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Effect of Different Filter Methods on Seawater Quality at a Marine Scallop Hatchery

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Abstract

The effect of using two different filtering methods in the main seawater inlet to a scallop (*Pecten maximus*) hatchery in Norway was tested. Seawater was filtered through active filter media (AFM) and a protein skimmer, or through a drum filter and a protein skimmer. Seawater quality was characterized and tested on algal growth rate, egg development and larval activity. Tests were performed under winter and spring conditions (March, April and May 2009).

Both seawater treatments reduced the dissolved organic carbon concentrations in the inlet seawater. The total bacterial number was stable in both seawater treatments, except for an increase in the drum filter in March. The bacterial community showed seasonal development: Actinobacteria and Alphaproteobacteria dominated in March, while Gammaproteobacteria dominated in April and May. In a cluster analysis, samples from both seawater treatments showed high similarity on similar sampling dates. *Vibrio* spp. occurred, but was never observed in seawater coming from the skimmer after the drum filter. This sampling point was often clustered as most similar to the incoming seawater.

The fraction of scallop eggs that developed into veliger larvae increased from 10% to 50% during the sampling period, and no significant differences were found between the two seawater treatments. The fraction of 8 day old active larvae was lowest in March, in experiments with both undiluted and diluted (1:10, 1:100) seawater from both treatments. No significant difference in activity was found between the treatments, except for undiluted (April) and 100-fold dilution (April and May) from the drum filter, when the larval activity was significantly higher. The effect of both seawater treatments was tested by growing the diatom *Chaetoceros muelleri* in small volumes for 4-5 days. Daily growth rates (μ) varied between 0.75 and 1.15, and were highest in May. No significant difference in cell concentration was found between the treatments. The results showed that the skimmer attached to the drum filter had the best performance overall in reducing dissolved organic carbon and potentially lethal bacteria. These findings have important implications for hatchery seawater management protocols.

Keywords: Seawater quality; Seawater treatment; Filter; Protein skimmer; Marine hatchery; Scallop

Introduction

Pecten maximus, the great scallop, is widely distributed in Norway, and is an aquaculture species with potential and high market values for human consumption [1-4]. The availability of spat is one of the main suppressing factors for aquaculture growth [5,6]. In Norway, production relies on spat produced in the hatchery [7-10]. Hatchery production has often been variable due to low larval survival associated with bacteria [11,12]). Larval survival has often been seasonal and associated with changes in seawater quality occurring during spring bloom conditions [13]. A number of investigations have shown that the deep seawater masses in the Atlantic Ocean contain large amounts of particulate material, also called marine snow [14-16]. Marine snow may contain the remains of different plankton organisms that have sedimented down from upper seawater masses, such as detritus or diatoms [15]. Investigations of marine snow have also shown that both the density and size of the particles vary greatly throughout the year and between different localities as the particles settle down to the seafloor [14,17].

The aggregation of small particles into larger ones increases the rate of settling down, which in turn affects the distribution of organic and inorganic matter in the seawater masses [17]. Turley (2002) reported on a build-up of marine snow in the North Atlantic during spring and autumn [16]. This organic matter often functions as a substrate for a number of marine bacteria, such as *Vibrio* spp. and *Listeria* spp. [14,18-20]. Plankton also produces organic carbons in the aquatic environment [21]. These organic carbons–particulate organic carbon (POC) and dissolved organic carbon (DOC)–in the seawater provide favourable growing conditions for the bacteria [22]. DOC is regarded as the organic carbon that passes through a 0.2-0.45 μm membrane filter, while POC is retained [14]. However, in Norway deep seawater is still used in order to secure a stable temperature and salinity throughout the year. The inlet seawater in the hatchery must be treated in order to prevent the outbreak of diseases, pathogens and toxic substances [23,24]. Different treatment methods (ozone, ultraviolet (UV) light, filtration, protein skimmers, disinfectant and antibiotics) are used in hatcheries [25,26].

The flow-through system has proved to be successful and cost-effective in larval rearing without the use of antibiotics and has shown success at the Scalpro AS hatchery [9]. However, the seawater quality is not stable. This affects spat and larval development and survival [9,13].

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Mechanical filters are often used to remove particles in the hatcheries as TOC accumulation has an impact on seawater quality and the efficiency of filters [27,28]. The AFM is composed of reprocessed glass with a surface area for bacterial growth and allows the removal of TOC. It operates both biologically and mechanically, as well as being self-sterilizing (www.afm.eu). The drum filter (DF) removes the suspended solids by rotating the drum while backwashing and removing the waste from the filter [29]. The DF is commonly used in the recirculating system for marine fish hatcheries more than in shellfish hatcheries [30]. The use of mechanical filters in marine shellfish hatcheries is less widely explored and studied [31]. Therefore, the use of a drum filter, however superficially explored, may serve as an alternative in treating the seawater and larval rearing [23]. Protein skimmers in marine hatcheries have proven to be efficient in reducing DOC and fine particles in seawater [32]. By using filters and protein skimmers in the hatchery, a larger amount of the organic material will be removed from the inlet seawater, at the same time reducing the threat of opportunistic bacteria.

