MICROBIAL COLONIZATION AND COMMUNITY SHIFTS IN A MARINE POST-SMOLT RAS, MONITORED THE FIRST YEAR OF OPERATION BY DEEP SEQUENCING ANALYSIS

by

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PREFACE

An application to the Norwegian Research Council in the fall of 2015 proposed to use deep sequencing to analyze the microbiology of Erko Settefisk AS's new marine post-smolt RAS plant in Sagvågen, Stord. The application was rejected, but the sampling from the facility still started in February 2016 to fulfill a Master in Fish Health. First of all, I would like to thank Rune Sandvik for allowing research at the Erko RAS, with access to biological material and measurement data for both the master and the large research project that came later. Your good mood and courage have been a great inspiration through thick and thin. Lise Øvreås and Kari Attramadal advised in advance for the master, and Lise confirmed my intuition about Heidrun Wergeland for supervision. Sampling for the master was carried out monthly between the studies in 2016. A revised NRF application was also prepared, now with Heidrun as project manager. This time the application came through the narrow eye of "frisk fisk", and the master's thesis and the test material were included as a part of the project. Irene Roalkvam became the closest support in my work, and together with Anita Fedøy at the CGB, I was given good instructions for the amplicon analysis. Håkon Dale also came with help on bioinformatics. Thanks to the three of you! Hanne Nielsen and Anne Berit Olsen from the Veterinary Institute also participated in the Monitor project, which has been a great pleasure and benefit. As the analysis results were eventually divided into four articles, we present in this thesis broadly the monitoring data. Main RAS events discussed in the Master are antibiotic treatment for wound epidemics, rapid changes in physio-chemical parameters, use of oxidants upon the biofilter, RAS washing, re-inoculation of the biofilter, shift in feed components, dominance of bacteria that alter the microbial commuity structure of the RAS, and the establishment of *Nitrospira* in the biofilter biofilm.

I would also like to thank Solveig Nygaard for exciting days with Fomas AS during the exercise part of the assignment, but also for participating in the Monitor project. Family, friend and colleagues for patience and support. Thanx! Finally, Heidrun, you can't be praised completely by words alone, so a big hug for you!

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ABBREVIATIONS, WORDS AND EXPRESSIONS

Post-smolt – the first period after smoltification of salmon, i.e.   the period after transfer from a freshwater to seawater tolerance.

Marine water – Atlantic Coastal sea water with salinity 32-35‰

RAS - Recirculating Aquaculture System

PC – Production cycle

Spedevann – Water in and out of RAS

Degree of recirculation – (Internal recirculation flow/(internal recirculation flow + new water intake)) x 100

Retention time – Time of stay of water in the RAS, i.e; Water volum/Flow rate

Pump sump – chamber bellow the CO_2 unit, gathering the degassed water

LOZ – Commercial available oxidative solution, containing mainly liquified ozone, hypochlorite and trace metals.

TAN – Total ammonium-nitrogen, (NH_4^+ og NH_3)

RNAlater – solution that preserves RNA from degradation during storage of samples.

PCR - Polymerase chain reaction

16S amplicon rDNA library – identifying the community structure by next generation sequencing. Here used equivalent to microbial deep sequencing

Ion Torrent PGM – Next generation Sequencing where addition of dNTP forms a proton that is registered.

OTU – Operational taxonomic unit or taxa

RA – Relative abundance, i.e. percent of a single taxa relative to all taxa in a sample

SECTION A

RAS DESCRIBTION

RAS DESCRIPTIONS

Erko Settefisk AS started their first post-smolt production in recirculating aquaculture system (RAS) September 2015. This was the first land-based marine RAS facility of its kind in Norway, and recycled seawater pumped from a depth of 70 meters (32-34‰.) The RAS module has a base area of 1490 m^2 and is built of steel and concrete, and holds 4 fishing tanks with 1100 m^3 water each. Each tank rears 100-125 000 post smolt from 100-500g in 3-4 months, i.e. annually 1.6-2 million salmon can be produced for sea stocking. In 2018 Erko Settefisk AS build a sister plant next to the first, doubled their post-smolt production capacity (Figure 1).

Figure 1: Two RAS modules at Erko Settefisk AS. The technology and module concept were delivery by Nofitech AS. Photo: Karine Drønen

Recirculation aquaculture system (RAS) is a closed system where most of the water is reused. A modern fresh water RAS has normally particle filtration, NH_4^+ removal by biofilter, CO_2 degassing and oxygenation as standard processes (Figure 2).   The marine RAS module at Erko settefisk AS is in principle like these, only that fresh water is replaced by sea water (Figure 3).
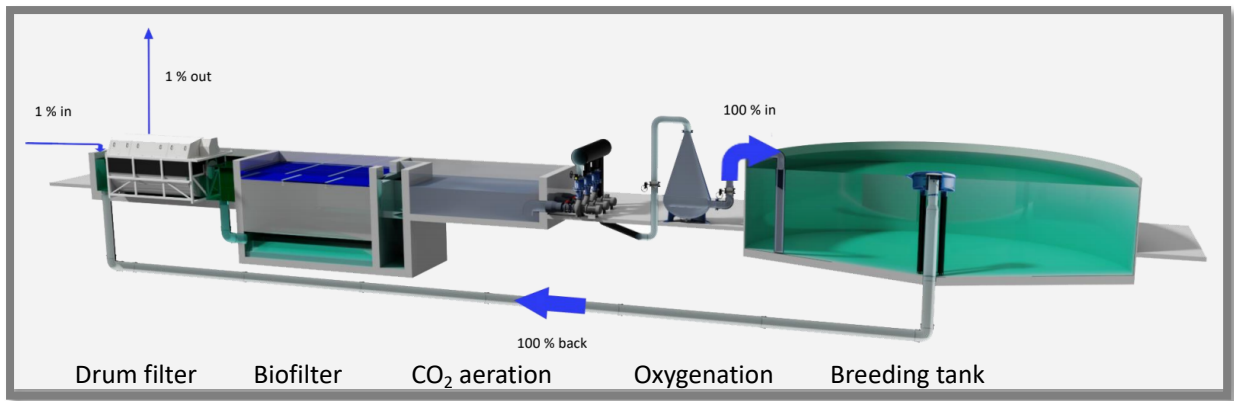
Figure 2. General sketch over a modern RAS plant (AquaGroup).

Such a shift is however a quantum leap in technology development, as sea-water brings in more corrosion problems, changed chemical equilibriums and new challenges in fish biology and microbial functionality.

RAS SPECIFICATIONS AND FLOW DIAGRAM

Tank volume	1100 m^3
Fishes stocked per tank	100-125 000
Biomass maximum	62.5 ton per tank
Water in and out	200-500 litre/min
"Spedevann"	100-250 litre/min
Retention time water in tank	1.5 timer
Degree of recirculation	97.8%
Water dilution rate	6-15%
Water operating temperature	14°C
Water operating salinity	26°C
Inlet water temperature.	8,5^{o}C
Inlet water salinity	32-34‰
Drum filter mesh	40 μm
Biofilter volume	300 m^3

To reduce the impact of external bacteria and pathogens upon the system, the inlet water was UV filtrated.

Figure 3: Flow diagram of the marine RAS modules at Erko Settefisk AS

PRINCIPLES

In RAS, chemistry, fish physiology and microbiology must function in a fine-tuned balance (Figure 4).
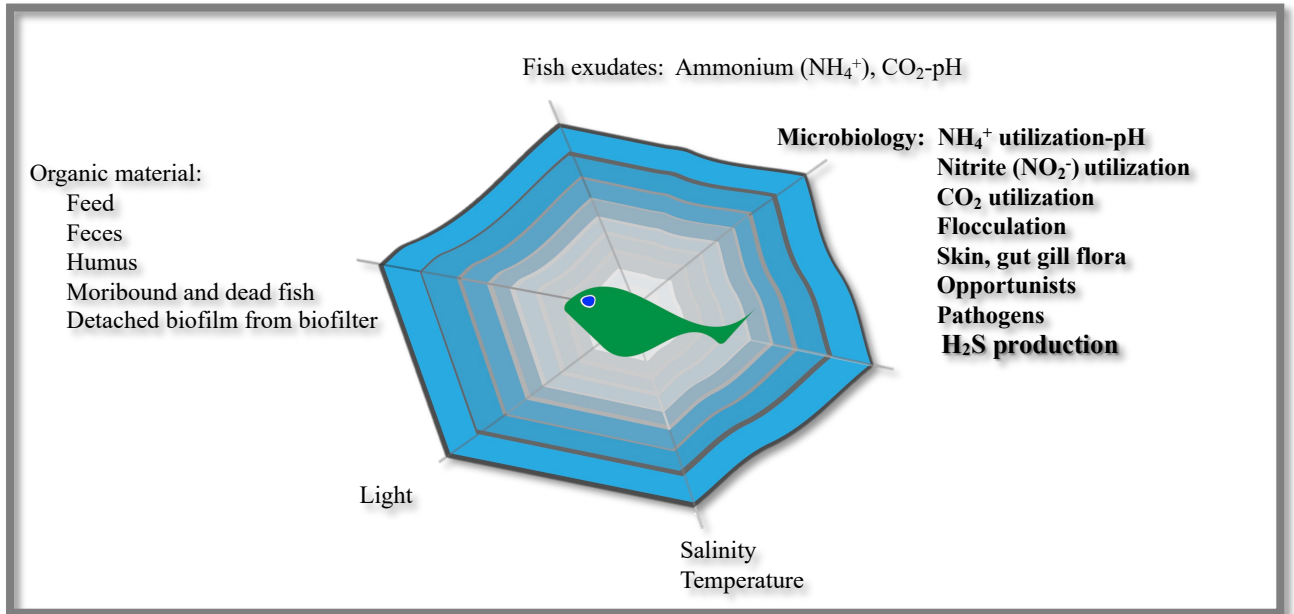
 Figure 4.   Main parameters working together in a RAS

Biofilter: Regarding RAS as an "organism", the biofilter will be the hearth that has to be kept sound and efficient. In line with the intake of fish feed, the fish will excrete ammonium (NH_4^+) and CO_2 over the gills, and these exudates must be removed from the water to avoid self-death. The main task of the biofilter is to transform ammonium into less toxic nitrate (NO_3^-) by biofilm microbes.   This is a microbial two step process[1], where the first group of microbes (ammonium oxidizers (AOB): Nitrosomonas, Nitrosococcus, Nitrosospira) oxidize ammonium into nitrite in the following reaction:

Step 1: $2NH_3 + 3O_2 \rightarrow 2NO_2^- + 2H^+ + 2H_2O$ ($\Delta G^{o\prime} = +16$ kJ/mol NH_3)

Another group of bacteria (nitrite oxidisers (NOB): Nitrococcus, Nitrobacter, Nitrospina, Nitrospira) oxidize nitrite to nitrate in a second step:

Step 2: $2NO_2^- + O_2 \rightarrow 2NO_3^-$ ($\Delta G^{o\prime} = -74$ kJ/mol NO_2^-)
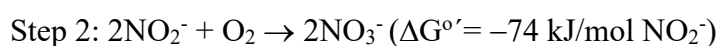
The biofilter chamber of the RAS module at Erko Settefisk was 300 m^3 large and filled with rotating biofilm carriers (Figure 5). Thus, offering an enormous surface for microbial biofilm

establishment and utilization (Figure 6). A commercial bacterial start culture (inoculum) was used initially to colonize the surface.

300 m³

Figur 5: Biofilm carriers. Photo: Karine Drønen   Figure 6: Visible part of the biofilter

The two nitrification processes operate best together at pH 7.5, thus, a primary goal during plant operation is to stabilize pH around this optimum (Figure 7).
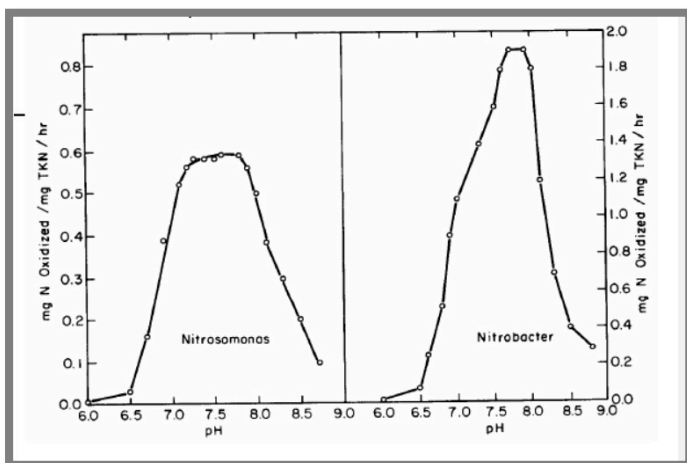
Figure 7: Optimum pH of the two microbial processes utilizing ammonium into nitrate. The figure is from teaching material at UiB.

Notable, two protons are relived in the first step of nitrification, that challenges the buffer capacity also of the marine system, and so do the CO_2 accumulation. pH is regulated chemically by the strong base sodium hydroxide (NaOH) and buffer capacity is increased by sodium bicarbonate ($NaHCO_3$, Figure 8). CO_2 is degassed from water using a vacuum fan in combination with risling.

Figure 8: Dosing tank for bicarbonate, releasing powder into the biofilter. Photo: Karine Drønen

Drum filtre: Mechanical removal of organic material such as feces and food residues is a prerequisite to balance the RAS plant and the caring capacity, and the concentration of total suspended solids (TSS) should be kept below 10 mg/L when farming salmonids, to minimize stress levels.[2] Screen filtration is the most common technology of particle removal in in modern RAS, and at Erko Settefisk AS, fish tanks outlet goes straight into drum filters (Hexfilter, Figure 9).

Figure 9: Hexfilters and their inlet. Photo: Karine Drønen

Screens are made of fine mesh material stretched on a rotating frame. Water passes through it, leaving small particles on the mesh surface. When lift above the water, this material is removed by backwashing using pressurized spray nozzles on the outer membrane. A sludge tray bellow the nozzels leads the material and washing water away from the filter and RAS flow line (Figure 6)[3].
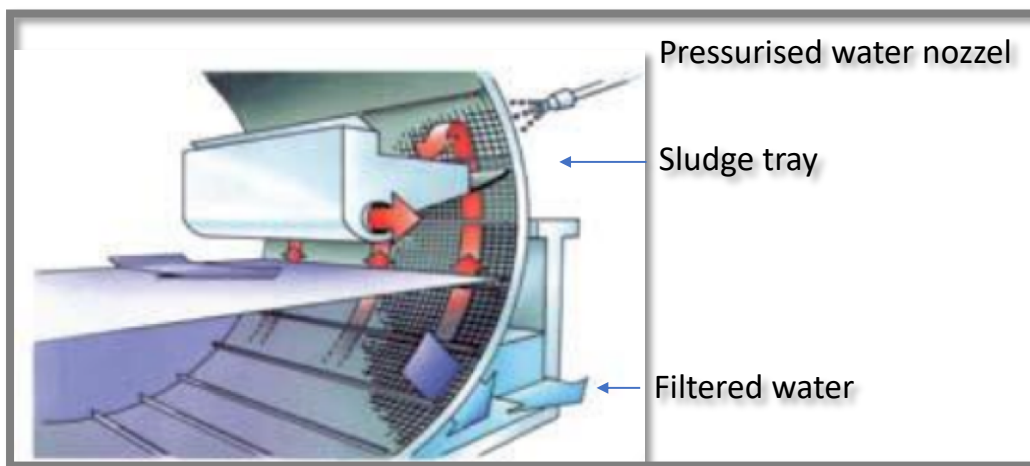
Figure 10: Backwashing of Hexfiltre.

LOZ-RAS: Use of LOZ is a second main tool in water purification, allowing further particle disintegration and microbial disinfection of the water. The product is developed by the Norwegian company LOZ AS in Trondheim and offers a more HMS friendly solution than traditional gassous ozon. The product is a mixture of liquified ozon (O_3), sodium hypoclorite (NaClO) and several metals and minerals. Thus, two very strong sources of free radicals are working together to widen the efficiency of microbial and viral removal. After filtration and LOZ treatment, the water will have ~10^5 cells/ml. The product is held in a pallet tank in the basement, side by side to the NaOH solution, and tubed to the first floor where it is injected on top of the CO_2 aeration section (Figure 11). LOZ is the main parameter to regulated the oxidative force in the system, as measured in mV. Consequently, nitrite can be chemically oxidized to nitrate by LOZ. Its addition to water increases pH.
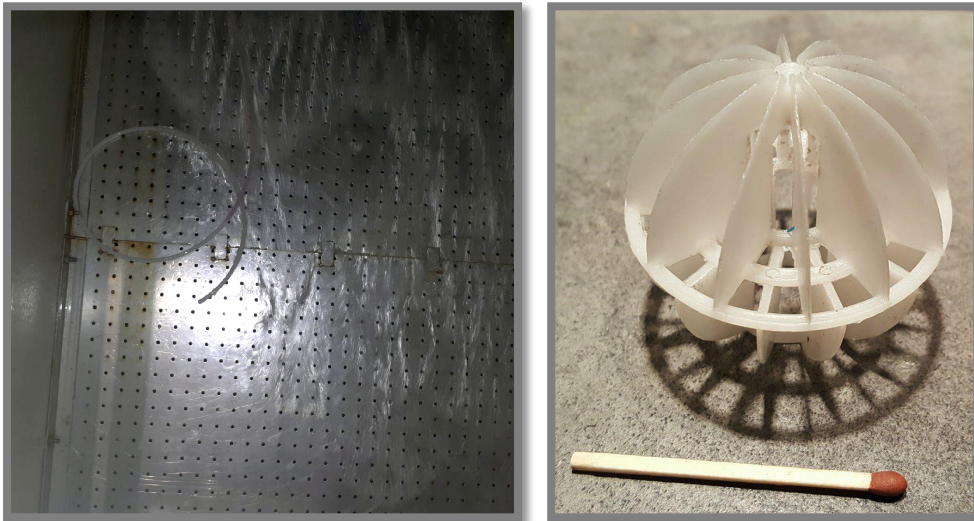
Figure 11: Water spread over perforated surface, LOZ injection and plastic balls in the CO_2 degasser unit. Photo: Liv Tori Selle og Karine Drønen

CO_2 stripping: Combined use of a large surface area and vacuum ensures diffusion of CO_2 gas from water to air at Erko Settefisk AS. Water flows above the surface and falls through the perforations (Figure 11). Below is a chamber filled with plastic balls. At this level a vacuum fan is installed. These fans ensure also that degassed CO_2 not enters the production hall. After sprinkling over the balls, the water passes another perforated plate, and falls down to the "pump sump".

Oxygenation: O$_2$ is added the rearing water by use of Oxygen cones at Erko Settefisk AS (Photo 9).   A separate circuit with pressurized water from the pump sump and liquified O$_2$ are mixed together at the top of the cone.   From the cone (one cone per tank), the oxygenated water enters the tank through a perforated vertical standing tube, A O$_2$ probe is installed distantly in the tank, to monitor the actual rearing level (80-100% saturation).
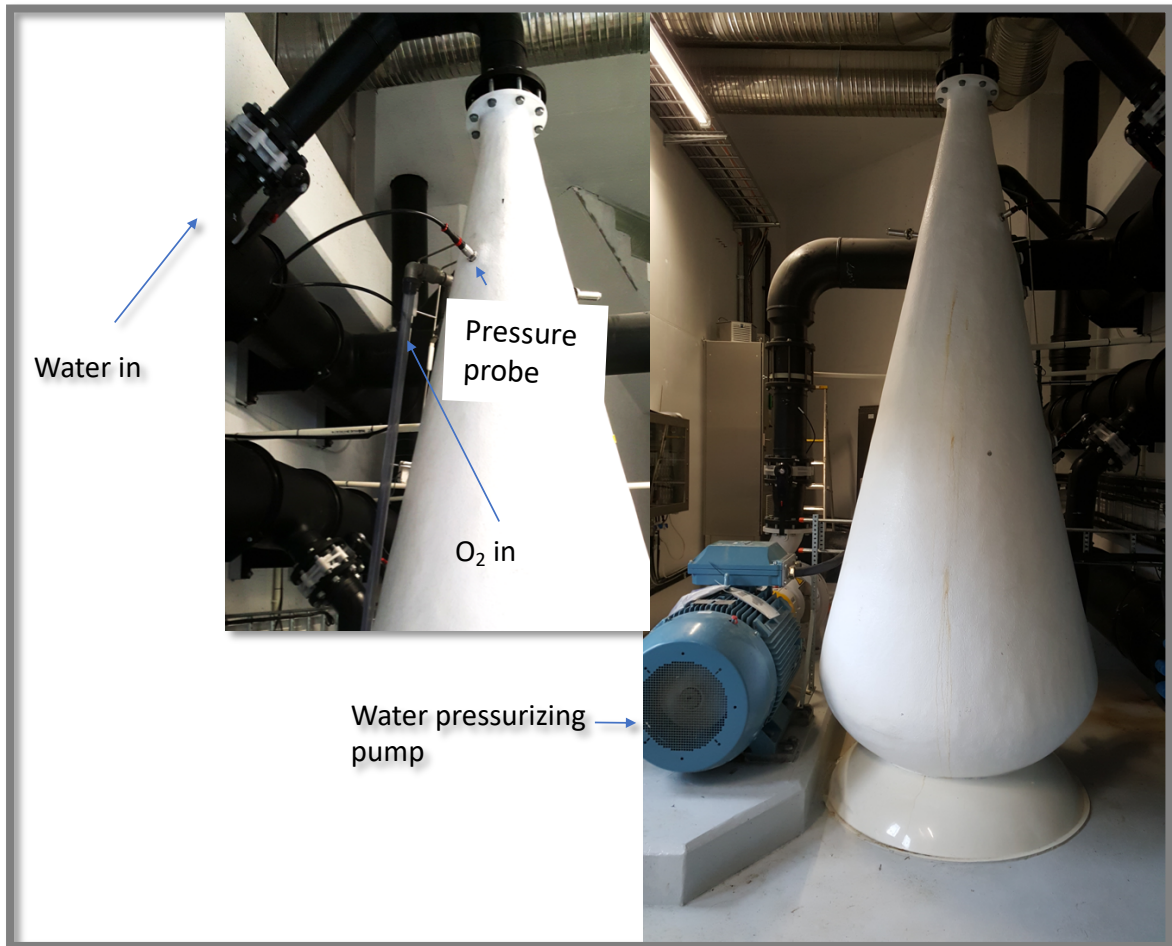
Figure 12: Oxygenation of water by mixing water and liquified O$_2$ in cones. Photo: Karine Drønen

Fresh water addition: The plant was without a fresh water infrastructure for sea water dilution, so a hose with public fresh water was inserted in via the biofilter hatch (Figure 13)
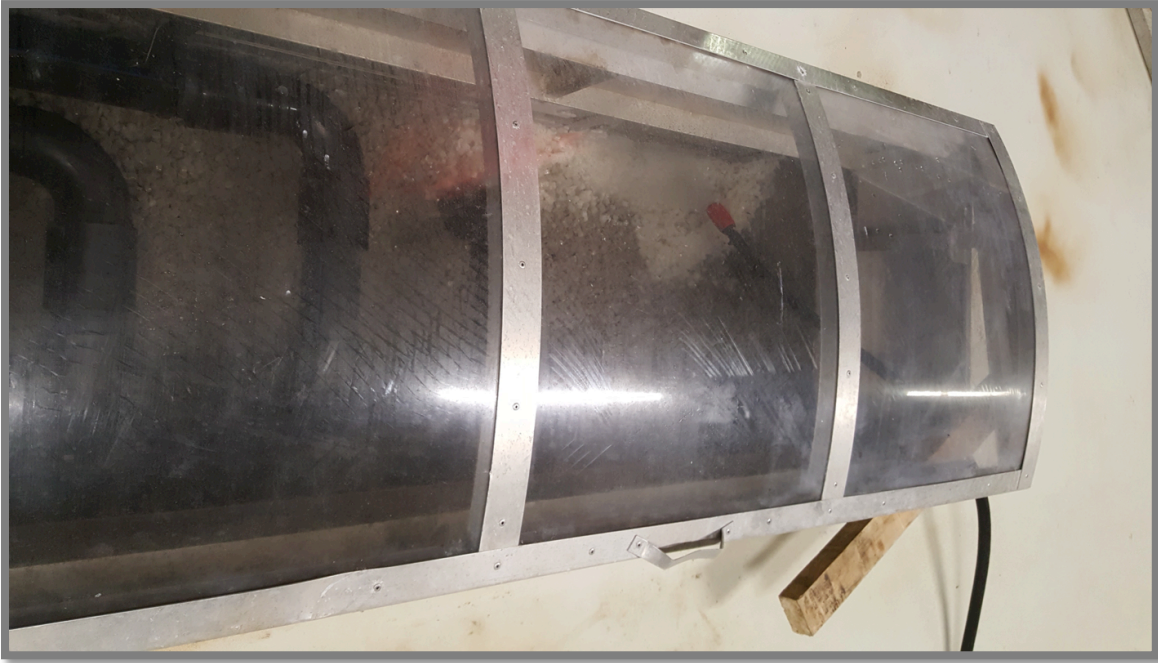
Figure 13: Fresh water injection into the biofilter chamber. Photo: Karine Drønen

RAS WORKING ROUTINES

The complexity of a RAS necessitates a number of daily routines, that is not commonly performed in the open sea environments rearing. This includes the daily measurements of the physiochemical parameters of ammonium, TAN (ammonium and ammonia), nitrate and nitrite, as well as regular cleaning of probes measuring continuously pH, CO_2, salinity, temperature and redox potential. The latter is in situ data that is fed into a computer and subjected an advanced alarm system calling up personnel. At any time, these data are available on screen, presented by the software Unitronics (Figure 14). The data collected are available also in an excel sheet, reporting one value per parameter per day.
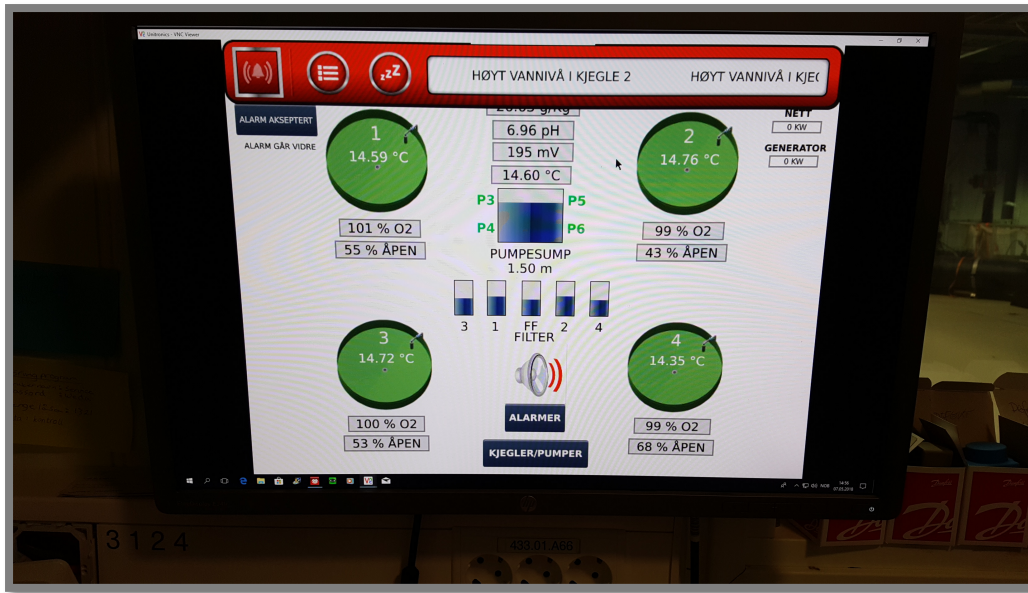
Figure 14: Software terminal window. Photo: Karine Drønen

pH, redox and conductivity probes were delivered by Schneider in an integrated probe and panel solution (Figure 15A). Temperature, O_2 and CO_2 probes were delivered by Oxy Guard. The O_2 probes are sited in the production tanks whereas the others were sited in a chamber with their own circuit from the from "pump sump" (Figure 15 B). Fouling will interfere with the measurements without regular cleaning of the different probes and their chamber.
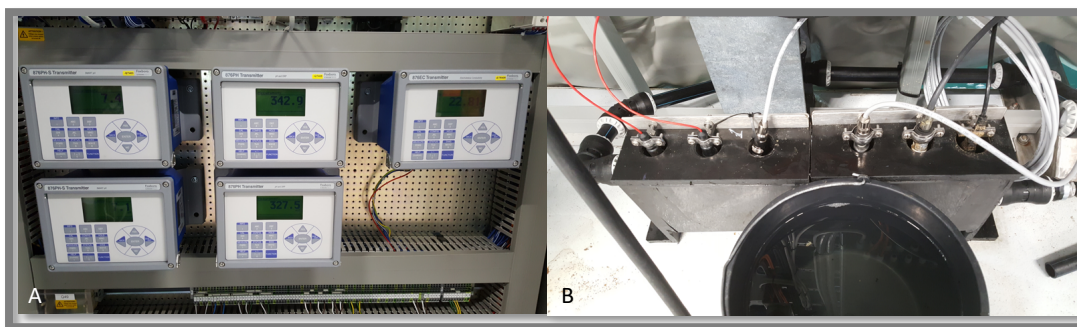
Figure 15: A) pH, redox and conductivity panels. B) pH, redox and conductivity probes installed in a chamber having water supply in its own circuit. Photo: Karine Drønen

Nitrite, nitrate, Tan and ammonium are measured manually every morning, and occasionally also in the afternoon in non-stabilized periods. These are spectrophotometric analysis, using the hand carried spectrophotometer ODEON produced by PONSEL Mesure (Figure 16). Water are then mixed with the reagent, that are delivered as tablets by ORCHIDIS

LABORATORY. TAN is the total amount of ammonium and ammonia, as pH determines the chemical equilibrium between these two compounds in water:

$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$

A chemical agent in the tablet will (strong acid) force the equilibrium to the right, so in practice TAN is measured as total NH_4^+. The concentration of each nitrogen species is notified in the daily journal.
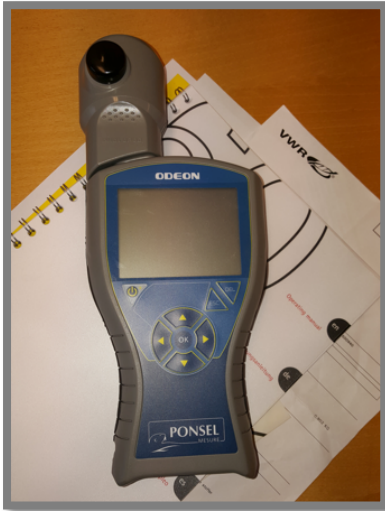
Figure 16: Spectrophotometer for quantification of nitrogen species. Photo: Karine Drønen

In the daily journal also the number of dead fishes the last 24 hours are noted, and if present, comments upon injuries and skin wounds.

Alkalinity is measured on daily basis using a titration kit, and is regulated with sodium bicarbonate powder that is dosed directly into the biofilter chamber (Figure 8).

Once a week 20 fishes are captured and weighted from each tank, to estimate the growth rate. These data are used to calculate the amount of feed to be handed out, using the program software Mercatus. Feeding is still based on appetite, and feed accumulating in the dead fish lock will be responded by reducing feed output. Daily consume of feed is notified in the daily journal. Refill of the feeders is one of the routines in the RAS. The feeding equipment is shown in Figure 17.

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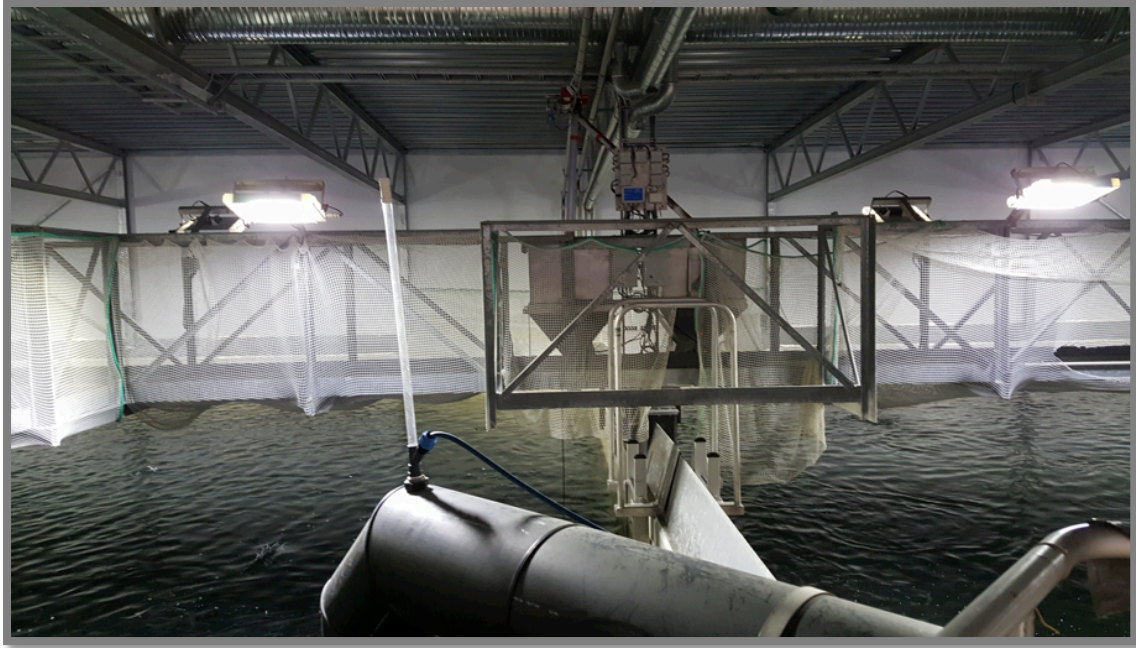
Figure 17: Feeding facilities. Photo: Karine Drønen

Other routines that are not on a daily basis, are washing of drum filter and refill of the tank with LOZ and NaOH.. The oxygenation cones do also have to be emptied after paucity, so that stagnant water is not mixed into the flow line.   Such a high tech system as RAS do also have a number of pumps, valvets and electrical equipment that constantly has to be checked. The amount of work peaks when fish goes in and out, every 3-4 month. More rearly, on an annual basis, the CO_2 stripping bowels has to be cleaned with respect to fouled material, otherwise the functionality might be lost.

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SECTION B

THESIS PAPER

ABSTRAKT PÅ NORSK

Resirkulering akvakultur systemer (RAS) teknologi for ferskvannsproduksjon av smolt ble for første gang brukt med marint vann for post-smolt påvekst, dvs. påvekst 100-500 gram i tre måneder. Mikrobiell dypsekvensering av produksjonsvann, fiskehud og biofilmer på biofilter og fisketankvegger ble utført gjennom fire produksjonssykluser (2-5), for å få innsikt i økologiske, patologiske og driftsmessige problemer, og som et første skritt for å utvikle et erfaringsbasert overvåkningsprogram for RAS. I tillegg ble et kommersielle inokulum som var brukt til kolonisering av biofilteret evaluert separat. Mikrobene fra inokulumet dominerte biofilmen i biofilteret frem til den femte produksjonssyklusen, og overflaten til biofilmbærerene ble kolonisert i samsvar med det som er tidligere rapportert for bruk av marint kystvann for overflatekolonisering. Imidlertid var modningstiden lang for steg to av den bakterielle nitrifikasjonsprosessen i biofilteret, og *Nitrospira* etablerte seg omtrent 15 måneder etter oppstart. Stabilisering av biofilmmatriksen av *Myxococccales* medlemmer kombinert med en saltholdighetsreduksjon i RAS-vannet til 22‰, bidro til at nitrifikasjonen kom i gang. Trolig var etableringen av *Myxococcales* og også *Vibrio* medlemmer i biofilmen forsinket av spesifikk predasjon, ixotrofi, utført av to første-kolonister fra *Bacteroidetes*. De ixotrofe stammene forsvant fra biofilmen da antibiotika ble gitt til fisken for å hindre hudsår (Produksjonssyklus 2) og når biofilmen ble behandlet direkte med hypokloritt / flytende ozon før neste syklus. Dominansen av *Myxococcales* i biofilterbiofilmen under den tredje produksjonssyklus forårsaket de største skiftene i de mikrobielle samfunnsstrukturene gjennom RASet i forsøksperioden. De fleste biofilmbakteriene ble redusert fire ganger av lysisaktiviteten til *Myxococcales*, og dessuten var det et skifte i produksjonsvannet fra *Colwellia* til en ikke-patogen *Francisella*-stamme, noe som indikerte at nekromasse var tilgjengelig i vannet. Saltholdigheten var høy i løpet av denne syklusen, og forhindret

sannsynligvis at *Nitrospira* utnyttet den åpne biofilmoverflaten og de biofilmforsterkende egenskapene til *Myxoococcales* til å etablere seg på dette tidspunktet.

