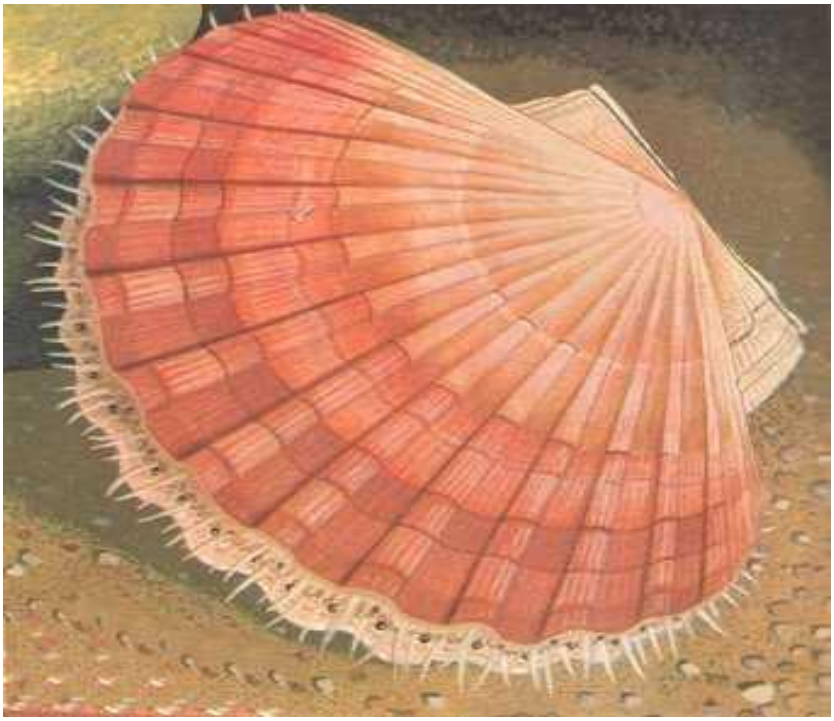
Effect of different filter methods on water quality to a marine scallop hatchery



Thesis for fulfilment of the degree

Master of Science in Aquaculture Biology

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Thank you very much "Ke le leboga go menagane"

Bergen, 30 May 2009
Malebo Hellen Moepi

Contents

Abstract

Water quality in marine hatcheries is a critical aspect in the production of spat. Different filtering methods have been used to treat the intake seawater in the hatcheries. The Scalpro AS hatchery uses two different types of filters to treat seawater, an active filter media and drum filter. The objective of this study was to establish a better knowledge on the effect of different filtering methods on marine water quality in the production of great scallop (*Pecten maximus*). The two filters were compared in relation to chemical and microbial properties, microbial communities and microalgal cell numbers. Egg development to day 3 larvae was performed by using water from both treatments. A larval activity experiment was conducted by exposing 8 days old larvae to different water treatments and concentrations.

There was no significant difference between treatments in dissolved oxygen (% and mg l^{-1}) while temperature at the skimmer after active filter media was significantly higher than other sampling points. Both treatments reduced the total, dissolved and particulate organic carbons concentrations from the intake water. The skimmer significantly reduced the total bacterial numbers from the drum filter in March. In March, April and May, no *Vibrios* spp. was found in other sampling points except the drum filter in March. The numbers of culturable heterotrophic bacteria in April were increased at the skimmer after active filter media from the intake water and reduced at the skimmer after drum filter. In May, both skimmers reduced the bacterial colonies from the intake water. Close similarities of microbial community were observed between the intake water and each treatment. From the denaturing gradient gels profiles it seemed that alpha proteobacteria dominated in March and gamma proteobacteria (opportunistic) dominated in April and May. Both treatments proved to be suitable for the egg development to larval stages. No significant differences were observed in larval activity between treatments in undiluted and 100-fold diluted water. In May, significant highest larval activity was found in the 10-fold diluted water from Bergen High Technology Centre and the lowest in the control (sterile seawater). From the microalgal growth experiment, the skimmer after drum filter had the highest number of microalgal cells than the skimmer after active filter media and water from Bergen High Technology Centre. The skimmer after drum filter performed better more than the skimmer after active filter media in reducing the organic carbons, bacterial cells and gave highest microalgal cells numbers.

1. Introduction

Pecten maximus, the great scallop, is an aquaculture species with potential and high market values for human consumption (Christophersen, 2000; Bergh and Strand, 2001; Brand, 2006; Christophersen and Magnesen, 2001). The great scallop is a widely distributed species and in Norway they are located on the western coast at 5 to more than 30 m depths and in the deeper water of the southern coast (Strand and Parsons, 2006). The scallops inhabit a different substrate of sand, gravel and mud in the wild (Bergh and Strand, 2001). The early juvenile "spat" availability is the main suppressing factor for aquaculture growth (Christophersen, 2000; Avendaño et al., 2007; Marshall et al., 2010). The production of scallop relies on the spat collected from the wild or produced in the hatchery (Christophersen, 2000; Magnesen and Christophersen, 2008; Magnesen et al., 2006). In Norway it is a common practice for divers to harvest wild spat (Bergh and Strand, 2001) and grow it until commercial size of 15 mm (Christophersen, 2000). However, this method is not reliable and numbers of spat collected vary each year (Anon, 2003).

Therefore, to ensure the on-going spat production to meet human demands and supplying spat to cultures outside the hatchery, the Scalpro AS hatchery was developed (Bergh and Strand, 2001; Christophersen and Magnesen, 2001; Christophersen and Lie, 2003; Marshall et al., 2010). The Scalpro AS is the only hatchery in Norway and only few others are existing in Europe. The adult scallop "broodstock" must be available at all times at the hatchery to ensure successful operation and continuous production of spat (Magnesen and Christophersen, 2008; Saucedo et al., 2007; Wilson et al., 1996). In the Scalpro hatchery, the broodstock are collected from the Hordaland (60 °N) and Trøndelag (64 °N) population, conditioned and spawned according to the protocol adapted within the hatchery (Bergh and Strand, 2001; Strand and Parsons, 2006). The hatchery grew the larvae up to 2 mm size (Magnesen et al., 2010). However, the production of spat is unstable and often low (Bergh and Strand, 2001). In addition, the larval growth may be disturbed by external environment such as microbial bacteria and thus leads to mortalities in the hatchery (Christophersen et al., 2006; Torkildsen et al., 2005).

In most of the Norwegian marine hatcheries, the intake water (raw untreated seawater) is taken from the deep water masses (50-180 m). It is economically convenient to extract water from the field because it allows the proper estimation of the required water capacity within the hatchery, thus preventing the extra costs of treating the over-estimated water capacity (Huguenin and Colt, 2002; Magnesen et al., 2010). Because of its physical, chemical and biological characteristics, deep seawater has several advantages such as it allows rearing of cold water species in tropical areas and the control of diseases occurrences (Nakasone and Akeda, 1999; Ku et al., 2009). However, a number of investigations have shown that the deep water masses in Atlantic ocean contains large amounts of particulate material, also called marine snow (Valiela, 1984; Alldredge et al., 1998; Turley, 2002; Danovaro et al., 2009). Marine snow may contain the remains of different plankton organisms that have sedimented down from upper water masses such as, detritus or diatoms (Alledredge et al., 1998). Investigations of marine snow have also shown that both the density and size of the particles varies much throughout the year and between different localities as the particles settles down to a seafloor (Valiela, 1984; Diercks and Asper, 1997).

Thus, incorporation of small particle matter to a larger one increases the rate of the settling down which in turn affects the distribution of organic and inorganic matter in the water masses (Diercks and Asper, 1997). Turley (2002) reported a build up of marine snow in North Atlantic ocean during spring and autumn. This organic matter often functions as substrate for a number of marine bacteria, such as *Vibrio* and *Listeria* spp. (Valiela, 1984; Urakawa et al., 1999; Lyon, 2001; Gram et al., 2002). In addition, phytoplanktons also produce the organic carbons in the aquatic environment (De Vittor et al., 2009). These organic carbons, particulate organic carbon (POC) and dissolved organic carbon (DOC) in the seawater provides favourable growing conditions to the bacteria (Fenchel and Jørgensen, 1977). The DOC is regarded as the organic carbon that passes 0.2-0.45 µm membrane filter while particulate organic carbon (POC) is retained (Valiela, 1984). However, deep seawater is still used in order to secure a stable temperature and salinity throughout the year. These two factors affect the growth and survival of marine species (Kumlu et al., 2000). Because of this, the quality of the intake water is usually considered satisfactory (A. Jacobsen, pers comment). To the hatchery, the water is pumped through polyethylene (PEH) pipes, filtered, aerated and transported to the different production lines.

The intake water in the hatchery must be treated in order to be able to prevent the outbreak of diseases, pathogens and toxic substances (Abasolo-Pacheco et al., 2009: Salvesen et al., 1999). Different treatment methods (ozone, ultraviolet (UV) light, filtration, protein skimmers, disinfectant, and antibiotics) are used in the hatcheries (Lekang and Kleppe, 2000; Dhert et al., 2001). The treatment used will determine the quality of water within the hatchery (Abasolo-Pacheco et al., 2009). The use of matured water is easily affected by the algal blooms in the intake water (Salvesen et al., 1999; Bergh and Strand, 2001). Therefore, flow-through system is commonly used in marine hatcheries culturing shellfish (Magnesen et al., 2006) and salmonid smolts (Bergheim and Brinker, 2003). This system ensures continuous flow of water which increases oxygen availability and removes accumulating organic substances (Christophersen et al., 2006). The flow-through system has proved to be successful in larval rearing without the use of antibiotics and being cost effective (Sarkis et al., 2006), and shown success at the Scalpro AS hatchery (Magnesen et al., 2006). However, the water quality is not stable (Franco-Nava et al., 2004). This affects spat and larval development and survival (Torkildsen and Magnesen, 2004; Magnesen et al., 2006). Therefore parameters like temperature, dissolved oxygen, salinity and pH, must to be carefully controlled to optimize scallops rearing conditions (Christophersen, 2000; Uriarte et al., 2001). In the hatchery the spat is grown at 15 °C (Christophersen and Magnesen, 2001). Salinities above 30 ‰ are suitable for spat growth (Christophersen and Magnesen, 2001; Christophersen and Strand, 2003).

The uses of mechanical filters (microscreen and sedimentation) are known to remove dissolved solids and even 40 µm sized particles within the water system in the hatcheries (Davidson et al., 2008). Thus, the active filter media (AFM) and drum filter (DF) are used in the Scalpro AS hatchery to remove total organic carbon (TOC) which consists of DOC and POC from the intake water. The TOC accumulation has an impact in the water quality and the efficiency of filters (Franco-Nava et al., 2004). Therefore, the AFM is composed of reprocessed glass with surface area for bacterial growth and allows the removal of TOC. It operates both biologically and mechanically, as well as self-sterilizing (www.afm.eu). The DF removes the suspended solids by rotating the drum while backwashing and removing the waste from the filter (Bergheim and Brinker, 2003). The DF is commonly used in the recirculating system for marine fish hatcheries more than in shellfish hatcheries (Sharrer et al., 2007).

In addition to filters, the use of a protein skimmer in marine hatcheries has proven to be efficient in removing the DOC and fine particles from the water before being decomposed into toxic substances (Brambilla et al., 2008). The principle of the skimmer is to add very fine air bubbles with a large surface area in an upwelling pipe system. The protein skimmer has ozone, enabling the DOC to adhere to the bubbles and be transported to the top of the skimmer as foam which can be removed (www.aqua-sander.de). By using filters and protein skimmers in the hatchery, a larger amount of the DOC and POC will be removed from the intake water and at the same time reducing the threat of opportunistic bacteria.

However, earlier investigations have shown that the spat is affected by the water after passing through the active filter media treatment in the Scalpro AS hatchery (Jacobsen and Magnesen, 2009). A biological film (biofilm) is been developed on the filter and covered by opportunistic heterotrophic bacteria as seen in Leonard et al. (2000). This may be caused by the DOC that is accumulating on the active filter media substrate and bacteria in the incoming water especially during algal blooms (Magnesen, 2000). Thus, opportunistic bacteria often dominate in the biofilm and provide unfavourable conditions to the larvae through the treated water (Bergh and Strand, 2001). These opportunistic bacteria occur naturally and live with other heterotrophic bacteria within the water treatment system (Sharrer et al., 2005). Results from Jacobsen and Magnesen (2009) at Scalpro AS hatchery showed a dramatic shift in the microbial community when it passes the active filter media, from a community naturally occurring in free water to a community of opportunistic bacteria. Therefore, avoiding blooming of opportunistic bacteria in marine hatcheries is of great importance, in order to secure a good and stable water quality throughout the production cycle (Jacobsen and Magnesen, 2009). The use of mechanical filters in marine shellfish hatcheries are less explored and studied (Borges et al., 2003). Therefore, the use of drum filter, however scarcely explored may serves as an alternative in treating the water and larval rearing (Abasolo-Pacheco et al., 2009).

This has led to undertake this project of comparing two different filter methods (active filter media and drum filter) to see the effects on water quality, microbial community, larval development and microalgal cell numbers. The main objective of the present study was to

establish a better knowledge on the effect of different water filtering methods on marine water quality used in the production of great scallop (*P. maximus*) larvae. This was achieved by testing the difference between the active filter media and drum filter water treatments on (i) chemical and microbial character of seawater (ii) larval activity (iii) egg development to day 3 larvae (iv) microalgal cell numbers. Based on the previous study conducted by Solberg (2009), several potential and harmful bacteria were found on the active filter media treatment, and some were toxic to the larvae. I am doing this study to see how the drum filter treatment will perform in comparison to the active filter media and as an alternative water treatment in the hatchery.

2. Materials and methods

The experiment took place at the scallop hatchery, Scalpro AS at Rong near Bergen (Øygarden, Hordaland, Norway) for approximately two weeks of each month from March, April and May, 2009 to see the effect of two different filtering systems. Scalpro AS hatchery has a good collaboration with the University of Bergen (UiB) in studying the effects of different filtration systems (Fig. 2.1) on water quality in marine hatcheries. The sampling and experiments for the whole experimental period were performed according to the following dates (Table 2.1).

Table 2.1

Sampling regime followed when performing the experiments using water from all sampling points (P1-P5) in March, April and May, 2009. The water quality parameters were measured from 10:30 am for all the months and followed by sampling.

		March	April	May
Water quality parameters				
Temperature (°C)		10.03	14.04	11.05
Salinity (‰)		10.03	14.04	11.05
Dissolved oxygen (%)		10.03	14.04	11.05
Dissolved oxygen (mg l^{-1})		10.03	14.04	11.05
Total organic carbon (mg l^{-1})		10.03	14.04	11.05
Dissolved organic carbon (mg l^{-1})		10.03	14.04	11.05
Microbial analyses				
Total bacterial numbers		10.03	14.04	11.05
Culturable heterotrophic bacteria				
Thiosulfate citrate bile sucrose	Plating	10.03	14.04	11.05
	Counting (48 hrs[a])	12.03	16.04	13.05
	Counting (6 days)	18.03	22.04	19.05
Marine agar	Plating	[b]	14.04	11.05
	Counting (48 hrs[a])	[b]	16.04	13.05
	Counting (6 days)	[b]	22.04	19.05
Microbial community		10.03	14.04	11.05
Larval activity experiment		11-13.03	21-23.04	19-21.05
Egg development to day 3 larvae		11-13.03	15-17.04	12-14.05
Microalgal cell numbers		16-20.03	20-25.04	18-23.05

[a] hrs means hours

[b] not performed. The experiment was not performed since it was not the initial idea to include it in the project. Due to the results we got from the TCBS in March we decided to include it to have a broader view of the results.

2.1 Experimental design and data collection

2.1.1 Water treatment methods

The Scalpro AS hatchery uses a flow-through water system (Magnesen et al., 2006). The main water intake at Scalpro AS is located in Hjeltefjorden, 1000 m outside the hatchery. The water intake is from 120 m depth but it is shifted to 60 m during unfavourable conditions such as toxic algal blooms and during production cycle from May to August.

From the water intake (P1), the water was separated into two different pipes where it was treated differently using active filter media (AFM) and drum filter (DF) (Fig. 2.1).

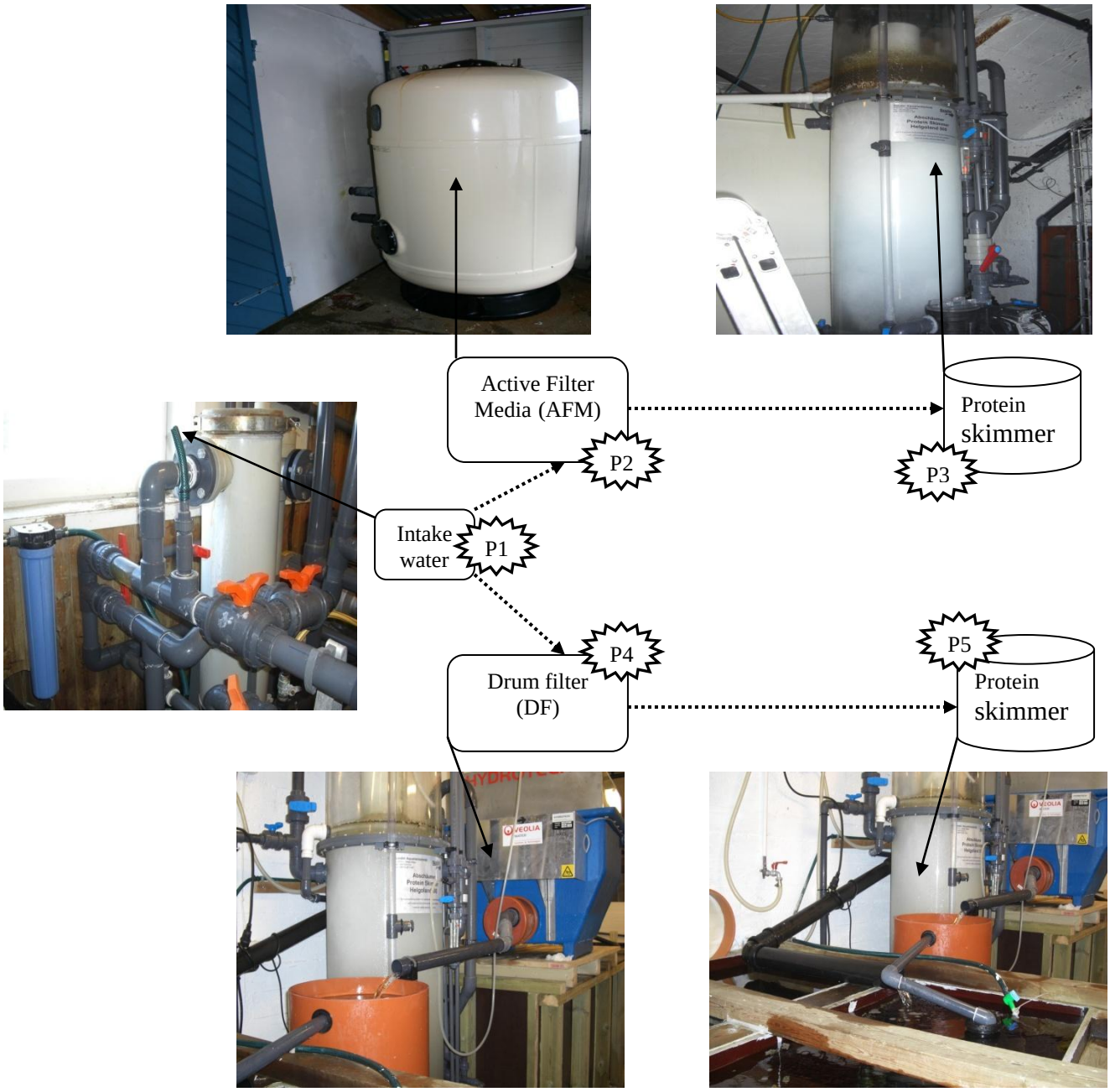

Fig. 2.1. The illustration of how the intake water is treated in the hatchery by using different filter methods (AFM and DF). The dotted arrows indicate the flow of water. The black arrows points different treatments, not where the samples were collected except for the intake water (P1). Illustration modified from Solberg (2009).

