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Published in:
Open Journal of Marine Science

DOI:
10.4236/ojms.2012.24020

Publication date:
2012

Document Version
Publisher's PDF, also known as Version of record

Link to publication

Citation for pulished version (APA):
Pleißner, D. (2012). Effect of salinity on growth of mussels, Mytilus edulis, with special reference to Great Belt (Denmark). *Open Journal of Marine Science*, *2*(4), 167-176. https://doi.org/10.4236/ojms.2012.24020

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Open Journal of Marine Science, 2012, 2, 167-176
http://dx.doi.org/10.4236/ojms.2012.24020 Published Online October 2012 (http://www.SciRP.org/journal/ojms)

Effect of Salinity on Growth of Mussels, *Mytilus edulis*, with Special Reference to Great Belt (Denmark)

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Received August 14, 2012; revised September 14, 2012; accepted September 28, 2012

ABSTRACT

The effects of salinities between 10 and 30 psu on the growth of blue mussels, *Mytilus edulis*, were studied in laboratory feeding experiments and compared to the growth of mussels suspended in net-bags in the brackish water Great Belt, Denmark. In the laboratory, 3 series of growth experiments were conducted: in Series #1, groups of mussels were exposed to 10, 15, 25 and 30 psu, in Series #2, two groups of mussels were exposed to 10 and 30 psu, respectively, for 15 days (first period) where upon the mussels were exposed to the reversed salinities for another 15 days (second period). In Series #3, two groups of mussels were initially exposed to 15 and 25 psu for 22 days whereupon the mussel groups were exposed to the reversed salinities for another 17 days. In the laboratory experiments there was a tendency towards reduced growth with decreasing salinity, reflected as reduced shell growth rate and decreasing weight specific growth rate with falling salinity. The shell growth rate was relatively low in the first feeding period compared to the second period, and mussels that were initially exposed to 10 psu, where the growth was low, exhibited fast growth when subsequently exposed to 30 psu, and reversed when 30 psu mussels were exposed to 10 psu. The study showed that mussels are able to adjust growth at changing salinities, and the observed effect of salinity could partly be explained by a temporary shell valve closure after a sudden change in salinity. The specific growth rate of mussels measured in laboratory experiments at salinities between 15 to 25 psu (4.2% to 4.8% d^{-1}) were comparable to the growth of mussels in the field experiment (3.2% to 4.0% d^{-1}) where the salinity varied between 24 and 13 psu during the growth period.

Keywords: Mussels; *Mytilus edulis*; Salinity Effects; Growth Rates; Condition Index; Doubling Time

1. Introduction

The blue mussel, *Mytilus edulis*, is a common member of the filter-feeding zoobenthos of coastal and estuarine intertidal areas where it may often encounter different salinities. Salinity is a key environmental factor that may directly influence the feeding behaviour and growth of mussels. Thus, constantly low salinities, frequency and amplitude of salinity changes, as well as the changing-rate of salinity will influence the behavioural response (siphon and shell valve closure), the filtration activity, actual growth rate, the maximal size, and the early development and survival of mussels [1-9].

Due to low salinities in the inner Baltic Sea (6 to 8 psu in the northern Baltic proper) *Mytilus edulis* is dwarfed in this area [10-12], and in the northern Baltic Sea along the southern coast of Finland where the salinity decreases from 6.5 psu in the west to become only 3 psu in the east, *M. edulis* lives at the margin of its salinity tolerance, and here a marked decline in mussel size can be observed along the decreasing salinity gradient [8]. Davenport [3] studied the response of both sudden and slowly falling seawater salinities on *M. edulis*' exhalant-siphon and shell valve closure. The behavioural response of the mussel was found to be dependent upon the rate of changes in salinity rather than being triggered by a fall to a critical salinity. At a changing rate of 16.75 psu·h^{-1} (which may be compared to 10 to 12 psu·h^{-1} in the Conway estuary, UK, [13]), Davenport [3,4] found it likely that oxygen depletion in the mantle water may be delayed "as a result of the mussels' partial isolation response to falling salinity". The effect of fluctuating salinity with a rate of 5.33 psu·h^{-1} (sinusoidal variation between 0 and 32 psu during 6 h) on shell growth of small *M. edulis* has been studied by Almada-Villela [5] who found that the growth was "markedly depressed". Further, Almada-Villela [5] studied the long term (38 d) effect on shell growth of different lowered salinities, and found that growth was significantly depressed at 6.4 and 16 psu, but not at 22.4 psu. Likewise, Hiebenthal *et al*. [14] recently found that *M. edulis* performed best at 25 psu and further that temperature effects may interact with salinity effects. However, acclimation to reduced salinities may take place [3,4,9,15-17], but due to shell valve closure

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and reduced filtration rate in the acclimation period, the resulting temporarily reduced growth may blur the actual ability of mussels to grow more or less unrestrained at low salinities, possibly down to 10 psu as studied in the present work.

Great Belt is one of the Danish Straits that form the transition between the tidal North Sea and the non-tidal Baltic Sea. The water exchange between the Baltic Sea and the open sea is driven both by the river run-off and by the meteorological conditions over the North Sea-Baltic Sea area [18-20]. In Great Belt the salinity varies according to changing flow situations; outflow of water from the Baltic Sea gives salinities down to less than 10 psu whereas inflow to the Baltic Sea gives salinities up to 27 psu in the upper layer [20-22]. Frequencies and amplitudes of salinity changes above the permanent halocline in the northern Great Belt appear from **Table 1** which shows data for the 10 year period 2000 to 2010. The mean salinity is about 17 psu, major salinity changes of about 8.5 psu take place with an annual frequency of about 10, a mean duration time of 21 d, and with a mean salinity changing rate of 0.4 psu·d^{-1}. Therefore, in order to evaluate the potential for future line-mussel cultivation in the Great Belt it has been of interest to know the possible effect of the frequently changing salinities on the growth rate of mussels, which nevertheless seem to be able to reach the full size of marine mussels (10 cm shell length of 5 years old line-mussels; Mads Anker van Deurs, Nordshell, personal communication).