Mindre intense ekstinksjons- og adhesjonsprosesser foregikk kontinuerlig i biofilteret, og et modnet biofilter ble ikke oppnådd i løpet av den eksperimentelle tidsperioden. Taxa inn og ut av biofilmen var i stor grad de samme. RAS-hendelser assosiert med reduksjon i biofilm taxa i biofilteret var skarpe pH /saltholdighetsfall, endring i oljekomponenter i fiskefôret og dominans av *Myxococcales*, mens antibiotikabehandling av fisken, behandling av biofilteret med oksiderende kjemikalier og re-inokulering økte antall taxa i biofilteret. Sistnevnte åpnet også for adhesjon av opportunistiske bakterier. Taxa som bare ble oppdaget i vannet eller biofilteret en gang, stammet hovedsakelig fra miljøet. Biofilmen i biofilteret og produksjonsvann var i motsatte diversitets faser, og vi tolker dette som at mikrober i vann i større grad enn mikrober i biofilm påvirkes av miljøendringer som skjer raskere enn mikrobiell adaptasjon i et RAS. Vi anbefaler bruk av mikrobiell dyp sekvensering for RAS-overvåking og har forslag til videreutvikling av tilnærmingen.

MICROBIAL COLONIZATION AND COMMUNITY SHIFTS IN A MARINE POST-SMOLT RAS DURING THE FIRST YEAR OF OPERATION AS ANALYZED BY DEEP SEQUENCING

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ABSTRACT

Salmon smolt recirculation aquaculture systems (RAS) fresh water producers used for the first-time marine water for post smolt production, growing the fish 100-500 grams in three months' time. Microbial deep sequencing of the production water, fish skin and biofilms on biofilter and fish tank walls was performed during production cycles 2-5, to address ecological, pathological and operational problems, and as a first step to develop an experience-based monitoring program for RAS. In addition, colonization of the RAS by microbes from a commercial brackish-water start culture was investigated separately for evaluation. These microbes dominated the biofilter biofilm until the fifth production cycle, and the surface of the biofilm carriers was colonized in accordance with the general colonization of coastal water surfaces. However, the maturation time of the biofilter biofilm with respect to second stage of nitrification by Nitrospira was long, about 15 months after start-up. Stabilization of the biofilm matrix by *Myxococcales* members combined with a salinity decrease in the production water to 22‰, contributed to this establishment. We further suggest that the establishment of the *Myxococcales* and also the *Vibrio´*s members into the biofilm was delayed by specific predation, ixotrophy, by two members of *Bacteroidetes*. The ixothropic strains disappeared from the biofilm when antibiotics were administered to the

fish to mitigate skin wounds (production cycle 2) and when the biofilm was treated directly with hypochlorite / liquid ozone before the next cycle. Dominance of the *Myxococcales* in the biofilter biofilm during the third production cycle caused the largest shifts in the microbial community structures in the RAS during the experimental period. Most biofilm bacteria were reduced four-fold in relative abundance from the lysing activity of *Myxococcales,* and furthermore, there was shift in the water dominance of *Colwellia* into a non-pathogenic *Francisella* strain, indicating that necromass was available in the water in large amounts. Notable, the salinity was high during this cycle, preventing probably *Nitrospira* to take advantage of the opened biofilm surface and biofilm strengthening properties of *Myxoococcales*. Less intense extinction and adhesion processes followed continuously in the biofilter biofilm over the experimental time period, and a matured biofilm was not obtained during the experimental time period. To a large extend it was the same taxa or close related bacteria that entered and disappeared from the biofilm during these changes. RAS events associated with decrease in taxa numbers in the biofilter biofilm were sharp pH/salinity drops, change in fish feed oil components and dominance by *Myxococcales*, whereas antibiotic treatment, treatment of the biofilter with oxidizing chemicals and re-inoculation increased taxa numbers in the biofilter.   The latter opened also for adhesion of opportunistic bacteria. Taxa that were detected in the water or biofilter only once, originated predominantly from the environment. Biofilter biofilm and production water were opposite in diversity phases, and we interpret this as water microbes to a greater extent than biofilm microbes are affected by environmental changes that occur faster than the microbial adaptation in a RAS. We recommend the use of microbial deep sequencing for RAS monitoring and have suggestions for further development of the approach.

INTRODUCTION

Farmed fish in the open sea experience problems due to sea lice infection, and these fishes represent a huge reservoir for lice production, which causes serious lice problems for the migrating wild salmon.[1] The chemical and mechanical treatment is stressful for farmed fish and chemicals used have adversely impact on the environment.[2] Sea lice are today regarded as the biggest bottleneck for further growth in Norwegian salmon production. Enhanced growth of post smolt (100-500 grams) in closed farms has been proposed as a possible solution to reduce this problem in Norwegian salmon farming.[3] The enhanced economic costs of using closed technology is balanced by shorter breeding time in sea, and consequently this will result in fewer delousing operations. However, use of new rearing technology then cause unforeseen challenges, and how these are solved will be crucial to the success of marine RAS.

Recirculation aquaculture systems (RAS) technology has been used successfully in salmon fresh water smolt production over the last 10 years. Such units are built on shore and recycle 99.5% of plant water, requiring UV-filtered dilution water on a scale of 300-600 l/min.[4] Water for reuse is treated in a flow line consisting of a drum filter for organic load removal, biofilter for ammonium removal, CO_2 degassing, chemical oxidation treatment and oxygenation. Ammonium and CO_2 are exudates from fish, and their excretion is linked to the fish feeding and growth. Ammonium is in pH balance with ammonia, that is highly toxic to fish, thus ammonium has to be removed instantly from the system by the biofilter. Microbial oxidation of ammonium to less toxic nitrate is a bacterial two-step process optimal only in a very narrow pH range. Therefore, in the daily operation of the facilities, it is a goal to keep the pH close to 7.5. During the first step of nitrification, when ammonium is oxidized into nitrite, a proton is released. Also the excreted CO_2 is a strong driver for souring, and CO_2

removal is essential to stabilize the pH. Various carbonate powders are used to increase the buffer capacity of the system and, if necessary, a base (NaOH) can also help. Thus, for a successful production, a fine-tuned balance must be established between the physicochemical parameters, microbiology and fish physiology. How this balance is influenced when fresh water is replaced by sea water, can only partly be addressed in advance. CO_2 removal is more energy demanding in seawater, since more CO_2 is in the form of bicarbonate (HCO_3^-), returning CO_2 to the water immediately after the degassing step.[5] High salinity ammonium oxidation rates have mainly been studied in the global oceans, i.e. environment with stable pH and very low ammonium concentrations.[6,7] Studies from marine aquariums show that high salinity results in a lower diversity in the ammonium-oxidizing population, whereas several studies from river plumes reports contradictionally upon efficacy versus salinity.[6,8] In a RAS the environmental factor that actually regulates nitrification will variate temporarily and spatially, and changes in physiochemical parameters will be faster than bacterial adaptability. Salinity will also influence upon pathogens selection and appearance.

Salmon is an anadrome fish that metamorphizes ahead of a transfer from freshwater to saline.[9] The changes embrace reorganisation of the major osmoregulatory organs, including the gills, gut and kidney. Still the inner osmotic environment is kept almost the same, i.e. ~300 mOsm/L in fresh water versus ~350 mOsm/L in sea water. Fresh water and sea water has osmotic concentrations around 0 and 1000 mOsm/L respectively. In nature the "post-smolt" spend some time in brackish water (~15‰) by the river mouth for final acclimatization, before it swims towards the open sea.[10] Given that the energy needed for ion pumping is smallest when outer and inner ion balance equalize in the fish, we expect brackish water to also have the highest growth yield. Studies so fare provides however not a generalised positive support for this expectation.[11,12,13] Normally, the smolt has been taken straight from fresh water to the open sea (32-35‰), though some smolt producers adds salt to

the feed or injects some sea water in the production line previous to the transfer. A positive effect of adaptation is however not evidented.[12,14]

Starting cultures for microbial inoculation of biofilm carriers prior to RAS biofilters operation are commercially available and are designed to operate in the salinity range of 0-15‰.[15,16] The use of starting cultures has several advantages, e.g. that unfavorable bacteria do not become first colonists and that the coating in the breeding tanks matches the selected microbes and repels invading bacteria from the environment. Given the uncertainty in nitrification efficiency for the start consortium in saline (32-34 ‰), existing starting cultures should be strengthened with more halotolerant species. In addition to the effective establishment of nitrification in the biofilter, the starting cultures should also prevent the establishment of sulfate-reducing bacteria (SRBs), which can be challenging at high salt content. So far, the microbiology of RAS has been unexplored, and process control mainly consists of regular measurements of physicochemical parameters in the water, especially the N-species. In order to further develop the marine RAS technology, a deeper understanding of the microbial ecological processes in RAS is needed. Furthermore, the interaction between fish, microbes and operational conditions must be understood and controlled in order to prioritize fish welfare.

In this study, we monitored the first commercial land-based marine post-smolt RAS plant in Norway using microbial deep sequencing. The measurements started in the second cycle, after the first cycle was terminated due to high nitrite and H_2S toxicity. The main events in the plant during the monitoring period were wound formation in fish skin, antibiotic medication, plant washing, biofilter treatment with oxidative chemicals, re-inoculation with new biofilter carriers, dominance of *Myxococcales* in the biofilter biofilm, changes in feed oil components and changes in the physiochemistry. A total of 4 production cycles were followed i.e. a timespan of a year. The main results of deep sequencing are reported and

discussed in this article, while issues such as sulfur cycling, pathogen reservoirs, and fish skin wound formation will be further explored in separate papers. The experience of using deep sequencing as a monitoring tool will be discussed in present study.

MATERIAL AND METHODS

RAS specifications

This study was performed in a Recirculation Aquaculture System (RAS) located at Stord, South of Bergen in the period 20.02.2016-23.2.2017. The plant belonged to Erko Settefisk AS and was built in concrete and steel and implemented 4 breeding tanks, each rearing 125000 post-smolt in $1100m^3$ coastal water from 70 meters depth (Figure 1).[17] The fish was grown 100-500g in 3-4 months at 14°C. The drum filters (Hex), the biofilter ($300m^3$) and CO_2 degassing (Aqua group) unit were located central between the breeding tanks (Figure 1).[4] A mixture of liquefied ozone and hypochlorite (LOZ) was injected in the degassing unit, obtaining a strong oxidative force that disinfected the water, it reduced the size of organic material and chemically oxidised nitrite to nitrate.[18] Some degassed water was circulated through oxygen cones with liquified ozone, and injected pressurized in the tanks through a perforated vertical pipeline. The biofilm carriers were squares of polyethylene $1x1cm^2$ with 5x5 inner grid, and rotated in the biofilm chamber. A microbial consortium (AVECOM AS) commercially available was used to inoculate the RAS biofilter prior to operation. Bicarbonate was injected as powder directly into the biofilter chamber to increase the water buffer capacity. NaOH was used for pH regulation and added after the water passed the drum filters ($40\mu m$). The dilution water made up 50-125 litre/min and was UV filtered. Retention time of the water in the tanks was 1.5 hour, and the degree of recirculation was 97.8%. White

LED light was on permanently in the RAS building. Blue light was used in one rearing tank, to increase growth. The first production cycle started September 15, 2015, and this study started the third week into the second production cycle (Table 1).
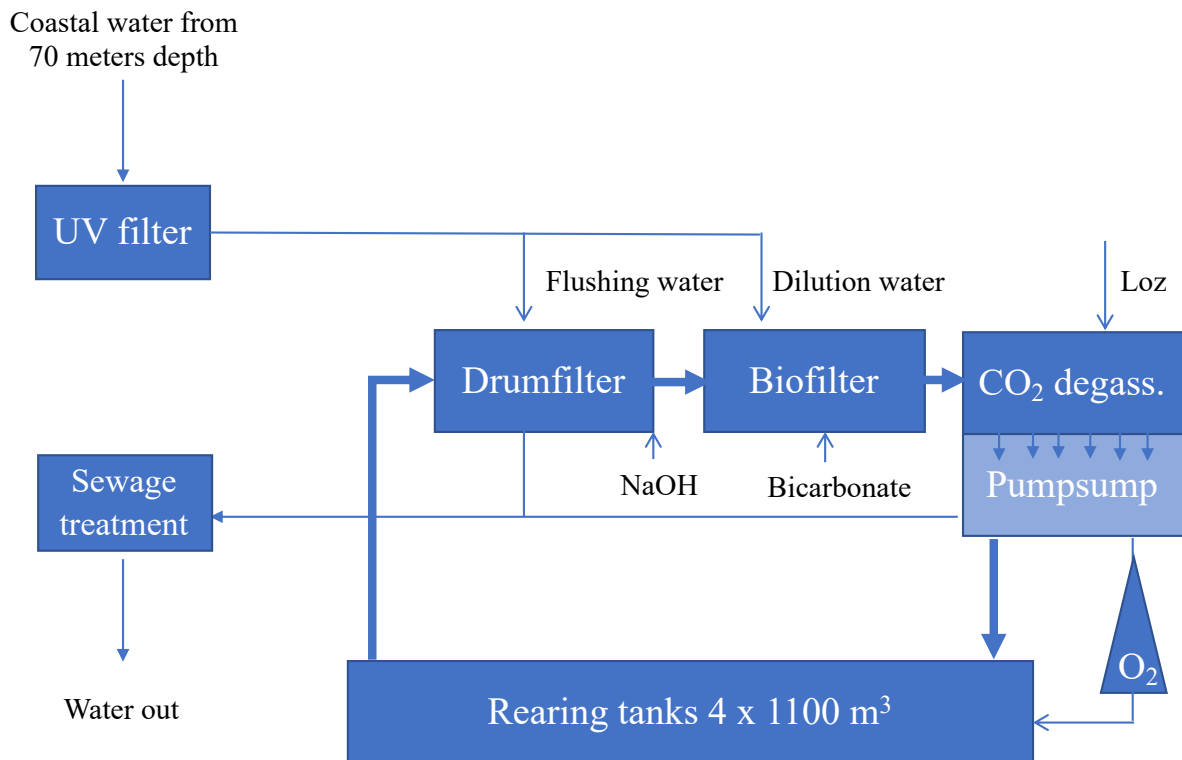
Figure 1. Simple flow diagram of the marine post smolt RAS. Dilution water and flushing water for the drum filter (60μm) were obtained from Atlantic costal water at 90 meters depth and UV treated before use. Rearing water was recirculated in a loop from tank to drum filter to biofilter to CO_2 degassing to tank again. Sites for addition of NaOH, bicrabonate and LOZ are shown. LOZ: Liquified ozon and hypochlorite.

Chemo-spectroscopic analysis of ammonium, TAN, nitrate and nitrite

Ammonium, TAN, nitrate and nitrite were measured on daily basis, using a hand hold spectrophotometer (ODEON by PONSEL Measure). The reagents were delivered by ORCHIDIS LABORATOIRE as tablets, and mixed directly with a specific amount of water. TAN is the total amount of ammonium and ammonia, and pH determines the chemical equilibrium between these two nitrogen species in water: $NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$. A

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chemical agent in the tablet (strong acid) forces the equilibrium to the right, so in practice TAN is measured as total NH_4^+. Ammonia was calculated as the difference between TAN and ammonium.

Probe based detection of pH, CO₂, salinity, temperature, O₂ and redox potential

The physiochemical parameters pH, CO_2, salinity, temperature, O_2 and redox potential were measured by probes that fed in situ data continuously to the software Unitronics. Thus, at any time, these data were available on screen and subjected an advanced alarm system calling up personnel. The pH, redox and conductivity probes were provided by Schneider, and the probes were in a chamber that had its own circuit from the flow line (from pump sump). O_2 and CO_2 probes were delivered by Oxy Guard and placed inside the breeding tanks and pump sump respectively. Regular cleaning of the probes and chambers was done to avoid fouling induced failures.

Table 1. Sampling times, sampling sites, and operational events in the RAS

	Fish stocked	Samling time (week)	Sample name	Sampling sites	Events
Production cycle 2	01.02.2016-30.04.2016	3	C2W3	R	
		6-7			Antibiotics added
					Fresh water added
		8	C2W8	R	
		13	C2W13	R	
Wash I		0	Wash I	R (No skin)	Pathogenes removal
					Re-inoculation
Production cycle 3	17.05.2016-01.08.2016	4	C3W4	R	
		8	C3W8	R	
Wash II		0	Wash II	Biofilter only	Tank biofilm removal
Production cycle 4	16.08.2016-23.11.2016	2	C4W2	R	
		3			Changes in feeds oil components
		5	C4W5	Biofilter, water	Yellow production water
		10	C4W10	R	
Production cycle 5	08.12.2016-01.04.2017	2	C5W2	R	
		10	C5W10	R+E	

R; Regular sampling sites: A) Biofilter biofilm, B) Tank 3 wall biofilm, C) Tank 3 water, D) Tank 3 skin samples
E; Extra ordinary sampling sites: Biofilter outer chamber wall biofilm, Biofilter biofilm depth profiles, Tanks 1,2 and 4 wall samples,

Sampling times, Sampling sites and major operational RAS events

Samples for deep sequencing of microbes were collected for the first time 20 days into the second fish production cycle and continued monthly for a year, covering 4 production cycles (Table 1). The sample times were named by the cycle number and week into the cycle, e.x. C2W3 for the first timepoint of sampling. The regular sampling sites were the biofilter biofilm and the tank enumerated 3. From the tank, samples were collected from the wall biofilm, from the water and from the fish skin. All samples overview is given in Table 1, including also the main operational events in the RAS. A severe outbreak of skin wounds in the fish population occurred in production cycle 2 (PC2), and wounded tissue was included in the sampling routines when present in the fish population. The antibiotics oxolinic acid and florfenicol were administrated to the fish through the feed during the outbreake. This was followed by fresh water addition to the rearing water, to lowering the salinity. Furthermore, to minimize pathogens, the RAS was extensively cleaned between PC2 and PC3, including also ozone treatment of the biofilter and a re-inoculation (Wash I). At re-inoculation consisted new biofilter pre-enriched in the same commercial start-culture (inoculum) as used prior to RAS start-up, were added. Another RAS wash (Wash II) was performed after the third cycle, though without biofilter ozonisation. Samples from the washing events were provided after water refill and only few hours prior to fish stocking. An unintended shift in the feeds oil components gave a diarrhea-like feces in the fish early in PC4, and the production water obtained a strong yellow colour at sampling time (C4W5). The biofilter carriers were also examined by microscopy upon sampling . More sampling sites than the regular was investigated in the end of the experimental period, including the biofilter outer chamber wall biofilm, biofilter biofilm depth profiles, tanks 1,2 and 4 wall samples, pump sump wall biofilm, water entering and leaving the UV filter and the dilution water line Bernaud filter biofilm (before UV filter).

Sampling methodology

Parallel samples were collected from the biofilter biofilm (3 parallels) and from the wall biofilm (3 parallels) and to less extend from water (1-2 parallels). Skin samples were not paralleled, except at time C2W8, when a triplicate was taken from the same wound. Tank 3 water microbes were sampled by filtering 240ml through a Milipore Sterivex™ filtre GV 0.22μm using a syringe (60ml, BD Plastipak™). The plastic caging was filled with RNAlater (http://patft.uspto.gov patent #6,528,641, 2mM EDTA, 25 mM sodium citrate, 5.3 μM ammonium sulphate pH 5.2) to enhance RNA and DNA stability during storage of the material gathered in the filter. Wall biofilm was collected by toothbrushes, new from box, that were taped to a long rod (2 meter). The brushes were pulled along the tank wall (10 meters), 15 cm below the water surface, giving visible organic matter. The brush head fell into a 50ml Falcon tube with 15ml RNAlater when cutted with a disinfected forceps (ethanol). Biofilter biofilm carriers were collected by an ethanol disinfected bean and transferred with a sterilized tweezer into a 50ml Falcon tube containing 10ml RNAlater. Fish skin samples (2x3cm) were cut out with a scalpel 1mm tick in the area laterally to the dorsal fin, and transferred with a sterile tweezer into a 50ml Falcon tube with 10ml RNA later. The samples were kept cold during transportation. Prior to -24°C storage, toothbrush and biofilm carriers were sonicated in water bath to assist the biofilm loosening. The brush heads and carriers were removed and the organic material pelleted by centrifugation 45min 5000g 7°C in Thermo Heraeus, Scientific Centrifuge (3SR+).

DNA extraction

Three commercial kits were used for all sample's DNA extraction, and processed in accordance with the producer's manual. Pelleted biofilter biofilm (0.05-0.2g) was processed

by the DNeasy PowerBiofilm Kit (Quiagen). This kit practices a combination of enzymatic cell lysis and mechanical disruption. All the kits processed lysed material by protein removal, DNA capture in columns, washing and eluation. Sterivex filters was released from its plastic caging using tongs and sterile scalpel, and carefully transferred to suitable bead beating tubes supported by the DNAeasy PowerWater kit (Quiagen). No bead beating step was necessary in the tissue DNA extraction kit delivered by Rocke (High Pure PCR Template Preparation). In this kit, the lysis was ensured by long time-high temperature exposure of 0.2g tissue, lysing the sample ahead of DNA capture, washing and eluation.

Microbial deep sequencing or amplicon libraries

16S amplicon rDNA libraries were generated in accordance with the protocol of Jørgensen & Zhao 2016, modified after the two-step amplification protocol recommended by Berry et al. (2011)[19,20]. This method allowed subsequent library sequencing in the Ion Torrent PGM Personal Genome Machine (PGM) platform technology (Life Technologies).[21] The primers 519f (5'-CAGCMGCCGCGGTAA-) and 805r (5'-GACTACHVGGGTATCTAATCC) were used for the first-step rDNA amplification, and the PCR mixture contained 12.5μl HotStarTaq Master Mix Kit (Qiagen), 2μl of each primer (100mM), 7.6μl dH$_2$O and 2μl DNA-template[20]. The thermal cycle program was: 95°C, 15min), 32 times repeating DNA melting (94°C, 30s); primer annealing (56°C, 30s); amplification (72°C, 30s). Positive PCR products were verified by 1% agorose gel electrophoresis (Agarose Electran, Cambrex Bio Science, 50V, 30min), using 1μl GelGreen Nucleic Acid Stain per 10ml agarose (BIOTIUM, VWR). DNA templates with positive product were triplicated in parallel PCR amplifications. Pooled triplicate DNA samples were purified by the bead based AMPure XP kit (Agencourt), in a 96 well square storage plate 1.2ml (Thermo scientific, AB-1127) adaptable to magnet (Alphaqua, 96S Super Magnetic

plate). The sample:bead ratio (0.7), ethanol washing solution (70%) and H_2O eluation

volume (20μl) was as in accordance with suppliers manual. The purified DNA was quantified

in the Fluorometer QUANTUS (Promega) using the QuantiFluor® dsDNA System mixture,

requiring 2μl DNA sample to the fluorochrome (0.5μl) and 1xTE buffer (197.5μl) . The kit

provided also a DNA standard (100ng/μl). Samples were diluted to a final concentration of

10ng/μl, based on the quantification information. Tagged primers were added the PCR

products in a second PCR: Forward tagged primer comprised both the "conventional" PCR

primer and a start site for the DNA polymerase, a barcode flag (Multiplex Identifiers, MIDs)

and a code for PGM calibration[21]. Reverse primer was added the "adapter B sequence" for

bead attachment in the PGM operation. The PCR mix of the second PCR was as following:

2.5μl HotStarTaq Master Mix Kit, 0.3μl H_2O, 2μl 519f MID primer (10μM) and 0.2μl 806r

B-key primer (100μM) and 10μl PCR product from initial PCR. In advance the latter

template DNA was diluted to approximately 10ng/μl. The PCR protocol was similar to initial

PCR, differing only in cycling number (7 cycles). The purification and agarose steps were

repeated as for the initial PCR, to ensure primers and primer-dimers absence. For final

sample normalization purpose, the purified product was quantified as previously described.

The normalization was performed in two steps, the first pooled 8 and 8 samples to their mean,

and then the 12 samples were pooled to a concentration of 0.1ng/μl . This stock was

quantified prior to the final dilution (40pM) the very day of sequencing.

Sequencing and bioinformatics

Resulting amplicons were sequenced on a PGM in the Laboratory of

Bioscience, University of Bergen, Norway. The down-stream 16S rRNA gene sequence

analysis includes the following steps: Filtering and clustering of sequences into operational

taxonomic units (OTUs) using USEARCH and UPARSE.[22,23] Quality filtering and trimming

to 250bp was performed with the '-fastq filter' command using options '-fastq_trunclen 250' and '-fastq maxee 1'. Chimeric sequences were detected and removed with the '-uchime_ref' command using the Gold database as reference (available from 'http://drive5.com/uchime/gold'). De novo OTU clustering was performed at a cut off of 97% nucleotide sequence similarity using the'-cluster_otus' command. Taxonomic classification was performed in QIIME, using the command 'summarize_taxa_through_plots.py' using Silva as reference database.[24] All libraries overview is given Table 2. When samples did not provide a library, the PCR amplification step was normally the failure.

Data handling

The data OTU text files were processed by the Microsoft Excel software to present relative abundance tables, taxa friendship diagram, statistical approaches or to prepare data for Sigma Plot13.0 (Systat Software, www.systatsoftware.com). Physiochemical data was provided from Erko settefisk AS in excel sheets and presented by SigmaPlot. Graphs from Sigma Plot were handled by Photoshop Illustrator CC 20.0.8 2018 for final figure presentation, or as with taxa flux data, Microsoft Power Point. Hierarchic cluster analysis of samples was done based on a Bray–Curtis distance matrix, using the ward algorithm (ward.D2) in R.[25] This analysis takes into account taxa presence/absence and their relative abundance. Shannon and Simpson indexes were reported from the same raw data, using VEGAN package, where Simpson index is 1-D and D is the Simpson value.

Table 2. 16S libraries obtained from the RAS samples and their use in different analysis.

Site	Time	Parallels	Comments	Libraries used in data analysis 1-15	Data analysis
				1 2 3 4 5 6 7 8 9 10 11 12 13 14 15	
Inoculum		A	Producers bottle		1 Seperates inoculums associated taxa from environmental taxa in all analysis
		B	Producers bottle		
		C	Producers bottle		
		D	Producers bottle		2 RAS total taxa number, inclusive:
			Enrichment tray		Biofilter biofilm total taxa number
Biofilter biofilm	C2W3	A			Wall biofilm total taxa number
	C2W3	B			Water total taxa number
	C2W3	C			Fish skin/Wound total taxa number
	C2W8	A			
	C2W8	B			Basis for calculating taxa in and out
	C2W8	C			of the RAS/biofilter biofilm system
	C2W8	D			
	C2W8	E			Basis for enumerating the taxa´s
	C2W8	F			times of appearance
	C2W13	A			
	C2W13	B			Basis for distribution of taxa in
	C2W13	C			fractions of relative abundance
	C3W4	A	Carrier new		
	C3W4	B	Carrier new		3 RAS total taxa as distributed on different
	C3W4	C	Carrier new		sampling sites the first day of deep
	C3W4	D	Carrier old		sequencing
	C3W8	A			
	C3W8	B			4 Taxa shift upon antibiotics addition
	C3W8	C			
	C4W2	A			5 Taxa shifts upon the steepest pH and
	C4W2	B			salinity drops
	C4W2	C			
	C5W10	A			6 Taxa shifts upon re-inoculation of the
	C5W10	B			biofilter
	Wash I	A	PC2-PC3		
	Wash I	B	PC2-PC3		7 Taxa shift after wash I and biofilter
	Wash II	A	PC3-PC4		LOZ treatment
	Wash II	B	PC3-PC4		
	Wash II	C	PC3-PC4		8 Taxa shift when *Myxococcales*
	C5W10		Bernaudfilter in water inlet		dominated the biofilms
Wall biofilm	C2W3	A/B			
	C2W8	A			9 Effect of Wash II
	C2W8	B			
	C2W13	A			10 Changes in feeds oil components and
	C2W13	B			yellow water formation
	C2W13	C			
	C3W4				11 Dominance of *Nitrospira* and salinity
	C3W8	A			drop
	C3W8	B			
	C3W8	C			12 All samples cluster analysis
	C4W2	A			
	C4W2	B			13 Shannon and Simpson indexes
	C4W10				
	C5W2	A			14 Comparing relative abundance in taxa
	C5W2	A			identified in both biofilm and water at
	C5W2	B			all comparable time points
	C5W10		K1 15cm Aeria with less flow		
	C5W10		K1 75cm		15 Samples relative abundance of taxa
	C5W10		K2 15cm Aeria with less flow		
	C5W10		K3 15cm		
	C5W10		K3 100cm		
	C5W10		K3 200cm		
	C5W10		K3 300cm		
	C5W10		K3 350cm		
	C5W10		K4 15cm		
	C5W10		K4 75cm		
	C5W10		K4 100cm		
	C5W10		Biofilter outer chamber biofilm		
	C5W10		Pump sump		
	C5W10		Wash I PC2-PC3 Black border		
	C5W10	A	Wash I PC2-PC3 Black border		
	C5W10	B	Wash I PC2-PC3		
Water	C2W3		K3		
	C2W8	A	K3		
	C2W8	B	K3		
	C2W13	A	K3		
	C2W13	B	K3		
	C3W4		K3		
	C3W8		K3		
	C4W2		K3		
	C4W10		K3		
	C5W10	A	K3		
	C5W10	B	K3		
	C5W10		UV in		
	C5W10		UV out		
	Wash I		PC2-PC3		
Fish skin	C2W3		Wound		
	C2W8	A	Wound		
	C2W8	B	Wound		
	C2W8	C	Wound		
	C2W13		Wound		
	C2W13		Skin next to wound		
	C2W13		Skin healthy fish		
	C3W4		Wound		
	C3W4		Skin next to wound		
	C3W8		Skin healthy fish		
	C3W8		Gill healthy fish		
	C4W10		Skin healthy fish		
	C4W10		Petichia skin		
	C4W10		Skin looser fish		

RESULTS

A fish farm for aquaculture (recirculation aquaculture system, RAS) was built on land to increase salmon smolt weight from 100-500 grams in 3-4 months in marine water. The physicochemical parameters were monitored regularly in accordance with the requirements of the Norwegian Food Safety Authority. To better understand the microbial events in the RAS, microbial deep sequencing was introduced for monitoring purpose. Monthly samples were taken from the biofilter biofilm, tank wall biofilm, tank water and fish skin. Here we report the microbial succession in the RAS from the second until the fifth breeding cycle, and connects these findings to a series of events in the farm. These events included changes in physiochemical parameters, antibiotic treatment towards fish skin wounds, washing, LOZ treatment, biofilter re-inoculation, dominance of *Myxococcales* in the biofilter biofilm and changed feed oil components.

Variation in physiochemical parameters

Salinity varied significantly during the experimental period, i.e. production cycles 2-5 (PC2-PC5), as measured by conductivity probes on daily basis (Figure 2). The major changes were caused by fresh water addition, aiming to reduce fish skin wound formation (PC2) and to increasing nitrate production in the biofilter (PC5). The salinity of the marine inlet water and the production water was in mean 32.1±1.7‰ and 27.7±3.8‰ respectively during the experimental period, and the extreme values in the production water was 34.8‰ and 21.6‰. From week 6 in PC2, the salinity dropped from 34.8‰ to 25.9‰ in a 25 days' time, when fresh water was added for wound mitigation. After that, the salinity was held between 26-28‰ to the end of the cycle. No fresh water was added during PC3, holding the mean salinity 31.1±0.8‰. In PC4 and PC5, the mean salinities were 26.5±1.3‰ and
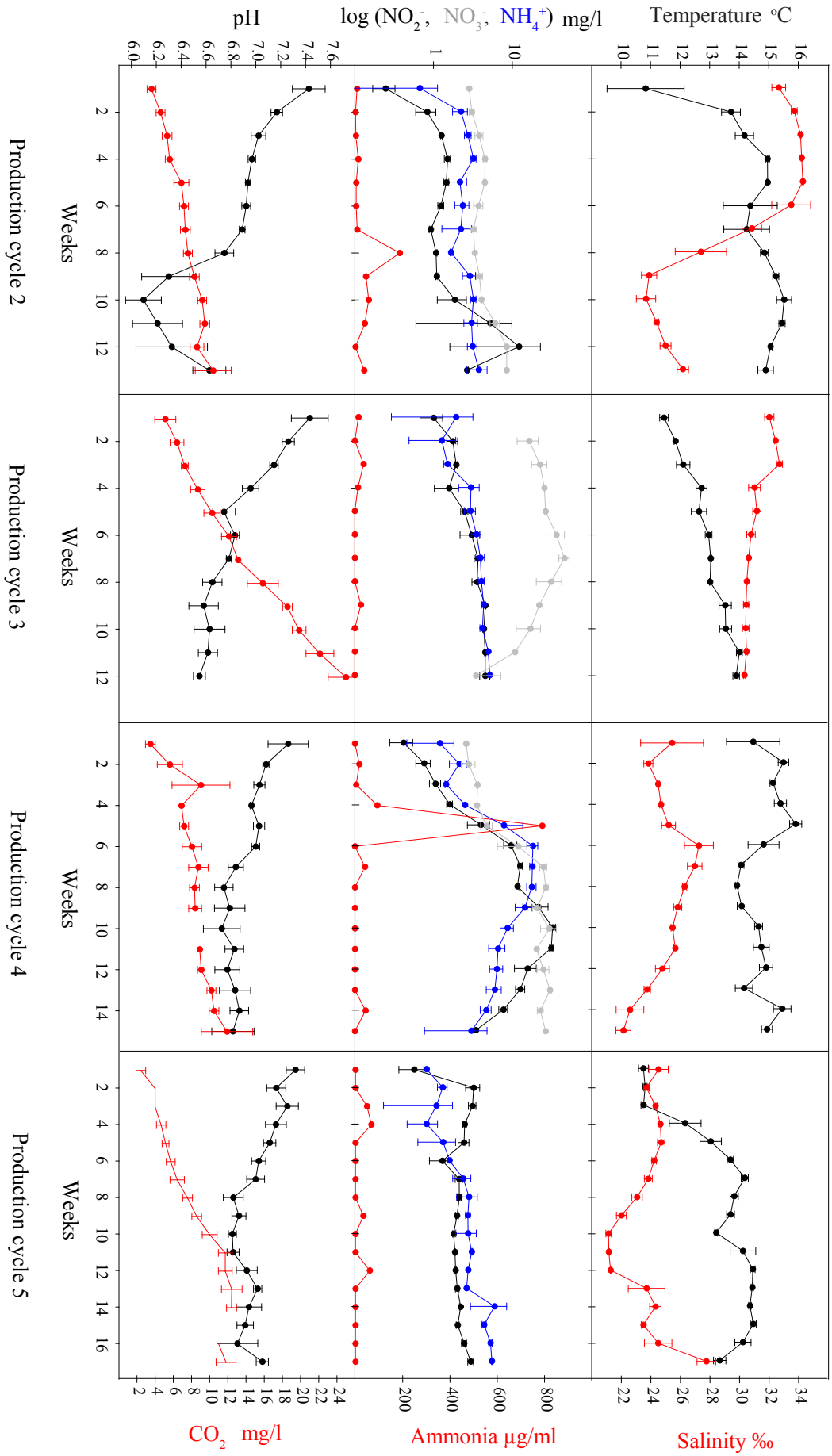
Figure 2. N-species, CO_2 and salinity in the marine RAS during four production cycluses. NO_2^-; Nitrite, NO_3^-; Nitrate, NH_4^-; Ammonium, NH_3; Ammonia, CO_2; Carbon dioxide.

