Report on Odense Harbour Mussel Project - September 2015

A pilot study to assess the feasibility of cleaning sea water in Odense harbour by means of filter-feeding blue mussels on suspended rope nets

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SUMMARY: The Odense Kommune initiated in May 2015 a five month long pilot study to evaluate the feasibility of bio-filtering blue mussels to clean the heavily eutrophicated water in the harbour of Odense and called for meetings with NordShell and the University of Southern Denmark. Over a period of 16 weeks the environmental conditions, mussel offspring, growth, and filtration capacity was investigated on a weekly basis. The results of the present pilot study show that the growth conditions for mussels in Odense harbour are reasonably good. Thus, the weight-specific growth rates of transplanted mussels in net-bags are comparable to other marine areas, such as the Great Belt and Limfjorden. Even if the chlorophyll a concentration is 5 to 10 times higher than the Chl a concentration in the reference station in the Kerteminde Fjord mussels do not grow with a factor of 5 to 10 faster. Changing salinities, which might influence the growth of mussels to a minor degree, rarely fall below the critical value of 10. The natural recruitment of the local mussel population which is mainly located up to 2 m below the water surface is sufficient for rope farming, and natural thinning of densely populated ropes leads to densities between 20 to 56 ind. per 10 cm rope. Filtration rate of mussels in harbour water was in one case shown to be lower than measured in lab experiments, however, blue mussel can take part in the cleaning process of highly eutrophicated sea water in Odense harbour without touching the basic reasons for the high phytoplankton biomass.

KEY WORDS: clearance rate \cdot veliger larva \cdot rope-farming \cdot eutrophication \cdot smart-farm unit \cdot bivalves \cdot weight-specific growth rate \cdot *Mytilus edulis*

INTRODUCTION

Odense Harbour shows a high degree of eutrophication due to sewage after heavy rainfalls (4-20 times per year). Since the abatement of ship traffic in the innermost part of the harbour Odense Kommune is looking for new ways of usage for this centrally located urban site. Touristic activities, such as cultural demonstrations, swimming, and water sports (e.g. kayak polo) are planned and partly already realised. To increase the possibilities of leisure activities the water clarity in this area shall be improved. Eutrophication and limited water exchange might be driving forces for the high turbidity and phytoplankton densities. Therefore, Odense Kommune called for meetings with NordShell and University of Southern Denmark (SDU) to develop a plan to improve the water quality.



Fig. 1. Examples of smart farm units (SFU): Left = Full scale SFU system in a Norwegian fjord (Smart Farm AS, Stavanger, Norway), Right = test SFU in Odense harbour (approximately 12 m long, 18 August 2015).

The main aim of the present 16 weeks long pilot study was to assess the feasibility of cleaning the sea water in Odense harbour by means of filter-feeding blue mussels (*Mytilus edulis*) on ropes/nets suspended from floating 'smart-farm units' (Fig. 1). The first step was to study the environmental conditions and determine the suitability for growing mussels. Following this the potential of recruitment of local mussel population was investigated in terms of sufficient newly settling mussels. Next part was to evaluate the weight-specific growth rate of mussels suspended in net bags and on farm ropes at selected sites within the harbour area. The question; to what degree the mussels are able to realise their growth potential at the prevailing highly variable salinity and high phytoplankton biomass (measured as chlorophyll *a* concentration) has been in focus. Further, the filtration rate under natural conditions was tested in mesocosm experiments and compared to laboratory values. Finally, the feasibility of cleaning the water by bio-filtering blue mussels has been discussed.

MATERIALS AND METHODS

Study area

Odense harbour is located in the northern centre of the city. The inner harbour (tides \pm 30 cm) is connected via the Odense Channel to the Odense Fjord which disembogues in the Great Belt (Fig. 2). The harbour consists out of three basins (Basin 1, Basin 2, Basin 3) and an outer part in where a sailing club with a pier is located. From Fig. 2 the sampling stations can be seen, which are mainly located in Basin 2 and one in the Odense Channel (#5). There are three inlets from rain water basins which run over 4 to 20 times per year (two in the harbour and one in the channel). The stream, Stavis Å, enters the Odense Channel north of the crossing bridge whereas the river Odense Å disembogues in the Odense Fjord. In 2015 a numerous amount of construction work was done in the harbour area (especially in the inner Basin 2) where sedimented nutrients probably are re-suspended.



Fig. 2. Locations for sampling sites in the Odense harbour and Kerteminde Fjord where environmental parameters, plankton samples, growth, and filtration studies have been taken and performed: #0 = Kerteminde Fjord reference station (55°26'59"N, 10°39'40"E), #1 = Inner Harbour (55°24'25"N, 10°22'56"E), #2 = Middle Harbour (55°24'31"N, 10°22'51"E), #3 = Mussel test unit (55°24'28"N, 10°22'50"E), #4 = Ruslandkaj (55°24'34"N, 10°22'42"E), #5 = Odense Channel reference station (55°25'13"N, 10°22'45"E).

Field investigations

Mussel collection

Blue mussels were collected in at the reference station in Kerteminde Fjord (Fig. 2) in May 2015, cleaned and placed overnight in bio-filtered sea water (from the inlet of Kerteminde Fjord (salinity between 18 and 22)) to allow them to empty their guts.

Environmental parameters

In the Odense harbour stations and the reference station in Kerteminde every week environmental parameters (i.e. temperature, salinity, chlorophyll *a* concentration) were measured from sub-surface down to the sea floor in 20 cm depth intervals using a YSI 650 (Yellowstone Scientific Instruments, Big Sky, Montana, USA). The high resolution monitoring of water chemical characteristics allowed an insight into hydrographical processes in the Odense harbour that is important to understand the study site dynamics. Construction work in the innermost part of Basin 2 (#1) did not allow a continuous work because the station was excluded from all environmental and mussel growth concerning considerations (see for position Fig. 2). The YSI 650 was lowered with 1 m distance to the harbour wall to minimise the effect of biomixing blue mussels on the water chemistry. For reading out the collected data the YSI 650 was connected to a computer with the software programme EcoWatch.

The Secchi Depth (SD, m) was measured weekly at all stations to cross check the readings from the YSI 650 about phytoplankton biomass (in terms of chlorophyll *a* concentration) using a standard Secchi disc.

