MarBioShell (2008-2012)

Final report 31 December 2012

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MarBioShell

The reorganized MarBioShell project (2009-2012)

The main theme in the reorganized MarBioShell project (2009-2012) is research aimed at production of line-mussels (*Mytilus edulis*) in the Great Belt (Denmark) to compensate for the present decrease in landings from Danish fjords and coastal waters. Methods are being developed to optimize mussel growth, and modelling tools are improved to guide dimensioning of mussel farms. In the laboratory, bioreactor technology is applied to provide controlled mass production of planktonic algae to be subsequently used in controlled growth experiments and bioenergetic and biochemical studies of food intake and assimilation in mussels. In the field, comparative studies of actual growth and production of mussels of different size in selected areas in and outside the Great Belt region are performed with special emphasis on the importance of salinity, current speed, and amount of phytoplankton (chl *a*) in the ambient water. Marked analyses are carried out to estimate whether product differentiation and consumer preferences contribute to the economic sustainability of the suggested new line-mussel production facilities in the Great Belt. Business network and branding, as well as local innovation systems are surveyed and used in future investment decisions. A research- and demonstration line-mussel farm has been established in the southern part of Kerteminde Bay, close to the Great Belt. The research- and demonstration facility has been a unique opportunity for all work packages within the MarBioShell project to work together in solving the main common task, namely to clarify the potential in the broadest sense of cultivating mussels in the Great Belt.

Hans Ulrik Riisgård

31 December 2012

WP0: Management

Management
Management of the project and organization of the network is ensured by the scientific leader, Hans Ulrik Riisgård, in close co-operation with key participants responsible for the different main fields of the project.

Steering committee
The committee consists of Hans Ulrik Riisgård (chairman, SDU-NAT), Flemming Møhlenberg (DHI), Eva Roth (SDU-SAMF), Niels T. Eriksen (AAU), Jens Honoré Walther (DTU). The steering committee meets at least once every year to discuss relevant issues within the project. The steering committee is collectively responsible for:

- Overall coordination at the network level to ensure progress of the project and budget coherence
- Approval of proposals for annual timetables and coordination with other relevant national and international research projects.
- In coordination with the relevant research schools, ensure research training, PhD-courses and collaboration with national and international companies, research teams and networks.
- Revise research plans.
- Observe all commitments to The Danish Council for Strategic Research.
- Administrate IPR and other legal aspects

Activities (Jan 2008 – Mar 2012)
Steering-committee meetings (scm), seminars (s), meeting with partners (mwp)

01 February 2008	Marine Biological Research Centre, Kerteminde (Kick-off meeting)
10 April 2008	Marine Biological Laboratory, KU, Helsingør. (scm)
06 May 2008	Sydfyns Linemuslinger, Svendborg (mwp)
25 September 2008	Musholm Lax, Gørlev (mwp)
31 October 2008	Danish Council for Strategic Research, Copenhagen (scm)
29 January 2009	LO-skolen, Helsingør (scm)
18 November 2009	Marine Biological Research Centre, SDU, Kerteminde (scm)
26 May 2010	SDU-Esbjerg, Esbjerg (s, scm)
28 June 2011	Marine Biological Research Centre, SDU, Kerteminde (scm)
01 March 2012	Marine Biological Research Centre, SDU, Kerteminde (scm)

WP1: Production of microalgae

Responsible
Assoc. Professor Niels T. Eriksen, Department of Biotechnology, Chemistry and Environmental Engineering, AAU

Aims
The aim of WP1 is to design and optimize cultures of 'feed-algae' with specific, nutritional compositions, and to develop heterotrophic microalgal cultures of high nutritional value in order to create sufficient productivity for quantitative feeding experiments with mussels at larger scale.

Scientific personnel
- Daniel Pleissner (DP) started his 3-year PhD-project on April 1, 2009. Daniel Pleissner is employed at SDU but will conduct approx. 18 months of research on microalgal cultures at AAU.
- Research assistant Kim Lundgreen (KL), SDU has contributed to the production of phototrophic microalgae for bivalve feeding at Marine Biological Research Centre, Kerteminde since mid-2008.
- Muge Isleten (MI), exchange PhD student from Ege University, Turkey has completed a 6 month research period working on lipid production in heterotrophic *Crypthecodinium cohnii* from 01.04.09-30.09.09 at AAU.
- Bjørn Lund Danielsen (BLD), 'diplomingeniørstuderende' at AAU, has completed a 30 ECTS BSc project on starch production in heterotrophic *Galdieria sulphuraria* from 01.03.09-31.10.09.
- Ana García (AG), exchange PhD student from University of Cádiz, Spain has carried out a 3 month project on gas exchange in phototrophic microalgae in photobioreactors, April-June 2010 at AAU
- Martin Malthe Borch (MMB), guest student from DTU has carried out a 5 ECTS project on controlled CO_2 additions in photobioreactors, Spring 2010 at AAU
- Döndü Yalçın (DY), MSc exchange student from Ege University, Turkey has carried out a project on isolation of heterotrophic microalgae from Feb-June, 2010 at AAU
- 15 MSc students in biotechnology have at their 5th semester carried out 16 ECST projects on growth, pigment and lipid formation in heterotrophic *Crypthecodinium cohnii*, *Chlorella saccharophila* and *Galdieria sulphuraria* during fall semesters 2008-2010, AAU.
  Associate Professor Niels T. Eriksen (NTE), AAU is acting as supervisor for the students involved in this project and participates in research ac*tivities.*
- Professor Hans Ulrik Riisgård (HUR), SDU participates in supervision and research activities.

Activities (Jan 2008 – Dec 2012)
- A novel 3 L photobioreactor for continuous production of *Rhodomonas* sp. under controlled conditions has been designed, optimised and installed in April 2008 at Marine Biological Research Centre, Kerteminde. Since mid-2008 the photobioreactor has been in use producing algae for mussel feeding experiments. A second reactor is currently used for optimisation of the design and studies of oxygen and carbon dioxide exchange. This reactor will later be used to increase the capacity for production of phototrophic feed algae.
- Heterotrophic *Galdieria sulphuraria* has been produced at Aalborg University and used for mussel feeding experiments at Marine Biological Research Centre, Kerteminde.
- Investigation on growth and lipid content in heterotrophic *Chlorella* spp. has been started (Fig. 1).

- Heterotrophic cultures of *Crypthecodinium cohnii* have been studied at Aalborg University, and culture conditions have been optimised with respect to growth, biomass productivity, and the lipid content of the cells (Fig. 1).
- Continuous flow cultures of *Crypthecodinium cohnii* subjected to carbon, nitrogen, or phosphorous limitation have been developed and characterised with respect to biochemical composition of the biomass (DP).
- Methods for lipid and fatty acid analysis in *Crypthecodinium cohnii*, based on gas chromatography and staining combined with fluorescence spectroscopy have been developed.
- A novel method for analysis of amino acids in growth media of high salinity has been developed.
- Continuous production of phototrophic 'feed-algae', *Rhodomonas* sp. in photobioreactors as feed in bioenergetics experiments with blue mussels (see WP2) feeding on phototrophic microalgae.
- Heterotrophic *Crypthecodinium cohnii* produced in processes developed at AAU have been used for feeding studies at Marine Biological Research Centre, Kerteminde.
- Collaborations between the original MarBioShell partners, Institute of Biology, SDU and Section of Biotechnology, AAU and a new partner, Institute of Chemical Engineering, Biotechnology and Environmental Technology, SDU (TekFak-SDU-KBM, former Odense Teknikum) have been formalised. The collaborations will focus on the production and consumption of phototrophic feed algae and the design of phototrophic algal cultures, and will supplement on-going MarBioShell activities on feeding in mussels and production of phototrophic as well as heterotrophic feed algae. The collaboration will involve students and researchers from all 3 institutions, and funding has been allocated to support research activities carried out by students from TekFak-SDU-KBM.

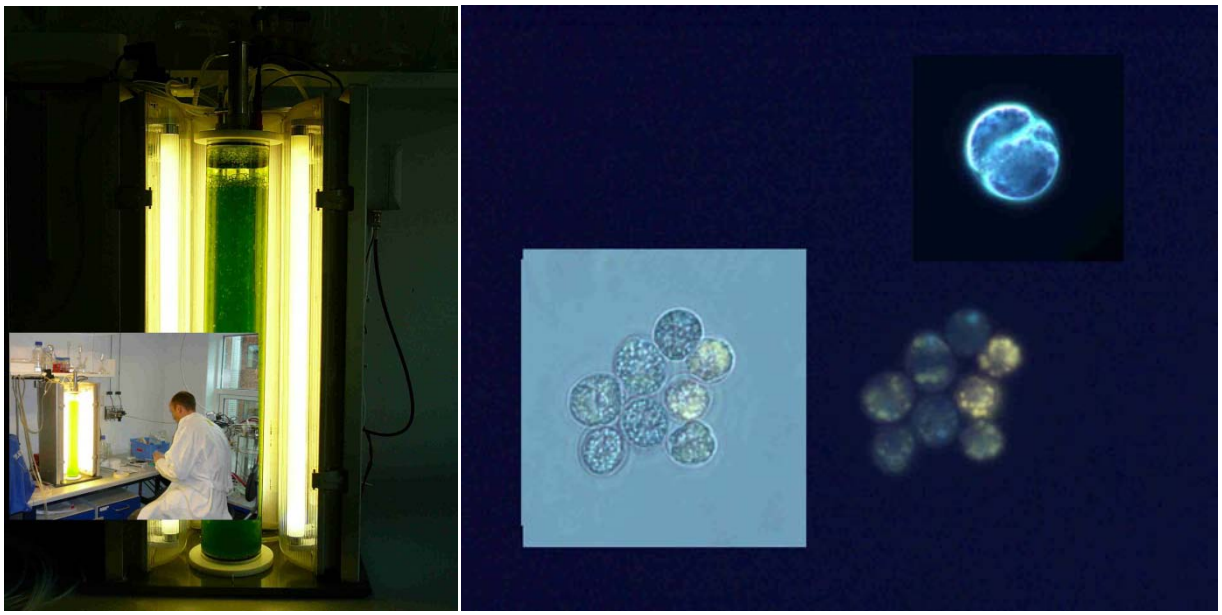

Fig. 1. Left. Photo-bioreactor designed for continuous production of phototrophic feed algae during initial optimisation tests using *Chlorella vulgaris*. Inset indicates scale (3 L). Right. Fluorescence micrograph of *Crypthecodinium cohnii* stained with Nile Red. Yellow fluorescence is from Nile Red dissolved in intracellular lipid droplets. Left inset shows same cells under bright field. Top inset shows cell under dark field.

Publications

Scientific articles (peer reviewed)

Eriksen NT (in press) Growth in photobioreactors, In Razeghifard R (ed) Natural and artificial photosynthesis. Pathways to clean, renewable energy. Wiley Publisher

Pleissner D, Eriksen NT (2012) Growth and biochemical composition of *Crypthecodinium cohnii* grown carbon-, nitrogen-, or phosphorous-limited in continuous flow culture. Biotechnology and Bioengineering 109: 2005-2016

Eriksen NT (2012). Heterotrophic microalgae in biotechnology, In Johansen MN (ed) Microalgae: Biotechnology, microbiology and energy. Nova Science Publishers, Inc. ISBN 987-1-61324-625-2, pp. 387-412.

Pleissner D, Wimmer R, Eriksen NT (2011) Quantification of amino acids in fermentation media by isocratic HPLC analysis of their α-hydroxy acid derivatives. Analytical Chemistry 83: 175-181.

Reports (PhD dissertations, M.Sc., Engineer, Bachelor)

Bjørn Lund Nielsen (2009) Analysis of the starch content in *Galdieria sulphuraria* - a potential feed in mussel farming. Graduation project (dip. ing.), Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University.

Daniel Pleissner (2012) Bio-production and bio-energetics: bioreactor production of microalgae and growth of filter-feeding bivalves. PhD dissertation, University of Southern Denmark.