In the present work, we studied the effect of different salinities (between 10 and 30 psu) on *Mytilus edulis* in laboratory feeding and growth experiments, and the obtained growth rates have been compared to the actual growth of mussels suspended in net-bags in Great Belt.

2. Materials and Methods

2.1. Laboratory Feeding and Growth Experiments

Blue mussels, *Mytilus edulis*, were collected in Kerteminde Bugt in the autumn of 2009 and transported to the nearby Marine Biological Research Centre (SDU) in Kerteminde, Denmark, where the feeding and growth experiments were conducted on groups of 20 or 30 mussels (mean shell length about 20 mm) in 16 l aquaria with seawater of different salinity (10, 15, 25 or 30 psu) adjusted by means of distilled water or salt (Read Sea Marine Salt) added to natural sea water, measured by means of a salinity meter (YSI 30). A dosing pump supplied the growth aquaria holding the experimental mussels with suspension of pure algae (the about 6 µm diameter flagellate *Rhodomonas salina* from a batch culture) which were kept homogeneous by strong aeration with 4 air stones. The growth experiments were carried out as 3 series, each performed at a well-defined algal concentration, adjusted by means of the dosing pump within the concentration interval where the mussels exploit their filtration capacity (*i.e.* above lower trigger concentration leading to closure of the valves, and below the satiation concentration where the mussels shut down [23-26] thus ensuring a steady-state between the addition and removal (feeding by mussels, wash out with through flow) of algal cells [27]. The algal concentration was measured by means of an electronic particle counter (Elzone 5380) several times a day during the experimental period to ensure that the concentration of *Rhodomonas salina* in the stock solution flask could be adjusted to be near constant.

Three series of growth experiments were conducted. In Series #1, 4 groups of mussels were exposed to 10, 15,

Table 1. Annual mean (S_{mean}), maximum (S_{max}) and minimum (S_{min}) salinities, frequency (f_S) of major (>4 psu) salinity changes, amplitude of major salinity changes (ΔS), duration time of major salinity changes (Δt), and salinity changing rates ($\Delta S_r = \Delta S/\Delta t$) in the period 2000 to 2010 measured above the permanent halocline in the northern Great Belt (STB 53, Fyn County 2001, Figure 8.4 therein) by the Danish Nature Agency, Danish Ministry of the Environment. Standard deviations (±) are indicated.

Year	S_{mean} (psu)	S_{max} (psu)	S_{min} (psu)	f_S (year^{-1})	ΔS (psu)	Δt (d)	ΔS_r (psu·d^{-1})
2000	17.2 ± 4.5	27.1	10.7	7	10.0 ± 3.4	15 ± 9	0.7
2001	16.3 ± 3.3	22.6	10.6	15	8.5 ± 1.2	22 ± 9	0.4
2002	16.1 ± 4.4	24.8	9.6	12	7.7 ± 2.3	17 ± 11	0.5
2003	17.2 ± 4.2	25.6	9.9	12	9.7 ± 2.1	21 ± 10	0.5
2004	17.4 ± 3.8	24.2	10.7	10	8.7 ± 2.1	29 ± 17	0.3
2005	16.0 ± 3.5	25.3	10.5	11	7.7 ± 2.7	18 ± 10	0.4
2006	16.7 ± 3.5	24.1	10.1	10	8.9 ± 2.1	20 ± 9	0.4
2007	16.4 ± 3.4	23.2	10.8	9	7.5 ± 2.3	21 ± 10	0.4
2008	16.8 ± 4.6	25.2	9.4	8	9.6 ± 2.1	28 ± 15	0.3
2009	17.4 ± 3.9	24.4	10.5	11	8.0 ± 2.1	23 ± 14	0.3
2010	15.4 ± 3.7	22.4	10.2	10	7.7 ± 1.8	16 ± 5	0.5
Mean	16.6 ± 0.6	24.4 ± 1.4	10.3 ± 0.5	10 ± 2	8.5 ± 0.9	21 ± 5	0.4 ± 0.1

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25 and 30 psu, respectively, for 14 days to allow the mussels to adjust, and subsequently during the following 17 days a mean steady-state algal concentration was established in order to compare the growth rate of the mussels. The shell length and the dry weight of soft parts were determined at the beginning and at the end of the experiment. In Series #2, two groups of mussels were exposed to 10 and 30 psu, respectively, for 15 days whereupon the 2 mussel groups were exposed to the reversed salinities for another 15 days. At regular intervals during the experiment, the shell length was measured, and 5 mussels were taken out on Day 15 for determination of body dry weight. In Series #3, 2 groups of 30 mussels were initially exposed to 15 and 25 psu for 22 days whereupon the 2 mussel groups were momentarily exposed to the reversed salinities for another 17 days. At regular intervals during the experiment, the shell length was measured, and 5 mussels were taken out on Day 10, 15, 22, 30, and 32 for determination of body dry weight. All experiments were conducted at 12˚C to 15˚C. No mortalities of experimental mussels were observed.

