Aquaculture and Fisheries. Science 1, Elsevier, Amsterdam, Netherlands, 224 pp

Langan, R., Horton, F. 2003. Design, operation and economics of submerged longline mussel culture in the open ocean. Bulletin of the Aquaculture Association of Canada, 103: 11–20

Lekang, O.-I., Stevik, T.K., Bomo, A.M. 2003. Evaluation of different combined collectors used in longlines for blue mussel farming. Aquacultural Engineering, 27(3): 89-104

McShane, P.E., Nylor, J.R. 1995. Depth can affect post-settlement survival of *Halotis iris* (Mollusca: Gastropoda).). Journal of Experimental Marine Biology and Ecology, 187: 1-12

Moeser, G.M., Leba, H., Carrington, E. 2006. Seasonal influence of wave action on thread production in *Mytilus edulis*. Journal of Experimental Biology, 209: 881-890

Moreno, C.A., 1995. Macroalgae as a refuge from predation for recruits of the mussel *Chloromytilus chorus* (Molina, 1782) in Southern Chile. Journal of Experimental Marine Biology and Ecology, 191: 181-193

Morrisey, D.J., Cole, R.G., Davey, N.K., Handley, S.J., Bradley, A., Brown, S.N., Madarasz, A.L. 2006. Abundance and diversity of fish on mussel farms in New Zealand. Aquaculture 252, 277-288

Nishida, A., Ohkawa, K., Ueda, I., Yamamoto, H. 2003. Green mussel *Perna vividis* L.: attachment behavior and preparation of antifouling surfaces. Biomolocular Engineering, 20: 381-387

Price, H.A. 1982. An analysis of factors determining seasonal variation in the byssal attachment strength of *Mytilus edulis* L.. Journal of the Marine Biological Association of the UK, 62: 147-155

Pulfrich, A. (1997). Seasonal variation in the occurrence of planktonic bivalve larvae in Schleswig-Holstein Wadden Sea. Helgoländer wissenschaftliche Meeresuntersuchungen, 51: 23-39

Ryan, J. 2005. Offshore aquaculture - Do we need it, and why is it taking so long? International Salmon Farmers Association (Ireland). Expert workshop on sustainable aquaculture, DG JRC European Commission, Institute for Prospective Technological Studies, 17–18 January 2005, Seville, Spain

Schiel, D.R. 2004. The structure and replenishment of rocky shore intertidal communities and biogeographic comparisons. Journal of Experimental Marine Biology and Ecology, 300: 309-342

Seaman, M.N.L., Ruth. M. 1997. The molluscan fisheries of Germany. NOAA Technical Reports NMFS 129, 57-84

Smaal, A.C. 2002. European mussel cultivation along the Atlantic coast: production status, problems and perspectives. Hydrobiologia 484, 89-98

Stickney, R.R. 1998. Joining forces with industry—open ocean aquaculture. Proceedings of the 3rd annual international conference, 10–15 May, Corpus Christi, Texas. TAMU-SG-99–103, Corpus Christi, Texas Sea Grant College Programme

Vogel, S. 1984. Drag and flexibility in sessile organisms. American Zoologist, 24(1): 37–44

Walter, U., Liebezeit, G. 2001. Nachhaltige Miesmuschel-Anzucht im niedersächsischen Wattenmeer durch die Besiedlung natürlicher und künstlicher Substrate. Forschungszentrum Terramare, Wilhelmshaven, Germany. Abschlussbericht der ersten Projektphase, 98 pp

Walter, U., Liebezeit, G. 2003. Efficiency of blue mussel (*Mytilus edulis*) spat collectors in highly dynamic tidal environments of the Lower Saxonian coast (southern North Sea). Biomolecular Engineering, 20: 407-411

Walter, U., Buck, B.H., Liebezeit, G. 2010. Larval occurrence and settlement in the German Bight: a trial to estimate potentials for *Mytilus edulis* culture in offshore areas, Aquaculture International (submitted)

Walters, L.J., Wethey, D.S. 1996. Settlement and early post-settlement survival of sessile marine invertebrates on topographically complex surfaces: importance of refuge dimensions and adult morphology. Marine Ecology Progress Series, 137: 161-171

Witman, J.D., Suchanek, T.H. 1984. Mussels in flow: drag and dislodgement by epizoans. Marine Ecology Progress Series, 16: 259-268

Chapter 07

ASSESSMENT OF THE CONSUMPTION SUITABILITY OF BLUE MUSSELS (*Mytilus edulis* L.) FROM OFFSHORE CULTIVATION AREAS - CONSEQUENCES FOR THE MUSSEL MONITORING

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Abstract

Due to strong tidal currents and harsh hydrographic conditions the German Bight is used only in the sheltered nearshore areas for on-bottom cultivation of blue mussels, where space, however, is limited by other stakeholder utilization e.g. shipping traffic, tourism and environmental protection interests. A further expansion of this food production sector within intertidal or subtidal areas of the coastal sea is not allowed due to legal restrictions. The potentials of additional longline cultivation within the planned offshore wind farms were assessed by a project coordinated by the Alfred Wegener Institute for Polar and Marine Research in Bremerhaven, Germany. In 2006 and 2007 blue mussels were cultivated in the area of the planned offshore wind farm *Nordergründe*. Samples were analysed by using biodiagnostic tools and by assessing microbial, toxicological and parasitological parameters to determine overall health status and consumption suitability of the mussels. In addition, the current regulatory framework of the official monitoring of mussel products was critically evaluated. First results show that an adjustment of the existing regulations and a differentiated approach according to the site specifics and cultivation method seems to be timely to improve consumer safety.

Keywords: blue mussels, *Mytilus edulis*, offshore windfarms, consumption suitability, health

UNTERSUCHUNGEN ZUR VERZEHRFÄHIGKEIT VON MIESMUSCHELN (*Mytilus edulis* L.) AUS OFFSHORE WINDPARKS – KONSEQUENZEN FÜR DIE BEHÖRDLICHE ÜBERWACHUNG

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Zusammenfassung

Die Nordsee kann aufgrund der ausgeprägten Gezeitenströmungen und Windverhältnisse nur im Küstenbereich durch die Besatzmuschelfischerei genutzt werden, wo jedoch die Nutzfläche durch Schiffsverkehr, Tourismus, und Naturschutz begrenzt ist. In einem vom Alfred-Wegener-Institut in Bremerhaven koordinierten Projektes wurde die Möglichkeit zusätzlicher Aufzuchtkapazitäten an Langleinen zwischen den Gründungsstrukturen von Offshore-Windkraftanlagen untersucht. Dazu wurden 2006 und 2007 Miesmuscheln im Gebiet des geplanten Windparks *Nordergründe* an Testanlagen kultiviert. Durch die Bestimmung von Wachstumsraten, den Einsatz biodiagnostische Methoden und durch die amtliche Analyse mikrobiologischer, toxikologischer und parasitologischer Parameter wurden der Gesundheitszustand und die Verzehrfähigkeit der Muscheln bestimmt. Darüber hinaus wurden die aktuell geltenden Richtlinien zur Überwachung von Muschelprodukten kritisch evaluiert. Erste Ergebnisse deuten darauf hin, dass eine Anpassung der Richtlinien und eine differenzierte Untersuchung der Verzehrfähigkeit von Muscheln, je nach Erzeugungsgebiet und Kultivierungsmethode, im Sinne eines verbesserten Verbraucherschutzes, erforderlich wären.

Schlüsselwörter: Miesmuscheln, *Mytilus edulis*, Offshore-Windparks, Verzehrfähigkeit, Gesundheit

1. Einleitung

Im Gebiet der Deutschen Nordsee werden Miesmuscheln seit Jahrhunderten zur Ernährung der Menschen genutzt. Dazu wurden sie bis etwa Mitte des letzten Jahrhunderts lediglich von ihren natürlichen eu- oder sublitoralen Wildbänken abgefischt. Erst mit Ende des zweiten Weltkrieges setzte sich eine Kombination aus Fischerei und Kultivierung bei der Miesmuschelerzeugung durch (Rosenthal & Hilge 2000). Bei dieser sogenannten Besatzmuschelfischerei werden juvenile Muscheln von 0.5 cm bis 4 cm Länge (Saatmuscheln) von ihren natürlichen Standorten abgefischt und auf Lizenzflächen, die auch bei Niedrigwasser mit Wasser bedeckt sind, wieder ausgebracht. Je nach Größe der Saatmuscheln werden die Kulturflächen mit Dichten von 30-100 t/ha bestückt, wo sie binnen 12 bis 24 Monaten zu marktreifen Muscheln von mindestens 50 mm Schalenlänge heran wachsen (Muschelfischer 2009). Aufgrund der ausgeprägten Gezeitenströmungen und Windverhältnisse kann die Nordsee jedoch nur im Küstenbereich durch diese Form der Muschelfischerei genutzt werden.
Von den vier Anrainerländern der deutschen Nordseeküste Niedersachsen, Bremen, Hamburg und Schleswig-Holstein haben nur Niedersachsen und Schleswig-Holstein Lizenzen für die Kultivierung von Muscheln vergeben. Auf den Flächen beider Länder wurden die Erträge der Besatzmuschelfischerei bis Ende der achtziger Jahre des letzten Jahrhunderts kontinuierlich von ca. 4.850 Tonnen von 1941-50 bis auf durchschnittlich 26.200 Tonnen in den Jahren 1981-90 gesteigert (Fischerblatt 2003a, 2003b, FAO 2004), wobei etwa 2/3 der durchschnittlichen Anlandungen von Muscheln auf schleswig-holsteinischen Kulturflächen erwirtschaftet wurden (FAO 1999). Seit 1990 fluktuieren die Anlandungen relativ stark, was meist auf große Schwankungen bei der Saatmuschelgewinnung zurückzuführen ist (Rosenthal & Hilge 2000). Heute liegen die durchschnittlichen Gesamterträge bei ca. 23.000 t pro Jahr.
Untersuchungen zur Reproduktionskapazität der Miesmuscheln ergaben, dass selbst in Jahren geringer Saatmuschelgewinnung immer ausreichend Muschellarven in der Wassersäule vorhanden waren (Walter & Liebezeit 2001). Die Gründe für die Ausfälle bei den Saatmuscheln liegen also nicht in einer veränderten oder reduzierten Reproduktionsfähigkeit der Miesmuschel. Vielmehr wird darüber spekuliert ob z. B. aufgrund ungünstiger Strömungsverhältnisse die Larven in die offene Nordsee verdriftet werden, wo sie keine Möglichkeit zur Anhaftung finden oder ob -als Folge warmer Winter-Fressfeinde der Muscheln, wie beispielsweise Seesterne in so großer Zahl überlebt haben, dass die neuangesiedelten Jungmuscheln umgehend gefressen werden. Möglicherweise ist aber auch das Nahrungsangebot für die jungen Stadien der Muscheln, bedingt durch den Rückgang der Eutrophierung (Nehls & Ruth 2004) oder durch die massive Ausweitung der Besiedlung durch die Pazifischen Austern (*Crassostrea gigas*), nicht mehr ausreichend.
Weltweit werden zurzeit durchschnittlich 420.000 t Miesmuscheln produziert. Die größten Produzenten von Miesmuscheln sind Kanada, Neuseeland, Spanien, Frankreich, Holland, Großbritannien und Dänemark. Im Vergleich zu den Erträgen der Hauptproduzenten fallen jedoch selbst erfolgreiche Jahre der deutschen Muschelfischer kaum ins Gewicht (FAO 2008, Muschelfischer 2009).

2. Potentiale der Muschelkultur in den Gewässern der Deutschen Nordsee

Einer Expansion der Besatzmuschelfischerei sind im deutschen Küstenbereich (12 Seemeilen-Zone) in der Konkurrenz zu anderen Nutzern wie Schifffahrt, Tourismus, Naturschutz, Marine, Fischerei sowie Sand- und Kiesgewinnung sehr enge Grenzen gesetzt (Buck 2002). Die drei Küstenanrainer Niedersachsen, Hamburg und Schleswig-Holstein haben darüber hinaus große Bereiche ihrer Küstengewässer unter Naturschutz gestellt und zu Nationalparks erklärt. Seitdem unterliegt die Muschelfischerei einer Kombination aus den einzelnen Landesfischereigesetzten (Bremisches Fischereigesetz [BremFiG 1991]; Hamburgisches Fischereigesetz [1986]; Niedersächsisches Fischereigesetz [Nds.FischG 1978] und Schleswig-Holstein: Landesfischereigesetzt [LFischG 1996] und den entsprechenden gesetzlichen Grundlagen der Nationalparke (Niedersächsisches Nationalparkgesetz [2001], Gesetz über den Nationalpark Hamburgisches Wattenmeer [1990] und dem Nationalparkgesetz Schleswig-Holstein [NPG 1999]. Unter strengen Auflagen ist die Besatzmuschelfischerei, als einzige kommerzielle Form der (Muschel-)Fischerei in allen drei

Nationalparks erlaubt. Neben Miesmuscheln dürfen lediglich Austern kultiviert werden (Boysen 1991). Die Zucht anderer potentieller Kandidaten wie Herz-, Kamm- oder Venusmuscheln ist verboten. Darüber hinaus haben sich die Muschelfischer nach langen und scharfen Auseinandersetzung insbesondere mit Vertretern des Naturschutzes 1997 darauf geeinigt, eine Obergrenze von 2.400 ha bei der Gesamtgröße der Kulturflächen zu akzeptieren (Ruth 1997), die nach Ablauf der bestehenden Lizenzen auf 2.000 ha reduziert wurden. Den zwangsläufigen wirtschaftlichen Einbußen wollen die Muschelfischer mit einer Produktionsintensivierung auf den verbleibenden Flächen begegnen. Damit kann jedoch auch im günstigsten Fall nur das (niedrige) Niveau der Anlandungen gehalten werden. Eine Expansion dieser traditionellen Form der Muschelzucht scheint so jedenfalls ausgeschlossen.

3. Muschelzucht auf offener See?

Durch den Bau von Offshore-Windkraftanlagen ergeben sich für die Muschelfischerei neue Perspektiven. Bis auf zwei Ausnahmen befinden sich alle bereits genehmigten Areale der zukünftigen Windparks fern ab der küstennahen Konkurrenz um Raum und Nutzungsformen (Abb. 1).

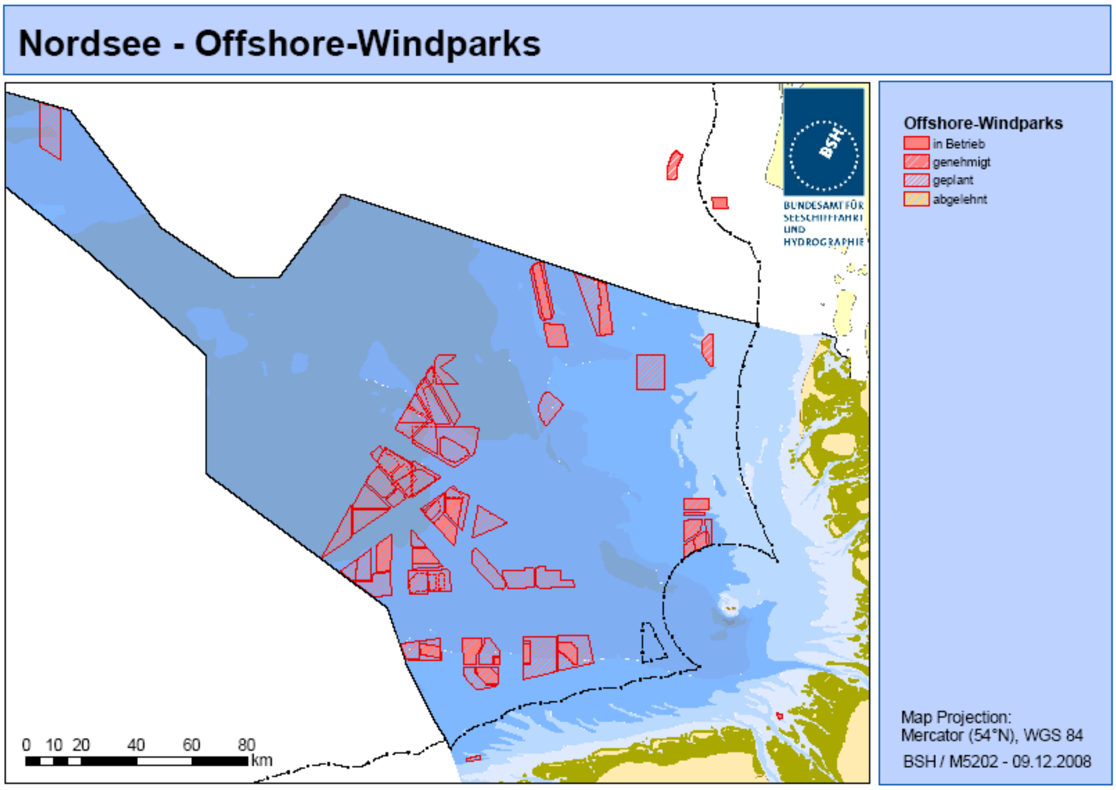

Abb. 1: Übersicht über die Deutsche Ausschließliche Wirtschaftszone (AWZ) mit den reservierten Flächen (rot) für offshore Windparks (BSH 2009).

Die Nutzungsformen sind dort weniger traditionell und nicht so zahlreich. Es dominieren große wirtschaftlich potente Stakeholder, wie z.B. die Handelsschifffahrt.
Die Meeresgebiete für die geplanten Windparks sind zwar in der Regel zu tief um mit den Methoden der Besatzmuschelfischerei bewirtschaftet zu werden, aber mit adaptierten Langleinen-Kulturverfahren könnten die entstehen Flächen sekundär genutzt werden (Abb. 2). Die Gründungsstrukturen der Windanlagen wären die idealen Befestigungen für die Kultursysteme. Bei der Langleinen Technik werden den Muschellarven, die im Frühjahr in der Wassersäule schweben, künstliche Substrate angeboten die senkrecht von einem starken Trägerseil hängen. Die Langleine wird in ruhigen Meeresgebieten mit entsprechenden Auftriebskörpern an der Oberfläche oder unter strömungs- bzw.

wellenreichen Bedingungen einige Meter abgesenkt gehalten. Das Verdriften der Anlage wird mit entsprechenden Verankerungen verhindert.

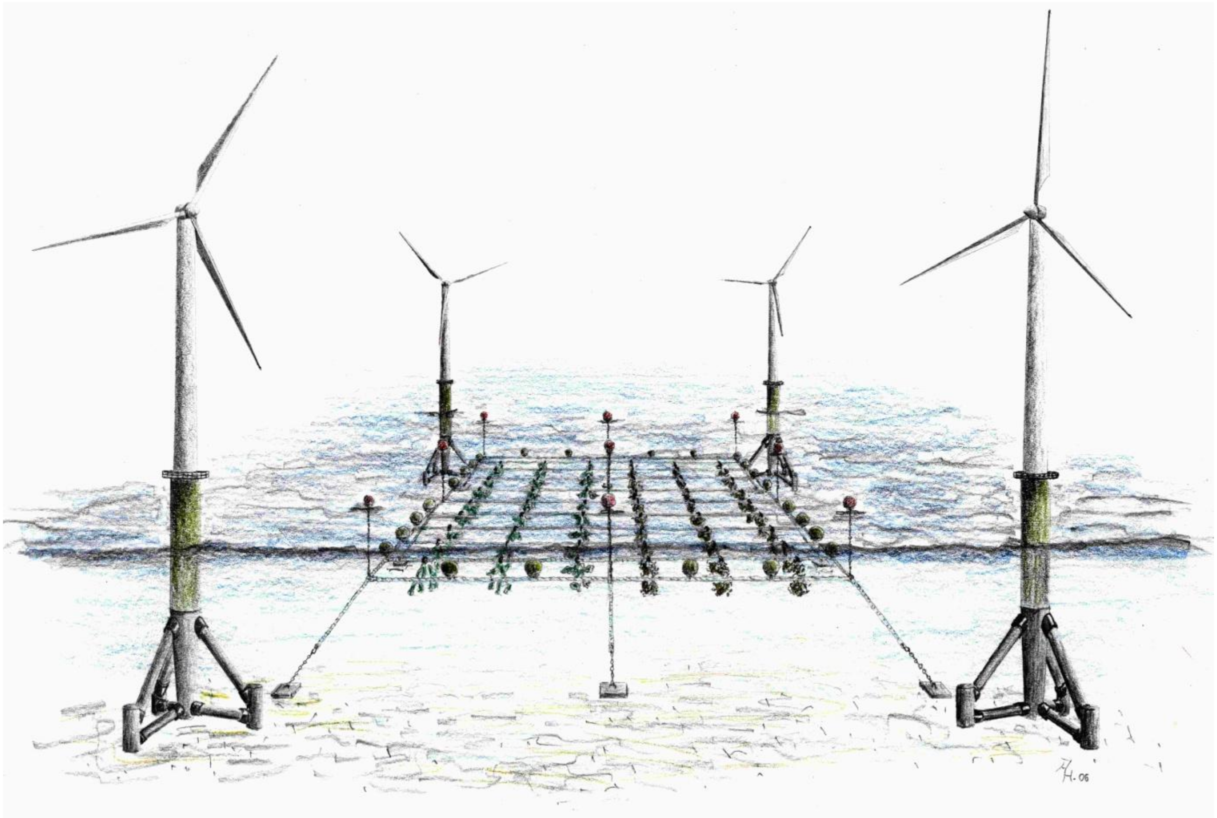

Abb. 2: Potentielle Co-Nutzung eines offshore Windparks durch Kultursysteme für Muscheln, Austern oder Makroalgen.

Untersuchungen von Buck (2007) ergaben, das in fast allen Bereichen der Deutschen Nordsee die Larvenkonzentration für eine Muschelkultivierung an Langleinen hoch genug ist. In einigen Gebieten wäre sogar eine Ausdünnung der Kulturseile nötig, damit die verbleibenden Muscheln mit ausreichend Platz entsprechend schnell wachsen. Überschüssige oder eigens zu diesem Zweck gewonnene Jungmuscheln könnten darüber hinaus zur Linderung der Engpässe bei der Saatmuschelgewinnung in der konventionellen Besatzmuschelfischerei Verwendung finden.
Neben der geringeren Konkurrenzsituation bieten küstenferne Standorte eine Reihe weiterer Vorteile. So ergaben vorangegangene Untersuchungen ein deutlich schnelleres Wachstum (Buck 2007) und eine bessere Versorgung mit Plankton sowie einen verbesserten Abtransport der Ausscheidungen durch die starke Strömung (Fréchette et al. 1989). Die Infizierung mit Makroparasiten nahm mit zunehmender Entfernung zur Küste ab, offshore kultivierte Muscheln waren sogar komplett frei von Makroparasiten (Buck et al. 2005, Brenner et al. 2009). Darüber hinaus stellen die Hauptfraßfeinde der Miesmuscheln, wie die Strandkrabbe (*Carcinus maenas*), der Seestern (*Asterias rubens*), der Austernfischer (*Haematopus ostralegus*), die Silbermöwe (*Larus argentatus*) und die Eiderente (*Somateria mollissima*) (Newell 1989) offshore eine wesentlich geringere Bedrohung dar, da die Muscheln in der Wassersäule hängen und zumindest für Strandkrabben und Seesterne nicht und für die meisten Vögel schlechter erreichbar sind. Seesterne können nur als Larve zusammen mit den Muscheln die Substrate erreichen. Dort müssen sie erst zu einer gewissen Größe heranwachsen, um zu einer Bedrohung für die Muscheln zu werden. Da die Leinen beim Abernten komplett gereinigt werden, können sich Seesterne aber auch Fouling-Organsimen nicht bzw. nur über den Zeitraum der Kultivierung einer Muschelgeneration etablieren.

Das größte Problem für eine sekundäre Bewirtschaftung der Windparkflächen sind die höheren Investitionskosten für die Zuchtanlagen. Da die Flächen der geplanten Windparks in der Regel zu tief für Bodenkulturen sind, müssen die teureren Langleinen- Kulturtechniken angewandt werden. Nur mit fundierten Kenntnissen über die biologischen Potentiale eines Meeresgebietes sind Wachstumsraten zu berechnen und Angaben zur Produktqualität und Marktfähigkeit der Zuchtmuscheln zu machen.

4. Einfluss des Klimawandels auf die Qualität der Muschelerzeugnisse

Seit ein paar Jahren verzeichnet die Wissenschaft um 2 °C höhere Durchschnittstemperaturen im Bereich der Deutschen Nordsee. Unabhängig davon, ob es sich dabei um Vorboten des Klimawandels oder lediglich um natürliche Schwankungen handelt, lassen sich bereits jetzt die gravierende Folgen einer Temperaturerhöhung ablesen. Für den Wildbestand der Miesmuscheln, die auch für die Rekrutierung von Zuchtmuscheln unverzichtbar ist, wird die stetige Ausbreitung der Pazifischen Auster (*Crassostrea gigas*) allmählich Existenz bedrohend (Nehls & Ruth 2004).
Fraglich ist ob auch neue Parasitenarten, aufgrund der Erwärmung in der Nordsee heimisch werden. Jüngste Funde (Wattreport 2008) des bisher nur aus der südlichen Nordsee bzw. aus dem Ärmelkanal bekannten Muschelwächters in der Deutschen Bucht belegen, dass die Nordsee inzwischen auch für andere Arten attraktiv geworden ist. Der Muschelwächter ist ein im Mantel der Muschel lebender parasitischer Krebs (*Pinnotheres pisum*). Ein Befall durch diesen erbsengroßen Parasit ist für den Konsumenten zwar unbedenklich, jedoch wirtschaftlich von erheblicher Bedeutung, da befallene Muscheln aus ästhetischen Gründen dramatisch an Wert verlieren und -wenn überhaupt. nur noch weiterverarbeitet (gekocht oder gefroren) zu vermarkten sind. Ein ähnliches Gefahrenpotential stellen intrazellulär vorkommende Mikroparasiten der Miesmuscheln dar. Der Befall durch *Marteilia refrigens* wird zusammen mit einer massiven Überfischung auch für das Aussterben der heimischen Auster *Ostrea edulis* verantwortlich gemacht (FAO 2009). Bei Miesmuscheln führt der Kontakt mit dem Erreger zwar nicht automatisch zum Tod, schwächt aber Kondition und Gesundheit der Tiere (OiE 2009). Bisher sind *Marteilia*-Befunde bei Miesmuscheln nur von der Atlantikküste Frankreichs, dem Ärmelkanal und von Großbritannien beschrieben.
In den betroffenen Ländern ist die Untersuchung auf Mikroparasiten durch die Überwachungsbehörden Routine. Unsere ersten Ergebnisse ergaben zwar keine Befunde an allen untersuchten Standorten in der Deutschen Bucht. Dennoch raten die Experten der ICES-Kommission (ICES 2008) zu einem koordinierten Monitoring der Muscheln im Raum Nordsee, um eventuell auftretende Vorkommen schnell zu detektieren. Motor der Verbreitung von Mikroparasiten ist jedoch nicht allein der Temperaturanstieg. Die Muschelfischer verfrachten seit Anfang 2007 Saatmuscheln aus Großbritannien -trotz Einspruchs der Naturschutzbehörden- als Ersatz für die ungenügende Rekrutierung in heimischen Gewässern (Wattreport 2008).

5. Hygienische Überwachung der Muscheln

Miesmuscheln filtrieren ihr Umgebungswasser. Neben ihrer eigentlichen Nahrung aus einzelligen Algen und partikulärem organischen Material nehmen sie dabei aber auch schnell und effektiv anthropogene Schadstoffe, Parasiten, Algentoxine, Bakterien und Viren auf.
Ähnlich wie bei den einzelnen Fischerei- und den Nationalparkgesetzen, liegt auch die Lebensmittelüberwachung und die Erstellung der entsprechenden Ausführungshinweisen (Sassen et al. 2005) in der Verantwortung der jeweiligen Bundesländer, wobei diese die wesentlichen Aufgaben hinsichtlich der Überwachung der hygienischen Beschaffenheit der Muscheln als Lebensmittel und der Eignung der Seegebiete, die der Muschelerzeugung für Lebensmittelzwecke dienen sollen, auf die Kreise und kreisfreien Städte übertragen haben.
Vorausgesetzt, die Offshore-Gebiete unterliegen ebenfalls dieser Zuständigkeitsregelung, müssten die entsprechenden Seegebiete basierend auf der Richtlinie EU 2006/113 des Europäischen Parlamentes und des Rates vom 12. Dezember 2006 über die Qualitätsanforderungen an Muschelgewässer und den Verordnungen EU 853/2004, EU 854/2004, EU 2073/2005 von den nationalen zuständigen Behörden einem umfangreichen Untersuchungs- und Bewertungsverfahren unterzogen werden. Zur Anleitung der nationalen Behörden hat die EU Working Group on the Microbiological Monitoring of

Bivalve Molluscs Harvesting Areas einen Community Guide to the Principles of Good Practice for the Microbiological Monitoring of Bivalve Molluscs Harvesting Areas with regard to Regulation 854/2004 (CEFAS 2007) erarbeitet.
Nach den Vorgaben der VO(EG) 854/2004 sind Muschelerzeugungsgebiete folgendermaßen zu überprüfen:

(1) Ermittlung aller tierischen und menschlichen Verschmutzungsquellen, die auch für Verunreinigungen des Erzeugungsgebietes relevant sein können.

(2) Überprüfung des Eintrages organischer Schadstoffe über den Saisonverlauf entsprechend der Variationen der menschlichen und tierischen Populationen im Einzugsgebiet und entsprechend der saisonalen Niederschlagsmengen.

(3) Bestimmung des Schadstoffkreislaufes eines Erzeugungsgebietes unter Berücksichtigung von Strömungsmustern, Tiefen und Gezeiten. Ziel der überarbeiteten Richtlinie des Rates ist es, die Gewässer einschließlich der Muschelgewässer vor Verunreinigung zu bewahren, um damit zur verbesserten Qualität der zum Verzehr gedachten Muschelerzeugnisse beizutragen.

(4) Erstellung eines Programms zur Probenahme für Muscheln im Erzeugungsgebiet, dass sich auf die Prüfung vorhandener Daten stützt, wobei die Zahl der Proben, die geographische Verteilung der Probenahmepunkte und die Probenahmehäufigkeit gewährleisten müssen, dass die Analyseergebnisse für das Gebiet so repräsentativ wie möglich sind.

Grundlage für die Bewertung der Eignung von Muschelerzeugungsgebieten und der Verzehr- und Verkehrsfähigkeit der Muscheln bleiben als Kriterien für eine mögliche fäkale Kontamination der Erzeugungsgebiete bis auf weiteres noch die Grenzwerte für *Echerichia coli* und Salmonellen gemäß VO(EG) 853/2004, 854/2004 sowie 2073/2005. Gleichwohl ist durch eine Reihe von Untersuchungen (Guyader et al. 2000, Croci et al. 2003, Formica-Cruz et al. 2002, Romalde et al. 2002) bereits nachgewiesen, dass die erwähnten Kriterien keine fundierte Aussage hinsichtlich einer Kontamination der Erzeugungsgebiete mit humanpathogenen Viren oder anderen Krankheitserregern zulässt.
Die Qualitätskriterien *E. Coli* und Salmonella werden für die Beurteilung der Eignung eines Erzeugungsgebietes bzw. der Verkehrsfähigkeit von geernteten Muscheln sicherlich noch für geraume Zeit eine Rolle spielen. Gleichwohl wurde bei der Konferenz der Nationalen Referenzlabore (NRL) für die Überwachung von Viren und Bakterien in zweischaligen Weichtieren in Galway (Irland) im Mai 2006 in den Beschlüssen -den wissenschaftlichen Erkenntnissen Rechnung tragend- gefordert, die Diagnostik in Bezug auf humanpathogene Viren und Vibrionen weiter zu entwickeln und die Rechtsetzung entsprechend anzupassen (Anonym 2006).
Alternativ zur Kultivierung der Muscheln bis zur Marktreife können die Zuchtsysteme auch zur Produktion von Saatmuscheln genutzt werden. Ein großer Vorteil dieses Ansatzes ist, dass eine Überwinterung der Muscheln an den Seilen auf hoher See nicht notwendig und damit die Gefahr Muscheln durch Winterstürme zu verlieren gering ist. Saatmuscheln könnten, da sie nicht für den direkten Verzehr bestimmt sind ohne Vorprüfungen auf den traditionellen Besatzmuschelflächen ausgebracht werden. Dies wäre eine sinnvolle Alternative zum problematischen Import von Muscheln aus anderen Seegebieten und böte darüber hinaus eine effektive Möglichkeit der Produktionsintensivierung auf traditionellen Besatzmuschelflächen.

