Universidade de Aveiro Departamento de Biologia
2018

Letícia Novaes Duarte

Microbiologia Molecular na Aquacultura: em busca de uma comunidade microbiana saudável

Molecular Microbiology in Aquaculture: the search for a healthy microbial community

Universidade de Aveiro Departamento de Biologia
2018

Letícia Novaes Duarte

Microbiologia Molecular na Aquacultura: em busca de uma comunidade microbiana saudável

Molecular Microbiology in Aquaculture: the search for a healthy microbial community

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Biologia, realizada sob a orientação científica do Doutor Newton Gomes, Investigador Principal do Departamento de Biologia da Universidade de Aveiro, do Doutor Daniel Cleary, Investigador Principal do Centro de Estudos do Ambiente e do Mar (CESAM) e do Doutor Francisco Coelho, Investigador de Pós Doutoramento.

Este trabalho foi financiado pelas unidades de investigação CESAM (UID/AMB/50017) e PROMAR (31-03-05-FEP-0016)

Letícia Duarte recebeu apoio financeiro do Conselho Nacional do Desenvolvimento da Ciência e Tecnologia do Brasil (CNPq) e CAPES através do Programa Ciência Sem Fronteiras (BEX 1287-13-3)

Dedico este trabalho aos meus pais, irmão, amigos e familiares pelo incansável apoio e confiança.

o júri

presidente

Prof. Doutor Nuno Miguel Gonçalves Borges de Carvalho
Professor Catedrático da Universidade de Aveiro

Prof. Doutor Rodrigo da Silva Costa
Professor Auxiliar do Instituto Superior Técnico da Universidade de Lisboa

Doutora Maria da Conceição Venâncio Egas
Investigadora Auxiliar do Centro de Neurociências e Biologia Celular da Universidade de Coimbra

Doutor Rodrigo Otávio de Almeida Ozório
Investigador Auxiliar do Centro Interdisciplinar de Investigação Marinha e Ambiental (CIIMAR)

Prof. Doutora Maria Ângela Sousa Dias Alves Cunha
Professora Auxiliar da Universidade de Aveiro

Doutor Newton Carlos Marcial Gomes
Investigador Principal do Centro de Estudos do Ambiente e do Mar

agradecimentos

Assim como na natureza, onde tudo está conectado, este trabalho não teria sido realizado de maneira individual e aqui deixo os meus mais sinceros agradecimentos aos principais colaboradores deste desafio. Em primeiro lugar, agradeço à Deus, por sua presença constante em minha vida que evidência cientifica nenhuma explica. Gostaria de agradecer aos meus orientadores pela amizade, cuidado e carinho com esta tese e comigo: Ao Professor Doutor Newton Gomes pela oportunidade que me proporcionou em participar do projeto AQUASAFE - Development of new technologies to anticipate and diagnose disease outbreaks in aquaculture (PROMAR 31-03-05-FEP-0016), pelo constante apoio ao longo destes anos sempre me conduzindo pelo melhor caminho e explorando o que havia de melhor em mim. A todo aconselhamento, orientação e apoio incondicional oferecidos pelo meu coorientador Dr. Francisco Coelho. Ao Dr. Daniel Cleary pela orientação e apoio durante a análise e tratamento dos dados. Gostaria de agradecer à toda equipe do LEMAM (Laboratório de Estudos Moleculares de Ambientes Marinhos), compartilhar com vocês a maior parte dos meus dias me tornou uma profissional e uma pessoa melhor, os admiro muito! O meu agradecimento total aos meus amigos e família pelo carinho, tolerância e apoio oferecido ao longo de todo o processo: pelas diversas vezes que acreditaram em mim quando eu já não acreditava. Minha infinita gratidão aos meus pais e irmão, de quem privei durante tantos anos a nossa convivência diária mas que não me privaram do seu amor e admiração: é por vocês! À Universidade de Aveiro e ao CESAM (Centro de Estudos de Ambientes Marinhos), por fornecerem as instalações para a realização deste trabalho. Aos proprietários das aquaculturas estudadas pela generosidade em nos disponibilizar os tanques de produção para amostragem. Ao apoio financeiro do Conselho Nacional do Desenvolvimento Tecnológico e Cientifico do Brasil (CNPq) e CAPES através do Programa Ciencia sem Fronteiras (BEX 1287-13-3).

palavras-chave Aquacultura, bacteria, microeucariontes, plancton, PCR, 18S, 16S, microbioma da aquacultura, manipulação microbiana.

resumo

As comunidades microbianas dos sistemas de aquacultura estão envolvidas na manutenção da saúde e crescimento dos organismos cultivados. Participam no ciclo dos nutrientes, nutrição, controle de doenças e qualidade da água do sistema e efluentes. Neste trabalho foram utilizadas técnicas independentes de cultivo (Denaturing Gradient Gel Electrophoresis e sequenciação) para caracterizar o microbioma da água de um sistema semi-intensivo e um sistema intensivo de aquacultura de peixes. Primeiro, investigamos a composição das comunidades bacterioplânctonicas de um sistema de aquacultura recirculante (SRA) utilizado para a produção de juvenis de linguado (*Solea senegalensis*). As ordens mais abundantes detectadas nas aquaculturas de liguado foram: Alteromonadales, Rhodobacterales, Oceanospirillales, Vibrionales e Flavobacteriales. Foram detetadas sequências com similaridade a espécies potencialmente patogénicas, assim como sequências com similaridade a grupos previamente descritos como probióticos. É discutido o papel destas ultimas na supressão dos potenciais patógenos de peixes e manutenção de um ambiente saudável (sem surtos de doenças). Numa aquacultura de adultos de linguados, a presença dos peixes foi descrita como um dos principais fatores determinantes na composição das comunidades bacterianas. Aqui, a água atuou como um importante banco de sementes para a colonização de populações bacterianas nos tanques do SRA, principalmente das relacionadas às bactérias probióticas. Este trabalho demonstra que a origem da água pode ter um papel relevante na manutenção de uma comunidade microbiana saudável, reforçando a sua importância em possíveis estratégias de manipulação/gestão microbiana das aquaculturas. Posteriormente, descrevemos a dinâmica sazonal e potenciais interações das comunidades de plâncton bacteriano e microeucariótico em uma aquicultura semi-intensiva para robalo (*Dicentrarchus labrax*) durante um ano. As classes bacterianas mais abundantes foram Gammaproteobacteria, Flavobacteriia e Alphaproteobacteria; enquanto a comunidades microeucariotica foi dominada pelos grupos Ochrophyta, Chlorophyta e Ciliophora. Aqui, além dos efeitos potenciais dos parâmetros abióticos no plâncton microbiano, houve correlação entre as populações de bactérias e microeucariotos o que pode ser uma indicação de interdependência trófica e / ou metabólica entre estes dois domínios. Estes estudos permitiram-nos descrever o microbioma normal de sistemas de aquacultura, suas interações ecológicas e os impactos exercidos pelos fatores ambientais com o intuito de fundamentar o desenvolvimento de estratégias para a manutenção de um ambiente produtivo e saudável.

keywords Aquaculture, bacteria, microeukaryote, plankton, PCR, 18S, 16S, aquaculture microbiome, microbial manipulation.

abstract

The microbial communities of aquaculture systems are involved in maintaining the health and growth of farmed organisms. They participate in nutrient cycling, nutrition, disease control and water quality of the system and effluents. We use DGGE fingerprint techniques and high-throughput sequencing analyzes to access the semi-intensive and intensive aquaculture microbiota. First, we investigated the composition of the bacterioplankton communities of a recirculating aquaculture system (RAS) used for the production of juveniles sole (*Solea senegalensis*). The most abundant orders detected in the aquaculture of sole were: Alteromonadales, Rhodobacterales, Oceanospirillales, Vibrionales and Flavobacteriales. OTUs related to potential fish pathogens in aquaculture systems were detected, as well as naturally occurring probiotic bacteria. These may have played a role in suppressing potential pathogens of fish, keeping the aquaculture free from disease. In an aquaculture of adult sole, the presence of fish was described as the main factor influencing bacterial composition. Here, supply water served as an important seed bank for the colonization of bacterial populations in the hatchery RAS tanks, mainly related to probiotic bacteria. The importance of this compartment for the maintenance of a healthy aquaculture and its importance in the development of strategies for microbial manipulation/management of aquaculture was reinforced. Subsequently, we describe the seasonal dynamics and potential interactions of bacterial and microeukaryotic plankton communities in a semi-intensive aquaculture for European sea bass (*Dicentrarchus labrax*) over a year. The most abundant bacterial classes were Gammaproteobacteria, Flavobacteriia and Alphaproteobacteria; while the microeukaryotic communities were dominated by the Ochrophyta, Chlorophyta and Ciliophora groups. Here, in addition to the potential effects of abiotic parameters on microbial plankton, there was a correlation between bacterial and microeukaryote populations which may be an indication of trophic and / or metabolic interdependence between these two domains. These studies allowed us to describe the normal microbiota of aquaculture systems, their ecological interactions and the impacts exerted by environmental factors in order to support the development of strategies for the maintenance of a productive and healthy environment.

Contents

List of Publications

Chapter I - Duarte LN, Coelho FJRC, Louvado AMO, Cleary DFR, Gomes NCM (2018) Exploring the aquaculture microbiome to improve fish health.

Chapter II - Duarte LN, Coelho FJRC, Oliveira V, Cleary DFR, Martins PT, Gomes NCM (2018) Characterization of Bacterial Communities from a Recirculating Aquaculture System for juvenile sole (*Solea senegalensis*) production. Submitted to PlosOne, PONE-S-18-14726

Chapter III - Duarte LN, Coelho FJRC, Cleary DFR, Bonifácio D, Martins PT, Gomes NCM (2018) Bacterial and microeukaryotic plankton communities in a semi-intensive aquaculture system of sea bass (*Dicentrarchus labrax*): a seasonal survey. Submitted to Aquaculture, AQUA_2018_796

List of Abbreviations

AXOS - Arabino-xylose oligosaccharides

BALOs - Bdellovibrionales and similar organisms

Bio - Biofilter tank

BLAST - Basic local alignment search tool

Br - Bromide

Cd – Cadmium

CFU - Colony-forming units

CU - Copper

DGGE - Denaturing gradient gel electrophoresis

DNA - Deoxyribonucleic acid

DO - dissolved oxygen

DSMP - dimethylsulfoniopropionate)

ETM - Estuarine turbidity maxima

EU - European Union

FOS - Fructose-oligosaccharides

GOS - Galacto-oligosaccharides

Hg - Mercury

HS - Humic substances

H_2SO_4 - Sulfuric acid

IMTA - Integrated multitrophic aquaculture

LAB - Lactic acid bacteria

MAST - marine Stramenopiles clade

MOS - Mannose- oligosaccharides

NCBI - National Center for Biotechnology Information

NH_4 - Ammonium

NO_2 - Nitrites

NO_3^- - Nitrates

OS - Oligosaccharide

OTU - Operational taxonomic units

Ozo - Ozonation tank

PCO - Principal coordinates analysis

PCR - Polymerase chain reaction

PHB - Poly-hydroxybutytrate

Pre - Pre-production tank

PS - Polysaccharides

QIIME - Quantitative insights into microbial ecology

RAS – Recirculating aquaculture system

RDP - Ribosomal database project

rRNA - Ribosomal ribonucleic acid

Sed - Sedimentation tank

SHIME - Simulator of the Human Intestinal Microbial Ecosystem

SRS - shallow raceway systems

Sup - Water supply

TAE - Tris Acetate EDTA buffer

TOC - Total organic carbon analysis

Zn - Zinc

List of Tables

List of Figures

Objectives

Objectives

The contribution of aquaculture to world food supply has been increasing over the past 10 years and currently this sector is responsible for providing more fish (73.8 million tonnes) for human consumption than wild-catch fisheries. In fact, fisheries and aquaculture supply 17% of global animal protein production and support the livelihoods of about 12% of the world's population. However, despite the recent technological advances of aquaculture systems for fish production, there is a lack of fundamental knowledge about their microbiome and strategies to prevent and manage disease outbreaks. The microbial metacommunities of aquacultures are involved in the productivity, nutrient cycling, nutrition of the cultured animals, water quality, disease control and environmental impact of the effluent (Martins, 2016), with a critical impact in the maintenance of fish health and growth. Recent studies suggest that fundaments of ecological theory could be used to support the development of sustainable microbial management methods to prevent diseases in aquaculture systems (Schryver and Vadstein, 2014). However, before applying any microbe-based strategy in aquaculture management to promote fish growth and health, one basic question needs to be answered: What is a healthy microbiome in a fish aquaculture system? In line with this question, this thesis aimed to provide fundamental base line information about the ecology, diversity and composition of microbial plankton communities in two distinct marine aquaculture systems: a recirculating aquaculture system (RAS) for production of sole (*Solea senegalensis*) juveniles and a semi-intensive aquaculture system for sea bass (*Dicentrarchus labrax*) located in the Portuguese coast. Among our specific goals we aimed to: 1) investigate the potential effects of environmental variables on the microbialplankton communities of semi-intensive and intensive aquaculture systems; 2) study the diversity and seasonal dynamics of prokaryotic and microeukaryotic plankton communities and their putative ecological interactions in a semi-intensive aquaculture system and 3) identify the core taxa of microbial communities and their potential relevance for fish health in the aquaculture systems studied.

Description of each chapter:

Chapter I Introduction - Exploring the aquaculture microbiome to improve fish health

In this chapter we presented an overview of aquaculture microbiome and discuss the importance of a better understand of the microbial metacommunities during fish production, their interactions and modulation to support the development of sustainable aquaculture pratices.

This chapter is a mini review article in preparation.

Chapter II Characterization of Bacterial Communities from a Recirculating Aquaculture System for juvenile sole (*Solea senegalensis*) production

In this chapter, we characterized the composition of bacterioplankton communities of a recirculating aquaculture system (RAS) for production of sole (*Solea senegalensis*) juveniles and compared the results obtained with the communities of a grow-out sole RAS that was characterized in a previous study (Martins et al., 2013). We used DGGE fingerprinting and high-throughput sequencing analyses to assess the bacterioplankton community. The importance of our findings was discussed in terms of water quality and fish health.

This chapter was submitted to the PlosOne journal with the following reference: PONE-S-18-14726

Chapter III Bacterial and microeukaryotic plankton communities in a semi-intensive aquaculture system of sea bass (*Dicentrarchus labrax*): a seasonal survey

In this chapter, we studied the seasonal variation of environmental parameters and bacterial and microeukaryotic plankton communities (16S and 18S rRNA gene high-throughput sequencing) of a semi-intensive estuarine aquaculture system over one-year period (January/2014 – November/2014). We discussed the ecological interactions between

prokaryotes and microeukaryotes and the impact of environmental factors on community structure during fish production.

This chapter was submitted to the Aquaculture journal with the following reference: AQUA_2018_796

Chapter I

Chapter I

Exploring the aquaculture microbiome to improve fish health

Duarte LN[1], Coelho FJRC[1], Louvado AMO[1], Cleary DFR[1], Gomes NCM[1]

[1]Department of Biology & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

Abstract

In order to support the increase demand for fish, aquaculture became one of the fastest growing food production sectors over the last years. The recent technological developments of aquaculture systems deal with lack of space (for expansion), water avaibility and pollution, but disease outbreaks still pose major threats to aquaculture production. In response to this problem, research on aquaculture microbiome has provided important knowledge on microbe interactions (microbe-microbe, -environment and -host interactions) and their role in the aquaculture systems. More studies in this field will contribute to produce fundamental and applied knowledge which will be key to the development of strategies to suppress the occurrence of fish diseases in aquaculture systems and improve fish production. In this review, we present an overview of aquaculture microbiome and discuss the importance of a better understanding of microbial metacommunities during fish production, their interactions and modulation and their potential to contribute for development of more sustainable aquaculture systems.

Aquaculture systems and global status

The increase of human population in the last decades has led to a substantial increase in the demand for fish, and subsequently decreases in natural stocks. According to FAO (2016), the world per capita food fish supply increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012. As a response to this growing demand, aquaculture has become one of the

fastest growing food production industries. Since 1980 up to 2012 aquaculture production increased at an average rate of 8.6 percent per year. In 12 years (from 2000 to 2012) world food fish aquaculture production more than doubled, increasing from 32.4 million tons to 66.6 million tons (FAO, 2016). In 2013, China produced 43.5 million tons of fish, being by far the largest producer in the world. Currently, aquaculture supplies more than 50 percent of the world demand for seafood (NOAA Fisheries). However, recent estimates indicate that the world will need another 40 million tons of seafood per year by 2030 just to support the consumption rates at this time (Hall et al., 2011).

Aquaculture systems are traditionally classified from extensive to semi-intensive, highly intensive and hyper-intensive. Intensification level depends on several factors, including the stocking density of the culture organisms, the level of inputs (food, feed additives) and the degree of management (Baluyut, 1989). Generally, extensive systems are characterized by low stocking densities and no supplemental feeding, whereas intensive systems use high densities of fishes and greatly rely on artificial feeds (Baluyut, 1989). Aquacultures can be located in water (cages, ponds, inshore/offshore) or in land (rainfed ponds, irrigated or flow-through systems, tanks and raceways). Architectural variations include recycling systems (high control enclosed systems, open pond based recirculation) and integrated farming systems (e.g. livestock-fish, agriculture and fish dual) (Funge-Smith and Phillips, 2001).

The variability of intensification and architecture has created a diversity of aquaculture systems and technologies. Among them, recirculating aquaculture systems (RAS) are used for intensive and super intensive fish production. Such systems can overcome some of the key issues related to aquaculture expansion such as the lack of space, the limited water availability or concerns over pollution (Badiola et al., 2012). RAS can continuously process and reuse the water, decreasing water pump and energy requirements while maintaining optimal environmental conditions for fish production with a minimum ecological impact (Labatut and Olivares, 2004; Summerfelt et al., 2009; Verdegem et al., 2006). This system, however, offers a higher risk to waterborne pathogens exposure, since high fish densities result in more rapid and severe disease outbreaks (Mennerat et al., 2010).

In a polyculture system, several species can be cultured together in the same compartments (for example, three species of finfish: salmon, cod and halibut) or in an integrated multitrophic aquaculture (IMTA) system, that combines the cultivation of fed aquaculture species (e.g. fish) with extractive aquaculture species (e.g. shellfish and kelp) (Yip

et al., 2017). IMTA systems aim to increase productivity and reduce the environmental impacts of aquaculture through the inclusion of organisms from various trophic levels, so the by-products of one become the inputs of another (European Commission). Not only it has obvious advantages from an environmental sustainability aspect, but it provides economic diversification reducing economic risks and increasing final productivity (Chopin, 2006). It has been shown that kelp and mussel production increases by 46 and 50%, respectively, when cultured in proximity to salmon sites. However, according to Guerrero and Cremades (2012), the main constrain of macroalgae cultures are the increase in fouling when in presence of fish and of submerged structures that reduces light radiation and increase sedimentation and recruitment processes. Different types of IMTA have different concerns and benefits. These systems can present lower productivity than fed monocultures and require specialized management to balance nutrient flows (Kinney, 2017).

Aquaculture microbiome and fish health

Microorganisms have central roles in marine food webs and global biogeochemical processes. In aquaculture settings, the outbreaks of parasitic, bacterial and fungal diseases often lead to high mortality rates and huge economic losses (Valladão et al., 2015). Disease outbreaks have reportedly cost the aquaculture industry tens of billions of dollars in the last 20 years (Ababouch et al., 2016). However, microbial communities are also responsible for nutrient cycling in aquaculture systems. This is of particular importance in intensive systems where water is treated in biofilters and recirculated (RAS). In these systems processes such nitrification, denitrification, dissimilatory nitrate reduction, anaerobic ammonium oxidation (anammox), sulphide oxidation and methanogenesis control nutrient levels and consequently, water quality (Schreier et al., 2010). Heterotrophic bacteria mineralize uneaten feeds, faeces and other organic matter released in RAS during fish production (Sugita et al., 2005). Microbes are also responsible for critical functions in fish gut and mucus (Wang et al., 2017; Romero et al., 2014). Mucus, besides has ability to self-repair, elasticity and viscosity (rheology), is the first physical, chemical and biological barrier from infection for trapping and immobilising pathogens (Bakshani et al., 2018; Benhamed et al., 2014). The comensal microbiota in aquatic animals contributes to nutrition and immune stimulation and provides protection by producing bacteriocins, competing for adhesion sites and altering of the gut physicochemical

environment, for example (Guarner and Malagelada, 2003; Stecher and Hardt, 2008). The aquaculture environmental microbiome (e.g. water and biofilms) may also play an important role suppressing the development of potential fish pathogens. For example, it is postulated that aquaculture bacterioplankton communities dominated by k-strategists will have a better performance (Attramadal et al., 2014). Fast growing opportunistic r-strategists are more likely to develop harmfull host–microbe interactions attacking young and stressed individuals.

In recent years, there has been an increasing interest in the potential development of technologies or strategies that would allow the modulation of microbial communities associated with fish and their surroundings. Microbial management of aquacultures offers a great potential to reduce the abundance of fish pathogens, circumventing the need to apply antibiotics, and improving the overall water quality (Bentzon-Tilia et al., 2016). However, the modulation of microbial metacommunities and application of the ecological concepts to manipulate these communities in aquaculture systems have been mainly focused on microbe-host interactions (e.g. probiotics). The implementation of microbiome management strategies and products is still in an earlier stage and in a 'hope for the best' perspective, with their mechanisms of action and impact on the overall microbial community not fully understood (Dittmann et al., 2017; Sharifuzzaman and Austin, 2017).

In gereral, intensive aquacultures can reach very high stocking densities, as long as oxygen levels, food and water quality are controlled accordingly. However, the utilization of high densities in these systems has resulted in more rapid and severe disease outbreaks and the development of emergent pathogens. Obviously, in contrast to natural environments, during intensive fish production, susceptible fish has a much higher risk to be exposed to waterborne pathogens. In fact fish production at high densities may cause selection towards increased virulence favoring the emergence of more aggressive fish pathogens and the emergence and spread of an increasing array of new diseases. Pulkkinen et al. (2010) showed that high stocking densities in a salmon aquaculture increase the occurrence of the bacterial fish disease *Flavobacterium columnare* by enhancing the transmission opportunities and selecting the most virulent strains. Similarly, the severity of the disease outbreaks in sole (*Solea senegalensis*) aquaculture (and other flat fishes) seems to be related with the increased intensification of the production (FAO 2014-2018). Photobacteriosis (*Photobacterium damselae* subsp. *piscicida*), vibriosis (*Vibrio harveyi* and other *Vibrio* spp.) and flexibacteriosis (*Tenacibaculum maritimum*) are the pathogens with the most frequent occurrence in Europe and they are limiting the

successful expansion of sole aquaculture (Martins et al., 2015; Medina et al., 2015). Currently, disease outbreaks are considered a limiting factor for the development of aquaculture worldwide, which may aggravate in consequence of global climatic changes (Jansen et al., 2012; Leung and Bates, 2013). In addition, problems with fish diseases have been aggravated due to the lack of adequate disease control measures for intensive aquaculture systems. For example, it is evident nowadays that the management of disease outbreaks poses specific challenges for intensive recirculating aquaculture systems. These systems produce fish indoor in tanks with high density under controlled environmental conditions and are highly dependent of microbial communities for water purification, quality and fish health (Tal et al., 2009).

Antibiotics are commonly used in the aquaculture to treat diseases and as antimicrobial prophylaxis. However, the utility of antibiotics (especially as a preventive measure) has been questioned due to the ability of the bacterial pathogen to develop resistance and horizontal transfer (e.g. plasmids, transposons, integrons and phages) of antibiotic resistance genes between other pathogens and bacterial populations within the organism and in the environment (Huddleston, 2014). According to the World Health Organization (WHO), the emergence and spread of antimicrobial resistance is an increasingly serious threat to global public health that requires action across all government sectors and society (Roca et al., 2015). Another problem to consider is that antibiotic treatment will result in changes in the diversity of microbial communities and adversely affect beneficial microbes with critical functions in fish gut and mucus. In addition, structural changes of fish microbial communities may facilitate the growth or invasion of opportunistic microorganisms which will occupy ecological niches which were previously unavailable to them (Roca et al., 2015). Due to the reasons listed above, the aquaculture sector urgently needs to gain a better understanding of the contribution of the aquaculture microbiome for fish health and to develop new methodologies to replace or to be used in alternation with antibiotics in order to maximize the treatment (when necessary) and improve fish health during aquaculture production.

Microbial communities inhabit fish host and environmental compartments

Fish (fish-microbe interactions)

The relationship between host and microbe is a delicate balance highly influenced by the environment, stress, host health and microbe ecological interactions such as competition for space, nutrients, production of inhibitory compounds and competitive exclusion. In general, microbes can attach to animal surfaces (skin, mucus and gills) or be ingested and colonize the intestine. The equilibrium between microbes that adhere to skin and the number that are present in healthy host can determine the 'normal skin microbiota' for a particular fish species (Larsen et al., 2013). However, they represent only a small fraction of the fish microbiome. The intestinal bacterial can count about 100 (during winter) to 1000 times higher (summer) comparing with skin bacterial community (Bisht et al., 2014). The majority of microorganisms that is ingested dies in stomach or is discarded with the feces. Those which manage to colonize the intestine can interact in a mutualistic, commensalistic or parasitic relationship and will play a key role in the fish development (starting from hatching). The microbiota can enhance the immunological functions of the host (Gómez and Balcázar, 2008; Montalban-Arques et al., 2015), stimulating the increase in the proportion of lymphocytes, macrophage number and phagocytic activity (Irianto and Austin, 2002), participate in the active competition against infections by creating a hostile environment to pathogens multiplication with the acids, bile salts and enzymes (Larsen et al., 2013) and improve the nutrient conversion (Montalban-Arques et al., 2015), specially in cholesterol metabolism (Nayak, 2010).

According to Elliott (2011), the fish skin serve in communication, sensory perception, locomotion, respiration, ion regulation, excretion and thermal regulation. The fish skin microbial community can be host species specificity (Larsen et al., 2013) and may also present specific compositional signatures according to their local of origin (Nguyen et al., 2008; Sheikha and Montet, 2014). The microbiota of fish body surface is the first line of protection against pathogens (Trivedi, 2012), they can increase fish resitance against diseases (Nayak, 2010; Montalban-Arques et al., 2015), participate in the epithelial development (Nayak, 2010) and secrete a range of antimicrobial substances (Nayak, 2010).

Biofilm and bio-filters

Biofilms are formed by microbial communities that are embedded in a self-produced matrix of extracellular polymeric substances (EPS). The EPS matrix composition comprises mainly polysaccharides, proteins, lipids and extracellular DNA which are self produced by the biofilm microbiome. The biofilm formed on the surface of solid substrates in biofilters plays a key role in the process of decontamination and nutrient cycling in recirculating aquaculture systems and during wastewater treatment (Li et al., 2017). A range of substrates with a large surface area such as silica sand, plastic rings or "bioballs" support biofilm formation in biofilters. In this environment, the presence of nitrifying bacteria is of paramount importance. These bacteria are extremely necessary to maintain the water quality of aquaculture since they are involved on water ammonia cycling which is the major metabolic waste produced in aquaculture systems. They participate in the transformation of a toxic product (ammonia) in less toxic forms as nitrite (Nitrosomonas) and nitrate (Nitrobacter). The facultative heterotrophic bacteria reduce nitrates and nitrites in gaseous nitrogen (N) and it leaves the system by aeration (DeLong and Losordo, 2012). The ammonium oxidizers and nitrite oxidizers need to coordinate their metabolisms to complete the nitrification process and avoid the accumulation of the intermediate nitrite (NO_2^-), which is toxic to fish. The excess of organic C has to be removed before the nitrifying process to prevent the slow-growing nitrifying biofilm to be overgrown by heterotrophs (Bentzon-Tilia et al., 2016). Environmental factors influence on nitrifying bacterial activity, in this way, the type of aquaculture influences bacterial activity (Martins, 2016). However, at the same time that biofilms can improve water quality in aquaculture, they can also represent a reservoir for opportunistic pathogens (*Aeromonas hydrophila*, *Edwardsiella ictaluri*, *E. tarda*, *E. piscicida, Flavobacterium columnare*, *Pseudomonas aeruginosa, Staphylococcus aureus*, *Staphylococcus epidermidis*).

Water

In aquaculture systems, water is the shared environment between microbes and animals. Recent studies provided evidences of a strong influence of cultured fish species on the microbiome of this biotope in aquacultures systems (Martins et al. 2013; Boaventura et al.

2018). Giatsis et al. (2015) further demonstrated that there is a transfer of microorganisms from rearing water into the fish gut, suggesting that water aquaculture microbes have a significant impact on the fish gut microbiome. Strategies that target the water compartment in aquacultures could potentially suppress the development of fish pathogens in the aquaculture system and improve fish health (Schryver and Vadstein 2014). Despite its importance, the majority of studies has overlooked the role of water microbiome for maintenance of a healthy aquaculture production system. General drivers of community composition in marine environments will certainly play a major role in modulating water microbial metacommunities. For example, bacterioplankton can be limited by resource supply, such as organic carbon or inorganic nutrients (often named 'bottom-up' control), and predation and mortality ('top-down' control) (Baltar et al. 2016). In most aquatic environments, these mechanisms are constantly at play. Aquacultures, however, have more limited spatiotemporal scales. Nonetheless, abiotic parameters have also been shown to be strong drivers of aquaculture bacterioplankton composition in a pound aquaculture for tilapia (Uddin and Al-Harbi, 2004). A recent study also highlighted the role of temperature in bacterial counts of common carp aquaculture that was significantly higher in summer than in winter (Bisht et al., 2014). Major nutrients such as phosphorus, that is critical for primary productivity and bacterial production (Jin et al., 2005; Yuan et al., 2011), have also been shown to play a role in the maintenance of a healthy aquaculture microbial community.

Water microbial community profile may also provide a reliable guidance in monitoring the water quality in aquaculture. For example, Xue et al. (2017) showed that Vibrionales and Flavobacteriales were the predominant strains in RAS-diseased samples with a relative abundance 50.5% and 36.5%, respectively. In contrast, the bacterial community in RAS-healthy samples contained 35.8% Vibrionales, 17.3% Alteromonadales, 10.7% Rhodobacterales, 7.43% Kordiimonadales, and 6.26% Oceanospirillales. Their results indicated that in a healthy RAS, the bacterial community was more diverse and balanced than in a RAS with occurrence of fish diseases. Therefore, the investigation of the diversity and dynamics of bacterial plankton communities can contribute to a better knowledge of biotic parameters which are relevant for the monitoring of diseases in aquaculture systems.

Microbiome modulation approaches

There is a growing understanding in the aquaculture research that naturally occurring microbes in the water and fish host can play a key role in suppressing pathogen development. Ecological interactions between microbes (e.g. competition, predation and mutualism) can be used to increase the abundance of harmless bacteria in the aquaculture microbiome which will in turn outcompete the opportunist harmful microbes (Bentzon-Tilia et al., 2016). Previous studies on the modulation of bacterial and microeukaryotic plankton communities and the fish gut microbiota suggest that there is a large untapped potential of these communities to promote fish health and productivity in the aquaculture systems.

Bacterioplankton communities

Bacterioplankton communities constitute an important component of the water microbiome and play a major role in the process of nutrient cycling, degradation of organic matter, fish health maintenance and are an important source of food for microbial grazers (Nevejan et al., 2018). However, members of the bacterioplankton communities may also cause fish disease and contribute for large economic losses in the aquaculture sector. Intensive aquacultures are more susceptible to disease outbreaks derived from pathogenic bacteria, viruses and parasites than other animal production facilities. It occurs because of the direct contact between fish and the environment microbiota (Wong and Rawls, 2012; Schryver and Vadstein, 2014). This contact is most problematic during the larval and juvenile phases when the immune system is undeveloped. Many bacterial infections are associated to opportunistic pathogens, bacteria that would normally coexist with fish could infect fish with a deteriorated immune system induced by the stressful conditions of intensive aquaculture conditions (e.g. high densities and high metabolic waste) (Schryver et al., 2012). The biocontrol of pathogenic bacteria in intensive aquacultures frequently relies on the physical and chemical suppression of the total bacterial density through the prophylactic administration of antibiotics, high dosages of UV radiation and/or ozonation (Defoirdt et al., 2011; Schryver et al., 2012). These strategies reduce the bacterial abundance in the water and can equally destroy pathogenic and beneficial microbes. Additionally, the constant input of nutrients (fish-feed and fish feces) in

aquaculture systems, can lead to a progressive eutrophication and stimulate the proliferation of r-strategist, including pathogenic opportunistic bacteria. A proposed alternative to these methods is to promote the stabilization of beneficial (including low growth k-strategist) bacteria in the aquaculture bacterioplankton (Attramadal et al., 2012) through the maturation of the bacterial community or through the addition of chemical substances with potential to modulate the bacterioplankton communities (prebiotics).

The microbial maturation concept was first proposed by Vadstein (1993) and is based on r/k ecological theory (Schryver and Vadstein, 2014). Through microbial maturation, slow-growing non-opportunists k-strategist bacteria are promoted through the filtration of recirculating rearing water (to remove organic suspended matter) and its posterior passage through a microbially mature and dense biofilter. By enriching the system's bacterioplankton with k-strategists bacteria, at carrying capacity similar to the rearing tanks, it is expected that, when organic matter rises, these will outcompete the emerging r-strategist bacteria and impede their proliferation (Schryver et al., 2012). Previous studies showed that microbially mature recirculating aquaculture systems tend to show more stable communities with high species richness, and lower abundance of r-strategist bacteria (Salvesen et al., 1999; Attramadal et al., 2012). In terms of fish production benefits, this method has been shown to enhance the survival of Atlantic cod (*Gadus morhua*) larvae (Attramadal et al., 2014), Atlantic halibut (*Hippoglossus hippoglossus*) and turbot (*Scophthalmus maximus*) eggs (Skjermo et al., 1997) and significantly improve growth of turbot (Salvesen et al., 1999).

Prebiotics are chemical substances that will influence the bacterial community in a beneficial way. Humic substances (HS) for example, are an interesting prebiotic for the bacterioplankton. HS is a heterogenous group of high molecular mass organic compounds derived from biological residues. HS do not have a defined structure but include various functional groups (e.g. aromatic rings, carboxylic and phenolic groups) that confer their distinctive chelating properties (Hammock et al., 2003). HS are known to reduce the concentration of dissolved heavy metal [Cd(II), Hg(II) and Zn(II) (Hammock et al., 2003)], unionized ammonia and nitrite levels (Meinelt et al., 2010) when added to freshwater aquaculture systems. In aquaculture, the HS constituent fulvic acid was shown to protect sea urchin (*Paracentrotus lividus*) from CU(II) toxicity (Lorenzo et al., 2006). HS addition in freshwater can also reduce the infection prevalence and intensity in infected guppy (*Poecilia reticulata)* (Yamin et al., 2017) and the survival rate of zebrafish (*Danio rerio*) embryos and larvae

(Meinelt et al., 2010). Yet, their impact on bacterioplankton remains unknown. Overall, there is a limited amount of information about the effect of prebiotics on the bacterioplankton communities of aquaculture systems.

Microeukaryotic plankton communities

Microeukaryotic structure and function in aquaculture systems has been largely ignored by most of the aquaculture microbiome studies. Microeukaryote activities and mediated processes can, however, be important drivers of aquaculture bacterioplankton community structure. It is already well known that in coastal seawater, protist predation can have a dramatic impact on bacterioplankton communities' structure. Together with viral-mediated lysis, grazing can be one of the main sources of microbial mortality in coastal seawater (Fuhrman and Noble, 1995). Recent theoretical models and experimental observations also suggest that microbial grazers could play an important role in controlling the abundance of pathogens in water. Through an epidemiological model, Merikanto et al. (2017) demonstrated that outside-host predation can influence disease dynamics, controlling pathogen populations before host infection. Experimentally, predation by protozoa has been shown to eliminate *Vibrio cholerae* from environmental water samples (Elena et al., 2004). Current knowledge thus appears to support the idea that microeukaryotic communities could play a relevant role in limiting the development of pathogens in aquacultures. If so, this would open the possibility of developing microbial modulation strategies to prevent disease outbreak in aquacultures that would act by limiting opportunistic pathogens that are able to grow in the outside-host environment.

The impact of microeukaryotic communities in aquaculture systems, however, is unknown and could be dependent of several factors. Predations as a driver of bacterioplankton structure are not straightforward. For example, in open or semi-enclosed aquaculture systems the impact of heterotrophic nanoflagelates predation depends on the trophic status of the water. Previous studies suggested that the impact of heterotrophic nanoflagelates grazing is related with the ecosystem overall productivity. Bacteria are limited by resources in eutrophic systems and predation-limited in oligotrophic conditions (Pernthaler, 2005). In nutrient-poor habitats the growth of heterotrophic nanoflagelates is limited by the availability of the prey. On the other hand, more nutrient rich eutrophic systems

can sustain a richer community of top predators that control bacterivorous nanoflagelates, releasing prokaryote community from predation pressure (Pernthaler, 2005). Other factors, can also determine the impact of protist predation on prokaryotic community structure. Recently, Baltar et al. (2016) showed that peaks in protist predation associated with phytoplankton blooms triggered strong changes in bacterial abundance and activity but not on their diversity. It is also known that the level of bacterivory of each group can change throughout the year, influenced by seasonal variations (Epstein, 1997).

The interest in this community however, goes far beyond their role in controlling bacterial communities. Microeukaryotes also play an interesting role as biogenic producers. Members of Labyrinthulea class (Stramenopiles division), commonly found as parasites on algae and seagrasses or as decomposers on dead plant material (Takao et al., 2005), have the ability to produce lipids that can be used as alternative source of the omega-3 in fish productions, increasing their growth rate (Atienza et al., 2012). Considering that the economic efficiency of aquaculture can be improved by the discovery of new by-products or the use of new substances to increase production, microeukaryotic role as biogenic producers could be of great interest.

With exception of their role as pathogens, there is also a considerable knowledge gap regarding the direct interaction of microeukaryotes with fish. As pathogens, they can cause a very significant impact. For example, the water mold *Saprolegnia parasitica* is one of the most important fish pathogens, especially on salmon and trout species, causing considerable economic losses (Torto-Alalibo et al., 2005). Other direct interactions such as their associations with fish gut, however, are still far from being understood. A recent study provided an interesting insight, suggesting that symbiotic microeukaryotic communities might be less prone to variation than prokaryotic ones. It was found that the intestines of four different larvae (reared in the same environment) contained distinct bacterial populations, while microeukaryotic communities were almost identical (Li et al., 2012). Overall, these studies highlight the importance of advancing fundamental knowledge of microeukaryotic ecology in aquaculture systems. Despite of the lack of studies on their diversity and function, microeukaryotes are an important component of the aquaculture microbiome with a potential critical role on the modulation prokaryotic communities in aquaculture systems.

Fish gut microbiota

The gastrointestinal tract of the fish is an important entry for pathogenic bacteria. There, the gut microbiota is the first barrier against pathogens, by producing antagonistic compounds, restricting surface attachment and competing for nutrients. Additionally, the local microbiota assists the digestive process, promotes a better assimilation of nutrients from feed and enhances the overall immunologic response of the fish. Overall, a well-established, stable and healthy microbiota contributes to an enhanced survival rate and growth of the fish in aquaculture conditions. In aquaculture systems, gut microbiota is modulated through diet, namely through the administration of microbially-derived feed (bioflocs) or through the supplementation of chemical (prebiotics) and cellular (probiotics) modulators in commercial fish feed.

Bioflocs is a common feed source in some aquaculture facilities (e.g. shrimp and tilapia fish farming). Bioflocs formations requires the addition of a carbon rich substrate (e.g. carbohydrates) to the inorganic nitrogen-rich RAS water in order to increase C:N ratio to an optimal 20:1 for heterotrophic bacteria (Avnimelech, 1999) and 10:1 for microalgae (Martínez-Córdova et al., 2015). This, in combination with an intensive aeration, will promote the rapid proliferation of microalgae and heterotrophic aerobic bacteria and the conversion of the added carbon and inorganic nitrogen into biomass. Subsequently, particulate matter is produced and used as a bacteria-enriched fish feed. The usage of bioflocs in aquaculture production can dually improve water quality, by reducing inorganic nitrogen, and reutilize unassimilated nutrients. Bioflocs are known to include various beneficial probiotic bacteria, namely polyhydroxybutyrate producing bacteria that, upon digestion, can release short chain fatty acids (Glencross 2009; Ekasari et al., 2010). Yet, bioflocs can also introduced pathogenic bacteria in the system (Martínez-Córdova et al., 2015; Cardona et al., 2016). To avoid this, biofloc microbial diversity and abundance can be modulated indirectly by altering the C:N ratio, carbon substrate and light intensity (Avnimelech 1999; Martínez-Córdova et al., 2015) or directly by adding probiotic bacteria to biofloc tank (e.g. Bacillus) (Crab et al., 2010). For example, the addition of smaller and more edible carbohydrates (e.g. sugars and alcohols) may induce a faster response in microbial abundance, but this will drastically increase oxygen requirements and may destabilize the microbial structure (Martínez-Córdova et al., 2015). The addition of more complex carbohydrates (e.g. starch and cellulose) may instead promote a

greater diversity of bacteria, provide a nucleation site for biofloc formation and prolong the fertilizing effect thus minimizing oxygen requirements in aeration tanks (Becerra-Dórame et al. 2012; Martínez-Córdova et al., 2015).

Other attempts to promote a stable and diverse gut microbiota of the commercial fish may rely on the addition of prebiotic and probiotic additives in fish feed. Prebiotics are not digested by teleost enzymes but are fermented by the gut microbiota (Llewellyn et al., 2014). Their addition may promote a higher diversity of bacteria and the proliferation of lactic acid bacteria (LAB) in the gut microbiota (Llewellyn et al., 2014). LAB will benefit gut microbiota by producing antagonist compounds such as bacteriocins and, through the fermentation of complex carbohydrates, they produce beneficial short chain fatty acids (e.g. formic, acetic, propionic, butyric and valeric acid) (Marcil et al., 2002; Geraylou et al., 2012). Overall, microbiota gut modulation may indirectly benefit the fish by inhibiting pathogen adhesion and spread; and by increasing fish innate immune response, ultimately enhancing biomass and survival rate during aquaculture production (Llewellyn et al., 2014). Various types of polymeric carbon substrates (e.g. oligosaccharides, polysaccharides and poly-β-hydroxybutyrates), naturally-derived or synthetic compounds with putative quorum-quenching properties (e.g. coumarin and cinnamaldehyde) and nutritional supplements (e.g. vitamins and essential fatty acids) can be used as prebiotics.