2.2. Field Growth Experiment

In the present study, the growth rate of *Mytilus edulis* sampled in Great Belt was compared with the growth of mussels collected in Limfjorden where the salinity is generally higher (about 25 to 28 psu in the central parts, e.g. [28,29]) than in the Great Belt surface water (about 17 to 21 psu during summer, [20], **Table 1**) in which the field growth experiment was performed. Small mussels (18 to 23 mm) *Mytilus edulis* were obtained from line-mussel farms in the Great Belt (near Svendborg) and in Limfjorden (Hvalpsund) two days before the onset of a field growth experiment conducted in the period 9 September to 30 October 2009 with 3 size groups of mussels in net bags at Stavreshoved (Great Belt, 55˚28.36'N, 10˚44.60'E, depth 8.5 m). The mussels were measured with a vernier gauge, sorted into size groups (Great Belt: shell length 18.6 ± 0.3 mm and 23.1 ± 0.3 mm; Limfjorden: 20.1 ± 0.3 mm) and put into net bags (Go Deep International Inc.) before they were transferred to the field location and hung up in a buoy system 1 m below the water surface, and weights were tight to the net bags to stabilize them in a vertical position. The net bags (widths of masks = 10 × 10 mm) were made of polypropylene fibres and cotton strings that disintegrate after about 1 week which result in an increase of mask width. The net bags were 50 cm long and placed approximately 1 m apart to avoid entanglement. Samples were subsequently collected with about 14 days interval from the buoy systems and transported to the Marine Biological Research Centre, Kerteminde, for analysis. Dry weight of shells and soft parts were measured by removing the soft parts from the shells, measure the wet weight and then drying the soft parts on pieces of tin foil in an oven for 24 h at 90˚C; shell lengths were measured with a vernier gauge.

2.3. Equations

The condition index (CI, mg·cm^{-3}) of the mussels was calculated from the dry weight of soft parts (W, mg) and the shell length (L, cm) according to the formula:

$$CI = \frac{W}{L^3} \tag{1}$$

The growth of an exponentially growing organism can be expressed by the equation: $W_t = W_o e^{\mu t}$ where W_o and W_t is the body dry weight on Day 0 and Day t, respectively, and thus the specific growth rate (μ, d^{-1}) can be calculated according to the equation:

$$\mu = \ln\left(\frac{W_t}{W_o}\right) t^{-1} \tag{2}$$

or obtained from slope of regression line for semi-ln plot of W versus time.

The time (t_2, d) for doubling the dry weight of soft parts of any given size of mussel is given by the equation:

$$t_2 = \frac{\ln 2}{\mu} \tag{3}$$

2.4. Statistical Analysis

Statistical analyses of impact of salinity on specific growth rate, doubling time and shell growth rate (**Tables 2-4**) were carried out by "One way repeated measures analysis of variance (*ANOVA*)" in Sigma Plot.

3. Results

3.1. Laboratory Studies

The measured increase in shell length, weight specific growth rate, and doubling time of mussels grown at different salinities in steady-state feeding experiments are shown in **Tables 2-4**. The experimental data from Series #2 are depicted in **Figures 1** and **2**. From **Figure 1** it appears that the mussels closed their shells when the salinities were changed from 10 to 30 psu and from 30 to 10 psu on Day 15 and that they remained more or less closed (as easily observed by eye) during the following week. On Day 22 the mussels were again fully open and filtering thus allowing the steady-state feeding condition to be re-established. It appears from the first growth period that the weight specific growth rate was low at 10 psu, μ =1.6% d^{-1}, compared to μ = 5.6% d^{-1} at 30 psu, and further, after transfer of mussels from high to low and *vice versa*, this resulted in increased and reduced growth at the high and low salinity, respectively (**Table 3**). **Fig-**

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Table 2. *Mytilus edulis*. Growth of mussels (Series #1) constantly exposed to 10, 15, 25 or 30 psu at maintained algal concentration (*C*) during the whole experimental time (*t*) inparallel steady-state experiments. L_o and L_t = initial and final shell length, respectively; W_o and W_t = initial and final body dry weight, respectively; $\Delta L/t$ = daily increase in shell length; μ [= $\ln(W_t/W_o)t^{-1} \times 100$] = weight specific growth rate; t_2 (=ln2/μ) = doubling time. Mean ± S.D., n = 20.

Series	S (psu)	t (d)	C (cells·ml^{-1})	L_o (mm)	L_t (mm)	$\Delta L/t$ (mm·d^{-1})	W_o (mg)	W_t (mg)	μ (% d^{-1})	t_2 (d)
#1A	10	17	2761 ± 910	20.0 ± 0.4	21.3 ± 0.5	0.071	22.9 ± 3.7	51.1 ± 8.1	4.7	14.8
#1B	15	17	2614 ± 1257	19.6 ± 0.3	21.3 ± 0.5	0.100	26.7 ± 2.8	40.7 ± 5.8	2.5	27.7
#1C	25	17	2353 ± 1212	19.5 ± 0.3	22.0 ± 0.7	0.147	26.7 ± 2.8	43.2 ± 5.4	2.8	24.8
#1D	30	17	2852 ± 722	20.0 ± 0.4	22.7 ± 0.7	0.159	22.9 ± 3.7	72.0 ± 7.7	6.7	10.4

Table 3. *Mytilus edulis*. Growth of mussels (Series #2) exposed to initially 10 and 30 psu whereupon the salinities were interchanged during the following 15 d at maintained algal concentration (*C*) during experimental time (*t*) in steady-state experiments. L_o and L_t = initial and final shell length, respectively; W_o and W_t = initial and final body dry weight, respectively; $\Delta L/t$ = daily increase in shell length; μ = weight specific growth rate (from slopes in Figure 2); t_2 = doubling time. Mean ± S.D., n = 20.