The main objective of the present study was to investigate the effect of different seawater treatments on the quality of marine seawater used in the hatchery production of great scallop (*P. maximus*) larvae. We used two treatments: active filter media and drum filter, both equipped with protein skimmers, and investigated the effect on the chemical and microbial characteristics of seawater, egg development to day 3 larvae, larval activity and growth of microalgae. The main background for comparing the two methods of seawater treatment was the occasional failure in scallop larval survival [13].

Materials and Methods

Samples for seawater characteristics were collected from the experimental set-up at the scallop hatchery Scalpro AS in Hordaland, Norway on 10 March, 14 April and 11 May 2009. The effect studies (egg development, larval activity and algal growth) were performed in the following weeks (11-20 March, 15-25 April and 12-23 May 2009). The main seawater inlet was located in Hjeltefjorden in Norway at a depth of 120 m in March and April and a depth of 60 m in May. Seawater samples were collected from the seawater inlet (sampling point P1). The seawater was separated into two different treatment lines. One consisted of a sand filter with active filter media (AFM, sampling point P2) followed by a protein skimmer (Sander Aquarietechnik, Helgoland 500, Uetze-Eltze, Germany, sampling point P3). The other line consisted of a drum filter (DF) with a 10 µm screen mesh (Hydrotech model HDF 1604-3H, Vellinge, Sweden, sampling point P4) followed by another Helgoland protein skimmer (sampling point P5).

Seawater quality parameters

Temperature (°C), salinity (‰) and dissolved oxygen (DO mg L^{-1} and %) were sampled from all sampling points (P1-P5). The measurements were taken once a month in March, April and May. The temperature, salinity and DO were all determined using a digital multimeter (WTW multi 197i Oximeter) following Standard Method 2810 [33]. DO was measured both as DO (mg L^{-1}) and as DO (%). Total organic carbon (TOC, n=1) and dissolved organic carbon (DOC, n=3) samples were taken from all sampling points (P1-P5). Samples for DOC were prefiltered using 0.45 µm GF/F membrane filters. Samples were analysed by Chemlab Services AS according to Norwegian Standard 1484 (1997) using a total organic carbon analyser (TOC-5000, Shimadzu) equipped with a sample exchanger (ASI-5000, Shimadzu) and TOC control (Shimadzu Corp, Version 1.05.01). Organic carbon was converted to carbon dioxide (CO_2) when heated at 680°C including the oxidizing platinum catalyst. The combustion product was transported by pure air passing through the inorganic carbon container into an infrared (IR) detector.

Total bacteria number was estimated from three replicate seawater samples (1.5 mL) collected from all sampling points (P1-P5). The samples were frozen in Eppendorf tubes at -80°C containing 40 µL glutaraldehyde (0.25% of final concentration). The samples were thawed and serially diluted with 0.2 µm filtered sterile seawater to 1:05, 1:10 and 1:50. The diluted samples were stained with molecular probes, SYBR Green I and analysed using CELLQuest software version 3.0 [34] and a FACSCalibur flow cytometer (Becton Dickinson). *Vibrio* spp. bacteria were cultured on Thiosulfate Citrate Bile Sucrose (TCBS) agar (specific for *Vibrio* spp.) (Merck Cat. No. 1.10263) and expressed as colony forming units (CFU ml^{-1}). Samples (in triplicates) were taken from all sampling points (P1-P5).

Bacterial composition was analysed from seawater samples (1 L) from all sampling points (P1-P5). The samples were filtered through 5 µm filters to remove larger particles and then filtered through 47 mm polycarbonate filters, pore size 0.2 µm (Whatman®, Schleider & Schuell, UK). The filtered samples were frozen at -20°C and analysed according to Sandaa et al. [35] Deoxyribonucleic acid (DNA) was extracted and the bacteria harvested were used as templates in a polymerase chain reaction (PCR) performed in a GeneAmp PCR System 2400 (Perkin Elmer) and thermal cycler (Applied Biosystems 2720). Denaturing gradient gel electrophoresis (DGGE) provided the profile of the bacterial community by analysing the PCR products. The sequencing was done at the SARS centre (Bergen, Norway) using Big-Dye protocol version 3.1 (http://seqlab.uib.no) on an ABI Prism 377 DNA sequencer, and further analysing the sequences using Invitrogen software. A cluster diagram was made for different sampling months and for comparisons of bacterial community composition.