2.1.1.1 Active filter media

First pipe goes to AFM (P2) composed of broken glass particles (Fig. 2.1). The Dryden aqua website (www.afm.eu) suggested that an AFM surface area is:

"Activated through high zeta and redox potential thus performs better in reducing the DOC concentrations and toxic substances found in the water, and saves up to 70% water through backwashing system"

The Dryden aqua catalogue "Drinking Water Treatment by AFM 30-80% improvement in sand filter performance Cryptosporidium problem and THM`s eliminated" (page 5) suggested that:

"The catalytic activity on the AFM occurs in the presence of dissolved oxygen. The dissolved oxygen dissociation increases the oxidation potential of the AFM thus making it a self sterilizing filter because no biofouling is occurring. Zeta potential creates a high charge density attracting charged particles while the slip zone, makes charged particles unavailable on the AFM substrate (Fig. 2.2). *Solids are trapped on the AFM but unable to make bonds with the surface. Therefore the water will be free from solids and be clean".*

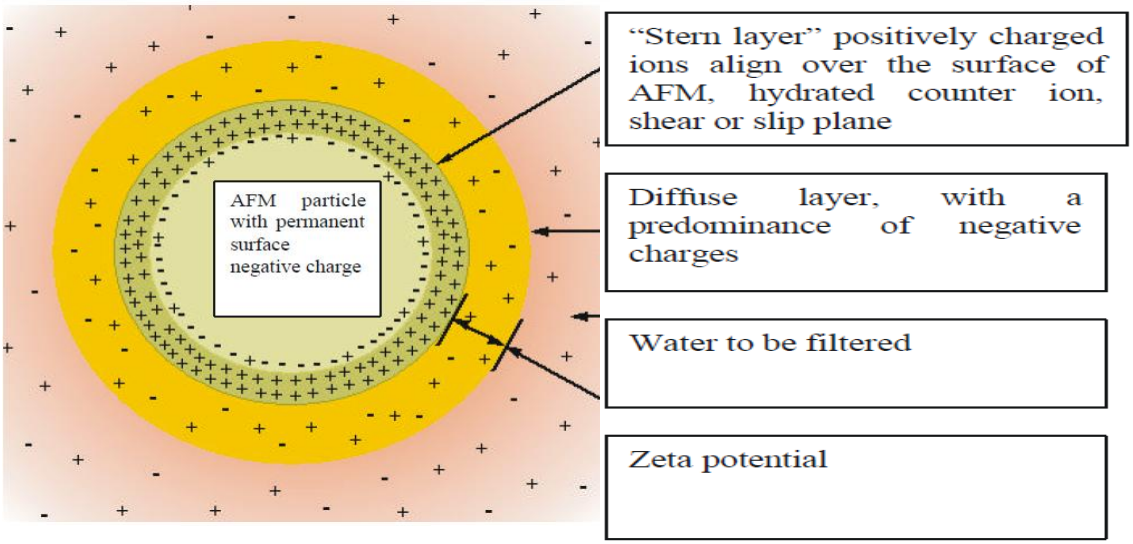

Fig. 2.2. The electrical charge on the AFM treatment. (Figure from Dryden aqua catalogue (2006) by Dr Howard Dryden).

2.1.1.2 Drum filter

The second pipe goes to the DF (P4) which consists of 10 µm screen mesh, Hydrotech model HDF 1604-3H, Vellinge, Sweden (Fig. 2.1). The Hydrotech drum filter catalogue (page 2) suggested that the DF is:

"A self-cleaning filter with low maintenance costs, high performance filtering system. It prevents particles or solids from fragmentation thus achieving high filtration efficiency. The water to be treated is released on the periphery of the rotating drum (Fig. 2.3)*. The special structures in the filter panels separate the suspended solids and trap them from the water. The separated solids are washed out into the solid collection tray and removed from the filter.*

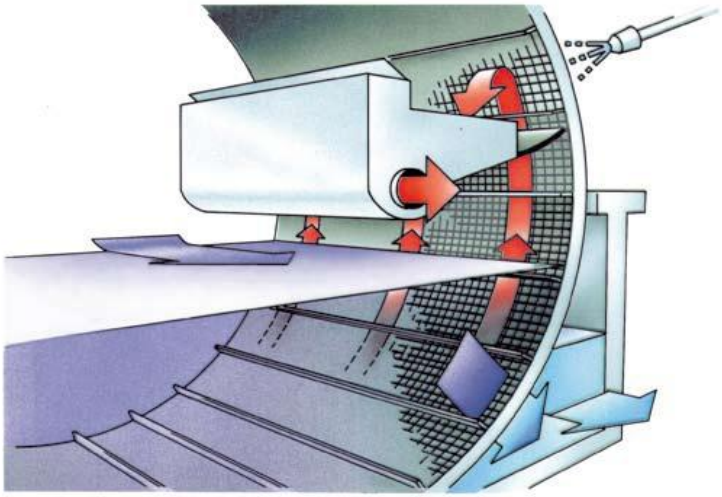

Fig. 2.3. How the intake water is treated inside the drum filter (Picture from Hydrotech drum filter catalogue).

2.1.1.3 Protein skimmers

Both AFM and DF pipes were followed by protein skimmers (P3 and P5, Fig. 2.1) (Sander Aquarietechnik, Helgoland: 500, Uetze-Eltze, Germany) before the water was distributed in the hatchery. The Sander Aquarientechnik (www.aqua-sander.de) suggested that:

"A protein skimmer removes and purifies the DOC from the water by continually water rinsing ring nozzle in the foam cup. The undissolved active surface of the organic matter is deposited between the water and air bubble thus accumulating in the foam (Fig. 2.4). *The non-surface, undissolved organic matter comes into contact with the dissolved compounds in the foam. Ozone can also be partially used on the dissolved matter to be able to make contact with air bubbles".*

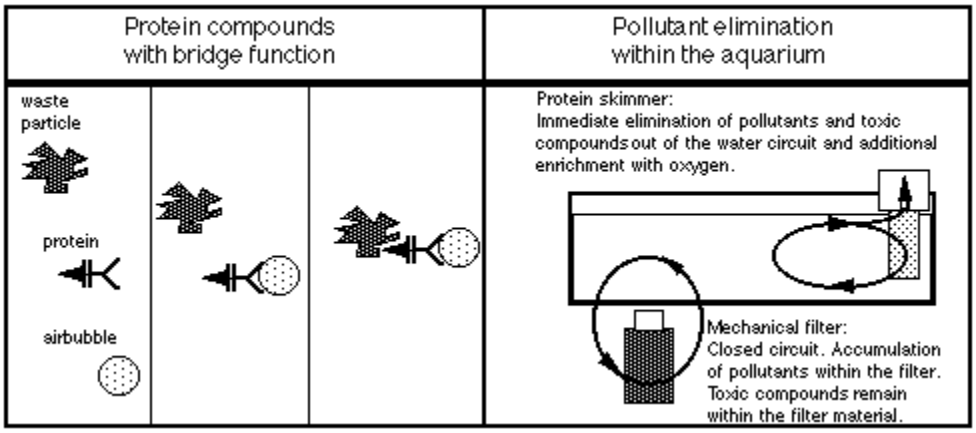

Fig. 2.4. The schematic illustration on how the dissolved organic carbon is being accumulated and eliminated in the foam cup of the protein skimmer (Figure from Sander Aquarientechnik website, www.aqua-sander.de).

2.2 Water quality parameters

Mainly three water quality parameters were measured from the hatchery namely temperature, salinity and dissolved oxygen. Temperature (°C), salinity (‰), and dissolved oxygen (DO, mg l^{-1} and %) were measured on site at the hatchery from all sampling points (P1-P5, Fig. 2.1). The measurements were taken once a month in March, April and May. The temperature, salinity and DO were all determined using a digital oximeter (WTW multi 197i Oximeter) following Standard Methods 2810 (Timmons et al., 2002). The dissolved oxygen was measured in two distinct calculation because DO (mg l^{-1}) is the absolute amount of oxygen in a medium and DO (%) concentration is relative to that when completely saturated at the temperature of the measurement depth and is therefore independent of temperature and salinity.

2.3 Total organic carbon (TOC), dissolved organic carbon (DOC) and particulate organic carbon (POC)

TOC and DOC (mg l^{-1}) samples were taken at the hatchery from all sampling points (P1-P5, Fig. 2.1). The TOC samples, one replicate and DOC samples, three replicates were taken in 100 ml brown medicine bottles. Different number of replicates for TOC and DOC was due to the high costs of the analysis and to the fact that DOC is the main constituents of TOC. The samples were stored in cooler box during transportation. Samples were brought to Chemlab Services AS for analysis the same day. In April, the Chemlab personnel recommended that were sent already filtered water samples for the DOC, and the samples were filtered using 0.45 µm GF/F membrane filter whilst the March and May samples were filtered by Chemlab.

Samples for May were not delivered to the laboratory the same day and were fixed with 0.5 ml hydrochloric acid (HCl) to preserve the samples. Samples for TOC and DOC were analyzed according to the Norwegian standard 1484 (1997), using a total organic carbon analyzer (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 platina catalyst. The combustion product was transported by pure air passing through the inorganic carbon container into infrared (IR) detector.

The TOC and DOC concentrations were obtained by measuring CO_2 against the calibration curve. The reduction percentage of the DOC from the intake water at the different treatments was calculated as follows:

$$DOC(\%) = \frac{P1 - (P3) or (P5)}{P1} \cdot 100 \quad (1)$$

In addition particulate organic carbon (POC) was determined from P1-P5 using Equation 2:

$$POC(\%) = \frac{TOC - DOC}{TOC} \cdot 100 \quad (2)$$

2.4 Microbial analyses

2.4.1 Total bacterial numbers (TBN)

Three replicates water samples (1.5 ml) were collected from all sampling points (P1-P5, Fig. 2.1). The samples were frozen in eppendorfs at -80 °C with 40 µl glutaraldehyde (0.25% final concentration) until further analysis. 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 analyzed using CELLQuest software version 3.0 (Marie et al., 2005). A FACS calibre flow cytometer (Becton Dickson) was used to perform the analysis. Total numbers of bacteria (cells ml^{-1}) were determined as in Equation 3:

$$N = F \cdot n \quad (3)$$

where: F is multiplication factor defined below in equation 4,

n is the raw cell counts.

The above multiplication factor (F) is mathematical determined as:

$$F = \frac{1000}{R} \bullet T \qquad (4)$$

where: 1000 is a constant in µl,

R is the flow rate (µl) and,

T is the time counted in minutes of sample (1 minute).

The flow rate (R) was therefore computed in µl min^{-1} as:

$$R = \frac{W_i - W_f}{T} \qquad (5)$$

where: W_i is the initial weight of sample tube,

W_f is the final weight of sample tube and,

T is the time (minutes) that the sample tube was running.

The initial weight of the sample tube filled with 0.2 µm filtered sterile seawater as in Equation 3 and recorded using the Mettler Toledo balance XS204 (Max 220 g and d=0.1 mg). The outer sleeve of the injection system in the Caliber flow cytometer was removed and the sample tube was placed. The sample tube was run for 10 minutes. New weight of the sample tube was recorded. The results were based on the 1:05 water dilutions because it was easy to count the bacterial cells.

2.4.2 Culturable heterotrophic bacteria

The number of culturable heterotrophic bacteria was expressed as colony forming units (CFU ml^{-1}). The medium for CFU culture was Marine agar (DifcoTM 2216) and Thiosulfate Citrate Bile Sucrose (TCBS) agar (specific for *Vibrio* spp.) (Merck Cat. No. 1.10263). Samples (in triplicates) were taken from all sampling points (P1-P5, Fig. 2.1). Samples of 100 µl were spread

on agar plates and the colonies were counted after 2 and 6 days in room temperature (approximately 20 °C). Dilution series of 1:10 and 1:100 were performed on marine agar for samples known to contain a lot of bacteria.

2.4.3 Microbial community

The water sample (1 l) was sampled from all sampling points (P1-P5, Fig. 2.1). The samples were also pre-filtered through 5 µm filter to remove larger particles then filtered through 47 mm polycarbonate filters, pore size of 0.2 µm (Whatman®, Schleider & Schuell, UK). The filtered samples were frozen at -20 °C for further analysis. The analysis was performed according to (Sandaa et al., 2003). Deoxyribonucleic acid (DNA) was extracted and bacteria harvested were taken as templates in polymerase chain reaction (PCR) performed in a gene Amp PCR system, 2400 Perkin Elmer thermal cycler (Applied Biosystems 2720).

The denaturing gradient gel electrophoresis (DGGE) provided the profile of the bacterial community by analyzing the PCR products. The sequencing was done at Bergen High Technology Centre (BHTC), SARS centre (Bergen, Norway) using Big-Dye protocol version 3.1 (http://seqlab.uib.no) on an ABI Prism 377 DNA sequencer, and further analyzing the sequences using Invitrogen software program. A cluster diagram was made for different sampling months and for comparisons of bacterial community composition.

2.5 Egg development to day 3 (D3) larvae

The *P. maximus* broodstock used in the experiment originated from Hardangerfjorden (Hordaland, Norway; 60 °N) for March and May experimental period and Kvitsøy (Rogaland country, Norway; 59 ° N) for April. The conditioning regime was the same for different broodstock populations. Broodstock was placed in a tank receiving water in a flow-through systems and conditioned according to the protocol within the hatchery for 6 to 8 weeks at 12-13 °C with light-dark phase for 12 hours, water flow rate of 2 l min^{-1}, salinity of 33 ‰ and fed *Isochrysis sp (T-iso), Pavlova lutheri, Chaetoceros muelleri* and *Skeletonema costatum.*

After conditioning process, the broodstock was placed in a bowl receiving water from the hatchery and induced to spawn by thermal stimulation (18 °C) according to (Gruffydd and Beaumont, 1970). The change in water colour indicated that the broodstock has spawned, then transferred to another bowl (Fig. 2.5A). Since the great scallops are hermaphrodites, they first release sperms (whitish colour) (Fig. 2.5B) into the water and then eggs (orange colour) (Fig. 2.5C) at a later stage. It is therefore important that the spawned scallops are moved to a different bowl or container before they self-fertilize the later released eggs.

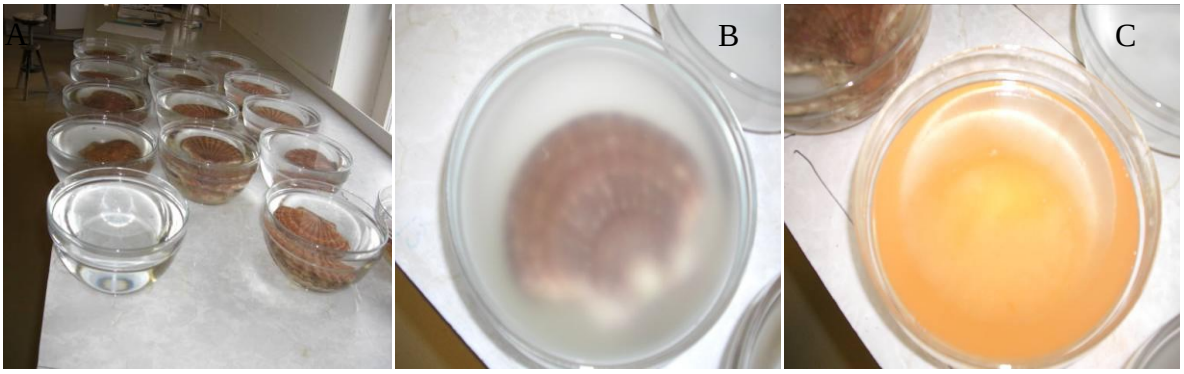

Fig. 2.5. The demonstration of how the broodstock spawn. (A) The broodstock are placed inside the bowl with water inside (B) The broodstock first releases the sperm into the water (C) thereafter, the broodstock was transferred into new water where it releases the eggs.

Experiments for testing egg development to D3 larvae were set up using water 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}. Three buckets each for March and five buckets each for April and May contained water from the AFM + skimmer (P3) and the DF + skimmer (P5) respectively. Air bubbles were provided to each bucket for 3 days. The D larvae were not fed.

Numbers of developed D3 larvae were counted in each bucket after 3 days. Larvae were filtered through two different screen mesh sizes; 60 µm to retain small, fragile D3 larvae and 45 µm to ensure everything was washed out properly. The retained D3 larvae were washed into a 40 ml container. A 50 µl sample of retained larvae was counted 5 times using an inverted stereoscopic microscope (Leitz DM IL).

Estimation of the total number of D3 larvae was calculated according to this formula:

$$T_{LR} = \mu \cdot V_t \quad (6)$$

where: T_{LR} is the total number of D3 larvae retained on 60 µm (%),

μ is the mean larvae per 50 µl x 20 and,

V_t is the *x* total volume (40 ml).

2.6 Larval activity experiment

The larval activity experiment was performed by exposing 8 days old larvae to different water qualities from the AFM + skimmer (P3), DF + skimmer (P5), sterile seawater (SSW) and the water from BHTC, HIB. The control (SSW) was prepared using the protocol from Kester et al. (1967) and had the salinity of 33 ‰. A larval activity experiment was performed according to (Sandlund et al., 2006) protocol. 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 firstly filled with 2 ml of SSW and approximately 20 to 30 larvae were placed in each well. Lastly the replicated wells were separately filled with 100 µl of undiluted water from AFM + skimmer (P3), DF + skimmer (P5) and HIB.

Dilution series of 1:10 and 1:100 were used for the experiment and also the unchallenged group (control) with SSW. Incubation of larvae was performed in a dark place at 18 °C in an air conditioned room according to the protocol. 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 by (Torkildsen et al., 2005) and (Sandlund et al., 2006). The wells were counted twice to reduce counting error because it was difficult to separate the active and in-active larvae when the larvae were swimming very fast.

2.7 Microalgal cell numbers

The microalgae, *C. muelleri* (CHM) a *Bacillariophyceae* were grown by using water from the AFM + skimmer (P3), DF + skimmer (P5) and BHTC (HIB). The starter culture was made by using water from Scalpro AS hatchery in March and HIB in April and May. The water samples were autoclaved at 120 °C for approximately 20 minutes before use. Stock cultures of *C. muelleri* were obtained from the Culture collection at the University of Bergen. 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 ± 1 °C and in Conway medium according to Laing (1991).

The stock cultures were not free of bacteria, but the bacteria concentrations were kept at a minimum. Starter cultures (2 l) were inoculated with stock cultures in good growth. The microalga was grown between 18-21 °C with continuous illumination by white fluorescent light (Osram L 58W/965 Biolux) at 100 µmol $m^{-2}s^{-1}$ and 20 °C ± 1. Aeration was added in the culture supplemented with carbon dioxide (CO_2) as flask necks with sterilised wads of cotton which also buffers the changes in pH. Live cells numbers (cells ml^{-1}) were determined using a Bürker counting chamber in a light microscope with phase contrast at x40 magnification. The following formula was used to calculate the cell numbers:

$$C_n = \frac{X}{40} \tag{7}$$

where: C_n is the cell numbers counted from 10 sub-fields on the Bürker counting chamber,

X is the number of the cells numbers counted and,

40 is the constant division factor according to Scalpro AS hatchery protocol.

Cell numbers were monitored according to the growth phase of microalgae (Fig. 2.6A). The microalgal phases were used to determine the various stages when counting. Counting was performed until the stationary phase and stopped when death phase was approached. The cells on the right and lower borders should not be counted while on the left and top borders should be counted according to the protocol by Lavens and Sorgeloos, 1996 (Fig. 2.6B).

