

#### Area-intensive bottom culture production of blue mussels, Mytilus edulis (L.)

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# Area-intensive bottom culture production of blue mussels, *Mytilus edulis* (L.)

Mussel bed structure and seed performance



Written by Helle Torp Christensen Defended 5 November 2012



# Area-intensive bottom culture production of blue mussels, *Mytilus edulis* (L.)

Mussel bed structure and seed performance

PhD Thesis by

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Defended 5<sup>th</sup> of November 2012

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## Abstract

The conflict between blue mussel, *Mytlius edulis* (Linnaeus, 1758), exploitation and other interest groups due to ecological effects of the fishery, was described ten years ago, and still exists today. To reduce the ecological effects and the conflicts between conservation and exploitation it is therefore necessary to focus on area-intensive production methods for mussel exploitation. For that purpose bottom culture production of blue mussels is relevant. Despite a long tradition for bottom culturing in Europe the method is still dependent on natural conditions, such as recruitment, food availability and predation. The major constrains for development of the production method is lack of recruitment to sustain the seed mussel source, and conflicts with nature conservation interests due to negative effects of dredging. The aim of the present PhD project was, therefore, to study how thorough insights in biological mechanisms can be used as a tool to develop and optimize bottom culture production methods. The study was addressed through the following questions: 1) How can the seed mussel source in bottom culturing be supported? 2) How can survival of mussels in bottom culturing be improved? And 3) How does bottom culture practice support an area-intensive exploitation of blue mussels? To answer the questions four experimental studies were conducted.

Results from a laboratory- and a manipulated field experiment showed that blue mussels collected on suspended long line cultures in the water column have the potential to become an alternative seed source for mussel production in bottom cultures. When compared to mussels collected from natural benthic mussel beds, suspended mussels had an active predator response by developing a significantly stronger attachment to the substrate and having a more pronounced aggregation behaviour. Bottom mussels exhibited a passive strategy by developing a thicker shell and larger relative size of the posterior adductor muscle. When comparing the performance of suspended and bottom seed mussels on complex and smooth substrate, respectively, originally suspended mussels aggregated significantly more than bottom originated mussels on smooth substrates. This indicated that suspended mussels are better in achieving the protection provided by group living compared to bottom mussels, since more aggregated mussels are more protected against predators. Thus, it is concluded that the use of suspended mussels in bottom culture production supplement, and possibly, secure the seed source in future blue mussel production in intensive bottom cultures.

From two manipulated field experiments it was concluded that substrate complexity stabilizes the structure of the mussel bed on micro-scale (< 1 m) resulting in an achievement of protection faster than without applying shell substrate to the seabed. On complex substrate, mussels have the protection from predators right after transplantation, due to more spatial refuges, in contrast to on smooth substrate where mussels need to aggregate to achieve the same protection. The stabilization is expressed by increased byssal strength and reduced aggregation activity within the mussels and is resulting in higher survival. However, the increased protection provided by the higher complexity also result in a trade-off between increased survival and reduced growth and lower condition index for the individual mussel. The production output was generally higher on complex substrate than on smooth substrate and it was therefore concluded that an increased substrate complexity has the potential to improve survival of mussels in bottom culture beds. Nevertheless, due to the trade-off between survival and growth, the degree of complexity is important in the planning of culture beds to secure that the reduction in growth and condition index do not eliminate the increased survival of the mussels.

To achieve a smaller impacted area, prerequisites concerning robust bed structures and seeding densities needs to be fulfilled. In a case study it was concluded that production of blue mussels in bottom cultures support an area-intensive exploitation, with off-set in Danish production practices. The robust structure and the seeding density of 3.5 kg m<sup>-2</sup> support an area-intensive exploitation of blue mussels in the case area. On macro-scale (> 100 m) it can be documented that macrostructure of the individual culture bed was similar to the original transplantation tracks established the year before. This indicates that bottom cultures can form robust structures, not affected by wave- or current induced transport. Seeding density of 1.5 and 3.5 kg m<sup>-2</sup> and the position of the individual mussel in the culture bed apparently did not adversely affect shell growth, suggesting that there was no detectable food limitation between transplantation tracks. Production of blue mussels in bottom culture beds may impact a smaller area compared to fishery on full-grown mussels from natural mussel beds, and can support an area-intensive production if the biomass/production ratio is higher than 0.5.

## Resumé

Udnyttelse af blåmuslinger, *Mytulis edulis* (Linnaeus, 1758) som ressource skaber konflikt mellem naturudnyttelse og naturbevarelse på grund af fiskeriets påvirkning af marine økosystemer. Til at mindske konflikten mellem naturbevarelse og naturudnyttelse, er et middel at udvikle areal-intensive metoder til muslingeproduktion. Til det formål er produktion af blåmuslinger i bundkultur relevant. På trods af en lang tradition for bundkulturproduktion i Europa, er metoden stadig afhængig af og ikke mindst styret af naturlige mekanismer, såsom rekruttering, fødetilgængelighed og prædation. De største udfordringer for udvikling af bundkulturproduktion er en ustabil tilgang til muslingeyngel og konflikten med naturbevarelsesinteressenter på grund af de negative effekter af fiskeriet. Formålet med nærværende Ph.d.-projekt var således, at undersøge hvordan en dybere forståelse af de biologiske mekanismer, kan benyttes som redskab til at udvikle og optimere produktionen af muslinger i bundkultur. Studiet tog udgangspunkt i tre spørgsmål: 1) Hvordan kan yngleressourcen i bundkulturproduktion blive sikret?, 2) Hvordan kan overlevelsen af muslinger i bundkulturer bliver forbedret? og 3) Hvordan understøtter produktionen i bundkulturer en arealintensiv udnyttelse af blåmuslinger? For at besvare spørgsmålene blev fire individuelle studier gennemført.

Resultater fra et laboratorie- og et manipuleret felteksperiment viste, at blåmuslingeyngel opsamlet på langliner i vandsøjlen havde potentiale til at blive en alternativ yngleressource til produktion i bundkultur. Sammenlignet med yngel opsamlet fra naturlige bentiske muslingbanker, havde linemuslinger et mere aktivt respons til prædation ved at fasthæfte sig bedre til substratet og have en mere udviklet aggregeringsad-færd. Bundmuslingerne viste en mere passiv strategi, med udvikling af tykkere skal og relativt større lukkemuskel. Sammenlignes line- og bundmuslinger placeret på komplekst og sandet substrat, aggregerede linemuslinger signifikant mere end bundmuslinger på sandet substrat. Dette resultat indikerer, at linemuslinger, sammenlignet med bundmuslinger, er bedre til at opnå den beskyttelse en aggregeret struktur giver, idet mere aggregerede muslinger er bedre beskyttede mod prædation. Det blev derfor konkluderet, at brugen af linemuslinger som yngleressource kan supplere, og eventuelt sikre yngelressourcen i en fremtidig intensiv produktion i bundkulturer.

Gennem to manipulerede felteksperimenter blev det konkluderet, at komplekst substrat stabiliserer strukturen i muslingebanken på mikroskala (< 1 m). Det komplekse substrat bidrager til, at muslinger hurtigere opnår beskyttelse end hvis de var placeret på sandet substrat på havbunden. På det komplekse substrat har muslingerne beskyttelsen mod prædatorer lige efter udlægningen pga. et øget antal af refugier, i modsætning til på sandet substrat, hvor muslingerne skal aggregerer, før de opnår beskyttelse. Men den øgede beskyttelse, der opnås på komplekst substrat, resulterer også i et trade-off mellem øget overlevelse og reduceret vækst og konditionsindeks for den enkelte musling. Produktionen var dog generelt højere på komplekst substrat, sammenlignet med hvad der blev opnået for muslinger på sandet substrat. Det blev derfor konkluderet, at øget substratkompleksitet har potentiale til at forbedre overlevelsen af de udlagte muslinger i bundkultur. På grund af trade-off´et mellem overlevelse og vækst er det vigtigt, at vurdere graden af kompleksitet, når kulturproduktionen planlægges, således at reduktionen i vækst og konditionsindeks ikke overgår den øgede overlevelse for muslingerne.

For at reducere det påvirkede areal af bundkulturproduktion, skal forudsætninger for en robust bankedannelse og udlægningstæthed af muslinger være opfyldt. I et case-studie blev det konkluderet, på baggrund af den robuste bankestruktur og udlægningstætheden på 3,5 kg m<sup>-2</sup>, at produktionen af blåmuslinger i bundkultur kan udgøre en areal-intensiv produktion i forhold til et fiskeri af muslinger på naturlige muslingebanker. På makro-skala (> 100 m) blev det dokumenteret, at makrostrukturen i bundkulturbanken var sammenfaldende med de oprindelige udlægningsspor etableret det foregående år. Det indikerer, at der i bundkulturer dannes robuste strukturer, der ikke blev ændret af bølge- og strømpåvirkning. Udlægningstætheden på 1,5 og 3,5 kg m<sup>-2</sup> og muslingernes placering i kulturbanken, havde ikke nogen signifikant effekt på muslingernes skalvækst, hvilket antyder, at der ikke var fødebegrænsning mellem udlægningssporerne. Hvis biomasse/produktionsforholdet er højere end 0,5, kan produktion af muslinger i bundkulturer påvirke et mindre areal, sammenlignet med fiskeri af muslinger på naturlige bentiske banker og dermed understøtte en areal-intensiv produktion af muslinger.

## Preface

Blue mussels, *Mytilus edulis* (Linnaeus, 1758), is a well-studied species. A literature search at Web of Science with *Mytilus edulis* in the search line, give a result of 9,483 hits. In comparison man's best friend, the dog, *Canis lupus* (Linnaeus, 1758), result in 1,719 hits. Blue mussels can be found and is known almost all over the world and its success is due to its adaptation to a broad range of conditions, salinity, temperature, substrates etc. (Reviewed in Gosling 1992). Blue mussels have achieved attention within an almost endless list of biological topics and applied uses. The species is described in relation to biological indicator species since they are available at many locations and are easy to collect (e.g. Hellou & Law 2003), the species and its function as filter feeder gives various opportunities within the area of habitat improvement (e.g. Leonard 1993), and its role as biogenic reef builder makes its functional role as habitat engineer interesting (e.g. Buschbaum et al. 2009). Furthermore, the species as a prey species has led to attention within especially studies of food availability for birds (e.g. Goss-Custard et al. 2004). Finally, the species is one of the most important aquaculture species in the world which means that the species is very well studied regarding survival, grazing, growth performance, diseases, etc.

Within the framework of the PhD project it is not possible or relevant to include all knowledge available on blue mussels. Therefore, it is important to emphasize that the framework of the present study is production of blue mussels in bottom culture with focus on the seed mussel performances and the effect of substrate complexity on mussel bed formation to reduce predator induced mortality. The study have basis in the major constrains of the production method including the absence of stability of the seed mussel source, survival of the transplanted mussels and an overall area-intensive exploitation of the blue mussel source. Attention has thus been on biological mechanisms including phenotypic plasticity, predatory responses, trade-off within species/habitat interactions and the prerequisites to achieve a small impacted area when exploiting blue mussels.

My main interest regarding production of blue mussels lies within how knowledge about biological mechanisms can be used to improve production. Shortly, it can be described as the use of natural ecological interactions and processes as an instrument in human food production. I believe that a detailed knowledge on the biological mechanisms behind the production will result in a sustainable long term development.

I am thankful for the opportunity to focus on this corner of research. It has been frustrating to recognize what a tiny part of the full picture within the world of blue mussels I have covered and at the same time extremely exciting to realize that I, what in the beginning of the study seemed like against all odds, have found a niche within the interdisciplinary subject of blue mussel production in on-bottom cultures with the use of natural conditions.

Helle Torp Christensen

14 September 2012

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## **Chapter 1 Introduction**

Exploitation of natural resources is often in conflict with nature conservation issues due to overlap in area of interest. Exploitation of blue mussels, Mytilus edulis (Linnaeus, 1758), is no exception (Cranford et al. 2012; Saurel et al. 2004; Kamermans & Small 2002). Non-Governmental Organisations (NGO), such as recreational and nature conservation organizations, also focus on the ecological effects and area-based conflicts of exploitation of mussels (CWSS 2008; Maguire et al. 2007). For example, Smaal (2002) reported that spatial competition between shellfish production and these interest groups leads to discussion of the ecological impact of mussel cultures. The conflict between mussel exploitation and the other interest groups was described ten years ago (Dolmer & Frandsen 2002; Kamermans & Smaal 2002; Smaal 2002; Jennings & Kaiser 1998), and still exists (Eriksson et al. 2010; Wolff et al. 2010). Dredging of the seabed is one of the most harmful fishing methods for benthic ecosystems (Collie et al. 2000). Dredging conducted as part of mussel exploitation results in local reduction of substrate and biodiversity (Neckles et al. 2005; Riis & Dolmer 2003; Dolmer 2002; Dolmer et al. 2001; Kaiser et al. 1998). Substantial differences in eelgrass, Zostera marina (Linnaeus, 1753) biomass in Maine, USA, persisted between dredged and not dredged sites up to seven years after fishery (Neckles et al. 2005). Dolmer et al. (2001) documented a reduction in number of benthic species in a mussel bed at least 40 days after dredging, and Riis & Dolmer (2003) showed that the density of sea anemone, Metridium senile (Linnaeus, 1761), was lower in dredged compared to not dredged areas. Due to adverse effects upon the habitat structure and the biodiversity, the area used for exploitation of blue mussels should be kept as limited as possible. To reduce the ecological effects and conflicts it is therefore necessary to focus on area- intensive production methods for mussel exploitation. For that purpose, bottom culture production of blue mussels is relevant (Anonymous 2012; Hoffman & Dolmer 2000). However, bottom culture production also has effects on infaunal communities (Ysebaert et al. 2009; Beadman et al. 2004; Smith & Shackley 2004), though only on local scale (0-10 m) and dependent on seeding density (Beadman et al. 2004). Despite a long tradition for bottom culturing in Europe (Smaal 2002) the method is still dependent on natural conditions, such as recruitment, food availability and predation. These biological mechanisms need to be clarified, in order to further develop the production method. The aim of the present PhD project was, therefore, to study how thorough insights in biological mechanisms can be used as a tool to develop and optimize bottom culture production methods.

#### 1.1 Blue mussel exploitation and management

Exploitation of blue mussels can be classified in two categories: on-bottom and off-bottom cultivation (Spencer 2002). On-bottom culture is based on transplantation of mussels from areas with high density seed beds to culture areas where the density is reduced to improve growth and condition of the mussels (Spenser 2002). On-bottom exploitation of natural mussel beds also takes place and is mainly practiced in Denmark (Smaal 2002). Off-bottom cultivation includes a range of methods e.g. bouchot, raft and long line cultures in the water column (Huntington et al. 2006; Smaal 2002; Spenser 2002).

In Europe, Spain is the most important mussel producing country followed by France, The Netherlands and United Kingdom (Table 1). In Spain, mussels, *Mytilus galloprovincialis* (Linnaeus, 1798), are cultured on ropes suspended in the water column and attached to rafts (Smaal 2002). In the rest of Europe the primary mussel species is *Mytilus edulis*. In France, mussels are grown on bouchots, long lines and on the bottom, while mussel culture in The Netherlands almost entirely consists of bottom culture. In England and Wales the major blue mussel culture consists of bottom culture, while in Scotland the production is conducted on

suspended long lines. Mussel production in Denmark comes mainly from fishery on natural beds. In Ireland blue mussels are cultured on bottom leased sites and on ropes, and fishing from wild beds also takes place, and in German blue mussel culture is conducted in bottom culture (Smaal 2002).

Table 1. Total production (ton) (culture production plus capture on natural beds) of mussels, *Mytilus edulis* and *Mytilus galloprovincialis*, in the seven most producing countries in Europe from 2005 to 2010. Source FAO FishStatJ statistics (www.fao.org).

Country	2005	2006	2007	2008	2009	2010
Spain	158,193	228,837	209,671	180,273	198,655	189,148
France	112,201	112,771	123,696	178,014	162,276	153,899
The Netherlands	69,615	61,703	96,502	96,328	77,705	91,767
United Kingdom	63,324	54,223	33,581	60,951	47,256	37,190
Denmark	69,318	55,318	58,388	37,119	39,264	29,564
Ireland	15,134	54,580	59,226	33,794	20,671	21,428
Germany	11,679	8,710	20,079	14,209	6,268	5,467

For more details on different production methods see e.g. Spencer (2002) or Smaal (2002). In Fig. 1 the production steps within the most common production methods are shown. The circle indicates on-bottom production, which can be divided into bottom culture production and fishery on natural mussel beds.



Fig. 1. Overview of production steps (Source: www.fao.org).

Increasing demand for coastal marine resources and solutions to solve conflicts concerning area use have led to development of different approaches and tools for management of human activities, such as Integrated Coastal Zone Management (ICZM), marine Ecosystem Based Management (EBM), Marine Spatial Planning (MSP) and System Approach Framework (SAF) (Anonymous 2012; Qiu & Jones 2011; Moksness et al. 2009). Management of blue mussel production is currently adapting to an increased focus on effects on ecosystems and demand and competition for space by moving towards more integrated, ecosystem based management, i.e. with the aim to maintain ecosystem functioning and sustain ecosystem goods and services for future exploitation as well as to identify potential for mutual opportunity with other sectors (Anonymous 2012; Cranford et al. 2012; Maguire et al. 2007).

Within the EU, production of blue mussels needs to adapt to the EU Marine Strategy Framework Directive, which aims to achieve "good environmental status" for all marine waters by 2020 (for an overview of the policy landscape in Europe see Qiu & Jones 2011). Furthermore, the Birds Directive and Habitat Directive require EU Member States to protect a range of sensitive species and habitats, e.g. through designation of Special Protection Areas and Special Areas of Conservation, which together form a network (the Natura 2000) of protected areas across Europe (Qiu & Jones 2011).

The prerequisites for a permit for fishery on natural mussel beds within Natura 2000 areas is an Environmental Impact Assessment, which documents that the fishery does not hinder 'favourable conservation status' of the habitats and species protected in the given area (Anonymous 2012). In the Netherlands, controversies between mussel producers, NGOs and the government have led to a shellfish management policy based on closure of areas for fishery, reservation of food for birds, co-management by the mussel industry, and a research program (Produs) to evaluate the effects of these initiatives (Smaal in Maguire et al. 2007). With basis in the controversies, an agreement was signed between the shellfish industry, NGOs and the Dutch government about a transition from conventional exploitation, including dredging for seed mussels, towards a more sustainable mussel exploitation (Nehls et al. 2009).

#### **1.2 Bottom culturing**

Production of blue mussels in bottom cultures is an extensive production method traditionally entirely depending on natural conditions for recruitment, food availability and predation (Smaal 2002). The production method depends on local conditions, traditions and procedures (Anonymous 2008; Kristensen & Lassen 1997; Korringa 1976). Regardless of local adaptations, the production method can be divided into three phases, the: 1) seed phase (shell length = 10-30 mm), 2) growth phase (shell length = 30-50 mm), and 3) harvest phase (shell length > 45 mm). In the seed phase, seed mussels are collected either from natural beds with a high density of mussel seed (Smaal 2002) or as under-sized (i.e. shell length < 45 mm) mussels from the dredge fishery industry (Kristensen & Lassen 1997). The collected seeds are transplanted to culture beds on the seabed in special appointed areas (Smaal 2002; Kristensen & Lassen 1997; Korringa 1976). Today, also additional or alternative methods for seed collection are being tested (Kamermans et al. 2009). Within the culture area, seed mussels are transplanted to the seabed by being flushed out from the sides of the transplantation vessel in tracks of a specific seeding density, with the aim to secure the food availability for the individual mussel at the culture bed (Smaal 2002; Kristensen & Lassen 1997; Korringa 1976). Seeding density is dependent on food availability, seed mussel size and hydrodynamics at the culture bed and is normally in the range of 2.5-3.0 kg m<sup>-2</sup> (Spencer 2002). In the seed phase, the seed survival during transplantation and sufficient supply of seeds to sustain production is critical.

Duration of the growth phase depends on the abiotic and biotic conditions in a given area, and the phase can vary from 0.5-3 years in the Netherlands (Maguire et al. 2007; Gosling 1992), 2 years in Denmark (Kristensen & Lassen 1997), 2-2.5 years in North Wales UK (Maguire et al. 2007) and 1.5-2 years in Ireland (Anonymous 2008). In bottom culturing of mussels, production output can be defined as survival plus individual growth of surviving mussels. Therefore, a high survival and individual growth is essential in the growth phase. Survival of seed mussels is however one of the major constrains in bottom culturing, since the transplantation process disturbs the mussel bed structure. Complex mussel bed structure can be described as a 3D matrix of aggregated mussels interconnected by their byssus threads (Tsuchiya & Nishihira 1985). The bed structure provides protection against predators, thus, disturbance of the structure makes the mussels more vulnerable to predation (Reimer & Tedengren 1997). A complex bed structure leads to increased search time for the predators and thereby reduces mortality from predation (Frandsen & Dolmer 2002). Furthermore, firm byssal attachment strength (Reimer & Tedengren 1997), as well as morphological adaptations, such as development of thicker shells (e.g. Beadman et al. 2003) and larger adductor muscle (Reimer & Tedengren 1996) protects blue mussels against predators by increasing the handling time for the predator. Search time is defined as the time it takes a predator to find a suitable prey (Dolmer & Frandsen 2002). In relation to predation on mussels it includes both, time to search for a prey within the preferred size range, and time to search for a prey within the right position (angle) that make it possible to handle. In bottom culture beds, seed mussels are within the same size range (Spencer 2002) and the search time for a prey of preferred size range might therefore be reduced, while the search time for a prey with the right position is expected to be similar to a natural mussel bed. More knowledge about predator response effects, including bed structure formation and substrate complexity on survival and growth of mussels, expectedly could improve establishing designs and mussel productivity of bottom culture beds.

When the mussels have reached commercial size they are harvested (shell length 45-55 mm). In the harvest phase, the final quality of the mussels, including shell thickness, fouling organisms, and condition index of the mussels is important with respect to the quality of the harvested mussels. These quality measures are dependent on the environmental condition of the seed phase and, in particular, the growth phase.

The main focus of the present PhD thesis will be the seed- and the growth phase because the major constrains for development of bottom culture production lies within these two important phases.

#### **1.3 Constrains in bottom culturing and development of the method**

The major constrains for development of blue mussel bottom culture production is lack of recruitment to sustain the seed mussel source (Kamermans et al. 2009; Anonymous 2008; Smaal 2002), and conflicts with nature conservation interests due to negative effects of dredging (Kamermans et al. 2009; Anonymous 2008; Dolmer 2002; Smaal 2002).

Bottom culture production is highly dependent on the natural stock of seed mussel, which is known to fluctuate greatly between years (Kamermans & Smaal 2002) (Fig. 2). Blue mussels follow a typical type III mor-



tality pattern (Krebs 2001) by producing a very large number of larvae of which only a very small proportion survive (McGrorty et al. 1990).

Fig. 2. Landings of blue mussels in the Wadden Sea 1965-2007 (tons wet weight) Denmark: DK, Schleswig-Holstein: SH, Niedersachen: Nds and The Netherlands: NL. In The Netherlands and Germany, the fishery is mainly on seed mussels, while fishery on full grown mussels takes place by the other nations (Nehls et al. 2009).