Current velocity and direction

When the present pilot study was started nothing was known about water movements, current situations, and driving forces of hydrographical motions in the harbour of Odense. Bio-filtering mussels can exert their greatest clearance activity when the water around or above them is moving slowing that time is sufficient to clear the water of particles (Lassen et al. 2006). In the opposite which the environment has low water movements, the water around the mussel bed or farm is cleared within few hours, but afterwards the mussels will starve because no new particles are transported towards them. The current situation in the harbour area was examined at station #3 during three 1 h study periods (30 June, 7 July, 15 July). Water

motions (i.e. current direction and speed) were recorded using a Mini Current Meter (model SD-6000, Sensordata a.s, Bergen, Norway) in two depths (1 and 3 m, respectively).

In situ growth study

Using a calliper rule total shell length of blue mussels was measured and individuals of approximately the same size $(22.5 \pm 1.6 \text{ mm}, \text{mean} \pm \text{SD})$ were selected (n = 360). Mussels for field growth experiments were transferred to commercially manufactured cylinder-shaped net bags (n = 30) made of polypropylene fibres with a mask width of 10 mm. The bags were placed in groups of three in stainless steel frames $(40 \times 40 \text{ cm})$ approximately 1 m below the water surface at three stations in Odense harbour and at a reference station in the Kerteminde Fjord. The frames were hold in position with a buoy and a concrete anchor at the sea floor (Fig. 3). Every 14 days the setup was lifted and one of the net bags containing 30 mussels was taken out carefully. The mussels were brought back to the Marine Biological Research Centre in Kerteminde (SDU) where they were frozen for 24 h at -20 °C and were measured in shell length and prepared for the weighing.



Fig. 3. Setup for mussel growth studies in the Odense harbour and Kerteminde Fjord. A steel frame with three net bags filled with mussels is hold in 1 m depth via buoy and anchor. Every second week the frame was lifted to the surface and one of the net bags was taken out to examine the mussels within.

Growth rate and condition index

Shell length (*L*, mm) and dry weight of soft parts (*DW*, mg) (24 h, 90 °C, Sartorius fine balance) were determined for representative groups of mussels (n = 30) on Day 0 and every second week during a period of 6 weeks. The condition index (*CI*, mg cm⁻³) was calculated according to the equation (Riisgård et al. 2013):

$$CI = DW/L^3$$
 Eq. (1)

The weight-specific growth rate (μ , % d⁻¹) of *Mytilus edulis* was determined using the equation (Clausen and Riisgård 1996):

$$\mu = \ln(W_t/W_0) \times t^{-1} \times 100$$
 Eq. (2)

Predation impact of mussels and ctenophores

Ctenophores are known predators of many zooplanktonic organisms including mussel veliger larvae. Therefore the predation impact by the gelatinous carnivores was quantified. The following relationship was used for converting oral-aboral length (L, mm) of *Mnemiopsis leidyi* to individual body volume (V, ml ind.⁻¹) (Riisgård et al. 2007):

$$V = 0.0226L^{1.72}$$
 Eq. (3)

The following equation (Riisgård et al. 2012a, based on Decker et al. 2004) was used to estimate the individual clearance rate (Cl_{ind} , 1 d⁻¹) of *M. leidyi* feeding on copepods as a function of their body volume (*V*, ml):

$$Cl_{ind} = 2.64V$$
 Eq. (4)

The volume-specific population clearance rates of mussels and ctenophores (Cl_{pop} , m³ water filtered by ctenophore population in one m³ water per day = m³ m⁻³ d⁻¹ and water filtered by mussel population per m² = m³ m⁻² d⁻¹) was estimated as the product of the individual clearance rate (Cl_{ind} , l d⁻¹) and the population density (D, ind. m⁻³) (Riisgård et al. 2012a):

$$Cl_{pop} = Cl_{ind} \times D/1000$$
 Eq. (5)

The time $(t_{1/2}, d)$ it takes for a population of ctenophores with known Cl_{pop} to reduce the concentration of prey organisms (copepods, respectively) in $V = 1 \text{ m}^3$ of water by 50 % (i.e. half-life time of prey) was estimated as (Riisgård et al. 2012a):

$$t_{1/2} = \ln 2/C l_{pop}$$
 Eq. (6)

The half-life time of phytoplankton organisms ($t_{1/2}$, d) in the water column was estimated as (Riisgård et al. 2004):

$$t_{1/2} = V_{total}/Cl_{pop} \times \ln 2 \qquad \text{Eq. (7)}$$

The filtration rate $(F, 1 h^{-1})$ of mussels in mesocosm experiments was determined from the exponential decrease in algal concentration as a function of time using the equation (Riisgård et al. 2013):

$$F = V \times b/n$$
 Eq. (8)

where V = volume of sea water, b = slope of regression line in a semi-ln plot for the reduction in algal concentration with time in the aquarium, n = number of mussels. The algal concentration was measured by means of a handheld fluorometer (Aquafluor, Turner Design). For evaluating their maximum filtration rate the flagellate *Rhodomonas salina* (Cryptophyta) was used in lab experiments. In mesocosm experiments newly pumped harbour water was taken minutes before the start from the surface.

Mesocosm filtration experiments

The filtration rate of blue mussels under natural conditions (i.e. *T*, *S*, Chl *a*) was studied in a number of mesocosm experiments in the Odense harbour. Thus, a holding tank was filled with sea water from the harbour. The water was collected with a pump positioned a few centimetres below the surface. Ten blue mussels were placed on a movable bottom plate and were allowed to acclimate for 30 min and to build up byssus threads. A transparent open plastic cylinder was placed on top of them and screwed at the bottom (remaining volume of 37 l). Air stones allowed sufficient water mixing. With an YSI 650 placed in the mid-volume (approximately 20 cm above the mussels) the decrease in chlorophyll *a* concentration over time was recorded. When the chl *a* concentration decreased below a certain level the cylindrical tube was lifted and new water from the surrounding could flow in. The chlorophyll *a* concentration was converted into algal concentration (*C*, cells ml⁻¹) using the empirical equation *Chl a* = 0.0008*C* + 1.0036. Following the exponential decrease in algal concentration and using Eq. 8 the individual filtration rate (*F_{ind}*, 1 h⁻¹) of a blue mussel in natural sea water was calculated.