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23.8±1.8‰ respectively. At the last sampling time (C5W10), the salinity was the lowest measured during the experimental period, having first dropped 4 units during the five preceding weeks.

The water pH is shown in Figure 2, and was strongly influenced by the fish's turnover of feed, i.e. respired CO_2 effected pH directly, while respired NH_4^+ effected pH indirectly through H^+ formation by microbiological oxidation of ammonium. The pH during PC2-5 was in mean 6.9±0.4 with the extremes 7.7 and 5.9. PC2 had the lowest mean pH, 6.7±0.4, in the experimental period, and the extreme values were 7.6 and 5.9. The steepest pH drop per time unit was also registered during PC2. The mean values increased by 6.8±0.3, 6.9±0.2 and 7.0±0.1 in the following cycles. In PC3 the extreme values were 7.7 and 6.4 whereas the pH in PC4 and PC5 spanned only 1.0 and 0.9 units respectively.

The temperature of the production water was intended to be 14°C (Figure 2). In mean, the temperature was 13.8±1.3°C during PC2-PC5, and the extreme values were 16.2°C and 9.5°C. PC4 had the highest mean temperature (14.8±0.7°C) and PC3 the lowest (12.9±0.8°C), spanning over 3.0°C and 2.8°C respectively. The largest temperature span was 6.1°C, attained in PC2. The mean value was here14.5±1.3°C. PC5 held 13.2±1.3°C in mean, with a temperature span of 4.1°C.

The lowest measurable ammonium concentration was 0.02mg/l. Values above this limit were in mean 2.0±2.7mg/l during PC2-PC5. Highest ammonium level, 12.8mg/l, was measured in PC4, six weeks into the cycle. PC4 held also the highest ammonium level in mean, 4.7±3.8mg/l, as compared to the other cycles: 0.8±0.35mg/l (PC2), 1.1±0.6mg/l (PC3) and 0.5±0.3mg/l (PC5).

The lower detection limit of nitrite was 0.03mg/l. The nitrite concentration during PC2-PC5 was in mean 2.6±4.8mg/l, peaking at 25.4mg/l in PC4 week 10 (Figure 2). A notable high peak of 22.8 mg/l nitrite was also measured in the end of PC2. However, fare

more nitrite was measured in mean in PC4 (6.5±7.3mg/l) than PC2 (2.4±4.3 mg/l). The other two cycles held, 1.0±0.5mg/l (PC3) and 0.6±0.2mg/l (PC5) nitrite in mean respectively. The sharpest increase in nitrite per time unit was observed in PC5 the first week of production (Figure 2).

Both chemical and microbial oxidation of nitrite influenced the formation of nitrate, and the measured nitrate values during the experimental period are shown in Figure 2. The nitrate level was 14.0±12.0 mg/l in mean during PC2-PC4, whereas the individual cycles held in mean 4.5±1.8mg/l (PC2), 22.3±12.6mg/l (PC3) and 16.2±10.7mg/l (PC4). Ammonia was not measured in the RAS except for two peaks, 200µg/l and 800µg/l each, in PC2 week 8 and PC3 week 5 respectively.

OTU`s or taxa in the RAS

The DNA extracted per areal or volume from the samples is presented in Table 4, and is the best indication of biomass quantity in this study. Microbial community structures were investigated using 16S rRNA amplicon library tools (deep sequencing) towards the template DNA. The data were presented in terms of operational taxonomic units (OTUs), also called taxa, where on taxon representing all sequences with > 97% similarity. RAS main taxa are presented in in Table 3 and the RAS total taxa overview is given in appendix.

RAS total taxa number and taxa flux. A microbial consortium commercially available was used to inoculate the RAS biofilter. This starting culture contained a total of 139 taxa when examined with deep sequence analysis. The DNA deep sequencing revealed totally145 taxa in the RAS the first sampling time (C2W3), of which 65 taxa where identical to those in the inoculum (inoculums associated taxa). Thus, 80 taxa originated from the external environment, that from now are named environmental taxa. At the last sampling time in the experimental period, the equivalent taxa numbers where 92 inoculums associated and 140

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Table 4. DNA yield from biofilter biofilm, water and wall biofilm.

Time	Water	Wall	Biofilm
	ng/ml	ng/cm^2	ng/cm^2
C2W3	1.5	0.1	101.6±117.1
C2W8	18.7±26.7	28.8±0.4	462.0±209.9
C2W13	5.6±36.2	27.7±0.7	704.9±15.8
C3W4	8.9	50.9±12.6	528.1±474.4
C3W8	1.0	56.0±1.5	344.5±441.7
C4W2	1.2	26.2±21.4	493.7±210.5
C4W10	2.2	32.3±1.9	768.3±44.1
C5W2	10.6	0.1±0.1	
C5W10	4.8±0.2	26±1.1	718.1±30.0

environmental taxa. RAS total taxa numbers were in mean of all the sampling times 211±31, of which 89±11 where inoculums associated and 122±23 environmental taxa. Also, out of the 139 taxa identified in the inoculum, 21 taxa never showed up in the RAS. Taxa appearing or leaving between sampling times, as given by the libraries, were defined as taxa flux in and out of a system. RAS and biofilm total taxa number and the fluxing taxa numbers are presented in Figure 3A and 3B. Environmental taxa flux in the RAS varied from 13-48 in and 17- 33 out at the different sampling times. Equivalently, inoculums associated taxa varied from 3-28 in and 1-16 out. Thus, environmental taxa demonstrated a relative higher instability, also when taking into account that they were generally most numerous. Important too, the flux in and out was often represented by re-appearing taxa, i.e. >50% for the inoculums associated and ~50% for the environmental taxa (Figure 3A and 3B). The occurrence of RAS events at different times of operation is summarized in Figure 3, as well as Table 1 and 2. Over all in the RAS, only the washing, LOZ and re-inoculation steps between PC2 and PC3 enforced an effect upon total taxa numbers, and then mainly upon the number of environmental taxa in the RAS.

Biofilter biofilm total taxa number and taxa flux. In mean 73±11 inoculums associated taxa and 76±22 environmental taxa were detected in the biofilter during the experimental

Table 3. Relative abundance of main taxa identified by deep sequencing in a marine RAS during one year of monitoring.

RELATIVE ABUNDANCE (%)

Column groups: Inoculum | BIOTFILTER BIOFILM (C2W3, C2W8, C2W13, Wash, C3W4, C3W8, Wash, C4W2, C5W10) | WALL BIOFILM (C2W3, C2W8, C2W13, Wash, C3W4, C3W8, C4W10, C5W2, C5W10) | TANK 3 WATER (C2W3, C2W8, C2W13, Wash, C3W4, C3W8, C4W2, C4W10, C5W2, C5W10) | FISH SKIN (C2W3*, C2W8*, C2W13*, C2W13^skin, C3W4*, C3W8^inf, C3W8^leg/ill, C4W10^phishing, C4W10^petechia, C4W10^inf)

OTU
Thaumarchaeota-
α− Rhizobiales-Bradyrhizobium
Cyanob.-MLE1-12
Acidob-Chloracidobacterium
TM6
B.d.-Sphingo-Lewinella
Other-Other
Plancto-Phycisphaerales-Phycisphaera
δ− Sh765B-TzT-29
γ− Family Incertae Sedis-Marinicella
B.d.-Chitinophagaceae
Chloroflexi- Anaerolineaceae
Chloroflexi-Caldilinea
Plancto-OM190
α− Rhodobacteraceae
B.d.-Sphingo-Saprospiraceae
δ− Myxococcales
δ− Myxococcales-Nannocystis
Planctom- Pir4 lineage
δ− Myxococcales-Sandaracinus
α− Rhodobacterales-Pseudorhodobacter
Planctom- Planctomyces
α− Rhodospirillales
δ− Desulfuromonadales-GR-WP33-58
β− Nitrosomonadales-Nitrosomonas
α− Rhodobacterales-Rhodobacter
α− Rhodobacterales-Other
Plancto-Phycisphaerales-SM1A02
γ− Alteromonadaceae-Marinobacter
B.d.-Flavob-Owenweeksia
B.d.- Cyto-Flexibacter
γ− Oceanospirillales-Alcanivorax
B.d.- Flavob-Aequorivita
γ− Alteromonadales-Colwellia
γ− Alteromonadales-BD1-7 clade
B.d.-Flavob-Other
B.d.- Flavob-Brumimicrobium
B.d.-Sphingo-Aureispira
Nitrospirae-Nitrospira
γ− Alteromonadales-Glaciecola
γ− Francisella
α− Rhizobiales-Ahrensia
Plancto-Rhodopirellula
α− Rhizobiales-Parvibaculum
γ− Xanthomonadales-JTB255 MBG
B.d.-Flavob-Muricauda
Ve- Verrucomicrobiales-Persicirhabdus
α− DB1-14
B.d.-Cyto-Leadbetterella
B.d.-Flavob-Lishizhenia
B.d.-Flavob-Lutibacter
B.d.-Flavob-Flavobacteriaceae
B.d.-Flavob-NS9 marine group
Planctom- Pirellula
α− Sneathiellales-Sneathiella
δ− Myxococcales-Nannocystaceae
γ− Alteromonadales-Idiomarina
γ− Other
γ− Pseudomonadales-Psychrobacter
Ve- Verrucomicrobiales-Rubritalea
Actinob-Corynebacteriales-Mycobacterium
BD1-5
B.d.- Cyto-Cytophaga
B.d.- Cyto-Ekhidna
B.d.- Cyto-Flexithrix
B.d.-Cyto-Reichenbachiella
B.d.- Flavob-Crocinitomix
B.d.- Flavob-Fluviicola
B.d.-Flavob-Kordia
B.d.-Flavob-Maribacter
B.d.-Flavob-Mesonia
B.d.-Flavob-Polaribacter
B.d.-Flavob-Tenacibaculum
B.d.-Flavob-Ulvibacter
B.d.- Flavob-Flavobacterium
Clostridia-Fusibacter
Fusobacteria-Psychrilyobacter
Gemmatimonadetes-BD2-11 TG
Plancto-028H05-P-BN-P5
Plancto-BD7-11
Plancto-Blastopirellula
AEGEAN-245
α− Rhizobiales-Maritalea
α− Rhizobiales-Cohaesibacter
α− Rhizobiales-Hoeflea
α− Rhodobacterales-Pseudovibrio
α− Rhodobacterales-Roseobacter NAC11-7 clade
α− Rhodobacterales-Roseovarius
α− Rhodospirillales-Thalassospira
α− Rickettsiales

α− SB1-18
α− Kiloniella
α− Sphingomonadales
α− Sphingomonadales-Sphingomonas
δ− Bdellovibrio
δ− Myxococcales
δ− Myxococcales-Sandaracinaceae
δ− Desulfovibrio
ε− Campylobacterales-Arcobacter
ε− Campylobacterales-Sulfurimonas
γ− Other-Other
γ− Alteromonadales-Alteromonadaceae
γ− Alteromonadales-Dasania
γ− Alteromonadales-OM60(NOR5) clade
γ− Alteromonadales-Thalassomonas
γ− Alteromonadales-Moritella
γ− Alteromonadales-Psychromonas
γ− Alteromonadales-Shewanella
γ− Chromatiales-Other
γ− Chromatiales-Thioalkalispira
γ− Order Incertae Sedis-Arenicella
γ− Pseudomonadales-Acinetobacter
γ− Thiotrichales-Pisciricketsiaceae
γ− Aliivibrio
γ− Oceanospirillales-CrystalBog021C3
γ− 34P16
Ve- Puniceicoccales-Pelagicoccus

Ve; *Verrucomicrobia*, B.d.: *Bacteriodetes*, Flovob-; *Flavobacteria*, α-β-,γ-, ε-; *Proteobacteria*, Plancto; *Planctomycetes*.
* ; Skin wound, s.n.w; Skin next to wound.; d.no.w; dead fish no wounds, hf; healthy fish.

>50%	20-50%	10-20%	5-10%
2.5-5%	1-2.5%	0.5-1%	
0.05-0.5%	<0.05%		

period. Similar to the RAS total situation (Figure 3A), there environmental taxa fluxed more in and out of the biofilter tan inoculums associated taxa. However, the biofilter biofilm taxa flux were in stronger phase with the RAS events than the RAS total situation. The biofilter biofilm taxa flux out were 17-22 upon the three RAS events pH/salinity drops, dominance of the *Myxococcales* in biofilm and changes in feed oil components giving yellow water formation (Figure 3B). This was counted between the sampling times C2W8-C2W13, C3W4-C3W8, and C4W2-C5W10. The biofilter biofilm taxa flux in was 22-30 upon the RAS events antibiotics addition, wash I and LOZ treatment as well as re-inoculation of the biofilter. The sampling times cowering these events were C2W3-C2W8, C2W13-Wash I-C3W4.

Water taxa numbers compared to biofilter biofilm taxa numbers. Biofilter biofilm and water had in mean the same number of taxa over all sampling times, i.e. 150±37 and 149±32, respectively. However, water had in mean more environmental taxa (86±24) than inoculums associated taxa (64±14), differing from the biofilter biofilm. To investigate further this finding, the taxa in the water and biofilter were compared for their times of appearance at the different sample times. The eight sampling times where water and biofilter biofilm taxa were compareble were: C2W3, C2W8, C2W13, Wash I, C3W4, C3W8, C4W2 and C5W10.

A) RAS total taxa number

Sample time: C2W3 C2W8 C2W13 Wash I C3W4 C3W8 C4W2 C5W10

Taxa in and out of RAS: 28 1 10 10 7 13 3 1 2 4 1 7 3 6 1 3 5 1 4 3 2 5 5

Inoculums associated taxa: 65 93 102 99 86 88 87 92 6

Environmental taxa: 80 104 116 140 114 151 138 140 6

15 39 5 16 25 8 5 12 28 4 9 14 19 7 5 3 7 12 27 10 10 7 22 4 10 1 1 4 14 8 8 11 5

Re-appearing taxa

B) Biofilter biofilm total taxa number

Sample time: C2W3 C2W8 C2W13 Wash 1 C3W4 C3W8 Wash 2 C4W2 C5W10

Taxa in and out of RAS: 2 32 2 22 2 18 16 7 1 6 5 4 15 7 1 1 3 10 2 1 6 6 5 1 9 10 3 2 2 1

Inoculums associated taxa: 49 79 57 80 82 62 78 84 67 4

Environmental taxa: 41 68 51 84 93 65 91 116 72 6

6 34 8 19 8 2 1 24 2 8 2 17 20 8 11 16 10 5 4 7 2 14 17 4 12 21 7 3 3 3 23 26 3 3 1 1 1

Re-appearing taxa

Figure 3. RAS and biofilm total taxa number and the fluxing taxa numbers as given by microbial deep sequencing at the different time points. Taxa fluxing out after only one observation are indicated by ⌐⌐, and if re-appearing later this is depicted by the dotted lines. Time point notifications: C; cycle, W; week, ☐; inoculum asociated taxa re-inoculated, ☐;environmental taxa re-inoculated. ● RAS events: 1. Antibiotics addition, 2. Wound outbreake peaking, 3. Steepest salinity drop in study, 4. Steepest pH drop in study, 5. Wash I and biofilter LOZ treatment, 6. Re-inoculation of the biofilter, 7. *Myxococcales* dominationg the biofilter biofilm, 8. Changes in feeds oil components and yellow water formation, 9. Dominance of *Nitrospira* in biofilter biofilm and salinity drop.

Taxa observed 8 times were largely inoculums associated and common between the water and the biofilter (Figure 4A). r. In contrast, the environmental taxa were over-represented in the

fraction of taxa that were counted only once, from now named "1-taxa" (Figure 4A). There were just as many "1-taxa" in both the biofilter and water samples, and only a few taxa were common between the two environments. However, taxa observed 2-5 times were more abundant in the water than biofilter biofilm.

We further elucidated this by investigating the tendency of non-shared taxa to occur more or less in the opposite environment (Figure 4B). Non-shared inoculums associated taxa
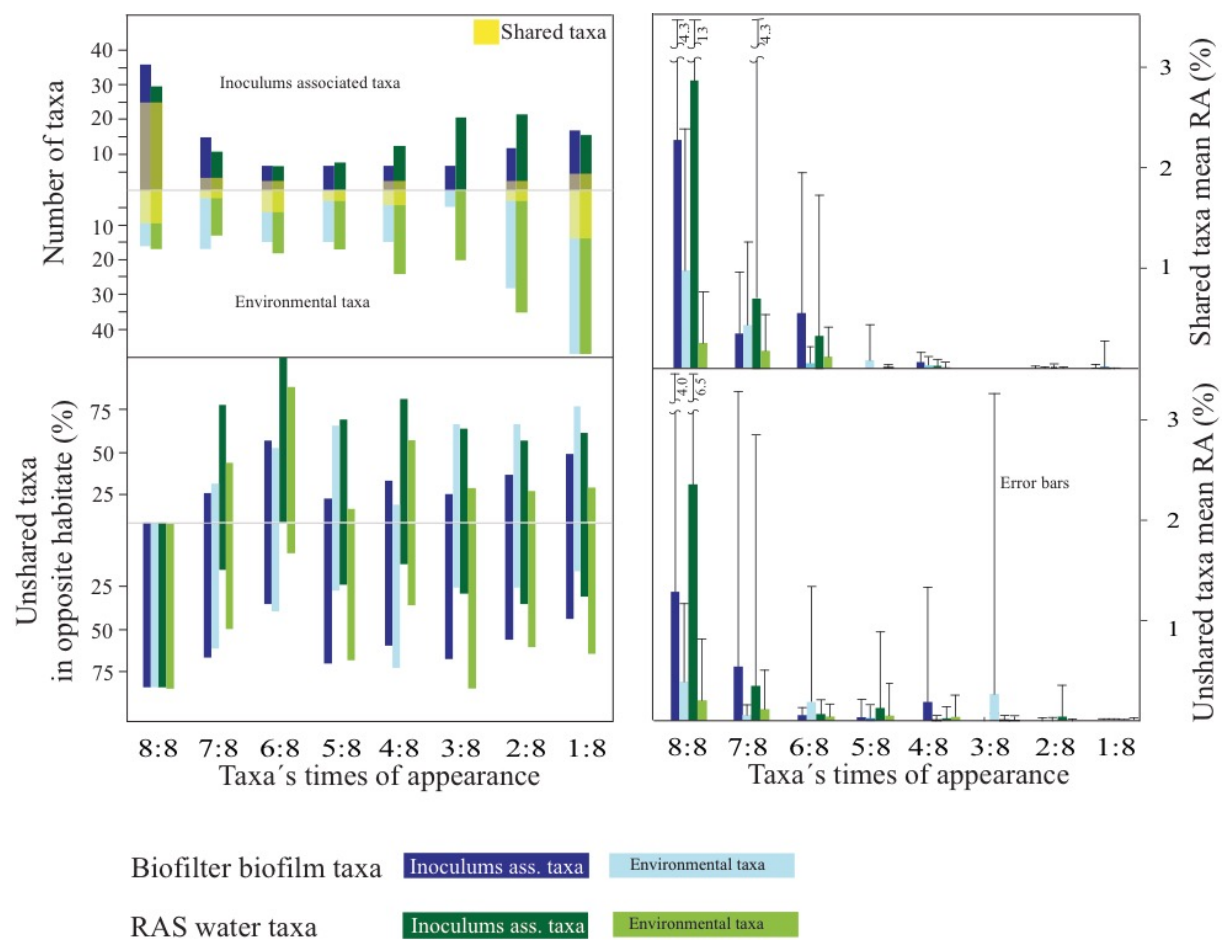
Figure 4. Comparing biofilter biofilm and water taxa by times of appearance, taxa shared and relative abundance (RA). The 8 sampling times with comparable deep sequencing data were C2W3, C2W8, C2W13, Wash I, C3W4, C3W8, C4W2, C5W10. A; Times of appearance of biofilter biofilm and tank 3 water taxa, B; The tendency of the unshared taxa to occure more or less in the oposite sampling site, C and D; The mean relative RA of shared (C) and unshared (D) biofilter biofilm and water taxa.

that were observed 2-7 times in the biofilter biofilms were less frequently seen in the water, whereas environmental taxa observed 1-3 times in the water tended to be rarer in the biofilter. However, water taxa observed 6-7 times in the water clearly tended to occur more frequently in the biofilm, thus, being permanently established there. Un-shared environmental "1-taxa" were equally numerous in the biofilter and water, but 50% of the biofilter biofilm "1-taxa" were detected >1x in the water, while <25% of environmental "1-taxa" in water were detected more frequently in the biofilter biofilm. Thus, the likelihood for environmental taxa to be observed in the biofilter 1x increased with the residence time of the taxon in the water.

Wall biofilm and fish skin total taxa numbers. The wall biofilm total taxa numbers were in mean fewer (124 ± 25) than the water and biofilter biofilm over the sampling times, and the even distribution between inoculum associated (62 ± 10) and environmental (62 ± 18) taxa resembled the biofilter biofilm. Most taxa fluxing in to the biofilter biofilm were also detected on the tank biofilm over the sampling times, but the residence time of the incoming taxa was normally shorter than in the biofilter. This pattern was observed for both inoculums associated and environmental taxa. Fish skin total taxa number resembled the production water situation, as in mean more environmental taxa (60 ± 8) were detected than inoculums associated taxa (47 ± 14). Fish skin samples had 107 ± 6 taxa in mean over the sampling times, that was lower than at the other sampling sites.

The RAS total taxa numbers distribution between the four sampling sites the first sampling time confirmed the enhanced abundancy of environmental taxa in the production water (Figure 5).

Taxa flux after LOZ treatment and re-inoculation between PC2 and PC3. The effect of the RAS events biofilter biofilm LOZ treatment and re-inoculation upon microbial community shifts was studied on basis of the Wash I and C3W4 samples. Re-inoculation involved the addition of new biofilm carriers pre-soaked in starting culture to the biofilter
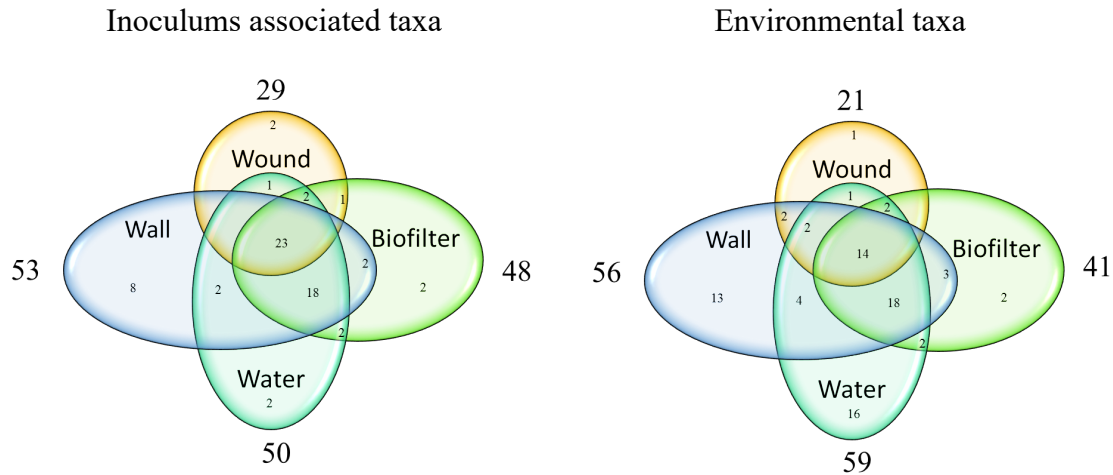
Figure 5. Distribution of taxa among sampling sites in the RAS at the first sampling time (C2W3), as given by microbial deep sequencing. Colored circles represent the sites and their reported taxa numbers in total are noted outside the circles . The shared taxa between sites are shown by overlapping circles

chamber. Samples were taken from the biofilter biofilm, wall biofilm and water shortly prior to fish stocking of PC3. For the RAS as a whole, the taxa flux in of inoculum associated taxa was low (7), and for the biofilter biofilm, in the normal range (23), thus un-effected by the re-inoculation (Figure 3A and 3B). However, the taxa flux in of environmental taxa, was in the upper range both for the RAS as a whole (41) and the biofilter biofilm (34) normal taxa flux. The flux out of re-inoculated taxa from the RAS was examined over the next sampling times, and eight months (C5W10) later, 12 taxa were still present in the RAS. Ten of these also in the biofilter biofilm, i.e. 4 inoculums associated taxa and 6 environmental taxa.

Taxa relative abundance (RA) at biofilm and water sampling sites

The number of reads affiliating to one operational taxonomic unit (OUT or taxa) relative to the total number of reads in the library gave the relative abundance (RA) of a taxon. For sample site comparison, biofilter biofilm, wall biofilm and water total taxa were sorted into 11 different RA categories, taking into account also inoculums associated and environmental taxa (Figure 6). All the three sampling sites bulked the inoculums associated

and the environmental taxa in the categories 0.1-0.5 %, 0.01-0.05% and <0.005%. The

production water was unique by having an inoculums associated taxa in the >50% RA at 7 out

of 9 sampling times, but bulked notable more environmental taxa in the 0.01-0.05% and

<0.005% fraction than the wall biofilm and biofilter biofilm sites.   The inoculums associated

taxa distributed more even between RA categories when antibiotics were administrated the

fish through the feed, especially in the wall biofilm. This was also observed in the biofilter

and wall biofilms when *Myxococcales* dominated the biofilter as a RAS event. This included

also the environmental taxa.

Shared and unshared taxa between the water and the biofilter biofilm sites were

compared for their mean RA (Figure 4 C and D). Inoculums associated taxa revealed in 8 of

8 possible cases where by fare the dominating bacteria whether shared or not, whereas

environmental taxa that appeared only once, had very low RA in mean.   The "1-time" taxa

peaked at the sampling times C2W8 and C4W2 in the biofilter biofilm, and at C5W10 in the

water. In 92% of the cases their RA was bellow <0.05% and in 40% of the cases <0.005%

RA. The highest RA registered by a "1-taxa" was 1.1%, obtained at time C4W2 in the

biofilm biofilter by an environmental taxon. High standard deviations of the taxa appearing 2-

7 times revealed that dominating bacteria did occur sporadic also in these fractions, especially

among the unshared taxa.

Bray-Curtis dissimilarity

The dissimilarity in the microbial community structures between the different sampling times

and sampling sites was investigated by all samples cluster analysis (Bryan-Curtis), that

accounted both for RA and presence or absence of taxa (Figure 7).   The analysis
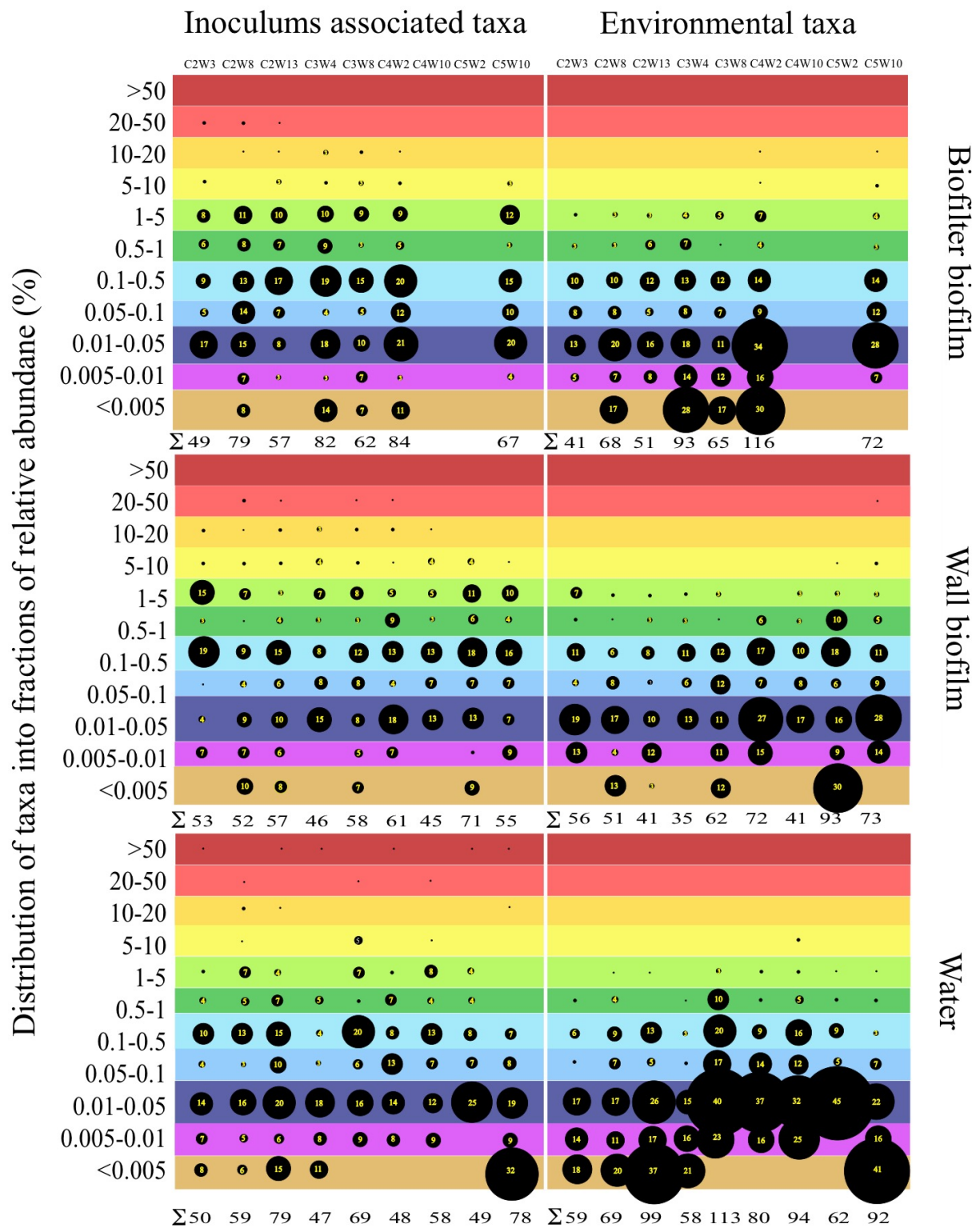
Figure 6. RAS total taxa number divided into 11 categories of relative abundance and sorted by sample time and sampling site. The bobble size is related to the number of taxa it represents.
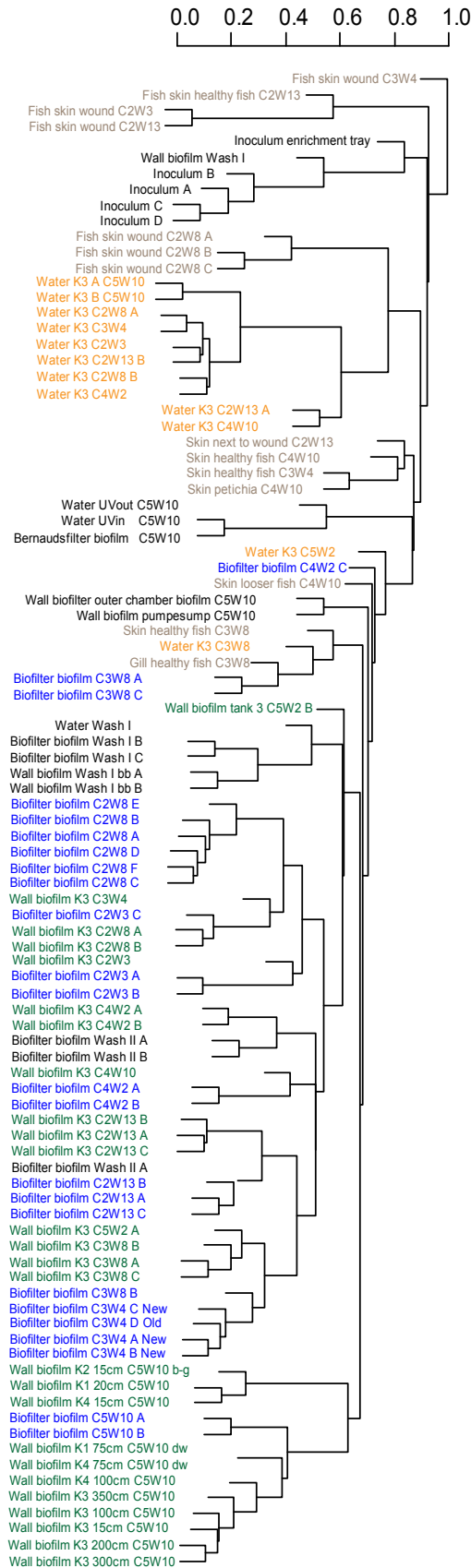
Figure 7. All samples cluster analysis. Based on the OTU`s and their relative abundance, using the Bray-Curtis matrix and Ward algorithm for hierarchical clustering. hf; healthy fish, bb; black boarder, b-g; black-green boarder, dw; dead water zone, K1-4; tank 1-4.

demonstrated a general strong similarity between the biofilter biofilm and the wall biofilm in time and space, whereas water samples largely gathered in its own cluster. The biofilm-wall main cluster was thus largely unaffected by the RAS events as significant shifts in physiochemical parameters and administration of antibiotics to the fish through the feed. The only RAS event that brought biofilm biofilter samples out of the biofilter-wall main cluster were the situation when *Myxococcales* dominated the biofilter biofilm (Figure 7). Out of the main cluster these samples were most like fish skin and water samples from the same sampling time (C3W8). In general, fish skin samples, wounded or not, clustered closer to water samples than the biofilm samples. Though, a mixed group of four skin samples made the most distant group in the cluster. The cluster analysis implemented also the extra ordinary samples. Between PC2 and PC3 the RAS was washed and the biofilter was LOZ treated and re-inoculated. Biofilter biofilm and water samples from Wash I gathered outside the large biofilm-wall cluster, forming a cluster along with a wall sample taken from a black border in the splash zone at the same sampling time. The normal tank 3 wall biofilm site at Wash I clustered along with the inoculum samples. After fish stocking, at sampling time C3W4, biofilms and water samples returned to the pre-washing situation where water and biofilter samples were separating in two distinct clusters. The Wash II event between PC3-PC4 did not cause notable changes in the microbial community structure of the biofilter. At the sampling time C5W10 a more thorough sampling was undertaken all the four rearing tanks. The biofilm and wall samples made a "sister" cluster on their own in the denogram, demonstrating some population shift compared to the other cycles. Also, the water sample clustered outside the large water cluster, closer to the water sample at time C3W8. Unique biofilm samples from the biofilter outer chamber and the "pump sump" wall clustered closely, but outside the large biofilter-wall clade, closer to biofilm from the water inlet tube (Bernaud filter) and water inlet and outlet of the UV-filter.
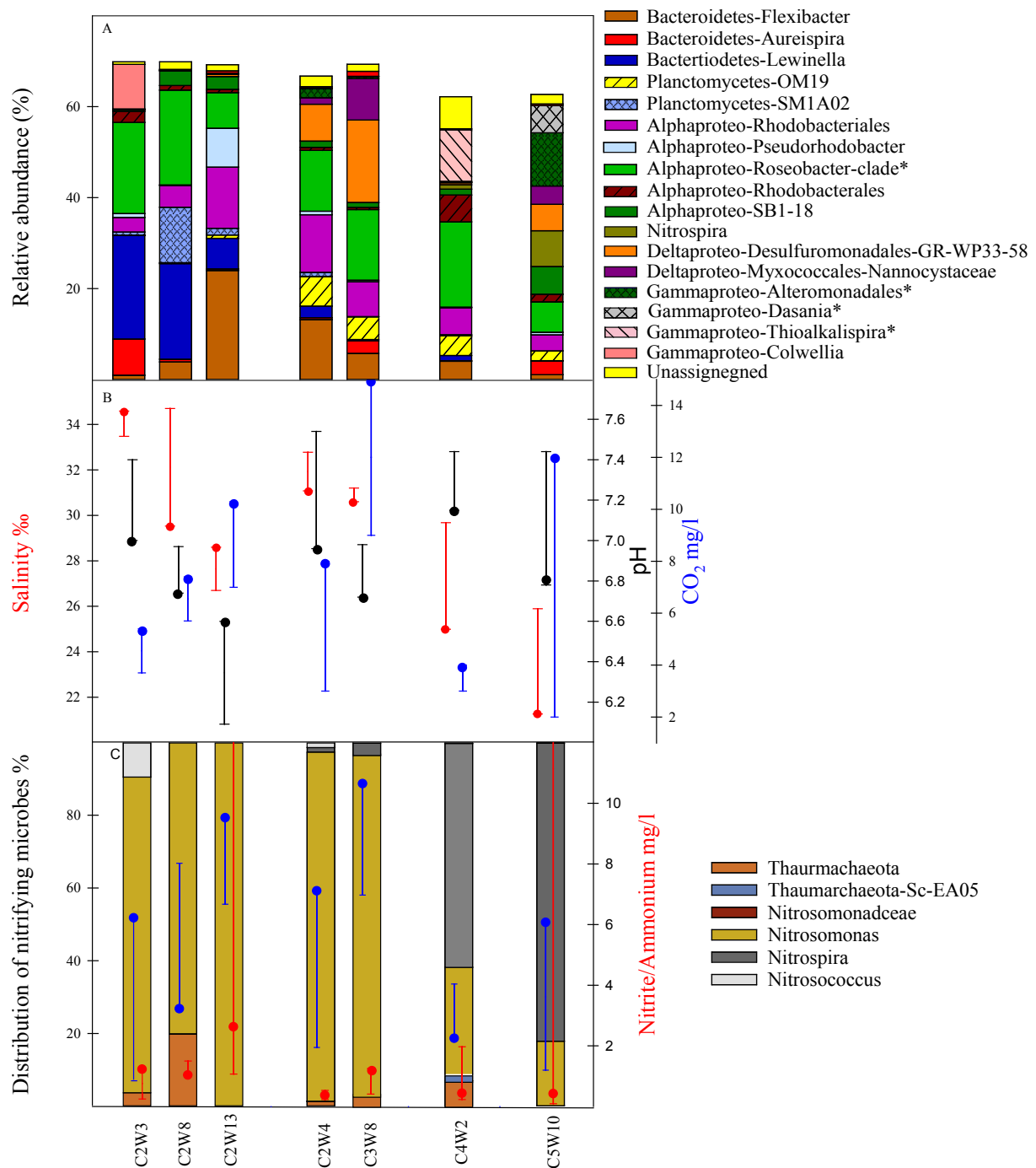
Figure 8. Dominating and nitrifying bacteria in the RAS biofilter during changing physiochemical situations. A; Taxa with relative abundance ≥5% once or several times during four rearing cycles (PC2-PC5) bacteria in the biofilter selected on their relative abundance. Dots notes measured values the sampling day, whereas lines marks highest and lowest registered value since previous sampling time point. B;. pH, CO_2 and salinity. C; Nitrifying microbes and their relative population distribution along with nitrite and ammonium concentrations. *;Environmental taxa.