Scallop egg development

Pecten maximus broodstock from western Norway was conditioned and spawned according to standard protocols. Experiments for testing egg development to 3 day old larvae (D3) were set up using seawater from the AFM+skimmer (P3) and the DF+skimmer (P5). Fertilized eggs were transferred to 10 L buckets at a density of approximately 100,000 bucket-1 without any algal feed, but gentle mixing with air bubbles, and counted after 3 days. A 50 µL sample of retained larvae was counted 5 times using an inverted stereo microscope.

Larval activity

Larval activity was tested using 8 day old larvae in seawater qualities from the AFM+skimmer (P3) and DF+skimmer (P5). The tests were replicated three times in order to account for variation in the number of larvae per well. The 12-well polystyrene multi-dish (Nunc) was filled with 2 mL of SSW, and approximately 20 to 30 larvae were placed in each well and then inoculated with 100 µL of undiluted seawater from the AFM+skimmer (P3) or DF+skimmer (P5). Dilution series of 1:10 and 1:100 were used. Incubation of larvae was performed in a dark place at 18°C in an air-conditioned room. After 48 hours, the number of moving or active larvae from all wells was counted using an inverted stereoscopic microscope (Leitz DM IL) according to the protocol in Torkildsen et al. [12] and Sandlund et al. [36]. The wells were counted twice to reduce counting errors.

Microalgal cell numbers

The microalgae, *Chaetoceros muelleri* (CHM–strain CCAP 1010/3), were grown in 2 L volume using seawater from the AFM+skimmer

(P3) and DF+skimmer (P5). The seawater samples were autoclaved at 120°C for approximately 20 minutes before use. The stock culture (30 mL) were grown and maintained under continuous white fluorescent light (Osram L 58W/965 Biolux) at 100 ± 2 µmol $m^{-2}s^{-1}$, 15°C ± 1°C and in Conway medium according to Laing [37]. The algae (2 L) were grown under the same light conditions, but at 20°C ± 1°C. Aeration was added in the culture supplemented with carbon dioxide. Algal growth was followed for 4-5 days.

Statistical analysis

Data were analysed using one-way analysis of variance (ANOVA) in STATISTICA 10. All statistical tests were carried out at a 0.05 significance level.

Results

Seawater quality parameters

The temperature of the incoming seawater increased from 7.4°C in March to 8.7°C in May. The mean temperature in the protein skimmers were significantly higher than in the other sampling points (p=0.00). The average temperature in protein skimmer after AFM (P3) was 12.7°C, while it in the protein skimmer after the DF-filter was 8.9°C (P5). Salinity was always between 34.9 and 35.0. Dissolved oxygen in incoming seawater was between 8.9 mg L^{-1} (75.7%) and 10.8 mg L^{-1} (88.0%), and increased in the protein skimmers to a maximum of 12.7 mg L^{-1} (110.6%). No significant differences were found in oxygen levels between the other sampling points measured as saturation (p=0.39) or concentration (p=0.06).

Total organic carbon (TOC) in the incoming seawater was dominated by content of dissolved organic carbon (DOC). TOC concentrations in the incoming seawater increased from 4.0 mg L-1 in March to 4.9 mg L^{-1} in May, and the DOC content constituted 3.5 (87 %) and 4.7 mg L^{-1} (98%). The DOC concentrations were usually highest in the inlet water and AFM filter, and significant reductions were found after the protein skimmers, particularly the skimmer after the drum filter (P5, Figure 1). In April there was a significant increase in DOC in the AFM filter. In May DOC was reduced with all treatments, and the DOC concentration at P5 was significantly (p=0.01) lower than at P3. On average, the DOC concentration present in inlet seawater was reduced by 31% by the AFM+skimmer (range 23-36%) and by 34% by the DF+skimmer (range 28-45%).

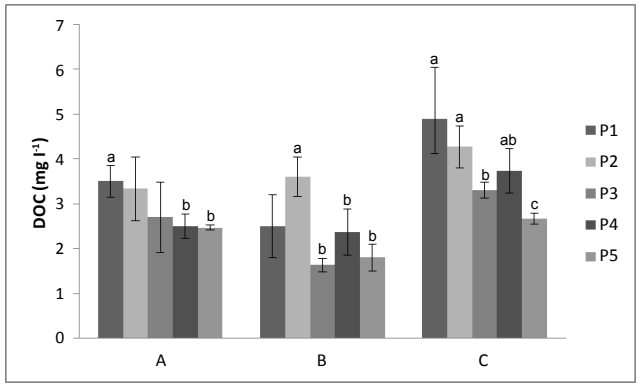

Figure 1: Concentration of dissolved organic carbon (DOC, mg l^{-1}) at five sampling points (P1-P5) in March (A), April (B) and May (C) 2009. Error bars indicate mean (n=3) ± STD. Bars with different letters are significant different (p<0.05).