In unexploited populations, individual blue mussel may live for >10 years. The species is dioecious and broad-cast spawning and the planktotrophic larvae develop within 3-4 weeks (Lutz & Hidu 1979). After larval settlement (including metamorphosis), the early juvenile (i.e. spat) may migrate longer distances (10-1,000s meters) by byssus-pelagic drifting (Sigurdsson et al. 1976; Bayne 1964). In most production areas, settling larvae and migrating spat are abundant between April-October (Bayne 1976), and are easily collected on lines suspended in the water column. The ability of byssus-pelagic drifting is lost in individuals of the common seed size (shell length >10 mm). However, individuals retain the ability to relocate themselves over shorter distances (0.01-10s meters) by pedal crawling (Theisen 1968). Understanding the mechanisms behind this behaviour is of key importance for further development of area-intensive production methods.

The recruitment success of mussels to the population a given year depend on a range of biotic and abiotic parameters such as temperature, food supply and especially predation (Gosling 1992). To secure a stable seed source, studies on the use of seeds collected from suspended cultures (hereafter suspended seed mussels) has, recently shown promising results, since the suspended mussels was not consumed at higher rates than wild littoral or sub-littoral seeds (Kamermans et al. 2009; Kamermans et al. 2002). The use of this method can secure or contribute to the seed source (Maguire et al. 2007; Saurel et al. 2004). To support development of the use of suspended seeds, knowledge of performance differences between suspended seed mussels and seed mussels collected from natural bottom mussel beds (hereafter bottom seed mussels) and the mechanisms behind, including predatory response, growth and survival, is needed.

The use of seeds collected on suspended cultures will not only contribute to meet the challenges of the fluctuating seed sources, it might also contribute to a smaller impacted area in mussel production. Collec-

tion of seed mussels on suspended cultures in the water column does not involve dredging. Although the method still affects the seabed by sedimentation (Reviewed in McKindsey et al. 2011), it allows for a higher seed production intensity within an area, than what can be obtained on natural seed mussel beds (Walter & Leeuw 2007). The latter, however, depends on equal or enhanced performance of seed from suspended seed cultures compared to that of bottom seed mussels.

Today bottom culture production of blue mussels includes dredging of the seabed. First, seed mussels are dredged to be transplanted to the culture bed and secondly, the full grown mussels are harvested by dredging. Bottom dredging of mussels affects the seabed for longer or shorter periods depending on substrate and hydrodynamic conditions in a given area (Wijnhoven et al. 2011; Foden et al. 2010; Dolmer 2002). For example, Foden et al. (2010) documented that recovery rates increased with sediment hardness.

Two approaches can be used to intensify the production with the result of limiting the impacted area in relation to bottom culturing. One approach is to reduce predation on the seed mussels transplanted to the culture bed. The biomass ratio between bottom seed mussels and harvestable mussels is relatively low, e.g. in the Dutch Wadden Sea, Kamermans & Smaal (2002) and Dankers (1987) reported ratios of 1:1.5 and 1:1, respectively. In Denmark ratios of 1:0.3 to 1:0.9 have been documented (Kristensen & Lassen 1979) and in Ireland the ratio in some areas can also be lower than 1:1 (Calderwood 2012). One reason for the relatively low ratio was heavy mortality caused by predation at the culture bed (Kamermans & Smaal 2002; Kristensen & Lassen 1997). Predator density is hard to control. Predator control with special dredges, rollers, mops (Korringa 1976) or fences (Spencer 2002; Davies et al. 1980 in Gosling 1992) had varying effects and often disproportional high effort. A second approach could be to increase mussel survival by increasing the search and handling time for predators by increasing substrate complexity. This is especially important for seed mussels because their smaller size makes them especially vulnerable to predation by crabs, Carsinus maenas (Linnaeus, 1758) (Smallegang & van der Meer 2003). If the ratio between transplanted seed and harvested mussels is increased, it can be expected that the same amount of mussels is produced in a smaller area, which will be of benefit for the area-based conflict between interest groups. However, knowledge on the direct effects on increased substrate complexity is needed before the approach can be included in the bottom culture practice.

#### **1.4 Outline of the thesis**

With the aim to study how biological mechanisms can be used to develop the blue mussel bottom culture production method, the constrains of bottom culturing was addressed through the following three research questions:

#### How can the seed mussel source in blue mussel bottom culture be supported?

#### How can survival of mussels in blue mussel bottom culture be improved?

#### How does bottom culture practice support an area-intensive exploitation of blue mussels?

#### 1.4.1 Supporting the seed mussel source in blue mussel bottom culture

The seed source is one of the limiting factors in blue mussel bottom culture production, hence alternative sources and practise for seed harvesting needs to be addressed. Kamermans et al. (2009) and Maguire et al. (2007) concluded that seed mussels from suspended seed cultures can be an additional source of seed for use in bottom culture production, based on their evaluation of the parameters predation rate and seed survival.

Phenotypic plasticity was documented in a great range of organisms (reviewed in Havel 1987), including blue mussels (Reimer & Harms-Ringdahl 2001; Reimer and Tedengren 1996). Blue mussels originating from suspended cultures in the water column are probably not to the same extent exposed to predation by ben-thic invertebrates such as adult crabs, *Carsinus maenas* (Linnaeus, 1758), and starfish, *Asteria rubens* (Linnaeus, 1758) (Gosling 1992), as is often the case for mussels settled on the seabed. Due to different origin of suspended and bottom seed mussels, it was expected that the two types of mussels perform differently when transplanted to the seabed. To study the general differences in predatory response between seed mussels collected on suspended long line cultures and natural bottom seed beds, respectively, and to test the performance of the two mussel types, the experimental studies reported in Chapter 2 and 3 were conducted. Chapter 2 focuses on differences in mussel predatory response to crabs, and the effects of mussel bed structure and time on aggregation and byssal strength in mussels is documented in Chapter 3.

#### 1.4.2 Improvement of survival of mussels in blue mussel bottom culture

Substrate complexity provides spatial refuges and leads to reduced mortality due to increased search (Frandsen & Dolmer 2002) and handling time for predators (Reimer & Tedengren 1997). A high complexity can be achieved through bed formation including aggregation and byssal attachment of mussels and by adding substrate that increase the heterogeneity of the seabed. However, increased complexity through an aggregated structure can also result in reduced growth of the individual mussels (Reimer & Tedengren 1996; Okamura 1986) leading to a trade-off between improved survival and reduced growth. In Chapter 3 and 4 the interaction between substrate complexity and predation was studied on small scale (< 1 m). Experimental results on effects of different substrate compositions on bed formation, loss and growth of mussels on the seabed (Chapter 3) and on migration, growth and condition of blue mussels living in a Pacific oyster, *Crassostrea gigas* Thunberg 1793, reef (Chapter 4) is presented with the aim to evaluate the effect of substrate complexity on survival, growth and on condition of mussels. Investigations focused on behavioural patterns and trade-offs suffered by blue mussels to enhance survival.

#### 1.4.3 Support of area-intensive exploitation of blue mussels

Bottom cultures have to be established using knowledge on best design of beds, optimal seeding-density and area characteristics to support a high production rate (Spenser 2002; Smaal et al. 2001). Bed structure has a great influence on growth and survival of mussels (van der Koppel et al. 2008; Svane & Ompi 1993; Okamura 1986). Since growth and survival are key parameters for production output (Bayne 1976), knowledge on bed structure (including macrostructure of bottom culture, individual mussel position in a bottom culture and seeding density) is studied in Chapter 5, with Danish culture practice as case. The aims of the study in Chapter 5 was therefore to describe the macrostructure (< 300 m) of a commercial bottom culture bed, to test blue mussel growth as a function of position in the bottom culture bed and to test the

effects of different seeding density on production. In model simulations, the area impacted by bottom culture production was analysed as a function of density of fished seed, efficiency of the mussel dredge, seeding density and population production/biomass ratio.

The four studies are presented in Chapter 2 to 5. The main findings in Chapter 2 to 5 are summarised and discussed and a conclusion is provided for each section in Chapter 6. My recommendations and visions upon future perspectives for blue mussel bottom culture production are presented in Chapter 7.

Chapter 2 Comparative study of predatory responses in blue mussels (*Mytilus edulis* L.) produced in suspended long line cultures or collected from natural bottom mussel beds (*Published in Helgoland Marine Research*)

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Blue mussels, Mytilus edulis, in bottom culture bed. Photo Per Dolmer.

## Comparative study of predatory responses in blue mussels (*Mytilus edulis* L.) produced in suspended long line cultures or collected from natural bottom mussel beds

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#### Abstract

Blue mussels (Mytilus edulis L.) are a valuable resource for commercial shellfish production and may also have uses as a tool in habitat improvement, because mussel beds can increase habitat-diversity and complexity. A prerequisite for both commercial mussel production and habitat improvement is the availability of seed mussels collected with minimum impact on the benthic ecosystem. To examine whether mussels collected in suspended cultures can be used for bottom culture production and as tool in habitat improvement, the differences in predatory defence responses between suspended and bottom mussels exposed to the predatory shore crab (Carcinus maenas L.) were tested in laboratory experiments and in the field. Predatory defence responses (byssal attachment and aggregation) and morphological traits were tested in laboratory, while growth and mortality were examined in field experiments. Suspended mussels had an active response in relation to the predator by developing a significantly firmer attachment to the substrate and a closer aggregated structure. Bottom mussels had a passive strategy by having a thicker shell and larger relative size of the adductor muscle. In a field experiment mussels originated from suspended cultures had a higher length increment and lower mortality when compared to bottom mussels. It is concluded that suspended mussels potentially is an alternative resource to bottom culture and can be used in habitat improvement of mussel beds, but that the use of suspended mussels have to be tested further in large scale field experiments.

#### Keywords

Predatory response, *Carcinus maenas, Mytilus edulis*, habitat improvement, bottom culture, long line culture.

#### 1. Introduction

Blue mussels form biogenic reefs, and thereby providing a complex structure to coastal habitats. In addition, blue mussels are a valuable resource for commercial shellfish production including fishery and culturing activities (Smaal 2002). The species also has perspectives as a tool in habitat improvement of costal habitats as the mussel beds increase habitat-diversity and complexity (McDermott et al. 2008). Habitat improvement using bivalves is mainly known in relation to restoration of oyster reefs (e.g. Coen and Luckenbach, 2000; Peterson et al. 2003; Coen et al. 2007), and the focus for many of the habitat improvement projects involving bivalves has typically been reduction of public health risk through improved water quality and to improve the harvest of bivalves for consumption (e.g. Leonard 1993). Only little work can be found in literature documenting the use of blue mussels as an improvement tool, though the species has proven to be suitable for the purpose (Szatybelko and Dubrawski 1999; McDermott et al. 2008). McDermott et al. (2008) reported that restoration of blue mussel beds can increase living marine resource utilization and species diversity within a degraded habitat. Biogenic reefs are included in the EU Habitat Directive and consequently mussel beds may be prioritised for protection in protected areas to fulfil Habitat Directive objectives.

The availability of seed mussels is fundamental for the success of both habitat improvement and culturing activities. The mussels for bottom culture are traditionally collected by dredging seeds on natural mussel beds and relaying the seed to an area with high primary productivity. In several European countries the exploitation of natural populations of blue mussels are restricted or locally banned due to declining populations and changes in management strategies. Alternative resources of seed have to be found if a constant European market supply is to be maintained, and the use of mussels in habitat improvement should have a large-scale potential. Alternative sources of mussel seed have included the development of methods for collection of seed from suspended cultures (Kamermans et al. 2002; Kamermans et al. 2009). This method eliminates the risks of spreading unintended organisms, since transfer of seeds from one area to another potentially involve the risk of spreading of harmful algae (Hégaret et al. 2008) or other harmful organisms.

Predation is the single most important source of mortality in mussels (Gosling 1992). However, mussels from suspended cultures are grown on ropes in the water column with minimum exposure to invertebrate predators (Gosling 2003). In contrast, bottom mussels are exposed to predators such as shore crabs and starfish. It is, therefore, reasonable to assume that mussels originating from bottom beds with predators are more robust to predation than mussels from suspended cultures. A study of blue mussels from the Baltic Sea and the North Sea found that blue mussels from the predator free Baltic Sea still exhibited inducible predator defence but the response was weaker than that exhibited by blue mussels from the North Sea (Reimer and Harms-Ringdahl 2001). In order to evaluate the growth and survival potential of mussels collected on suspended cultures knowledge of their predatory response is central.

Blue mussels use a variety of defence responses to reduce predation risk (Beadman et al. 2003). The mussel can induce both shell thickening (Reimer and Tedengren 1996; Leonard et al. 1999) and shell lip thickening (Smith and Jennings 2000) in response to predatory exposure. Furthermore, development of strong attachment of byssal threads to the substrate, or even to the predator, can occur when there is a high predation rate (Côté 1995; Dolmer 1998; Leonard et al. 1999; Farrell and Crowe 2007). Living in aggregations does also reduce predation (Bertness and Grosholz 1985; Okamura 1986; Reimer and Tedengren 1997) since it prolongs predator search time (Frandsen and Dolmer 2002). The shore crab is one of the most important predators of blue mussels (Davies et al. 1980) and may thus have a large influence of the success of mussels relayed on the sea bed.

In order to test whether mussels produced on suspended cultures can be used for on-growing on the sea bed, laboratory and field experiments were used to explore differences in predatory defence responses between suspended and bottom mussels. Byssal attachment strength, aggregation behaviour and differences in morphological traits were tested in laboratory, while growth and mortality was examined in field experiments.

#### 2. Materials and Methods

#### 2.1 Laboratory experiments

Mussels used in the experiments were collected in the central part of Limfjorden, Denmark (N56 40 E8 45). Mussels used in the experiments were produced on suspended line systems (suspended mussels) and mussels collected from natural mussel beds (bottom mussels). Bottom mussels were marked to make it possible to distinguish them from suspended blue mussels. Shore crabs were collected in cage traps and held unfed in running seawater in the laboratory for four days to standardize level of hunger. Information on collection and sizes of mussels and shore crabs is given in Table 1.

Attachment strength, aggregation behaviour and predation of bottom and suspended mussels were tested using shore crabs placed inside cages with mussels. The cages were 48 cm in diameter and submerged. In each cage, a PVC-plate divided into 36 squares of 5×5 cm was placed on the bottom of the cage, with one mussel placed in each square. Six cages contained 36 bottom blue mussels (separate bottom cages), six cages contained 36 suspended blue mussels (separate suspended cages), and six cages contained a mix of 18 bottom blue mussels and 18 suspended blue mussels (mixed cages). In three cages of each treatment shore crabs were introduced after 48 hours (exposed) and in the other three cages no shore crabs were introduced (controls). After another 48 hours the crabs were removed, their sex was determined and carapace width was measured, and the experimental parameters of the mussels measured (Table 1). Running sea water (salinity 30-32) supplied the cages with fresh seawater during the experiment. The experiment was conducted once, in August 2007 and repeated again in September 2007. Identical protocols were used in both laboratory experiments. After the first experiment in August the size of adductor muscle and shell density for the two types of mussels was determined.

#### 2.1.1 Aggregation behaviour

After each experiment the PVC-plate with mussels attached was removed from the cage and the distribution of mussels photographed. Numbers of bottom mussels and suspended mussels on each plate were counted. Distribution of mussels was quantified by recording the number of mussels in each of the 36 squares. Squares where umbo was located were recorded as a placement square. Coefficient of Variance (CV) of density of mussels was used as measure for aggregation.

#### 2.1.2 Attachment

Mussel attachment was measured with a spring scale attached to individual mussels with a clamp. Attachment was given as the maximum weight (g) the byssal threads held before the mussels detached. Attachment was quantified for 10 randomly chosen mussels from each cage. In cages with a mixture of bottom and suspended mussels of 10 of each mussel type were measured.

#### 2.1.3 Predation

Predation rate (M<sub>pre</sub>) was estimated as the number of eaten mussels per day, and calculated as:

$$\mathsf{M}_{\mathsf{pre}} = \mathsf{In}\left(\frac{N_{t0}}{N_{t1}}\right) \times \mathsf{t}^{-1}$$

where  $N_{t0}$  is the number of mussels at start of experiment,  $N_{t1}$  is the number of mussels at the end of the experiment, and t is the duration of the experiment in days (Frandsen and Dolmer 2002). All factors were assumed to be independent of density, and rates were estimated to be constant throughout the experiments.

#### 2.1.4 Adductor muscle

To investigate differences in the shell closure strength of bottom mussels and suspended mussels the posterior adductor muscle diameter was quantified from mussels used in the August experiment. The posterior adductor muscle was exposed by cutting the muscle along the plane of the shell edge, and measuring the diameter (mm) under a dissection microscope. To obtain the relative size of the posterior adductor muscle mean muscle diameter (m) was related to the length (I) of the shell (Hancock, 1965):

Relative adductor muscle size =  $\left(\frac{m}{l}\right)$ 

#### 2.1.5 Shell index

A sub sample of 10 mussels was used to quantify shell density before the experiment. An index of shell density was calculated from shell ash weight and surfaces area. Ash weight (AW<sub>shell</sub>) was determined via furnace ignition (4 h at 550 °C) followed by weighing. The surface of the mussel shell can be described as a cylinder with an elliptical cross section (Reimer and Tedengren 1996). Surface area was calculated via:

$$A=I\times\sqrt{h^2+w^2}\times\pi/2$$

Where I was shell length, h was shell height and w was shell width given in mm.

Index of shell density was given as:

Shell density =  $\frac{AW_{shell}}{A}$ 

#### 2.1.5 Statistical analysis

Data on attachment strength was tested in a three-way ANOVA as a function of mussel type, presence of shore crab and experiment run. In *a posterior* test the factor Time was excluded from the analysis due to a non-significant effect, and attachment was tested in a reduced ANOVA model with only mussel type and presence of shore crabs. The tests were conducted on the mean attachment strength of bottom or suspended mussels in each cage, as the measurements in each cage may not be independent. Prior to the analysis, data was tested for normality distribution and variance homogeneity. Pair wise multiple comparison Tukey tests were used to establish significant differences between separate groups of data.

Coefficient of variation of aggregation behaviour (CV) was measured together with information on predation, mussel and time for 24 cases. The possible linear relationship was investigated using the linear model E(CV)=Mussel+Predation+Time+Predation:Time. The results of the model are given in Table 2. The table shows that the interaction effect is non-significant on a 95 % confidence level. Hence this effect is then removed from the model and the reduced model E(CV)=Mussel+Predation+Time was analysed (Table 3). Results indicated that the effect of Predation was just non-significant and the model was reduced to the final model E(CV)=Mussel+Time. A Q-Q plot indicates that observations may be considered to be normally distributed.

The predation of mussel types in separate and mixed cages was tested by use of one-way ANOVA. Data on adductor muscle and shell density did not meet the assumptions of normal distribution and variance homogeneity. Data was divided into the different treatments and tested separately using nonparametric student t-tests and Mann-Whitney rank sum tests.

#### 2.2 Field experiment

A field experiment was conducted in Sallingsund (5-6 metres of water depth) in the central part of Limfjorden (N56 42 E8 48) from August to November (105 days) 2007. In August bottom and suspended mussels were relayed in 10 open frames of 2×2 m. In each frame, 25 kg of bottom or suspended mussels were relayed (6.25 kg m<sup>-2</sup>).

#### 2.2.1 Growth, density, mortality, biomass and condition index

Before the mussels were placed in the frames, length was measured from 60 randomly chosen mussels in each frame. Mean length ( $\pm$ SE) of mussels were 40.6  $\pm$ 0.6 mm for bottom mussels and 43.0  $\pm$ 0.8 mm for suspended mussels.

After the experiment, all mussels in three smaller subsample frames (0.25 m<sup>2</sup>) within each of the 10 frames were collected. Mussels were counted, weighed and length measured and shell length increment (mm) was estimated.

Since mortality due to predation and mortality due to other factors could not be distinguished in the field experiment, the rate of total mortality (M<sub>total</sub>) was used as a measure of predation. M<sub>total</sub> was calculated as:

$$\mathsf{M}_{\mathsf{total}} = \mathsf{In}\left(\frac{N_{t0}}{N_{t1}}\right) \times \mathsf{t}^{-1}$$

where  $N_{t0}$  is the number of mussels at the start of the experiment,  $N_{t1}$  is the number of mussels at the end of the experiment, and t is the duration of the experiment in days. All factors were assumed to be independent of density, and rates were estimated to be constant throughout the experiments.

As a measure of intraspecific competition the condition index (CI) for mussels was estimated as:

$$Cl = \left(\frac{Biomass_{end}}{Length_{end}^3}\right) \times 1000$$

where  $Biomass_{end}$  is the wet weight (g) of mussels at the end of the experiment,  $Length_{end}$  is the shell length (mm) of the mussels at the end of the experiment.

#### 2.2.2 Statistical analysis

Differences in density and growth measured as shell length increment for bottom and suspended mussels were tested with student t-test. A one-way ANOVA was used to test for differences in mortality between the two mussel types.

#### 3. Results

#### 3.1 Laboratory experiments

#### 3.1.1 Aggregation behaviour

The experiments showed that suspended blue mussels form significantly more aggregated bed structure in cages than bottom mussels (Linear model P=0.03) (Fig. 1; Table 4). The presence of predators did not affect aggregation (P=0.08; Table 3).

#### 3.1.2 Attachment

For both bottom and suspended mussels the attachment strength was significantly stronger when predators were present compared to when no predators were present (Fig. 2; Table 5). When exposed to predators suspended mussels ( $624 \pm 34$  g) had a significantly stronger byssal attachment ( $\pm$ SE) than both bottom mussels separately ( $365 \pm 24$  g) (Tukey test P=0.002) and bottom mussels from cages mixed with suspended mussels ( $407 \pm 25$  g) (Tukey test P=0.012).

#### 3.1.3 Predation

Mortality due to predation varied between 0.07-0.25 for bottom mussels, 0.07-0.41 for suspended mussels, 0.09-0.29 for bottom mussels in mixed cages and 0.16-0.41 for suspended mussels in mixed cages. Due to large variation in mortality rates within treatments no differences were observed in predation of suspended mussels or bottom mussels, either when mussels were kept separately or when mixed (One way ANOVA P=0.134) (Fig. 3; Table 6). Mortality rates in control cages varied between 0-0.01 for bottom mussels, 0-0.09 for suspended mussels, 0-0.06 for bottom mussels in mixed cages and 0-0.03 for suspended mussels in mixed cages. There were no significant differences in mortality between suspended mussels or bottom mussels were kept separately or when mixed (Kruskal-Wallis test P=0.636) (Fig. 3; Table 6)

#### 3.1.4 Adductor muscle

Comparison of adductor muscle size (±sd) between bottom and suspended mussels showed that bottom mussels (4.6 ±0.1) had a significantly larger relative size of adductor muscle than suspended mussels (4.2 ±0.1) (Mann Whitney Rank Sum Test P <0,001). There were no significant differences between treatments exposed or not exposed to predators for either bottom mussels (exposed 4.7 ±0.7, not exposed 4.5 ±0.5; Student t-test P=0.173) or suspended mussels (exposed 4.2 ±0.3, not exposed 4.3 ±0.5; Mann Whitney Rank Sum Test P = 0.084).

#### 3.1.5 Shell density

Comparisons of relative shell density (mg mm<sup>-2</sup> ±sd) between bottom mussels and suspended blue mussels show that bottom mussels (1.04 ±0.22) had a significantly higher shell density than suspended mussels (0.84 ±0.08) (Mann Whitney Rank Sum Test P<0.001). There were no significant differences between treatments exposed or not exposed to predators for either bottom mussels (exposed 1.04 ±0.25, not exposed 1.04 ±0.21; Mann Whitney Rank Sum Test P=0.818) or suspended mussels (exposed 0.87 ±0.06, not exposed 0.81 ±0.10; Student t-test P=0.209).