Conference contributions

Eriksen NT (2012) Physiology of phototrophic and autotrophic microalgal cultures under nutrient-limited conditions. Algae Workshop, Ås, Norway 15-16/11 2012

Borch MM, Eriksen NT, Bjerre AB (2011) Development and screening of sustainable algae fermentation medium. 4th Congress of the International Society for Applied Phycology, Halifax, Canada 19-24/6 2011

Pleissner D, Eriksen NT (2011) Derivatization of amino acids to corresponding α-hydroxy acids and their analysis in fermentation broths. 6th Danish Conference on Biotechnology and Molecular Biology. Synthetic biology and cell factories, Vejle, Denmark 26-27/5 2011

Eriksen NT (2010) Heterotrophic microalgal cultures and the synthesis of pigments of poly-unsaturated fatty acids. Workshop on algal biotechnology and cultivation - a Nordic perspective. Gothenburg, Sweden 25/11 2010

Pleissner D, Eriksen NT (2010) Production and control of biochemical composition of the heterotrophic dinoflagellate *Crypthecodinium cohnii* in continuous cultures. Workshop on algal biotechnology and cultivation - a Nordic perspective. Gothenburg, Sweden 25/11 2010

Eriksen NT, Pleissner D, Isleten M (2010) Pigment and lipid synthesis in heterotrophic microalgal cultures. Vth International Bioengineering Congress, Izmir, Turkey 16-19/6 2010

Pleissner D, Eriksen NT (2010) Continuous culture of the heterotrophic dinoflagellate *Crypthecodinium cohnii*. 8th European Workshop Biotechnology of Microalgae, Potsdam, Germany 7-10/6 2010

WP2: Growth and bioenergetics of filter-feeding mussels

Responsible
Professor Hans Ulrik Riisgård, Marine Biological Research Centre, University of Southern Denmark

Aims
The overall aim of WP2 is to provide the necessary growth energetic information that is needed in WP3 for construction of an easy-to-use production model and objectives are to study growth and bioenergetics in mussels living on diets of heterotrophic 'feed-algae' and to establish relationships between feed availability and growth in blue mussels of all sizes in the laboratory and in the field.

Scientific personnel
- Professor Hans Ulrik Riisgård (HUR) participates in research activities and is acting as supervisor for a number of Ph.D. and master students involved in this project.
- Associate Professor Niels T. Eriksen (NTE), AAU participates in supervision and research activities.
- Daniel Pleissner (DP) started his 3-year PhD-project on April 1, 2009. Daniel Pleissner will conduct approx. 18 months of research on feeding and bioenergetics in blue mussels at Marine Biological Research Centre (SDU), Kerteminde while the remaining time of his PhD project is allocated to WP2.
- Research assistant Kim Lundgreen, SDU works full-time on MarBioShell, carrying out all-round work in lab and field, and he assists the scientific leader Hans Ulrik Riisgård in the administration of MarBioShell.
- Inma Martín Arnan from University of Murcia, Spain has worked for 6 months in 2008-2009 within MarBioShell as a Leonardo da Vinci grant trainee.
- Ana Orts Pérez from University Miguel Hernandez of Elche, Alicante, Spain worked as a Leonardo da Vinci grant trainee for 6 month from August 2009 to January 2010.
- Isabel Barreiro Saavedra from University Alcalá de Henares, Madrid, Spain worked as a Leonardo da Vinci grant trainee from January 2010 to July 2010. She was afterwards employed on the project for 4 month to work as a research assistant from September 2010 to December 2010.
- Sandrine Serre from National Agronomic Engineering Post Graduate School Toulouse, France worked within the MarBioShell project as a research assistant for 4 month, from September to December 2010.
- Line Bøttiger, master student studied the effect of salinity on growth of mussels. Graduated (M.Sc.) in March 2011.
- Parnuna Egede, master student studied food uptake and growth of blue mussels. Graduated (M.Sc.) in September 2010.
- Gorm Rønved Larsen, M.Sc. worked 2 months (October and November 2010) as a consultant obtaining and treating environmental monitoring data on phytoplankton biomass in Great Bet and Limfjorden for the last 20 years.
- Lærke Arentoft Johansen, bachelor student who worked with outreach within the project to different target groups. Project finished September 2010.
- Sabrina Maria Nothlev Sørensen, bachelor student who worked with outreach within the project to different target groups. Project finished September 2010.
- Jakob Sten Knudsen, bachelor student studied the effects of extremely high algal concentrations on filtration rate and digestion in blue mussels. Project finished in November 2011.
- Maria Àngeles Provencio López from University of Murcia, Spain, is currently working as a Leonardo da Vinci grant trainee from November 2011 until May 2012.

- Florian Lüskow, B.Sc. from University of Bremen, Germany, will be working on effects of salinity-changing rates on feeding behaviour of blue mussels in the period March to June 2012.

Activities (Jan 2008 – Dec 2012)

- Investigations of growth and bioenergetics in blue mussels fed phototrophic *Rhodomonas* sp. at Marine Biological Research Centre, Kerteminde (Fig. 2).
- Investigations of growth and bioenergetics in blue mussels fed heterotrophic *Galdieria sulphuraria* during spring 2009 at Marine Biological Research Centre, Kerteminde (Fig. 2).
- Design and test of experimental set-up for automatic control of algal concentration and continuous detection of filtering activity and feeding rates in blue mussels at Marine Biological Research Centre, Kerteminde (Fig. 2).
- Feeding and growth experiments using phototrophic *Rhodomonas* sp. and heterotrophic *Galdieria sulphuraria* have been carried out at the Marine Biological Research Centre, Kerteminde from mid-2008 to November 2010 (Fig. 2).
- Two students (Lasse Tellerup Hansen & Michala Karlsen Møller, Tek-Fak-SDU KBM) finished their projects on blue mussels (7. semester afgangsprojekt) by the end of 2008.
- Master student Parnuna Egede, SDU, has carried out growth experiments for determination of satiation degree in blue mussels fed different concentrations of phototrophic *Rhodomonas* sp.
- Kim Lundgreen has together with Mads van Deurs, Nordshell company partner within the MarBioShell project, and several students carried out field growth experiments with mussels suspended in net-bags at different locations in the Great Belt and Limfjorden (reference area) during 2008, and 2010 (Fig. 4 and Fig. 5).
- A survey of mussel larvae density has been conducted at 4 sites (Svendborg, Musholm, Horsens, Kerteminde) during 2008, and in Kerteminde Bugt during spring and summer of 2009 and 2010.
- Kim Lundgreen and others have together with Bo Hoffmann Jørgensen and Francesca Storti, DTU-Aqua, carried out particle image velocimetry (PIV) measurements for determination of the exhalant jet velocity of mussels to be used in future computational modelling (Fig. 3).
- During the summer and autumn of 2011 data from field growth experiments in the Great Belt and Limfjorden from 2009 and 2010/2011 was analyzed and presented in two manuscripts that were submitted in the autumn 2011 (Fig. 4 and Fig. 5).
- Growth of small mussels from settling on farm-ropes in late spring of 2011 throughout the season until December 2011 was followed to determine growth rate of juvenile mussels (<10 mm) and to determine the growth potential of a whole season (Fig. 5 and Fig. 6).
- Investigations of growth and bioenergetics in blue mussels fed heterotrophic *Crypthecodinium cohnii* during spring 2011 at Marine Biological Research Centre, Kerteminde.
- Short term growth experiments were conducted in Oct and Nov 2011 to determine growth of mussels in relation to chl *a* concentrations and possible influence of current speeds measured simultaneously with equipment set out by DHI on location.
- In autumn 2011 and spring 2012 investigation of growth and bioenergetics in the laboratory with mussels fed with *Rhodomonas* at different concentrations (to simulate different chl *a* concentrations) was continued as part of earlier experiments with the aim to cover growth rate of mussels exposed to low and up to very high algal concentrations.
- For high concentration laboratory growth experiments a fluorometer controlled apparatus was used and a computer program was specifically designed in spring 2011 to be able maintain high algal concentration through feedback regulation.
- Nov 2010 - Dec 2011: Growth and bioenergetics using phototrophic microalgae (see WP1) as feed for blue mussels (KL, DP).

- Nov 2010 - Oct 2011: Growth and bioenergetics using heterotrophic ‘feed-algae’ of different species and with predetermined biochemical composition (see WP1) as feed for blue mussels of all sizes (DP, KL).
- Nov 2010 - Oct 2011: Characterisation of biochemical and fatty acid composition of blue mussels feeding on phototrophic or heterotrophic microalgae (DP).
- Importance of heterotrophic relative to phototrophic plankton organisms in blue mussel diets in the field has been studied (NTE, DP, HUR).
- May-Dec 2011: Field growth experiments with different size groups of mussels in net-bags at the MarBioShell mussel farm in the Great Belt and other sites in the Great Belt region have been carried out in order to measure the weight specific growth rate of different size groups of mussels (HUR, KL).
- Due to frequently changing salinities in the Great Belt the effect of salinity on growth of mussels has been studied in controlled laboratory feeding experiments (HUR, KL).
- 2011-2012: Based on combined laboratory and field studies on mussels, weight specific growth rates have been used to evaluate the production potential of line mussels in the Great Belt region.

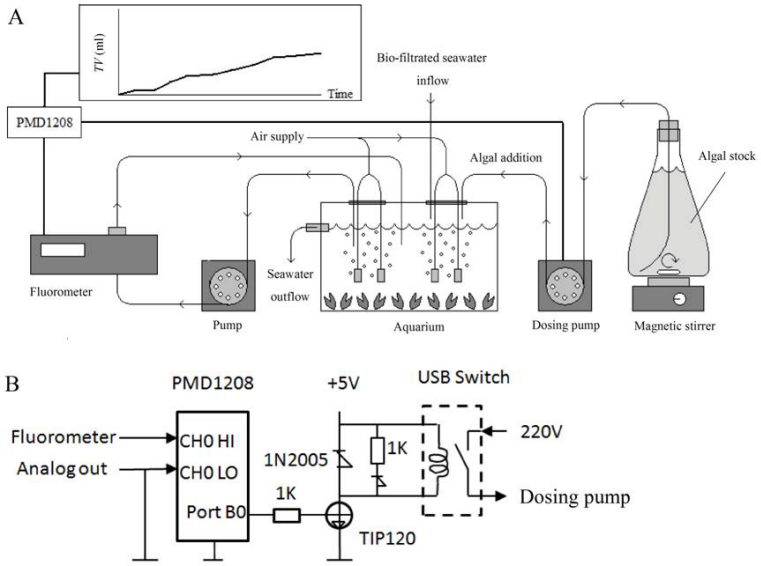

Fig. 2. Fluorometer-controlled apparatus for continuous measurement of filtration rate of mussels at constant algal concentration.

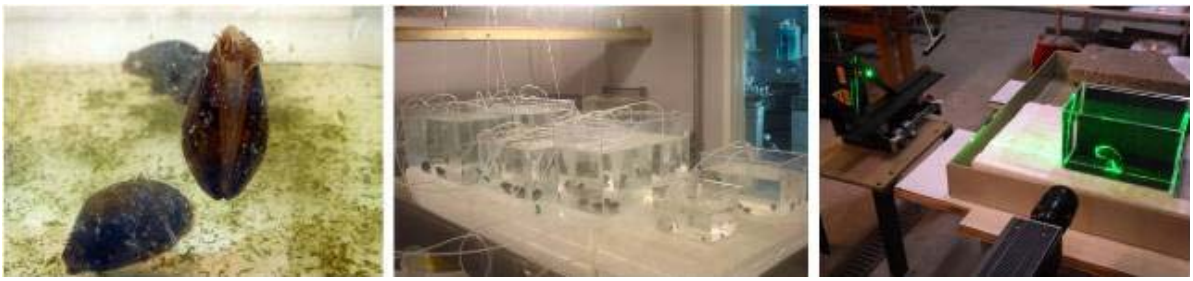

Fig. 3. Blue mussels (left), experimental set-up for mussels-growth experiments in the laboratory (middle), and set up for PIV-measurements in Kerteminde within WP2 in collaboration with WP3 & WP4.