6. Potentiale der Offshore-Kultivierung

Der Eintrag von Schadstoffen, Fäkalkeimen oder human pathogener Viren geschieht mit Ausnahme von Einträgen durch die Schifffahrt und Meerestieren immer von Land aus und wird sich mit entsprechendem Abstand zur Küste durch Verdünnung verringern. Es ist also sehr wahrscheinlich, dass Offshore-Standorte generell eine bessere Wasserqualität aufweisen als küstennahe Standorte, die wie in Deutschland ausschließlich der Fall, in unmittelbarer Nähe zu dichter Besiedlung und intensiver landwirtschaftlicher oder industrieller Nutzung liegen. Deswegen sollten die Belastungen

potentieller Offshore-Muschelfarmen durch Bakterien und human pathogener Viren vernachlässigbar gering sein. Ausnahmen bei der Wasserqualität bilden dabei lokale „hot-spots“, wie Gebiete mit verklappter Munition oder Bereiche in denen z.B. Ölförderung betrieben wird. Durch entsprechende Vorversuche sind diese Einflüsse jedoch zu erkennen und für die Produktion von Lebensmittel auszuschließen.
Anders sieht die Situation bei der Belastung der Muschelprodukte durch Algentoxine aus. Obwohl auch in der Nordsee etwa 20 Algenarten vorkommen die Toxine bilden können, sind Blüten von Toxinbildnern selten (Elbträchter 1996). Die Blüten gefährlicher Algen bilden sich vornehmlich in strömungsarmen Gebieten mit stabiler Wasserschichtung (Nehring et al. 1995). Oft rekrutieren sie sich dabei aus Cysten, die über Jahre im Sediment überdauern können (Tillmann & Rick 2003). Im Ärmelkanal und an den Küsten Großbritanniens, Skandinaviens und Dänemarks treten toxische Algenblüten regelmäßig auf (Smaal 2002). Die Deutsche Bucht und die Bereiche des Wattenmeeres scheinen hingegen aufgrund ihrer Hydrographie und hohen Sedimentfracht eher ungeeignet, für das Massenauftreten problematischer Algen zu sein. In Zuchtanlagen auf offener See können Muscheln jedoch unter Umständen mit voll intakten und vitalen Toxin produzierenden Algen in Kontakt kommen und die gefährlichen Substanzen in entsprechend hohen Konzentrationen akkumulieren. Da die Grenzwerte für alle bekannten Algentoxine und speziell auch für das einzige je in der Deutschen Bucht nachgewiesene Toxin DSP so hoch sind, dass selbst erwachsene und gesunde Menschen nach dem Verzehr belasteter Muscheln Symptome zeigen werden ist neben einer deutlichen Korrektur der Grenzwerte auch ein der realen Gefahr angepasstes Monitoring erforderlich. In Irland, in dessen küstennahen Erzeugungsgebieten Algentoxine häufig sind und dort wiederholt zu mehrmonatigen Schließungen von Farmen geführt haben, wurde bereits 2005 angeregt die Grenzwerte für die Biotoxine deutlich nach unten zu korrigieren (McMahon 2005).
Auch die European Food Safety Authority (EFSA 2008) als wissenschaftliches Beratungsgremium schlägt in seinen neuen Gutachten zu den Toxinen des Okadasäurekomplexes (DSP) und zu Azaspiraziden (AZA) eine drastische Absenkung der Grenzwerte vor. Begründet wird dieses mit der Feststellung, dass bei derzeitiger Rechtslage auch bei Einhaltung der gültigen Grenzwerte eine Erkrankung der Konsumenten nicht ausgeschlossen werden kann.
In küstennahen Bereichen können Toxin bildende Algen, neben der direkten Kontamination der Muschelprodukte auch zu nachhaltigen Kontaminationen der Kultivierungsflächen führen. Zysten als Überdauerungsstadien können über Jahre hinweg zu einer Belastung der Muscheln werden, so dass inzwischen sogar einige Kultivierungsgewässer aufgegeben werden mussten. Sollte der wissenschaftlichen Auffassung der EFSA in Bezug auf Grenzwerte für bestimmte Algentoxine in zukünftigen Rechtssetzungsverfahren nur annähernd gefolgt werden, ist mit dem Ausfall von weiteren Erzeugungsgebieten für Muscheln zu rechnen.
Aufgrund des ausgeprägten Strömungsregimes und der größeren Wassertiefen, wäre bei küstenfernen Standorten eine vergleichsweise günstige Situation hinsichtlich der Belastung mit Überdauerungsstadien von Toxin bildenden Algen zu erwarten.

Seit April 2006 wurden dazu im Rahmen eines Kooperationsprojektes (*MytiFit*) des Alfred-Wegener-Institutes für Polar und Meeresforschung in Bremerhaven (Buck et al. 2007) Miesmuscheln im Weserästuar im Gebiet des geplanten Windparks *Nordergründe* an Testanlagen in der Wassersäule hängend kultiviert. Allen beschriebenen Parametern und Einflüssen Rechnung tragend, wurden die Muscheln nicht nur nach den amtlichen Vorschriften mikrobiologisch auf ihre Verzehrfähigkeit hin untersucht (Institut für Fische und Fischereierzeugnisse (LAVES), Cuxhaven), sondern darüber hinaus virologisch (LAVES) und parasitologisch getestet sowie mit biodiagnostischen Methoden auf ihren allgemeinen Gesundheitszustand hin untersucht. Das zentrale Mittel der biodiagnostischen Untersuchungen ist der Lysosomen-Membran-Stabilitätstest mit dem die Funktionsfähigkeit der Mitteldarmdrüse, als zentrales Stoffwechselorgan für Nahrungsaufnahme und -verwertung der Muschel, analysiert wird. Bei negativen Testergebnissen können durch detaillierte toxikologische Analysen auch die Gründe für die Belastung festgestellt werden.
Ziel des Projektes ist es eine Datenbasis zu schaffen, die als Bewertungsgrundlage von potenziellen Betreibern einer Offshore-Muschelfarm an Windkraftanlagen für eine rentable und qualitätskontrollierte Miesmuschelzucht genutzt werden kann.

7. Ausblick

Erste Ergebnisse zeigen, dass offshore kultivierte Muscheln hohe Wachstumsraten und einen hohem Fleischanteil haben, mikrobiologisch, toxikologisch und virologisch einwandfrei sind und keinerlei Parasiten aufweisen (Brenner et al. 2009). Darüber hinaus hätte die Verlagerung der Muschelproduktion in Offshore-Gebiete keine lebensmittelrechtlichen Konsequenzen. Im Gegenteil, die homogenen hydrographischen Bedingungen und die zu erwartende geringe Belastung mit coliformen Keimen sprächen eher für eine Verlagerung der Produktion in küstenferne Gebiete. Andere potentielle Gefahren für die Konsumenten von offshore produzierten Muscheln, wie eine Vergiftung durch Algentoxine, werden jedoch nicht im Rahmen des vorgeschriebenen Monitorings untersucht. Deswegen erscheint insgesamt eine differenziertere Überwachung von Muscheln- und auch Austernprodukten angebracht zu sein. Die Grenzwerte für Algentoxine sollten auf eine Größenordnung abgesenkt werden, die dem Verbrauchschutz dienlich sind. Die derzeitigen Grenzwerte für DSP und Azaspiraicide erfüllen diese Voraussetzung nicht. Auch der derzeitige rechtliche Rahmen bei der mikrobiolgischen Einstufung von Muscheln und Muschelerzeugungsgebieten – obwohl durch VO(EG) 853 und 854/2004 bereits verschärft – bietet bei Rohverzehr von zweischaligen Weichtieren keinen ausreichenden Verbraucherschutz, da lediglich das Freisein von Salmonellen und die Einhaltung von Grenzwerten in Bezug auf *E. Coli* zu überprüfen ist. Ein wirksamer Schutz – beispielsweise – vor humanpathogenen Viren ist hiermit nicht zu erreichen. Hierzu wären weit umfangreichere Untersuchungen als die derzeit praktizierten erforderlich. Andererseits ist das derzeitige Schutzniveau bei ausreichender Erhitzung der Muscheln vor dem Verzehr bei weitem ausreichend. Möglicherweise sollte überlegt werden, ein intensives Schutzniveau lediglich auf die zweischaligen Weichtiere zu beschränken, die für den Rohverzehr bestimmt sind.

Literatur

Anonym (2006). Resolutions of the 6th workshop of NRLs for bacteriological and viral contamination of bivalve molluscs, Galway, 15.-17. May 2006

Boysen H-O (1991). Standpunkt der Fischereiverwaltung Schleswig-Holstein. Volume 50-52 In Dethlefsen V (Ed.). Probleme der Muschelfischerei im Wattenmeer, SDN-Kolloquium. Wilhelmshaven, Schriftenreihe der Schutzgemeinschaft Deutsche Nordseeküste e.V.

BremFiG 1991. Bremisches Fischereigesetz vom 17. September 1991, Brem.GBI. S. 309. Geändert durch Art. 1 des Gesetzes vom 1. Juni 1999, Brem.GBI. S. 139 und § 23 Abs. 6 des Gesetzes vom 21. November 2000, Brem.GBI. S. 437

Brenner M, Ramdohr S, Effkemann S, Stede M (2009). Key parameters for the consumption suitability of offshore cultivated blue mussels (*Mytilus edulis* L.) in the German Bight. European Food Research and Technology (eingereicht)

BSH (2009). Bundesamt für Seeschifffahrt und Hydrographie, Hamburg. http://www.bsh.de/de/Meeresnutzung/Wirtschaft/CONTIS-Informationssystem/Contis/Karten/NordseeOffshoreWindparksPilotgebiete.pdf, Stand 20. März 2009

Buck BH (2002). Open Ocean Aquakultur und Offshore-Windparks. Eine Machbarkeitsstudie über die multifunktionale Nutzung von Offshore-Windparks und Offshore-Marikultur im Raum Nordsee. Berichte zur Polar- und Meeresforschung, 412: 252pp

Buck BH, Thieltges DW, Walter U, Nehls G, Rosenthal H (2005). Inshore offshore comparison of parasite infestation in *Mytilus edulis*: implications for open ocean aquaculture. Journal of Applied Ichthyology, 21: 107-113

Buck BH (2007). Experimental trials on the feasibility of offshore seed production of the mussel Mytilus edulis in the German Bight: installation, technical requirements and environmental conditions. Helgoland Marine Research, 61: 87-101

Buck BH, Koehler A, Brenner, M, Stede, M (2007). Eignung des Seegebietes am geplanten Offshore-Windpark *Nordergründe* für die Zucht von Miesmuscheln: Fitness, Parasitisierung und Substratwahl. Endbericht, 91 pp

CEFAS (2007). Microbiological monitoring of bivalve mollusc harvesting areas – Guide to good practice: Technical application. European Community Reference Laboratory for monitoring bacteriological and viral contamination of bivalve mollusc. The centre of environment, fisheries and aquaculture science (CEFAS) 3, 67 pp

Croci L, De Medici D, Ciccozzi M, Di Pasquale S, Suffredini E, Toti L (2003). Contamination of mussels by hepatitis A virus: a public-health problem in southern Italy. Food control, 14: 559-563

EFSA (2008). Anonym: Marine biotoxins in shellfish – Azaspiracid group[1] - Scientific Opinion of the Panel on Contaminants in the Food chain und Marine Biotoxine in Schalentieren - Okadasäure und Analoga - Gutachten des Wissenschaftlichen Gremiums für Kontaminanten in der Lebensmittelkette)

Elbträchter M (1996). Toxische Algen in der Nordsee. Deutsche Hydrographische Zeitschrift, Suppl. 6: 37-44

EU 854/2004. Verordnung des Europäischen Parlaments und des Rates vom 29. April 2004 mit besonderen Verfahrensvorschriften für die amtliche Überwachung von zum menschlichen Verzehr bestimmten Erzeugnissen tierischen Ursprungs (Abl. L 139 vom 30.4.2004). ABl. der EG Nr. L 226 S. 0083 - 00127

EU 853/2004. Verordnung des Europäischen Parlaments und des Rates vom 29. April 2004 mit spezifischen Hygienevorschriften für Lebensmittel tierischen Ursprungs. ABl. der EG Nr. L 139 S. 0055 - 0205

EU 2073/2005. Verordnung der Kommission vom 15. November 2005 über mikrobiologische Kriterien für Lebensmittel. ABl. der EG Nr. L 338 S. 0001 - 0026

EU 2006/113. Richtlinie des Europäischen Parlaments und des Rates vom 12. Dezember 2006 über die Qualitätsanforderungen an Muschelgewässer

FAO (1999). Aquaculture Production Statistics 1988-1997. FAO Fisheries Circulars, Food and Agriculture Organisation of the United Nation, Rome, Italy

FAO (2004). FAO-Fisheries Department, Fishery information, Data and Statistics Unit. FISHSTAT Plus. Universal Software for fishery statistical time series. Version 2.3. last updated March 2004. Food and Agriculture Organisation of the United Nation, Rome, Italy

FAO (2008). State of World Fisheries and Aquaculture - Food and Agriculture Organization of the United Nations, Rome, Italy, 176 pp

FAO (2009). http://www.fao.org, Stand 15.02.2009.

Fréchette M, Butman CA, Geyer WR (1989). The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnology and Oceanography, 34: 19-36

Fischerblatt (2003a). Die Kleine Hochsee- und Küstenfischerei Schleswig-Holsteins im Jahre 2002. Fischerblatt, 3: 61-81

Fischerblatt (2003b). Die Kleine Hochsee- und Küstenfischerei Schleswig-Holsteins im Jahre 2002. Fischerblatt, 3: 81-102

Formica-Cruz M, Tofino-Quesada G, Bofill-Mas S, Lees DN, Henshilwood K, Allard AK, Conden-Hansson A–C, Hernroth BE, Vantarakis A, Tsibouxi A, Papapetropoulou M, Furones MD, Girones R (2002). Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden and the United Kingdom. Applied and Environmental Microbiology, 68: 5990-5998

Gesetz über den Nationalpark Hamburgisches Wattenmeer (1990). Hamburgisches Gesetz und Verordnungsblatt, Nr. 11/1990, 9. April 1990, 64-66

Guyader F le, Haugarreau L, Miossec L, Dubois E, Pommepuy M (2000). Three-year study to assess human enteric viruses in shellfish. Applied and Environmental Microbiology, 66: 3241-3248

Hamburgisches Fischereigesetz (1986). Hamburgisches Fischereigesetz vom 22. Mai 1986, Hbg.GVBI. S. 95. Geändert durch Gesetz vom 9. April 1990, Hbg.GVBI. S. 63

ICES (2008). Report of the Working Group on Marine Shellfish Culture (WGMASC), 1-3 April 2008, Aberdeen, UK. ICES CM 2008/MCC:02: 71 pp

LFischG. (1996). Fischereigesetz für das Land Schleswig-Holstein (Landesfischereigesetz) vom 10. Februar 1996, GVOBI. Schleswig-Holstein. S. 211. Geändert durch Gesetz vom 12. Dezember 1997, GVOBI. Schleswig-Holstein, S. 471

McMahon T (2005). Evolution of Irish Programme and EU Policies on Biotoxin Management. Proceedings of the 6th Irish Shellfish Safety Workshop, Galway, 1. December 2005, Galway, Ireland, 82-88

Muschelfischer (2009). http://muschelfischer.de, Stand 15.02.2009

Nds.FischG. (1978). Niedersächsisches Fischereigesetz vom 1. Februar 1978, Nieders.GVBI. S. 81. Geändert durch Art. 34 des Gesetzes vom 22. März 1990, Nieders.GVBI. S. 321

Nehls G, Ruth M (2004). Miesmuschelmonitoring und Miesmuschelmanagement im Nationalpark „Schleswig-Holsteinisches Wattenmeer“. Bericht, Landesamt für den Nationalpark „Schleswig-Holsteinisches Wattenmeer“

Nehring S, Hesse K-J, Tillmann U (1995). The German Wadden Sea: A problem area for nuisance blooms? In: Lassus P, Arzul G, Erard E, Gientien P, Marcaillou C (eds): Harmful Marine Algal Blooms, Lavoisier, Intercept Ltd., Paris, New York, 199–204

Newell RIE (1989). Species Profile of the Blue Mussel, *Mytilus edulis*. U.S. Fish and Wildlife Service - Biological Report. U.S. Army Corps of Engineers, 82(11)

Niedersächsisches Nationalpark Gesetz (2001)

NPG (1999). Nationalparkgesetz von Schleswig-Holstein vom 17. Dezember 1999

OiE (2009). Manual of diagnostic tests for aquatic animals. World Organisation for Animal Health. 7pp. http://www.oie.int/eng/normes/fmanual/A_index.htm. , Stand Januar 2009

Romalde JL, Area E, Sánchez G, Ribao C, Torrado I, Abad X, Pintó RM, Barja JL, Bosch A (2002). Prevalence of enterovirus and hepatitis A virus in bivalve molluscs from Galicia (NW Spain): inadequacy of the EU standards of microbiological quality. International Journal of Food Microbiology, 74: 119-130

Rosenthal H, Hilge V (2000). Aquaculture production and environmental regulations in Germany. Journal of Applied Ichthyology, 16: 163-166

Ruth M (1997). Zukunft der Muschelfischerei im Schleswig-Holsteinischen Wattenmeer: Ausgleich zwischen Ökologie und Ökonomie? In: SDN-Kolloquium, Zukunft der Muschelfischerei im Schleswig-Holsteinischen Wattenmeer. Dethlefsen V (Ed), 9-17

Sassen K, Velleuer R, Feldhusen F, Stede M, Effkemann S, Graf K, Zander HD, Pohlenz F, Heyken F, Hagena W, Hanslik M (2005). Niedersächsische Ausführungshinweise für die Überwachungsbehörden zur Durchführung der Muschelhygieneüberwachung. Niedersächsisches Ministerium für den ländlichen Raum, Ernährung, Landwirtschaft und Verbraucherschutz, Hannover, Germany, 18pp

Smaal A (2002). European mussel cultivation along the Atlantic coast: production status, problems and perspectives. Hydrobiologia, 484: 89-98

Tillmann U, Rick H-J (2003). North Sea phytoplankton: a review. Senckenbergiana maritima, 33: 1-69

Walter und Liebezeit (2001). Nachhaltige Miesmuschel-Anzucht im niedersächsischen Wattenmeer durch die Besiedlung künstlicher Substrate. Abschlussbericht der ersten Projektphase, 98 pp

Wattreport 2008. Nationalpark Wattenmeer – (k)ein Raum für ungestörte Natur? Berichte von Meer und Küste für Förderer und Freunde der Schutzstation Wattenmeer. http://schutzstation-wattenmeer.de , Stand August 2008

Chapter 08

KEY PARAMETERS FOR THE CONSUMPTION SUITABILITY OF OFFSHORE CULTIVATED BLUE MUSSELS (*MYTILUS EDULIS* L.) IN THE GERMAN BIGHT

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Abstract
The occurrence and composition of toxic algae, and presence of viruses and other human microbial pathogens in production areas of mussels are factors determining suitability of mussel products for human consumption. As bivalves feed by filtering large volumes of water, potentially toxic viruses, algae, and bacteria as well as phytoplankton are ingested. With the expansion of mussel aquaculture and subsequent increase in human consumption of mussel products, improved risk management is required for consumer protection. For example, shifting production to offshore areas (e.g. wind farms) can decrease the hazards of infection due to dilution of contaminants, and increase overall health of mussels. In addition, the deployment of off-bottom cultivation methods such as longlines increases the condition index, growth, and aesthetic appearance of mussels. However, other hazards like algal toxins not yet monitored on a regular basis, may play a more important rule offshore. Here we present an analysis of biological, economic, and consumer health-related aspects of mussel cultivation under near- and offshore conditions.

Keywords: blue mussel, offshore cultivation, site selection, parasites, viruses, biotoxins, microbial load

1. Introduction

In their natural environment, at in- and nearshore inter- and subtidal areas, mussels can be exposed to high concentrations of pollutants, pesticides, near surface agents, and estuarine runoff. High nutrient values in marine waters, particularly in densely populated coastal areas, provide an ideal environment for potential explosive growth of algae and bacteria. Even in regions with strict regulation of wastewater treatment, contamination with human pathogenic microbes can be found, which accumulate in filter feeding mussels. Many different kinds of bacteria and viruses, which are transmitted through the faecal-oral route, can occur in high numbers in sewage and cause illness, such as gastroenteritis. This route has been recognized as one of the most clearly identified health risks associated with urban wastewater (WHO 1987) and the concern remains that sewage treatment does not remove all pathogens from the effluent. Most cultivated and wild bivalves, for example mussels, oysters and clams, thrive in nearshore areas and are commonly consumed raw, or slightly cooked. As these organisms are exposed to a wide array of contaminants, clearly a serious hazard to human health exists. Numerous outbreaks of shellfish-transmitted infections have been recorded (Lees 2000). In contrast to nearshore areas, offshore waters offer a much cleaner environment due to the effects of dilution. Thus, the shifting of mussel production from intertidal or nearshore to offshore cultivation areas would reduce the general risks of infection and contamination. Yet other hazards, such as algal toxins, can occur more frequently offshore (Smaal 2002).

The European Community has outlined specific rules for products of animal origin in the Regulation (EC) 854/2004. According to this, contamination with parasites should be generally avoided. However, since mussel parasites are non- pathogenic to consumers, moderate infestations are tolerated. This practice is also applied to other marine fishery products (e.g. nematods in wild salmon), since a strict interpretation of the regulation would have a severe impact on sales and distribution of these products.
Although including parameters such as human pathogen viruses or the *Vibrio* species into a regular monitoring of bivalves remains a suggestion (Guyader et al. 2000, Formica-Cruz et al. 2002, Croci et al. 2003) as inferences from the microbial load to these contaminants are not evident (Romalde et al. 2002), the European Commission has designated only coliform bacteria i.e. *Escherichia coli* as an indicator for faecal contamination. Even the most recent EU guidelines (CEFAS 2003) for the control of mussel products expand the monitoring of the production areas only to all relevant influences on the microbial environment (runoffs, shipping zones, wild animals and other potential factors of contamination). However the focus remains on the analysis of *E. coli* and *Salmonella* as the principle parameters when defining suitability for consumption.

1.1. Bacterial infections

The survival of bacteria in seawater and its exposure to bivalves varies due to environmental factors such as temperature and salinity, and is influenced on seasonal and spatial scales (Hernroth 2003). The bivalves' response towards ingested microbes is to eliminate them. However, it has been shown that *Salmonella typhimurium* can survive more than two weeks after being injected into the circulating system of mussels (Hernroth 2003). *Salmonella* species can cause enterocolitis, enteric fevers such as typhoid fever, and septicemia with metastatic infections in humans. Seawater is the natural habitat of the *Vibrio* bacteria, feared as pathogens in fish and shellfish (Shao 2001). *Vibrio* can also cause severe infections in humans after consumption of raw or undercooked shellfish and contaminated food. A special hazard is caused by *V. vulnificus,* where severe infections can occur through skin lesions (Blake et al. 1979).

1.2. Viral infections

Like bacteria, viruses are predominantly concentrated in the digestive glands, but can also be absorbed through the gills (Abad et al. 1997) of mussels. Certain viruses such as the Norovirus are even more persistent and can remain infectious for weeks to months in seawater or in sediment (Gantzer et al. 1998)]. Although they are inherently unable to multiply in bivalves, shellfish are efficient vehicles for transmission of pathogenic viruses to humans. Epidemiological studies have revealed that human enteric viruses are the most common pathogens transmitted by consumption of bivalve shellfish (Lees 2000, Lipp & Rose 1997). Among these, HAV is the most serious viral infection linked to the consumption of bivalves. In Italy, recent estimates suggest that approximately 70 % of HAV cases are caused by shellfish consumption (Salamina & D' Argenio 1998). The relatively long incubation period following initial infection (average 4 weeks), complicates the traceability of the viral source. Thus, HAV infections caused through shellfish consumption are probably underreported or even remain undiscovered. Norovirus and serotypes of the adenovirus group are associated with gastroenteritis. These viruses have been recorded in seawater and shellfish in many countries (Formica-Cruz et al. 2002). In particular overall viral infections caused by the Norovirus (gene group II) have shown a remarkable increase, as registered by the Robert-Koch Institute (RKI 2000). This increase however, may be because Norovirus infections must be reported by law. However, the rapid course of the illness within a few hours complicates appropriate countermeasures.

1.3. Algae toxins

The main food source for bivalves is phytoplankton and here the potential for accumulating algal toxins is high. Several human diseases have been reported to be associated with many toxin-producing species of dinoflagellates, diatoms, nanoflagellates and cyanobacteria that occur in the marine environment (CDC 1997). Marine algal toxins become a problem primarily because they may concentrate in shellfish and fish that are subsequently eaten by humans (CDR 1991, Lehane 2000), causing syndromes including paralytic shellfish poisoning (PSP), diarrhetic shellfish poisoning (DSP), amnesic shellfish poisoning (ASP), neurotoxic shellfish poisoning (NSP), and azaspirasid shellfish poisoning (AZP). Beside NSP all other syndromes can also be traced to contaminated shellfish in European coastal waters. These shellfish-caused illnesses compromise human health, resulting in fishery closures, commercial losses, and serious concern over seafood safety and environmental quality. A regular monitoring system covering the risks according to site, which is able to detect problematic mussel products, is therefore a prerequisite to protect consumers.

1.4. Parasites

All known micro and macro parasites of the European coastal waters are -in contrast to other mentioned organisms- not pathogenic to consumers, but may have negative condition effects and cause higher mortalities of infested hosts. Parasites of blue mussels occur largely in intertidal and nearshore areas. Buck et al. (2005) have shown that mussels grown offshore are free of macro parasites. Infestation rates increased from sites towards the shore, where in particular intertidal mussels showed the highest numbers of parasites. In this study we focused on abundant species commonly regarded as harmful (e.g. Odlaug 1946, Meyer & Mann 1950, Cole & Savage 1951, Dethlefsen 1975, Kent 1979, Kent 1981, Taskinen 1998, Thieltges 2006) or cadging (Davey & Gee 1988) to the host living in the tissues, organs, or shell of the mussel. Species living in the mantle cavern or on tissues were not considered, since any impact on the energy household of the mussel is unlikely.

The only micro parasite known to be associated with *M. edulis* along the European Atlantic coast is *Marteilia refrigens,* causing the Marteilliosis or Aber disease (Le Roux et al. 2001). From the North Sea, infested populations of mussels have been reported from the British Isles, whereas the eastern regions including the German Bight are regarded as micro parasite free. Marteilliosis in mussels is generally associated with a poor condition index, exhaustion of energy reserves (e.g. glycogen) and high mortalities (Grizel et al. 1974). Mass infections with *M. refrigens* can have a severe economic impact, e.g. oyster farmers in France lost approximately 440 million Euros in two years (1980 and 1983) due to Marteilliosis.

1.5. Shell commensals

Many of these organisms use mussels, or any other suitable hard substrate, to settle on. Since these organisms do not depend on mussels to fulfill their reproduction cycle, they are commonly regarded as commensals (Cheng 1967). Unsoiled mussel shells, due to their aesthetic appearance, demand the highest prices on the market since they can be sold alive without being extensively cleaned before processing or selling. Information on the health effects of shell commensals on host organisms is still scarce, since the measurement and evaluation of the impact of parasites or commensal species and their influence on single hosts or host populations is difficult to determine (Zens 1999). However, studies have shown that massive covering hamper feeding, increase flow resistance, and reduce growth (Laudien & Wahk 2004, Buschbaum & Saier 2001, Dittmann & Robles 1991).

1.6. Economics

From the economic point of view mussels should not contain microbes, or be at least clearly under legal thresholds. Mussels should grow fast, have a good meat-shell ratio and should look aesthetically pleasing to achieve the highest price on the market. In the traditional nearshore on-bottom cultivation grounds in the Wadden Sea of Germany, not all of these preconditions for maximum growth, microbial purity, and aesthetic demands are fulfilled. Mussels cultivated off-bottom using longlines grow faster and have higher meat/ shell ratios than on-bottom cultivated mussels (Buck 2007).

1.7. Offshore Production

Although the market for mussel products in Germany is not saturated and mussels are imported, an expansion of the on-bottom production sector within intertidal and subtidal areas of the coastal sea is not allowed due to restrictions on the number of licenses, environmental protection and stakeholder conflicts (Buck et al. 2004). However the development the offshore wind farming industry offers a unique opportunity to co-use large marine areas with submerged culture systems for blue mussels and other candidates (Buck 2002). Estuarine runoffs result in a high concentration of contaminants in the Wadden Sea. In contrast offshore areas are far enough away from sources of urban sewage and estuarine runoff that waters are clean with continuously good O_2-conditions. Organisms living under good water conditions accumulate fewer toxins and have a less stressed immune system. Mussels grown under offshore conditions should be in better health than mussels grown in near- and inshore areas. Healthier mussels mean faster growth rates and a qualitatively better product for human consumption. In addition rapid growth and a better quality of product compensate for the higher investment costs incurred by the new culture systems compared to traditional bottom culture techniques.

In the present study we have examined mussels from six different sites of the German Bight, including an offshore and a nearshore testing area both equipped with submerged culture systems. Samples were assessed according to the actual legislation and guidelines of the EU, Germany and its States (bacteriological load, viruses and algae toxins). Parameters relevant for growth (macro- and micro parasites and to some extent shell commensals) and those influencing the marketability of mussel products (calcareous fouling organisms, meat content) were also investigated.

2. Material and Methods

Five locations along the coast of the German Bight were sampled to test and analyse mussels grown under different conditions (Fig. 1). Three areas were natural beds of mussels near Neuharlingersiel (NH, upper intertidal, Position 53° 42’ 10’’ N; 007° 43’ 50’’ E), Bordumer Sand (BS, upper intertidal, Position 53° 30’ 00’’ N; 008° 06’ 00’’ E) and from the Lister Strand from the Island of Sylt (SY, lower intertidal, Position 55° 01’ 32’’ N; 008° 26’ 43’’ E).
Two locations were specially designed testing areas, where mussels were grown suspended on an artificial substrate: the nearshore location on the *Niedersachsenbrücke*, an approx. 1.300 m long cargo bridge, at the Jade estuary (JD, Position 53° 35’ 05’’ N; 008° 09’ 14’’ E) near the city of Wilhelmshaven, and under offshore conditions an area called *Roter Sand* (RS, Position 53° 51’ 00’’ N; 008° 04’ 20’’ E) situated in the Weser estuary ca. 17 nautical miles northwest of the city of Bremerhaven. Throughout 2007 four consecutive sampling cycles in March, May, August, and November were conducted to test for site and seasonal influences on assessed parameters. Each sampling cycle was completed within 10 days and all parameters were analysed for each site and sample cycle. Intertidal areas (NH, BS and SY) were sampled at low water, whereas RS had to be sampled at slack water with a team of scuba divers operating from a research vessel. The JD site is accessible without any tidal constraints all year round. At each sampling site ca. 5 kg of mussels were collected for all investigations.

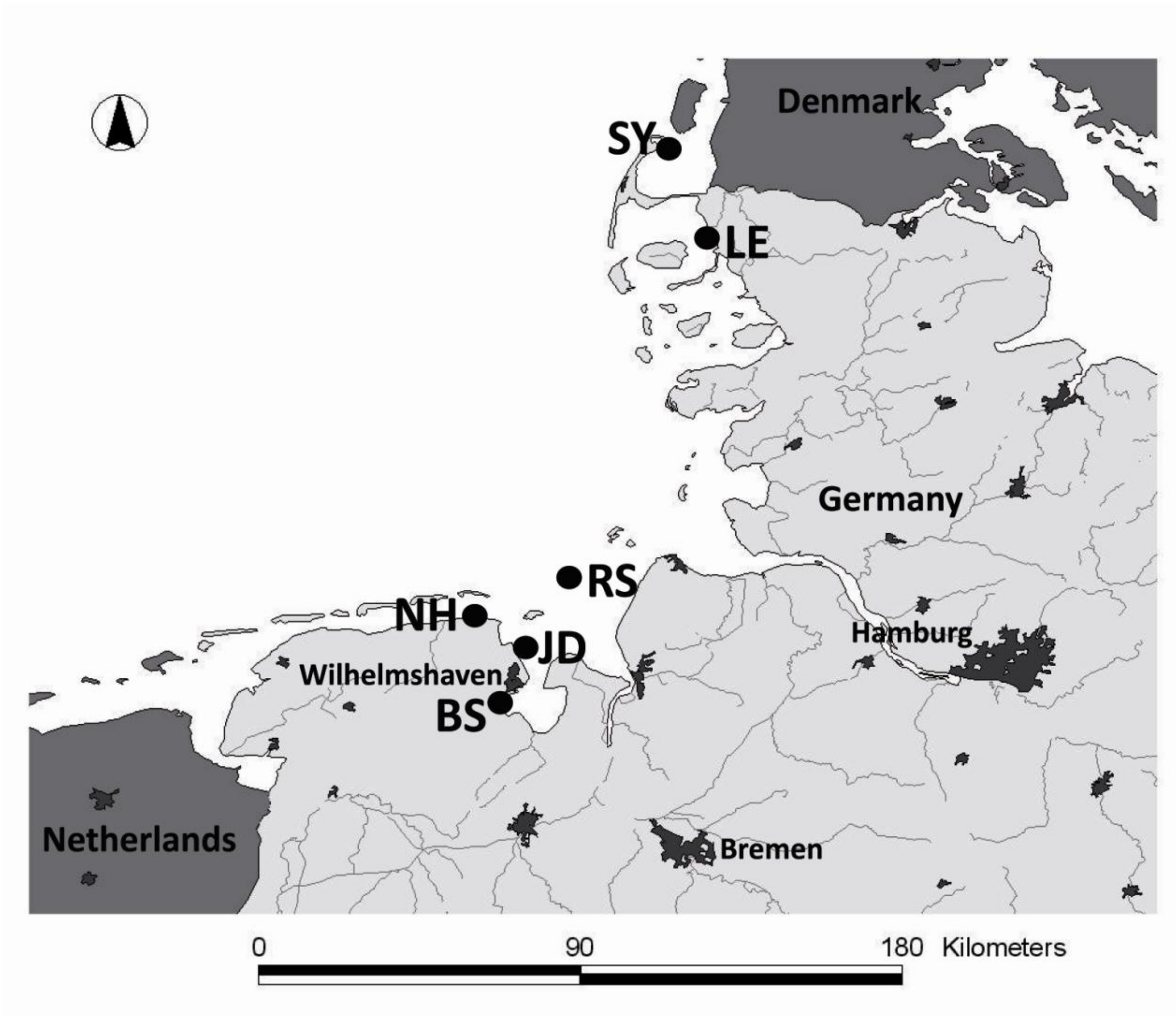

Fig.1: Map of the German Bight showing the sample sites. Three intertidal sampling locations at Neuharlingersiel (NH), Bordumer Sand (BS) and Lyster Strand at the island of Sylt (SY) and two suspended hanging cultures at the *Niedersachsenbrücke* (nearshore) near Wilhelmshaven in the Jade (JD) estuary and offshore at the entranceof the Weser estuary near the lighthouse *Roter Sand* (RS) were sampled in the year 2007. The on-bottom cultivation (subtidal) area at *Eidumstief* (LE) was sampled once in winter 2009.