Dominating microbes in the biofilter biofilm and the water

Taxa with >5% RAS were termed as dominating. Totally 18 biofilter taxa were recognized in this fraction during PC2-PC5, and 13 out of these taxa were inoculums associated. Most prevailing were four different strains of *Rhodobacteriales* (α-*Proteobacteria*), and one out of these was permanently in the >5% fraction, independently of the changing RAS events (Figure 8A and B). The genera *Flexibacter* (*Bacteriodetes*) was also present in high relative abundance at all timepoints, being strongest when pH was low (Figure 8B). In general, *Bacteriodetes* taxa were prevailing in the system, especially the first cycles. Together, the *Rhodobacteriales* and *Bacteriodetes* taxa made up > 60% of the RA in PC2 and PC3. Notable, the >>5% fraction declined by time (Figure 3A). The Phylum *Planctomycetes* was also abundant during PC2-PC5 and peaked along with the fish skin wound outbrake in PC2, and two taxa reached the >5% fraction during the experimental period. The δ-*Proteobacteria* was dominating by a taxon from *Desulfuromonadales* and a taxon from *Myxococcales*, each reaching the >5% fraction firstly in PC3. Three environmental taxa that affiliated to the γ-*Proteobacteria* were dominating during the last two cycles. One of these, the genus *Thioalkalispira*, did have a very strong onetime appearance in the >5% fraction at C4W10. The other two affiliated to the order *Alteromonadales*. Other genera with onetime appearance in the >5% fraction was *Aureispira*, *Pseudorhodobacter* and *Nitrospira*. The latter bloomed during PC4 and PC5 when salinity declined (Figure 8A and B). Interestingly, a taxon not possible to classify at all was successful in the biofilter all through production cycles PC2-PC5.

The water samples were largely dominated by one taxon, the genus *Colwellia* (γ-*Proteobacteria*), having in mean 50±36% RA. Two major RA drops down to 5% and 0.6% were observed for this taxon at the times C3W8 and C5W2 (Table 3). At timepoint C3W8, the inoculums associated taxa; *Francisella* (27%), *Rhodobacteriales* (8%),

Desulfuromonadales (7%), *Glaciecola* (8%), and *Myxoccocales* (5%) were the dominating water taxa. The *Rhodobacteriales* taxa was the one being dominating in the biofilter biofilm at all sampling times. The second main drop in the *Colwellia* taxon RA was seen at the sampling time C5W2, and this time the very *Rhodobacteriales* taxon replaced *Colwellia* almost in sole (78%). The second most common taxon in the production water at this sampling time was the *Owenweeksia* (*Flavobacterium*, 4.9%). The *Colwellia* taxa did also do minor drops at time C2W4 and C4W10, and again taxa common in the biofilter biofilm increased their RA in the production water. Notable, several environmental taxa were prominent in the water at C4W10, i.e. an uknown γ-*Proteobacteria* (7%), *Kiloniella* (*Shingomonadales,* 6%) and the *Hoeflea* (*Rhizobiales,* 4%). Most prominent still (7.5%), was the unassigned but inoculums associated taxa that was also ubiquitous in the biofilm. Despite the generally strong dominance of *Colwellia* in the production water, the reminding bacterial community was fare more unstable than the biofilm community. Still, 42 of the water taxa were detected at all the 10 sampling times, including the Wash I sample collected prior to fish stocking.

Biofilter biofilm and water taxa that were shared at both sampling sites 8 out of 8 times, where compared for their mean RA (Figure 9). The shared biofilter biofilm taxa had in mean 3.8 times higher RA than equivalent water taxa. Out of the 35 shared taxa, 11 had RA > 5% once or more during the experimental period. Biofilter biofilm taxa exceeding this RA value where largely *Rhodobacteriales* and *Bacetroidetes* strains.

Dominating bacteria on the fish skin

Environmental taxa dominated in fish skin wounds, being mainly *Aliivibrio* at sampling time C2W4 (94%) and C2W13 (99%) or *Moritella* (97%) at C3W4 (Table 3). At
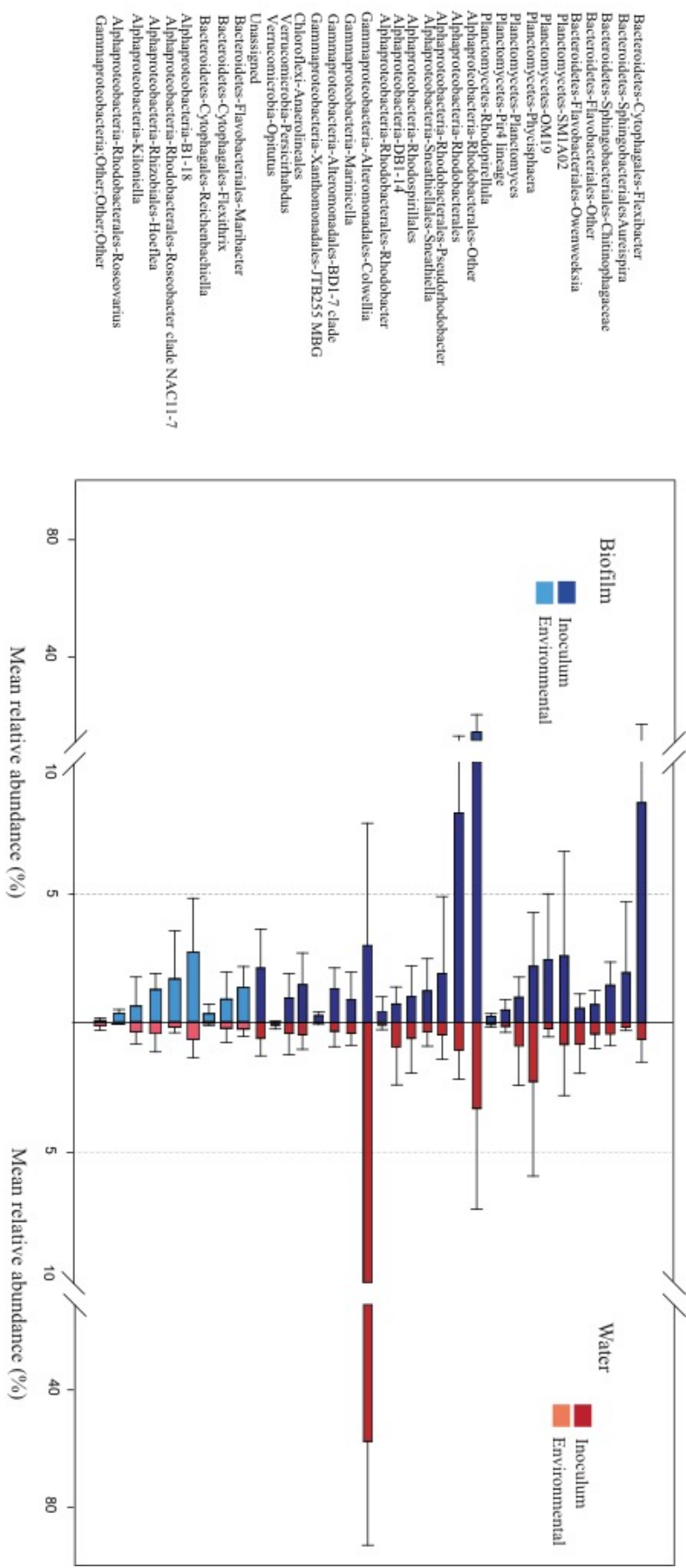
Figure 9. Mean relative abundance of shared taxa identified in both biofilm and water at 8 sampling times. Standard deviation is shown with error bars

time C2W8, when the wound outbreake was peaking and antibiotica administrated the fish by the feed, *Arcobacter* (42%) and *Tenacibaculum* (18%) dominated in the wound. Inoculums associated taxa dominated in fish skin otherwise, though *Aliivibrio*, *Moritella* and *Arcobacter* where still common. Dominating inoculums associated bacteria were *Psychrobacter* (9-15%), *Aureispira* (12-20%), *Glaciecola* (8-14%) and *Colwellia* (6-10%). At time C3W8, when *Myxococcales* dominated the biofilter biofilm, *Lishizenia* (10%), *Rhodobacteriales* (10%), and *Psychrobacter* (9%) were detected on skin, whereas *Myxococcales* (12%), and *Desulfuromonadales* (9%) was dominating in the gill sample.

Nitrifying bacteria in the RAS biofilter biofilm

A taxon within the genus *Nitrosomonas* made up 80-100% of the nitrifying population in the biofilter during PC2 and PC3. This ammonium oxidizing genus had in mean $1.0\pm0.4\%$ RA during PC2-PC5, with max. and min. RA of $1.7\pm0.3\%$ (C5W10) and $0.2\pm0.1\%$ (C2W3). A taxon with the nitrite oxidizing genus *Nitrospira*, was observed in the RAS biofilter for the first time 4 weeks into the third breeding cycle (C3W4), thus after the re-inoculation step (Figure 8C). The same sequence was detected in 2 of 5 parallel samples with the start culture (inoculum), and then at very low RA. The *Nitrospira* taxon increased significantly the RA in the biofilter biofilm during PC4 and PC5. At the final sampling time point C5W10 the total nitrifying taxa were distributed into 18% *Nitrosomonas* and 82% *Nitrospira*. This corresponded to 9.6% RA of the two taxa in the biofilter. The *Nitrosomonas* and *Nitrospira* taxa were also associated with the wall biofilm, although delayed in time of appearance and at lower RA than in the biofilter biofilm. The nitrifying *Archaea* identified in the commercial inoculum was only present in very low RA in the biofilter biofilm (Table 1), but detected at higher RA once, in a darkly colored biofilm sampled from the splash zone after re-

inoculation. When detected in the biofilter or the wall, these *Archaea* taxa were always along with the taxa *Bradyrhizobium*, *Cand. Chloracidobaterium* and a *Cyanobacteria*.

Microbial shifts when *Myxococcales* dominated the biofilm

The microbial shift when the taxon *Nannocystaceae* (*Myxococcales*) were dominating in the biofilter biofilm at sampling time C2W8, is summarized in Figure 10. Most classes and taxa where reduced 4-fold from time C2W4 into C2W8, whereas ~13 taxa were selected and ~17 were extincted.
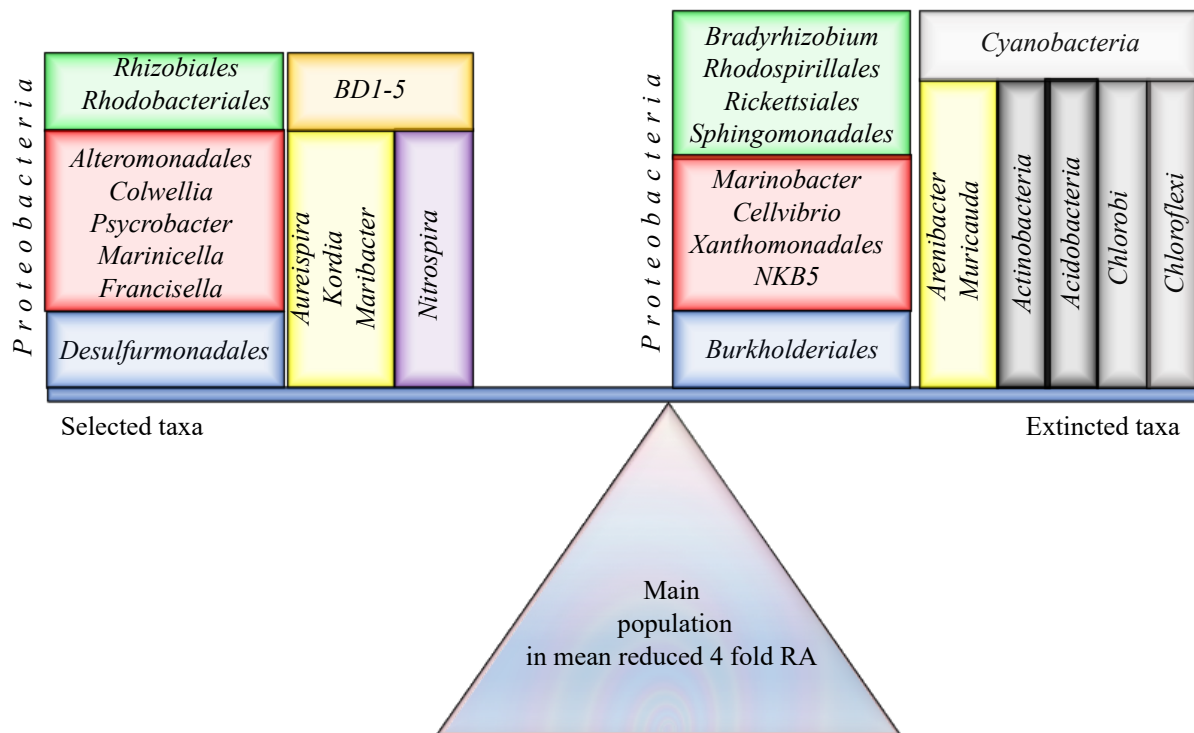
Figure 10. Taxa shift from time C3W4 to C3W8 in the biofilter biofilm, a period with dominance of *Myxococcales*. Yellow color denotes *Flavobacteria*. BD1-5; non-cultured group of *Bacteria*, RA; Relative abundance.

Diversity indexes based upon OTU data

Simpson and Shannon's diversity indexes include both specie richness and evenness, and were calculated based on the 16S library data (Figure 10). A higher value represents
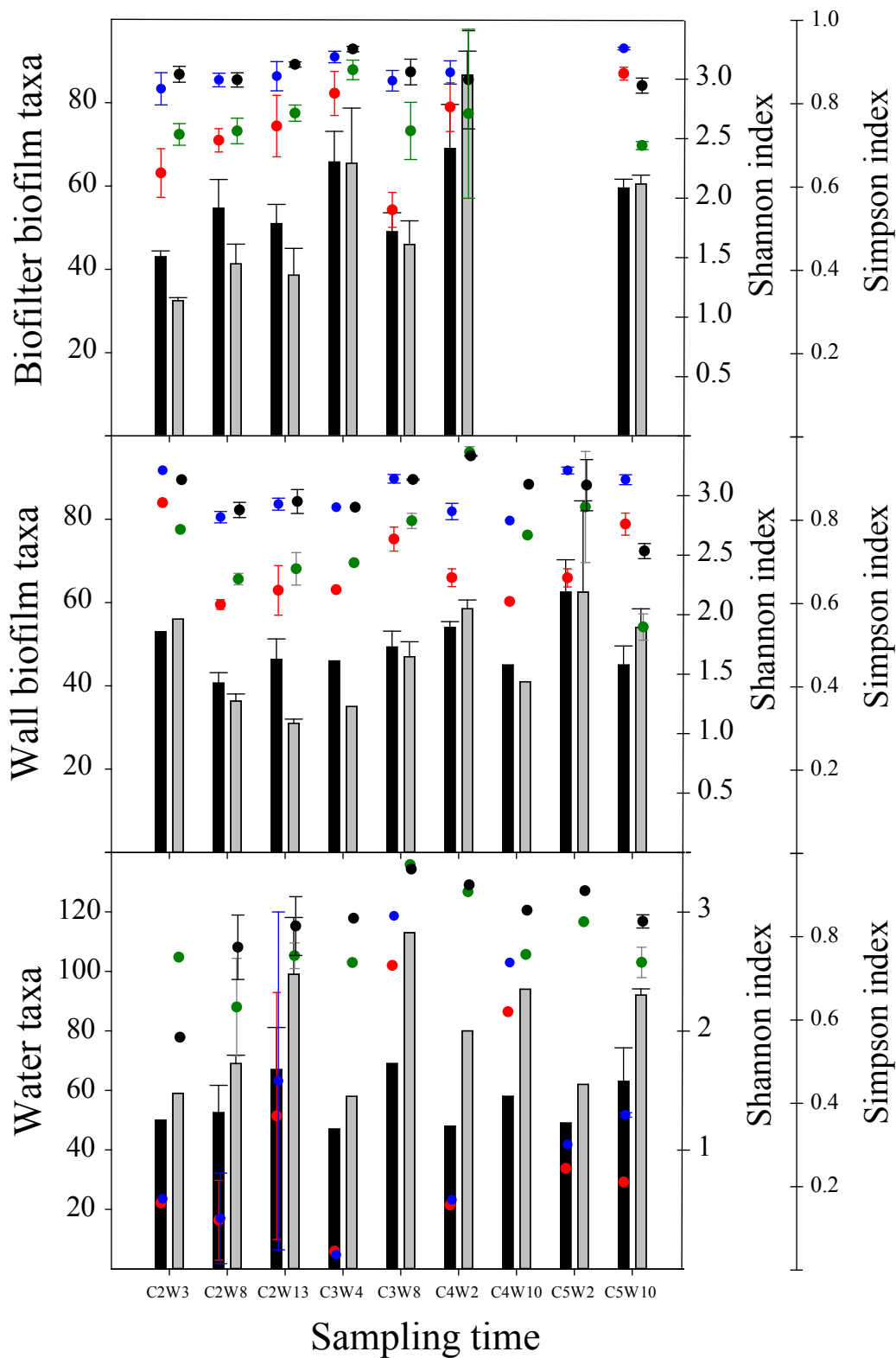
Figure 11. Shannon and Simpson diversity indexes in RAS biofilter, wall and water, based on 16S library data from four production cycles. ■; number of inoculums associated taxa, ▨; number of taxa with environmental origin. ●; Shannon index of inoculums associated taxa, ●; Simpson index for environmental taxa. ●; Shannon index for environmental taxa, ●; Simpson index of inoculums associated taxa. Bars shows the standard deviation.

higher diversity. The indexes differ in that the Simpson index is more weighted on the

dominating bacteria. The biofilter biofilm, wall biofilm and water indexes for environmental

and inoculums associated taxa at the different sampling times are presented together with the

average taxa number in Figure 11.

Biofilms. The Simpson index was stable for both the environmental and the inoculums

associated fraction of the biofilter biofilm community during PC2-PC5, with values in the

range 0.8 and 0.9 with one exception. The wall biofilm was slightly in opposite phase with

the biofilter, still operating in the 0.8-0.9 range. The Shannon index increased steadily in both

the inoculums associated and environmental taxa fractions of the biofilter biofilm from time

C2W3 until time C3W4 (Figure 10). At time C3W8, there was a sudden fall in the index, in

particular in the inoculums associated fraction (0.82 to 0.55). The index values were back to

C2W13 level at the next sampling point. The Shannon index pattern was slightly different for

the wall samples, mainly following from a drop in the index values from the first and the

second sampling timepoint. The index drop in the biofilter biofilm at time C3W8 was not

observed, but at time C4W2 the value droped for the inoculum fraction and increased to its

maxima value for the environmental fraction (0.88).

In the production water. Both indexes showed large differences between the fractions

of environmental and inoculums associated taxa in the production water. The index values

for the inoculums associated taxa were erratic between the sampling times, being most <0.6,

but peaked >0.6 at time C3W8 and C4W10, strongly correlated to the RA of the taxon

Colwellia (see above). The index values for environmental taxa in the production water were

fare close to the wall biofilm and biofilter biofilm sampling sites, though the Simpson value

was low the first sampling time (C2W3). Another difference was the sampling time for

reaching the highest Simpson value.

Variance equality between the wall and the biofilter

The general similarity between the wall and the biofilter shown by the cluster analysis was further investigated by several F-tests to verify equality in taxa variance between the two populations. The null hypothesis anticipated equality between two normally distributed populations. First, a sample set of six parallel biofilm carriers from pre re-inoculation (C2W8) were tested against a set of 4 parallel biofilm carriers from post re-inoculation (C3W8). Means were 70 and 93 OTU´s respectively with corresponding variances σ^2_{C2W8}=68 and σ^2_{C3W8}=226, giving $F > F_{crit}$ one-tail and rejection of the null hypothesis. Secondly, two set of triplicates from wall (C2W8 and C2W13) were compared with two set of biofilter triplicates at the same time points. Both sampling points were pre re-inoculation. Again the null hypothesis was rejected ($\bar{x}_{carrier}$=64, $\sigma^2_{carrier}$=269, $\bar{x}_{wall}$=57, σ^2_{wall}=14). In a third variance comparison, 12 biofilter samples from post re-inoculation, including replicates, were compared with 20 wall samples post re-inoculation. The latter included also a depth profile of the tank K3 (25, 100,200,350 cm) and 25cm and 100 cm profiles in the other breeding tanks at sampling point C5W10. The F ratio was now lower than prior to re-inoculation ($\bar{x}_{carrier}$=91, $\sigma^2_{carrier}$=413, $\bar{x}_{wall}$=73, σ^2_{wall}=134), but still the null hypothesis was rejected. Thus, statistical analysis stated that biofilm carriers had a larger OTU variance than the wall.

Correlation between parallel samples

Six triplicate sample set from wall and biofilter at the time points C2W8, C2W13 and C3W8 were compared to illuminate methodical biases. In mean ($\bar{x}$), 86 ±10 ng/µl wall DNA and 74±35 ng/µl biofilter DNA was obtained by the kit. The upper and lower yield was 2.4 ng/µl and 121 ng/µl for the samples to be compared, and only two samples made up the extremes. All distinct OTU´s in the data set (S) of three parallels were summarized. Taxa in

S were sorted by their occurrence in 3 out of 3, 2 out of 3 and 1 out of 3 parallels, and the percentage distribution was as follow: $\overline{x}_{3of3\ wall}$=59.0±5.4%, $\overline{x}_{2of3\ wall}$=14.9±4.0%, $\overline{x}_{1of3\ wall}$=26.1±3.3%, $\overline{x}_{3:3\ biof}$=58.6±4.0%, $\overline{x}_{2:3\ biof}$=16.5±3.8%, $\overline{x}_{1:3\ biof}$=24.9±6.4%. This consistency in percent distribution between wall and biofilter samples, was further evaluated towards the relative abundance. A pattern was seen, as 3 out of 3 successes were only obtainable for taxa with relative abundance ≳0.015%, 2 out of 3 cases in the range 0.006-0.02% and 1out of 3 cases when fivefold lower than this again. The probability that any sequence in one sample was revealed in a parallel was thus ~77%.

The sorted data set S were further divided into the origin of the strains, either from the environment or by the inoculum. As much as 30% of environmental taxa occurred only in 1 parallel in both wall and biofilter samples, whereas corresponding for inoculated taxa were 18% in the biofilter and 0.5% in the wall.

The correlation between replication success and relative abundance was further examined by statistical correlation tools. Totally 8 categories were correlated i.e. sampling site (wall, biofilter), occurrence in parallels (1 to 3) and origin of the strains (inoculum, environmental). The relative abundance of affiliating taxa was summarized for each of the 8 categories. Summed relative abundance made up one matrix, and were aligned towards matrix 2, contain OTU´s percent distribution into 1, 2 or 3 parallels. The correlation coefficient for the data set was 0.83, that indicated a positive correlation.

DISCUSSION

Salmon smolt RAS fresh water technology was transferred to marine post smolt RAS, but the water salinity shift was challenging, and the initial cycle was terminated. The N-

species and physicochemical parameters were monitored regularly in the production water following the requirements of the Norwegian Food Safety Authority. Prior to RAS operation, the biofilm carriers were colonized by microbes from a commercial start culture (inoculum). To better understand the microbial events in the RAS, deep microbial sequencing was used to monitor the RAS from the second production cycle (PC2) to the fifth (PC5). The most important events in the RAS during the survey were rapid changes in physicochemical parameters, antibiotic treatment of fish, washing and use of chemicals between cycles, biofilter re-inoculation and dominance of of *Myxococcales* in the biofilter biofilm.

RAS biofilter biofilm colonization versus general colonization of surfaces in marine water

Inoculums associated taxa were dominating in the biofilter biofilm the three first cycles of the survey, and at all sampling times, Rhodobacteriales taxa dominated as a group, having 20-30% RA (Figure 8).   One member, *Rhodobacteriales*-others, was at >5% RA over the entire experimental period, demonstrating an impressive adaptability towards alternations in salinity, pH, redox potential, alkalinity and not at least biological competitors. The role of *Rhodobacteriales* in colonization of surfaces has been observed previously for marine costal waters and explained by their efficiency in carbon utilization, intercellular communication, the production of antibacterial components and even the ability to dispersed preestablished biofilms formed by others.[26]   No known pathogens to salmon affiliates to the *Rhodobacteriales* or α- *Proteobacteria* in general, and these bacteria are indeed wanted in fish rearing environments. In particular the genus *Roseobacter* has been used for probiotic purposes in aquaculture, and reported as a common member of RAS biofilters biofilm.[27,28]

The order *Bacteroidetes* was also prominent as group in the young biofilter biofilm, having >30% RA during PC2, but declining during PC3, and only 4.2% RA was observed in

PC5. Three taxa, *Flexibacter*, *Aureispira* and *Lewinella,* were once or more in the >5% RA

fraction. Dang et al. 2000 suggested *Bacteroidetes* as an efficient bacterial group to fill the

niche of secondary colonization.[26] Accumulation of EPS and polymers from the primary

colonizer, changes the surface properties significantly, allowing other microbes than

Rhodobacteriales to grow, and *Bacteroidetes* members are regarded as specialists for the

degradation of high molecular weight organics.[29,26]   The order of *Bacteriodetes* is associated

with native marine fish species, and alternation between environment and animal gut has been

noted.[30,31] However, a biofilter-gut relation has still to be proven for these bacteria in RAS.

Differently from the *Rhodobacteriales*, several fish pathogens affiliates to the *Bacteroidetes*.[32]

Three *Alteromonadales* (γ-*Proteobacteria*) taxa appeared once in the >5% RA fraction

of the biofilter biofilm (Figure 8).   Dang et al. 2000 assume that during primary

colonization, γ-*Proteobacteria* as a group are held down by the *Rhodobacteriales*, and if

present it is most likely the *Alteromonadales* that succeded.[1] It has been suggested that high

production of external polysaccharides forms the most important colonizing properties of

Alteromonadales species.[8] The *Alteromonadales* taxon *Colwellia* that dominated in

production water, was at >5% RA also in the biofilter biofilm at the first sampling time

C2W3. The presence of *Colwellia* in the biofilm biotope has been reported previously in the

context of high nitrate in water, and some species are even reported to utilize nitrite into

nitrogen.[9,10] The two other *Alteromonadales* taxa, (*Dasania* (6%) and an unknown genera

(12%), were environmental taxa, and dominated in the biofilter biofilm at sample time

C5W10. *Alteromonadales taxa* were also present in the biofilter biofilm in the 1-5% RA

fraction prior to PC5, represented by *Glaciecola* and the BD1-7 clade. Over all,

Alteromonadales taxa were the dominating γ-*Proteobacteria* in the biofilter biofilm during the

experimental period, increasing in RA by time, but also the order *Chromatiales* were

represented by the genera *Thioalkalispira* with 10% RA in PC4. *Thioalkalispira* is a genus

associated with sulfur cycling and is discussed in detail in a separate paper.[33]   *Bacteroidetes*

taxa declined, thus, confirming the theories about *Bacteriodetes* holding down γ-

Proteobacteria.[1] Most of the common fish pathogens affiliates to the γ-*Proteobateria* e.g.

Vibrio, *Aeromonas*, *Pasteurella, Klebsiella* and *Yersinia* and even in the order of

Alteromonadales, *Pseudoalteromonas* and *Shewanella* are genera recognized as fish

patogenes.[32]

Planctomycetes taxa totaled between 2.5-10% RA in the biofilter biofilm during the

course of study, and two inoculums associated taxa were detected in the >5% RA fraction.

This group of bacteria has recently been recognized to be among the early colonizers of

surfaces in marine water in general.[34,35] Recent genomic studies have shown the presence of

adherence genes in *Planctomycetes* members, and *Planctomycetes* species are common in

microbial aggregates.[36] They are frequently observed in RAS and industrial sewage

biofilms.[28,37,38]   Here they are doing anaerobe ammonium oxidation, anammox, oxidizing

ammonium to nitrogen by nitrite. This is slow growing bacteria and their contribute to the

total ammonia and nitrite utilization is unclear in the RAS. Their presence indicates however

an anaerobe environment in the inner of the biofilter biofilm.   In this respect, it was

interesting that these taxa were common also in the wall biofilm of the rearing tank.

Microbial shifts and diversity in the RAS during operation

Theoretical biofilm models of microbial succession have consensus on the following:

Community diversity rapidly increases in the first phase of biofilm establishment, before

dropping again in the intermediate stage when some first colonists are extinct locally.

Diversity increases again in the third phase of biofilm development. Furthermore, the total

biomass will increase with time in all phases, but cannot grow unbounded.[39] During the

experimental period the dominating bacteria changed from four taxa in the >5% RA fraction,

averaging 15% RA per taxon the first sampling time, to 6 taxa averaging 7% RA in the end, indicating an increase in diversity over time. However, the quantitative DNA yield did not increase steadily over time and was not at its highest in the end (Table 4). The development of the RAS biofilter was therefore complicated by the RAS events, and has to be discussed cycle by cycle.