Polymeric carbon substrates can provide a relatively edible carbon substrate, which will enhance bacterial abundance and, consequently, promote a healthier microbial community, when applied at optimal dosage. Oligosaccharide (OS)-supplemented feed [e.g arabino-xylose oligosaccharides (AXOS), galacto-oligosaccharides (GOS), fructose-oligosaccharides (FOS) and mannose- oligosaccharides (MOS)] have been found to enhance survival rate and growth of multiple freshwater and saltwater fish aquacultures (Dimitroglou et al., 2010; Geraylou et al., 2012; Torrecillas et al., 2012; Hoseinifar et al., 2013; Hoseinifar et al., 2014; Hoseinifar et al., 2016) when added at a 1-2% w/w dosage. OS supplementation, by increasing gut mucus production, villi surface and microvilli length, will hinder or reduce the pathogen adhesion at gut epithelium and increase nutrient assimilation (Dimitroglou et al. 2010). The impact of OS supplementation on gut microbiota's structure is poorly studied. Geraylou et al. (2012) showed that 2% (w/w) AXOS supplementation promoted the proliferation of lactic acid bacteria (Eubacteriaceae, Clostridiaceae, Streptococcaceae and Lactobacillaceae) in the hindgut of Siberina sturgeon (*Acipenser baerii*). AXOS supplementation

also increased the concentration of short-chain fatty acids and suppressed the growth of putative pathogenic *Aeromonas* sp., *Citrobacter freundii* and *E. coli* bacteria. MOS-supplemented diet was also shown to suppress the development of the pathogen *Clostridium botulinum* (Burr et al., 2010).

In addition to OS, various polysaccharides (PS) supplementation experiments have been conducted. Comparatively to OS, PS was shown to have a more profound effect on the bacterial community of gut microbiota in human microbiota (Van De Wiele et al., 2007). Their higher degree of polymerization promotes a slower fermentation rate and a gradual release of energy is obtained throughout the gut in comparison to OS (Van De Wiele et al., 2007). Previous studies showed that carp juveniles (*Cirrhina mrigala*) feed with β-glucan- , inulin, chitosan- or chitin-supplements, and later exposed to microbial pathogens presented significantly better growth, higher survival rate and an overall healthier physiological and immunological status (Misra et al., 2006; Shanthi Mari et al., 2014; Raffic Ali et al., 2016). Inulin supplementation has been shown alter the structure of the bacterial community and to increase short-chain fatty acid by approximately 30% in Simulator of the Human Intestinal Microbial Ecosystem (SHIME) (Van De Wiele et al., 2007). Another polymeric carbon substrate frequently tested is poly-hydroxybutytrate (PHB), which is a natural polymer synthetized by some bacteria and plants to store energy and through bacterial metabolism it breaks down into small soluble short-chain fatty acid monomers (Najdegerami et al., 2012; Hoseinifar et al., 2016). Overall, PHB-supplementation in feed seem to enhance growth and survival rate of Siberian sturgeon fingerlings (*Acipenser baerii*) (Najdegerami et al., 2012), giant freshwater prawn (*Macrobrachium rosenbergii*) larvae (Nhan et al., 2010) and, in live feed *Artemia franciscana* nauplii, it improved survival in pathogen-infected tanks (Defoirdt et al., 2007).

Antagonist prebiotics include naturally derived and bioactive and quorum-quenching compounds. Quorum-quenching compounds such as coumarin and cinnamaldehyde (Ali et al., 2005; Walasek et al., 2015) can be used as interesting alternatives to highly effective but toxic synthetic brominated furanones and lactones (Defoirdt et al., 2011). For example, in comparison to synthetic brominated furanone and lactones, the administration of cinnamaldehyde in water effectively inhibit pathogenic bacteria *Vibrio harveyi*, *Aeromonas samonicida* and *A. hydrophila* at a similar dosage, but cinnamaldehyde was significantly less toxic to fish (Natrah et al., 2012). Cinnamaldehyde powder and oil supplementation in feed enhanced fish growth, survival rate and physiological parameters (Santos et al., 2016). While,

coumarin supplementation exhibited some type quorum-quenching activity against three representatives of quorum-sensing bacteria and against opportunistic human pathogens bacteria *Stenotrophomonas maltophilia*, *Burkholderia cepacia* and *Pseudomonas aeruginosa* (Gutiérrez-Barranquero et al., 2015). In aquaculture experiments, coumarin reduce the expression of virulence genes, infection related symptoms and increased survival rate in *Vibrio splendidus*-infected sea cucumbers (*Apostichopus japonicus*) (Zhang et al., 2017). Also, the supplementation of humic substances in fish diet enhanced the survival rate and significantly reduced skin lesions in common carp (*Cyprinus carpio*) and ayu fish (*Plecoglossus altivelis*) challenged with a virulent strains of *Alteromonas salmoncida* and *Flavobacterium psychrophilum*, respectively (Kodama and Nakagawa, 2007). Yet the most interesting results could be obtained through the administration of prebiotic cocktails. For example, PHB supplementation synergy with other prebiotics has been tested and seem to improve overall fish health in comparison to each prebiotic individually and to control (Defoirdt et al., 2007), also a supplementation cocktail seem to increase survival rate of rainbow trout (*Oncorhynchus mykiss*) under low infection pressure of *Yersinia ruckeri* (Jaafar et al., 2013).

The use of probiotics is also a resourceful tool to increase the viability and quality of livestock in high production facilities. Probiotics are "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (Fuller 1989; FAO/WHO, 2001). The allochthonous supplementation of probiotics in fish feed has repeatedly been hypothesized and tested with usually positive results. However, until now only one probiotic strain (*Pediococcus acidilactici* CNCM MA18/5M) has been approved under EU regulation for aquaculture purposes (European Union, 2018), but in other markets (e.g. Asia and United States) various strains have been approved and many commercial probiotic formulations are available (Martínez Cruz et al., 2012). Probiotics most commonly tested and/or available belong to phenotypic group of LAB [genera Lactobacillus, Pedicoccus, Streptococcus, Lactococcus, Leuconostoc, Bifidobacterium, Carnobacterium and some strains of Bacillus (Holzapfel and Wood, 2012)] or to yeast of genera Saccharomyces (Martínez Cruz et al., 2012). LAB can be beneficial, since they produce a variety of antagonist compounds (e.g. bacteriocins) (Ribeiro et al., 2014) that act against fish pathogens (Ringø and Gatesoupe, 1998).

Probiotics supplementation in fish feed has been shown to inhibit pathogenic infection, enhance immune response, water quality and stress tolerance of fish, improve gut

function, namely digestion and nutrient assimilation and enhance reproduction and survival rates in freshwater and seawater aquacultures (Vine et al., 2004; EL-Haroun et al., 2006; Hidalgo et al., 2006; Ghosh et al., 2007; Lalloo et al., 2007; Merrifeld et al., 2010; Doroteo et al., 2018). Some probiotic bacteria will directly enhance survival of infected fish through the production of bactericidal or bacteriostatic substances (Tovar et al., 2002) or by impeding the adhesion of pathogenic bacteria (Vine et al., 2004). In the fish digestive tract, well-established probiotics will synthetize various extracellular enzymes (Tovar et al., 2002) and growth factors (e.g. vitamins, fatty acids and aminoacids) (Martínez Cruz et al., 2012). These will subsequently lead to a cascade of benefits to the fish: higher protein digestibility, a higher feed conversion rate, a better nutrition and an overall enhanced fish fitness (De Schrijver and Ollevier, 2000; Lara-Flores et al., 2003; Martínez Cruz et al., 2012). By promoting a better nutrition and synthesizing growth factors, probiotic supplementation can indirectly improve the native immune responses of fish to disease outbreak (Taoka et al., 2006a; Taoka et al., 2006b) and by increasing feed conversion and enhancing fish growth it can compensate the additional cost of probiotics and even increase total net return of an aquaculture production facility (EL-Haroun et al., 2006).

Conclusions and Future Perspectives

Recent advances in DNA sequencing technologies have allow us to uncover microbial community's diversity with an unprecedented level of detail. Increase in DNA sequencing throughput and cost reduction have made feasible to sequence community DNA in environmental samples without cloning or cultivation. In line with other studies that have characterized microbial diversity in different environments, rRNA gene surveys have also been applied to characterize microbial communities in aquacultures, especially in the gastrointestinal tract of fishes (Tarnecki et al., 2017). Much less studies have characterized the water aquaculture microbiome structure and function (Rud et al., 2017; Martins et al., 2018). Overall, the use microbiome data for use in the development strategies for better aquaculture practices and sustainability is still in its infancy. More studies are necessary in order to improve our understating on what constitutes a healthy aquaculture microbiome and how we can manipulate the microbial communities in aquaculture systems.

The use of bioinformatics technologies and biostatistics approaches that have been recently developed can also increase our knowledge of the aquaculture microbiome. The increased number of studies based on high-throughput sequencing technologies has fostered the development of new bioinformatics approaches and biostatistics analysis for characterization of the structure of complex microbial communities and function. One of such techniques that has become popular in recent years is correlation network analysis. Microorganisms do not exist in isolation, they form complex ecological interaction webs that can have a positive, negative or no impact on the species involved (Faust and Raes, 2012). As referred, antagonistic interactions such as inter-specific competition between microbes and predation can influence disease dynamics (Merikanto et al., 2017). The construction and analysis of networks could elucidate which taxa occur together in water aquaculture and identify the direction of interactions between taxa or groups. This would not only help to elucidate key ecological principles but also be used as a tool to guide prebiotic and probiotic selection and application. For example, the construction of correlation networks in human and mouse models helped identify *Clostridium scindens* as exhibiting a negative correlation pattern with *C. difficile* infection. Transfer of *C. scindens* was then experimentally shown to increase resistance to *C. difficile* infection in mouse models (Buffie et al., 2014).

In recent years, it has become clear that the study of the diversity and ecological interactions of microbial communities in aquaculture systems will provide the foundation to develop environmentally friendly approaches to prevent or influence fish pathogen development and will support the development of sustainable fish farming practices. Research on aquaculture microbiome has started to allow us to understand microbe interactions (microbe-microbe, -environment and -host interactions) and their role in the aquaculture environment. Due to the rapid technological development of high-throughput sequencing technologies and reduction in their operating costs, it is likely that in the future they will be used for a rapid assessment of environmental microbial communities and support strategies for microbiome modulation in aquaculture systems.

References

Ababouch L, Vannuccini S, Chomo V (2016) 2.2 Fisheries, aquaculture utilization and trade: challenges and opportunities. In Fish Trade – part 2, Trade in sustainable fisheries and aquaculture. Food and Agriculture Organization of the United Nations (FAO). Available at: http://unctad.org/en/PublicationChapters/ditcted2016d3_part2_en.pdf. Acessed: apr/2018

Ali SM, Khan AA, Ahmed I, Musaddiq M, Ahmed KS, Polasa H, Rao LV, Habibullah CM, Sechi LA, Ahmed N (2005) Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen Helicobacter pylori. Annals of Clinical Microbiology and Antimicrobials 4: 20. doi: 10.1186/1476-0711-4-20

Atienza GAMV, Arafiles KHV, Carmona MCM, Garcia JPC, Macabago AMB, Peñacerrada BJDC, Cordero PRF, Bennett RM, Dedeles GR (2012) Carotenoid analysis of locally isolated Thraustochytrids and their potential as an alternative fish feed for *Oreochromis niloticus* (Nile tilapia). Mycosphere 3: 420-428. doi: 10.5943/mycosphere/3/4/5

Attramadal KJK, Salvesen I, Xue R, Øie G, Størseth TR, Vadstein O, Olsen Y (2012) Recirculation as a possible microbial control strategy in the production of marine larvae. Aquacultural Engineering 46: 27-39. doi: 10.1016/j.aquaeng.2011.10.003.

Attramadal KJK, Truong TMH, Bakke I, Skjermo J, Olsen Y, Vadstein O (2014) RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. Aquaculture 432: 483-490. doi:10.1016/j.aquaculture.2014.05.052

Avnimelech Y (1999) Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 176: 227-235. doi: 10.1016/S0044-8486(99)00085-X.

Badiola M, Mendiola D, Bostock J (2012) Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. Aquacultural Engineering 51: 26-35. doi:10.1016/j.aquaeng.2012.07.004

Bakshani CR, Morales-Garcia AL, Althaus M, Wilcox MD, Pearson JP, Bythell JC, Burgess JG (2018) Evolutionary conservation of the antimicrobial function of mucus: a first defence against infection. npj Biofilms and Microbiomes 4 (1), 14. doi: 10.1038/s41522-018-0057-2.

Baltar F, Palovaara J, Unrein F, Catala P, Horňák K, Šimek K, Vaqué D, Massana R, Gasol JM, Pinhassi J (2016) Marine bacterial community structure resilience to changes in protist predation under phytoplankton bloom conditions. The ISME Journal 10 (3):568-81. doi: 10.1038/ismej.2015.135.

Baluyut EA (1989) Aquaculture Systems and Practices: A Selected Review. United Nations Development Programme, Food and Agriculture Organization Of The United Nations (FAO), ADCP/REP/89/43. FAO Corporate Document Repository, Produced by Fisheries and Aquaculture Departament. Available in: http://www.fao.org/docrep/t8598e/t8598e05.htm. Acessed in: aug/2017.

Becerra-Dórame MJ, Martínez-Porchas M, Martínez-Córdova LR, Rivas-Vega ME, Lopez-Elias JA, Porchas-Cornejo MA (2012) Production Response and Digestive Enzymatic Activity of the Pacific White Shrimp *Litopenaeus vannamei* (Boone, 1931) Intensively Pregrown in Microbial Heterotrophic and Autotrophic-Based Systems. The Scientific World Journal 2012: 723654. doi:10.1100/2012/723654.

Benhamed S, Guardiola FA, Mars M, Esteban MÁ (2014) Pathogen bacteria adhesion to skin mucus of fishes. Veterinary Microbiology 25, 171(1-2):1-12. doi: 10.1016/j.vetmic.2014.03.008.

Bentzon-Tilia M, Sonnenschein EC, Gram L (2016) Monitoring and managing microbes in aquaculture – Towards a sustainable industry. Microbial Biotechnology 9: 576-584. doi: 10.1111/1751-7915.12392

Bisht A, Singh UP, Pandey NN (2014) Comparative study of seasonal variation in bacterial flora concomitant with farm raised fingerlings of *Cyprinus carpio* at tarai region of Uttarakhand. *Journal of Environmental Biology* 35(2):363-367. ISSN: 0254-8704.

Boaventura CM, Coelho FJRC, Martins PT, Pires ACC, Duarte LN, Uetanabaro APT, Cleary DFR, Gomes NCM (2018) Micro-eukaryotic plankton diversity in an intensive aquaculture system for production of Scophthalmus maximus and Solea senegalensis. Aquaculture 490, 321-328. doi: 10.1016/j.aquaculture.2018.02.033

Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A, Littmann E, van den Brink MR, Jenq RR, Taur Y, Sander C, Cross JR, Toussaint NC, Xavier JB, Pamer EG (2014) Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. Nature 8; 517(7533):205-8. doi: 10.1038/nature13828.

Burr G, Hume M, Ricke S, Nisbet D, Gatlin D (2010) In Vitro and In Vivo Evaluation of the Prebiotics GroBiotic®-A, Inulin, Mannanoligosaccharide, and Galactooligosaccharide on the Digestive Microbiota and Performance of Hybrid Striped Bass (*Morone chrysops* × *Morone saxatilis*). Microbial Ecology 59: 187-198. doi: 10.1007/s00248-009-9597-6.

Cardona E, Gueguen Y, Magré K ... Pierrat F, Noguier F, Saulnier D (2016) Bacterial community characterization of water and intestine of the shrimp *Litopenaeus stylirostris* in a biofloc system. BMC Microbiology 16: 157. doi: 10.1186/s12866-016-0770-z.

Chopin T (2006) Integrated Multi-Trophic Aquaculture. What it is and why you should care and don't confuse it with polyculture. Commentary in Northern Aquaculture, July/August 2006 page 4.

Crab R, Chielens B, Wille M, Bossier P, Verstraete W (2010) The effect of different carbon sources on the nutritional value of bioflocs, a feed for Macrobrachium rosenbergii postlarvae. Aquaculture Research 41: 559-567. doi: 10.1111/j.1365-2109.2009.02353.x.

De Schrijver R, Ollevier F (2000) Protein digestion in juvenile turbot (*Scophthalmus maximus*) and effects of dietary administration of *Vibrio proteolyticus*. Aquaculture 186: 107-116. doi: 10.1016/S0044-8486(99)00372-5.

Defoirdt T, Halet D, Vervaeren H, Boon N, Van de Wiele T, Sorgeloos P, Bossier P, Verstraete W (2007) The bacterial storage compound poly-β-hydroxybutyrate protects *Artemia franciscana* from pathogenic *Vibrio campbellii*. Environmental Microbiology 9(2): 445-452. doi:10.1111/j.1462-2920.2006.01161.x

Defoirdt T, Sorgeloos P, Bossier P (2011) Alternatives to antibiotics for the control of bacterial disease in aquaculture. Current Opinion in Microbiology 14: 251-258. doi: 10.1016/j.mib.2011.03.004.

DeLong DP, Losordo TM (2012) How to Start a Biofilter. Southern Regional Aquaculture Center, SRAC Publication No. 4502.

Dimitroglou A, Davies SJ, Sweetman J, Divanach P, Chatzifotis S (2010) Dietary supplementation of mannan oligosaccharide on white sea bream (*Diplodus sargus* L.) larvae: effects on development, gut morphology and salinity tolerance. Aquaculture Research 41: e245-e251. doi: 10.1111/j.1365-2109.2010.02513.x.

Dittmann KK, Rasmussen BB, Castex M, Gram L. Bentzon-Tilia M (2017) The aquaculture microbiome at the centre of business creation. Microbial Biotechnology 10: 1279-1282. doi: 10.1111/1751-7915.12877.

Doroteo AM, Pedroso FL, Lopez JDM, Apines-Amar MJS (2018) Evaluation of potential probiotics isolated from saline tilapia in shrimp aquaculture. Aquaculture International. doi: 10.1007/s10499-018-0270-2.

Ekasari J, Crab R, Verstraete W (2010) Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. HAYATI Journal of Biosciences 17: 125-130. doi: 10.4308/hjb.17.3.125.

Elena MPM, Miroslav M, Teresa CGM (2004) Do protozoa control the elimination of *Vibrio cholerae* in brackish water? International Review of Hydrobiology 89: 215-227. doi: 10.1002/iroh.200310644.

EL-Haroun ER, Goda AM, Kabir Chowdhury MA (2006) Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquaculture Research 37: 1473-1480. doi: 10.1111/j.1365-2109.2006.01584.x.

Elliott, D (2011) The skin. The Many Functions of Fish Integument. Encyclopedia of fish physiology: from genome to environment. 1. 471-475. doi:10.1016/B978-1.

Epstein SS (1997) Microbial Food Webs in Marine Sediments. II. Seasonal Changes in Trophic Interactions in a Sandy Tidal Flat Community. Microbial Ecology, 34 (3): 199–209. doi: 10.1007/s002489900049

European Comission. Aquaculture methods. Available in: https://ec.europa.eu/fisheries/cfp/aquaculture/aquaculture_methods_en

European Union (2018) European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003.

FAO/WHO (2001) Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. ISSN: 0254-4725

FAO (2016). The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

FAO (2014-2018) Cultured Aquatic Species Information Programme. *Solea solea*. Cultured Aquatic Species Information Programme. Text by Colen, R., Ramalho, A., Rocha, F, Dinis, M.T. In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 18 February 2014. [Cited 24 May 2018]

Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Micro 10: 538-550. doi:10.1038/nrmicro2832.

Fernandez S, Goldsby KA (2015) Construction and Implementation of a Bench-Top Aquaponic System. Honors Theses, Department of Biological Science. The Florida State Universitycollege of Arts & Sciences.

Fuhrman JA, Noble RT (1995) Viruses and protists cause similar bacterial mortality in coastal seawater. Limnology and Oceanography 40 (7): 1236-1242 . doi: 10.4319/lo.1995.40.7.1236

Fuller R (1989) Probiotics in man and animals. Journal of Applied Bacteriology 66(5): 365-378.

Funge-Smith S, Phillips MJ (2001) Aquaculture systems and species. In R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery and J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000. pp. 129-135. NACA, Bangkok and FAO, Rome.

Geraylou Z, Souffreau C, Rurangwa E, D'Hondt S, Callewaert L, Courtin CM, Delcour JA, Buyse J, Ollevier F (2012) Effects of arabinoxylan-oligosaccharides (AXOS) on juvenile Siberian sturgeon (*Acipenser baerii*) performance, immune responses and gastrointestinal microbial community. Fish & Shellfish Immunology 33 (4): 718-724. doi: 10.1016/j.fsi.2012.06.010

Ghosh S, Sinha A, Sahu C (2007) Effect of probiotic on reproductive performance in female livebearing ornamental fish. Aquaculture Research 38: 518-526. doi: 10.1111/j.1365-2109.2007.01696.x.

Giatsis C, Sipkema D, Smidt H, Heilig H, Benvenuti G, Verreth J and Verdegem M (2015) The impact of rearing environment on the development of gut microbiota in tilapia larvae. Scientific Reports 5:18206. doi: 10.1038/srep18206.

Glencross BD (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture 1: 71-124. doi: 10.1111/j.1753-5131.2009.01006.x.

Gómez GD, Balcázar JL (2008) A review on the interactions between gut microbiota and innate immunity of fish. FEMS Immunology & Medical Microbiology 52 (2): 145–154. doi: 10.1111/j.1574-695X.2007.00343.x.

Guarner F, Malagelada JR (2003) Gut flora in health and disease. Lancet 361(9356):512–519. doi: 10.1016/S0140-6736(03)12489-0.

Guerrero S, Cremades J (2012) Integrated Multi-trophic Aquaculture (IMTA): A sustainable, pioneering alternative for marine cultures in Galicia. J. Regional Government of Galicia (Spain), pp.111, 13:978-84-695-3260-7. <hal-00743395>

Gutiérrez-Barranquero JA, Reen FJ, McCarthy RR, O'Gara F (2015) Deciphering the role of coumarin as a novel quorum sensing inhibitor suppressing virulence phenotypes in bacterial pathogens. Applied Microbiology and Biotechnology 99: 3303-3316. doi: 10.1007/s00253-015-6436-1.

Hall SJ, Delaporte A, Phillips MJ, Beveridge M, O'Keefe M (2011) Blue Frontiers: Managing the Environmental Costs of Aquaculture. The World Fish Center, Penang, Malaysia.

Hammock D, Huang CC, Mort G, Swinehart JH (2003) The Effect of Humic Acid on the Uptake of Mercury(II), Cadmium(II), and Zinc(II) by Chinook Salmon (*Oncorhynchus tshawytscha*) Eggs. Archives of Environmental Contamination and Toxicology 44: 0083-0088. doi: 10.1007/s00244-002-1261-9.

Hidalgo MC, Skalli A, Abellán E, Arizcun M, Cardenete G (2006) Dietary intake of probiotics and maslinic acid in juvenile dentex (*Dentex dentex* L.): effects on growth performance, survival and liver proteolytic activities. Aquaculture Nutrition 12: 256-266. doi: 10.1111/j.1365-2095.2006.00408.x.

Holzapfel W, Wood BJ (2012) The genera of lactic acid bacteria. vol 2 Springer Science & Business Media.

Hoseinifar SH, Khalili M, Khoshbavar Rostami H, Esteban MÁ (2013) Dietary galactooligosaccharide affects intestinal microbiota, stress resistance, and performance of Caspian roach (*Rutilus rutilus*) fry. Fish & Shellfish Immunology 35: 1416-1420. doi: 10.1016/j.fsi.2013.08.007.

Hoseinifar SH, Soleimani N, Ringø E (2014) Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp (*Cyprinus carpio*) fry. British Journal of Nutrition 112: 1296-1302. doi: 10.1017/S0007114514002037.

Hoseinifar SH, Ringø E, Shenavar MA, Esteban MÁ (2016) Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. Reviews in Aquaculture 8: 89-102. doi: 10.1111/raq.12082.

Huddleston JR (2014) Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infection and Drug Resistance 7: 167-176. doi: 10.2147/IDR.S48820.

Irianto A, Austin B (2002) Use of probiotics to control furunculosis in rainbow trout *Oncorhynchus mykiss* (Walbaum). Journal of Fish Disease 25 (6): 333–342. doi: 10.1046/j.1365-2761.2002.00375.x

Jaafar R, Kania PW, Larsen A, Nielsen DS, Fouz B, Browdy C, Buchmann K (2013) Gut microbiota changes in rainbow trout, *Oncorhynchus mykiss* (Walbaum), during organic acid feed supplementation and *Yersinia ruckeri* infection. Journal of Fish Diseases 36: 599-606. doi: 10.1111/jfd.12047.

Jansen PA, Kristoffersen AB, Viljugrein H, Jimenez D, Aldrin M, Stien A (2012) Sea lice as a density-dependent constraint to salmonid farming. Proceedings of the Royal Society B: Biological Sciences. doi: 10.1098/rspb.2012.0084.

Jin X, Wang S, Pang Y, Zhao H, Zhou X (2005) The adsorption of phosphate on different trophic lake sediments. Colloids and Surfaces A, 254: 241–248

Kinney H (2017) Aquaculturists' Perceptions of Integrated Multi-Trophic Aquaculture (IMTA). University of Rhode Island. Open Access Master's Theses, paper 1024. http://digitalcommons.uri.edu/theses/1024

Kodama H, Nakagawa T (2007) Protection against atypical *Aeromonas salmonicida* infection in carp (*Cyprinus carpio* L.) by oral administration of humus extract. Journal of veterinary medical science 69: 405-408. doi: 10.1292/jvms.69.405.

Labatut RA, Olivares JF (2004) Culture of turbot (*Scophthalmus maximus*) juveniles using shallow raceways tanks and recirculation. Aquacultural Engineering, 32(1):113-127. doi:10.1016/j.aquaeng.2004.05.008.

Lalloo R, Ramchuran S, Ramduth D, Görgens J, Gardiner N (2007) Isolation and selection of *Bacillus* spp. as potential biological agents for enhancement of water quality in culture of ornamental fish. Journal of Applied Microbiology 103: 1471-1479. doi: 10.1111/j.1365-2672.2007.03360.x.

Lara-Flores M, Olvera-Novoa MA, Guzmán-Méndez BzE, López-Madrid W (2003) Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture 216: 193-201. doi: 10.1016/S0044-8486(02)00277-6.

Larsen A, Tao Z, Bullard SA, Arias CR (2013) Diversity of the skin microbiota of fishes: evidence for host species specificity. *FEMS Microbiology Ecology* 85, 483–494. doi: 10.1111/1574-6941.12136.

Leung TLF, Bates AE (2013) More rapid and severe disease outbreaks for aquaculture at the tropics: implications for food security. Journal of Applied Ecology 50: 215-222. doi: 10.1111/1365-2644.12017.

Li X, Yu Y, Feng W, Yan Q, Gong Y (2012) Host species as a strong determinant of the intestinal microbiota of fish larvae. The Journal of Microbiology 50: 29-37. doi: 10.1007/s12275-012-1340-1.

Li Z, Che J, Xie J, Wang G, Yu E, Xia Y, Yu D, Zhang K (2017) Microbial succession in biofilms growing on artificial substratum in subtropical freshwater aquaculture ponds. FEMS Microbiology Letters 364 (4): fnx017. doi: 10.1093/femsle/fnx017.

Llewellyn MS, Boutin S, Hoseinifar SH, Derome N (2014) Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. Frontiers in Microbiology 5. doi: 10.3389/fmicb.2014.00207.

Lorenzo JI, Nieto O, Beiras R (2006) Anodic stripping voltammetry measures copper bioavailability for sea urchin larvae in the presence of fulvic acids. Environmental Toxicology and Chemistry 25: 36-44. doi: 10.1897/05-236R.1.

Marcil V, Delvin E, Seidman E, Poitras L, Zoltowska M, Garofalo C, Levy E (2002) Modulation of lipid synthesis, apolipoprotein biogenesis, and lipoprotein assembly by butyrate. American Journal of Physiology-Gastrointestinal and Liver Physiology 283: G340-G346. doi: 10.1152/ajpgi.00440.2001.

Martínez Cruz P, Ibáñez AL, Monroy Hermosillo OA, Ramírez Saad HC (2012) Use of Probiotics in Aquaculture. ISRN Microbiology 2012: 916845. doi: 10.5402/2012/916845.

Martínez-Córdova LR, Emerenciano M, Miranda-Baeza A, Martínez-Porchas M (2015) Microbial-based systems for aquaculture of fish and shrimp: an updated review. Reviews in Aquaculture 7: 131-148. doi: 10.1111/raq.12058.

Martins P, Cleary DFR, Pires ACC, Rodrigues AM, Quintino V, Calado R, Gomes NCM (2013) Molecular analysis of bacterial communities and detection of potential pathogens in a recirculating aquaculture system for *Scophthalmus maximus* and *Solea senegalensis*. Plos One, 8 (11):e80847. doi: 10.1371/journal.pone.0080847.

Martins P, Navarro RVV, Coelho FJRC, Gomes NCM (2015) Development of a molecular methodology for fast detection of *Photobacterium damselae* subspecies in water samples. Aquaculture 435: 137-142. doi: 10.1016/j.aquaculture.2014.09.028.

Martins P (2016) Molecular characterization of microbial communities in aquaculture. PhD Thesis. University of Aveiro.

Medina A, Mancera JM, Martínez-Manzanares E, Moriñigo MA, Arijo S (2015) Identification of *Vibrio harveyi* proteins involved in the specific immune response of Senegalese sole (*Solea senegalensis*, Kaup). Fish & Shellfish Immunology 47: 377-380. doi: 10.1016/j.fsi.2015.09.031.

Meinelt T, Kroupova H, Stüber A, Rennert B, Wienke A, Steinberg CEW (2010) Can dissolved aquatic humic substances reduce the toxicity of ammonia and nitrite in recirculating aquaculture systems? Aquaculture 306: 378-383. doi: 10.1016/j.aquaculture.2010.06.007.

Mennerat A, Nilsen F, Ebert D, Skorping A (2010) Intensive farming: evolutionary implications for parasites and pathogens. Evolutionary Biology 37(2-3):59-67. doi: 10.1007/s11692-010-9089-0.

Merikanto I, Laakso JT, Kaitala V (2017) Outside-host predation as a biological control against an environmental opportunist disease. Ecological Modelling, 347: 85-93. doi: 10.1016/j.ecolmodel.2017.01.002

Merrifeld DL, Bradley G, Baker RTM, Davies SJ (2010) Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) II. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria postantibiotic treatment. Aquaculture Nutrition 16: 496-503. doi: 10.1111/j.1365-2095.2009.00688.x.

Misra CK, Das BK, Mukherjee SC, Pattnaik P (2006) Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. Aquaculture 255: 82-94. doi: 10.1016/j.aquaculture.2005.12.009.

Montalban-Arques A, Schryver P, Bossier P, Gorkiewicz G, Mulero V, Gatlin DM, Galindo-Villegas J (2015) Selective Manipulation of the Gut Microbiota Improves Immune Status in Vertebrates. Frontiers in Immunology 6: 512. doi: 10.3389/fimmu.2015.00512

Najdegerami EH, Tran TN, Defoirdt T, Marzorati M, Sorgeloos P, Boon N, Bossier P (2012) Effects of poly-β-hydroxybutyrate (PHB) on Siberian sturgeon (*Acipenser baerii*) fingerlings performance and its gastrointestinal tract microbial community. FEMS Microbiology Ecology 79: 25-33. doi: 10.1111/j.1574-6941.2011.01194.x.

Natrah FM, Alam MI, Pawar S, Harzevili AS, Nevejan N, Boon N, Sorgeloos P, Bossier P, Defoirdt T. (2012) The impact of quorum sensing on the virulence of *Aeromonas hydrophila* and *Aeromonas salmonicida* towards burbot (*Lota lota* L.) larvae. Veterinary Microbiology 159: 77-82. doi: 10.1016/j.vetmic.2012.03.014.

Nayak SK (2010) Role of gastrointestinal microbiota in fish. Aquaculture Research 41: 1553-1573. doi: 10.1111/ j.1365 -210 9.2010.02546. x

Nevejan N, Schryver P, Wille M, Dierckens K, Baruah K, Stappen GV (2018) Bacteria as food in aquaculture: do they make a difference? Reviews in Aquaculture 10, 180–212. doi: 10.1111/raq.12155.

Nguyen DDL, Ngoc HH, Dijoux D, Loiseau G and Montet D (2008) Determination of fish origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: An application on Pangasius fish from Viet Nam. Food Control 19: 454–460. doi:10.1016/j.foodcont.2007.05.006

Nhan DT, Wille M, De Schryver P, Defoirdt T, Bossier P, Sorgeloos P (2010) The effect of poly β-hydroxybutyrate on larviculture of the giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture 302: 76-81. doi: 10.1016/j.aquaculture.2010.02.011.

NOAA Fisheries. https://www.fisheries.noaa.gov/topic/aquaculture

Pernthaler J (2005) Predation on prokaryotes in the water column and its ecological implications. Nature Reviews Microbiology 3: 537. doi: 10.1038/nrmicro1180.

Pulkkinen K, Suomalainen L-R, Read AF, Ebert D, Rintamäki P, Valtonen ET (2010) Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. Proceedings of the Royal Society B: Biological Sciences 277: 593-600. doi:10.1098/rspb.2009.1659

Raffic Ali SS, Ambasankar K, Nandakumar S, Praveena PE, Syamadayal J (2016) Effect of dietary prebiotic inulin on growth, body composition and gut microbiota of Asian seabass (*Lates calcarifer*). Animal Feed Science and Technology 217: 87-94. doi.org/10.1016/j.anifeedsci.2016.04.011.

Ribeiro SC, Coelho MC, Todorov SD, Franco BDGM, Dapkevicius MLE, Silva CCG (2014) Technological properties of bacteriocin-producing lactic acid bacteria isolated from Pico cheese an artisanal cow's milk cheese. Journal of Applied Microbiology 116: 573-585. doi: 10.1111/jam.12388.

Ringø E, Gatesoupe F-J (1998) Lactic acid bacteria in fish: a review. Aquaculture 160: 177-203. doi: 10.1016/S0044-8486(97)00299-8.

Roca I, Akova M, Baquero F ... Tacconelli E, Wellington E, Vila J (2015) The global threat of antimicrobial resistance: science for intervention. New Microbes and New Infections 6: 22-29. doi: 10.1016/j.nmni.2015.02.007.

Romero J, Ringø E, Daniel LM (2014). The Gut Microbiota of Fish. In book: Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics, Edition: 1, Chapter: 4, Publisher: John Wiley & Sons, Ltd, Chichester, UK., Editors: eds Merrifield D and Ringø E. doi: 10.1002/9781118897263.ch4.

Rud I, Kolarevic J, Holan AB, Berget I, Calabrese S, Terjesen BF (2017) Deep-sequencing of the bacterial microbiota in commercial-scale recirculating and semi-closed aquaculture systems for Atlantic salmon post-smolt production. Aquacultural Engineering. doi:10.1016/j.aquaeng.2016.10.003

Salvesen I, Skjermo J, Vadstein O (1999) Growth of turbot (*Scophthalmus maximus L.*) during first feeding in relation to the proportion of r/K-strategists in the bacterial community of the rearing water. *Aquaculture* 175 (3–4): 337-350. doi:10.1016/S0044-8486(99)00110-6.

Santos WM, de Brito TS, Prado SdA, de Oliveira CG, De Paula AC, de Melo DC, Ribeiro PA (2016) Cinnamon (*Cinnamomum* sp.) inclusion in diets for Nile tilapia submitted to acute hypoxic stress. Fish & Shellfish Immunology 54: 551-555.

Schreier HJ, Mirzoyan N, Saito K (2010) Microbial diversity of biological filters in recirculating aquaculture systems. Current Opinion in Biotechnology 21 (3): 318-325. doi: 10.1016/j.copbio.2010.03.011.

Schryver P, Defoirdt T, Boon N, Verstraete W, Bossier P (2012) 15 - Managing the microbiota in aquaculture systems for disease prevention and control A2 - Austin, B (ed) Infectious Disease in Aquaculture, Prevention and Control. p. 394-418. Woodhead Publishing. ISBN 978-0-85709-016-4

Schryver P, Vadstein O (2014) Ecological theory as a foundation to control pathogenic invasion in aquaculture. The ISME Journal 8: 2360–2368. doi:10.1038/ismej.2014.84

Shanthi Mari LS, Jagruthi C, Anbazahan SM, Yogeshwari G, Thirumurugan R, Arockiaraj J, Mariappan P, Balasundaram C, Harikrishnan R (2014) Protective effect of chitin and chitosan enriched diets on immunity and disease resistance in *Cirrhina mrigala* against *Aphanomyces invadans*. Fish & Shellfish Immunology 39(2): 378-385. doi: 10.1016/j.fsi.2014.05.027

Sharifuzzaman SM, Austin B (2017) Probiotics for disease control in aquaculture. Diagnosis and Control of Diseases of Fish and Shellfish. John Wiley & Sons, Ltd. 189-222. doi: 10.1002/9781119152125.ch8.

Sheikha AF, Montet D (2014) How to Determine the Geographical Origin of Seafood? Critical Reviews in Food Science and Nutrition 56 (2): 306-317. doi: 10.1080/10408398.2012.745478

Skjermo J, Salvesen I, Øie G, Olsen Y, Vadstein O (1997) Microbially matured water: a technique for selection of a non-opportunistic bacterial flora in water that may improve performance of marine larvae. Aquaculture International 5(1): 13–28.

Stecher B, Hardt WD (2008) The role of microbiota in infectious disease. Trends in Microbiology 16:107–114. doi: doi: 10.1016/j.tim.2007.12.008.

Sugita H, Nakamura H, Shimada T (2005) Microbial communities associated with filter materials in recirculating aquaculture systems of freshwater fish. Aquaculture 243: 403-409. doi: 10.1016/j.aquaculture.2004.09.028.

Summerfelt ST, Sharrer MJ, Tsukuda SM, Gearheart M (2009) Process requirements for achieving full-flow disinfection of recirculating water using ozonation and UV irradiation. Aquacultural Engineering 40, 17–27. doi: 10.1016/j.aquaeng.2008.10.002.

Takao Y, Nagasaki K, Mise K, Okuno T, Honda D (2005) Isolation and Characterization of a Novel Single-Stranded RNA Virus Infectious to a Marine Fungoid Protist, *Schizochytrium* sp. (Thraustochytriaceae, Labyrinthulea). Applied Environmental Microbiology 71 (8): 4516-4522. doi: 10.1128/AEM.71.8.4516-4522.2005.

Tal Y, Schreier HJ, Sowers KR, Stubblefield JD, Place AR, Zohara Y (2009) Environmentally sustainable land-based marine aquaculture. Aquaculture 286 (1–2), 28-35. doi:10.1016/j.aquaculture.2008.08.043.

Taoka Y, Maeda H, Jo J-Y, Kim S-M, Park S-I, Yoshikawa T, Sakata T (2006a) Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*. Fisheries Science 72: 755-766. doi: 10.1111/j.1444-2906.2006.01215.x

Taoka Y, Maeda H, Jo JY, Jeon MJ, Bai SC, Lee WJ, Yuge K, Koshio S (2006b) Growth, stress tolerance and non-specific immune response of Japanese

flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. Fisheries Science 72(2): 310-321. doi: 10.1111/j.1444-2906.2006.01152.x

Tarnecki AM, Burgos FA, Ray CL, Arias CR (2017) Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. Journal of Applied Microbiology 123: 2-17. doi: 10.1111/jam.13415.

Torrecillas S, Makol A, Caballero MJ, Montero D, Dhanasiri AKS, Sweetman J, Izquierdo M (2012) Effects on mortality and stress response in European sea bass, *Dicentrarchus labrax* (L.), fed mannan oligosaccharides (MOS) after *Vibrio anguillarum* exposure. Journal of Fish Diseases 35: 591-602. doi: 10.1111/j.1365-2761.2012.01384.x.

Torto-Alalibo T, Tian M, Gajendran K, Waugh ME, van West P, Kamoun S (2005) Expressed sequence tags from the oomycete fish pathogen *Saprolegnia parasitica* reveal putative virulence factors. BMC Microbiology 5: 46. doi: 10.1186/1471-2180-5-46.

Tovar D, Zambonino J, Cahu C, Gatesoupe FJ, Vázquez-Juárez R, Lésel R (2002) Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 204: 113-123. doi: 10.1016/S0044-8486(01)00650-0.

Trivedi B (2012) Microbiome: The surface brigade. Nature 492: S60-S61. doi:10.1038/492S60a.

Uddin MN, Al-Harbi AH (2004) Seasonal Variation of Bacterial Flora in Ponds in Saudi Arabia Used for Tilapia Aquaculture. Journal of Applied Aquaculture, Vol. 16(1/2). doi: 10.1300/J028v16n01_04

Vadstein O (1993) A strategy to obtain microbial control during larval development of marine fish. Fish Farmig Technology 69-75.

Valladão GMR, Gallani SU, Pilarski F (2015) Phytotherapy as an alternative for treating fish disease. Journal of Veterinary, Pharmacology and Therapeutics 38: 417-428. doi: 10.1111/jvp.12202

Van De Wiele T, Boon N, Possemiers S, Jacobs H, Verstraete W (2007) Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. Journal of Applied Microbiology 102: 452-460. doi: 10.1111/j.1365-2672.2006.03084.x.