Bacterial communities

The total bacterial cell numbers (TBN) were lowest in March, and there were small variations in April and May (Figure 2). In March the bacterial cell number (2.3×10^5 cells mL^{-1}) in the inlet seawater (P1) increased after the DF (P4), but was significantly reduced after the skimmer (P5, p=0.01). In April and May both treatments (AFM and DF) maintained similar bacterial cell numbers (3.7×10^5 cells mL^{-1}) to the inlet seawater (P1), and there was no significant difference between treatments (p=0.28 (P3) and p=0.11 (P5).

TCBS samples were taken in triplicate in March, April and May from all sampling points (P1-P5). *Vibrio* spp. colonies (Table 1) varied between 0 and more than 70 CFU mL^{-1}. In March *Vibrio* spp. was only found in one sample from the drum filter. In April and May it was found at most sampling points. No colonies were observed in seawater coming from the skimmer after the drum filter (P5).

Based on the denaturing gradient gel electrophoresis (DGGE–not shown), a profile of the bacterial community was produced by analysing the PCR products from all sampling points. The strongest bands were

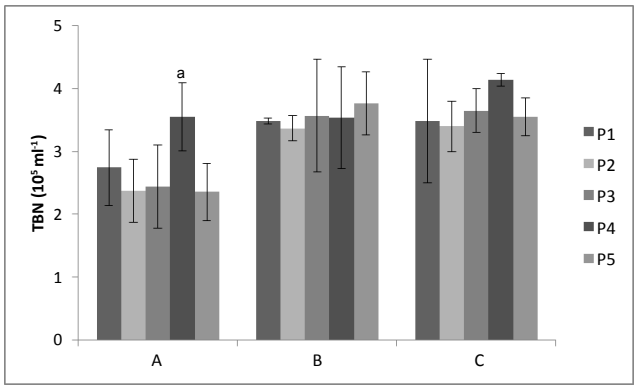

Figure 2: Total bacterial numbers (TBN, 105 ml-1) in five sampling points (P1-P5) in March (A), April (B) and May (C). Error bars indicate mean (n=10) ± STD. Bars with different letters are significant different (p<0.05).

		March	April	May
1	a,b,c	0,0,0	0,20,20	0,0,10
2	a,b,c	0,0,0	0,40,>70	0,20,20
3	a,b,c	0,0,0	20,40,70	0,0,10
4	a,b,c	0,0,40	10,10,20	0,10,20
5	a,b,c	0,0,0	0,0,0	0,0,0

Table 1: Numbers of CFU ml^{-1} on TCBS agar in samples (a, b, c) at sampling points 1 to 5 on 10 March, 14 April and 11 May 2009.

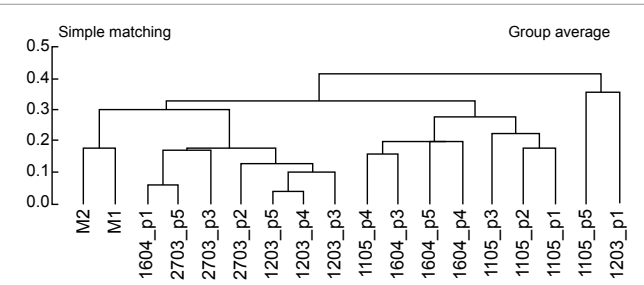

Figure 3: Cluster-analyses of DGGE gel with samples from the Scalpro AS hatchery. Dates are given. M1 and M2 are markers. The analyses were made only from bands that were good from different sampling points at different months.

used for sequencing and cluster analysis (Figure 3). Commonly found sequences found in bands from the DGGE gel were compared by simple matching from Genbank (Table 2). A marine bacterioplankton community commonly found in natural seawater could be seen. Many of the sequences belong to Alpha-and Gammaproteobacteria and to Actinobacteria. Most sequences were found in samples from P3 and P5. More sequences of Actinobacteria were found in seawater passing through the AFM (P3) than through the drum filter (P5). Also, the Actinobacteria were most prominent in March (A) compared to the Gammaproteobacteria and other bacterial strains found.