Administration of oxolinic acid and florfenicol between sampling time C2W3 and C2W8 in PC2. Biofilter biofilm DNA yield and microbial diversity increased steadily over PC2. The microbial deep sequencing analysis revealed a flux in of 57 taxa in the biofilter biofilm from sampling times C2W3 to C2W8 (Figure 3B), a period where antibiotics were administrated the fish in feed to mitigate a wound outbreake. Noteworthy, the comparison of parallel biofilter samples showed that each of three parallels had 25% unique taxa. The number of parallel samples was 2 and 6 at C2W3 and C2W8 respectively (Table 3), and the difference in the taxa number could have been due to the use of parallel samples. However, "non-repeatable" taxa were largely associated with environmental taxa with low RA, and the "cut-off" for replicated detection in parallel biofilter samples was > 0.015% in present study. If we ignore the two lower RA categories (<0.01%) from the taxa distribution (Figure 6), the net flux into the biofilter was 15 inoculums associated and 8 environmental taxa, and we can conclude that the species richness increased during medication. Furthermore, a higher evenness was detected in the distribution of inoculums associated taxa between the RA categories compared to environmental taxa (Figure 6). This was consistent with a larger increase in the Shannon diversity index for the former fraction (Figure 10). The diversity indexes were calculated for each parallel biofilter sample and averaged for each time point, reducing the effect of the diversification of parallel samples.   Antibiotics in the feed are metabolized in the fish to less potent forms, but non-metabolized antibiotics will leak from the feed and feces into the water.[40] Compounds that are continuously released in RAS will

accumulate in the system, if not actively removed.[41] Antibiotics may also stay in the RAS system bound to microparticles (<60 um), and no strong oxidants with particle reducing capacity were added the rearing water at the time of medication. The biofilm matrix protects inner biofilm bacteria from antibiotics, as the antibiotic concentration will fall towards the biofilm carrying surface.[42] Peripheral biofilm bacteria are also believed to be most susceptible as they have higher metabolic activity and cell divisions.[43] Microbes with glycosphingolipids (GLS) in their outer membrane (OM) has strong hydrophobic domains, and it is anticipated that such microbes has enhanced susceptibility towards hydrophobic antibioitics.[44] The oxolinic acid and florfenicol, that were used in the RAS, are both amphiphilic and only weekly hydrophic.[45] Bacterial members of *Sphingobacteriia* and *Sphingomonadaceae* are known to have GLS in their OM. Prior to the medication of the RAS, at sampling time C2W3, *Sphingobacteriia* was readily represented in the biofilter by the ixotrophic genera *Lewinella* (21%) and *Aureispira* (8%). However, their family *Saprospiraceae* do not have GSL forming members, despite its affiliation to the *Sphingobacteriia*.[46] Interestingly, in the end of medication, at time C2W8, *Lewinella* remained it´s RA. whereas *Aureispira* was reduced ten times. These taxa differs in that *Aureispira* produces large amounts of arachidonic acid (AA).[47,48] AA has been studied in the context of antibiotic resistance combatment, i.e. facilitated transport over OM, but clear answers are lacking.[49,50,51] A recent study indicates that an alginate derivative represented the sticky sub- stance present in the initial step of the ixotrophy of *Aureispira*.[47] Flagellated bacteria that are selectively captured in this matrix are further preyed via enzymatic lysis.[46] Alginate is important in marine bacteria's attachment to pristine surfaces as well as the biofilm matrix in general.[52,53] A constructed alginate oligomere potentiated a broad specter of antibiotics significantly when influencing the biofilm matrix.[54] *Colwellia* was the second taxa markedly reduced in the biofilter after the medication, and genera members are known to

produce PUFA and AA-like fatty acids.[55,56] *Colwellia* is not reported to be predated by

ixotrophy, but are among the marine microbes associated with alginate lyase activity.[57] Thus,

an entailing thought is that *Colwellia* was living in the viscinity of *Aureispira* and *Lewinella*,

and that the combination alginate/AA enhanced the antibiotic potency to *Colwellia* and

Aureispira versus *Lewinella*. Their collective disappearance was also seen in the wall

biofilm. *Colwellia* was not influenced by the medication in the water, strengthening the

assumption that the eradication seen in the biofilms was directed. The *Aureispira* and

Colwellia taxa were tightly connected and dominating in skin samples after medication (not

wound), making fish mucus as the main reservoir of *Aureispira* in the RAS (0.7-20%). Two

more taxa followed the *Aureispira* fluctuations in skin, water or wall samples. These were the

taxa *Owenweeksia* (*Flavobacteriales*) and the totally unassignet taxon affiliating to the

dominating biofilter population. Alginate lyase activity in *Owenweeksia* is likely according to

reported genome annotations.[58] However, *Colwellia* (18%) thrived also close to the

Arcobacter taxon (42%) in the fish wounds during medication (C2W8), and a close

connection and dominance by these taxa was seen in the biofilm grown on the tube wall after

the UV-filter unit in the inlet water (Table 3).[59] This suggest that *Arcobacter* formed alginate,

a suggestion hinted as well by the genomic analysis of *Arcobacter butzleri*.[60] Wound and

water clustered relative densely in the all samples cluster analysis at sampling time C2W8

(Figure 7), and the wound taxa were indeed among the most common water taxa. Most

prominent in the water, after *Colwellia*, were the two taxa suggested to utilize alginate above.

These taxa strengthen their RA also in the biofilter biofilm, although *Lewinella* was

unchanged in RA and *Aureispira* were reduced (Table 3). All of these observations indicated

that wound biofilm with alginate was shaded to the water, being the water main substrate.

Shading of biofilm from fish wounds matched the enhanced DNA yield obtained in the water

at this sampling timepoint (Table 4). The long-standing dominating position of *Colwellia* in

the production water suggested a permanent and less competed growth source, a niche probably given by alginate from the biofilter (Figure 9). The RA of the *Aureispira* taxon in water was unchanged after medication, which precludes dispersion as the escape mechanism from the biofilm.[61] On the contrary, adhesion was an ongoing process in the biofilter at time (C2W8), indicating an EPS formation phase.[62] The adhering taxa were or affiliated mainly to *Actinobacteria*, *Sphingobacteriia* and *Bradyrhizobium* that are bacteria with anticipated specific attachment domains, and previously reported to adhere to biofilm during antibiotic selection.[61,63,64] Interestingly, subinhibitory concentrations of florfenicol enhanced the adherence of Staphylococcus to epithelial cells due to expression of adhesins towards host lectins.[65,66] Biofilm from activated sludge was shown to react with hemagglutinins, and Bap1 is an EPS epipolysaccharide matrix (EPS) protein with lectin domains shown to participating in marine bacterial adhesion.[34,64] It would be interestingly to biomine the biofilm matrix for the lectin proteins associated with resistant bacterial specific adhesion or fish pathogens. In opposite to the biofilter biofilm, the wall biofilm did have a netto loss of taxa during medication. In addition to the extinction, a few dominating taxa grew out. This lowered the diversity indexes, despite a flattering in the lower RA categories (Figure 6 and 10). The extinction followed probably from a stronger exposure to the antibiotics on the wall as compared to the biofilter bofilm, given a thinner wall biofilm, strongly hydrophobic wall coating and direct feed exposure. The increased eveness in the lower RA categories corresponded to a reduction in the outer biofilm bacteria by antibiotics, mainly represented by taxa of the *Flavobacteriales* and *Acidobacteria*. The space fried was occupied by a few taxa, in particular outgrowth of a taxon in *Saprospiraceae* (2%). The very taxon adhered simultaneously in the biofilter biofilm and became detectable in the water. Thus, in this case the wall was a taxa reservoir for the biofilter and the water. Regarding both biofilms biotopes over all, *Sphingobacteriia* and *Planctomycetes* taxa increased most in RA during medication.

A possible explanation for the success of the *Planctomycetes* was a temporary thinner biofilm that allowed more ammonium to diffusing to the inner of the biofilm.

Salinity and pH drops between the sampling times C2W8 and C2W13 in PC2.The antibiotic medication was followed by an ordered salinity drop (10 units) for improved wound healing. As fish recovered, more feed was given. This resulting in a sharp and temporary pH drop from week 8 to 10, as ammonium from the fish formed protons in the biofilter when oxidized to nitrite (Figure 2). The effect of this physiochemical shift was measured by the deep sequencing data from timepoints C2W8 and C2W13. Two major fluctuations were seen in the >5% RA fraction in the biofilter biofilm. In the *Bacteroidetes*, the taxon *Flexibacter* increased the RA by ~20% on the expense of the *Lewinella*. A study by McEldowney and Fletcher 1988, reported that *Flexibacter* sp. adhered increasingly to polystyrene surfaces with lowering pH, although growth was at maximum at neutral pH.[67] Probably the *Flexibacter* taxon adhered when pH was at the lowest and propagated as pH rised again. Enhanced RA for the *Flexibacter* taxon was seen several times after pH drops, in particulare in the wall biofilm (Table 3, Figure 2). The second fluctuation was in the *Rhodobacteriales*, where *Pseudorhodobacter* did a one-time appearance in the >5% RA fraction, while the abundant taxon *Rhodobacteriales*-other declined accordingly. A marine *Pseudorodobacter* strain was compared to several *Rhodobacter* strains, all of which grew without NaCl and had growth optimum at 0.5% NaCl.[68] Thus, the salinity drop was probably not the selective force for the shift. The pH optimum was not compared, but most *Pseudorhodobacter* strains grew in the pH range 5.5-9.5, and best at neutral pH as reported by Lee et al. 2016.[69]   Thus, neither the pH drop could explain the high RA of the taxon.

The biofilms diversity indexes increased modest from time C2W8 to C2W13, and the shifts observed was 4-6 taxa more in the 0.5-1% RA categories of inoculums associated taxa and a more even distribution in the dominating taxa. The lower number of total OUT´s at

C2W13 confirmed the theoretical biofilm modelling telling that extinction follows after adhesion, thus anticipating a lowering in the diversity by the model. In contrast to the biofilms, a dramatic increase in the diversity indexes was seen in the water for both the inoculum and environmental fraction, following from more taxa in all RA categories (Figure 6 and 10). So why responded the water and biofilm so differently to the physiochemical changes? In a RAS, the physiochemical changes occur in a much shorter time frame than the bacterial adaption. The biofilm matrix buys however times for its fixed members, and has members demands upon quorums sequencing system and extracellular polymeric substances (EPS)/outer membrane (OM) sugar, fatty acid and protein chemistry. Thus, to a much higher degree, water members are continuously competed while adapting, resulting in fewer dominating taxa. The fact that the inoculum originally was enriched from brackish water, more taxa found probably their optimums as salinity dropped. Out of 56 taxa fluxing in to the water at time C2W13, 17 taxa were re-appearers from prior the use of antibiotics, 10 taxa were one-time appearers and 26 taxa were registered for the first time, but recorded 2-6 times later. The latter group had increasing chance to be detected also in the biofilter, in particular if associated with the inoculum (Figure 4B). In the group we detected *Myxococcales*, *Desufuromonadales* and *Nitrospirales* for the first time, taxa that later dominated in the biofilter.

RAS cleaning, biofilter biofilm LOZ treatment and re-inoculation between the sampling times C2W13-Wash I-C3W4. The RAS was washed comprehensively between PC2 and PC3, including "back washing" of the bioflter with a strongly oxidative LOZ solution.[18] Secondly, new bio filter carriers pre-soaked in start culture, substituted a portion of the established biofilter carriers. Taxa of *Flavobacteriales* and *Saprospiraceae* (*Lewinella*) had the strongest reduction in RA after the biofilter treatment (Table 3), indicating that these bacteria were most exposed to the LOZ solution. Microscoping fresh biofilm at low solution

demonstrated normally filamentous bacteria stretching out of the biofilm into the flowing water, a morphology in consistence with the *Flavobacteriales and Saprospiracea.* The changed biofilm surface and new bio carriers fried area for bacterial adhesion, and new and re-appearing taxa was identified on the biofilm carriers the day prior to fish stocking of PC3. Several of the new once were among the taxa registered in the water for the first time at C2W13 (*Myxococcales*, *Desufuromonadales* and *Nitrospirales*), but also other taxa (*Chloroflexi*, *Chloroacidobacterium*, *Arthrobacter)* with the potential to utilize halogenated biproducts from the ozon and hypochlorite treatment (LOZ).[70,71] Regular LOZ addition to the production water started after time C2W8. Among the re-appearing taxa with inoculums association, *Actinobacteria*, *Sphingobacteriia* and *Bradyrhizobium* adhered as also seen during the antibiotica treatment, strengthen the assumption that these taxa was able to attach specifically when biofilm surfaces were interrupted. [61,63,64] Also the *Aureispira* taxon re-appeared in the biofilter at low RA. Only re-appearing taxa became permanently established in the biofilter after the re-inoculation step. Besides *Aureispira,* these were five environmental taxa of γ-*Proteobacteria*, several known from human and animal bacteriology, including *Aliivibrio*.[72] The establishment of *Vibrio′*s and *Myxococales* taxa are likely to be correlated to the weakening of the taxa *Lewinella*, as they are bacteria reported to be predated by ixotrophy. [47,46] Overall, the biofilter LOZ wash and re-inoculation step did only influence the microbiology in the biofilter by supporting more sites for microbial attachment.

Dominance of *Myxococcales* in the biofilter biofilm, PC3and PC5. A major shift in the microbial community structure occurred in the biofilter biofilm during PC3, a cycle characterized by the entrance of the *Nannocystaceae* (*Myxococcales*) and *Desulfuromondales* taxa in the >5% RA fraction. The first taxa twice as abundant than the second. *Myxococcales* members forms channels in the EPS, and these channels are also attachment cites for their philia used for gliding movment.[73] The close relation upon phili development between the

Desulfuromonadales and *Myxococcales* (δ-*Proteobacteria*) may explain their co-occurrence, as also seen at their synchronized appearance in the >5% fraction in PC5. The *Desulfuromonadales* taxa was probably selected by sulfur in the RAS, being an important participant in the RAS sulfur cycling.[33] *Myxococcales* taxa has previously been reported to stabilize biofilm, resulting in better N-cycling by *Nitrosomonas* and *Nitrospira*.[74] These bacteria are poor EPS formers, and seems to need extracellular DNA around their biofilm cells, more than many other bacteria.[75] Not unlikely, the stabilizing factor of *Myxococcales* previously reported was due to insertion extracellular DNA in the biofilm. In present study, *Nitrospira* establish at high RA in the biofilter when the second peaking of *Myxococcales* was registered in PC5. High abundancy of the *Myxococcales* taxon resulted also in a green coloring of the RAS rearing water and off-flavor smell. *Myxococcales* performs cellular lysing and grows upon the lysate (necromass) of other microbes in the biofilter biofilm. Most taxa where reduced 4-fold from time C2W4 into C2W8. In the same time span, the biomass in the biofilter biofilm dropped markedly (Table4), and released necro mass selected probably for the *Francisella* taxon that replaced *Colwellia* in the production water.[33] This pattern repeated in a less scale also at time C5W10. Thus, the entrance of the *Myxococcales* into the biofilter influenced the ecology of the RAS more than any of the other RAS events reported in the experimental period. *Aureispira, Colwellia and Francisella* were enriched in the biofilter by the presence of *Myxococcales* (Figure 11). The extincted taxa were the same as the adhering one in the next breeding cycle, and very much the same groups and taxa being extincted and adhered during medication and LOZ treatment. The microbial community shifts registered at C3W8, were reflected also in the diversity indexes, and at sampling time C4W2, production water and biofilter biofilm are again in their opposite phases with respect to diversity (Figure 10).

<u>Wash II between PC3 and PC4, changes in feeds oil component in PC4 and salinity drop in PC5.</u> The RAS wash, Wash II, between PC3 and PC4 reduced strongly the RA of the anaererobe and sulfur-reducing taxon *Desulfuromonadales* in the biofilter biofilm.   Two sulfur-oxidizing taxa, ie *Thioalkalispira* and a *Rhodobacteriales*-clade were replacing, verifying the presence of sulfur in the biofilter (Figure 8).[33,76] Fish feed with vegetable oils from a new supplier was used in the time after C4W2, resulting in a diarrhea-like feces from the fish, and the water turned bright yellow. The missing microbiological biofilter biofilm data at the time of C4W8 is difficult to interpret from the wall data, since this biofilm was in a phase of M*yxococcales* predation. If the biofilter biofilm was also predated by *Myxococcales*, then the biofilter biofilm biomass would probably not have increase as it did (Table 3). The final breeding cycle monitored in this survey was PC5, and after more than a year of operation *Nitrospira* establish with significance in the biofilter, likely by the help of the *Myxococcales* and the lowering of salinity bellow 22‰. Unfortunately, nitrate was not measured for this cycle, so the level of nitrate production after establishment was not possible to compare with the pre-establishment situation. In absence of Nitrospira or other nitrite oxidizers, chemical oxidation by LOZ was anticipated as the main utilizer of nitrite

Evaluating deep sequencing as a monitoring tool

A RAS plant is excellent for studying ecological processes, as the changes are rapid and the processes are enlarged. In this study we have been able to assess the characteristics of a commercially available starting culture. We have been able to discuss the effects of medication with antibiotics, washing with LOZ , re-inoculation and changes in physicochemical parameters. We have seen that the second steps of nitrification can take a long time to establish as a process in the biofilter biofilm . All of this is applied information that can either be used directly by the farmer or developed further, even into pure basic

research. Equally important, we have been able to identify possible indicator microbes, which are necessary for faster interpretation of data if the methodology is to be used for regular monitoring. With faster analysis time and digitization of data management, deep sequencing can become a good monitoring tool in the future. As a monitoring tool, we make the following recommendations. For regular monitoring, water and biofilter are most important, and sampling can be at somewhat higher frequency than 1 month. DNA amplification problems can easily arise and the data gap period can be long. Generally, only small amounts of Prokaryote DNA was possible to obtain from fish skin samples of healthy fish, but should not be omitted from analysis as our data shows that important microbiological processes can taking place on the fish surface in RAS. It is also important that each 16S analysis plate includes enough parallels to determine the set's RA "cutoff" value. We used this on parallel samples, but it is also important to include several analyzes from the same DNA extract to distinguish better between environmental and methodological variations.

Evaluating the inoculum.

The microbial consortium in the start culture had good colonization properties over a wide salinity gradient. Among the first colonists were two taxa with known ixotrophy, which probably prevented the growth of *Vibrio´*s and *Myxococcales*. However, *Myxococcales* had an important role in biofilm stabilization and the establishment of the nitrite oxidation process. This process might have been established earlier if the salinity was lower from the beginning. Furthermore, it prompts the needed of biomining new nitrite oxidizing bacteria with better colonization properties and functionality at high salinity. Possible, it would be an advantage if *Desulfuromonadels* was replaced with sulfur oxidizing bacteria in the start culture. *Myxococcales* as a biofilm stabilizer are disadvantageous with respect to off flavor production.

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SECTION C

APPENDIX

APPENDIX

C2W3		C2W8		C2W13		Washl		C3W4		C3W8		C4W2		C5W10	
11	22,784	11	21,068	86	24,075	86	14,294	71	13,398	134	18,150	71	18,786	106	7,907
71	20,072	71	20,898	34	13,454	34	13,071	86	13,267	71	15,547	12	7,047	71	6,707
95	9,837	80	12,228	56	8,552	95	11,229	34	12,709	65	9,120	34	5,971	134	5,810
104	7,989	34	4,806	71	7,875	71	6,792	134	8,131	34	7,697	108	4,562	65	4,045
13	4,762	86	3,930	11	6,757	56	3,821	30	6,535	86	5,803	30	4,355	34	3,512
94	3,336	22	2,615	120	2,215	61	3,762	11	2,526	30	4,976	86	4,069	13	3,290
34	3,202	133	2,441	22	1,952	133	3,491	12	2,318	108	3,733	18	2,047	42	3,040
22	3,006	38	2,346	133	1,865	120	2,648	18	2,029	126	3,562	13	1,337	104	3,017
18	2,876	1	2,269	18	1,830	57	2,232	108	2,013	13	3,199	57	1,263	22	2,668
17	2,621	57	1,905	66	1,652	17	2,150	96	1,782	104	2,795	61	1,165	108	2,490
96	2,176	12	1,675	124	1,605	80	2,006	85	1,717	70	1,774	11	1,152	96	2,390
99	1,295	120	1,411	80	1,494	124	1,848	13	1,639	96	1,646	96	1,018	30	2,142
86	0,996	13	1,411	12	1,349	99	1,548	65	1,410	12	1,543	106	0,974	12	2,112
56	0,872	99	1,013	61	1,021	11	1,477	66	1,343	95	1,097	54	0,786	66	1,660
80	0,688	66	0,992	126	1,006	66	1,135	54	1,251	17	0,736	133	0,715	86	1,124
124	0,583	18	0,908	57	0,959	131	1,057	61	0,917	66	0,721	38	0,654	18	0,978
12	0,561	124	0,745	99	0,733	12	0,689	80	0,909	85	0,619	16	0,634	85	0,958
70	0,548	17	0,633	30	0,714	54	0,684	38	0,841	56	0,322	128	0,490	56	0,584
115	0,333	104	0,603	128	0,648	128	0,620	17	0,835	11	0,293	22	0,475	57	0,496
133	0,284	96	0,566	55	0,601	25	0,576	56	0,757	128	0,284	134	0,453	128	0,385
119	0,226	61	0,564	13	0,546	13	0,572	57	0,655	18	0,273	116	0,450	54	0,383
66	0,177	94	0,502	134	0,501	18	0,557	133	0,653	54	0,266	66	0,407	38	0,377
116	0,174	111	0,429	95	0,480	85	0,471	55	0,595	55	0,235	99	0,389	139	0,357
111	0,158	2	0,360	96	0,465	138	0,443	128	0,500	110	0,212	17	0,330	126	0,349
108	0,137	32	0,222	54	0,363	83	0,411	22	0,474	99	0,202	25	0,305	124	0,343
76	0,132	85	0,216	94	0,362	94	0,371	70	0,456	22	0,197	124	0,296	61	0,343
61	0,125	25	0,214	104	0,347	55	0,341	116	0,453	57	0,180	72	0,273	95	0,337
9	0,099	108	0,205	25	0,298	111	0,338	104	0,424	38	0,172	85	0,269	116	0,321
30	0,090	30	0,194	38	0,237	91	0,337	111	0,337	61	0,143	41	0,252	72	0,228
57	0,087	95	0,179	131	0,235	96	0,318	41	0,331	111	0,117	1	0,235	133	0,206
137	0,081	54	0,172	17	0,234	22	0,294	25	0,321	115	0,117	64	0,231	110	0,182
120	0,080	119	0,161	110	0,229	30	0,294	95	0,310	133	0,112	111	0,220	120	0,173
85	0,035	116	0,148	111	0,224	16	0,215	120	0,306	124	0,097	95	0,220	48	0,099
83	0,035	70	0,145	70	0,175	9	0,210	99	0,280	41	0,088	80	0,199	11	0,095
67	0,035	9	0,119	85	0,164	130	0,199	48	0,271	120	0,084	104	0,148	68	0,085
27	0,032	131	0,096	116	0,139	119	0,187	115	0,256	16	0,082	120	0,117	99	0,079
65	0,026	76	0,096	42	0,139	29	0,187	16	0,254	80	0,060	56	0,103	27	0,073
129	0,026	6	0,091	29	0,115	115	0,175	124	0,252	106	0,045	136	0,099	109	0,067
131	0,026	128	0,090	91	0,114	116	0,147	6	0,229	25	0,044	78	0,096	16	0,058
32	0,026	56	0,083	32	0,075	1	0,119	126	0,225	36	0,040	130	0,088	111	0,058
25	0,020	29	0,073	65	0,067	36	0,116	42	0,195	127	0,034	65	0,083	46	0,057
110	0,020	7	0,072	119	0,067	136	0,114	110	0,184	6	0,027	70	0,082	17	0,053
6	0,020	115	0,067	83	0,066	70	0,093	67	0,111	109	0,019	6	0,077	91	0,046
15	0,020	67	0,064	115	0,060	76	0,073	91	0,091	130	0,016	115	0,072	70	0,038
62	0,020	129	0,060	76	0,059	126	0,059	131	0,085	139	0,016	42	0,070	41	0,035
16	0,016	42	0,058	16	0,057	42	0,059	29	0,080	42	0,015	110	0,064	78	0,034
42	0,016	5	0,057	139	0,048	38	0,055	94	0,079	67	0,013	112	0,062	44	0,034
54	0,016	27	0,056	41	0,047	129	0,054	27	0,038	94	0,010	36	0,058	2	0,026
1	0,016	15	0,051	15	0,033	134	0,046	78	0,026	131	0,010	48	0,055	132	0,026
36	0,000	8	0,043	9	0,029	110	0,045	9	0,026	1	0,008	55	0,045	1	0,024
38	0,000	16	0,046	36	0,027	41	0,040	119	0,025	19	0,008	129	0,042	9	0,023
41	0,000	10	0,045	27	0,020	65	0,036	36	0,022	27	0,007	83	0,041	19	0,017
55	0,000	126	0,037	129	0,015	139	0,034	136	0,020	72	0,007	46	0,038	3	0,017
109	0,000	134	0,034	107	0,014	109	0,031	106	0,019	83	0,007	67	0,035	25	0,017
126	0,000	110	0,025	138	0,008	78	0,031	1	0,015	78	0,004	126	0,026	36	0,014
128	0,000	3	0,023	109	0,006	72	0,027	130	0,013	46	0,004	2	0,024	55	0,014
134	0,000	55	0,021	20	0,006	27	0,026	129	0,013	136	0,004	5	0,024	117	0,014
138	0,000	78	0,019	1	0,000	2	0,023	32	0,012	138	0,003	76	0,022	101	0,014
78	0,000	35	0,015	6	0,000	107	0,022	76	0,012	48	0,003	4	0,020	94	0,012
139	0,000	14	0,015	67	0,000	104	0,020	72	0,011	7	0,003	27	0,019	131	0,012
2	0,000	58	0,015	78	0,000	32	0,019	127	0,011	129	0,002	32	0,018	6	0,012
19	0,000	107	0,015	108	0,000	64	0,016	83	0,011	9	0,000	3	0,018	127	0,012
46	0,000	130	0,015	2	0,000	121	0,012	109	0,011	15	0,000	131	0,015	129	0,006
48	0,000	20	0,011	19	0,000	15	0,010	107	0,010	116	0,000	94	0,015	15	0,006
72	0,000	21	0,008	46	0,000	89	0,009	3	0,010	32	0,000	109	0,014	138	0,006
107	0,000	31	0,008	48	0,000	5	0,007	139	0,009	2	0,000	139	0,014	114	0,006
130	0,000	40	0,008	72	0,000	23	0,007	46	0,007	107	0,000	24	0,014	80	0,000
3	0,000	43	0,008	130	0,000	135	0,007	2	0,006	3	0,000	15	0,014	32	0,000
5	0,000	37	0,007	3	0,000	6	0,006	24	0,004	5	0,000	7	0,012	67	0,000
29	0,000	109	0,006	5	0,000	67	0,006	121	0,004	4	0,000	119	0,011	107	0,000
91	0,000	41	0,005	106	0,000	46	0,004	63	0,003	24	0,000	19	0,010	130	0,000
106	0,000	83	0,004	127	0,000	49	0,003	125	0,002	49	0,000	49	0,008	5	0,000
127	0,000	24	0,004	24	0,000	63	0,003	58	0,002	63	0,000	9	0,006	136	0,000
136	0,000	23	0,004	4	0,000	19	0,001	5	0,001	68	0,000	107	0,005	4	0,000
4	0,000	26	0,004	7	0,000	48	0,001	4	0,001	121	0,000	39	0,004	7	0,000
7	0,000	28	0,004	24	0,000	4	0,001	49	0,001	20	0,000	121	0,004	24	0,000
24	0,000	65	0,003	49	0,000	39	0,000	35	0,001	23	0,000	127	0,004	49	0,000
49	0,000	36	0,003	63	0,000	112	0,001	117	0,001	35	0,000	123	0,004	63	0,000
63	0,000	138	0,002	68	0,000	123	0,001	15	0,001	37	0,000	63	0,002	121	0,000
68	0,000	139	0,000	121	0,000	47	0,001	138	0,001	39	0,000	89	0,002	20	0,000
121	0,000	19	0,000	23	0,000	108	0,000	19	0,001	58	0,000	138	0,001	23	0,000
20	0,000	46	0,000	35	0,000	3	0,000	68	0,001	64	0,000	68	0,001	35	0,000
23	0,000	48	0,000	37	0,000	106	0,000	7	0,000	89	0,000	37	0,001	37	0,000
35	0,000	72	0,000	39	0,000	127	0,000	20	0,000	112	0,000	132	0,001	39	0,000

Legend:
- >50
- >20
- 10-20
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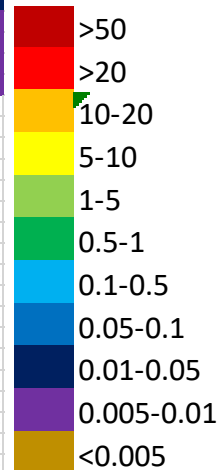
APPENDIX -2: Environmental taxa in biofilter biofilm.

C2W3		C2W8		C2W13		Washl		C3W4		C3W8		C4W2		C5W10	
158	2,430	187	3,246	187	2,861	187	5,244	233	1,998	150	3,109	248	11,392	233	11,712
64	1,719	153	1,267	153	2,338	45	3,187	64	1,497	64	1,869	158	5,917	187	6,087
190	0,879	158	1,012	55	1,364	153	1,819	45	1,429	62	1,404	190	3,303	236	5,997
232	0,643	64	0,836	150	0,862	72	0,939	187	1,360	218	1,187	237	2,007	64	2,824
153	0,626	55	0,550	158	0,681	55	0,835	218	0,884	187	1,067	153	1,456	158	1,665
187	0,380	237	0,528	45	0,599	123	0,681	27	0,827	153	0,812	187	1,289	153	1,251
55	0,368	51	0,274	64	0,542	158	0,536	150	0,737	233	0,466	152	1,134	50	1,035
159	0,344	70	0,236	224	0,519	64	0,384	158	0,673	158	0,450	45	1,030	21	0,840
50	0,194	138	0,216	124	0,505	190	0,293	123	0,564	159	0,410	64	1,022	124	0,808
45	0,174	45	0,212	237	0,421	218	0,265	72	0,543	124	0,345	191	0,980	19	0,671
224	0,158	123	0,199	218	0,388	232	0,259	153	0,508	45	0,324	50	0,768	188	0,486
222	0,152	42	0,128	72	0,321	159	0,248	159	0,461	190	0,271	21	0,717	237	0,476
72	0,114	78	0,121	138	0,226	237	0,235	230	0,309	50	0,178	159	0,539	159	0,440
174	0,110	157	0,115	236	0,193	150	0,226	182	0,260	237	0,177	123	0,346	283	0,378
236	0,109	190	0,113	159	0,190	224	0,219	138	0,252	224	0,160	72	0,286	224	0,368
78	0,097	50	0,112	50	0,179	51	0,215	237	0,252	182	0,120	113	0,273	27	0,350
280	0,097	310	0,096	51	0,150	135	0,187	50	0,179	27	0,107	124	0,263	193	0,299
70	0,094	182	0,096	123	0,147	154	0,163	54	0,171	70	0,106	145	0,262	113	0,277
237	0,091	159	0,083	162	0,114	138	0,155	124	0,165	230	0,077	54	0,256	240	0,246
29	0,074	72	0,075	29	0,105	124	0,137	135	0,158	72	0,076	150	0,250	138	0,234
218	0,071	150	0,068	41	0,103	78	0,093	236	0,146	123	0,067	19	0,238	306	0,231
144	0,059	38	0,067	154	0,091	70	0,077	19	0,140	236	0,063	51	0,218	42	0,167
192	0,035	236	0,062	30	0,072	227	0,075	21	0,139	21	0,056	218	0,190	45	0,165
253	0,032	124	0,060	182	0,072	162	0,072	44	0,133	51	0,054	236	0,154	245	0,125
205	0,029	62	0,045	40	0,057	19	0,068	190	0,087	19	0,050	138	0,142	182	0,095
156	0,026	167	0,039	156	0,055	256	0,065	224	0,083	44	0,048	182	0,130	10	0,089
193	0,026	192	0,038	135	0,045	71	0,064	113	0,079	113	0,036	267	0,112	218	0,084
154	0,020	171	0,030	157	0,043	236	0,055	2	0,068	54	0,034	70	0,090	38	0,082
246	0,020	241	0,029	167	0,034	66	0,053	78	0,064	138	0,032	2	0,082	18	0,073
40	0,016	135	0,029	78	0,031	41	0,052	221	0,062	156	0,020	251	0,081	114	0,072
47	0,016	170	0,026	222	0,028	188	0,046	167	0,061	204	0,017	41	0,068	204	0,070
163	0,016	19	0,026	19	0,027	98	0,044	235	0,051	175	0,013	146	0,066	131	0,068
221	0,016	29	0,025	190	0,022	283	0,044	154	0,037	69	0,013	18	0,063	230	0,063
230	0,016	154	0,024	230	0,022	233	0,039	204	0,037	162	0,013	245	0,057	164	0,059
243	0,016	43	0,023	306	0,020	167	0,039	162	0,036	283	0,012	276	0,055	78	0,052
256	0,016	218	0,020	204	0,019	204	0,032	62	0,034	235	0,010	135	0,052	162	0,052
18	0,010	178	0,020	221	0,019	182	0,032	38	0,034	285	0,010	204	0,045	150	0,047
42	0,010	156	0,016	235	0,019	21	0,030	70	0,025	295	0,010	235	0,041	55	0,041
51	0,010	144	0,014	276	0,019	50	0,025	119	0,025	57	0,008	233	0,041	144	0,038
58	0,010	205	0,013	139	0,015	40	0,024	205	0,024	55	0,008	62	0,038	206	0,035
235	0,010	142	0,012	65	0,013	230	0,023	51	0,022	2	0,007	167	0,036	192	0,031
1	0,000	305	0,012	227	0,013	111	0,023	188	0,022	18	0,007	281	0,035	222	0,026
2	0,000	283	0,012	188	0,013	142	0,022	42	0,016	135	0,007	230	0,033	246	0,023
3	0,000	306	0,011	18	0,008	229	0,022	156	0,016	144	0,007	239	0,033	2	0,020
4	0,000	230	0,010	38	0,008	240	0,021	246	0,016	154	0,007	295	0,032	190	0,018
5	0,000	253	0,007	70	0,006	306	0,019	222	0,015	243	0,006	119	0,032	305	0,017
6	0,000	233	0,007	71	0,006	65	0,019	18	0,014	267	0,006	178	0,031	135	0,014
7	0,000	172	0,006	142	0,006	278	0,017	232	0,013	80	0,005	224	0,031	154	0,014
8	0,000	162	0,006	178	0,006	235	0,016	194	0,012	98	0,005	188	0,029	169	0,014
9	0,000	40	0,005	192	0,006	192	0,015	139	0,011	222	0,005	78	0,028	238	0,014
10	0,000	232	0,005	305	0,006	243	0,014	245	0,010	65	0,004	162	0,027	256	0,014
11	0,000	224	0,005	1	0,000	246	0,014	65	0,010	167	0,004	27	0,025	71	0,012
12	0,000	30	0,004	2	0,000	30	0,013	55	0,009	188	0,004	136	0,023	174	0,012
13	0,000	54	0,004	3	0,000	221	0,012	227	0,009	221	0,004	144	0,023	267	0,012
14	0,000	4	0,003	4	0,000	194	0,010	178	0,008	241	0,004	44	0,022	310	0,012
15	0,000	222	0,003	5	0,000	253	0,010	239	0,008	245	0,004	210	0,022	29	0,009
16	0,000	269	0,003	6	0,000	241	0,009	142	0,007	246	0,004	114	0,021	72	0,009
17	0,000	58	0,003	7	0,000	69	0,009	71	0,007	116	0,003	238	0,021	134	0,009
19	0,000	146	0,003	8	0,000	295	0,009	29	0,006	174	0,003	203	0,020	178	0,009
20	0,000	163	0,003	9	0,000	54	0,007	295	0,006	227	0,003	131	0,018	194	0,009
21	0,000	21	0,002	10	0,000	42	0,007	243	0,006	282	0,003	205	0,018	205	0,009
22	0,000	44	0,002	11	0,000	43	0,007	276	0,006	30	0,002	222	0,017	253	0,009
23	0,000	264	0,002	12	0,000	52	0,007	306	0,006	58	0,002	29	0,016	287	0,009
24	0,000	27	0,002	13	0,000	193	0,007	199	0,006	304	0,002	192	0,015	301	0,009
25	0,000	46	0,002	14	0,000	207	0,007	41	0,005	310	0,002	20	0,014	40	0,006
26	0,000	221	0,002	15	0,000	228	0,007	256	0,005	1	0,000	240	0,013	62	0,006
27	0,000	246	0,002	16	0,000	248	0,007	137	0,004	3	0,000	30	0,011	85	0,006
28	0,000	248	0,002	17	0,000	282	0,007	69	0,004	4	0,000	259	0,011	89	0,006
30	0,000	1	0,000	20	0,000	157	0,006	310	0,004	5	0,000	69	0,011	139	0,006
31	0,000	2	0,000	21	0,000	215	0,006	269	0,004	6	0,000	38	0,011	157	0,006
32	0,000	3	0,000	22	0,000	16	0,004	277	0,003	7	0,000	65	0,009	170	0,006
33	0,000	5	0,000	23	0,000	29	0,003	144	0,003	8	0,000	174	0,009	243	0,006
34	0,000	6	0,000	24	0,000	296	0,003	173	0,003	9	0,000	194	0,009	1	0,000
35	0,000	7	0,000	25	0,000	305	0,003	283	0,003	10	0,000	148	0,009	3	0,000
36	0,000	8	0,000	26	0,000	18	0,001	1	0,003	11	0,000	306	0,008	4	0,000
37	0,000	9	0,000	27	0,000	35	0,001	66	0,003	12	0,000	175	0,008	5	0,000
38	0,000	10	0,000	28	0,000	38	0,001	157	0,002	13	0,000	268	0,007	6	0,000
39	0,000	11	0,000	31	0,000	102	0,001	20	0,002	14	0,000	170	0,007	7	0,000
41	0,000	12	0,000	32	0,000	139	0,001	278	0,002	15	0,000	310	0,007	8	0,000
43	0,000	13	0,000	33	0,000	143	0,001	241	0,002	16	0,000	149	0,006	9	0,000
44	0,000	14	0,000	34	0,000	156	0,001	253	0,002	17	0,000	253	0,006	11	0,000
46	0,000	15	0,000	35	0,000	174	0,001	58	0,001	20	0,000	154	0,006	12	0,000
48	0,000	16	0,000	36	0,000	259	0,001	102	0,001	22	0,000	202	0,006	13	0,000
49	0,000	17	0,000	37	0,000	276	0,001	145	0,001	23	0,000	283	0,006	14	0,000
52	0,000	18	0,000	39	0,000	1	0,000	146	0,001	24	0,000	40	0,005	15	0,000
53	0,000	20	0,000	42	0,000	2	0,000	280	0,001	25	0,000	256	0,005	16	0,000
54	0,000	22	0,000	43	0,000	3	0,000	286	0,001	26	0,000	243	0,005	17	0,000

Legend:

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- 10-20
- 5-10
- 1-5
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- 0.1-0.5
- 0.05-0.1
- 0.01-0.05
- 0.005-0.01
- <0.005

APPENDIX -3: Inoculums associated taxa in wall biofilm.