In addition, mussels from a licensed area (LE, subtidal, Position 54° 46‘, 66’’N, 008°, 18’, 72’’ E [Fig. 1]) for on-bottom cultivation at *Eidumstief* near Emmelsbüll-Horsbüll, Germany, were sampled once in winter 2009 by the local fishermen. The spat for these mussels was collected on vertical nets at the Jade estuary at May 2007, transferred in October 2007 to the LE (N 37) and harvested there in February 16th 2009. For these mussels weights and shell lengths were determined, but they were only analysed for macro parasites.

2.1. Macro parasites

To ensure that all mussels were of a comparable age range, 15 mussels were selected according to a shell length between 25 to 50 mm. These represent specimens of similar physiology, also used in standardized bioassays (Ernst et al. 1991). Mussels bigger than 50 mm originated from the offshore sampling site of RS (August and November 2007) where growth rates are high and mussels reached sizes up to 65 mm within 15 months. Mussels from suspended offshore and nearshore sites were of a defined age since deployment of the artificial substrate took place in April 2006 at both sites. Raw mussel were frozen and stored at -20 °C. After defrosting at room temperature (approx. 20-30 min) mussels were analyzed immediately.

First, the area covered by calcareous shell commensals of all mussels was estimated. Length and width of each selected mussel was measured according to Seed (1968) to the nearest 0.1 mm using a vernier calliper. Mussels were opened, briefly drained on absorbent paper, and subsequently total wet weight was determined. Then, the soft body was removed and both shell and soft body were weighed (± 0.01 g) separately. The soft body was then placed on the bottom of a glass compressorium and the mantle, gills, food, adductor muscle and other tissues were dissected carefully and dispersed. The digestive gland was pulled apart and squeezed together with the other tissues using the cover glass of the compressorium.
The preparations were examined under a stereo magnifying glass (10-50 magnification) with transmitting light for the presence of macro parasites. Parasite species were identified according to descriptions from the literature (e.g. Dethlefsen 1970; 1972, Lauckner 1975, Watermann et al. 1998) and infested organs listed. As freezing of the samples does not affect size of a trematod's metacercaria (Lepitzki et al. 1994), identification of trematodes was also reliable using frozen samples. In a final step all shells of the analysed mussels were inspected for the presence of shell-boring polychaets using the stereo magnifying glass.
In addition 15 mussels from the winter sample of LE were also analysed according to the same scheme described above.

2.2. Micro parasites
Forty mussels (30-50 mm) per sample site were analysed each sampling cycle to assess potential infestations with intracellular micro parasites, of the genus *Marteilia*. Fresh meat of 20 mussels was removed from the shell and glued separately on aluminium chucks before being frozen at –20 °C for kryostat-sectioning. To ensure a representative overview of potential infested organs, the frozen softbody was trimmed until digestive gland, gills, and palps appeared together in one tissue sections of the sample. Soft bodies of additional 20 mussels were removed and cut transversally according to international standard methods (Ifremer 2008) and subsequently used for smear preparations. Tissue sections and smear preparations were stained using *Haemacolor®* (Merck) before assessed by light microscopy.

2.3. Condition Index and shell length-weight relation
30 mussels were used to calculate the condition index (CI) for all testing sites (data of 15 mussels used for macro parasite assessment added by 15 additional mussels to increase sample size). For a direct comparison of CI and the parasite load only wet weights of tissues and shells could be used for the calculation (see below). An additional comparison is provided with all winter samples including mussels from LE. Here, also 30 individuals were used for CI.

$$CI = \frac{Wet\ meat\ weight\ [g]}{Shell\ weight\ [g]} \text{ x } 100 \qquad (1)$$

Since shell thickness and strength strongly depends on natural conditions and the cultivation method of the mussel, a shell length-weight (dry weights) correlation of winter samples including the licensed area was established. Mussels were sorted into three groups containing each a minimum of 45 individuals: intertidal (n= 45), off-bottom (n=60), and on-bottom (n=45).

2.4. Bacterial count, *E. coli*, *Salmonella*, *Clostridia* and *Vibrio*
The mussels from each sampling site were examined at the Institute for Fish and Fishery Products of the State Office for Consumer Protection and Food Safety of Lower Saxony (LAVES). Prior to bacterial investigation the mussels were cleaned, opened and prepared under sterile conditions.

2.4.1. Total aerobe bacterial number
The method used corresponded to the standardized method DIN 10161 which describes the drop plating procedure. According to this method an initial solution of 5 g of the homogenized sample was

diluted decimally over six steps, and then incubated separately on culture media (plate count). The result (colony forming unit [cfu/g]) was calculated based on the formula for the "weighed arithmetic mean" (DIN 10161).

2.4.2. *Escherichia coli*
The MPN-method (Most Probable Number) used here corresponds to the "Generic Standard Operating Procedures for the Enumerations of *E. coli* in Molluscan Bivalve Shellfish", issued by the European Community Reference Laboratory for Monitoring Bacteriological and Viral Contamination of Bivalve Mollusks CEFAS/CRL, Weymouth, UK) (CEFAS/CRL 2008). The initial solution of 15 g of the homogenized sample was dispensed to a 5-tube-3-dilution- scheme. The combination of the tubes with a confirmed growth of *E. coli* revealed the Most Probable Number of cfu of *E. coli*/100 g.

2.4.3. *Salmonella*
The method corresponds to the international norm DIN EN ISO 6579 (2003). The initial solution of the 25 g of homogenized sample was enriched twice in culture media and then plated on selective agar plates, allowing the identification of cfu of *Salmonella*.

2.4.4. *Clostridia* and *Vibrio*
The method of detecting *Clostridia* corresponds to the standardized norm DIN EN ISO 7937. The initial solution of 5 g of the homogenized sample was incubated in selective culture media under anerobic conditions. For *Vibrio* only qualitative approaches were conducted for identification, using 25 g of the homogenized sample according to ISO 21872 standard.

2.5. Viruses
Prior to viral examination the mussels were cleaned, opened and prepared under sterile conditions. Then 6 g meat of mussels of each sample was homogenized under PCR-clean conditions, and then analyzed using the Real Time Reverse Transcriptase–Polymerase Chain Reaction (RT PCR).
The method for the qualitative detection of Norovirus (gene group II) corresponds to the reference method (Höhne & Schreier 2004), issued by the National Reference Laboratory (NRL) for Viral Contaminations of Bivalve Molluscs at the Federal Institute for Risk Assessment (BfR) in Berlin, Germany.

2.6. Algae toxins/ Shellfish poisons
The monitoring of algal toxins is organized by the States according to EC 854/2004, specified by the regulations of the responsible public surveillance authorities [e.g. Sassen et al. 2005). Concentration limits for biotoxins in shellfish products are listed in EC 853/2004. The applied methodologies for the analysis of algal toxins are according to EC 2074/2005, however, without using any mouse bioassays since the use of animals in food analysis is not allowed by law in Germany. Alternatively chemical approaches such as High Performance Liquid Chromatography (HPLC) were used for detection of algal toxins. Prior to the examination the mussels were cleaned, opened and prepared under sterile conditions. Then ca. 100 g meat of mussels of each sample was homogenized and analyzed using three different methodological adaptions of the HPLC-Method. For the detection of DSP a Liquid Chromatography with mass spectrometric detection (LC-MS/MS) was applied, whereas ASP was examined using an adapted HPLC-Method according to Quilliam et al. (1989). PSP was detected by using the method of Lawrence and Menard (1991).

3. Results

3.1. Condition Index and shell length-weight relationship

According to the condition index values (CI) sites are divided into two groups (Fig. 2). Low CIs (CI 27.39 to 39-47) are found throughout the year with only moderate variances at the intertidal areas, whereas high indices (CI 61.21 to 113.79) are found at both culture sites. While the nearshore hanging culture JD showed an overall peak already in spring 07 (CI 113.79) followed by a decrease of the CI down to 61.21 in autumn, the values of the offshore site stayed rather stable from spring to autumn with a minimum in winter time (CI 66.20). The mean values calculated for the whole sampling season showed the highest numbers for RS (94.5 ± 21.5 SD), followed by JD (82.97 ± 24.88 SD), NH (34.76 ± 5.56 SD), BS (32.58 ± 8.96 SD) and SY (31.38 ± 7.83 SD) (Fig. 2).

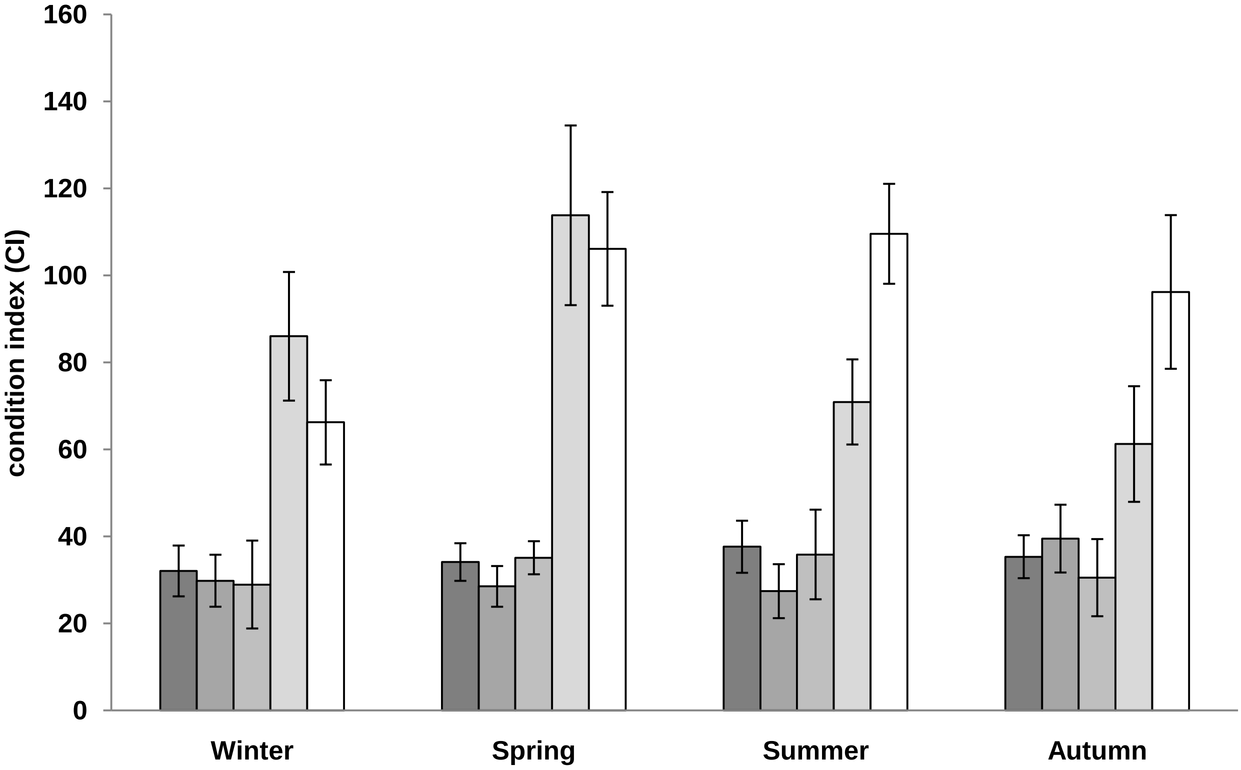

Fig. 2: Condition indices [CI] of blue mussels from five different sampling sites (NH [black], SY [dark grey], BS [grey], JD [light grey] and RS [white] over the season 2007 in the German Bight (n=15 per site and sample cycle).

All winter samples, including the mussels from the on-bottom culture plot, were sorted according to their culture method and tidal regime. On-bottom cultivated mussels had the best CI (LE, 88.95 ± 12.67 SD, n=45) followed by nearshore cultivated (JD, 88.19 ±12.98 SD, n=45) and offshore-cultivated (RS, 70.81 ± 11.63 SD, n=30) mussels. The mussels from the three intertidal areas (each n=45): BS 34.34 ± 11.61 SD, NH 33.70 ± 8.02 and SY 33.66 ± 7.24 had lowest CI.

The shell length-weight relationship (Fig. 3) showed that intertidal mussels (n=45) had the heaviest shells in relation to their length. The shells of the on-bottom cultured mussels (n=45) had an intermediate weight whereas the hanging cultivated mussels (RS & JD, n=60) developed the lightest shells.

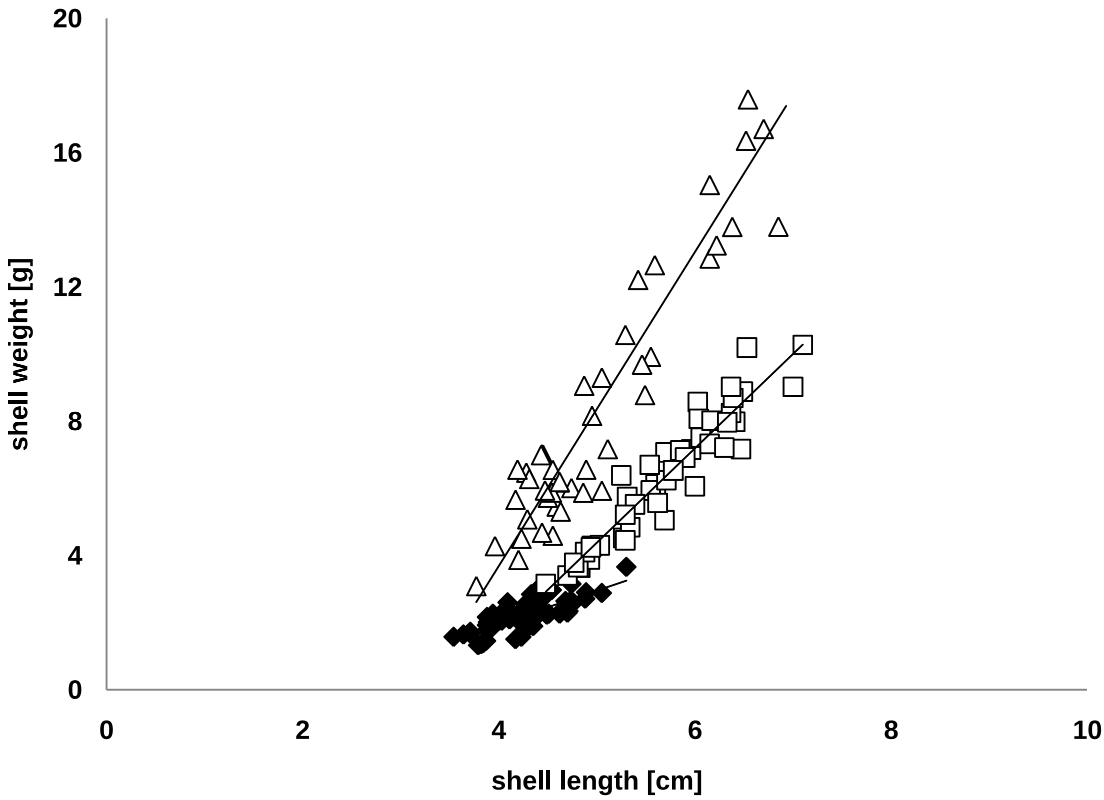

Fig. 3: Relationship of shell length [cm] and shell weight [g] of wild intertidal (white triangle, n=45), on-bottom cultivated (white square, n=45) and off-bottom cultivated (black rhombus, n=60) mussels from 6 different sample sites of the German Bight of winter 2007/09.

3.2. Macro and micro parasites

Most macro parasites found in the tissues and organs of *M. edulis* belonged to four different native species (Krakau et al. 2006): *Mytilicola intestinalis* a copepod living as juvenile and adult individual in the digestive gland, two trematod species *Renicula roscovita* and *Himastla elongata* occurring as metacercarias in the gills, mouth palps and tubuli of the digestive gland or in the foot and other muscles, respectively. And last the Polychaet *Polidora ciliata* living in self drilled ducts of the shell of mussels. Other candidates such as *Modiolicula insgnis* and species of the genus *Gymnophallus* occurred in less than 1 % of the cases and are not displayed. With the deployed sampling method (using a glass compressorium under a stereo magnifying glass) only adult *M. intestinalis* of >2.5 mm were found in the digestive gland.

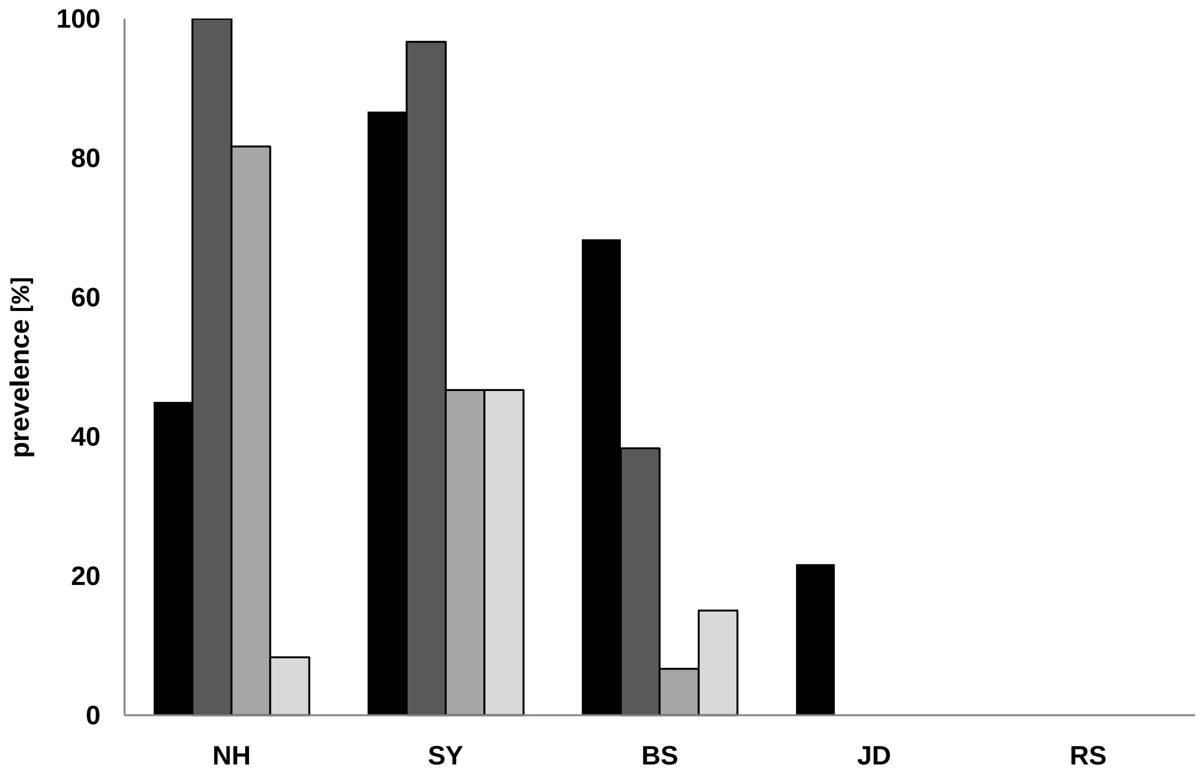

Fig. 4: Prevalence [%] of macro parasites *M. intestinalis* (black), *R. roscovita* (dark grey), *H. elongate* (grey) and *P. ciliata* (light grey) found in blue mussel according to five sampling site (n=60 per site) in the German Bight during the season 2007.

The most common macro parasites showed a high prevalence of up to 100 % at the intertidal areas whereas the cultivated mussels were hardly infested (nearshore) or free of parasites (offshore) (Fig. 4). Prevalence of *M. intestinalis* from intertidal samples ranged from 45.0 % (NH), 68.33 % (BS) up to 86.67 % at SY (Fig. 4) with a mean intensity spreading from 0.87 ± 1.20 SD, 3.30 ± 2.30 SD and 3.22 ± 2.76 SD individuals per mussel, respectively (Fig. 5b). At the nearshore cultivation area JD about 21.67 % of the mussels were infested by *M. Intestinalis* (Fig. 4) with an average of 0.33 ± 0.73 SD individuals (Fig. 5b).

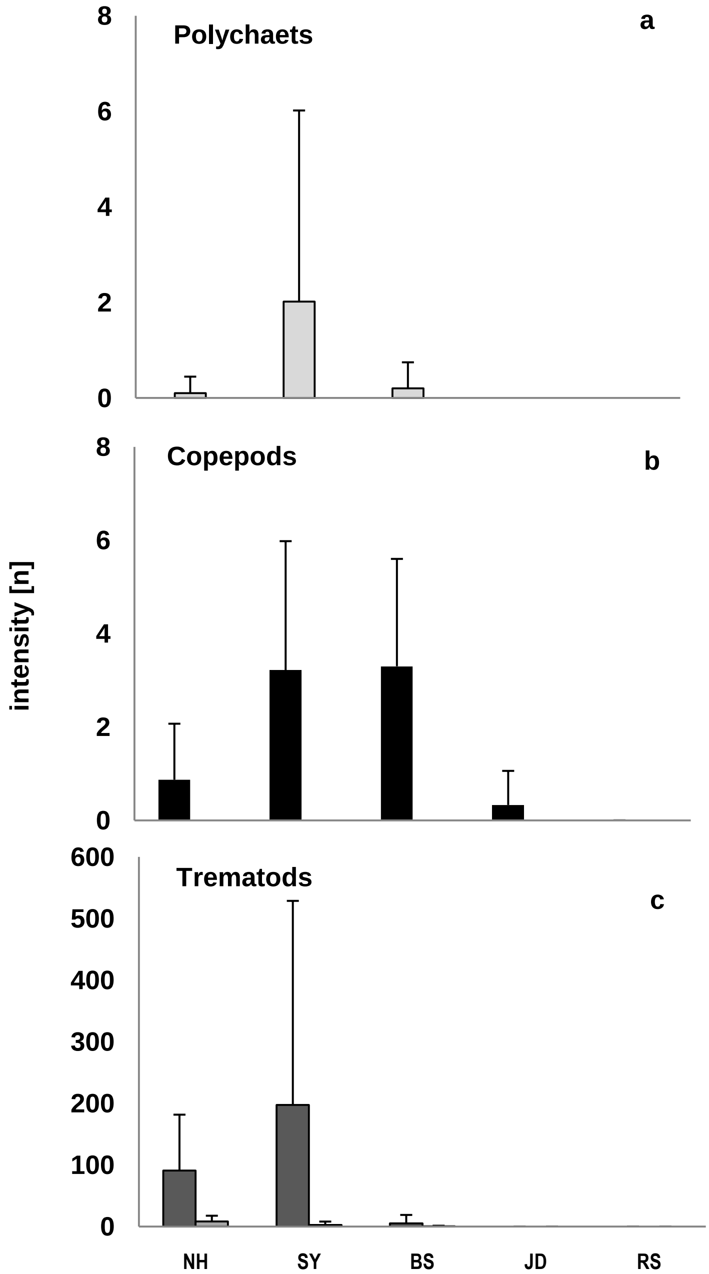

Fig. 5a-c: Intensity [n] of macro parasites (a shell boring Polychaet/ *P.ciliata* [light grey], b Copepod/ *M. intestinalis* [black], c Trematods/ *R. roscovita* [dark grey]; *H. elongate* [light grey]) in blue mussels of five sampling sites (n=60 per site) in the German Bight in the year 2007.

Trematods occurred in two species in intertidal areas. There, *R. roscovita* exhibited a prevalence up to 96.67 % at SY and 100 % at NH (Fig. 4) together with high mean intensities of 90.52 ± 91.05 SD and 197.28 ± 331.40 SD individuals per mussel, respectively (Fig. 5c). At the SY sampling site mass infestations with >1000 *R. roscovita* were also observed. BS showed low intensities of an average of 5 ± 13.80 SD metacercarias of *R. roscovita* per mussel in about 38.33 % of the samples (Fig. 4 & 5c). *Himastla elongata* the second trematod specie found as metacercarias occurred, similarly to *R. roscovita,* only at intertidal sites. In this case prevalences were highest in NH (81.67 %), followed by SY (46.67 %) and BS with 6.67 % of infested mussels (Fig. 4). Intensities were low and ranged from 8.28 ± 9.22 (NH), to 2.67 ± 5.34 SD (SY) and 0.22 ± 1.04 SD at BS (Fig. 4 & 5c).
Similarly to the three other parasite species, *P. ciliata* occurred only at intertidal sites. Prevalence was high in SY (46.67 %), moderate at BS (15.00 %) and low at NH (8.33 %) (Fig. 4). Intensities were also

low and ranged between 0.10 ± 0.35 SD at NH, 2.02 ± 4.00 SD at SY and 0.20 ± 0.55 SD at the sample site of BS (Fig. 5a).
The winter sample of LE showed high prevalence of *M. Intestinalis* (86.67 %) at a moderate average intensity of 2.73 ± 2.09 individuals per mussel. Other species of macro parasites were absent in the mussels from the subtidal on-bottom cultivation area.
Adult *M. intestinalis* inhabit only the hind gut of the digestive gland, whereas *R. roscovita* occurred in the tubuli of the digestive gland (59 %) and in the gills or pulps (35 %) of the mussel. The second trematod *H. elongata* is found mainly in the foot (78 %) and in other muscular tissues (15 %) (Tab. 1).

Tab.1: Infestation [%] of mussel (n=300) organs by most common parasites of blue mussels from five sampling sites of the German Bight (2007).

	Digestive gland	Gills/Palps	Foot	Muscle	Shell
M. intestinalis	100	-	-	-	-
R. roscovita	59	35	3	3	-
H. elongata	6	1	78	15	-
P. ciliata	-	-	-	-	100

The most invested organs by macro parasites were the digestive gland, where *M. intestinalis* and *R. roscovita* were found, mouth palps and gills infested by *R. roscovita* and the foot infested by mainly *H. elongata* and to a certain extent also *R. roscovita* (Tab. 1).
All organs and tissues of the investigated samples from all five different sample sites were free of *M. refrigens* throughout the year 2007.

3.3. Shell commensals
Many organisms use mussel shells as a hard substrate to attach to and live on. Four taxa which build up calcareous parts were found in samples at all sites: the barnacle *Balanus* spp., the pacific oyster *Crassostrea gigas*, the Bryozoa *Flustra foliacea* and the common slipper snail *Crepidula fornicate*. Especially at intertidal sites (NH & BS) *Balanus* spp. covered 30.88 % and 32.28 % of the shell surface, respectively. At SY and at JD only 6.72 % and 5.45 % were covered by barnacles. *Flustra foliacea* became more abundant except for in the intertidal areas at the nearshore (JD 13.53 %) and offshore cultivation sites (RS 10.23 %). Beside bryozoes, offshore cultivated mussels were free of calcareous fouling organisms. *Crepidula fornicate* and *C. gigas* were found only infrequently at intertidal areas on the shells of mussel.
The winter samples of LE were covered by *Balanus* spp. at an average of 1.87 % and by *F. foliacea* at 23.67 % of shell surface.

3.4. Microbial assessment
Throughout the seasons of 2007 a microbial assessment was conducted for all sites and samples with a focus on the total aerobic microbial load and the contamination with *E. coli*, and *Salmonella*. Besides *E. coli*, three specimens of *Clostridia* (*C. perfringens, C. butyricum, and C. botulinum*) (Fig. 6a-d) and four different *Vibrio* (qualitative approach) species (*V. parahämolyticus, V. alginolyticus, V. cholera,* and *V. fluvialis)* were detected (Tab. 2). *Salmonella* subspecies were not found in any of the investigated samples.

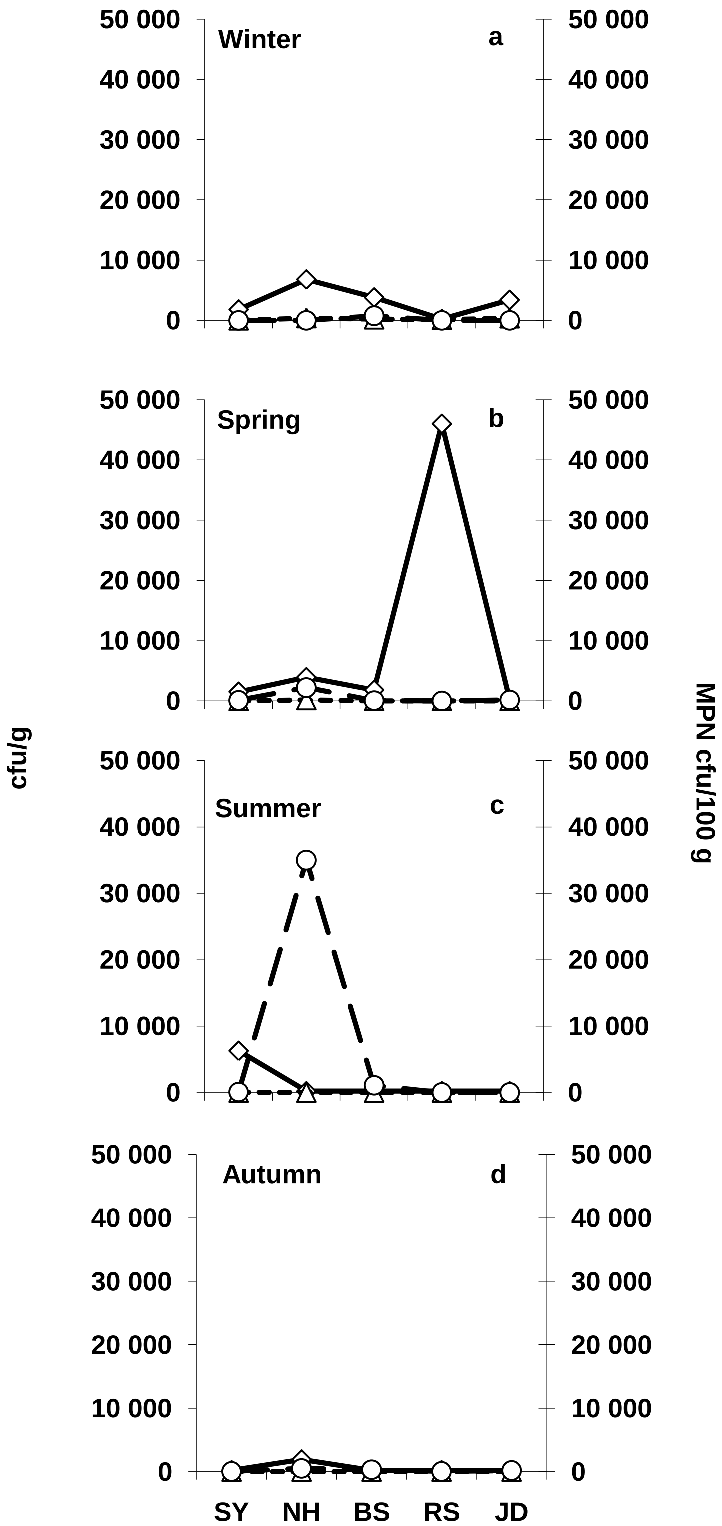

Fig. 6a-d: Variances of the total microbial load [cfu/g]) (rhombus) and the presence of *E. coli* (circle) and *Clostridium* spp. (triangle) (both MPN [cfu/100 g]) in mussels of five different sampling locations of the German Bight during the season (a/ winter, b/ spring, c/ summer and d/ autumn) 2007.

In 19 out of 20 samples the total microbial load varied between 200 and 6800 colony forming units [cfu/g] (Fig. 6a-d). In spring a single peak was detected at 46.000 cfu/g at the offshore location RS (Fig. 6b). A similar pattern was found when assessing the Most Probable Number [MPN] of *E. coli* [cfu/100 g] at the five different sample sites. In 19 out of 20 samples the contamination with *E. coli* bacteria varied between 20 (lower detection limit) and 1100 MPN [cfu/100 g] (Fig. 6a-d). One summer sample of the intertidal area near NH showed the maximum load of 35.000 MPN [cfu/100 g] of *E. coli* (Fig. 6c).

Colony forming units of *Clostridium* spp. (10-377 [cfu/g]) were found throughout the year at all sites (65 cfu/g ± 114) (Fig. 6a-d). In spring stations showed highest average contamination of *Clostridium* spp. (203 cfu/g ± 158), consisting only of *C. perfringens*. In spring and summer two other species, *C. butyricum* and *C. botulinum* (no botoxin detectable), were found in low concentrations (154 and 6 cfu/g, respectively) at NH. For the remaining spring and summer samples and all samples from the autumn a qualitative analysis was not possible.

BS was the only site where all samples were contaminated by *Vibrio* species throughout the year. At JD all four *Vibrio* species occurred, in the autumn sample even *V. cholera* but without cholera toxins. The summer sampling showed *Vibrio* at all sites and in autumn four (NH, BS, JD and RS) out of five sites were contaminated. In winter and spring *Vibrio* were detected only at two sites. Winter (NH, SY and BS) and spring (BS and JD) samples showed fewer sites contaminated with *Vibrio* (Tab. 2).