Fig. 4. Mesocosm setup for mussel filtration experiments with natural sea water from the Odense harbour. Left = testing tube with 10 mussels, aeration, and YSI 650, Right = Experimental tank and project sign at station #3 in the background.

Larval settlement and young mussel growth

The progress of mussel larval population was followed throughout the study period. Zooplankton samples were taken every week at the five harbour stations and the reference station (#0) in Kerteminde. Samples were collected from 1 m depth by using a water sampler. Five litres of sea water were filtered through an 80 μ m filter in order to collect the zooplankton and have been preserved in 1.5 ml Lugol's solution (6 % iodine-potassium, 4 % iodine solution). Samples were brought back and counted using sediment chambers and an inverted microscope (Uthermöhl's cell counting technique) in the Marine Biological Research Centre in Kerteminde (SDU).

The second growth experiment was focused on growing newly settled young mussels (spat) on farm ropes. The existing mussel farm unit at station #3 was used to collect a small piece of rope every week (Fig. 5) from approximately 1 m depth. Therein the number and shell length of small mussels was examined on a standardised single string section (L = 10 cm). The rope was brought back in a bucket filled with sea water to the Marine Biological Research Centre and processed under a stereo microscope (25-fold magnification).



Fig. 5. Farm rope with Mytilus edulis collected from mussel farm unit at station #3 on 18 August 2015.

Sediment sampling

For estimating the size of the mussel 'background' population (natural population in the harbour) which also takes part in the cleaning process of the harbour water sediment samples have been taken to evaluate the bottom living mussel population. In terms of getting representative samples sediment hauls were conducted in the three harbour basins using a Van Veen Grab Sampler of board a small vessel (skipper Erik Østergård). The taken samples were

not quantitative but nevertheless gave an impression about the bottom living community and the sediment oxygenation.

Harbour water volume estimation

The first step to come up with an estimate of the total water volume (V_{total} , m³) of the Odense harbour was to calculate the harbour area using the FreeMapTools software (<u>http://www.freemaptools.com/</u>). Therein, the harbour was separated from the channel at the narrowest point (behind Halmstadsgade). Following this the depth of the different areas was needed to estimate the water volume. Therefore, the harbour was divided into four areas: Basin 1, Basin 2, Basin 3, Outer Basin. Depth measurements (ten measurements in total) onboard a small vessel (skipper Erik Østergård) were used as basis for an average depth of cuboid shaped basins (simplification). Summing the four basin volumes up gave an estimated V_{total} for the Odense harbour.

Due to limited time and resources only a few depth measurements could be done. Using cuboids as basic shape for the basins is a simplifications and could lead to overestimate of water volume because the basins are shallower close to the walls and deeper in the middle parts (trial to overcome this with averaged depth values).

Statistical analysis and data presentation

Analysis of variance (ANOVA) with repeated measures was carried out using R software (R Core Team 2013) for the investigation of temporal weight differences between the stations (Tab. 1, Fig. 11). Hypothesis of significance was accepted for P < 0.05. All calculations and creation of plots have been done in Microsoft Excel 2010.

RESULTS AND DISCUSSION

Environmental parameters

Vertical profiles of abiotic parameters (i.e. *T*, *S*, Chl *a*) have been taken on a weekly basis throughout the study period from May to August 2015. Values recorded in 1 m depth were used to generate development plots for the different stations as shown in Fig. 7. In Fig. 6 profiles of temperature, salinity, and chlorophyll *a* are shown at station #3 on 16 June. It appears obvious that between 1.5 and 2.0 m there is a halocline in all readings. The chlorophyll *a* concentration drops from 9.5 to 5.3 μ g l⁻¹ within 20 cm (see Fig. 6). Similar changes are also present in salinity (increase from 16.9 to 18.7) and less pronounced for temperature (increase from 15.9 to 16.2 °C). The halocline that can be identified in most of the other 88 vertical profiles varies with respect to the tide between 1.5 and 2.0 m, which could be caused by one of two things. on the one hand that light, due to high phytoplankton biomass in the surface, becomes limiting and sharply decreases or on the other hand, that

counter drifting water masses with different salinities and chlorophyll *a* concentrations are superimposed.



Fig. 6. Vertical profile of environmental parameters at station #3 on 16 June 2015: T = temperature, S = salinity, Chl a = chlorophyll a concentration.

The temporal development of environmental parameters is shown in Fig. 7. The typical pattern for a temperate coastal ecosystem can be seen in Fig. 7A (Kerteminde Fjord). The dashed line indicates the Danish coastal mean chlorophyll *a* concentration of 5.1 μ g l⁻¹. The Chl *a* concentrations in the oligotrophic fjord are generally below this and only exceeded once the mean (18 August 2015) after intense rainfalls. On the contrary, the fluctuations in salinity and chlorophyll *a* concentration in the harbour of Odense and the Odense Channel (7B and 7C, respectively) are by far bigger. With occasionally Chl *a* concentration > 20 μ g l⁻¹ the harbour might be characterised as eutrophicated and a highly variable study site. Probably due to intense rainfalls salinity reductions from 16.6 to 9.2 within one week are frequently recorded and might have effects on water exchange dynamics and inherent animals, such as blue mussels (Riisgård et al. 2013). Laboratory studies with blue mussels from the Great Belt and field observations revealed that the faster the salinity is either increasing or reducing, the more pronounced is the change in filtration and growth rate (Pleissner et al. 2013, Landes et al. 2015).



Fig. 7. Progress of environmental parameters throughout the study period measured in 1 m depth: T = temperature, S = salinity, Chl a = chlorophyll a concentration at (A) station #0 (Kerteminde), (B) station #3 (Chl

 $a = 86.2 \ \mu g \ l^{-1}$ on 18 August has been excluded) and (C) station #5. The dotted line in (7A) indicates the Danish coastal mean chlorophyll *a* concentration (DCMC).