C2W3		C2W8		C2W13		C3W4		C3W8		C4W2		C4W10		C5W2		C5W10	
71	14,277	71	29,968	86	24,328	34	26,187	86	13,667	30	32,421	71	33,164	34	11,75	71	11,555
13	10,427	34	20,219	34	18,067	11	20,384	11	13,475	11	12,211	86	15,518	30	7,7161	66	5,4567
86	8,0191	11	13,373	71	15,902	71	12,247	34	13,124	71	10,766	12	10,95	96	7,3363	96	4,38
95	7,9296	96	6,6366	96	9,7611	96	7,0971	71	8,7863	86	7,7418	66	8,1702	71	6,3802	34	3,1027
96	4,6364	80	5,0001	11	5,2047	30	5,5896	30	8,4231	96	7,7281	34	3,9642	11	5,6727	86	2,9444
11	4,2483	86	3,0128	80	4,2262	13	3,2551	134	6,3106	34	3,3159	134	3,6742	85	4,9166	30	2,5902
133	2,945	18	3,0073	66	3,2465	38	2,7481	13	6,041	38	2,3379	96	2,6831	86	3,4109	12	2,5096
94	2,9151	13	1,7601	18	2,4471	22	2,1745	96	4,7657	13	2,0715	104	2,1513	13	2,3358	13	1,9198
80	2,8355	22	1,6401	56	0,8871	12	1,6275	108	2,0049	16	1,437	85	1,7646	22	2,3353	106	1,691
18	2,6167	66	1,3963	124	0,8374	86	1,5208	12	1,8786	12	1,3407	13	0,9911	12	2,2905	85	1,5862
99	2,6167	108	1,0574	95	0,7766	70	1,3074	65	1,6499	134	0,9349	133	0,7252	65	2,1476	22	1,2848
22	2,0595	38	1,0268	61	0,516	54	1,1206	66	1,5955	18	0,896	106	0,5318	124	1,9531	139	1,1452
34	2,0396	57	0,5086	22	0,4778	65	0,8805	57	1,3561	108	0,7163	30	0,4109	134	1,8162	38	0,8267
119	1,6615	120	0,4892	13	0,4669	111	0,707	22	1,1818	99	0,6247	54	0,3384	128	1,4927	18	0,7759
56	1,4625	12	0,4205	12	0,4521	57	0,627	85	1,0354	54	0,6114	116	0,3384	18	1,3438	78	0,676
116	1,2835	124	0,333	133	0,4106	18	0,4936	99	0,7917	22	0,6055	11	0,2901	120	1,284	11	0,6195
124	1,0546	99	0,1979	99	0,3951	134	0,1868	126	0,7225	55	0,6048	128	0,2901	110	0,9096	80	0,457
57	1,0447	111	0,1942	30	0,3281	108	0,1734	54	0,683	57	0,594	61	0,2417	108	0,7898	104	0,418
12	1,0148	30	0,1848	55	0,3051	56	0,1601	38	0,458	85	0,5177	57	0,2175	38	0,765	124	0,3952
111	0,8258	61	0,1841	120	0,2533	95	0,1467	18	0,4533	61	0,4956	99	0,2175	139	0,7162	108	0,3596
137	0,8059	119	0,1487	119	0,2265	42	0,1334	16	0,3561	41	0,4193	38	0,1934	57	0,7081	61	0,3413
17	0,5572	54	0,1448	57	0,2175	128	0,1334	42	0,2555	17	0,4013	124	0,1934	54	0,626	42	0,3097
25	0,4477	133	0,0946	128	0,1615	99	0,1201	104	0,2547	116	0,3668	56	0,1692	66	0,4701	134	0,2844
61	0,4378	55	0,0747	54	0,147	55	0,08	55	0,2406	95	0,3178	95	0,145	99	0,4097	54	0,2311
30	0,3781	15	0,072	111	0,1451	104	0,08	95	0,2087	130	0,292	108	0,1209	127	0,3886	133	0,2309
27	0,3482	95	0,0508	134	0,1253	67	0,0667	70	0,1995	133	0,1899	16	0,0967	136	0,3721	126	0,1594
120	0,3283	129	0,0421	110	0,1164	110	0,0667	111	0,1781	120	0,1862	111	0,0967	16	0,2887	65	0,1585
70	0,3184	27	0,0287	126	0,0966	16	0,0534	56	0,1363	111	0,1583	126	0,0967	70	0,281	16	0,1582
115	0,3184	131	0,0287	85	0,0826	29	0,0534	120	0,1346	80	0,1347	130	0,0967	133	0,2777	56	0,1512
85	0,2388	29	0,025	108	0,0826	124	0,0534	17	0,1078	66	0,1344	17	0,0725	56	0,2645	57	0,1436
76	0,2288	85	0,0216	131	0,0766	136	0,0534	61	0,0988	124	0,1176	22	0,0725	116	0,246	95	0,1179
104	0,189	17	0,0199	29	0,0539	80	0,04	67	0,0966	128	0,1077	139	0,0725	111	0,2293	27	0,1029
9	0,1791	70	0,0164	38	0,0512	133	0,04	128	0,0828	106	0,0878	18	0,0483	126	0,1931	64	0,0864
67	0,1791	115	0,0122	139	0,0416	1	0,0267	133	0,0786	70	0,0804	70	0,0483	55	0,1766	46	0,0718
109	0,1592	110	0,012	104	0,041	17	0,0267	41	0,0728	115	0,0701	115	0,0483	42	0,1634	128	0,0716
66	0,1492	134	0,0093	65	0,0348	66	0,0267	27	0,0686	67	0,0568	138	0,0483	61	0,1552	116	0,0704
131	0,1492	6	0,0088	27	0,0331	85	0,0267	124	0,0547	109	0,0472	1	0,0242	80	0,1547	91	0,0626
108	0,1293	42	0,0086	115	0,0244	126	0,0267	91	0,0542	83	0,0431	41	0,0242	95	0,1441	41	0,0557
6	0,1094	76	0,0086	32	0,0235	2	0,0133	116	0,044	136	0,0431	42	0,0242	17	0,1196	120	0,0555
38	0,1094	56	0,0083	1	0,02	6	0,0133	115	0,0428	48	0,0428	46	0,0242	104	0,1128	99	0,0477
54	0,1094	65	0,0081	6	0,0175	41	0,0133	6	0,0425	65	0,041	55	0,0242	41	0,094	109	0,032
32	0,0597	78	0,0079	130	0,0156	61	0,0133	80	0,0287	104	0,0372	83	0,0242	91	0,0663	70	0,0317
64	0,0398	62	0,0047	91	0,0104	116	0,0133	29	0,0282	64	0,0354	109	0,0242	115	0,0663	111	0,0315
36	0,0298	128	0,0047	16	0,0096	120	0,0133	110	0,0282	94	0,0236	110	0,0242	6	0,0611	132	0,0311
78	0,0199	130	0,0047	78	0,0096	127	0,0133	109	0,0209	56	0,0233	120	0,0242	67	0,0554	48	0,0237
107	0,0199	2	0,0041	83	0,0096	130	0,0133	32	0,0187	1	0,0214	2	0	106	0,0527	3	0,0155
3	0,0099	7	0,0041	94	0,0096	3	0	127	0,0087	46	0,0214	3	0	64	0,0519	72	0,0081
15	0,0099	94	0,0041	67	0,0052	4	0	131	0,0071	110	0,0196	4	0	130	0,0487	83	0,0081
39	0,0099	16	0,0039	76	0,0052	5	0	15	0,0065	72	0,0177	5	0	131	0,0487	112	0,0081
48	0,0099	32	0,0039	2	0,0044	7	0	1	0,0051	36	0,0155	6	0	36	0,0386	1	0,008
58	0,0099	67	0,0039	17	0,0044	8	0	48	0,0051	42	0,0155	7	0	44	0,0386	55	0,008
110	0,0099	126	0,0039	33	0,0044	9	0	89	0,0036	25	0,0137	8	0	94	0,0266	6	0,0078
129	0,0099	1	0	58	0,0044	10	0	119	0,0036	29	0,0137	9	0	72	0,0241	9	0,0078
1	0	3	0	62	0,0044	14	0	138	0,0036	76	0,0137	10	0	1	0,0221	17	0,0078
2	0	4	0	70	0,0044	15	0	72	0,0029	6	0,0078	14	0	76	0,0209	131	0,0078
4	0	5	0	109	0,0044	19	0	77	0,0029	19	0,0078	15	0	32	0,0193	2	0
5	0	8	0	136	0,0044	20	0	78	0,0029	62	0,0078	19	0	27	0,0156	4	0
7	0	9	0	3	0	21	0	139	0,0029	91	0,0078	20	0	29	0,0144	5	0
8	0	10	0	4	0	23	0	2	0	129	0,0078	21	0	109	0,012	7	0
10	0	14	0	5	0	24	0	3	0	32	0,0059	23	0	78	0,0108	8	0
14	0	19	0	7	0	25	0	4	0	139	0,0059	24	0	101	0,008	10	0
16	0	20	0	8	0	26	0	5	0	2	0	25	0	129	0,008	14	0
19	0	21	0	9	0	27	0	7	0	3	0	26	0	48	0,0048	15	0
20	0	23	0	10	0	28	0	8	0	4	0	27	0	83	0,0048	19	0
21	0	24	0	14	0	31	0	9	0	5	0	28	0	2	0,0036	20	0
23	0	25	0	15	0	32	0	10	0	7	0	29	0	15	0,0036	21	0
24	0	26	0	19	0	33	0	14	0	8	0	31	0	19	0,0024	23	0
26	0	28	0	20	0	35	0	19	0	9	0	32	0	121	0,0024	24	0
28	0	31	0	21	0	36	0	20	0	10	0	33	0	49	0,0012	25	0
29	0	33	0	23	0	37	0	21	0	14	0	35	0	119	0,0012	26	0
31	0	35	0	24	0	39	0	23	0	15	0	36	0	135	0,0012	28	0

Legend:
- >50
- >20
- 10-20
- 5-10
- 1-5
- 0.5-1
- 0.1-0.5
- 0.05-0.1
- 0.01-0.05
- 0.005-0.01
- <0.005

APPENDIX -4: Environmental taxa in wall biofilm.

C2W3		C2W8		C2W13		C3W4		C3W8		C4W2		C4W10		C5W2			C5W10
45	3,960	187	2,944	187	2,984	187	3,895	187	1,848	187	0,707	64	2,635	153	6,697	233	24,522
187	2,905	62	1,685	153	1,248	218	1,107	182	1,205	237	0,684	50	2,345	187	2,946	236	8,85
153	1,821	237	0,641	158	0,743	64	0,734	150	1,176	54	0,623	153	1,015	150	2,293	187	8,2359
64	1,482	158	0,491	55	0,640	123	0,667	218	0,917	153	0,570	159	0,822	158	1,727	50	1,4354
55	1,224	123	0,430	123	0,516	135	0,534	204	0,320	64	0,551	158	0,701	267	0,921	138	1,2328
50	1,065	64	0,405	218	0,480	44	0,494	64	0,309	224	0,524	45	0,604	19	0,896	153	1,019
158	1,065	138	0,369	64	0,304	182	0,454	138	0,265	182	0,446	237	0,435	21	0,724	159	0,756
190	0,637	153	0,311	124	0,242	138	0,320	237	0,187	45	0,354	190	0,411	167	0,697	124	0,6512
40	0,507	182	0,112	50	0,212	27	0,307	153	0,164	113	0,320	222	0,338	159	0,658	245	0,5414
78	0,378	70	0,098	236	0,207	237	0,240	205	0,159	236	0,269	236	0,266	64	0,653	64	0,5046
230	0,338	157	0,072	237	0,191	150	0,227	159	0,158	218	0,241	123	0,145	182	0,559	2	0,5011
159	0,298	310	0,071	138	0,174	62	0,147	158	0,132	158	0,236	21	0,121	218	0,538	158	0,4372
167	0,219	135	0,069	233	0,152	236	0,147	124	0,130	190	0,224	182	0,121	50	0,533	182	0,374
236	0,219	260	0,068	150	0,099	158	0,120	190	0,111	138	0,217	230	0,121	144	0,516	237	0,3426
253	0,169	159	0,067	224	0,097	159	0,120	44	0,103	21	0,209	240	0,121	224	0,423	193	0,2863
232	0,139	78	0,058	159	0,059	205	0,120	236	0,100	159	0,178	248	0,121	204	0,388	188	0,221
235	0,129	190	0,055	52	0,046	124	0,093	224	0,099	267	0,146	150	0,097	239	0,387	218	0,158
192	0,119	236	0,050	78	0,045	153	0,093	78	0,094	222	0,144	205	0,097	237	0,387	19	0,1495
218	0,109	171	0,046	182	0,043	224	0,080	27	0,091	19	0,131	221	0,097	190	0,383	78	0,1344
224	0,109	124	0,043	45	0,039	50	0,067	123	0,089	245	0,123	235	0,097	281	0,313	224	0,1194
237	0,099	50	0,041	222	0,035	78	0,053	135	0,083	72	0,117	243	0,097	222	0,307	114	0,1118
246	0,080	192	0,039	135	0,034	204	0,053	50	0,078	188	0,113	245	0,097	44	0,299	157	0,102
135	0,060	224	0,034	157	0,033	19	0,040	70	0,068	150	0,104	187	0,073	138	0,286	192	0,0872
222	0,060	29	0,032	154	0,024	45	0,040	54	0,065	221	0,084	191	0,073	135	0,203	113	0,0869
19	0,050	42	0,022	156	0,016	72	0,040	233	0,061	135	0,076	138	0,048	124	0,201	256	0,0864
62	0,050	218	0,021	71	0,016	157	0,027	167	0,061	204	0,063	204	0,048	123	0,192	27	0,0786
239	0,050	305	0,021	29	0,010	222	0,027	21	0,055	124	0,061	218	0,048	78	0,162	21	0,0642
280	0,050	222	0,020	30	0,010	29	0,011	222	0,053	192	0,055	8	0,024	45	0,147	204	0,0563
29	0,040	253	0,017	54	0,010	71	0,011	230	0,038	205	0,055	46	0,024	205	0,145	306	0,0553
72	0,040	205	0,017	167	0,010	114	0,013	19	0,038	203	0,051	55	0,024	85	0,141	222	0,0552
163	0,040	150	0,017	40	0,010	154	0,011	221	0,035	44	0,047	65	0,024	139	0,129	267	0,0552
221	0,040	44	0,013	72	0,010	175	0,011	235	0,035	123	0,045	70	0,024	54	0,126	42	0,0479
269	0,040	55	0,013	290	0,009	233	0,013	72	0,034	167	0,043	113	0,024	27	0,099	295	0,0479
281	0,040	19	0,013	62	0,005	264	0,013	113	0,029	50	0,041	114	0,024	245	0,067	87	0,0477
174	0,030	139	0,009	230	0,005	310	0,011	142	0,029	51	0,033	124	0,024	240	0,065	150	0,0398
193	0,030	30	0,008	69	0,005	1	0,000	188	0,024	18	0,031	145	0,024	18	0,053	18	0,0396
205	0,030	167	0,008	188	0,005	2	0,000	45	0,024	70	0,031	161	0,024	70	0,051	135	0,0396
306	0,030	144	0,008	205	0,005	3	0,000	85	0,014	42	0,031	164	0,024	113	0,050	305	0,0321
42	0,020	156	0,005	51	0,004	4	0,000	246	0,012	78	0,024	170	0,024	29	0,049	40	0,0319
70	0,020	175	0,005	144	0,004	5	0,000	171	0,009	181	0,023	175	0,024	62	0,048	162	0,0315
71	0,020	178	0,005	190	0,004	6	0,000	192	0,009	38	0,021	309	0,024	221	0,041	240	0,0315
142	0,020	232	0,005	1	0,000	7	0,000	178	0,009	174	0,018	1	0,000	72	0,040	275	0,0315
283	0,020	161	0,004	2	0,000	8	0,000	276	0,009	230	0,018	2	0,000	233	0,037	310	0,0313
4	0,010	174	0,004	3	0,000	9	0,000	80	0,008	233	0,018	3	0,000	114	0,036	246	0,0241
27	0,010	227	0,004	4	0,000	10	0,000	156	0,008	30	0,016	4	0,000	230	0,034	287	0,0236
65	0,010	251	0,004	5	0,000	11	0,000	145	0,006	157	0,016	5	0,000	40	0,034	85	0,0235
111	0,010	43	0,004	6	0,000	12	0,000	157	0,006	114	0,014	6	0,000	236	0,033	190	0,0235
113	0,010	45	0,004	7	0,000	13	0,000	18	0,005	119	0,014	7	0,000	306	0,033	29	0,016
139	0,010	72	0,004	8	0,000	14	0,000	38	0,005	131	0,014	9	0,000	188	0,028	55	0,016
140	0,010	154	0,004	9	0,000	15	0,000	267	0,005	243	0,014	10	0,000	203	0,025	134	0,016
144	0,010	230	0,004	10	0,000	16	0,000	41	0,004	246	0,014	11	0,000	235	0,022	230	0,016
154	0,010	1	0,000	11	0,000	17	0,000	42	0,004	253	0,014	12	0,000	51	0,019	205	0,016
156	0,010	2	0,000	12	0,000	18	0,000	51	0,004	85	0,012	13	0,000	287	0,016	164	0,0158
170	0,010	3	0,000	13	0,000	20	0,000	119	0,004	139	0,012	14	0,000	269	0,014	45	0,0157
172	0,010	4	0,000	14	0,000	21	0,000	169	0,004	154	0,012	15	0,000	10	0,008	86	0,0157
241	0,010	5	0,000	15	0,000	22	0,000	181	0,004	235	0,012	16	0,000	60	0,008	131	0,0157
1	0,000	6	0,000	16	0,000	23	0,000	256	0,004	307	0,012	17	0,000	69	0,008	171	0,0157
2	0,000	7	0,000	17	0,000	24	0,000	29	0,003	43	0,008	18	0,000	86	0,008	38	0,0155
3	0,000	8	0,000	18	0,000	25	0,000	65	0,003	178	0,008	19	0,000	156	0,008	206	0,0155
5	0,000	9	0,000	19	0,000	26	0,000	229	0,003	227	0,008	20	0,000	195	0,008	46	0,0081
6	0,000	10	0,000	20	0,000	28	0,000	305	0,003	241	0,008	22	0,000	295	0,008	143	0,0081
7	0,000	11	0,000	21	0,000	30	0,000	310	0,003	255	0,008	23	0,000	142	0,007	154	0,0081
8	0,000	12	0,000	22	0,000	31	0,000	1	0,000	276	0,008	24	0,000	271	0,006	156	0,0081
9	0,000	13	0,000	23	0,000	32	0,000	2	0,000	29	0,006	25	0,000	4	0,005	241	0,0081
10	0,000	14	0,000	24	0,000	33	0,000	3	0,000	66	0,006	26	0,000	71	0,005	210	0,008
11	0,000	15	0,000	25	0,000	34	0,000	4	0,000	82	0,006	27	0,000	137	0,005	239	0,008
12	0,000	16	0,000	26	0,000	35	0,000	5	0,000	142	0,006	28	0,000	157	0,005	248	0,008
13	0,000	17	0,000	27	0,000	36	0,000	6	0,000	144	0,006	29	0,000	191	0,005	309	0,008
14	0,000	18	0,000	28	0,000	37	0,000	7	0,000	239	0,006	30	0,000	192	0,005	10	0,0078
15	0,000	20	0,000	31	0,000	38	0,000	8	0,000	240	0,006	31	0,000	246	0,005	144	0,0078
16	0,000	21	0,000	32	0,000	39	0,000	9	0,000	264	0,006	32	0,000	310	0,005	191	0,0078
17	0,000	22	0,000	33	0,000	40	0,000	10	0,000	295	0,006	33	0,000	30	0,004	258	0,0078
18	0,000	23	0,000	34	0,000	41	0,000	11	0,000	1	0,000	34	0,000	178	0,004	307	0,0078
20	0,000	24	0,000	35	0,000	42	0,000	12	0,000	2	0,000	35	0,000	181	0,004	1	0
21	0,000	25	0,000	36	0,000	43	0,000	13	0,000	3	0,000	36	0,000	241	0,004	3	0
22	0,000	26	0,000	37	0,000	46	0,000	14	0,000	4	0,000	37	0,000	251	0,004	4	0
23	0,000	27	0,000	38	0,000	47	0,000	15	0,000	5	0,000	38	0,000	256	0,004	5	0
24	0,000	28	0,000	39	0,000	48	0,000	16	0,000	6	0,000	39	0,000	259	0,004	6	0
25	0,000	31	0,000	41	0,000	49	0,000	17	0,000	7	0,000	40	0,000	20	0,002	7	0
26	0,000	32	0,000	42	0,000	51	0,000	20	0,000	8	0,000	41	0,000	169	0,002	8	0
28	0,000	33	0,000	43	0,000	52	0,000	22	0,000	9	0,000	42	0,000	253	0,002	9	0
30	0,000	34	0,000	44	0,000	53	0,000	23	0,000	10	0,000	43	0,000	265	0,002	11	0
31	0,000	35	0,000	46	0,000	54	0,000	24	0,000	11	0,000	44	0,000	276	0,002	12	0
32	0,000	36	0,000	47	0,000	55	0,000	25	0,000	12	0,000	47	0,000	280	0,002	13	0
33	0,000	37	0,000	48	0,000	56	0,000	26	0,000	13	0,000	48	0,000	305	0,002	14	0
34	0,000	38	0,000	49	0,000	57	0,000	28	0,000	14	0,000	49	0,000	2	0,001	15	0
35	0,000	39	0,000	53	0,000	58	0,000	30	0,000	15	0,000	51	0,000	38	0,001	16	0
36	0,000	40	0,000	56	0,000	59	0,000	31	0,000	16	0,000	52	0,000	87	0,001	17	0
37	0,000	41	0,000	57	0,000	60	0,000	32	0,000	17	0,000	53	0,000	146	0,001	20	0
38	0,000	46	0,000	58	0,000	61	0,000	33	0,000	20	0,000	54	0,000	164	0,001	22	0
39	0,000	47	0,000	59	0,000	63	0,000	34	0,000	22	0,000	56	0,000	171	0,001	23	0
41	0,000	48	0,000	60	0,000	65	0,000	35	0,000	23	0,000	57	0,000	275	0,001	24	0
43	0,000	49	0,000	61	0,000	66	0,000	36	0,000	24	0,000	58	0,000	285	0,001	25	0

Legend:
- >50
- >20
- 10-20
- 5-10
- 1-5
- 0.5-1
- 0.1-0.5
- 0.05-0.1
- 0.01-0.05
- 0.005-0.01
- <0.005

APPENDIX -5: Inoculums associated taxa in water.

C2W3		C2W8		C2W13		C3W4		C3W8		C4W2		C4W10		C5W2		C5W10	
95		95		95		95		109	27,355	95		95	16,1..	71		95	
18	1,345	12	1,561	138	16,240	85	0,253	71	8,062	71	1,969	12	7,549	85	4,936	109	19,424
11	1,135	85	0,667	57	1,667	96	0,230	108	8,031	12	1,672	13	3,412	34	3,317	104	0,311
13	0,985	133	0,447	109	1,634	124	0,127	65	6,844	109	0,799	71	3,345	12	1,234	139	0,310
71	0,967	99	0,425	124	1,528	6	0,121	134	5,276	124	0,503	108	3,067	124	1,011	71	0,268
94	0,581	6	0,396	120	1,015	71	0,121	95	5,005	108	0,487	34	2,897	44	0,900	12	0,250
22	0,523	57	0,269	71	0,965	30	0,112	12	2,801	34	0,280	124	2,305	78	0,769	34	0,190
99	0,429	120	0,208	12	0,951	86	0,098	85	2,367	99	0,265	86	2,268	95	0,627	65	0,169
12	0,353	124	0,207	56	0,933	34	0,095	13	2,025	64	0,254	99	1,760	13	0,526	124	0,118
104	0,335	80	0,199	34	0,926	104	0,086	96	1,800	30	0,180	104	1,204	30	0,303	120	0,118
137	0,246	71	0,197	85	0,738	54	0,046	34	1,575	18	0,148	134	0,859	120	0,243	13	0,093
86	0,241	32	0,182	54	0,701	109	0,043	127	1,226	104	0,132	85	0,817	133	0,162	126	0,082
80	0,226	22	0,167	18	0,616	12	0,040	30	1,001	85	0,111	139	0,726	6	0,121	85	0,081
96	0,216	61	0,150	131	0,479	99	0,037	126	0,916	86	0,090	66	0,635	96	0,121	1	0,074
17	0,147	34	0,138	61	0,444	127	0,037	124	0,621	13	0,085	96	0,569	86	0,111	96	0,070
133	0,147	86	0,118	22	0,389	13	0,035	66	0,574	61	0,085	109	0,569	109	0,111	80	0,070
34	0,135	54	0,078	99	0,364	65	0,026	22	0,551	129	0,085	17	0,538	57	0,101	86	0,055
56	0,086	18	0,075	13	0,259	108	0,026	70	0,535	17	0,079	120	0,423	80	0,091	134	0,051
119	0,086	15	0,067	30	0,200	110	0,026	86	0,388	54	0,074	130	0,387	65	0,081	132	0,048
85	0,079	67	0,063	86	0,195	126	0,026	120	0,341	38	0,069	57	0,351	127	0,081	6	0,040
124	0,063	111	0,063	32	0,173	57	0,020	17	0,326	76	0,069	128	0,351	139	0,081	108	0,039
115	0,038	38	0,062	139	0,169	61	0,020	6	0,318	96	0,069	133	0,333	56	0,051	133	0,037
25	0,036	13	0,057	128	0,165	111	0,020	57	0,310	120	0,064	106	0,315	108	0,051	110	0,037
70	0,036	129	0,052	110	0,163	134	0,020	18	0,272	136	0,064	54	0,206	134	0,051	30	0,036
116	0,033	11	0,037	111	0,149	17	0,017	111	0,264	57	0,053	111	0,200	16	0,040	66	0,032
57	0,030	128	0,036	130	0,137	18	0,017	38	0,233	139	0,053	61	0,194	18	0,040	57	0,029
111	0,023	66	0,027	133	0,128	22	0,012	99	0,209	6	0,048	30	0,157	19	0,040	11	0,029
61	0,018	104	0,022	29	0,111	80	0,012	104	0,202	22	0,042	126	0,145	99	0,040	106	0,027
30	0,015	27	0,021	94	0,092	56	0,009	11	0,194	80	0,042	83	0,139	116	0,040	61	0,027
108	0,015	126	0,018	104	0,088	67	0,009	56	0,194	83	0,042	16	0,127	11	0,030	111	0,025
66	0,013	138	0,015	15	0,085	120	0,009	61	0,186	27	0,037	70	0,091	106	0,030	18	0,021
67	0,013	131	0,012	80	0,084	130	0,009	55	0,178	134	0,037	18	0,085	111	0,030	22	0,021
27	0,010	29	0,011	6	0,082	66	0,006	54	0,171	106	0,026	19	0,073	17	0,020	17	0,016
32	0,010	119	0,010	11	0,067	70	0,006	128	0,155	133	0,026	6	0,067	22	0,020	78	0,015
120	0,010	110	0,009	27	0,066	115	0,006	133	0,140	70	0,021	15	0,067	110	0,020	15	0,013
109	0,008	116	0,009	126	0,059	133	0,006	83	0,124	126	0,021	116	0,060	126	0,020	99	0,012
110	0,008	130	0,008	55	0,055	16	0,003	110	0,124	16	0,016	56	0,054	130	0,020	54	0,011
130	0,008	17	0,008	91	0,052	27	0,003	130	0,101	78	0,016	76	0,030	1	0,010	27	0,009
9	0,005	30	0,007	65	0,045	38	0,003	78	0,078	11	0,011	127	0,030	38	0,010	138	0,008
76	0,005	56	0,006	76	0,041	41	0,003	138	0,078	128	0,011	65	0,024	42	0,010	64	0,008
78	0,005	1	0,005	66	0,037	42	0,003	15	0,062	32	0,005	112	0,024	46	0,010	42	0,007
83	0,005	83	0,005	67	0,031	78	0,003	115	0,062	36	0,005	114	0,024	54	0,010	56	0,006
1	0,003	78	0,004	17	0,030	83	0,003	16	0,054	46	0,005	22	0,018	61	0,010	116	0,006
6	0,003	137	0,003	70	0,029	89	0,003	80	0,054	56	0,005	38	0,018	62	0,010	70	0,006
35	0,003	58	0,003	83	0,026	107	0,003	125	0,047	111	0,005	37	0,012	66	0,010	72	0,006
42	0,003	76	0,003	123	0,026	116	0,003	27	0,039	116	0,005	41	0,012	91	0,010	3	0,006
54	0,003	96	0,003	36	0,025	139	0,003	37	0,039	130	0,005	55	0,012	101	0,010	55	0,005
131	0,003	89	0,002	115	0,023	1	0,000	67	0,039	138	0,005	94	0,012	128	0,010	2	0,005
134	0,003	2	0,002	78	0,021	2	0,000	62	0,031	1	0,000	110	0,012	129	0,010	16	0,005
138	0,003	107	0,002	134	0,019	3	0,000	137	0,031	2	0,000	2	0,006	2	0,000	38	0,005
2	0,000	9	0,002	96	0,016	4	0,000	82	0,023	3	0,000	11	0,006	3	0,000	128	0,004
3	0,000	36	0,002	129	0,015	5	0,000	106	0,023	4	0,000	48	0,006	4	0,000	5	0,003
4	0,000	94	0,002	16	0,015	7	0,000	116	0,023	5	0,000	80	0,006	5	0,000	8	0,003
5	0,000	7	0,001	25	0,015	8	0,000	123	0,023	7	0,000	119	0,006	7	0,000	25	0,003
7	0,000	109	0,001	107	0,012	9	0,000	139	0,023	8	0,000	129	0,006	8	0,000	36	0,003
8	0,000	3	0,001	58	0,012	10	0,000	7	0,016	9	0,000	131	0,006	9	0,000	68	0,003
10	0,000	4	0,001	1	0,011	11	0,000	19	0,016	10	0,000	132	0,006	10	0,000	94	0,003
14	0,000	44	0,001	19	0,011	14	0,000	41	0,016	14	0,000	135	0,006	14	0,000	101	0,003
15	0,000	62	0,001	116	0,008	15	0,000	42	0,016	15	0,000	1	0,000	15	0,000	67	0,003
16	0,000	115	0,001	38	0,007	19	0,000	46	0,016	19	0,000	3	0,000	20	0,000	83	0,003
19	0,000	127	0,001	41	0,007	20	0,000	29	0,008	20	0,000	4	0,000	21	0,000	9	0,002
20	0,000	135	0,001	89	0,007	21	0,000	48	0,008	21	0,000	5	0,000	23	0,000	10	0,002
21	0,000	5	0,000	2	0,005	23	0,000	53	0,008	23	0,000	7	0,000	24	0,000	14	0,002
23	0,000	8	0,000	46	0,005	24	0,000	72	0,008	24	0,000	8	0,000	25	0,000	23	0,002
24	0,000	10	0,000	42	0,004	25	0,000	77	0,008	25	0,000	9	0,000	26	0,000	32	0,002
26	0,000	14	0,000	37	0,004	26	0,000	91	0,008	26	0,000	10	0,000	27	0,000	49	0,002
28	0,000	16	0,000	62	0,004	28	0,000	94	0,008	28	0,000	14	0,000	28	0,000	62	0,002
29	0,000	19	0,000	9	0,003	29	0,000	129	0,008	29	0,000	20	0,000	29	0,000	73	0,002
31	0,000	20	0,000	64	0,003	31	0,000	136	0,008	31	0,000	21	0,000	31	0,000	89	0,002
33	0,000	21	0,000	48	0,003	32	0,000	1	0,000	33	0,000	23	0,000	32	0,000	91	0,002
36	0,000	23	0,000	106	0,003	33	0,000	2	0,000	35	0,000	24	0,000	33	0,000	114	0,002
37	0,000	24	0,000	118	0,003	35	0,000	3	0,000	37	0,000	25	0,000	35	0,000	129	0,002
38	0,000	25	0,000	3	0,001	36	0,000	4	0,000	39	0,000	26	0,000	36	0,000	130	0,002
39	0,000	26	0,000	4	0,001	37	0,000	5	0,000	40	0,000	27	0,000	37	0,000	7	0,001
40	0,000	28	0,000	7	0,001	39	0,000	8	0,000	41	0,000	28	0,000	39	0,000	19	0,001
41	0,000	31	0,000	50	0,001	40	0,000	9	0,000	42	0,000	29	0,000	40	0,000	48	0,001
43	0,000	33	0,000	121	0,001	43	0,000	10	0,000	43	0,000	31	0,000	41	0,000	63	0,001
44	0,000	35	0,000	135	0,001	44	0,000	14	0,000	44	0,000	32	0,000	43	0,000	112	0,001
45	0,000	37	0,000	136	0,001	45	0,000	20	0,000	45	0,000	33	0,000	45	0,000	4	0,000

Legend:

- >50
- >20
- 10-20
- 5-10
- 1-5
- 0.5-1
- 0.1-0.5
- 0.05-0.1
- 0.01-0.05
- 0.005-0.01
- <0.005
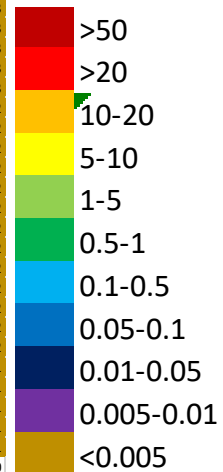
APPENDIX -6: Environmental taxa in water.