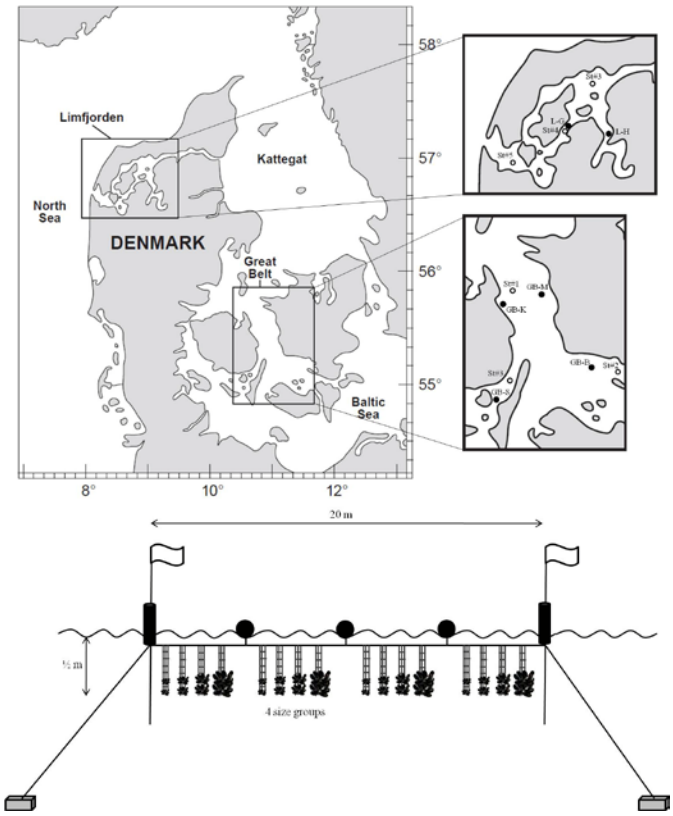

Fig. 4. Map of Denmark showing the locations for field growth experiments with *Mytilus edulis* hung up in net bags at different localities in Great Belt and in Limfjorden. Buoy-system with mussels in net-bags.

Fig. 5. Mussels in net-bags used for growth experiments at the research and demonstration farm.


Fig. 6. Line mussels (left) and 'Smart-Farm' nets (middle and right) in the MarBioShell research- and demonstration farm in Great Belt (Kerteminde Bay, Denmark).

Publications

Scientific articles (peer reviewed)

Riisgård HU, Jørgensen BH, Lundgreen K., Storti F, Walther JH, Meyer KE, Larsen PS (2011). The exhalant jet of mussels, *Mytilus edulis*. Mar. Ecol. Prog. Ser. Vol. 437:147-164

Riisgård HU, Egede PP, Saavedra IB (2011). Threshold algal concentrations for pseudofaeces production and incipient saturation reduction of filtration rate of mussels (*Mytilus edulis*). Journal of Marine Biology 2001:1-13 (Published online, DOI: 10.1155/2011/312459)

Larsen PS, Riisgård HU (2011). Validation of the flow-through chamber method (FTC) and steady-state method (SS) to measure clearance rates in mussels. Biology Open (Published online, DOI: 10.1242/bio.2011011, 1-6)

Riisgård HU, Lundgreen K, Larsen PS (2012). Field data and growth model for mussels *Mytilus edulis* in Danish waters. Marine Biology Research 8: 683-700

Riisgård HU, Bøttiger L, Pleissner D (2012). Effect of salinity on growth of mussels, *Mytilus edulis*, with special reference to Great Belt (Denmark). Open Journal of Marine Science 2012 (2):167-176

Riisgård HU**,** Pleissner D, Lundgreen K, Larsen PS (2012). Growth of mussels *Mytilus edulis* at algal (*Rhodomonas salina*) concentrations below and above saturation levels for reduced filtration rate. Marine Biology Research 00:1-13 (in press)

Pleissner D, Eriksen NT, Lundgreen K, Riisgård HU (2012). Biomass composition of blue mussels, *Mytilus edulis*, is affected by living site and species of ingested microalgae. ISRN Zoology (published online: Volume 2012, Article ID 902152, 12 pages; doi:10.5402/2012/902152)

Larsen PS, Lundgreen K, Riisgård HU (2012). Bioenergetic model predictions of actual growth and allometric transitions during ontogeny of juvenile blue mussels *Mytilus edulis*. In: Mussels: Ecology, Life Habits and Control. Nova Science Publishers, Inc. (in press)

Riisgård HU**,** Lundgreen K, Larsen PS (2012). A preliminary evaluation of potential for line-mussel production in the Great Belt (Denmark) based on actual growth of young mussels *Mytilus edulis*. (submitted)

Pleissner D, Lundgreen K, Riisgård HU (2012). A new fluorometer controlled apparatus designed for long-term algal-feeding experiments with mussels. (submitted)

Riisgård HU**,** Lüskow F, Pleissner D, Lundgreen K, López MAP (2012). Effect of salinity on filtration rates of mussels *Mytilus edulis* with special emphasis on dwarfed mussels from the low saline Central Baltic Sea. (submitted)

Reports (PhD dissertations, M.Sc., Engineer, Bachelor)

Lasse Tellerup Hansen (2008). Blåmuslingers potentiale ved oprensning af havmiljø. Graduation project (dip. ing.). Institute of Chemical Engineering, Biotechnology and Environmental Technology, Technical Faculty, University of Southern Denmark.

Michala Karlsen Møller (20 08). Anvendelse af blåmuslingen, *Mytilus edulis*, i foder. Graduation project (dip. ing.). Institute of Chemical Engineering, Biotechnology and Environmental Technology, Technical Faculty, University of Southern Denmark.

Annette Høyvald (2009). Fedtsyrer i muslinger. Graduation project (dip. ing.)). Institute of Chemical Engineering, Biotechnology and Environmental Technology, Technical Faculty, University of Southern Denmark.

Daniel Pleissner (2009). Chemostat-aquarium-system (CAS) for long term investigation of mussel filtration and growth. Individual Ph.D. study activity report, August 2009. Institute of Biology, University of Southern Denmark.

Lærke Arentoft Johansen (2010). "Faglig formidling i praksis – Formidling af MarBioShell-projektet til tre målgrupper." Bachelor project finished September 2010. Institute of Biology, University of Southern Denmark.

Sabrina Maria Nothlev Sørensen (2010). "Faglig formidling i praksis – Formidling af MarBioShell-projektet til tre målgrupper." Bachelor project finished September 2010. Institute of Biology, University of Southern Denmark.

Parnuma P. Egede (2010). Food uptake and growth of blue mussels (*Mytilus edulis*) - a combined laboratory and field study. M.Sc. thesis finished September 2010. Institute of Biology, University of Southern Denmark.

Line Bøttiger (2011). Growth of blue mussels, *Mytilus edulis*, and effects of salinity. M.Sc. thesis finished March 2011. Institute of Biology, University of Southern Denmark.

Jakob Sten Knudsen (2011). Fødeoptagelse og vækst under høje koncentrationer af alger hos blåmuslingen, *Mytilus edulis* - fysiologisk regulering af filtrationsraten versus overbelastning af fordøjelsessystemet. Bachelor project finished November 2011. Institute of Biology, University of Southern Denmark.

Daniel Pleissner (2012) Bio-production and bio-energetics: bioreactor production of microalgae and growth of filter-feeding bivalves. PhD dissertation, University of Southern Denmark.

WP3: Mussel farm models

Responsible
Head of Innovation Flemming Møhlenberg, DHI & Associate Professor Jens Honoré Walther, DTU

Aims
The overall aim of the research in WP3 is to develop easy-to-use tools that can aid planning and design of offshore mussel farms. The objectives and activities to achieve this are as follows:

- Develop concepts, methods and ultra-fine scale models for description of the flow around individual mussels and smaller clumps of mussels
- Develop methods and fine scale models for description of the flow around mussel growth lines and nets established in a farm under varying current and wave conditions
- Upscale ultra- and fine-scale models by parameterization of horizontal dispersion and mixing and implement refined mussel growth formulations (from WP2) in meso-scale models to be integrated seamless with coupled larger-scale hydrodynamic-ecological models
- Calibrate and verify mussel farm models using dedicated lab and field campaigns

Scientific personnel

- Head of Innovation Flemming Møhlenberg, DHI
- Head of Innovation Erik Damgaard Christensen, DHI
- Lars Yde, DHI
- Mads Joakim Birkeland, DHI
- Johannes Tophøj Rasmussen, DTU
- Francesca Storti, DTU (PhD-student: Sept 2009 - April 2010)
- Bo Hoffmann Jørgensen, DTU (Post Doc)
- Associate Professor Jens Honoré Walther, DTU

Activities (Jan 2008 – Dec 2012)

- Meso-scale 2-dimensional models (1000 × 500 m) have been established to model current speed and transports of algae into a section of a mussel farm. Mussel growth lines (‘droppers’) are implemented as “piers” where the diameter and flow resistance can be varied by the user. Spatial resolution (grid cell sizes) has been varied between 1 m and 0.25 m. Mussel growth is implemented using a “simple” saturation-function between algal carbon concentration and net growth rate that further are scaled to shell length using an allometric function. Grazing loss of phytoplankton is implemented by multiplying growth rate by 3-4 assuming gross growth efficiency between 20 and 33%. The range in resistance and dispersion has been set using a rather comprehensive Norwegian data set (Strohmeier et al. 2005; raw data submitted), but neither current speed (< 5 cm/s) nor chl *a* (1-3 µg/l) are representative for the Great Belt.
- Through an external grant (€ 34.000) from the European Fisheries Fund) the current model set-up has been used in 2008 to predict the potential for mussel production near to three Danish fish farms. Although the model still needs to be improved (see above) we believe that even in its current state this tool is superior to most other tools in use.
- During winter and spring 2009 the 2-D model has been transferred to 3-D models allowing for modelling of mussel farms in stratified waters, characterized by 2-way flows, and depth gradients in flow speed and phytoplankton. Models have tested thoroughly for mass conservation and numerical stability, and at present the model tool appears robust.

- An external FTP project "Multiscale Simulations Using Particle Vortex Methods with Application to Bluff Body Flow" (FTP grant 274-08-0258) has allowed us to develop a large scale three-dimensional detailed model of the flow in complex geometries, including flows in biological systems. The goal of the FTP project is to study the aerodynamic stability of long suspension bridges, but we have used this opportunity to perform validation of the software on biological relevant flows - here the flow through a mussel (Fig. 7).
- Detailed measurements of the flow rate and the exhalent jet from mussels have been performed (Fig. 8 and Fig. 9). The measurements were conducted at the Marine Biological Research Center (SDU) in Kerteminde. The flow measurements were used to provide boundary conditions for ultra-fine scale models. Preliminary simulations have been conducted and compare with the measurements, and the work has been published.
- Azur Hodzic and Peter Johatan Jensen, bachelor students studied elliptic and triangular jets in a laboratory experiment at DTU MEK. Project finished in December 2011.
- In 2012, using measured hydrographical and realised mussel growth data from WP7, the fine-scale model initially applied was simplified to allow for model simulation of one growth season in less than 24 h. Hence, despite simplifications the farm model meets the requirements of a fast and reliable Decision Support Tool for assessing the production capacity of a given mussel farm. In the Kerteminde model-setup the farm is 240 m long, 48 m wide and 8 m deep. The model elements each measured 4×2×2 m. The production lines in the model are represented with piers, with a diameter of 20 cm, extending from the surface to the seabed. Piers are introduced to represent local resistance to flow in accordance to measured reduction of flow during field campaign in 2010 (ca 23% flow reduction). The piers are placed in 8 rows representing the long-lines in the mussel farm. The long-lines are separated by 5 m (Fig. 10). The model is executed for the period from June-December 2012.
- HD modelling: A detailed provides forcing data to describe currents, water level, salinity, temperature at the model boundaries. The model is calibrated with model data from 2005 and validated with measurements from 2011 (see Fig. 11).
- Mussel model: Mussels as a cohort are introduced in an area of 104 × 48 m with 8 long-lines 208 drop-lines in total. Food consists of phytoplankton (entire fraction available), while only a fraction of zooplankton and detritus are available. The food items are introduced at the southern and northern boundary and the food concentration within the farm area is controlled by the current patterns between piers and mussel consumption of food. The mussel farm model simulates the production potential in the different sections (horizontal and vertical) of the farm. The mussel model describes the horizontal and vertical differences in production capacity in the mussel farm together with the potential for the entire farm. Fig. 12 presents examples of the model results.