Case study heavy rainfalls

Three rain water basins that flow over 4 to 20 times per year are situated around the Odense harbour. The change in environmental parameters (i.e. temperature, salinity, chlorophyll a concentration) after one intense rain event between 12 and 18 August 2015 has been shown in Fig. 8. The salinity dropped from 18.0 to 11.5 which can frequently be seen in the harbour. The chlorophyll *a* concentration was elevated during this event to supernatural high levels compared to eutrophic normal conditions (Chl a = 86.2 and 7.1 µg l⁻¹, respectively). Short term changes in nutrient availability might have caused the drastic increase in phytoplankton biomass seen in that week. Inner parts of Limfjorden, such as the Lovns Broadening experience comparably high phytoplankton biomasses (Chl $a = 47.3 \pm 28.2 \ \mu g \ l^{-1}$, mean \pm SD) where blue mussels still growth with comparable growth rates ($\mu = 5.0-5.9 \ \text{\%} \ \text{d}^{-1}$) (Landes et al. 2015). This 'boom and crash dynamic' seems to be characteristic for the Odense harbour and in certain weeks when rain falls have been intense phytoplankton might be out of control from mussels. Assuming the reason for these tremendous additions of nutrients is caused by sewage outlets and rain water basin overflows mussels may not always be able to buffer such nutrient inputs. On the other hand, these phytoplankton blooms last rather short because nutrients get limited after a few days and show high dynamics in the semi-enclosed study site.



Fig. 8. Progress of environmental parameters at station #3 before and after an intense rainfall between 12 and 18 August 2015 measured in 1 m depth: T = temperature, S = salinity, Chl a = chlorophyll a concentration.

The Secchi Depth was measured every week at all harbour stations. On 2 August 2015 the Secchi Depth was measured in the Great Belt to compare with the eutrophic conditions in the

harbour. In the Kerteminde harbour no Secchi Depth could be measured because the bottom was reached before the disc became invisible (Fig. 9). The general pattern of shallow Secchi Depths in the Odense harbour is persistent throughout the study period with a mean $SD = 2.2 \pm 0.5$ m (\pm SD). The Great Belt station revealed a Secchi Depth of 5.7 m. On basis of all measurements in the harbour it might be expected that the visibility in the Kerteminde Fjord is 2-3 times higher than in the eutrophicated Odense harbour. The extraordinarily low *SD* on 18 August 2015 can be traced back to heavy rainfalls in the days before (1.2 ± 0.4 m, mean \pm SD) which led to freshwater inflows from the rain basins and resuspension of sedimented nutrients which might gave rise to phytoplankton.



Fig. 9. Secchi Depth at four stations measured weekly throughout the study period. In case of #0 the bottom was reached with the Secchi disc for what reason there is no Secchi Depth. One measurement taken in the Great Belt (also Great Belt water at #0) allows a comparison between both study sites.

Water movements

Water currents were measured three times during 1 h study periods. Results suggest that the current direction in 1 and 3 m, respectively is opposed. For example on 30 June 2015 in the time between 2 and 4 p.m. the sea water was moving in the sub-surface in SW direction, whereas the current in 3 m depth was directed into NE. This pattern could be shown during the three short-term study periods. Vertical profiles of temperature, salinity, and chlorophyll *a* concentration already suggested that environmental parameters are changing in approximately 2 m depth. Combined with the flow direction data it might be that a density driven current is dominating the harbour with high saline bottom water moving in one direction and lower saline sea water floating in the opposite direction.

Additionally, the current speed was recorded by the Mini Current Meter. Unfortunately, in almost all cases the flow velocity was displayed as 1.0 m s^{-1} which is the lower trigger level of the instrument. This result can have two explanations. Either the current speed was around

that value or more probably the flow velocity was lower and automatically set to 1.0 m s^{-1} . In any case this result suggests that the harbour water is only moving with a low speed which should give the filter-feeding mussels time to reduce the amount of phytoplankton in the passing water mass.

Based on the present examination of water movements it is impossible to say if the water in Odense harbour is mainly mixing or stagnating and only pushed forth and back by the tides. A long-term study in the interface between channel and harbour could answer the question if there is a water exchange with the fjord and higher current velocities. A water body without internal movements would be difficult to clean for mussels which create a mixing (biomixing) themselves (Lassen et al. 2006) but not sufficient for a water volume of this size.

Harbour wall mussel population

It was assumed that along the harbour walls a self-maintaining blue mussel population was existing but at the start of the pilot study there was no information on densities and size of these mussels. Using a LH underwater camera system (Mocro Pocket DVRI video recorder) vertical video recording has been performed and allowed an insight into the wall growing mussel population. In Fig. 9 pictures from such a vertical transect vicarious at station #3 can be seen. Starting below the surface and going down to the bottom a clear change in population density can be recognised. While at the surface and above 2.5 m blue mussels build up a dense population which is initially overgrown by filamentous green algae and barnacles they gain importance when light gets limited (as assumed from high chlorophyll *a* concentrations at the surface). Below 2.5 m depth the number of mussels decreases drastically and soon no more bivalves growing at the wall can be seen (Fig. 10, Appendix).

This first step investigation about the natural harbour mussel population clarified that blue mussels are able to grow to big size and build up dense populations in the upper 2 m of the harbour. Unfortunately, the applied method is not quantitative and it cannot be stated a mean density or shell length. Nevertheless, assuming that mussels grow in the same pattern along all harbour walls as at station #3 growth conditions can be estimated as liveable. A vertical diver transect for sampling mussels in a small defined area would allow a better overview about the mussel population structure.

Sediment observations

Sediment samples were taken in all three harbour basins to estimate the bottom living community and sediment oxygenation. The samples consisted almost completely of anoxic sediment which was confirmed visually (see Fig. 11) and olfactory. Only very few mussel shells could be found in samples, whereas no living specimens were detected. One small common crab and polychaete were the only living organisms found in the basin 1 sample. Based on the almost complete absence of oxygen in the sediment and rarely found animals it

can be concluded that the bottom-based mussel background filtration capacity is extremely small, if not zero.

Estimates about the dissolved oxygen concentration in the water column (from surface to bottom) would give essential insights into the living conditions in the open water and close to the sea floor (depths between 3.5 and 7.5 m).



Fig. 11. Sediment samples lifted with a Van Veen Grab Sampler in the harbour of Odense: Left = Sample taken in Basin 2, Right: = Samples taken in Basin 3.