Verdegem MCJ, Bosma RH, Verreth JAJ (2006) Reducing water use for animal production through aquaculture. International Journal of Water Resources Development, 22(1):101-113. doi: 10.1080/07900620500405544.

Vine NG, Leukes WD, Kaiser H, Daya S, Baxter J, Hecht T (2004) Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. Journal of Fish Diseases 27: 319-326. doi: 10.1111/j.1365-2761.2004.00542.x.

Walasek M, Grzegorczyk A, Malm A, Skalicka-Woźniak K (2015) Bioactivity-guided isolation of antimicrobial coumarins from *Heracleum mantegazzianum*. Sommier & Levier (Apiaceae) fruits by high-performance counter-current chromatography. Food Chemistry 186: 133-138. doi: 10.1016/j.foodchem.2015.02.011.

Wang AR, Chao Ran C, Ringø E, Zhou ZG (2017) Progress in fish gastrointestinal microbiota research. Reviews in Aquaculture 0, 1–15. doi: 10.1111/raq.12191.

WHO. Report of a WHO consultation with participation of FAO. Geneva. 29-31 May 1995. WHO/FNU/FOS/95.7 Geneva, WHO. Pp. 45] In: Almeida A, Cunha A, Santos L, Salvador S, Gomes A. 2009. Evaluation of the impact of two aquaculture systems on communities of the estuarine system Ria de Aveiro. In: Current Research Topics in Applied Microbiology and Microbial Biotechnology: Proceedings of the II International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld2007) Ed Mendez-Vilas A. World Scientific, 788p ISBN 9812837558, 9789812837554.

Wong S, Rawls JF (2012) Intestinal microbiota composition in fishes is influenced by host ecology and environment. Molecular Ecology 21: 3100-3102. doi: doi.org/10.1111/j.1365-294X.2012.05646.x.

Xue S, Xu W, Wei J, Sun J (2017) Impact of environmental bacterial communities on fish health in marine recirculating aquaculture systems. *Veterinary Microbiology* 203, 34–39. doi: 10.1016/j.vetmic.2017.01.034.

Yamin G, Zilberg D, Levy G, van Rijn J (2017) The protective effect of humic-rich substances from monogenean parasites infecting the guppy (*Poecilia reticulata*). Aquaculture 479: 487-489. doi: 10.1016/j.aquaculture.2017.06.022.

Yip W, Knowler D, Haider WG, Trenholm R (2017)Valuing the Willingness-to-Pay for Sustainable Seafood: Integrated Multitrophic versus Closed Containment Aquaculture. Canadian Journal Of Agricultural Economics-Revue Canadienne D Agroeconomie 65 (1):93-117. doi: 10.1111/cjag.12102.

Yuan XC, Yin KD, Harrison P, He L, Xu J (2011) Variations in apparent oxygen utilization and effects of P addition on bacterial respiration in subtropical Hong Kong waters. Estuaries and Coasts, 34: 536–543. doi: 10.1007/s12237-010-9329-7.

Zhang S, Liu N, Liang W, Han Q, Zhang W, Li C (2017) Quorum sensing-disrupting coumarin suppressing virulence phenotypes in *Vibrio splendidus*. Applied Microbiology and Biotechnology 101: 3371-3378. doi: 10.1007/s00253-016-8009-3.

Chapter II

Chapter II

Characterization of bacterioplankton communities from a hatchery recirculating aquaculture system (RAS) for juvenile sole (*Solea senegalensis*) production

Duarte LN[1], Coelho FJRC[1], Oliveira V[1], Cleary DFR[1], Martins P[1], Gomes NCM[1]

[1]Department of Biology & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

Abstract

There is a growing consensus that future technological developments of aquaculture systems should account for the structure and function of microbial communities in the whole system and not only in fish guts. In this study, we aimed to investigate the composition of bacterioplankton communities of a hatchery recirculating aquaculture system (RAS) used for the production of Senegalese sole (*Solea senegalensis*) juveniles. To this end, we used a 16S rRNA gene based denaturing gradient gel electrophoresis (DGGE) and pyrosequencing analyses to characterize the bacterioplankton communities of the RAS and its supply water. Overall the most abundant orders were Alteromonadales, Rhodobacterales, Oceanospirillales, Vibrionales, Flavobacteriales, Lactobacillales, Thiotrichales, Burkholderiales and Bdellovibrionales. Although we found a clear distinction between the RAS and the supply water bacterioplankton communities, most of the abundant OTUs (≥50 sequences) in the hatchery RAS were also present in the supply water. These included OTUs related to Pseudoalteromonas genus and the Roseobacter clade, which are known to comprise bacterial members with activity against Vibrio fish pathogens. Overall, in contrast to previous findings for sole grow-out RAS, our results suggest that the supply water may influence the

bacterioplankton community structure of sole hatchery RAS. Further studies are needed to investigate the effect of aquaculture practices on RAS bacterioplankton communities and identification of the key drivers of their structure and diversity.

Introduction

The world population is expected to reach approximately 9.7 billion in 2050 (FAO 2016). As population increases, so will the demand for food, which will have to increase by 70% by 2050 (FAO's Director-General on How to Feed the World in 2050, 2009). The increase in demand will require substantial technological advances in food production. At present, aquaculture is undergoing rapid technological development and is emerging as a major food production sector. The demand for higher sustainability, reduced production costs and food safety has continuously driven the development of new and innovative aquaculture systems. Technologies such as recirculating aquaculture systems (RAS) with shallow raceway systems (SRS) allow more controlled and cost-effective production conditions, while having a reduced environmental impact. RAS is an advanced approach that reuses water in the production system with mechanical and biological filters (Bregnballe, 2015). SRS contribute for an optimized hydrodynamic performance over common raceways, allowing a lower water level and plug-flow pattern that enables high fish stocking densities, improving overall productivity (Labatut and Olivares, 2004). RAS technology with shallow raceways continuously processes and recycles water, reducing water pump requirements while maintaining optimal environmental conditions for fish production (Labatut and Olivares, 2004). However, the utilization of high fish densities during production may result in more rapid and severe disease outbreaks (Pulkkinen et al., 2010). In fact, currently, there is a growing understanding that improvements in the prevention and management of disease

outbreaks requires a deeper knowledge of the ecology of microbial communities in aquaculture systems. Outbreaks of parasitic, bacterial, and fungal diseases are among the most important limiting factors for the success of aquaculture production, leading to high mortality rates and important economic losses (Valladao et al., 2015). For example, the production of Senegalese sole (*Solea senegalensis*), a species of considerable commercial value, is strongly limited by its sensitivity to infectious diseases such as pasteurellosis (caused by *Photobacterium damselae* subsp *piscicida*), vibriosis (caused by various species of the genus Vibrio, especially *Vibrio anguillarum*) and flexibacteriose (caused by *Tenacibaculum maritimum*) (Howell et al., 2009). However, despite the deleterious effects of fish pathogens, the aquaculture water microbiome is essential for maintaining water quality (nutrient recycling) and fish health during intensive fish production (Tal et al., 2009; Blancheton et al., 2013). For example, nitrogen and phosphorus are recycled through the activity of heterotrophic decomposers (Moriarty, 1997). The presence of beneficial microbes was also shown to reduce colony-forming units (CFU) of pathogenic bacterial species (Ramachandran, 2016). Naturally occurring or introduced beneficial bacteria (probiotics) may contribute to improve water quality, inhibit the development of fish pathogens, improve the fish immune system and promote the balance of the fish bacterial flora (Blancheton et al., 2013; Martins et al., 2013; Kesarcodi-Watson et al., 2008).

In previous studies, we showed that *S. senegalensis* appears to influence the bacterial communities in a grow-out RAS and that, despite the presence of several potential fish pathogens, no diseased fish were observed during the study period. Our findings indicated that the water in grow-out RAS was dominated by naturally occurring beneficial microbes (antagonistic populations), which may have played an important role in suppressing the development of putative pathogens (Martins et al., 2013; Martins et al., 2015). However, we could not determine if such a trend would also be detectable in RAS systems used for

production of juvenile specimens (hatchery), which are supplied with seawater from a different collection point. Here, we aimed to investigate bacterioplankton community composition and diversity in the water of a commercial hatchery operating a RAS for the production of sole (*Solea senegalensis*) juveniles and compare results with those previously recorded for sole grow-out RAS (Martins et al. 2013). We also evaluated our results in light of the putative function of bacterioplankton populations in the hatchery RAS.

Material and Methods

Study site and Experimental design

Fieldwork was conducted in October 2013 in a RAS at a hatchery employing SRS for juvenile Senegalese sole with a capacity to produce more than 1 million juveniles per year that are stocked from hatching until they reach approximately 40 g. The fish hatchery employed water recirculation at a renewal rate of <5% of total system volume per day. Briefly, the water supply reservoir (Sup) is filled with seawater pumped through an inlet pipe from the ocean and is ozonized in a tank connected to a protein skimmer (Ozo) before entering the pre-production reservoir (Pre) (hatchery containing juvenile sole weighing approximately 4 g and densities with about 3.7 kg/m^2). Water from Pre is recycled by passing through a sedimentation tank (Sed) where mechanical filtration is also carried out. After mechanical filtration, water flows to a biofilter tank (Bio) for biological filtration and is subsequently pumped back to Ozo where it reenters the system. A simplified scheme of the system is shown in Figure 1. Water samples for bacterial community analysis and chemical characterization were collected in triplicate from all 5 different compartments (Sup, Ozo, Pre, Sed and Bio).

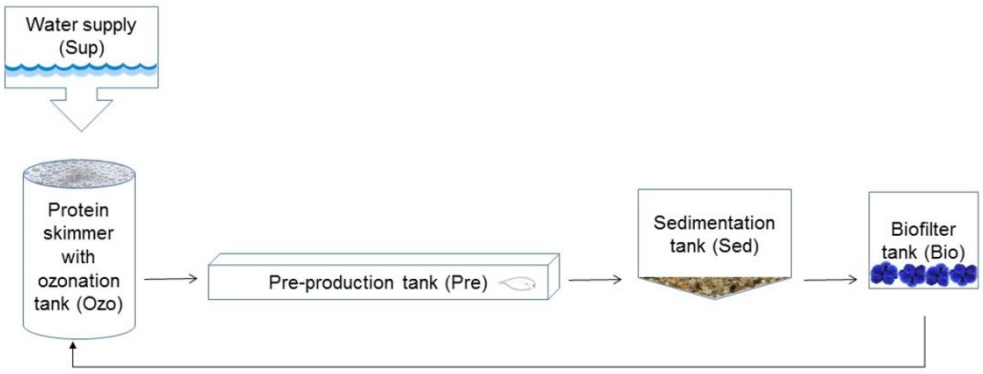

Figure II-1 - Schematic representation of the hatchery Recirculated Aquaculture System (RAS) surveyed in the present study. System components: Sup - water supply, Ozo - ozonation tank, Pre - pre-production tank, Sed - sedimentation tank and Bio - biofilter tank. Adapted from Martins et al. (2013).

Water chemistry analysis and bacterial communities

Chemical analysis

Ammonium (NH_4^+), nitrites (NO_2^-) and nitrates (NO_3^-) were determined for each water sample collected following the NP 730, EPA 300.1 and NP EN 26777 methods, respectively. Bromide (Br-) was determined according to EPA Method 300.1. Total organic carbon analysis (TOC) in the water was performed according to the European Norm 1484. Conventional physicochemical parameters, namely, temperature, pH, dissolved oxygen (DO) and salinity were also measured.

DNA analysis

Water samples were transported to the laboratory and immediately processed for DNA extraction. Briefly, 250 ml of water were filtered through 0.2μm pore size polycarbonate membranes (Poretics, Livermore, CA, USA) and total DNA was extracted from each filter using the E.Z.N.A. Soil DNA Extraction kit (Omega Bio-Tek, USA) according to the manufacturer's instructions. Bacterial community composition was compared among samples using DGGE fingerprinting in combination with a more-in-depth barcoded pyrosequencing analysis of composite samples (Cleary et al., 2012). Amplified 16S rRNA gene fragments suitable for bacterial DGGE fingerprints of total microbial community DNA samples were obtained using a nested approach following Gomes et al. (2008). In the first PCR, amplicons of the bacterial 16S rRNA gene were obtained using bacteria specific primers 27F and 1494R (21 PCR cycles) (Gomes et al. 2001). For DGGE analyses, the second PCR (21 PCR cycles) used the primers 968GC - 1378R (Nübel et al. 1996), with a GC clamp attached to the 5' end to prevent complete melting of double-stranded DNA during DGGE. DGGE was performed on a DCode Universal Mutation Detection System (Bio-Rad, Hercules, CA, USA), in 1x Trisacetate-EDTA (TAE) with a denaturing gradient ranging from 40% to 58% (100% denaturant contains 7 M urea and 40% formamide) and performed at 58 °C at 160 V during 16 hours onto 8% (w/v) polyacrylamide gels. DGGE gels were silver stained as described by Byun et al. (2009), except for the stop solution that was replaced by a Na_2CO_3 0.75% solution. The image was acquired using an Epson perfection V700 Photo Scanner. Digitalized DGGE gels were analysed with the software package GelCompar (version 4.0; Applied Maths), as described by Smalla et al. (2001). Briefly, both band position and intensity were processed in a spreadsheet. The data matrix of relative abundance (band positions and their corresponding intensities) per sample was $\log_{10}$ (x +1) transformed, and a distance matrix was constructed

using the Bray-Curtis similarity coefficient with the vegdist() function in the vegan package (Oksanen, 2011) in R (version 3.1.1; http://www.r-project.org/). Variation in bacterial composition among compartments was visually assessed with principal coordinates analysis (PCO) using the cmdscale() function in R using the Bray-Curtis distance matrix as input.

For compositional analysis, DNA from the three replicates of each compartment were pooled to obtain one DNA library per compartment. The V3-V4 regions of the 16S rRNA gene were amplified using barcoded fusion primers V3 Forward (5´ -ACTCCTACGGGAGGCAG-3') and V4 Reverse (5´ -TACNVRRGTHTCTAATYC-3') (Wang and Qian, 2009). The amplified fragments were purified (Agencourt Ampure beads, Agencourt Bioscience Corporation, MA, USA) and then sequenced using a Roche 454 FLX Titanium pyrosequencer (Brandford, CT, USA) following manufacturer's guidelines. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater,TX, USA).

The barcoded pyrosequencing libraries were processed using the QIIME (Quantitative Insights Into Microbial Ecology; (Caporaso et al. 2010)) software package (http://qiime.org; accessed 15/03/2014) according to published recommendations (Kuczynski et al., 2011) and following previously described methods (Cleary et al., 2015; Coelho et al., 2015), with the exception of the OTU picking step (97% threshold), where the UPARSE (Edgar, 2013) clustering method and chimera check were used, and the most recent Greengenes database (ftp://greengenes.microbio.me/greengenes_release/gg_13_5/gg_13_8_otus.tar.gz) for OTU picking and taxonomic assignment. Full details about the UPARSE steps can be found in Cleary et al. (2015). Finally, the make_otu_table.py script was used to produce an OTU by sample table containing the abundance and taxonomic assignment of all OTUs. After removal of non-bacteria, chloroplasts and mitochondria sequences, this table was uploaded to R software (version 3.1.1; http://www.r-project.org/) for statistical computing and graphics.

Rarefaction curves were made for each sampling compartment using a self-written function in R (Gomes et al., 2010). Variation in OTU composition was visualized using principal coordinates analysis (PCO) with the cmdscale() function in R. Variation in the relative abundance of the most abundant bacterial taxa was assessed using barplot graphs. In addition to this, OTUs taxonomically classified into genera known to be fish pathogens were selected and representative sequences compared with those available in GenBank. We used BLAST search (GenBank ® Nucleotide Databases Searched http:// www.ncbi.nlm.nih.gov/) to obtain the closest relatives of selected OTUs (pathogens and abundant taxa, i.e., number of sequences ≥ 50). Sequences were, furthermore, aligned using ClustalW and a phylogenetic tree was constructed in Mega7 (http://www.megasoftware.net/) using the Maximum Composite Likelihood method with a gamma distribution (five categories) and 1000 bootstraps to compute evolutionary distances. The iTOL v3 (http://itol.embl.de/) server was used to annotate the phylogenetic tree (Letunic and Bork, 2016). DNA sequences generated in this study have been submitted to the NCBI SRA (Accession number SRP095444).

Results and Discussion

The physicochemical characteristics of the water in each compartment are summarized in Table 1. The most notable differences were between Sup and the hatchery RAS compartments. There was a slight increase in pH and fairly low levels of nutrients in the Sup compartment when compared to RAS compartments (Table 1).

Table II-1 - Physico-chemical parameters in the pre-production RAS for each sampling point.

	Temperature °C	pH	DO mg/L	Salinity	Ammonium $mgNH_4^+/L$	Nitrite $mgNO_2^-/L$	Nitrate $mgNO_3./L$	Bromide mgBr/L	TOC mg/L
Sup	19.1	7.95 ± 0.03	7.82	35	0.57 ± 0.51	< 1.00 *	0.97 ± 0.87	0.00	1.30 ± 0.10
Pre	20.2	7.18 ± 0.00	16.86	35	0.90 ± 0.00	4.40 ± 0.00	19.40 ± 0.69	0.06	4.67 ± 1.15
Sed	20.3	7.23 ± 0.02	9.77	35	0.60 ± 0.53	4.50 ± 0.00	19.20 ± 0.36	0.07	4.67 ± 1.15
Bio	20.3	7.30 ± 0.03	7.90	35	0.73 ± 0.06	4.63 ± 0.06	19.93 ± 0.40	0.07	4.00 ± 0.00
Ozo	20.3	7.33 ± 0.00	20.00	35	0.67 ± 0.06	4.43 ± 0.11	20.03 ± 0.32	0.09	4.00 ± 0.00

Sup - water supply, Ozo - ozonation tank, Bio - biofilter tank, Pre - pre-production (hatchery) tank and Sed - sedimentation tank.
* concentration below the limit of quantification

DO concentration ranged from 7.82 mg/L in Sup to 20 mg/L in Ozo. Ammonia concentration was lowest in Sup (0.57±0.51 mg/L) and highest in Pre (0.90mg/L). Nitrite and nitrate concentrations were lower in Sup (<1 and 0.97±0.87 mg/L, respectively) when compared to RAS compartments, (average of 4.49±0.10 mg NO_2/L and 19.64±0.40 $mgNO_3$/L). We did not detect bromide in the Sup compartment and its concentration was stable in the hatchery system (average of 0.07±0.01 mg/L). TOC concentration was lower in Sup (1.3±0.10 mg/L) than in the other compartments (average of 4.33±0.78 mg/L). Overall, the concentration of nutrients in the sole hatchery was much lower than in the sole grow-out RAS characterized in our previous study (Martins et al, 2013). Such a difference in nutrient levels may be expected, as juvenile fish are grown to adulthood in the grow-out RAS and, therefore, the system is exposed to higher loads of non-eaten feed and fish excretion.

The DGGE analysis of bacterioplankton communities showed that, despite the young age of fishes and their relatively short period in the tanks (45 days), there was a significant separation between supply water and RAS compartments (adonis; $F_{4,14}$ = 2.831, R^2 =0.531, P=0.003) (Figure 2). The communities of RAS compartments defined by DGGE also tended to cluster together (S1 Figure). The in depth pyrosequencing analysis of these communities

yielded a total of 14451 sequences that varied between 1858 in Sed to 4336 in the Ozo compartment. To examine changes in bacterial richness, rarefaction curves were generated for all compartments (Figure 3). Controlling for sampling size (n=1700), OTU richness in the Sup compartment was 35.79±1.02. In the aquaculture tanks, richness was lowest in Sed (69.84±1.74) and highest in Ozo (92.88±4.82). The high diversity detected in Ozo may be due to an important fraction of dead microorganisms that accumulate in this compartment naturally derived from supply water and fish and feed waste from Pre tank and from the bacteria that proliferate in the biofilter. The introduction of ozone into a recirculation system is used to inactivate fish pathogens, remove accumulated organic residues and nitrite (NSW Government, 2016). Ozonation has been showed to kill or inactivate fish pathogens and total heterotrophic bacterial loading (Kasai et al., 2002; Powell et al., 2015). The effectiveness of ozone treatment, however, depends on ozone concentration, duration of ozone exposure, pathogen loads and levels of organic matter (NSW Government, 2016); microorganisms able to persist following ozone treatment may again enter and grow in the system. DNA based analyses performed in this study, however, cannot provide any information on cell viability. Therefore, we cannot provide any information about the efficiency of ozone treatment on bacterial cell viability.

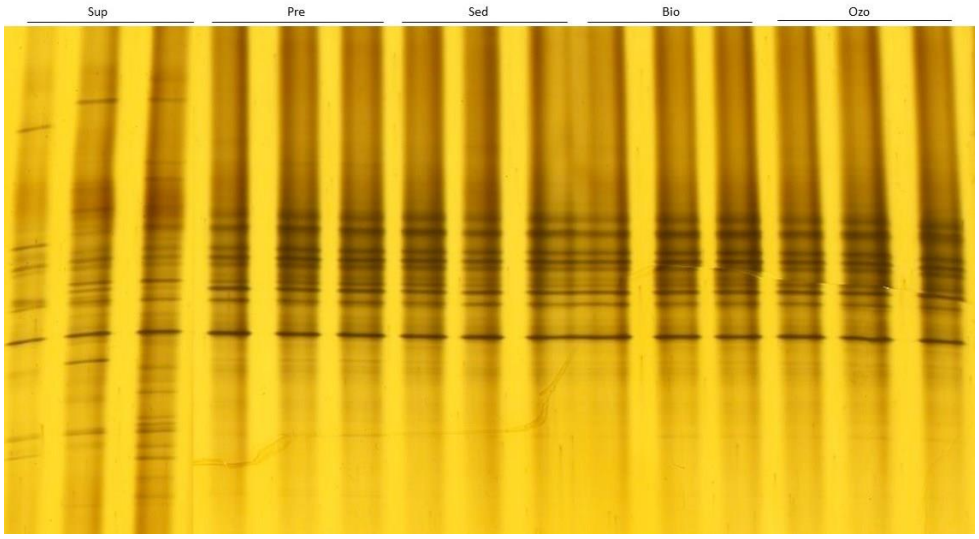

Figure II - S1 - DGGE profiles of 16S rRNA gene amplified from total community DNA extracted from three replicates of water supply (Sup), ozonation tank (Ozo), biofilter tank (Bio), pre-production (hatchery) tank (Pre) and sedimentation tank (Sed)

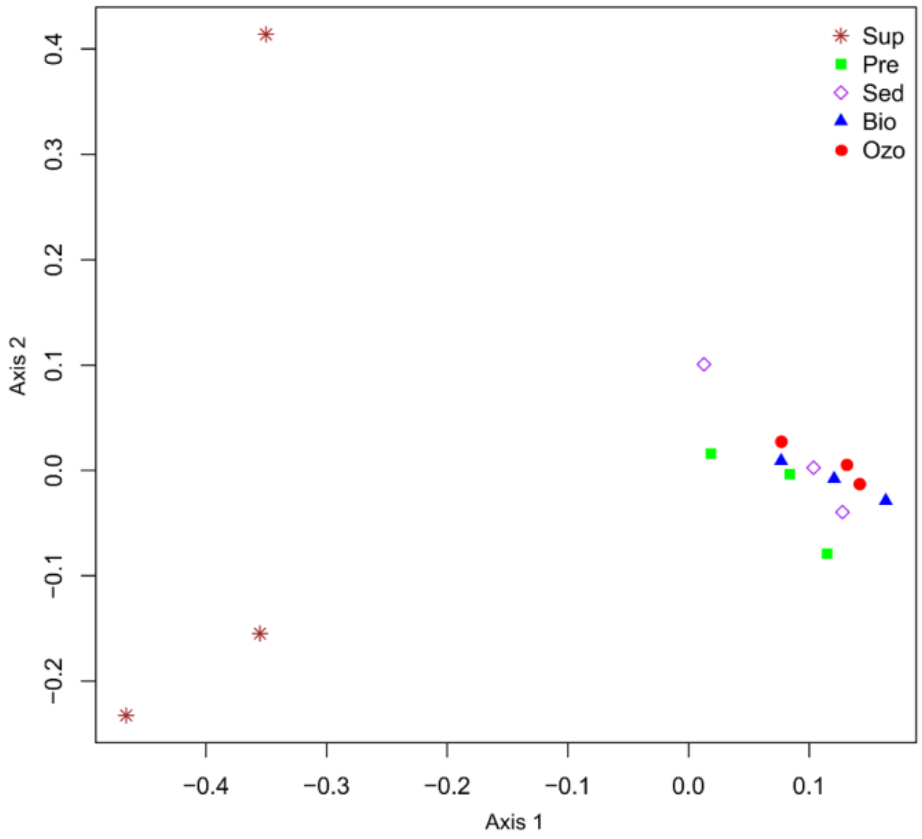

Figure II-2 - Principal Coordinates Analysis (PCO) of bacterial DGGE profiles. The first two explanatory axes are shown. Sup - water supply, Ozo - ozonation tank, Bio - biofilter tank, Pre - pre-production (hatchery) tank and Sed - sedimentation tank.

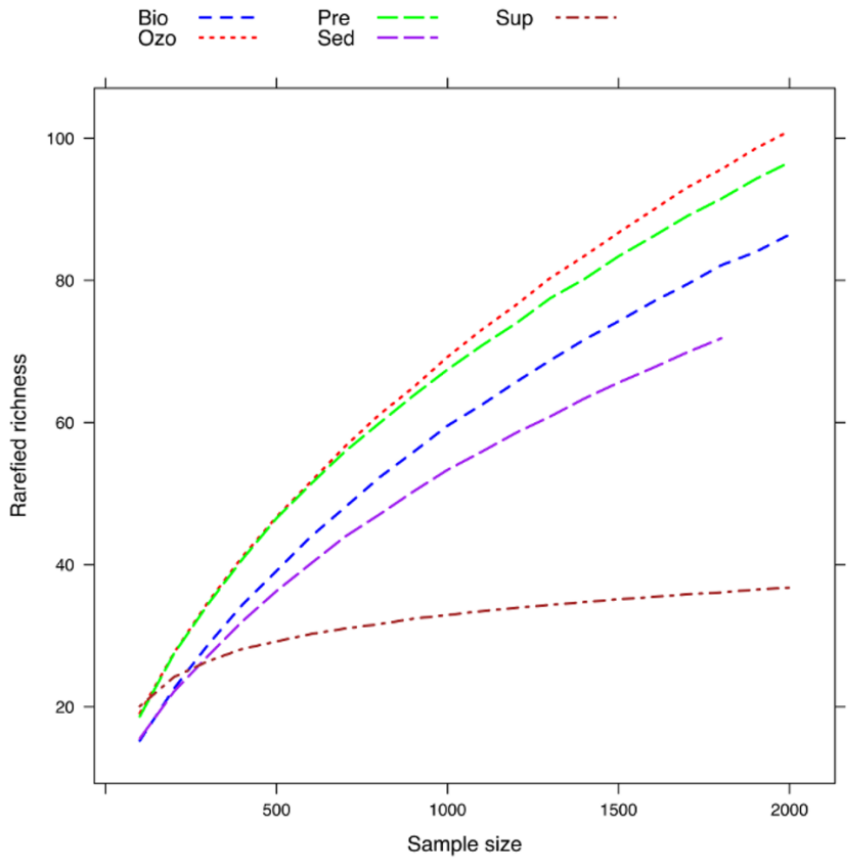

Figure II- 3 - Rarefied OTU richness in all sampling compartments. Sup- water supply, Ozo - ozonation tank, Pre - pre-production tank, Sed - sedimentation tank and Bio - biofilter tank.

In line with the DGGE and richness analysis, the PCO ordination of OTU composition showed marked differences between supply water and RAS compartments (Figure 4). Along the first PCO axis, the Sup compartment separated from RAS compartments with a range of dominant OTUs shared by all compartments. These results indicate that, despite the fact that the bacterioplankton communities in the supply water were clearly distinct from RAS tanks, several dominant bacterial communities in the hatchery tanks were originally introduced in the system through the supply water. This finding is in contrast

with the results obtained for sole grow-out RAS (Martins et al, 2013), where only few bacterial OTUs were found to be dominant in the water supply and fish tanks. Probably, due to the early life stage development of the fish in this study, gut microbes released to the environment via feces may have had lower influence on hatchery water bacterioplankton than in grow-out RAS. However, no fish gut samples were taken during this experiment, which hamper our ability to evaluate the contribution of fish microbiome to the hatchery bacterioplankton composition (and vice versa). Nevertheless, in line with this hypothesis, Giatsis et al (2015) showed that variations in gut bacterial community composition during Nile tilapia larvae (*Oreochromis niloticus*, Linnaeus) development were highly correlated with shifts in the bacterioplankton communities. Providing evidences that intestinal microbiota of the fish juveniles may share more similarities with their respective water bacterial communities.

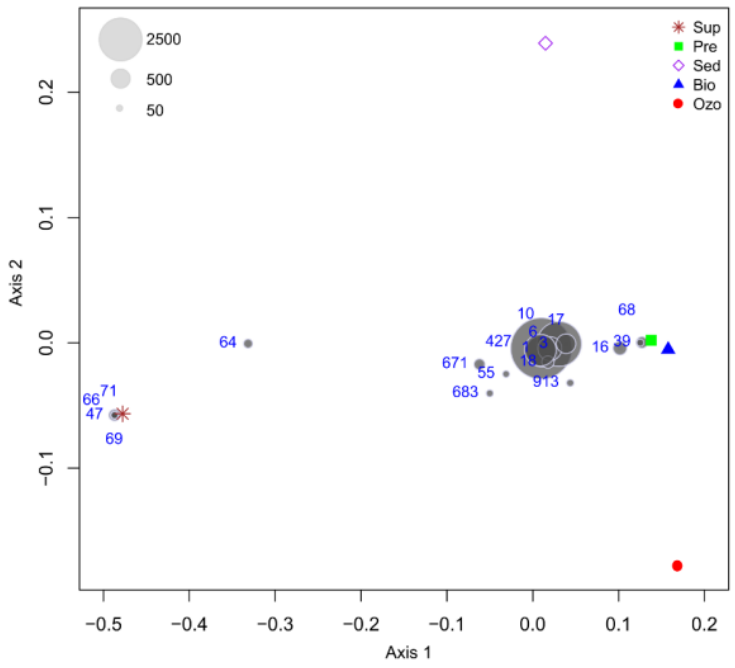

Figure II- 4 - Ordination showing the first two axes of the Principal Coordinates Analysis (PCO) of bacterial OTU composition. The light gray symbols represent most abundant OTUs (≥50 sequences) with symbol size representing their abundance in the entire data set. Sup- water supply, Ozo - ozonation tank, Pre - pre-production tank, Sed - sedimentation tank and Bio - biofilter tank.

In this study, we used the RDP classification to obtain taxonomic information about the most abundant OTUs (≥50 sequence reads - Figure 5) and phylogenetic analyses to identify ecotypes related to these OTUs in different RAS compartments (Figure 6, Table S1). This approach allowed us to better understand the composition and putative ecological role of the dominant bacterial populations in the RAS bacterioplankton.

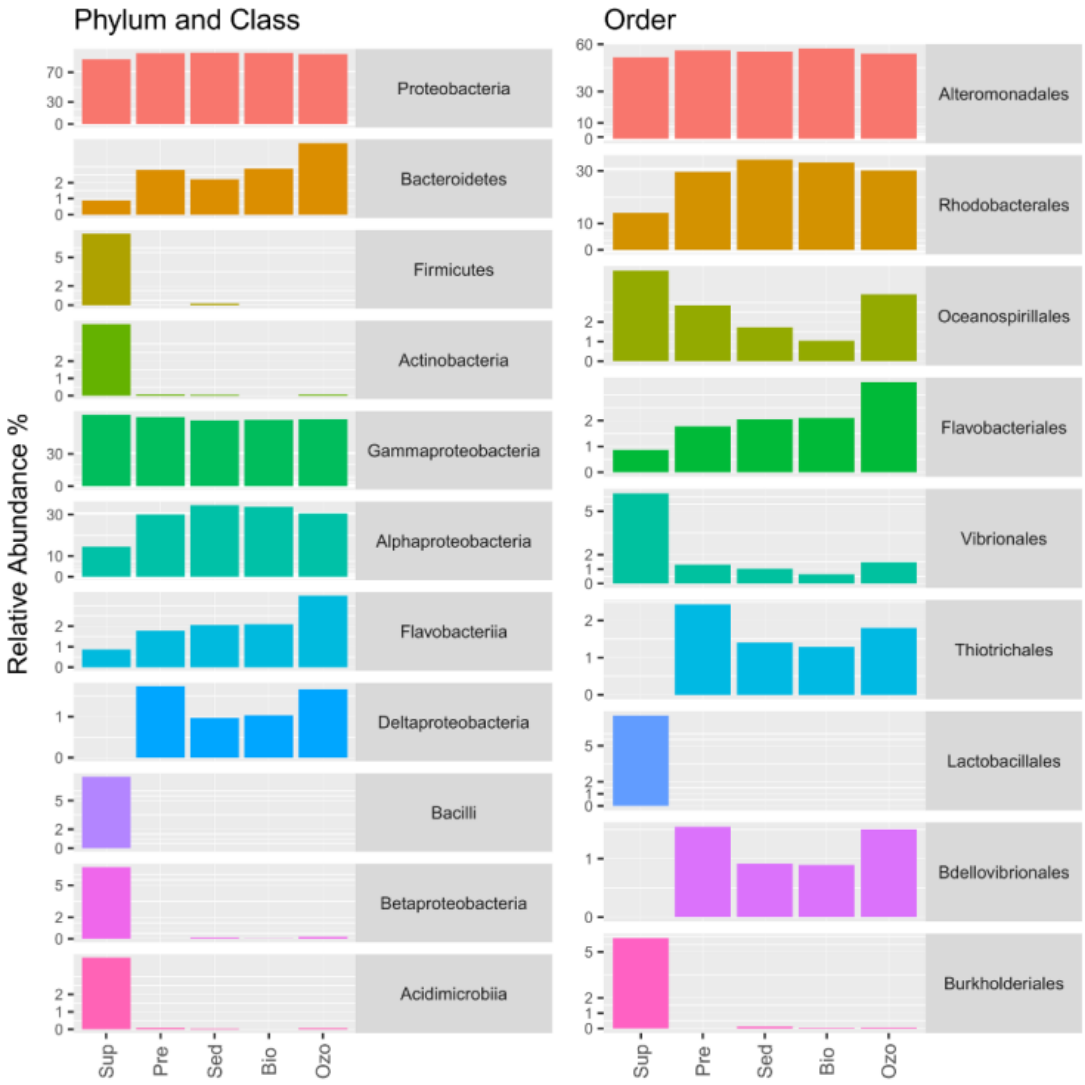

Figure II -5 - Relative abundance of the most dominant bacterial groups (4 phyla, 7 classes, 9 orders) in each sampling compartment.

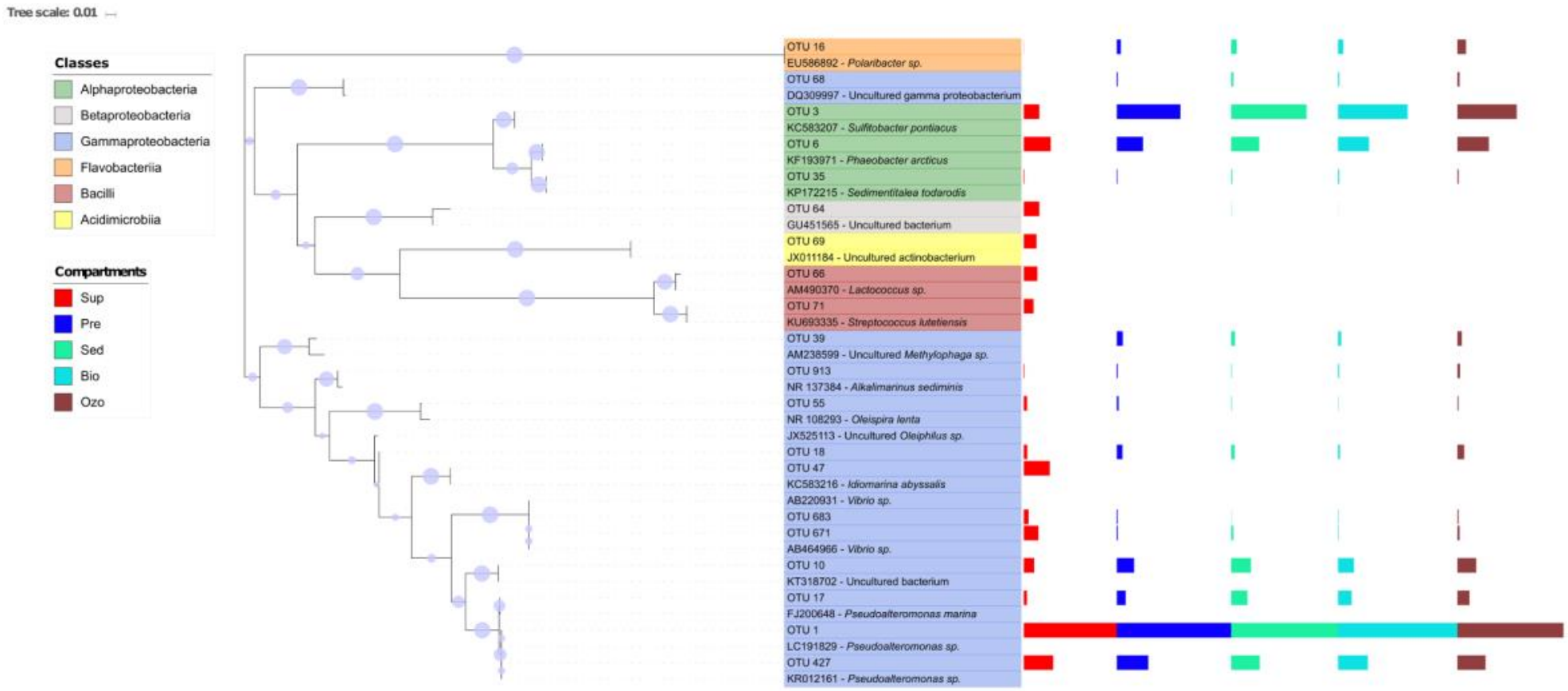

Figure II-6 - Phylogenetic tree of the most abundant OTUs (≥ 50 sequences) and their closest relatives in the sole hatchery including their closest relatives and GenBank accession numbers. The bar plots indicate the abundance of each OTU; with each compartment aligned with the maximum value of the previous compartment. Node confidence (1000 bootstrap replicates) higher than 50% is shown with symbol size (○) scaled to reflect support levels. Sup - supply water, Ozo - ozonation tank, Pre - pre-production tank, Sed - sedimentation tank and Bio - biofilter tank.

The overall taxonomic analyses showed that Proteobacteria was the most abundant bacterial phylum in all RAS compartments (average relative abundance 94.60±4.10%), followed by Bacteroidetes (average relative abundance 2.65±1.30%) (Figure 5). The phyla Firmicutes and Actinobacteria were more abundant in the supply water (7.50% and 4.13%, respectively) than in the hatchery RAS (0.04% and 0.05%, respectively). The most abundant orders detected in this study were Alteromonadales (54.98±2.16%), Rhodobacterales (28.22±8.17%), Oceanospirillales (2.73±1.41%), Vibrionales (2.14±2.32%), Flavobacteriales (2.05±0.94%), Lactobacillales (1.50±3.35%), Thiotrichales (1.38±0.89%), Burkholderiales (1.22±2.62%) and Bdellovibrionales (0.97±0.62%) (Figure 5). Only 1.79±0.28% OTUs remained unclassified at the order level. Interestingly, the most abundant orders detected in the hatchery (Alteromonadales, Rhodobacterales, Oceanospirillales, Vibrionales, Flavobacteriales and Thiotrichales) were also the most abundant groups in our previous study on sole grow-out RAS (Martins et al., 2013). In both studies, Alteromonadales was by far the most abundant order in the bacterioplankton. This order comprises copiotroph bacteria with wide distribution in marine environments (Bowman and McMeekin, 2005). In line with the higher concentration of nitrate in the hatchery tanks, previous studies suggest that Alteromonadales have a relevant environmental role in the uptake of nitrate in marine environments (Wawrik et al., 2012). Probably, members of this order were enriched in the RAS due to high nutrient inputs from fish feed and fish exudates during intensive fish production. Most of the OTUs assigned within the Alteromonadales belonged to the Pseudoalteromonas genus (47.39±4.44%). Members of this genus include a large and cosmopolitan group of marine bacteria that are usually found in association with marine eukaryotes (Emami et al., 2016). The genus Pseudoalteromonas contains numerous marine species that synthesize biologically active molecules and produce anti-bacterial products (Holmstrom and Kjelleberg, 1999). They have also been shown to exhibit specific activity

against *Vibrio* spp. in aquaculture systems (Uchida et al., 1997; Kesarcodi-Watson et al., 2012; Rodrigues et al., 2015; Skjermo et al., 2015; Wesseling et al., 2015) and previous studies propose that members of this genus may comprise valuable biocontrol strains for application in aquaculture (Holmstrom and Kjelleberg, 1999; Richards et al., 2017).