The cluster diagram grouped sampling points sampled on the same date and over time. The bacterial composition changed over time (Figure 3) in all sampling points. A succession both through the season and between treatments could be observed. It seemed that sampling point P5 was most similar to the seawater inlet samples (P1) and less similar to the other samples. The samples from March were grouped together with inlet seawater in April, while the other samples from April formed one group and subsequently grouped with the May samples. The highest matching was found between inlet seawater in March and after the drum filter and skimmer (P5) in May.

In sample	Similar to	Accession no
P1 (B)	Uncultured gamma proteobacterium clone PM1-27 (98%); *Oceaniserpentilla haliotidis* (94%)	EF215799 AM747817
P1 (B)	*Colwellia maris* strain ABE-1(92%) Uncultured bacterium clone HC-8 (92%)	NR_024635 AY529875
P1 (B)	Uncultured bacterium clone HF130_D6_P1 (94%)	DQ300613
P1 (C)	Uncultured gamma proteobacterium clone PM1-27	EF215799
P2 (A)	Unidentified alpha proteobacterium OM75 (90%) *Nisaea nitritireducens* strain DR41_18 (88%)	U70683 DQ665839
P2 (A, B)	Uncultured Chloroflexaceae group bacterium Arctic96BD-6 (93%)	AF355053
P2 (A)	Uncultured gamma proteobacterium clone PM1-27 (99%); Oceaniserpentilla haliotidis (94%)	EF215799 AM747817
P2 (A)	Uncultured organism clone ctg_NISAA66 (99%) Uncultured Candidatus *Microthrix* sp. clone BATS136-250-93	DQ396300 FJ960805
P2 (C)	Uncultured bacterium clone HF130_D6_P1 (94%)	DQ300613
P2 (C)	Uncultured bacterium clone Mann16S_G10B (100%)	FJ952689
P3 (A)	Uncultured SAR11 cluster alpha proteobacterium	AM748185
P3 (A)	Uncultured SAR11 cluster alpha proteobacterium	DQ186916
P3 (A)	Uncultured marine bacterium clone BM1-F-105 (82%)	FJ826203
P3 (A)	Uncultured marine bacterium clone BM1-8-74 (99%)	FJ826062
P3 (A)	Uncultured gamma proteobacteriumFFW81 (99%)	AY830024
P3 (A)	Uncultured actinobacterium clone HF4000_16H14 (98%)	EU361019
P3 (B)	Uncultured gamma proteobacterium clone GC21V_AD (95%)	AY701419
P3 (C)	Uncultured bacterium clone Mann16S_G10B (100%)	FJ952689
P4 (B, C)	Uncultured gamma proteobacterium clone GC21V_AD (95%)	AY701419
P4 (C)	Uncultured bacterium clone Mann16S_G10B (100%)	FJ952689
P5 (B)	Uncultured gamma proteobacterium clone GC21V_AD (95%)	AY701419
P5 (C)	*Sphingomonas melonis* strain PR-3	FJ605424
P5 (C)	Uncultured marine bacterium clone BM1-8-74 (97%)	FJ826062
P5 (C)	*Colwellia rossensis* strain ANT9279 (98%)	AY167311
P5 (C)	Uncultured bacterium clone Mann16S_G10B (100%)	FJ952689
P5 (C)	Uncultured *Rhodobacteraceae* bacterium clone IG3E05	FJ718205
P5 (C)	Uncultured marine bacterium clone ArtRif4-2	FJ594812
P5 (C)	Uncultured alpha proteobacterium clone HF130_15B09; Uncultured bacterium clone 2C228359	EU361386 EU800311

Table 2: Dominant sequences found in bands from DGGE-gel (not shown) with simple matching from Genbank in samples from sampling points P1-P5 in March (A), April (B) and May (C).

Egg development and larval activity

Egg development, as the fraction of D3 larvae from eggs, increased from March to May (Figure 4). It was low in March and April (<20%), but high (>50%) in May in both treatments, but no significant differences was found. Larval activity was lowest in March and showed some increase in April and May (Figure 5). In March no significant difference was found between the two treatments. In April significant higher larval activities were observed in undiluted (p=0.02) and 100-fold diluted (p=0.03) seawater samples coming from the protein skimmer after the drum filter (P5). In May the same significant (p=0.04) increase was found in the 100-fold diluted sample.

Algal growth

Chaetoceros muelleri was grown in March, April and May using seawater from skimmer P3 and skimmer P5. The cell numbers were counted from day 1 until the exponential phase finished (Figure 6). Growth rates (μ) increased from March to May, being 0.75-0.83, 0.95-1.14 and 1.10-1.15, respectively. No significant difference in algal concentration was found between the two seawater treatments during the first 4-5 days.