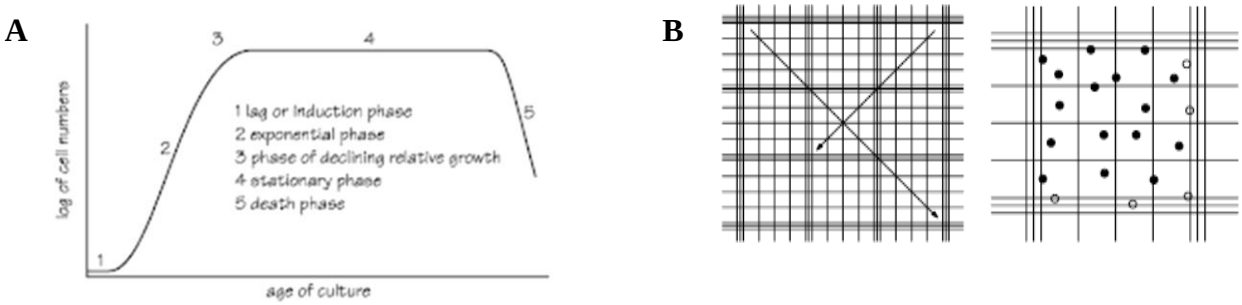

Fig. 2.6. (A) The microalgal growth phases in culture (B) counting methods (Figure from Lavens and Sorgeloos, 1996).

2.8 Statistical analysis

Data were analyzed using the software of SPSS® (Version 15.0, 2009; SPSS, USA). One-Sample Kolmogorov-Smirnov Test (Sokal and Rohlf, 1997) was used to test for the sample normality, evaluated by test of homogeneity of variances carried out by Levene statistic. When violated, Welch and Brown-Forsythe were used to display the alternative version of F-statistic and the results were used (Sokal and Rohlf, 1997). Welch and Brown-Forsythe are robust tests of equality of means. One way analysis of variance (ANOVA) was used to perform the analysis (Sokal and Rohlf, 1997). The post-hoc Tukey honest significant difference (HSD) (Sokal and Rohlf, 1997) and Games-Howell (Games and Howell, 1976) multiple comparison were used to determine the significant differences between treatments Tukey tests assume population variances and sample size are equal while Games-Howell does not (Sokal and Rohlf, 1997).

Figures were produced using Microsoft excel (Version 1997-2003). All statistical tests were carried out at a 0.05 significance level.

3. Results

3.1 Water quality parameters

The mean for all the water quality parameters was calculated for all sampling months and used for plotting graphs and statistical analysis (Table 3.1).

Table 3.1

Water quality parameters measured from all sampling points (P1-P5) at the Scalpro AS hatchery for March, April and May, 2009, and the mean (μ) of the parameters at different sampling points.

Water quality parameters	P1	P2	P3	P4	P5
Temperature (°C)					
March	7.4	8.6	13.4	7.7	8.3
April	7.8	9.7	11.6	8.2	8.9
May	8.7	8.5	13.2	8.6	9.4
μ	8.0	8.9	12.7	8.2	8.9
Salinity (‰)					
March	35.0	35.0	35.0	35.0	35.0
April	35.0	35.0	35.0	35.0	35.0
May	34.9	34.9	34.9	34.9	34.9
μ	34.9	34.9	34.9	34.9	34.9
Dissolved oxygen (%)					
March	75.7	76.5	90.8	80.2	88.7
April	88.0	90.0	106.0	93.0	105.0
May	84.4	88.7	110.7	93.7	109.0
μ	82.7	85.1	102.5	89.0	100.9
Dissolved oxygen (mg l^{-1})					
March	8.96	8.95	9.34	9.42	10.18
April	10.60	10.00	11.50	11.00	12.20
May	10.82	10.85	11.81	11.13	12.72
μ	10.13	9.93	10.88	10.52	11.70

3.1.1 Temperature and salinity

The mean temperature varied between the sampling points (Fig. 3.1). Maximum mean temperature (12.7 °C) was registered in the AFM + skimmer (P3), while the minimum mean temperature (8.0 °C) was registered in the intake water (P1). Mean temperature at AFM + skimmer (P3) was significant higher than other points (P=0.000, Table 12, Appendix B). Salinity was constant (34.9 ‰) throughout the sampling period for all sampling points.

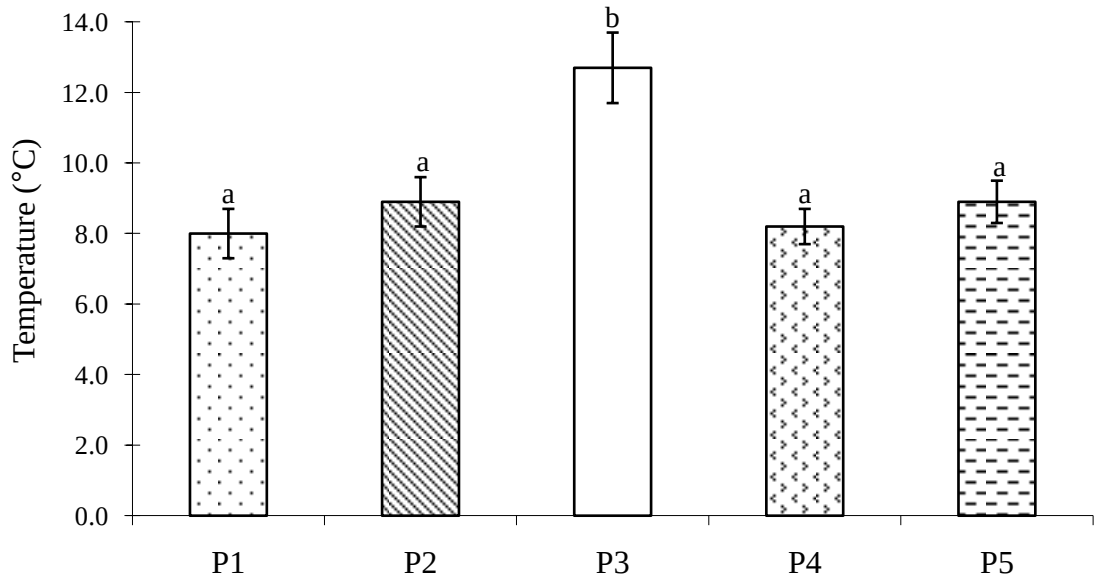

Fig. 3.1. Mean temperature (°C) measured from all sampling points (P1-P5) at the Scalpro AS hatchery for March, April and May (see Table 1, Appendix A). The arrow bars indicate mean ± S.D (standard deviation). Bars with different letters are significant at P<0.05.

3.1.2 Dissolved oxygen (DO, % and mg l^{-1})

The mean DO concentration (%) increased after passing through the AFM + skimmer (P3) and the DF + skimmer (P5) (Fig. 3.2). DO (%) ranged from 82.7% at the intake water (P1) to 102.5% at the AFM + skimmer (P3) throughout the experiment. No significant difference were observed between the treatments (P=0.057, Table 13, Appendix B). The mean DO concentrations were also higher when measured as mg l^{-1} after passing through the AFM + skimmer (P3) and the DF + skimmer (P5) (Fig. 3.3). The DO concentration ranged between 9.93 mg l^{-1} at the AFM (P2) and 11.70 mg l^{-1} at the DF + skimmer (P5) throughout the experiment. There was no significant difference between treatments (P=0.393, Table 14, Appendix B).

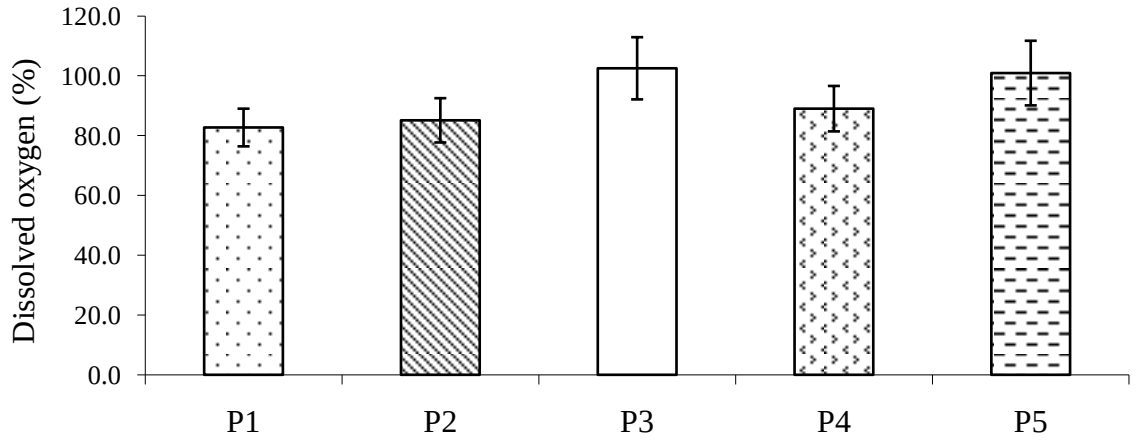

Fig. 3.2. Mean (n=3) dissolved oxygen (%) concentrations measured from all sampling points (P1-P5) at the Scalpro AS hatchery for March, April and May (see Table 1, Appendix A). The arrow bars indicate mean ± S.D.

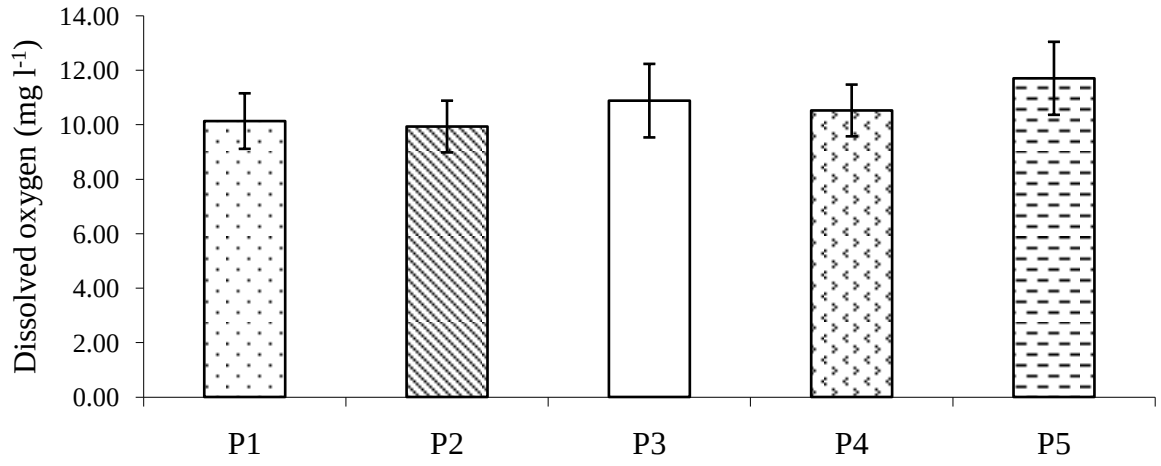

Fig. 3.3. Mean (n=3) dissolved oxygen (mg l^{-1}) concentrations measured from all sampling points (P1-P5) at the Scalpro AS hatchery for March, April and May (see Table 1, Appendix A). The arrow bars indicate mean ± S.D.

3.2 Total organic carbon (TOC), dissolved oxygen carbon (DOC) and particulate organic carbon (POC)

In general, the TOC concentrations were reduced from the intake water (P1) at the AFM + skimmer (P3) and the DF + skimmer (P5). The reductions were low in March and highest in May.

In March, the TOC concentrations were reduced (4.0 mg l^{-1}) from the intake water (P1) at the AFM + skimmer (P3) (3.5 mg l^{-1}) and the DF + skimmer (P5) (3.3 mg l^{-1}) (Fig. 3.4A). In April, the TOC concentration (0.9 mg l^{-1}) from the intake water (P1) were increased (2.5 mg l^{-1}) at the AFM + skimmer (P3) and (1.3 mg l^{-1}) at the DF + skimmer (P5) (Fig. 3.4B). In May, the TOC concentrations were reduced (4.7 mg l^{-1}) from the intake water (P1) at the AFM + skimmer (P3) (3.2 mg l^{-1}) and the DF + skimmer (P5) (2.6 mg l^{-1}) (Fig. 3.4C).

In March, April and May, the DOC concentrations from the intake water (P1) were reduced at the AFM + skimmer (P3) and the DF + skimmer (P5).

The DOC concentrations in March were reduced from the intake water (P1) (3.5 mg l^{-1}) at the AFM + skimmer (P3) (2.7 mg l^{-1}) and the DF + skimmer (P5) (2.5 mg l^{-1}) respectively (Fig. 3.5A), but the reduction was not significant (P=0.075, Table 16, Appendix B).

In April, the DOC concentrations (3.6 mg l^{-1}) at AFM (P2) from the intake water (P1) were significantly reduced (1.6 mg l^{-1}) at the AFM + skimmer (P3) (Fig. 3.5B) (P=0.003, Table 17, Appendix B).

In May, the DOC concentrations from the intake water (P1) were reduced at both treatments (Fig. 3.5C). The DF + skimmer (P5) significantly reduced (2.7 mg l^{-1}) the DOC concentration from the intake water (P1, 4.9 mg l^{-1}) more than the AFM + skimmer (P3) with 3.3 mg l^{-1} (P=0.011, Table 19, Appendix B).

In addition, the percentage reduction of the DOC concentration from the intake water (P1) was calculated at the AFM and DF after skimmers (Table 3.2). Overall, the percentage reduction from the intake water was on average slightly higher at the DF + skimmer (34%) compared to the AFM + skimmer (31%).

Table 3.2

The percentage reduction (%) of the DOC (mg l^{-1}) by both treatments from the intake water.

	March	April	May	Average
DOC (mg l^{-1}) at the intake water (P1)	3.5	2.5	4.9	
% reduction (AFM + skimmer)	23	36	33	31
% reduction (DF + skimmer)	29	28	45	34

In March, the POC concentrations from the intake water (P1) were increased at the AFM + skimmer (P3) and DF + skimmer (P5). The POC concentration ranged between 12.5 mg l^{-1} at the intake water (P1) and 32.4 mg l^{-1} at the DF (P4). In April (except AFM + skimmer with 36 mg l^{-1}) and May, the POC concentration gave negative values (Results not shown).

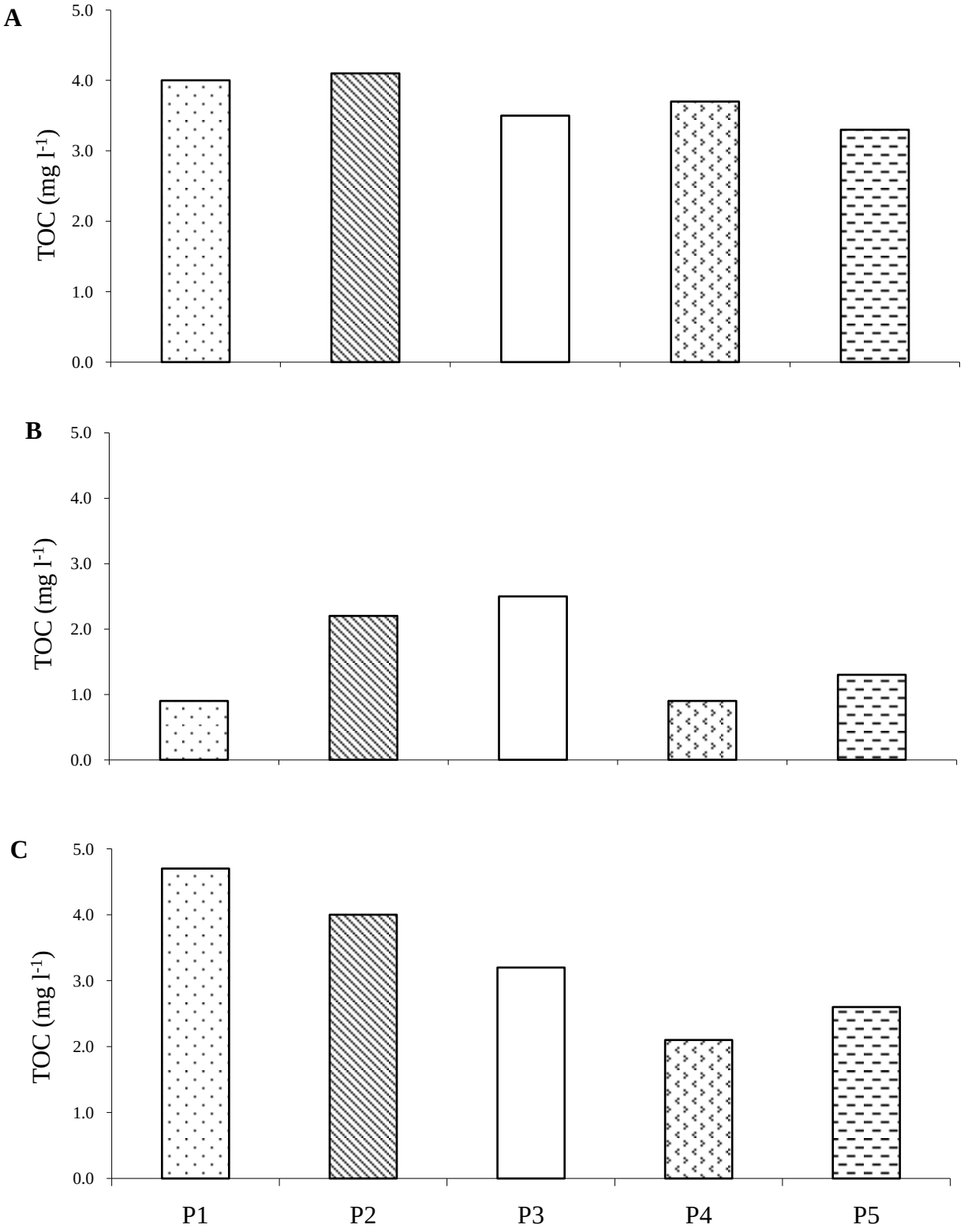

Fig. 3.4. TOC (mg l^{-1}) concentrations measured from all sampling points (P1-P5) at the Scalpro AS hatchery for (A) March, (B) April and (C) May.

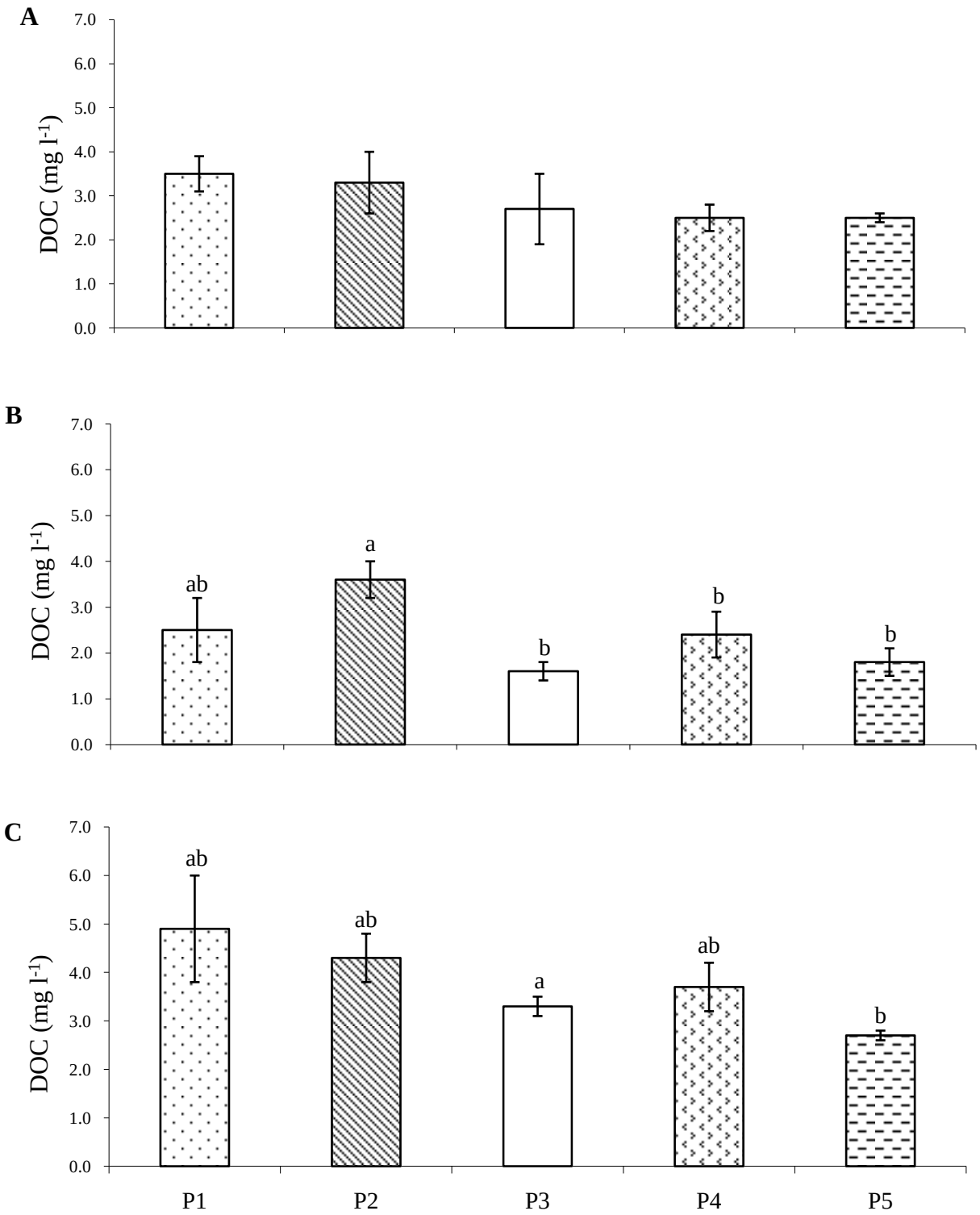

Fig. 3.5. Mean (n=3) DOC (mg l^{-1}) concentrations measured from all sampling points (P1-P5) at the Scalpro AS hatchery for (A) March (B), April and (C) May (see Table 2, Appendix A). The arrow bars indicate mean ± S.D. Bars with different letters are significant at $P<0.05$.