Series	S (psu)	t (d)	C (cells·ml^{-1})	L_o (mm)	L_t (mm)	$\Delta L/t$ (mm·d^{-1})	W_o (mg)	W_t (mg)	μ (% d^{-1})	t_2 (d)
#2A	10	15	2978 ± 1684	19.5 ± 0.3	19.6 ± 0.3	0.01	16.8 ± 2.8	21.5 ± 4.2	1.6	43.3
#2B	30	15	1934 ± 874	19.5 ± 0.3	20.4 ± 0.4	0.06	16.8 ± 2.8	39.5 ± 6.3	5.6	12.4
#2C	10 to 30	15	3212 ± 1000	19.5 ± 0.3	21.3 ± 0.2	0.12	21.5 ± 4.1	66.3 ± 10.3	6.2	11.2
#2D	30 to 10	15	2578 ± 720	20.3 ± 0.4	21.3 ± 0.4	0.06	39.5 ± 6.3	69.8 ± 8.6	3.1	22.4

Table 4. *Mytilus edulis*. Growth of mussels (Series #3) exposed to initially 15 and 25 psu during 22 d whereupon the salinities were interchanged during the following 11 d at maintained algal concentration (*C*) during experimental time (*t*) in steady-state experiments. L_o and L_t = initial and final shell length, respectively; W_o and W_t = initial and final body dry weight, respectively; $\Delta L/t$ = daily increase in shell length; μ = weight specific growth rate (from slopes in Figure 5); t_2 = doubling time. Mean ± S.D., n = 20.

Series	S (psu)	t (d)	C (cells·ml^{-1})	L_o (mm)	L_t (mm)	$\Delta L/t$ (mm·d^{-1})	W_o (mg)	W_t (mg)	μ (% d^{-1})	t_2 (d)
#3A	15	22	3351 ± 1407	19.6 ± 0.3	20.0 ± 0.5	0.02	12.5 ± 2.2	33.4 ± 8.1	4.6	15.1
#3B	25	22	3185 ± 1371	19.5 ± 0.3	20.3 ± 0.6	0.04	12.5 ± 2.2	34.8 ± 6.6	4.8	14.4
#3C	15 to 25	11	2959 ± 961	20.0 ± 0.5	21.5 ± 0.9	0.14	33.4 ± 8.1	54.0 ± 9.9	4.0	16.5
#3D	25 to 15	11	3533 ± 1697	20.3 ± 0.6	21.6 ± 0.8	0.12	34.8 ± 6.6	52.8 ± 10.1	4.2	17.3

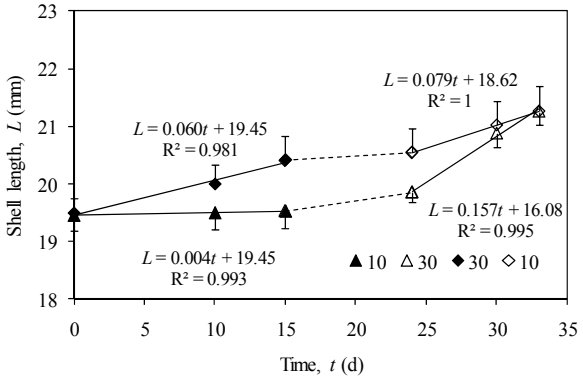

Figure 1. *Mytilus edulis*. Mean (± SD) increase in shell length (*L*) of mussels in Series #2 (Table 3) where the mussels in 2 parallel growth experiments were exposed to 10 and 30 psu, respectively, during the first 15 days, whereupon the salinities were changed from 10 to 30, and from 30 to 10 psu, respectively. The data are split into 2 growth periods: Period I with closed symbols for 10 and 30 psu, Period II with open symbols for mussels transferred from 10 to 30 psu, and from 30 to 10 psu, respectively. Linear regression lines and their equations are shown. The slopes of the lines indicate the daily increase in shell length (mm·d^{-1}). During the time period indicated with dotted lines the mussels were closed or not fully open.

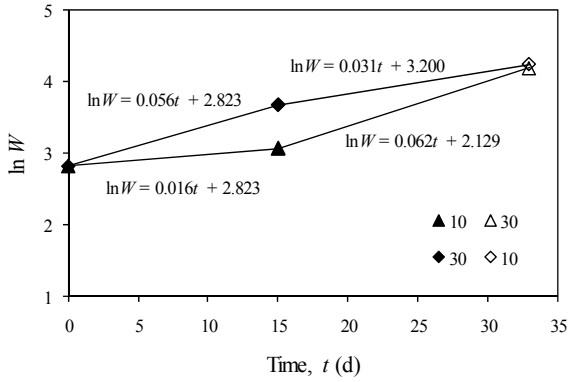

Figure 2. *Mytilus edulis*. The natural logarithm (ln) of mean dry weight of soft parts (*W*, mg) of mussels in Series #2 (Table 3). The data are split into 2 growth periods: Period I with closed symbols, Period II with open symbols. Linear regression lines and their equations are shown. The slopes of the lines indicate the weight specific growth rate (μ, d^{-1}).

ure 3 shows that the condition index increased during the growth period, from 2 to about 7 mg·cm^{-3}. The growth data from Series #3 are depicted in **Figures 4** and **5**, and it is notable that the weight specific growth rate was near constant, between 4.0% and 4.8% d^{-1}, during the whole