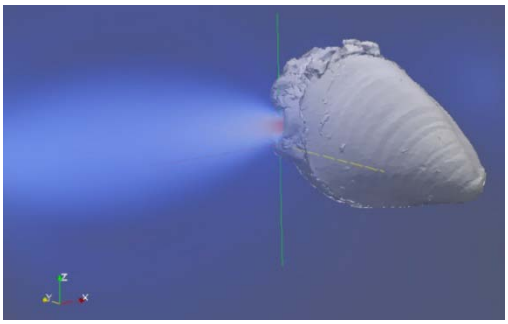

Fig. 7. Numerical simulation of the exhalant flow from a mussel. The simulations were performed using an in-house 3D vortex method code. The picture shows the flow speed. From report by Florian Willerval and Charles Thouny made during their visit to DTU MEK.

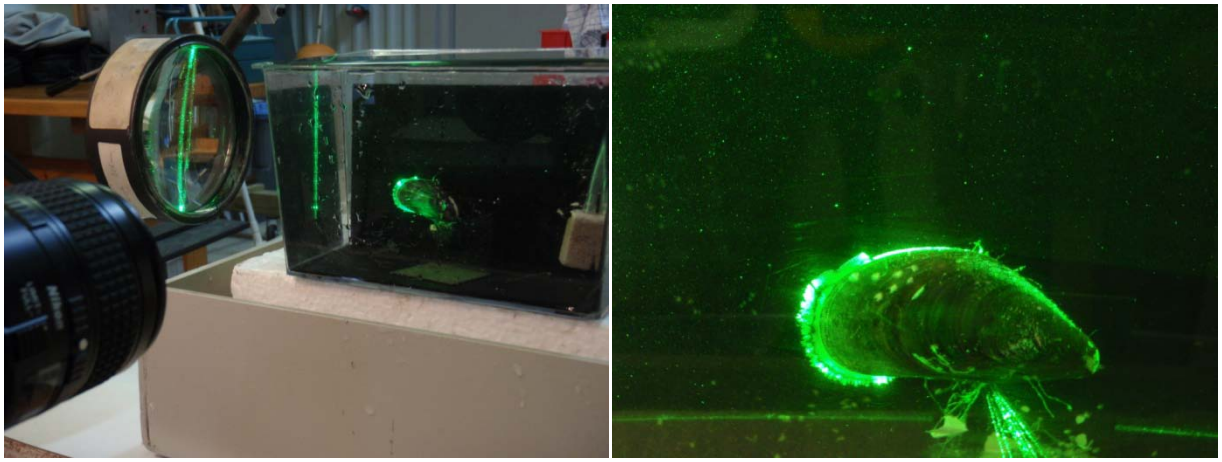

Fig. 8. PIV experiments where a laser, a camera and computer software was used to estimate the velocity of the jet stream from the exhalent siphon of a blue mussel.

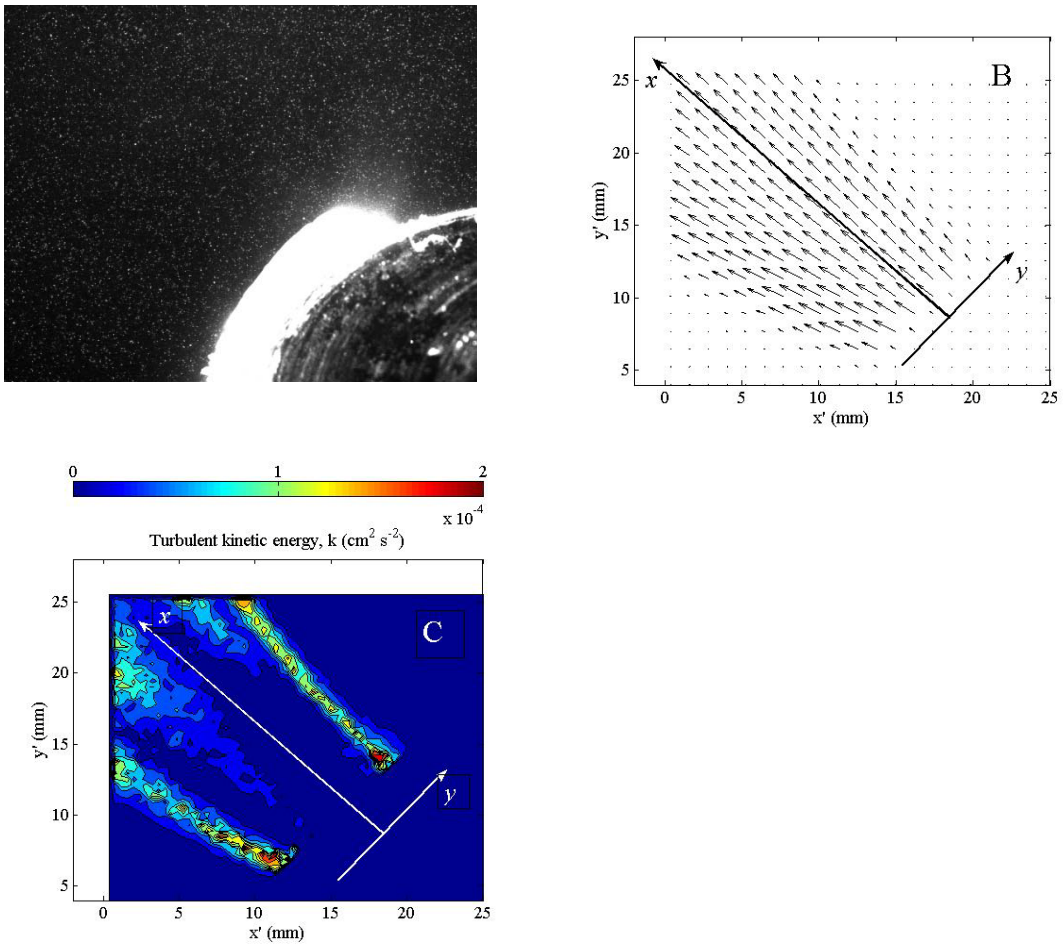

Fig. 9. *Mytilus edulis*. (A) PIV image of captured field showing seeding particles in the jet flow, the exhalant siphon and part of the shell to the lower right. (B) Averaged vector field of the exhalant jet. (C) Contour plot of turbulent kinetic energy showing jet spreading by shear layers.

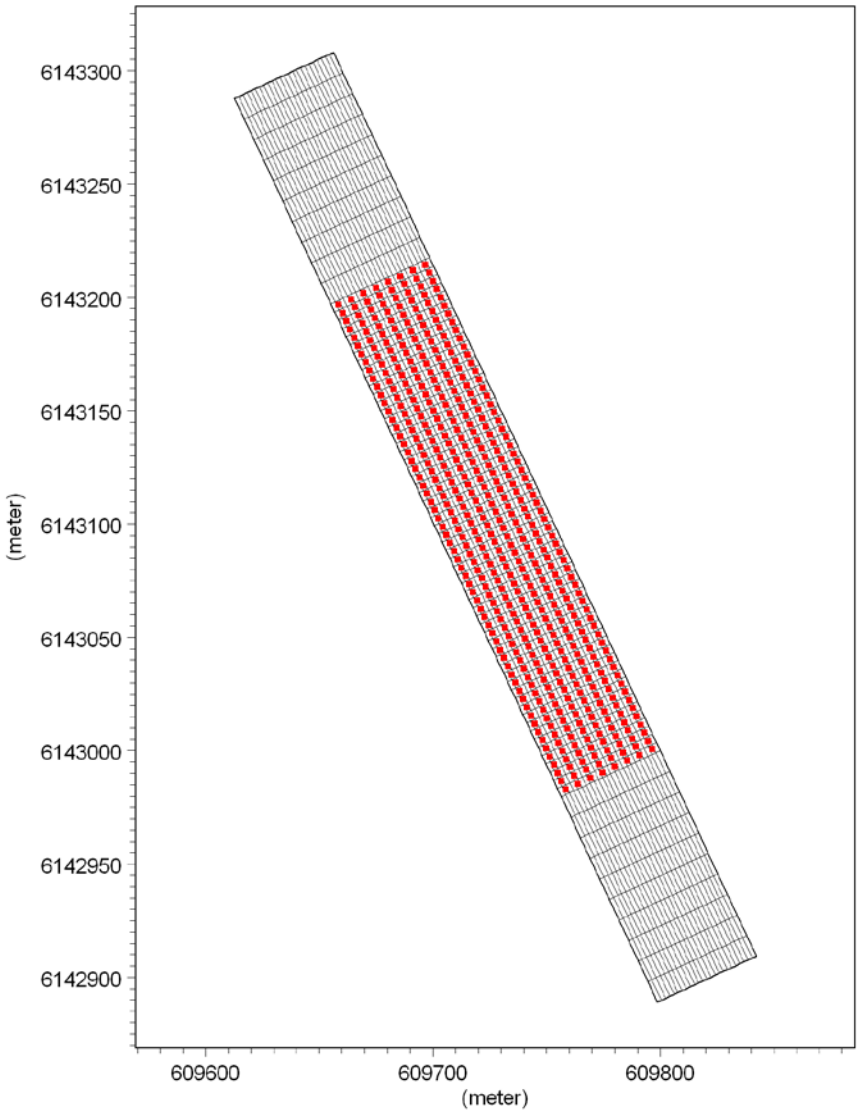

Fig. 10. The applied model mesh and pier representation.

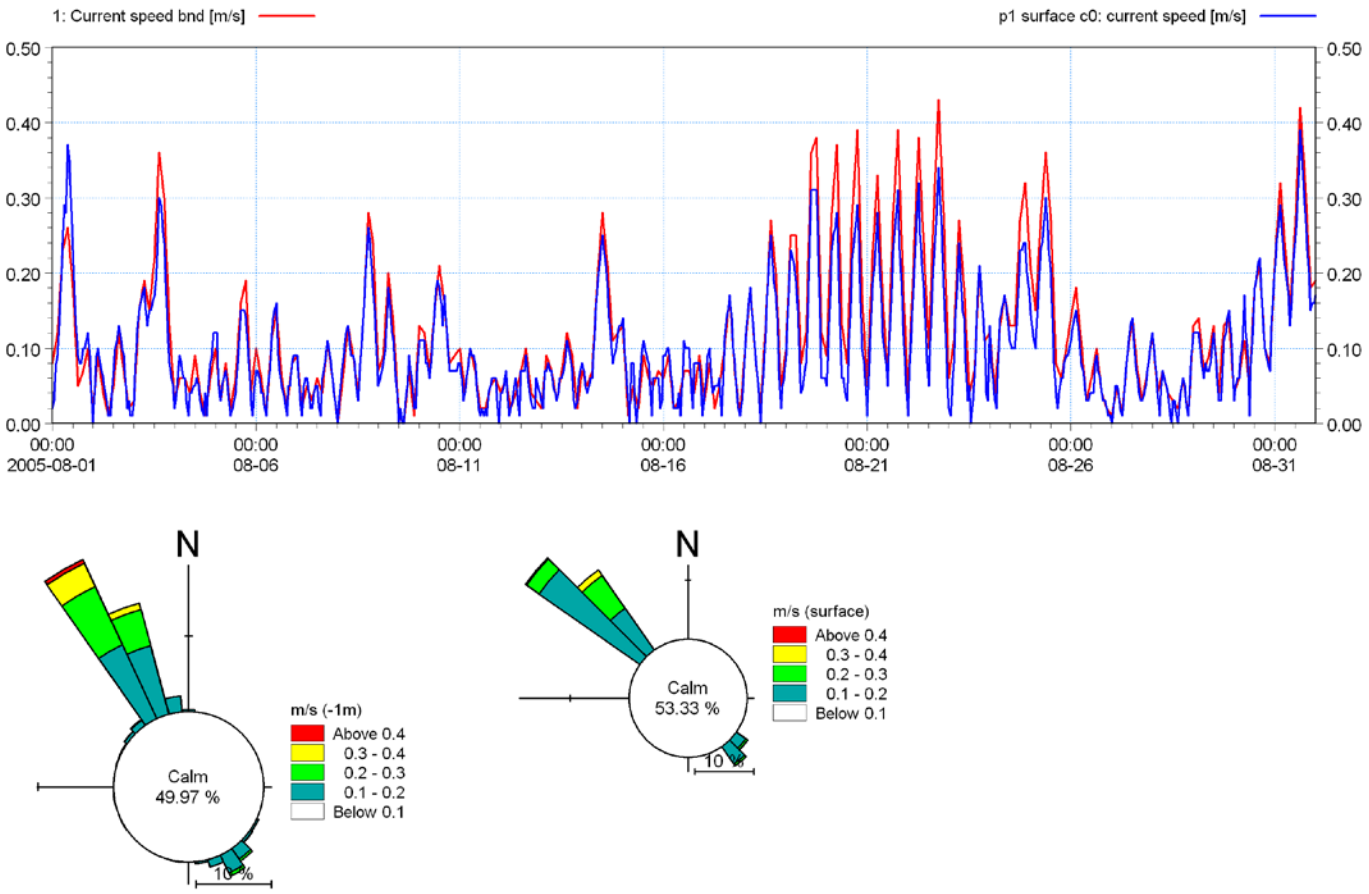

Fig. 11. Modelling of current speed and direction.