In similarity to our previous study (Martins et al., 2013), a much higher abundance of Rhodobacterales was observed in the RAS compartments. Members of this order are well known for their metabolic versatility (e.g. photosynthesis, CO_2 and nitrogen fixation and sulfur oxidation) which can significantly contribute for nutrient cycling and improve water quality (Gupta and Mok, 2007; Voget et al., 2015). Previous studies suggest that the Roseobacter clade (Rhodobacterales) may play an important role against the development of fish pathogens in aquaculture systems (Hjelm et al., 2004; Martins et al., 2018). For example, D'Alvise et al. (2010) showed that a Vibrio-antagonistic Roseobacter (producer of tropodithietic acid, TDA), was able to suppress the development of the fish pathogen *Vibrio anguillarum* in model systems simulating a fish larval aquaculture environment. The most abundant OTUs assigned to Rhodobacterales (OTUs 3, 6 and 35) were present in all RAS compartments including supply water (Figure 6). However, OTU 3, the second most abundant OTU in the aquaculture system, was more abundant inside the hatchery tanks (21.40±2.40%) than in the supply water (4.95%). This OTU was similar to an organism previously identified as *Sulfitobacter pontiacus* (sequence similarity 100%, Table S1). This species is specialized in sulfite oxidation and was detected for the first time in the Black Sea (Sorokin, 1995). Several studies have reported on the occurrence of Sulfidobacteria in aquacultures, or nearby water, highlighting the potential importance of members of this genus in the sulfur cycling within these systems (Bourne et al., 2004; McIntosh et al., 2008). Interestingly, Sharifah and Eguchi (2012) showed that, in the presence of the phytoplankton *Nannochloropsis oculata*, *Sulfitobacter* sp. showed inhibitory activity towards *Vibrio anguillarum*. OTUs 6 and 35 showed

close phylogenetic relationship to *Phaeobacter arcticus* and *Sedimentitalea todarodis* and were abundant in the supply water (8.60% and 0.24%, respectively) and in the hatchery tanks (average relative abundance 9.34±0.78% and 0.36±0.06%, respectively) (Figure 6 and Table S1, sequence similarities 100%). These bacteria belong to the Roseobacter clade and have been shown to be active against *Vibrio* spp. (Michaud et al., 2009; D'Alvise et al., 2012). These species are described as psychotrophic bacteria previously isolated from Artic marine sediment (*P. arcticus*) and from the intestinal tract of a squid (*S. todarodis*) (Zhang et al., 2008; Kim et al,. 2016). Curiously, a previous study also detected these bacteria as abundant members of a marine RAS (Lee et al., 2016), however, there is no previous information about their putative role in aquaculture systems.

The variation in the relative abundance of the phylum Firmicutes was mainly related to OTUs 66 and 71 that were similar to organisms retrieved from a fish farm and from fish gut (sequences similarity = 99% and 100%, respectively) (Figure 6 and Table S1). OTU 66 was assigned to the genus Lactococcus and OTU 71 to the genus Streptococcus. Members of these genera belong to the lactic acid bacteria group and are often found in fish guts (Merrifield and Carnevali, 2014). Their ability to produce bacteriocins may inhibit pathogenic bacteria colonization in the gastrointestinal tract (Merrifield and Carnevali, 2014). In this study, they were only detected in the supply water (OTU 66 – 4.37% and OTU 71 – 3.12%), which could indicate limited ability to colonize the water of hatchery RAS. The Actinobacteria phylum was dominated by OTU 69 (close related to uncultured actinobacterium from seawater) and was only detected in the supply water (Figure 6 and Table S1).

Table II-S1 - List of most abundant bacterial OTUs across the dataset (≥50 sequences) and their relative abundance in each hatchery RAS compartment. The table includes the taxonomic assignment, the closest related organisms using BLAST, their accession numbers, the sequence similarity of the closest matches with our representative OTU sequences (Seq. Sim.) and the source of these organisms

OTU	SUP	PRE	SED	BIO	OZO	PHYLUM	CLASS	ORDER	FAMILY	GENUS	GI	SEQ	SOURCE
1	29.70	36.55	34.18	38.08	33.90	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	LC191829	100	seawater, Japan
3	4.95	20.34	24.11	22.18	18.96	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Sulfitobacter	KC583207	100	seawater from Rio Grande Rise Region, South Atlantic
6	8.60	8.41	8.99	9.87	10.08	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Phaeobacter	KF193971	100	gastrointestinal tract of cultured olive flounder (*Paralichthys olivaceus*), South Korea
10	3.32	5.56	6.35	5.05	6.02	Proteobacteria	Gammaproteobacteria	Alteromonadales	Unclassified	Unclassified	KT318702	100	ocean water from northeastern Gulf of Mexico, USA (after exposure to oil and dispersant)
16	0.05	1.31	1.78	1.62	2.77	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Polaribacter	EU586892	100	RAS seawater, Portugal
17	1.01	2.86	5.22	4.30	3.87	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	FJ200648	100	seawater from Turkey: eastern Aegean Sea
18	1.11	1.81	1.13	0.56	2.19	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oleiphilaceae	uncultured Oleiphilus	JX525113	99	surface water from the Southern ocean (iron fertilization experiment), India
35	0.24	0.27	0.38	0.36	0.42	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Sedimentitalea	KP172215	100	Japanese flying squid (*Todarodes pacificus),* South Korea
39	0.00	1.93	1.24	0.98	1.45	Proteobacteria	Gammaproteobacteria	Thiotrichales	Piscirickettsiaceae	uncultured Methylophaga	AM238599	96	sea water enriched with dimethylsulfide, Atlantic Ocean: Pensacola Pier
47	8.31	0.00	0.00	0.00	0.00	Proteobacteria	Gammaproteobacteria	Alteromonadales	Idiomarinaceae	Idiomarina	KC583216	100	Oceanic water from Rio Grande Rise Region, Atlantic Ocean, Brazil
55	1.06	0.62	0.22	0.11	0.35	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Oleispira	NR108293	99	Coastal seawater from Yellow Sea
64	4.95	0.00	0.11	0.03	0.00	Proteobacteria	Betaproteobacteria	Burkholderiales	Unclassified	Unclassified	GU451565	97	macroalgal surface
66	4.37	0.00	0.00	0.00	0.00	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Lactococcus	AM490370	99	fish and the fish farm environment
68	0.00	0.42	0.70	0.36	0.71	Proteobacteria	Gammaproteobacteria	Unclassified	Unclassified	Unclassified	DQ309997	99	associated with the red seaweed, *Delisea pulchra. Australia*
69	4.08	0.00	0.00	0.00	0.00	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	Unclassified	JX011184	100	Marine sample, China
71	3.12	0.00	0.00	0.00	0.00	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	KU693335	100	Lactic acid bacteria from fish gut, Thailand
427	9.42	10.07	9.15	9.48	8.99	Proteobacteria	Gammaproteobacteria	Alteromonadales	Pseudoalteromonadaceae	Pseudoalteromonas	KR012161	100	Deep-sea sediment from the Pacific Ocean
671	4.66	0.42	0.70	0.17	0.71	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Aliivibrio	AB464966	100	Senegal sole (*Solea senegalensis*) intestine, Spain: Cadiz
683	1.54	0.35	0.11	0.14	0.39	Proteobacteria	Gammaproteobacteria	Vibrionales	Vibrionaceae	Unclassified	AB220931	100	Intestine of japanese flounder (Paralichthys olivaceus), Japan
913	0.24	0.39	0.22	0.33	0.85	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oleiphilaceae	Alkalimarinus	NR_137384	99	Marine sediment, China: Weihai coast

Members of the Oceanospirillales order were present in all compartments (including supply water) and were mainly represented by OTUs 18 (1.35±0.6%) and 55 (0.47±0.38%) (Figure 6, Table S1). Members of the Oceanospirillales are often described as halotolerant and halophilic, aerobic, microaerophilic or facultative chemoorganotrophs and are widespread in marine environments (Garrity et al., 2005). OTU 55 was similar to an organism previous identified as *Oleispira lenta* (sequence similarity = 99%) (Figure 6, Table S1). Members of this species have been described as mesophilic hydrocarbon degraders (Wang et al., 2012). A recent study reported on the dominance of an OTU assigned to the genus *Oleispira* associated with salmon skin (Lokesh and Kiron, 2016), which could indicate their ability to colonize fish skin.

Flavobacteriales were more abundant in the hatchery RAS (2.32±0.77%) than in the supply water (0.89%). Flavobacteriales was mainly represented by OTU 16 (average relative abundance of 1.87±0.63% inside the RAS), which was assigned to the Flavobacteriaceae family and was similar to an organism previously identified as *Polaribacter* sp. (Figure 6, Table S1, sequence similarity 100%) obtained from aquaculture water. This OTU was also present in the supply water but showed much higher abundance in the RAS tanks. Members of this genus have been found in RAS compartments in different geographic locations (Martins et al. 2013; Matos et al., 2011; Rud et al., 2017). Rud et al. (2017), specifically, found a higher abundance of *Polaribacter* sp. in tank biofilms when compared to water in a RAS system. Members of the Flavobacteriales are known for their ability to form biofilms on surfaces in marine environments (Nocker et al., 2004; Webster and Negri, 2006). Such an ability may improve their capacity to colonize the RAS environment.

The orders Thiotrichales and Bdellovibrionales were only detected inside the hatchery RAS (average relative abundance of 1.72±0.51% and 1.21±0.36%, respectively) (Figure 5). The order Thiotrichales was mainly represented by OTU 39 (average relative abundance of

1.40±0.40% inside of the RAS), which was assigned to the Piscirickettsiaceae family (Table S1). This OTU was only 96% similar to its closest relative in the GenBank database, an uncultured *Methylophaga* sp. (Figure 6). Members of this genus have been described as aerobic methylotrophs involved in denitrification in marine environments, seawater aquariums and aquacultures (Bourne et al., 2004; Auclair et al., 2010). The high abundance of Bdellovibrionales (Figure 5) is also noteworthy, since members of this order prey exclusively on other bacteria including potential fish pathogens (Schoeffield and Williams, 1990; Welsh et al., 2016). Bdellovibrionales and similar organisms (BALOs) isolated from fish ponds have been shown to reduce disease incidence caused by the fish pathogens *Aeromonas hydrophila* and *Vibrio alginolyticus* (Kandel et al., 2014 and references therein). The orders Vibrionales and Burkholderiales were both moderately abundant in the supply water (6.25% and 5.91%, respectively), however, their abundance was reduced in the hatchery compartments (1.11±0.36% and 0.05±0.04%, respectively). Overall, our results showed that, with exception of OTUs 39 and 68, all dominant OTUs detected in the hatchery tanks were originally present in the supply water before entering the RAS.

In order to evaluate the composition of potential fish pathogens in the hatchery RAS we also specifically searched for OTUs related to bacterial genera which are often comprising known fish pathogens (S2 Table). OTUs 49 and 198 (0.17±0.14% and 0.02±0.02%, respectively) were assigned to *Vibrio ichthyoenteri* (S2 Table, sequence similarities of 100%). This species was previously reported to be a pathogen of flounder (*Paralichthys olivaceus*) (Ishimaru et al., 1996). Likewise, OTU 70 (relative abundance 0.06±0.03%) was similar to a microorganism identified as *Vibrio anguillarum* (S2 Table), a pathogen that causes vibriosis in approximately 50 species of fish (Actis et al., 2011). However, it should be noted that despite the 16S rRNA gene can be used for classification of Vibrio at genus level, this gene may not have enough resolution for Vibrio at the species level (Thompson et al., 2005; Martins et al., 2013) and

must be carefully considered when used to interpret the diversity of *Vibrio* communities. Interestingly, despite the relatively high abundance of members of the Vibrionales order in the supply water, only a few members of this genus found favorable conditions inside the hatchery RAS (Figure 5). OTUs 59 and 290 were assigned to *Serratia marcescens* and *Francisella philomiragia*, respectively, two known fish pathogens (S2 Table, sequences similarities of 100%). These OTUs occurred in low abundance inside the RAS and only *F. philomiragia* was detected in the fish compartment (Pre). This species is an opportunistic waterborne pathogen able to cause disease in a range of animals, including finfish species (Birkbeck et al., 2011; Kreitmann et al., 2015). However, in line with our previous study (Martins et al., 2013) and despite the presence of potential pathogens, no diseased fish were detected in the hatchery RAS during this study. Although in the present study we did not show a direct causal relationship between the activity of putative antagonistic bacterial populations and pathogen development, it is reasonable to assume that naturally occurring probiotic bacteria may play a role in the suppression of potential fish pathogens in the hatchery RAS.

Table II-S2 - Values of relative abundance (%) of potential fish pathogens detected in water supply (Sup), sole pre-production tank (Pre), sedimentation tank (Sed), biofilter tank (Bio) and ozone tank (Ozo) and their closest relatives (accession number, classification and source)

OTU	*SUP*	*PRE*	*SED*	*BIO*	*OZO*	*CLASS*	*ORDER*	*FAMILY*	*GENBANK*	*GI*	*SEQ*	*SOURCE*
49	0.00	0.39	0.11	0.20	0.16	Gamma	Vibrionales	Vibrionaceae	*Vibrio ichthyoenteri*	AM181658	100	digestive tract of *Paralichthys olivaceus*
59	0.00	0.00	0.00	0.03	0.00	Gamma	Enterobacteriales	Enterobacteriaceae	*Serratia marcescens*	KT215434	100	freshwater
70	0.00	0.08	0.05	0.03	0.09	Gamma	Vibrionales	Vibrionaceae	*Vibrio anguillarum*	KR270138	100	gut of *Apostichopus japonicus*
198	0.00	0.04	0.00	0.03	0.05	Gamma	Vibrionales	Vibrionaceae	*Vibrio ichthyoenteri*	HG931133	100	cultured Sparus aurata
208	0.00	0.00	0.05	0.00	0.00	Gamma	Vibrionales	Vibrionaceae	*Vibrio s*p.	EU253597	100	Mediterranean Sea surface water
290	0.00	0.04	0.00	0.00	0.00	Gamma	Legionellales	Francisellaceae	*Francisella philomiragia*	EF364047	100	cultured Atlantic cod
544	0.00	0.00	0.00	0.03	0.02	Gamma	Vibrionales	Vibrionaceae	*Vibrio splendidus*	KF009796	100	Portugal seawater
671	4.66	0.42	0.70	0.17	0.71	Gamma	Vibrionales	Vibrionaceae	*Vibrio* sp.	AB464966	100	sole intestine
683	1.54	0.35	0.11	0.14	0.39	Gamma	Vibrionales	Vibrionaceae	*Vibrio* sp.	AB220931	100	coastal seawater
1140	0.00	0.00	0.00	0.03	0.00	Flavo	Flavobacteriales	Flavobacteriaceae	*Flavobacterium* sp.	KT284905	97	soil of rhizosphere seepweed

Conclusion

Exploring the potential of naturally occurring microorganisms as biocontrol agents in aquacultures is not a new concept (Salvesen et al., 1999; Hjelm et al., 2004; Michaud et al., 2009; Attramadal et al., 2014). The development of microbial management or modulation approaches should be based on a fundamental knowledge about the aquaculture microbiome. This study provides baseline information about the bacterioplankton community composition and diversity of a commercial hatchery RAS for the production of juvenile Senegalese sole. Our results showed that despite the differences in relative abundance, the most abundant orders detected in the hatchery RAS (Alteromonadales, Rhodobacterales, Oceanospirillales, Vibrionales and Flavobacteriales) were also the most abundant detected in the sole grow-out RAS characterized in our previous study (Martins et al., 2013). Curiously, in contrast to our findings for grow-out RAS, our results indicated that the bacterial assemblage of the supply water played an important role for the colonization of bacterial populations [e.g. *Pseudoalteromonas* sp., members of the Roseobacter clade (*Phaeobacter arcticus* and *Sedimentitalea todarodis*) and Sulfidobacteria] in the hatchery RAS. Most remarkable, here supply water seems to contribute for a strong colonization of *Pseudoalteromonas* sp. in the tanks, which in turn may play a role in suppressing the development of potential fish pathogens in the aquaculture system (Uchida et al., 1997; Holmstrom and Kjelleberg, 1999; Kesarcodi-Watson et al., 2012; Rodrigues et al., 2015; Skjermo et al., 2015; Wesseling et al., 2015). Our findings suggest that the bacterial composition of the water supply may influence the bacterioplankton community structure of sole hatchery RAS. However, taking in consideration the results obtained for sole grow-out RAS (Martins et al., 2013), the contribution of water supply to shape RAS bacterioplankton communities may vary between different RAS. Further studies are needed to

investigate the effect of reared fish species and aquaculture practices for identification of the key drivers of RAS bacterioplankton communities.

Acknowledgments

We thank Ricardo Calado for helpful discussions. Letícia N. Duarte was supported by the CNPq, National Council of Scientific and Technological Development - Brazil and CAPES on CsF Program (BEX 1287-13-3). Francisco J.R.C. Coelho was supported by a postdoctoral scholarship (SFRH/BPD/92366/2013) financed by the Portuguese Foundation for Science and Technology (FCT). This study has been carried out with the financial support of the project AQUASAFE (PROMAR 31-03-05-FEP-0016) funded by PROMAR (Operational Programme for Fisheries) and AquaHeal (MAR-02.01.01-FEAMP-0031) funded by MAR2020, Operational Programme for the European Maritime and Fisheries Fund (EMFF). We are also grateful for the financial support to CESAM (UID/AMB/50017), to FCT/MEC through national funds, and co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020.

References

Actis LA, Tolmasky ME, Crosa JH (2011) Vibriosis (Chapter 15). In: Woo PTK, Bruno DW (ed) Fish diseases and disorders, vol. 3: viral, bacterial, and fungal infections, 2nd ed. CABI International, pp 570–605. ISBN: 781845935542. doi:10.1079/9781845935542.0000.

Attramadal KJK, Truong TMH., Bakke I, Skjermo J, Olsen Y, Vadstein O (2014) RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. Aquaculture. doi:10.1016/j.aquaculture.2014.05.052

Auclair J, Lépine F, Parent S, Villemur R (2010) Dissimilatory reduction of nitrate in seawater by a Methylophaga strain containing two highly divergent narG sequences. The ISME Journal. doi:10.1038/ismej.2010.47.

Birkbeck TH, Feist SW, Verner-Jeffreys DW (2011) Francisella infections in fish and shellfish. Journal of Fish Diseases. doi: 10.1111/j.1365-2761.2010.01226.x.

Blancheton JP, Attramadal KJK, Michaud L, Roque D'orbcastel E, Vadstein O (2013) Insight into the bacterial population in aquaculture systems and its implication. Aquacultural Engineering. doi:10.1016/j.aquaeng.2012.11.009.

Bourne DG, Young N, Webster N, Payne M, Salmon M, Demel S, Hall M (2004) Microbial community dynamics in a larval aquaculture system of the tropical rock lobster, Panulirus ornatus. Aquaculture. doi:10.1016/j.aquaculture.2004.08.047.

Bowman JP, McMeekin TA (2005) Order X. Alteromonadales ord. nov. Bergey's Manual of Systematic Bacteriology, Vol 2 (Brenner DJ Krieg NR, Stanley JT, Garrity GM , eds), pp. 443 –491. Springer, New York, NY

Bregnballe J (2015) A Guide to Recirculation Aquaculture - An introduction to the new environmentally friendly and highly productive closed fish farming systems. Food and Agriculture Organization of the United Nations (FAO) and EUROFISH International Organisation.

Byun SO, Fang Q, Zhou H, Hickford JGH (2009) An effective method for silver-staining DNA in large numbers of polyacrylamide gels. Analytical Biochemistry. doi: 10.1016/j.ab.2008.10.024.

Caporaso JG, Kuczynski J, Stombaugh J … Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nature Methods. doi:10.1038/nmeth.f.303.

Cleary DFR, Smalla K, Mendonça-Hagler L, Gomes NCM (2012) Assessment of variation in bacterial composition among microhabitats in a mangrove environment using DGGE and barcoded pyrosequencing. PLoS One. doi:10.1371/journal.pone.0029380.

Cleary DFR, Becking LE, Polónia ARM, Freitas RM, Gomes, NCM (2015) Composition and predicted functional ecology of mussel-associated bacteria in Indonesian marine lakes. Antonie van Leeuwenhoek. doi:10.1007/s10482-014-0375-1.

Coelho FJRC, Cleary DFR, Rocha RJM … Costa R, Pereira R, Gomes NCM (2015) Unraveling the interactive effects of climate change and oil contamination on laboratory simulated estuarine benthic communities. Global Change Biology. doi:10.1111/gcb.12801.

D'alvise PW, Melchiorsen J, Porsby CH, Nielsen KF, Gram L (2010) Inactivation of *Vibrio anguillarum* by attached and planktonic Roseobacter cells. Applied and Environmental Microbiology. doi 10.1128/AEM.02717-09

D'Alvise PW, Lillebø S, Prol-Garcia MJ, Wergeland HI, Nielsen KF, Bergh O, Gramet L (2012) *Phaeobacter gallaeciensis* reduces *Vibrio anguillarum* in cultures of microalgae and rotifers, and prevents vibriosis in cod larvae. PLoS One. doi:10.1371/journal.pone.0043996

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods. doi:10.1038/nmeth.2604.

Emami K, Nelson A, Hack E, Zhang J, Green DH, Caldwell GS, Mesbahi E (2016) MALDI-TOF mass spectrometry discriminates known species and marine environmental isolates of Pseudoalteromonas. Frontiers in Microbiology. doi:10.3389/fmicb.2016.00104.

FAO (2016) The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Food and Agriculture Organization of the United Nations. Rome. 200 pp. ISBN 978-92-5-109185-2.

FAO's Director-General on How to Feed the World in 2050 (2009) Population and Development Review. doi:10.1111/j.1728-4457.2009.00312.x.

Garrity GM, Bell JA, Lilburn T (2005) Class II. Betaproteobacteria class. nov. (Chapter) In: Bergey's Manual of Systematic Bacteriology (Book). Springer US. ISBN 978-0-387-29298-4. doi:10.1007/978-0-387-29298-4_2.

Giatsis C, Sipkema D, Smidt H, Heilig H, Benvenuti G, Verreth J, Verdegem M (2015) The impact of rearing environment on the development of gut microbiota in tilapia larvae. Scientific Reports 2:18206. doi: 10.1038/srep18206.

Gomes NCM, Heuer H, Schönfeld J, Costa R, Mendonça-Hagler L, Smalla K (2001) Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant and Soil. doi: 10.1023/A:1010350406708.

Gomes NCM, Borges LR, Paranhos R, Pinto FN, Mendonça-Hagler LCS, Smalla K (2008) Exploring the diversity of bacterial communities in sediments of urban mangrove forests. FEMS Microbiology Ecology. doi:10.1111/j.15746941.2008.00519.x.

Gomes NCM, Cleary DFR, Pinto FN, Egas C, Almeida A, Cunha A, Mendonça-Hagler LCS, Smalla K (2010) Taking Root: Enduring Effect of Rhizosphere Bacterial Colonization in Mangroves. Plos One. doi:10.1371/journal.pone.0014065.

Gupta RS, Mok A (2007) Phylogenomics and signature proteins for the alpha proteobacteria and its main groups. BMC Microbiology. doi: 10.1186/1471-2180-7-106.

Hjelm M, Bergh O, Riaza A, Nielsen J, Melchiorsen J, Jensen S, Duncan H, Ahrens P Birkbeck H, Gram L (2004) Selection and identification of autochthonous potential probiotic bacteria from turbot larvae (*Scophthalmus maximus*) rearing units. Systematic and Applied Microbiology. doi:10.1078/0723-2020-00256

Holmstrom C, Kjelleberg S (1999) Marine Pseudoalteromonas species are associated with higher organisms and produce biologically active extracellular agents. Fems Microbiology Ecology. doi: 10.1111/j.1574-6941.1999.tb00656.x

Howell B, Conceição L, Prickett R, Cañavate P, Mañanos E (2009) Sole farming: nearly there but not quite?! A report of the 4th workshop on the cultivation of soles. Aquaculture Europe 34: 24-27. In: Aquaculture Europe: Magazine of the European Aquaculture Society (EAS). ISSN 0773-6940.

Ishimaru K, Akagawa-Matsushita M, Muroga K (1996) *Vibrio ichthyoenteri* sp. nov., a pathogen of japanese flounder (*Paralichthys olivaceus*) larvae. International Journal Of Systematic Bacteriology, 46(1):155-159.

Kandel PP, Pasternak Z, van Rijn J, Nahum O, Jurkevitch E (2014) Abundance, diversity and seasonal dynamics of predatory bacteria in aquaculture zero discharge systems. FEMS Microbiology Ecology. doi: 10.1111/1574-6941.12342.

Kasai H, Yoshimizu M, Ezura Y (2002) Disinfection of water for aquaculture. Fisheries Science. ISSN 0919-9268.

Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L (2008) Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. Aquaculture. doi:10.1016/j.aquaculture.2007.11.019.

Kesarcodi-Watson A, Miner P, Nicolas JL, Robert R (2012) Protective effect of four potential probiotics against pathogen-challenge of the larvae of three bivalves: Pacific oyster (*Crassostrea gigas*), flat oyster (*Ostrea edulis*) and scallop (*Pecten maximus*). Aquaculture. doi:10.1016/j.aquaculture.2012.02.029.

Kim HS, Hyun DW, Lee JY, Kim PS, Whon TW, Kang W, Bae JW (2016) *Sedimentitalea todarodis* sp. nov., isolated from the intestinal tract of a Japanese flying squid.

International Journal of Systematic and Evolutionary Microbiology. doi: 10.1099/ijsem.0.001188

Kreitmann L, Terriou L, Launay D, Caspar Y, Courcol R, Maurin M, Lemaître N. (2015) Disseminated Infection Caused by *Francisella philomiragia*, France, 2014. Emerging Infectious Diseases. doi: 10.3201/eid2112.150615.

Kuczynski J, Stombaugh J, Walters WA, González A, Caporaso JG, Knight R (2011) Using QIIME to analyze 16S rRNA gene sequences from microbial communities In: Current Protocols in Bioinformatics, chapter 10. doi:10.1002/0471250953.bi1007s36.

Labatut RA, Olivares JF (2004) Culture of turbot (*Scophthalmus maximus*) juveniles using shallow raceways tanks and recirculation. Aquacultural Engineering. doi:10.1016/j.aquaeng.2004.05.008.

Lee DE, Lee J, Kim YM, Myeong JI, Kim KH (2016) Uncultured bacterial diversity in a seawater recirculating aquaculture system revealed by 16S rRNA gene amplicon sequencing. Journal of Microbiology. doi: 10.1007/s12275-016-5571-4

Letunic I, Bork P (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Research. doi:10.1093/nar/gkw290.

Lokesh J, Kiron V (2016) Transition from freshwater to seawater reshapes the skin-associated microbiota of Atlantic salmon. Scientific Reports. doi:10.1038/srep19707.

Martins P, Cleary DFR, Pires ACC, Rodrigues AM, Quintino V, Calado R, Gomes NCM (2013) Molecular analysis of bacterial communities and detection of potential pathogens in a recirculating aquaculture system for *Scophthalmus maximus* and *Solea senegalensis*. Plos One. doi: 10.1371/journal.pone.0080847.

Martins P, Navarro RVV, Coelho FJRC, Gomes NCM (2015) Development of a molecular methodology for fast detection of *Photobacterium damselae* subspecies in water samples. Aquaculture. doi.org/10.1016/j.aquaculture.2014.09.028.

Martins P, Coelho FJRC, Cleary DFR, Pires ACC, Marques B, Rodrigues AM, Quintino V, Gomes NCM (2018) Seasonal patterns of bacterioplankton in a semi-intensive European seabass (*Dicentrarchus labrax*) aquaculture system. Aquaculture. doi: 10.1016/j.aquaculture.2018.02.038

Matos A, Borges MT, Peixe C, Henriques I, Pereira CM, Castro PML (2011) A molecular and multivariate approach to the microbial community of a commercial shallow

raceway marine recirculation system operating with a Moving Bed Biofilter. Aquaculture Research. doi:10.1111/j.1365-2109.2010.02719.x.

McIntosh D, Ji B, Forward BS, Puvanendran V, Boyce D, Ritchie R (2008) Culture-independent characterization of the bacterial populations associated with cod (*Gadus morhua* L.) and live feed at an experimental hatchery facility using denaturing gradient gel electrophoresis. Aquaculture. doi:10.1016/j.aquaculture.2007.12.021

Merrifield DL, Carnevali O (2014) Probiotic modulation of the gut microbiota of fish, Chapter 8. In: Merrifield D, Ringø E (ed) Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics, First Edition. John Wiley & Sons, pp.185-222. ISBN: 978-0-470-67271-6.

Michaud L, Lo Giudice A, Troussellier M, Smedile F, Bruni V, Blancheton JP (2009) Phylogenetic characterization of the heterotrophic bacterial communities inhabiting a marine recirculating aquaculture system. Journal of Applied Microbiology. doi:10.1111/j.1365-2672.2009.04378.x.

Moriarty DJW (1997) The role of microorganisms in aquaculture ponds. Aquaculture. doi:10.1016/S0044-8486(96)01487-1.

Nocker A, Lepo JE, Snyder RA (2004) Influence of an oyster reef on development of the microbial heterotrophic community of an estuarine biofilm. Applied and Environmental Microbiology, 70(11):6834-45.

NSW Government, Department of Primary Industries. Ozone in recirculating aquaculture systems. Available in: http://www.dpi.nsw.gov.au. Accessed December 2016.

Nübel U, Engelen B, Felske A, Snaidr J, Wieshuber A, Amann RI, Ludwig W, Backhaus H (1996) Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. Journal of Bacteriology. doi:10.1128/jb.178.19.5636-5643.1996.

Oksanen J (2011) Vegan: ecological diversity. Available: http://cran.rproject.org/web/packages/vegan/vignettes/diversity-vegan.pdf. Accessed February 2014.

Powell A, Chingombe P, Lupatsch I, Shields RJ, Lloyd R (2015) The effect of ozone on water quality and survival of turbot (*Psetta maxima*) maintained in a recirculating aquaculture system. Aquacultural Engineering. doi:10.1016/j.aquaeng.2014.11.005.

Pulkkinen K, Suomalainen LR, Read AF, Ebert D, Rintamaki P, Valtonen ET (2010) Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. Proceedings of The Royal Society B. doi:10.1098/rspb.2009.1659.

Ramachandran K (2016) Beneficial microbes for the sustainable management of shrimp aquaculture. Guybro Chemicals Pvt Ltd, Corporate Office, (www.guybro.com). Available:
http://www.ctaquaculture.tn/index.php?id=45&L=3%2F%27&tx_ttnews%5Btt_news%5D=608&cHash=9f5e86bd53fbd4451e31c0f83e01dffa at 22/11/2016. Accessed: November 2016.

Richards GP, Watson MA, Needleman DS, Uknalis J, Boyd EF, Fay JP (2017) Mechanisms for *Pseudoalteromonas piscicida*-induced killing of vibrios and other bacterial pathogens. Applied and Environmental Microbiology. doi: 10.1128/AEM.00175-17

Rodrigues S, Paillard C, Dufour A, Bazire A (2015) Antibiofilm activity of the marine bacterium *Pseudoalteromonas* sp. 3J6 against *Vibrio tapetis*, the causative agent of Brown Ring Disease. Probiotics and Antimicrobial Proteins. doi:10.1007/s12602-014-9173-3.

Rud I, Kolarevic J, Holan AB, Berget I, Calabrese S, Terjesen BF (2017) Deep-sequencing of the bacterial microbiota in commercial-scale recirculating and semi-closed aquaculture systems for Atlantic salmon post-smolt production. Aquacultural Engineering. doi:10.1016/j.aquaeng.2016.10.003

Salvesen I, Skjermo J, Vadstein O (1999) Growth of turbot (*Scophthalmus maximus* L.) during first feeding in relation to the proportion of r/K-strategists in the bacterial community of the rearing water. Aquaculture. doi:10.1016/S0044-8486(99)00110-6.

Schoeffield AJ, Williams HN (1990) Efficiencies of recovery of Bdellovibrios from brackish- water environments by using various bacterial species as prey. Applied and Environmental Microbiology, 56, 230-236.

Sharifah EN, Eguchi M (2012) Mixed cultures of the phytoplankton *Nannochloropsis oculata* and the marine bacterium *Sulfitobacter* sp. RO3 inhibit the growth of virulent strains of the major fish pathogen Vibrio anguillarum. Aquaculture Science. doi: 10.11233/aquaculturesci.60.39

Skjermo J, Bakke I, Dahle SW, Vadstein O (2015) Probiotic strains introduced through live feed and rearing water have low colonizing success in developing Atlantic cod larvae. Aquaculture. doi: 10.1016/j.aquaculture.2014.12.027.

Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Applied and Environmental Microbiology. doi:10.1128/AEM.67.10.4742-4751.2001.

Sorokin DY (1995) *Sulfitobacter pontiacus* gen. nov., sp. nov.: a new heterotrophic bacterium from the Black Sea specialized on sulfite oxidation. Microbiology 64(3):295-305.

Tal Y, Schreier HJ, Sowers KR, Stubblefield JD, Place AR, Zohar Y (2009) Environmentally sustainable land-based marine aquaculture. Aquaculture. doi:10.1016/j.aquaculture.2008.08.043.

Thompson FL, Gevers D, Thompson CC, Dawyndt P, Naser S, Hoste B, Munn CB, Swings J (2005) Phylogeny and molecular identification of Vibrios on the basis of multilocus sequence analysis. Applied Environmental Microbiology. doi:10.1128/AEM.71.9.5107-5115.2005.

Uchida M, Nakata K, Maeda M (1997) Conversion of Ulva fronds to a hatchery diet for *Artemia nauplii* utilizing the degrading and attaching abilities of *Pseudoalteromonas espejiana*. Journal of Applied Phycology. doi: 10.1023/A:1007940005528

Valladao GMR, Gallani SU, Pilarski F (2015) Phytotherapy as an alternative for treating fish disease. Journal of Veterinary Pharmacology and Therapeutics. doi:10.1111/jvp.12202.

Voget S, Wemheuer B, Brinkhoff T … Billerbeck S, Daniel R, Simon M (2015) Adaptation of an abundant Roseobacter RCA organism to pelagic systems revealed by genomic and transcriptomic analyses. The ISME Journal. doi: 10.1038/ismej.2014.134

Wang Y, Qian PY (2009) Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One. doi: 10.1371/journal.pone.0007401.

Wang Y, Yu M, Austin B, Zhang X-H (2012) *Oleispira lenta* sp. nov., a novel marine bacterium isolated from Yellow sea coastal seawater in Qingdao, China. Antonie van Leeuwenhoek. doi: 10.1007/s10482-011-9693-8.

Wawrik B, Boling WB, Van Nostrand JD, Xie J, Zhou J, Bronk DA (2012) Assimilatory nitrate utilization by bacteria on the west Florida shelf as determined by stable isotope probing and functional microarray analysis. FEMS Microbiology Ecology. doi: 10.1111/j.1574-6941.2011.01226.x

Webster NS, Negri AP (2006) Site-specific variation in Antarctic marine biofilms established on artificial surfaces. Environmental Microbiology. doi: 10.1111/j.1462-2920.2006.01007.x

Welsh RM, Zaneveld JR, Rosales SM, Payet JP, Burkepile DE, Thurber RV (2016) Bacterial predation in a marine host-associated microbiome. The ISME journal. doi:10.1038/ismej.2015.219.

Wesseling W, Wittka S, Kroll S, Soltmann C, Kegler P, Kunzmann A, Riss HW, Lohmeyer M (2015) Functionalised ceramic spawning tiles with probiotic Pseudoalteromonas biofilms designed for clownfish aquaculture. Aquaculture. doi: 10.1016/j.aquaculture.2015.04.017

Zhang DC, Li HR, Xin YH, Liu HC, Chi ZM, Zhou PJ, Yu Y (2008) *Phaeobacter arcticus* sp. nov., a psychrophilic bacterium isolated from the Arctic. International Journal of Systematic and Evolution Microbiology. doi: 10.1099/ijs.0.65708-0

Chapter III

Chapter III

Bacterial and microeukaryotic plankton communities in a semi-intensive aquaculture system of sea bass (*Dicentrarchus labrax*): a seasonal survey

Duarte LN[1], Coelho FJRC[1], Cleary DFR[1], Bonifacio D[1], Martins P[1], Gomes NCM[1]

[1]Department of Biology & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal

Abstract

The importance of microbial diversity and their role in the maintenance of fish health in aquaculture systems has been increasingly recognized in recent years. However, there is still a major knowledge gap regarding the ecology, composition and dynamics of microbial plankton assemblages during fish production. In this study, we aimed to investigate the seasonal dynamics and potential interactions of bacterial and microeukaryotic plankton communities in a semi-intensive aquaculture for European sea bass (*Dicentrarchus labrax*) cultured together with low density of gilthead sea bream (*Sparus aurata*) over a one-year period (January/2014 – November/2014). While the most abundant bacterial classes were Gammaproteobacteria, Flavobacteriia and Alphaproteobacteria; microeukaryotic communities were dominated by Ochrophyta, Chlorophyta and Ciliophora groups. Temperature and salinity were identified as significant drivers of the overall microbial community composition, which varied congruently along the seasons. However, while the dominant (more abundant) groups of bacteria occurred in the warmest months, the dominant groups of microeukaryotes occurred in the coldest months. There was also an inverse relationship between abundances of grazers and bacterial OTUs. Overall, besides the potential effects of the abiotic parameters on the microbial plankton communities, the correlation between bacteria and microeukaryotic

populations observed here may be an indication of trophic and/or metabolic interdependence between these two domains. Future studies should focus on the underlying mechanisms of this interdependence for a better understand of the impact of microeukaryotic communities on aquaculture bacterioplankton structure and function. In addition, this knowledge could be of interest in the development of microbial management strategies for aquaculture systems.

Introduction

Finfish farming represents the major activity in the global aquaculture sector (FAO, 2017). These farms rely on different production methods; such as extensive, semi-intensive and intensive systems (Soliman and Yacout, 2016). Traditional extensive fish farming is practiced throughout Europe. This method consists in the maintenance of ponds (natural or artificial) for the development of target species. In traditional systems, lagoons are fertilized to stimulate aquatic vegetation and, consequently, increase the abundance of microorganisms and small invertebrates that form the base of the aquatic food pyramid. This promotes the development of the cultivated species at a higher density than that observed in natural ecosystems (DG Fisheries, 2017). In a semi-intensive system, farmed organisms are kept at higher densities than in extensive aquaculture (and less than intensive aquaculture). The semi-intensive aquaculture is interesting for small producers to increase their fish production and to improve family income without substantial investment (Edwards et al., 2000). This production method is increasingly becoming an important source of animal protein in some developed nations in Asia (Golden et al., 2017).

Aquaculture production, however, is currently facing several serious obstacles such as limitations associated to the use of natural resources (water and land), pollution of coastal

zones and significant losses in the fish farming industry due to disease outbreaks caused by known and newly emerging pathogens (Aly and Albutt, 2014; Yue and Wang, 2017). Diseases in aquaculture have led to the loss of several billions dollars per year (Yue and Wang, 2017). Semi-intensive aquacultures, furthermore, depend on tidal flow and are directly influenced by the environmental conditions. High temperatures and elevated nutrient concentrations for example, can increase the occurrence of phytoplankton blooms and influence the density of potential pathogens and virulence factor activation (Barg, 1992; Kinnula et al., 2017).

In recent years the concept of the active management of microbial communities as a means to decrease disease and optimize animal production is gaining strength (Schryver and Vadstein, 2014, Bruijin et al. 2018). Microorganisms occupy central roles in marine food webs and global biogeochemical processes. In aquaculture settings, besides having direct effects on fish health and quality, microbial communities also influence fundamental processes such as nutrient cycling and water purification (Tal et al., 2009; Rurangwa and Verdegem, 2015). However, fundamental baseline information concerning the microbial dynamics of these systems and how ecological interactions can be used to modulate microbial assemblages are still scarce. In a previous study, we have shown that potential fish pathogens and naturally occurring putative antagonistic bacterial groups are influenced by changes in environmental variables in aquaculture systems (Martins et al., 2018). Recently, we have shown that microeukaryotic plankton communities in turbot and sole recirculating aquaculture systems (RAS) were dominated by bacterial grazers and represented by a large fraction of unknown organisms whose taxonomy and function have yet to be determined (Boaventura et al., 2018). Our findings highlighted that the ecology of micro-eukaryotes in aquaculture systems are poorly understood, limiting our ability to understand their role in these systems. Interactions between different microbial domains are fundamental components of the food web and functioning of aquatic ecosystems (Faust and Raes, 2012; Fuhrman et al., 2015 and Hennessy

et al., 2017). For example, antagonistic interactions such as predator–prey interactions (Microeukaryotes and Bacteria) can play a crucial role in controlling pathogens in aquatic environments (Feichtmayer et al., 2017). Integrated analysis of several domains is, therefore, fundamental to further advance our understanding of the aquaculture microbiome structure and function. In this study, for the first time, we investigated the seasonal dynamics of bacterial and microeukaryotic plankton communities in a semi-intensive aquaculture used for raising European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) over a one-year period. These fish species are often reared together and are among the most important marine finfish reared in Europe (Oliva-Teles, 2000; Theodorou, 2002; Califano et al., 2017).

Material and methods

Study site, sampling and DNA extraction

This study was carried out in an European sea bass (*Dicentrarchus labrax*) semi-intensive aquaculture production co-cultured with sea bream (*Sparus aurata*) (10%), located in Aveiro, Portugal. The aquaculture is composed of earthen ponds, which receives natural water from the Ria de Aveiro estuarine system. Water samples were collected from three different tanks in the aquaculture system at six sampling events throughout the year of 2014: 15th January (winter), 11st March (end of winter), 5th May (spring), 8th July (beginning of summer), 16th September (end of summer) and 18th November (autumn). Tanks had very similar characteristics including the fish density and weight and the exact same date of introduction of juveniles (approximately 6g/fish introduced in June 2013). Water samples were transported to

the lab and immediately processed. For DNA extraction, 250 ml of water was filtered through a 0.2 μm pore polycarbonate membrane (Poretics, Livermore, CA, USA) and total DNA was extracted directly from each filter using the E.Z.N.A. Soil DNA Extraction kit (Omega Bio-Tek, USA) according to the manufacturer's instructions.

Several physicochemical parameters were measured in the tanks. Water samples were collected with a sterilized glass vessel, in triplicate, in the middle of each tank. They were kept at 4ºC until analysis. Levels of NH_3+NH_4, NO_2^-, NO_3^- and PO_4^{3-} were determined colorimetrically with a segmented flow analyzer (Skalar Sanplus), using the following methods: M461-318 (EPA 353.2), M155- 008R (EPA 350.1) and M503-555R (Standard Method 450-P I), respectively. Water was kept in acid (H_2SO_4) until analysis to total organic carbon (TOC) that was performed according to the European Norm 1484. Other parameters such as temperature, pH, salinity and dissolved oxygen (DO) were evaluated in surface water *in situ*.