In situ growth study

The growth of medium sized blue mussels ($L_0 = 22.5 \pm 1.6$ mm, mean \pm SD) was followed in field experiments over a time of 42 days in June and July 2015 (Fig. 12, Tab. 1). The increase in dry weight of soft parts was compared between the eutrophic harbour of Odense and the oligotrophic Kerteminde Fjord was expressed as weight-specific growth rate (μ , % d⁻¹) (Tab. 2). The calculated growth rates for the three stations in Odense harbour where mussels have been brought out in net bags are in good agreement to each other (see Fig. 12, Tab. 2) and are slightly higher than the growth rate of blue mussels placed in the Kerteminde Fjord. A difference between them of 5 to 10 times what could be expected on the basis of chlorophyll *a* concentrations was not found (indicated by Landes et al. 2015) even if the differences in growth between the harbour of Odense and the Kerteminde Fjord are two stations significant (ANOVA, $F_{(1.447)} = 6.88$, P = 0.01).

The Condition index (*CI*, mg cm⁻³) was calculated to increase in all four stations during the 42 d study period from 6.9 mg cm⁻³ of at least 0.4 mg cm⁻³ (Tab. 1). The pattern of increase and decrease in *CI* is not that clear as in case of μ . However, the *CI* rather displaces growth conditions which are considered as nearly identically good in the Odense and Kerteminde harbours. The increase in dry weight of mussel soft parts (ΔDW) is a better marker for growth than the increase in shell length (ΔL) because bivalves first gain in weight before they

increase their shell length which makes the *DW* a more sensitive character in terms of growth characterisation (see *CI* in Tab. 1). In Tab. 3 growth rates based on dry weights from different locations are listed. However, the actual weight-specific growth rates measured in the present study are in good agreement with them even if they are in the lower end of the range. The highest μ reported by Riisgård et al. (2014a) from growth experiments in the Great Belt are taken in a comparably short study period and with slightly smaller mussels. Apart from this the growth rates are persistent in a chlorophyll *a* concentration range of Δ Chl *a* = 15 µg Γ^1 . Therefore, the growth conditions in the harbour of Odense can be considered in relation to the Kerteminde Fjord and other brackish water locations as suitable for blue mussels.



Fig. 12. Natural logarithm (ln) of dry weight of soft parts (*DW*, mg) of *Mytilus edulis* as a function of time (d) from three stations in the Odense harbour (#2, #3, #4) and the reference station in the Kerteminde harbour (#0). * indicates a significant difference (P = 0.01) to dry weight increases at the Kerteminde harbour station.

Tab. 1. Growth conditions of *Mytilus edulis* at three stations in the Odense harbour (#2, #3, #4) and at the reference station in the Kerteminde harbour (#0): t = growth period, *Chl a* = mean chlorophyll *a* concentration (± SD), L = mean shell length (± SD), DW = mean dry weight of soft tissues (± SD), CI = mean condition index (± SD), according to Eq. (1), S = number of survived mussels.

#	<i>t</i> (d)	Chl a (µg l ⁻¹)	L (mm)	DW (mg)	$CI (\mathrm{mg}\mathrm{cm}^{-3})$	<i>S</i> (ind.)				
Kerteminde Harbour										
0	0		22.5 ± 1.6	75.3 ± 24.9	6.9 ± 2.9	30				
	14		23.8 ± 1.8	98.9 ± 30.7	7.2 ± 1.5	29				
0	28		25.8 ± 1.7	127.6 ± 30.7	7.3 ± 1.3	27				
	42	2.8 ± 0.5	27.4 ± 1.9	205.5 ± 51.9	10.1 ± 2.9	28				
	Odense Harbour									
	0		22.5 ± 1.6	75.3 ± 24.9	6.9 ± 2.9	30				
r	14		25.8 ± 1.6	148.6 ± 34.6	8.6 ± 1.6	29				
2	28		28.5 ± 2.0	162.3 ± 30.2	7.1 ± 1.5	28				
	42	10.4 ± 6.3	31.5 ± 2.9	335.1 ± 68.0	7.3 ± 1.8	28				
	0		22.5 ± 1.6	75.3 ± 24.9	6.9 ± 2.9	30				
3	14		25.1 ± 1.9	106.3 ± 29.4	6.7 ± 1.6	30				
	28		28.9 ± 1.8	224.5 ± 51.5	9.4 ± 2.0	28				
	42	8.0 ± 3.8	31.4 ± 2.7	222.1 ± 42.8	10.9 ± 2.6	26				
4	0		22.5 ± 1.6	75.3 ± 24.9	6.9 ± 2.9	30				
	14		25.3 ± 1.8	133.5 ± 39.0	8.2 ± 2.3	27				
	28		28.2 ± 2.6	199.4 ± 48.9	9.0 ± 2.2	29				
	42	10.9 ± 5.1	31.8 ± 2.2	271.2 ± 56.6	8.5 ± 2.0	24				

Tab. 2. Growth of *Mytilus edulis* at three stations in the Odense harbour (#2, #3, #4) and at the reference station in the Kerteminde harbour (#0): Mean chlorophyll *a* concentration (\pm SD), Regression equation is taken from linear regression lines in Fig. 7, R^2 = correlation coefficient, μ = weight-specific growth rate based on slopes of linear regressions in Fig. 7 and Eq. (2).