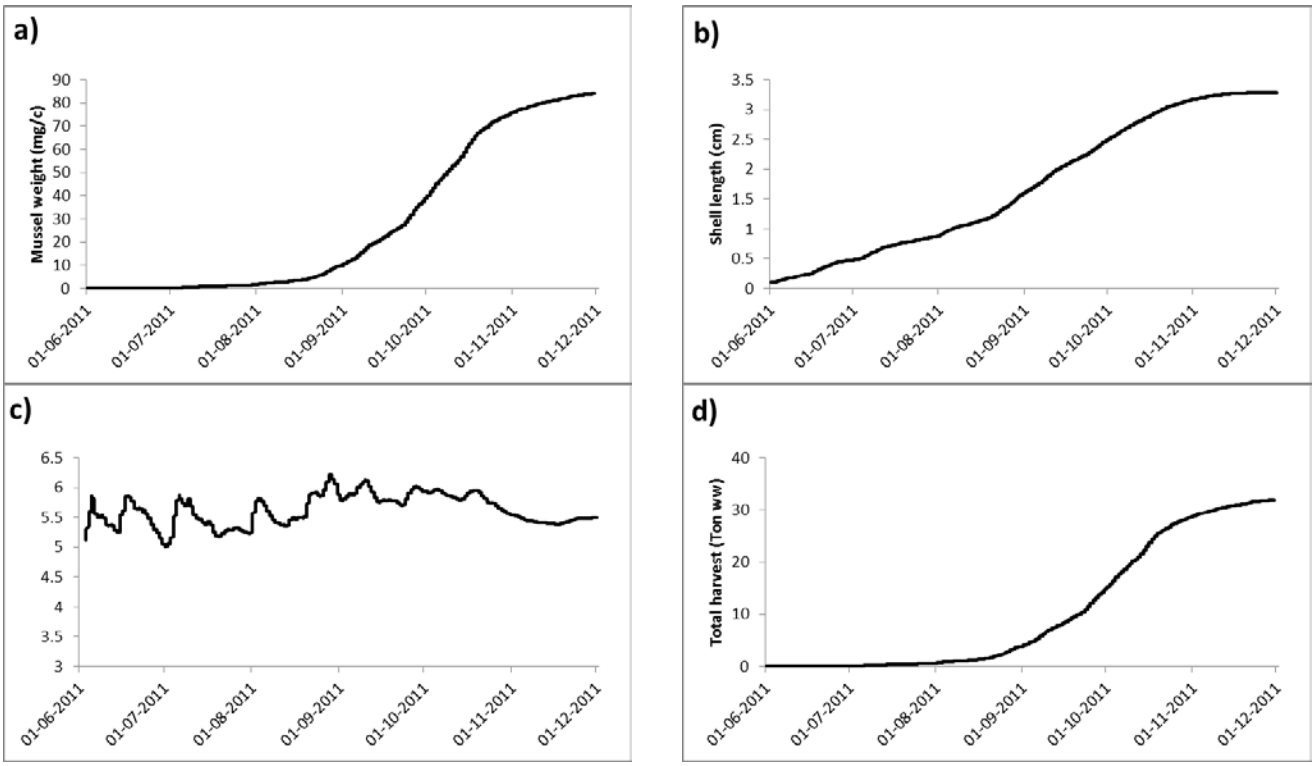

Fig. 12. Modelled weight (a), shell length (b) and condition index (c) of an average mussel in the model farm as a function of time. d) depicts the development of the total harvestable mussel biomass in the farm.

Publications

Scientific articles (peer reviewed)

Riisgård HU, Jørgensen BH, Lundgreen K, Storti F, Walther JH, Meyer KE, Larsen PS (2011). The exhalant jet of mussels (*Mytilus edulis*). Marine Ecology Progress Series 437: 147-164

Reports (PhD dissertations, M.Sc., Engineer, Bachelor)

Thouny C, Willerval F (2010). Numerical simulation of the exhalent jet flow from the blue mussel *Mytilus edulis*. Student report, Technical University of Denmark.

Poulsen M (2009). Undersøgelse af rentable muligheder for dyrkning og høst af muslinger dyrket off-shore: Et casestudie. M.Sc. thesis, University of Copenhagen.

Hodzic A, Jensen PJ (2011). Måling på model af strømning ud af en musling. B.Sc. Thesis, Technical University of Denmark.

Fourteu, CN, Pellerin, T, (2012). Instability of jets with a non-circular cross section. Student report, Technical University of Denmark.

WP4: Mussel farm design and harvest

Responsible
Head of Innovation Flemming Møhlenberg, DHI Water-Environment-Health, Hørsholm

Aims
The overall aim is to develop settling lines and production ropes that prevent crowding of mussels and reduce loss of production. A related aim is to test various methods in accordance with the selected designs.

Scientific personnel
- Flemming Møhlenberg, DHI
- Mads Joakim Birkeland, DHI

Activities (Jan 2008 – Dec 2012)
A research- and demonstration line-mussel farm was established in the southern part of Kerteminde Bay, close to the Great Belt in spring 2010 (Fig. 13). The aim of the establishment of the line-mussel farm in 2010 was to test various methods and achieve valuable experience to be implemented in the farm design prior to the activities in 2011-2012. Further, two ADCPs was deployed at the bottom within the farm for quantifying depth gradients of flows inside and outside of mussel farm. The farm was slightly modified in 2011 with fewer long-lines and drop-lines consisting of bands of trawl-net that proved most successful in 2010 trial. During the field campaign in 2011 ADCP was supplemented with self-recording CTD and a fluorometer deployed 20 m east of the farm to provide continuous recording of food availability.

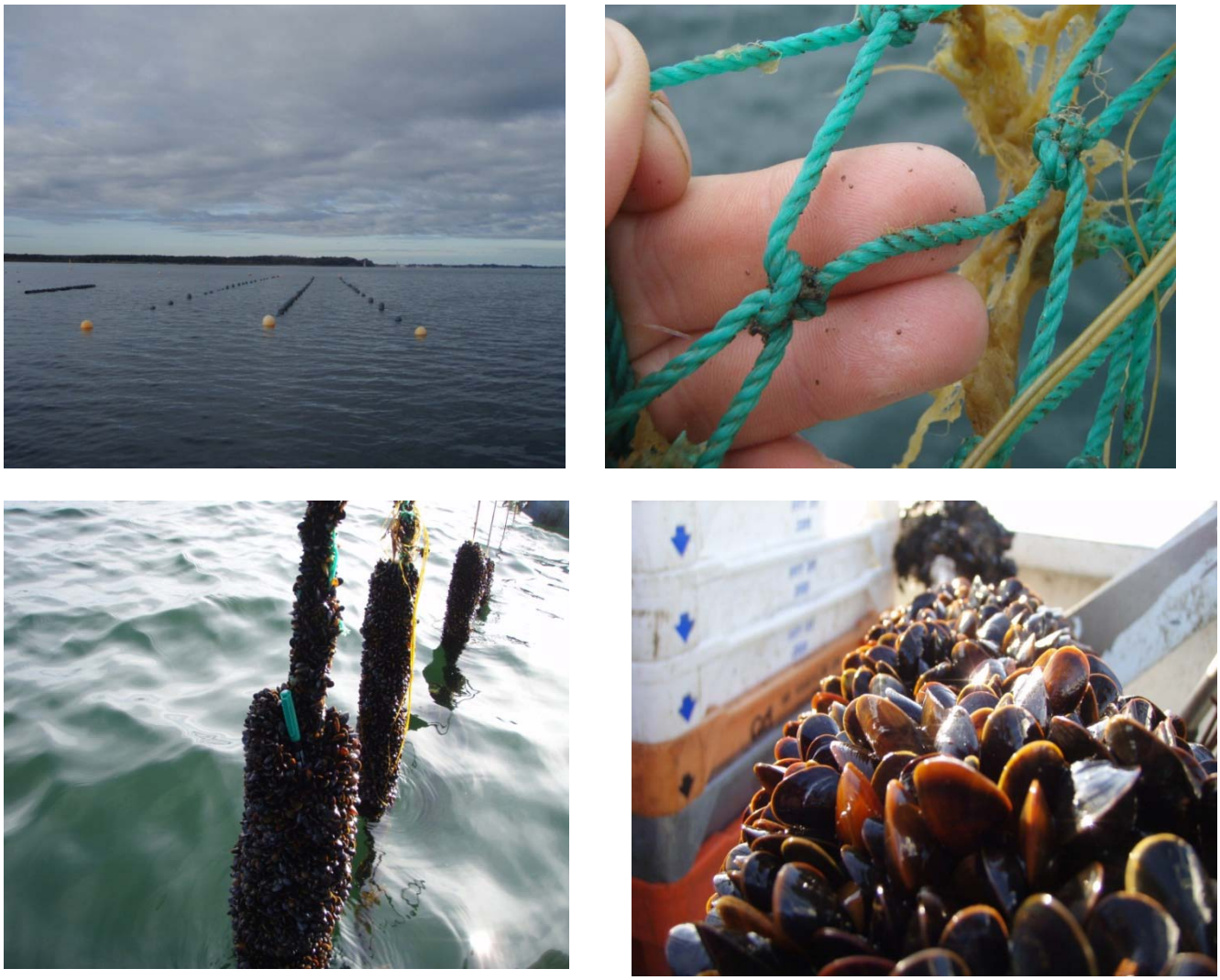

Fig. 13. Pictures from the MarBioShell research- and demonstration line-mussel farm in the southern part of Kerteminde Bay (Great Belt). From the early stages of farm establishment, settlement of larvae to high density lines and harvested mussels.

Publications

Scientific articles (peer reviewed)

Møhlenberg F, Birkeland M et al. (2013). Capacity for growing mussels in the Great Belt, Denmark – two years field study supported by numerical modelling (in prep., to be submitted to Aquaculture in March 2013)

WP5: Market analysis

Responsible
Eva Roth, Associate Professor, Department of Environmental and Business Economics, SDU, Esbjerg

Aims
The group is responsible for carrying out part of the socio-economic research of the project. The aim falls within 3 integrated studies:
1. Market analyses, what are the market structure, consumer preference and demand in the EU for blue mussel?
2. Ascertain mussel farmer's risk perception and risk management strategies.
3. Model the real economic risk of HAPs, E-coli and oxygen depletion incidents for mussel aquaculture (in cooperation with the SPICOSA project).