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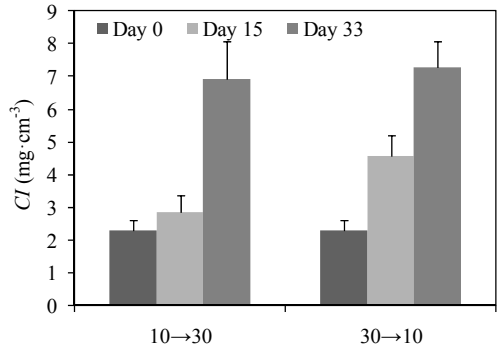

Figure 3. *Mytilus edulis*. Condition index (*CI*) for mussels in two parallel growth experiments, Series #2 (Table 4) where the salinity was changed from 10 to 30 psu, and decreased from 30 to 10 psu, respectively. Mean + S.D., $n = 20$.

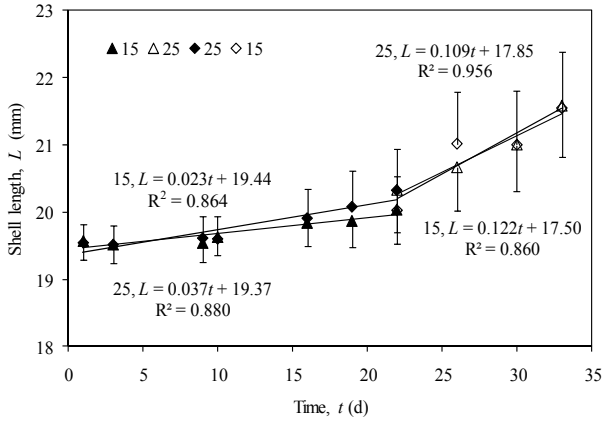

Figure 4. *Mytilus edulis*. Mean (±S.D.) increase in shell length (*L*) of mussels in Series #3 (Table 4). The data are split into 2 growth periods: Period I with closed symbols, Period II with open symbols. Linear regression lines and their equations are shown. The slopes of the lines indicate the daily increase in shell length (mm·d^{-1}).

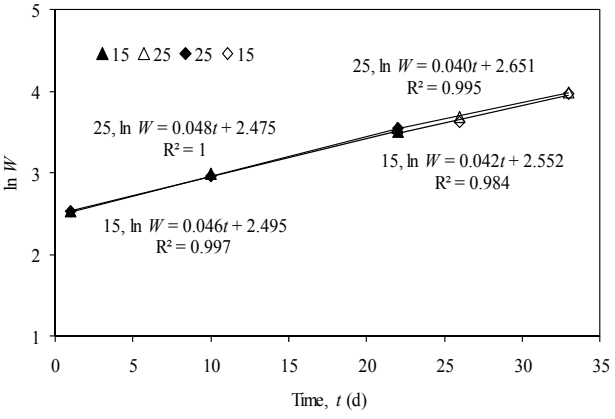

Figure 5. *Mytilusedulis*. The natural logarithm (ln) of mean dry weight of soft parts (*W*, mg) of mussels in Series #3 (Table 4). The data are split into 2 growth periods: Period I with closed symbols, Period II with open symbols. Linear regression lines and their equations are shown. The slopes of the lines indicate the weight specific growth rate (μ, d^{-1}).

growth period irrespective of the salinity, whereas the increase in shell length was relatively low, 0.02 to 0.04 mm·d^{-1}, during the first period, where the condition index increased increased from less than 2 to about 6 mg·cm^{-3} (**Figure 6**). The doubling times of body dry weight for mussels grown at different salinities in all the experiments are shown in **Figure 7**. Although there was a tendency of longer doubling times at the lower salinities, it is remarkable that the most pronounced effect of salinity wasa temporary closure of the shells which lasted for several days (cf. **Figure 1**) when the salinity was suddenly changed.

Figure 8 shows the growth rate of shells of *Mytilus edulis* exposed to different salinities. It is seen that the growth rate of shell length increased with increasing salinity when the salinity was kept constant, whereas the growth was lower in the first period of growth experiments in which the salinity was either increased or decreased in the middle of the experiment. The weight spe-

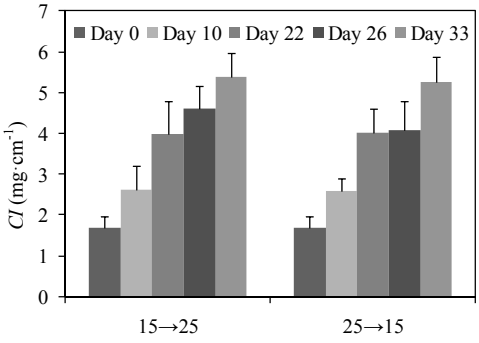

Figure 6. *Mytilus edulis*. Condition index (*CI*) for mussels in two parallel growth experiments, Series #3 (Table 4) where the salinity was changed from 15 to 25 psu, and from 25 to 15 psu, respectively. Mean + S.D.