The classification of cultures in plots is based on the Regulation (EC) 854/2004. Class A plots should have *E. coli* values below 230 cfu/100 g MPN, whereas B-class plots can reach values up to 4600 cfu/100 g MPN. Mussels from B-class plots must be transferred and purified, whereas A-class mussels can be sold alive. C-class plots (values above 46.000 cfu/100 g) risk loss of the cultivation license.

Tab. 2: *Vibrio* spp. infestations of blue mussels of five different sites of the German Bight throughout the season 2007.

Season 2007	Site	*Vibrio* spp. [qual]
Winter	NH	*V. alginolyticus*
	SY	*V. alginolyticus*
	BS	*V. fluvialis*
	JD	n.n.
	RS	n.n.
Spring	NH	n.n.
	SY	n.n.
	BS	*V. parahämolytikus*
	JD	*V. parahämolytikus*
	RS	n.n.
Summer	NH	*V. parahämolyticus*
	SY	*V. parahämolyticus*
	BS	*V. parahämolyticus*
	JD	*V. alginolyticus*
	RS	*V. parahäm/alginolyt*
Autumn	NH	*V. alginolyticus*
	SY	n.n.
	BS	*V. parahämolyticus*
	JD	*V.chol*/parahäm/alginol*
	RS	*V. alginolyticus*

3.5. Virus contamination and Shellfish poisons

In no sample from the five different sites biotoxins reached a critical level. Only a sporadic presence of DSP in marginal concentrations was detected. No ASP or PSP was found during the sampling period throughout the season 2007. Viruses were also absent in all samples.

4. Discussion

Our data show that offshore-suspended cultivated mussels from the location *Roter Sand* fulfil all official requirements for edibles. They were free of *E. coli* and parasites, grew fast, and reached market size within 15 months. Maximum CIs of mussels investigated over the whole sampling season were achieved in spring and summer by the hanging cultures. In winter, however, the CIs of on-bottom and nearshore cultivated mussels were higher than intertidal and hanging cultivated mussels from both sites. High numbers of *E. coli* were found once at the intertidal area NH. However, offshore cultivated mussels contained high bacterial loads in spring and were detected as carriers of two *Vibrio* species. Hence, the greater distance to shore at our offshore site provided no guarantee for microbial purity of the mussels. This indicates that dilution, normally providing better water quality in terms of microbes, occurs even further out from the coast of the German Bight. It is possible that, as the offshore area of the *Roter Sand* is near the entrance of the Weser estuary, it is exposed to the last discharges of black water by trading ships just about to enter Bremerhaven harbour. Other potential hazards for offshore sites may result from local “hot spots” such as munition discharge areas, oil spills, pipelines and platforms. Together with natural sources of contamination and pollution such as large bird or seal colonies from islands or other exposed areas, these hazards should be of concern during site selection and observed during production time.

4.1. Parasite, virus and bacteria infestation

Due to (i) the absence of first intermediate trematod hosts (e.g. *Littorina* spp.), which thrive in nearshore waters habitats, (ii) the distance from the host populations, resulting in dilution effects, which might be an explanation for the absence of shell-boring polychaetes and parasitic copepods, and (iii) the poor swimming capacities of planktonic stages of *M. intestinalis* (Davey & Gee 1988), offshore mussels are free of macro parasites. In contrast, intertidal mussels show the highest infestations rates regarding number of parasites and number of species. The on-bottom cultivated mussels were only infested by *M. intestinalis,* but to a high degree.

The potentials of off-bottom and offshore cultivation methods are most obvious in the case of macro parasites. Hanging cultivation reduces the risk of infestation drastically, both in prevalence and intensity. Additionally, the spectrum of species is reduced by off-bottom cultivation methods. Even in the vicinity of highly infested intertidal areas, nearshore hanging cultures showed low infestations. In the case of *M. intestinalis* the poor swimming capacities of the larvae is perhaps the reason for the low infestation rates of hanging cultures near- or offshore. Whether a similar mechanism also holds for trematodes and shell boring polychaets, completely absent in the suspended culture areas, remains speculative. However, only the combination of off-bottom cultivation and a long distance to shore prevented contamination by parasites.

The role and effects of macro parasites on the health status of their hosts is still debated intensively. Older studies have shown that *M. intestinalis,* although living in the hind gut, have a severe negative impact on the condition of their hosts (Odlaug 1946, Meyer & Mann 1950), hence reducing the meat yield of the mussel (Cole & Savage 1951, Dethlefsen 1975) and reducing the resistance and adaptability of the mussel in its environment (Calvo-Ugarteburu & McQuaid 1998); whereas in a more recent 10-year study from Davey and Gee (1988), *M. intestinalis* was interpreted more as a commensal organism feeding on unutilized fractions in the hint gut of mussels. Although *M. intestinalis* appears not to be the epizootic hazard for mussels as described in earlier publications (Meyer & Mann 1950), it is hard to believe that its existence has no negative consequences for the energy budget of its host, particularly since infections occur in the digestive gland which is the central organ for energy metabolism. Together with the impediments caused by trematods’ metacercarias,

the holes caused by *P. ciliata*, and high loads of bacteria and viruses, it can be assumed that the overall health and growth performance of mussels is negatively impacted.
This is also displayed in the low condition indices of intertidal mussels correlating with the highest parasite infestation rates, whereas mussels with low infestations had the highest condition values. Thus evidence strongly indicates that the negative condition values are caused by parasites. Offshore mussels showed condition values at least twice as high over the whole sampling season compared to mussels from intertidal areas. Since hanging cultivated mussels produce a lighter shell, these differences may overestimate the impact of parasites, however, it remains most likely that parasites are responsible for low condition values.
Viruses were not observed in any sample collected for this study. Other problematic microbes, such as *Clostridium* spp., were present in all samples, however, in low numbers. Additionally, four different species of *Vibrio* were proven at all sampling sites. The results for *Clostridium* spp. and *Vibrio* spp. correspond with the findings from Lhafi (2006) who surveyed different on-bottom mussel production areas in the German Bight in 2005. However, Lhafi (2006) also detected Noro- and Rota-Viruses in 30 % and 2.2 % of the samples, respectively.
The findings for *Vibrio* spp. and *Clostidium* spp. in this study were independent of the solely registered high values of *E. coli* at NH in the summer sample. Thus supporting the frequently pronounced suggestion (Guyader et al. 2000, Formica-Cruz et al. 2002, Croci et al. 2003, Romalde et al. 2002) of including human pathogenic viruses and bacteria into a regular survey, since focusing on coliform bacteria or *Salmonella* will not exclude these mussels from consumption (Rehnstam-Holm & Hernroth 2005).

4.2. Influence of distance to shore and cultivation method

High condition indices, good growth rates, low parasite infestation rates, and a minimal number of calcareous fouling organisms on the shells characterize mussels cultivated off-bottom and exposed to sustained inundation. An increased distance to shore would further decrease parasite infestation rates and most likely lead to minimal microbial and viral infestations. In this study evidence was supplied only in the case of parasites. Future studies should operate offshore testing sites further off the coast. Distance to shore for the offshore site followed the definition of Ryan (2005) and Buck (2004). Even at this distance however, the strong estuarine run-offs of the Elbe and Weser rivers impact the quality of these marine waters. Perhaps dilution effects, decreasing the microbial load, set in further off the coast of the German Bight.
Trend lines of the shell length-weight relationship for intertidal on-bottom and off-bottom mussels show that off-bottom cultivated mussels invest the least energy in their shells. The shell is thin and weak, causing problems during the harvesting process. In contrast, on-bottom- cultured and shore-exposed intertidal mussels invest much more energy for building up their shells. Thicker shells allow a better handling during harvesting, processing and transport, however reduce the energy available for growth and build-up of meat content.
Calcareous shell commensals follow the same pattern as mussel parasites. Intertidal mussels are densely covered with various species, whereas subtidal on-bottom and nearshore off-bottom cultivated mussels showed a reduced spectrum of specimens occurring in low numbers. Offshore cultivated mussels were, besides some bryozoes, essentially free of shell commensals. Since market price is highly dependent on growth, meat yield, shell condition and aesthetic issues such as extent of parasites and the cleanliness of the shell, hanging cultivated mussels should achieve higher market prices. However, harvesting and processing equipment has to be adapted to the thin shells of the mussel to reduce loss.

4.3. Implications for regulation and monitoring

Toxin- producing algae are found only seldom in low concentrations in the German Bight, where harsh conditions and high sediment loads prevent algae from blooming. In Danish and English waters, however, these algae are commonly found. A shift of mussel production from on-bottom nearshore areas to off-bottom offshore areas would increase potential contact of mussels with toxic algae (Smaal 2002).

Another potential hazard stems from the recently registered warming in the North Sea. Parasites formerly known only in warmer regions, such as the parasitic crabs of the genus *Pinotheres* spp., are migrating north and have been sporadically seen in mussels in the German Bight (Wattreport 2008). They inhabit the mantle cavity of the mussel and reach sizes up to 1 cm. This parasite is not pathogenic to consumers, but extraordinarily problematic with regard to marketability as the price for such infested mussels is low. A similar temperature effect is likely to affect the distribution of *M. refrigens*, where conditions could begin to favour sporulation (OiE 2003). Therefore, the ICES report (2008) on marine shellfish cultivation has already recommended including *M. refirgens* into routine monitoring.
Today even the updated versions of the EC regulations focus primarily on nearshore hazards. Since the majority of potential hazards to mussels differ between both seasons and among areas, a uniform monitoring program is insufficient to protect all consumers at all times. It should be recognized that analysis of risk must entail seasonal and geographical differences, and include plans for dealing with potential threats associated with global warming.

5. Conclusion and outlook

Our data show that offshore locations are a good alternative for traditional mussel cultivation. The microbial findings of offshore cultivated mussels are clearly under the legal threshold and mussels are free of parasites. However as species of *Vibrio* and *Clostridium* were also found in offshore samples, this type of production does not offer a complete guarantee of microbial purity and an absence of human pathogens. Future investigations should focus on potential cultivation sites even further off the coast, to determine the distance to shore necessary for microbial purity in the German Bight.
It is recommended that the currently existing regulatory framework, focusing only on nearshore requirements, should be expanded to cover site specific risks. Further, we suggest shifting of monitoring focus for offshore sites from coliform bacteria to e.g. algal toxins and concerning the recent warming of the North Sea since a migration of commercially relevant micro and macro parasites into the German Bight seem possible.

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References

Abad FX, Pinto RM, Gajardo R, Bosch A (1997). Viruses in mussels: public health implications and depuration. Journal of Food Protection, 60: 677-681

Blake PA, Merson MH, Weaver RE, Hollis DG, Heublein PC (1979). Disease caused by a marine vibrio: clinical characteristics and epidemiology. New England Journal of Medicine, 300: 1-5

Buck BH (2002). Open Ocean Aquaculture und Offshore-Windparks: Eine Machbarkeitsstudie über die multifunktionale Nutzung von Offshore-Windparks und Offshore-Marikultur im Raum Nordsee. Reports on Polar and Marine Research, Bremerhaven, 412: 252 pp

Buck BH (2004). Farming in a high energy environment: potentials and constraints of sustainable offshore aquaculture in the German Bight (North Sea). Dissertation. University of Bremen, Bremen, Germany, 258 pp

Buck BH (2007). Experimental trials on the feasibility of offshore seed production of the mussel *Mytilus edulis* in the German Bight: installation, technical requirements and environmental conditions. Helgoland Marine Research, 61: 87-101

Buck BH, Krause G, Rosenthal H (2004). Multifunctional use, environmental regulations and the prospect of offshore co-management: potential for and constraints to extensive open ocean aquaculture development within wind farms in Germany. Ocean & Coastal Management, 47: 95-122

Buck BH, Thieltges DW, Walter U, Nehls G, Rosenthal H (2005). Inshore-offshore comparison of parasite infestation in *Mytilus edulis*: implications for open ocean aquaculture. Journal of Applied Ichthyology, 21(2): 107-113

Buschbaum C, Saier B (2001). Growth of the mussel *Mytilus edulis* L. in the Wadden Sea affected by tidal emergence and barnacle epibionts. Journal of Sea Research, 45: 27-36

Calvo-Ugarteburu G, McQuaid CD (1998). Parasitism and invasive species: effects of digenetic trematodes on mussels. Marine Ecology Progress Series, 169: 149-163

CDC (1997). Results of the public health response to *Pfiesteria*-Workshop – Atlanta, Georgia, September 29–30, 1997. Morbidity and Mortality Weekly Report, 46(40): 951–952

CDR (1991). Paralytic shellfish poisoning. Communicable Disease Report, 1(22): 1

CEFAS (2007). Microbiological monitoring of bivalve mollusc harvesting areas – Guide to good practice: Technical application. European Community Reference Laboratory for monitoring bacteriological and viral contamination of bivalve mollusc. The centre of environment, fisheries and aquaculture science, 3: 67 pp

CEFAS/CRL (2008). Generic Standard Operating Procedure. The Centre of Environment, Fisheries and Aquaculture Science, Weymouth Laboratory, Weymouth, UK

Cheng TC (1967). Marine Molluscs as host for Symbiosis. In: Advances Marine Biology (5). FS Russel (ed). Academic Press, London, New York, 424 pp

Cole A, Savage RE (1951). The effect of the parasitic copepod *Mytilicola intestinalis* (Steuer) upon the condition of mussels. Parasitology, 41: 156-161

Croci L, De Medici D, Ciccozzi M, Di Pasquale S, Suffredini E, Toti L (2003). Contamination of mussels by hepatitis A virus: a public-health problem in southern Italy. Food control, 14: 559-563

Davey JT, Gee JM (1988). *Mytilicola intestinalis*, a copepod parasite of blue mussels. American Fisheries Society, Special Publications, 18: 64-73

Dethlefsen V (1970). On the parasitology of *Mytilus edulis* (L. 1758). International Council for the Exploration of the sea (ICES) C.M. 1970/K: 16, Hamburg, 11pp

Dethlefsen V (1972). Zur Parasitologie der Miesmuschel (*Mytilus edulis* L., 1758). Berichte der Deutschen wissenschaftlichen Kommission für Meeresforschung, 22: 344-371

Dethlefsen V (1975). The influence of *Mytilicola intestinalis* (Steuer) on the meat content of the mussel *Mytilus edulis*. Aquaculture, 6: 83-97

DIN (German Industry Norm) 10161. Estimation of aerobic microbial number using drop plating procedure. German Institute for Standardisation e.V., Berlin, Germany

DIN (German Industry Norm) EN (European Norm) ISO (International Organisation of Standardisation) 6579 (2003). Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Salmonella* spp.. German Institute for Standardisation e.V., Berlin, Germany

DIN EN ISO 7937. Microbiology of edibles and food stuff – Horizontal Procedure to count *Clostridia* – Colony-Counting-Method. German Institute for Standardisation e.V., Berlin, Germany

Dittmann D, Robles C (1991). Effect of algae epiphytes on the mussel *Mytilus californianus*. Ecology, 72 (1): 286-296

EC Regulation (EC) 853/2004. Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific hygiene rules for food of animal origin, 61 pp

EC Regulation (EC) 854/2004. Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption, 139 pp

EC Regulation (EC) 2074/2005. Commission regulation of 5 December 2005 laying down implementing measures for certain products under Regulation (EC) No 853/2004 of the European Parliament and of the Council and for the organisation of official controls under Regulation (EC) No 854/2004 of the European Parliament and of the Council and Regulation (EC) No 882/2004 of the European Parliament and of the Council, derogating from Regulation (EC) No 852/2004 of the European Parliament and of the Council and amending Regulations (EC) No 853/2004 and (EC) No 854/2004

Ernst W, Weigelt S, Rosenthal H; Hansen PD (1991). Testing bioconcentration of organic chemicals with the common mussel (*Mytilus edulis*). In: Bioaccumulation in aquatic systems: contributions to the assessment. L. Nagel and R. Loskill (Eds). Proceeding of International Workshop, Berlin, 1990. VCH-Verlag, Weinheim, New York, Basel, Cambridge, 99-131

Formica-Cruz M, Tofino-Quesada G, Bofill-Mas S, Lees DN, Henshilwood K, Allard AK, Conden-Hansson A–C, Hernroth BE, Vantarakis A, Tsibouxi A, Papapetropoulou M, Furones MD, Girones R (2002). Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden and the United Kingdom. Appl. Environ. Microbiology, 68: 5990-5998

Gantzer C, Dubois E, Crance JM, Billaudel S, Kopecka H, Schwartzbrod L, Pommepuy M, Le Guyader F (1998). Influence of environmental factors on the survival of enteric viruses in seawater. Acta Oceanologia, 21: 983-992

Grizel H, Comps M, Bonami JR, Cousserans F, Duthoit JL, Le Pennec MA (1974). Recherche sur l'agent de la maladie de la glande digestive d'*Ostrea edulis* Linne. Bulletin de l'Institute des Pêches Maritimes, 240: 7-30

Guyader F le, Haugarreau L, Miossec L, Dubois E, Pommepuy M (2000). Three-year study to assess human enteric viruses in shellfish. Applied Environmental Microbiology, 66: 3241-3248

Hernroth B (2003). Factors influencing bactericidal activity of blue mussel (*Mytilus edulis*) haemocytes against *Salmonella typhimurium*. Fish and Shellfish Immunology, 14: 93-104

Höhne M, Schreier E (2004). Detection and Characterization of Norovirus Outbreaks in Germany: Application of a One-Tube RT-PCR Using a Fluorogenic real-time Detection System. Journal of Medical Virology, 72: 312-319

ICES (2008). Report of the Working Group on Marine Shellfish Culture (WGMASC), 1-3 April 2008, Aberdeen, UK. ICES CM 2008/MCC:02: 71pp

Ifremer (2008). Standard Operating Procedures and Quality – Molluscs processing for diagnostics by histology. Institut Français de Recherche pour l'Exploitation de la Mer. 7 pp. http://www.ifremer.fr/crlmollusc/page_labo/SOPs. , accessed January 2009

ISO 21872. Microbiology of edibles and food stuff – Horizontal Procedure to detect potential enteropathogene *Vibrio* spp.

Kent RML (1979). The influence of heavy infestations of *Polydora ciliata* on the flesh content of *Mytilus edulis*. Journal of the Marine Biological Association of the U.K., 59: 289-297

Kent RML (1981). The effect of *Polydora ciliata* on the shell strength of *Mytilus edulis*. Journal of the international Council for the Exploration of the Sea. 39: 252-255

Krakau M, Thieltges DW, Reise K (2006). Native parasites adopt introduced bivalves of the North Sea. Biological Invasions, 8: 919-925

Lauckner G (1983). Diseases of Mollusca: Bivalvia. In: Diseases of marine animals. Introduction, Bivalvia to Scaphopoda. O. Kinne (Ed.). Biologische Anstalt Helgoland, Hamburg, Westholsteinische Verlagsdruckerei Boyens & Co., Heide, 477-961

Laudien J, Wahl M (2004). Associational resistance of fouled blue mussels (*Mytilus edulis*) against starfish (*Asterias rubens*) predation: relative importance of structural and chemical properties of the epibionts. Helgoland Marine Research, 58: 162-167

Lawrence JF, Menard C (1991). Liquid Chromatography determination of paralytic shellfish poisons in shellfish after pre-chromatographic oxidation. Journal of the Association of Analytical Chemistry, 74: 1006-1012

Le Roux F, Lorenzo G, Peyret P, Audemard C, Figueras A, Vivarès C, Gouy M, Berthe FCJ (2001). Molecular evidence for the existence of two species of Marteilia in Europe. Journal of Eukaryotic Microbiology, 48 (4): 449-454

Lees D 2000. Viruses in bivalve shellfish. Int. Journal of Food Microbiology, 59: 81-116

Lehane L (2000). Paralytic Shellfish Poisoning: A review. National Office of Animal and Plant Health, Agriculture, Fisheries and Forestry. Canberra, Australia

Lepitzki DAW, Scott ME, McLaughlin JD (1994). Influence of storage and examination methods on the recovery and size of metacercaria of *Cerastoderma edule* (L.) from commercial beds of the lower Thames estuary. Zeitschrift für Parasitenkunde, 56: 1-11

Lhafi SK (2006). Untersuchungen zum bakteriellen und viralen Kontaminationsstatus von Miesmuscheln (*Mytilus edulis*) deutscher Herkunft. Dissertation. Institut für Lebensmittelqualität und –sicherheit, Tierärztliche Hochschule Hannover, 137pp

Lipp EK, Rose JB (1997). The role of seafood in foodborne diseases in the United States of America. Rev Sci Tech. 16: 620-40

Meyer PF, Mann H (1950). Beiträge zu Epidemiologie und Physiologie der parasitischen Copepoden *Mytilicola intestinalis*. Archiv für Fischereiwissenschaften 2: 120-134

Odlaug TO (1946). The effects of copepod *Mytilicola orientalis* upon the Olympia oyster *Ostrea lurida*. Trans American Microscopic Society, 65: 311-317

OiE (2003). Manual of diagnostic tests for aquatic animals. World Organisation for Animal Health, 7 pp. http://www.oie.int/eng/normes/fmanual/A_index.htm , accessed January 2009

Quilliam MA, Sim PG, McCulloch AW, McInnes AG (1989). High Performance Liquid Chromatography of Domoic Acid, a Marine Neurotoxin, with Application to Shellfish and Plankton. International Journal of Environmental Analytical Chemistry, 36: 139-154

Rehnstam-Holm A-S, Hernroth B (2005). Shellfish and Public Health: A Swedish Perspective. Ambio, 34(2): 139-144

RKI-Bulletin 2000. Epidemiologisches Bulletin (ongoing). Robert Koch Institute, Berlin, Germany. http://www.rki.de/DE/Content/Infekt/EpidBull/epid__bull__node.html , accessed January 2009

Romalde JL, Area E, Sánchez G, Ribao C, Torrado I, Abad X, Pintó RM, Barja JL, Bosch A (2002). Prevalence of enterovirus and hepatitis A virus in bivalve molluscs from Galicia (NW Spain): inadequacy of the EU standards of microbiological quality. International Journal of Food Microbiology, 74: 119-130

Ryan J (2005). Offshore aquaculture - Do we need it, and why is it taking so long? International Salmon Farmers Association (Ireland). Expert workshop on sustainable aquaculture, DG JRC European Commission, Institute for Prospective Technological Studies, 17–18 January 2005, Seville, Spain

Salamina G, D´Argenio P (1998). Shellfish consumption and awareness of risk of acquiring hepatitis A among Neapolitan families - Italy, 1997. European Surveillance, 3: 97-98

Sassen K, Velleuer R, Feldhusen F, Stede M, Effkemann S, Graf K, Zander HD, Pohlenz F, Heyken F, Hagena W, Hanslik M (2005). Niedersächsische Ausführungshinweise für die Überwachungsbehörden zur Durchführung der Muschelhygieneüberwachung. Niedersächsisches Ministerium für den ländlichen Raum, Ernährung, Landwirtschaft und Verbraucherschutz, Hannover, Germany, 18pp

Seed R (1968). Factors influencing shell shape in the mussel *Mytilus edulis*. Journal of the Marine Biological Association of the U.K., 48: 561-584

Shao ZJ (2001). Aquaculture pharmaceuticals and biologicals: current perspectives and future possibilities. Advanced drug delivery reviews, 50: 229-243

Smaal A (2002). European mussel cultivation along the Atlantic coast: production status, problems and perspectives. Hydrobiologia, 484: 89–98

Taskinen J (1998). Influence of trematode parasitism on the growth of a bivalve host in the field. International Journal of Parasitology, 28: 599-602

Thieltges DW (2006). Effect of infection by the metacercarial trematodes *Renicola roscovita* on growth in the intertidal blue mussel *Mytilus edulis*. Marine Ecology Progress Series, 319: 129-134

Watermann B, Die I, Liebe S (1998). Krankheiten der Miesmuschel (*Mytilus edulis*) an der ostfriesischen Küste: VII. Tagung der Deutschen Sektion der European Association of Fish Pathologists (EAFP) - Krankheiten der Aquatischen Organismen. 23.-25. September 1998, Schmallenberg-Grafschaft, 177-187

Wattreport (2008). Nationalpark Wattenmeer – (k)ein Raum für ungestörte Natur? Berichte von Meer und Küste für Förderer und Freunde der Schutzstation Wattenmeer. http://schutzstation-wattenmeer.de , accessed August 2008

World Health Organisation and United Nations Environmental Programme (UNEP). 1987. Environmental quality criteria for shellfish-growing waters and shellfish in the Mediterranean. WHO/EURO document EUR/ICP/CEH 051. World Health Organization/ Europe, Copenhagen

Zens M (1999). Der Einfluss von Parasiten auf Vitalität und Bestandentwicklung der Miesmuschel (*Mytilus edulis* L.). Dienstbericht der Forschungsstelle Küste, Niedersächsisches Landesamt für Ökologie, 31 pp

Chapter 09

HEALTH AND GROWTH PERFORMANCE OF BLUE MUSSELS (*Mytilus edulis* L.) FROM TWO DIFFERENT HANGING CULTIVATION SITES IN THE GERMAN BIGHT: A NEARSHORE - OFFSHORE COMPARISON

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Abstract
The health status of cultivated blue mussels from an offshore site at the outer Weser estuary and from a nearshore site at the Jade estuary were compared (lysosomal membrane stability (LMS), condition index, parasite infestation), as well as the growth performance during 10 months of sampling in the 2007 season. Abiotic and biotic site characteristics were investigated by water analysis for each sampling cycle. In addition, data of contaminant loads of both sites were provided by the Federal Marine Hydrographical Agency (BSH). The results show that positive offshore effects, due to contaminant dilution were not evident for all parameters analysed. Condition indices were high and parasite not present offshore, but the central health parameter LMS and growth performance were better in nearshore cultivated mussel. These findings are corresponding with the contamination loads being higher at the offshore testing site. Values of lysosomal membrane stability were generally low (6-8 min) at both sites, indicating that environmental conditions for blue mussels in the German Bight are generating stress even up to the outer border of the 12 nautical miles zone.

Keywords: blue mussel, lysosomal membrane stability, offshore cultivation, stress response, scope of growth

1. Introduction

Estuarine and marine coasts over much of the globe, and particularly in Europe, have become densely settled. Estimates indicate that as many as half the total European population lives alongside or in close proximity to coastal areas (COM 2006). This human migration to coastal regions, resulting in significantly increased urbanization and industrialization, has led to contamination of marine environments. As a result, the coastal environment has become contaminated by sewage, industrial and agricultural effluents, accidental spills of fuels or different chemicals and atmospheric precipitation. Native ecosystem and natural habitat destruction has and continues to occur in areas that are not only biologically sensitive, but also vital economically (Moore et al. 2004). In the past the effects of this kind of contamination were commonly addressed only when final, irreversible damage had occurred, ie a total elimination of a habitat or species. Today, to prevent this dynamic, biomarkers have been developed which measure harmful, sublethal effects, and monitoring programs use early warning tools to mark changes in the marine environment (Livingstone et al. 1990). Since the beginning of these monitoring programs mussels (*Mytilus* spp.) were deployed as bioindicators to assess the effects of aquatic contaminants (Goldberg 1975, Widdows & Donkin 1992, Salazar & Salazar 1995). In addition to a wide geographic distribution, mussels are abundant, stationary, easy to sample and they are known to exhibit a range of biological responses when exposed to contaminants (Goldberg et al. 1978, Rasmussen 1982, O'Connor 1998, Widdows et al. 2002). Due to their widespread application in many monitoring programmes over the years (e.g. Marigómez et al. 1996, Da Ros et al. 2002, Castro et al. 2004, Petrovic et al. 2004) mussels offer considerable potential for the comparison of international data on the health status of our seas and oceans.

A possible tool for assessing the health status of mussels cultivated under certain conditions at a certain site is the test on lysosomal membrane stability (LMS). The lysosomal responses in molluscan digestive cells constitute one of the most accepted biomarkers, world-wide, for assessing the health status of the species and its environment (ICES 2005; 2008, UNEP 2007). This tool, integrating various classes of pollutants and indicating toxicant-induced cell pathologies, can provide evidence of the degree of stress or disease affecting the organism (Allen & Moore 2004). Lysosomes respond to exposure to pollutants with significant increases in size (Lowe et al. 1981, Moore 1988, Cajaraville et al. 1989, Lowe & Clarke 1989, Marigómez et al. 1989, Viarengo et al. 1992, Regoli et al. 1998, Koehler et al. 2002, Marigómez & Baybay-Villacorta 2003) and with a labilisation of their membranes (Moore et al. 1978; 1979; Nott & Moore 1987, Viarengo et al. 1992, Krishnakumar et al. 1994, Regoli et al. 1998, Marigómez et al. 2005). In addition to pollutants, natural stress factors such as temperature, salinity, availability and quality of food, tidal cycle and habitat conditions may influence the stability of lysosomal membranes (Moore 1976, Marigómez et al. 1991, Tremblay & Pellerin-Massicotte 1997). Seasonal influences can alter site-specific parameters or vary between sites (Petrovic et al. 2004). In non-polluted sites lysosomal membrane stability in mussel digestive gland might be highly variable through the year, whereas in a polluted site values are lower and more constant between seasons (Regoli 1992). Also seasonal effects have been described as affecting gonad development (Caceres- Martinez & Figueras 1998) and scope for growth (Wong & Cheung 2003).

In the digestive gland of mussels lysosomes are responsible -beside their role in detoxification processes- for food uptake, transport and storage of reserve substances and therewith a substantial element in the energy metabolism. Stressed lysosomes will lead to a lower overall energy status of the organism, affecting growth. The scope for growth is linearly correlated with the lysosomal stability of digestive gland epithelial cells, indicating that lysosomal stability is also a suitable indicator of the health of the organism (Moore 1992; 2002, Widdows et al. 1982).

The ability to track the health of blue mussels can be also used for commercial interest if suitably sites for cultivation purpose are wanted. Following this approach mussels are deployed in different marine areas with farming potentials. An exposure of mussels for weeks, months or even up to a year will provide information about water quality and cover also seasonal influences, such as food availability, hydrographical amplitude (e.g. storm events, current peaks), and temperature ranges of a specific site and their impacts on the mussels. These data can be specifically used to compare growth and health of mussels as a culture candidate, or to investigate general suitability for the cultivation of any potential culture candidate (e.g. macro algae, fish).

Along the Germany Bight, most of the suitable nearshore areas for mussel cultivation are e.g. natural reserves, recreational areas, and shipping routes. An expansion of marine aquaculture in suitable coastal areas is limited, since many stakeholders with vital interest compete for the same space (Buck 2007). A shifting of the mussel production off the coast would decrease spatial conflicts (Buck et al. 2004), but would require different, more cost-intensive farming techniques. The development of a new stakeholder, the offshore wind farms industry, offers a unique opportunity to co-use large marine areas with submerged culture systems for various candidates (Buck 2002, Brenner et al. 2007). In addition to the spatial cooperation ideas were proposed to share the solid groundings of windmills to attach the culture systems (Buck et al. 2006). However, more substantial data on the site criteria of a culture plot are needed to calculate economic risks.

Following the definition of Ryan (2005) and Buck (2007) an offshore testing site was developed to cultivate mussels under exposed conditions. In addition a second cultivation plot was deployed nearshore. An extensive support program was organized to investigate seasonal variations in abiotic hydrographical parameters, nutrients and biotic conditions e.g. parasite loads and chlorophyll availability of the two areas, to evaluate growth rates and lysosomal membrane stability. These data were completed by values of contaminant loads of both areas as measured under supervision of the Federal Marine Hydrographical Agency (BSH), at mess points in the vicinities of our testing areas.

2. Material and Methods

2.1. Origin of mussels used for the experiment

The experiments were conducted in the years 2006 and 2007 with cultivated blue mussels (*M. edulis*) settled on suspended artificial spat collectors at two sampling sites in the German Bight (Fig. 1). These testing areas were located nearshore on the *Niedersachsenbrücke*, an approx. 1300 m long cargo bridge, at the outer Jade estuary (JD, Position 53° 35' 05'' N; 008° 09' 14'' E) near the city of Wilhelmshaven (Fig. 2), and offshore (Ryan 2005) near the lighthouse *Roter Sand* (RS, Position 53° 51' 00'' N; 008° 04' 20'' E) situated at the entrance of the Weser estuary about 17 nautical miles northwest of the city of Bremerhaven (Fig. 3).