#### 3.2 Field experiment

#### 3.2.1 Growth

Difference in mean lengths of bottom mussel and suspended mussels was significant both before (Student t-test P=0.016) and after (Student t-test P<0.001) the experiment (Fig. 4). Shell length increment ( $\pm$ SE) during the experiment was significantly higher for suspended mussels (5.2  $\pm$ 0.4 mm) compared to bottom mussels (3.5  $\pm$ 0.5 mm) (Student t-test P=0.026).

#### 3.2.2 Density, mortality and biomass

Mean density of mussels was reduced during the experiment. Initial mean density of suspended blue mussels was 803 mussels m<sup>-2</sup>, which decreased to 259 mussels m<sup>-2</sup> at the end of the experiment. In contrast, mean density for bottom blue mussels was 978 mussels m<sup>-2</sup> at the start of experiment and 147 mussels m<sup>-2</sup> at the end of experiment. Estimates of mortality rates (day<sup>-1</sup>), showed that suspended mussels (0.01 ±0.0) had a significantly lower mortality than bottom mussels (0.02 ±0.0) (One way ANOVA P=0.006; Table 7). Despite the significant differences in mussel shell length before the experiment there was no significant effect of initial shell length on mortality (One way ANOVA P=0.151).

At the start of the experiment the biomass of both bottom and suspended mussels in the frames were 6.25 kg m<sup>-2</sup>. When the experiment ended the biomass ( $\pm$ SE) of suspended mussels was 2.8  $\pm$ 0.3 kg m<sup>-2</sup> and the biomass of bottom mussels was 1.2  $\pm$ 0.2 kg m<sup>-2</sup>, a reduction of 55 % and 81 % of the total relayed biomass, respectively. The biomass of suspended mussels was significantly higher than the biomass of bottom mussels after the experiment (Student t-test P=<0.001).

#### 3.2.3 Condition index

After the experiment there was no significant difference in condition index between bottom (0.10  $\pm$ 0.01) and suspended mussels (0.10  $\pm$ 0.00) (Student t-test P=0.249).

#### 4. Discussion

The present study found that mussels produced on suspended long lines and mussels collected from natural mussel beds responded differently in relation to predatory defence, growth and mortality. In the laboratory experiment, suspended mussels had a more active predator response in relation to aggregation behaviour and attachment compared to bottom mussels. Bottom mussels have been adapted to predators over time and showed a more passive predator response by their significantly thicker shell density and adductor muscle diameter. The field experiment showed that shell growth was significantly higher for suspended mussels compared to bottom mussels. Furthermore, mortality of bottom mussels was higher than the mortality of suspended mussels, and in total biomass of suspended mussels was larger when contrasted to bottom mussels.

Formation of mussel beds by byssal attachment and aggregation may include a trade off between advantages in relation to reduction of predation and a cost of increased intraspecific competition for food depending on where in the bed structure the mussel is attached (Okamura 1986). In the centre of a mussel bed, individuals reduce the risk of predation but intraspecific competition for food is likely to be greatest (Bertness and Grosholz 1985). In a field experiment Frandsen and Dolmer (2002) observed, that mussels had an increased survival in complex substrates, but a lower growth rate due to a reduced transport of food particles and intraspecific competition. Intraspecific competition does not occur to the same extend on the edge of a mussel bed, where mussels are more exposed to predators (Okamura 1986; Auster 1988). However, in our study there was no significant difference in condition index between bottom and suspended mussels, indicating that there was no intraspecific competition between mussels during the experimental period.

#### 4.1 Aggregation behaviour

Comparison of bottom and suspended mussels showed that suspended mussels formed significantly denser bed structure than bottom mussels. Living in aggregations can reduce the rate of predation on individuals (Bertness and Grosholz 1985; Okamura 1986; Côté and Jelnikar 1999). Okamura (1986) found that blue mussels in the centre of a mussel bed suffer lower predation than mussels on the edge of the bed. The tendency for blue mussels to clump is enhanced under risk of predation (Côté and Jelnikar 1999). The same pattern is known from for example zebra mussel (*Dreissena polymorpha*). In the presence of predators, attachment strength and the tendency to from aggregations increased among small and medium sized zebra mussels (Kobak and Kakareko 2009).

#### 4.2 Attachment

Both bottom and suspended blue mussels increased byssal attachment when exposed to predators. This response to predators was also reported by Leonard et al. (1999), where both mussels from field populations and from cultures had significant higher attachment strength in a habitat with a high density of predators compared to a habitat with a low predator density. In the present experiment, suspended mussels showed significantly stronger attachment and established a more dense bed structure than bottom mussels. Attachment strength was significantly higher for suspended mussels than for both bottom mussels either in separated or mixed populations. The opposite was found in Kirk et al. (2007), where bottom mussels from intertidal beds in general exhibited stronger byssal attachment than suspended mussels. The experiments by Kirk et al. (2007) were conducted on mussels collected from intertidal habitats and long line

mussel farms. Mussels from the intertidal may be exposed to wave surge and therefore may form more byssal threads for attachment than the bottom mussels used in the present experiment.

Byssal treads are not produced continuously but respond to e.g. wave activity (Witman and Suchanek 1984; Young 1985) and predation (Côté 1995; Dolmer 1998; Leonard et al. 1999). Côté (1995) observed that byssal treads become shorter and stronger on mussels exposed to predators. The greater byssal attachment strength of suspended blue mussels in the present study may therefore indicate that suspended blue mussels have developed a strong byssal attachment because they are hanging from ropes exposed to waves in contrast to bottom mussels (Kirk et al. 2007) or suspended mussels are compensating for a lack of a thick shell as a predatory defence.

#### 4.3 Adductor muscle

Bottom mussels had a significantly larger adductor muscle relative to shell length compared to suspended mussels. Due to the relatively short time span of our experiment and the uncertainties of the measurements it would not be possible to detect differences in relative adductor muscles size between mussels exposed and not exposed to predation. This was confirmed since there were no significant differences between either bottom or suspended mussels exposed and not exposed to predation. Differences are assumed to be a result of the origin of the two types of mussels. Field studies from Limfjorden have shown that blue mussels on smooth substrate develop a significantly larger adductor muscle than mussels on a more complex substrate due to higher predator exposure (Frandsen and Dolmer 2002). The relative size of the adductor muscle in our laboratory experiment is comparable to the adductor muscle size from field experiments where mussels were laid on a smooth substrate and exposed to predators (Frandsen and Dolmer 2002). Reimer and Harms-Ringdahl (2001) showed that mussels can adjust their predatory defences depending on the type of predator and their prey handling. Larger adductor muscles are developed when mussels are exposed to starfish (Reimer and Tedengren 1996). Contrary, thicker shells are developed when mussels are exposed to shore crabs (Reimer and Harms-Ringdahl 2001).

#### 4.4 Shell density

Lack of, or reduced predator exposure of suspended mussel cultures may result in a reduced development of inducible shell density, compared to bottom mussels exposed to predators (Kirk et al. 2007). This result was also evident in the present study. The mean shell density (±SE) for bottom mussels in our experiment was 1.03 ±0.28 mg mm<sup>-2</sup> are comparable to the values given by Reimer and Tedengren (1996), where blue mussels exposed to predators on a wave exposed rocky shore had a shell thickness of 99 mg cm<sup>-2</sup>. Suspended mussels in our experiment also exhibited thinner shell relative to length, which is comparable to results from controls in the study by Reimer and Tedengren (1996) and Kirk et al. (2007). Development of thicker shells was observed in mussels exposed to predation by crabs or cues from crab predators (Leonard et al. 1999; Reimer and Harms-Ringdahl 2001). The thinner shells in suspended mussels are also a result of faster growth rates when the mussels are suspended in the water column (Garen et al. 2004).

Suspended mussels are reported to have lower shell thickness and weaker byssal attachment due to differences in environmental conditions and predation regimes (Kirk et al. 2007). Because of the relatively short time span of our experiment, it would not have been possible to detect any change in shell density as a result of predator exposure. This was also confirmed by the non significant difference between bottom and suspended mussels exposed and not exposed to predation. The significant difference between suspended and bottom mussel is therefore assumed to be due to the differences in origin as shown in Kirk et al. (2007).

#### 4.5 Mortality

In the present field experiment the mortality rates were at same level as previously estimated rates of mortality due to predation by shore crab on a smooth substrate in Limfjorden (Frandsen and Dolmer 2002). The increased aggregation by suspended mussels observed in current study increases the substrate complexity, and may therefore explain the difference in predation or mortality. In the field experiment the mortality rate of bottom mussels was higher than the rate for suspended mussels, whereas no differences in mortality were seen in the laboratory experiment. Measured predation rates may be influenced by different initial sizes of suspended and bottom mussels (Reimer and Tedengren 1996; Kirk et al. 2007). However, statistical test stated that there were no significant effects of shell length on predation. In the laboratory experiment, mussels were exposed to predation in an experimental setup without a natural bed structure, as was the case in the field experiments. Therefore, predation rates from laboratory experiments can only be used to compare the two types of mussels and should not be used as an exact measure of mortality due to predation.

#### 4.6 Active versus passive predator response

Analysis of byssal thread attachment and aggregation showed that suspended mussels develop a more predation resistant bed structure than bottom mussels. In contrast, bottom mussels had a thicker shell density and a larger adductor muscle. The predation rate of suspended mussels in field experiments was significantly lower for suspended mussels compared to bottom mussels.

The field experiment differs from the laboratory experiment in not controlling the density and species composition of the naturally present predators. An unknown number, species and size composition and activity of shore crabs (*Carsinus maenas*) and starfish (*Asterias rubens*) predated on mussels. Defence responses against the different predatory species can differ since the presence of starfish often facilitates a larger adductor muscle and shore crabs is known to increase mussel attachment (Côte 1995; Leonard et al. 1999). Attempts to estimate the density of these two predators failed and therefore it is not possible to distinguish the effect of the natural predators. In a controlled cage study by Kamermans et al. (2009) consumption of mussel seed by starfish was much lower than by crabs, which is why we assume that the main predation in the field experiment was from crabs.

Selection of prey by a predator can be influenced by the size and the morphology of the prey items (Kirk et al. 2007). Parameters as growth rate, byssal thread strength and shell thickness of mussels can affect both predation attempts and success (Norberg and Tedengren 1995; Reimer and Tedengren 1996). In a study by Reimer and Harms-Ringdahl (2001), where blue mussels from the North Sea and the Baltic Sea were compared in terms of predator inducible changes, it was concluded that inducible plasticity was still present in blue mussels from the Baltic Sea even though they were not naturally exposed to predatory crabs and star-fish. Correspondingly, suspended mussels in our experiment still show predatory defence mechanisms despite their origin on predator free suspended long lines.

#### 4.7 Use of suspended blue mussels in relaying of mussels

Both laboratory and field experiments demonstrated that blue mussels produced on suspended systems represents a viable alternative to bottom mussels both in relation to bottom culturing activities and for relay in relation to habitat improvement. It should be noted that mussels used in present study was generally (about 20 mm) larger than the typical size of mussels used for culturing and habitat improvement activities. However, since the predatory responses we have reported in our experiments also have been reported in smaller mussels (20-45 mm Norberg and Tedengren 1995; 16-33 mm Côté and Jelnikar 1999; 15-30 mm Reimer and Harms-Ringdahl 2001) we expect that our results are also valid for mussels in the size range typically used for relay and transplantation. Survival and growth in the field experiment was higher for suspended mussels due to a more adaptive predator defence response, which included higher byssal production and higher aggregation activity compared to bottom mussels. This conclusion is supported by Kamermans et al. (2009) who tested the applicability of mussels from seed collectors as a seed source in bottom culture production in relation to predation loss caused by crabs and starfish. The use of suspended mussels as seed for bottom cultures and habitat improvement require a full scale test comparing the two types of mussel over time.

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#### **Figure legends**



Fig. 1. Aggregation of mussels (*M. edulis*) measured as Coefficient of Variance ( $\pm$ SE) of density of bottom and suspended mussels, when exposed and not exposed to shore crab (*C. maenas*) (n=6 replicates per treatment).



Fig. 2. Difference in strength of byssal treads (g) ( $\pm$ SE) for bottom and suspended mussels (*M. edulis*), in separate and mixed cages exposed and not exposed to shore crab (*C. maenas*) (n=6 replicates per treatment).


Fig. 3. Predation rate ( $\pm$ SE) in laboratory experiment on bottom and suspended mussels (*M. edulis*) in separate and mixed mussel cages, respectively. Predation rates are estimated as total mortality in each cage. Due to large variation in predation rates within the treatments statistical test showed no significant differences between treatments.



Fig. 4. Initial and end shell length (mm) (±SE) of mussels collected from natural mussel beds and suspended cultures.

# **Table legends**

Table 1. Overview of number of days blue mussels were caught before experiment, mean length of mussels used and mean carapace width of shore crabs used in the experiments.

Experiment	Origin of blue mussels ( <i>M.</i> <i>eđulis</i> ) (bottom/ suspended)	Number of days bottom/suspended blue mussels ( <i>M. edulis</i> ) were col- lected before experiment	Mean length (± sd) of bottom/ suspended blue mussels ( <i>M.</i> <i>eđuiis</i> ) (mm)	Number of days shore crab ( <i>C.</i> <i>maenas</i> ) were collected before experiment	Mean carapace width (± sd) for shore crab (C. maenas) (mm). N=18
August	Kaas Bredning/	17/10	37.9±1.8/	5	$65.2 \pm 3.4$
	Sallingsund		$34.9 \pm 2.2$		
September	Commercial	2/20	37.0±1.9/	5	$62.6 \pm 2.6$
-	fishery/		$40.2 \pm 2.4$		
	Sallingsund				

Table 2. Results of linear model on aggregation behaviour in bottom and suspended mussels given as Coefficient of Variance (CV). The interaction effect is non-significant on a 95 % confidence level. Hence the effect was removed from the model.

Coefficients	Estimate	SE	t-value	Р
(Intercept)	1.821	0.164	11.08	9.8e-10
Mussel	0.362	0.147	2.46	0.036
Predation	0.164	0.208	0.79	0.4405
Time	-0.635	0.208	-3.05	0.0065
Predation:Time	0.214	0.294	0.73	0.4763

Table 3. Results of linear model on aggregation behaviour in bottom and suspended mussels given asCoefficient of Variance (CV). Predation was non-significant on a 95 % confidence level. Hence the effectwasremovedfromthemodel.

Coefficients	Estimate	SE	t-value	Р
(Intercept)	1.768	0.145	12.17	1.06e-10
Mussel	0.362	0.145	2.49	0.0217
Predation	0.271	0.145	1.86	0.0772
Time	-0.528	0.145	-3.63	0.0017

Table 4. Results of linear model on aggregation given as Coefficient of Variance (CV) with the parameters type of mussel (Mussel) and experimental run (Time).

Coefficients	Estimate	SE	t-value	Р
(Intercept)	1.9032	0.1330	14.313	2.65e-12
Mussel	0.3616	0.1535	2.355	0.0283
Time	-0.5278	0.1535	-3.438	0.00247

Table 5. Results of Two ways ANOVA on byssal attachment strength of bottom and suspended mussels.

Comparison	DF	SS	MS	$\mathbf{F}$	Р
Mussel	3	320475.227	106825.092	7.937	< 0.001
Predation	1	463512.948	463512.948	34.436	< 0.001
Mussel×Predation	3	24008.114	8002.705	0.595	0.622
Residual	40	538398.737	13459.968		
Total	47	1346395.076	28646.704		

Table 6. Results of one way ANOVA on differences in mortality due to predation on bottom and suspended mussels.

Comparison	$\mathbf{DF}$	SS	MS	$\mathbf{F}$	Р	
Mussel	3	0.0291	0.00971	1.214	0.330	-
Residual	20	0.160	0.00799			
Total	23	0.189				

Table 7. Results of a one way ANOVA on difference in mortality due to predation of bottom and suspended mussels.

Comparison	DF	SS	MS	$\mathbf{F}$	Р
Mussel	1	1.501	1.501	14.116	0.006
Residual	8	0.851	0.106		
Total	9	2.352			

Chapter 3 Aggregation and attachment responses of Blue mussels, *Mytilus edulis* - impact of substrate composition, time scale and source of mussel seed *(Submitted to Aquaculture)* 



Experimental setup to study aggregation and attachment strength of blue mussels, Mytilus edulis, as a function of substrate composition, time scale and seed mussel source. Photo Per Dolmer.

# Aggregation and attachment responses of Blue mussels, *Mytilus edulis* - impact of substrate composition, time scale and source of mussel seed

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# Abstract

Survival after transplantation of mussel seeds is crucial for the production output of blue mussels (Mytilus edulis L.) in bottom cultures. Hence, an understanding of the interactions between bed formation, habitat structure and performance of mussel seed with different origins can contribute to an optimization of the production. The effect of substrate composition and timing of formation of a mussel bed in relation to aggregation and attachment of mussels were investigated with mussel seeds obtained from two different sources: mussel seed dredged from a natural mussel bed and mussel seed collected from a suspended long line culture. The mussels was applied to experimental unites of complex and smooth substrate on the sea bed. Data on aggregation (day 0-3), attachement strength (day 2 and 30), loss (day 3 and 30) and growth (day 30) of mussels was collected during the experiment. The results showed that complex substrate indeed had a stabilizing effect on the mussel structure resulting in less aggregation and increased attachment strength. The 3D matrix forming a mussel bed was achieved faster on complex substrate, and led to reduced mortality of transplanted mussels. Furthermore, suspended mussels aggregated more and faster and had a stronger and more rapid attachment as compared to bottom mussels. Consequently, it was concluded that when transplanting mussels, seeding with substrate increases surface complexity on the seabed and can increase survival of the mussels. To this end suspended mussels could be a promising alternative to the traditionally used bottom mussel seeds.

Key words: *Mytilus edulis*, aggregation, attachment, substrate, bottom culture

# Introduction

Blue mussels, *Mytilus edulis* (L.), live in robust aggregated structures stabilized by byssal threads. The mussel beds form a 3D matrix consisting of 1) a physical matrix of interconnected living and dead mussel shells, 2) a bottom layer of accumulated sediments, mussel faeces and pseudo-faeces, organic detritus and shell debris and 3) a taxonomically diverse assemblage of associated flora and fauna (Suchanek 1979 (*Mytilus californianus*); Tsuchiya and Nishihira 1985). The structure provides stability and protection against predation and other disturbances for the individual mussel (Bertness and Grosholz 1985). A number of studies document that the bed formation in relation to aggregation and attachment strength is enhanced if blue mussels are exposed to predators (e.g. Reimer and Tedengren 1997; Schneider et al. 2005; Christensen et al. 2012). Young (1985), demonstrated that byssal threads are produced by the individual mussel in response to a number of contributing factors, of which agitation was the most powerful effect observed. Disturbance from e.g. wave activity or currents have also been shown to increase aggregation and attachment in blue mussels (Witman and Suchanek 1984; Bertness and Grosholz 1985) (*Geukenia demissa*); Dolmer and Svane 1994). The aggregated structure of closely attached mussels is therefore important at both individual and population level.

The firmness and resilience of the mussel bed to physical disturbance are determined by the aggregation and attachment of the blue mussels. Studies of aggregation and attachment have mostly focused on the effect of predation (e.g. Reimer and Tedengren 1997; Reimer and Harms-Ringdahl 2001; Farrell and Crowe 2007; Christensen et al. 2012), whereas studies of the combined effect of substrate composition and time scale is absent in literature. When closely aggregated and firmly attached to the substrate and to other mussels, both search time (Frandsen and Dolmer 2002) and handling time for the predators increase (Reimer and Tedengren 1997). Thus, mussels which are more aggregated and have stronger byssal threads exhibit higher survival due to reduced predation (Bertness and Grosholz 1985 (*Geukenia demissa*); Okamura 1986).

When transplanting blue mussels to bottom cultures on the sea bed for production purposes, knowledge on the bed formation process and the effects of substrate composition is highly relevant for determining the method to use in order to optimize production. Ultimately, survival and growth of seeded mussels determine the final harvest output. In bottom cultures, the ratio between biomass of seeded mussels and harvested mussels is reported to be 1:1.5 and 1:0.3-0.9 in the Netherlands (Kamermans and Smaal 2002) and in Denmark (Kristensen and Lassen 1997), respectively. A field study during four months in 2007 demonstrated that the ratio was 1:2 and that approximately 50 % of the biomass production was lost from bottom cultures due to mortality (Dolmer et al. submitted). The causes for mortality were not revealed and studies on how survival can be increased are therefore crucial for optimizing the harvest output.

One way to obtain mussel seed for transplantation to bottom cultures is from dredge fishery on natural seed mussel beds (bottom mussels). Another source is to produce mussel seeds on suspended long line systems in the water column (suspended mussels) (Kamermans et al. 2009). Studies have shown that the two types of mussels respond differently in relation to predatory defence, growth and mortality (Kamermans et al. 2009; Christensen et al. 2012). Suspended mussels have lower shell thickness and compensate for this by an active response where they develop a significantly firmer attachment to the substrate and a closer aggregated structure, while bottom mussels have a more passive response by having a thicker shell and larger relative size of the adductor muscle (Christensen et al. 2012). An evaluation of the mussel seed source used in bottom cultures and methods to improve survival is therefore essential.

The present study examin the effect of substrate composition and the time-course of formation of a mussel bed in relation to the aggregation and attachment of mussel seeds from two different sources: mussel seeds dredged from a natural mussel bed and mussel seeds collected from a suspended long line culture. A field experiment tested the hypothesis that 1) mussels on complex substrate (composed of mussel shells) would aggregate less compared to mussels on smooth substrate (composed of sand), 2) mussels attach more strongly to the complex substrate compared to mussels on smooth substrate, 3) aggregation and attachment strength change with time (<30 days) after transplantation, and 4) suspended mussels aggregate more and achieve a stronger attachment compared to bottom mussels.

## Materials and methods

#### Study area and experimental design

The field study was conducted from September to October 2010 in Helnæs Bight (10 7.17E 55 9.10N) south west of the Island of Funen, Denmark. The estuary is a protected bay with two connecting sounds to Lille Belt. The mean water depth is 5.5 m and the maximum depth is 12 m (Rask et al. 2000). The present experiment was conducted at a water depth of approximately 7 m. Water temperature during the experiment was 14°C and salinity ranged from 12 to 20 PSU at the study site.

Mussels were collected from a suspended long line system (suspended mussels) in Helnæs Bight and from natural mussel beds (bottom mussels) in the adjacent Lille Belt north east of Helnæs Bight, at a depth of 10 m. Growth conditions at the two collection sites were similar regarding water temperature and salinity. Mussels were collected two days before experiment start and kept out of water in a cool room (5-8 °C) during preparation of the experiment.

Initial shell length (to the nearest 0.1 mm), condition index, size of adductor muscle relative to shell length and shell index of mussels (n = 60 for both suspended and bottom mussels) was measured (Table 1). Experimental units consisted of fibre cement tile panels of  $50 \times 50$  cm. Eight different treatments were tested including the parameters: smooth versus complex substrate, suspended versus bottom mussels, and time. Panels with a stretched out fishnet and sand embedded in cement represented smooth substrate and panels with empty shells embedded in cement represented complex substrate. The different experimental panels were placed randomly by diver in a 2 × 24 matrix in the experimental area. Thereafter, 50 mussels were randomly distributed on each panel.