Sequencing

DNA samples from all three tanks, obtained in each time point, were combined into one composite sample before sequencing. Therefore, one DNA library representing the aquaculture plankton microbiome was analyzed per sampling time (Jan, Mar, May, Jul, Sep, Nov). For bacterial community analysis, the V3-V4 regions of 16S rRNA gene were amplified using barcoded fusion primers V3 Forward (5´ -ACTCCTACGGGAGGCAG-3') and V4 Reverse (5´ -TACNVRRGTHTCTAATYC-3') (Wang and Qian, 2009). The amplified fragments were purified (Agencourt Ampure beads, Agencourt Bioscience Corporation, MA, USA) and then sequenced using a Roche 454 FLX Titanium pyrosequencer (Brandford, CT, USA) following manufacturer's guidelines. For microeukaryotic communities analysis, 18S

rDNA gene fragments were amplified using primers TAReuk454FWD1 (5'-CCAGCASCYGCGGTAATTCC-3') and TAReukREV3 (5'- ACTTTCGTTCTTGATYRA-3') (Stoeck et al., 2010). The amplified fragments were purified (Ampure XP beads, Beckman Coulter, Life Sciences, IN, USA) and sequenced on a MiSeq sequencing platform following standard Illumina protocols. Both sequencing were performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA).

Sequence analysis

Both barcoded libraries (bacterial and microeukaryotic) were processed using the Quantitative Insights Into Microbial Ecology (QIIME) software package (http://qiime.org; last checked 2017-01-20) according to the published recommendations (Kuczynski et al., 2011) and following previously described methods (Cleary et al., 2015; Coelho et al., 2015) with the exception of the OTU picking step, where the UPARSE (Edgar, 2013) clustering method and chimera check was used. Full details about the UPARSE were described elsewhere (Cleary et al., 2015). For bacteria, reference sequences of OTUs were assigned taxonomies using default arguments in the assign_taxonomy.py script in QIIME with the RDP method (Wang et al., 2007). The Greengenes database (ftp://greengenes.microbio.me/greengenes_release/ gg_13_5/gg_13_8_otus.tar.gz) was used for OTU picking and taxonomic assignment. For microeukaryotes, reference sequences of OTUs were assigned taxonomies using the assign_taxonomy.py with the uclust method with a confidence threshold of 0.8. The PR2 database (http://ssu-rrna.org/pr2) was used following PR2 taxonomic descriptors (structured using eight unique terms) (Guillou et al., 2013).

Statistical analysis

The make_otu_table.py script was used to produce two OTU-by-sample tables containing the abundance and taxonomic assignment of bacterial and microeukaryotic OTUs. After removal of unassigned and singleton OTUs, chloroplast and mitochondrial sequences, the tables were further analysed in R software (version 3.1.1; http://www.r-project.org) for statistical computing and graphics. Observed OTU richness was assessed using rarefaction analysis with a self-written function in R (Gomes et al., 2010). Shannon diversity indice was calculated with the diversity() function in vegan (Oksanen et al., 2016). Variation in OTU composition among sampling events was assessed with PCO (Principal Coordinates Analysis) ordination using the cmdscale() function in R and the Bray–Curtis distance matrix as input. Environmental parameters were then fit onto PCO ordinations of OTU composition using the envfit() function in vegan. Using the envfit() function, we also tested for significant relationships between these variables and OTU ordination using 999 permutations; all other arguments in the function were left as default. The procrustes() function in vegan was used to assess congruence among bacterial and microeukaryotic PCO ordinations. In addition to the procrustes() function, the protest() function in vegan was used to estimate the significance of the procrustes statistic. The number of permutations in the protest() function was set to 999. Pearson correlations between the most abundant bacterial orders and microeukaryotic divisions [$\log_e(x + 1)$ transformed] were computed using rcorr() from the Hmisc package (Harrel et al., 2016) and plotted using the corrplot R package (Wei and Simko, 2016). BLAST search (http://www.ncbi.nlm.nih.gov/) was used to obtain the closest relatives of the most abundant OTUs (≥ 100 sequences for bacteria and ≥ 200 sequences for microeukaryotes) using command line "blastn" tool with the -db argument set to nt (Zhang et al., 2000). We

used blastn to query representative sequences of selected taxa against the online NCBI nucleotide database. The DNA sequences generated in this study were submitted to the NCBI SRA (Accession number SRP095459).

Results

Environmental Data

During this study, water temperature varied from 13.23±0.35 in November to 20.30±0.40 in July (Table 1). The highest temperature was observed in July followed by September and May. The pH values were relatively constant throughout the year (from 7.26±0.18 in May to 7.93±0.12 in September). The highest values of salinity and TOC were registered in the end of summer (September) (37.33±0.58 and 6.87±7.04 mg l^{-1}, respectively). The lowest salinity values were registered in November (21.67± 2.08), while the lowest TOC values were registered in March (1.5±0.1 mg l^{-1}). Ammonia concentrations were lowest in March (0.23±0.06 mg l^{-1}) and highest in July (0.99±0.05 mg l^{-1}). Nitrate concentrations varied from 0.43±0.12 mg l^{-1} in September to 2.21±0.22 mg l^{-1} in January. Nitrite concentrations were relatively stable with a peak in July (0.11±0.06 mg l^{-1}). Oxygen concentrations were also relatively constant during the year, with an increase in March (11.30±0.56 mg l^{-1}). The concentration of phosphate was highest level in January (0.30±0.12 mg l^{-1}), gradual decreasing during the year up to 0.16±0.10 mg l^{-1} in November.

Table III-1 - Mean values and standard deviation of temperature, pH, salinity, dissolved oxygen (DO), ammonium, nitrites, nitrates, phosphates, total organic carbon (TOC) and weight and size of cultured fishes in the semi-intensive aquaculture system in January, March, May, July, September and November of 2014

	Temperature	**pH**	**Salinity**	**DO**	**Ammonium**	**Nitrite**	**Nitrate**	**Phosphate**	**TOC**	**Fish Weight**	**Fork Length**
	°C			**mg/L**	**mgNH_3+NH_4/L**	**mgNO_2/L**	**mgNO_3/L**	**mgPO_4/L**	**mgC/L**	**g**	**cm**
Jan	13.37±0.15	7.58±0.13	25.67±1.15	7.30±0.46	0.53±0.29	0.09±0.01	2.21±0.22	0.30±0.12	NA	NA	NA
Mar	15.47±1.02	7.86±0.08	27.33±0.58	11.30±0.56	0.23±0.06	0.03±0.00	1.20±0.10	NA	1.50±0.10	60.00±8.66	16.67±0.60
May	17.10±0.78	7.26±0.18	32.67±1.15	5.47±0.95	0.91±0.27	0.07±0.02	0.81±0.01	0.26±0.14	2.10±0.56	78.33±20.82	17.43±1.91
Jul	20.30±0.40	7.42±0.10	35.00±0.00	6.67±0.29	0.99±0.05	0.11±0.06	0.79±0.24	0.27±0.02	4.00±0.00	101.70±5.77	20.67±0.58
Sep	19.53±1.20	7.93±0.12	37.33±0.58	5.47±0.31	0.76±0.22	0.07±0.03	0.43±0.12	0.19±0.04	6.87±7.04	140.00±22.91	22.43±0.95
Nov	13.23±0.35	7.38±0.16	21.67±2.08	6.40±1.11	0.69±0.35	0.07±0.01	1.37±0.21	0.16±0.10	3.67±0.58	133.73±17.64	21.87±0.32

Variation in bacterioplankton composition

In total, 14131 bacterial sequences were obtained from all sampled months, which were assigned to 1333 bacterial OTUs. Overall OTU richness differed between sampling times (Figure III S1). Controlling for sample size (n = 1200 individual sequences), OTU richness had its lowest value in May (139±6.59 OTUs) and peaked in January (393.77±9.18 OTUs. It should be noted that rarefaction curves did not reach an asymptote, indicating that a significant amount of diversity remained undetected (Figure S1), in particular for January and November. Major patterns of variation, however, can be recovered even if sampling doesn´t covers all the diversity. Although in a different context (animal gut), coverages of approximately 1000 sequences/sample have been found to provide a good balance between number of samples and depth of sampling (Hamady and Knight, 2009).

The PCO analysis of bacterial OTU composition showed that the first axis separated samples from May, July and September in a cluster apart, with a tendency to show higher dominance of abundant OTUs (>1000 reads) in these months (Figure 1a). This dominance trend is consistent with lower Shannon diversity values for these months. January, March and November had the highest Shannon index values (5.10, 4.21 and 4.98, respectively), with May, July and September registering the lowest values (2.88, 3.89 and 3.67, respectively). The second axis separated samples collected in November from samples collected in March, with January occupying an intermediate position. There was a significant association between temperature (envfit for 1st and 2nd axes: P = 0.04) and salinity (envfit for 1st and 2nd axes: P = 0.03) with May, July and September. Nitrate, on the other hand, was significantly associated with January (envfit for 1st and 2nd axes: P = 0.02). There were no significant associations

between pH, dissolved oxygen, ammonium, nitrite, phosphate, TOC and the ordination of the 1st and 2nd axes.

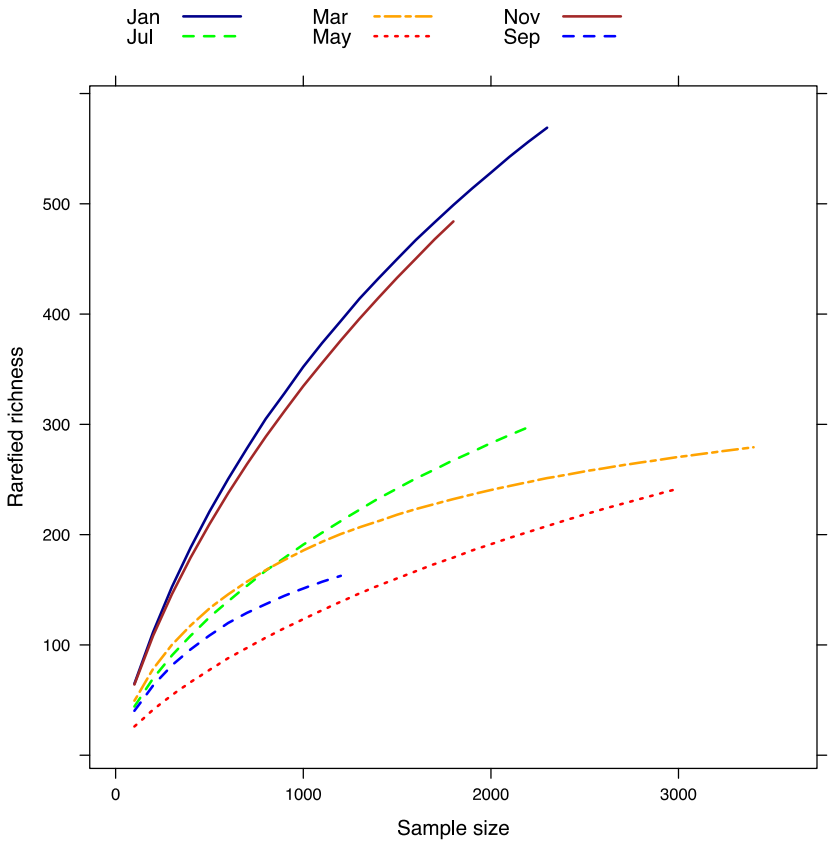

Figure III-A1 - Rarefied bacterial OTUs richness in all sampling events (January (Jan), March (Mar), May (May), July (Jul), September (Sep) and November (Nov) of 2014).

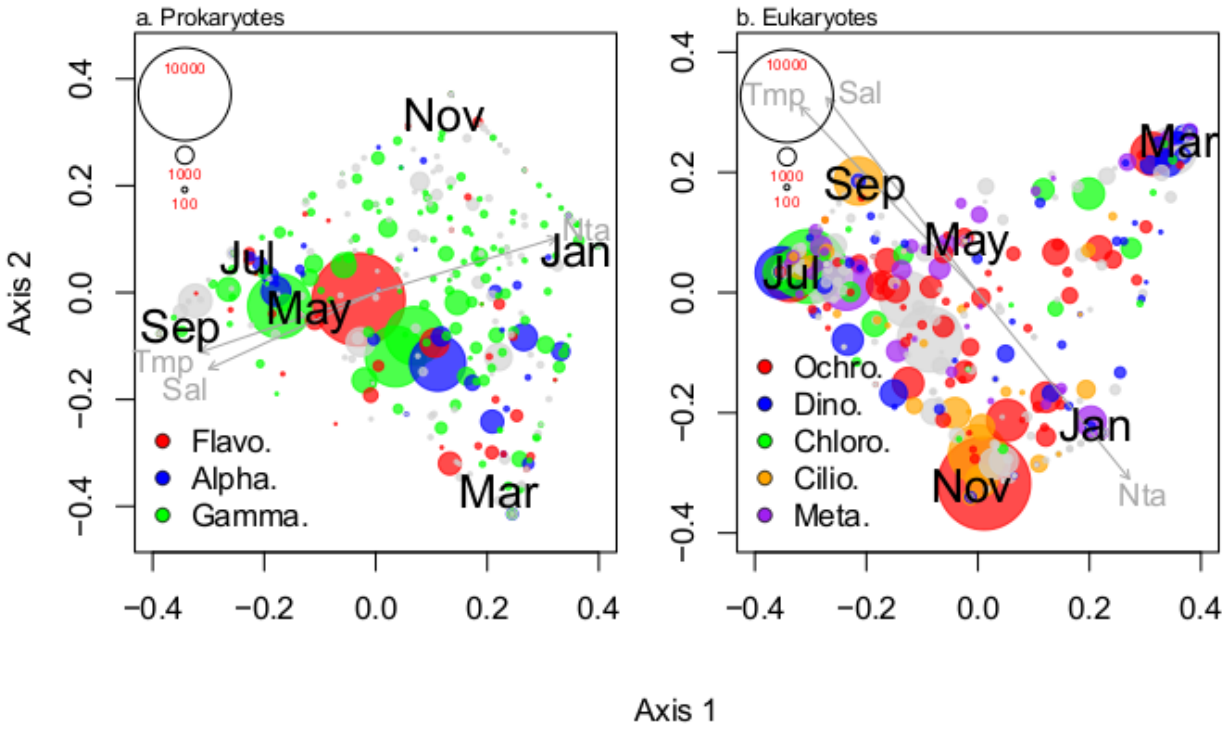

Figure III- 1 - Principal Coordinates Analysis (PCO) of operational taxonomic unit composition of the most abundant bacterial (a) and microeukaryotic (b) classes based on a matrix of OTU composition from January (Jan), March (Mar), May (May), July (Jul), September (Sep) and November (Nov). The color symbols represent OTUs with symbol size representing the number of reads in the entire data set. The closest relatives of the most abundant OTUs were further identified using the NCBI Basic Local Alignment Search Tool (BLAST). Environmental variables with significant associations [temperature (Tmp), salinity (Sal) and nitrate (Nta)] were fit onto the PCO ordinations using the envfit() function in vegan.

The taxonomic analysis of the bacterioplankton communities showed that *Proteobacteria* was the most abundant phylum (average relative abundance of 62.80±5.11%), followed by *Bacteroidetes* (22.04±6.90%), *Firmicutes* (5.42±8.31%) and *Actinobacteria* (3.56±0.88%) (Figure 2). The relative abundance of *Proteobacteria* varied from 68.86% in March to 55.02% in September. *Bacteroidetes* varied from 31.15% in May to 14.29% in September. The abundance of *Firmicutes* appeared to vary inversely to that of *Bacteroidetes* showing the lowest value in May (0.20%) and

the highest in September (22.20%). *Actinobacteria* varied from 4.70% in January to 2.12% in May. Together, these five phyla represented more than 93.82% of all sequences. The most abundant bacterial classes were Gammaproteobacteria (41.73±4.71%), Flavobacteriia (20.79±7.26%), Alphaproteobacteria (13.55±5.26%), Bacilli (5.00±8.24%), Deltaproteobacteria (3.14±2.05%) and Betaproteobacteria (2.62±2.43%) (Figure 2). The variation in the relative abundance of dominant OTUs (represented with ≥100 sequence reads) through the sampling months can be further visualized in Figure 3. Sequence similarity with related organisms identified using BLAST is detailed in Table S3.

The compositional analysis of dominant OTUs (represented with ≥100 sequence reads) showed that OTU-3 was the most dominant OTU in the bacterioplankton with a fairly stable relative abundance through all the year (Figure 3). This OTU was assigned to the *Flavobacteriaceae* family (Flavobacteriia) and had high similarity to an uncultured bacterium previously detected in Norwegian oil-contaminated water (Table A1, sequence similarity 99%). The OTUs 13 and 362, also related with Flavobacteriia class, were present during all year and were assigned with Cryomorphaceae family. They had high similarity with organisms retrieved from the northwestern coast of the USA (Table A1, sequence similarities of 99%). The OTUs 4, 7 and 11 also showed strong dominance and a relatively stable abundance all over the year. OTUs 4 and 11, were assigned to the Alteromonadales order and the *Rhodobacteraceae* family, respectively, and had high similarity to organisms obtained from coastal seawater in Chinese marine waters (Table A1, sequence similarities 100 and 99%). OTU-4 was found to be associated with the oligotrophic marine Gammaproteobacteria group that includes sequences exclusively from marine environments (Na et al., 2011). OTU-7 was also assigned to Alteromonadales order and was related to an uncultured *Glaciecola* sp. found in all treatments of a carbon source enrichment experiment in Mediterrean Sea (Table A1, sequence similarity 99%).

Table III-A1 - List of abundant bacterial OTUs (≥ 100 sequences) including: OTU-numbers; number of total reads (Sum); taxonomic affiliation of OTU; GenInfo sequence identifiers of closely related organisms identified using BLAST (GI); Sequence similarity of these organisms with our representative (Seq) OTU sequences and Isolation source of organisms identified using BLAST

OTU	Sum	Phylum	Class	Order	Family	Genus	GI	Seq	Source
2	285	Firmicutes	Bacilli	Bacillales	Paenibacillaceae	Paenibacillus	EU363688	99	river water: China
3	2203	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Sediminicola	KJ139654	99	oil-contaminated seawater: Norway
4	870	Proteobacteria	Gammaproteobacteria	Alteromonadales	HTCC2188	HTCC	GU061024	100	intertidal beach water, Yellow Sea: China
7	937	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	Glaciecola	HQ836381	99	carbon source enrichment experiment from Bay of Blanes: Spain
10	1051	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Unclassified	EF092617	99	bacterioplankton sample of Guanabara Bay: Brazil
11	803	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Octadecabacter	KU173771	99	surface seawaters from the East China Sea
13	207	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Unclassified	JN591936	99	surface seawater, Puget Sound: USA
17	140	Proteobacteria	Gammaproteobacteria	Chromatiales	Unclassified	Unclassified	KC006261	99	estuarine water from Jiulong River: China
19	187	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	KT720393	99	skin of frog *Pelophylax perezi*, Salreu: Portugal
21	154	Proteobacteria	Gammaproteobacteria	Alteromonadales	OM60	Unclassified	FR647885	100	seawater, 2 m depth, Baltic Sea
22	133	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Unclassified	KR492890	99	isolated from the Pacific green alga *Ulva fenestrata*
24	189	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	RS62	EU167389	99	surface water, Sapelo Island, Georgia: EUA
30	212	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Candidatus Aquiluna	EU878153	100	Mesocosm experimente, Baltic Sea
31	150	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oleiphilaceae	Unclassified	EF491299	100	steel surfaces immerged in marine water of Qingdao Coast: China
63	221	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Octadecabacter	JN625570	99	estuarine plankton communities from Patagonia
362	131	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Unclassified	JN591936	99	surface seawater, Puget Sound, Washington: USA
500	157	Proteobacteria	Gammaproteobacteria	Oceanospirillales	Oceanospirillaceae	Unclassified	KR077451	99	seawater from Shandong, China

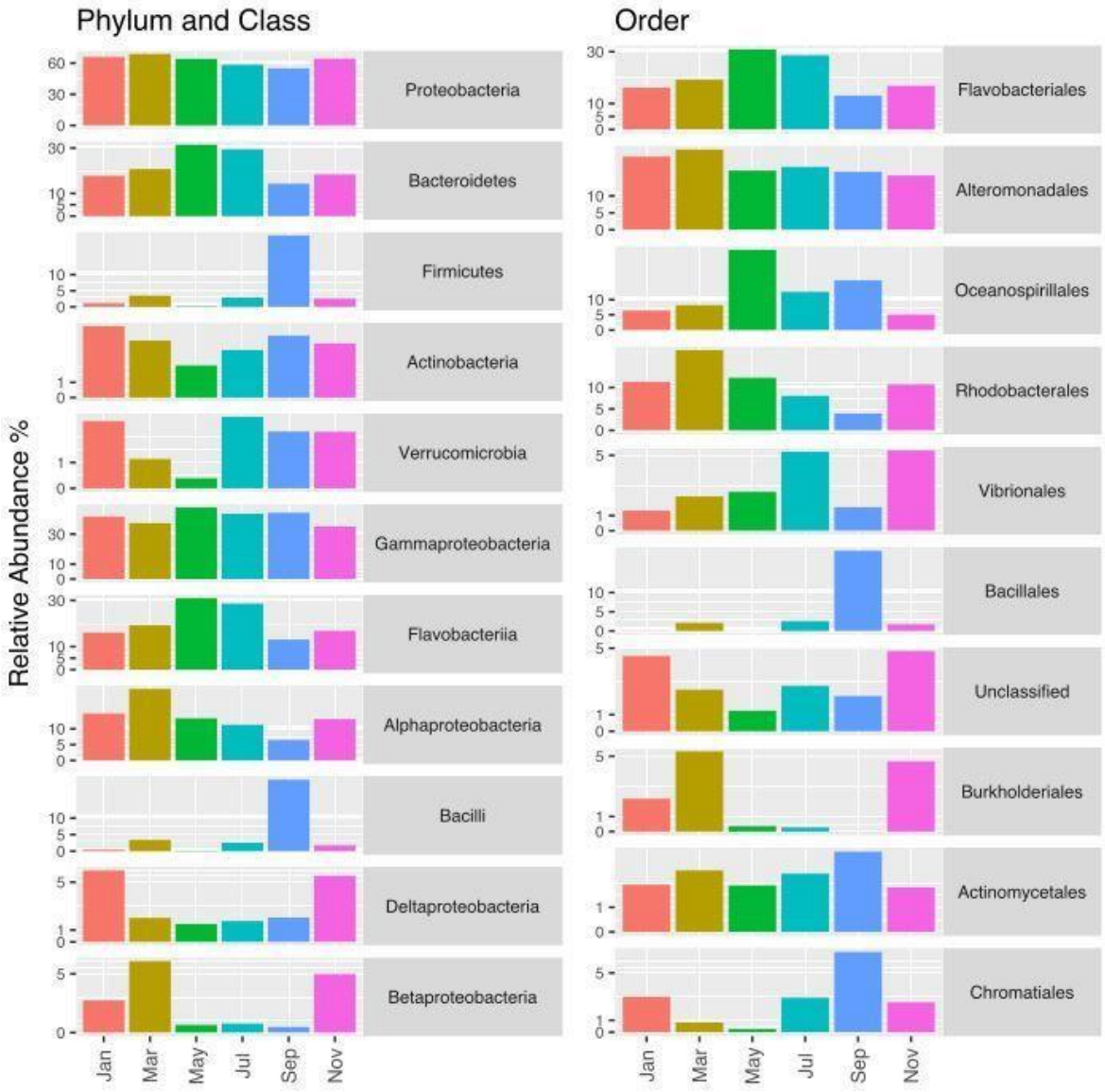

Figure III-2 - Mean relative abundance of the most abundant bacterial phyla, classes and orders.

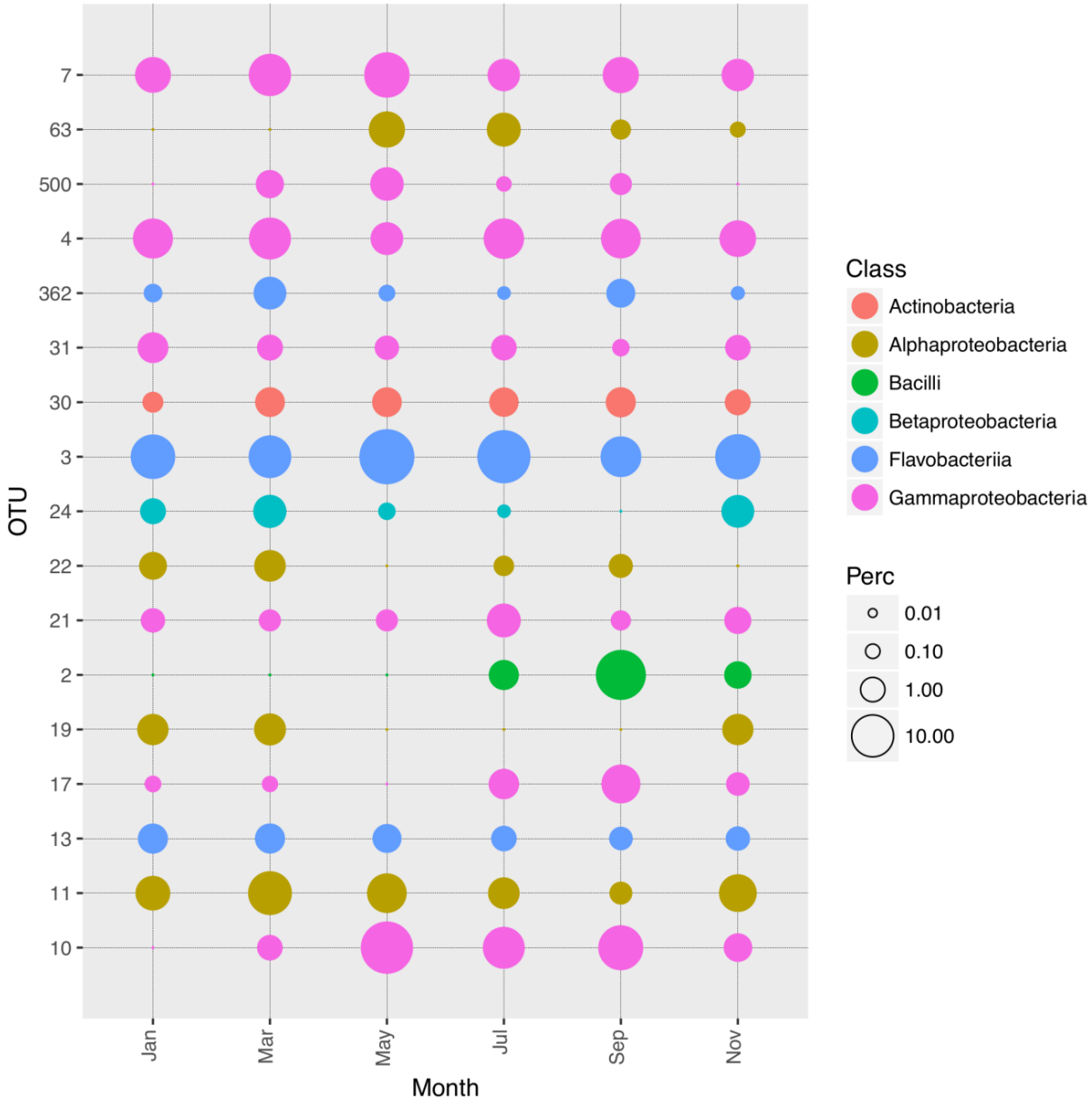

Figure III-3 - Relative abundance of the most abundant (>100 sequences) bacterial OTUs (class level). The size of the circles denotes the total sequence abundance (after square root transformation).

The dominant OTUs 10 and 500 peaked in May (Figure 3) and were assigned to the *Oceanospirillaceae* family and had similarity to an organism obtained from inside a polluted estuarine system in Brazil (Table A1, sequence similarities 99%), and with an uncultured bacterium clone from seawater from Shandong, China (Table A1, sequence similarities 99%),

respectively. OTU-63, assigned to the *Rhodobacteraceae* family, peaked in May and July and had similarity to an uncultured bacterium found in a microcosm experiment with estuarine water from Patagonia (Table A1, sequence similarity 99%). OTU-21 also peaked in July (Figure 3) and was assigned to the order Alteromonadales. This OTU was related to an uncultured gammaproteobacterium found in seawater from the Baltic Sea (Table A1, sequence similarity 100%).

OTUs 2 and 17 registered their highest relative abundance in September (Figure 3) and were assigned to the *Paenibacillaceae* family and Chromatiales order, respectively. Both had high similarity to uncultured organisms obtained from Chinese rivers (Table A1, sequence similarities 99%). OTU-31, assigned to *Oleiphilaceae* family, peaked in January (Figure 3). This OTU had high similarity to an uncultured bacterium found on steel surfaces immersed in marine water (Table A1, sequence similarity 100%). Among others, this order contributes to initial formation and development of surface biofilms (Dang et al., 2011).

OTU-24 showed increased relative abundance during colder months (January, March and November) and was the only dominant OTU assigned to the *Betaproteobacteria* class (Figure 3). This OTU was similar to an uncultured Comamonadaceae bacterium obtained from North Atlantic Ocean (Table A1, sequence similarities 99%). OTU-19 was assigned to the Rhodobacteraceae family and also showed relatively high abundance in the coldest months (Figure 3). This OTU was related to an uncultured organism found on the skin of a frog (*Pelophylax perezi*) from Portugal (Table A1, sequence similarity 99%) as well in cold places as glacier in Canada (DQ628964), China (JX949604) and Antartic soil (NR_148653, KM9780762, KY476581). OTU-22 was not detected in May and November. This OTU was also assigned to the Rhodobacteraceae family and related with a novel species of the genus Amylibacter (*Amylibacter ulvae* sp. nov.) isolated from the green alga *Ulva fenestrate*

(Nedashkovskaya et al., 2016*)* (Table A1, sequence similarity 99%). The OTU-30 was the only one that belonged to the class Actinobacteria among the most dominant bacterial OTUs. It was present throughout all year and was related an uncultured actinobacterium (Table A1, sequence similarity 100%).

Variation in microeukaryotic plankton composition

The total amount of microeukaryote sequences retrieved in this study was 20545 that were assigned to 833 OTUs. Controlling for sample size (n = 1500 individual sequences), OTU richness varied from 134.82±3.13 OTUs in March to 261.23±4.50 OTUs in January (Figure S2). Similar to the bacterioplankton analysis, the rarefaction curves did not reach an asymptote in any of the months, suggesting that a significant amount of diversity was not detected (Figure S2). Shannon diversity index values varied between 3.38 in November and 4.64 in May (Table S2). Also in line with the bacterioplankton analysis, the PCO ordination of microeukaryotic communities showed that the first axis separated samples from May, July and September in a cluster apart (Figure 1b). However, July tended to show a higher dominance of abundant OTUs (>1000 reads) belonging to Dinophyta and Chlorophyta groups. The second axis showed that, while samples collected in November and January tend to share more similarities, March was placed apart from all other samples. Dominant OTUs belonging to the Ochrophyta group (>1000 reads) were detected in March and November (Figure 1b). Also in line with the bacterioplankton analysis, temperature (envfit for 1st and 2nd axes: P = 0.01) and salinity (envfit for 1st and 2nd axes: P = 0.03) were significantly associated with May, July and September samples. Nitrate was significantly associated with January (envfit for 1st and 2nd axes: P = 0.04).

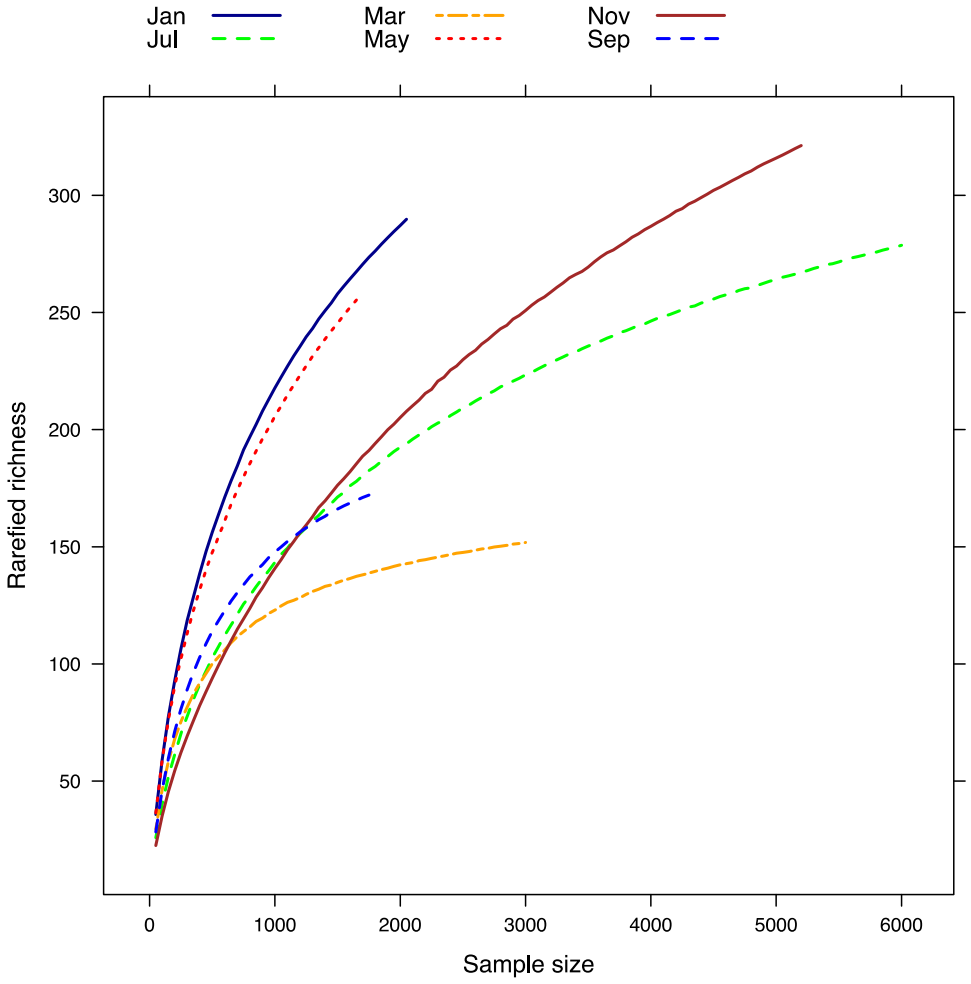

Figure III-A2 - Rarefied microeukaryotic OTUs richness in all sampling events (January, March, May, July, September and November of 2014).

The overall taxonomic analysis of the microeukaryotic plankton communities showed that Stramenopiles was the most abundant higher taxon (average relative abundance of 30.40±9.50%), followed by Alveolata (23.55±8.44%), Opisthokonta (18.53±8.66%), Archaeplastida (12.67±9.44%), Hacrobia (9.73±3.73%) and Rhizaria (4.56±3.33%) (Figure 4). Together, these groups made up more than 99% of all sequences. The Stramenopiles, was consistently the most abundant higher taxon throughout the year, although abundance varied from a high of 45.57% in November to a low of 21.39% in September. Interestingly, their

relative abundance clearly decreased in the warmest months (May, Jul, Sep). The abundance of Alveolata peaked in September (38.50%) and was lowest in May (14.37%). Opisthokonta abundance was highest in March and May (26.48 and 26.60%, respectively) and subsequently declined throughout the year to a low of 4.15% in November. The abundance of Archaeplastida was highest in the warmer months. The most abundant microeukaryotic divisions were Ochrophyta (26.47±6.88%), Chlorophyta (11.62±9.18%), Ciliophora (12.99±11.07%), Dinophyta (10.03±6.21%) and Metazoa (11.75±5.19%) (Figure 4). The variation in the relative abundance of dominant OTUs (represented with ≥200 sequence reads) through the sampling months can be further visualized in Figure 5. Sequence similarity with related organisms identified using BLAST is detailed in Table S4.

The compositional analysis of the most dominant microeukaryotic OTUs (represented with ≥200 sequence reads) showed an increase in the abundance of Ochrophyta OTUs 2, 14 and 15 during colder months (January, March and November) (Figure 5). The OTU-2 was the most abundant OTU in November and was assigned to the Pedinellales order. This OTU had high similarity to an uncultured dictyochophyte clone retrieved from water in the Columbia River estuary (Table A2, sequence similarity = 100%). OTU-14 registered its higher abundance in March (Figure 5) and showed strong similarity to an uncultured eukaryote found in water from Ross Sea (Table A2, sequence similarity = 100%). OTU-15 was one of the most dominant OTUs in January. This OTU was assigned to algal group (Chrysophyceae-Synurophyceae class), and had similarity to an uncultured *Chrysolepidomonas* sp. found in Central Baltic Sea (Table A2, sequence similarity = 100%). With lowest relative abundance but also belonging to Ochrophyta, OTU-21 was well distributed throughout the year, with a slight higher density in November (Figure 5). This OTU was assigned to the Bacillariophyta and related with the diatom *Nitzschia draveillensis* cloned from a Spanish river (Table A2, sequence similarity = 100%). OTU-7 was the only dominant OTU belonging to Ochrophyta, showing

increased abundance during the warmest period (July) (Figure 5). This OTU was also assigned to a diatom (Bacillariophyta) and was similar to an uncultured *Navicula cryptocephala* var. veneta found in river from northern Germany (Table A2, sequence similarity = 99%).

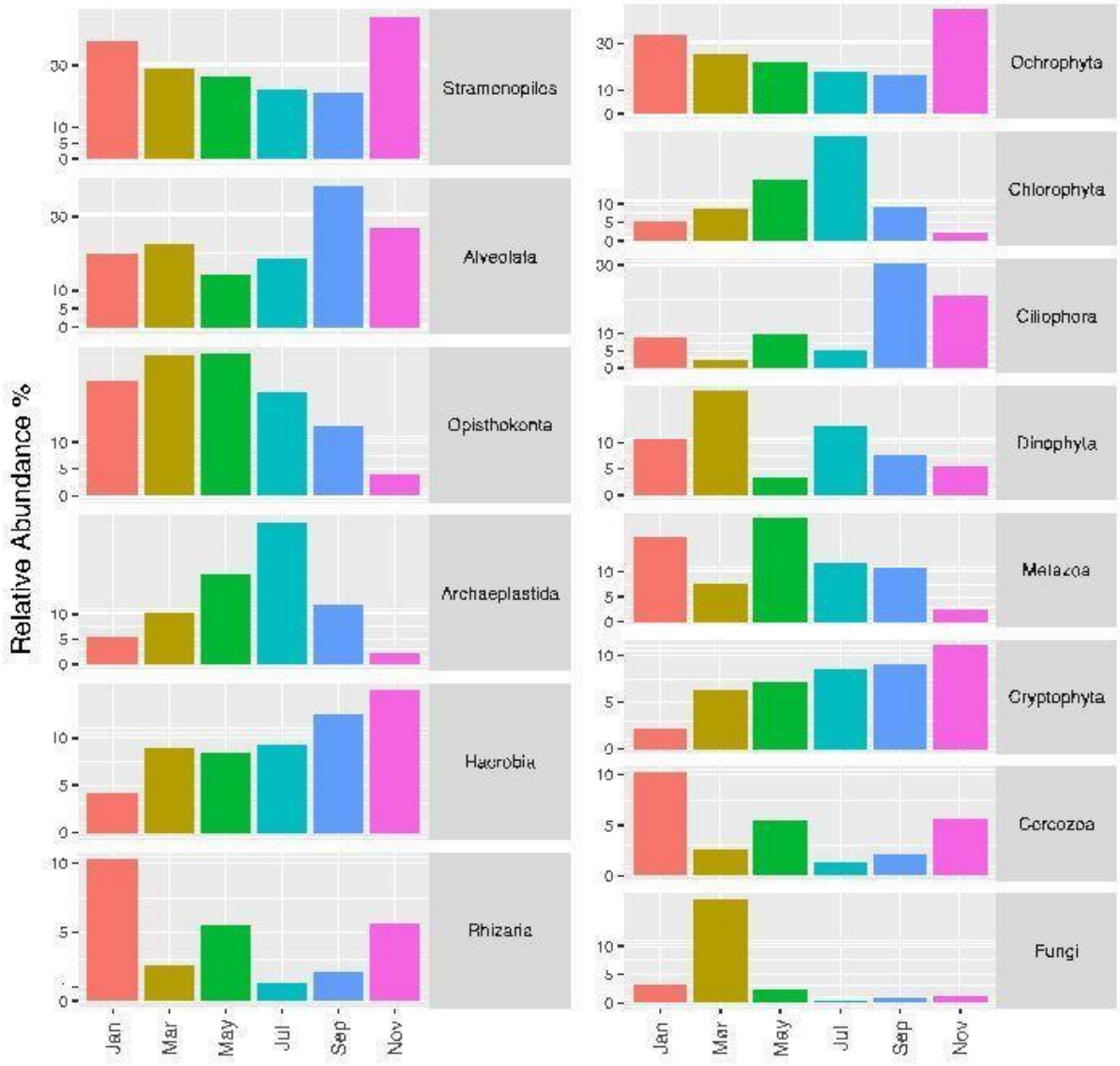

Figure III- 4 - Mean relative abundance of the most abundant microeukaryotic groups.