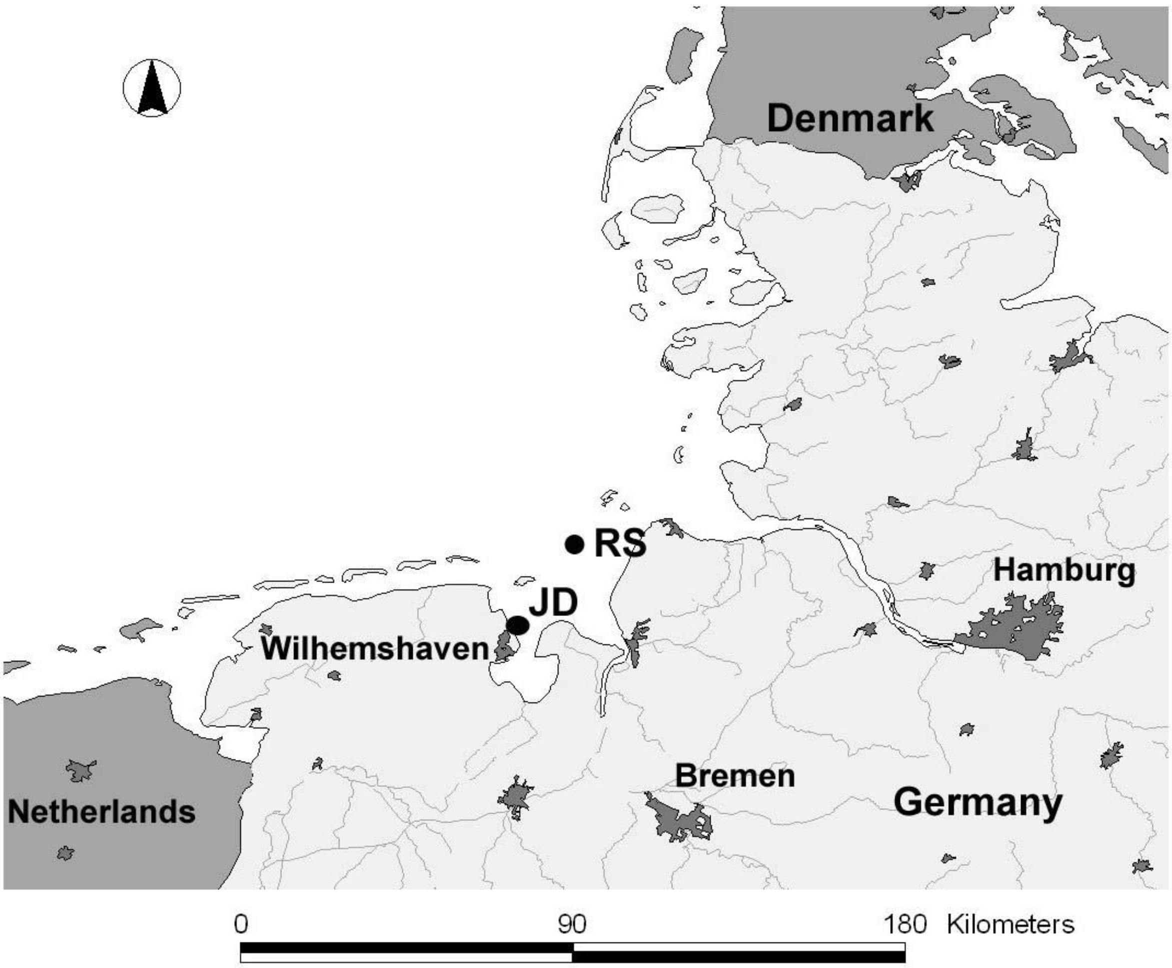

Fig.1: Map of the German Bight showing the two sampling sites equiped with suspended hanging cultures at the *Niedersachsenbrücke* (nearshore) near the city of Wilhelmshaven in the Jade (JD) estuary and at the entrance of the Weser estuary (offshore) near the lighthouse *Roter Sand* (RS) sampled in the year 2007.

At the *Niedersachsenbrücke* the artificial substrates were attached to 10 harnesses hanging from the bridge allowing a distance of 20 m between harnesses. Each harness consisted of a 20 mm polypropylene rope with an iron plate (5 kg) at its distal end, weighing down the substrates into the water column even at strong current velocities. The upper part of the harness was attached to a steel beam between the pillars of the bridge. The polypropylene (pp) rope was strengthened with two swirls and shackles to prevent entanglement. The lower swirl was placed one meter above the mean high water (mHW) level to prevent corrosion and fouling. To insure that the cultivated mussels were submerged during the whole experiment, samples of artificial test collectors were fixed to the pp-rope from one to approximately three meters below mean low water (mLW) level using sea water- and uv-resistant plastic binders. The deployed substrate samples were about 15 cm in length and fixed every 20 cm to the rope.

Fig. 2: Cargo bridge *Niedersachsenbrücke* in the Jade (JD) estuary where mussels were cultivated nearshore on suspended substrates using harnesses hanging from the bridge (2007).

At the offshore location RS three modified buoyancies (3.6 m in height; 1.1 m in diameter) were deployed to cultivate the mussels. All buoyancies were equipped with an anchor stone of 1.5 tons and a 30 m steel chain in between. Buoy and chain were connected by a swirl to prevent entanglement. All buoys were equipped with three steel rings, welded to each buoy by three 25 cm long crossbeams. Rings were placed at water surface level, 1.5 m and 3 m in depth. A 10 mm nylon rope was tied up vertically between the three steel rings. Similar to the constructions at the *Niedersachsenbrücke* substrate samples were attached at 20 cm intervals to the pp-rope using plastic binders.

Fig. 3: Modified testing buoyancy equipped with suspended artificial substrates at the offshore sampling site RS at the entrance of the Weser estuary in the German Bight (Brenner et al. 2007).

2.2. Sampling procedure

The set up of the artificial substrates was conducted in April at both sites, before the expected spawning time of mussels in early May (Pulfrich 1997). Harnesses at JD and buoys at RS from the season 2006 were inspected regularly between May and November and maintained if necessary. In 2007 depending on the schedule of the research vessel FK *Uthörn* simultaneous (maximum time between sampling 10 days) excursions from February to November to both testing sites were organized. Sampling at RS had to be sampled at slack water with a team of scuba divers operating from the research vessel, whereas JD was accessible without any tidal constraints all year round. Water samples were taken every month. Mussels growing on the substrates since May 2006 were sampled in February, May, August and November 2007 and analysed for lysosomal membrane stability (LMS), condition index (CI) and macro parasites. By February 2007 sampled mussels were about 3 cm in length, ensuring sufficient sample tissue for analysis and allowing appropriate handling while sectioning.

Growth rates were calculated using mussels settled in April 2007. Sampling started in May 2007 approx. 4 weeks after the substrates where placed at the testing sites. Here, sampling was conducted again monthly during the main growing phase between May and August and ended in November 2007.

2.3. Assessing lysosomal membrane stability

Mussels between 3 and 5 cm in length were collected from the substrates and transferred immediately dry in cool boxes to the lab. The length and width of 15 mussels was measured to the nearest 0.1 mm using a vernier calliper. The mussels were opened; drained and total wet weight was determined (± 0.01 g). The soft body was then dissected, weighed (± 0.01 g) and put in cryo-vials each filled with 1 ml fish gelatine. Samples were controlled for remaining air bubbles and then frozen in liquid nitrogen. Subsequently, shells were weighed (± 0.01 g).

Frozen soft body samples were fixed on pre-frozen aluminium chucks for subsequent cryostat-sectioning. Tissue sections of 10 µm were obtained using a cryotome (Microm, HM 500) with chamber temperature of -25 °C. Sections were stored for a maximum of 24 hours at -20 °C until processed for histochemistry.

The lysosomal membrane stability test was performed according to Moore et al. (2004). Serial cryostat sections were incubated at 37 °C in a 0.1 M citrate buffer, pH 4.5, containing 3 % NaCl to destabilize the membrane for increasing time intervals (2–50 min). After this acid labilisation, sections were incubated for 20 min at 37 °C in a medium containing the substrate Naphthol AS-BI N-acetyl β-D-glucosamide (Sigma) dissolved in 2-methoxy ethanol and low-viscosity polypeptide (Polypep, Sigma) dissolved in 0.1 M citrate buffer, pH 4.5 with 3 % NaCl. The lysosomal hydrolase N-acetyl ß-D hexosamidase catalyses the release of the Naphthol AS-BI group which undergoes a post-coupling reaction with the diazonium salt Fast Violet B (Sigma) dissolved in 0.1 M phosphate buffer (pH 7.4) leading to an insoluble bright violet reaction product. Following the colour reaction, samples were rinsed in running tap water, fixed in Baker's Formol, rinsed in distilled water and dried overnight in the dark at room temperature. Subsequently, slights were mounted in Kaiser's glycerine-gelatine.

The maximum reaction product for N-acetyl β-D-Hexosamidase was determined by automatic measurement of number and percentage of dark stained lysosomes in the digestive tubules by the use of computer assisted image analysis (Zeiss, KS 300) combined with a light microscope (Zeiss, Axioskop) at 400 fold magnification. A long destabilization period indicates high lysosomal membrane integrity and vice versa. Unclear results of labilisation series (no clear peaks, only 1 peak) were re-measured or excluded from further analysis.

2.4. Growth rates

Simultaneous samplings from both sites were organized in May, June, August and November to assess growth rates. Therefore, one substrate sample per site overgrown with mussel post larvae or juveniles was used for further examination. In the lab all juvenile mussels were removed from the substrate. The substrates were then carefully brushed over a sieve (mesh size 100 µm) to peel off post larvae. Finally cleaned substrates were controlled for remaining post larvae using a stereo magnifying glass. Depending on the number of removed juveniles and post larvae a Folsom-Splitter (Dubischar et al. 2002) was deployed to split the samples down to 50-100 individuals. Conglomerates of juveniles were teased apart and single individuals were transferred to the splitter. Larvae were washed from the sieve into the splitter. Tap water was added until mussels and larvae could spread evenly over the whole bottom of the splitter. Subsequently, the sample was divided into two subsamples using the two chambers of the Folsom-Splitter.

The splitting procedure was repeated until a subsample of 50 to 100 juvenile mussels remained in one chamber. Juveniles >1 cm in length were collected and length, height and width were measured using a vernier calliper. Juveniles less than 1 cm in length and remaining larvae were transferred to the 100 µm sieve and eventually split again before being examined under a stereo magnifying glass. There, larvae were counted and measured using the ocular scale.

2.5. Condition Index

Data from August and November samples prepared for LMS and macro parasite assessment was also used to calculate the condition index (CI). For a direct comparison of CI with LMS only wet weights of tissues and shells could be used for the calculation (see below).

$$CI = \frac{Wet\ meat\ weight\ [g]}{Shell\ weight\ [g]} \text{ x } 100 \qquad (1)$$

Mussels samples from August and November 2007 from the growth experiment were additionally used to calculate CI based on dry weights (see below). Dissected soft bodies and shells were dried for 24 h at 60 °C in drying cabinet before being weighed (± 0.001 g) separately.

$$CI = \frac{Dry\ meat\ weight\ [g]}{Dry\ shell\ weight\ [g]} \text{ x } 100 \qquad (2)$$

2.6. Analysis of macro parasites

Mussels were selected according to a shell length ranging from 25 to 60 mm, stored at -20 °C and immediately analyzed after defrosting. First, the length and width of each selected mussel were measured to the nearest 0.1 mm using a vernier calliper. Mussels were opened, drained and wet weight was determined. The soft body was removed and both shell and soft body were weighed (± 0.01 g) separately. The soft body was then placed on the bottom of a glass compressorium and the mantle, gills, food, adductor muscle and other tissues were dissected carefully and dispersed. The digestive gland was pulled apart and squeezed together with the other tissues using the cover glass of the compressorium.

The preparations were examined under a stereo magnifying glass (10-50 fold magnification) with transmitting light for the presence of macro parasites. Parasite species were identified according to descriptions from the literature (e.g. Dethlefsen 1970; 1972, Lauckner 1983, Waterman et al. 1998) and organs infested with parasites were listed. In a final step all shells of the analysed mussels were inspected for the presence of shell-boring polychaets using the stereo magnifying glass.

2.7. Heavy metals and pollutants

Data on contaminants and pollutants in the water column of both testing areas were provided by the Marine Environmental Data Base (MUDAB) of the Federal Maritime and Hydrographic Agency, Hamburg, Germany (BSH 2009).

2.8. Biological support programme

Data describing temperature, salinity, chlorophyll contents (*a, b, c* and phaeophytin *a*), carotinoids, nitrate+nitrite (NOX), particulate organic carbon (POC), and particulate organic nitrogen (PON) were obtained by taking monthly water samples between March and October 2007 at both sampling sites. The results were added with values from the MUDAB data base for phosphate and ammonium concentrations measured in August and October 2007 (BSH 2009).

To determine the above mentioned parameters seawater from both sites was pumped from a depth of between 1-2 m. Temperature and salinity were measured immediately, using a conductivity meter (Microprocessor Conductivity Meter (LF 3000, Jürgens).

C/N-ratio was assessed by filtering 3 times 1L of seawater over precombusted (450 °C, min. 5 h) glass fibre filters (Whatman GF/C), and dried subsequently for 24 h at 60 °C in a drying cabinet. After weight determination the samples were acidified with 1 M HCl to remove inorganic carbonates and then redried. C/N-ratio was analysed by high temperature combustation (Elemental Analyzer, Euro Vector), using acetanilide as calibration standard.

To determine the nutrient content (NOX), 50 ml seawater was poisoned using 80 µl of $HgCl_2$. The fixed water samples were filtered over 0.45 µm wetting agent free cellulose acetate (Sartorius) syringe filters and placed in acid-cleaned polyethylene bottles before double measured using a continuous flow analyser (Evolution III, Alliance Instruments).

To measure chlorophyll (chl *a, b, c_1, c_2*) (Jeffrey & Humphrey 1975) carotenoids (Richard & Thompsen 1952) and phaephytin *a* content, 3 times 1 L of seawater was filtered over glass fibre filters (Whatman GF/C). Filters were rolled and stored in 2 ml Eppendorf cups at -20 °C. Subsequently frozen filters were transferred to test tubes filled with 10 ml aqueous acetone (90 %) and treated with an ultrasonic device (Ultra Sonifier (W 250), Branson) for 20 seconds while cooled in crushed ice. Samples were incubated at 4 °C in the dark for two hours and then centrifugated for 10 min at 4000 rpm. The extinction was measured (cuvette length 5 cm) at 750 (turbidity), 663 (chl *a*), 647 (chl *b*), 630 (chl *c*), 510 and 480 (both carotenoids) nm with a spectrophotometer (Milton Roy Genesys) using aqueous acetone (90 %) as reference. Then, 0.1 ml HCL was added and the measurements at 750 and 663 nm were repeated to calculate phaeophytin *a*. After corrections of the extinction for all wavelengths by the extinction at 750 nm the chlorophyll contents were calculated. Carotenoid concentrations were determined by subtracting extinction values from 480 nm – 510 nm (Richard & Thompsen 1952).
Low chlorophyll concentrations (extinction < 0.1) were measured with a fluorometer (TD-700, Gamma Analysen Technik) using aqueous acetone (90 %) as reference. The fluorescence is measured before and after adding 2 drops of 1 M HCL. Phaeophytin *a* concentrations are calculated from the difference of both values.

2.9. Statistical analysis
Means and standard deviations (mean ± SD) of data of the biological support program, growth rates, macro parasite intensities and condition indices were calculated by using Microsoft Office Excel 2007. Box plots displaying LMS were calculated using Statistica 5.0. software. Differences of LMS between sites and season were tested for significances using SigmaPlot 11.0 software.

3. Results

3.1. Environmental conditions

At both testing sites salinity and temperature were comparable over the sampling season 2007 and follow both the same pattern (Tab. 1). Salinity varied moderately between 28 ppt and 32 ppt at RS (LW and HW) and between 29 ppt and 31 ppt at JD (only HW). At both sites temperature ranged between 7 °C in winter and 21 °C in summer, whereas the nearshore area JD remained slightly cooler throughout the year compared to the offshore area RS.

Tab. 1: Data of tide, salinity, temperature, carotenoids and phaephytin *a* at the areas RS and JD during sampling season 2007.

RS	tide	salinity [g/L]	temperature °C	carotenoids [µg/l]	phaeophytin *a* [µg/l]
Mar-07	HW	28.60	7.40	0.39	1.05
Apr-07	LW	31.50	12.20	1.93	0.90
May-07	HW	32.10	16.60	4.26	30.16
Jun-07	LW	23.10	18.50	4.02	22.75
Jul-07	LW	30.20	21.10	5.25	31.57
Aug-07	LW	29.40	19.70	2.32	14.17
Oct-07	HW	31.60	13.10	-	3.22
JD					
Mar-07	HW	29.30	7.30	0.61	2.47
Apr-07	HW	29.10	10.30	0.86	1.70
May-07	HW	31.10	13.50	7.52	9.89
Jun-07	HW	31.40	18.40	2.72	5.97
Jul-07	HW	30.70	20.40	2.26	14.05
Aug-07	HW	30.50	19.50	2.96	11.61
Oct-07	HW	29.30	10.60	-	-

Chlorophyll (total, *a, b,* c_1 and c_2), carotinoids and phaeophythin *a* concentration varied more between the sites (Tab. 1 and Fig. 4a/b). At JD total chlorophyll reached its maximum in May (30.5 µg/l) followed by the normal summer depletion in August (8.9 µg/l) whereas at RS (May 24.5 µg/l) a second peak was registered in July (23.8 µg/l) (Fig. 4a/b). The analysis of phaeophytin *a* showed the same pattern*:* two peaks at higher concentrations at RS in May and July (30 µg/l and 31 µg/l) and only one peak at a lower concentration at JD in July (14 µg/l). Carotinoids varied between 0.4 µg/l and 5.2 µg/l at RS and 0.6 µg/l and 7.5 µg/l at JD.

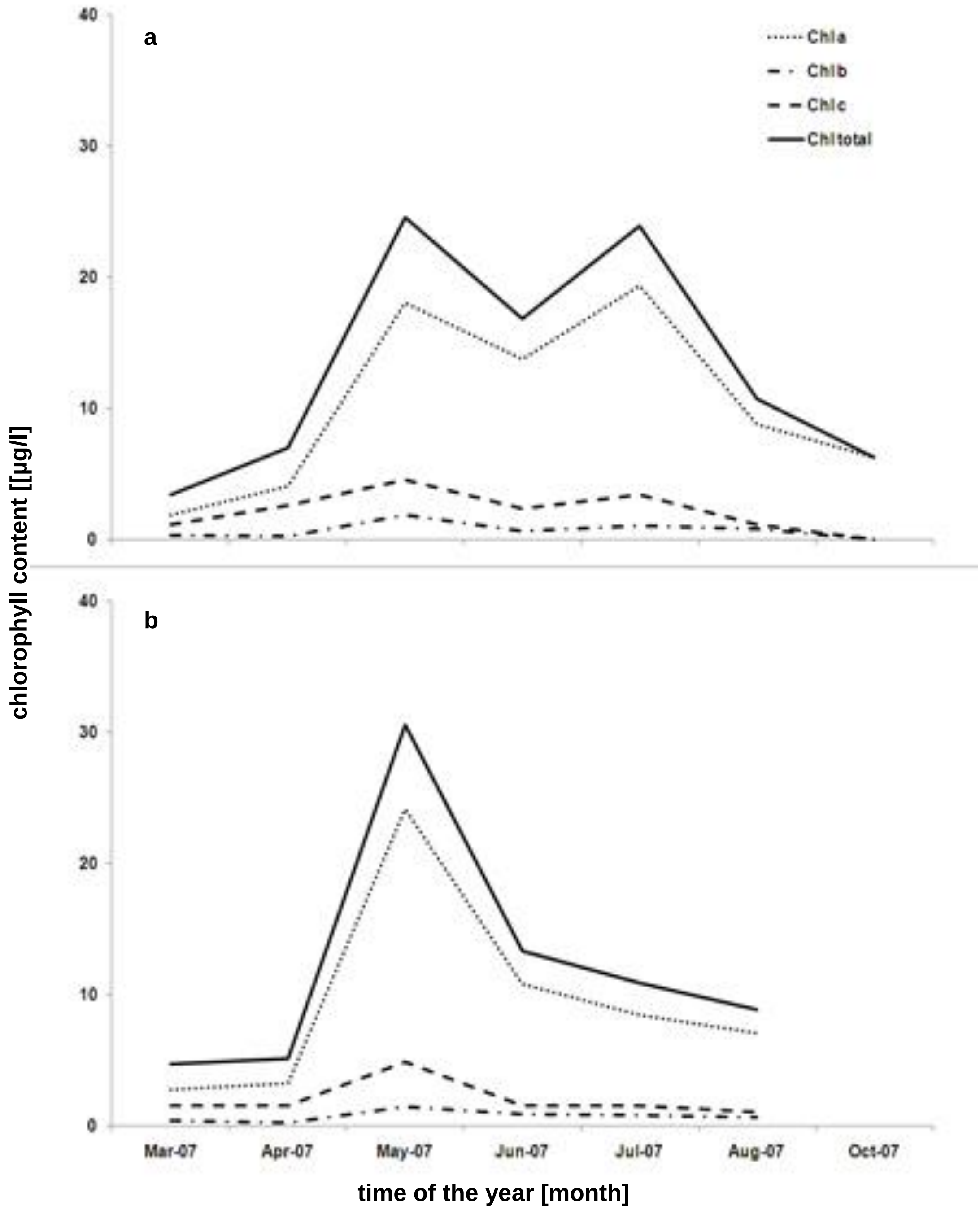

Fig. 4a/b: Chlorophyll contents in the water at RS (a) and at JD (b) during sampling season 2007.

Nitrogen (NOX = sum of nitrate and nitrite) was measured in comparable amounts at both sites (2.17 µmol/l – 64.95 µmol/l at RS; 5.87 µmol/l – 71.66 µmol/l at JD) (Tab. 2). The highest concentrations were found in spring followed by a significant reduction in summer. Particular organic carbon (POC) and nitrogen (PON) showed decreasing concentration for POC at both sites over the season (1045 µg/l – 18 µg/l at RS; 2269 µg/l – 63 µg/l at JD) and summer peaks of PON at both sites (RS June 1518 µg/l; JD July 1005 µg/l).

Tab. 2: Data of POC/ PON and nutrients at the areas RS and JD during sampling season 2007 (* data from MUDAB database BSH 2009).

RS	POC [µg/l]	PON [µg/l]	NOX [µmol/l]	phosphate [µmol/l]	amonium [µmol/l]
Mar-07	769.36	70.64	64.95	-	-
Apr-07	1045.80	118.59	38.15	-	-
May-07	117.69	952.63	2.17	-	-
Jun-07	176.31	1518.63	7.72	-	-
Jul-07	80.03	475.26	7.07	-	-
Aug-07	87.00	456.60	7.99	0.43 - 0.44*	0.7*
Oct-07	18.45	130.00	7.06	1.02 - 1.05*	4.6 - 4.7*
JD					
Mar-07	2269.07	211.32	55.63	-	-
Apr-07	1402.93	149.42	71.66	-	-
May-07	1505.03	235.64	16.95	-	-
Jun-07	114.08	678.63	5.87	-	-
Jul-07	137.77	1005.17	14.51	-	-
Aug-07	136.60	934.85	16.58	-	-
Oct-07	63.36	828.61	26.08	1.39 - 2.19*	3.71 - 7.00*

3.2. Contaminant loads of the water column

The concentrations of metals and contaminants in the water column of the German Bight were assessed by the BSH and other cooperating public authorities. Table 3 shows the average concentrations of eight different metals and four exemplary groups of pollutants (polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDTs), hexachlorobenzene (HCBs) and hexachlorohexane (HCHs)) measured at different stations in the vicinity of both testing sites. The concentrations of pollutants in both areas are comparable. In the case of metals higher concentrations are found at RS (Tab. 3). Data about polycyclic aromatic hydrocarbons (PAHs) were only available for August of the years 2006 and from August and October 2008 for the outer Weser estuary. Here, values varied between 5.2 ng/l in August 2006 and 3.51 ng/l and 1.26 ng/l in August and October 2008 (BSH 2009).

Tab. 3: Heavy metals and pollutants (polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDTs), hexachlorobenzene (HCBs) and hexachlorohexane (HCHs)) measured in the respective area around the two sampling sites in winter and autumn 2007 (BSH 2009).

	area RS		area JD		
pollutant	**Winter**	**Autumn**	**Winter**	**Autumn**	**unit**
Arsenic	1300 - 1400	1900 - 2900	1500 - 2100	1900 - 2500	ng/l
Cadmium	50 - 260	50 - 1300	50 - 200	110 - 350	ng/l
Chromium	500 - 2000	500 - 2900	700 - 2100	500 - 2900	ng/l
Copper	1.1 - 2.9	1.1 - 4.1	1.1 -2.9	2.2 - 4.1	µg/l
Lead	32 - 2800	34 - 4300	810 - 2200	1300 - 2700	ng/l
Mercury	5.0 -11.0	0.6 – 13.0	5.0 – 11.0	7.0 – 13.0	ng/l
Nickel	0.9 - 2.5	0.4 - 2.5	1.5 - 2.5	1.3 - 1.9	µg/l
Zinc	2.2 - 12.0	0.9 – 12.0	3.3 – 12.0	4.7 - 6.6	µg/l
PCBs	<0.1 - <0.4	<0.1 - <0.4	<0.1 - <0.4	<0.1 - <0.4	ng/l
DDTs	<0.1	<0.1	<0.1	<0.1	ng/l
HCBs	<0.06	<0.06	<0.06	<0.06	ng/l
HCHs	<0.07 - <0.2	<0.07 - <0.2	<0.07 - <0.2	<0.07 - <0.2	ng/l

The distribution of three exemplary pollutants (lead (Pb), mercury (Hg) and HCB) over the German Bight from 2006 is displayed in Figure 5a-c. The maps show that at both sampling sites concentrations of Pb and Hg are in a similar range. HCB is even more concentrated at the offshore area RS.

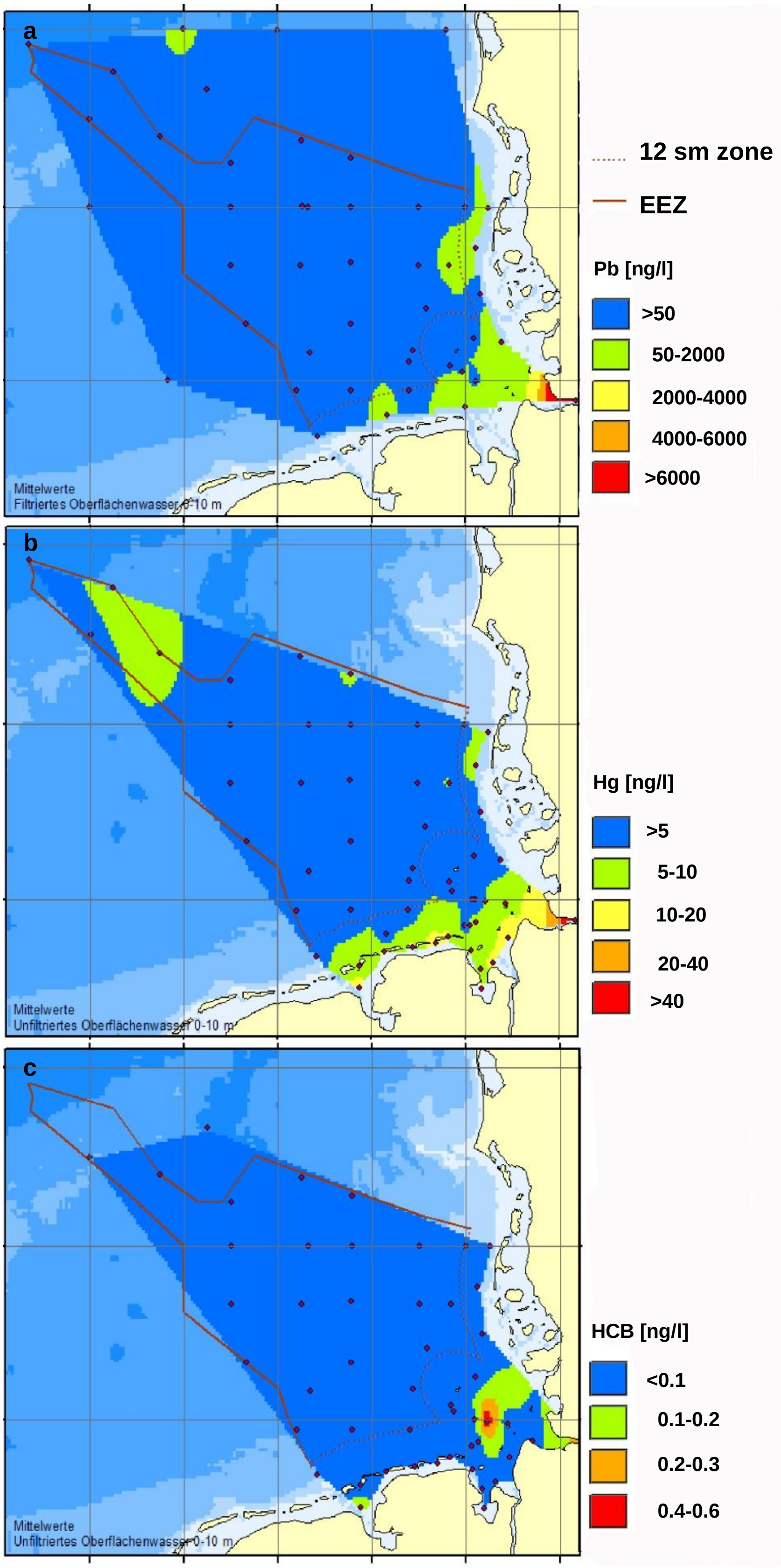

Fig. 5a-c: Distribution of three exemplary pollutants (a) lead (Pb), (b) mercury (Hg) and (c) hexachlorobenzene (HCB) over the German Bight in the year 2006 (BSH 2009).

The accumulation of pollutants in the tissues of blue mussels from different sites in the German Bight is displayed in Figure 6a/b. PCB is accumulated in higher concentrations in tissues of samples from the East Frisian Wadden Sea, than in samples from North Frisian sites. The highest values are measured in the Elbe estuary (Fig 6a). Gamma-HCH (Lindane) is found mostly in the East Frisian samples. North Frisian tissues had only low Lindane concentrations (Fig. 6b).

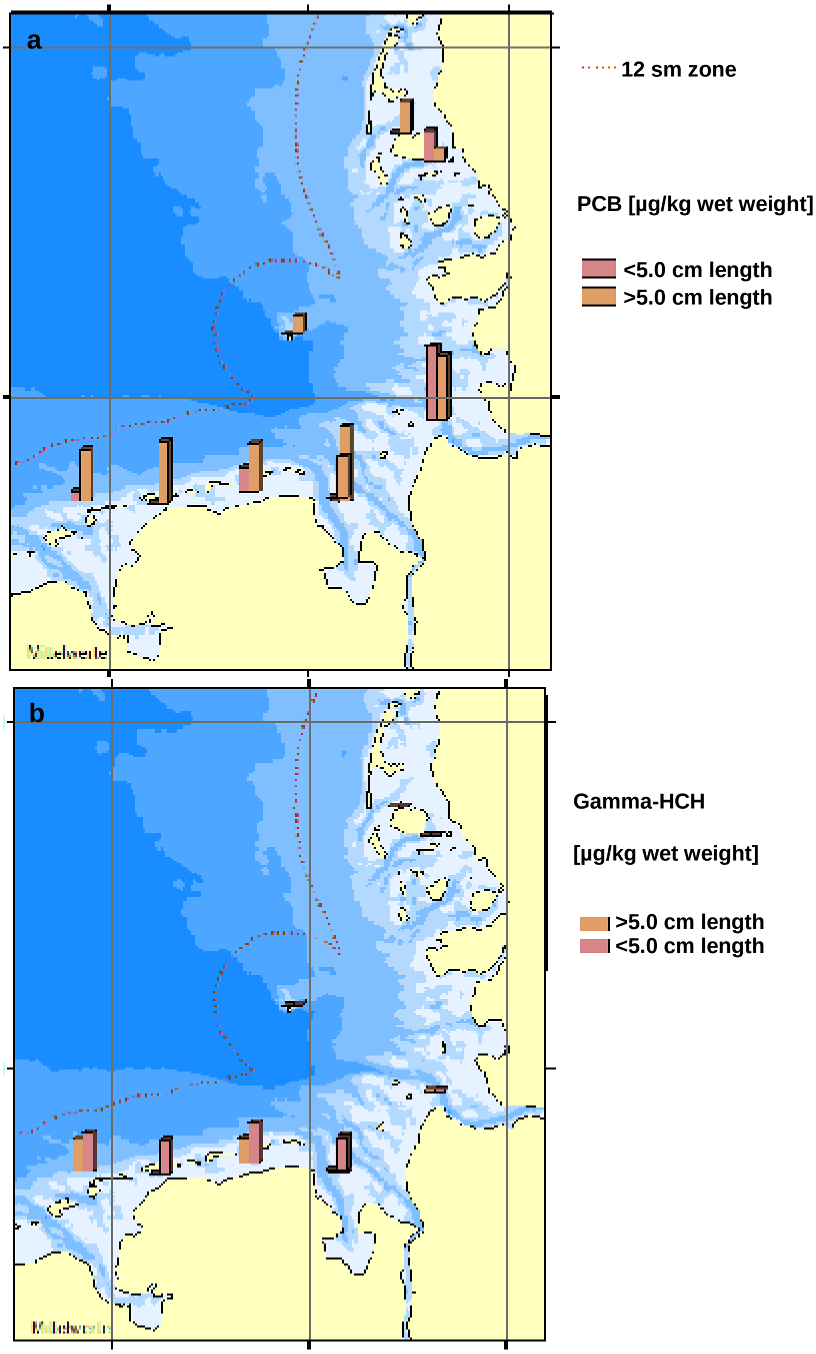

Fig. 6a/b: Concentrations of (a) PCBs and (b) Lindane in the tissue of blue mussels at different sites of the German Bight in the year 2006 (BSH 2009).

3.3. Parasite infestation

Only one species of macro parasite was identified in the samples from both sites throughout the sampling season 2007. This parasitic copepod *Mytilicola intestinalis* was found in the digestive gland of the mussels with increasing prevalence and intensity from February to November in samples of the nearshore cultivation site JD. In February 6 % of the mussels were infested with an average of 0.07 ± 0.26 copepods. At the end of season already 60 % of the tested mussels were affected by an average of 0.8 ± 0.77 parasites. In the offshore samples macro parasites were absent (Tab. 4).