#### Aggregation and attachment strength

After establishment, each experimental panel was photographed to document initial distribution (d0) of mussels as a baseline for aggregation, and photos were taken after 1 day (d1) and 2 days (d2) (n = 12 replicates).

Distribution of mussels was quantified by marking the umbo of all mussels in each digital photograph using Adobe Photoshop 8.0.1. Afterwards, the panel in each photograph was divided into 25 equally sized squares and the number of marked umbos in each square was counted. Coefficient of variance (CV) of density was used as a measure for aggregation. To be able to compare results, data was corrected for loss:

$$N_{corr} = N_{square} / N_{total} \times N_{initial}$$

where  $N_{square}$  is the number of mussels in a specific square,  $N_{total}$  is the total number of mussels left in the panel and  $N_{initial}$  is the initial number of mussels in the panel (n = 50). After correction the CV was calculated for each panel.

Data on attachment strength was collected two days (d2) and 30 days (d30) after experimental start (n = 6 replicates). The experimental panels were collected from the seabed by SCUBA diver. Immediately after the collection mussel attachment strength was measured, using a spring scale attached to individual mussels with a clamp. Attachment of 10 mussels per replicate was measured to secure that only mussels from un-

disturbed structures were included in the measurements. Mussels connected to the spring scale were pulled in a 90° angle from the substrate until they detached. Attachment was given as the maximum weight (g) held before mussels detached (Lee et al. 1990; Dolmer and Svane 1994; Christensen et al. 2012).

#### Loss of mussels and specific growth rate

The number of mussels left on the experimental panel was registered at day 3 (d3) and day 30 (d30) of the experiment (n = 6 replicates). The rate of loss is given as:

$$Loss = \ln(N_0/N_t) \times t^{-1}$$

where  $N_0$  is the number of mussels at the experimental unit at the start of the experiment,  $N_t$  is the number of mussels at a given time and t is the time. The rate expresses the sum of predation and migration of mussels.

Dry weight of mussels on the experimental units was measured (n= 6 replicates) before (n = 30) and after (n = 11 to 30 per treatment) the experiment. Soft tissue from the mussels was dried at 90  $^{\circ}$ C until weight was stable. The specific growth was calculated as:

$$\mu = \ln(dw_t/dw_0) \times t^{-1}$$

where  $dw_t$  is dry weight (mg) of soft parts a given time,  $dw_0$  is the dry weight (mg) of soft parts at the start of the experiment and t is the time.

#### **Statistical analysis**

Data on aggregation, attachment strength and rate of lost mussels were tested as a function of substrate (complex, smooth), time (d0, d1, d2 for aggregation; d2, d30 for attachment) and mussel (suspended, bottom) (Three Way ANOVA). The unadjusted Holm-Sidak method was used as post hoc analysis to test for interactions between the independent factors. Specific growth rate was tested as a function of mussel type and substrate (Two Way ANOVA). Prior to the analyses, data were tested for normality and variance homogeneity. Data on loss rate did not meet the assumptions of normal distribution and was thus log10 transformed. All statistical tests was analysed in SigmaPlot 11.0.

# Results

#### Aggregation and attachment strength

Substrate composition, time, and mussel seed source had a significant effect on mussel aggregation (Three Way ANOVA, p = 0.001) and there was a significant interaction between substrate composition and time (p < 0.001) (Fig. 1; Table 2). Aggregation increased significantly between each sampling on smooth substrate (Holm-Sidak method d0 to d1 p < 0.001; d1 to d2 p = 0.014). On complex substrate aggregation also increased with time, but the increase was only significant between the first (d0) and third (d2) sampling (Holm-Sidak method p = 0.002). Analyses of the interaction between substrate composition and time showed that mussels were significantly more aggregated on complex than on smooth substrate at the start of the experiment (d0) (Holm-Sidak method p = 0.002). However, at d1 and d2 mussels on smooth substrate were significantly more aggregated than mussels on complex substrate (Holm-Sidak method p < 0.001). On both smooth and complex substrate aggregation activity was significantly higher between first and second sampling compared to second and third sampling. The significant effect of mussel type showed that suspended mussels aggregated significantly more than bottom mussels (Three Way ANOVA, p = 0.001) (Fig. 1; Table 2).

Attachment strength was significantly higher on complex than on smooth substrate, and attachment increased significantly with time. Moreover, attachment strength was significantly higher for suspended than for bottom mussels (Three Way ANOVA, substrate composition (p < 0.001), time (p < 0.001) and mussel type (p = 0.002) (Fig. 2; Table 3)).

### Loss of mussels and specific growth rate

Substrate composition, time and mussel type significantly affected the loss rate of mussels on the experimental panels (Three Way ANOVA, p < 0.05) (Fig. 3; Table 4). The rate of loss was significantly higher on smooth compared to complex substrate. The rate of lossdecreased significantly with time and the loss rate was additionally significantly higher for suspended mussels compared to bottom mussels.

Similarly, specific growth rate of mussels was significantly affected by mussel type and substrate composition (Two Way ANOVA, p < 0.05). The specific growth rate was significantly higher on smooth substrate compared to complex substrate (p = 0.001), and bottom mussels had significantly higher specific growth rate in contrast to suspended mussels (p < 0.001) (Fig. 4; Table 5).

Thus, results showed that mussels generally form a stabilized aggregation faster and become stronger attached on complex compared to smooth substrate. Besides, suspended mussels were more aggregated and had stronger attachment strength compared to bottom mussels. The loss of mussels was lower among bottom mussels and they achieved a higher specific growth rate.

# Discussion

The substrate composition of the seabed is important in relation to the production of mussel beds as the complexity modulates interactions between the blue mussels and their predators (Moksnes et al. 1998; Frandsen and Dolmer 2002). Hence, the self-organisation of mussel beds in relation to aggregation and attachment increases habitat complexity and affects the population dynamic of the species (van de Koppel et al. 2008) increasing the fitness of the mussel population.

# Aggregation

The reduced aggregation response of mussels on complex substrate compared to smooth substrate, corresponded with the results reported in Frandsen and Dolmer (2002). They documented that mussels on complex substrate are more protected from predation by shore crabs (*Carcinus maenas*) as compared to mussels on smooth substrate (Frandsen and Dolmer 2002), since more complex substrate increase the number of spatial refuges (Moksnes et al. 1998), that increase search time. Mussels aggregate as a result of disturbance by predators and wave activity (Reimer and Tedengren 1997; Côté and Jelnikar 1999; Kobak et al. 2010 (*Dreissena polymorpha*); Christensen et al. 2012). The more the mussels aggregate the better they are protected against disturbances (Okamura 1986). However, substrate complexity seems to reduce the need to aggregate.

The temporal development is central when evaluating how fast transplanted mussel seeds are resistant to predation, since the predatory response of mussels change with time when adapted to the environment (Reimer and Harms-Ringdahl 2001 (duration: 28 days, predator-induced plasticity); Beadman et al. 2003 (duration: three months, shell thickening to resist predation); Yanick et al. 2003 (duration: 16 months, effect on survival and growth)). The findings in present study that the level of aggregation increased within a time scale of two days and that the increase in aggregation over time was less on complex substrate than on smooth substrate indicate that mussels on complex substrate stabilize the bed forming response faster than on smooth substrate. On the complex substrate mussels have the protection from predators from the substrate right after transplantation due to more spatial refuges, in contrast to on smooth substrate where

mussels need to aggregate to achieve the same protection, indicating an increased risk of predation on smooth substrate.

Since aggregated mussels are more protected against predators than solitary mussels (Bertness and Grosholz 1985), the higher aggregation of suspended mussels indicates that these mussels are better in achieving the protection provided by group living compared to bottom mussels. The results are supported by Christensen et al. (2012), where suspended mussels on smooth substrate had a more active predator response including more aggregated structures compared to bottom mussels which were protected by a thicker shell and larger adductor muscle. The more active response among suspended mussels is explained in the origin of the mussels on the suspended long lines where they develop a strong byssal attachment because they are hanging from ropes exposed to waves in contrast to bottom mussels (Kirk et al. 2007) and in a compensation for a lack of a thick shell as a predatory defence (Christensen et al. 2012). The study thereby confirmed the hypothesis that mussels on complex substrate aggregated less than on smooth substrate, since it was demonstrated that mussels on complex substrate are more protected right after transplantation, since the bed structure is faster stabilized.

#### Attachment strength

The measured attachment strength in the present study was in the same range as registered *in situ* in Dolmer (1998). As earlier described does mussel increase their byssal attachment in response to disturbances (e.g. Christensen et al. 2012). The enhanced attachment strength on complex substrate compared to smooth substrate was expected as the complex substrate forms a 3D structure with both horizontal and vertical dimensions in contrast to the smooth substrate with only horizontal dimensions. The smooth substrate will not achieve the 3D structure until mussels have aggregated. The extra dimension of the complex substrate allow a more complex attachment and increases the stability of the structure. In the present study the number and angle of byssal threads was not quantified, but it has earlier been documented how mussels secrete more and thicker byssal threads as a result of disturbances (predation) (Côté 1995; Reimer and Tedengren 1997; Dolmer 1998 (*Asteria rubens*); Farrell and Crowe 2007; Kobak et al. 2009 (*Dreissena polymorpha*)). However, the results in present study indicate that substrate also has an effect on byssal attachment.

The significant increase of attachment strength with time indicates that mussel bed structure stabilise with time. High attachment strength increase predator handling time (Reimer and Tedengren 1997) and resistance to wave disturbance (Witman and Suchanek 1984; Dolmer and Svane 1994). From the literature it is unclear how the secretion of byssal threads changes with mussel size (Lee et al. 1990; Clarke and McMahon 1996; Babarro et al. 2008). In the present experiment suspended mussels were significantly smaller than bottom mussels. However, the mean size difference between suspended and bottom mussels was 4.1 mm and the mussels were considered as being in the same size class (Lee et al. 1990; Babarro et al. 2008). The difference in mussel size is therefore not expected to interfere with our results.

On smooth substrate the mussels depend on attachment to other mussels to form the complex mussel bed. The mussel beds are dynamic as mussels move in competition for food and protection (Gosling 1992). Thus, attachment to other mussels will not be as constant and firm as on complex substrate where mussels aggregate less. In addition, the dead shells providing the more complex substrate also stabilize the structure. The results can be compared with mussels in bed formation. Mussels in the centre of a mussel bed are more fixed in their position due to the greater entanglement of the neighbouring mussels compared to mussels on the edge of a group (Okamura 1986).

Suspended mussels were significantly stronger attached to the substrate compared to bottom mussels. From an earlier study by Christensen et al. (2012) it was demonstrated that the development of high attachment strength was part of a more active response to predation compared to bottom mussels. Christen-

sen et al. (2012) described how suspended mussels compensated for a thinner shell and smaller adductor muscle by producing higher attachment strength and increased aggregation activity. We thereby confirmed the hypothesis that a complex substrate result in a stronger attached mussels which are better protected in the presence of predators. Compared to bottom mussels suspended mussels were even more protected due to their significantly stronger attachment.

#### Loss of mussels

The lower specific loss rate on complex compared to smooth substrate indicate that the substrate protective effect which also corresponds with the reduced aggregation and stronger attachment on the complex substrate. More complex and heterogenic substrate increased the number of spatial refuges (Moksnes et al. 1998) which provided protection against disturbances. This was also confirmed by Frandsen and Dolmer (2002) who demonstrated that mussels on complex substrate were more protected compared to smooth substrate. Hence, the significantly lower rate of loss documented on complex substrate in the present study support that mussels are more protected on this substrate.

The declining rate of loss with time can be a result of two different mechanisms. Firstly, since mussels become more aggregated and stronger attached to the substrate with time, they are also more protected. Secondly, a functional response in the predators feeding success results in decreasing loss with decreasing prey density. As the number of prey decrease the search time increases resulting in a decline in rate of loss. The significantly higher loss of suspended mussels compared to bottom mussels was contrary to what was documented by Kamermans et al. (2009) who found that mussels collected on suspended systems did not suffer higher mortality due to predation than bottom mussels. In the present study, the rate of loss included both mortality due to predation and loss from the area due to migration of mussels. The higher aggregation activity among suspended mussels. On complex substrate, aggregation activity was significantly lower and there were no significant differences between the number of suspended and bottom mussels lost during the experiment in present study. Frandsen and Dolmer (2002) interpreted the reduced predatory response (adductor muscle and shell thickness) on complex substrate to be a result of decreased physical disturbance, reduction of water circulation and thereby the transport of chemical cues or a combination of the two.

#### Specific growth rate

The significantly higher specific growth rate on the blue mussels on smooth substrate indicates that food availability is reduced on complex substrate. Eschweiler and Christensen (2011) documented that mussels living in the protective interspaces of a Pacific oyster, *Crassostrea gigas*, reef also suffer reduced growth compared to mussels living on top of the reef. The flow of water is affected by the substrate complexity and thereby affects the flow of food particles to the mussels positioned on the bottom of the reef. Frandsen and Dolmer (2002) also demonstrated that complexity can have a negative effect on growth rate due to limited food supply caused by decreased water flow in the cavities of the complex substrates which correspond with the results in present study.

The higher specific growth for bottom mussels contrasted to suspended mussels can be explained in the significantly higher condition index (test not shown) of suspended mussels from the start of the experiment. Condition index for suspended mussels was around three times higher as for bottom mussels (Table 1). The difference between the two mussel types is due to the higher food availability in the water column (Garen et al. 2004) on the suspended long lines. When suspended mussels are transplanted to the sea bed, food availability is significantly reduced resulting in reduction in growth rate and condition index. Thus, the negative growth documented for suspended mussels on complex substrate can most likely be explained by an insufficient food availability to sustain the high condition index of the suspended mussels. The initial condition index of both suspended and bottom mussels correspond however to those (range in condition

index: 1.79-7.35 mg cm<sup>-3</sup>) documented in a growth and filtration study conducted by Clausen and Riisgård (1996) one meter below water surface in a Danish estuary system.

#### The use of substrate and suspended mussels

A higher production output can be expected when transplanting mussels to complex substrate. With the protection provided by the complex substrate, there is a potential for substrate to substitute higher densities of mussels and yet achieve the same level of protection as documented as one of the advantages of living in aggregated structures (Bertness and Grosholtz 1985). Lower mussel density would reduce a possible intra-specific competition for food and thus increase growth. The fewer mussels in a group and the closer to the edge mussels are placed, the lower the intra-specific competition (Okamura 1986), resulting in a higher growth (Svane and Ompi 1993). Contrary, the advantage of group living is reduced mortality due to predation and other disturbances (Bertness and Grosholtz 1985). We document in the present study that the complex substrate had a negative effect on specific growth rates. Thus, the protective structure of the complex substrate and the exposed smooth substrate introduced a trade-off, since both loss rate and specific growth rate was reduced on complex substrate. However, estimates of the development in mussel biomass (data not shown) during the experiment documented that the total biomass production was higher when mussels were placed on complex substrate. Potentially, seeding with substrate can therefore reduce the seeding density of mussels and thus reduce intra-specific competition, while still maintaining the level of protection provided by the complexity of the substrate instead of by the neighbouring transplanted mussels.

The use of suspended mussels as source of seed for bottom cultures has on basis of present and other studies (Kamermans and Smaal 2002; Kamermans et al. 2009; Christensen et al. 2012) perspectives in relation to development of a more sustainable and stable bottom culture production. Increased focus on the environmental policies in EU and an aim to be able to withstand the fluctuating availability of mussel seeds makes suspended mussel seeds an ecological feasible alternative to bottom mussel seeds. The traditional mussel dredging for seeds affects the sea bed in the areas where the seeds are fished (Collie et al. 2000; Dolmer and Frandsen 2002; Riis and Dolmer 2003). Contrary, seeds produced on suspended long lines are harvested directly from the long lines and dredging of the sea bed is not included. While not affected by dredge fishery the production of seeds still has an effect beneath and around the long line system (McKindsey et al. 2011). However, the use of mussel seeds collected on suspended long line systems makes it possible to manage the total bottom culture production including both seed source and grow out area with respect to nature conservation and seed source stability. This way the impacted area in the exploitation of mussels can be reduced (Dolmer et al. submitted).

#### Conclusion

The hypothesis on the effect of complex substrate on aggregation and attachment was confirmed since blue mussels on complex substrate aggregateed less and were more strongly attached to the substrate compared to mussels on smooth substrate, and the effect increased with time. The complex substrate had a stabilizing effect on the mussel structure resulting in less aggregation and increased attachment strength. The 3D matrix which forms a mussel bed was achieved faster on complex substrate, which led to reduced mortality of the transplanted mussels. Furthermore, the hypothesis that suspended mussels aggregated more and faster and had a stronger and more rapid attachment compared to bottom mussels was confirmed, resulting in a lower loss rate. Thus, when transplanting mussels to the sea bed suspended mussels proved to be a promising alternative to bottom mussels.

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# **Tables and figures**

Table 1. Data on initial shell length (mm), condition index (mg cm<sup>-3</sup>), adductor muscle relative to shell length and shell index (mg mm<sup>-2</sup>) as measure for shell thickness of mussels used in the experiment.

Mussel	Shell length (mm) (±sd)	Cl (±sd)	Relative adductor muscle (±sd)	Shell index (mg mm <sup>-2</sup> ) (±sd)
Suspended (n=60)	25.9 ±1.9	7.2 ±1.8	0.13 ±0.02	0.71 ±0.20
Bottom (n=60)	30.0 ±2.4	2.1 ±0.8	0.13 ±0.02	1.02 ±0.18

Table 2 Result of Three Way ANOVA on aggregation as a function of mussel type (suspended, bottom), substrate (smooth, complex) and time (d0, d1, d2).

Source of Variation	DF	SS	MS	F	Р
Mussel	1	0,048	0.048	10.849	0.001
Substrate	1	0.076	0.076	17.252	<0.001
Timer	2	0.548	0.274	62.504	<0.001
Mussel x Substrate	1	0.005	0.006	1.360	0.246
Mussel x Time	2	0.006	0.003	0.694	0.502
Substrate x Time	2	0.202	0.101	23.048	<0.001
Mussel x Substrate x Time	2	0.016	0.008	1.822	0.166
Residual	132	0.578	0.004		
Total	143	1.479	0.010		

# Table 3. Result of Three Way ANOVA on attachment as a function of mussel type (suspended, bottom), substrate (smooth, complex) and time (d2, d30).

Source of Variation	DF	SS	MS	F	Р
Mussel	1	0.185	0.185	11.047	0.002
Substrate	1	0.472	0.472	28.101	<0.001
Timer	1	3.105	3.105	185.017	<0.001
Mussel x Substrate	1	< 0.001	< 0.001	0.019	0.889
Mussel x Time	1	0.003	0.003	0.208	0.651
Substrate x Time	1	0.014	0.014	0.871	0.357
Mussel x Substrate x Time	1	0.043	0.043	2.610	0.115
Residual	35	0.587	0.016		
Total	42	5.087	0.121		

# Table 4. Result of Three Way ANOVA on loss of mussels as a function of mussel type (suspended, bottom), substrate (smooth, complex) and time (d3, d30).

Source of Variation	DF	SS	MS	F	Р
Mussel	1	0.637	0.637	17.656	<0.001
Substrate	1	0.937	0.937	25.987	<0.001
Timer	1	1.120	1.120	31.063	<0.001
Mussel x Substrate	1	0.001	0.001	0.017	0.898
Mussel x Time	1	< 0.001	< 0.001	0.011	0.918
Substrate x Time	1	0.033	0.033	0.928	0.342
Mussel x Substrate x Time	1	0.001	0.001	0.012	0.912
Residual	35	1.262	0.036		
Total	42	4.686	0.112		

bottom), substrate (smooth, complex) and time (ds0).							
Source of Variation	DF	SS	MS	F	P		
Mussel	1	0.002	0.002	54.338	<0.001		
Substrate	1	0.001	0.001	15.077	0.001		
Mussel x Substrate	1	< 0.001	< 0.001	0.0871	0.772		
Residual	16	0.001	< 0.001				
Total	19	0.004	< 0.001				

Table 5. Result of Two Way ANOVA on specific growth rate for mussels as a function of mussel type (suspended, bottom), substrate (smooth, complex) and time (d30).







Fig. 2. Byssal attachment strength (±SE) of mussels as a function of substrate composition and mussel type (suspended vs. bottom) in two different samplings; after two days (d2) and after 30 days (d30) (n = 6 replicates).



Fig. 3. Rate of loss ( $d^{-1} \pm SE$ ) of mussels as a function of substrate composition and mussel type (suspended vs. bottom) in two different samplings; after two days (d2) and after 30 days (d30) (n = 6 replicates).



Fig. 4. Specific growth rate (±SE) of mussels as a function of substrate composition and mussel type (suspended vs. bottom) (n = 6 replicates).

Chapter 4 Trade-off between increased survival and reduced growth for blue mussels living on Pacific oyster reefs (*Published in Journal of Experimental Marine Biology and Ecology*)

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Experimental setup up to study blue mussel, Mytilus edulis, development in growth rate and condition index as a function of substrate end predation. Photo Helle Torp Christensen.

# Trade-off between increased survival and reduced growth for blue mussels living on Pacific oyster reefs

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#### Abstract

Pacific oysters *Crassostrea gigas* (Thunberg 1793) have been introduced into the Wadden Sea (North Sea, Germany) in the mid of the 1980s and have invaded native blue mussel *Mytilus edulis* (L.) beds. The latter turned into oyster reefs where mussels seem to be relegated to the bottom in between the much larger oysters. By combining field and laboratory experiments, we reveal how mussels react to cohabitation with the invasive oysters. Mussels subjected to direct contact with crabs *Carcinus maenas* migrate from top to bottom positions between oysters in both field and laboratory experiments within 22 days. Shell growth was significantly reduced for mussels placed on the bottom compared to mussels at the top of an oyster reef. Condition index was lower for mussels on the bottom of the reef irrespective of whether placed between dead or living oysters. We conclude that mussels experience a trade-off between survival and food supply and prefer to take refuge from predation even when this decreases growth and condition. This mechanism may have facilitated the take-over of *C. gigas* on *M. edulis* beds in the European Wadden Sea.

Key words: trade-off, Crassostrea gigas, Mytilus edulis, growth, biological invasion, Wadden Sea

#### Introduction

Species coexistence in communities is often accompanied by trade-offs in species behavior that entails numerous different effects on living conditions or population dynamics of a species (Boyce 1984, Stearns, 1989, Gleeson and Tilman, 1990, Zera and Harshman, 2001, Kneitel & Chase, 2003). Non-indigenous species have to cope with their new environment, and native species have to cope with their new neighbors and potential competitors entailing benefits, disadvantages and trade-offs.

Invasive species are not predetermined to have a negative effect on coastal systems nor do they necessarily pose a threat to species diversity (Reise et al., 2006). In coastal environments, aliens are not known to have caused any extinction of native species (Gurevitch and Padilla, 2004).

The invasive Pacific oyster *Crassostrea gigas* with its origin at the Japanese coast has been introduced into the northern Wadden Sea (German Bight, North Sea) by aquaculture in 1986 (Reise 1998). Already five years later the first free living *C. gigas* could be found on an intertidal blue mussel bed a few kilometers from the oyster farm. With their thick, hard-edged shells it seems to be almost impossible for predators like birds, crabs or starfish to feed on these oysters after they have reached a length of about 4 cm (Diederich 2005b).