Discussion

Environmental factors

The results obtained in this study did not show any significant changes in egg development, larval activity or growth of microalgae that could be related to the different water treatments. We observed, however, a clear seasonal change in the results reflecting the environmental changes that occurred during early spring events. Significant changes due to water treatments were also observed in the content of DOC and the occurrence of potential lethal bacterial.

In scallop hatcheries, one of the main problems is larval survival

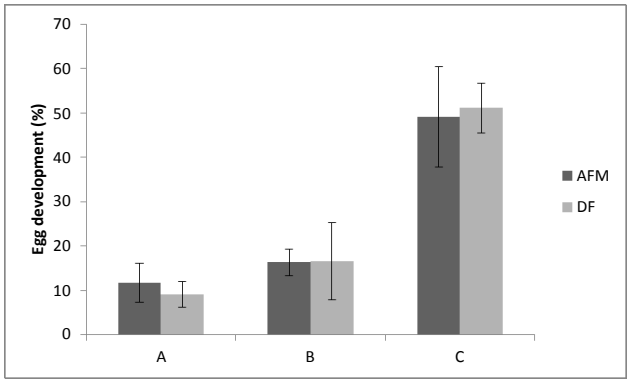

Figure 4: Egg development to day 3 larvae (%) in March (A), April (B) and May (C) 2009. AFM=incubated in water from AFM+skimmer, DF=incubated in water from drum filter and skimmer. Error bars indicate mean (n=5) ± STD.

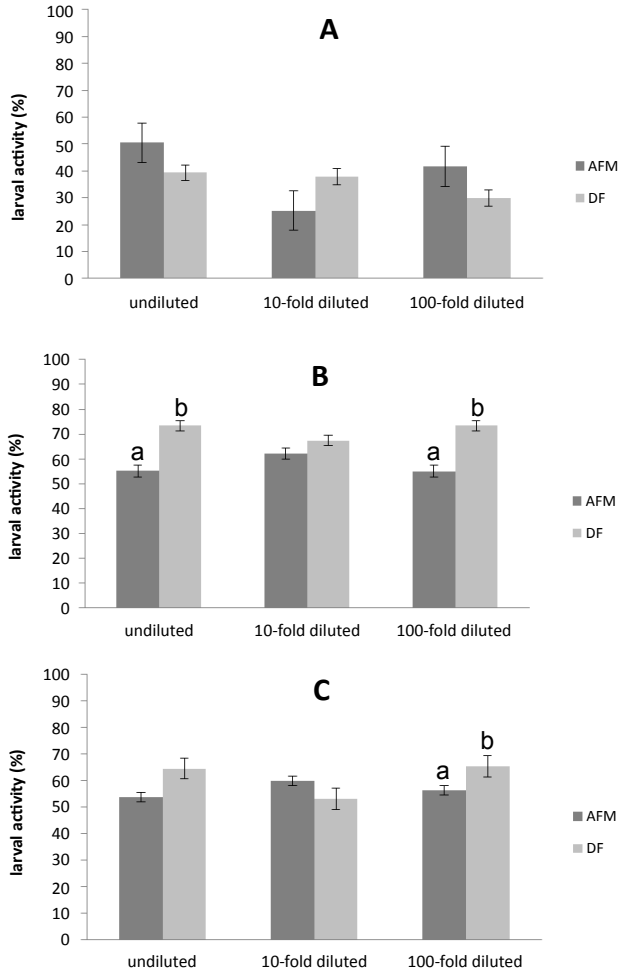

Figure 5: Larval activity (%) of scallop larvae after 48 hours exposure to undiluted, 10-fold diluted and 100-fold diluted water from AFM and drum filter (DF) in March (A), April (B) and May (C) 2009. Error bars indicate mean (n=3) ± STD. Bars with different letters are significant different ($p<0.05$).

that may limit the production of spat [13]. Mortalities are often associated with opportunistic bacteria, but may also be due to traces of inhibitory or toxic substances sporadically occurring in the water supply [11,12,38-41]. Bacterial blooms benefit from increased organic carbon concentrations and the major bacteria sources are inlet water, algal production and brood stock [42]. Seawater quality is given the highest importance and is the single most important factor for hatchery site selection, and ideally solids and dissolved substances should be absent [43]. It should, however, also be recognized that dissolved organic material may be utilized by bivalve larvae Manahan [44], bacteria are an important source Wakeham et al. [45], and bacteria may also be an important part of the larval diet [46].

Marine hatcheries located in Norway usually have the deep water inlet located at a depth of 60-180 m. The main reason is to avoid seasonal variations in temperature, salinity and organic production occurring in the surface layer. Dissolved organic carbon (DOC) compounds in deep water are less than half that in surface layers, but little is known of their composition and accumulation [47-49]. In the Trondheimsfjord in Norway, DOC in deep water accumulated during the productive season and had maximum concentrations in spring and summer [50].