Tab. 4: Infestation of mussels with the parasite (*Mytilicola intestinalis*) from RS and JD sampled during the season 2007.

M. intestinalis	**RS**			
	Feb	**May**	**Aug**	**Nov**
prevelence [%]	0.00	0.00	0.00	0.00
intensity	0.00	0.00	0.00	0.00
M. intestinalis	**JD**			
	Feb	**May**	**Aug**	**Nov**
prevelence [%]	6.67	13.33	13.33	60.00
intensity	0.07 ± 0.26	0.13 ± 0.35	0.33 ± 1.05	0.80 ± 0.77

3.4. Condition index

The condition indices of mussels from both testing areas are displayed in Table 5. Indices of 2 year old mussel used for LMS and macro parasite analysis based on wet weights and condition indices calculate from mussels from the season 2007 used for calculation of growth rates based on dry weights showed similar trends. At JD and RS CIs were higher in August than in November and higher at RS than at JD (70 and 61 at JD; 109 and 96 at RS) (Tab. 5).

Tab. 5: Condition indices of blue mussels based on wet weights (individuals used to calculate lysosomal stability) and dry weights (individuals used for calculating growth) from RS and JD sampled in August and November 2007.

condition-index	**RS**		**JD**	
	Aug	**Nov**	**Aug**	**Nov**
wet weights	109.54 ± 11.49	96.17 ± 17.67	70.89 ± 9.79	61.21 ± 13.29
dry weights	23.91 ± 0.50	13.94 ± 1.28	12.29 ± 3.70	11.66 ± 2.50

3.4. Growth and length distribution

The length distributions of mussel post larvae and juveniles on the artificial substrates deployed at RS and JD are displayed in Figure 7a-d. In both testing areas 3 recruitment cycles were detected throughout the sampling period. Growth rates were calculated using the mean lengths of the first recruitment group of both sites.

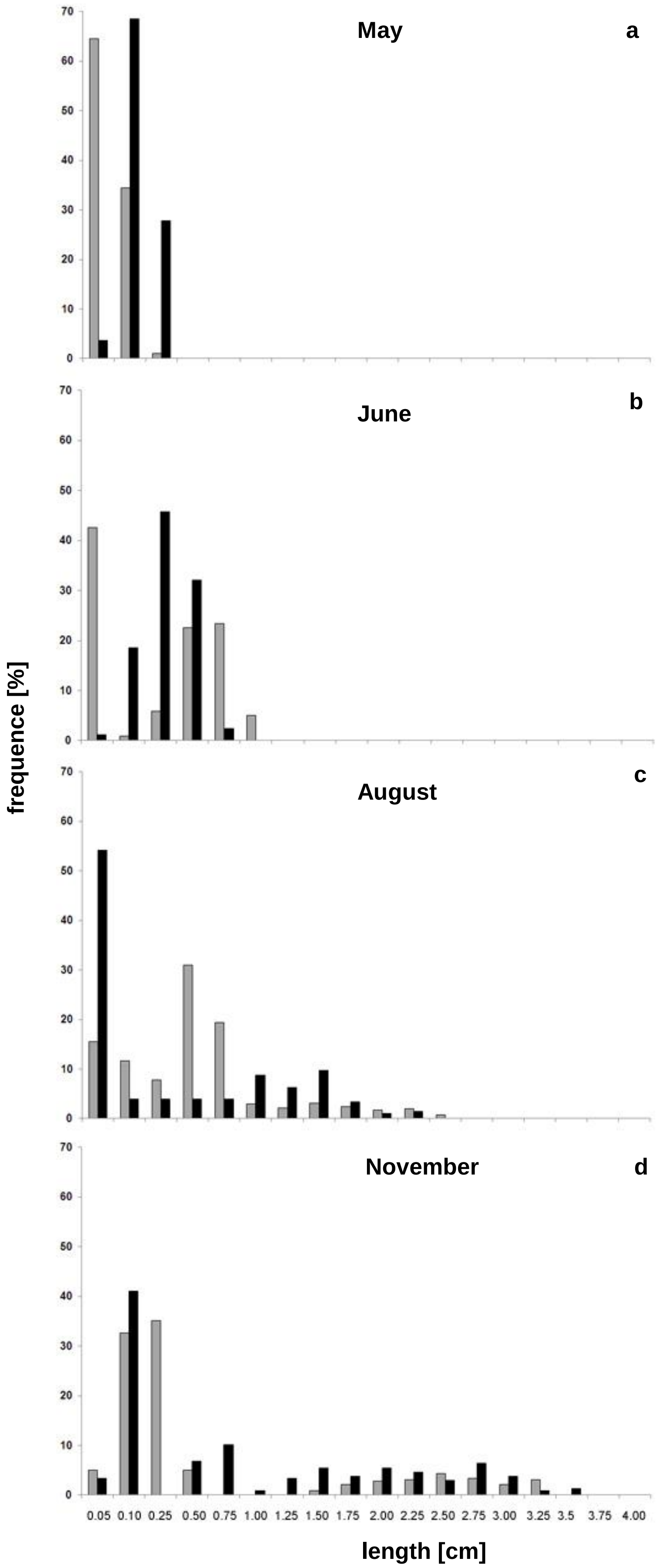

Fig. 7a-d: Length frequencies of blue mussels settled on artificial substrates at JD (gray bars) and RS (black bars) in May (a), June (b), August (c) and November (d) 2007.

At both sites growth rates are displayed as mean values for the total time of substrate exposure (6.4 months) and for each time period between the four consecutive sampling cycles conducted at both sites (Tab. 6). Growth rates for all phases between the samplings were higher at the nearshore site JD, resulting in a higher total growth based on the entire duration of the experiment at JD (2.6 cm) than at RS (2.3 cm) (Tab. 6).

Tab. 6: Growth rates of mussels cultivated at RS and JD between May and November 2007.

growth	phase 1 [cm/month]	phase 2 [cm/month]	phase 3 [cm/month]	mean [cm/month]	total cm in 6.4 months
RS	0.16	0.57	0.34	0.36	2.30
JD	0.28	0.77	0.31	0. 41	2.60

3.5. Lysosomal membrane stability

The results of the LMS-analysis for both sampling locations are shown in Figure 8a-d. The mean labilisation times for peak 1 were low at JD (7.70 min ± 3.49) and at RS (6.06 min ± 3.26) and did not differ significantly due to seasonal influences or between sites. The values for peak 2 showed a similar pattern with an average labilisation time of 23.03 min ± 9.51 at JD and 19.72 min ± 8.63 at RS. However, both sites and both peaks display comparable trends showing the lowest labilisation values in spring followed by an increase in summer. Differences between spring and summer were significant at both sites (JD p= 0.028; RS p=0.032). Autumn and winter sample at both sites stayed mostly stable on intermediate levels. Significant differences between the two sampling sites according to season could not be detected.

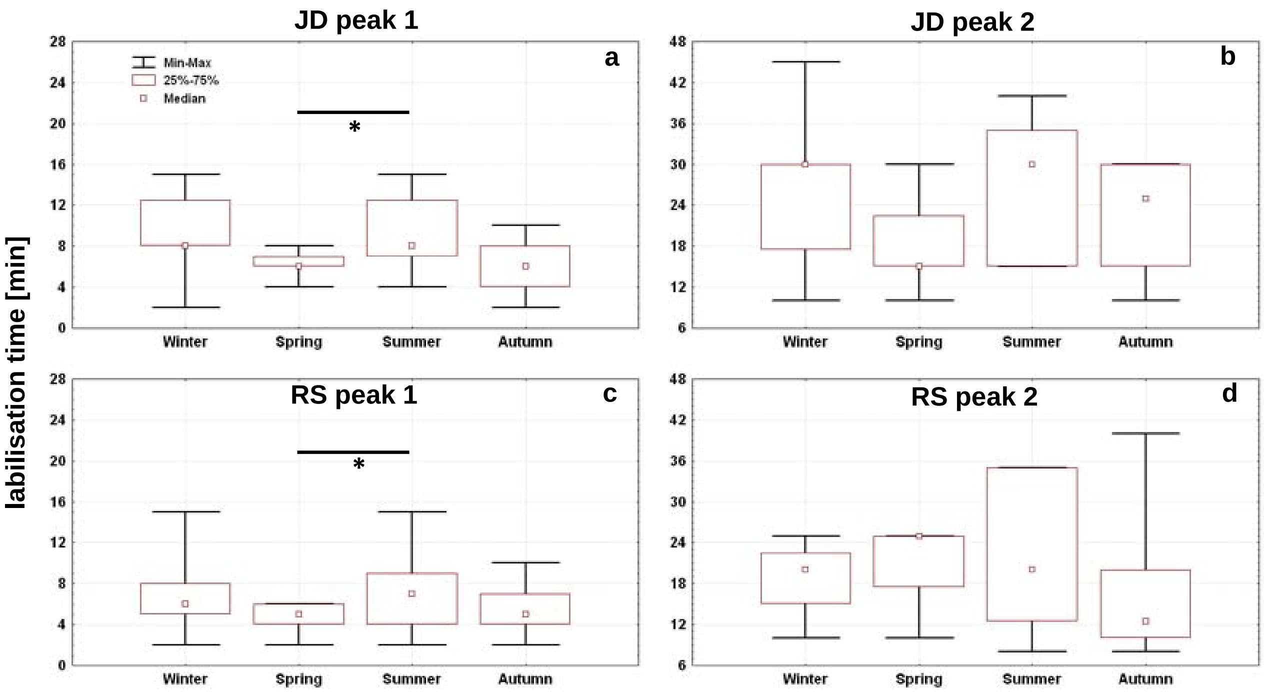

Fig 8a-d: Box-Whisker plots of JD peak 1 (a) and 2 (b) and RS peak 1 (c) and 2 (d) of four consecutive sampling cycles of the season 2007 from the German Bight. Differences for peak 1 between Spring and Summer at JD (a) and RS (c) are significant (p < 0.05) (*).

4. Discussion

It is widely believed that cultivation of organisms under offshore condition will lead to increased growth and better product quality since e.g. a better water quality, less temperature variability, better O_2-conditions and lower microbial or contaminant loads can be expected due to the distance to shore and the resulting dilution effects. The definition of the term offshore focuses on hydrographical or socio economic prerequisites: (1) the full exposure to all environmental conditions (Ryan 2005) and (2) a minimum distance of eight nautical miles to shore (Buck 2007) and reduced stakeholder conflicts (Dahle et al. 1991). Our data show, however, that a differentiated perspective is necessary in marine areas such as the German Bight. The test area RS fulfils the offshore definition for exposure and distance to shore, however, significant site specific differences concerning the central fitness parameters of mussels cultivated there for the 10 months duration time of the experiment could not be detected.

The results of the support programme measured throughout the whole sampling time revealed comparable properties at both site regarding salinity, temperature and nutrients, whereas organic matter and chlorophylls differed between the sites. Chlorophyll showed a more balanced distribution over the year with higher concentrations in the summer at RS. Concentrations of chlorophylls serve as an equivalent for the potentially available food for suspension feeders like mussels. Interestingly, the normal depletion of chlorophyll in the summer did not occur offshore leading to a prolonged feeding time at RS in the year 2007. According to status reports from the Federal Maritime Hydrographical Agency (BSH 2005), the German Bight continues to be burdened with substantial nutrient concentrations, particularly on the eastern side of the German Bight, leading to oxygen depletion at ground level. The production of biomass during summer is not limited by nutrients in the coastal areas, since estuarine run-offs compensate for the biomass production with dissolved nitrogen and phosphate compounds (BSH 2005). This trend, reflected by our, however, punctual measurements is still ongoing as displayed in data about the sampling year 2007 (BSH 2009).

Pollutants and heavy metals were found in the water column and suspended matter of both areas, however, in higher concentrations at RS especially in autumn 2007. An explanation for these findings might be that the offshore site RS is located at the south-west edge of an intermixing zone of estuarine run-offs of the rivers Weser and Elbe (BSH 2009). Due to the more extensive drainage area of the Elbe the river is burdened with higher loads of contaminants (BSH 2005, UBA 2009), leading to the paradox situation that pollutant concentrations are higher offshore than nearshore in this region. This effect is more pronounced in autumn, since run-off is increased by higher precipitation. According to OSPAR (2008) the southern part of the North Sea including the German Bight remains in an unacceptable status, showing concentration levels of heavy metals and organic pollutants in the sediment and in biota with assumed risks to the environment at population, and community level. Although some pollutants (e.g. PCB) and heavy metals (e.g. lead and mercury) show trends of decrease in concentrations, the Southern North Sea including the German Bight is still one of the most polluted marine areas of the OSPAR regions (OSPAR 2008).

Infestations with parasites in the German Bight, as determined in this study, followed the pattern described in other publications (e.g. Buck et al. 2005, Brenner et al. 2009). Macro parasites were absent in offshore mussels but present in -over the sampling season- increasing prevalence and intensities in nearshore samples. The offshore situation can be explained by: (i) the absence of intermediate hosts (e.g. *Littorina* spp. for trematod species), which are restricted to nearshore water habitats, (ii) the distance from the host populations, resulting in dilution effects and (iii) the poor swimming capacities of parasites' planktonic reproduction stages (e.g. *M. intestinalis*) (Davey and Gee 1988). According to the findings of Brenner et al. (2009) intertidal individuals from wild mussel beds showed high infestations rates regarding number of parasites and number of parasitic species. The mussels from the nearshore area JD cultivated off-bottom in the vicinity of these highly affected populations were infested moderately from the second year on, however only by the copepod *M. intestinalis*.

The prolonged feeding period at RS together with the complete absence of macro parasites might be an explanation for the higher condition indices at RS from August until November 2007. However,

these higher condition indices are not reflected in the growth rates calculated for both sites. Here, the nearshore site JD displayed higher rates at all phases and in total throughout the sampling period.
Forces generated by waves and currents are affecting the mussels and will increase exponentially with the size of the mussels (Harger 1970). Both locations are exposed to high current velocity up to 1.5 m/s, but only the offshore site RS is additionally exposed to pronounced wave forces (Buck 2007, BSH 2009). These wave forces may lead to an increased detachment of especially big mussels, reducing the calculated growth rates for these particular sites. Previous studies conducted in the same areas (Buck 2007, Walter et al. 2009) have revealed reverse results with higher growth rates at RS. Mussels used for these experiments were placed in nylon nets (Buck pers. comm.) similar to caging experiments and could not get lost during exposure even during storm events. The mussels used for this study at both sites, however, were unprotected against detachment. Regrettably, there are only few data sets about current speeds and no data about sea states including wave heights of the two sites for 2007 available.
Looking at all data available to describe the properties of the two testing sites it gets obvious that the German Bight is in an area up to the border of the 12 nautical miles zone a relatively homogenous water body. Thus, is reflected in the comparable results of the lysosomal membrane stability a sensitive parameter for environmental impacts and stress on organisms (e.g. Lowe et al. 1981, Moore 1988). The fact that the differences between the sites (e.g. parasites infestation, condition index, concentration of pollutants) found during the sampling season did not affect the LMS significantly could be explained as (i) the differences measured did not affect the fitness and the health of blue mussels or (ii) -more likely- the affects mutually compensated since sites were affected alternately. However, LMS and growth performance showed better results for the nearshore area most probably correlated with the higher concentrations of pollutants offshore.
Mussels living suspended hanging in the water column can feed permanently. The good food supply at both sampling sites explains the rapid growth rates and high condition indices, however, also leads to an increased accumulation of pollutants incorporated together with the food. Thus, may explain the surprisingly low means of LMS labilisation values for the first peak varying between 6 and 8 min. According to Viarengo et al. (2007) these animals are considered as severely stressed, already exhibiting pathologies. The results of this study correspond with findings from an experiment conducted with mussel samples from the same (JD) area in the year 2008 (Brenner et al. 2010) and with data on intertidal mussels from different wild banks of the German Bight in the year 2007 (Brenner unpublished data). The fact that all investigated mussels regardless of their habitat conditions, sampling season or year from all over the German Bight exhibit similar low LMS values provides strong evidence that the cause for the indicated stress is the same for all sampling sites. The described relatively homogenous water body burdened with contaminants is the most conceivable reason for these findings. If this is true, the coastal water up to 12 nautical miles distance to shore in the German Bight poses a severe risk to the health of blue mussels.

5. Conclusions and outlook

The results of this study show that offshore cultivation of mussel, according to the definitions, does not offer a guarantee of superior health and quality of cultivated organisms. The example of the German Bight shows that high concentrations of pollutants are not limited to nearshore waters. Contaminants are transported by air or currents up to the outer border of the 12 nautical miles zone. Future testing sites should therefore be located further offshore to ensure clearly different habitat conditions in terms of water quality and contaminant loads. The results concerning the health status of investigated mussels are worrisome, since impacts of environmental conditions of the German Bight are already detectable in young mussels (12-16 months). Accumulation effect during their whole life span will increase measured negative impacts.

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References

Allen JI, Moore MN (2004). Environmental prognostics: is the current use of biomarkers appropriate for environmental risk evaluation. Marine Environmental Research, 58: 227-232

Brenner M, Buck BH, Koehler A (2007). New concept combines offshore wind farms, mussel cultivation. Global Aquaculture Advocate, 10(1): 79-81

Brenner M, Ramdohr S, Effkemann S, Stede M (2009). Key parameters consumption suitability of offshore cultivated blue mussels (*Mytilus edulis* L.) in the German Bight. European Food Research and Technology, 230: 255-267

Brenner M, Wilhelm C, Broeg K, Koehler A (2010). Effect of air exposure on lysosomal membrane stability of *Mytilus edulis* L. from intertidal wild banks and culture ropes: implications for sampling practices (in prep.)

BSH (2005). System Nordsee – Zustand 2005 im Kontext langzeitlicher Entwicklung. Federal Maritime Hydrographic Agency, Hamburg, Germany, Report, 44: 270 pp

BSH (2009). Federal Maritime Hydrographic Agency. Marine environmental database (MUDAB). Hamburg, Germany. Online data acquisition http://www.bsh.de

Buck BH (2002). Open Ocean Aquaculture und Offshore-Windparks: Eine Machbarkeitsstudie über die multifunktionale Nutzung von Offshore-Windparks und Offshore-Marikultur im Raum Nordsee. Reports on Polar and Marine Research. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, 412: 252 pp

Buck BH (2007). Farming in a high energy environment: potentials and constraints of sustainable offshore aquaculture in the German Bight (North Sea). Reports on Polar and Marine Research. Alfred Wegener Institute for Polar and Marine Research, Bremerhaven, 543: 235 pp

Buck BH, Krause G, Rosenthal H (2004). Multifunctional use, environmental regulations and the prospect of offshore co-management: potential for and constraints to extensive open ocean aquaculture development within wind farms in Germany. Ocean Coastal Management, 47: 95–122

Buck BH, Thieltges DW, Walter U, Nehls G, Rosenthal H (2005). Inshore-offshore comparison of parasite infestation in *Mytilus edulis*: implications for open ocean aquaculture. Journal of Applied Ichthyology, 21(2): 107-113

Buck BH, Berg-Pollack A, Assheuer J, Zielinski O, Kassen D (2006). Technical realization of extensive aquaculture constructions in offshore wind farms: consideration of the mechanical loads. In: Proceedings of the 25th international conference on offshore mechanics and Arctic engineering, OMAE 2006: presented at the 25th International conference on offshore mechanics and Arctic engineering, 4.–9. June 2006, Hamburg, Germany/ American Society of Mechanical Engineers, 1–7

Cáceres-Martínez J, Figueras A (1998). Long-term survey on wild and cultured mussels (*Mytilus galloprovincialis* Lmk) reproductive cycles in the Ría de Vigo (NW Spain). Aquaculture, 162: 141-156

Cajaraville MP, Marigómez JA, Angulo E (1989). A stereological survey of lysosomal structure alterations in *Littorina littorea* exposed to 1-naphtol. Comparative Biochemistry and Physiology, 93C: 231-237

Castro M, Santos MN, Monteiro NM, Vieira N (2004). Measuring lysosomal stability as an effective tool for marine coastal environmental monitoring. Marine Environmental Research, 58: 741-745

COM (2006). Commission of the European Communities of 7th June 2006: Green Paper. Towards a future Maritime Policy for the Union: A European vision for the oceans and seas. “How inappropriate to call this planet Earth when it is quite clearly Ocean” attributed to Arthur C. Clarke. Brussels, Belgium, 275 pp

Da Ros L, Meneghetti F, Nasci C (2002). Field application of lysosomal destabilization indices in the mussel *Mytilus galloprovincialis* (L) as biomarkers of environmental stress. Journal of Experimental Marine Biology and Ecology, 170: 179-195

Dahle LA, DePauw N, Joyce J (1991). Offshore aquaculture technology – possibilities and limitations. Aquaculture and Environment, 14: 83-84

Davey JT, Gee JM (1988). *Mytilicola intestinalis*, a Copepod Parasite of Blue Mussels. American Fisheries Society, Special Publications, 18: 64-73

Dethlefsen V (1970). On the parasitology of *Mytilus edulis* (L. 1758). International Council for the Exploration of the sea (ICES) C.M. 1970/K: 16, Hamburg, 11 pp

Dethlefsen V (1972). Zur Parasitologie der Miesmuschel (*Mytilus edulis* L., 1758). Berichte der Deutschen wissenschaftlichen Kommission für Meeresforschung, 22: 344-371

Dubischar CD, Lopes RM, Bathmann UV (2002). High summer abundances of small pelagic copepods at the Antarctic Polar Front-implications for ecosystem dynamics. Deep-Sea Research II, 3871-3887

ICES (2005). Report of the Working Group on Biological Effects of Contaminants (WGBEC), 18.-22. April 2005, Reykjavik, Iceland. ICES CM 2005/E:08. 94 pp

ICES (2008). Report of the Fourth ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea Areas (WKIMON IV), 5.-7. February 2008, ICES, Copenhagen, Denmark. ICES CM 2008/ACOM:49. 82 pp

Jeffrey SW, Humphrey GF (1975). New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae, and natural phytoplankton. Biochemie und Physiologie der Pflanzen, 107: 191-194

Goldberg EG (1975). The mussel watch: a first step in global marine monitoring. Marine Pollution Bulletin, 6(7): 111

Goldberg ED, Bowen VT, Farrington JW, Harvey G and others (1978). The mussel watch. Environmental Conservation, 5: 101-125

Harger JRE (1970). The effect of wave impact on some aspects of the biology of mussels. Veliger, 12: 401-414

Koehler A, Wahl E, Soeffker K (2002). Functional and morphological changes of lysosomes as prognostic biomarkers of toxic liver injury in a marine flatfish (*Platichthys flesus* L.). Environmental Toxicology and Chemistry, 21(11): 2434–2444

Krishnakumar PK, Casillas E, Varanasi U (1994). Effects of environmental contaminants on the health of *Mytilus edulis* from Puget Sound, Washington, USA. I. Cytochemical measures of lysosomal response in the digestive cells using automatic image analysis. Marine Ecology Progress Series, 106: 249-261

Lauckner G (1983). Diseases of Mollusca: Bivalvia. In: Kinne O (Ed). Diseases of marine animals. Introduction, Bivalvia to Scaphopoda. Biologische Anstalt Helgoland, Hamburg, Westholsteinische Verlagsdruckerei Boyens & Co., Heide, 477-961

Livingstone DR, García-Martínez P, Michel X, Narbonne JF, O'Hara S, Ribera D, Winston GW (1990). Oxyradical production as a pollutionmediated mechanism of toxicity in the common mussel, *Mytilus edulis* L., and other molluscs. Functional Ecolology, 4: 415-424

Lowe DM, Moore MN, Clarke KR (1981). Effects of oil in the digestive cells in mussels: quantitative alterations in cellular and lysosomal structure. Aquatic Toxicology, 1: 213-226

Lowe DM, Clarke KR (1989). Contaminant induced changes in the structure of the digestive epithelium of *Mytilus edulis*. Aquatic Toxicology, 15: 345-358

Marigómez JA, Vega MM, Carajaville MP, Angulo E (1989). Quantitative response of the digestive-lysosomal system of winkles to sublethal concentrations of cadmium. Cellular and Molecular Biology, 35: 555-562

Marigómez I, Soto M, Angulo E (1991). Responses of winkles digestive cells and their lysosomal system to environmental salinity changes. Cellular and Molecular Biology, 37: 29-39

Marigómez I, Orbea A, Olabarrieta I, Etxeberria M, Cajaraville MP (1996). Structural changes in the digestive lysosomal system of sentinel mussels as biomarkers of environmental stress in mussel-watch programs. Comparative Biochemistry and Physiology, 113C: 291-297

Marigómez I, Baybay-Villacorta L (2003). Pollutant-specific and general lysosomal response in digestive cells of mussels exposed to model organic chemicals. Aquatic Toxicology, 64: 235-257

Marigómez I, Iekube X, Carajaville MP, Domouhtsidou G, Dimitriadis V (2005). Comparison of cytochemical procedures to estimate lysosomal biomarkers in mussels digestive cells. Aquatic Toxicology, 75: 86-95

Moore MN (1976). Cytochemical demonstration of latency of lysosomal hydrolases in the digestive cells of the common mussel, *Mytilus edulis*, and changes induced by thermal stress. Cell and Tissue Research, 175: 279-287

Moore MN (1988). Cytochemical response of the lysosomal system and NADPH-ferrihemoprotein reductase in molluscan digestive cells to environmental and experimental exposure to xenobiotics. Marine Ecology Progress Series, 46: 81-89

Moore MN (1992). Molecular cell pathology of pollutant-induced liver injury in flatfish: use of fluorescent probes. Marine Ecology Progress Series, 91: 127-133

Moore MN (2002). Biocomplexity: the post-genome challenge in ecotoxicology. Aquatic Toxicology, 59: 1-15

Moore MN, Lowe DM, Fieth PEM (1978). Lysosomal responses to experimentally injected anthracene in the digestive cells of *Mytilus edulis*. Marine Biology, 48: 297-302.

Moore MN, Lowe DW, Moore SL (1979). Induction of lysosomal destabilization in marine bivalve molluscs exposed to air. Marine Biology Letters, 1: 47-57

Moore MN, Lowe DM, Koehler A (2004) Biological effects of contaminants: Measurements of lysosomal membrane stability. ICES Techniques in Marine Environmental Sciences (TIMES), Vol. 36. ICES, Copenhagen

Nott JA, Moore MN (1987). Effects of polycyclic hydrocarbons on molluscan lysosomes and endoplatic reticulum. Histochemical Journal, 19: 357-368

O`Connor TP (1998). Mussel Watch results from 1986 to 1996. Marine Pollution Bulletin, 37: 14-19

OSPAR (2008). 2006/2007 CEMP Assessment - Trends and concentrations of selected hazardous substances in the marine environment. OSPAR Commission, 2007, London, UK, 63 pp

Petrovic S, Semencic L, Ozretic B, Ozretic M (2004). Seasonal variations of physiological and cellular biomarkers and their use in the biomonitoring of North Adriatic coastal waters (Croatia). Marine Pollution Bulletin, 49: 713-720

Pulfrich A (1997). Seasonal variation in the occurance of planktic bivalve larvae in the Schleswig-Holstein Wadden Sea. Helgoländer wissenschaftliche Meeresuntersuchungen, 51: 23-49

Rasmussen L (1982). Light microscopical studies of the acute toxic effects of *N*-Nitrosodimenthylamine on the marine mussel, *Mytilus edulis*. Journal of Invertebrate Pathology, 48: 117-123

Regoli F (1992). Lysosomal responses as a sensitive stress index in biomonitoring heavy metal pollution. Marine Ecology Progress Series, 84: 63-69

Regoli F, Nigro M, Orlando E (1998). Lysosomal and antioxidant response to metals in the Antartic scallop *Adamussium colbecki*. Aquatic Toxicology, 40: 375-392

Richards FA, Thompson TG (1952). Estimation and characterization of plankton populations by pigment analysis. II. Spectrophotometric method for the estimation of plankton pigments. Journal of Marine Research, 11:156-172

Ryan J (2005). Offshore Aquaculture – Do we need it, and why is it taking so long? International Salmon Farmers Association (Ireland). Expert workshop on “Sustainable Aquaculture”, DG JRC European Commission, Institute for Prospective Technological Studies, 17.-18. January 2005, Seville, Spain

Salazar MH, Salazar SM (1995). *In situ* bioassays using transplanted mussels. I. Estimating chemical exposure and bio-effects with bioaccumulation and growth. In: Hughes JS, Biddinger GR, Mones E (eds) Environmental toxicology and risk assessment, Vol 3. STP 2118, American Society for Testing and Materials, Philadelphia, PA, 216-241

Tremblay R, Pellerin-Massicotte J (1997). Effect of the tidal cycle on lysosomal membrane stability in the digestive gland of *Mya arenaria* and *Mytilus edulis* (L.). Comparative Biochemistry and Physiology, 117A: 99-104

UBA (2009). Umwelt Bundesamt, Dessau-Roßlau, Germany. http://www.umweltbundesamt-umwelt-deutschland.de/umweltdaten/public/ assessed 01.07.2009

UNEP (2007). MED POL - Biological Effects Monitoring Program: Achievements and Future Orientations: Proceedings of the Workshop, Alessandria, Italy, 20.-21. December 2006. Mediterranean Action Plan. MED POL, Athens, Greece

Viarengo A, Moore MN, Pertica M, Mancinelli G, Accomando R (1992). A simple procedure for evaluating the protein degradation rate in mussel tissues and its application in a study of phenanthrene effects on protein catabolism. Comparative Biochemistry and Physiology, 103B: 27-32

Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007). The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comparative Biochemistry Physiology, 146C: 281-300

Walter U, Buck BH, Liebezeit G (2009). Larval occurrence and settlement in the German Bight: a trial to estimate potentials for *Mytilus edulis* culture in offshore areas. Aquaculture International (submitted)

Watermann B, Die I, Liebe S (1998). Krankheiten der Miesmuschel (*Mytilus edulis*) an der ostfriesischen Küste: VII. Tagung der Deutschen Sektion der European Association of Fish Pathologists (EAFP) - Krankheiten der Aquatischen Organismen. 23.-25. September 1998, Schmalenberg-Grafschaft, 177-187

Widdows, J., Bakke, T., Bayne, B. L., Donkin, P., Livingstone, D. R., Lowe, D. M., Moore,M. N., Evans, S. V., & Moore, S. L. (1982). Responses of *Mytilus edulis* on exposure to the water-accommodated fraction of North Sea oil. Marine Biology, 67: 15–31

Widdows J, Donkin P (1992). Mussels and environmental contaminants: bioaccumulation and physiological aspects. In: Gosling E (Ed) The mussel *Mytilus*: ecology, physiology, genetics and culture. Elsevier, Amsterdam, 383–424

Widdows J, Donkin P, Staff FJ, Matthiessen P, Law RJ, Allen YT, Thain JE, Allchin CR, Jones BR (2002). Measurement of stress effects (scope of growth) and contaminant levels in mussels (*Mytilus edulis*) collected from the Irish Sea. Marine Environmental Research, 53: 327-356

Wong WH, Cheung SG (2003). Seasonal variation in the feeding physiology and scope of growth of green mussels, *Perna viridis* in estuarine Ma Wan, Hong Kong. Journal of the Marine Biological Association, UK, 83: 543-552

Chapter 10

EFFECT OF AIR EXPOSURE ON LYSOSOMAL MEMBRANE STABILITY OF *Mytilus edulis* L. FROM INTERTIDAL WILD BANKS AND SUBMERGED CULTURE ROPES

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Abstract

Blue mussel from suspended culture ropes and three intertidal wild banks of different areas of the German Bight were tested for their ability to cope with air exposure. All mussels showed high tolerance against air exposure with moderate mortalities up to 48 h exposure period. Labilisation values of lysosomal membranes in intertidal mussels changed notably between a minimum after 12 hours and a maximum after 24 hours of aerial exposure. Labilisation times of mussels from the hanging culture increased constantly up to 48 hours of exposure. Intertidal mussels from the island of Helgoland exhibited a significant decrease in membrane stability after 72 hours of air exposure correlating with highest mortalities. The regeneration of mussels under hypoxic conditions within the first 24 hours is discussed under the focus of anoxic fermentation and enhanced autophagy as energy producing processes. Whereas differences in the labilisation time at the end of the experiment may derive from varying infestations with parasites. The acclimatisation of intertidal mussels to aerial exposure had no influence under long term exposure conditions, since samples from suspended culture showed similar pattern in mortalities and even higher lysosomal membrane stability during the experiment.