3.3 Microbial analyses

3.3.1 Total bacterial numbers (TBN)

The bacterial cell numbers were lowest in March and there was a small variation in April and May. In March, the bacterial cell numbers (2.3 x 10^5 cells ml^{-1}) from the intake water (P1) were slightly increased (2.5 x 10^5 cells ml^{-1}) at the AFM + skimmer (P3) while the DF + skimmer (P5) maintained the same bacterial cell numbers (2.3 x 10^5 cells ml^{-1}) with the intake water (P1) (Fig. 3.6A). There was a significant increase in bacterial cell numbers at the DF (P4) from the intake water (P1) in March, but significantly reduced at the DF + skimmer (P5) (P=0.010, Table 20, Appendix B).

In April, both treatments maintained the same bacterial cell numbers (3.7 x 10^5 cells ml^{-1}) as with the intake water (P1) (Fig. 3.6B). However, there was no significant difference between treatments (P=0.276, Table 22, Appendix B).

In May, the bacterial cell numbers from the intake water (P1) (3.4 x 10^5 cells ml^{-1}) were the same (3.6 x 10^5 cells ml^{-1}) at the AFM + skimmer (P3) and the DF + skimmer (P5) (Fig. 3.6C). No significant difference was found between treatments (P=0.105, Table 23, Appendix B).

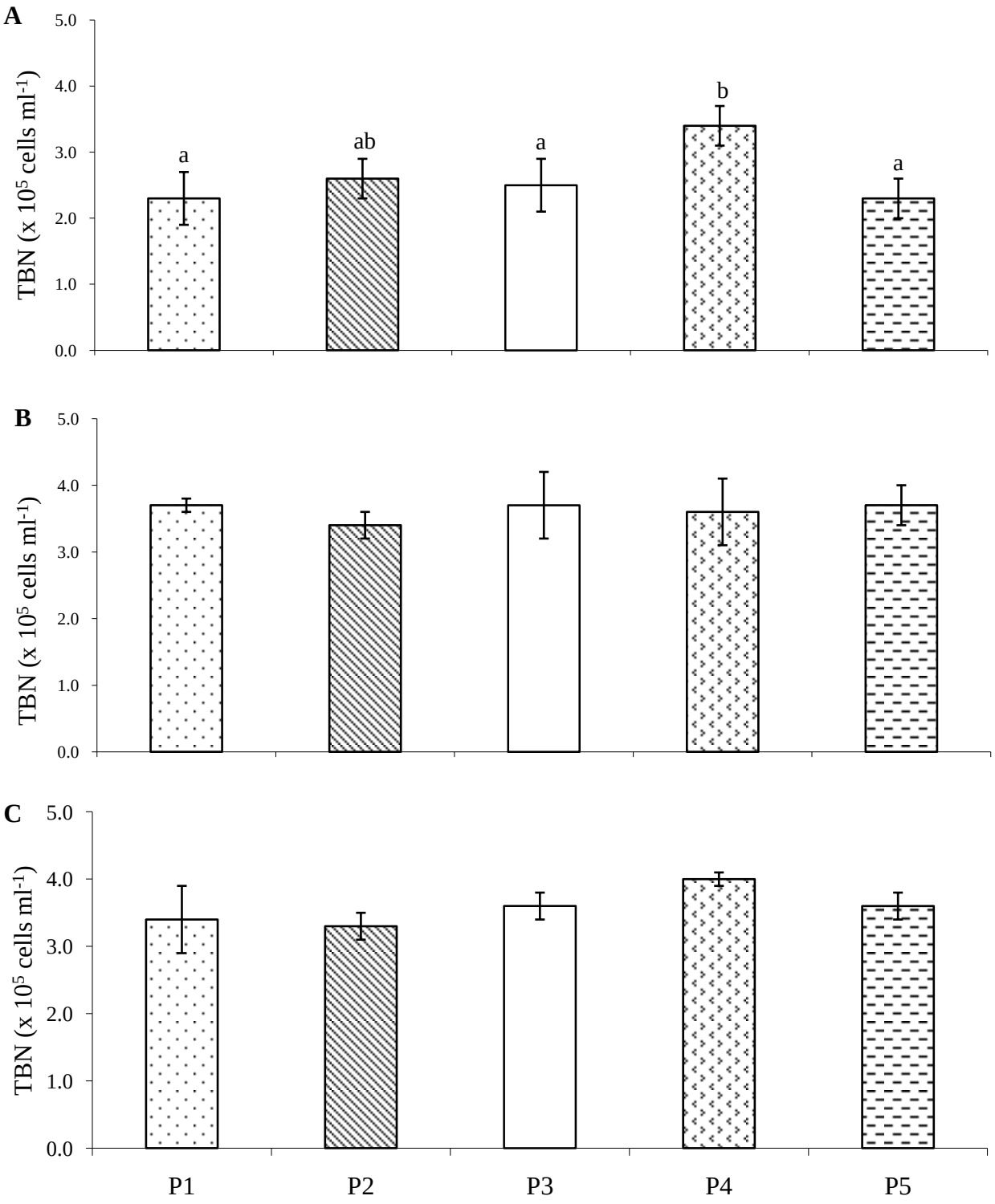

Fig. 3.6. Mean (n=3) TBN (x 10^5 cells ml^{-1}) recorded when analysing the water samples from P1-P5 on the flow cytometer for (A) March, (B) April and (C) May (see Table 3, Appendix A). The samples were analyzed on the 25 and 26 October 2009 for March and April, and 27 October 2009 for May. The arrow bars indicate mean ± S.D. Bars with different letters are significant at $P<0.05$.

3.3.2 Culturable heterotrophic bacteria

3.3.2.1 Thiosulfate citrate bile sucrose (TCBS)

TCBS samples were taken in March, April and May for all sampling points (P1-P5). In March, no colonies were found, except the DF (P4) with 3 CFU ml^{-1}. The colony found was yellow in colour indicating *Vibrio* spp. In April and May, no *Vibrio* colonies were detected (results not shown).

3.3.2.2 Marine agar

In general, the numbers of colonies were higher in April more than May. In April, the CFU from the intake water (P1) (13 CFU ml^{-1}) were increased (43 CFU ml^{-1}) at the AFM + skimmer (P3), but the DF + skimmer had no colonies present (0 CFU ml^{-1}) (Fig. 3.7A). However, there was no significant difference between treatments (P=0.241, Table 24, Appendix B).

In May, the numbers of colonies from the intake water (P1) were slightly increased (5 CFU ml^{-1}) at the AFM + protein skimmer (P5) but no colonies (0 CFU ml^{-1}) were detected at the DF + skimmer (P5) (Fig. 3.7B). There was no significant difference between treatments (P=0.570, Table 25, Appendix B).

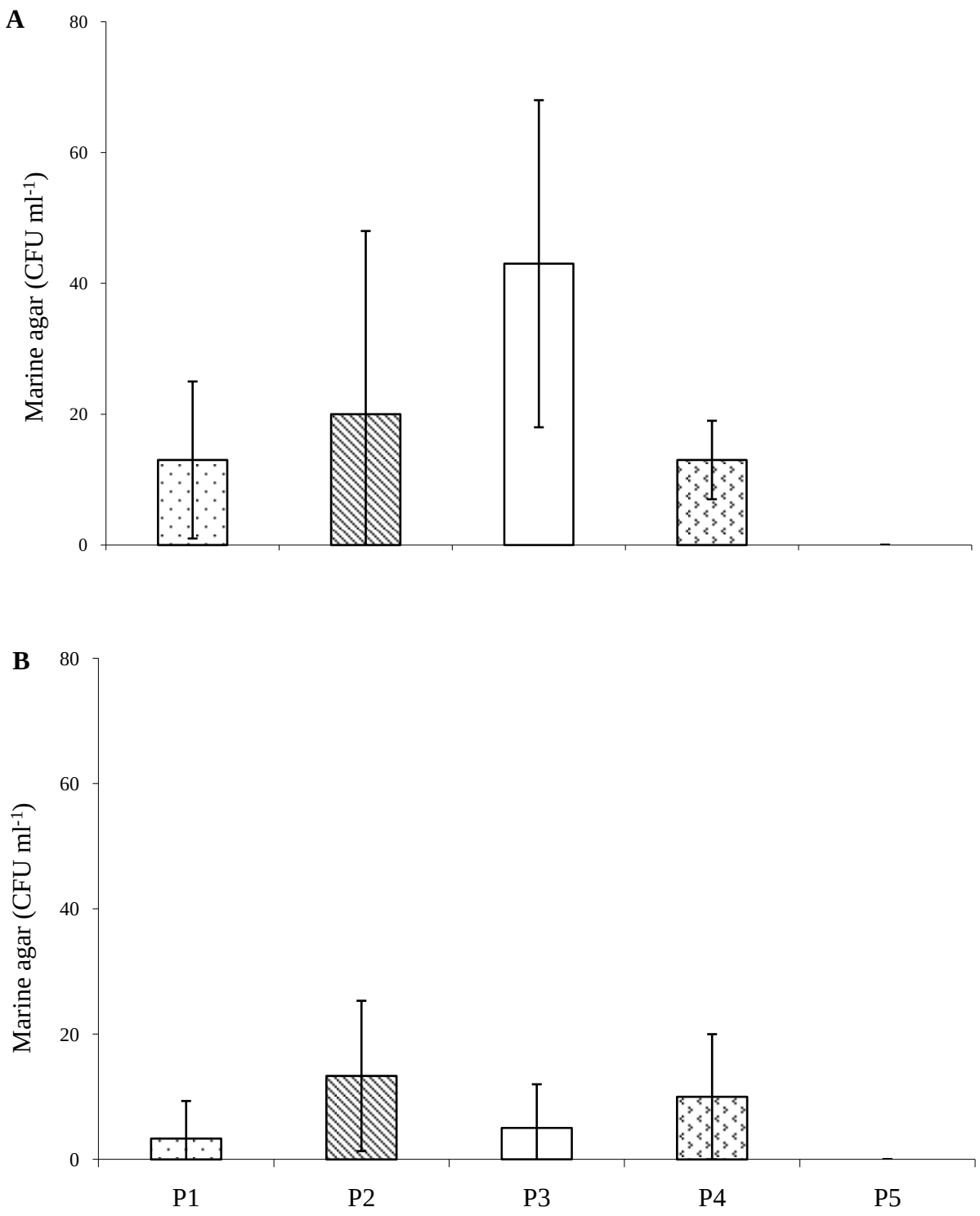

Fig. 3.7. Culturable heterotrophic bacteria counts on marine agar plates measured from all sampling points (P1-P5) at the Scalpro AS hatchery for (A) April and (B) May after 48 hours (see Table 4, Appendix A). The arrow bars indicate mean (n=3) ± S.D.

3.3.3 Microbial community

Denaturing gradient gel showed a mobility of different microbial species represented by a band (Fig. 3.8). Each band has a specific 16S rDNA. The denaturing gel gradient contained 20 wells. All the five sampling points from March, April and May were investigated. Only the strongest bands were used for sequencing and cluster analysis. The band from the third and eleventh well (from the left) in March and April from the AFM (P2) was weak that it was not used for the cluster analyses. The samples dated 27/3 were from the hatchery water treatment points. The numbers on the different sampling points shows where the bands were taken for cluster analysis and sequencing.

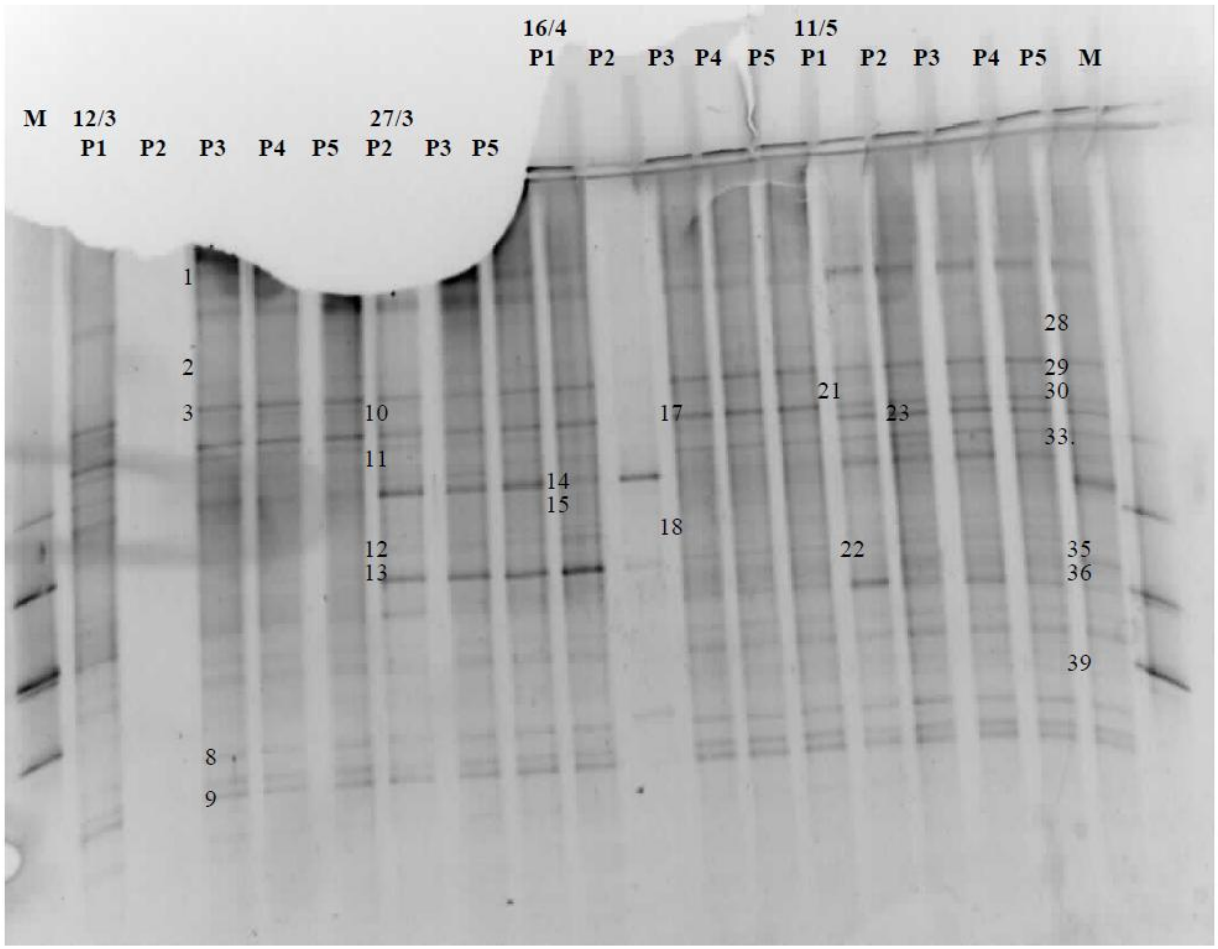

Fig. 3.8. The DGGE gel with bands showing different sampling points (P1-P5) for March, April and May. Both Markers (M) and samples dated 27 March 2009 served as control in this experiment (Performed by Torill Vik Johannessen).

Many of the bacteria hits from the database were clones from seawater, some from bacterioplankton blooms or that are commonly found in the natural water masses (Table 3.3). Some of the sequences looked like clones found associated with different marine animals and one sequence showed similarity to bacteria associated with a toxin producing dinoflagellate (Saxotoxin). The bacterial composition changed over time in all of the sampling points.

Table 3.3

Sequences found in bands from DGGE-gel in Figure 3.11 with simple matching from Genbank (Performed by Torill Vik Johannessen).

Sequence nr	band	Sample from	In sample	Similar to	Accession nr	Comment
TJ708	1	P3 (12/3)	All	Uncultured SAR11 cluster alpha proteobacterium	AM748185	
709	2	P3 (12/3)	All	Uncultured SAR11 cluster alpha proteobacterium Uncultured marine bacterium clone BM1-F-105 (82%)	DQ186916 FJ826203	
726	3	P3 (12/3)	All	Uncultured marine bacterium clone BM1-8-74 (99%) Uncultured gamma proteobacteriumFFW81 (99%)	FJ826062 AY830024	
727	8	P3 (12/3)	All	Uncultured actinobacterium clone HF4000_16H14 (98%)	EU361019	Bacterioplankton
646	9	P3 (12/3)		Uncultured actinobacterium clone HF4000_16H14 (82%)		
711	10	P2 (27/3)	All (11/5)	Unidentified alpha proteobacterium OM75 (90%) *Nisaea nitritireducens* strain DR41_18 (88%)	U70683 DQ665839	
712	11	P2 (27/3)	All (27/3) & P2 (16/4)	Uncultured Chloroflexaceae group bacterium Arctic96BD-6 (93%)	AF355053	
728	12	P2 (27/3)	All (27/3) & P1 (16/4)	Uncultured gamma proteobacterium clone PM1-27 (99%) Oceaniserpentilla haliotidis (94%)	EF215799 AM747817	Schlösser, A., 2008
713	13	P2 (27/3)		Uncultured organism clone ctg_NISAA66 (99%) Uncultured Candidatus *Microthrix* sp. clone BATS136-250-93	DQ396300 FJ960805	

729	14	P1 (16/4)		Uncultured gamma proteobacterium clone PM1-27 (98%) *Oceaniserpentilla haliotidis* (94%)	EF215799 AM747817	
715	15	P1 (16/4)		*Colwellia maris* strain ABE-1(92%) Uncultured bacterium clone HC-8 (92%)	NR_024635 AY529875	Diseases in corals, Bourne, DG coral reefs, 2005
730	17		P3-P5 (16/4) & P1-P5 (11/5)	Uncultured bacterium clone HF130_D6_P1 (94%)	DQ300613	Chloroflexi
731 (654)	18	P3 (16/4)	P3-P5 (16/4) & P2-P4 (11/5)	Uncultured gamma proteobacterium clone GC21V_AD (95%)	AY701419	Bacterial community associated with the paralytic shellfish toxin producing dinoflagellate *Gymnodinium catenatum*
732	21			*Colwellia rossensis* strain ANT9279 (97%)	AY167311	
655	22	P1 (11/5)	All (11/5)	Uncultured gamma proteobacterium clone PM1-27	EF215799	Dang, H., 2008
733	23	P2 (11/5)		Uncultured bacterium clone HF130_D6_P1 (94%)	DQ300613	Chloroflexi
657	28	P5 (11/5)	All	*Sphingomonas melonis* strain PR-3	FJ605424	
734	29	P5 (11/5)	All	Uncultured marine bacterium clone BM1-8-74 (97%)	FJ826062	
735	30	P5 (11/5)	All (11/5)	*Colwellia rossensis* strain ANT9279 (98%)	AY167311	
737	33	P5 (11/5)	P2-P5 (11/5)	Uncultured bacterium clone Mann16S_G10B (100%)	FJ952689	Associated with corals
664	35	P5 (11/5)		Uncultured *Rhodobacteraceae* bacterium clone IG3E05	FJ718205	
665	36	P5 (11/5)		Uncultured marine bacterium clone ArtRif4-2	FJ594812	
668	39	P5 (11/5)	All	Uncultured alpha proteobacterium clone HF130_15B09 Uncultured bacterium clone 2C228359	EU361386 EU800311	

It appeared like sampling time had an effect on the bacterial community composition both in the intake water and water passing through the filters (Fig. 3.9). There was a succession in the bacterial composition through season and sampling months were grouping together. There was a change in March (1203) and May (1105) from the intake water (P1) and the DF + skimmer (P5) to the other treatments. In May, the bacteria community in the AFM (P2) was similar to the intake water (P1) while the AFM + skimmer (P3) was less similar to the intake water (P1). In April, the DF + skimmer (P5) was similar to the DF (P4) and less similar to the AFM + skimmer (P3) in April and DF (P4) in May.