Table III- A2 - List of abundant microeukaryotic OTUs (≥ 200 sequences) including: OTU-numbers; Number of total reads (Sum); Taxonomic affiliation of OTU; GenInfo sequence identifiers of closely related organisms identified using BLAST (GI); Sequence similarity of these organisms with our representative OTU sequences (Seq) and Isolation source of organisms identified using BLAST

OTU	Sum	Phylum	Division	Class	Order	Family	Genus	GI	Seq	Source
2	1775	Stramenopiles	Ochrophyta	Dictyochophyceae	Dictyochophyceae_X	Pedinellales	Pedinellales_X	JF275796	100	Water from estuary south channel; Columbia River: USA
3	1132	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Bathycoccaceae	Ostreococcus	CP000592	100	Guillard Culture Collection of Marine Phytoplankton: CCMP2514 & water from Pacific Ocean coastal site bound by the California Current
4	881	Hacrobia	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Teleaulax	AB471786	100	Seawater, Funka Bay, Hokkaido: Japan
5	693	Alveolata	Ciliophora	Spirotrichea	Choreotrichia	Choreotrichia_X	Unassigned	KC911784	100	Surface brackish water, Segura River coastal zone Continuum: Spain
6	583	Opisthokonta	Metazoa	Platyhelminthes	Monogenea	Monopisthocotylea	Pseudorhabdosynochus	FJ797060	96	Fish: *Epinephelus* sp.; aquaculture in Vietnam
7	571	Stramenopiles	Ochrophyta	Bacillariophyta	Bacillariophyta_X	Raphid-pennate	Navicula	AM501970	99	River; northern Germany
8	559	Alveolata	Dinophyta	Dinophyceae	Unassigned	Unassigned	Unassigned	HG005134	95	Seawater; Masan Bay: Korea
9	634	Hacrobia	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Cryptomonadales_X	JQ420121	100	Brown tide; Qinhuangdao coast: China
10	474	Archaeplastida	Chlorophyta	Chlorodendrophyceae	Chlorodendrales	Chlorodendrales_X	Unassigned	KT007553	100	Culture Collection
11	480	Opisthokonta	Fungi	Ascomycota	Saccharomycotina	Saccharomycetales	Saccharomyces	CP009950	100	Microbial Type Culture Collection (MTCC)
12	480	Alveolata	Ciliophora	Spirotrichea	Choreotrichia	Strobilidiidae	Pelagostrobilidium	JQ781699	99	Seawater; Coastal Northeastern Taiwan
13	538	Opisthokonta	Unassigned	Unassigned	Unassigned	Unassigned	Unassigned	KF177726	99	Great Salt Plains, Oklahoma: USA
14	386	Stramenopiles	Ochrophyta	Unassigned	Unassigned	Unassigned	Unassigned	KJ757884	100	Seawater; Ross Sea 20m
15	359	Stramenopiles	Ochrophyta	Chrysophyceae-Synurophyceae	Chrysophyceae-Synurophyceae_X	Clade-C	Clade-C_X	KX431556	100	Suboxic and anoxic waters; Landsort Deep: Central Baltic Sea
17	244	Archaeplastida	Chlorophyta	Ulvophyceae	Oltmansiellopsidales	Oltmansiellopsidales_X	Oltmannsiellopsis	KT072980	99	River, Canal de Nantes a Brest a Nort-sur-Erdre: France
18	200	Hacrobia	Katablepharidophyta	Katablepharidaceae	Katablepharidales	Katablepharidales_X	Katablepharidales_XX	JF275678	100	Water from estuary south channel; Columbia River: USA
21	201	Stramenopiles	Ochrophyta	Bacillariophyta	Bacillariophyta_X	Raphid-pennate	Bacillariophyta	KC736635	100	River; Spain
23	213	Alveolata	Ciliophora	Spirotrichea	Choreotrichia	Strobilidiidae	Strobilidiidae_X	FJ939033	99	Freshwater lake; China
551	203	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Bathycoccaceae	Ostreococcus	AY329635	100	Enclosed shallow oyster production lagoon; Mediterranean Sea

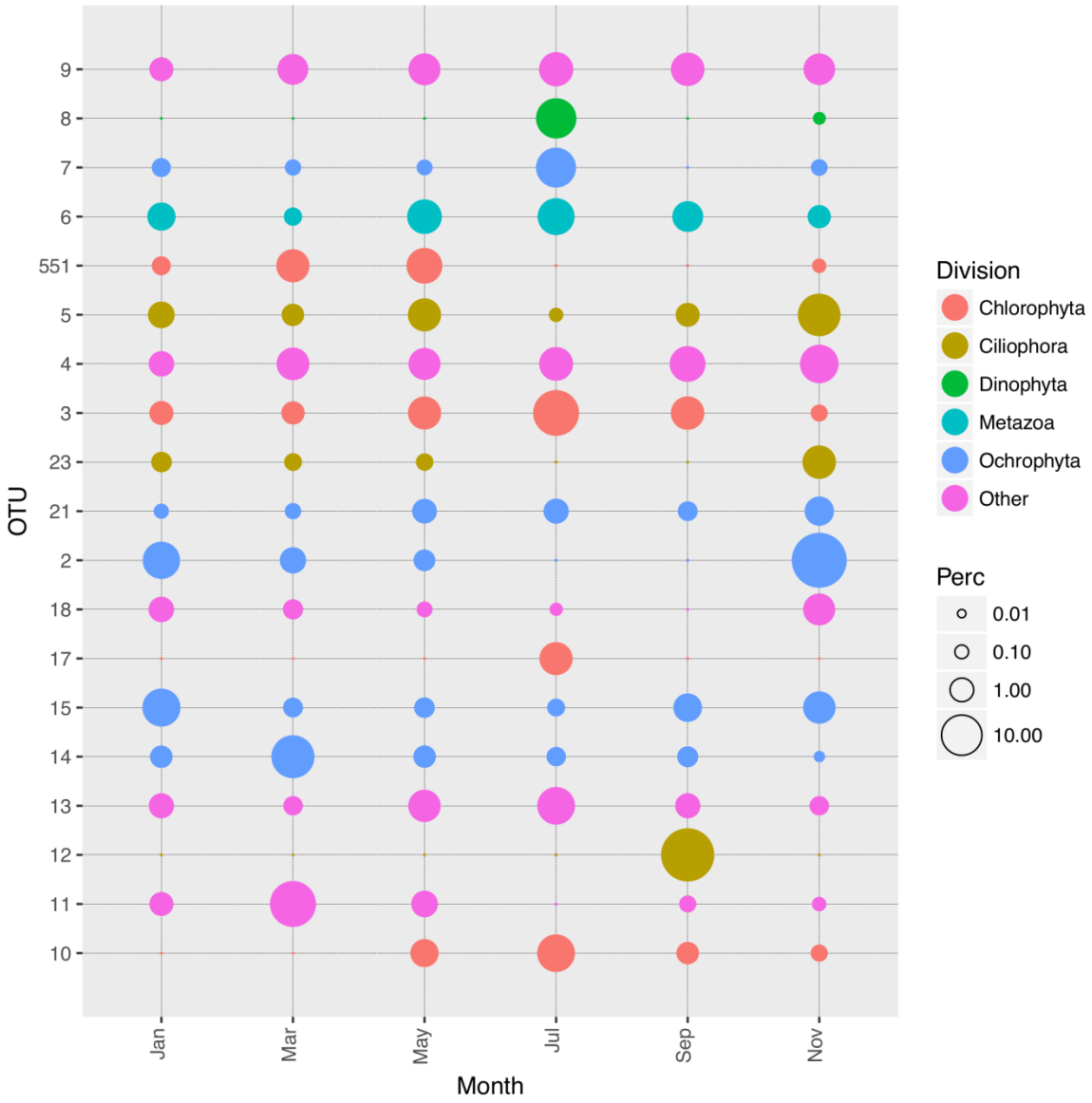

Figure III- 5 - Relative abundance of the most abundant (>200) microeukaryotic OTUs. The size of the circles denotes the total sequence abundance (after square root transformation).

Other OTUs, however, peaked in July. OTUs 3, 10 and 17 showed increased abundance in July and were all assigned to the Chlorophyta (Figure 5). OTU-3 was further assigned to the family Bathycoccaceae and had similarity to *Ostreococcus lucimarinus* previously isolated from Pacific Ocean (San Diego, EUA) (Table A2, sequence similarity = 100%). OTU-10 was assigned within the Chlorodendrales order and had similarity to *Tetraselmis* sp.

previously isolated from Napoli (Italy) (Table A2, sequence similarity = 100%). OTU-17 was further assigned to the order Oltmansiellopsidales and was similar to organisms retrieved from water in a French river (Table A2, sequence similarity = 99%). Not all the dominant Chlorophyta OTUs, however, showed higher abundance levels in July. OTU-551, assigned to the Bathycoccaceae family and similar to *Ostreococcus tauri* from an enclosed shallow oyster production lagoon (Thau lagoon, France) (Table A2, sequence similarity = 100%), registered its highest relative abundance value in May and was absent in the warmest months (July and September) (Figure 5). OTU-6 was assigned with Platyhelminthes class and was similar to *Pseudorhabdosynochus sp.* previously found in Vietnam (Table A2, sequence similarity = 96%). This parasite was detected all year but with higher abundance in warmer months such as in cultured groupers in South China Sea (Luo and Yang, 2010). Also found with higher abundance in summer was the OTU-8, from Dinophyta division. This was related with a heterotrophic dinoflagellate *Stoeckeria algicida* isolated from the coastal waters of Korea (Jeong et al., 2014) (Table A2, sequence similarity = 95%).

Interestingly, dominant OTUs belonging to the Ciliophora (OTUs 5 and 23) also showed clear decrease in their relative abundance during the warmest months. OTU-5 was assigned to Choreotrichia order and was similar to an uncultured ciliate previously detected in surface brackish water (Table A2, sequence similarity = 100%). OTU-23 was assigned to the Strobilidiidae family and was similar to an uncultured organism found in freshwater lake from China (Table A2, sequence similarity = 99%). Worthy of note was the high dominance of OTU-12 in September (Figure 5) and its absence in the other months. This OTU had similarity with an uncultured *Pelagostrobilidium* sp. previously found in the coastal waters of northeastern Taiwan (Chen et al., 2017) (Table A2, sequence similarity = 99%).

The OTUs identified in Figure 5 as "Other" belong to four different groups. OTUs 4 and 9 were assigned to Cryptomonadales (Hacrobia pylum, Cryptophyta division) and were present all year. OTU-4 was related with *Teleaulax acuta* (Table A2, sequence similarity = 100%) isolated from Japanese coastal waters (Nishitani et al., 2010). OTU-9 had similarity with an uncultured phytoplankton clone from Chinese waters (Yanghekou Harbor - outside the algae bloom area) (Table A2, sequence similarity = 100%). The OTU-11 was assigned to Fungi group. This OTU had higher density in March and had high similarity to an organism classified as *Saccharomyces cerevisiae* (strain NCIM3107) (Table A2, sequence similarity = 100%). This strain is a moderate producer of bioethanol and was obtained from Microbial Type Culture Collection (Chandigarh, India) (Ulaganathan et al., 2015). OTU-18 was only absent in September with highest abundance in November. This OTU was assigned within the flagellate Katablepharidales order and was similar to an uncultured katablepharis obtained from estuarine water (Table A2, sequence similarity = 100%). They were discovered in association with the ETM (Estuarine Turbidity Maxima) event in Columbia River (Herfort et al., 2011). OTU-13 was classified as an unassigned division that was related with a *Bacillariophyta* sp. from a terrestrial hypersaline environment (Table A2, sequence similarity = 99%).

Integrated analysis of bacterial and microeukaryotic plankton communities

In this study we used procrustes analysis to assess the congruence among PCO ordinations of bacterial and microeukaryotic plankton communities inhabiting the aquaculture system during a one year period. This analysis revealed a highly significant association between both communities (procrustes correlation; R=0.98, P= 0.001; Figure A3). We further tested the correlation between the most abundant bacterial orders and microeukaryotic divisons

(Figure 6). Among others, there was a significant negative correlation between the Ciliophora division and the bacterial orders Alteromonadales (pearson correlation; R=-0.89, P=0.01) and Actinomycetales (pearson correlation; R=-0.91, P=0.009). A significant positive correlation was found between the Haptophyta division and Alteromonadales (pearson correlation; R=0.82, P=0.04), Rhodobacterales (pearson correlation; R=0.88, P=0.01) and Burkholderiales (pearson correlation; R=0.89, P=0.02) orders. A significant positive correlation was also found between Fungi and Burkholderiales (pearson correlation; R=0.84, P=0.03) and Rhizobiales (pearson correlation; R=-0.86, P=0.03) orders.

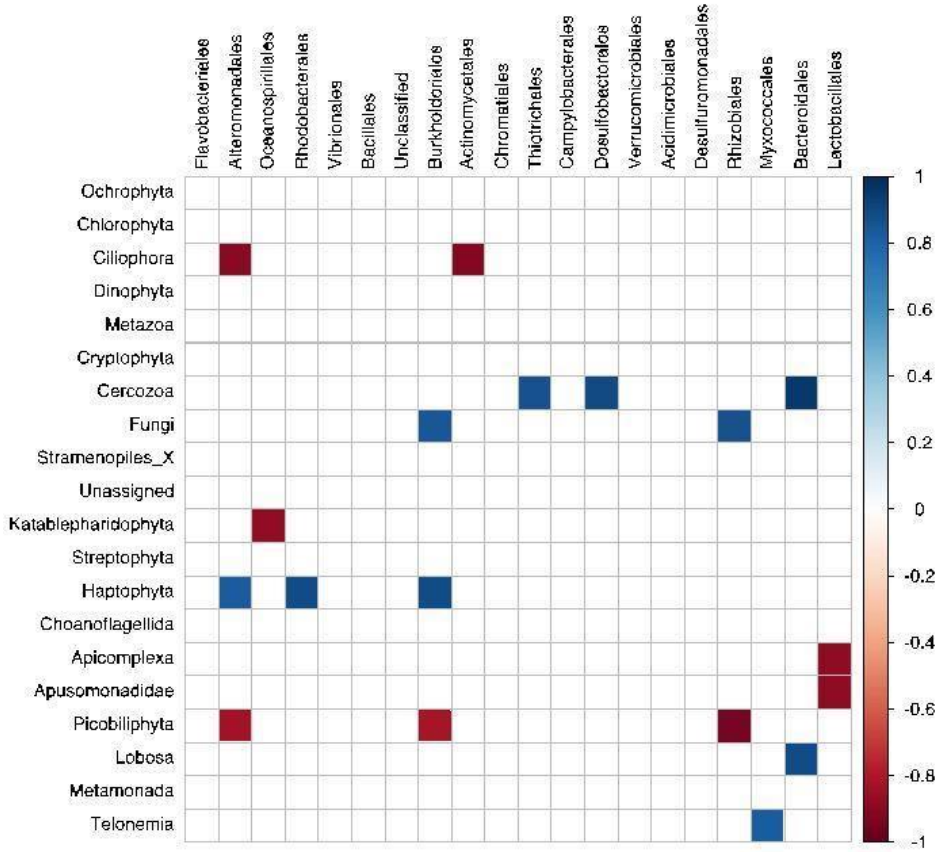

Figure III- 6 - Correlation matrix based on Pearson's correlation between most abundant bacterial orders and microeukaryotic groups. The intensity of color for each square represents the strength of the correlation; blue illustrate positive correlation and red negative correlation coefficients. Only significant ($p<0.05$) correlations are show.

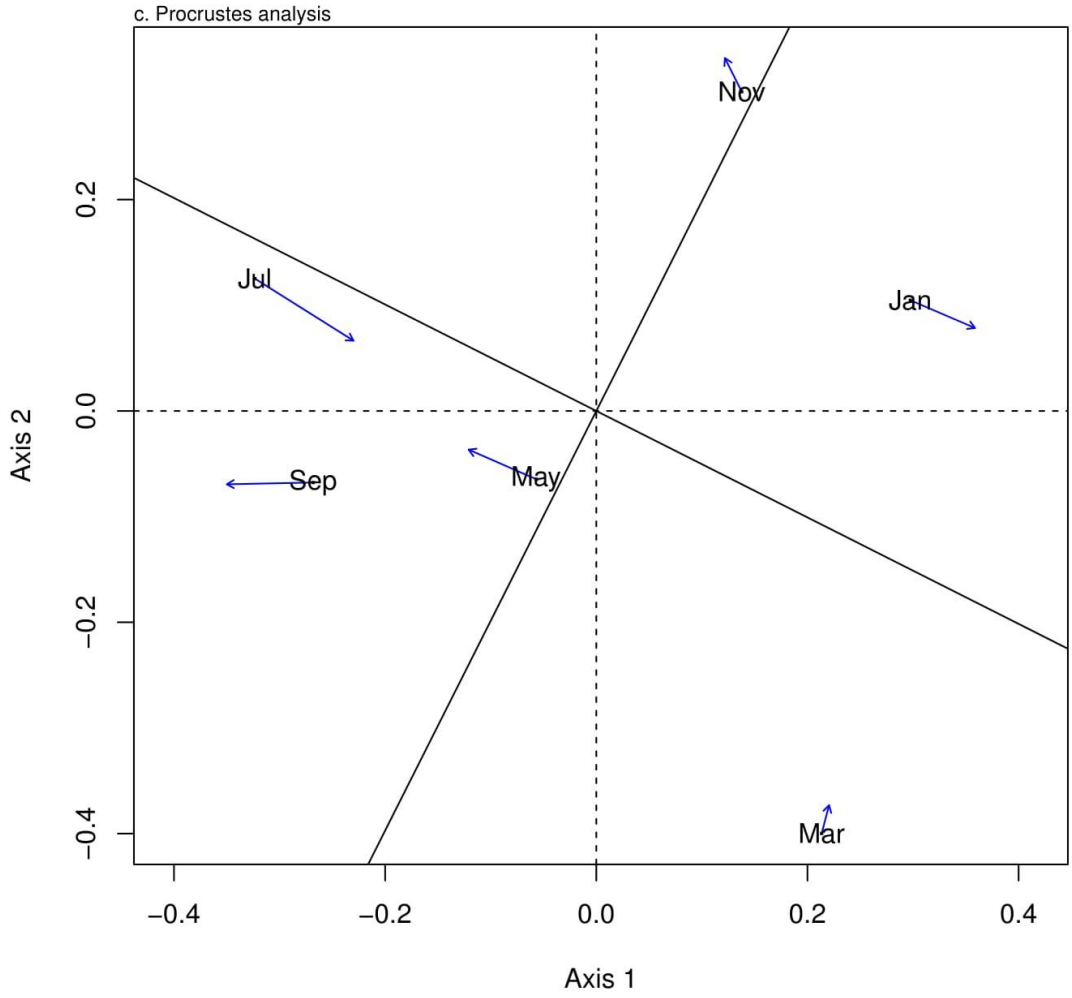

Figure III-A3 - Procrustes analysis comparing bacterial (Figure 3) and microeukaryotic (Figure 5) OTU composition (arrow base indicates the corresponding positions of the samples in the bacterial map while arrowhead indicates the corresponding positions of the samples in the microeukaryotic map).

Discussion

Semi-intensive sea bass aquaculture is susceptible to variations of environmental and biological parameters such as temperature, salinity, nutrients and the influence of other organisms which enter the production systems through water inlets. Variations in these parameters may influence the microbial communities, leading to disease outbreaks and proliferation of parasites and pathogens. Previous studies showed that the seasonal variability of chemical and physical environmental parameters and biological interactions (e.g predator–prey and microbial competition) can influence bacterioplankton dynamics at different spatiotemporal scales (Strom, 2008; Bunse and Pinhassi, 2017). However, there is a scarcity of knowledge on the potential effects of seasonal variation of these parameters on the dynamic of microbial plankton communities in estuarine aquaculture ponds (Pereira et al., 2011; Martins et al., 2018). Overall, our results showed that seasonality impacted both the bacterial and microeukaryotic plankton communities of the aquaculture system studied. The bacterial community analysis showed that Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria dominated the bacterioplankton during all sampling period. With exception of Firmicutes, the dominance of these groups is in agreement with our previous study on the dynamics of the bacterioplankton in this same aquaculture farm in a different year (2012) (Martins et al., 2018). Furthermore, although there were some differences between the years (2012 and 2014), the main groups showed similar changes in their relative abundance in response to seasonal variation. For example, Proteobacteria showed increased relative abundance in colder months and registered its lowest values in warmer months. Members of this phylum play important roles in several biogeochemical processes such as aerobic denitrification (Zehr and Ward, 2002), autotrophic nitrification (Bentzon-Tilia et al., 2016) or sulfate reduction (Amaral-Zettler

et al., 2010). Bacteroidetes registered the lowest values in colder months and a clear increase on its relative abundance in the warmest months. Members of this phylum are known degraders of polymeric organic matter and are found in a range of habitats that include marine environments and the gastrointestinal tract of animals (Thomas et al., 2011). In this study, most of the members of the phylum Bacteroidetes were assigned to the Flavobacteriales order, which is often associated with phytoplankton blooms (Buchan et al., 2014). This association could explain their higher abundance in warmer months. Concurrent variations were also found within the relative abundance of Chromatiales that peaked in September in aquaculture tanks in both studies (Martins et al., 2018). In agreement with Martins et al. (2018), Actinobacteria was also among the most abundant phyla, with the majority of its members being assigned to the Actinomycetales order. Members of this order are frequently found in fish and are likely characteristic of fish intestinal microbiotas (Schmidt et al*.,* 2016).

Regarding the characterization of microeukaryotic communities, few studies have focused on the structure of these communities in aquaculture systems (Boaventura et al., 2018). Here we observed a dominance of microeukaryotes belonging to Stramenopiles, Alveolata, Opisthokonta, Archaeplastida, Hacrobia and Rhizaria groups all over the year in the aquaculture ponds. Interestingly, the relative abundance of Stramenopiles and Archaeplastida related with temperature and salinity. While Stramenopiles relative abundance tended to decrease in the warmest months, Archaeplastida showed a clear increase. The in depth community composition analysis at lower taxonomic ranks showed that the variations observed for Stramenopiles and Archaeplastida phyla were mainly due to changes in the relative abundance of Ochrophyta (Stramenopiles) and Chlorophyta (Archaeplastida). Most OTUs assigned to Stramenopiles belonged to the marine Stramenopiles (MAST) clade. This group includes heterotrophic nanoflagellates, thought to be important grazers of bacteria and picophytoplankton (Lin et al., 2012). Nanoflagellates have been previously showed to control

bacterial abundance in the plankton and, to form an important link in aquatic food webs between bacteria and zooplankton organisms (such as ciliates, rotifers and small crustaceans) (Fenchel, 1984; Sanders et al., 1989). Here, OTU-2, one of the most abundant microeukaryote, was classified within the Pedinellales order (Ochrophyta) that includes mixotrophic nanoflagellate. In general, the abundance of this OTU and other Ochrophyta's OTUs showed correlation with lower salinity and higher levels of nitrate during the coldest months (November and January). Probably, stormwater runoff and river waters inflow contributed for higher levels of nitrate and lower salinity during this period and consequently, the increased abundance of the Ochrophyta group. In line with this hypothesis, Piwosz and Pernthaler (2010) observed that members of this group formed short-lived blooms during a period of decreased salinity after riverine freshwater influx in coastal surface waters. Nitrate, in addition, is an important source of nitrogen for the phytoplankton and is considered a key nutrient for primary production in aquatic environments (Dugdale and Goering, 1967). Archaeplastida was represented by OTU 3, one of the most abundant OTUs belonging to the Bathicoccaceae family. Members of this family play a key ecological role in marine environments as primary producers (Lara et al., 2017). The increase abundance of this group during warming period is related with increase phytoplankton growth rates, nutrient uptake and overall metabolic activity (Litchman et al., 2007).

An interesting finding of this study was the congruent response of both bacterial and microeukaryotic communities to seasonal changes of environmental parameters. Among others, the basis of this relationship could be related to the direct effects of abiotic parameters on each domain. Temperature and salinity were significantly associated with the ordination analysis, with the formation of clusters in both domains that grouped warmer months with the highest salinity levels (May, July and September). Both these parameters have been found to be strong drivers of bacterial and microeukaryotic community variations. For example, shifts

in bacterial community structure associated to changes in salinity are well described in estuarine systems (Kirchman et al., 2005; Bouvier and del Giorgio, 2002). By analyzing 16S rRNA gene sequences compiled from 111 studies with diverse physical environments, Lozupone and Knight (2007) identified salinity as the major environmental determinant of prokaryotic community composition in several habitats. Recently, in a survey in the Baltic Sea, Hu et al. (2016) found that besides being a strong driver of bacterial community variation, salinity is also a major factor affecting microeukaryotic community assemblages. Salinity was also identified as a significant driver of microeukaryotic communities composition in a 2.5-year time series conducted in Mobile Bay along the Alabama continental shelf (Brannock et al., 2016).

In this study, nitrate concentration was also a significant predictor of both bacterial and microeukariotic communities. Interestingly, despite of previous indications that high nitrate concentrations can cause eutrophication, which may lead to harmful algal blooms and reduction in biodiversity (Washbourne et al., 2011), here, higher concentrations of nitrate were related with high diversity of both bacterial and microeukaryotic communities. We have previously shown that variations in inorganic nitrogen compounds can play an important role in structuring the bacterial community in a semi-intensive European seabass (*D. labrax*) aquaculture system and in a turbot (*Scophthalmus maximus*) and sole (*Solea senegalensis*) recirculating aquaculture system (Martins et al., 2013; Martins et al., 2018; Duarte et al., unpub. data). We also identified nitrogen compounds as an important driver of micro-eukaryotic communities in a turbot and sole recirculating aquaculture system (Boaventura et al., 2018). Taken together, our studies indicate that inorganic nitrogen species are important drivers of the aquaculture microbiome, irrespective of fish species culture or system architecture.

Besides the potential effects of the abiotic parameters on bacterial and microeukaryotic communities, the trophic and/or metabolic interdependence between these two domains may have contributed to the seasonal variations observed in this study. For example, we found a strong negative correlation between ciliated protozoa (Ciliophora group) and the orders Alteromonadales and Actinomycetales, which may suggest a trophic interdependence. Although heterotrophic nanoflagellates are usually the primary grazers of bacteria, ciliates can be important consumers of bacteria in eutrophic freshwater and coastal systems (Sherr and Sherr, 2002). Previous studies have found evidences that specific bacterial lineages, including *Alteromonas*, might be a preferred target for selective predation. Many ciliates and heterotrophic nanoflagellates selectively prey for larger-sized bacteria (Gonzalez et al., 1990). Beardsley et al. (2003), found a negative correlation between heterotrophic nanoflagellates and the bacterial lineages *Alteromonas*, *Pseudoalteromonas* and *Vibrio*, whose cell size range was significantly larger than the community average.

Among the other significant correlations, it is worth mentioning the positive correlation between the variation of Rhodobacterales and brown algae (Haptophyta). In our study, several of the most abundant Rhodobacterales OTUs (OTUs 11, 19, 63) belonged to the Roseobacter, a group commonly found in marine environments (Moran et al., 2007) whose role as fish pathogen antagonists in aquaculture systems is being increasingly recognized (Hjelm et al., 2004; D'Alvise et al., 2010; D'Alvise et al., 2012; Martins et al., 2013; Martins et al., 2018). Their abundance is often associated with algal blooms since they are thought to promote algal growth by biosynthesizing and secreting antibiotics and growth stimulants (Seyedsayamdost et al., 2011). Members of Roseobacter are among a select group of marine bacterial lineages that have the ability to metabolize dimethylsulfoniopropionate (DSMP), a volatile sulfur compound produced in abundance by dinoflagellates and

coccolithophorids (Luo and Moran, 2014). DMSP can act as a specific chemical that attracts chemotactic bacteria, such as members of the Roseobacter group (Jackson, 1987).

In this study, we also detect a positive correlation between Fungi and the Burkholderiales and Rhizobiales orders. Members of the Burkholderiales order have been repeatedly associated with fungi in soils. It has been suggested that many *Burkholderia* strains have beneficial effects on fungi and can, among other things, use several fungal exudates as nutrients (Stopnisek et al., 2016). Members of the Rhizobiales order (Alphaproteobacteria) are known for their beneficial interactions with many higher plants, algae, lichens and soil fungi (Vessey, 2003; Frey-Klett et al., 2011; Erlacher et al., 2015; Ramanan et al., 2016). However, there is a lack of information about their interaction with marine fungi.

Conclusions

Overall, our results showed that seasonality impacted both the bacterial and microeukaryotic plankton communities of a semi-intensive aquaculture system for sea bass production. Temperature, salinity and nitrate were identified as key drivers of these communities, which varied congruently along the seasons. Besides the potential effects of the physical-chemical parameters on microbial plankton communities, the strong correlation between bacteria and microeukaryote populations observed in this study may be an indication that trophic and/or metabolic interdependence between these two domains can contribute to seasonal variations of these communities in aquaculture systems. Such an interaction may have consequences on the structural composition and function (eg. nutrient cycling) of the microbial plankton community during fish production. Besides the ecological implications, this apparent interdependence could be used to develop microbial management strategies for

aquaculture systems. For example, previous studies suggest that outside-host predation, can influence disease dynamics and can be used to control pathogen populations before host infection (Guedes and Malcata, 2012; Merikanto et al., 2017). However, more in depth studies under controlled conditions are necessary for a better understanding of cause-and-effect relationships between compositional variability of bacterial and microeukaryotic plankton communities in aquaculture systems.

Acknowledgements

Thanks are due for the financial support to CNPq, National Council of Scientific and Technological Development - Brazil and CAPES on CsF Program (BEX 1287-13-3) within the Ministry of Education of Brazil. Francisco J.R.C. Coelho was supported by a postdoctoral scholarship (SFRH/BPD/92366/2013) financed by the Portuguese Foundation for Science and Technology (FCT). This study has been carried out with the financial support of the project AQUASAFE - Development of new technologies to anticipate and diagnose disease outbreaks in aquaculture (PROMAR 31-03-05-FEP-0016) (PROMAR, a Portuguese instrument for the sectors of fisheries and aquaculture funded by the European Fisheries Fund). We are also grateful for the financial support to CESAM (UID/AMB/50017), to FCT/MEC through national funds, and co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020.

References

Aly SM, Albutti A (2014) Antimicrobials Use in Aquaculture and their Public Health Impact. J Aquac Res Development 5:247. doi: 10.4172/2155-9546.1000247

Amaral-Zettler L, Artigas LF, Baross J, Boetius AA, Chandramohan D, Herndl G, Kogure K, Neal P, Pedrós-Alió C, Ramette A, Schouten S, Stal LJ, Thessen A, De Leeuw JW, Sogin M (2010) A global census of marine microbes. In: McIntyre A (ed) Life in the World's Oceans: Diversity, Distribution, and Abundance. Hoboken, NJ: Wiley-Blackwell. doi: 10.1002/9781444325508.ch12

Barg UC (1992) Guidelines for the promotion of environmental management of coastal aquaculture development. FAO Fisheries Technical Paper 328

Beardsley C, Pernthaler J, Wosniok W, Amann R (2003) Are Readily Culturable Bacteria in Coastal North Sea Waters Suppressed by Selective Grazing Mortality? Appl Environ Microbiol 69 (5): 2624-2630. doi: 10.1128/AEM.69.5.2624-2630.2003

Bentzon-Tilia M, Sonnenschein EC, Gram L (2016) Monitoring and managing microbes in aquaculture – Towards a sustainable industry. Microb Biotechnol 2020, 576-584. doi: 10.1111/1751-7915.12392

Boaventura CM, Coelho FJRC, Martins PT, Pires ACC, Duarte LN, Uetanabaro APT, Cleary DFR, Gomes NCM (2018) Micro-eukaryotic plankton diversity in an intensive aquaculture system for production of *Scophthalmus maximus* and *Solea senegalensis*. Aquaculture 490, 321-328. doi: 10.1016/j.aquaculture.2018.02.033

Bouvier TC, del Giorgio PA (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol Oceanogr 47 (2): 453-470. doi: 10.4319/lo.2002.47.2.0453

Brannock PM, Ortmann AC, Moss AG, Halanych KM (2016) Metabarcoding reveals environmental factors influencing spatio-temporal variation in pelagic micro-eukaryotes. Mol Ecol 25 (15): 3593-3604. doi: 10.1111/mec.13709

Bruijin I, Liu Y, Wiegertjes GF, Raaijmakers JM (2018) Exploring fish microbial communities to mitigate emerging diseases in aquaculture. FEMS Microbiology Ecology, 94 (1): 1-12. doi: 10.1093/femsec/fix161

Buchan A, LeCleir GR, Gulvik CA, González JM (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. Nat Rev Microbiol 12 (10): 686-98. doi: 10.1038/nrmicro3326

Bunse C, Pinhassi J (2017) Marine bacterioplankton seasonal succession dynamics. Trends Microbiol 25(6):494-505. doi: 10.1016/j.tim.2016.12.013

Califano G, Castanho S, Soares F, Ribeiro L, Cox CJ, Mata L, Costa R (2017) Molecular Taxonomic Profiling of Bacterial Communities in a Gilthead Seabream (*Sparus aurata*) Hatchery. Frontiers in Microbiology, 8: 204. doi: 10.3389/fmicb.2017.00204

Chen PC, Chiang KP, Tsai SF (2017) *Pelagostrobilidium liui* n. sp. (Ciliophora, Choreotrichida) from the Coastal Waters of Northeastern Taiwan and an Improved Description of *Pelagostrobilidium minutum* Liu et al., 2012. J Eukaryot Microbiol 64 (5): 579-587. doi: 10.1111/jeu.12392

Cleary DFR, Becking LE, Polónia ARM, Freitas RM, Gomes, NCM (2015) Composition and predicted functional ecology of mussel-associated bacteria in Indonesian marine lakes. Antonie van Leeuwenhoek 107 (3): 821-34. doi:10.1007/s10482-014-0375-1

Coelho FJRC, Cleary DFR, Rocha RJM … Costa R, Pereira R, Gomes NCM (2015) Unraveling the interactive effects of climate change and oil contamination on laboratory simulated estuarine benthic communities. Glob Change Biol 21(5): 1871-1886. doi:10.1111/gcb.12801

D'Alvise PW, Melchiorsen J, Porsby CH, Nielsen KF, Gram L (2010) Inactivation of *Vibrio anguillarum* by Attached and Planktonic Roseobacter Cells. Appl Environ Microbiol 76 (7): 2366-2370

D'Alvise PW, Lillebø S, Prol-Garcia MJ, Wergeland HI, Nielsen KF, Bergh Ø, Gram L (2012) *Phaeobacter gallaeciensis* reduces *Vibrio anguillarum* in cultures of microalgae and rotifers, and prevents vibriosis in cod larvae. PLoS One 7(8): e43996. doi:10.1128/AEM.02717-09

Dang H, Chen R, Wang L, Shao S, Dai L, Ye Y, Guo L, Huang G, Klotz MG (2011) Molecular characterization of putative biocorroding microbiota with a novel niche detection of Epsilon- and Zetaproteobacteria in Pacific Ocean coastal seawaters. Environ Microbiol 13(11): 3059-74. doi:10.1111/j.1462-2920.2011.02583

DG Fisheries (2017) The common fisheries policy (CFP). European Commission - Directorate-General for Maritime Affairs and Fisheries. Available: https://ec.europa.eu/fisheries/cfp/aquaculture/aquaculture_methods_en. Acessed: March 2017.

Duarte LN, Coelho FJRC, Oliveira V, Cleary DFR , Martins P, Gomes NCM (*submitted*) Characterization of Bacterial Communities from a Recirculating Aquaculture System for juvenile sole (*Solea senegalensis*) production.

Dugdale RC, Goering JJ (1967) Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol Oceanogr 12, (2): 196-206. doi: 10.4319/lo.1967.12.2.0196

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10 (10): 996-8. doi:10.1038/nmeth.2604

Edwards P, Lin CK, Yakupitiyage A (2000) Semi-intensive pond aquaculture. In: Beveridge MCM, McAndrew BJ (eds) Tilapias: Biology and Exploitation. Fish and Fisheries Series, vol 25. Springer, Dordrecht

Erlacher A, Cernava T, Cardinale M, Soh J, Sensen CW, Grube M, Berg G (2015) Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of *Lobaria pulmonaria L.* Front Microbiol 6: 53. doi: 10.3389/fmicb.2015.00053

FAO (Food and Agriculture Organization of the United Nations) Aquaculture Newsletter FAN 56, March 2017.

Feichtmayer J, Deng L, Griebler C (2017) Antagonistic Microbial Interactions: Contributions and Potential Applications for Controlling Pathogens in the Aquatic Systems. Front Microbiol 8: 2192. doi: 10.3389/fmicb.2017.02192

Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10(8): 538-50. doi:10.1038/nrmicro2832

Fenchel T (1984) Suspended marine bacteria as a food source. In: Fasham MJR (ed) Flows of energy and materials in marine ecosystems: Theory and Practice. Springer US

Fuhrman JA, Cram JA, Needham DM (2015) Marine microbial community dynamics and their ecological interpretation. Nat Rev Microbiol 13 (3): 133-46. doi:10.1038/nrmicro3417

Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A (2011) Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiol Mol Biol Rev 75 (4): 583-609. doi: 10.1128/MMBR.00020-11

Golden CD, Seto KL, Dey MM, Chen OL, Gephart JA, Myers SS, Smith M, Vaitla B, Allison EH (2017) Does Aquaculture Support the Needs of Nutritionally Vulnerable Nations? Front Mar Sci 4: 159. doi: 10.3389/fmars.2017.00159

Gomes NCM, Cleary DFR, Pinto FN, Egas C, Almeida A, Cunha A, Mendonça-Hagler, Smalla K (2010) Taking root: enduring effect of rhizosphere bacterial colonization in mangroves. PLoS One 5 (11): e14065. doi:10.1371/journal.pone.0014065

Gonzalez JM, Sherr EB, Sherr BF (1990) Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Appl Environ Microbiol 56(3): 583–589

Guedes AC, Malcata FX (2012) Nutritional Value and Uses of Microalgae in Aquaculture. In: Muchlisin ZA (ed) Aquaculture. IntechOpen Limited, UK. doi: 10.5772/1516

Guillou L, Bachar D, Audic S ... Vaulot D, Zimmermann P, Christen R (2013) The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41:(D) 597-604. doi: 10.1093/nar/gks1160

Hamady M, Knight R. Microbial community profiling for human microbiome projects: Tools, techniques, and challenges. Genome Research. 2009;19(7):1141-1152. doi:10.1101/gr.085464.108

Harrel FE Jr with contributions from C Dupont and many others (2016) *Hmisc: Harrell miscellaneous*. R package version *3.17-4*

Hennessy RC, Glaring MA, Olsson S, Stougaard P (2017) Transcriptomic profiling of microbe–microbe interactions reveals the specific response of the biocontrol strain *P. fluorescens* In5 to the phytopathogen *Rhizoctonia solani*. BMC Res Notes 10 (1): 376. doi:10.1186/s13104-017-2704-8

Herfort L, Peterson TD, McCue LA, Zuber P (2011) Protist 18S rRNA gene sequence analysis reveals multiple sources of organic matter to turbidity maxima of the Columbia River estuary. Mar Ecol Prog Ser 438: 19-31. doi: 10.3354/meps09303

Hjelm M, Bergh O, Riaza A, Nielsen J, Melchiorsen J, Jensen S, Duncan H, Ahrens P, Birkbeck H, Gram L (2004) Selection and identification of autochthonous potential probiotic bacteria from turbot larvae (*Scophthalmus maximus*) rearing units. Syst Appl Microbiol 27 (3): 360-371. doi: 10.1078/0723-2020-00256

Hu YO, Karlson B, Charvet S, Andersson AF (2016) Diversity of Pico- to Mesoplankton along the 2000 km Salinity Gradient of the Baltic Sea. Front Microbiol 12 (7): 679. doi: 10.3389/fmicb.2016.00679

Jackson GA (1987) Simulating chemosensory responses of marine microorganisms. Limnol Oceanogr 32 (6): 1253-1266. doi: 10.4319/lo.1987.32.6.1253

Jeong HJ, Kang NS, Moestrup O, Yoo YD, Potvin E (2014) Description of the new heterotrophic dinoflagellate *Stoeckeria changwonensis* n. sp. from Korean coastal waters and emended description of the genus Stoeckeria and the type species *Stoeckeria algicida*. Harmful algae 36: 38-56. doi: 10.1016/j.hal.2014.04.017

Kinnula H, Mappes J, Valkonen JK, Pulkkinen K , Sundberg LR (2017) Higher Resource Level Promotes Virulence in an Environmentally Transmitted Bacterial Fish Pathogen. Evol Appl 10 (5): 462-470. doi: 10.1111/eva.12466

Kirchman DL, Dittel AI, Malmstrom RR, Cottrell MT (2005) Biogeography of major bacterial groups in the Delaware Estuary. Limnol Oceanogr 50 (5): 1697-1706. doi: 10.4319/lo.2005.50.5.1697

Kuczynski J, Stombaugh J, Walters WA, González A, Caporaso JG, Knight R (2011) Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Curr Protoc Bioinformatics 10: 10.7. doi: 10.1002/0471250953.bi1007s36

Lara E, Fernández LD, Schiaffino MR, Izaguirre I (2017) First freshwater member ever reported for the family Bathycoccaceae (Chlorophyta; Archaeplastida) from Argentinean Patagonia revealed by environmental DNA survey. European Journal Protistology 60:45-49. doi: 10.1016/j.ejop.2017.05.008.