Scientific personnel
- Associate Professor Eva Roth.
- Professor Hans Stubbe Solgaard
- PhD student Dewan Ali Ahsan
- PhD student Thong Tien Nguyen
- Post.Doc, PhD Lars Ravn Jonsen

Activities (Jan 2008 - Dec 2012)
The activities of the working package including four research activities:
- February 2009-December 2009:
    - Nguyen Tien Thong (PhD student) conducted interview with mussel producers and scientists in Limfjord area and DTU about the mussels production and market;
    - Nguyen Tien Thong travelled to the Netherland for investigating the auction market activities, discussing with consultant and producers about mussels markets and productions, and collecting data.
    - Analysing secondary data to investigate the consumer preference for mussels characteristics;
- 2010:
    - Collecting data of mussel production in European countries;
    - Writing articles on implicit price of mussels in auction market;
    - Constructing model of consumer demand for mussel in European countries;
    - Visiting French markets, presenting research results in conference, and group interview of consumers about mussels consumption;
    - Preparing questionnaire and choice experiments for seafood consumption study;
- 2011:
    - Collecting data on seafood consumption in French markets to investigate the consumer preference and demand for mussel at individual level;
    - Analysing data and writing articles

Publications
Scientific articles (peer reviewed)
Ahsan D, Roth E (2010). Farmers' risk perception and risk management strategies in an emerging mussel aquaculture industry in Denmark. Marine Resource Economics 25:309-323.

Thong NT (2011). Implicit price of mussels characteristics in auction market. Journal of Aquaculture International (doi: 10.1007/s10499-011-9489-x).

Dinesen GE, Timmermann K, Roth E, Markager S, Ravn-Jonsen L, Hjorth M, Holmer M, Støttrup JG. (2012). Mussel production and WFD targets in the Limfjord, Denmark: an integrated assessment for use in system-based management. Ecology & Society (submitted)

Thong NT (2012). Inverse almost ideal demand system for mussels in the European countries. (Submitted)

Thong NT, Wolfgang H, Solgaard HS, Roth E (2012). Demand structure and segmentation of seafood: stated choice approach. (In prep.)

Thong NT, Wolfgang H, Solgaard HS, Roth E, Lars RJ (2012). Valuing seafood attributes: Stated choice model. (In prep.)

Non-peer reviewed articles
Støttrup JG, Dinesen GE, Timmermann K, Markager S, Roth E, Ravn-Jonsen L. 2010. Integrated assessment for use in system based management: WFD nutrient targets and mussel production in the Limfjord, Denmark. ICES CM2010/B:09: 1-14. [Printed online September 2010]

Reports (PhD dissertations, M.Sc., Engineer, Bachelor)
Ahsan Dewan (2011) Risk perception and Risk Management Strategies among Aquaculture Farmers. PhD dissertation, University of Southern Denmark.

Conference contributions
Posters
Dinesen GE, Ahsan D, Dolmer P, Holmer, Hjort M, Jarlbæk H, Hoffman E, Markager S, Roth E, Sverdrup-Jensen S, Petersen JK, Timmermann K, Støttrup JG. 2010. The Limfjord, Denmark. SPICOSA study site 5. [Presented at the SPICOSA SAF meeting, Istanbul 2010]

Dinesen GE, Timmermann K, Markager S, Roth E, Ravn-Jonsen L, Hjort M, Petersen JK, Holmer, M, Støttrup JG. 2010. The Limfjord, Denmark. SPICOSA study site 5. [Presented at the Littoral Conference, London 2010]

Dinesen GE, Timmermann K, Markager S, Roth E, Ravn-Jonsen L, Hjort M, Holmer M, Støttrup JG. 2010. The Limfjord, Denmark. SPICOSA study site 5. [Presented at the SPICOSA SAF meeting, Malta 2010]

WP6: Networks, branding and regional development

Responsible

Professor Flemming Just, Professor, Head of Department, Department of Environmental and Business Economics, University of Southern Denmark, was responsible until he left his position in mid-2011. In March 2012, Professor Anne-Mette Hjalager, Danish Centre for Rural Research (CLF), University of Southern Denmark, became responsible for WP6.

Aims

To contribute to the development of organization, brands and innovation system for multifunctional off shore mussel production in Denmark, WP6 will in cooperation with business partners and research (MarBioShell WPs) use the suggested research- and demonstration mussel farm at Kerteminde to investigate and organize a multifunctional mussel production that is indented to be integrated with tourism and experience-economy in the area (tours, gastronomy, etc.). There is a need for organizing cooperation with the municipality, authorities, business partners and researchers, organization of a local company (cooperative or similar), development of business plans, cooperation with retail and consumers, and the tourism industry etc. The specific project enables the development of models for establishment of multifunctional off-shore mussel production on a national scale by collection of experiences from line-mussel production in Limfjorden, Denmark and development strategies to be applied specifically in the Wadden Sea, Denmark.

Research questions: How to create an economically sustainable off-shore mussel production? How to create demand for mussels and associated experience economy? How to organize businesses along the value chain and across sectors?

Scientific personnel

- Flemming Just, Professor, IME, University of Southern Denmark. WP responsible and engaged in network and innovation studies and regional policy. From 2008 to mid-2011.
- Bodil Stilling Blichfeldt, Associate Professor, TIC, University of Southern Denmark University. Engaged in branding analysis and marketing strategy. From 2008 to February 2011.
- Klaus Lindegaard, Associate Professor, CLF, University of Southern Denmark. Engaged in organization and innovation analysis and regional policy. From 2008 to February 2011.
- Anne-Mette Hjalager, Professor, CLF, University of Southern Denmark. New responsible for WP6 from March 2012 to present.

Activities (Jan 2008 - Dec 2012)

Because all participants had left the project by mid-2011, the remaining part of WP6 was allocated to Professor Anne-Mette Hjalager in March 2012. The aim of the slightly revised WP6 will provide prospective knowledge contributions in terms of networks, branding and regional economic development.

MarBioShell is embedded in a local, regional and national aquaculture community and in the experience economy set-up, and the project enjoys relations with a number of actors in these fields. As the category of mussels produced and their attributes deviate significantly from existing species and types on the market, there is essential to establish supportive narratives and to disseminate information to relevant stakeholders. The research in WP6 has addressed the role of museums and science centres as mediating the information flows on mussels across scientific, business and entertainment boundaries. An experiment was undertaken by a Danish marine science centre, Fjord & Belt. It was found that mussels represent a gripping topic of interpretation with many aspects, mussels as food items included. However, guests are generally very conservative in their feedback.

The benefits of involving and observing guests lie in the phenomenological insight into the social contexts of food, particularly in the relationships between children and their parents. This research illustrates the potentials of interpretative elements in more comprehensive regional line-mussel production and branding initiatives in the region both the science centre and wider, and it suggests that further advanced phenomenological studies can be informative for all actors in the regional mussels which includes also regional business actors and university partners.

In addition, WP 6 has approached the innovation and branding issues from a lead user angle. A lead user experiment has been performed in the autumn 2012 which involved eight experts from different professional domains in the development of recipes with blue mussels. In addition the experiment aimed at extracting narratives about the mussels that might be used to propagate this particular food item in a cultural and regional context. Throughout the experiment the lead users succeeded in widening the interpretation of mussels, and they address sensory, technological, cultural, scientific, nutritional and environmental aspects and interesting combinations hereof. They delivered useful inputs for the marketing of the mussels and the branding of regions that produce the mussels. The interactive part of lead user experiment on Facebook was less successful, as food specialists claimed to be mainly orally and hands-on oriented people. In total the experiment demonstrates the feasibility of lead user approaches in innovation in the case of blue mussels in a regional context.

Publications

Scientific articles (peer reviewed)

Hjalager A-M , Wahlberg M (2012). Museum guests as contributors to local food innovation. Museum Management and Curatorship (Submitted).

Hjalager A-M, Heike Johansen P, Rasmussen B (2012). Informing regional food development through lead user experiments. The case of blue mussels. Journal of Product Innovation Management (Submitted).

Non-peer reviewed articles

Stilling-Blichfeldt B (2010). “Town of Mussels”. A Danish case study on place branding, food festivals and community identity. CLF Working Paper No. 08/2010.

WP7: Cross-cutting issue: Research and demonstration mussel farm

Responsible
Professor Hans Ulrik Riisgård, Marine Biological Research Centre, University of Southern Denmark

Aims
In 2010 the MarBioShell-project established a research and demonstration farm for cultivation of off-shore line mussels in Kerteminde Bay (Great Belt) in collaboration with a current partner, the commercial mussel company Nordshell. The plan is to construct and operate a 25% full-scale experimental mussel farm during the years 2010-2011 (cost around 200,000 DKR per year minus anticipated income from the sale of mussels together with tourism activities and eventually sale of the plant when the project period is terminated). The research- and demonstration farm is a unique opportunity for all work packages within MarBioShell to work together in solving an important common task, namely to throw light on the potential, in the broadest sense, to cultivate off-shore line mussels in a rational manner in the Great Belt, thus opening the way for a new trade and employment in this region.

Scientific leaders and participating WPs
- Hans Ulrik Riisgård (WP2)
- Flemming Møhlenberg (WP3 & WP4)
- Jens Honoré Walther ((WP3)
- Eva Roth (WP5)
- Anne-Mette Hjalager (WP6)
- Niels T. Eriksen (WP1)

Activities (Nov 2009 - Dec 2012)
In close collaboration with the company Nordshell the following activities on the research and demonstration mussel farm has been performed:
- Reconnaissance and construction of the farm and carrying lines was performed in autumn 2009 and spring 2010 (Fig. 14).
- Growth lines were set out in spring 2010 in order to test for optimization of mussel farm design, calibration of mussel farm models, impact of thinning out on growth and grazing impact and for testing harvest methods. These issues were continued in 2011.
- Net-bags with mussels were hung out and collected from July to October 2010 to investigate bioenergetics and to evaluate the growth potential for line-mussel farming in the Great Belt.
- Mussels were collected from farm-ropes during the winter of 2010/2011 to investigate the effect of temperature on growth and lipid composition and concentration.
- Mussel larvae settling on farm-ropes in the spring 2011 and subsequent growth throughout the season of 2011 has been investigated to supplement our research for evaluation of the potential for line-mussel farming in the area.
- In October and November 2011 growth of mussels in net-bags was followed simultaneously with *in situ* measurements of current flow and chl *a* levels in collaboration with DHI to look at the effect of these parameters on growth of mussels and the effect of farm design on the parameters.
- Phyto- and zooplankton data from the last 10 years is being analysed in order to evaluate the importance of heterotrophic relative to phototrophic plankton organisms in blue mussel diets in the field.
- Organization, innovation, branding and regional development

- The concept of growing mini-mussels within one season (June-December) is being evaluated as a potential strategy for producing line-mussels in the Great Belt. The results from modelling (WP 3), measurements of currents and the potential yield of mussels show that high growth rate of mussels at least to a depth of 6-7 m even in a dense farm results in very high area-production capacity; among the highest – if not the highest – recorded. The main reasons are: high current speeds uniform over depth (Fig. 15) will allow exploiting the water column to at least 8 m, which is almost 3 times larger than at the farms established in the Limfjord.
- Outcome of harvest in 2010 and 2011 showed that the predicted biomass of 4 kg/m was exceeded 3.5 times; hence in November 2011 between 11 kg and 14 kg wet weight was present per meter rope, from surface to at least 6 m depth. Converting to the area occupied by a farm up to 1500 g of dry meat weight and 12 kg drained wet weight of mussels can potentially (neglecting loss during harvest) be harvested in the Great Belt (Fig. 16 and Fig. 17).
- Based on the recorded growth in 2010 and 2011 the potential yield at harvest in November will vary according to length of growth season and food availability (i.e. the level of autumn bloom). In 2010 low temperatures in early summer and low concentration of chlorophyll in late autumn resulted in lower growth than during 2011, when temperatures were higher in May and concentration of chlorophyll *a* in November was twice as high as in 2010. The growth was especially fast during autumn and was fuelled by high currents (average at 13 cm/s in surface waters) and high concentration of chlorophyll *a* in surface waters from the western Baltic Sea. In November mussels larger than 30 mm accounted for 75% of the biomass (Fig. 18). Ash free dry weight constituted 15% of wet weight (normally 6% in natural populations) underlining that meat content was very high. The capacity of Great Belt to support mussel growth in rope cultures relies on a consistent and long-lasting autumn bloom in the western Baltic Sea. Monitoring in the Great Belt in 2009 and 2010 did show increasing chlorophyll *a* levels from early August to early December averaging 3 µg/l. High frequency measurement at the mussel farm showed that high chlorophyll *a* levels were associated with falling salinities (i.e. higher contribution of waters from the western Baltic Sea) and decreasing levels at rising salinities (from Kattegat) (Fig. 19).