Oyster larvae require hard substratum for settlement which is rare in the sand flat dominated Wadden Sea. Shells of native blue mussels *M. edulis* represent a suitable hard substrate at proper locations for *C. gigas*. Once they are settled, Pacific oysters are attached to the substrate for their entire life. This can result in a nearly complete overgrowth by the invader (Diederich et al., 2005, Nehls et al., 2006, Nehls and Büttger, 2007, Büttger et al., 2008) and a potential take-over of the role of mussels as ecosystem engineers. As both species are epibenthic suspension feeders and have an overlapping niche the question arises, what effect the oysters have on the M. edulis population. Predation is expected to be one of the main reasons for the decline of *M. edulis* in the Wadden Sea due to a prolonged absence of cold winters (Nehls et al., 2006). Mild winters promote an earlier arrival of predators onto the tidal flats, thus predators and mussel larvae encounter simultaneously (Strasser and Günther 2001; Beukema and Dekker 2005). Substrate complexity improves protection of M. edulis against predation and other physical disturbances (Frandsen and Dolmer, 2002). Both M. edulis and C. gigas reefs provide a highly complex substrate (Kochmann et al., 2008). Thus, the presence of C. gigas may be of benefit for the survival of M. edulis. An increased presence of M. edulis in the interspaces provided by the C. gigas reef rather than on top of the reef could be observed in the field (own obs.). In order to investigate migration of mussels exposed to predation and those not exposed to predation, both a field experiment and a laboratory experiment were conducted ascertaining whether M. edulis react to the presence of predators via migration and which aspects of this predator presence might be the most crucial. Furthermore, a comprehensive field experiment was established to determine the effects on growth and condition indices in M. edulis as a function of position, predation and live and dead C. gigas to ascertain a possible food competition between the two so far co-occurring bivalve species.

#### **Material and Methods**

#### **Experimental site**

Investigations were conducted on a mixed bed with native *M. edulis* and invasive *C. gigas*, as it is currently characteristic for the two species, in the List tidal basin in the northern Wadden Sea. The basin covers an area of about 400 km<sup>2</sup> and is located between the German island of Sylt and the Danish island of Rømø (54°50`- 55°10`N and 08°20`- 08°40`E). In the north and south the basin is closed by dams and protected from prevailing westerly winds and waves by sand dune barriers. The only connection to the North Sea is a tidal inlet of 2.8 km width. Tides are semi-diurnal and the mean tidal range is about 2 m. Average salinity is 30 and mean annual water temperature is about 9°C (winter 4°C, summer 15°C). More detailed descriptions of geology, hydrography and sediments of the area are given by Reise (1985), Reise et al. (1994) and Gätje and Reise (1998). Within the List tidal basin beds of *M. edulis* are currently overgrown by *C. gigas*, and both mussel and oysters now occur in mixed reefs at adult densities of up to 2000 individuals per m<sup>2</sup> (Nehls and Büttger, 2007).

#### Position of M. edulis in a C. gigas reef

To investigate the position of *M. edulis* in a Pacific oyster reef, the height of each mussel (> 20 mm) above the bottom of a reef was measured to the nearest cm on randomly selected sections of the oyster reef (n=25). Since bottom height varied with sedimentation across the *C. gigas* reef, the bottom of the reef was defined as the sediment surface between the oyster shells in a given section.

#### Effect of predator occurrence on *M. edulis* migration and loss

To ascertain why mussels occur primarily in the interspaces of *C. gigas* reefs instead of on top of the reef, a field experiment was conducted in June 2009. The experimental setup consisted of four treatments with four replicates: 1) Mussels on the bottom, between oysters and with predators excluded, 2) mussels on top of oysters with access for predators, 3) mussels on top of oysters and with predators excluded, and 4) mussels on the bottom, between oysters, with access for predators. Therefore, mussel position (initially top vs. bottom of the oyster reef) was crossed with predator access (cage exclosure vs. no cage). One replicate (50 x 50 cm) of each treatment was established on four square plots of 1.44 m<sup>2</sup> of an oyster reef at low tide. Plots were cleaned, except for *C. gigas* and remaining sessile organisms on the shells, to allow precise quantification of migrated mussels at the end of the experiment. 30 mussels (40 mm  $\pm$  2.8 mm) were placed on 0.25 m<sup>2</sup> polyethylene mesh wire (mesh size 1.0 cm), located either on top of oyster clumps or underneath them. Holes (12.25 cm<sup>2</sup>, n= 4) were cut into the mesh to enable downward migration of mussels. For predator exclusion, treatments were fenced and covered with a 1-cm mesh. After 22 days, loss of individuals and the position of mussels were recorded by counting remaining and migrated live *M. edulis* in each treatment.

#### Migration of M. edulis due to physical and chemical cues of C. maenas

To test migration response of *M. edulis* as a function of chemical or physical stimulation by the presence of the shore crab *C. maenas*, a laboratory experiment was conducted in July 2009. The experimental setup consisted of three treatments with six replicates. Five-liter basins were filled with cleaned oyster clumps (50 individuals), arranged in a natural matrix, and 10 individuals of *M. edulis* (approximately 4 cm shell

length) were added to the top of the oyster matrix. In treatment 1, mussels were exposed to physical contact with one crab. Crabs were unable to prey on *M. edulis* because their pincers were taped. In treatment 2, mussels were exposed to a crab, which was placed behind a barrier of perforated acrylic glass permeable to water. Treatment 3 was a control without crabs. Basins were supplied with running sea water (10 °C). After 15 days, mussels were counted in two categories: between 0 and 8 cm from the bottom, and higher than 8 cm from the bottom.

#### Growth and condition index of M. edulis

A second field experiment was conducted in July/August 2009 to examine differences in growth and condition index for mussels placed in different heights (on top and in interspaces) of a *C. gigas* reef. Mussels used in the experiment were collected on a mussel bed nearby and shell length of each mussel was measured to the nearest 1mm (mean shell length (± sd)  $32.9 \pm 3.2$  mm, n = 2050). Initial condition index of *M. edulis* was determined from a subsample of 50 individuals prior to the experiment. Soft tissue of mussels was dried at 70 °C until weight was stable (70 h). Condition index was determined as CI= DW/L<sup>3</sup>, where DW is dry weight of soft parts and L is shell length (Petersen et al. 2005).

In the field the experimental setup consisted of eight treatments with five replicates, each placed just above low tide line. Prior to the experiment at five plots, epibenthic structures were removed to eliminate any possible influence on mussel growth due to e.g. overgrowth by algae. Previously removed oysters were placed back on the plots to maintain natural oyster density (approximately 600 ind./m<sup>2</sup>). Plots were subdivided into eight squares of equal size (50×50 cm). Each square represented one of the eight different treatments. Three factors were manipulated in a completely crossed split-plot design: mussel position (top vs. bottom of an oyster reef), oyster matrix (live oysters vs. shells of oysters) and predator (crabs) access (cage exclosure vs. no cage). For the latter, four treatments of each block were fenced with polyethylene mesh wire (mesh size 1.0 cm) on all sides to exclude predation by crabs. The other four treatments of each block remained freely accessible for crabs. Within fenced and accessible treatments of each of the five blocks, position of replicates was randomized. The cage/no cage treatments represented the main plot, which were split into smaller varietal sub-plots (layer and oyster matrix). All experimental areas were covered with mesh wire to prevent feeding by birds. To control position of mussels in the experiment, mussels were placed in mesh bags (5×10 cm) made of polyethylene mesh wire (mesh size 1.0 cm). Each of the five replicates comprised five bags with ten mussels in each.

Treatments with mussels on the bottom were secured with wire. Treatments with mussels above the *C. gigas* reef were fixed on piles with water-resistant wooden plates (15x15 cm) on top. Holes in each corner of the plates were used to fix mussel bags with cable ties, to make them freely hanging just above the *C. gigas* reef without any contact to the plates. After 48 days all bags were collected. Growth of *M. edulis* was measured as shell increment during the experimental period. Condition index after the experiment was determined the same way as prior to the experiment (see above).

#### **Statistical analysis**

Results are given as arithmetic means with standard deviation (sd). Variances were tested for homoscedasticity using Levene Median test and for normal distribution using Kolmogorov-Smirnov test. Significance levels, F-statistics with degrees of freedom for effect and error (F<sub>x,y</sub>) and mean square errors (MS<sub>error</sub>) are given for all results. As one level of a main factor (bottom layer) in the migration experiment had a mean of zero with no variation, analysis using Two Way ANOVA was inappropriate. Therefore, data of migration dependent on layer and loss of mussels were analyzed by using non-parametrical Mann-Whitney Rank Sum Test. Data of migration dependent on predation were analyzed by using t-test. Migration of mussels in the laboratory experiment was analyzed by using non-parametric analyses of variance on ranks (Kruskal-Wallis ANOVA (because of the heterogeneity of variances), with physical and chemical cues by crabs or without crab as fixed factors and migration as dependent factor). Due to multiple treatments within each caged and not caged block of the growth and condition index experiment, a split-plot design (nested design) was applied, with cage/no cage as main plot being split into smaller varietal sub-plots (layer and oyster matrix). The error structure is defined in the Error term, with the plot sizes listed from left to right, from largest to smallest: growth/condition index - cage \* layer \* oyster matrix + error (block/cage). The smallest plot size (layer/oyster matrix) does not need to appear in the error term (software package R 2.12.2, by R Foundation for statistical computing). Differences in shell length increment and condition index of mussels in the different layers between dead and alive oysters and exposed and not exposed to predators were tested using analyses of variance (Three Way ANOVA, with position in the reef, between dead or alive oysters and access for predators or not as fixed factors and growth and condition index as dependent factors), including nested error, followed by Holm-Sidak method for pairwise multiple comparisons of the different treatments. Effects were considered to be statistically significant if p < 0.05.

#### Results

#### Natural position of *M. edulis* in a *C. gigas* reef

Measurements of mean height ( $\pm$  sd) of *M. edulis* in the *C. gigas* reef prior to the field experiments were 2.0  $\pm$  0.2 cm above bottom. Mean height ( $\pm$  sd) of the *C. gigas* reefs where *M. edulis* was present was 9.0 cm  $\pm$  2.0 cm.

#### Effects of predators on *M. edulis* loss and migration

Loss of mussels ( $20 \pm 15$  %) occurred exclusively among mussels on top of the oyster reef exposed to predation by crabs and birds (Mann-Whitney Rank Sum Test, p < 0.05). *M. edulis* migrated only from top to bottom layer and not vice versa (Mann-Whitney Rank Sum Test, p < 0.001). In treatments with predator access most of the migration occurred (Fig. 1). Approximately five times more mussels ( $\pm$  sd) migrated downwards (58  $\pm$  18 %) than in treatments not exposed to predation ( $12 \pm 6$  %) (t-test, p = 0.003).



Fig. 1. Loss and migration (±sd) of mussels (*M. edulis*) from top to bottom layer with and without access to predators in a mixed intertidal *M. edulis/C. gigas* bed. Mean percentage of migrated mussels and loss of *M. edulis* (n = 120) per treatment (4 replicates).

### Migration of *M. edulis* due to physical and chemical cues of *C. maenas*

The fraction of mussels migrating downwards over 15 days in the absence of crabs, with chemical cues, or in the presence of crabs could not be distinguished statistically (Kruskal-Wallis ANOVA, p = 0.15). However, trends in response were observed. Approximately twice as many *M. edulis* were found in the lower layer after 15 days when crabs were present than when they were not or only chemical cues were present (Fig. 2).



Fig. 2. Mean number (±sd) of migrated mussels (*M. edulis*) per treatment (6 replicates with together with 60 individuals) with direct physical contact, exposed to chemical cues and with absence of the shore crab *C. maenas* in a laboratory experiment after 15 days in July 2009.

#### Growth and condition index of M. edulis

There was a significant main effect of the layer within the reef (Three Way ANOVA, F  $_{1,23}$  = 33.118, MS  $_{error}$  = 0.07325, p < 0.001). Mean shell length increment (± sd) for *M. edulis* was significantly higher on top (1.3 ± 0.2 mm) of the *C. gigas* reef than in the interspaces at the bottom of the reef (0.8 ± 0.3 mm). An interaction between layer and oyster matrix treatments occurred (Three Way ANOVA, F  $_{1,23}$  = 4.9946, MS  $_{error}$  = 0.7325, p < 0.05). Mussels placed down at the bottom in between dead oysters showed significantly higher length increment (1.0 ± 0.2 mm) than mussels placed in between live oysters (0.6 ± 0.2 mm) (Holm-Sidak method, p = 0.005) (Fig. 3A). There was no significant difference in length increment for *M. edulis* placed on top of the oyster reef in treatments with dead and living *C. gigas* (Holm-Sidak method, p = 0.90). Presence of predators had no significant main effect on shell length increment (Three Way ANOVA, F  $_{1,23}$  = 0.0054, MS  $_{error}$  = 0.03967, p = 0.95). There was a main effect of layer on condition index (Three Way ANOVA, F  $_{1,23}$  = 1.0054, MS  $_{error}$  = 0.0757, p < 0.001) (Fig. 3B). Mean condition index (± sd) of mussels was significantly higher for *M. edulis* on top (4.6 ± 0.2) than on bottom (3.6 ± 0.1). Neither placement between dead or living *C. gigas* (Three Way ANOVA, F  $_{1,24}$  = 0.002, MS  $_{error}$  = 0.0757, p = 0.95) of M. *edulis* on top (4.6 ± 0.2) than on bottom (3.6 ± 0.1). Neither placement between dead or living *C. gigas* (Three Way ANOVA, F  $_{1,24}$  = 0.0002, MS  $_{error}$  = 0.0757, p = 0.15) of M. *edulis*.



Fig. 3A. Mean shell length increment ( $\pm$  sd) of mussels (*M. edulis*) subject to layer (top/bottom) and dead or alive oysters (*C. gigas*). B. Condition index ( $\pm$  sd) of mussel (*M. edulis*) subject to layer (top/bottom) and dead or alive oysters (*C. gigas*). Number of analyzed *M. edulis* was 50 per replicate with 5 bags of 10 each for 7 weeks in July-August 2009.

#### Discussion

In the present study, loss of the blue mussel *M. edulis* was only observed among individuals on top of the *C. gigas* reef exposed to predation by crabs and birds. From there *M. edulis* migrated downwards, indicating that migration was induced by presence of predators. This was supported by the fact that mussels not exposed to predation migrated significantly less compared to mussels exposed to predation. *M. edulis* had a significantly higher shell length increment and condition index when located at the top of a *C. gigas* reef compared to *M. edulis* located in the interspaces at the bottom of the reef. At the bottom between the oysters, shell length increment was significantly higher when oysters were dead compared to *M. edulis* 

located between live *C. gigas*, while no such difference occurred in the top layer. The investigations identify a trade-off for blue mussels between a reduced predation pressure when migrating into the interspaces provided by the much larger oysters vs. reduced growth in this refuge compared to a position on top of the oyster reef.

#### Food limitation for *M. edulis* as consequence of migration

In previous studies by Bayne (1976) it was ascertained that length increment primarily is dependent on food availability, therefore we expected that *M. edulis* on the top of the reef would have a greater shell length increment than mussels located at the bottom between C. gigas. Growth of M. edulis in our experiment was relatively low, but in the same range as reported earlier from an adjacent location by Buschbaum and Saier (2001). These authors found length increments of 3.3 ±1.8 mm for a 12 week period during July/August, compared to our findings of an increment of 1.3 ±0.4 mm and 0.8 ±0.3 mm for M. edulis of the same size for a 7 week period at the top and bottom, respectively. Food availability may be distorted due to differences in filtration capacity and position in the C. gigas reef. A large sized M. edulis (50-70 mm shell length) can filter 1-7 | h<sup>-1</sup> (Riisgård, 2001) compared to a medium sized *C. gigas* (90-100 mm shell length) that can filter 30 | h<sup>-1</sup> (Quayle, 1988). A comparison of filtration rates of similar sized oysters and mussels showed that filtration rates of C. gigas are two to three folds higher (Walne, 1972). Differences in filtration rates of bivalve species are also presumed due to differences in size, growth rate and reproduction of the population (Powell et al. 1992). Therefore, M. edulis may be disadvantaged in contrast to oysters, because of decreased size and therefore less filtration rates when placed inside the mixed bed. However, in Bougrier et al. (1997) it was assumed that M. edulis and C. gigas are not necessarily strong competitors due to different feeding behavior and possibly also different food sources. In a recent study by Troost et al. (2009) it was found that, despite significant differences between species (including M. edulis and C. gigas), the absolute differences in feeding current characteristics were small and were not expected to lead to significant differences in feeding efficiency. Even though it is argued that M. edulis and C. gigas are not necessarily strong competitors for food on an individual level, we detected that the presence of C. gigas still significantly influences the shell length increment of mussels. M. edulis placed in the interspaces between dead oyster shells had significantly higher shell length increment compared to M. edulis between live C. gigas. The length increment was not significantly different between mussels placed on top of live and dead C. gigas, but significantly higher than near the bottom, indicating that mussels perform better in the absence of oysters. Reduced food availability for *M. edulis* caused by decreased current rates and increased sedimentation at the bottom between the much larger oysters might be responsible for differences in mussel growth between the different layers. Correspondingly, the individual condition index was significantly higher for mussels located on the top of the reef compared to M. edulis in interspaces between C. gigas at the bottom.

Unfavorable feeding positions leading to low condition index for *M. edulis* may negatively affect the reproductive output of *M. edulis* (see Bayne et al., 1978). Okamura (1986) found that *M. edulis* at the center of beds grew more slowly and had a lower reproductive output. Food availability and food uptake control the energy budget of bivalves, in turn affecting storage of glucogen and reproductive output (Schlüter and Josefsen, 1994; Loo and Rosenberg, 1996). Consequently, the reduction in food uptake and condition index may have implications for survival and reproduction, as well as the overall population growth of the species.

#### Migration as a response to predation

Highly complex substratum compared to smooth substrate provides enhanced protection for *M. edulis* against predators (Frandsen and Dolmer, 2002). Mussel beds in the Wadden Sea provide such a complex substratum, where mussels occur in two layers (Kochmann et al., 2008). In the newly developed mixed beds of mussels and oysters, a shift may have taken place from intraspecific food limitation to interspecific food limitation, i.e., from hiding places provided by the top layer of mussels to the mussels below - to refuges provided by large oysters to the much smaller mussels in-between.

For oysters this may be different. Young oysters tend to attach to the upper parts of adult oysters (pers. obs.) and thus may not be subject to the same predation as the mussels. Moreover, young oysters attach over their whole length to shells of adults, making it difficult for crabs to crush them. When M. edulis were located in the interspaces of the C. gigas reef, we recorded no loss or mortality in M. edulis, indicating that mussels were more protected from dislodging and/or predation, as it was shown for mussels living in close proximity to conspecifics (Okamura, 1986). M. edulis in the center of a bed suffer lower predation than M. edulis at the edge of the bed (Okamura, 1986). This corresponds to results in the present study. Due to absence of mesh fences in exposed treatments, waves may also induce dislodgement of mussels (Dolmer and Svane 1994). However, the experiment was located at a sheltered site and lasted only 22 days in summer. Strong waves due to rough weather did not occur. Moreover, mussels living on the edge tend to be more strongly attached than mussels in deeper regions of the reef and therefore may be more resistant to flow forces (Witman and Suchanek 1984). Beds of empty shells and mussel beds provide suitable hard substrate for oyster larvae to settle on (Reise, 1998). Both M. edulis and C. gigas are known to settle on conspecifics. Diederich (2005a) found that when oyster reefs are present, C. gigas larvae prefer to settle on conspecifics over M. edulis, while mussels do not show any preference for either C. gigas or M. edulis as substrate. Suchanek (1978) notices that subtidal mussels, which are subject to intense predation, survived only in crevices and other interspaces providing structural refuges. Studies have also shown that more exposed mussels, e.g. growing at the edge of patches or in smaller patches, suffer a relatively higher mortality to crabs (Bertness and Grosholz, 1985, Okamura, 1986).

The present study indicates that survival of mussels is higher in the interspaces of the oyster reef. Moreover, *M. edulis* migrated only from top to bottom and not vice versa, with most individuals migrating in treatments with exposure to predators. Mussels as prey have become increasingly scarce for the predators, which might contribute to the strong response of mussels to take a refuge among the non-edible big oysters. Migration is assumed to have been induced by predation, since *M. edulis* with physical contact to *C. maenas* was observed more frequently at the bottom layer, compared to *M. edulis* exclusively exposed to chemical cues or with no contact to *C. maenas*. Although only a small number of mussels escaped to the lower layer in the basins, presumably due to minor predator cues (only one crab in each treatment), than under natural conditions (5.6 ± 2.8 adult *C. maenas* m<sup>-2</sup>, Eschweiler et al. 2009), migration of the mussels is suggested to be initiated more by direct physical contact with a crab rather than by exposure to chemical cues released by a crab. Scent from the predator was not enough to provoke a migration response among

*M. edulis*, since migration in treatments with separated crabs was not evidently different from the control without predators. The lack of response to scents can be explained by too weak scents, since just one individual of *C. maenas* was used per aquarium. Reimer and Tedengren (1997) documented that *M. edulis* exposed to predators such as crabs or starfish developed a stronger byssal attachment and sought structural refuges more often. Chemical cues of predators or damaged conspecifics could as well provoke the development of thicker shells, which occurred in littorinids (Raffaelli, 1982; Trussel and Nicklin, 2002). In our experiment, cues from damaged *M. edulis* could not have induced the migration behavior, since crabs with taped pincers were unable to prey on mussels.

#### The cost of predatory protection

M. edulis in aggregated beds attain an advantage from group living by being better protected against predators (Frandsen and Dolmer, 2002). From our field experiment it was clear that mussels have significantly reduced growth and condition index when positioned in the interspaces between C. gigas. Our results indicate that the cost of growth is paid to secure protection. Utne et al. (1993) ascertained a strongly reduced food intake combined with a considerably higher use of shelter by the goby Gobiusculus flavescens when predators are present in contrast to treatments without predators. Hence, gobies seem to prioritize survival over feeding. Frequently, these trait-mediated indirect effects emerge because of effects of predation risk on prey traits (Werner and Peacor, 2003, Schmitz et al., 2004, Trussell et al., 2006), such as waterborne cues released by predators like the shore crab C. maenas provoking reduced feeding rates in its snail prey (Nucella lapillus and Littorina littorea) (Trussell et al., 2003). This trade off is also documented in studies of organisms living in aggregations (patches, reefs, beds etc) in general (Fréchette et al., 1989, Svane and Ompi, 1993). Exposing a smaller surface area and restricting attack angles available to predators will most likely make the predators less efficient in opening the shells (Reimer and Tedengren, 1997). Mussels benefit from aggregation with conspecifics by achieving substrate complexity and stability which reduce predation (McGrorty et al., 1990, Frandsen and Dolmer, 2002), but increase intraspecific competition e.g. food competition (Fréchette et al., 1989, Asmus and Asmus, 1990). The success of M. edulis aggregation in general, however, suggests that the costs of group living are less than the advantages gained by individuals in the group (Bertness and Grosholz, 1985). When living in association with C. gigas, M. edulis benefit from the protection and stability the structurally more complex oyster reef provides. Encounter rates of mussels and predators, such as crabs or starfish, and accessibility for birds may be strongly reduced (Bartholomew et al. 2000), Grabowski 2004), since mussels are covered by the oyster aggregates. In the future, a switch to alternative appropriate food sources for the predators may be necessary, for instance, by changing their sizeand species- selective feeding (Elner and Hughes 1978; Ameyaw-Akumfi & Hughes 1987; Mascaró and Seed 2001) and learning how to optimize handling of the invasive oyster.