Litchman E, Klausmeier CA, Schofield OM, Falkowski PG (2007) The role of functional traits and trade-offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. Ecol Lett 10 (12): 1170-1181. doi: 10.1111/j.1461-0248.2007.01117.x

Lin YC, Campbell T, Chung CC, Gong GC, Chiang KP, Wordenb AZ (2012) Distribution Patterns and Phylogeny of Marine Stramenopiles in the North Pacific Ocean. Appl Environ Microbiol 78 (9): 3387–3399. doi:10.1128/AEM.06952-11

Lozupone CA, Knight R (2007) Global patterns in bacterial diversity. PNAS 104 (27): 11436-11440. doi:10.1073/pnas.0611525104

Luo H, Moran MA (2014) Evolutionary Ecology of the Marine Roseobacter Clade. Microbiol Mol Biol Rev 78 (4): 573-87. doi:10.1128/MMBR.00020-14

Luo YF, Yang TB (2010) Seasonal population dynamics of the monogeneans *Pseudorhabdosynochus coioidesis* and *P. serrani* on wild versus cultured groupers in Daya Bay, South China Sea. Helminthologia 47 (4): 241-250. doi: 10.2478/s11687-010-0038-3

Martins P, Cleary DFR, Pires ACC, Rodrigues AM, Quintino V, Calado R, Gomes NCM (2013) Molecular analysis of bacterial communities and detection of potential pathogens in a recirculating aquaculture system for *Scophthalmus maximus* and *Solea senegalensis*. PLoS One 8 (11): e80847. doi: 10.1371/journal.pone.0080847

Martins P, Coelho FRC, Cleary DFR, Pires ACC, Marques B, Rodrigues AM, Quintino V, Gomes NCM (2018) Seasonal patterns of bacterioplankton in a semi-intensive European seabass (*Dicentrarchus labrax*) aquaculture system. Aquaculture 490: 240-250. doi: 10.1016/j.aquaculture.2018.02.038

Merikanto I, Laakso JT, Kaitala V (2017) Outside-host predation as a biological control against an environmental opportunist disease. Ecol Modell 347:85-93. doi: 10.1016/j.ecolmodel.2017.01.002

Moran MA, Belas R, Schell MA … Thompson LS, Saunders E, Buchan A (2007) Ecological Genomics of Marine Roseobacters. Appl Environ Microbiol 73 (14): 4559–4569. doi: 10.1128/AEM.02580-06

Na H, Kim OS, Yoon SH, Kim Y, Chun J (2011) Comparative approach to capture bacterial diversity of coastal waters. J Microbiol 49 (5): 729-40. doi: 10.1007/s12275-011-1205-z

Nedashkovskaya OI, Kukhlevskiy AD, Zhukova NV, Kim SB (2016) *Amylibacter ulvae* sp. nov., a new alphaproteobacterium isolated from the Pacific green alga *Ulva fenestrate*. Arch Microbiol 198 (3): 251-6. doi: 10.1007/s00203-015-1185-1

Nishitani G, Nagai S, Baba K, Kiyokawa S, Kosaka Y, Miyamura K, Nishikawa T, Sakurada K, Shinada A, Kamiyama T (2010) High-Level Congruence of *Myrionecta rubra* Prey and Dinophysis Species Plastid Identities as Revealed by Genetic Analyses of Isolates from

Japanese Coastal Waters. Appl Environ Microbiol 76 (9): 2791-2798. doi: 10.1128/AEM.02566-09

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2016). vegan: Community Ecology Package. R package version 2.4-1. https://CRAN.R-project.org/package=vegan

Oliva-Teles A (2000) Recent advances in European sea bass and gilthead sea bream nutrition. Aquacult Int 8 (6): 477-492. doi: 10.1023/A:1009236517555

Pereira C, Salvador S, Arrojado C, Silva Y, Santos AL, Cunha A, Gomes NCM, Almeida A (2011) Evaluating seasonal dynamics of bacterial communities in marine fish aquaculture: A preliminary study before applying phage therapy. J Environ Monit 13 (4): 1053-1058. doi: 10.1039/c0em00434k

Piwosz K, Pernthaler J (2010) Seasonal population dynamics and trophic role of planktonic nanoflagellates in coastal surface waters of the Southern Baltic Sea. Environ Microbiol 12 (2): 364-77. doi:10.1111/j.1462-2920.2009.02074.x

Ramanan R, Kim BH, Cho DH, Oh HM, Kim HS (2016) Algae-bacteria interactions: Evolution, ecology and emerging applications. Biotechnol Adv 34 (1): 14-29. doi: 10.1016/j.biotechadv.2015.12.003

Rurangwa E, Verdegem MCJ (2015) Microorganisms in recirculating aquaculture systems and their management. Reviews in Aquaculture 7 (2): 117-130. doi:10.1111/raq.12057

Sanders RW, Porter KG, Bennett SJ, Debiase AE (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol Oceanogr 34 (4): 673-687. doi: 10.4319/lo.1989.34.4.0673

Schmidt V, Amaral-Zettler L, Davidson J, Summerfelt S, Good C (2016) Influence of Fishmeal-Free Diets on Microbial Communities in Atlantic Salmon (*Salmo salar*) Recirculation Aquaculture Systems. Appl Environ Microbiol 82 (15): 4470-4481. doi: 10.1128/AEM.00902-16

Schryver P, Vadstein O (2014) Ecological theory as a foundation to control pathogenic invasion in aquaculture. ISME J 8 (12): 2360-8. doi:10.1038/ismej.2014.84

Seyedsayamdost MR, Case RJ, Kolter R, Clardy J (2011) The Jekyll-and-Hyde chemistry of Phaeobacter gallaeciensis. Nat Chem 3(4): 331-5. doi:10.1038/nchem.1002

Sherr EB, Sherr BF (2002) Significance of predation by protists in aquatic microbial food webs. Antonie van Leeuwenhoek 81 (1-4): 293-308. doi: 10.1023/A:1020591307260

Soliman NF, Yacout DMM (2016) Aquaculture in Egypt: status, constraints and potentials. Aquacult Int 24, 1201-1227. doi: 10.1007/s10499-016-9989-9

Stoeck T, Bass D, Nebel, M, Christen R, Jones MDM, Breiner H-W, Richards TA (2010) Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol 19 (1): 21-31. doi: 10.1111/j.1365-294X.2009.04480.x

Stopnisek N, Zühlke D, Carlier A, Barberán A, Fierer N, Becher D, Riedel K, Eberl L, Weisskopf L (2016) Molecular mechanisms underlying the close association between soil Burkholderia and fungi. ISME J 10 (1): 253-64. doi:10.1038/ismej.2015.73

Strom SL (2008) Microbial ecology of ocean biogeochemistry: a community perspective. Science 320 (5879): 1043-5. doi: 10.1126/science.1153527

Tal Y, Schreier HJ, Sowers KR, Stubblefield JD, Place AR, Zohara Y (2009) Environmentally sustainable land-based marine aquaculture. Aquaculture 286 (1): 28-35. doi: 10.1016/j.aquaculture.2008.08.043

Theodorou JA (2002) Current and Future Technological Trends of European Seabass-Seabream Culture. Rev Fish Sci 10 (3-4): 529-543. doi: 10.1080/20026491051776

Thomas F, Hehemann JH, Rebuffet E, Czjzek M, Michel G (2011) Environmental and gut bacteroidetes: the food connection. Front Microbiol 2: 93. doi: 10.3389/fmicb.2011.00093

Ulaganathan K, Goud BS, Reddy MM, Kumar VP, Radhakrishna S, Balsingh J (2015) Genome Sequence of *Saccharomyces cerevisiae* NCIM3107, Used in Bioethanol. Genome Announc 3 (1): e01557-14. doi: 10.1128/genomeA.01557-14

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255 (2): 571-586. doi: 10.1023/A:1026037216893

Yue GH, Wang L (2017) Current status of genome sequencing and its applications in aquaculture. Aquaculture 468: 337-347. doi:10.1016/j.aquaculture.2016.10.036

Wang Q, Garrity GM, Tiedje JM, Cole, JR (2007) Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73 (16): 5261–5267. doi: 10.1128/aem.00062-07

Wang Y, Qian PY (2009) Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One 4 (10): e7401. doi: 10.1371/journal.pone.0007401

Wei T, Simko V (2016) *corrplot:* Visualization of a Correlation Matrix. *R package version 0.77*, https://cran.r-project.org/web/packages/corrplot/index.html

Washbourne IJ, Crenshaw CL, Baker MA (2011) Dissimilatory nitrate reduction pathways in an oligotrophic aquatic ecosystem: spatial and temporal trends. Aquat Microb Ecol 65: 55-64. doi: 10/3354/ame01538

Zehr JP, Ward BB (2002) Nitrogen Cycling in the Ocean: New Perspectives on Processes and Paradigms. Appl Environ Microbiol 68 (3) 1015-1024. doi: 10.1128/AEM.68.3.1015-1024.2002

Zhang Z, Schwartz S, Wagner L, Miller WA (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7: 1-2. doi: 10.1089/10665270050081478

Conclusion and Final Remarks

Conclusion and Final Remarks

Global population will reach more than 10 billion by 2100. Consequently, food production will have to double during this period. Currently aquaculture is one of the fastest growing food-producing sector and is considered a strategic sector for animal protein supply for human consume at global scale and especially relevant in developing regions. According to the Food and Agriculture Organization of the United Nations (FAO), since 2014 the aquaculture sector is responsible for providing more fish (73.8 million tonnes) for human consumption than wild-catch fisheries and it is expected that fish production will double in 2030. However, despite of the recent developments of the aquaculture sector, disease outbreaks are considered a limiting factor for the development of aquaculture worldwide, which may also face higher incidence of diseases due to climate change. In response to these challenges, chemical and cellular modulators known as prebiotics and probiotics (respectively) can be used to explore host-microbe interactions to influence fish immunity and disease resistance. More recently, there is a growing understand that the use of these modulators, aligned with strategies based on ecological principles, can be used to promote microbiome modulation in aquaculture environment (water and biofilm) and prevent or influence fish pathogen development (as opposed to antibiotic use). Furthermore, there is an increase demand for cleaner and eco-friendly production systems, in addition to healthy fish reared without chemical additives or antibiotics. Strategies that take advantage of the potential of microbial communities would be well aligned with environmental concerns and contribute for development of environmentally friendly and sustainable fish farming practices. However, priory microbial modulation strategies can be used in fish farming environment; it is of paramount importance to develop baseline knowledge about the diversity and ecology of microbial communities in the aquaculture systems.

In chapter 1 we presented an overview of aquaculture status, the need for growth in the aquaculture sector, their challenges and major risks. Currently, there is a growing interest on the microbial communities in aquacultures, especially on the structure and function of fish gut microbiome and probiotics. In less intensity, but still highly relevant, different studies have investigated the contribution of microbial plankton communities and biofilms for maintenance of aquaculture water quality and fish health. Curiously, no study has investigated the aquaculture microbiome as a whole up to now (water, biofilms and fish microbial communities). Although pathogenic microorganisms are a major constraint in the aquaculture industry, microbial communities are fundamental to its functioning since they participate in important environmental processes such as the cycling of nutrients to maintain water quality and fish health. In addition, strategies to manipulate/modulate aquaculture microbiome can be an alternative to antibiotics and contribute to maintain a healthier aquaculture environment. However, the use microbiome data for the development of better aquaculture practices is still in its infancy. Research on aquaculture microbiome has started to allow us to understand microbe interactions (microbe-microbe, -environment and -host interactions) and their role in the aquaculture environment. However, more studies are necessary in order to improve our understating on what constitutes a healthy aquaculture microbiome and how we can use this knowledge to promote more environmentally friendly and sustainable fish production systems.

In Chapter 2, we characterized the composition of bacterioplankton communities of a RAS for production of sole juveniles and compared the results obtained with the communities of a grow-out sole RAS that was characterized in a previous study (Martins et al., 2013). Interistingly, our results showed that despite the differences in relative abundance, the orders Alteromonadales, Rhodobacterales, Oceanospirillales, Vibrionales and Flavobacteriales were the most abundant bacterial groups in both aquaculture systems. However, in contrast to our

findings for grow-out RAS, our results indicated that the bacterial assemblage of the supply water played an important role as a 'seed' bank for the colonization of bacterial populations [e.g. Pseudoalteromonas sp., members of the Roseobacter clade (*Phaeobacter arcticus* and *Sedimentitalea todarodis*) and Sulfidobacteria] in the hatchery RAS. Most remarkable, supply water seems to contribute for a strong colonization of Pseudoalteromonas genus and the Roseobacter clade in the hatchery RAS, these groups are known to comprise bacterial members with activity against Vibrio fish pathogens. Our findings suggest that the bacterial composition of the water supply may influence the composition of the bacterioplankton of sole hatchery RAS. However, taking in consideration the results obtained for sole grow-out RAS (Martins et al., 2013), the intensity of this effect may vary between different RAS. Our results emphasize the importance of the water supply on the composition of the aquaculture microbiome and highlight its importance as seed bank for the colonization of bacterial populations with putative antagonism activity against fish pathogens in the RAS.

In Chapter 3 we described, for the first time, the seasonal dynamics and potential interactions of bacterial and microeukaryotic plankton communities in a semi-intensive aquaculture for European sea bass cultured together with low density of gilthead sea bream over one-year period. This study demonstrated that while the most abundant bacterial classes were Gammaproteobacteria, Flavobacteriia and Alphaproteobacteria; microeukaryotic communities were dominated by Ochrophyta, Chlorophyta and Ciliophora groups. Temperature, salinity and nitrate were the environmental parameters that had the higher influence in both bacterial and microeukaryotic communities. Interestingly, besides the potential effects of the abiotic parameters on the plankton microbiome, there was a strong correlation in the temporal variation of bacterial and microeukaryotic communities. Therefore, suggesting trophic and/or metabolic interdependence between these two domains during fish production. Previous studies showed that the presence of some microeukaryotes can provide

good conditions for beneficial bacteria, causing the bacteria to proliferate or become active against pathogen (Elena et al, 2014; Merikanto et al., 2017). In addition, microeukaryotes can prey on both bacterial pathogens and probiotic bacteria and can significantly influence the composition and abundance of the bacterial communities in the system (Fuhrman and Noble, 1995). Therefore, complex interactions between microeukaryotes and prokaryotes in aquaculture systems may have different impacts on the function, water quality and fish host health. Overall, this thesis provides a baseline characterization of the diversity and putative role of bacterial and microeukaryotic plankton communities in intensive and semi-intensive aquaculture systems. Our results showed that the study of the diversity and ecological interactions of microbial communities in aquaculture systems could provide the bases to develop strategies to prevent or influence fish pathogen development, with potential to support the development of more sustainable fish farming practices.

We emphasized that water should be the main target to manage and maintain fish health since the microorganisms present in it interact directly with the host's microbiota as a whole (skin, gills, gut, contaminants, food and feces). The search for the aquaculture site as well as the balance of water renewal in RAS is crucial since supply water has proved to be a relevant source of naturally probiotic bacteria. Chemical, physical and microbiological quality of water is important for aquaculture systems. Microorganisms do not exist in isolation, the complex interactions they exert may have different impacts on the environment or host. Further studies on these interactions should be performed in the laboratory before being applied in aquaculture enterprises. Microcosm systems may be the key to bringing this information quickly to commercial use as they would simulate small-scale interactions beneficial or not that could occur in these systems, avoid diseases outbreaks and searching for a healthy and eco-friendly aquaculture pratices. In addition, the next steps towards the knowledge of the function and ecology of microbial communities will benefit from recent advances in computer

and biostatistical tools that can be used to guide prebiotic and probiotic selection in the aquaculture sector.

Literature cited

Ababouch L, Vannuccini S, Chomo V (2016) 2.2 Fisheries, aquaculture utilization and trade: challenges and opportunities. In Fish Trade – part 2, Trade in sustainable fisheries and aquaculture. Food and Agriculture Organization of the United Nations (FAO). Available at: http://unctad.org/en/PublicationChapters/ditcted2016d3_part2_en.pdf. Acessed: apr/2018

Actis LA, Tolmasky ME, Crosa JH (2011) Vibriosis (Chapter 15). In: Woo PTK, Bruno DW (ed) Fish diseases and disorders, vol. 3: viral, bacterial, and fungal infections, 2nd ed. CABI International, pp 570–605. ISBN: 781845935542. doi:10.1079/9781845935542.0000.

Ali SM, Khan AA, Ahmed I, Musaddiq M, Ahmed KS, Polasa H, Rao LV, Habibullah CM, Sechi LA, Ahmed N (2005) Antimicrobial activities of Eugenol and Cinnamaldehyde against the human gastric pathogen Helicobacter pylori. Annals of Clinical Microbiology and Antimicrobials 4: 20. doi: 10.1186/1476-0711-4-20

Aly SM, Albutti A (2014) Antimicrobials Use in Aquaculture and their Public Health Impact. J Aquac Res Development 5:247. doi: 10.4172/2155-9546.1000247

Amaral-Zettler L, Artigas LF, Baross J, Boetius AA, Chandramohan D, Herndl G, Kogure K, Neal P, Pedrós-Alió C, Ramette A, Schouten S, Stal LJ, Thessen A, De Leeuw JW, Sogin M (2010) A global census of marine microbes. In: McIntyre A (ed) Life in the World's Oceans: Diversity, Distribution, and Abundance. Hoboken, NJ: Wiley-Blackwell. doi: 10.1002/9781444325508.ch12

Atienza GAMV, Arafiles KHV, Carmona MCM, Garcia JPC, Macabago AMB, Peñacerrada BJDC, Cordero PRF, Bennett RM and Dedeles GR (2012) Carotenoid analysis of locally isolated Thraustochytrids and their potential as an alternative fish feed for *Oreochromis niloticus* (Nile tilapia). Mycosphere 3: 420-428. doi: 10.5943/mycosphere/3/4/5

Attramadal KJK, Salvesen I, Xue R, Øie G, Størseth TR, Vadstein O, Olsen Y (2012) Recirculation as a possible microbial control strategy in the production of

marine larvae. Aquacultural Engineering 46: 27-39. doi: 10.1016/j.aquaeng.2011.10.003.

Attramadal KJK, Truong TMH, Bakke I, Skjermo J, Olsen Y, Vadstein O (2014) RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. Aquaculture 432: 483-490. doi:10.1016/j.aquaculture.2014.05.052

Auclair J, Lépine F, Parent S, Villemur R (2010) Dissimilatory reduction of nitrate in seawater by a Methylophaga strain containing two highly divergent narG sequences. The ISME Journal. doi:10.1038/ismej.2010.47.

Avnimelech Y (1999) Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture 176: 227-235. doi: 10.1016/S0044-8486(99)00085-X.

Badiola M, Mendiola D, Bostock J (2012) Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. Aquacultural Engineering 51: 26-35. doi:10.1016/j.aquaeng.2012.07.004

Bakshani CR, Morales-Garcia AL, Althaus M, Wilcox MD, Pearson JP, Bythell JC, Burgess JG (2018) Evolutionary conservation of the antimicrobial function of mucus: a first defence against infection. npj Biofilms and Microbiomes 4 (1), 14. doi: 10.1038/s41522-018-0057-2.

Baltar F, Palovaara J, Unrein F, Catala P, Horňák K, Šimek K, Vaqué D, Massana R, Gasol JM, Pinhassi J (2016) Marine bacterial community structure resilience to changes in protist predation under phytoplankton bloom conditions. The ISME Journal 10 (3):568-81. doi: 10.1038/ismej.2015.135.

Baluyut EA (1989) Aquaculture Systems and Practices: A Selected Review. United Nations Development Programme, Food and Agriculture Organization Of The United Nations (FAO), ADCP/REP/89/43. FAO Corporate Document Repository, Produced by Fisheries and Aquaculture Departament. Available in: http://www.fao.org/docrep/t8598e/t8598e05.htm. Acessed in: aug/2017.

Barg UC (1992) Guidelines for the promotion of environmental management of coastal aquaculture development. FAO Fisheries Technical Paper 328

Beardsley C, Pernthaler J, Wosniok W, Amann R (2003) Are Readily Culturable Bacteria in Coastal North Sea Waters Suppressed by Selective Grazing Mortality? Appl Environ Microbiol 69 (5): 2624-2630. doi: 10.1128/AEM.69.5.2624-2630.2003

Becerra-Dórame MJ, Martínez-Porchas M, Martínez-Córdova LR, Rivas-Vega ME, Lopez-Elias JA, Porchas-Cornejo MA (2012) Production response and digestive enzymatic activity of the Pacific white shrimp *Litopenaeus vannamei* (Boone, 1931) Intensively pregrown in microbial heterotrophic and autotrophic-based systems. The Scientific World Journal 2012: 723654. doi:10.1100/2012/723654.

Benhamed S, Guardiola FA, Mars M, Esteban MÁ (2014) Pathogen bacteria adhesion to skin mucus of fishes. Veterinary Microbiology 25, 171(1-2):1-12. doi: 10.1016/j.vetmic.2014.03.008.

Bentzon-Tilia M, Sonnenschein EC, Gram L (2016) Monitoring and managing microbes in aquaculture – Towards a sustainable industry. Microb Biotechnol 2020, 576-584. doi: 10.1111/1751-7915.12392.

Birkbeck TH, Feist SW, Verner-Jeffreys DW (2011) Francisella infections in fish and shellfish. Journal of Fish Diseases. doi: 10.1111/j.1365-2761.2010.01226.x.

Bisht A, Singh UP, Pandey NN (2014) Comparative study of seasonal variation in bacterial flora concomitant with farm raised fingerlings of *Cyprinus carpio* at tarai region of Uttarakhand. Journal of Environmental Biology 35(2):363-367. ISSN: 0254-8704.

Blancheton JP, Attramadal KJK, Michaud L, Roque D'orbcastel E, Vadstein O (2013) Insight into the bacterial population in aquaculture systems and its implication. Aquacultural Engineering. doi:10.1016/j.aquaeng.2012.11.009.

Boaventura CM, Coelho FJRC, Martins PT, Pires ACC, Duarte LN, Uetanabaro APT, Cleary DFR, Gomes NCM (2018) Micro-eukaryotic plankton diversity in an intensive aquaculture system for production of *Scophthalmus maximus*

and *Solea senegalensis*. Aquaculture 490, 321-328. doi: 10.1016/j.aquaculture.2018.02.033

Bourne DG, Young N, Webster N, Payne M, Salmon M, Demel S, Hall M (2004) Microbial community dynamics in a larval aquaculture system of the tropical rock lobster, *Panulirus ornatus*. Aquaculture. doi:10.1016/j.aquaculture.2004.08.047.

Boutin S, Bernatchez L, Audet C and Derome N (2013) Network Analysis Highlights Complex Interactions between Pathogen, Host and Commensal Microbiota. Plos One 8(12): e84772. doi:10.1371/journal.pone.0084772.

Bouvier TC, del Giorgio PA (2002) Compositional changes in free-living bacterial communities along a salinity gradient in two temperate estuaries. Limnol Oceanogr 47 (2): 453-470. doi: 10.4319/lo.2002.47.2.0453

Bowman JP, McMeekin TA (2005) Order X. Alteromonadales ord. nov. Bergey's Manual of Systematic Bacteriology, Vol 2 (Brenner DJ Krieg NR, Stanley JT, Garrity GM , eds), pp. 443 –491. Springer, New York, NY

Brannock PM, Ortmann AC, Moss AG, Halanych KM (2016) Metabarcoding reveals environmental factors influencing spatio-temporal variation in pelagic micro-eukaryotes. Mol Ecol 25 (15): 3593-3604. doi: 10.1111/mec.13709

Bregnballe J (2015) A Guide to Recirculation Aquaculture - An introduction to the new environmentally friendly and highly productive closed fish farming systems. Food and Agriculture Organization of the United Nations (FAO) and EUROFISH International Organisation.

Bruijin I, Liu Y, Wiegertjes GF, Raaijmakers JM (2018) Exploring fish microbial communities to mitigate emerging diseases in aquaculture. FEMS Microbiology Ecology, 94 (1): 1-12. doi: 10.1093/femsec/fix161

Buchan A, LeCleir GR, Gulvik CA, González JM (2014) Master recyclers: features and functions of bacteria associated with phytoplankton blooms. Nat Rev Microbiol 12 (10): 686-98. doi: 10.1038/nrmicro3326

Buffie CG, Bucci V, Stein RR, McKenney PT, Ling L, Gobourne A, No D, Liu H, Kinnebrew M, Viale A, Littmann E, van den Brink MR, Jenq RR, Taur Y, Sander C, Cross JR, Toussaint NC, Xavier JB, Pamer EG (2014) Precision microbiome reconstitution restores bile acid mediated resistance to *Clostridium difficile*. Nature 8; 517(7533):205-8. doi: 10.1038/nature13828.

Bunse C, Pinhassi J (2017) Marine bacterioplankton seasonal succession dynamics. Trends Microbiol 25(6):494-505. doi: 10.1016/j.tim.2016.12.013

Burr G, Hume M, Ricke S, Nisbet D, Gatlin D (2010) In Vitro and In Vivo Evaluation of the Prebiotics GroBiotic®-A, Inulin, Mannanoligosaccharide, and Galactooligosaccharide on the Digestive Microbiota and Performance of Hybrid Striped Bass (*Morone chrysops* × *Morone saxatilis*). Microbial Ecology 59: 187-198. doi: 10.1007/s00248-009-9597-6.

Byun SO, Fang Q, Zhou H, Hickford JGH (2009) An effective method for silver-staining DNA in large numbers of polyacrylamide gels. Analytical Biochemistry. doi: 10.1016/j.ab.2008.10.024.

Califano G, Castanho S, Soares F, Ribeiro L, Cox CJ, Mata L, Costa R (2017) Molecular Taxonomic Profiling of Bacterial Communities in a Gilthead Seabream (Sparus aurata) Hatchery. Frontiers in Microbiology, 8: 204. doi: 10.3389/fmicb.2017.00204

Caporaso JG, Kuczynski J, Stombaugh J … Yatsunenko T, Zaneveld J, Knight R (2010) QIIME allows analysis of high-throughput community sequencing data. Nature Methods. doi:10.1038/nmeth.f.303.

Cardona E, Gueguen Y, Magré K ... Pierrat F, Noguier F, Saulnier D (2016) Bacterial community characterization of water and intestine of the shrimp *Litopenaeus stylirostris* in a biofloc system. BMC Microbiology 16: 157. doi: 10.1186/s12866-016-0770-z.

Chen PC, Chiang KP, Tsai SF (2017) *Pelagostrobilidium liui* n. sp. (Ciliophora, Choreotrichida) from the Coastal Waters of Northeastern Taiwan and an Improved Description of *Pelagostrobilidium minutum* Liu et al., 2012. J Eukaryot Microbiol 64 (5): 579-587. doi: 10.1111/jeu.12392

Chopin T (2006) Integrated Multi-Trophic Aquaculture. What it is and why you should care and don't confuse it with polyculture. Commentary in Northern Aquaculture, July/August 2006 page 4.

Cleary DFR, Smalla K, Mendonça-Hagler L, Gomes NCM (2012) Assessment of variation in bacterial composition among microhabitats in a mangrove environment using DGGE and barcoded pyrosequencing. PLoS One. doi:10.1371/journal.pone.0029380.

Cleary DFR, Becking LE, Polónia ARM, Freitas RM, Gomes, NCM (2015) Composition and predicted functional ecology of mussel-associated bacteria in Indonesian marine lakes. Antonie van Leeuwenhoek. doi:10.1007s10482-014-0375-1.

Coelho FJRC, Cleary DFR, Rocha RJM … Costa R, Pereira R, Gomes NCM (2015) Unraveling the interactive effects of climate change and oil contamination on laboratory simulated estuarine benthic communities. Global Change Biology. doi:10.1111/gcb.12801.

Crab R, Chielens B, Wille M, Bossier P, Verstraete W (2010) The effect of different carbon sources on the nutritional value of bioflocs, a feed for *Macrobrachium rosenbergii* postlarvae. Aquaculture Research 41: 559-567. doi: 10.1111/j.1365-2109.2009.02353.x.

D'alvise PW, Melchiorsen J, Porsby CH, Nielsen KF, Gram L (2010) Inactivation of Vibrio anguillarum by attached and planktonic Roseobacter cells. Applied and Environmental Microbiology. doi 10.1128/AEM.02717-09

D'Alvise PW, Lillebø S, Prol-Garcia MJ, Wergeland HI, Nielsen KF, Bergh O, Gramet L (2012) Phaeobacter gallaeciensis reduces Vibrio anguillarum in cultures of microalgae and rotifers, and prevents vibriosis in cod larvae. PLoS One. doi:10.1371/journal.pone.0043996

Dang H, Chen R, Wang L, Shao S, Dai L, Ye Y, Guo L, Huang G, Klotz MG (2011) Molecular characterization of putative biocorroding microbiota with a novel niche detection of Epsilon- and Zetaproteobacteria in Pacific Ocean coastal seawaters. Environ Microbiol 13(11): 3059-74. doi:10.1111/j.1462-2920.2011.02583

De Schrijver R, Ollevier F (2000) Protein digestion in juvenile turbot (*Scophthalmus maximus*) and effects of dietary administration of *Vibrio proteolyticus*. Aquaculture 186: 107-116. doi: 10.1016/S0044-8486(99)00372-5.

Defoirdt T, Halet D, Vervaeren H, Boon N, Van de Wiele T, Sorgeloos P, Bossier P, Verstraete W (2007) The bacterial storage compound poly-β-hydroxybutyrate protects *Artemia franciscana* from pathogenic *Vibrio campbellii*. Environmental Microbiology 9(2): 445-452. doi:10.1111/j.1462-2920.2006.01161.x

Defoirdt T, Sorgeloos P, Bossier P (2011) Alternatives to antibiotics for the control of bacterial disease in aquaculture. Current Opinion in Microbiology 14: 251-258. doi: 10.1016/j.mib.2011.03.004.

DeLong DP, Losordo TM (2012) How to Start a Biofilter. Southern Regional Aquaculture Center, SRAC Publication No. 4502.

DG Fisheries (2017) The common fisheries policy (CFP). European Commission - Directorate-General for Maritime Affairs and Fisheries. Available: https://ec.europa.eu/fisheries/cfp/aquaculture/aquaculture_methods_en. Acessed: March 2017.

Dimitroglou A, Davies SJ, Sweetman J, Divanach P, Chatzifotis S (2010) Dietary supplementation of mannan oligosaccharide on white sea bream (*Diplodus sargus* L.) larvae: effects on development, gut morphology and salinity tolerance. Aquaculture Research 41: e245-e251. doi: 10.1111/j.1365-2109.2010.02513.x.

Dittmann KK, Rasmussen BB, Castex M, Gram L and Bentzon-Tilia M (2017) The aquaculture microbiome at the centre of business creation. Microbial Biotechnology 10: 1279-1282. doi: 10.1111/1751-7915.12877.

Doroteo AM, Pedroso FL, Lopez JDM, Apines-Amar MJS (2018) Evaluation of potential probiotics isolated from saline tilapia in shrimp aquaculture. Aquaculture International. doi: 10.1007/s10499-018-0270-2.

Duarte LN, Coelho FJRC, Oliveira V, Cleary DFR , Martins P, Gomes NCM (submitted) Characterization of Bacterial Communities from a Recirculating Aquaculture System for juvenile sole (*Solea senegalensis*) production.

Dugdale RC, Goering JJ (1967) Uptake of new and regenerated forms of nitrogen in primary productivity. Limnol Oceanogr 12, (2): 196-206. doi: 10.4319/lo.1967.12.2.0196

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat Methods 10 (10): 996-8. doi:10.1038/nmeth.2604

Edwards P, Lin CK, Yakupitiyage A (2000) Semi-intensive pond aquaculture. In: Beveridge MCM, McAndrew BJ (eds) Tilapias: Biology and Exploitation. Fish and Fisheries Series, vol 25. Springer, Dordrecht

Ekasari J, Crab R, Verstraete W (2010) Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. HAYATI Journal of Biosciences 17: 125-130. doi: 10.4308/hjb.17.3.125.

Elena MPM, Miroslav M, Teresa CGM (2004) Do protozoa control the elimination of *Vibrio cholerae* in brackish water? International Review of Hydrobiology 89: 215-227. doi: 10.1002/iroh.200310644.

EL-Haroun ER, Goda AM, Kabir Chowdhury MA (2006) Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquaculture Research 37: 1473-1480. doi: 10.1111/j.1365-2109.2006.01584.x.

Elliott, D (2011) The skin. The Many Functions of Fish Integument. Encyclopedia of fish physiology: from genome to environment. 1. 471-475. doi:10.1016/B978-1.

Emami K, Nelson A, Hack E, Zhang J, Green DH, Caldwell GS, Mesbahi E (2016) MALDI-TOF mass spectrometry discriminates known species and marine environmental isolates of Pseudoalteromonas. Frontiers in Microbiology. doi:10.3389/fmicb.2016.00104.

Epstein SS (1997) Microbial Food Webs in Marine Sediments. II. Seasonal Changes in Trophic Interactions in a Sandy Tidal Flat Community. Microbial Ecology, 34 (3): 199–209. doi: 10.1007/s002489900049

Erlacher A, Cernava T, Cardinale M, Soh J, Sensen CW, Grube M, Berg G (2015) Rhizobiales as functional and endosymbiontic members in the lichen symbiosis of *Lobaria pulmonaria* L. Front Microbiol 6: 53. doi: 10.3389/fmicb.2015.00053

European Comission. Aquaculture methods. Available in: https://ec.europa.eu/fisheries/cfp/aquaculture/aquaculture_methods_en

European Union (2018) European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003.

FAO/WHO (2001) Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotics in Food including Powder Milk with Live Lactic Acid Bacteria. ISSN: 0254-4725

FAO's Director-General on How to Feed the World in 2050 (2009) Population and Development Review. doi:10.1111/j.1728-4457.2009.00312.x.

FAO (2016). The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

FAO (Food and Agriculture Organization of the United Nations) Aquaculture Newsletter FAN 56, March 2017.

FAO (2014-2018) Cultured Aquatic Species Information Programme. *Solea solea*. Cultured Aquatic Species Information Programme. Text by Colen, R, Ramalho, A, Rocha, F, Dinis, MT In: FAO Fisheries and Aquaculture Department [online]. Rome. Updated 18 February 2014. [Cited 24 May 2018]

Faust K, Raes J (2012) Microbial interactions: from networks to models. Nat Rev Microbiol 10(8): 538-50. doi:10.1038/nrmicro2832

Feichtmayer J, Deng L, Griebler C (2017) Antagonistic Microbial Interactions: Contributions and Potential Applications for Controlling Pathogens in the Aquatic Systems. Front Microbiol 8: 2192. doi: 10.3389/fmicb.2017.02192

Fenchel T (1984) Suspended marine bacteria as a food source. In: Fasham MJR (ed) Flows of energy and materials in marine ecosystems: Theory and Practice. Springer US

Fernandez S, Goldsby KA (2015) Construction and Implementation of a Bench-Top Aquaponic System. Honors Theses, Department of Biological Science. The Florida State Universitycollege of Arts & Sciences.

Frey-Klett P, Burlinson P, Deveau A, Barret M, Tarkka M, Sarniguet A (2011) Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiol Mol Biol Rev 75 (4): 583-609. doi: 10.1128/MMBR.00020-11

Fuhrman JA, Cram JA, Needham DM (2015) Marine microbial community dynamics and their ecological interpretation. Nat Rev Microbiol 13 (3): 133-46. doi:10.1038/nrmicro3417

Fuhrman JA, Noble RT (1995) Viruses and protists cause similar bacterial mortality in coastal seawater. Limnology and Oceanography 40 (7): 1236-1242 . doi: 10.4319/lo.1995.40.7.1236

Fuller R (1989) Probiotics in man and animals. Journal of Applied Bacteriology 66(5): 365-378.

Funge-Smith S, Phillips MJ (2001) Aquaculture systems and species. In R.P. Subasinghe, P. Bueno, M.J. Phillips, C. Hough, S.E. McGladdery and J.R. Arthur, eds. Aquaculture in the Third Millennium. Technical Proceedings of the Conference on Aquaculture in the Third Millennium, Bangkok, Thailand, 20-25 February 2000. pp. 129-135. NACA, Bangkok and FAO, Rome.

Garrity GM, Bell JA, Lilburn T (2005) Class II. Betaproteobacteria class. nov. (Chapter) In: Bergey's Manual of Systematic Bacteriology (Book). Springer US. ISBN 978-0-387-29298-4. doi:10.1007/978-0-387-29298-4_2.

Geraylou Z, Souffreau C, Rurangwa E, D'Hondt S, Callewaert L, Courtin CM, Delcour JA, Buyse J, Ollevier F (2012) Effects of arabinoxylan-oligosaccharides (AXOS) on juvenile Siberian sturgeon (*Acipenser baerii*) performance, immune

responses and gastrointestinal microbial community. Fish & Shellfish Immunology 33 (4): 718-724. doi: 10.1016/j.fsi.2012.06.010

Ghosh S, Sinha A, Sahu C (2007) Effect of probiotic on reproductive performance in female livebearing ornamental fish. Aquaculture Research 38: 518-526. doi: 10.1111/j.1365-2109.2007.01696.x.

Giatsis C, Sipkema D, Smidt H, Heilig H, Benvenuti G, Verreth J, Verdegem M (2015) The impact of rearing environment on the development of gut microbiota in tilapia larvae. Scientific Reports 5:18206. doi: 10.1038/srep18206.

Glencross BD (2009) Exploring the nutritional demand for essential fatty acids by aquaculture species. Reviews in Aquaculture 1: 71-124. doi: 10.1111/j.1753-5131.2009.01006.x.

Golden CD, Seto KL, Dey MM, Chen OL, Gephart JA, Myers SS, Smith M, Vaitla B, Allison EH (2017) Does Aquaculture Support the Needs of Nutritionally Vulnerable Nations? Front Mar Sci 4: 159. doi: 10.3389/fmars.2017.00159

Gomes NCM, Heuer H, Schönfeld J, Costa R, Mendonça-Hagler L, Smalla K (2001) Bacterial diversity of the rhizosphere of maize (*Zea mays*) grown in tropical soil studied by temperature gradient gel electrophoresis. Plant and Soil. doi: 10.1023/A:1010350406708.

Gomes NCM, Borges LR, Paranhos R, Pinto FN, Mendonça-Hagler LCS, Smalla K (2008) Exploring the diversity of bacterial communities in sediments of urban mangrove forests. FEMS Microbiology Ecology. doi:10.1111/j.15746941.2008.00519.x.

Gomes NCM, Cleary DFR, Pinto FN, Egas C, Almeida A, Cunha A, Mendonça-Hagler, Smalla K (2010) Taking root: enduring effect of rhizosphere bacterial colonization in mangroves. PLoS One 5 (11): e14065. doi:10.1371/journal.pone.0014065

Gómez GD, Balcázar JL (2008) A review on the interactions between gut microbiota and innate immunity of fish. FEMS Immunology & Medical Microbiology 52 (2): 145–154. doi: 10.1111/j.1574-695X.2007.00343.x.

Gonzalez JM, Sherr EB, Sherr BF (1990) Size-selective grazing on bacteria by natural assemblages of estuarine flagellates and ciliates. Appl Environ Microbiol 56(3): 583–589

Guarner F, Malagelada JR (2003) Gut flora in health and disease. Lancet 361(9356):512–519. doi: 10.1016/S0140-6736(03)12489-0.

Guedes AC, Malcata FX (2012) Nutritional Value and Uses of Microalgae in Aquaculture. In: Muchlisin ZA (ed) Aquaculture. IntechOpen Limited, UK. doi: 10.5772/1516

Guerrero S, Cremades J (2012) Integrated Multi-trophic Aquaculture (IMTA): A sustainable, pioneering alternative for marine cultures in Galicia. J. Regional Government of Galicia (Spain), pp.111, 13:978-84-695-3260-7, <hal-00743395>.

Guillou L, Bachar D, Audic S ... Vaulot D, Zimmermann P, Christen R (2013) The Protist Ribosomal Reference database (PR2): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Res 41:(D) 597-604. doi: 10.1093/nar/gks1160

Gupta RS, Mok A (2007) Phylogenomics and signature proteins for the alpha proteobacteria and its main groups. BMC Microbiology. doi: 10.1186/1471-2180-7-106.

Gutiérrez-Barranquero JA, Reen FJ, McCarthy RR, O'Gara F (2015) Deciphering the role of coumarin as a novel quorum sensing inhibitor suppressing virulence phenotypes in bacterial pathogens. Applied Microbiology and Biotechnology 99: 3303-3316. doi: 10.1007/s00253-015-6436-1.

Hall SJ, Delaporte A, Phillips MJ, Beveridge M, O'Keefe M (2011) Blue Frontiers: Managing the Environmental Costs of Aquaculture. The World Fish Center, Penang, Malaysia.

Hammock D, Huang CC, Mort G, Swinehart JH (2003) The Effect of Humic Acid on the Uptake of Mercury(II), Cadmium(II), and Zinc(II) by Chinook Salmon (*Oncorhynchus tshawytscha*) Eggs. Archives of Environmental Contamination and Toxicology 44: 0083-0088. doi: 10.1007/s00244-002-1261-9.

Harrel FE Jr with contributions from C Dupont and many others (2016) Hmisc: Harrell miscellaneous. R package version 3.17-4

Hennessy RC, Glaring MA, Olsson S, Stougaard P (2017) Transcriptomic profiling of microbe–microbe interactions reveals the specific response of the biocontrol strain *P. fluorescens* In5 to the phytopathogen *Rhizoctonia solani*. BMC Res Notes 10 (1): 376. doi:10.1186/s13104-017-2704-8

Herfort L, Peterson TD, McCue LA, Zuber P (2011) Protist 18S rRNA gene sequence analysis reveals multiple sources of organic matter to turbidity maxima of the Columbia River estuary. Mar Ecol Prog Ser 438: 19-31. doi: 10.3354/meps09303

Hidalgo MC, Skalli A, Abellán E, Arizcun M, Cardenete G (2006) Dietary intake of probiotics and maslinic acid in juvenile dentex (*Dentex dentex* L.): effects on growth performance, survival and liver proteolytic activities. Aquaculture Nutrition 12: 256-266. doi: 10.1111/j.1365-2095.2006.00408.x.

Hjelm M, Bergh O, Riaza A, Nielsen J, Melchiorsen J, Jensen S, Duncan H, Ahrens P Birkbeck H, Gram L (2004) Selection and identification of autochthonous potential probiotic bacteria from turbot larvae (*Scophthalmus maximus*) rearing units. Systematic and Applied Microbiology. doi:10.1078/0723-2020-00256

Holmstrom C, Kjelleberg S (1999) Marine Pseudoalteromonas species are associated with higher organisms and produce biologically active extracellular agents. Fems Microbiology Ecology. doi: 10.1111/j.1574-6941.1999.tb00656.x

Holzapfel W, Wood BJ (2012) The genera of lactic acid bacteria. vol 2 Springer Science & Business Media.

Hoseinifar SH, Khalili M, Khoshbavar Rostami H, Esteban MÁ (2013) Dietary galactooligosaccharide affects intestinal microbiota, stress resistance, and performance of Caspian roach (*Rutilus rutilus*) fry. Fish & Shellfish Immunology 35: 1416-1420. doi: 10.1016/j.fsi.2013.08.007.