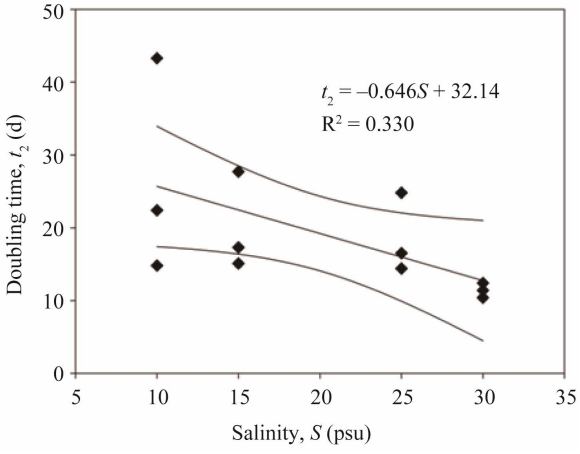

Figure 7. *Mytilus edulis*. Doubling time of body dry weight for mussels grown at different salinities during 11 to 22 days on *Rhodomonas salina* cells. Regression line, its equation, and the 95% confidence interval (dashed lines) are indicated. Data from Tables 2-4.

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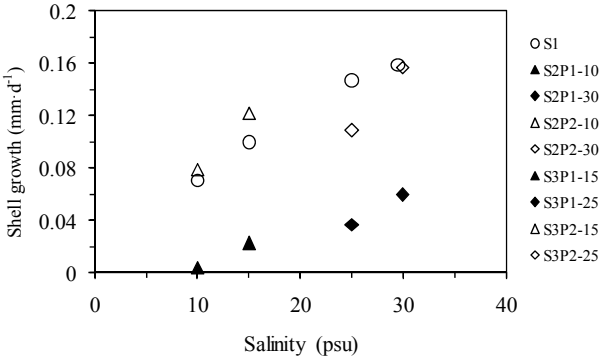

Figure 8. *Mytilus edulis*. Increase rate of shell length of mussels fed with algal cells and exposed to different salinities. Symbols: S1 = Series #1; S2P1-10 = Series #2, Period I, 10 psu, etc.

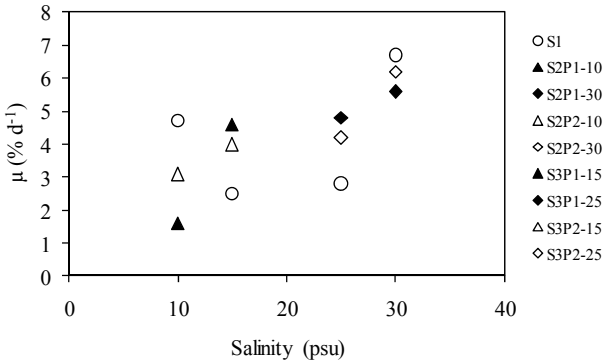

Figure 9. *Mytilus edulis*. Weight specific growth rate (*μ*) of mussels in Series #1 (S1) at different constant salinities, and in Series #2 & #3 with high/low salinities during first period (PI), and second period (PII) with reversed salinities, see Figures 2 & 5. Symbols: S2P1-10 = Series #2, Period I, 10 psu, etc.

cific growth rates of mussels exposed to different salinities in the various growth experiments are shown in **Figure 9** which indicate that there was a tendency of reduced growth with decreasing salinity, and it is striking that mussels exposed to 10 psu in the first period were growing slowly (1.6% d^{-1}) but when the water was changed to 30 psu, the mussels obtained a fast growth rate (6.2% d^{-1}). Likewise, mussels that were initially exposed to 30 psu had a fast growth rate of 5.6% d^{-1}, but when the salinity was reduced to 10 psu, the growth rate decreased to 3.1% d^{-1}.

One way repeated measures *ANOVA* revealed that the specific growth rate ($P = 0.034$) and shell growth rate ($P = 0.028$) are statistically significant different and the hypothesis of reduced specific growth rate and shell growth rate with decreasing salinity (**Tables 2** and **3**, at 10 and 30 psu, and change from 30 to 10 psu and reverse) can be accepted. Doubling times, however, are not statistically significant different ($P = 0.190$). Further, one way repeated measures *ANOVA* revealed that the weight specific growth rates ($P = 0.580$), shell growth rates ($P = 0.084$), and doubling times ($P = 0.178$) are not statistically significant different, and likewise not between 15 and 25 psu (**Tables 2** and **4**, at 15 and 25 psu, and for change from 25 to 15 psu and reverse). Thus, the statistical analysis indicate significant decrease of weight specific growth rate and shell growth rate when salinity was changed from 30 to 10 psu and reverse, but not when changed from 25 to 15 psu and reverse.

3.2. Field Studies

The growth rates of mussels in net bags in the field are shown on **Figures 10** and **11**. The increase in shell length is expressed by the slope of regression lines (**Figure 10**), and for Great Belt mussels the growth rate was 0.177 and 0.128 mm·d^{-1} for the initially 18.6 and 23.1 mm mussels, respectively, while the shell growth rate of the 20.1 mm

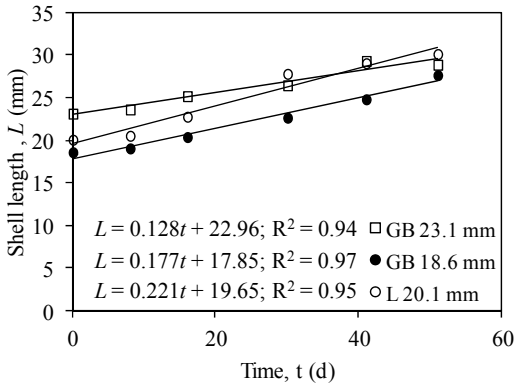

Figure 10. *Mytilus edulis*. Increase in mean (±S.D.) shell length as a function of time of 3 size classes of mussels transferred from Limfjorden (L) and Great Belt (GB) and hung up in a buoy system at Stavreshoved (Great Belt). The initial shell length of the 3 size classes are indicated.