C2W3		C2W8		C2W13		C3W4		C3W8		C4W2		C4W10		C5W2		C5W10	
232	0,828	190	1,847	256	2,959	27	0,622	243	1,816	21	1,392	230	7,368	60	1,376	233	1,545
244	0,536	187	0,622	232	0,492	232	0,392	150	1,466	232	1,085	190	5,928	281	0,799	232	0,628
45	0,269	232	0,460	150	0,401	150	0,141	187	1,304	70	0,783	153	4,162	21	0,738	21	0,567
64	0,236	157	0,212	55	0,332	187	0,115	70	0,970	244	0,556	240	1,204	187	0,475	187	0,198
190	0,208	42	0,183	187	0,314	230	0,089	190	0,900	158	0,492	158	0,968	230	0,465	64	0,195
187	0,170	55	0,182	153	0,282	233	0,086	227	0,861	243	0,392	64	0,919	158	0,364	236	0,149
55	0,142	38	0,164	66	0,265	244	0,045	64	0,854	187	0,201	191	0,817	237	0,303	27	0,084
158	0,112	153	0,162	21	0,211	64	0,035	229	0,799	283	0,201	306	0,653	27	0,273	256	0,079
70	0,076	64	0,131	64	0,143	204	0,033	283	0,691	237	0,180	237	0,502	153	0,162	29	0,076
153	0,074	227	0,083	135	0,134	138	0,026	218	0,636	227	0,169	174	0,466	190	0,142	158	0,064
224	0,033	138	0,050	230	0,122	54	0,023	167	0,628	64	0,148	21	0,442	19	0,131	193	0,061
230	0,030	181	0,041	224	0,116	218	0,023	21	0,590	150	0,122	123	0,442	204	0,131	153	0,057
243	0,030	150	0,035	158	0,114	229	0,023	230	0,535	190	0,101	232	0,436	283	0,081	55	0,057
50	0,025	124	0,035	276	0,107	21	0,020	27	0,411	230	0,095	170	0,308	64	0,071	70	0,047
174	0,023	170	0,033	124	0,095	70	0,017	204	0,372	153	0,090	27	0,260	241	0,071	38	0,042
283	0,023	51	0,030	41	0,092	153	0,017	153	0,326	276	0,085	150	0,248	29	0,061	283	0,035
178	0,020	29	0,023	227	0,078	243	0,015	50	0,264	19	0,079	146	0,188	239	0,061	240	0,034
222	0,015	70	0,019	138	0,074	62	0,015	116	0,225	136	0,074	227	0,169	124	0,040	230	0,026
29	0,013	230	0,017	72	0,063	264	0,014	267	0,209	167	0,074	145	0,157	144	0,040	57	0,022
40	0,013	123	0,016	190	0,049	19	0,012	256	0,202	181	0,074	229	0,157	236	0,040	50	0,019
72	0,013	241	0,015	157	0,048	158	0,012	233	0,194	27	0,064	50	0,151	280	0,040	171	0,019
218	0,013	229	0,015	123	0,044	8	0,009	224	0,186	2	0,058	256	0,145	224	0,030	178	0,018
42	0,010	175	0,012	93	0,043	72	0,009	124	0,140	62	0,058	236	0,121	233	0,030	275	0,016
56	0,010	71	0,012	159	0,034	157	0,009	178	0,140	253	0,058	204	0,115	2	0,020	18	0,015
71	0,010	310	0,011	45	0,033	227	0,009	19	0,132	178	0,053	248	0,115	18	0,020	190	0,015
78	0,010	19	0,011	204	0,033	45	0,006	54	0,124	204	0,053	224	0,097	38	0,020	237	0,014
159	0,010	305	0,010	218	0,030	55	0,006	182	0,124	240	0,053	187	0,091	50	0,020	241	0,014
144	0,008	167	0,009	19	0,029	111	0,006	158	0,116	18	0,048	243	0,091	70	0,020	162	0,013
236	0,008	171	0,009	50	0,027	124	0,006	282	0,116	251	0,048	38	0,085	89	0,020	19	0,012
253	0,008	116	0,009	30	0,027	135	0,006	80	0,101	45	0,042	167	0,079	111	0,020	170	0,012
280	0,008	192	0,008	42	0,021	159	0,006	111	0,101	229	0,042	238	0,079	159	0,020	124	0,011
281	0,008	238	0,008	162	0,019	161	0,006	119	0,101	119	0,037	43	0,073	171	0,020	150	0,011
18	0,005	162	0,007	29	0,019	167	0,006	159	0,101	274	0,037	152	0,073	193	0,020	144	0,010
156	0,005	62	0,007	27	0,019	182	0,006	8	0,085	123	0,037	29	0,067	240	0,020	191	0,010
163	0,005	159	0,007	178	0,018	280	0,006	44	0,078	124	0,037	55	0,067	256	0,020	244	0,010
192	0,005	163	0,007	78	0,018	281	0,006	45	0,078	256	0,037	159	0,067	259	0,020	138	0,009
221	0,005	158	0,006	167	0,014	298	0,006	298	0,078	144	0,026	277	0,067	268	0,020	188	0,009
225	0,005	237	0,006	243	0,014	2	0,003	40	0,070	267	0,026	36	0,042	306	0,020	224	0,009
227	0,005	30	0,005	277	0,014	29	0,003	235	0,070	268	0,026	70	0,042	6	0,010	85	0,009
235	0,005	50	0,005	38	0,014	38	0,003	138	0,062	308	0,026	19	0,036	40	0,010	238	0,009
256	0,005	154	0,005	154	0,014	50	0,003	263	0,062	113	0,023	45	0,036	42	0,010	42	0,009
19	0,003	36	0,004	194	0,012	89	0,003	270	0,062	192	0,021	222	0,036	54	0,010	280	0,009
20	0,003	72	0,004	51	0,013	119	0,003	29	0,054	235	0,021	235	0,036	71	0,010	84	0,008
30	0,003	4	0,004	71	0,013	123	0,003	145	0,054	277	0,021	135	0,030	84	0,010	204	0,008
38	0,003	45	0,004	192	0,013	133	0,003	237	0,054	281	0,021	164	0,030	87	0,010	2	0,007
43	0,003	33	0,003	188	0,010	162	0,003	241	0,054	298	0,021	178	0,030	136	0,010	265	0,007
47	0,003	178	0,003	111	0,008	178	0,003	268	0,054	29	0,018	192	0,030	138	0,010	157	0,006
99	0,003	306	0,003	306	0,008	186	0,003	269	0,054	50	0,018	205	0,030	150	0,010	62	0,006
105	0,003	41	0,003	4	0,008	188	0,003	296	0,054	54	0,018	245	0,030	164	0,010	245	0,006
142	0,003	111	0,003	40	0,008	190	0,003	304	0,054	82	0,018	111	0,024	178	0,010	306	0,006
162	0,003	174	0,003	265	0,007	215	0,003	41	0,047	111	0,018	142	0,024	185	0,010	40	0,006
173	0,003	182	0,003	235	0,007	235	0,003	98	0,047	159	0,018	212	0,024	191	0,010	72	0,005
182	0,003	281	0,003	142	0,007	241	0,003	188	0,047	38	0,018	297	0,024	202	0,010	113	0,005
205	0,003	98	0,002	283	0,007	248	0,003	238	0,047	41	0,018	60	0,018	205	0,010	131	0,005
237	0,003	215	0,002	170	0,007	256	0,003	264	0,047	51	0,018	114	0,018	227	0,010	243	0,005
264	0,003	283	0,002	171	0,007	273	0,003	277	0,047	72	0,018	139	0,018	229	0,010	258	0,005
282	0,003	27	0,002	182	0,007	277	0,003	42	0,039	98	0,018	171	0,018	245	0,010	45	0,004
298	0,003	137	0,002	270	0,007	284	0,003	99	0,039	162	0,018	265	0,018	258	0,010	175	0,004
306	0,003	256	0,002	237	0,006	1	0,000	113	0,039	173	0,011	9	0,012	263	0,010	8	0,003
1	0,000	278	0,002	238	0,006	3	0,000	232	0,039	203	0,011	30	0,012	264	0,010	66	0,003
2	0,000	43	0,002	61	0,005	4	0,000	236	0,039	225	0,011	98	0,012	265	0,010	89	0,003
3	0,000	135	0,002	137	0,005	5	0,000	285	0,039	241	0,011	113	0,012	293	0,010	167	0,003
4	0,000	236	0,002	43	0,004	6	0,000	31	0,031	265	0,011	119	0,012	1	0,000	174	0,003
5	0,000	243	0,002	18	0,004	7	0,000	33	0,031	296	0,011	175	0,012	3	0,000	192	0,003
6	0,000	91	0,001	70	0,004	9	0,000	57	0,031	30	0,005	202	0,012	4	0,000	218	0,003
7	0,000	142	0,001	145	0,004	10	0,000	108	0,031	49	0,005	228	0,012	5	0,000	242	0,003
8	0,000	242	0,001	193	0,004	11	0,000	161	0,031	78	0,005	263	0,012	7	0,000	268	0,003
9	0,000	248	0,001	221	0,004	12	0,000	215	0,031	108	0,005	267	0,012	8	0,000	152	0,003
10	0,000	251	0,001	222	0,004	13	0,000	225	0,031	131	0,005	278	0,012	9	0,000	267	0,003
11	0,000	298	0,001	241	0,004	14	0,000	18	0,023	145	0,005	2	0,006	10	0,000	26	0,002
12	0,000	35	0,001	244	0,004	15	0,000	55	0,023	154	0,005	6	0,006	11	0,000	31	0,002
13	0,000	119	0,001	263	0,004	16	0,000	62	0,023	157	0,005	58	0,006	12	0,000	54	0,002
14	0,000	139	0,001	144	0,003	17	0,000	89	0,023	176	0,005	71	0,006	13	0,000	78	0,002
15	0,000	144	0,001	296	0,003	18	0,000	123	0,023	193	0,005	72	0,006	14	0,000	87	0,002
16	0,000	145	0,001	82	0,003	20	0,000	170	0,023	202	0,005	85	0,006	15	0,000	123	0,002
17	0,000	172	0,001	119	0,003	22	0,000	175	0,023	210	0,005	96	0,006	16	0,000	134	0,002
21	0,000	253	0,001	172	0,003	23	0,000	240	0,023	224	0,005	101	0,006	17	0,000	135	0,002
22	0,000	296	0,001	225	0,003	24	0,000	280	0,023	233	0,005	108	0,006	20	0,000	139	0,002
23	0,000	304	0,001	236	0,003	25	0,000	281	0,023	275	0,005	116	0,006	22	0,000	159	0,002
24	0,000	1	0,000	240	0,003	26	0,000	38	0,016	284	0,005	118	0,006	23	0,000	161	0,002
25	0,000	2	0,000	242	0,003	28	0,000	84	0,016	1	0,000	162	0,006	24	0,000	173	0,002
26	0,000	3	0,000	298	0,003	30	0,000	112	0,016	3	0,000	173	0,006	25	0,000	198	0,002
27	0,000	5	0,000	16	0,001	31	0,000	203	0,016	4	0,000	216	0,006	26	0,000	227	0,002
28	0,000	6	0,000	46	0,001	32	0,000	222	0,016	5	0,000	218	0,006	28	0,000	264	0,002
31	0,000	7	0,000	73	0,001	33	0,000	228	0,016	6	0,000	253	0,006	30	0,000	272	0,002
32	0,000	8	0,000	81	0,001	34	0,000	231	0,016	7	0,000	264	0,006	31	0,000	277	0,002
33	0,000	9	0,000	113	0,001	35	0,000	245	0,016	8	0,000	268	0,006	32	0,000	292	0,002
34	0,000	10	0,000	141	0,001	36	0,000	275	0,016	9	0,000	281	0,006	33	0,000	16	0,001
35	0,000	11	0,000	173	0,001	37	0,000	284	0,016	10	0,000	282	0,006	34	0,000	145	0,001
36	0,000	12	0,000	174	0,001	39	0,000	306	0,016	11	0,000	283	0,006	35	0,000	229	0,001
37	0,000	13	0,000	215	0,001	40	0,000	2	0,008	12	0,000	294	0,006	36	0,000	281	0,001
39	0,000	14	0,000	228	0,001	41	0,000	5	0,008	13	0,000	296	0,006	37	0,000	310	0,001
41	0,000	15	0,000	253	0,001	42	0,000	32	0,008	14	0,000	298	0,006	39	0,000	1	0,000
44	0,000	16	0,000	261	0,001	43	0,000	36	0,008	15	0,000	307	0,006	41	0,000	3	0,000
46	0,000	17	0,000	267	0,001	44	0,000	66	0,008	16	0,000	1	0,000	43	0,000	4	0,000
48	0,000	18	0,000	269	0,001	46	0,000	88	0,008	17	0,000	3	0,000	44	0,000	5	0,000
49	0,000	20	0,000	271	0,001	47	0,000	110	0,008	20	0,000	4	0,000	45	0,000	6	0,000
51	0,000	21	0,000	282	0,001	48	0,000	135	0,008	22	0,000	5	0,000	46	0,000	7	0,000
52	0,000	22	0,000	310	0,001	49	0,000	136	0,008	23	0,000	7	0,000	47	0,000	9	0,000
53	0,000	23	0,000	1	0,000	51	0,000	139	0,008	24	0,000	8	0,000	48	0,000	10	0,000
54	0,000	24	0,000	2	0,000	52	0,000	144	0,008	25	0,000	10	0,000	49	0,000	11	0,000
57	0,000	25	0,000	3	0,000	53	0,000	149	0,008	26	0,000	11	0,000	51	0,000	12	0,000
58	0,000	26	0,000	5	0,000	56	0,000	176	0,008	28	0,000	12	0,000	52	0,000	13	0,000
59	0,000	28	0,000	6	0,000	57	0,000	194	0,008	31	0,000	13	0,000	53	0,000	14	0,000
60	0,000	31	0,000	7	0,000	58	0,000	202	0,008	32	0,000	14	0,000	55	0,000	15	0,000
61	0,000	32	0,000	8	0,000	59	0,000	207	0,008	33	0,000	15	0,000	56	0,000	17	0,000
62	0,000	34	0,000	9	0,000	60	0,000	244	0,008	34	0,000	16	0,000	57	0,000	20	0,000
63	0,000	37	0,000	10	0,000	61	0,000	251	0,008	35	0,000	17	0,000	58	0,000	22	0,000
65	0,000	39	0,000	11	0,000	63	0,000	272	0,008	36	0,000	18	0,000	59	0,000	23	0,000
66	0,000	40	0,000	12	0,000	65	0,000	273	0,008	37	0,000	20	0,000	61	0,000	24	0,000
67	0,000	44	0,000	13	0,000	66	0,000	297	0,008	39	0,000	22	0,000	62	0,000	25	0,000
68	0,000	46	0,000	14	0,000	67	0,000	303	0,008	40	0,000	23	0,000	63	0,000	28	0,000
69	0,000	47	0,000	15	0,000	68	0,000	310	0,008	42	0,000	24	0,000	65	0,000	30	0,000

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- 0.005-0.01
- <0.005

APPENDIX -7: Inoculums associated taxa in fish skin wounds.

C2W3		C2W8		C2W13		C3W4	
11	1,127	95	17,634	104	0,073	34	0,023
95	0,336	11	2,870	95	0,036	12	0,009
71	0,164	71	1,949	85	0,032	1	0,005
18	0,101	99	0,830	12	0,024	22	0,005
104	0,090	34	0,646	34	0,024	95	0,005
13	0,082	104	0,607	71	0,024	99	0,005
17	0,078	83	0,496	65	0,016	104	0,005
12	0,060	120	0,473	61	0,012	108	0,005
99	0,056	12	0,426	99	0,012	109	0,005
96	0,052	139	0,423	18	0,008	2	0,000
1	0,049	86	0,420	120	0,008	3	0,000
94	0,045	2	0,383	1	0,004	4	0,000
22	0,037	109	0,290	25	0,004	5	0,000
34	0,022	108	0,230	30	0,004	6	0,000
85	0,022	96	0,223	56	0,004	7	0,000
137	0,022	1	0,193	86	0,004	8	0,000
25	0,011	61	0,190	106	0,004	9	0,000
111	0,011	38	0,180	108	0,004	10	0,000
138	0,011	133	0,140	126	0,004	11	0,000
56	0,007	134	0,140	138	0,004	13	0,000
65	0,007	70	0,137	2	0,000	14	0,000
70	0,007	85	0,137	3	0,000	15	0,000
86	0,007	3	0,097	4	0,000	16	0,000
134	0,007	25	0,097	5	0,000	17	0,000
7	0,004	106	0,097	6	0,000	18	0,000
66	0,004	124	0,097	7	0,000	19	0,000
80	0,004	22	0,093	8	0,000	20	0,000
89	0,004	30	0,093	9	0,000	21	0,000
133	0,004	47	0,093	10	0,000	23	0,000
2	0,000	49	0,093	11	0,000	24	0,000
3	0,000	65	0,093	13	0,000	25	0,000
4	0,000	126	0,093	14	0,000	26	0,000
5	0,000	18	0,086	15	0,000	27	0,000
6	0,000	66	0,086	16	0,000	28	0,000
8	0,000	13	0,043	17	0,000	29	0,000
9	0,000	80	0,043	19	0,000	30	0,000
10	0,000	115	0,043	20	0,000	31	0,000
14	0,000	130	0,043	21	0,000	32	0,000

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	5-10
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	0.1-0.5
	0.05-0.1
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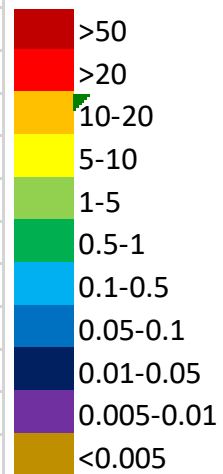
APPENDIX -8: Environmental taxa in fish skin wounds.

C2W3		C2W8		C2W13		C3W4	
262	94,497	206	42,040	262	99,575	220	97,689
50	2,593	50	18,213	20	0,045	50	1,446
169	0,142	211	2,099	206	0,036	262	0,709
43	0,119	220	1,504	137	0,008	206	0,074
211	0,075	43	1,289	197	0,008	43	0,005
49	0,056	49	1,048	51	0,004	157	0,005
34	0,011	262	0,703	92	0,004	169	0,005
137	0,011	41	0,503	132	0,004	216	0,005
201	0,011	166	0,477	161	0,004		
123	0,007	51	0,233	222	0,004		
206	0,007		0,193				
24	0,004	6	0,190				
26	0,004	169	0,173				
41	0,004	24	0,097				
138	0,004	63	0,097				
153	0,004	132	0,097				
197	0,004	161	0,097				
203	0,004	204	0,097				
212	0,004	251	0,097				
220	0,004	34	0,093				
222	0,004	248	0,093				
		137	0,043				
		138	0,043				
		197	0,043				
		212	0,043				
		219	0,043				
		221	0,043				

Legend:
- >50
- >20
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- 1-5
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- 0.1-0.5
- 0.05-0.1
- 0.01-0.05
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- <0.005

APPENDIX -9: Inoculums associated taxa in fish skin.

C2W13$_{s.n.w}$		C2W13$_{d.n.w}$		C3W4$_{s.n.w}$		C3W8$_{hf}$		C3W8$_{hf\,gill}$		C4W10$_{lashing}$		C4W10$_{petechia}$		C4W10$_{hf}$	
138	15,272	104	12,530	104	20,880	127	10,441	134	12,627	65	29,504	104	37,575	98	22,944
95	4,422	95	10,362	108	14,498	71	10,232	71	11,895	34	16,471	48	5,944	113	20,584
104	3,559	99	4,699	95	6,475	138	8,892	65	9,192	94	7,962	99	5,057	99	4,573
34	3,387	34	4,170	83	5,735	34	7,290	108	8,671	86	6,409	71	3,957	95	3,162
99	2,071	18	4,098	126	4,053	99	5,657	12	4,476	71	5,931	129	3,558	138	2,899
71	1,186	12	2,659	34	2,754	126	4,808	85	3,941	83	4,213	126	3,106	108	2,525
85	1,143	120	2,295	99	1,511	108	4,126	96	3,252	12	2,666	12	2,209	83	1,220
12	0,992	71	1,930	71	1,299	104	3,726	95	3,069	85	2,585	95	1,972	129	1,194
139	0,733	56	1,493	65	0,689	85	3,660	34	2,829	114	2,582	108	1,912	104	0,856
18	0,690	126	1,421	12	0,462	103	2,320	126	2,675	103	2,397	34	1,350	34	0,770
37	0,539	85	0,874	134	0,319	18	2,284	13	2,660	99	1,951	98	1,296	48	0,617
120	0,431	124	0,656	96	0,305	134	1,997	30	2,013	128	1,896	86	1,221	139	0,536
126	0,410	110	0,619	85	0,263	95	1,800	127	1,365	89	1,825	128	0,756	102	0,506
128	0,324	86	0,492	124	0,226	83	1,734	120	1,140	118	1,553	38	0,738	71	0,482
17	0,259	22	0,437	76	0,217	12	1,728	86	1,126	139	0,835	17	0,531	86	0,298
11	0,237	11	0,419	127	0,199	139	1,645	11	1,056	18	0,796	130	0,395	12	0,295
70	0,173	139	0,346	86	0,148	120	1,639	70	0,929	96	0,764	11	0,354	85	0,243
54	0,108	128	0,328	17	0,143	70	1,208	104	0,774	138	0,508	139	0,246	130	0,234
65	0,108	130	0,291	18	0,125	96	1,053	38	0,732	133	0,443	85	0,228	17	0,232
86	0,108	115	0,273	30	0,120	38	0,909	22	0,577	17	0,404	83	0,186	126	0,208
96	0,108	17	0,255	37	0,102	65	0,658	54	0,535	76	0,382	119	0,181	103	0,200
49	0,086	32	0,237	110	0,088	128	0,640	17	0,521	115	0,356	134	0,175	1	0,191
56	0,086	138	0,200	54	0,083	118	0,628	18	0,479	95	0,343	124	0,171	119	0,137
57	0,086	57	0,182	139	0,083	130	0,610	66	0,479	124	0,265	138	0,149	65	0,080
118	0,086	94	0,146	115	0,074	110	0,490	128	0,465	130	0,223	113	0,145	128	0,063
124	0,086	30	0,127	48	0,069	78	0,431	57	0,408	122	0,210	18	0,119	38	0,059
130	0,086	70	0,127	57	0,051	11	0,377	56	0,310	108	0,142	66	0,103	13	0,054
30	0,065	96	0,127	70	0,051	13	0,299	78	0,282	104	0,110	13	0,073	18	0,046
32	0,065	13	0,109	13	0,042	17	0,281	99	0,239	13	0,087	54	0,055	89	0,046
83	0,065	54	0,091	56	0,042	57	0,233	55	0,211	134	0,087	57	0,045	66	0,044
16	0,043	111	0,091	11	0,037	115	0,215	83	0,141	135	0,049	120	0,041	124	0,041
22	0,043	133	0,091	22	0,032	76	0,209	109	0,113	70	0,036	30	0,030	112	0,037
91	0,043	66	0,073	6	0,028	135	0,197	80	0,099	110	0,036	102	0,026	54	0,033
110	0,043	129	0,073	1	0,023	54	0,173	111	0,084	120	0,036	70	0,019	70	0,022
111	0,043	29	0,055	38	0,023	30	0,167	6	0,070	11	0,032	133	0,015	57	0,019
131	0,043	76	0,055	120	0,023	56	0,167	16	0,070	37	0,023	111	0,013	30	0,013
1	0,022	78	0,055	128	0,023	86	0,155	19	0,070	80	0,019	55	0,011	134	0,013
15	0,022	127	0,055	55	0,018	37	0,150	42	0,070	126	0,019	96	0,009	76	0,011
38	0,022	16	0,036	67	0,018	124	0,144	61	0,070	1	0,016	103	0,009	11	0,009
61	0,022	27	0,036	89	0,018	89	0,138	130	0,070	38	0,013	112	0,009	115	0,007
103	0,022	55	0,036	111	0,018	114	0,138	115	0,056	57	0,013	115	0,008	133	0,007
129	0,022	106	0,036	16	0,014	109	0,120	124	0,056	2	0,010	65	0,006	94	0,006
134	0,022	108	0,036	61	0,014	132	0,114	133	0,056	54	0,010	89	0,004	55	0,004
135	0,022	131	0,036	66	0,009	111	0,102	41	0,042	78	0,010	56	0,002	22	0,000
2	0,000	1	0,018	118	0,009	19	0,078	132	0,042	106	0,010	76	0,002	56	0,000
3	0,000	5	0,018	129	0,009	66	0,048	36	0,028	111	0,010	1	0,000	96	0,000
4	0,000	15	0,018	133	0,009	6	0,042	48	0,028	112	0,010	22	0,000	111	0,000
5	0,000	38	0,018	2	0,005	131	0,030	67	0,028	116	0,010	94	0,000	120	0,000
6	0,000	61	0,018	27	0,005	133	0,030	89	0,028	129	0,010	127	0,000	127	0,000
7	0,000	65	0,018	32	0,005	22	0,024	110	0,028	132	0,010	2		2	
8	0,000	67	0,018	46	0,005	55	0,024	125	0,028	19	0,006	3		3	
9	0,000	80	0,018	49	0,005	94	0,018	138	0,028	91	0,006	4		4	
10	0,000	83	0,018	80	0,005	9	0,012	139	0,028	10	0,003	5		5	
13	0,000	103	0,018	94	0,005	16	0,012	1	0,014	16	0,003	6		6	
14	0,000	109	0,018	116	0,005	42	0,012	2	0,014	22	0,003	7		7	
19	0,000	135	0,018	135	0,005	1	0,006	29	0,014	25	0,003	8		8	
20	0,000	2	0,000	136	0,005	2	0,006	32	0,014	46	0,003	9		9	
21	0,000	3	0,000	3	0,000	5	0,006	46	0,014	53	0,003	10		10	
23	0,000	4	0,000	4	0,000	32	0,006	106	0,014	56	0,003	14		14	
24	0,000	6	0,000	5	0,000	49	0,006	129	0,014	64	0,003	15		15	
25	0,000	7	0,000	7	0,000	61	0,006	131	0,014	109	0,003	16		16	
26	0,000	8	0,000	8	0,000	80	0,006	3	0,000	119	0,003	19		19	
27	0,000	9	0,000	9	0,000	121	0,006	4	0,000	3	0,000	20		20	
28	0,000	10	0,000	10	0,000	122	0,006	5	0,000	4	0,000	21		21	
29	0,000	14	0,000	14	0,000	129	0,006	7	0,000	5	0,000	23		23	

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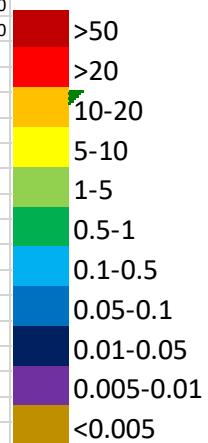
APPENDIX -10: Environmental taxa in fish skin.

C2W13$_{s.n.w}$		C2W13$_{d.n.w}$		C3W4$_{s.n.w}$		C3W8$_{hf}$		C3W8$_{hf\ gill}$		C4W10$_{lashing}$		C4W10$_{petechia}$		C4W10$_{hf}$	
243	58,302	283	41,668	241	20,422	64	2,978	243	4,251	190	1,178	70	2,890	283	0,823
64	1,553	150	0,765	283	9,922	283	1,836	283	3,252	64	0,802	63	2,043	70	0,513
227	0,539	64	0,692	227	4,561	243	1,674	64	1,886	240	0,615	158	1,432	72	0,495
72	0,453	227	0,692	233	0,615	227	1,621	227	1,450	66	0,573	237	0,760	158	0,417
269	0,302	55	0,619	64	0,582	66	1,041	70	0,901	158	0,466	152	0,452	63	0,397
70	0,173	187	0,437	224	0,564	267	0,825	218	0,859	243	0,434	69	0,433	166	0,345
283	0,173	153	0,419	38	0,236	229	0,664	241	0,802	159	0,262	55	0,331	71	0,323
41	0,151	72	0,255	150	0,203	242	0,628	187	0,760	237	0,207	248	0,305	243	0,263
242	0,108	224	0,200	243	0,166	54	0,431	150	0,563	271	0,165	164	0,188	65	0,124
55	0,086	51	0,146	27	0,129	222	0,359	190	0,479	29	0,139	267	0,175	237	0,117
98	0,086	124	0,146	232	0,116	70	0,293	229	0,380	153	0,120	153	0,166	152	0,106
150	0,065	159	0,109	153	0,111	224	0,293	124	0,338	70	0,110	71	0,090	55	0,093
241	0,065	29	0,091	190	0,106	150	0,263	237	0,296	55	0,065	166	0,053	69	0,056
66	0,043	158	0,073	230	0,092	190	0,257	267	0,253	204	0,065	72	0,043	267	0,030
71	0,043	218	0,073	158	0,083	80	0,245	159	0,239	189	0,049	154	0,036	154	0,028
135	0,043	71	0,055	187	0,079	57	0,233	224	0,225	224	0,036	27	0,026	241	0,019
138	0,043	222	0,055	159	0,055	233	0,191	182	0,211	236	0,036	150	0,024	164	0,017
153	0,043	42	0,036	62	0,051	55	0,138	158	0,197	261	0,029	243	0,023	153	0,013
232	0,043	45	0,036	70	0,037	187	0,132	50	0,183	306	0,026	283	0,017	27	0,011
236	0,043	50	0,036	44	0,032	111	0,114	233	0,183	61	0,023	65	0,008	168	0,007
256	0,043	75	0,036	71	0,032	72	0,102	111	0,127	194	0,023	241	0,006	156	0,006
29	0,022	123	0,036	123	0,032	159	0,102	153	0,127	27	0,019	168	0,004	150	0,004
30	0,022	190	0,036	237	0,032	153	0,084	232	0,113	139	0,019	124	0,000	248	0,004
40	0,022	233	0,036	138	0,028	218	0,084	19	0,099	193	0,016	155	0,000	124	0,000
42	0,022	40	0,018	236	0,018	27	0,078	27	0,099	310	0,016	156	0,000	155	0,000
75	0,022	41	0,018	21	0,014	158	0,072	55	0,099	19	0,013	161	0,000	161	0,000
157	0,022	65	0,018	54	0,014	135	0,066	72	0,070	78	0,013	233	0,000	233	0,000
158	0,022	66	0,018	72	0,014	50	0,054	113	0,070	230	0,013	240	0,000	240	0,000
229	0,022	82	0,018	124	0,014	241	0,054	178	0,070	21	0,010	1	0,000	1	0,000
278	0,022	84	0,018	222	0,014	44	0,042	204	0,070	45	0,010	2	0,000	2	0,000
1	0,000	135	0,018	242	0,014	230	0,042	282	0,070	74	0,010	3	0,000	3	0,000
2	0,000	138	0,018	19	0,009	41	0,036	21	0,056	178	0,010	4	0,000	4	0,000
3	0,000	141	0,018	85	0,009	78	0,036	41	0,056	242	0,010	5	0,000	5	0,000
4	0,000	191	0,018	102	0,009	237	0,036	45	0,056	50	0,006	6	0,000	6	0,000
5	0,000	192	0,018	135	0,009	240	0,036	222	0,056	54	0,006	7	0,000	7	0,000
6	0,000	236	0,018	204	0,009	270	0,036	18	0,042	93	0,006	8	0,000	8	0,000
7	0,000	241	0,018	215	0,009	94	0,030	44	0,042	145	0,006	9	0,000	9	0,000
8	0,000	269	0,018	218	0,009	124	0,030	51	0,042	164	0,006	10	0,000	10	0,000
9	0,000	276	0,018	240	0,009	204	0,030	167	0,042	192	0,006	11	0,000	11	0,000
10	0,000	1	0,000	20	0,005	310	0,030	54	0,028	235	0,006	12	0,000	12	0,000
11	0,000	2	0,000	78	0,005	108	0,024	114	0,028	307	0,006	13	0,000	13	0,000
12	0,000	3	0,000	141	0,005	178	0,024	135	0,028	20	0,003	14	0,000	14	0,000
13	0,000	4	0,000	154	0,005	304	0,024	154	0,028	72	0,003	15	0,000	15	0,000
14	0,000	5	0,000	167	0,005	19	0,018	236	0,028	85	0,003	16	0,000	16	0,000
15	0,000	6	0,000	178	0,005	29	0,018	259	0,028	149	0,003	17	0,000	17	0,000
16	0,000	7	0,000	182	0,005	71	0,018	295	0,028	171	0,003	18	0,000	18	0,000
17	0,000	8	0,000	229	0,005	228	0,018	29	0,014	175	0,003	19	0,000	19	0,000
18	0,000	9	0,000	244	0,005	266	0,018	42	0,014	187	0,003	20	0,000	20	0,000
19	0,000	10	0,000	265	0,005	100	0,012	85	0,014	222	0,003	21	0,000	21	0,000
20	0,000	11	0,000	269	0,005	116	0,012	94	0,014	227	0,003	22	0,000	22	0,000
21	0,000	12	0,000	306	0,005	139	0,012	101	0,014	232	0,003	23	0,000	23	0,000
22	0,000	13	0,000	1	0,000	236	0,012	116	0,014	238	0,003	24	0,000	24	0,000
23	0,000	14	0,000	2	0,000	244	0,012	119	0,014	241	0,003	25	0,000	25	0,000
24	0,000	15	0,000	3	0,000	261	0,012	123	0,014	248	0,003	26	0,000	26	0,000
25	0,000	16	0,000	4	0,000	2	0,006	138	0,014	262	0,003	28	0,000	28	0,000
26	0,000	17	0,000	5	0,000	18	0,006	145	0,014	1	0,000	29	0,000	29	0,000
27	0,000	18	0,000	6	0,000	51	0,006	156	0,014	2	0,000	30	0,000	30	0,000
28	0,000	19	0,000	7	0,000	58	0,006	175	0,014	3	0,000	31	0,000	31	0,000
31	0,000	20	0,000	8	0,000	96	0,006	188	0,014	4	0,000	32	0,000	32	0,000
32	0,000	21	0,000	9	0,000	138	0,006	193	0,014	5	0,000	33	0,000	33	0,000
33	0,000	22	0,000	10	0,000	141	0,006	228	0,014	6	0,000	34	0,000	34	0,000
34	0,000	23	0,000	11	0,000	154	0,006	230	0,014	7	0,000	35	0,000	35	0,000
35	0,000	24	0,000	12	0,000	167	0,006	235	0,014	8	0,000	36	0,000	36	0,000
36	0,000	25	0,000	13	0,000	174	0,006	240	0,014	9	0,000	37	0,000	37	0,000
37	0,000	26	0,000	14	0,000	194	0,006	245	0,014	10	0,000	38	0,000	38	0,000
38	0,000	27	0,000	15	0,000	225	0,006	281	0,014	11	0,000	39	0,000	39	0,000
39	0,000	28	0,000	16	0,000	232	0,006	296	0,014	12	0,000	40	0,000	40	0,000
43	0,000	30	0,000	17	0,000	253	0,006	298	0,014	13	0,000	41	0,000	41	0,000
44	0,000	31	0,000	18	0,000	256	0,006	1	0,000	14	0,000	42	0,000	42	0,000
45	0,000	32	0,000	22	0,000	265	0,006	2	0,000	15	0,000	43	0,000	43	0,000
46	0,000	33	0,000	23	0,000	295	0,006	3	0,000	16	0,000	44	0,000	44	0,000

Legend:
- >50
- >20
- 10-20
- 5-10
- 1-5
- 0.5-1
- 0.1-0.5
- 0.05-0.1
- 0.01-0.05
- 0.005-0.01
- <0.005