Hoseinifar SH, Soleimani N, Ringø E (2014) Effects of dietary fructo-oligosaccharide supplementation on the growth performance, haemato-immunological parameters, gut microbiota and stress resistance of common carp

(*Cyprinus carpio*) fry. British Journal of Nutrition 112: 1296-1302. doi: 10.1017/S0007114514002037.

Hoseinifar SH, Ringø E, Shenavar MA, Esteban MÁ (2016) Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. Reviews in Aquaculture 8: 89-102. doi: 10.1111/raq.12082.

Howell B, Conceição L, Prickett R, Cañavate P, Mañanos E (2009) Sole farming: nearly there but not quite?! A report of the 4th workshop on the cultivation of soles. Aquaculture Europe 34: 24-27. In: Aquaculture Europe: Magazine of the European Aquaculture Society (EAS). ISSN 0773-6940.

Hu YO, Karlson B, Charvet S, Andersson AF (2016) Diversity of Pico- to Mesoplankton along the 2000 km Salinity Gradient of the Baltic Sea. Front Microbiol 12 (7): 679. doi: 10.3389/fmicb.2016.00679

Huddleston JR (2014) Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infection and Drug Resistance 7: 167-176. doi: 10.2147/IDR.S48820.

Irianto A, Austin B (2002) Use of probiotics to control furunculosis in rainbow trout *Oncorhynchus mykiss* (Walbaum). Journal of Fish Disease 25 (6): 333–342. doi: 10.1046/j.1365-2761.2002.00375.x

Ishimaru K, Akagawa-Matsushita M, Muroga K (1996) *Vibrio ichthyoenteri* sp. nov., a pathogen of japanese flounder (*Paralichthys olivaceus*) larvae. International Journal Of Systematic Bacteriology, 46(1):155-159.

Jaafar R, Kania PW, Larsen A, Nielsen DS, Fouz B, Browdy C, Buchmann K (2013) Gut microbiota changes in rainbow trout, *Oncorhynchus mykiss* (Walbaum), during organic acid feed supplementation and *Yersinia ruckeri* infection. Journal of Fish Diseases 36: 599-606. doi: 10.1111/jfd.12047.

Jackson GA (1987) Simulating chemosensory responses of marine microorganisms. Limnol Oceanogr 32 (6): 1253-1266. doi: 10.4319/lo.1987.32.6.1253

Jansen PA, Kristoffersen AB, Viljugrein H, Jimenez D, Aldrin M, Stien A (2012) Sea lice as a density-dependent constraint to salmonid farming. Proceedings of the Royal Society B: Biological Sciences. doi: 10.1098/rspb.2012.0084.

Jeong HJ, Kang NS, Moestrup O, Yoo YD, Potvin E (2014) Description of the new heterotrophic dinoflagellate *Stoeckeria changwonensis* n. sp. from Korean coastal waters and emended description of the genus Stoeckeria and the type species *Stoeckeria algicida*. Harmful algae 36: 38-56. doi: 10.1016/j.hal.2014.04.017

Jin X., Wang S, Pang Y, Zhao H, Zhou X (2005) The adsorption of phosphate on different trophic lake sediments. Colloids and Surfaces A, 254: 241–248

Kandel PP, Pasternak Z, van Rijn J, Nahum O, Jurkevitch E (2014) Abundance, diversity and seasonal dynamics of predatory bacteria in aquaculture zero discharge systems. FEMS Microbiology Ecology. doi: 10.1111/1574-6941.12342.

Kasai H, Yoshimizu M, Ezura Y (2002) Disinfection of water for aquaculture. Fisheries Science. ISSN 0919-9268.

Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L (2008) Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. Aquaculture. doi:10.1016/j.aquaculture.2007.11.019.

Kesarcodi-Watson A, Miner P, Nicolas JL, Robert R (2012) Protective effect of four potential probiotics against pathogen-challenge of the larvae of three bivalves: Pacific oyster (*Crassostrea gigas*), flat oyster (*Ostrea edulis*) and scallop (*Pecten maximus*). Aquaculture. doi:10.1016/j.aquaculture.2012.02.029.

Kim HS, Hyun DW, Lee JY, Kim PS, Whon TW, Kang W, Bae JW (2016) *Sedimentitalea todarodis* sp. nov., isolated from the intestinal tract of a Japanese flying squid. International Journal of Systematic and Evolutionary Microbiology. doi: 10.1099/ijsem.0.001188

Kinney H (2017) Aquaculturists' Perceptions of Integrated Multi-Trophic Aquaculture (IMTA). University of Rhode Island. Open Access Master's Theses, paper 1024. http://digitalcommons.uri.edu/theses/1024

Kinnula H, Mappes J, Valkonen JK, Pulkkinen K, Sundberg LR (2017) Higher Resource Level Promotes Virulence in an Environmentally Transmitted Bacterial Fish Pathogen. Evol Appl 10 (5): 462-470. doi: 10.1111/eva.12466

Kirchman DL, Dittel AI, Malmstrom RR, Cottrell MT (2005) Biogeography of major bacterial groups in the Delaware Estuary. Limnol Oceanogr 50 (5): 1697-1706. doi: 10.4319/lo.2005.50.5.1697

Kodama H, Nakagawa T (2007) Protection against atypical *Aeromonas salmonicida* infection in carp (*Cyprinus carpio* L.) by oral administration of humus extract. Journal of Veterinary Medical Science 69: 405-408. doi: 10.1292/jvms.69.405.

Kreitmann L, Terriou L, Launay D, Caspar Y, Courcol R, Maurin M, Lemaître N. (2015) Disseminated Infection Caused by *Francisella philomiragia*, France, 2014. Emerging Infectious Diseases. doi: 10.3201/eid2112.150615.

Kuczynski J, Stombaugh J, Walters WA, González A, Caporaso JG, Knight R (2011) Using QIIME to analyze 16S rRNA gene sequences from microbial communities In: Current Protocols in Bioinformatics, chapter 10. doi:10.1002/0471250953.bi1007s36.

Labatut RA, Olivares JF (2004) Culture of turbot (*Scophthalmus maximus*) juveniles using shallow raceways tanks and recirculation. Aquacultural Engineering, 32(1):113-127. doi:10.1016/j.aquaeng.2004.05.008.

Lalloo R, Ramchuran S, Ramduth D, Görgens J, Gardiner N (2007) Isolation and selection of *Bacillus* spp. as potential biological agents for enhancement of water quality in culture of ornamental fish. Journal of Applied Microbiology 103: 1471-1479. doi: 10.1111/j.1365-2672.2007.03360.x.

Lara E, Fernández LD, Schiaffino MR, Izaguirre I (2017) First freshwater member ever reported for the family Bathycoccaceae (Chlorophyta; Archaeplastida)

from Argentinean Patagonia revealed by environmental DNA survey. European Journal Protistology 60:45-49. doi: 10.1016/j.ejop.2017.05.008

Lara-Flores M, Olvera-Novoa MA, Guzmán-Méndez BzE, López-Madrid W (2003) Use of the bacteria *Streptococcus faecium* and *Lactobacillus acidophilus*, and the yeast *Saccharomyces cerevisiae* as growth promoters in Nile tilapia (*Oreochromis niloticus*). Aquaculture 216: 193-201. doi: 10.1016/S0044-8486(02)00277-6.

Larsen A, Tao Z, Bullard SA, Arias CR (2013) Diversity of the skin microbiota of fishes: evidence for host species specificity. *FEMS Microbiology Ecology* 85, 483–494. doi: 10.1111/1574-6941.12136.

Lee DE, Lee J, Kim YM, Myeong JI, Kim KH (2016) Uncultured bacterial diversity in a seawater recirculating aquaculture system revealed by 16S rRNA gene amplicon sequencing. Journal of Microbiology. doi: 10.1007/s12275-016-5571-4

Letunic I, Bork P (2016) Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. Nucleic Acids Research. doi:10.1093/nar/gkw290.

Leung TLF, Bates AE (2013) More rapid and severe disease outbreaks for aquaculture at the tropics: implications for food security. Journal of Applied Ecology 50: 215-222. doi: 10.1111/1365-2644.12017.

Li X, Yu Y, Feng W, Yan Q, Gong Y (2012) Host species as a strong determinant of the intestinal microbiota of fish larvae. The Journal of Microbiology 50: 29-37. doi: 10.1007/s12275-012-1340-1.

Li Z, Che J, Xie J, Wang G, Yu E, Xia Y, Yu D, Zhang K (2017) Microbial succession in biofilms growing on artificial substratum in subtropical freshwater aquaculture ponds. FEMS Microbiology Letters 364 (4): fnx017. doi: 10.1093/femsle/fnx017.

Lin YC, Campbell T, Chung CC, Gong GC, Chiang KP, Wordenb AZ (2012) Distribution Patterns and Phylogeny of Marine Stramenopiles in the North Pacific Ocean. Appl Environ Microbiol 78 (9): 3387–3399. doi:10.1128/AEM.06952-11

Litchman E, Klausmeier CA, Schofield OM, Falkowski PG (2007) The role of functional traits and trade-offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. Ecol Lett 10 (12): 1170-1181. doi: 10.1111/j.1461-0248.2007.01117.x

Llewellyn MS, Boutin S, Hoseinifar SH, Derome N (2014) Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. Frontiers in Microbiology 5. doi: 10.3389/fmicb.2014.00207.

Lokesh J, Kiron V (2016) Transition from freshwater to seawater reshapes the skin-associated microbiota of Atlantic salmon. Scientific Reports. doi:10.1038/srep19707.

Lorenzo JI, Nieto O, Beiras R (2006) Anodic stripping voltammetry measures copper bioavailability for sea urchin larvae in the presence of fulvic acids. Environmental Toxicology and Chemistry 25: 36-44. doi: 10.1897/05-236R.1.

Lozupone CA, Knight R (2007) Global patterns in bacterial diversity. PNAS 104 (27): 11436-11440. doi:10.1073/pnas.0611525104

Luo YF, Yang TB (2010) Seasonal population dynamics of the monogeneans *Pseudorhabdosynochus coioidesis* and *P. serrani* on wild versus cultured groupers in Daya Bay, South China Sea. Helminthologia 47 (4): 241-250. doi: 10.2478/s11687-010-0038-3

Luo H, Moran MA (2014) Evolutionary Ecology of the Marine Roseobacter Clade. Microbiol Mol Biol Rev 78 (4): 573-87. doi:10.1128/MMBR.00020-14

Marcil V, Delvin E, Seidman E, Poitras L, Zoltowska M, Garofalo C, Levy E (2002) Modulation of lipid synthesis, apolipoprotein biogenesis, and lipoprotein assembly by butyrate. American Journal of Physiology-Gastrointestinal and Liver Physiology 283: G340-G346. doi: 10.1152/ajpgi.00440.2001.

Martínez Cruz P, Ibáñez AL, Monroy Hermosillo OA, Ramírez Saad HC (2012) Use of Probiotics in Aquaculture. ISRN Microbiology 2012: 916845. doi: 10.5402/2012/916845.

Martínez-Córdova LR, Emerenciano M, Miranda-Baeza A, Martínez-Porchas M (2015) Microbial-based systems for aquaculture of fish and shrimp: an updated review. Reviews in Aquaculture 7: 131-148. doi: 10.1111/raq.12058.

Martins P, Cleary DFR, Pires ACC, Rodrigues AM, Quintino V, Calado R, Gomes NCM (2013) Molecular analysis of bacterial communities and detection of potential pathogens in a recirculating aquaculture system for *Scophthalmus maximus* and *Solea senegalensis*. Plos One, 8 (11):e80847. doi: 10.1371/journal.pone.0080847.

Martins P, Navarro RVV, Coelho FJRC, Gomes NCM (2015) Development of a molecular methodology for fast detection of *Photobacterium damselae* subspecies in water samples. Aquaculture 435: 137-142. doi: 10.1016/j.aquaculture.2014.09.028.

Martins P (2016) Molecular characterization of microbial communities in aquaculture. PhD Thesis. University of Aveiro.

Martins P, Coelho FJRC, Cleary DFR, Pires ACC, Marques B, Rodrigues AM, Quintino V, Gomes NCM (2018) Seasonal patterns of bacterioplankton in a semi-intensive European seabass (*Dicentrarchus labrax*) aquaculture system. Aquaculture. doi: 10.1016/j.aquaculture.2018.02.038

Matos A, Borges MT, Peixe C, Henriques I, Pereira CM, Castro PML (2011) A molecular and multivariate approach to the microbial community of a commercial shallow raceway marine recirculation system operating with a Moving Bed Biofilter. Aquaculture Research. doi:10.1111/j.1365-2109.2010.02719.x.

McIntosh D, Ji B, Forward BS, Puvanendran V, Boyce D, Ritchie R (2008) Culture-independent characterization of the bacterial populations associated with cod (*Gadus morhua* L.) and live feed at an experimental hatchery facility using denaturing gradient gel electrophoresis. Aquaculture. doi:10.1016/j.aquaculture.2007.12.021

Medina A, Mancera JM, Martínez-Manzanares E, Moriñigo MA, Arijo S (2015) Identification of *Vibrio harveyi* proteins involved in the specific immune response of Senegalese sole (*Solea senegalensis*, Kaup). Fish & Shellfish Immunology 47: 377-380. doi: 10.1016/j.fsi.2015.09.031.

Meinelt T, Kroupova H, Stüber A, Rennert B, Wienke A, Steinberg CEW (2010) Can dissolved aquatic humic substances reduce the toxicity of ammonia and nitrite in recirculating aquaculture systems? Aquaculture 306: 378-383. doi: 10.1016/j.aquaculture.2010.06.007.

Mennerat A, Nilsen F, Ebert D, Skorping A (2010) Intensive farming: evolutionary implications for parasites and pathogens. Evolutionary Biology 37(2-3):59-67. doi: 10.1007/s11692-010-9089-0.

Merikanto I, Laakso JT, Kaitala V (2017) Outside-host predation as a biological control against an environmental opportunist disease. Ecological Modelling, 347: 85-93. doi: 10.1016/j.ecolmodel.2017.01.002

Merrifeld DL, Bradley G, Baker RTM, Davies SJ (2010) Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) II. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria postantibiotic treatment. Aquaculture Nutrition 16: 496-503. doi: 10.1111/j.1365-2095.2009.00688.x.

Merrifield DL, Carnevali O (2014) Probiotic modulation of the gut microbiota of fish, Chapter 8. In: Merrifield D, Ringø E (ed) Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics, First Edition. John Wiley & Sons, pp.185-222. ISBN: 978-0-470-67271-6.

Michaud L, Lo Giudice A, Troussellier M, Smedile F, Bruni V, Blancheton JP (2009) Phylogenetic characterization of the heterotrophic bacterial communities inhabiting a marine recirculating aquaculture system. Journal of Applied Microbiology. doi:10.1111/j.1365-2672.2009.04378.x.

Misra CK, Das BK, Mukherjee SC, Pattnaik P (2006) Effect of long term administration of dietary β-glucan on immunity, growth and survival of *Labeo rohita* fingerlings. Aquaculture 255: 82-94. doi: 10.1016/j.aquaculture.2005.12.009.

Montalban-Arques A, Schryver P, Bossier P, Gorkiewicz G, Mulero V, Gatlin DM, Galindo-Villegas J (2015) Selective Manipulation of the Gut Microbiota Improves Immune Status in Vertebrates. Frontiers in Immunology 6: 512. doi: 10.3389/fimmu.2015.00512

Moran MA, Belas R, Schell MA … Thompson LS, Saunders E, Buchan A (2007) Ecological Genomics of Marine Roseobacters. Appl Environ Microbiol 73 (14): 4559–4569. doi: 10.1128/AEM.02580-06

Moriarty DJW (1997) The role of microorganisms in aquaculture ponds. Aquaculture. doi:10.1016/S0044-8486(96)01487-1.

Na H, Kim OS, Yoon SH, Kim Y, Chun J (2011) Comparative approach to capture bacterial diversity of coastal waters. J Microbiol 49 (5): 729-40. doi: 10.1007/s12275-011-1205-z

Najdegerami EH, Tran TN, Defoirdt T, Marzorati M, Sorgeloos P, Boon N, Bossier P (2012) Effects of poly-β-hydroxybutyrate (PHB) on Siberian sturgeon (*Acipenser baerii*) fingerlings performance and its gastrointestinal tract microbial community. FEMS Microbiology Ecology 79: 25-33. doi: 10.1111/j.1574-6941.2011.01194.x.

Natrah FM, Alam MI, Pawar S, Harzevili AS, Nevejan N, Boon N, Sorgeloos P, Bossier P, Defoirdt T. (2012) The impact of quorum sensing on the virulence of *Aeromonas hydrophila* and *Aeromonas salmonicida* towards burbot (*Lota lota* L.) larvae. Veterinary Microbiology 159: 77-82. doi: 10.1016/j.vetmic.2012.03.014.

Nayak SK (2010) Role of gastrointestinal microbiota in fish. Aquaculture Research 41: 1553-1573. doi: 10.1111/ j.1365 -210 9.2010.02546. x

Nedashkovskaya OI, Kukhlevskiy AD, Zhukova NV, Kim SB (2016) *Amylibacter ulvae* sp. nov., a new alphaproteobacterium isolated from the Pacific green alga *Ulva fenestrate*. Arch Microbiol 198 (3): 251-6. doi: 10.1007/s00203-015-1185-1

Nevejan N, Schryver P, Wille M, Dierckens K, Baruah K, Stappen GV (2018) Bacteria as food in aquaculture: do they make a difference? Reviews in Aquaculture 10, 180–212. doi: 10.1111/raq.12155.

Nguyen DDL, Ngoc HH, Dijoux D, Loiseau G and Montet D (2008) Determination of fish origin by using 16S rDNA fingerprinting of bacterial communities by PCR-DGGE: An application on Pangasius fish from Viet Nam. Food Control 19: 454–460. doi:10.1016/j.foodcont.2007.05.006

Nhan DT, Wille M, De Schryver P, Defoirdt T, Bossier P, Sorgeloos P (2010) The effect of poly β-hydroxybutyrate on larviculture of the giant freshwater prawn *Macrobrachium rosenbergii*. Aquaculture 302: 76-81. doi: 10.1016/j.aquaculture.2010.02.011.

Nishitani G, Nagai S, Baba K, Kiyokawa S, Kosaka Y, Miyamura K, Nishikawa T, Sakurada K, Shinada A, Kamiyama T (2010) High-Level Congruence of *Myrionecta rubra* Prey and Dinophysis Species Plastid Identities as Revealed by Genetic Analyses of Isolates from Japanese Coastal Waters. Appl Environ Microbiol 76 (9): 2791-2798. doi: 10.1128/AEM.02566-09

NOAA Fisheries. https://www.fisheries.noaa.gov/topic/aquaculture

Nocker A, Lepo JE, Snyder RA (2004) Influence of an oyster reef on development of the microbial heterotrophic community of an estuarine biofilm. Applied and Environmental Microbiology, 70(11):6834-45.

NSW Government, Department of Primary Industries. Ozone in recirculating aquaculture systems. Available in: http://www.dpi.nsw.gov.au. Accessed December 2016.

Nübel U, Engelen B, Felske A, Snaidr J, Wieshuber A, Amann RI, Ludwig W, Backhaus H (1996) Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. Journal of Bacteriology. doi:10.1128/jb.178.19.5636-5643.1996.

Oksanen J (2011) Vegan: ecological diversity. Available: http://cran.rproject.org/web/packages/vegan/vignettes/diversity-vegan.pdf. Accessed February 2014

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2016). vegan: Community Ecology Package. R package version 2.4-1. https://CRAN.R-project.org/package=vegan

Oliva-Teles A (2000) Recent advances in European sea bass and gilthead sea bream nutrition. Aquacult Int 8 (6): 477-492. doi: 10.1023/A:1009236517555

Pereira C, Salvador S, Arrojado C, Silva Y, Santos AL, Cunha A, Gomes NCM, Almeida A (2011) Evaluating seasonal dynamics of bacterial communities in marine fish aquaculture: A preliminary study before applying phage therapy. J Environ Monit 13 (4): 1053-1058. doi: 10.1039/c0em00434k

Pernthaler J (2005) Predation on prokaryotes in the water column and its ecological implications. Nature Reviews Microbiology 3: 537. doi: 10.1038/nrmicro1180.

Piwosz K, Pernthaler J (2010) Seasonal population dynamics and trophic role of planktonic nanoflagellates in coastal surface waters of the Southern Baltic Sea. Environ Microbiol 12 (2): 364-77. doi:10.1111/j.1462-2920.2009.02074.x

Powell A, Chingombe P, Lupatsch I, Shields RJ, Lloyd R (2015) The effect of ozone on water quality and survival of turbot (*Psetta maxima*) maintained in a recirculating aquaculture system. Aquacultural Engineering. doi:10.1016/j.aquaeng.2014.11.005.

Pulkkinen K, Suomalainen LR, Read AF, Ebert D, Rintamaki P, Valtonen ET (2010) Intensive fish farming and the evolution of pathogen virulence: the case of columnaris disease in Finland. Proceedings of The Royal Society B. doi:10.1098/rspb.2009.1659.

Raffic Ali SS, Ambasankar K, Nandakumar S, Praveena PE, Syamadayal J (2016) Effect of dietary prebiotic inulin on growth, body composition and gut microbiota of Asian seabass (*Lates calcarifer*). Animal Feed Science and Technology 217: 87-94. doi.org/10.1016/j.anifeedsci.2016.04.011.

Ramachandran K (2016) Beneficial microbes for the sustainable management of shrimp aquaculture. Guybro Chemicals Pvt Ltd, Corporate Office, (www.guybro.com). Available: http://www.ctaquaculture.tn/index.php?id=45&L=3%2F%27&tx_ttnews%5Btt_news%5D=608&cHash=9f5e86bd53fbd4451e31c0f83e01dffa at 22/11/2016. Accessed: November 2016.

Ramanan R, Kim BH, Cho DH, Oh HM, Kim HS (2016) Algae-bacteria interactions: Evolution, ecology and emerging applications. Biotechnol Adv 34 (1): 14-29. doi: 10.1016/j.biotechadv.2015.12.003

Ribeiro SC, Coelho MC, Todorov SD, Franco BDGM, Dapkevicius MLE, Silva CCG (2014) Technological properties of bacteriocin-producing lactic acid bacteria isolated from Pico cheese an artisanal cow's milk cheese. Journal of Applied Microbiology 116: 573-585. doi: 10.1111/jam.12388.

Richards GP, Watson MA, Needleman DS, Uknalis J, Boyd EF, Fay JP (2017) Mechanisms for *Pseudoalteromonas piscicida*-induced killing of vibrios and other bacterial pathogens. Applied and Environmental Microbiology. doi: 10.1128/AEM.00175-17

Ringø E, Gatesoupe F-J (1998) Lactic acid bacteria in fish: a review. Aquaculture 160: 177-203. doi: 10.1016/S0044-8486(97)00299-8.

Roca I, Akova M, Baquero F ... Tacconelli E, Wellington E, Vila J (2015) The global threat of antimicrobial resistance: science for intervention. New Microbes and New Infections 6: 22-29. doi: 10.1016/j.nmni.2015.02.007.

Rodrigues S, Paillard C, Dufour A, Bazire A (2015) Antibiofilm activity of the marine bacterium *Pseudoalteromonas* sp. 3J6 against *Vibrio tapetis*, the causative agent of Brown Ring Disease. Probiotics and Antimicrobial Proteins. doi:10.1007/s12602-014-9173-3.

Romero J, Ringø E, Daniel LM (2014). The Gut Microbiota of Fish. In book: Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics, Edition: 1, Chapter: 4, Publisher: John Wiley & Sons, Ltd, Chichester, UK., Editors: eds Merrifield D and Ringø E. doi: 10.1002/9781118897263.ch4.

Rud I, Kolarevic J, Holan AB, Berget I, Calabrese S, Terjesen BF (2017) Deep-sequencing of the bacterial microbiota in commercial-scale recirculating and semi-closed aquaculture systems for Atlantic salmon post-smolt production. Aquacultural Engineering. doi:10.1016/j.aquaeng.2016.10.003

Rurangwa E, Verdegem MCJ (2015) Microorganisms in recirculating aquaculture systems and their management. Reviews in Aquaculture 7(2): 117–130. doi:10.1111/raq.12057.

Salvesen I, Skjermo J, Vadstein O (1999) Growth of turbot (*Scophthalmus maximus L.*) during first feeding in relation to the proportion of r/K-strategists in the bacterial community of the rearing water. Aquaculture 175 (3–4): 337-350. doi:10.1016/S0044-8486(99)00110-6.

Sanders RW, Porter KG, Bennett SJ, Debiase AE (1989) Seasonal patterns of bacterivory by flagellates, ciliates, rotifers, and cladocerans in a freshwater planktonic community. Limnol Oceanogr 34 (4): 673-687. doi: 10.4319/lo.1989.34.4.0673

Santos WM, de Brito TS, Prado SdA, de Oliveira CG, De Paula AC, de Melo DC, Ribeiro PA (2016) Cinnamon (*Cinnamomum* sp.) inclusion in diets for Nile tilapia submitted to acute hypoxic stress. Fish & Shellfish Immunology 54: 551-555.

Schmidt V, Amaral-Zettler L, Davidson J, Summerfelt S, Good C (2016) Influence of Fishmeal-Free Diets on Microbial Communities in Atlantic Salmon (*Salmo salar*) Recirculation Aquaculture Systems. Appl Environ Microbiol 82 (15): 4470-4481. doi: 10.1128/AEM.00902-16

Schoeffield AJ, Williams HN (1990) Efficiencies of recovery of Bdellovibrios from brackish- water environments by using various bacterial species as prey. Applied and Environmental Microbiology, 56, 230-236.

Schreier HJ, Mirzoyan N, Saito K (2010) Microbial diversity of biological filters in recirculating aquaculture systems. Current Opinion in Biotechnology 21 (3): 318-325. doi: 10.1016/j.copbio.2010.03.011.

Schryver P, Defoirdt T, Boon N, Verstraete W, Bossier P (2012) 15 - Managing the microbiota in aquaculture systems for disease prevention and control A2 - Austin, B (ed) Infectious Disease in Aquaculture, Prevention and Control. p. 394-418. Woodhead Publishing. ISBN 978-0-85709-016-4

Schryver P, Vadstein O (2014) Ecological theory as a foundation to control pathogenic invasion in aquaculture. The ISME Journal 8: 2360–2368. doi:10.1038/ismej.2014.84

Seyedsayamdost MR, Case RJ, Kolter R, Clardy J (2011) The Jekyll-and-Hyde chemistry of Phaeobacter gallaeciensis. Nat Chem 3(4): 331-5. doi:10.1038/nchem.1002

Shanthi Mari LS, Jagruthi C, Anbazahan SM, Yogeshwari G, Thirumurugan R, Arockiaraj J, Mariappan P, Balasundaram C, Harikrishnan R (2014) Protective effect of chitin and chitosan enriched diets on immunity and disease resistance in *Cirrhina mrigala* against *Aphanomyces invadans*. Fish & Shellfish Immunology 39(2): 378-385. doi: 10.1016/j.fsi.2014.05.027

Sharifah EN, Eguchi M (2012) Mixed cultures of the phytoplankton *Nannochloropsis oculata* and the marine bacterium *Sulfitobacter* sp. RO3 inhibit the growth of virulent strains of the major fish pathogen Vibrio anguillarum. Aquaculture Science. doi: 10.11233/aquaculturesci.60.39

Sharifuzzaman SM, Austin B (2017) Probiotics for disease control in aquaculture. Diagnosis and Control of Diseases of Fish and Shellfish. John Wiley & Sons, Ltd. 189-222. doi: 10.1002/9781119152125.ch8.

Sheikha AF, Montet D (2014) How to determine the geographical origin of seafood? Critical Reviews in Food Science and Nutrition 56 (2): 306-317. doi: 10.1080/10408398.2012.745478

Sherr EB, Sherr BF (2002) Significance of predation by protists in aquatic microbial food webs. Antonie van Leeuwenhoek 81 (1-4): 293-308. doi: 10.1023/A:1020591307260

Skjermo J, Salvesen I, Øie G, Olsen Y, Vadstein O (1997) Microbially matured water: a technique for selection of a non-opportunistic bacterial flora in water that may improve performance of marine larvae. Aquaculture International 5(1): 13–28.

Skjermo J, Bakke I, Dahle SW, Vadstein O (2015) Probiotic strains introduced through live feed and rearing water have low colonizing success in developing Atlantic cod larvae. Aquaculture. doi: 10.1016/j.aquaculture.2014.12.027.

Smalla K, Wieland G, Buchner A, Zock A, Parzy J, Kaiser S, Roskot N, Heuer H, Berg G (2001) Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. Applied and Environmental Microbiology. doi:10.1128/AEM.67.10.4742-4751.2001.

Soliman NF, Yacout DMM (2016) Aquaculture in Egypt: status, constraints and potentials. Aquacult Int 24, 1201-1227. doi: 10.1007/s10499-016-9989-9

Sorokin DY (1995) *Sulfitobacter pontiacus* gen. nov., sp. nov.: a new heterotrophic bacterium from the Black Sea specialized on sulfite oxidation. Microbiology 64(3):295-305.

Stecher B, Hardt WD (2008) The role of microbiota in infectious disease. Trends in Microbiology 16:107–114. doi: doi: 10.1016/j.tim.2007.12.008.

Stoeck T, Bass D, Nebel, M, Christen R, Jones MDM, Breiner H-W, Richards TA (2010) Multiple marker parallel tag environmental DNA sequencing reveals a highly complex eukaryotic community in marine anoxic water. Mol Ecol 19 (1): 21-31. doi: 10.1111/j.1365-294X.2009.04480.x

Stopnisek N, Zühlke D, Carlier A, Barberán A, Fierer N, Becher D, Riedel K, Eberl L, Weisskopf L (2016) Molecular mechanisms underlying the close association between soil Burkholderia and fungi. ISME J 10 (1): 253-64. doi:10.1038/ismej.2015.73

Strom SL (2008) Microbial ecology of ocean biogeochemistry: a community perspective. Science 320 (5879): 1043-5. doi: 10.1126/science.1153527

Sugita H, Nakamura H, Shimada T (2005) Microbial communities associated with filter materials in recirculating aquaculture systems of freshwater fish. Aquaculture 243: 403-409. doi: 10.1016/j.aquaculture.2004.09.028.

Summerfelt ST, Sharrer MJ, Tsukuda SM, Gearheart M (2009) Process requirements for achieving full-flow disinfection of recirculating water using ozonation and UV irradiation. Aquacultural Engineering 40, 17–27. doi: 10.1016/j.aquaeng.2008.10.002.

Takao Y, Nagasaki K, Mise K, Okuno T, Honda D (2005) Isolation and Characterization of a Novel Single-Stranded RNA Virus Infectious to a Marine Fungoid Protist, *Schizochytrium* sp. (Thraustochytriaceae, Labyrinthulea). Applied Environmental Microbiology 71 (8): 4516-4522. doi: 10.1128/AEM.71.8.4516-4522.2005.

Tal Y, Schreier HJ, Sowers KR, Stubblefield JD, Place AR, Zohara Y (2009) Environmentally sustainable land-based marine aquaculture. Aquaculture 286 (1–2), 28-35. doi:10.1016/j.aquaculture.2008.08.043.

Taoka Y, Maeda H, Jo J-Y, Kim S-M, Park S-I, Yoshikawa T, Sakata T (2006a) Use of live and dead probiotic cells in tilapia *Oreochromis niloticus*. Fisheries Science 72: 755-766. doi: 10.1111/j.1444-2906.2006.01215.x

Taoka Y, Maeda H, Jo JY, Jeon MJ, Bai SC, Lee WJ, Yuge K, Koshio S (2006b) Growth, stress tolerance and non-specific immune response of Japanese flounder *Paralichthys olivaceus* to probiotics in a closed recirculating system. Fisheries Science 72(2): 310-321. doi: 10.1111/j.1444-2906.2006.01152.x

Tarnecki AM, Burgos FA, Ray CL, Arias CR (2017) Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. Journal of Applied Microbiology 123: 2-17. doi: 10.1111/jam.13415.

Theodorou JA (2002) Current and Future Technological Trends of European Seabass-Seabream Culture. Rev Fish Sci 10 (3-4): 529-543. doi: 10.1080/20026491051776

Thomas F, Hehemann JH, Rebuffet E, Czjzek M, Michel G (2011) Environmental and gut bacteroidetes: the food connection. Front Microbiol 2: 93. doi: 10.3389/fmicb.2011.00093

Thompson FL, Gevers D, Thompson CC, Dawyndt P, Naser S, Hoste B, Munn CB, Swings J (2005) Phylogeny and molecular identification of Vibrios on the basis of multilocus sequence analysis. Applied Environmental Microbiology. doi:10.1128/AEM.71.9.5107-5115.2005.

Torrecillas S, Makol A, Caballero MJ, Montero D, Dhanasiri AKS, Sweetman J, Izquierdo M (2012) Effects on mortality and stress response in European sea bass, *Dicentrarchus labrax* (L.), fed mannan oligosaccharides (MOS) after *Vibrio anguillarum* exposure. Journal of Fish Diseases 35: 591-602. doi: 10.1111/j.1365-2761.2012.01384.x.

Torto-Alalibo T, Tian M, Gajendran K, Waugh ME, van West P, Kamoun S (2005) Expressed sequence tags from the oomycete fish pathogen *Saprolegnia parasitica* reveal putative virulence factors. BMC Microbiology 5: 46. doi: 10.1186/1471-2180-5-46.

Tovar D, Zambonino J, Cahu C, Gatesoupe FJ, Vázquez-Juárez R, Lésel R (2002) Effect of live yeast incorporation in compound diet on digestive enzyme activity in sea bass (*Dicentrarchus labrax*) larvae. Aquaculture 204: 113-123. doi: 10.1016/S0044-8486(01)00650-0.

Trivedi B (2012) Microbiome: The surface brigade. Nature 492: S60-S61. doi:10.1038/492S60a.

Uchida M, Nakata K, Maeda M (1997) Conversion of Ulva fronds to a hatchery diet for *Artemia nauplii* utilizing the degrading and attaching abilities of *Pseudoalteromonas espejiana*. Journal of Applied Phycology. doi: 10.1023/A:1007940005528

Uddin MN, Al-Harbi AH (2004) Seasonal Variation of Bacterial Flora in Ponds in Saudi Arabia Used for Tilapia Aquaculture. Journal of Applied Aquaculture, Vol. 16(1/2). doi: 10.1300/J028v16n01_04

Ulaganathan K, Goud BS, Reddy MM, Kumar VP, Radhakrishna S, Balsingh J (2015) Genome Sequence of *Saccharomyces cerevisiae* NCIM3107, Used in Bioethanol. Genome Announc 3 (1): e01557-14. doi: 10.1128/genomeA.01557-14

Vadstein O (1993) A strategy to obtain microbial control during larval development of marine fish. Fish Farmig Technology 69-75.

Valladão GMR, Gallani SU, Pilarski F (2015) Phytotherapy as an alternative for treating fish disease. Journal of Veterinary, Pharmacology and Therapeutics 38: 417-428. doi: 10.1111/jvp.12202

Van De Wiele T, Boon N, Possemiers S, Jacobs H, Verstraete W (2007) Inulin-type fructans of longer degree of polymerization exert more pronounced in vitro prebiotic effects. Journal of Applied Microbiology 102: 452-460. doi: 10.1111/j.1365-2672.2006.03084.x.

Verdegem MCJ, Bosma RH, Verreth JAJ (2006) Reducing water use for animal production through aquaculture. International Journal of Water Resources Development, 22(1):101-113. doi: 10.1080/07900620500405544.

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255 (2): 571-586. doi: 10.1023/A:1026037216893

Vine NG, Leukes WD, Kaiser H, Daya S, Baxter J, Hecht T (2004) Competition for attachment of aquaculture candidate probiotic and pathogenic bacteria on fish intestinal mucus. Journal of Fish Diseases 27: 319-326. doi: 10.1111/j.1365-2761.2004.00542.x.

Voget S, Wemheuer B, Brinkhoff T … Billerbeck S, Daniel R, Simon M (2015) Adaptation of an abundant Roseobacter RCA organism to pelagic systems revealed by genomic and transcriptomic analyses. The ISME Journal. doi: 10.1038/ismej.2014.134

Walasek M, Grzegorczyk A, Malm A, Skalicka-Woźniak K (2015) Bioactivity-guided isolation of antimicrobial coumarins from *Heracleum mantegazzianum*. Sommier & Levier (Apiaceae) fruits by high-performance counter-current chromatography. Food Chemistry 186: 133-138. doi: 10.1016/j.foodchem.2015.02.011.

Wang AR, Chao Ran C, Ringø E, Zhou ZG (2017) Progress in fish gastrointestinal microbiota research. Reviews in Aquaculture 0, 1–15. doi: 10.1111/raq.12191.

Wang Q, Garrity GM, Tiedje JM, Cole, JR (2007) Naïve bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol 73 (16): 5261–5267. doi: 10.1128/aem.00062-07

Wang Y, Qian PY (2009) Conservative fragments in bacterial 16S rRNA genes and primer design for 16S ribosomal DNA amplicons in metagenomic studies. PLoS One 4 (10): e7401. doi: 10.1371/journal.pone.0007401.

Wang Y, Yu M, Austin B, Zhang X-H (2012) *Oleispira lenta* sp. nov., a novel marine bacterium isolated from Yellow sea coastal seawater in Qingdao, China. Antonie van Leeuwenhoek. doi: 10.1007/s10482-011-9693-8.

Washbourne IJ, Crenshaw CL, Baker MA (2011) Dissimilatory nitrate reduction pathways in an oligotrophic aquatic ecosystem: spatial and temporal trends. Aquat Microb Ecol 65: 55-64. doi: 10/3354/ame01538

Wawrik B, Boling WB, Van Nostrand JD, Xie J, Zhou J, Bronk DA (2012) Assimilatory nitrate utilization by bacteria on the west Florida shelf as determined by stable isotope probing and functional microarray analysis. FEMS Microbiology Ecology. doi: 10.1111/j.1574-6941.2011.01226.x

Webster NS, Negri AP (2006) Site-specific variation in Antarctic marine biofilms established on artificial surfaces. Environmental Microbiology. doi: 10.1111/j.1462-2920.2006.01007.x

Wei T, Simko V (2016) *corrplot:* Visualization of a Correlation Matrix. *R package version 0.77*, https://cran.r-project.org/web/packages/corrplot/index.html

Welsh RM, Zaneveld JR, Rosales SM, Payet JP, Burkepile DE, Thurber RV (2016) Bacterial predation in a marine host-associated microbiome. The ISME journal. doi:10.1038/ismej.2015.219.

Wesseling W, Wittka S, Kroll S, Soltmann C, Kegler P, Kunzmann A, Riss HW, Lohmeyer M (2015) Functionalised ceramic spawning tiles with probiotic Pseudoalteromonas biofilms designed for clownfish aquaculture. Aquaculture. doi: 10.1016/j.aquaculture.2015.04.017

WHO. Report of a WHO consultation with participation of FAO. Geneva. 29-31 May 1995. WHO/FNU/FOS/95.7 Geneva, WHO. Pp. 45] In: Almeida A, Cunha A, Santos L, Salvador S, Gomes A. 2009. Evaluation of the impact of two aquaculture systems on communities of the estuarine system Ria de Aveiro. In: Current Research Topics in Applied Microbiology and Microbial Biotechnology: Proceedings of the II International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld2007) Ed Mendez-Vilas A. World Scientific, 788p ISBN 9812837558, 9789812837554.

Wong S, Rawls JF (2012) Intestinal microbiota composition in fishes is influenced by host ecology and environment. Molecular Ecology 21: 3100-3102. doi: doi.org/10.1111/j.1365-294X.2012.05646.x.

Xue S, Xu W, Wei J, Sun J (2017) Impact of environmental bacterial communities on fish health in marine recirculating aquaculture systems. Veterinary Microbiology 203, 34–39. doi: 10.1016/j.vetmic.2017.01.034.

Yamin G, Zilberg D, Levy G, van Rijn J (2017) The protective effect of humic-rich substances from monogenean parasites infecting the guppy (*Poecilia reticulata*). Aquaculture 479: 487-489. doi: 10.1016/j.aquaculture.2017.06.022.

Yip W, Knowler D, Haider WG, Trenholm R (2017) Valuing the Willingness-to-Pay for Sustainable Seafood: Integrated Multitrophic versus Closed Containment Aquaculture. Canadian Journal Of Agricultural Economics-Revue Canadienne D Agroeconomie 65 (1):93-117. doi: 10.1111/cjag.12102.

Yuan XC, Yin KD, Harrison P, He L, Xu J (2011) Variations in apparent oxygen utilization and effects of P addition on bacterial respiration in subtropical Hong Kong waters. Estuaries and Coasts, 34: 536–543. doi: 10.1007/s12237-010-9329-7.

Yue GH, Wang L (2017) Current status of genome sequencing and its applications in aquaculture. Aquaculture 468: 337-347. doi:10.1016/j.aquaculture.2016.10.036

Zehr JP, Ward BB (2002) Nitrogen Cycling in the Ocean: New Perspectives on Processes and Paradigms. Appl Environ Microbiol 68 (3) 1015-1024. doi: 10.1128/AEM.68.3.1015-1024.2002

Zhang Z, Schwartz S, Wagner L, Miller WA (2000) A greedy algorithm for aligning DNA sequences. J Comput Biol 7: 1-2. doi: 10.1089/10665270050081478

Zhang DC, Li HR, Xin YH, Liu HC, Chi ZM, Zhou PJ, Yu Y (2008) *Phaeobacter arcticus* sp. nov., a psychrophilic bacterium isolated from the Arctic. International Journal of Systematic and Evolution Microbiology. doi: 10.1099/ijs.0.65708-0

Zhang S, Liu N, Liang W, Han Q, Zhang W, Li C (2017) Quorum sensing-disrupting coumarin suppressing virulence phenotypes in *Vibrio splendidus*. Applied Microbiology and Biotechnology 101: 3371-3378. doi: 10.1007/s00253-016-8009-3.