#	Chl a (µg l ⁻¹)	Regression equation	R^2	$\mu \ (\% \ d^{-1})$		
Kerteminde Harbour						
0	2.8 ± 0.5	$\ln DW = 0.0233t + 4.2825$	0.976	2.33		
		Odense Harbour				
2	10.4 ± 6.3	$\ln DW = 0.0326t + 4.3716$	0.932	3.26		
3	8.0 ± 3.8	$\ln DW = 0.0285t + 4.3523$	0.889	2.85		
4	10.9 ± 5.1	$\ln DW = 0.0303t + 4.3916$	0.981	3.03		

Location	<i>t</i> (d)	$L_0 (\mathrm{mm})$	Chl a (µg l ⁻¹)	μ (% d ⁻¹)	References
Laboratory	35	26.6 ± 0.8	2.4	1.90	Clausen and Riisgard 1996
Great Belt	21	16.5	2.7	7.00	Riisgard et al. 2014a
Great Belt	69	21.1 ± 0.2	3.0 ± 0.2	3.05	Riisgard et al. 2014b
Limfjorden	41	21.0 ± 0.1	3.4 ± 0.2	4.15	Riisgard et al. 2014b
Nørrefjord	50	19.7 ± 0.4	17.2 ± 5.2	3.80	Landes et al. 2015
Odense Harbour	42	22.5 ± 1.6	9.8 ± 5.0	3.05	this study
Kerteminde Fjord	42	22.5 ± 1.6	2.8 ± 0.5	2.33	this study

Tab. 3. Weight-specific growth rates (μ , % d⁻¹) of *Mytilus edulis* in various laboratory and field experiments next to L_0 = mean initial shell length (\pm SD), t = study period, Chl a = mean chlorophyll a concentration (\pm SD).

Larval settlement

The seasonal pattern of larval density in the harbour of Odense followed the typical trend in temperate regions, with a pronounced early summer abundance peak, a much smaller peak in autumn, and comparably low mussel larval densities in between (Fig. 13). In the Kerteminde harbour (station #0) there was a twice as high larvae population in early spring than in the Odense harbour (262 and 126 ind. $5 \ 1^{-1}$, respectively). This might be due to a bigger spawning mussel population which is located in the entrance of the Kerteminde Fjord (Riisgård et al. 2006), due to assumed higher flow velocities in the fjord, or caused by a higher predation of mussel veliger larvae by jellyfish and ctenophores.

Riisgård et al. (2015a) systematically tried to gain an overall understanding of the year-round veliger larvae population (between 1989 and 2010) which is mostly attributed to *Mytilus edulis* in the inner Danish waters. In most years the spring density peak (typically in May) and subsequent lower autumn peak could be seen in Skive Fjord, Limfjorden with larval densities up to 319 ± 260 ind. 1^{-1} (10 to 100 times lower peak densities in several other locations). The reported observations show that mussel larvae are omnipresent in the studied areas, whereas the numbers may vary conspicuously between areas due to differences in mussel population size and hydrography (Riisgård et al. 2015a). The larval population in the inner Danish water might be sufficient to sustain a healthy young mussel population on harbour walls and other submerged surfaces.

Even if the presented maximum spring peak abundances (Fig. 13) are lower than the reported values in the heavily eutrophicated Skive Fjord they are still in the range of Kerteminde Bay spring larval bloom densities (4.9 ± 2.0 ind. 1^{-1} , study period between 2008-2011, Riisgård et al. 2015). The all-season persistently low mussel larval densities still allow a sufficient recruitment in the Odense harbour which is also reflected in the high densities of newly settled pediveliger on farm ropes (Fig. 14).



Fig. 13. Development of mussel veliger larvae (*Mytilus edulis*) densities at five stations during the study period between May and August 2015: #0 = Kerteminde Harbour, #2-4 = Odense Harbour, #5 = Odense Channel.

Case study: Ctenophore predation

Ctenophores (or comb jellies) can exert pronounced predation impacts on zooplankton species, including mussel larvae. Therefore, ctenophore sampling has been performed to characterise their predatory influence on veliger larvae in the harbour of Odense. Ctenophore hauls (three replicates) using a 500 μ m meshed plankton net (0.25 m² mouth area, KC Denmark A/S, haul length: 5 m, sub-surface) were performed in the Odense harbour on 14 July 2015 on station #2 and at the reference station in Kerteminde. After each haul, ctenophores were gently rinsed off the plankton net and subsequently measured for oral-aboral length (*L*, mm) to the nearest mm directly in the harbour. No *Aurelia aurita* have been caught during this procedure.

Predation on veliger larvae is not only exerted by the common jellyfish *Aurelia aurita* (predominant in May and June) but also by the invasive ctenophore *Mnemiopsis leidyi* (predominant in July and August). For a better understanding of the driving forces for the differences in mussel larval population in the Kerteminde Fjord compared to the Odense harbour the predation impact (i.e. half-life time $t_{1/2}$, d) of the introduced ctenophore was estimated on 4 August 2015. The predatory effect of *M. leidyi* on larvae of blue mussels varies between the two locations. Whereas in the Kerteminde Fjord a $t_{1/2}$ was found to be 12.2 d (using Eqs. 3-6), it was only 4.3 d in the harbour of Odense.

Nevertheless, the ctenophore sampling on 4 August is just a snapshot it reveals differences in population structures (i.e. size and abundance) of *M. leidyi*. The applied equations 4 and 6 are formulated for copepods and might be therefore an underestimation of the actual predation impact (different modes of mobility). The calculation clearly points out a strong control of veliger larvae population by the ctenophore (compared to the Kerteminde Fjord) which partly can explain the lower larval densities throughout the study period.

Growth of young mussels

Fig. 14 shows the population density and increasing mean shell length of newly settled *Mytilus edulis* spat on 10 cm farm rope. After an intense condensation phase up to 104 ind. 10 cm rope⁻¹ the number of young mussels decreases to a minimum of only 20 individuals and stabilises afterwards in intermediate densities. The natural thinning can be attributed to intraspecific competition for space. The growth of mussel could only be followed since 7 July 2015 because before the spat have been too small for length measurements. The equation for the linear regression of the spat growth (both not shown in Fig. 9) L = 0.2322t - 0.5686 is used to calculate the shell growth (ΔL , mm mo⁻¹). The slope of the regression line therein indicates the daily increase in shell length (mm d⁻¹) that in the respective study period (t = 52 d) was $\Delta L = 6.97$ mm mo⁻¹.



Fig. 14. Development of newly settled *Mytilus edulis* spat density per 10 cm single string and increase of mean shell length (\pm SD) of young mussels.