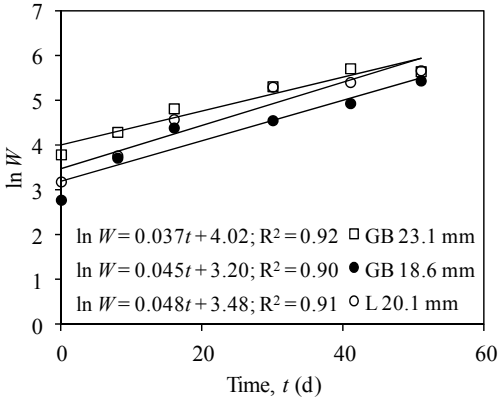

Figure 11. *Mytilus edulis*. Natural logarithm (ln) to dry weight of soft parts (*W*, mg) as a function of time of 3 size classes of mussels transferred from Limfjorden (L) and Great Belt (GB) and hung up in a buoy system at Stavreshoved (Great Belt). The initial shell length of the 3 size classes are indicated.

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mussels from Limfjorden was 0.221 mm·d^{-1}. Likewise, the weight specific growth rates are expressed by the slope of regression lines (**Figure 11**). Thus, the weight specific growth rate of 20.1 mm mussels from Limfjorden was 4.8% d^{-1} which may be compared with 4.5 and 3.7% d^{-1} for 18.6 and 23.1 mm Great Belt mussels, respectively. From **Figure 12** it appears that the initial condition index was low, <3 mg·cm^{-3}, but rapidly increased during especially the first 30 days to become high, about 9 to 12 mg·cm^{-3}. The mussels from Limfjorden had the shortest body-dry weight doubling time of t_2 = (ln2/0.048 =) 14.4 d, compared to 15.4 and 18.7 d for the 18.6 and 23.1 mm Great Belt mussels, respectively. Clearly, the mussels from Limfjorden were not impeded by being transferred to the lower saline Great Belt.

4. Discussion

In all the laboratory mussel-growth experiments there was a tendency towards reduced growth with decreasing salinity, reflected as increasing doubling time of the body dry weight (**Figure 7**), reduced shell growth (**Figure 8**), and as decreasing weight specific growth rate with falling salinity (**Figure 9**). However, it is notable that the shell growth rate was relatively low in the first feeding period compared to the second period (**Figure 8**). This phenomenon is likely to be due physiological and behavioural adaptation correlated with shell valve opening-closing [1,3,4,16], possibly combined with an increasing condition index during the experiments (**Figures 3** and **6**). It is also striking that mussels initially exposed to 10 psu where the growth was low (1.6% d^{-1}) exhibited a faster growth when exposed to 30 psu, and reversed, when 30 psu mussels were exposed to 10 psu (**Figure 2**, **Table 3**).

The cumulative shell length increase of small (15 mm)

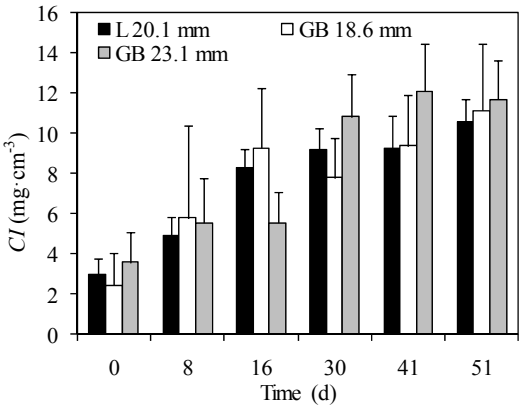

Figure 12. ***Mytilus edulis*****. Condition index (*CI*, mean + S.D.) as a function of time of 3 size classes of mussels transferred from Limfjorden (L) and Great Belt (GB) and hung up in a buoy system at Stavreshoved (Great Belt). The initial shell lengths of the 3 size classes are indicated.**

Mytilus edulis at different constant lowered salinities, between 12.8 and 32 psu, was studied in a long-term experiment by Almada-Villela [5] using a laser technique. It was found that the growth was initially delayed at 12.8 and 16 psu but recovered eventually, and mussels in the range 19.2 to 32 psu grew at similar rates. The delay of growth was overcome rapidly for mussels in 16 psu whereas those in 12.8 psu "suffered severely from low salinity" (*i.e*. shell closure for 8 to 9 days, only 50% of the mussels survived more than 12 days). It was suggested by Almada-Villela [5] that acclimation of shell growth does take place and that the threshold for shell growth is close to 12.8 psu. Further, Hiebenthal *et al*. [14] found that effects of salinity on both growth and mortality may be influenced by temperature. In the present work no mortalities were observed at 10 psu where a considerable shell growth was observed in Series #1 and Series #S2P2-10 (**Figure 8**). However, the present study confirms that mussels are able to adjust shell growth at reduced salinities, and the observed tolerance of the brackish water Great Belt mussels to lower salinities than experienced by Almada-Villela [5] may be explained by the high saline (32 psu) mussels used by the last-mentioned [7,30,31]. The growth rate and maximum size of *M. edulis* is much lower in the Baltic Sea (7 psu) than in the North Sea (28 psu), and reciprocally transplanted mussels were found by Kautsky *et al*. [7] to grow at rates similar to those of native mussels at each site, and therefore the authors suggested that the observed variation should be explained by "physiological differences due to environmental salinity". The mortality was very high (>90%) among North Sea mussels transferred to the Baltic Sea, whereas the survival of Baltic mussels transferred to the North Sea was very high, and therefore Kautsky *et al*. [7] suggested that the different survival rates may have "genetic causes", and differences between Baltic and North Sea mussels may thus be due to "differential selection" as suggested by Tedengren *et al*. [30] and Johannesson *et al*. [31].