Fig. 14. The MarBioShell research- and demonstration farm for line-mussels was established in 2010 in Kerteminde Bay (Great Belt).

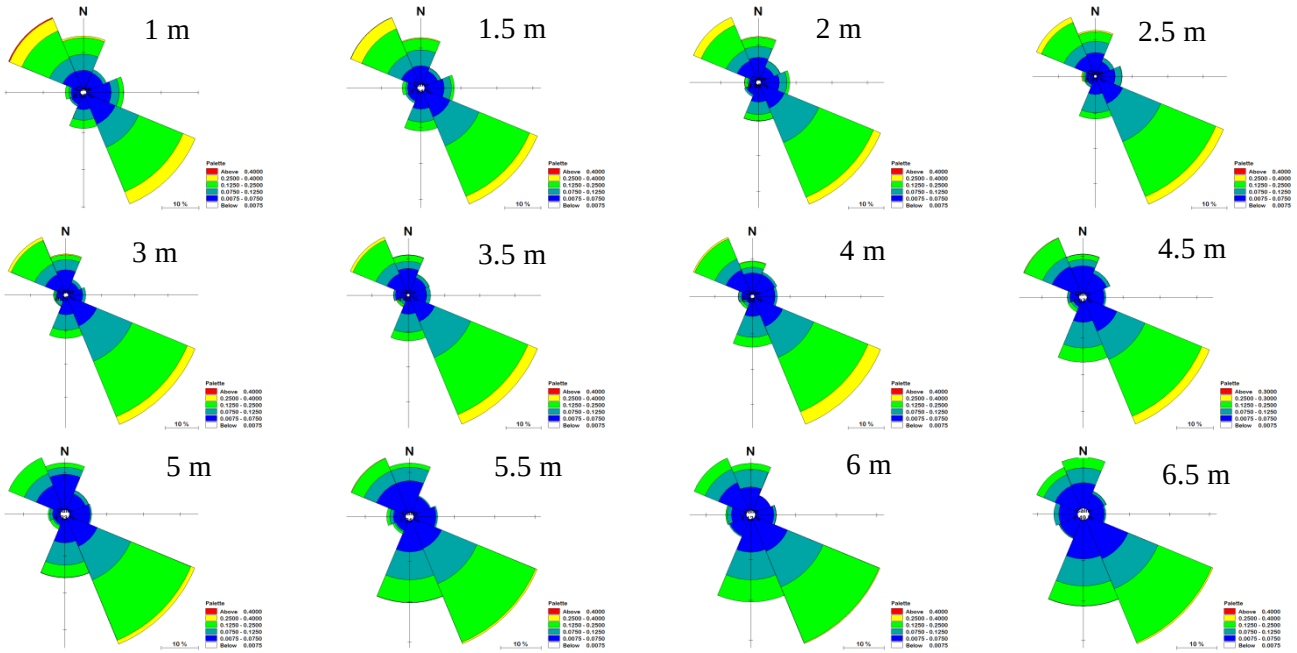

Fig. 15. Current direction and speed in 0.5 m depth intervals from surface (1 m) to 6.5 m (2 m above the seabed).Values averaged over the field campaign in 2011 (11 – 23 November).

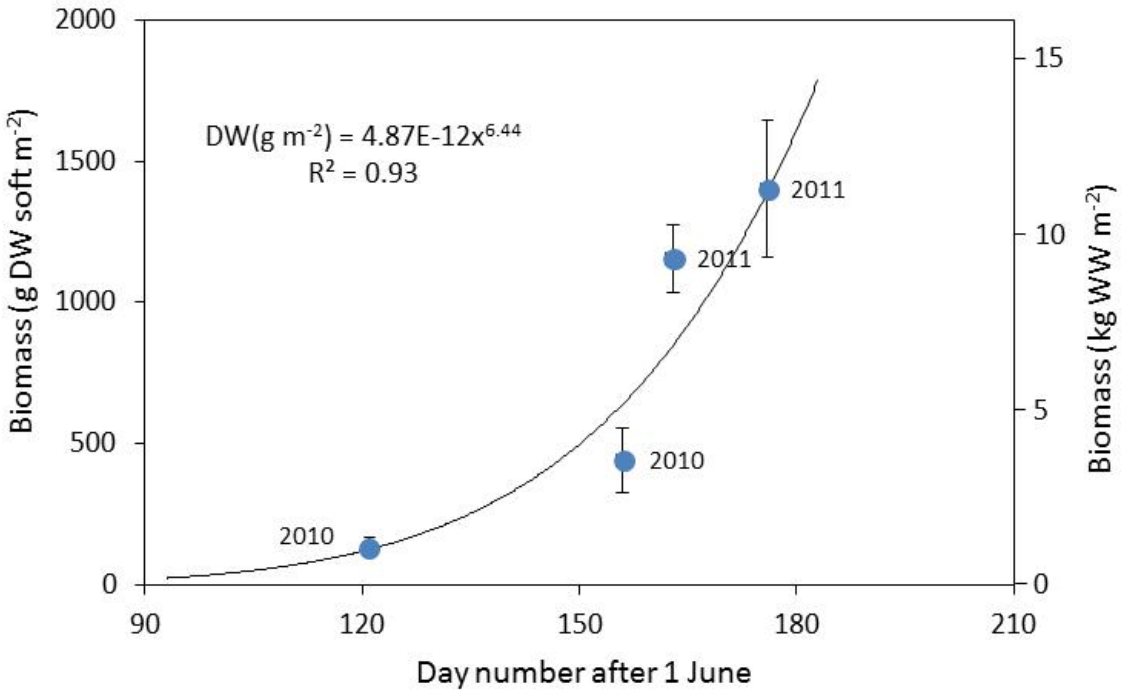

Fig. 16. Calculated dry meat weight in September 2011 through November 2011 available for harvest per m^2 of mussel farm in the Great Belt. Each value (point) is based on measurements (length and biomass) of 1250 to 2210 individual mussels sampled at 8 to 12 positions in the farm.

Fig. 17. Farm-ropes with mussels in the MarBioShell farm, October 2011.

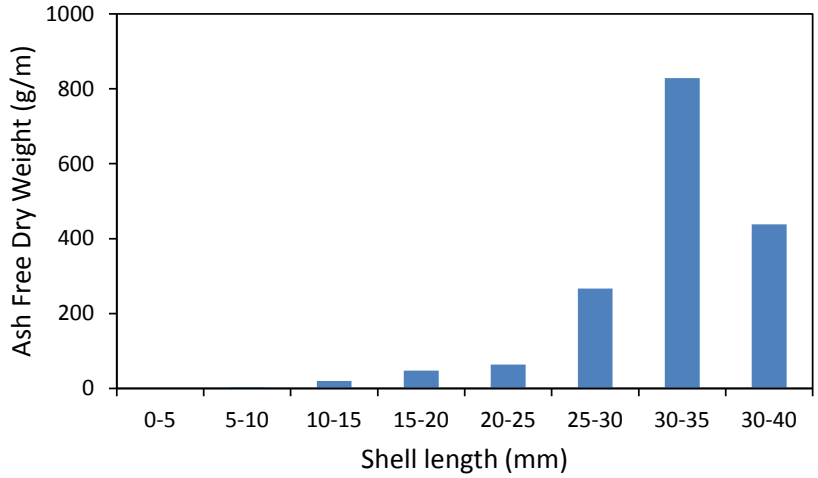

Fig. 18. Ash free dry weight (without shells) of mussels harvested in November 2011.

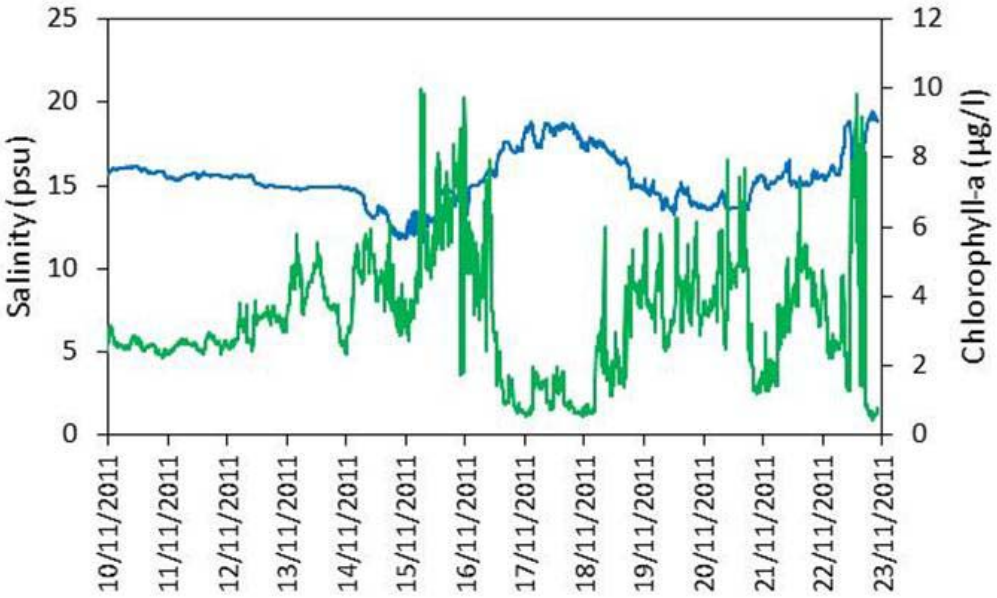

Fig. 19. Temporal variation in salinity and chlorophyll *a* in the mussel farm during 14 days in November 2011.

Highlights and recommendations

Research highlights

- Continuous algae cultivation and harvest of phytoplankton components. The heterotrophic dinoflagellate *Crypthecodinium cohnii* was tested with emphasis on continuous cultivation in bioreactors and with the opportunity to manipulate the biochemical composition of the biomass by selective nutrient limitation. A defined medium containing glucose, glutamic acid and phosphate as carbon, nitrogen and phosphorous sources, respectively was developed and used. Results from continuous flow cultures showed that the content in lipids in *C. cohnii* did not only increase when cultures are nitrogen limited but also during phosphorous limitation. Carbon limitation did lead to a decrease in starch but the lipid content was unaffected (1). The opportunities to cultivate *C. cohnii* in continuous flow cultures and to manipulate the biomass composition opened up for the use of the biomass in experiments for investigation of the uptake and accumulation of micro-algal (phytoplankton) components in *Mytilus edulis*. It was found that *M. edulis* can be considered as a highly efficient "harvester" of phytoplankton components in particular omega-3 and omega-6 fatty acids from a highly diluted environment (2).
- The potential for line-mussel production in the Great Belt region has been studied with special emphasis on testing a new bioenergetic growth model for the blue mussel, *Mytilus edulis* of shell lengths > 20 mm. The mussel-growth model predicts that the weight specific growth rate (μ) as a function of dry weight of soft parts (*W*) under optimal conditions can be expressed as a power function $\mu = aW^b$ with an exponent $b = -0.34$ and a constant *a* that depends on the phytoplankton biomass (expressed as chlorophyll *a*) at the growth site. The actual growth rates of *M. edulis* in suspended net-bags were measured in terms of shell length and dry weight of soft parts during extended periods in the productive season in series of field experiments, including sites in Great Belt and in Limfjorden. Growth results appeared to be close to the theoretical predictions at the prevailing chl *a* concentration observed near sites, both in rate of growth at any size and in rate of decrease of growth with increasing size. Based on these results, calculated time to double dry weight of soft parts versus mussel size, and supplementary long-term records of salinity, temperature, chl *a* concentration and current, an evaluation has been made for the potential of line-mussel farming in Great Belt region (3, 4, 5).
- Effects of salinity on growth of mussels, *Mytilus edulis*, were studied in laboratory feeding experiments and compared to the growth of mussels suspended in net-bags in the brackish-water Great Belt. The study showed that mussels are able to adjust growth at changing salinities, and the observed effect of salinity could partly be explained by a temporary shell valve closure after a sudden change in salinity. The specific growth rate of mussels measured in laboratory experiments at salinities between 15 to 25 psu were comparable to the growth of mussels in the field experiment where the salinity varied between 24 and 13 psu during the growth period. The shell-closure effect observed in the laboratory when the salinity was suddenly changed and which lasted for several days does not represent the normal environmental conditions in the Great Belt where the salinity-changing rate is relatively low and where *M. edulis* may therefore be able to adjust (acclimate) to the occasionally low salinities (down to about 10 psu) in such a way that the growth rate remains rather unaffected. Statistical analysis indicate significant decrease of weight specific growth rate and shell growth rate when salinity was suddenly changed from 30 to 10 psu and reverse, but not when changed from 25 to 15 psu and reverse. However, more studies on the effect of Great Belt-relevant salinity changing rates on feeding behaviour as well as growth experiments with mussels that are well-adjusted to low salinities (i.e. fully open valves and exhalant siphon) are needed to separate the acute shell-closing effect on food uptake and growth from possible physiological effects of low salinities resulting in reduced growth (6).