Keywords: blue mussel, lysosomal membrane stability, hypoxia, stress response

1. Introduction

Since the early 1970s when the first biomonitoring programmes were initiated, blue mussels are internationally accepted indicator organisms for the assessment of contamination levels and their harmful effects on marine and estuarine environments (Goldberg 1975). In addition to a wide geographic distribution, blue mussels are abundant, stationary, and easy to sample. They respond to anthropogenic stress and are therefore used as indicator species in monitoring programs which include the measurement of biological effects (UNEP 2007). Nonetheless, they also respond to common abiotic (salinity, tidal variation, temperature etc.) and biotic (pathogens, parasites, food availability, reproduction) stress. As intertidal sessile organisms, mussels have to cope with harsh and rapidly changing physical conditions during tidal cycles, which include thermal stress (Helmuth & Hofmann 2001), desiccation (Moore et al. 1979), anoxia (Hole et al. 1995), and reduced food availability during periods of emersion. In contrast, subtidal mussels and mussels cultured on submerged ropes inhabit a less changing environment (Hunt & Scheibling 2001) although differences between high and low tide such as food availability might also affect their physiology.
As a result, some intertidal bivalve species like the ripped mussel *Geukensia demissa* (Dillwyn) exhibit a higher capacity to adapt to environmental changes more rapidly than subtidal organisms (Charles & Newel 1997). According to Moore et al. (2006a) animals living in a fluctuating environment are more tolerant towards pollutants since changes in salinities and food availability as well as periodical hypoxia are enhancing autophagic processes. The lysosomal autophagy, a process of degradation of cellular components, is a highly conserved evolutionary mechanism found in diverse groups of animals including nematodes, flies and mammals (Cuervo 2004). It is up-regulated in stress situations or physiological change and allows cells to temporarily self-sustain in periods when nutrients are

restricted (Bergamini et al. 2003). The required energy is produced by the recycling of proteins and organelles (Levine 2005). In addition to the role of autophagy as a survival strategy (Moore et al. 2006b) it is also discussed as an important response to remove oxidatively damaged proteins and organelles (Bergamini et al. 2003, Cuervo 2004).

The stability of lysosomal membranes of invertebrate digestive cells and the vertebrate liver are widely used as biomarkers in monitoring programmes for the assessment of marine and coastal environmental quality (Marigómez et al 1996, ICES 2006; 2007; 2008, UNEP 2007). Lysosomes respond to a broad range of pollutants with a significant increase in size (e.g. Lowe et al. 1981, Cajaraville et al. 1989, Moore 1988, Viarengo et al. 1992) and a labilisation of their membranes (Moore et al. 1978, Viarengo et al. 1992, Koehler et al. 2002, Marigómez & Baybay-Villacorta 2003). It has been shown that this response is correlated to site-specific contamination levels (Kagley et al. 2003, Broeg et al. 1999; 2002, Schiedeck et al. 2006, Sturve et al. 2005), In addition to chemical pollutants, natural stress factors such as temperature, salinity, availability and quality of food, tidal cycle and habitat conditions may also influence the stability of lysosomal membranes (e.g. Marigómez et al. 1991, Abele et al. 1998, Tremblay & Pellerin-Massicotte 1997, McVeigh et al. 2006). A decrease in the labilisation time and lysosomal enlargement in mussels has also been shown to occur under combined hypoxic and hyperthermic conditions (Tremblay & Pellerin-Massicotte 1997). In addition to anthropogenic and natural stressors also metabolic processes like reproductive status or disease may influence lysosomal properties (Bayne et al. 1985, Bignell et al. 2008). Izagirre (2007) showed changes of lysosomal membrane stability in mussels from the high intertidal, starting after aerial exposure and discussed these results as a response to digestion processes.

Since numerous factors may affect size and membrane stability of lysosomes, appropriate experimental designs and a fundamental knowledge of basic responses are necessary to facilitate interpretations of results with respect to the anthropogenic or natural causes of the observed effects.

Many bivalve species have developed strategies to cope with oxygen depletion in their natural environments. Intertidal organisms are exposed to air every tidal cycle during low tide, whereas animals living in subtidal areas may have to cope with periodic oxygen depletion in their habitat due to hydrographic (e.g. reduced water exchange) or anthropogenic influences (e.g. increased oxygen demanding degradation processes due to euthrophication) (Oeschger 1990). The species *Arctica islandica,* inhabiting the muddy bottoms of the Baltic Sea, for example, undergo self-induced anaerobiosis, since they dig burrows in oxygen depleted sediments. In experimental trials *A. islandica* survived for 55 days in oxygen deficient sea water (Theede et al. 1969). Some intertidal acclimatized bivalves close their valves, maintaining a small gap through which oxygen can diffuse into the mantle cavity. Species like *Mytilus californicus* reach values up to 80 % of the immersed rate of oxygen uptake by gaping under air exposure, whereas *M. edulis* generally close the valves during time of aerial exposure (Widdows et al. 1979). Closing valves lead to oxygen deprivation in the tissues, followed by a profound depression of energy demand and a change from aerobic to anerobic metabolism (Eertman et al. 1993). Under anaerobic conditions the fermentation of carbohydrates such as glycogen becomes the main source for energy (de Zwaan & Wijsman 1976). This anaerobic fermentation process is less efficient than the aerobic oxidation but may still exceed 12 % of aerobic ATP production under normal submerged conditions (Widdows et al. 1979). By these strategies blue mussel can survive in oxygen depleted water for up to 35 days (Theede et al. 1969) and under aerial exposure up to 20 days (Babarro & de Zwaan 2008). Natural or anthropogenic stressors increase the metabolic rates of mussels and concomitantly the energy demand, thus, reducing aerial survival time compared to unstressed mussels (de Zwaan & de Kock 1988).

The aim of this experimental study was to obtain deeper insights into the influence of long-term aerial exposure on the lysosomal membrane stability of digestive gland cells of *M. edulis* depending on (i) sampling site (ii) habitat conditions (intertidal/ submerged) and (iii) acclimatisation to tidal related aerial exposure.

2. Materials and Methods

Mussels were sampled at four locations along the coast of the German Bight (Fig. 1). Sampling was conducted between the 20th of April and the 5th of May 2007. Three areas were wild mussel beds: Bordumer Sand (BS, high intertidal, Position 53° 30' 00'' N; 08° 06' 00'' E), island of Helgoland (HL, intertidal, Position 54° 11' 00'' N; 07° 54' 09'' E) and Lister Strand from the Island of Sylt (SY, low intertidal, Position 55° 01' 10'' N; 08° 26' 50'' E).

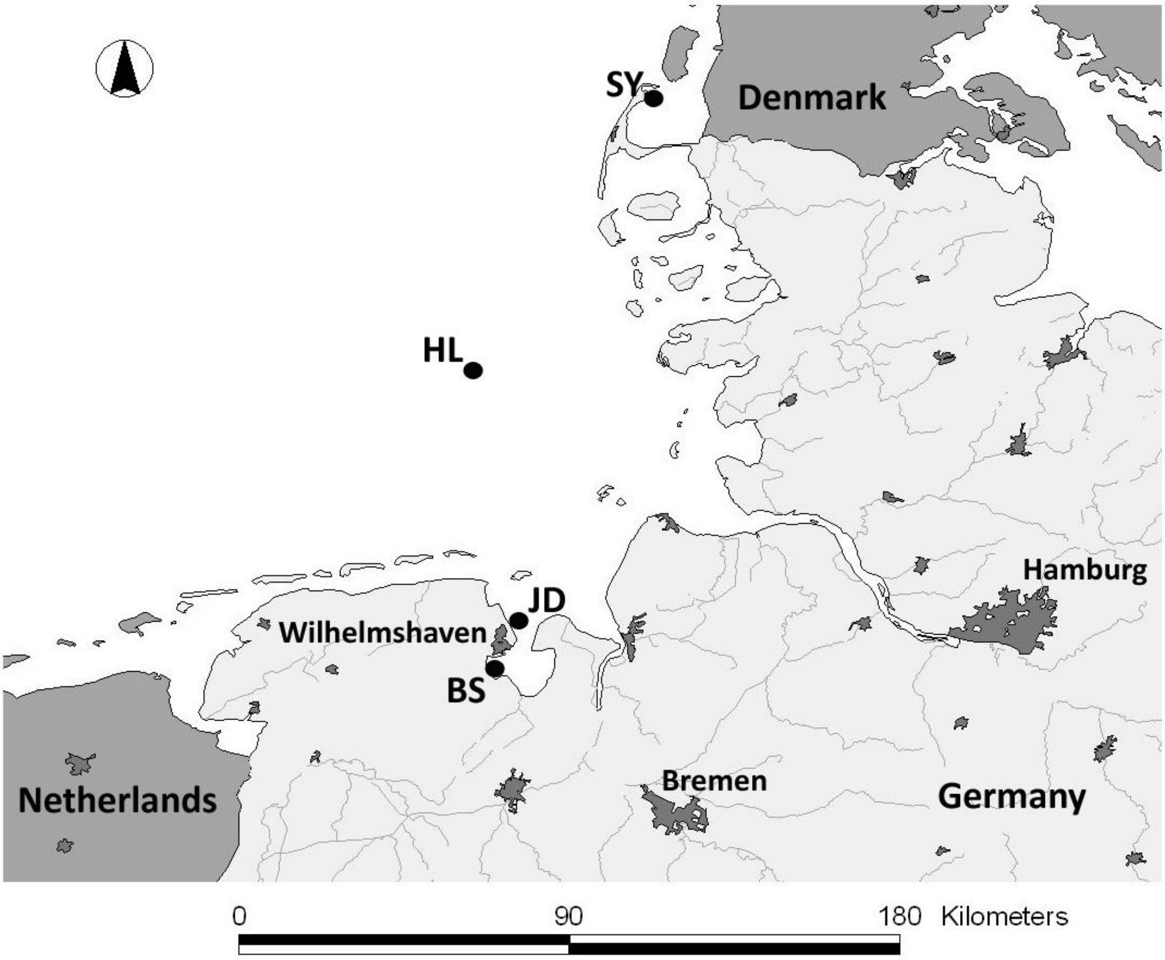

Fig.1: Map of the German Bight showing the sampling sites. Three intertidal sampling sites at Bordumer Sand (BS), Lyster Strand at the island of Sylt (SY) and from the dune of the island of Heligoland (HL) and one suspended hanging cultures at the *Niedersachsenbrücke* (nearshore) near Wilhelmshaven in the Jade (JD) estuary were sampled in April/ May 2008.

The intertidal areas were sampled during low tide. The fourth location was a test facility where mussels grew nearshore on suspended artificial substrates at the *Niedersachsenbrücke*, an approx. 1,300 m long cargo bridge located in the Jade estuary (JD, Position 53° 35' 00 N; 08° 09' 00 E). The artificial substrates were attached to 10 harnesses hanging from the bridge allowing a distance of 20 m between harnesses. Each harness consisted of a 20 mm polypropylene rope with an iron plate (5 kg) at its distal end, weighing down the substrates into the water column even at strong current velocities. The upper part of the harness was attached to a steel beam between the pillars of the bridge. The polypropylene (pp) rope was strengthened with two swirls and shackles to prevent entanglement. The lower swirl was placed one meter above the mean high water (mHW) level to prevent corrosion and fouling. To insure that the cultivated mussels were submerged during the whole experiment, samples of artificial test collectors were fixed to the pp-rope from one to approximately three meters below mean low water (mLW) level using sea water- and uv-resistant plastic binders. The deployed substrate samples were about 15 cm in length and fixed every 20 cm to the rope.

All sampling sites showed typical nearshore salinities between 28 ‰ and 31 ‰ throughout the year during high tide (Brenner et al. 2010).

2.1. Sampling design

Approximately eighty mussels between 3 to 5 cm shell lengths were transported in a cool box without water (dry) from each sampling location into the laboratory. Times necessary to transport mussels from the sampling location to the lab varied between 2 hours (BS and JD), 6 hours HL and 12 hours from the island of Sylt (SY). In the lab, all mussels from each sampling site were stored separately in aerated 30 l sea water aquaria (salinity 31 ‰) at natural water temperature (10 °C) illuminated for 12 h. Mussels were kept for seven days in the aquaria and were fed at the second and fifth day (5 ml, DT`s Plankton Form). The day after feeding water of the aquaria was exchanged.

After seven days, all mussels of one sampling site were taken out of their aquarium and kept dry under air exposure at a constant temperature of 10 °C. Ten mussels were analyzed immediately (00 h). The length and width of each mussel was measured to the nearest 0.1 mm using a vernier calliper. Mussels were opened, drained and total wet weight determined. Then the digestive gland was dissected, weighed, put in cryo-vials and directly frozen in liquid nitrogen. Subsequently, soft body was removed and both shell and soft body were weighed (± 0.01 g) separately. The procedure was repeated with another ten mussels still kept under air exposure after 12 h, 24 h, 48 h and 72 h. Mortality was calculated following daily inspection prior to preparation. Mussels were considered dead and excluded from the experiment, when they were open and squeezing did not lead to any response. Mortality was calculated after subtracting the ten mussels used for preparation and analysis after 00 h, 12 h, 24 h, 48 h and 72 h, respectively.

Mortality [%] = *dead animals* / (*number of mussels survived* - 10)*100 (1)

2.2. Sectioning and histochemical analysis of lysosomal membrane stability

Samples of frozen digestive glands were fixed in line on pre-frozen aluminium chucks for subsequent cryostat-sectioning. Tissue sections of a constant thickness of 10 µm were obtained using a cryotome (Microm' HM 500) with chamber temperature of -25 °C. Sections were stored for a maximum of 24 hours at -20 °C until processed for histochemistry.

The lysosomal membrane stability test was performed according to the method of Moore et al. (2004). Serial cryostat sections were incubated at 37 °C in a 0.1 M citrate buffer, pH 4.5, containing 3 % NaCl to destabilize the membrane for increasing time intervals (2–50 min). After this acid labilisation, sections were incubated for 20 min at 37 ° C in a medium containing the substrate Naphthol AS-BI N-acetyl β-D-glucosaminide (Sigma) dissolved in 2-methoxy ethanol and low-viscosity polypeptide (Polypep, Sigma) dissolved in 0.1 M citrate buffer, pH 4.5 with 3 % NaCl. During incubation, the lysosomal acid hydrolase N-acetyl ß-D-hexosaminidase catalyses the release of the Naphthol AS-BI group. In the next step, the Naphthol AS-BI group undergoes a post-coupling reaction with the diazonium salt Fast Violet B (Sigma) dissolved in 0.1 M phosphate buffer (pH 7.4) leading to an insoluble bright violet reaction product. Following the colour reaction, samples were rinsed in running tap water, fixed in Baker's Formalin, rinsed twice in distilled water, and dried overnight in the dark. Subsequently slides were mounted in Kaiser's glycerine-gelatine.

2.3. Lysosomal membrane stability (LMS)

The maximum reaction product for N-acetyl β-D-hexosaminidase (peak) was determined by automatic measurement of number and percentage of dark stained lysosomes in the digestive tubules by the use of computer assisted image analysis (Zeiss, KS 300) combined with a light microscope (Zeiss, Axioskop) at 400 fold magnification according to Broeg et al. (in prep). A long destabilization period indicates high lysosomal membrane integrity and vice versa.

Unclear results of labilisation period results (no clear peaks, only 1 peak) were re-measured or excluded from further analysis.

2.4. Statistical analysis of data

All statistical analyses were conducted using the SigmaPlot 11.0 and Statistica 5.0 software. The non-parametric Kruskal-Wallis ANOVA (SigmaPlot) was deployed to compare time of air exposure (00 h, 12 h, 24 h, 48 h and 72 h) for peak 1 and 2 at all sites. This test was followed by an all pair-wise multiple comparison procedure (Dunn's Method) to describe differences between the sites for peak 1 and 2. In addition initially dissected mussels (00 h) were compared for peak 1 and 2 according to the four different sites. This procedure was repeated at the end of the experiment (72 h). Box –Whisker plots and correlation matrix were calculated for all sites and for both peaks using Statistica 5.0 software. Means and standard deviations (mean ± SD) of data were calculated by using Microsoft Office Excel 2007.

3. Results

3.2. Lysosomal membrane stability (LMS)

At the beginning of the experiment (00 h, Fig. 2a/b) different values for peak 1 and 2 were detected between the sites. SY showed highest labilisation values for peak 1 and 2, followed by BS. Values for JD and HL were lowest at the beginning of the experiment. All differences at 00h were, however, not significant (Fig. 2a/b). Figure 3a-h show the results for all air exposure times (00 h, 12 h, 24 h, 48 h and 72 h) of each site (peak 1 and peak 2). At all intertidal sites (BS, SY and HL) values for peak 1 and 2 were highest after 24 h of air exposure (Fig. 3c-h). At JD highest labilisation values were achieved even later after 48 h of air exposure (peak 1 and 2) (Fig. 3a/b). Differences were significant only for HL peak 2 (Fig. 3h) between HL 00 vs. HL 24 ($p<0.05$), HL 12 vs. HL 48 ($p<0.05$) and HL 48 vs. HL 72 ($p<0.01$). All other differences were not statistically significant. Intertidal sites showed lowest labilisation values after 72 hours of exposure, whereas mussels from JD showed a minor decrease for peak 1 and even constant values between 48 and 72 hours after exposure for peak 2. Mean labilisation times for peak 1 and 2 at the beginning of the experiment were at JD 3.50 min ± 1.77/ 13.38 min ± 5.45, BS 5.78 min ± 3.80/ 17.78 min ± 6.67, SY 7.89 min ± 4.01/ 22.89 min ± 10.97 and HL 5.50 min ± 3.16/ 16.00 min ± 5.55. Figure 4a/b show the LMS for all sites at the end (72 h) of the experiment. A significant difference was detected for peak 2 between JD vs. BS ($p<0.05$).

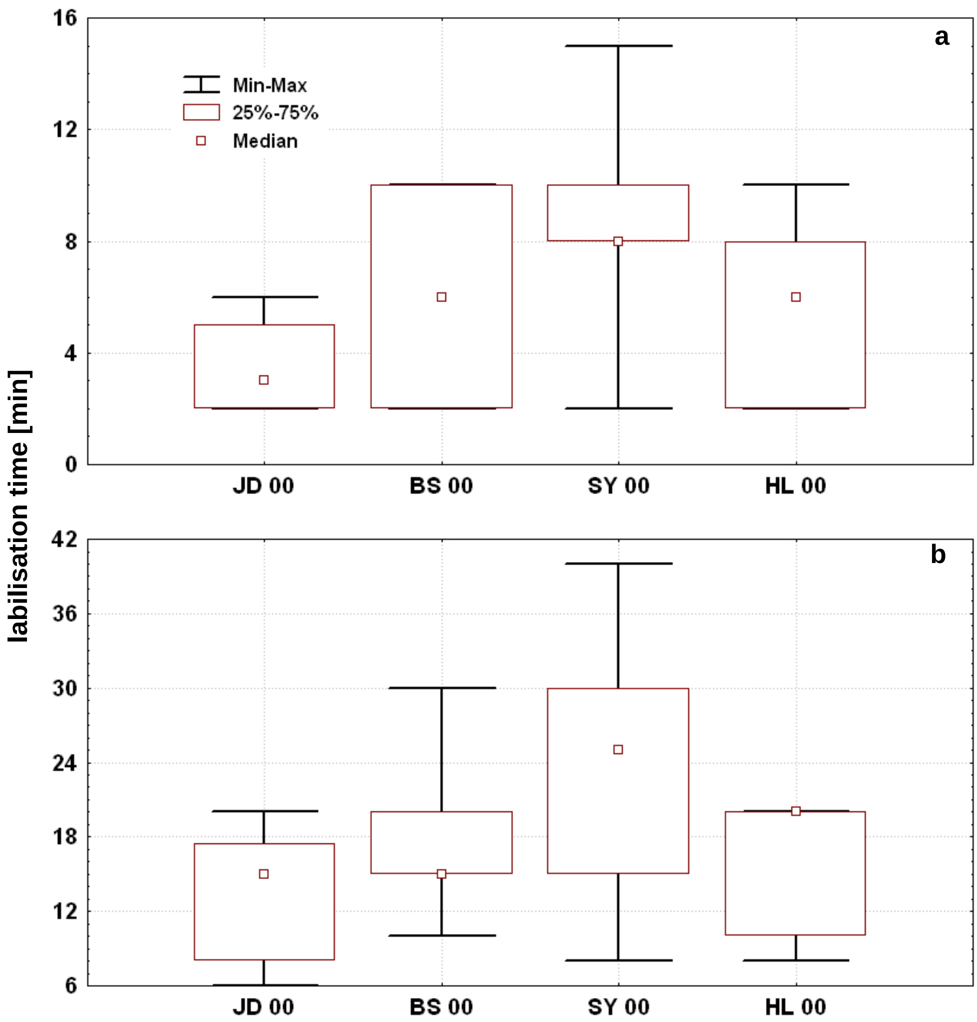

Fig. 2a/b: Box-Whisker plots comparing (a) peak 1 and (b) peak 2 of all sampling sites at the beginning of the test (T 00h).

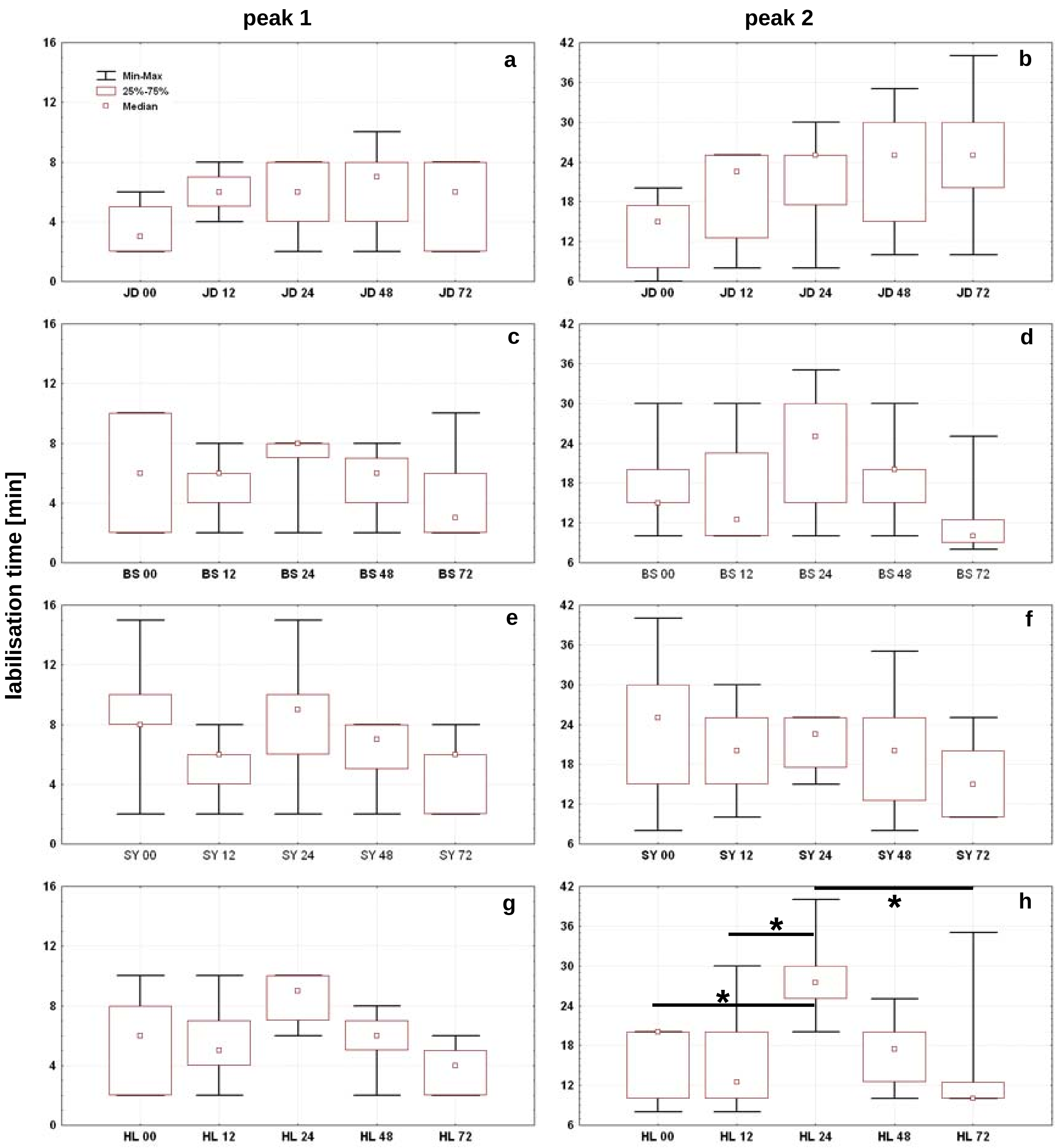

Fig.3a-h: Box-Whisker plots of peak 1 and peak 2 of all sampling sites (a) JD peak 1, (b) JD peak 2, (c) BS peak 1, (d) BS peak 2, (e) SY peak 1, (f) SY peak 2, (g) HL peak 1and (h) HL peak 2 comparing tested times of hypoxia (00h, 12h, 24h, 48, and 72h). Differences for peak 2 between HL 00 vs HL 24 ($p < 0.05$), HL 12 vs HL 24 ($p < 0.05$), and HL 48 vs HL 72 ($p < 0.01$), are significant (*).

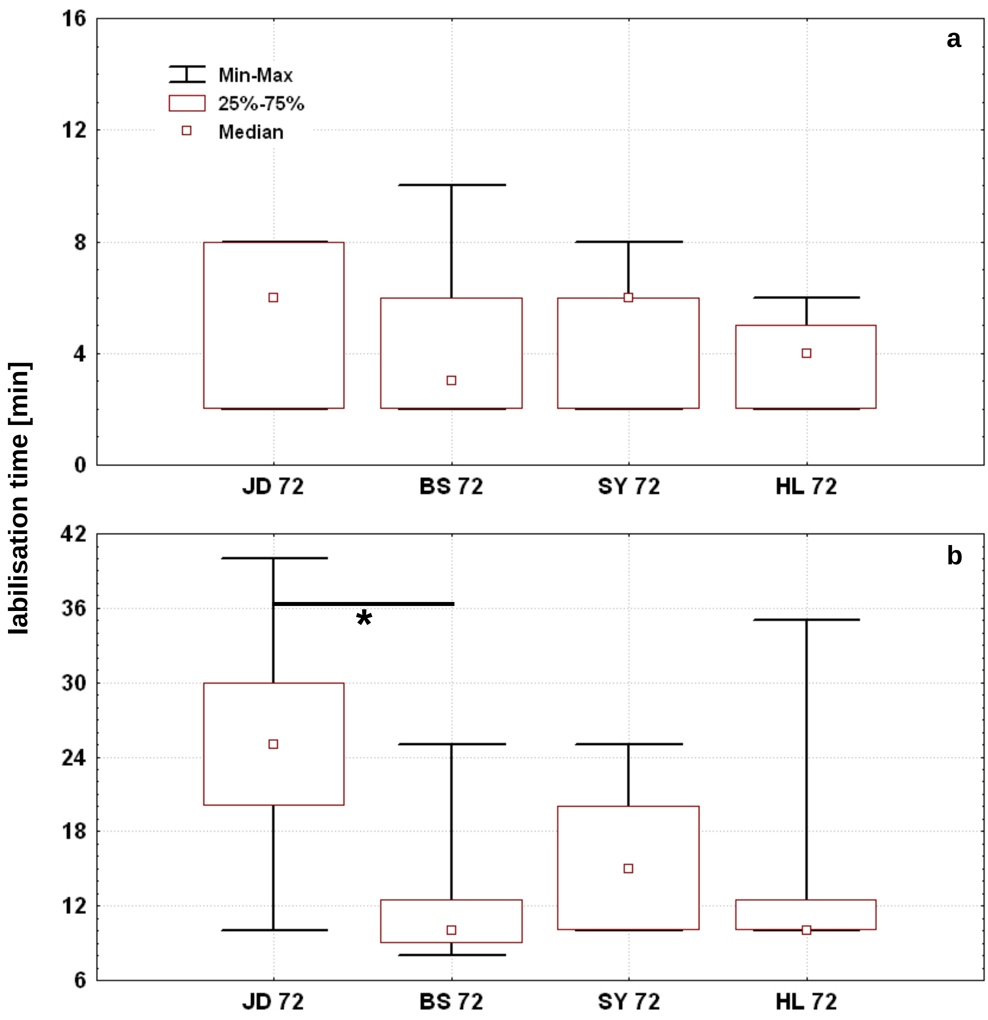

Fig. 4a/b: Box-Whisker plots comparing (a) peak 1 and (b) peak 2 of all sampling sites at the end of the experiment (T 72h). Difference of peak 1 (a) between BS vs. Hl and SY vs. Hl are significant (*). For peak 2 differences between JD and BS are significantly ($p < 0.05$) (*).

3.3. Mortality

At the start of the experiment n=66 (JD), n=85 (BS), n=80 (SY) and n=80 (HL) mussels were exposed to air. Until 24 h of exposure the mortality was relatively low at all sites and varied between 3 % (BS) and 11 % (SY) (Fig. 5). After 48 h of exposure, mortalities increased and varied between 5 % (BS) and 24 % (HL). At the end of the experiment after 72 h, more than 50 % of the mussels from HL were dead, whereas the mortality at BS was only 20 %. JD and SY showed intermediate mortalities after 72 h of 38 % and 32 % (Fig. 5). The number of mussels which died during exposure time is listed for each site in Tab. 1.

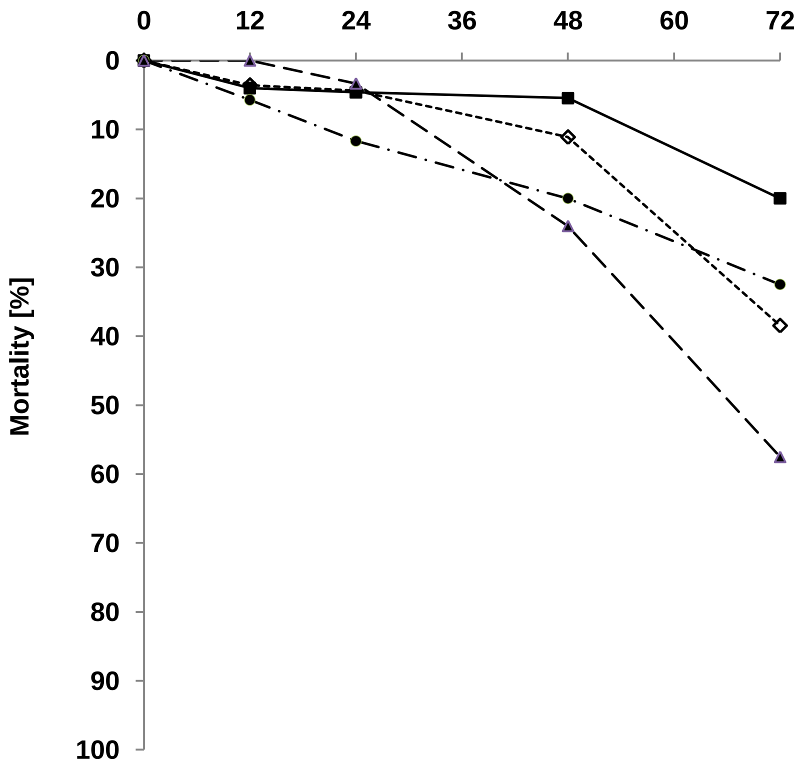

Fig. 5: Mortality in % of mussel during air exposure time (0h-72h) of the different sampling sites JD (white rhombus), BS (black squares), SY (black circles) and HL (black triangles) four different sampling sites of the German Bight April/May 2008.

Tab. 1: Initial number of mussels and number of mussel died during air exposure (0h-72h) of four different sampling sites of the German Bight April/May 2008.

	00 h	12 h	24 h	48 h	72 h
JD (n=66)	0	2	2	4	10
BS (n=85)	0	3	3	3	9
SY (n=80)	0	4	7	10	13
HL (n=80)	0	0	2	24	23

4. Discussion

Mussels have specialized mechanisms to cope with reduced oxygen availability due to air exposure in intertidal areas or oxygen depletion in the water column. Under aerial exposure some species are able to reduce oxygen deficiency by gaping, but in general bivalves reduce their energy demand and switch from aerobic processes to anaerobic metabolism to maintain a minimum of energy supply (de Zwaan & Wijsman 1976, Eertman et al. 1993). With this metabolic switch, some species e.g. *Artica islandica* are able to survive for weeks under anoxic conditions (Oeschger & Storey 1993). Blue mussels, whose natural habitat includes sub- and intertidal areas, emerge periodically during low tide and are able to survive for days under air exposure or even weeks in oxygen depleted waters (Theede et al. 1969). In addition to metabolic changes, lysosomal autophagy is increased under hypoxic conditions (Hipkiss 2006, Moore et al. 2007) helping to generate energy by the recycling of proteins and organelles during nutritional deprivation (Levine 2005, Moore et al. 2006c).

This investigation covered a large geographic area encompassing different types and sizes of water bodies and habitats; thereby it could be expected that a broad spectra of mussels of varying health status, due to different exposures to contaminants and with different acclimatisation levels to tide-related aerial exposure, would be covered. According to the database (MUDAB) of the Federal Maritime Hydrographic Agency (BSH 2009), the coastal and nearshore sites (BS, SY and JD) showed comparably high contamination loads in the water column and suspended particles, whereas concentrations around the island of Helgoland further offshore were lower.

In addition to the pollutant levels, parasite infestations also varied between sites along the coast of the German Bight. The intertidal mussels of the island of Helgoland showed the highest infestation rates with mass infestations of up to several thousand individuals of a single parasite species per mussel (Voss 2006), as there exists here all parasitic hosts needed to fulfil the life cycles of the different parasitic species. The two remaining intertidal areas in this study were analysed for parasites in 2007 (Brenner et al. 2009). As showed in previous investigations (Buck et al. 2005, Thieltges et al. 2006) these areas displayed high infestation rates combined with a large variety of parasitic species, whereas hanging cultivated mussels had low infestation rates with only a single parasitic species (Brenner et al. 2009).