Both markers were similar to March samplings including controls and the intake water in May. In March, the control (P5) was similar to the intake water (P1) in April and less similar to the control (P3) in March. In March, AFM (P2) was less similar to AFM + skimmer (P3). while the DF (P4) and DF + skimmer (P5) were similar to AFM + skimmer (P3). There was a little similarity between sampling points over time because the bacterial composition changed with the season.

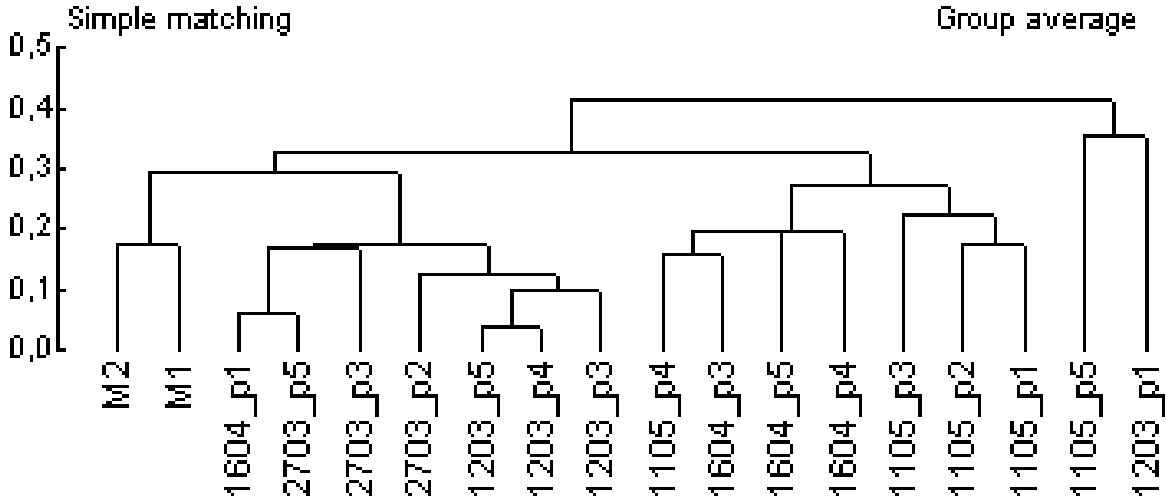

Fig. 3.9. Cluster-analyses of DGGE gel with samples from Scalpro AS hatchery. Dates are given. M1 and M2 are markers. The analyses were made only from bands that were good from different sampling points at different months (Performed by Torill Vik Johannessen).

3.4 Egg development to day 3 (D3) larvae

The highest fraction of D3 larvae was achieved in May followed by April and lastly March. In May, more than 50% of the eggs reached D3 larvae in the DF + skimmer (P5).

In March, the AFM + skimmer (P3) gave the highest (11.8%) larval fraction more than the DF + skimmer (P5) with 9.2% (Fig. 3.10A). The AFM + skimmer (P3) performed better than the DF + skimmer (P5) in March by having a 2.6% increase in egg development to D3 larvae, however no significant differences were observed between treatments (P=0.438, Table 26, Appendix B).

In April, the highest (16.6%) larval fraction was found in the DF + skimmer (P5) and the lowest (16.3%) in the AFM + skimmer (P3) (Fig. 3.10B). There were no significant differences between treatments (P=0.952, Table 27, Appendix B).

In May, the highest (51.1%) larval fraction was found at the DF + skimmer (P5) and the lowest (49.1%) at the AFM + skimmer (P3) (Fig. 3.10C). There was no significant differences between treatments (P=0.729, Table 28, Appendix B). In April and May, the DF + skimmer (P5) increased the larval fraction by 0.3% and 2.0% as compared to the AFM + skimmer (P3) respectively. Overall, the larval development averaged approximately 26.0% for both treatments.

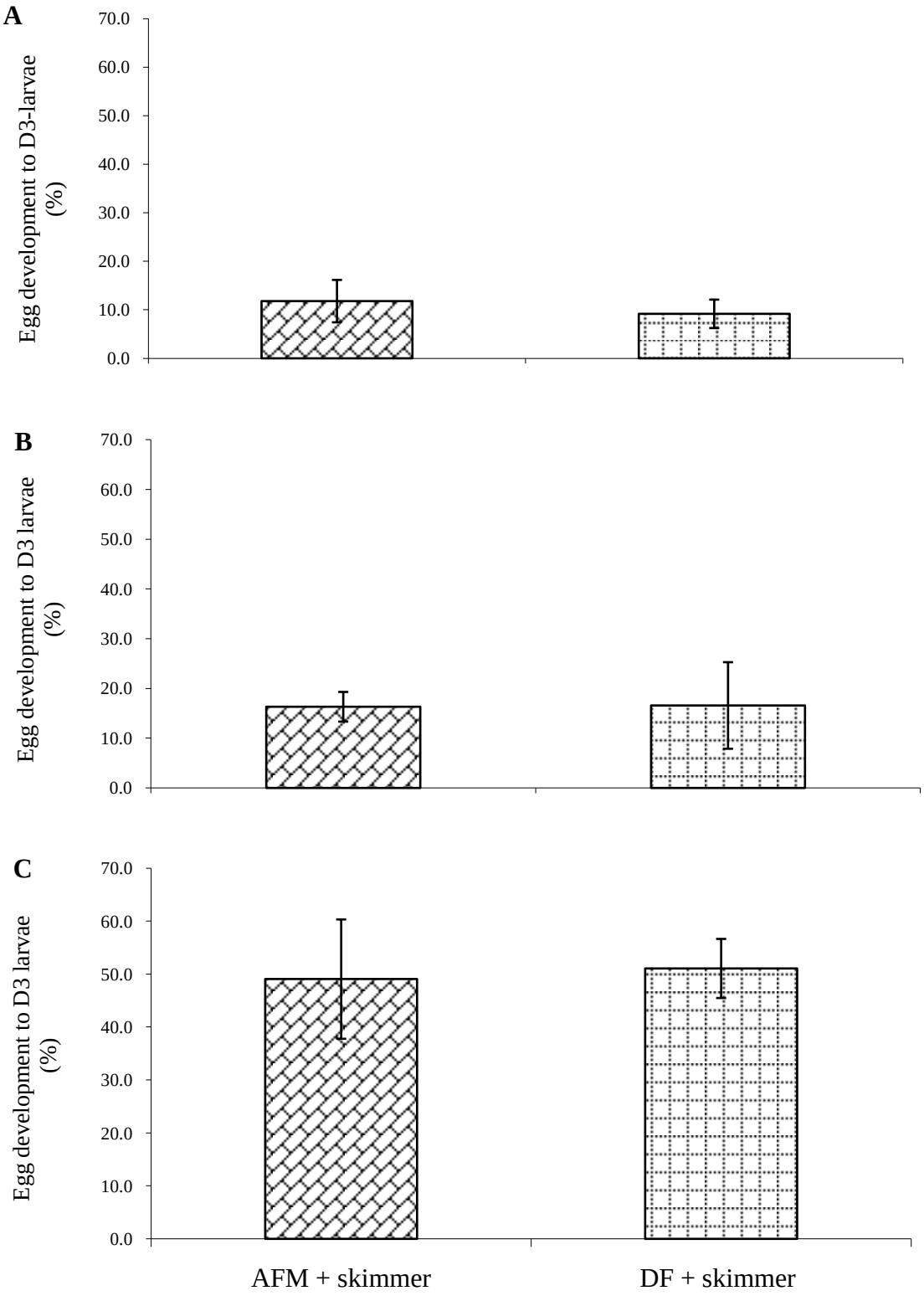

Fig. 3.10. Mean egg development to D3 larvae (%) experiment performed using water from AFM + skimmer (P3) and DF + skimmer (P5) for (A) March (n=3), (B) April (n=5) and (C) May (n=5) (see Table 5, Appendix A). The arrow bars indicate mean ± S.D.

3.5 Larval activity experiment

There was no significant increase in larval activity in undiluted and 100-fold diluted water for March, April and May. In May, there was a significant increase in the larval activity in the 10-fold diluted water.

3.5.1 Undiluted samples

In March, the larval activity was highest (50.5%) at the AFM + skimmer (P3) and lowest (39.4%) at the DF + skimmer (P5) (Fig. 3.11A) but there was no significant differences between treatments (P=0.439, Table 29, Appendix B).

In April, the larval activities were highest (73.4%) at the DF + skimmer (P5) and lowest (55.2%) at the AFM + skimmer (P3) (Fig. 3.11B). SSW (64.9%) and the HIB water (59.0%) performed better than the AFM + skimmer (P3) with 55.2%. However no significant difference was observed between treatments (P=0.299, Table 30, Appendix B).

In May, the larval activity was highest (64.5%) at the DF + skimmer (P5) and lowest (48.4%) in the HIB water (Fig. 3.11C). SSW had higher (57.5%) larval activity than AFM + skimmer (P3) with 53.8%. There was no significant differences between treatments (P=0.078, Table 31, Appendix B).

3.5.2 10-fold diluted samples

In March, the larval activity was highest (37.8%) at the DF + skimmer (P5) and lowest (25.3%) at the AFM + skimmer (P3) (Fig. 3.12A). No significant difference was observed between treatments (P=0.430, Table 32, Appendix B).

In April, the highest (67.3%) larval activity was achieved at the DF + skimmer (P5) and HIB water had the lowest (57.7%) activity (Fig. 3.12B). SSW had higher (64.0%) larval activity

compared to AFM + skimmer (P3) with 62.1%. There was no significant differences between treatments (P=0.543, Table 33, Appendix B).

In May, the larval activity ranged between 61.4% at the HIB water and 48.2% at the SSW (Fig. 3.12C). SSW had significantly higher larval activity compared to the HIB water (P=0.002, Table 35, Appendix B). The larval activity was higher (59.9%) at the AFM + skimmer (P3) compared to 53.1% found at the DF + skimmer (P5).

3.5.3 100-fold diluted samples

In March, the larval activity was highest (41.7%) at the AFM + skimmer (P3) and lowest (29.9%) at the DF + skimmer (P5) (Fig. 3.13A). No significant difference was observed between treatments (P=0.407, Table 36, Appendix B).

In April, the highest (73.3%) larval activity was achieved at the DF + skimmer (P5) and the lowest (55.0%) at the AFM + skimmer (P3) (Fig. 3.13B). There was no significant differences between treatments (P=0.165, Table 38, Appendix B).

In May, the larval activity was highest (65.3%) at the DF + skimmer (P5) and SSW had the lowest (49.5%) activity (Fig. 3.13C). HIB water had higher (60.6%) larval activity as compared to the AFM + skimmer (P3) with 56.4%. No significant difference was observed between treatments (P=0.356, Table 39, Appendix B).

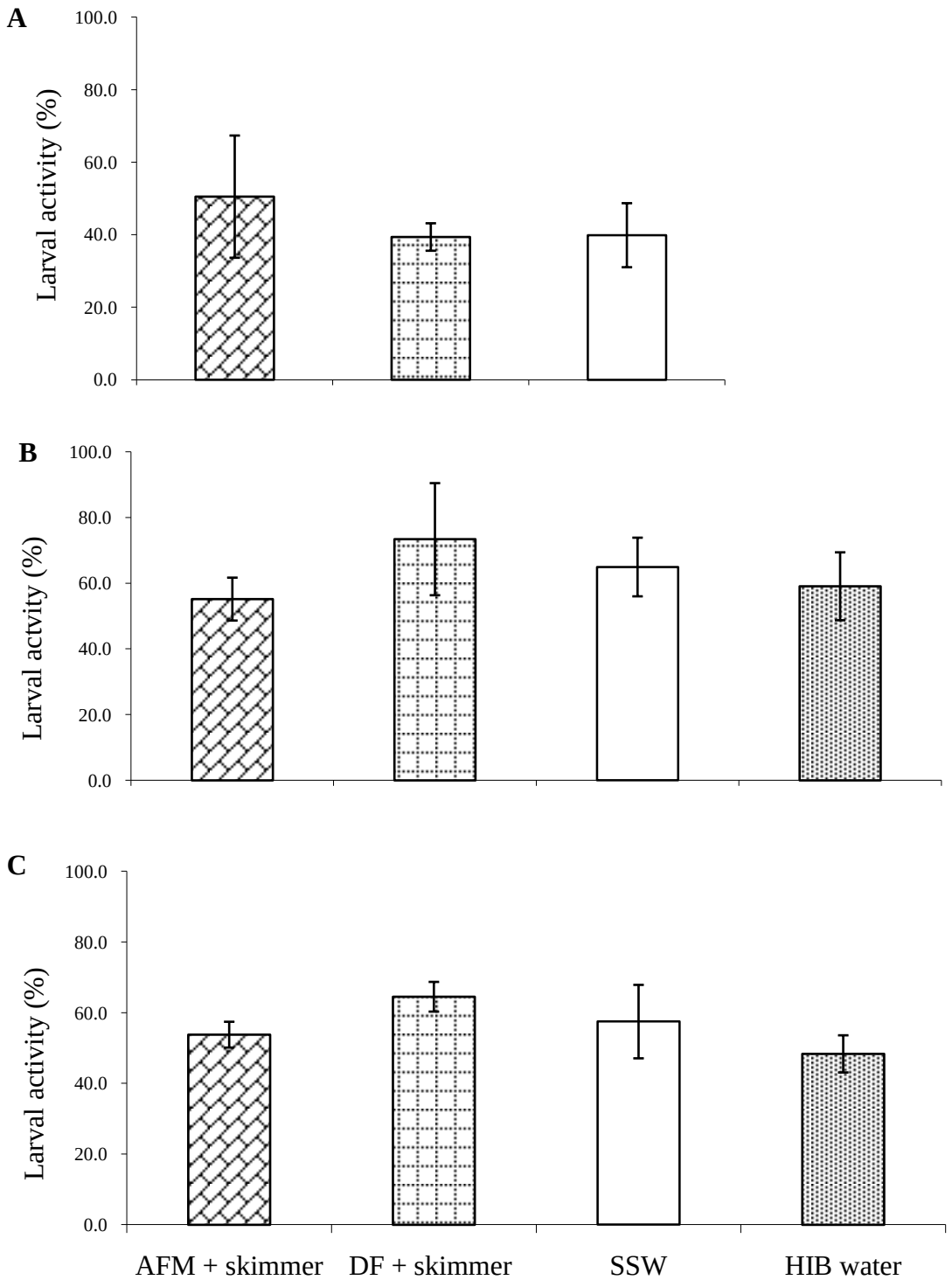

Fig. 3.11. Activity (%) of scallop larvae after 3 days of exposure to undiluted water from AFM + skimmer (P3), DF + skimmer (P5), HIB water and SSW for (A) March, (B) April and (C) May (see Table 6, Appendix A). The arrow bars indicate mean (n=3) ± SD.

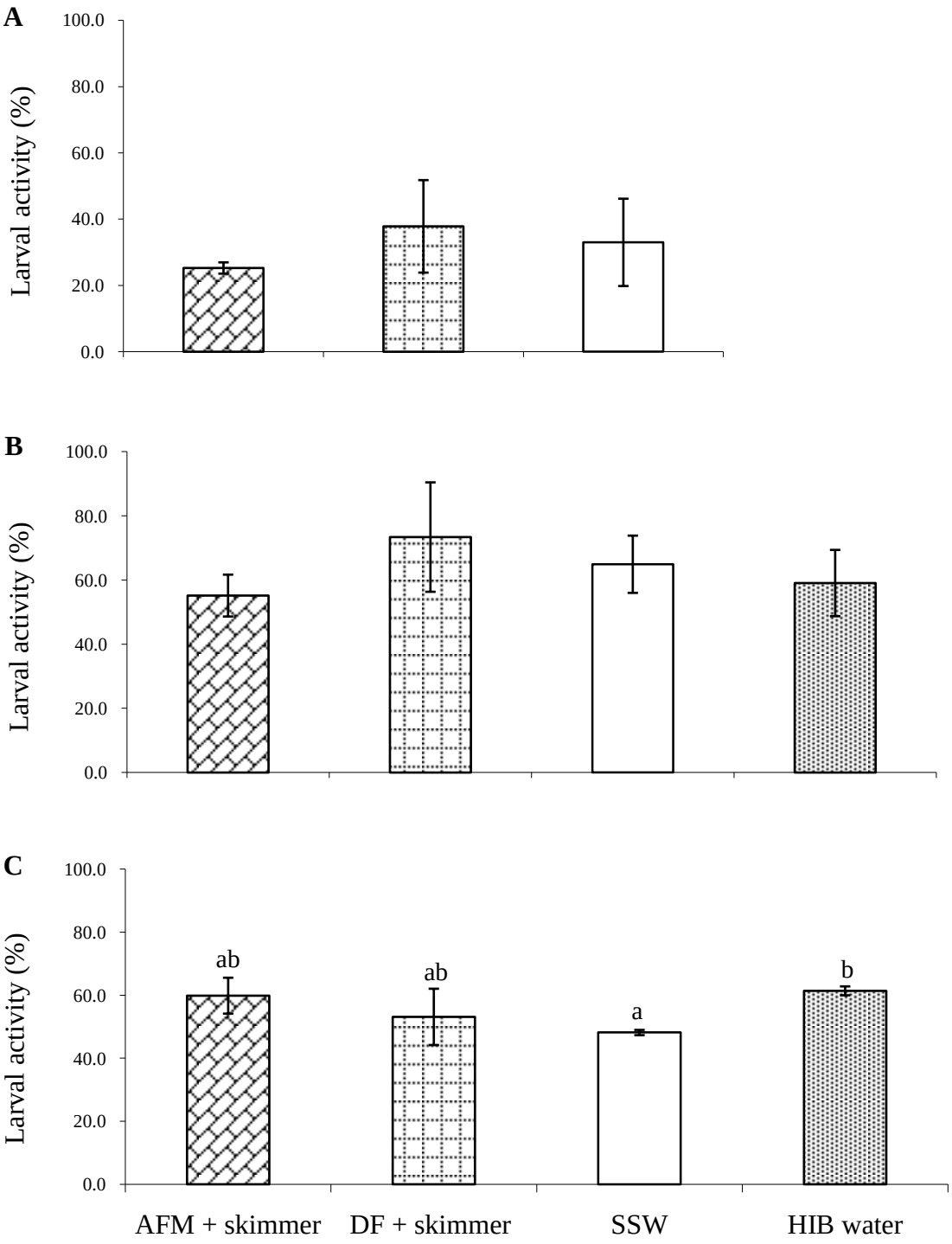

Fig. 3.12. Activity (%) of scallop larvae after 3 days of exposure to 10-fold water dilutions from AFM + skimmer (P3), DF + skimmer (P5), HIB water and SSW for (A) March, (B) April and (C) May (see Table 7, Appendix A). The arrow bars indicate mean (n=3) ± SD. Bars with different letters are significant at $P<0.05$.