However, on long term the cost can be more important for the mussels. Beds of *M. edulis* in the Wadden Sea are in many areas replaced by *C. gigas* reefs (Reise, 1998; Diederich, 2006). The replacement has occurred gradually over a period of about 20 years. Nevertheless, recruits of *M. edulis* are still settling in *C. gigas* reefs (Diederich, 2005a). Beds have changed from mussel beds to reefs dominated by 95 % *C. gigas* biomass (Nehls and Büttger, 2007). A mature oyster reef consists of a complex matrix of *C. gigas* cemented together in large units forming interspaces supporting a diverse assemblage of epibenthos, including *M.* 

*edulis* (Kochmann et al. 2008; Markert et al. 2010). In the reefs, mussels are only observed in the interspaces of the reef. *M. edulis* is characterized by its wide niche and ability to adapt to different substrates, salinities and temperatures (Bayne, 1976). In the Wadden Sea with its high density of predators and high transport rates of food, the trade-off may explain coexistence between the two bivalve species. Increased numbers of refuges for blue mussels may sustain or stabilize the population. Due to decreased food availability the carrying capacity for mussels may be lower. However, the interspaces of an oyster reef as an alternative habitat for *M. edulis* may imply a reduced but more stable population of blue mussels. Whether in the long term *M. edulis* will be subject to competitive exclusion by *C. gigas* or whether the two species will coexist depends on many factors, such as responses to temperature change and larval recruitment success (Strasser et al. 2001a, b, Diederich 2006), larviphagy (Troost 2009), parasitism (Thieltges 2006; Elsner et al., in press) and finally the balance between all positive effects of predatory protection and the consequences of reduced growth in *M. edulis*.

#### Conclusion

In the present study *M. edulis* migrated downwards to hide among large *C. gigas* as a response to predator exposure. The significantly higher number of *M. edulis* moving downwards when predators were present indicated that *M. edulis* uses this strategy to escape predation. The strategy results in a trade-off between higher survival and reduced growth compared to conspecifics positioned at the top of a *C. gigas* reef. *M. edulis* leverage the presence of *C. gigas* which provide a structurally more complex substrate for settlement and protection (Bartholomew et al. 2000; Grabowski 2004). This protection is at the cost of growth and condition which might lead to reduced overall fitness, a smaller size and altered population performance.

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# Chapter 5 Bottom culture of blue mussels, *Mytilus edulis*, in a micro-tidal estuary: Production and area of impacted sea bottom (*Published in Aquaculture Environment Interactions*)

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Side scan map of bottom culture bed. Horizontal light green tracks are the mussel transplantation tracks.

# Area-intensive bottom culture of blue mussels, *Mytilus edulis,* in a micro-tidal estuary

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#### Abstract

Dredge fishery for blue mussels, Mytilus edulis (L.), impacts the benthic ecosystem. Two different field studies in 2007 and 2009, tested the productivity of bottom culture of blue mussels, and whether a shift from dredging of full-grown blue mussels to production of blue mussels in bottom culture could reduce area of impacted sea bottom. In the first study the macrostructure of a commercial bottom culture was analysed by side scan mapping, and the growth of blue mussels was recorded on a transect from the edge to the central part of the bottom culture. In a second field experiment the effect of seeding density 1.5 and 3.5 kg  $m^{-2}$  on mussel production was analysed. The measured production was used to model impacted area when producing blue mussels in bottom culture. It was demonstrated that the macrostructure of the culture bed formed during the transplantation of mussel seed was not changed one year after transplantation, indicating that the transplantation supported a robust blue mussel bed formation. Shell growth showed no spatial variability from the edge to the central part of the commercial bottom culture suggesting that growth was not reduced by density dependent food limitation. The population production/biomass ratio (P/B) on the experimental bottom cultures during the production period from April to August was 1.0 and showed no significant effect of seeding density. Model-simulations indicated that the impacted area when producing blue mussels in bottom culture is smaller than the impacted area in a fishery of full-grown blue mussels if P/B is higher than 0.5.

# Key words

Bottom culture, ecosystem impact, production/biomass, shell growth, *Mytilus edulis*, blue mussels, transplantation

# Introduction

In Europe there is a long tradition for production of blue mussels, *Mytilus edulis* (L), in bottom culture (Smaal 2002, Gosling 2003). Traditionally, blue mussel seed for bottom culture are fished by dredge from natural mussel beds in areas with high production of seed. After collection the seed are transplanted to bottom culture beds in areas supporting a high growth rate and a low mortality rate (Spenser 2002). In Denmark, the main part of the blue mussel production is based on dredging of full-grown mussels on natural populations (Dolmer & Frandsen 2002, Smaal 2002). Dredging is one of the most harmful fishing methods for benthic ecosystems (Jennings & Kaiser 1998, Collie et al. 2000, Kaiser et al. 2006). The dredging activities for mussels in subtidal areas may change marine ecosystems in relation to benthic organisms (Dolmer et al. 2001, Dolmer 2002, Neckles et al. 2005) and substrate (Dolmer 2002) and may induce cascade effects on higher trophic levels, including birds (Atkinson et al. 2010).

Aquaculture is identified as a production form that meets the growing demand for shellfish and an extended knowledge on the ecosystem impact was established during the last decade (Cranford et al. 2007, Dumbauld et al. 2009, McKindsey et al. 2011). Production of blue mussels in bottom culture is reported to change the structure of the ecosystem affecting several trophic levels (Dankers & Zuidema 1995). Bottom culture may change the composition of the benthic community with a decreased number of species and individuals (Smith & Shackley 2004, Beadman et al. 2004). Otherwise, also positive effects have been documented including increased biodiversity due to biodepositions from filtering mussels and the trapping effect of the mussel bed changing the sedimentary environment due to the introduction of a complex mussel matrix that offers a habitat for epibenthic organisms (Ysebarth et al. 2009). Furthermore, Common eider, Somateria mollissima, may benefit from an increased source of food in areas with bottom culture of blue mussels (Smaal et al. 2010). With reference to implementation of Nature 2000, Marine Strategy Framework Directive (MSFD) and Water Framework Directive (WFD) legislations there is a political request to adapt new, more sustainable production methods with reduced ecosystem impact. Use of bottom culture as production platform may reduce ecosystem impact in contrast to traditional mussel dredging. Estimates of areas impacted by different production forms are thus a prerequisite for deciding management strategies.

Few studies on the productivity of bottom cultures are reported (e.g., Dankers 1987, Kristensen & Lassen 1997, Kamermans & Smaal 2002). In Limfjorden, the productivity of transplanted blue mussels was shown to be 2-7 times higher than in the natural blue mussel beds (Kristensen & Lassen 1997). However, Yanick et al. (2003) showed that natural populations of *Mytilus trossulus* perform better than transplanted mussels on the east coast of Vancouver Island in Canada. Regardless of whether blue mussels are situated in natural or culture beds they will affect downstream food availability within and above the mussel beds (Frechette & Bourget 1985a, b, Frechette et al. 1988, Butman et al. 1994, Saurel et al. 2007) and continuous availability of food is one of the most important factors determining the growth of blue mussels (Seed 1976, Suchanek 1981). Hence, a balanced ratio between mussel abundance and food availability is necessary to ensure a high production and avoid growth limitation in the central part of a mussel bed (Okamura 1986, Svane & Ompi 1993).

Production of mussels in bottom culture is based on transplantation of mussels from areas with high seed density to culture areas where density is reduced to improve production (Spenser 2002). Thus, bottom culture has to be established using knowledge on best design of beds, optimal seeding-density and area characteristics to support a high production rate. Since growth is a key parameter for production, knowledge on impact of macrostructure of bottom culture, impact of individual mussel position in a bottom culture and impact of seeding density are crucial when planning a production. The aims of the present study were therefore to describe the macrostructure of a commercial bottom culture bed and to test blue mussel growth as a function of position in the bottom culture bed. Furthermore, in a field experiment bot-

tom cultures were created with different seeding density to test impact on production. In model simulations the impacted area, when producing mussels in bottom culture, was analysed as a function of production ratios and seeding density.

# Materials and methods

#### Study area

Field observations conducted in spring 2009 of macrostructure and shell growth on a commercial bottom culture bed of blue mussels and production experiments conducted in spring 2007 on bottom cultures with two different initial densities of seed were conducted in Kaas Broad (N 56° 40′ E 08° 45′) in Limfjorden, Denmark, in two separate field studies (Fig. 1). The estuary is a 1,575 km<sup>2</sup> sound open to the North Sea in the west and to the Kattegat in the east. The salinity ranges from 32 psu in the western part to 22 psu in the eastern part and is controlled by the predominantly west to east current. Barometric forcing induces water exchange and circulation, whereas tidal pumping is insignificant because of its low amplitude (0.1 - 0.2 m) (Dolmer 2000). The average water depth in Kaas Broad is approximately six meters. The mean Chl *a* concentrations for Kaas Broad were during spring and summer 8.5 and 5-9 mg Chl *a* m<sup>3</sup> (Markager et al. 2009).

# Macrostructure of commercial bottom culture

A commercial culture bed was mapped in April 2009 using side scan sonar (Humminbird 1197c SI Combo) (Blondel 2009) (Fig. 1A). The side scan map was compared to the tracks registered from 'm/s Limfjorden' during the transplantation (Fig. 1B). The commercial bottom culture bed was established (N 56° 40.3' E 08 ° 45.5') by the vessel 'm/s Limfjorden' in April to June 2008. The blue mussel seed were transplanted in straight tracks with bare strips of 15 - 20 m in between. Mussel seed were pumped out from both sides of the vessel and settled at the bottom in two tracks of about two m in width and about five m apart (shown as white and light grey tracks in Fig. 1B). The mean shell length of the transplanted mussels ( $\pm$  SD; n = 150) at station 1 to 4 was at the time of transplantation 16.0  $\pm$  3.8 mm, 28.2  $\pm$  5.0 mm, 25.5  $\pm$  3.4 mm and 20.6  $\pm$  2.6 mm, respectively. The seed derived from natural blue mussel beds and were deposited with a biomass density of 3.5 kg m<sup>-2</sup> across the entire culture bed (Fig. 1A).

#### Growth on a commercial bottom culture

In order to describe the growth of the mussels at the commercial bottom culture, mussels were sampled at four stations along a transect ranging from southwest to northeast from the edge of the bed, to the middle of the bed (Fig. 1A), since this is the dominant water current direction in Kaas Broad (Wiles et al. 2006). There was approximately 300 m between each station.

At each station frame samples  $(0.25 \text{ m}^2)$  (st. 1 and 4 n = 20; st. 2 and 3 n = 10) of mussels was collected randomly by SCUBA diver. The total number of mussels in each sample was counted and shell lengths were recorded using a digital calliper (0.1 mm). Station 2 to 4 had two distinct size classes while at station 1 only one broad size class was identified. The age of mussels was recorded from visual inspection of growth rings on the shells (Bayne 1976) on each station (n = 20). This indicated a mixed population of two year old transplanted and one year old settled mussels at all four stations. Assuming two cohorts, the statistics (mean shell length, SD and number of individuals) at each station was determined for each cohort. According to the cohort analysis, transplanted mussels were defined as mussels larger than the mean initial shell length plus 1 standard deviation. Mussels settled after transplantation were defined as mussels less than the mean of initial shell length of transplanted mussels. Shell lengths and percentage of mussels in each cohort are given in Table 1.

Due to differences in initial shell lengths of transplanted blue mussels, the cohort of one year old mussels settled after the establishment of the bottom culture was used to test if growth differed between the sta-

tions due to food limitation, assuming that settled mussels were settled synchronously. The growth of the two year old transplanted mussels was measured in order to contrast production conditions at the study site to other production areas. The dry weight of tissue  $(dw_1)$  of sampled mussels was measured (st. 1 n = 199; st. 2 n = 123; st. 3 n = 69; st. 4 n = 95) at the four stations. The tissue was dissected out and dried for 24 hours at 105 °C. Thereafter they were left to cool in a dessicator for 30 minutes before the dry tissue weight (g) was weighed. The condition index (CI) was calculated for transplanted mussels for each station as:  $CI = dw/L^3$ , where dw was dry weight of tissue (mg) and L was shell length (cm) (Lucas & Beninger 1985, Petersen et al. 2005).

# *Production as function of seeding density in experimental bottom cultures*

Two experimental bottom culture beds with seeding densities of 1.5 kg m<sup>-2</sup> (site A), and 3.5 kg m<sup>-2</sup> (site B) were established (N 56 40.1' E 8 44.0 to 8 45.5') in March to May 2007 by the vessel 'm/s Limfjorden' (Fig. 1A). The culture beds were  $300 \times 300$  m and 300 m apart. All blue mussel seed were harvested in Løgstør Broad, NE of the experimental cultures. Seeding densities were estimated from fishery reports to the fishery authorities and from observations of the fishery. During the transplantation process 8 - 10 samples of 10 I from each cargo were sampled in order to record the biomass of mussels in the catch. Furthermore, shell lengths of the blue mussels were measured. One cargo of 135 t (mean shell length ± SD: 22.4 ± 44.6 mm) of mussel seed was transplanted to site A in March 2007. Three cargos with a total of 315 t (mean shell length: 23.5 ± 7.4 mm in March and 26.4 ± 5.3 mm in May) were transplanted to site B from early March to early May.

In June and August 2007 five frame samples of 1 m<sup>2</sup> were collected by SCUBA diver at three stations at site A and B, respectively. Blue mussel densities, biomass density and shell length were measured. At site A the blue mussel biomass density was also monitored at one station (n=5) in April 2007, 40 days after the transplantation in order to test if the transplanted biomass density of mussels corresponded to estimated biomass density from data on ship cargoes of seed mussels. All sampling were conducted on mussels transplanted in March to April 2007. The ratio between the production and the initial biomass (P/B) in a bottom culture during a production period can be measured based on growth of individual mussels or growth of the population. The production/biomass ratio (P/B) estimated on individual growth in relation to biomass of individual blue mussels (P/B-ind) corresponds to production/biomass ratio estimated for the population. The population (P/B-pop) subtracted a loss, due to mortality in the population. The population production/biomass ratio multiplied by seeding density estimates the amount of harvestable blue mussels.

The population production/biomass ratio (P/B-pop) from April to August was calculated as biomass production ((biomass<sub>end</sub>-biomass<sub>start</sub>)/biomass<sub>start</sub>) in relation to initial biomass density (1.5 and 3.5 kg m<sup>-2</sup>, respectively) in April. In May 2007, site B was sampled by dredge and shell length measured.

The relation between shell length (cm) and wet weight (g) was due to the uniform size of the blue mussels calculated as the relation between mean shell length and mean wet weight (WW). The relation was described by the power function:  $WW = 0.049L^{3.51}$  (R<sup>2</sup> = 0.97, p < 0.0001). The average individual production/biomass ratios (P/B-ind) in April to August were calculated as ( $WW_{August}$ - $WW_{April}$ )/  $WW_{April}$ , where  $WW_{April}$  and  $WW_{August}$  are the average wet weights (g) of individual blue mussels in April and August, respectively.

Loss of production due to mortality was estimated as the difference between the individual production/biomass ratio (P/B-ind) and the population production/biomass ratio (P/B-pop).

# Model of impacted area

Production of blue mussels in bottom culture includes collection of seed on natural blue mussel beds and harvest of the full-grown mussels. Both processes include dredging. The total impacted area per harvest unit, when producing blue mussels in bottom culture ( $A_{cult}$ ), can be calculated as the sum of area used for collection of seed (S), and the area used for bottom culture (B), divided by the weight of commercial harvest (H):

$$A_{cult} (m^2 \text{ kg}^{-1}) = \frac{S+B}{H} = \frac{d+f_{seed}}{0.67 f_{seed} d(1+P/B-pop)}$$

Where d is seeding density (kg m<sup>-2</sup>) at the bottom culture,  $f_{seed}$  is mussel seed biomass density at seed fishery site (kg m<sup>-2</sup>), P/B-pop is population production ratio, 0.67 is the efficiency of the dredge (Eigaard et al. 2011).

The impacted area (A<sub>com</sub>) when fishing full-grown blue mussels for commercial sale (> 4.5 cm) can be calculated as:

$$A_{com} (m^2 \text{ kg}^{-1}) = \frac{1}{0.67 f_{com}}$$

where  $f_{com}$  is the biomass density of blue mussels at fishery site and 0.67 is the efficiency of the dredge.

# Scenarios based on interview with mussel producers

In order to estimate the impacted area, when producing blue mussels in bottom culture and when fishing full-grown mussels, three fishermen from the local producer association were interviewed on production practice. The fishermen specify that on average for Limfjorden  $f_{seed} = 5 \text{ kg m}^{-2}$ ; d = 2.5 to 3 kg m<sup>-2</sup> and  $f_{com} = 2.5 \text{ kg m}^{-2}$ . Based on this information and the model of impacted area, different scenarios were tested in the model as a function of production/biomass ratios (P/B-pop) and seeding density (d).

#### Statistical analysis

Data was tested for normality of distribution and homogeneity of variances and if requirements were met, parametric tests were used otherwise a non-parametric test was used. For comparison of densities between the four stations at the commercial bottom culture a parametric One way ANOVA and post hoc Tukey test was used. Difference in shell growth and condition index between stations was tested in One way ANOVA or the non-parametric Kruskal-Wallis tests followed by post hoc Dunn's method. Differences between settled and transplanted mussels were tested in separate Kruskal-Wallis tests.

In the field experiment separate one sample t-tests were used to test test if the densities of blue mussels at site A and B in April, June and August were different from the estimated seeding density. The production ratio ((biomass<sub>end</sub>-biomass<sub>start</sub>)/initial biomass) was tested in an One way ANOVA on ranks as a function of site. Changes in shell lengths were tested on linear regressions as a function of time. Differences in shell length as a function of time and site were tested in Two way ANOVAs.

# Results

# Macrostructure of a commercial bottom culture

A visual comparison of the transplantation tracks in the commercial bottom culture bed (Fig. 1A), the results from the side scan sonar mapping (Fig. 1B) and reports from the SCUBA diver in April 2009 confirmed that the tracks in which the blue mussels were transplanted to the commercial bottom culture in 2008 were clearly identifiable one year after transplantation. The results indicated that the transplantation practice on the commercial culture bed supported the formation of a robust bed structure.

#### Growth on commercial bottom culture

In April 2009, one year after transplantation, a significant difference was observed in density between stations (One way ANOVA, p = < 0.001). The density (mean ± SD) of blue mussel increased from the edge (st. 1: 545 ± 704 ind. m<sup>-2</sup>) across the bed (st. 2: 1,225 ± 426 and 3: 1,348 ± 1,208 ind. m<sup>-2</sup>) to the middle (st. 4: 2,175 ± 1,027 ind. m<sup>-2</sup>). The increase was significant between stations 1 and 4 and 2 and 4 (Tukey test st. 1 vs 4 p < 0.001; st. 2 vs st. 4 p = 0.040).

The growth in shell length of the transplanted mussels ranged from 1.1 to 2.2 mm month<sup>-1</sup> and the growth of the settled mussels ranged from 2.5 to 3.5 mm month<sup>-1</sup>. The tests of shell growth showed significant differences between stations (transplanted: Kruskal-Wallis, p<0.001; settled: One way ANOVA, p<0.001). However, the pairwise comparison showed no systematic difference in growth comparing stations positioned at the edge and central in the commercial bottom culture (Fig. 2). A Kruskal-Wallis test showed a significant difference in shell growth between transplanted and settled mussels (p<0.001). Mussels settled after the transplantation had significantly higher growth compared to transplanted mussels (Fig. 2).

The mean condition index (CI) ( $\pm$  SD) of the transplanted blue mussels ranged from 3.8 ( $\pm$  1.0) to 5.2 ( $\pm$  0.9) mg cm<sup>-3</sup> with significant difference between stations (Kruskal-Wallis test, p < 0.001; Fig. 3). Pairwise comparison of stations showed that CI was significantly higher at station 1 compared to station 2, 3 and 4 (Dunn's method p < 0.05).

#### *Production as function of seeding density in experimental bottom cultures*

The biomass density of blue mussels was sampled at site A, 40 days after transplantation in 2007. The mean biomass density ( $\pm$  SD) was 1.76  $\pm$  1.31 kg m<sup>-2</sup> and not significantly different from the estimated seeding density (one sample t-test, p = 0.68), indicating that the transplanted biomass density corresponded to the estimated biomass density from data on ship cargos of seed mussels of 1.5 kg m<sup>-2</sup>.

At site A, the biomass density increased to 2.52 kg m<sup>-2</sup> in June (Fig. 4), not significantly different from the estimated seeding density (one sample t-test, p = 0.26). In August the biomass density was 3.10 kg m<sup>-2</sup> which was significantly different from the seeding density (one sample t-test, p < 0.05). At site B the biomass densities increased to 5.69 kg m<sup>-2</sup> in June, and 6.69 kg m<sup>-2</sup> in August 2007. In both months the biomass densities had increased significantly (one sample t-test, June p = 0.02; August p = 0.03). The population production/biomass ratio (P/B-pop) from April to August 2007 corresponded to 1.1 and 1.0 and was not significantly different between sites (One way ANOVA, site: P = 0.901). The mean P/B-pop ratio (± SD) for the two sites was 1.0 ± 1.7.

At both sites, shell length increased significantly (Fig. 5). At site A the mean shell length ( $\pm$  SD) increased from 22.4  $\pm$  4.7 mm in March to a mean length of 39.9  $\pm$  5.2 mm in August 2007. At site B the mean shell length increased from 23.5  $\pm$  7.4 mm in March to 38.2  $\pm$  4.8 mm in August 2007. At the two sites the shell growth rate of the transplanted mussels was 3.4 and 3.1 mm month<sup>-1</sup>, respectively, independent of seeding density. The shell lengths were significantly larger at site A compared to site B in June (Two way ANOVA, p = 0.003), whereas no differences were observed in April and August 2007.

The individual production/biomass ratio (P/B-ind) estimated as production of individual blue mussels from April to August 2007 was 1.0 at both sites, and P/B-pop constituted 40 % and 50 % of the individual production at site A and B, respectively, and consequently 60 to 50 % of the production was lost due to mortality.

# Estimate of impacted area

The area impacted by production of blue mussels in bottom culture ( $A_{cult}$ ) and the area impacted by dredging full-grown blue mussels from natural mussel beds ( $A_{com}$ ) were modelled for different scenarios (Fig. 6). The model results showed that the fishery of full-grown blue mussels at a biomass density of 2.5 kg m<sup>-2</sup> would impact 0.59 m<sup>2</sup> kg<sup>-1</sup> (large grey arrow at Fig. 6) corresponding to a production in bottom culture with a population production ratio (P/B-pop) of 0.5. At a P/B-pop ratio of 1.0 the impacted area would be 0.44 m<sup>2</sup> kg<sup>-1</sup> (large black arrow at Fig. 6). The scenarios revealed that the area impacted by bottom culture was smaller than the area dredged in the fishery of full-grown blue mussels if the seeding density at the bottom culture bed was larger than the present practice (> 2.5 kg m<sup>-2</sup>), or if the population production ratio (P/Bpop) was larger than 0.5 (Fig. 6).