- Feeding behaviour of mussels. Under optimal conditions, mussels tend to filter the ambient water at a maximum rate, but under sub-optimal environmental conditions, including low or very high algal concentrations, the filtration rate is reduced. The upper algal concentration at which the blue mussel, *Mytilus edulis*, exploits its filtration capacity over an extended period of time was identified by stepwise raising the algal (*Rhodomonas salina*) concentration in steady-state laboratory experiments above the threshold for continuous high filtration rate. The duration time before incipient saturation reduction decreased with increasing algal concentration, and the threshold concentration for incipient saturation reduction of filtration activity was found to be between about 5,000 and 8,000 cells ml^{-1}, equivalent to 6.3 and 10.0 µg chl *a* l^{-1}, respectively, which may be compared to a mean annual concentration of 2.5 ± 1.2 µg chl *a* l^{-1} in the Great Belt (7).
- Evaluation of methods for measurements of filtration rates of mussels. To obtain precise and reliable laboratory filtration rate measurements with the so-called "flow-through chamber method" (FTC) the design must ensure that only inflow water reaches the bivalve's inhalant aperture and that exit flow is fully mixed. For the "steady-state method" (SS), the design must ensure that inflow water becomes fully mixed with the bivalve's excurrent flow to establish a uniform chamber concentration prevailing at its incurrent flow and at the chamber outlet. The fundamental differences between the FTC and the SS methods and practical guidelines for their use have been pointed out, and new data on filtration rate for the blue mussel, *Mytilus edulis*, illustrate a design and use of the SS method which may be employed in e.g. long-term growth experiments at constant algal concentrations, as done in a number of subsequent MarBioShell studies (8).
- Fluorometer-controlled apparatus for feeding experiments with mussels. The principle used is feedback regulation of the algal concentration based on continuous monitoring of the fluorescence intensity of chlorophyll *a* in water pumped through the apparatus from an aquarium with mussels (see Fig. 14). The new apparatus has been used to measure the filtration rates of blue mussels (*Mytilus edulis*) at algal concentrations both near and above the incipient saturation level for reduced filtration activity. The algal concentration at which *M. edulis* eventually reacts by partial valve-closure and reduced filtration rate was found to be between 6,000 and 7,000 *Rhodomonas salina* cells ml^{-1} (9).
- Exhalant jet velocity of mussels. The detailed fluid mechanics of the mussel-near flow generated by the exhalant jet has hitherto been uncertain although this flow in conjunction with currents and/or other mussels may strongly influence their grazing impact. Computational modelling of this phenomenon depends on knowledge of the velocity distribution near the exhalant siphon aperture of mussels to provide appropriate boundary conditions for numerical flow models, and to be useful such information should be available for a range of mussel shell lengths. Therefore, a detailed study has been performed on fully open mussels (*Mytilus edulis*) in terms of filtration rate, exhalant siphon-aperture area and jet velocity, gill area, dry body weight, all as function of shell length. Scaling laws for these parameters in terms of size by shell length have been presented. The exhalant jet velocity was determined by three methods: I) measured filtration rate divided by exhalant aperture area, II) manual particle tracking velocimetry (PTV) using video-microscope recordings, and III) particle image velocimetry (PIV). The latter has provided detailed two-component velocity distributions near the exhalant siphon in 5 planes parallel to the axis of the jet and the major axis of the oval aperture, hence estimates of momentum and kinetic energy flows in addition to mean velocity. Here, data obtained on particles inside the exhalant jet of filtered water was ensured by the use of TiO_2 seeding particles which were de-agglomerated by ultrasound to size-range 0.7 to 2 µm prior to addition to avoid retention by the gill-filter of the mussels. Notably it was found that the exhalant jet velocity is essentially constant, about 8 cm s^{-1}, and independent of shell length. Based on geometric similarity and scaling of pump-system characteristics of the mussel it was found that

these characteristics coincide approximately for all sizes when expressed as pressure head versus volume flow divided by shell length squared (10).

- Mussels as a food item with an under-utilized narrative power and branding opportunity The study demonstrates the complex and interrelated narratives of mussels, which have a potential for a wider utilisation in regional branding of the Great Belt areas and its business and tourism facilities. The traditional *sensory approach* addresses the sweetness-acid and other contrast of the mussels as a food item and operates with different elements of combined fragrances and levels taste experiences. The *sensory approach* could be supplemented with narratives about mussel dishes that comprise crispness, softness, fullness, fluidness – things that fill the mouth and activate the face muscles, chaw etc. Narratives for the consumers and visitors to a region can include also the *scientific approach* which demonstrates how research knowledge and transforms and transfers it for technical perfection or for taste optimization purposes. There are significant *ethical* narrative potentials which take into consideration the environmental issues and issues connected to the location of food supplies, terroir in the specific regional contexts. A *food culture approach* recognizes the food heritage and attempts to reinvent and proceed along the historical traditions. *The nutritional approach* has a focus on how mussels can benefit people suffering from modern lifestyle health problems.

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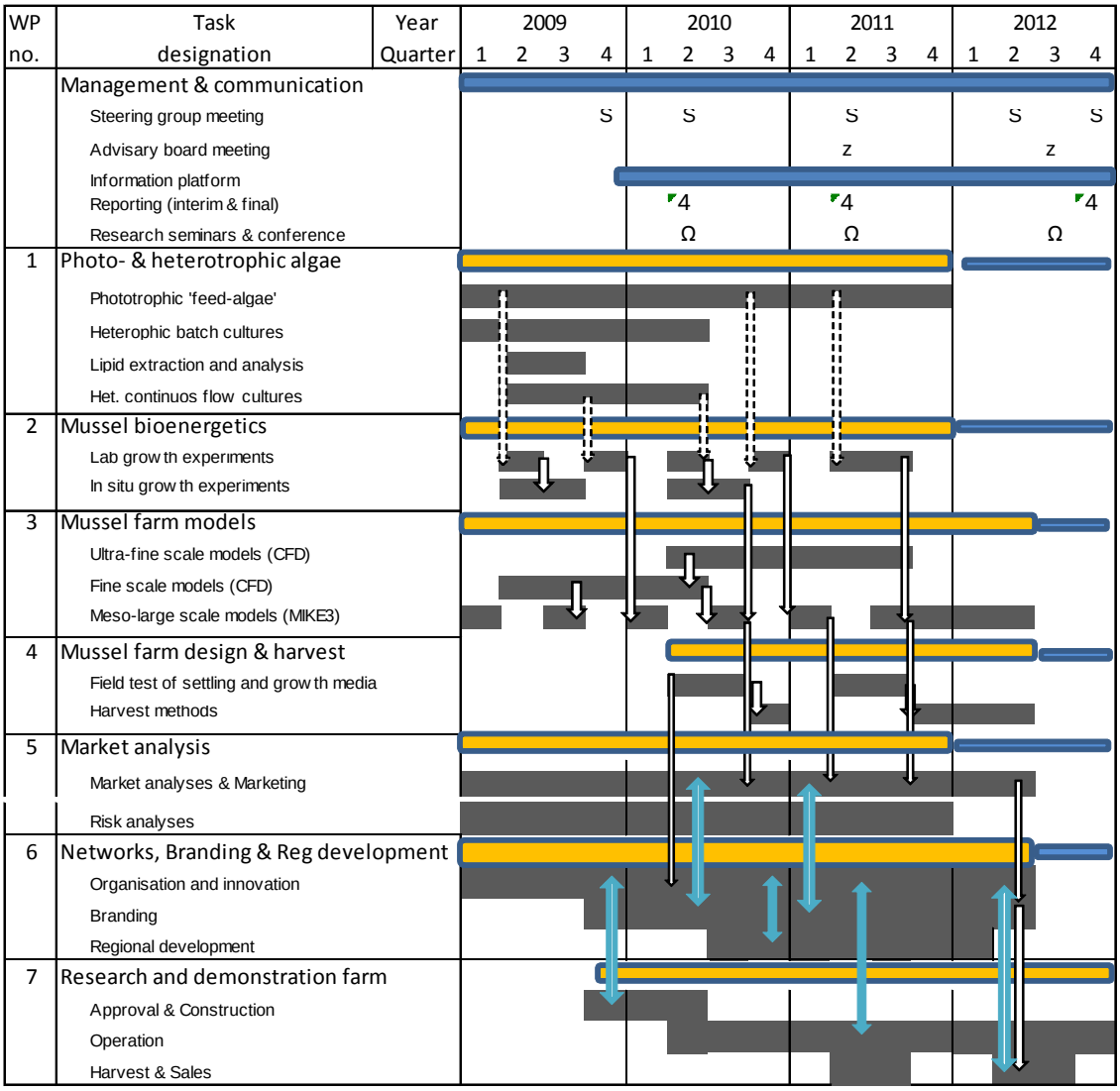
Recommendations

- Mini-mussels. Regarding future blue mussel farming in the Great Belt region the studies have shown that mussels can grow from settlement in spring to 30 mm in shell length in November. However, to reach the traditional consumer size of at least 45 mm it will probably take about 18 months, because of the winter period with weight loss and subsequent re-growth during the next season. Therefore it is recommended to consider a new approach of line farming of 30 mm mini-mussels during one growth season, from early spring to November, recovering all equipment at the time of harvest and re-establishing it at the beginning of the next season and thus protecting the equipment from the often damaging weather of the Danish winter season. The new, smaller sized consumer product should be attractive in its own right. Finally, the steady increase in condition index of mini-mussels from early spring to November is associated to an increase in glycogen, fatty acids and in particular

polyunsaturated fatty acids. The steady accumulation of phytoplankton components is an advantage to big mussels and makes mini-mussels to a promising alternative.

Timetable

MarBioShell timetable of reconstructed project 2009-2012. Time course for start and end of the different research activities and their interconnectivity are shown in the diagram below.



Publicity - press & media about MarBioShell

- Fyens Stiftidende 30 January 2008: "Millioner til muslinge-projekt"
- Ny Viden April 2008: "Muslinger - en ny delikatesse"
- Kjerteminde Avis 12 February 2009: "Muslinger i alle afskygninger"
- Kjerteminde Avis 13 August 2009: "Fremtidens muslingeopdræt som forsøg
- Mussel -theme with Fjord&Belt during the winter holiday 2009
- Forskningens Døgn 25 April 2009
- Forskningens Døgn 24 April 2010
- Fyens Stifttidende 18 March 2010: "Muslingeforskere bruger praktiker"
- Kjerteminde Avis 23 June 2010: "Muslingeprojekt skal nu stå prøven på vandet"