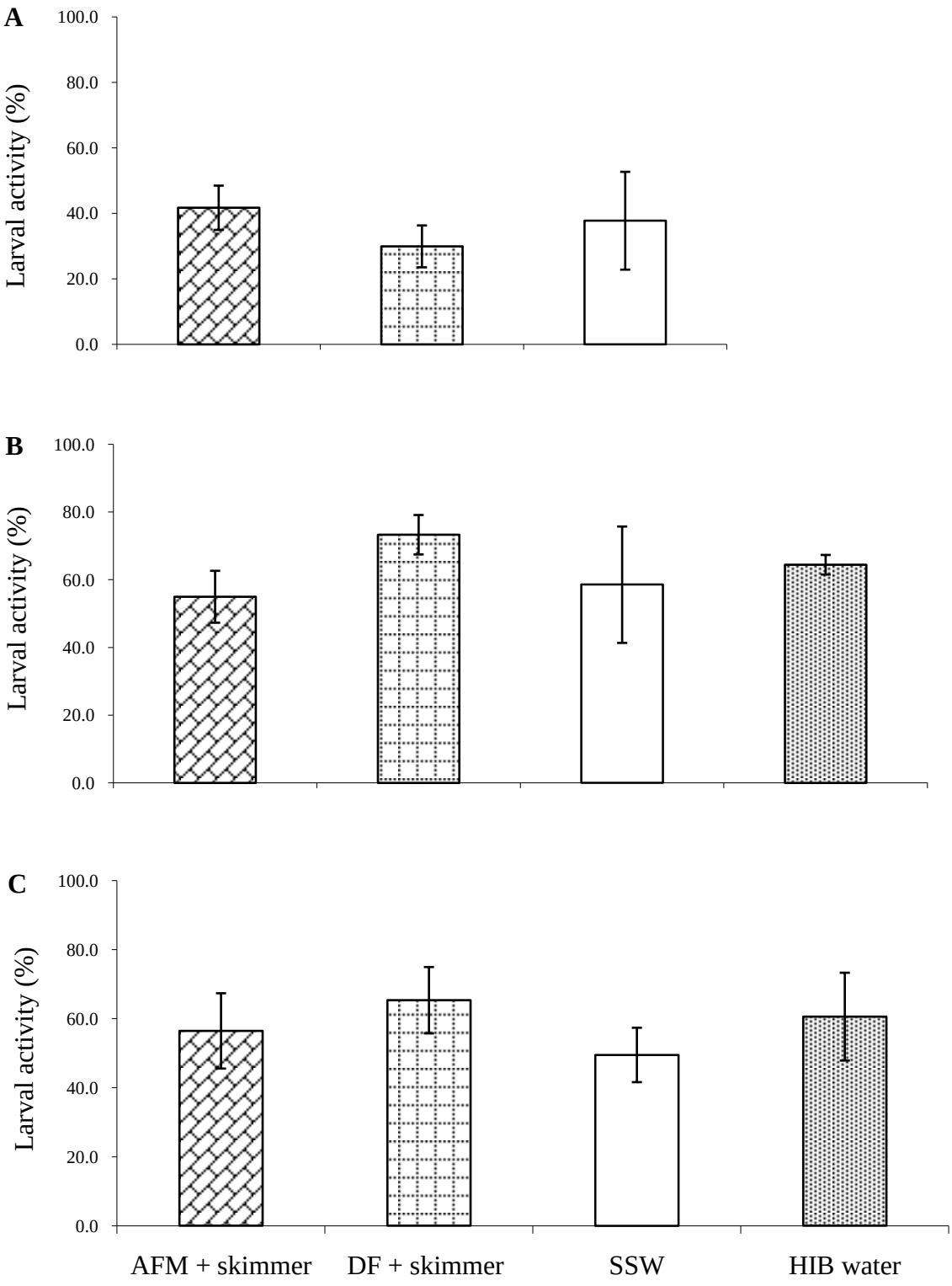

Fig. 3.13. Activity (%) of scallop larvae (D11) after 3 days of exposure to 100-fold water dilutions from AFM + skimmer (P3), DF + skimmer (P5), HIB and SSW for (A) March, (B) April and (C) May (see Table 8, Appendix A). The arrow bars indicate mean (n=3) ± SD.

3.6 Microalgal cell numbers

C. muelleri was grown in March, April and May using water from the AFM + skimmer (P3), DF + skimmer (P5) and HIB. The cell numbers were counted from day 1 till the day the culture collapsed.

In March at the peak day (day 3), the highest (17 x 10^6 cells ml^{-1}) cell numbers were achieved at the DF + skimmer (P5), and the lowest (15 x 10^6 cells ml^{-1}) at the AFM + skimmer (P3) (Fig. 3.14A). In general, the DF + skimmer (P5) had the highest cell numbers. There was no significant difference in cell numbers between the treatments for all the days (P=0.471, Table 42, Appendix B).

In April, at the peak day (day 3), the DF + skimmer (P5) had the highest (13 x 10^6 cells ml^{-1}) cell numbers and the water from HIB had the lowest (9 x 10^6 cells ml^{-1}) (Fig. 3.14B). There was a significant increase in cell numbers at the AFM + skimmer (P3) and the DF + skimmer (P5) than the water from HIB (P=0.003, Table 46, Appendix B). There was a significant increase in the cell numbers at day 4 (P=0.025, Table 47, Appendix B) and day 5 (P=0.022, Table 48, Appendix B) at the DF + skimmer (P5) than the HIB water.

In May, the highest (11 x 10^6 cells ml^{-1}) cell numbers were achieved at day 4 at the DF + skimmer (P5) and the lowest cell numbers (8 x 10^6 cells ml^{-1}) were obtained at the water from HIB (Fig. 3.14C). The DF + skimmer (P5) had significantly higher cell numbers than HIB (P=0.035, Table 51, Appendix B).

The highest cell numbers were found in March but were not significant between different treatments and the lowest in May. In April and May, there was a significant in cell numbers at peak days.

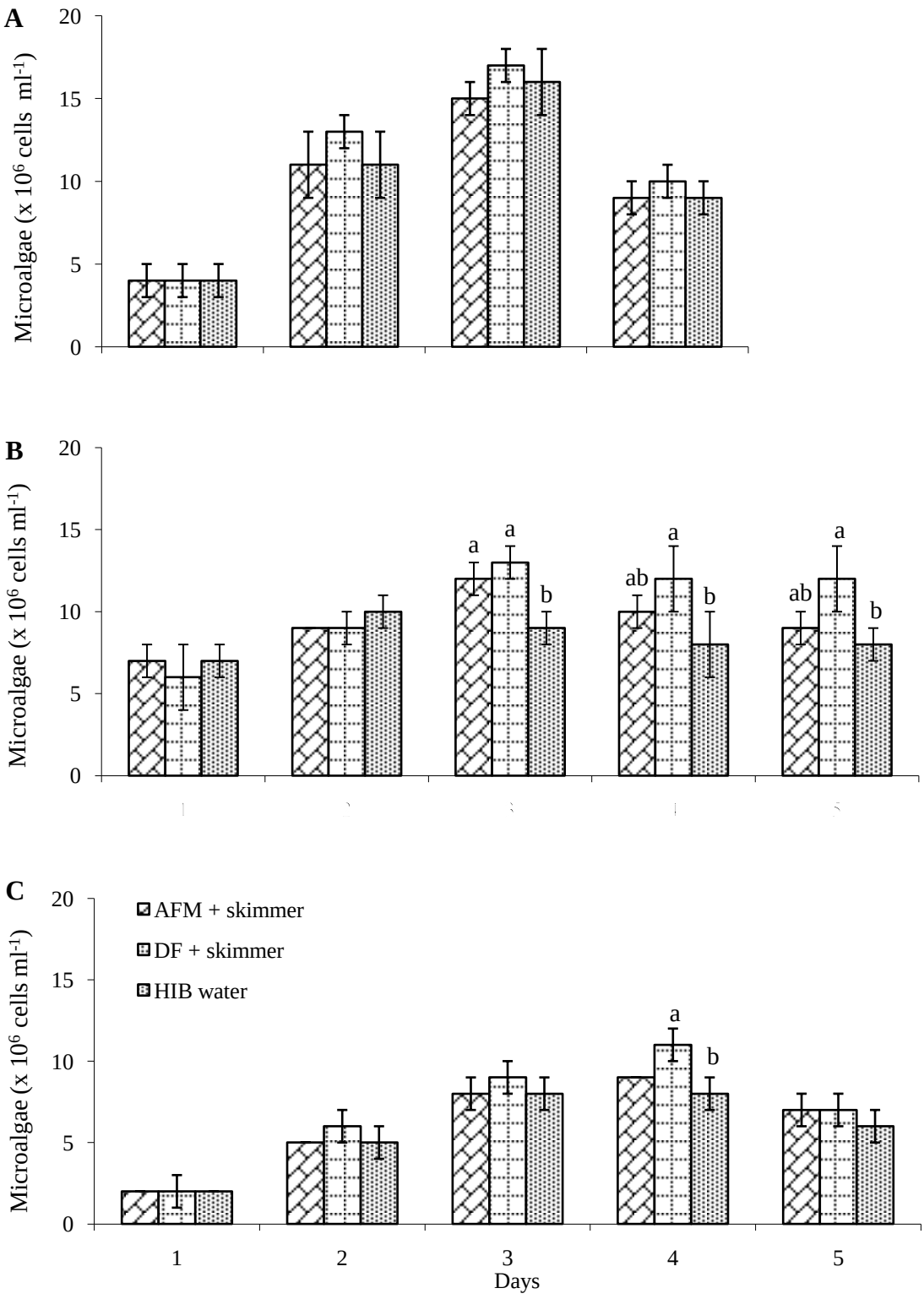

Fig. 3.14. Mean (n=3) microalgal cells numbers (x 10^6 cells ml^{-1}) in three different water qualities (DF + skimmer, AFM + skimmer and HIB water) at three different months (A) March (see Table 9, Appendix A), (B) April (see Table 10, Appendix A) and (C) May (see Table 11, Appendix A). The arrow bars indicate mean ± S.D. Bars with different letters are significant at $P<0.05$.

Table 3.4

The variables (mean ± S.D) measured and performed during sampling months (March, April and May). The different sampling points and treatments are shown.

Variables and sampling month	Sampling points				
	Intake water (P1)	AFM (P2)	AFM + skimmer (P3)	DF (P4)	DF + skimmer (P5)
Temperature	8.0 ± 0.7[a]	8.9 ± 0.7[a]	12.7 ± 1.0[b]	8.2 ± 0.5[a]	8.9 ± 0.6[a]
Dissolved oxygen (%)	82.7 ± 6.3	85.1 ± 7.4	102.5 ± 10.4	89.0 ± 7.6	100.9 ± 10.8
Dissolved oxygen (mg l^{-1})	10.13 ± 1.02	9.93 ± 0.95	10.88 ± 1.35	10.52 ± 0.95	11.70 ± 1.34
DOC, March	3.5 ± 0.4	3.3 ± 0.7	2.7 ± 0.8	2.5 ± 0.3	2.5 ± 0.1
DOC, April	2.5 ± 0.7[ab]	3.6 ± 0.4[a]	1.6 ± 0.2[b]	2.4 ± 0.5[b]	1.8 ± 0.3[b]
DOC, May	4.9 ± 1.1[ab]	4.3 ± 0.5[ab]	3.3 ± 0.2[a]	3.7 ± 0.5[ab]	2.7 ± 0.1[b]
TBN, March	2.3 ± 0.4[a]	2.6 ± 0.3[ab]	2.5 ± 0.4[a]	3.4 ± 0.3[b]	2.3 ± 0.3[a]
TBN, April	3.7 ± 0.1	3.4 ± 0.2	3.7 ± 0.5	3.6 ± 0.5	3.7 ± 0.3
TBN, May	3.4 ± 0.5	3.3 ± 0.2	3.6 ± 0.2	4.0 ± 0.1	3.6 ± 0.2
Marine agar, April	13 ± 12	20 ± 28	43 ± 25	13 ± 6	
Marine agar, May	3 ± 6	13 ± 12	5 ± 7	10 ± 10	

	Treatments			
	AFM + skimmer (P3)	DF + skimmer (P5)	SSW (control)	HIB water
Egg development to D3 larvae, March	11.8 ± 4.4	9.2 ± 2.9		
Egg development to D3 larvae, April	16.3 ± 3.0	16.6 ± 8.7		
Egg development to D3 larvae, May	49.1 ± 11.3	51.1 ± 5.6		
Larval activity undiluted, March	50.5 ± 16.8	39.4 ± 3.8	39.9 ± 8.8	
Larval activity undiluted, April	55.2 ± 6.5	73.4 ± 17.1	64.9 ± 8.9	59.1 ± 10.3
Larval activity undiluted, May	53.8 ± 3.7	64.5 ± 4.2	57.5 ± 10.4	48.4 ± 5.3
Larval activity 10-fold dilutions, March	25.3 ± 1.7	37.8 ± 14.0	33.0 ± 13.2	
Larval activity 10-fold dilutions, April	62.1 ± 4.6	67.3 ± 11.1	64.0 ± 9.7	57.7 ± 3.6
Larval activity 10-fold dilutions, May	59.9 ± 5.7[ab]	53.1 ± 8.9[ab]	48.2 ± 0.8[a]	61.4 ± 1.4[b]
Larval activity 100-fold dilutions, March	41.7 ± 6.8	29.9 ± 6.4	37.8 ± 14.9	
Larval activity 100-fold dilutions, April	55.0 ± 7.7	73.3 ± 5.8	58.6 ± 17.2	64.5 ± 2.9
Larval activity 100-fold dilutions, May	56.4 ± 10.9	65.3 ± 9.6	49.5 ± 7.9	60.6 ± 12.7

Common superscript letters shows no significant difference ($P<0.05$) between sampling points and different treatments.DOC = dissolved organic carbon and TBN = total bacterial numbers.

Table 3.5

The microalgal cell numbers (mean ± S.D) counted at different days during different sampling months (March, April and May). The different treatments (AFM + skimmer, DF + skimmer and HIB water) are shown.

Days	March			April			May		
	AFM + skimmer (P3)	DF + skimmer (P5)	HIB water	AFM + skimmer (P3)	DF + skimmer (P5)	HIB water	AFM + skimmer (P3)	DF + skimmer (P5)	HIB water
Day 1	4 ± 1	4 ± 1	4 ± 1	7 ± 1	6 ± 2	7 ± 1	2 ± 0	2 ± 1	2 ± 0
Day 2	11 ± 2	13 ± 1	11 ± 2	9 ± 0	9 ± 1	10 ± 1	5 ± 0	6 ± 1	5 ± 1
Day 3	15 ± 1	17 ± 1	16 ± 2	12 ± 1^{a}	13 ± 1^{a}	9 ± 1^{b}	8 ± 1	9 ± 1	8 ± 1
Day 4	9 ± 1	10 ± 1	9 ± 1	10 ± 1^{ab}	12 ± 2^{a}	8 ± 2^{b}	9 ± 0	11 ± 1^{a}	8 ± 1^{b}
Day 5				9 ± 1^{ab}	12 ± 2^{a}	8 ± 1^{b}	7 ± 1	7 ± 1	6 ± 1

Common superscript letters shows no significant difference ($P<0.05$) between treatments.

4. Discussion

4.1 Discussion of the methods

4.1.1 Water intake

The water intake in the hatchery is normally from 120 m, but was shifted to 60 m in May, in order to avoid the remains of the spring bloom coming with the intake water. This is common practice at the hatchery and is required in order to follow the normal production cycle. As water depth was not the same in May, comparison of treatments between March and April to May must be taken lightly. Differences in the intake water may affect organic loading in the incoming water as natural organic loading differs with depth in the sea (Sharp et al., 1982; Keizer et al., 1989; Kasai et al., 2004). If there is more organic carbon within the intake water, this may affect the treatment efficiency of the filters. Different filters, may respond differently.

4.1.2 Water quality parameters

It was not possible to measure the temperature, salinity and dissolved oxygen directly at the sampling points. These parameters were measured by taking a 5 l plastic measuring jug filling it with water one sampling point at a time and a digital oximeter (WTW multi 197i Oximeter) was used to record measurements. As measurements were taken immediately, this practice was not thought to affect results.

4.1.3 Total organic carbon (TOC), dissolved organic carbon (DOC) and particulate organic carbon (POC)

The April and May TOC results were lower than the DOC levels. As there were no replicates (due to cost limitations), it is difficult to establish if this was the actual level, or if there was a problem in the sampling.

For April, the DOC samples were filtered using 0.45 µm GF/F membrane filters and the filtrate were sent to Chemlab for analysis. In March and May, the samples were not pre-filtered. There was no difference in DOC measurements with these two protocols.

4.1.4 Culturable heterotrophic bacteria

In March, only TCBS plates were inoculated. A single colony was found in the DF (P4) and based on the results I included marine agar in April and May to get an overview of the bacterial community. In March, the total bacterial numbers (TBN) results suggested that there was normal bacterial numbers, and marine agar at that time would have confirmed this.

There were no colonies found on marine agar for DF + skimmer (P5) in April and May. There can be a number of potential explanations for the lack of growth. It could be that the concentration was not high enough; however, this seemed unlikely as the sample was undiluted. Secondly, the bacteria may have been killed by the spreader after being used too quickly after burning. Thirdly, the plates may have been forgotten during inoculation and the lack of growth is probably due to human error. Finally, the filter may have indeed reduced the colonies; however this is an unlikely situation in marine environment.

4.1.5 Microbial community

The cluster analysis and sequencing of the microbial community was made from the identified bands from the denaturing gradient gel. Due to the melting of the samples on the gel, the good bands were used for clustering and sequencing of the microbial community. For clustering the bands from AFM in March and April was poor and it was not used in the analysis, but there was no difference in the results. Few bands from sampling points were used for the sequencing. In March, the AFM + skimmer and the control bands were used. In April, the intake water and AFM + skimmer bands were selected for the analysis. In May the bands from the intake water, AFM and DF + skimmer were included. Those selected bands served as the representative of the treatments and no differences in microbial community were found.

4.1.6 Larval activity experiment

The larval activity experiment was designed following a protocol by Sandlund et al. (2006). After the experiment was being set up, the prepared well were put under the table and covered with black plastic bag. The lights in the room in which the experiment was performed were switched off. This was done in order to prevent the larvae from any access to light.

The 12-well polystyrene multi-dish was used, it has four horizontal and three vertical components. There were three replicates for each treatment. In March, the water samples for each treatment and larvae were placed inside one 12-well. In April and May, different treatments were randomly placed in one four horizontal component of the well. This was done in order to eliminate the differences in larval activity between wells. There were no differences found in the larval activity for different designs.

There were difficulties in placing approximately 20 to 30 larvae in this experiment and as also experienced by Sandlund et al. (2006) in each well due to their size. Because of that, the initial number of larvae within each well differed. This was done in order to avoid stress and handling of the larvae. There were no differences in the larval activity in each treatment independent of the initial larval numbers.

4.1.7 Statistical analysis

In this experiment, the samplings were performed in a random and independent manner to meet the basic assumptions of ANOVA (Sokal and Rohlf, 1997). The samples normality was tested by one-sample Kolmogorov-Sminov (K-S) test (Sokal and Rohlf, 1997). The samples were normally distributed in this experiment except for the larval activity experiment in May at the 10-fold diluted water and in April at the 100-fold diluted water that expressed some deviation from normality (Table 53, Appendix C). Practically, the non-parametric tests (Mann-Whitney) in this case should have been used. However, since the sampling was random and independent, ANOVA was used and it is more powerful than non-parametric tests (Sokal and Rohlf, 1997).

Multiple comparisons were tested by carrying Tukey test as it is parametric and assumes population variances and sample sizes are equal while Games-Howell does not (Sokal and Rohlf, 1997; Games and Howell, 1976). The samples homogeneity was tested by Levene statistic. There were some violation in the dissolved organic carbon in March and May, and total bacterial numbers in April (Table 54, Appendix D), but the robust tests of equality of means (Welch and Brown-Forsythe) displayed the alternative version of F-statistics and the results were used (Sokal and Rohlf, 1997).