# Discussion

# Macrostructure of commercial bottom culture

The present study of bottom culture showed that the mussel industry with high precision can transplant seed mussel of a predefined biomass density, to form robust bottom culture, that are not affected by wave- or current induced transport. In 2009, the macrostructure of the culture bed was similar to the original transplantation tracks established the previous year. The blue mussels can be assumed to aggregate within the tracks (van der Koppel et al. 2005, 2008, Christensen et al. 2012) but not at a scale that change the macrostructure of the tracks. The mussel industry has thus developed a knowledge and practice enabling formation of bottom culture of blue mussels.

#### Growth conditions in the bottom culture – food limitation?

In the commercial bottom culture the growth in shell length was 1.1 to 3.5 mm month<sup>-1</sup>, with no systematic difference between edge and the more central part of the bottom culture. In the experimental bottom cultures established with a seeding density of 1.5 and 3.5 kg m<sup>-2</sup> shell growths were estimated to be 3.4 and 3.1 mm month<sup>-1</sup>, respectively. The results from the commercial and the experimental bottom cultures indicated that the used densities of mussels did not affect shell growth adversely. The estimated shell growth rates for bottom cultures are in the same range as natural mussel beds in Limfjorden (Kristensen & Lassen 1997, Dolmer 1998, Christensen et al. 2012) and other eutrophic Danish estuaries (Petersen et al. 1997). Hence, the present study of shell growth in the commercial and the experimental bottom cultures indicated no food limitation between transplantation tracks. On the other hand, this study did not focus on food limitation within single tracks. Using a model, Van de Koppel et al. (2005, 2008) demonstrated that self-organised spatial heterogeneity of blue mussels improves productivity, when compared to a completely homogeneous bed of blue mussels at the same density. It appears that the present procedure for transplanting blue mussels in tracks or long beds provide a sufficient heterogeneity to avoid a decline in growth across the culture bed.

The condition index (CI) of mussels at the commercial mussel culture differed between stations indicating that food availability was higher at the edge of the culture bed compared to within the bed. The CI was in the same range as reported for blue mussels of approximately similar sizes on natural mussel beds in May to June (Clausen & Riisgård 1996). Condition index increases during the period of energy storage and game-togenesis, and decreases with the main spawning event (Gosling 2003). During the field campaign only a few spawning mussels were observed, indicating that the main spawning event had not yet taken place. Thus, the relatively high CI in the commercial bottom culture is most likely due to the fact that the mussels

had not yet spawned. During the last 2 - 3 years, the Danish blue mussel producers have reported a low quality of the blue mussels in bottom culture. Self-regulation implemented by the fishery, ensures no harvest of the natural populations and bottom culture if the meat content (proportion of meat in a mussel when cooked) is < 14 %. This regulation has, in periods, terminated the harvest of blue mussels due to low quality.

Investigations on the physical regime in the Limfjord in relation to transport of seston to a mussel culture, indicated that the water column switched between stratification due to a thermocline and mixing due to wind forcing, whereas turbulence due to current was of minor importance (Wiles et al. 2006). Recorded Chl *a* concentration in the area (Markager et al. 2009) should support high specific growth rates (Riisgaard & Clausen 1996) of suspended mussels, whereas blue mussels located at the sea bed may be limited due to reduced food depletion in the boundary layer (Frechette & Bourget 1985a, b, Frechette et al. 1988, Butman et al. 1994, Saurel et al. 2007) and during stratification (Møhlenberg 1995, Dolmer 2000). In relation to food limitation across the bottom culture, shell growth showed no effect, whereas an increased CI on the edge indicated food limitation. Shell growth is a long-term response of food conditions, whereas CI reflects variations in short-term food conditions due to specific growth rates of tissue in mussels of same size up to 9 % d<sup>-1</sup> (Clausen & Riisgård 1996). Periods of low wind mixing may result in food limitation during short periods (days, weeks) that can be monitored in the CI in the central part of the bottom culture.

#### Production/biomass ratios

The population production/biomass ratio (P/B-pop) from April to August in the experimental bottom culture at site A and B was 1.0, and no difference was observed between sites. A seeding density up to 3.5 kg m<sup>-2</sup> may then be recommended to the mussel industry. The measured P/B-pop ratio corresponded to a ratio between seed and harvested biomass of 1:2. At the bottom cultures of commercial value Kristensen & Lassen (1997) calculated ratios between seeded biomass and harvestable biomass ranged from 1:0.3 to 1:0.9. The Danish mussel producers have reported that due to reduced growth rates and low meat content of blue mussels in bottom culture the last few years, the production time in bottom culture may have increased to several years, before the mussels have a quality for harvest. In the Dutch Wadden Sea, Dankers (1987) and Kamermans & Smaal (2002) reported a ratio of 1:1 and 1:1.5, respectively. Hence, the production estimated from the present study is equal to or slightly higher than previously reported from Limfjorden and other areas.

Comparison of the individual production/biomass ratio and the population production/biomass ratio indicated that 50-60 % of the growth production is lost due to mortality. Loss of production from bottom cultures due to predation and other factors is well known (e.g., Kristensen & Lassen 1997). Kristensen & Lassen (1997) studied production of four bottom cultures in Limfjorden. One of the cultures never produced blue mussels of commercial value due to an invasion of starfish, *Asterias rubens*, consuming and eliminating the mussel population.

#### Impacted area – management perspectives

Production in bottom culture requires that the mussels are dredged two times (i.e., dredging of seed and dredging of full-grown mussels for sale). The impacted areas when producing blue mussels with the two different methods were modeled, based on the production parameters measured in the present study, the efficiency of the mussel dredge and information from the fishery on production practice for bottom culture production, and in the dredging fishery of full-grown blue mussels. In the model a constant efficiency of the mussel dredge (0.67) is assumed based on investigations at a single site at blue mussel dredge used in the present model. In 2011 the mussel producers decided to implement the mussel dredge used in the present model. The simulations indicated that the impacted area when producing in bottom culture was smaller than the dredged area of full-grown blue mussels if the seeding density was larger than in the

present practice (> 2.5 kg m<sup>-2</sup>) or if the production/biomass ratio (P/B-pop) were larger than 0.5. Hence, in the present study bottom culture site with the lower seeding density (site A) affected a larger area ( $A_{cult} = 0.64 \text{ m}^2 \text{ kg}^{-1}$ ) than dredging of full grown mussels ( $A_{com} = 0.59 \text{ m}^2 \text{ kg}^{-1}$ ), whereas the bottom culture site with the higher seeding density (site B) affected a smaller area ( $A_{cult} = 0.36 \text{ m}^2 \text{ kg}^{-1}$ ). This calculation assumed, that the seeds transplanted to site A and B were fished from a biomass density of 5 kg m<sup>-2</sup>. Moreover, the areas affected by production of blue mussels in bottom culture can be reduced further by using seed produced on suspended collectors (Kamermans et al. 2002, 2009, Christensen et al. 2012), although the suspended cultures also may impact benthic habitats by affection both pelagic and benthic habitats (McKindsey et al. 2011).

#### Planning of bottom culture production

Limfjorden is the most important area for the exploitation of blue mussels in Denmark (Kristensen & Lassen 1997, Dolmer & Frandsen 2002). According to the landing statistics registered by the Danish Ministry of Food, Agriculture and Fishery, the annual landings of blue mussels from Danish waters amounts to 25-35,000 t including 5-10,000 t produced in bottom culture (fd-statweb.fd.dk). The statistics do not include the fishery for seed. Currently, there are two sources of seed for blue mussel bottom culture; transplanted seed from natural beds with high densities and low production rates and re-laid undersized mussels (i.e., shell length < 4.5 cm) discarded from the industrial processing at the blue mussel industries. The present study demonstrated that a shift from dredging full-grown blue mussels from natural mussel beds to production of blue mussels in bottom culture in a eutrophic micro-tidal area can reduce the bottom area impacted by the production. Ecosystem impact of bottom culture production is reduced further if the transplantation exports blue mussels originating from 1) areas with high densities of mussels with a low growth rate or even with high mortality rate due to food limitation, and 2) areas with a high frequency of events of oxygen depletion and mass mortality. The production would then exploits a resource that left untouched would partly disappear. The transplantations move extracted nutrients accumulated in blue mussel biomass (Gren et al. 2009) to new areas, where the blue mussels will continue extracting nutrients and improve transparency of the water by filtering seston until harvest, where the nutrients are exported from the ecosystem. Eutrophication and reduced transparency in the water column are key issues in the management plans for Natura 2000 and WFD in Limfjorden. Bottom culture planned in accordance to conservation targets will intensify the blue mussel production in robust habitats leaving more sensitive habitats to be permanently closed to mussel dredging, conserving the benthic flora and fauna in these areas. In order to implement the EU-legislation (Natura 2000, MSFD, WFD) production of mussels by dredging seed from high-density beds and transplant them to bottom culture may be a production method that can reduce the impacted area in relation to a dredge fishery on natural populations.

From a producer point of view, improvements of 1) reduced mortality due to predation from e.g. crabs, *Carsinus maenas*, starfish, *Asterias rubens*, and 2) food conditions in relation to seston concentration and transport of seston to the sea bed can significantly increase the production in bottom culture and reduce impacted area in relation to production. A future development of the bottom culture production could focus on transplantations to more shallow water areas in eutrophic estuaries where the Chl-*a* concentrations often are higher and the salinity is lower, which may reduce the density of starfish and thereby loss of mussel biomass due to predation (Rasmussen 1973). In relation to conservation objectives, development of bottom culture in eutrophic, shallow water depths may pose a trade-off between different conservation targets e.g. distribution of *E. marina*. A spatial planning approach of blue mussel production in bottom culture is therefore central in order to optimize blue mussel production and ecosystem services and to minimize negative impact on conservation objectives. Therefore, further research should focus on development of a method to identification of suitable areas for bottom culture production which includes benefits for both production-, ecological- and social interest.

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# **Tables and figures**

# Table 1. Shell lengths (mm ± SD) and percentage of mussels in each cohort of blue mussels, Mytilus edulis, in a commercial bottom culture.

	station 1		Station 2		Station 3		Station 4	
	Length		Length		Length		Length	
Mussels	(mm ± SD)	(%)						
Settled	17 ± 1	12	24 ± 2	21	19 ± 2	45	19 ± 2	24
Transplanted	28 ± 3	88	41 ± 1	79	44 ± 2	55	35 ± 4	76



Figure 1A - B. Map of the study area. 1A: The original vessel GPS-tracks generated during transplantation of mussels in 2008. White circles indicate the four sample stations on the commercial culture bed in 2009 (edge of culture bed: station 1; middle of culture bed: station 4 and two stations (station 2 and 3) between the edge and middle stations). White boxes indicate experimental culture sites A and B for experiment conducted in 2007. The white arrow indicates the predominant direction of water current. 1B: Side scan map of the culture bed one year after transplantation (2009). White and light grey horizontal lines show hard substrate (i.e. live and dead mussels). The darker area between the white and light grey horizontal tracks is soft seabed. Magnification of four parallel transplantation tracks is shown in top right corner.



Figure 2. Mean shell length growth ( $\pm$ SD) for blue mussels, *Mytilus edulis*, transplanted to a commercial bottom culture in April to June 2008 and for blue mussels settled at the culture bed after transplantation. The result of the pairwise comparison (Duncan method) of shell growth of transplanted mussels show that St3 > St1 = St2 = St4 and for settled mussels St2 > St3 = St4 > St1.







Figure 4. Biomass density ( $\pm$ SD) of blue mussels, *Mytilus edulis*, transplanted to experimental mussel beds (site A and B) in June and August 2009. Initial mussel biomass density at bed A and B was 1.5 and 3.5 kg m<sup>-2</sup> indicated by horizontal lines. The biomass densities are tested against initial seeding density and \* indicate that p < 0.05.



Figure 5. Shell length of blue mussels, *Mytilus edulis*, as a function of day when transplanted ( $\blacklozenge$ ) and at experimental mussel beds ( $\blacklozenge$ ) and at site A (dark) and site B (grey). Linear regressions (site A: R<sup>2</sup> = 0.87, P < 0.001; site B: R<sup>2</sup> = 0.95, P < 0.001) showed that the growth rates measured as slopes were 3.4 and 3.1 mm month<sup>-1</sup> at site A and B, respectively.



Figure 6. Model simulation of impacted area, when producing blue mussels, *Mytilus edulis*, in bottom culture and dredging of full-grown blue mussels. Impacted area in bottom culture is simulated based on a constant efficiency of the mussel dredge (E= 0.67), a population production/biomass density ratio (P/B) of 0.5 and 1.0 and a seeding density (d) of 2.5 or 3.5 kg m<sup>-2</sup>. The fishery (light grey line) is simulated based on a constant efficiency of the dredge (E = 0.67) and the blue mussel density at the fishery site ( $f_{com}$ ) (for further details see text). The small black arrow indicate present production practice in relation to fishery of seed ( $f_{seed} = 5.0$  kg m<sup>-2</sup>) and the large black arrow point the impacted area at a seeding density at 2.5 kg m<sup>-2</sup> and a P/B of 1.0. The small gray arrow indicate present production practice in relation to fishery of full-grown blue mussels ( $f_{com} = 2.5$  kg m<sup>-2</sup>) and the large gray arrow point the corresponding impacted area.

# **Chapter 6 Discussion**

To approach the major constraints for bottom culture production of blue mussels, Mytilus edulis (Linnaeus, 1758) in European waters, the goal was to study how biological mechanisms can be used to develop and optimize the bottom culture production method through knowledge on seed mussel performance and bed structure. Knowledge on the seed mussel source will support the bottom culture production and might even have the potential to reduce the total impacted area in bottom culture production. In the present PhD-project it was demonstrated that the use of seed mussels, collected on suspended long line cultures in the water column in bottom culturing have the potential to secure a stable seed source for the bottom culture production (Chapter 2) in the future. The use of shell substrate enhances complexity of the seabed surface structure and therefore improve the survival (Chapter 3 and 4), and with that, the output of the culture beds. Furthermore, it was documented that the transplantation practice in a commercial bottom culture bed supported the formation of stable mussel bed macro structure one year after transplantation of seed mussels and, that production of blue mussels in bottom culture beds can under certain conditions reduce the impacted area used for exploitation compared to fishery on natural beds (Chapter 5). A more area-intensive exploitation will lead to a reduction of impacted area, which will support both nature conservation interests and the increased focus from the environmental policies in the EU (Kamermans & Smaal 2002; Smaal 2002). In the present chapter, the performance of suspended and bottom seed mussels, the effect of increased substrate complexity on survival, and growth and reduction of impacted area are discussed, and conclusions regarding the three research questions are drawn.

# 6.1 Supporting the seed mussel source in blue mussel bottom culture

Morphological differences in suspended and bottom mussels, and the consequences regarding growth performance and survival of the differences, are important parameters in the evaluation of suspended mussels as a sustainable seed mussel source, since this affect production output.

# 6.1.1 Effect of origin in suspended and bottom mussels

The morphological differences in suspended and bottom seed mussel individuals documented in Chapter 2 and 3 can be explained as phenotypic plasticity. Phenotypic plasticity in blue mussels has also earlier been documented (Reimer & Harms-Ringdahl 2001; Reimer & Tedengren 1996). Swedish studies showed that mussels cultured in field enclosures exposed and not exposed to predatory starfish, *Asterias rubens* (Linnaeus, 1758), after four weeks were morphologically different. The predator exposed mussels had significantly smaller outer shell (shell length, height and width), and had at the same time developed at significantly lager posterior adductor muscle, thicker shell and more tissue per shell volume (Reimer & Tedengren 1996).

The three main parameters that lead to morphological differences between seed mussels originating from suspended cultures and natural bottom mussel beds, respectively, are exposure to physical forces (Kirk et al. 2007), exposure to predation (Gosling 2003), and food availability (Garen et al. 2004; Fréchette & Grant 1991). In the suspended cultures, seed mussels are exposed to water currents and wave action that calls for strong attachment for the individual mussel (Kirk et al. 2007), while they are not, to the same extend as bottom mussels, exposed to invertebrate predators (Gosling 1992). Seed mussels on suspended cultures

have higher food availability than bottom dwelling mussels, due to their position in the water column, which support high growth rates (Garen et al. 2004; Camacho et al. 1995) that result in higher tissue content and thinner shells compared to bottom mussels (Kirk et al. 2007). On the seabed, however, mussels grow more slowly due to lower food availability and are indeed exposed to benthic invertebrate predators. The slow growth and the predation induce the development of a thick shell (Smith & Jennings 2000) and large adductor muscles (Reimer & Tedengren 1996). In Table 2 the differences documented in Chapter 2 and 3 in morphological parameters for the seed mussels from the two different origins are summarised.

Table 2. Overview of the differences in the morphological parameters of suspended and bottom seed mussels, documented in the studies reported upon in Chapter 2 and 3. Shell index is defined as the shell ash weight divided by the shell surface area (Reimer & Tedengren 1996). Relative adductor muscle is given as the posterior adductor muscle diameter related to the length of the mussel (Hancock 1965). Attachment strength was measured with a spring scale and given as the maximum weight (Chapter 2). Coefficient of Variance (CV) of density of mussels was used as measure for aggregation. Data marked with '+ ' indicate that there are significant differences between suspended and bottom seed mussels.

	Chapter 3 (incl. strate)	smooth and co	mplex sub-	Chapter 2 (incl. smooth substrate only)			
Parameter	Suspended mussels	Bottom mussels	Scale of differ- ence (%)	Suspended mussels	Bottom mussels	Scale of differ- ence (%)	
Length (mm)	26 ±2	30 ±2	13	43 ±1	41 ±1	5	
Density (ind. m <sup>-2</sup> )	200	200	0	803	978	$18^{+}$	
Shell index (mg mm <sup>-2</sup> )	0.71 ±0.20	1.02 ±0.18	30 <sup>+</sup>	0.84 ± 0.08	1.04 ±0.22	19 <sup>+</sup>	
Relative adductor muscle	0.125 ±0.018	0.132 ±0.016	$5^+$	0.122 ±0.015	0.120 ±0.013	2	
Attachment strength (g)	910 ±208	683 ±291	25 <sup>+</sup>	624 ±34	365 ±24	42 <sup>+</sup>	
Aggregation (CV)	1.3 ±0.3	1.2 ±0.3	8 <sup>+</sup>	2.0 ±0.6	1.6 ±0.3	20 <sup>+</sup>	

The thinner shell and smaller adductor muscles of suspended mussel individuals would expectedly make them more vulnerable to predators (Reimer & Tedengren 1996). However, when exposed to predation the consequence was not a higher mortality for suspended mussels compared to bottom mussels (Chapter 2). The stronger attachment strength and greater ability to aggregate for suspended mussels contrasted to bottom mussels may partly explain why suspended mussels in general do not exhibit higher mortality than bottom mussels.

Disturbance responses in blue mussels depend on the type of disturbance e.g. wave action induce byssal production to secure the mussel in its position (Young 1985), while predation additional to increased byssal production also induce formation of larger aggregates (Reimer & Tedengren 1997), development of thicker shells, and increased size of the adductor muscle (Reimer & Tedengren 1996). Response to predators changes with the type of predator. When predated by crabs, *Carcinus maenas*, mussel individuals develop thicker shells and seek spatial refuge (Reimer & Tedengren 1997). A thick shell protects the individual mussel against the crabs crushing technique and the spatial refuge reduces the number of attack angles which complicates the handling of the mussels by the predator (Reimer & Harms-Ringdahl 2001; Reimer & Tedengren 1997). Predation by starfish, *Asteria rubens*, has been documented to not only induce a thicker shell in the prey mussel, but also development of larger adductor muscles (Reimer & Harms-Ringdahl 2001; Reimer & Tedengren 1996). A larger adductor muscle makes it harder for the starfish to open the mussel and insert

its digesting stomach (Nordberg & Tedengren 1995). Water born cues, as well as direct contact with the predator induce the development of such responses (Chapter 2; Leonard et al. 1999; Reimer & Tedengren 1996, 1997; Côté 1995). Predator-induced defences may evolve when the prey suffers large, variable and unpredictable predation pressure (Havel 1987), which is the case for blue mussels, especially when located on the seabed. Due to the morphological differences between suspended and bottom mussels, the response to predation come into action in different strategies. Suspended mussels had an active response in relation to the predator by developing a significantly firmer attachment to the substrate and a closer aggregated structure, while bottom mussels had a passive strategy by having a thicker shell and larger relative size of the adductor muscle (Chapter 2).

# 6.1.2 Production output of suspended and bottom mussels

Despite differences in performance and predator response, suspended seed mussels are evaluated as a good alternative or additional source of seed for bottom mussel culturing (Chapter 2 and 3; Kamermans et al. 2009). Related to this evaluation, especially growth and mortality from predation are two essential parameters for the mussel production output, because the output of these two parameters defines success of the production.

Growht. Data on growth rates of mussels within the first period after transplantation of seed mussels are very scarce in the literature (Kristensen & Lassen 1997). It can, however, be expected that mussels, dependent on their initial condition, experience reduced growth rates until they have acclimatised to their new environment (e.g. food availability, structure stability, predator pressure). This is partly supported by Yanick et al. (2003) who documented potential for local adaptation effects between populations. The assumption corresponds with the results of specific growth rates obtained in Chapter 3, in which suspended mussels had a negative specific growth rate and, compared to bottom mussels, had a significantly lower specific growth rate. The negative and lower growth rates are explained by an insufficient food availability to sustain the higher condition index of the suspended mussels (Chapter 3). This conclusion is further supported by the slightly lower specific growth rates of mussels in Chapter 3 compared to the results in Frandsen & Dolmer (2002). This indicate that food availability in the study area in Chapter 3 was lower compared to that of the study area reported upon in Frandsen & Dolmer (2002), since temperature was within the same level (15 °C) during the two studies. The time scale is important when evaluating growth performance of mussels because of the expected reduction in growth after transplantation. In Chapter 2, the duration of the field experiment was 105 days and the results showed that suspended mussels at the end of the experiment had a significantly higher growth (given as shell increment) than bottom mussels. The discrepancy in the growth results in Chapter 2 and 3 can be expected to be due to the difference in duration of the two experiments since, in comparison, the experiment in Chapter 3 lasted for only 30 days. In Chapter 3, the results of the expected acclimating phase after transplantation were documented, while Chapter 2 presented the results of a more than three months growth period, which gives a more realistic picture of the growth performance and, with that, what can be expected of a final production output.

In Table 2 the response pattern regarding shell index, adductor muscle, attachment strength and aggregation between suspended and bottom seed mussels is similar within the studies in Chapter 2 and 3. Initial seeding density and shell length of the mussels, do not follow a pattern across the two studies. These two parameters, may therefore also be part of the explanation for the discrepancy in growth between the studies in Chapter 2 and 3. Seeding density in Chapter 2 was four times higher than the seeding density in Chapter 3, and within Chapter 2, the seeding density for bottom mussels was 18 % higher than for suspended mussels (Table 2). A high seeding density can result in growth limitations caused by intra-specific competition. A seeding density of 6.25 kg m<sup>-2</sup> is considered a high density compared to densities found in the natural mussel beds adjacent to the study area, where the experiment was conducted and the mean seeding density of 2.5-3.0 kg m<sup>-2</sup> in bottom cultures (Spencer 2002). It is therefore reasonable to assume that the intra-specific competition for food partly explain the higher growth rates among suspended mussels that experienced lower seeding density than the bottom seed mussels.