The high fraction of DOC (70-90 %) was reasonable Chrzanowski et al. [51] , but the values may seem a bit higher than what are considered typical values of DOC in oxygen-bearing water (1.0-1.2 mg L^{-1}, Adams & Richards 1968) [52], in the deep water masses of the Trondheimsfjord (<1.2 mg L^{-1}, Børsheim et al. [50] and in other north Norwegian fjords (1.5-2.5 mg L^{-1}, A. Jacobsen pers. comm.). The organic load may, however, differ with depth and location [53]. The presence of particulate material serves as an important substrate for bacterial attachment (Garneau et al. [54], and DOC as an important source for bacterial production [55,56]. Both seawater treatments significantly reduced the content of DOC. On average, the content was reduced by 30-35%. These figures were low compared to what may be obtained in recirculation systems where as much as 60-100% may be removed, but higher than observed by Park et al. [57] who used addition of ozone to increase DOC removal efficiency [28,32].

The total available organic carbon in a hatchery should be kept low without large fluctuations. In our experiment, DOC increased from March to May. This is the period when the local spring phytoplankton bloom occurs in surface water [58]. Both of the water treatments used

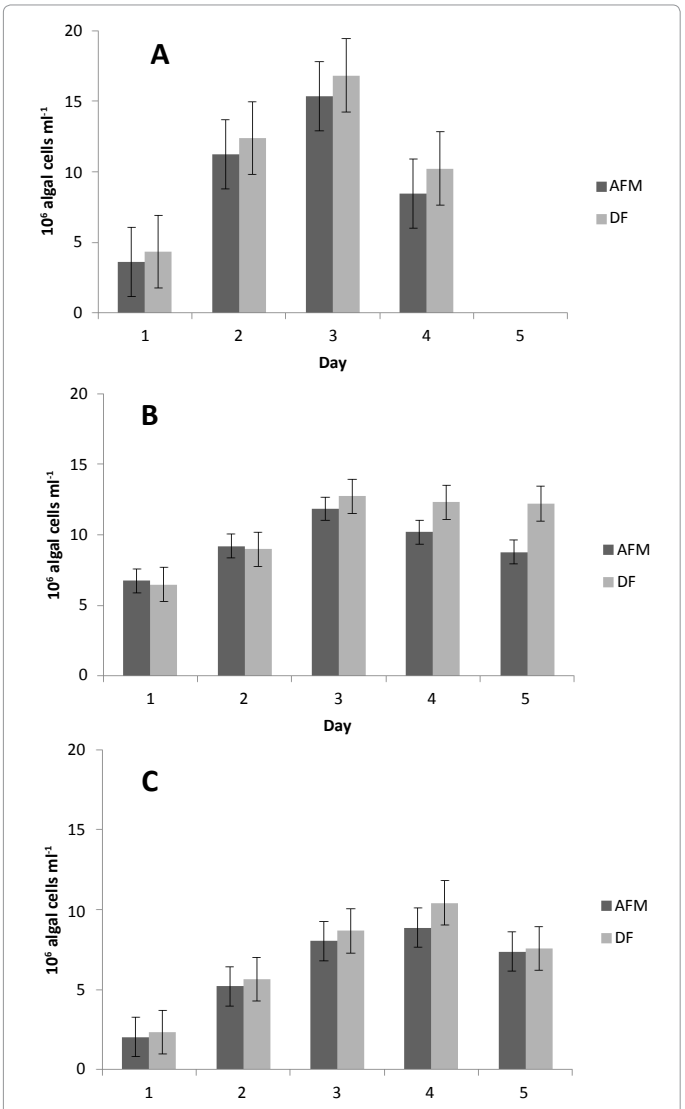

Figure 6: Algal cell numbers (10^6 ml^{-1}) has grown in different water qualities (AFM and DF) in March (A), April (B) and May (C). Error bars indicated mean (n=3) ± S.E. Bars with different letters are significant different ($p<0.05$).

significantly decreased the DOC content and evidently the use of a drum filter and protein skimmer was the most effective. This could be explained by the backwashing action of the drum filter [59]. The drum filter is known to remove both small and large components of the organic material, and even large or small solids from the water [27,28]. The use of a biofilter such as Active Filter Media (AFM) in the flow-through system may serve as a major substrate for heterotrophic bacteria in the hatchery because it traps DOC and POC on the filter [60]. In May, the low reduction of DOC by the AFM+skimmer may have favoured opportunistic bacteria as seen from the DGGE profile, where the Gamma proteobacteria became dominant.