4.2 Discussion of the results

4.2.1 Temperature and salinity

In this experiment from March to May, the temperature ranged between 8.0 °C at the intake water and 12.7 °C at the AFM + skimmer. The water was heated at 17 °C after the AFM + skimmer but the water recirculated back to the treatment (AFM + skimmer) to stabilise the temperature. This explains the higher temperature found at that point. The water was heated before going to the larval room because marine hatcheries constantly require high temperature to have high growth rates for the species cultured (Blancheton, 2000; Christophersen, 2000). Scallop larvae achieve higher growth and survival at 15-16 °C (Gruffydd and Beaumont, 1972). Temperatures above 10 °C increased the feeding and hence the growth of *P. maximus* larvae in the nursery (Laing, 2000). This experiment was performed in a temperature stabilized (17-18 °C) environment. The temperature was within the growing limits for scallop larvae. This environment can improve the larval production in the hatchery while the costs of heating the water is minimised.

The salinity was stable throughout the experimental period, which is the case for deep water (Torkildsen and Magnesen, 2004). The salinity was within the limits for larval growth. The salinity was the same for both 60 and 120 m water intake depths, and was consistent with levels found in Magnesen et al. (2006). Previous studies have found high mortalities at lower salinities (Strand et al., 1993; Laing, 2000, 2002; Bergh and Strand, 2001; Christophersen and Magnesen,

2001). Utilization of deep seawater into the hatchery has an advantage in maintaining the stable environment for the growing larvae by increasing the larval growth (Urakawa et al., 1999).

4.2.2 Dissolved oxygen (% and mg l^{-1})

Dissolved oxygen ranged from 82.7 at the intake water to 102.5% at the AFM + skimmer (or 9.93 mg l^{-1} at the AFM to 11.7 mg l^{-1} at the DF + skimmer). The dissolved oxygen concentration (% and mg l^{-1}) were higher after the protein skimmers. This may be due to oxygen enrichment by the protein skimmers. Scallops are sensitive to low oxygen concentration (Taylor et al., 1983), however, there is a lack of information specifically regarding the *P. maximus* oxygen requirements. The dissolved oxygen (mg l^{-1}) found in this experiment was higher than concentration needed for *Argopecten irradians* (Taylor et al., 1983). Survival (100%) of *Chlamys farreri* was achieved at dissolved oxygen ranging from 6.5 to 8.5 mg l^{-1} (Chen et al., 2007). The acceptable level of oxygen for *Pinctada mazatlanica* was averaged to 6 mg l^{-1} in spring and 4 mg l^{-1} in summer (Saucedo et al., 2007). The impact of dissolved oxygen supersaturation must be taken into account since it leads to hyperoxia conditions (Colt, 2006). The AFM + skimmer had the higher (102.5%) dissolved oxygen concentrations more than DF + skimmer (100.9%).

4.2.3 Total organic carbon (TOC), dissolved organic carbon (DOC) and particulate organic carbon (POC)

The reduction of TOC from the intake water was highest in May and lowest in March after the AFM + skimmer and the DF + skimmer. In March, the AFM and DF alone did not reduce the TOC concentration as much as in May. This showed that the addition of a protein skimmer after the filter reduces the TOC concentration. The TOC concentration was within the normal range (0.3 to 1000 mg l^{-1}) for seawater (AOAC, 1973). Solberg (2009) found lower concentrations of TOC in the intake water and the AFM in May compared to the current experiment. Theoretically, the TOC concentration has to exceed the DOC levels. In this experiment, low TOC concentrations were found in April and May. This may be explained by the analysis performed at the laboratory, as explained above in the discussion of the method. In March, April and May, the

DF + skimmer reduced the TOC concentration from the intake water more than AFM + skimmer.

The percentage reduction of DOC from the intake water was on average slightly higher at the DF + skimmer (34%) compared to the AFM + skimmer (31%). In May, after the spring bloom, the loading of DOC concentrations was higher at the intake water. However, the DF + skimmer treatment significantly reduced (2.7 mg l^{-1}) the DOC from the intake water (4.9 mg l^{-1}) more than the AFM + skimmer (3.3 mg l^{-1}) treatment. This could be explained by backwashing action of the drum filter (D'orbcastel et al., 2009). The drum filter is known to remove both small and large components of the DOC, and even large or small solids from the water (Franco-Nava et al., 2004; Davidson et al., 2008). The DOC concentrations found in my experiment were within safe limits for fish, as illustrated by Laudon et al. (2001), their model suggested that surface water containing 10 mg l^{-1} DOC concentrations caused fish mortality (pH of 5.0).

The use of a biofilter (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 (Leonard et al., 2000). In May, the low reduction of DOC by the AFM + skimmer may have contributed to the increase of food for the bacteria in the system, favouring opportunistic bacteria by this treatment. This can also be seen from the DGGE profile, where the gamma proteobacteria becomes dominating. Skjermo et al. (1997) found that increased DOC concentration in turbot rearing caused proliferation of opportunistic bacteria, consistent with results from this experiment. In March, April and May, the DF + skimmer reduced the DOC concentrations from the intake water more than the AFM + skimmer.

In March, the POC concentrations from the intake water (P1) were increased at the AFM + skimmer (P3) and the DF + skimmer (P5). This was the case in Chrzanowski et al. (1983) in which the DOC was averaged 70% of the TOC pool. In April (except AFM + skimmer) and May both treatments gave the negative POC values.

The presence of POC serves as a substrate for bacterial attachment (Garnaeu et al., 2009). Franco-Nava et al. (2004) in a recirculating system found drum filter to be removing the POC 60% of carbon concentrations.

The use of drum filter has several advantages over the active filter media in reducing the total and dissolved organic carbon from the incoming water. The drum filter can reduce even the finer dissolved substances more than the active filter media. Therefore, the use of drum filter will benefit the hatchery in improving the water quality thereby increasing the larval survival and production. The use of drum filter can prevent the search of having more sophisticated ways of treating the seawater in the hatchery. It prevents unnecessary costs of replacing the active filter media with the new equipment. The total organic carbon (mainly dissolved organic carbon) may create unsuitable environment for the growing larvae.

4.2.4 Total bacterial numbers (TBN)

In March, the bacterial cell numbers were lower than in April and May, which could reflect a seasonal trend (Jacobsen and Magnesen, 2009). In March, the AFM + skimmer slightly increased (2.5×10^5 cells ml^{-1}) the bacterial cell numbers from the intake water (2.3×10^5 cells ml^{-1}) while the DF + skimmer (2.3×10^5 cells ml^{-1}) maintained the same number. Both treatments in April maintained the same (3.7×10^5 cells ml^{-1}) bacterial cell numbers as in the intake water. Slight increases in bacterial cell numbers within the treatments were seen in the AFM + skimmer in March and both treatments in May. In April and May, the treatment seemed to maintain the same number of bacterial cells from the intake water. This shows consistency of both filters in maintaining the bacterial cell numbers within the range. In addition, there was no difference in bacterial cell numbers between treatments in April and May. The bacterial cell numbers found in this experiment were within the normal range (10^5-10^8 cells ml^{-1}) of bacteria found in natural waters. The bacterial cell numbers were still low as compared to Solberg (2009) and Garneau et al. (2009), but comparable to the numbers found in Liu and Han (2004). In March, the DF + skimmer reduced the total bacterial numbers from the intake water more than AFM + skimmer. In April and May, both filters maintained the same number of bacterial cells from the intake water.

4.2.5 Culturable heterotrophic bacteria

The TCBS agar gave the number of *Vibrio* spp. colonies present from the sampling points. In this experiment, the number of colonies on TCBS plates was generally low, except for after the drum filter in March, and colony numbers declined with sampling month. No *Vibrio* spp. colonies were found in the intake seawater, consistent with Solberg (2009) and Andersen et al. (2000). In contrast in other marine hatchery, *Vibrio* spp. colonies were detected in the intake water by Sainz-Hernández and Maeda-Martínez (2005). The flow-through system has been found to maintain very low the bacterial numbers in hatcheries especially during algal blooms (Andersen et al., 2000). An increase in the *Vibrio* spp. bacteria has been found in the sand-filtered seawater and surface seawater but the numbers were still within acceptable limits (100×10^3 CFU ml^{-1}) (Sainz-Hernández and Maeda-Martínez, 2005; Saucedo et al., 2007; Abasolo-Pacheco et al., 2009). The low or no numbers of *Vibrio* spp. within the water in the hatchery provides a suitable environment for the growing scallop larvae. This lack of bacteria will enhance the larval growth and survival in the hatchery. However, the scallop larvae are also known to feed on bacteria (Hovgaard, et al., 2001).

In March, the AFM + skimmer had the highest (43 CFU ml^{-1}) number of colonies found on marine agar. The treatment did not reduce the culturable heterotrophic bacteria from the intake water. In April and May, there were no colonies found in the DF + skimmer (0 CFU ml^{-1}). This could be due to sampling error, as previously discussed. The number of colonies found in this experiment was lower compared to Jorquera et al. (2004), Michaud et al. (2006) and Solberg (2009). Due to sampling error, no proper conclusion can be made between treatments. The heterotrophic bacteria found in this experiment were low that would not affect the larval production in the hatchery.

4.2.6 Microbial community

Different bands were sequenced to identify the microbial community found in the different sampling points. The bacterial community found was the same as the one found in natural water

masses. From the DGGE profiles it seemed that alpha proteobacteria dominated in March and gamma proteobacteria (opportunistic) dominated in April and May. The microbial community changed seasonally. This has also been seen in samples from the same location where scallop larvae performed badly around May and improving in late June, suggested that some toxic substances built up slowly in the treatments (Jacobsen and Magnesen, 2009). The bacterial species composition affected the larval development rather than the bacterial numbers found within water treatments (Douillet and Pickering, 1999). The microbial community in both treatments followed the seasonal trend, and was not different between treatments.

Many of the bacteria sequences contained unculturable clones. These unculturable bacteria have been detected in Sandaa et al. (2003) and classified as Cyanobacteria group. The marine agar plates cannot detect these unculturable bacteria (Solberg, 2009). *Vibrio splendidus, V. pectenicida* and *Pseudoalteromonas* spp. are known to cause mortalities in *P. maximus* larvae (Nicolas et al., 1996; Lambert, 1998; Sandaa et al., 2003). These pathogenic bacteria were not found in this experiment. *Colweliia rossensis, C. maris* and *Oceanserpentilla* spp. were also detected. These bacteria were also found in the hatchery by Sandaa et al. (2003). In April, the AFM + skimmer contained bacteria associated with paralytic shellfish toxin producing dinoflagellate, *Gymnodinium catenatum*. This is known to be toxic to human beings when consuming the poisoned shellfish (Martins et al., 2003). Saxitoxin (like paralytic shellfish poisoning) are also known to have a detrimental effect on larval development (Lefebvre et al., 2005).

The cluster analysis was made from the identified denaturing gradient gel bands. The cluster analysis showed the similarities of the microbial communities between sampling points for March, April and May. Close similarities were observed between the intake water and each treatment. In March and April, the DF and DF + skimmer were similar to each other. In May, the AFM and the AFM + skimmer were closely related. This showed that the bacterial compositions of each treatment were closely related. This also indicates high bacterial stability within the treatments (Sandaa et al., 2003).

4.2.7 Egg development to day 3 (D3) larvae

In the present study the development of D3 larvae was assessed by comparing the two different water systems in the hatchery. There was an increase in D3 larval fraction according to the sampling months; the lowest fraction was found in March and the highest in May. In March, the larval fraction was high (11.8%) in the AFM + skimmer and low (9.2%) in the DF + skimmer, April the highest (16.6%) larval fraction was found in the DF + skimmer and the lowest (16.3%) in the AFM + skimmer, and in May, the DF + skimmer had the highest (51.1%) larval fraction and the AFM + skimmer had the lowest (49.1%). It was found that water treatment had no effect on egg development to D3 larvae. All water treatments proved to be acceptable for the egg development to larval stages. However, in March and April, the percentage of development of D3 larvae (larval fraction) was quite low as compared to May. This discrepancies found between sampling months may result in significant differences in the larval production in the hatchery over time. Magnesen and Christophersen (2008) suggested spawning to be successful if more than 20% of the eggs developed into D3 larvae. The low larval fraction obtained in March and April relates with the study conducted by Magnesen et al. (2006) in which the number of competent, ready-to-settle larvae were low due to poor water quality. The different sampling months may have implications on the production of D3 larvae in the hatchery. It is more feasible to produce the D3 larvae in the hatchery in May more than March and April independent of the treatment.

In March and April, I found low (9-17%) fraction of larval development than found by Magnesen and Christophersen (2008), where fraction was 18% in November and 36% in January. In May, better larval development was observed in DF + skimmer (51.1%) and DF + skimmer (49.1%). In March, the AFM + skimmer had higher development success than the DF + skimmer. In April and May, the DF + skimmer performed better than the AFM + skimmer.

In my experiment development fraction averaged approximately 26% independent of treatment. Magnesen et al. (2006) found an average of 36.7% in winter and spring. Andersen and Ringvold (2000) reported the higher (51-69%) fraction in winter.

4.2.8 Larval activity experiment

There was no significant difference between treatments in the larval activity from the undiluted water in March, April and May. The larval activity was high in April and lowest in March. In March, the AFM + skimmer had the highest (50.5%) larval activity compared to DF + skimmer (39.9%). In March, the AFM + skimmer performed better more than the DF + skimmer. The numbers of active larvae found in the DF + skimmer were still higher than numbers found in the *Ostrea edulis* challenged with seawater extracts and heat treated seawater extracts (DiSalvo et al., 1978). In April and May, the highest (73.4 and 64.5%) larval activity was achieved in the DF + skimmer and the lowest (55.2 and 48.4%) in the AFM + skimmer and HIB water respectively. Sandlund et al. (2006) found no difference in the larval mortality challenged with different bacterial strains; and, the mortality was averaged 25%.

In the 10-fold diluted water, April had the highest activity and March had the lowest. In March and April, the larval activity was high (37.8 and 67.3%) in the DF + skimmer and lowest (25.3 and 57.7%) in the AFM + skimmer and HIB water respectively. However, no significant differences were observed. The DF + skimmer performed better more than other treatments. There was a significant difference in larval activity in May. No significant difference was observed in the AFM + skimmer (59.9%) and the DF + skimmer (53.1%) but the water from HIB (61.4%) had significantly higher activity than SSW (48.2%). Both treatments performed better than the control.

No significant difference was observed between treatments in the 100-fold diluted water for March, April and May. April had the highest larval activity and March had the lowest. In March, the AFM + skimmer (41.7%) gave the highest larval activity than DF + skimmer (29.9%). The AFM + skimmer performed better more than DF + skimmer. In April and May, the DF + skimmer (73.3 and 65.3%) performed better than the AFM + skimmer (55.0 and 56.4%) respectively.

The accumulation of bacterial toxins inside the water may prevent the larvae from swimming (DiSalvo et al., 1978). This caused the aggregation of the larvae in the bottom of the tank known

as “spotting” (DiSalvo et al., 1978), thus the aggregation may increase the probability of the larvae being infected by the bacteria since organic matter, faeces and detritus all accumulates at the bottom of the tank (DiSalvo et al., 1978). To prevent the accumulation of disease in the hatchery, the water must be treated on a daily basis (Abasolo-Pacheco et al., 2009).

4.2.9 Microalgal cell numbers

In the microalgal growth experiment, the highest cell numbers were counted in March and lowest in May. In March, all treatments performed almost the same with the DF + skimmer having the highest (17 x 10^6 cells ml^{-1}) cell numbers. The starter culture for March was made with the water from the hatchery and it had no effect on the performance of the treatments. In April, the DF + skimmer (13 x 10^6 cells ml^{-1}) had higher cell numbers than the AFM + skimmer (12 x 10^6 cells ml^{-1}) and water from HIB (9 x 10^6 cells ml^{-1}). In May, the DF + skimmer (11 x 10^6 cells ml^{-1}) had the highest cell numbers than the AFM + skimmer (9 x 10^6 cells ml^{-1}) and the water from HIB (8 x 10^6 cells ml^{-1}). Surprisingly, in April and May, the DF + skimmer performed significantly better than the water from HIB. In addition, the starter culture was made using the water from HIB water. The DF + skimmer performed better independent of the origin of the starter culture. The water filtered using the drum filter can benefit the hatchery in growing the microalgae. In this experiment, the drum filtered water had the highest microalgae cell numbers. Therefore, large amount of microalgae can be produced within the shorter period even though there was no significant difference observed between the drum filter and the active filter media. However, microalgal cultures grew better in the water from DF + skimmer.

Microalgae is used in hatcheries as feed for the spat and larvae (Robert and Gérard, 1999). These microalgae must be rich with high levels of polyunsaturated fatty acids to meet the demands of the growing spat and larvae in the hatcheries (Jacobsen et al., in press). However, the growth of microalgae is affected by poor water quality (A. Jacobsen, pers comment). The algal bacterial load between different treatments was not performed in this experiment. It has been found that high counts of opportunistic and haemolytic bacterial species were associated with *Bacillariophyceae*, the family in which *C. muelleri* belongs (Salvesen et al, 2000). In March, April and May, the DF + skimmer had the highest number of microalgal cells compared to AFM + skimmer.

5. Conclusions

The two different treatments (active filter media and drum filter) gave different results. In general, the AFM worked more effectively than the DF in March while the DF performed better in April and May experiments. The DF + skimmer treatment seemed to reduce the DOC levels and bacterial cell numbers more in April and May. Water treatment had no effect on the number of culturable heterotrophic bacteria and egg development to D3 larvae. The DF + skimmer had the highest larval activity in April and May. In March, AFM + skimmer had the highest larval activity in undiluted and 100-fold diluted water. SSW and the water from HIB performed unpredictable in larval activity experiment. The microalgal cell numbers were not very different between the AFM and DF treatments. However, the DF + skimmer gave the highest cell numbers and the water from HIB did not favour the increase in cell numbers.

It seems that the AFM treatment is not able to cope with higher organic loading during spring blooms in April and May. In March, there was a reduced stress on the AFM treatment and it was equally effective. In April and May, the DF treatment was able to reduce the organic carbons from the intake water more than the AFM treatment. It is recommended that the DF treatment may be used in the hatchery throughout the year.