In Tab. 4 shell growth rates from other study sites and laboratory experiments are summarised. Even if there is an extremely high chlorophyll *a* concentration in the harbour of Odense the shell growth of young mussels is not 5 to 10 times higher as it could be expected from the higher density of phytoplankton organisms in the water (estimated on basis of chlorophyll *a* concentrations). For the conversion of algal cell number per ml to chl *a* concentration the empirical equation *Chl a* = 0.0008C + 1.0036 has been used. Growth studies on blue mussels conducted with comparably low chlorophyll *a* concentrations (Clausen and Riisgård 1996, Riisgård et al. 2012b, 2014a, b) and comparable pelagic phytoplankton biomass (Landes et al. 2015) showed that independently of the chlorophyll *a* concentration ΔL varies between 4.73 and 8.31 mm mo⁻¹ (6.97 mm mo⁻¹ in this study). The low shell growth rate reported by Riisgård et al. 2012 might be due to the short study period (only 15 days). Young mussels growing in the Odense harbour might therefore be in the ordinary growth range empirically proven in a number of other locations (Tab. 4). Even if it has been shown

that the shell growth is not as good as increase in soft tissue weight as growth parameter it allows an estimation about the general growth conditions (see *CI* in Tab. 1). In case of the initial very small mussel in this study (0.8 ± 0.1 mm, mean shell length \pm SD) taking out soft parts for the dry weight determination was not possible which is why ΔL has been used as growth parameter.

Location	<i>t</i> (d)	$L_0 (\mathrm{mm})$	Chl a (µg l ⁻¹)	$\Delta L (\mathrm{mm \ mo^{-1}})$	Reference
Laboratory	35	26.6 ± 0.8	2.4	4.7	Clausen and Riisgård 1996
Laboratory	15	19.5 ± 0.3	2.5	1.8	Riisgård et al. 2012b
Great Belt	21	14.8 ± 0.4	2.7 ± 0.6	5.0	Riisgård et al. 2014a
Great Belt	76	4.4 ± 1.6	3.1 ± 0.8	8.3	Riisgård et al. 2014b
Limfjorden	30	19.0 ± 0.1	17.6 ± 20.2	6.9	Landes et al. 2015
Nørrefjord	50	19.7 ± 0.4	17.2 ± 5.2	5.7	Landes et al. 2015
Odense Harbour	52	0.8 ± 0.1	18.4 ± 27.8	7.0	this study
Odense Harbour	42	22.5 ± 1.6	9.8 ± 5.0	6.5	this study
Kerteminde Fjord	42	22.5 ± 1.6	2.8 ± 0.4	3.6	this study

Tab. 4. Shell growth rates (ΔL , mm mo⁻¹) of *Mytilus edulis* in various laboratory and field experiments next to L_0 = mean initial shell length (\pm SD), t = study period, Chl a = mean chlorophyll a concentration (\pm SD).

Clearance rates and mesocosm experiments

Filtration experiments under natural conditions have been conducted to evaluate the *in situ* clearance potential. Fig. 15 shows a typical result of a filtration experiment in which the exponential decrease in algal concentration (by means of chlorophyll *a* concentration) is followed over time. In Tab. 5 the filtration rates of blue mussels under artificial and natural conditions are presented. Before (pre-study) and after (post-study) the field examination maximum filtration rates were measured in order to show healthiness of individuals. The individual filtration rates that were calculated in pre- and post-study experiments have been compared to literature values using the model equation $F = 0.0022L^2$ (Riisgård et al. 2011) and the mean shell length of 63.6 mm to be $F_{ind} = 8.9 \text{ l h}^{-1}$. Rates measured with acclimated mussels before and after the study lay in that order ($F_{ind} = 6.9 \pm 0.5$ and $5.6 \pm 1.8 \text{ l h}^{-1}$, respectively).

From Tab. 5 it appears that filtration rates measured under natural conditions were much lower than under lab conditions using monocultures of the unicellular dinoflagellate *Rhodomonas salina* and compared to model rates (Riisgård et al. 2011). For this discrepancy there might be several reasons. Fluctuating phytoplankton biomasses in the Odense harbour might have effects on the feeding rate of filter-feeding mussels. Especially after intense rainfalls the chlorophyll *a* concentrations can be extremely high (see Fig. 8). But also occasionally changes in the salinity can be observed regularly in the eutrophic study site (compare Fig. 7).



Fig. 15. Decreasing chl *a* concentration (Chl *a*, μ g l⁻¹) due to filtratory activity of ten blue mussels (mean shell length L = 63.6 mm) over time in a semi-ln plot in the pre-study period. The linear regression lines and their equations for the different experiments are shown. See also Tab. 4.

Tab. 5. Individual filtration rate (F_{ind} , 1 h⁻¹) of *Mytilus edulis* calculated from clearance experiments: V = volume (= 37 l), t = experimental time (= 30 min, every 5 min one sample was taken), # = time period, Chl a = mean chlorophyll a concentration (± SD), L = mean shell length (± SD), n_m = number of mussels, n_e = number of filtration experiments, F_{ind} = mean individual filtration rate (± SD). In the first study scenario mussels from the Odense harbour have been just. In the other cases mussels were brought from Kerteminde.

#	Chl a (µg l ⁻¹)	L (mm)	n_m	n _e	Prey item	F_{ind} (l h ⁻¹)
pre-study	5.3 ± 0.3	63.6 ± 2.5	10	4	Rhodomonas salina	6.9 ± 0.5
study	86.2 ± 0.0	48.4 ± 2.2	1	6	natural sea water	0.5 ± 0.2
study	4.8 ± 0.9	63.6 ± 2.5	10	3	natural sea water	1.7 ± 1.5
post-study	3.1 ± 0.6	63.6 ± 2.5	10	6	Rhodomonas salina	5.6 ± 1.8

The present measurements of reduced filtration rates cannot be associated with depressed growth rates as the weight-specific growth rates in the harbour are in good agreement with μ in other study sites in Denmark. Rather reduced assimilation efficiencies in terms of production of pseudo-faeces might be the reason for the comparably low filtration rate. Fig. 16 (Appendix) shows a blue mussel of about 60 mm producing a string of undigested algae of about the same length. During one of the filtration experiments 3 out of 10 mussels started producing pseudo-faeces (2 cm in 5 min, Chl $a = 4.8 \pm 0.9 \ \mu g \ \Gamma^1$ (mean \pm SD). The inability of digesting completely natural phytoplankton species composed food at already intermediate algal concentrations might indicate the reason for even lower filtration rates at extraordinarily high Chl *a*. The valve opening (i.e. shell opening degree, *SOD*) could not be quantified in the mesocosm experiments but lowered filtration rates in field studies can be correlated to reduced valve openings at intermediate and almost valve closure at extremely high chlorophyll *a* concentrations (own observations).