The weight specific growth rates measured in the laboratory growth studies on *Mytilus edulis* exposed to salinities between 15 to 25 psu (**Figure 5**) are comparable to the growth rates obtained in the field growth experiment (**Figure 11**). Thus, the doubling times of body dry weight in the laboratory experiments varied between 10.4 and 24.8 d (**Tables 2-4**), which may be compared with 14.4 to 18.7 d for mussels in the field, and this inspires confidence to both relevance and credibility of the laboratory growth experiments.

The chlorophyll *a* concentration in Great Belt is routinely monitored by the Danish Nature Agency, Danish Ministry of the Environment. The mean (±S.D.) concentration measured during the field-growth period in the northern Great Belt (55˚30.46'N, 10˚51.72'E, 12 km NE

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of Stavreshoved) was 3.0 ± 1.1 µg·chl*a*·l^{-1} which is equivalent to (3.0/1.251 × 10^3 =) 2400 *Rhodomonas salina* cells·ml^{-1} [32]. This value is fairly close to the mean algal concentration used in the laboratory feeding experiments, and this indicates that the observed effects of salinity on growth may also apply for mussels in the field. Here, it should be mentioned that available monitoring data on the biomass of autotrophic and heterotrophic plankton in the Great Belt has recently been examined by Riisgård *et al*. [33] who found that the heterotrophic biomass generally accounts for less than 10% of the total pelagic biomass, and that it therefore can be concluded that the autrotrophic plankton, which can be quantified by measurement of the chl*a* concentration, generally dominates the pelagic microplankton in the Great Belt. In the actual field-growth period for mussels, the salinity varied between 24.4 and 13.0 psu (**Figure 13**), and the salinity decreased from 23.3 to 13.3 psu in the period 13 to 26 October, thus resulting in a decrease of ([23.3 – 13.3]/13 =) 0.8 psu·d^{-1}. This salinity changing rate may be compared with the values listed in **Table 1** from which it appears that the typical salinity changing rate in the Great Belt is about 0.4 psu·d^{-1} and that major salinity changes take place about10 times per year with at duration time of about 21 d.

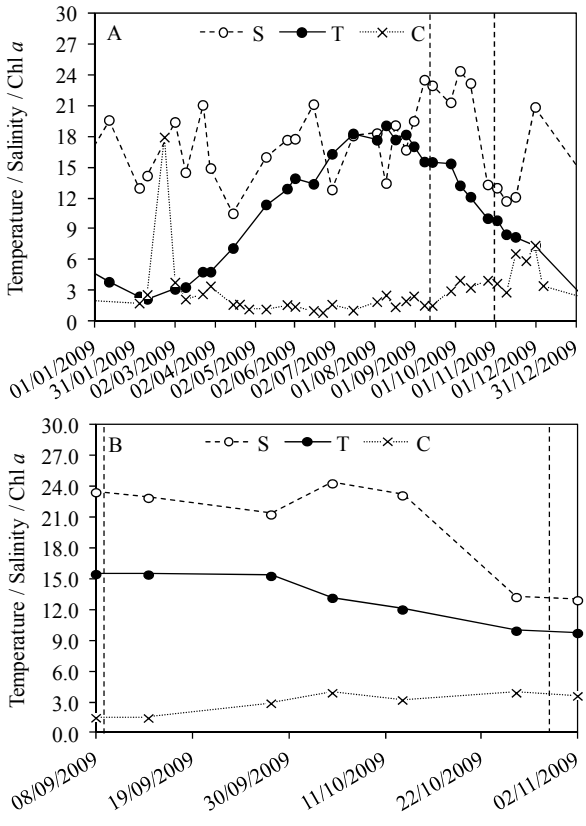

Figure 13. Salinity (S, psu), temperature (T, ˚C), and chlorophyll *a* (C, µg·l^{-1}) in the northern Great Belt (STB53, close to the experimental net-bag mussel growth site) (A) in 2009, and (B) during the mussel growth period (marked by broken vertical lines). Based on data provided by the Danish Nature Agency, Danish Ministry of the Environment.

The shell-closure effect observed in the laboratory when the salinity was suddenly changed and which lasted for several days (**Figure 1**) does not represent the normal environmental conditions in the Great Belt (**Table 1**, **Figure 13**) where the salinity-changing rate is relatively low and where *Mytilus edulis* may therefore be able to adjust (acclimate) to the occasionally high (up to about 25 psu) and low salinities (down to about 10 psu) in such a way that the growth rate remains rather unaffected. More studies on the effect of Great Belt-relevant salinity changing rates on feeding behaviour as well as growth experiments with mussels that are well-adjusted to low salinities (*i.e*. fully open valves and exhalant siphon, cf. [3]) are needed to separate the acute shell-closing effect on food uptake and growth from the possible physiological effect of low salinities on the metabolism and excretion of nitrogen [11,34,35] resulting in reduced growth.

5. Acknowledgements

This work formed part of the MarBioShell project supported by the Danish Agency for Science, Technology and Innovation for the period January 2008 to December 2012. Thanks are due to the Danish Nature Agency, Danish Ministry of the Environment, for providing monitoring data from the Great Belt, and to Kim Lundgreen for comments on the manuscript.

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