**Mortality.** A small initial shell length makes mussel individuals more vulnerable to predation after transplantation as small mussels are an easier target for the predators (Smallegange & van der Meer 2003; Paine 1976). When predating on small mussels, crabs, *Carcinus maenas*, reduces the risk of claw damage, which outweigh energy optimization (Smallegange & van der Meer 2003). This corresponds with the results in Chapter 2 and 3, since small mussels in the two studies had a higher mortality than larger mussels. In Chapter 3 it was assumed that the difference in shell length did not interfere with the ability of mussels to attach. However, regarding mortality due to predation, individual mussel size is important (section 6.2.2). This corresponds well with the fact that in both Chapter 2 and 3 the smallest mussels suffer the highest mortality independent of seed mussel origin (i.e. bottom and suspended cultures). Because of the smaller size of bottom mussels in Chapter 3 and of suspended mussels in Chapter 2, they suffer higher mortality, indicating that the size and not the origin of the mussels determines the mortality rate. Initial shell length of suspended mussels was 13 % smaller than of bottom mussels in Chapter 3, while it was 5 % larger for suspended mussels than bottom mussels in Chapter 2.

Another explanation for the contradicting results may be the experimental design, since the designs are not directly comparable. Although both studies were carried out in the field, the study described in Chapter 2 was conducted on the seabed and was thus designed for natural conditions, though it was manipulated. The experiment described in Chapter 3 was conducted on artificial bed structure. In Chapter 3, the loss rate included both mortality and migration (i.e. immigration and emigration) of mussel individuals. The loss rate was therefore, not directly comparable with the mortality rates found in Chapter 2, that included only mortality due to predation. Furthermore, the scale of difference in shell index between suspended and bottom mussels were larger in Chapter 3 than in Chapter 2. The larger difference in shell thickness index may also be part of the explanation to why the suspended mussels suffer a higher loss rate than bottom mussels in Chapter 3, as thin shelled mussels are more vulnerable to predation (Reimer & Harms-Ringdahl 2001).

Despite the discrepancy in growth and mortality in the study in Chapter 3 and Chapter 2, both studies showed that based on the production output at the end of the experiments, suspended blue mussels transplanted to the seabed is a viable seed source for bottom culture production due to lower mortality and well developed predatory defences (Chapter 2 and 3).

To be an additional or alternative source of seed, suspended seed mussels do not necessarily have to perform better than the natural bottom seed mussel. As long as suspended seeds result in a comparable output and quality of harvestable mussels, they are an interesting alternative. Regarding the seed source two things are equally important, 1) collection of enough seeds to secure the production and, 2) ability to control seed sampling and transplantation with a minimum influence on the seabed. Already in the seed phase suspended mussels have a great potential to impact a smaller seabed area compared to the traditional dredged bottom mussels seeds due to the higher seed density of the suspended lines (Murray et al. 2007a). Furthermore, the position of the seed collectors can be planned so that ecological-, economic- and social interests are accounted for, opposite to collection of seed mussels on natural benthic mussel beds that have to take place on natural established seed beds.

# 6.1.3 Conclusion

Blue mussels collected on suspended long line cultures in the water column have the potential to become an alternative seed source for mussel production in bottom cultures. When compared to mussels collected from natural benthic mussel beds, suspended mussels had an active predator response by developing a significantly stronger attachment to the substrate and a more pronounced aggregation behaviour. Bottom mussels exhibited a passive strategy by possessing a thicker shell and larger relative size of the posterior adductor muscle. When comparing the performance of suspended and bottom seed mussels transplanted on complex and smooth substrate, respectively, suspended mussels aggregated significantly more than bottom mussels on smooth substrates. This indicates that suspended mussels are better in achieving the protection provided by group living compared to bottom mussels, since more aggregated mussels are more protected against predators. Despite different strategies, comparison of production output between suspended and bottom seed mussels also show similar results. Thus, it is concluded that the use of suspended mussels in bottom culture production supplement, and possibly, secure the seed source in future blue mussel production in intensive bottom cultures.

# 6.2 Improvement of survival of mussels in blue mussel bottom cultures

# 6.2.1 Bed formation as predatory response

The aggregated structure of a mussel bed, where mussels live closely together and attached in a 3D matrix is expected to be one of the keys to their success, as the aggregated bed structure provides protection against biological and physical challenges (Dame 2012; Côté & Jelnikar 1999; Reimer & Tedengren 1997). Distinct larval settlement preferences and adult behaviour lead to the formation of the aggregated structures (Seed 1969), and the structures are stabilized by byssal treads. Stability of substrate is an ecological benefit for mussels to form aggregations or beds. The stable mussel bed is formed by the aggregated mussels interconnected and entangled by their byssal threads (Gosling 1992).

In Chapter 4, it was documented that mussels exposed to predation migrated significantly more downwards (i.e. vertically) in the oyster reef contrary to mussels not exposed to predators. Evidence that predation at the micro scale is one of the main causes of the closely aggregated bed structure is provided by a number of studies that show active aggregation behaviour and increased byssal production in response to variation in risk of predation (e.g. Chapter 2, 3 and 4; Kobak & Kakereko 2009 (*Dreissena polymorpha* (Pallas, 1771)); Côté & Jelnikar 1999; Reimer & Tedengren 1997). Laboratory experiments of aggregation response, however, failed to document an effect of predators on the aggregation behaviour of mussels, while the byssal attachment strength of mussels increased significantly when exposed to predators (Chapter 2). A power analysis revealed that with the number of samples available in the study, there was only a 30 % chance of the insignificant effect of predation to be true (Type II error). The result should, therefore, be interpreted with the necessary caution. Furthermore, Reimer & Tedengren (1997) have earlier shown that mussels exposed to predating starfish, *Asterias rubens*, and shore crab, *Carsinus maenas*, form larger aggregates, migrated less, and sought spatial refuges more often than mussels not exposed to predation. In the literature several authors have also documented a predatory effect on byssus attachment strength (Farrell & Crowe 2007; Dolmer 1998b; Reimer & Tedengren 1997; Côté 1995). A field experiment, in which mussels collected from low predation sites were transplanted to low and high predation sites, respectively, documented that mussels transplanted to high predation sites produced significantly more byssal threads and, thus, had significantly higher dislodgement force than mussels transplanted to low predation sites (Leonard et al. 1999).

Living in closely aggregated structures reduced blue mussel mortality due to predation (Chapter 3 and 4). Individuals in the centre of the mussel bed suffered lower predation than those positioned at the edge of the bed, probably because the latter are easier to grab and handle for a predator (Okamura 1986). Bertness & Grosholz (1985) found a reduction in mortality by both crab predation and winter ice scour when the Northern Ribbed mussel, Geukensia demissa (Dillwin, 1817), grew at high density (1,600 ind. m<sup>-2</sup>). When the surface complexity increased, so did the search time of the predating shore crab (Frandsen & Dolmer 2002), since a more complex surface provide an increased number of spatial refuges (Moksnes et al. 1998). This was also confirmed by Suchanek (1979), who documented that surface complexity increased with the density of California mussel, Mytilus californianus (Conrad, 1837). The protective effects of living close together, however, come at a cost. The aggregated structure is documented to negatively affect growth (Chapter 3; Reimer & Tedengren 1996 (6 % less growth); Okamura 1986), fecundity (Bertness & Grosholz 1985 (Geukensia demissa)) and pre- and post-settling mortality of conspecific larvae (Dolmer & Stenalt 2010; Troost et al. 2008) of mytilid mussels. Mussel aggregating behaviour can affect fecundity both positively and negatively. Living close together can improve the fertilisation success between individuals of a dioecious, broadcast species, whereas on the other hand and probably with a larger effect, fecundity can be adversely affected if growth of individual mussels is limited by food competition (Okamura 1986). Settling of conspecific larvae in a mussels bed results in a trade-off because the filtering mussels not only increase the mortality of larvae, but also serve as an important substrate, reducing post-settling predation from benthic predators (Dolmer & Stenalt 2010).

Retaining the advantages of the aggregated structure of a mussel bed without the cost in growth due to intra-specific competition, would therefore provide the bottom culture production method with a tool that reduced predation while still maintaining growth. Use of mussel shell substrate to increase substrate complexity might therefore be feasible in relation to production.

# 6.2.2 Effects of increased substrate complexity

Mussels are vulnerable to predation at all stages of the culturing process. Small mussels (shell length < 25-30 mm) are especially vulnerable to predation by crabs, *Carcinus maenas* (reviewed in Murray et al. 2007b). Predatory starfish, *Asterias vulgaris* (Linnaeus, 1758) prefer mussels smaller than 35 mm (O'Neill et al. 1983) and birds such as oystercatcher, *Haematopus ostralegus* (Linnaeus, 1758), typically feed on larger mussels (30-60 mm) (Gross-Custard et al. 2004), while eider duck, *Somateria mollissima* (Linnaeus, 1758), feed on mussels with a mean size of 18 mm (Milne & Dunnet 1972).

As described in Chapter 6.2.1, increased substrate complexity leads to increased survival among mussels (Chapter 3 and 4; Frandsen & Dolmer 2002), due to increased number of spatial refuges (Moksnes et al. 1998), and the firmer attachment to the substrate (Chapter 3). Hence, to reduce the intra-specific competi-

tion, which is a consequence of group living (Okamura 1986), mussel shells can substitute live mussels and still maintain the protective complex structure (Chapter 3). In Chapter 3 is was indicated that the effect of increased substrate complexity in a culture bed area may reduce loss of mussels, and that mussels formed a firm bed structure on this substrate faster than on smooth substrate. The results indicate that mussels on complex substrate stabilize the bed forming response faster than on smooth substrate. Thus, on the complex substrate, mussels have the protection from predators from the substrate right after transplantation due to more spatial refuges, in contrast to on smooth substrate where mussels need to aggregate to achieve the same protection (Chapter 3).

The density of mussels reported upon in Chapter 3 was not different between treatments with and without complex substrate. Thus, from the study it was possible to conclude that adding shell substrate to a certain density of mussels would reduce the loss rate of mussels. It was, however, not possible to evaluate if the complex substrate could substitute mussels and, thereby reduce mussel density with a reduction of intra-specific competition as a result.

Besides the positive effect of increased substrate complexity on survival, it was documented that the specific growth rates were reduced when substrate complexity was increased (Chapter 3). This indicated that food supply was reduced in complex substrate, since the most important factor explaining growth is the food supply (Thompson 1984). This trade-off was supported in Chapter 4 where mussels located within the protective interspaces of oyster, *Crassostrea gigas* (Thunberg, 1793), shells also suffered from reduced growth, but gained in terms of increased survival. The structures of the oyster reef studied in Chapter 4 and the treatments with complex substrate in Chapter 3 are however not directly comparable due to the significant larger size of oyster shells compared to mussel shells. Both studies, however, documented that increased substrate complexity results in increased survival and reduced growth of mussels. The question is how density of mussels should be adjusted to overcome the reduction of growth among mussels. In bottom cultures an overall high survival of the transplanted mussels and a high growth/condition index of the individual mussel are both essential for the production output. Knowledge on the interactions between food availability, substrate complexity and mussel density is therefore needed to successfully use complex substrate designs in culture beds in order to increase survival of mussels.

# 6.2.3 Conclusion

Substrate complexity stabilizes the structure of the mussel bed on micro-scale (< 1 m) resulting in an achievement of protection faster than without applying shell substrate to the seabed. On complex substrate, mussels have the protection from predators right after transplantation, due to more spatial refuges, in contrast to on smooth substrate where mussels need to aggregate to achieve the same protection. The stabilization is expressed by increased byssal strength and reduced aggregation activity within the mussels and is resulting in higher survival. However, the increased protection provided by the higher complexity also results in a trade-off between increased survival and reduced growth and lower condition index for the individual mussel. The production output is generally higher on complex substrate than on smooth sub-strate, and it is therefore concluded that an increased substrate complexity has the potential to improve survival of mussels in bottom culture beds. Nevertheless, due to the trade-off between survival and growth, the degree of complexity is important in the planning of culture beds to secure that the reduction in growth and condition index do not eliminate the increased survival of the mussels.

# 6.3 Support of area-intensive exploitation of blue mussels

To achieve smaller impacted area, prerequisites concerning robust bed structures and seeding densities needs to be fulfilled. A robust bed structure support mussel survival (e.g. Chapter 2; 3; 4; Reimer & Tedengren 1997; McGrorty et al 1990) and food availability (van de Kopple et al. 2005) while seeding density potentially impact the biomass production (Okamura 1986). The case study from the Limfjorden, Denmark, of bottom culture production presented in Chapter 5 indicates, using model simulations, that bottom cultures under certain conditions can impact a smaller area than fishery of full-grown blue mussels on natural beds, although the production includes both dredging of seed mussels on natural seed beds and dredging of the harvestable mussels.

# 6.3.1 Robust structures

In Chapter 5 it is documented using side scan sonar that the macrostructure of the individual culture bed was similar to the original transplantation tracks established the year before, indicating that the transplantation practice support the formation of stable mussel beds not affected by waves- or currents. The blue mussels can be assumed to aggregate within the tracks (Chapter 2 and 3; van der Koppel et al. 2008, 2005) but not at a scale that change the macrostructure of the tracks, and it is indicated that the procedure for transplanting blue mussels in tracks provide a sufficient heterogeneity to avoid a decline in growth across the culture bed (Chapter 5).

With the establishment of the bed structure in bottom culture described in Chapter 5, it is possible to sustain a fishable mean biomass (kg m<sup>-2</sup>) in the culture bed that corresponds to the biomass of mussels in what is quantified as a typical high biomass area in Limfjorden (DTU Aqua (2012), Fig. 3). Fig. 3 presents the distribution of densities of mussels in the Limfjorden in the last 10 years except 2005.



Fig. 3. Interpolated mussel densities in Limfjorden the last 10 years except 2005 where the survey was not conducted (Source: DATA: DTU Aqua (2012), MAP: Kerstin Geitner).

In the last 10 years the highest biomass of mussels in the Limfjorden has been 5.6 kg m<sup>-2</sup> (DTU Aqua 2012). However, the usual biomass for the fishery to consider as high biomass is 2.5-3.0 kg m<sup>-2</sup> (Fig. 3) (Dolmer et al. 2011). In comparison, the recorded harvestable biomass in the culture areas was 6.7 kg m<sup>-2</sup> (initial seed-ing density 3.5 kg m<sup>-2</sup>) after five months (Chapter 5). When comparing impacted area in culture production with fishery on natural stock, the seed fishery for the culture production also has to be included. According

to Chapter 5, seed fishery takes place on biomass of 5.0 kg m<sup>-2</sup>. However, if seeds are produced on suspended cultures, a biomass of 2.0 to 9.0 kg m<sup>-2</sup> equal to 4,500 to 20,300 individuals m<sup>-2</sup> has been documented (Walter & Leeuw 2007). The total impacted area using suspended mussel seeds for bottom culturing would thus be further reduced.

Blue mussel beds impact the distribution of phytoplankton in the water column (e.g. Nielsen & Maar 2007; Dolmer 2000; Fréchette & Bourget 1985). Dolmer (2000) documented that mussels deplete the phytoplankton concentration in the water column up to a couple of meters above the bed (Fig. 4).



Fig. 4. Vertical algal concentration profiles as a function of height (z) above mussels beds at three stations in Limfjorden, Denmark (From Dolmer 2000).

Hence, the amount of food available for the mussels depends very much on the vertical mixing of the water column by current, waves, wind and seabed complexity (e.g. Dame 2012; Nielsen & Maar 2007; Fréchette & Bourget 1985). Also bio-mixing where food-depleted jets of water expelled through the exhalent opening of a mussel affects the food availability, especially during low levels of current and wind induced mixing of the water column (Lassen et al. 2006). To secure food availability the bed structure is thus important when constructing bottom culture beds on both a small (Chapter 3) and large scale (Wiles et al. 2006; van de Koppel et al. 2005). Knowledge on food availability as a function of seabed complexity across constructed bottom culture beds is absent in literature. However, in flume experiments, Butman et al. (1994) demonstrated that turbulent mixing was three to ten times higher over a mussel bed compared to over smooth flume bottom. And on natural mussel beds, forming ribbed patterns similar to transplantation tracks in culture beds, van de Koppel et al. (2008; 2005) have documented that the bed structure impacted the food availability for the mussels. The patterns in young mussel beds are potentially not only explained by the interaction of a small-scale positive feedback but also a large-scale negative feedback to the growth of juvenile mussels. The positive feedback is a result of an aggregated structure that leads to protection against waves and water current, while the negative feedback is a result of depletion of algal food sources on a large scale (van de Koppel et al. 2005). It was thus concluded that self-organized spatial heterogeneity of blue mussels improves productivity, when compared to a completely homogeneous bed of blue mussels at the same density (van de Koppel et al. 2005), which also was concluded on small scale in Chapter 3.

#### 6.3.2 Seeding density

Beside a robust macro structure of the culture bed, also seeding density is of great importance to secure optimal growth and condition of the cultured mussels. Patch size and with that density dependent competi-

tion among mussels is one of the controlling factors for growth (Svane & Ompi 1993; Newell 1990; Okamura 1986), since intra-specific competition between mussels can lead to reduced growth and condition index (Svane & Ompi 1993; Newell 1990). The optimal seeding density for a given location depends on seed mussel size (Korringa 1976) and the hydrodynamics and with that the food availability of the system (Fréchette & Bourget 1985).

However, specific studies of the combined effects of seed density and seed size on growth of mussels in bottom cultures are absent in the literature. Lauzon-Guay et al. (2005) studied the effects on suspended cultures and documented that seed mussels in very high densities generally had a lower tissue-to-shell ratio on short term (first spring), and survival of small (13.6  $\pm$  0.4 mm) seed generally decreased with increasing initial density, while survival of large (28.5  $\pm$  0.3 mm) seed was not affected by initial density. Furthermore, the study showed that small seeds grew faster than large seeds while large seeds generally had a higher survival. The same response can be expected for mussels produced on the seabed, where density dependent responses, are also a well-known phenomenon (Asmus & Asmus 1991; Fréchette & Bourget 1985). The response might be more pronounced on the seabed, since food availability is reduced compared to food availability in the water column (Garen et al. 2004; Fréchette & Grant 1991).

In Chapter 5 it was demonstrated that seeding densities of 1.5 and 3.5 kg m<sup>-2</sup> (with mean initial shell length of 24 mm) did not affect shell growth, indicating that there was no detectable long term food limitation between transplantation tracks with either of the two seeding densities. The estimated shell growth rates for bottom cultures was in the same range as reported for natural mussel beds in Limfjorden (Chapter 2; Dolmer 1998a; Kristensen & Lassen 1997) and other eutrophic Danish estuaries (Petersen et al. 1997).

A study of impact of bottom culture beds on species richness, recommended to use lower seeding density, 2-3 kg m<sup>-2</sup>, in culture beds and to establish larger culture beds contrasted to smaller, to reduce the edge effects of the cultivated beds (Beadman et al. 2004). In that study it was synthesised that the presence of mussel beds change the infaunal community, at local scale (0-10 m), while the effect was not detectable at large scale (10-100 m) (Beadman et al. 2004). The recommended seeding density corresponds with the general practise in Europe of 2.5-3.0 kg m<sup>-2</sup> (Spencer 2002). However, the exact density is dependent on local conditions regarding food availability, seed mussel size and hydrodynamics in a given area.

# 6.3.3 Conclusion

It is concluded that production of blue mussels in bottom cultures support an area-intensive exploitation, with off-set in Danish production practices. The robust structure and the seeding density of 3.5 kg m<sup>-2</sup> support an area-intensive exploitation of blue mussels in the case area. On macro-scale (> 100 m) it can be documented that macrostructure of the individual culture bed was similar to the original transplantation tracks established the year before. This indicates that bottom cultures can form robust structures, not affected by wave- or current induced transport. Seeding density of 1.5 and 3.5 kg m<sup>-2</sup> and the position of the individual mussel in the culture bed apparently did not adversely affect shell growth, suggesting that there was no detectable food limitation between transplantation tracks. Production of blue mussels in bottom culture beds impact a smaller area compared to fishery on full-grown mussels. Bottom cultures will support an area-intensive production if the population production ratio is higher than 0.5.

# **Chapter 7 Recommendations and future perspectives**

Recommendations for the development of bottom culture production of blue mussels, *Mytilus edulis* (Linnaeus, 1758), in Europe are presented based on the results of the research questions addressed in the present PhD project. In addition, several important issues are still to be addressed to further develop bottom culture production in the future.

# 7.1 Supporting the seed mussel source in blue mussel bottom culture

# 7.1.1 Recommendations

• The use of blue mussel seeds collected from suspended long line cultures in the water column are recommended as an alternative or supplementary seed source in bottom culture production.

# 7.1.2 Future research and perspectives

Methodologies used today in bottom culture production are based on knowledge from research results and practical experience concerning seed size, seed density, optimal transplantation season, and optimal area for production (Spenser 2002; Korringa 1976). Based on the documented morphological differences between seed mussels collected on suspended cultures and from natural seed mussel beds (Chapter 2 and 3), it is expected that these differences can lead to adjustments valuable for the production. A full scale study on production output when using seed mussels collected on suspended long lines would provide the practical knowledge that is needed in order to evaluate the viability of a partly or full implementation of this seed source. To further fulfil the targets regarding development of area-intensive mussel exploitation, planning of optimal areas for seed production should be included in the study. The study should, with respect to spatial management and carrying capacity models, include both production of suspended seeds and the on-growth of the seeds on the seabed in a given area.

# 7.2 Improvement of survival of mussels in blue mussel bottom culture

# 7.2.1 Recommendations

It is recommended to develop the use of increased substrate complexity to support bed formation
of mussels with the aim to reduce predation on transplanted mussels. By increasing the substrate
complexity, predation can be reduced due to a faster bed formation of the transplanted mussels.
However, the increased complexity also led to a reduction in mussel growth rate.

# 7.2.2 Future research and perspectives

A prerequisite to full scale implementation of increased substrate complexity in bottom culture is identification of the balance of the trade-offs documented in Chapter 3 and 4, between optimal survival, growth rate and condition of mussels. A large scale experiment including both size of culture beds and time span of growth phase, on the effect of adding substrate to transplanted mussels, would provide the insight needed to achieve an optimal production output. The large scale experiment should include the different procedures in bottom culturing, such as the timing of applying the substrate (e.g. before transplantation of seeds or during transplantation), the amount of substrate needed, and the type and composition of the substrate that should be applied.

# 7.3 Support of area-intensive exploitation of blue mussels

# 7.3.1 Recommendations

 With the increased focus on ecosystem effects from use of spatial resources for different purposes, in relation to ecosystem based management, it is recommended to use bottom mussel culturing as a tool to intensify the blue mussel production in robust habitats leaving more sensitive habitats to be permanently closed. With bottom culture production it is possible to plan where to place the impacted area with respect to both ecologic-, and economic sustainability.

# 7.3.2 Future research and perspectives

Bottom mussel culture production should be included in future ecosystem based management. This would involve the development of a method for identification of suitable areas for bottom culture production that benefits production-, ecological- and social interests. Limitations for production regarding food availability for mussels, needs to be identified to secure productivity of the culture bed. Since bottom culture production will occupy specific areas for a shorter or longer period, potential conflicts and collaborations with other activities and stakeholders should be addressed and solved in order for the planning to be sustainable on the long term. Carrying capacity determinations, both ecologic- and social carrying capacity (Cranford et al. 2012), are needed to address the conflict between interest groups, when area-intensive production is planned.
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