Reduced filtratory capacity was shown in a number of mesocosm experiments based in the Odense harbour. For the basic calculation of the total number of mussels that have to be brought in the harbour basins to control the phytoplankton biomass the model equation from Riisgård et al. (2011) was used because of unpredictable fluctuations of salinity and chlorophyll a concentration.

Harbour water volume and estimated amount of mussels

The harbour area was calculated using the FreeMapTools software to be 139,932 m² (= 0.140 km²). Depth measurements conducted in all four basins are the basis for the volume calculations and are shown in Fig. 17. Summing up the four basin-wise volumes the V_{total} can be estimated as 867,578 m³. For calculating the half-life time of phytoplankton as an indicator for the ability of mussels to control the algal bloom a standard mussel has to be defined: L = 40 mm, $F_{ind} = 3.5 \text{ l} \text{ h}^{-1} = 84 \text{ l} \text{ d}^{-1}$ (Riisgård et al. 2011). The calculation has been done on the assumption that the water body in the harbour is completely mixed. To what degree this assumption can be considered as realistic is at the end of the present pilot study not yet clear. Based on Eqs. (5) and (7), the calculated total water volume, the standard parameters for a mussel, and the assumptions of full water mixture the total number of needed mussels can be calculated as:

$$V_{total}/F_{ind} = 867,578 \times 10^{3} \, \text{l/84 l d}^{-1} = \underline{10.3 \times 10^{6} \text{ ind.}}$$
$$t_{1/2} = V_{total}/F_{pop} \times \ln 2 = 867,578 \times 10^{3} \, \text{l/(84 l d}^{-1} \times 10.3 \times 10^{3} \text{ ind.}) \times \ln 2 = \underline{0.69 \text{ d}}$$

The half-life time of phytoplankton indicates that mussels can control the algal biomass. The local mussel population takes part in the cleaning process, as well, and has to be subtracted from the amount of mussel that had to be brought in via suspended farm ropes (i.e. on SFU). Thus, the harbour mussel population will be defined for an area of 2 m height and 4,032 m length (length of harbour walls, FreeMapTools) with an estimated density of 200 ind. m² not taking into account mussels settled on ship hulls:

$$n_{local} = 2 \text{ m} \times 4,032 \text{ m} \times 200 \text{ ind. } \text{m}^2 = \underline{1.6 \times 10^6 \text{ ind.}}$$

 $n_{SFU} = n_{total} - n_{local} = 10.3 \times 10^6 \text{ ind.} - 1.6 \times 10^6 \text{ ind.} = \underline{8.7 \times 10^6 \text{ ind.}}$



Fig. 17. Measured depths (m) in the different harbour areas (source: Google Maps).

Conclusion

The results of the present pilot study show that the growth conditions for mussels in Odense harbour are reasonably good. Thus, the weight-specific growth rates of transplanted mussels in net-bags are comparable to other marine areas ($\mu = 2.85$ to 3.26 % d⁻¹), such as the Great Belt and Limfjorden. Even if the chlorophyll *a* concentration is 5 to 10 times higher than the Chl *a* concentration in the reference station in Kerteminde the mussels do not grow with a factor of 5 to 10 faster. Changing salinities, which might influence the growth of mussels to a minor degree, rarely fall below the critical value of 10. The natural recruitment of the local mussel population which is mainly located up to 2 m below the water surface is sufficient for rope farming and natural thinning of densely populated ropes leads to densities between 20 to 56 ind. per 10 cm rope. Filtration rate of mussels in harbour sea water was in one case shown to be lower than measured in lab experiments, however, blue mussel can take part in the cleaning process of highly eutrophicated sea water in Odense harbour without touching the basic reasons for the high phytoplankton biomass.

Possible future follow up studies

The vertical transect recorded by the underwater camera system along the harbour walls at station #3 could not reveal precise population characteristics (i.e. sizes and density of naturally growing mussel population). Divers could extend the gained knowledge about the background mussel population and could also quantify the presents of predators, such as sea stars or crabs.

It should be identified how nutrient rich (i.e. nitrogen and phosphorus) the sewage outlets in the harbour area are and if the blue mussel clearance capacity is appropriate to control phytoplankton biomasses that increase drastically within a few days. Evaluation of heavy metal loads, their elimination, and bacterial (i.e. *Escherichia coli*) concentrations are so far not considered in the project and could be part of a subsequent study.

Sediment samples revealed the absence of oxygen in the bottom layer but at the end of the pilot study the concentration of dissolved oxygen, its vertical profile and perhaps absence below a certain depth is not known. The driving force for the absence of blue mussels along the harbour wall below 2 m is still unclear. This pattern could be also seen in smart farm unit nettings. As maybe the most important abiotic factor for the presence of life in the sea sensing dissolved oxygen levels should be included in a follow up study.

To quantify the filtratory effect of mussels settled on suspended ropes of a smart farm unit the incoming chlorophyll *a* concentration could be measured before entering the vertical mussel bed as well as the out-coming filtered sea water chlorophyll *a* concentration.

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SUPPLEMENTARY DATA

In total 89 vertical profiles of temperature, salinity, and chlorophyll *a* concentration have been taken in the harbour and at the reference station in the time between 11 May and 28 August 2015 of which only one profile (i.e. Fig. 6) is shown in the report (contact: florian.lueskow@web.de).

The plots of progress of environmental parameters (as in Fig. 7) in 1 m depth at stations #1, #2, and #4 have not been shown in the report due to redundancy but display comparable patterns (contact: <u>florian.lueskow@web.de</u>).





Fig. 10. Vertical transect along harbour wall at station #3 showing the mussel population density in (A) 0.0 m, (B) 0.5 m, (C) 1.0 m, (D) 1.5 m, (E) 2.0 m, (F) 2.5 m, (G) 3.0 m, (H) 3.5 m, (I) 4.0 m, (J) 4.5 m, (K) 5.0 m, (L) 5.5 m depth.



Fig. 16. Mytilus edulis producing pseudo-faeces in natural sea water (mussel shell length about 60 mm).