The total bacterial numbers found in this study were within the typical range found in seawater (10^3-10^6 cells mL^{-1}) and marine hatcheries [61,62]. The total bacterial number increased from 2.5 to 3.5 10^5 mL^{-1} from March to April, and no significant decrease was obtained with the water treatments. Instead we found an elevated number of bacteria in the drum filter in March, but this was significantly reduced by the skimmer. Even though the overall bacterial number did not change, a change in bacterial composition may have occurred [63], as also seen from the cluster analysis in our study. The cluster analysis reflected a seasonal change in bacterial composition and clearly separated samples collected in March from the April and May samples. The samples collected from water after skimmer and drum filter showed least similarity to the other samples. This was particularly obvious in May when grouped together with a sample obtained from the water inlet in March. Low bacterial numbers may not always be beneficial in marine larval production systems [62]; they are more dependent on an even and diverse microbial community structure [62,64].

The most common sources of *Vibrio* spp. in the hatchery are algal cultures and introduction with brood stock [42]. We found *Vibrio* spp. colonies in March associated with the maximum total bacterial number in the drum filter. In April and May *Vibrio* spp. were found in the water inlet, AFM, DF and the skimmer after the AFM, but never in water coming from the skimmer after the drum filter. All figures must be considered low. Lewis et al. [65] suggested that seawater must contain less than 100,000 CFU mL^{-1} for safe use in bivalve hatcheries. This was supported by Abasolo-Pacherco et al. [23], who found little effect of *Vibrio* spp. on larval survival, but specific studies with *V. alginolyticus* have shown significant effects at 0.002 cells mL^{-1} [66]. Increased scallop larval mortality has been observed when challenged with *V. splendidus* at higher concentrations (10^7-10^9 cells mL^{-1} [36].

DGGE analysis has previously been used to characterize bacterial communities in a scallop hatcher[35], who found a change in composition in samples from a pipe inlet but a high stability in composition in larval tanks. A high fraction of the bacteria (53%) were similar to the gamma subclass of Proteobacteria, as observed by Jorquera et al. [62] in hatcheries in Chile. Brunvold et al. [67] concluded that DGGE was a suitable method for characterizing bacterial communities in hatcheries, but genes other than 16 rDNA should be used for the discrimination of closely related taxa, like different *Vibrio* spp.

Effects on egg development, larval activity and algal growth

The development from egg to D3 larvae (<20% in March and April) were low compared to the large-scale development of eggs in the hatchery's commercial spawning groups during the same period (35-46%, T. Magnesen, pers. comm.). Normally about 30% of the eggs will develop into D3 larvae [10]. The differences could be due to the selection of a small number of eggs coming from only a few broodstock. We did not find any effect of water treatment on the fraction of eggs that developed into D3 veliger larvae at any sampling date, indicating that water quality did not affect their development.

In this study, no significant changes were observed in larval activity related to the different water treatments, except for undiluted (April) and 100-fold dilution (April and May) from the drum filter, when larval activity was significant higher. The higher larval activity detected with water passing through the drum filter could therefore indicate better water quality for the young larvae from this water treatment. This method for studying larval activity was also used by Sandlund et al. [36] and was found to be useful in a challenge test with pathogenic *Vibrio* bacteria. Sandlund et al. [36] also found no difference in larval mortality challenged with different bacterial strains, with mortality averaging 25%.

We found no significant effect of the seawater treatments on microalgal growth growth rates decreased as spring progressed. This may be due to inhibitory substances secreted by other spring bloom species [68] or algal-associated bacteria. It has been found that high counts of opportunistic and haemolytic bacterial species were associated with *Bacillariophyceae*, the class to which *C. muelleri* belongs [69]. Hattenrath-Lehmann and Gobler [70] also found that growth of diatoms was inhibited by the dinoflagellate *Alexandrium fundyense*. Species from the genus *Alexandrium* have been found to have an allelopathic effect on the natural phytoplankton bloom in Norwegian waters by decreasing the growth rate [71]. It has also been shown that polyunsaturated aldehydes derived from *Skeletonema marinoi*, a common spring bloom species in Norwegian waters, may also trigger the declining phase of algal growth [72].

Conclusion

We therefore conclude that the use of drum filter and protein skimmer was most effective in reducing dissolved organic carbon and *Vibrio* spp. bacteria in the seawater and increased larval activity. We did, however, not find any significant effects from the different water treatments on egg development or growth of microalgae. A seasonal variation from winter to spring was found for egg development and larval activity (increasing), and algal growth rates (decreasing). Reducing dissolved organic substances and potentially lethal bacteria is an important prerequisite for hatchery management.

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