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Appendix

Appendix A. Descriptive statistics

Table 1: Descriptive statistics of temperature (°C), dissolved oxygen (%) and dissolved oxygen (mg l^{-1}) measured in March, April and May from all sampling points (P1-P5). Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	Temperature					Dissolved oxygen (%)					Dissolved oxygen (mg l^{-1})				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
P1	3	8.0	0.7	7.4	8.7	3	82.7	6.3	75.7	88.0	3	10.13	1.02	8.96	10.82
P2	3	8.9	0.7	8.5	9.7	3	85.1	7.4	76.5	90.0	3	9.93	0.95	8.95	10.85
P3	3	12.7	1.0	11.6	13.4	3	102.5	10.4	90.8	110.7	3	10.88	1.35	9.34	11.81
P4	3	8.2	0.5	7.7	8.6	3	89.0	7.6	80.2	93.7	3	10.52	0.95	9.42	11.13
P5	3	8.9	0.6	8.3	9.4	3	100.9	10.8	88.7	109.0	3	11.70	1.34	10.18	12.72

Table 2: Descriptive statistics of dissolved organic carbon (mg l^{-1}) measured in March, April and May from all sampling points (P1-P5). Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	March					April					May				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
P1	3	3.5	0.4	3.1	3.8	3	2.5	0.7	2.1	3.3	3	4.9	1.1	3.6	5.7
P2	3	3.3	0.7	2.7	4.1	3	3.6	0.4	3.3	4.1	3	4.3	0.5	3.9	4.8
P3	3	2.7	0.8	1.8	3.1	3	1.6	0.2	1.5	1.8	3	3.3	0.2	3.2	3.5
P4	3	2.5	0.1	2.3	2.8	3	2.4	0.5	1.8	2.8	3	3.7	0.5	3.2	4.2
P5	3	2.5	0.6	2.4	2.5	3	1.8	0.3	1.5	2.1	3	2.7	0.1	2.6	2.8

Table 3: Descriptive statistics of total bacterial numbers (TBN, x 10^5 cells ml^{-1}) performed on flow cytometer from all sampling points (P1-P5) in March, April and May. Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	March					April					May				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
P1	3	2.3	0.4	1.9	2.7	3	3.7	0.1	3.6	3.8	3	3.4	0.5	2.9	3.9
P2	3	2.6	0.3	2.3	2.8	3	3.4	0.2	3.2	3.5	3	3.3	0.2	3.2	3.6
P3	3	2.5	0.4	2.1	2.8	3	3.7	0.5	3.1	4.0	3	3.6	0.2	3.4	3.8
P4	3	3.4	0.3	3.2	3.7	3	3.6	0.5	3.0	4.0	3	4.0	0.1	3.9	4.1
P5	3	2.3	0.3	2.0	2.6	3	3.7	0.3	3.4	3.9	3	3.6	0.2	3.4	3.7

Table 4: Descriptive statistics of culturable heterotrophic bacteria (CFU ml^{-1}) from sampling points (P1-P5) in April and May. Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	April					May				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max
P1	3	13	12	0	20	3	3	6	0	10
P2	2	20	28	0	40	3	13	12	0	20
P3	3	43	25	20	70	2	5	7	0	10
P4	3	13	6	10	20	3	10	10	0	20
P5	3	0	0	0	0	3	0	0	0	0

Table 5: Descriptive statistics of egg development to day 3 larvae (%) performed by using water from the AFM + skimmer and DF + skimmer in March, April and May. Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	March					April					May				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
AFM + skimmer	3	11.8	4.4	7.8	16.5	3	16.3	3.0	12.5	19.8	3	49.1	11.3	35.0	61.8
DF + skimmer	3	9.2	2.9	6.9	12.5	3	16.6	8.7	6.7	29.0	3	51.1	5.6	44.5	59.8

Table 6: Descriptive statistics of larval activity experiment (%) performed by using undiluted water from the AFM + skimmer, DF + skimmer, SSW and HIB water in March, April and May. HIB water was not included in March experiment. Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	March					April					May				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
AFM + skimmer	3	50.5	16.8	34.8	68.3	3	55.2	6.5	47.7	59.8	3	53.8	3.7	50.9	57.9
DF+ skimmer	3	39.4	3.8	35.9	43.4	3	73.4	17.1	55.6	89.6	3	64.5	4.2	60.0	68.3
SSW	3	39.9	8.8	29.9	46.7	3	64.9	8.9	58.0	75.0	3	57.5	10.4	46.4	67.0
HIB water						3	59.1	10.3	47.8	68.1	3	48.4	5.3	44.3	54.3

Table 7: Descriptive statistics of larval activity experiment (%) performed by using 10-fold diluted water from the AFM + skimmer, DF + skimmer, SSW and HIB water in March, April and May. HIB water was not included in March experiment. Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	March					April					May				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
AFM + skimmer	3	25.3	1.7	23.6	27.0	3	62.1	4.6	56.8	65.4	3	59.9	5.7	54.1	65.5
DF+ skimmer	3	37.8	14.0	24.0	51.9	3	67.3	11.1	57.4	79.3	3	53.1	8.9	42.9	59.4
SSW	3	33.0	13.2	22.0	47.6	3	64.0	9.7	54.0	73.3	3	48.2	0.8	47.5	49.1
HIB water						3	57.7	3.6	54.1	61.3	3	61.4	1.4	59.8	62.5

Table 8: Descriptive statistics of larval activity experiment (%) performed by using 100-fold diluted water from the AFM + skimmer, DF + skimmer, SSW and HIB water in March, April and May. HIB water was not included in March experiment. Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	March					April					May				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
AFM + skimmer	3	41.7	6.8	37.2	49.5	3	55.0	7.7	46.5	61.4	3	56.4	10.9	47.8	68.7
DF+ skimmer	3	29.9	6.4	25.8	37.3	3	73.3	5.8	68.5	79.8	3	65.3	9.6	54.4	72.4
SSW	3	37.8	14.9	24.6	54.0	3	58.6	17.2	39.1	71.7	3	49.5	7.9	40.6	55.7
HIB water						3	64.5	2.9	62.6	67.8	3	60.6	12.7	46.1	70.0

Table 9: Descriptive statistics of microalgal cell numbers experiment (x 10^6 cells ml^{-1}) performed by using water from the AFM + skimmer, DF + skimmer and HIB water in March. Cell numbers were counted until when the culture collapsed. Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Appendix.

	Day 1					Day 2					Day 3				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
AFM + skimmer	3	4	1	3	4	3	11	2	10	13	3	15	1	15	16
DF+ skimmer	3	4	1	4	5	3	13	1	12	14	3	17	1	16	17
HIB water	3	4	1	3	4	3	11	2	10	13	3	16	2	14	18
	Day 4														
Sampling points	N	Mean	S.D	min	max										
AFM + skimmer	3	9	1	8	9										
DF+ skimmer	3	10	1	9	11										
HIB water	3	9	1	9	10										

Table 10: Descriptive statistics of microalgal cell numbers experiment (x 10^6 cells ml^{-1}) performed by using water from the AFM + skimmer, DF + skimmer and HIB water in April. Cell numbers were counted until when the culture collapsed. Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	Day 1					Day 2					Day 3				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
AFM + skimmer	3	7	1	6	7	3	9	0	9	9	3	12	1	11	13
DF+ skimmer	3	6	2	5	8	3	9	1	9	10	3	13	1	12	13
HIB water	3	7	1	7	8	3	10	1	9	11	3	9	1	9	10

	Day 4					Day 5				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max
AFM + skimmer	3	10	1	10	11	3	9	1	8	10
DF+ skimmer	3	12	2	11	14	3	12	2	11	14
HIB water	3	8	2	7	10	3	8	1	7	9

Table 11: Descriptive statistics of microalgal cell numbers experiment (x 10^6 cells ml^{-1}) performed by using water from the AFM + skimmer, DF + skimmer and HIB water in May. Day 1 was not included in the Appendix because of 0 S.D value. Cell numbers were counted until when the culture collapsed. Number of observation (N), mean, standard deviation (S.D), minimum (Min) and maximum (Max) values are indicated in the Table.

	Day 1					Day 2					Day 3				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max	N	Mean	S.D	min	max
AFM + skimmer	3	2	0	2	2	3	5	5	5	5	3	8	1	7	9
DF+ skimmer	3	2	1	2	3	3	6	1	5	6	3	9	1	8	10
HIB water	3	2	0	2	2	3	5	1	5	6	3	8	1	7	9
	Day 4					Day 5				
Sampling points	N	Mean	S.D	min	max	N	Mean	S.D	min	max
AFM + skimmer	3	9	0	8	9	3	7	1	6	8
DF+ skimmer	3	11	1	10	11	3	7	1	6	8
HIB water	3	8	1	7	9	3	6	1	5	7

Appendix B. One-way ANOVA and robust tests of equality of means

Table 12: One-way ANOVA calculated for temperature (°C).

Temperature					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	45.500	4	11.375	24.032	0.000
Within Groups	4.733	10	0.473		
Total	50.233	14			

Table 13: One-way ANOVA calculated for dissolved oxygen (%).

Dissolved oxygen					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	999.656	4	249.914	3.314	0.057
Within Groups	754.073	10	75.407		
Total	1753.729	14			

Table 14: One-way ANOVA calculated for dissolved oxygen (mg l^{-1}).

Dissolved oxygen					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.882	4	1.470	1.139	0.393
Within Groups	12.912	10	1.291		
Total	18.793	14			

Table 15: One-way ANOVA calculated for dissolved organic carbon (mg l^{-1}) in March.

Dissolved organic carbon					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.807	4	0.702	2.665	0.095
Within Groups	2.633	10	0.263		
Total	5.440	14			

Table 16: Robust test of equality of means for dissolved organic carbon (mg l^{-1}) in March.

Robust Tests of Equality of Means				
	Statistic	df1	df2	Sig.
Welch	4.795	4	4.154	0.075
Brown- Forsythe	2.665	4	5.357	0.148

Table 17: One-way ANOVA calculated for dissolved organic carbon (mg l^{-1}) in April.

Dissolved organic carbon					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	7.191	4	1.798	8.588	0.003
Within Groups	2.093	10	0.209		
Total	9.284	14			

Table 18: One-way ANOVA calculated for dissolved organic carbon (mg l^{-1}) in May.

Dissolved organic carbon					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.889	4	2.222	6.139	0.009
Within Groups	3.620	10	0.362		
Total	12.509	14			

Table 19: Robust test of equality of means for dissolved organic carbon (mg l^{-1}) in May.

Robust Tests of Equality of Means				
	Statistic	df1	df2	Sig.
Welch	12.130	4	4.584	0.011
Brown- Forsythe	6.139	4	3.683	0.061

Table 20: One-way ANOVA calculated for total bacterial numbers (TBN) (x 10^5 cells ml^{-1}) in March.

TBN					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.497	4	0.624	6.003	0.010
Within Groups	1.040	10	0.104		
Total	3.537	14			

Table 21: One-way ANOVA calculated for total bacterial numbers (TBN) (x 10^5 cells ml^{-1}) in April.

TBN					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.220	4	0.055	0.444	0.775
Within Groups	1.240	10	0.124		
Total	1.460	14			

Table 22: Robust tests of equality of means for total bacterial numbers (TBN) (x 10^5 cells ml^{-1}) in April.

Robust Tests of Equality of Means				
	Statistic	df1	df2	Sig.
Welch	1.789	4	4.654	0.276
Brown- Forsythe	0.444	4	5.404	0.774

Table 23: One-way ANOVA calculated for total bacterial numbers (TBN) (x 10^5 cells ml^{-1}) in May.

TBN					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.783	4	0.196	2.552	0.105
Within Groups	0.767	10	0.077		
Total	1.549	14			

Table 24: One-way ANOVA calculated for culturable heterotrophic bacteria (CFU ml^{-1}) in April.

Marine agar					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1818.182	3	606.061	1.768	0.241
Within Groups	2400.000	7	342.857		
Total	4218.182	10			

Table 25: One-way ANOVA calculated for culturable heterotrophic bacteria (CFU ml^{-1}) in May.

Marine agar					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	180.303	3	60.101	0.721	0.570
Within Groups	583.333	7	83.333		
Total	763.636	10			

Table 26: One-way ANOVA calculated for egg development to day 3 larvae (%) in March.

Egg development to day 3 larvae					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.244	1	10.244	0.740	0.438
Within Groups	55.381	4	13.845		
Total	65.626	5			

Table 27: One-way ANOVA calculated for egg development to day 3 larvae (%) in April.

Egg development to day 3 larvae					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.164	1	0.164	0.004	0.952
Within Groups	338.565	8	42.321		
Total	338.729	9			

Table 28: One-way ANOVA calculated for egg development to day 3 larvae (%) in May.

Egg development to day 3 larvae					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	10.161	1	10.161	0.129	0.729
Within Groups	632.371	8	79.046		
Total	642.532	9			

Table 29: One-way ANOVA calculated for larval activity (%) in undiluted water in March.

Larval activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	237.269	2	118.634	0.946	0.439
Within Groups	752.193	6	125.366		
Total	989.462	8			

Table 30: One-way ANOVA calculated for larval activity (%) in undiluted water in April.

Larval activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	565.949	3	188.650	1.451	0.299
Within Groups	1039.840	8	129.980		
Total	1605.789	11			

Table 31: One-way ANOVA calculated for larval activity (%) in undiluted water in May.

Larval activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	414.497	3	138.166	3.317	0.078
Within Groups	333.233	8	41.654		
Total	747.730	11			

Table 32: One-way ANOVA calculated for larval activity (%) in 10-fold diluted water in March.

Larval activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	241.087	2	120.543	0.974	0.430
Within Groups	724.193	6	123.699		
Total	983.280	8			

Table 33: One-way ANOVA calculated for larval activity (%) in 10-fold diluted water in April.

Larval activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	144.700	3	48.233	0.769	0.543
Within Groups	502.040	8	62.755		
Total	646.740	11			

Table 34: One-way ANOVA calculated for larval activity (%) in 10-fold diluted water in May.

Larval activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	339.529	3	113.176	3.934	0.054
Within Groups	230.140	8	28.768		
Total	569.669	11			

Table 35: Robust tests of equality of means for larval activity exposed to 10-fold diluted water in May.

Robust Tests of Equality of Means				
	Statistic	df1	df2	Sig.
Welch	49.713	3	3.858	0.002
Brown- Forsythe	3.934	3	3.560	0.123

Table 36: One-way ANOVA calculated for larval activity (%) exposed to 100-fold diluted water in March.

Larval activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	216.336	2	108.168	1.048	0.407
Within Groups	619.420	6	103.237		
Total	835.756	8			

Table 37: One-way ANOVA calculated for larval activity (%) exposed to 100-fold diluted water in April.

Larval activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	577.449	3	192.483	1.940	0.202
Within Groups	739.760	8	99.220		
Total	1371.209	11			

Table 38: Robust tests of equality of means for larval activity exposed to 100-fold diluted water in April.

Robust Tests of Equality of Means				
	Statistic	df1	df2	Sig.
Welch	2.929	3	3.939	0.165
Brown- Forsythe	1.940	3	3.418	0.283

Table 39: One-way ANOVA calculated for larval activity (%) exposed to 100-fold diluted water in May.

Larval activity					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	406.883	3	135.628	1.246	0.356
Within Groups	870.647	8	108.831		
Total	1277.530	11			

Table 40: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 1 in March.

Cell numbers at day 1					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.889	2	0.444	1.333	0.332
Within Groups	2.000	6	0.333		
Total	2.889	8			

Table 41: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 2 in March.

Cell numbers at day 2					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.667	2	2.333	1.050	0.406
Within Groups	13.333	6	2.222		
Total	18.000	8			

Table 42: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 3 in March.

Cell numbers at day 3					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.667	2	1.333	0.857	0.471
Within Groups	9.333	6	1.556		
Total	12.000	8			

Table 43: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 4 in March.

Cell numbers at day 4					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.222	2	2.111	3.167	0.115
Within Groups	4.000	6	0.667		
Total	8.222	8			

Table 44: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 1 in April.

Cell numbers at day 1					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.556	2	0.778	0.778	0.501
Within Groups	6.000	6	1.000		
Total	7.556	8			

Table 45: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 2 in April.

Cell numbers at day 2					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.556	2	0.778	1.750	0.252
Within Groups	2.667	6	0.444		
Total	4.222	8			

Table 46: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 3 in April.

Cell numbers at day 3					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	18.667	2	9.333	16.800	0.003
Within Groups	3.333	6	0.556		
Total	22.000	8			

Table 47: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 4 in April.

Cell numbers at day 4					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	24.000	2	12.000	7.200	0.025
Within Groups	10.000	6	1.667		
Total	34.000	8			

Table 48: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 5 in April.

Cell numbers at day 5					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	27.556	2	13.778	7.750	0.022
Within Groups	10.667	6	1.778		
Total	38.222	8			

Table 49: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 2 in May.

Cell numbers at day 2					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.167	1	0.167	0.500	0.519
Within Groups	1.333	4	0.333		
Total	1.500	5			

Table 50: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 3 in May.

Cell numbers at day 3					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1.556	2	0.778	0.636	0.562
Within Groups	7.333	6	1.222		
Total	8.889	8			

Table 51: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 4 in May.

Cell numbers at day 4					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	8.167	1	8.167	9.800	0.035
Within Groups	3.333	4	0.833		
Total	11.500	5			

Table 52: One-way ANOVA calculated for microalgal cell numbers (x 10^6 cells ml^{-1}) at day 5 in May.

Cell numbers at day 5					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.556	2	1.778	1.455	0.305
Within Groups	7.333	6	1.222		
Total	10.889	8			

Appendix C. Kolmogorov-Smirnov tests for normality

Table 53: Normality tests results conducted by one-sample Kolmogorov-Smirnov test. Number of observation and Kolmogorov Sminorvoz (K-S) Z value are indicated in the Appendix.

	N	K-S
Temperature	15	0.996
Dissolved oxygen (%)	15	0.674
Dissolved oxygen (mg l^{-1})	15	0.461
Dissolved organic carbon, March	15	0.540
Dissolved organic carbon, April	15	0.908
Dissolved organic carbon, May	15	0.495
Total bacterial numbers, March	15	0.601
Total bacterial numbers, April	15	0.600
Total bacterial numbers, May	15	0.418
Culturable heterotrophic bacteria, April	11	0.929
Culturable heterotrophic bacteria, May	11	0.929
Egg development to D3-larvae, March	6	0.586
Egg development to D3-larvae, April	10	0.413
Egg development to D3-larvae, May	10	0.516
Larval activity undiluted water, March	9	0.631
Larval activity undiluted water, April	12	0.730
Larval activity undiluted water, May	12	0.439
Larval activity 10-fold diluted water, March	9	0.782
Larval activity 10-fold diluted water, April	12	0.695
Larval activity 10-fold diluted water, May	12	0.690
Larval activity 100-fold diluted water, March	9	0.598
Larval activity 100-fold diluted water, April	12	0.688
Larval activity 100-fold diluted water, May	12	0.616
Microalgal cell numbers, March, day 1	9	1.053
Microalgal cell numbers, March, day 2	9	0.600
Microalgal cell numbers, March, day 3	9	0.500
Microalgal cell numbers, March, day 4	9	1.008
Microalgal cell numbers, April, day 1	9	0.771
Microalgal cell numbers, April, day 2	9	1.189
Microalgal cell numbers, April, day 3	9	0.635
Microalgal cell numbers, April, day 4	9	0.641
Microalgal cell numbers, April, day 5	9	0.618
Microalgal cell numbers, May, day 2	6	0.782
Microalgal cell numbers, May, day 3	9	0.626
Microalgal cell numbers, May, day 4	6	0.500
Microalgal cell numbers, May, day 5	9	0.509

Appendix D. Levene statistic for homogeneity of variances

Table 54: Tests results of homogeneity of variances from Levene statistic. Levene statistic value, degree of freedom (df) 1 and 2, and significance (Sig) level are indicated.

	Levene statistic	df1	df2	Sig
Temperature	1.081	4	10	0.416
Dissolved oxygen (%)	0.611	4	10	0.664
Dissolved oxygen (mg l^{-1})	0.445	4	10	0.774
Dissolved organic carbon, March	3.625	4	10	0.045
Dissolved organic carbon, April	2.488	4	10	0.110
Dissolved organic carbon, May	5.389	4	10	0.014
Total bacterial numbers, March	0.299	4	10	0.872
Total bacterial numbers, April	3.786	4	10	0.040
Total bacterial numbers, May	2.157	4	10	0.148
Culturable heterotrophic bacteria, April	2.811	3	7	0.118
Culturable heterotrophic bacteria, May	0.769	3	7	0.547
Egg development to D3-larvae, March	0.448	1	4	0.540
Egg development to D3-larvae, April	5.143	1	8	0.053
Egg development to D3-larvae, May	3.037	1	8	0.120
Larval activity undiluted water, March	2.172	2	6	0.195
Larval activity undiluted water, April	0.864	3	8	0.498
Larval activity undiluted water, May	1.531	3	8	0.280
Larval activity 10-fold diluted water, March	2.229	2	6	0.189
Larval activity 10-fold diluted water, April	1.341	3	8	0.328
Larval activity 10-fold diluted water, May	4.747	3	8	0.035
Larval activity 100-fold diluted water, March	1.740	2	6	0.253
Larval activity 100-fold diluted water, April	4.315	3	8	0.044
Larval activity 100-fold diluted water, May	0.465	3	8	0.715
Microalgal cell numbers, March, day 1	0.000	2	6	1.000
Microalgal cell numbers, March, day 2	0.462	2	6	0.651
Microalgal cell numbers, March, day 3	1.684	2	6	0.263
Microalgal cell numbers, March, day 4	2.667	2	6	0.148
Microalgal cell numbers, April, day 1	2.400	2	6	0.171
Microalgal cell numbers, April, day 2	2.800	2	6	0.138
Microalgal cell numbers, April, day 3	0.364	2	6	0.709
Microalgal cell numbers, April, day 4	1.333	2	6	0.332
Microalgal cell numbers, April, day 5	1.273	2	6	0.346
Microalgal cell numbers, May, day 2	0.000	1	4	1.000
Microalgal cell numbers, May, day 3	0.235	2	6	0.797
Microalgal cell numbers, May, day 4	3.200	1	4	0.148
Microalgal cell numbers, May, day 5	0.235	2	6	0.797