Although mussels from all sampling sites were acclimatised for seven days in aquaria with clean sea water prior to the experiment, lysosomal membrane stability differed notably between the sites. Membranes of mussels taken from the intertidal areas were more stable at the beginning of the experiment than those taken from the subtidal cultivation site. At the end of the experiment, this pattern was reversed and mussels from the subtidal cultivation site showed higher membrane stabilities, even though the differences were not statistically significant. A possible explanation for the initial higher labilisation periods in intertidal mussels is given by Moore et al. (2007). The authors concluded that a stressful fluctuating environment triggers autophagic events with the consequence that altered proteins or damaged cellular constituents are removed and the formation as well as

accumulation of peroxidation endproducts like lipofuscin is minimised. In contrast the lysosomes in mussels which have fed continuously are overburdened by the continual demand of digestion, resulting in a steady generation and accumulation of lipofuscin (Moore et al. 2007).
During the experiment, labilisation periods decreased at all intertidal sites between 00 and 12 hours of aerial exposure followed by an increase of the labilisation periods between 12 and 24 hours. Significant changes of lysosomal membrane stabilities shortly after emersion were also reported by Izagirre (2007). He concluded that digestion processes i.e. intracellular digestion, triggered by tide, might be responsible for the decreased lysosomal membrane stability during the time of air exposure. The increase of membrane stability at all intertidal sites between 12 and 24 hours of air exposure is followed by a decline of values until the end of the experiment. This pattern corresponds with the results of Moore et al. (2007), where a marked decrease in lysosomal membrane stability was detected after 24 hours of air exposure.
In contrast to the results from the intertidal sites, mussels from the cultivation plot showed a different pattern during the time of aerial exposure. Here, a constant increase in labilisation values is displayed until 48 hours of exposure, before membranes then began to become instable (72 hours of exposure). Interestingly, mussels from all sites reached the maximum of membrane stability during the period of aerial exposure. Labilisation values after 24 hours (intertidal site) and 48 hours (cultivation site) are even higher than the values from the beginning of the experiment. If this pattern is verifiable mussels are not only able to sustain a minimum energy level for surviving phases of prolonged hypoxia by using anaerobic metabolic pathways or autophagic processes, but they are also able to regenerate under anaerobic conditions.
In this experimental approach however it is difficult to evaluate the rule and importance of the each metabolic process relevant for each of the different phases delineated in this experiment. However, it can be hypothesised that intertidal mussels which are acclimatised to hypoxic conditions first begin to reduce their metabolic rate once they are exposed to air. Only under prolonged hypoxic conditions - a twelve hour period is almost twice the time of a tidal cycle in the German Bight (approx. 6 hours 20 min) - alternative processes are activated to generate the necessary energy. Cultivated mussels, never having been previously exposed to air, might switch immediately to the genetically defined alternative pathways.
At the end of the experiment the negative impacts of anoxic conditions induced the decrease of LMS. All three intertidal sites have the lowest labilisation values of the entire experiment after 72 hours of air exposure. Mussels from the cultivation site show, however, only a minor trend of decreasing labilisation time. After 72 hours, membranes at JD continued to be more stable than at the beginning of the experiment. The notable increase in the mortality rate of the mussels at all sites during the experiment was reflected consistently in the results of lysosomal membrane stability at all intertidal sites and to a certain extent also at the cultivation site. At HL the high mortality correlated with a decrease of membrane stability for peak 2 between 24 hours and 72 hours of aerial exposure even on a significant level. However, since blue mussels can survive even longer than 72 hours under hypoxic conditions (Theede et al. 1969, Babarro & de Zwaan 1983) a significant decrease in membrane stabilities at all other sites might occur even later.
A possible explanation for the differences of LMS obtained at the end of the experiment between intertidal sites and the cultivation site could be the different infestation of mussels with parasites. Parasites function as a trigger of the mussels' immune system. According to Veldhuizen-Tsoerkan et al. (1991) and Eertman et al. (1993) survival in air is reduced under additional stressful environmental conditions, as mussels which are subject to toxic substances or natural stress have an increased metabolic rate (de Zwaan & de Kock 1988), leading to higher mortalities under aerial exposure (stress on stress response) (Smaal et al. 1991, Viarengo et al. 1995). Mussels from Helgoland have better water conditions concerning pollutants (BSH 2009) but suffer, especially since they were collected at the bank of tetra pods on the dune of Helgoland, under mass infections of parasites (Voss 2006). The same, although on an intermediate level, is true for BS and SY. The hanging cultivated mussels from the Jade estuary are less burdened by parasites, thus potentially leading to a lower stress on stress response.
This study shows that acclimatisation to aerial exposure of intertidal mussels does not offer a relevant advantage under prolonged exposure conditions, as mussels from suspended cultures showed even

higher labilisation values compared to intertidal mussels after 48 and 72 hours. These findings indicate that the ability to cope with long term anoxia is genetically determined rather than a question of phenotypic acclimatisation. This is supported by the fact that the two metabolic pathways to generate energy under anaerobic conditions - anaerobic fermentation and autophagy – discussed in this study are common mechanisms used by different bivalve species (e.g. de Zwaan & Wijsman 1976, Eertman et al. 1993, Oeschger & Storey 1993, Moore et al. 2006c). Autophagy has been shown to be characteristic of many groups of animals from nematodes to mammals (Cuervo 2004), giving strong evidence that it is an evolutionary conserved mechanism (Moore et al. 2006a).
Interestingly, all mussels regardless of origin and habitat properties showed low membrane labilisation values of 3 to 8 min for peak one, which did not differ significantly between sites. According to Viarengo et al. (2007) these animals would be considered as severely stressed, already exhibiting pathologies. Following this general classification our results indicate that the quality of the coastal waters of the German Bight -including the area around the island of Helgoland- poses a risk to the health of its populations of blue mussels. Low labilisation times were also found in mussels from the same sampling site (JD) over the whole year of 2007 (Brenner et al. 2010) and in mussels from intertidal wild banks (including BS and SY) of the German Bight in the year 2007 (Brenner unpublished data).

Conclusions and outlook

The results of this study show that intertidal organisms, such as blue mussels, have developed robust strategies to cope with anoxia without suffering severe metabolic stress. Even under long term anoxic conditions mussels are able to maintain the integrity of lysosomal membranes, since a sudden release of high amounts of hydrolytic enzymes due to membrane leakage, would result in self-digestion and death of the organisms. Thus the key function of lysosomal membrane stability for the viability of the organism is highlighted. Furthermore, it could be demonstrated that mussels are even able to regenerate under anaerobic conditions using autophagy and/or fermentation processes to produce energy.

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References

Abele D, Burlando B, Viarengo A, Pörtner HO (1998). Exposure to elevated temperatures and hydrogen peroxide elicits oxidative stress and antioxidant response in the Antarctic intertidal limpet *Nacella concinna.* Comparative Biochemistry and Physiology, 120B: 425-435

Barbarro JMF, de Zwaan A (2008). Influence of abiotic factors on bacterial proliferation and anoxic survival of the sea mussel *Mytilus edulis* L. Journal of Experimental Marine Biology and Ecology, 273: 33-49

Bayne BL, Brown DA, Burns K, Dixon DR, Ivanovici A, Livingstone DR, Lowe DM, Moore MN, Stebbing ARD, Widdows J (1985). The effects of stress and pollution on marine animals. Praeger, New York, USA

Bergamini E, Cavallini G, Donati A, Gori Z (2003). The anti-ageing effects of caloric restriction may involve stimulation of macroautophagy and lysosomal degradation, and can be intensified pharmacologically. Biomedicine and Pharmacotherapy, 57: 203–208

Bignell JP, Dodge MJ, Feist SW, Lyons B, Martin PD, Taylor NGH, Stone D, Travalent L, Stentiford GD (2008). Mussel histopathology: effects of season, disease and species. Aquatic Biology, 2: 1-15

Brenner M, Ramdohr S, Effkemann S, Stede M (2009). Key parameters for the consumption suitability of offshore cultivated blue mussels (*Mytilus edulis* L.) in the German Bight. European Food Research and Technology, 230: 255-267

Brenner M, Buchholz C, Buck BH, Koehler A (2010). Health and growth performance of blue mussels (M*ytilus edulis* L.) from two different hanging cultivation sites in the German Bight: a nearshore - offshore comparison. (in prep.)

Broeg K, Zander S, Diamant A, Körting W, Krüner G, Paperna I, Westernhagen H v (1999). The use of fish metabolic, pathological and parasitological indices in pollution monitoring I North Sea. Helgoland marine research, 53: 171-194

Broeg K, Koehler A, Westernhagen H. von (2002). Disorder and recovery of environmental health monitored by means of lysosomal stability in liver of European flounder (*Platichthys flesus* L.). Marine Environmental Research, 54 (3-5): 569-573

BSH (2009). Federal Maritime Hydrographic Agency. Marine environmental database (MUDAB). Hamburg, Germany. Online data acquisition http://www.bsh.de

Buck BH, Thieltges DW, Walter U, Nehls G, Rosenthal H (2005). Inshore-offshore comparison of parasite infestation in *Mytilus edulis*: implications for open ocean aquaculture. Journal of Applied Ichthyology, 21(2): 107-113

Cajaraville MP, Marigómez JA, Angulo E (1989). A stereological survey of lysosomal structure alterations in *Littorina littorea* exposed to 1-naphtol. Comparative Biochemistry and Physiology, 93C: 231-237

Charles F, Newell RIE (1997). Digestive physiology of the ribbed mussel *Geukensia demissa* (Dillwyn) held at different tidal heights. Journal of Experimental Marine Biology and Ecology, 209: 201-213

Cuervo AM (2004). Autophagy: in sickness and in health. Trends in cell biology, 14: 70-77

Eertman RHM, Wagenvoort AJ, Hummel H, Smaal A (1993). “Survival in air” of blue mussel *Mytilus edulis* L. as a sensitive response to pollution-induced environmental stress. Journal of Experimental Marine Biology and Ecology, 170: 179-195

Goldberg EG (1975). The mussel watch: a first step in global marine monitoring. Marine Pollution Bulletin, 6 (7): 111

Helmuth BST, Hofmann GE (2001). Microhabitats, thermal heterogenity, and patterns of physiological stress in the rocky intertidal zone. Biological Bulletin, 201: 374-384

Hipkiss A (2006). Accumulation of altered proteins and ageing: causes and effects. Experimental Gerontology, 41: 464-473

Hole LM, Moore MN, Bellamy D (1995). Age-related cellular and physiological reactions to hypoxia and hyperthermia in marine mussels. Marine Ecology Progress Series, 122: 173-178

Hunt HL, Scheibling RE (2001). Predicting wave dislodgment of mussels: Variation in attachment strength with body size, habitat, and season. Marine Ecology Progress Series, 213:157-164

ICES (2006). Report of the Working Group on Biological Effects of Contaminants (WGBEC), 27.–31. March 2006, ICES Headquarters, Copenhagen, Denmark. ICES CM 2006/MHC:04. 79 pp

ICES (2007). Report of the Working Group on Biological Effects of Contaminants (WGBEC),19.-23. March 2007, Alessandria, Italy. CM 2007/MHC:03. 129 pp

ICES (2008). Report of the Fourth ICES/OSPAR Workshop on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea Areas (WKIMON IV), 5.–7. February 2008, ICES, Copenhagen, Denmark. ICES CM 2008/ACOM:49. 82 pp

Izagirre U (2007). Contribution to the interpretation of lysosomal biomarkers in marine organisms based on the mechanistic understanding of the lysosomal responses to pollutants. Dissertation. Universidad del País Vasco, Spain, 318 pp

Kagley AN, Snider RG, Krishnakumar PK, Casillas E (2003). Assessment of seasonal variability of cytochemical responses to contaminant exposure in the blue mussel *Mytilus edulis* (Complex). Archives of Environmental Contamination and Toxicology, 44: 43-52

Koehler A, Wahl E, Soeffker K (2002). Functional and morphological changes of lysosomes as prognostic biomarkers of toxic liver injury in a marine flatfish (*Platichthys flesus* L.). Environmental Toxicology and Chemistry, 21(11): 2434–2444

Levine B (2005). Eating oneself and uninvited guests: Autophagy-related pathways in cellular defense. Cell, 120: 159-162

Lowe DM, Moore MN, Clarke KR (1981). Effects of oil in the digestive cells in mussels: quantitative alterations in cellular and lysosomal structure. Aquatic Toxicology, 1: 213-226

Marigómez I, Baybay-Villacorta L (2003). Pollutantspecific and general lysosomal responses in digestive cells of mussels exposed to model organic chemicals. Aquatic Toxicology, 64: 235-257

Marigómez I, Orbea A, Olabarrieta I, Etxeberria M, Cajaraville MP (1996). Structural changes in the digestive lysosomal system of sentinel mussels as biomarkers of environmental stress in mussel-watch programs. Comparative Biochemistry and Physiology, 113C: 291-297

Marigómez I, Soto M, Angulo E (1991). Responses of winkles digestive cells and their lysosomal system to environmental salinity changes. Cell and Molecular Biology, 37: 29-39

McVeigh A, Moore MN, Allen JI, Dyke P (2006). Lysosomal response to nutritional and contaminant stress in mussel hepatopancreatic digestive cells: A modelling study. Marine Environmental Research, 62: 433-438

Moore MN (1988). Cytochemical responses of the lysosomal system and NADPH-ferrihemoprotein reductase in molluscan digestive cells to environmental and experimental exposure to xenobiotics. Marine Ecology Progress Series, 46: 81-89

Moore MN, Lowe DM, Fieth PEM (1978). Lysosomal responses to experimentally injected anthracene in the digestive cells of *Mytilus edulis*. Marine Biology, 48: 297-302.

Moore MN, Lowe DW, Moore SL (1979). Induction of lysosomal destabilization in marine bivalve molluscs exposed to air. Marine Biology Letters, 1: 47-57

Moore MN, Lowe DM, Koehler A (2004). Biological effects of contaminants: Measurements of lysosomal membrane stability. ICES Techniques in Marine Environmental Sciences (TIMES), vol. 36. ICES, Copenhagen

Moore MN, Allen JI, Somerfield PJ (2006a). Autophagy: Role in surviving environmental stress. Marine Environmental Research, 62: 420-425

Moore, MN, Allen JI, McVeigh A (2006b). Environmental prognostics: An integrated model supporting lysosomal stress responses as predictive biomarkers of animal health status. Marine Environmental Research, 61: 278-304

Moore MN, Allen JI, McVeigh A, Shaw J (2006c). Lysosomal and autophagic reactions as predictive indicators of environmental impact in aquatic animals. Autophagy, 2:3 217-220

Moore MN, Viarengo A, Donkin P, Hawkins AJS (2007). Autophagic and lysosomal reactions to stress in the hepatopancreas of blue mussels. Aquatic Toxicology, 84: 80-91

Oeschger R (1990). Long-term anerobiosis in sublittoral marine invertebrates from the Western Baltic Sea: *Halicryptus spinulosus* (Priapulida), *Astarte borealis* and *Artica islandica* (Bivalvia). Marine Ecology Progress Series, 59: 133-143

Oeschger R, Storey KB (1993). Impact of anoxia and hydrogen sulphide on the metabolism of *Artica islandica* L. (Bivalvia). Journal of Experimental Marine Biology and Ecology, 170: 213-226

Schiedek D, Broeg K, Baršienė J, Lehtonen KK, Gercken J, Pfeifer S, Vuontisjärvi H, Vuorinen PJ, Dedonyte V, Koehler A, Balk L, Schneider R (2006). Biomarker responses as indication of contaminant effects in blue mussel (*Mytilus edulis*) and female eelpout (*Zoarces viviparus*) from the southwestern Baltic Sea. Marine Pollution Bulletin, 53 (8-9): *387-405*

Smaal AC, Wagenvoort A, Hemelraad J, Akkerman I (1991). Response to stress of mussels (*Mytilus edulis*) exposed in Dutch tidal waters. Comparative Biochemistry and Physiology, 100C: 197-200

Sturve J, Berglund Å, Balk L, Broeg K, Böhmert B, Massey S, Savva D, Parkkonen J, Stephensen E, Koehler A, Förlin L (2005). Effects of dredging in Göteborg Harbor, Sweden, assessed by biomarkers in eelpout (*Zoarces viviparus*). Environmental Toxicology and Chemistry, 24 (8): 1951-1961

Theede H, Ponat A, Hiroki K, Schlieper C (1969). Studies on the resistance of marine bottom invertebrates to oxygen-deficiency and hydrogen sulphide. Marine Biology, 2: 325-337

Thieltges DW, Krakau M, Andresen H, Fottner S, Reise K (2006). Macroparasite community in molluscs of a tidal basin in the Wadden Sea. Helgoland Marine Research, 60: 307-316

Tremblay R, Pellerin-Massicotte J (1997). Effect of the tidal cycle on lysosomal membrane stability in the digestive gland of *Mya arenaria* and *Mytilus edulis* (L.). Comparative Biochemistry and Physiology, 117A: 99-104

UNEP (2007). MED POL - Biological Effects Monitoring Program: Achievements and Future Orientations: Proceedings of the Workshop, Alessandria, Italy, 20.-21. December 2006. Mediterranean Action Plan. MED POL, Athens, Greece

Veldhuizen-Tsoerkan MB, Holwerda DA, Zandee DI (1991). Anoxic survival time and metabolic parameters as stress indices in the sea mussel exposed to cadmium or polychlorinated biphenyls. Archives of Environmental Contamination and Toxicology, 20: 259-265

Viarengo A, Moore MN, Pertica M, Mancinelli G, Accomando R (1992). A simple procedure for evaluating the protein degradation rate in mussel tissues and its application in a study of phenanthrene effects on protein catabolism. Comparative Biochemistry and Physiology, 103B: 27-32

Viarengo A, Canesi L, Pertica M, Mancinelli G, Accomando R, Smaal AC, Orunesu M (1995). Stress on stress response: A simple monitoring tool in the assessment of a general stress syndrome in mussels. Marine Environmental Research, 39: 245-248

Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007). The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comparative Biochemistry Physiology, 146C: 281-300

Voss D (2006). Parasitierungsgrad der Miesmuschel (*Mytilus edulis*) in Abhängigkeit zur Tiefenzonierung und Entfernung zur Küste. Bachelor-Thesis. University of Applied Science, Bremerhaven, Germany, 55 pp

Widdows J, Bayne BL, Livingstone DR, Newell RIE, Donkin P (1979). Physiological and biochemical responses of bivalve molluscs to exposure to air. Compendium of Biochemical Physiology, 62A: 301-308

de Zwaan A, Wijsman TCM (1976). Anaerobic metabolism in bivalvia (Mollusca). Compendium of Biochemical Physiology, 54B: 313-324

de Zwaan A, de Kock WC (1988). The development of a general biochemical stress index. Marine Environmental Research, 24: 254-255

Chapter 11

Synthesis

Mussels of the family Mytilidae have traditionally been an important food source for populations living along coastal areas where mussels are naturally distributed. Initially only fished from wild banks, these mussels began to be cultivated nearshore using different methods and techniques (e.g. Dardignac-Corbeil 1979, Walter & Liebezeit 2001, Smaal 2002) which varied between countries and habitat conditions. Along the German coast of the North Sea, the blue mussels *Mytilus edulis* is the only existing indigenous species of the family Mytilidae which is cultivated extensively in its natural environment (Rosenthal & Hilge 2000).
Besides their function as a traditional food source, mussel species have been intensively used in marine science (Gosling 1992). Since mussels are widely distributed, abundant, stationary, and easy to obtain, they are nowadays one of the best investigated marine organisms worldwide. Particularly as marine ecologists have begun to focus on environmental protection, mussels have been used intensively for monitoring programmes to test the status and development of marine habitats (e.g. Goldberg 1975, Smolders et al. 2003, ICES 2006).
Due to their importance as a food source, the cultivation of mussels has increased substantially in the last decades on a global scale (Smaal 2002). The development of new cultivation techniques has played a role in this intensification of production. One of these new methods, the longline system, involves a shift from on-bottom to off-bottom cultivation, and a change from shallow to deeper waters where traditional mussel cultivation would be difficult or even impossible (e.g. Kamerman et al. 2002, Walter & Liebezeit 2003). Adapted longline systems withstand harsh offshore conditions and can help to shift production from less competitive areas with good water conditions to further off the coast (Langan & Horton 2003). These offshore culture systems combined with the solid groundings of the planned offshore wind mills might result in a achievable alternative for nearshore production of mussels or other candidates (Buck 2004).
However, these systems will clearly be more costly and therefore detailed data are needed to calculate economic potential, and risks, of areas being considered for mussel farming.

1.1. Experimental approach

For this study three testing sites were initially planned and constructed. The main testing site was chosen in accordance with the affected fishermen and the involved fishing and shipping authorities in the outer Weser estuary. Following the offshore definitions of Ryan (2005) and Buck (2004) the site was situated at approximately 8 nautical miles off the coast. At this site all parameters would be assessed throughout the duration of the experiment. In addition both a near- and another offshore site were chosen and equipped with artificial mussel collectors. Unfortunately the second offshore site situated 14 nautical miles west of the island of Sylt could not be sampled by scuba divers due to consistently bad weather conditions. High waves and strong currents finally led to the destruction and loss of the system. Since the testing site was not reconstructed only the remaining two sites were used to obtain all necessary samples and data.
The aim of this thesis was to assess, using biodiagnostic tools developed originally for biomedical purposes and for environmental monitoring programmes, the health of the cultivated organisms, or in economic terms, the quality of the product. Other parameters including condition index, parasite infestation, and microbial or viral loads, were investigated in addition to evaluating the principle drivers of health and consumption suitability of the mussels. Using the blue mussel as the culture candidate of choice it was assured that a well investigated organism was deployed to assess sites for potential cultivation.
Besides a well-established data base on mussel physiology and response to environmental conditions, other reasons were important in the decision to use blue mussels. Previous investigations had shown that larval concentrations and environmental conditions at both testing sides were suitable, so that mussels would attach themselves in adequate numbers on the offered substrates and grow to appropriate sizes within the duration time of experiment (Buck 2007, Walter et al. 2009).

1.2. Technical improvements

The experimental design for both testing areas also allowed work on technical issues, such as the efficiency of the farming devices themselves. Previous investigations had revealed results on construction of the longline and mooring systems (Buck 2004), but there was no focus on details such as suitable offshore substrates. Healthy mussels will reach market size in offshore conditions only if they are firmly attached to their artificial substrate. As mussels growing on suspended substrates need about 15 months (Buck 2007) on average to reach market size, they must survive one winter and withstand storm events producing wave heights up to several meters (Loewe et al. 2003). Continuing investigations on the health and quality of market sized mussels would be moot if mussels failed to stay attached to substrate gear.

Most available substrates are designed and deployed for nearshore use under calm water condition. This study has found that improvment for construction of new collectors can be achieved. New substrates should have felt-like structures around the core of collector for larval attraction and long appendices in high density to interweave the mussel conglomerates with the substrate. Future investigations should focus further on the fabrication and testing of a prototype of this collector, concerning the results of this study. Besides providing optimal larval attraction and attachment for juvenile mussels even under winter conditions the new substrate should proof its durability under conditions of a daily farming routine. This would include mechanical thinning, harvesting processes, and tests on the reusability of the material.

1.3. Mussels' health

This study is the first approach to integrate methods used in environmental monitoring into marine aquaculture. In combination with the assessment of parasites and data concerning consumption suitability, the results should be used to aid in selection of offshore cultivation sites. The results of this study showed, however, that water quality regarding the concentrations of pollutants is comparable in the German Bight up to the 12 nautical mile zone border. Lysosomal membrane stability was relatively low at all tested near- and offshore sites. Interestingly, growth rates of the hanging cultivated mussels were not affected by this low fitness parameter. Other health parameters such as parasite infestation rates followed, however, a clear near/ offshore pattern where mussels from offshore site were free of parasites whereas nearshore mussels were highly infested. Future investigations should try again to include testing sites further offshore, where low concentrations of pollutants have been proven in advance. By this means the assumed trend towards healthier mussels when cultivated in offshore sites might be detectable, aiding in the calculation of economic potential for offshore cultivation of blue mussels.

1.4. Consumption suitability

Cultivated mussels are intended for human consumption and are therefore subject to a complex regulatory framework supervised by public authorities. Since all analyses to evaluate the consumption suitability of mussels have to be conducted using standardised methods only official or certificated laboratories are involved in processing (CEFAS 2007). Therefore the assessment of bacteriological load, viruses and algae toxins were determined in close cooperation with the State Office for Consumer Protection and Food Safety (LAVES) of Lower Saxony, Cuxhaven, Germany. Like this, mussels from the testing sites were processed like official samples. Although EU regulations (EC 854/2004) include only the analysis of *E. coli* and *Salmonella* as obligatory for mussels and mussel products, all parameters relevant for consumption suitability were analysed in our study. Using this approach a preferably broad spectrum of the potential hazards to human consumption could be covered. As other studies have shown before (e.g. Lhafi 2006), dangerous human pathogens such as *Vibrio* and *Clostridia* were found independently of the solely registered high values for of *E. coli*, thus thwarting the oft pronounced assumption that these pathogens occur only in combination with *E. coli*. However, offshore cultivated mussels were no guarantee for microbial purity. This finding denoted, similarly to the results of the lysosomal membrane stability tests, that the deployed offshore site is still strongly affected by the estuarine runoffs of the rivers Weser and Elbe.

1.5. Legal issues

Currently existing EU regulation for hygienical control is focused strongly on faecal contaminations (EC 854/2004). A review of the most recent publications on mussel contamination with human pathogens has shown, however, that a differentiated approach according to site specifics and cultivation methods is needed for both nearshore and offshore cultivation areas (Guyader et al. 2000, Formicca-Cruz et al. 2002, Romalde et al. 2002). Further, in no European states have legal issues affecting potential offshore cultivation nor official supervisional responsibilities been clarified.

1.6. Monitoring practices

To avoid any impact on stability of the lysosomal membrane, most experts suggest a direct dissection of mussels after sampling to guarantee an *in situ* approach. Due to these specifications sampling and field work conducted for this study was highly complex and labour intensive, since transport times needed to be short, or dissection had to be conducted partly during excursions under unfavourable conditions. Knowing however, that intertidal organisms have the capacity to cope with anoxic conditions, the idea arose to verify the effects of hypoxia on the lysosomal membrane stability of wild and cultivated mussels. The results of this laboratory experiment showed that, the lysosomal membrane stability undergoes remarkable changes under aerial exposure. Intertidal mussels display minimum and maximum labilisation values within the first 24 hours of air exposure, whereas hanging cultivated mussels seem to regenerate constantly up to 48 hours of exposure. This suggests that organisational efforts are justified even for large monitoring programmes with many sampling sites.

1.7. Conclusions and perspectives

The multifactorial and interdisciplinary approach used for this study revealed valuable results and insight in various fields of mussel cultivation. Beside the first successful implementation of biomarkers into aquaculture, it was shown that technical requirements could be improved and discussion initiated on supervisional control and regulation. These results will aid in the development of a functioning and efficient offshore cultivation of mussels in the North Sea, since sustained economic success can only be achieved if cultured organisms of optimal quality are cultivated for human consumption and market needs of the products are seriously considered.

The deployed experimental design allowed sampling of additional parameters e.g. sex, gonad status, neutral lipid and lipofuscin concentrations and investigation of authophagy processes which were not examined yet but will certainly complete the picture about useful tools for site selection. A following evaluation of all applied parameters should result in a list of most effective and efficient parameters for selection of cultivation sites near- or offshore. These criteria may further help to improve and supplement the limited conventional EU guidelines (CEFAS 2007). Issues which may affect the economics of mussel cultivation such as reproduction, shell strength, and degree of shell fouling according to site, season, and cultivation method need further analysis, and thereby improvement of the marketability of offshore cultivated mussels can be expected. In a prospective step, the significance and comparability of the deployed parameters should be tested by extending the area to the European Atlantic coast and the Mediterranean Sea using a variety of relevant aquaculture species like *Crassostrea gigas*, *Ostrea edulis* and *Mytilus galloprovincialis* with the aim to develop an intercalibrated and internationally approved controlling system for cultivated organisms and cultivation sites. Further, a transfer of obtained knowledge for diagnostic purposes at existing aquaculture sites where quality or health problems of cultivated organisms arouse seem to realisable. Globally applicable standards for diagnostic and site selection can be exported to and used in areas outside of European legislation. Potential users are stakeholders e.g. in Korea, China, Thailand as well as countries in South-America.

The technical improvements presented in this study should be used to develop new larvae collecting and grow out gear for offshore purpose. In close cooperation with specialised manufactures development of prototypes, on-road tests, and marketing, appears promising.

Finally, this work should promote the necessary critical discussion on the regulatory framework suitable for mussel products in general and in particular concerning products related to offshore cultivated mussels to uncover solutions balanced between consumer safety, farming and processing.

References

Buck BH (2004). Farming in a high energy environment: potentials and constraints of sustainable offshore aquaculture in the German Bight (North Sea). Dissertation. University of Bremen, Bremen, Germany, 258 pp

Buck BH (2007). Experimental trials on the feasibility of offshore seed production of the mussels *Mytilus edulis* in the German Bight: installation, technical requirements and environmental conditions. Helgoland Marine Research, 61:87–101

CEFAS (2007). Microbiological monitoring of bivalve mollusc harvesting areas – Guide to good practice: Technical application. European Community Reference Laboratory for monitoring bacteriological and viral contamination of bivalve mollusc; The centre of environment, fisheries and aquaculture science, 3: 67 pp

Dardignac-Corbeil MJ (1979). Manuel de la conchyliculture. La mytiliculture. Rev. Trav. Inst. Pêches Marit., 43: 105-128

EC 854/2004. Regulation of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organization of official controls on products of animal origin intended for human consumption, 139 pp

Formica-Cruz M, Tofino-Quesada G, Bofill-Mas S, Lees DN, Henshilwood K, Allard AK, Conden-Hansson A–C, Hernroth BE, Vantarakis A, Tsibouxi A, Papapetropoulou M, Furones MD, Girones R (2002). Distribution of human virus contamination in shellfish from different growing areas in Greece, Spain, Sweden and the United Kingdom. Applied and Environmental Microbiology, 68: 5990-5998

Goldberg ED (1975). The Mussel Watch - a first step in global marine monitoring. Marine Pollution Bulletin, 6: 111

Gosling EG (Ed) (1992). The mussel *Mytilus*: Ecology, Physiology, Genetics and Culture. Population and community ecology of *Mytilus*. New York, Tokyo, Elsevier, 589 pp

Guyader F le, Haugarreau L, Miossec L, Dubois E, Pommepuy M (2000). Three-year study to assess human enteric viruses in shellfish. Applied and Environmental Microbiology, 66: 3241-3248

ICES (2006) Report of the Working Group on Biological Effects of Contaminants (WGBEC), 27-31 March 2006, Copenhagen, Denmark. ICES CM 2006/MHC: 04: 79 pp

Izagirre U (2007). Contribution to the interpretation of lysosomal biomarkers in marine organisms based on the mechanistic understanding of the lysosomal responses to pollutants. Dissertation. Universidad del País Vasco, 318 pp

Kamermans P, Brummelhuis E , Smaal A (2002). Use of spat collectors to enhance supply of seed for bottom culture of blue mussels (*Mytilus edulis*) in the Netherlands. World Aquaculture, 33(3): 12-15

Lhafi SK (2006). Untersuchungen zum bakteriellen und viralen Kontaminationsstatus von Miesmuscheln (*Mytilus edulis*) deutscher Herkunft. Dissertation. Institut für Lebensmittelqualität und –sicherheit, Tierärztliche Hochschule Hannover, Germany, 137 pp

Langan R, Horton F (2003). Design, operation and economics of submerged longline mussel culture in the open ocean. Bulletin of the Aquaculture Association of Canada, 103: 11–20

Loewe P, Becker G, Brockmann U, Frohse A, Herklotz K, Klein H, Schulz A (2003). Nordsee und Deutsche Bucht 2002 - Ozeanographischer Zustandsbericht. Berichte des Bundesamt für Seeschifffahrt und Hydrographie Nr. 33/2003, Bundesamt für Seeschifffahrt und Hydrographie, Hamburg, Germany

Romalde JL, Area E, Sánchez G, Ribao C, Torrado I, Abad X, Pintó RM, Barja JL, Bosch A (2002). Prevalence of enterovirus and hepatitis A virus in bivalve molluscs from Galicia (NW Spain): inadequacy of the EU standards of microbiological quality. International Journal of Food Microbiology, 74: 119-130

Rosenthal H, Hilge V (2000). Aquaculture production and environmental regulations in Germany. Journal of Applied Ichthyologie, 16: 163-166

Ryan J (2005). Offshore aquaculture - Do we need it, and why is it taking so long? International Salmon Farmers Association (Ireland). Expert workshop on sustainable aquaculture, DG JRC European Commission, Institute for Prospective Technological Studies, 17.–18. January 2005, Seville, Spain

Smaal AC (2002). European mussel cultivation along the Atlantic coast: production status, problems and perspectives. Hydrobiologia, 484: 89–98

Smolders R, Bervoets L, Wepener V, Blust R (2003). A conceptual framework for using mussels as biomonitors in whole effluent toxicity. Human and Ecology Risk Assessment, 9: 741-760

Walter U, Liebezeit G (2001). Nachhaltige Miesmuschel-Anzucht im niedersächsischen Wattenmeer durch die Besiedlung natürlicher und künstlicher Substrate. Abschlußbericht des ersten Projektjahres, 98 pp

Walter, U. & Liebezeit, G. (2003). Efficiency of blue mussel (*Mytilus edulis*) spat collectors in highly dynamic tidal environments of the Lower Saxonian coast (southern North Sea). Biomolecular Engineering, 20: 407-411

Walter U, Buck BH, Liebezeit G (2009). Larval occurrence and settlement in the German Bight – a trial to estimate potentials for *Mytilus edulis* culture in offshore areas. Aquaculture International (submitted)

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