APPENDIX-11: Number code for inoculums associated taxa

1 Archaea;__Thaumarchaeota;__Soil_Crenarchaeotic_Group(SCG);__o;__f;__g
2 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Bradyrhizobiaceae;__Bradyrhizobium
3 Bacteria;__Cyanobacteria;__MLE1-12;__o;__f;__g
4 Archaea;__Thaumarchaeota;__Sc-EA05;__o;__f;__g
5 Bacteria;__Acidobacteria;__Acidobacteria;__Candidatus_Chloracidobacterium;__f;__g
6 Bacteria;__TM6;__c;__o;__f;__g
7 Bacteria;__Actinobacteria;__Acidimicrobiia;__Acidimicrobiales;__OCS155_marine_group;__g
8 Bacteria;__Deinococcus-Thermus;__Deinococci;__Deinococcales;__Trueperaceae;__Truepera
9 Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Arenibacter
10 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Xanthobacteraceae;__Pseudolabrys
11 Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__Saprospiraceae;__Lewinella
12 Unassigned;Other;Other;Other;Other;Other
13 Bacteria;__Planctomycetes;__Phycisphaerae;__Phycisphaerales;__Phycisphaeraceae;__Phycisphaera
14 Bacteria;__Actinobacteria;__Acidimicrobiia;__Acidimicrobiales;__TM214;__g
15 Bacteria;__Proteobacteria;__Gammaproteobacteria;__NKB5;__f;__g
16 Bacteria;__Proteobacteria;__Deltaproteobacteria;__Sh765B-TzT-29;__f;__g
17 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Order_Incertae_Sedis;__Family_Incertae_Sedis;__Marinicella
18 Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__Chitinophagaceae;__g
19 Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__NS11-12_marine_group;__g
20 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Caulobacterales;__Hyphomonadaceae;__Woodsholea
21 Bacteria;__Chlorobi;__Ignavibacteria;__Ignavibacteriales;__PHOS-HE36;__g
22 Bacteria;__Chloroflexi;__Anaerolineae;__Anaerolineales;__Anaerolineaceae;__g
23 Bacteria;__Chloroflexi;__TK10;__o;__f;__g
24 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Pseudomonadales;__Pseudomonadaceae;__Cellvibrio
25 Bacteria;__Chloroflexi;__Caldilineae;__Caldilineales;__Caldilineaceae;__Caldilinea
26 Bacteria;__Gemmatimonadetes;__Gemmatimonadetes;__Gemmatimonadales;__Gemmatimonadaceae;__g
27 Bacteria;__Verrucomicrobia;__Opitutae;__Opitutales;__Opitutaceae;__Opitutus
28 Bacteria;__Proteobacteria;__Betaproteobacteria;__Nitrosomonadales;__Nitrosomonadaceae;__g
29 Bacteria;__Planctomycetes;__Planctomycetacia;__Planctomycetales;__Planctomycetaceae;__g
30 Bacteria;__Planctomycetes;__OM190;__o;__f;__g
31 Bacteria;__Planctomycetes;__Phycisphaerae;__Phycisphaerales;__Phycisphaeraceae;Other
32 Bacteria;__Verrucomicrobia;__OPB35_soil_group;__o;__f;__g
33 Bacteria;__Candidate_division_WS3;__c;__o;__f;__g
34 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodobacterales;__Rhodobacteraceae;__g
35 Bacteria;__Chloroflexi;__Gitt-GS-136;__o;__f;__g
36 Bacteria;__Actinobacteria;__Acidimicrobiia;__Acidimicrobiales;__Iamiaceae;__Iamia
37 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Pseudomonadales;__Pseudomonadaceae;__Pseudomonas
38 Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__Saprospiraceae;__g
39 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Xanthomonadales;__Sinobacteraceae;__g
40 Bacteria;__Chloroflexi;__Thermomicrobia;__JG30-KF-CM45;__f;__g
41 Bacteria;__Bacteroidetes;__Cytophagia;__Order_II_Incertae_Sedis;__Rhodothermaceae;__g
42 Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Sorangiineae;__g
43 Bacteria;__Armatimonadetes;__c;__o;__f;__g
44 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Legionellales;__Coxiellaceae;__Aquicella
45 Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__env.OPS_17;__g
46 Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__0319-6G20;__g
47 Bacteria;__Proteobacteria;Other;Other;Other;Other
48 Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Nannocystaceae;__Nannocystis
49 Bacteria;__Proteobacteria;__Betaproteobacteria;__Burkholderiales;__Comamonadaceae;__Pelomonas
50 Bacteria;__Chloroflexi;__Thermomicrobia;__AKYG1722;__f;__g
51 Bacteria;__Chloroflexi;__KD4-96;__o;__f;__g
52 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Xanthomonadales;__Xanthomonadaceae;__Pseudofulvimonas
53 Bacteria;__Proteobacteria;__Gammaproteobacteria;__aaa34a10;__f;__g
54 Bacteria;__Planctomycetes;__Planctomycetacia;__Planctomycetales;__Planctomycetaceae;__Pir4_lineage
55 Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Sandaracinaceae;__Sandaracinus
56 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodobacterales;__Rhodobacteraceae;__Pseudorhodobacter
57 Bacteria;__Planctomycetes;__Planctomycetacia;__Planctomycetales;__Planctomycetaceae;__Planctomyces
58 Bacteria;__Candidate_division_TM7;__c;__o;__f;__g
59 Bacteria;__Planctomycetes;__Planctomycetacia;__Planctomycetales;__Planctomycetaceae;__Gemmata
60 Bacteria;__Chloroflexi;__Caldilineae;__Caldilineales;__Caldilineaceae;__g
61 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__g
62 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__Rickettsiaceae;__g
63 Bacteria;__Proteobacteria;__Betaproteobacteria;__Burkholderiales;__Comamonadaceae;Other
64 Bacteria;__Proteobacteria;__Deltaproteobacteria;__Bdellovibrionales;__Bacteriovoracaceae;__Peredibacter
65 Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfuromonadales;__GR-WP33-58;__g
66 Bacteria;__Proteobacteria;__Betaproteobacteria;__Nitrosomonadales;__Nitrosomonadaceae;__Nitrosomonas
67 Bacteria;__Verrucomicrobia;__Verrucomicrobiae;__Verrucomicrobiales;__DEV007;__g
68 Bacteria;__Actinobacteria;__Acidimicrobiia;__Acidimicrobiales;__uncultured;__g
69 Bacteria;__Acidobacteria;__Acidobacteria;__DA023;__f;__g
70 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodobacterales;__Rhodobacteraceae;__Rhodobacter
71 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodobacterales;__Rhodobacteraceae;Other
72 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__Rhodovibrio
73 Bacteria;__Chloroflexi;__S085;__o;__f;__g
74 Bacteria;__Elusimicrobia;__Elusimicrobia;__Lineage_IIb;__f;__g
75 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__wr0007;__g
76 Bacteria;__Chlorobi;__Chlorobia;__Chlorobiales;__OPB56;__g
77 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__EF100-94H03;__g
78 Bacteria;__Cyanobacteria;__Chloroplast;__Chloroplast;__Chloroplast;__Chloroplast
79 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Hyphomicrobiaceae;__Hyphomicrobium
80 Bacteria;__Planctomycetes;__Phycisphaerae;__Phycisphaerales;__Phycisphaeraceae;__SM1A02
81 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Order_Incertae_Sedis;__Family_Incertae_Sedis;__Thiohalophilus
82 Bacteria;__Chloroflexi;__JG30-KF-CM66;__o;__f;__g
83 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;__Marinobacter
84 Bacteria;__Planctomycetes;__Planctomycetacia;__Planctomycetales;__Planctomycetaceae;__Singulisphaera
85 Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Cryomorphaceae;__Owenweeksia
86 Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Cytophagaceae;__Flexibacter
87 Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__Sphingobacteriaceae;__Pedobacter
88 Bacteria;__Proteobacteria;__Betaproteobacteria;__TRA3-20;__f;__g
89 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__Alcanivoracaceae;__Alcanivorax
90 Bacteria;__Actinobacteria;__Thermoleophilia;__Solirubrobacterales;__480-2;__g
91 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sphingomonadales;__Erythrobacteraceae;__Erythrobacter
92 Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__f;__g
93 Bacteria;__WCHB1-60;__c;__o;__f;__g
94 Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Aequorivita
95 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Colwelliaceae;__Colwellia
96 Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;__BD1-7_clade
97 Bacteria;__Proteobacteria;__SC3-20;__o;__f;__g
98 Bacteria;__Firmicutes;__Bacilli;__Bacillales;__Thermoactinomycetaceae;Other
99 Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;Other
100 Bacteria;__Proteobacteria;__Deltaproteobacteria;__Syntrophobacterales;__Syntrophaceae;__Smithella

101	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Legionellales;__Legionellaceae;__Legionella
102	Bacteria;__Chlorobi;__Chlorobia;__Chlorobiales;__SJA-28;__g
103	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Cryomorphaceae;__Brumimicrobium
104	Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__Saprospiraceae;__Aureispira
105	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__uncultured;__g
106	Bacteria;__Nitrospirae;__Nitrospira;__Nitrospirales;__Nitrospiraceae;__Nitrospira
107	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;Other
108	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;__Glaciecola
109	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__Francisellaceae;__Francisella
110	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Phyllobacteriaceae;__Ahrensia
111	Bacteria;__Planctomycetes;__Planctomycetacia;__Planctomycetales;__Planctomycetaceae;__Rhodopirellula
112	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Rhodobiaceae;__Parvibaculum
113	Bacteria;__Acidobacteria;__Holophagae;__NS72;__f;__g
114	Bacteria;__Bacteroidetes;__Cytophagia;__Order_III_Incertae_Sedis;__Family_Incertae_Sedis;__Balneola
115	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodobacterales;__Rhodobacteraceae;__Paracoccus
116	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Xanthomonadales;__Sinobacteraceae;__JTB255_marine_benthic_group
117	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Phyllobacteriaceae;Other
118	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__MBAE14;__g
119	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Muricauda
120	Bacteria;__Verrucomicrobia;__Verrucomicrobiae;__Verrucomicrobiales;__Verrucomicrobiaceae;__Persicirhabdus
121	Bacteria;__Actinobacteria;__Actinobacteria;__Micrococcales;__Micrococcaceae;__Arthrobacter
122	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Cyclobacteriaceae;__Cyclobacterium
123	Bacteria;__Fibrobacteres;__Fibrobacteria;__Fibrobacterales;__Fibrobacteraceae;__g
124	Bacteria;__Proteobacteria;__Alphaproteobacteria;__DB1-14;__f;__g
125	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__Candidatus_Odyssella;__g
126	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Cytophagaceae;__Leadbetterella
127	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Cryomorphaceae;__Lishizhenia
128	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Lutibacter
129	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__g
130	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__NS9_marine_group;__g
131	Bacteria;__Planctomycetes;__Planctomycetacia;__Planctomycetales;__Planctomycetaceae;__Pirellula
132	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__mitochondria;__g
133	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sneathiellales;__Sneathiellaceae;__Sneathiella
134	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Nannocystineae;__Nannocystaceae
135	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Idiomarinaceae;__Idiomarina
136	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__Alcanivoracaceae;__Kangiella
137	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Order_Incertae_Sedis;__Family_Incertae_Sedis;Other
138	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Pseudomonadales;__Moraxellaceae;__Psychrobacter
139	Bacteria;__Verrucomicrobia;__Verrucomicrobiae;__Verrucomicrobiales;__Rubritaleaceae;__Rubritalea

APPENDIX-12: Number code for environmental taxa.

1	Archaea;__Euryarchaeota;__Halobacteria;__Halobacteriales;__Deep_Sea_Hydrothermal_Vent_Gp_6(DHVEG-6);__Candidatus_Parvarchaeum
2	Archaea;__Euryarchaeota;__Halobacteria;__Halobacteriales;__Deep_Sea_Hydrothermal_Vent_Gp_6(DHVEG-6);__g
3	Archaea;__Euryarchaeota;__Halobacteria;__Halobacteriales;__Halobacteriaceae;__g
4	Archaea;__Euryarchaeota;__Methanococci;__Methanococcales;__Methanococcaceae;__Methanococcus
5	Archaea;__Euryarchaeota;__Thermoplasmata;__Thermoplasmatales;__Marine_Group_II;__g
6	Archaea;__Euryarchaeota;__Thermoplasmata;__Thermoplasmatales;__Marine_Group_III;__g
7	Archaea;__Thaumarchaeota;__Marine_Benthic_Group_A;__o;__f;__g
8	Archaea;__Thaumarchaeota;__Marine_Group_I;__o;__f;__g
9	Bacteria;Other;Other;Other;Other;Other
10	Bacteria;__Acidobacteria;__Acidobacteria;__PAUC26f;__f;__g
11	Bacteria;__Acidobacteria;__Holophagae;__Acanthopleuribacterales;__Acanthopleuribacteraceae;__Acanthopleuribacter
12	Bacteria;__Acidobacteria;__Holophagae;__CA002;__f;__g
13	Bacteria;__Acidobacteria;__Holophagae;__NKB17;__f;__g
14	Bacteria;__Acidobacteria;__Holophagae;__Sva0725;__f;__g
15	Bacteria;__Acidobacteria;__RB25;__o;__f;__g
16	Bacteria;__Actinobacteria;__Acidimicrobiia;__Acidimicrobiales;Other;Other
17	Bacteria;__Actinobacteria;__Acidimicrobiia;__Acidimicrobiales;__Acidimicrobiaceae;Other
18	Bacteria;__Actinobacteria;__Acidimicrobiia;__Acidimicrobiales;__Candidatus_Microthrix;__g
19	Bacteria;__Actinobacteria;__Acidimicrobiia;__Acidimicrobiales;__Sva0996_marine_group;__g
20	Bacteria;__Actinobacteria;__Actinobacteria;__Corynebacteriales;__Corynebacteriaceae;__Corynebacterium
21	Bacteria;__Actinobacteria;__Actinobacteria;__Corynebacteriales;__Mycobacteriaceae;__Mycobacterium
22	Bacteria;__Actinobacteria;__Actinobacteria;__Micrococcales;__Micrococcaceae;__Micrococcus
23	Bacteria;__Actinobacteria;__Actinobacteria;__Micromonosporales;__Micromonosporaceae;Other
24	Bacteria;__Actinobacteria;__Actinobacteria;__PeM15;__f;__g
25	Bacteria;__Actinobacteria;__Thermoleophilia;__Solirubrobacterales;__Elev-16S-1332;__g
26	Bacteria;__Actinobacteria;__Thermoleophilia;__Solirubrobacterales;__TM146;__g
27	Bacteria;__BD1-5;__c;__o;__f;__g
28	Bacteria;__BHI80-139;__c;__o;__f;__g
29	Bacteria;__Bacteroidetes;Other;Other;Other;Other
30	Bacteria;__Bacteroidetes;__AMV16;__o;__f;__g
31	Bacteria;__Bacteroidetes;__BD2-2;__o;__f;__g
32	Bacteria;__Bacteroidetes;__Bacteroidia;__Bacteroidales;__Bacteroidaceae;__Bacteroides
33	Bacteria;__Bacteroidetes;__Bacteroidia;__Bacteroidales;__Marinilabiaceae;__g
34	Bacteria;__Bacteroidetes;__Bacteroidia;__Bacteroidales;__Porphyromonadaceae;__Paludibacter
35	Bacteria;__Bacteroidetes;__Bacteroidia;__Bacteroidales;__Porphyromonadaceae;__g
36	Bacteria;__Bacteroidetes;__Bacteroidia;__Bacteroidales;__Rikenellaceae;__RC9_gut_group
37	Bacteria;__Bacteroidetes;__Class_Incertae_Sedis;__Order_Incertae_Sedis;__Family_Incertae_Sedis;__Prolixibacter
38	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;Other;Other
39	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Cytophagaceae;__Adhaeribacter
40	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Cytophagaceae;__Cytophaga
41	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Cytophagaceae;__Microscilla
42	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;Other
43	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;__Candidatus_Amoebophilus
44	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;__Ekhidna
45	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;__Flexithrix
46	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;__Marinoscillum
47	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;__Marivirga
48	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;__Persicobacter
49	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;__Rapidithrix
50	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;__Reichenbachiella
51	Bacteria;__Bacteroidetes;__Cytophagia;__Cytophagales;__Flammeovirgaceae;__g
52	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;Other;Other
53	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Cryomorphaceae;Other
54	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Cryomorphaceae;__Crocinitomix
55	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Cryomorphaceae;__Fluviicola
56	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Cryomorphaceae;__NS10_marine_group
57	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Cryomorphaceae;__NS7_marine_group
58	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Cellulophaga
59	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Croceibacter
60	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Flavobacterium
61	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Gramella
62	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Kordia
63	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Leeuwenhoekiella
64	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Maribacter
65	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Maritimimonas
66	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Mesonia
67	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__NS4_marine_group
68	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__NS5_marine_group
69	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Pibocella
70	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Polaribacter
71	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Tenacibaculum
72	Bacteria;__Bacteroidetes;__Flavobacteria;__Flavobacteriales;__Flavobacteriaceae;__Ulvibacter
73	Bacteria;__Bacteroidetes;__SB-1;__o;__f;__g
74	Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__Chitinophagaceae;__Hydrotalea
75	Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__Sphingobacteriaceae;__Sphingobacterium
76	Bacteria;__Bacteroidetes;__Sphingobacteriia;__Sphingobacteriales;__WCHB1-69;__g
77	Bacteria;__Bacteroidetes;__VC2.1_Bac22;__o;__f;__g
78	Bacteria;__Candidate_division_BRC1;__c;__o;__f;__g
79	Bacteria;__Candidate_division_OP3;__c;__o;__f;__g
80	Bacteria;__Candidate_division_SR1;__c;__o;__f;__g
81	Bacteria;__Chlamydiae;__Chlamydiae;__Chlamydiales;__Simkaniaceae;__Candidatus_Fritschea
82	Bacteria;__Chlamydiae;__Chlamydiae;__Chlamydiales;__Simkaniaceae;__g
83	Bacteria;__Chloroflexi;Other;Other;Other;Other
84	Bacteria;__Chloroflexi;__SAR202_clade;__o;__f;__g
85	Bacteria;__Cyanobacteria;__ML635J-21;__o;__f;__g
86	Bacteria;__Cyanobacteria;__SHA-109;__o;__f;__g
87	Bacteria;__Cyanobacteria;__SM2F09;__o;__f;__g
88	Bacteria;__Deferribacteres;__Deferribacteres;__Deferribacterales;__PAUC34f;__g
89	Bacteria;__Deferribacteres;__Deferribacteres;__Deferribacterales;__SAR406_clade(Marine_group_A);__g
90	Bacteria;__Fibrobacteres;__Fibrobacteria;__P.palm_C70;__f;__g
91	Bacteria;__Firmicutes;__Bacilli;__Bacillales;__Bacillaceae;__Bacillus
92	Bacteria;__Firmicutes;__Bacilli;__Bacillales;__Bacillaceae;__Marinococcus
93	Bacteria;__Firmicutes;__Bacilli;__Bacillales;__Family_XII_Incertae_Sedis;__Exiguobacterium
94	Bacteria;__Firmicutes;__Bacilli;__Lactobacillales;__Lactobacillaceae;__Lactobacillus
95	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Clostridiaceae;__Clostridium
96	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Family_XIII_Incertae_Sedis;Other
97	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Family_XIII_Incertae_Sedis;__Incertae_Sedis
98	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Family_XII_Incertae_Sedis;__Fusibacter
99	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__JTB215;__g
100	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Lachnospiraceae;__Incertae_Sedis
101	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Lachnospiraceae;__Pseudobutyrivibrio
102	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Lachnospiraceae;__g
103	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Peptococcaceae;__Peptococcus

104	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Peptococcaceae;__g
105	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Peptostreptococcaceae;__Incertae_Sedis
106	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Ruminococcaceae;__Faecalibacterium
107	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Ruminococcaceae;__g
108	Bacteria;__Firmicutes;__Clostridia;__Clostridiales;__Veillonellaceae;__Phascolarctobacterium
109	Bacteria;__Firmicutes;__Erysipelotrichi;__Erysipelotrichales;__Erysipelotrichaceae;__Turicibacter
110	Bacteria;__Fusobacteria;__Fusobacteria;__Fusobacteriales;__Fusobacteriaceae;__Propionigenium
111	Bacteria;__Fusobacteria;__Fusobacteria;__Fusobacteriales;__Fusobacteriaceae;__Psychrilyobacter
112	Bacteria;__Fusobacteria;__Fusobacteria;__Fusobacteriales;__Leptotrichiaceae;__g
113	Bacteria;__Gemmatimonadetes;__Gemmatimonadetes;__BD2-11_terrestrial_group;__f;__g
114	Bacteria;__Gemmatimonadetes;__Gemmatimonadetes;__Gemmatimonadales;__Gemmatimonadaceae;__Gemmatimonas
115	Bacteria;__Gemmatimonadetes;__Gemmatimonadetes;__PAUC43f_marine_benthic_group;__f;__g
116	Bacteria;__Lentisphaerae;__Lentisphaeria;__Lentisphaerales;__Lentisphaeraceae;__Lentisphaera
117	Bacteria;__Lentisphaerae;__Lentisphaeria;__MSBL3;__f;__g
118	Bacteria;__Lentisphaerae;__Lentisphaeria;__R76-B128;__f;__g
119	Bacteria;__Lentisphaerae;__Lentisphaeria;__Victivallales;__Victivallaceae;__g
120	Bacteria;__Lentisphaerae;__Lentisphaeria;__WCHB1-41;__f;__g
121	Bacteria;__Lentisphaerae;__Lentisphaeria;__c5LKS8;__f;__g
122	Bacteria;__Nitrospirae;__Nitrospira;__Nitrospirales;__Nitrospiraceae;__Leptospirillum
123	Bacteria;__Planctomycetes;__028H05-P-BN-P5;__o;__f;__g
124	Bacteria;__Planctomycetes;__BD7-11;__o;__f;__g
125	Bacteria;__Planctomycetes;__Phycisphaerae;__CCM11a;__f;__g
126	Bacteria;__Planctomycetes;__Phycisphaerae;__MSBL9;__f;__g
127	Bacteria;__Planctomycetes;__Phycisphaerae;__Phycisphaerales;__Phycisphaeraceae;__CL500-3
128	Bacteria;__Planctomycetes;__Phycisphaerae;__Phycisphaerales;__Phycisphaeraceae;__FS140-16B-02_marine_group
129	Bacteria;__Planctomycetes;__Phycisphaerae;__Phycisphaerales;__Phycisphaeraceae;__JL-ETNP-F27
130	Bacteria;__Planctomycetes;__Phycisphaerae;__Phycisphaerales;__Phycisphaeraceae;__Urania-1B-19_marine_sediment_group
131	Bacteria;__Planctomycetes;__Phycisphaerae;__Pla1_lineage;__f;__g
132	Bacteria;__Planctomycetes;__Phycisphaerae;__SHA-43;__f;__g
133	Bacteria;__Planctomycetes;__Pla3_lineage;__o;__f;__g
134	Bacteria;__Planctomycetes;__Pla4_lineage;__o;__f;__g
135	Bacteria;__Planctomycetes;__Planctomycetacia;__Planctomycetales;__Planctomycetaceae;__Blastopirellula
136	Bacteria;__Planctomycetes;__SGST604;__o;__f;__g
137	Bacteria;__Planctomycetes;__vadinHA49;__o;__f;__g
138	Bacteria;__Proteobacteria;__AEGEAN-245;__o;__f;__g
139	Bacteria;__Proteobacteria;__Alphaproteobacteria;Other;Other;Other
140	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Caulobacterales;__Caulobacteraceae;__Amorphus
141	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Caulobacterales;__Caulobacteraceae;__g
142	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Caulobacterales;__Hyphomonadaceae;Other
143	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Caulobacterales;__Hyphomonadaceae;__Hyphomonas
144	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Caulobacterales;__Hyphomonadaceae;__Robiginitomaculum
145	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Caulobacterales;__Hyphomonadaceae;__g
146	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Kordiimonadales;__Kordiimonadaceae;__Kordiimonas
147	Bacteria;__Proteobacteria;__Alphaproteobacteria;__MNG3;__f;__g
148	Bacteria;__Proteobacteria;__Alphaproteobacteria;__OCS116_clade;__f;__g
149	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Bradyrhizobiaceae;__Rhodopseudomonas
150	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Hyphomicrobiaceae;__Maritalea
151	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__KF-JG30-B3;__g
152	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Phyllobacteriaceae;__Cohaesibacter
153	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Phyllobacteriaceae;__Hoeflea
154	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Phyllobacteriaceae;__Nitratireductor
155	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhizobiales;__Rhodobiaceae;__Rhodobium
156	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodobacterales;__Rhodobacteraceae;__Litoreibacter
157	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodobacterales;__Rhodobacteraceae;__Pseudovibrio
158	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodobacterales;__Rhodobacteraceae;__Roseobacter_clade_NAC11-7_lineage
159	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodobacterales;__Rhodobacteraceae;__Roseovarius
160	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Acetobacteraceae;Other
161	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__AEGEAN-169_marine_group
162	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__Defluviicoccus
163	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__Nisaea
164	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__Pelagibius
165	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__Rhodospirillum
166	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__Thalassobaculum
167	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__Thalassospira
168	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rhodospirillales;__Rhodospirillaceae;__Tistrella
169	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;Other;Other
170	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__Candidatus_Captivus;__g
171	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__Candidatus_Hepatincola;__g
172	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__Family_Incertae_Sedis;__Caedibacter
173	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__Ho(lab);__g
174	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__Holosporaceae;__Holospora
175	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__LWSR-14;__g
176	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__RB446;__g
177	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__Rickettsiaceae;__Candidatus_Cryptoprodotis
178	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__Rickettsiaceae;__Rickettsia
179	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__S25-593;__g
180	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__SAR116_clade;__g
181	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__SM2D12;__g
182	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Rickettsiales;__TK34;__g
183	Bacteria;__Proteobacteria;__Alphaproteobacteria;__S26-47;__f;__g
184	Bacteria;__Proteobacteria;__Alphaproteobacteria;__SAR11_clade;__Deep_1;__g
185	Bacteria;__Proteobacteria;__Alphaproteobacteria;__SAR11_clade;__Surface_1;__g
186	Bacteria;__Proteobacteria;__Alphaproteobacteria;__SAR11_clade;__Surface_4;__g
187	Bacteria;__Proteobacteria;__Alphaproteobacteria;__SB1-18;__f;__g
188	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sphingomonadales;__Erythrobacteraceae;__Altererythrobacter
189	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sphingomonadales;__Erythrobacteraceae;__g
190	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sphingomonadales;__Family_Incertae_Sedis;__Kiloniella
191	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sphingomonadales;__GOBB3-C201;__g
192	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sphingomonadales;__Sphingomonadaceae;Other
193	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sphingomonadales;__Sphingomonadaceae;__Novosphingobium
194	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sphingomonadales;__Sphingomonadaceae;__Sphingomonas
195	Bacteria;__Proteobacteria;__Alphaproteobacteria;__Sphingomonadales;__Sphingomonadaceae;__Sphingopyxis
196	Bacteria;__Proteobacteria;__Betaproteobacteria;__Burkholderiales;__Alcaligenaceae;__Sutterella
197	Bacteria;__Proteobacteria;__Betaproteobacteria;__Hydrogenophilales;__Hydrogenophilaceae;__Thiobacillus
198	Bacteria;__Proteobacteria;__Betaproteobacteria;__Methylophilales;__Methylophilaceae;__OM43_clade
199	Bacteria;__Proteobacteria;__Betaproteobacteria;__Neisseriales;__Neisseriaceae;__g
200	Bacteria;__Proteobacteria;__Class_Incertae_Sedis;__Order_Incertae_Sedis;__Family_Incertae_Sedis;__Mariprofundus
201	Bacteria;__Proteobacteria;__Deltaproteobacteria;Other;Other;Other
202	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Bdellovibrionales;__Bacteriovoracaceae;Other
203	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Bdellovibrionales;__Bacteriovoracaceae;__g
204	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Bdellovibrionales;__Bdellovibrionaceae;__Bdellovibrio
205	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Bdellovibrionales;__Bdellovibrionaceae;__OM27_clade
206	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfarculales;__Desulfarculaceae;__g

207	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfobacterales;__Desulfobacteraceae;__Desulfofrigus
208	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfobacterales;__Desulfobacteraceae;__SEEP-SRB1
209	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfobacterales;__Desulfobulbaceae;__Desulfobulbus
210	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfobacterales;__Desulfobulbaceae;__Desulfocapsa
211	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfobacterales;__Desulfobulbaceae;__Desulforhopalus
212	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfobacterales;__Desulfobulbaceae;__g
213	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfobacterales;__Nitrospinaceae;__Nitrospina
214	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfobacterales;__Nitrospinaceae;__g
215	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfovibrionales;__Desulfovibrionaceae;__Desulfovibrio
216	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfuromonadales;__Desulfuromonadaceae;__Desulfuromusa
217	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Desulfuromonadales;__Sva1033;__g
218	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Cystobacterineae;__g
219	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Haliangiaceae;__Haliangium
220	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Nannocystaceae;Other
221	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Nannocystaceae;__Enhygromyxa
222	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Nannocystaceae;__g
223	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Nannocystineae;__g
224	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Myxococcales;__Sorangiineae;__Sandaracinaceae
225	Bacteria;__Proteobacteria;__Deltaproteobacteria;__SAR324_clade(Marine_group_B);__f;__g
226	Bacteria;__Proteobacteria;__Deltaproteobacteria;__Sva0485;__f;__g
227	Bacteria;__Proteobacteria;__Epsilonproteobacteria;__Campylobacterales;__Campylobacteraceae;__Arcobacter
228	Bacteria;__Proteobacteria;__Epsilonproteobacteria;__Campylobacterales;__Campylobacteraceae;__Sulfurospirillum
229	Bacteria;__Proteobacteria;__Epsilonproteobacteria;__Campylobacterales;__Helicobacteraceae;__Sulfurimonas
230	Bacteria;__Proteobacteria;__Gammaproteobacteria;Other;Other;Other
231	Bacteria;__Proteobacteria;__Gammaproteobacteria;__34P16;__f;__g
232	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;Other;Other
233	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;Other
234	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;__Agarivorans
235	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;__C1-B045
236	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;__Dasania
237	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;__OM60(NOR5)_clade
238	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;__Simiduia
239	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Alteromonadaceae;__g
240	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Colwelliaceae;__Thalassomonas
241	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Moritellaceae;__Moritella
242	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Pseudoalteromonadaceae;__Pseudoalteromonas
243	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Psychromonadaceae;__Psychromonas
244	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Alteromonadales;__Shewanellaceae;__Shewanella
245	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Chromatiales;Other;Other
246	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Chromatiales;__Chromatiaceae;__Nitrosococcus
247	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Chromatiales;__Ectothiorhodospiraceae;__Acidiferrobacter
248	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Chromatiales;__Ectothiorhodospiraceae;__Thioalkalispira
249	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Halothiobacillaceae;__Halothiobacillus
250	Bacteria;__Proteobacteria;__Gammaproteobacteria;__E01-9C-26_marine_group;__f;__g
251	Bacteria;__Proteobacteria;__Gammaproteobacteria;__EC3;__f;__g
252	Bacteria;__Proteobacteria;__Gammaproteobacteria;__KI89A_clade;__f;__g
253	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Legionellales;__Coxiellaceae;__Coxiella
254	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Legionellales;__Coxiellaceae;__Rickettsiella
255	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Legionellales;__Coxiellaceae;__g
256	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Legionellales;__Legionellaceae;__g
257	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Methylococcales;__Crenotrichaceae;__Crenothrix
258	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Methylococcales;__Methylococcaceae;__Methylosoma
259	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;Other;Other
260	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__CrystalBog021C3;__g
261	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__Halomonadaceae;__Salinicola
262	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__OM182_clade;__g
263	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__Oceanospirillaceae;__Marinospirillum
264	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__SAR86_clade;__g
265	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__SS1-B-06-26;__g
266	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Oceanospirillales;__Saccharospirillaceae;__Saccharospirillum
267	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Order_Incertae_Sedis;__Family_Incertae_Sedis;__Arenicella
268	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Order_Incertae_Sedis;__Family_Incertae_Sedis;__Sedimenticola
269	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Pseudomonadales;__Moraxellaceae;__Acinetobacter
270	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Pseudomonadales;__Moraxellaceae;__Enhydrobacter
271	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Salinisphaerales;__Salinisphaeraceae;__Salinisphaera
272	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Salinisphaerales;__Salinisphaeraceae;__ZD0417_marine_group
273	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;Other;Other
274	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__1G93;__g
275	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__CHAB-XI-27;__g
276	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__EV818SWSAP88;__g
277	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__Family_Incertae_Sedis;__Caedibacter
278	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__Piscirickettsiaceae;__Cycloclasticus
279	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__Piscirickettsiaceae;__Methylophaga
280	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__Piscirickettsiaceae;__Piscirickettsia
281	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__Piscirickettsiaceae;__g
282	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Thiotrichales;__Thiotrichaceae;__Candidatus_Thiopilula
283	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Vibrionales;__Vibrionaceae;__Aliivibrio
284	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Vibrionales;__Vibrionaceae;__g
285	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Xanthomonadales;__Xanthomonadaceae;__Arenimonas
286	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Xanthomonadales;__Xanthomonadaceae;__Luteibacter
287	Bacteria;__Proteobacteria;__Gammaproteobacteria;__Xanthomonadales;__Xanthomonadaceae;__g
288	Bacteria;__Proteobacteria;__JTB23;__o;__f;__g
289	Bacteria;__Proteobacteria;__Milano-WF1B-44;__o;__f;__g
290	Bacteria;__Proteobacteria;__Skagenf62;__o;__f;__g
291	Bacteria;__Proteobacteria;__TA18;__o;__f;__g
292	Bacteria;__RF3;__c;__o;__f;__g
293	Bacteria;__Spirochaetes;__Spirochaetes;__LK-44f;__f;__g
294	Bacteria;__Spirochaetes;__Spirochaetes;__MSBL8;__f;__g
295	Bacteria;__Spirochaetes;__Spirochaetes;__Spirochaetales;__Leptospiraceae;__Turneriella
296	Bacteria;__Spirochaetes;__Spirochaetes;__Spirochaetales;__Spirochaetaceae;__Spirochaeta
297	Bacteria;__Spirochaetes;__Spirochaetes;__Spirochaetales;__Spirochaetaceae;__Treponema
298	Bacteria;__Tenericutes;__Mollicutes;__Anaeroplasmatales;__Anaeroplasmataceae;__Anaeroplasma
299	Bacteria;__Tenericutes;__Mollicutes;__Mycoplasmatales;__Mycoplasmataceae;Other
300	Bacteria;__Verrucomicrobia;__Arctic97B-4_marine_group;__o;__f;__g
301	Bacteria;__Verrucomicrobia;__Opitutae;Other;Other;Other
302	Bacteria;__Verrucomicrobia;__Opitutae;__A714019;__f;__g
303	Bacteria;__Verrucomicrobia;__Opitutae;__MB11C04_marine_group;__f;__g
304	Bacteria;__Verrucomicrobia;__Opitutae;__Puniceicoccales;__Puniceicoccaceae;Other
305	Bacteria;__Verrucomicrobia;__Opitutae;__Puniceicoccales;__Puniceicoccaceae;__Cerasicoccus
306	Bacteria;__Verrucomicrobia;__Opitutae;__Puniceicoccales;__Puniceicoccaceae;__Pelagicoccus
307	Bacteria;__Verrucomicrobia;__Opitutae;__Puniceicoccales;__Puniceicoccaceae;__marine_group
308	Bacteria;__Verrucomicrobia;__Spartobacteria;__Chthoniobacterales;__FukuN18_freshwater_group;__g
309	Bacteria;__Verrucomicrobia;__Spartobacteria;__Chthoniobacterales;__Xiphinematobacteraceae;__Candidatus_Xiphinematobacter
310	Bacteria;__Verrucomicrobia;__Verrucomicrobiae;__Verrucomicrobiales;__Verrucomicrobiaceae;__Roseibacillus