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PII: S0044-8486(18)30899-8
DOI: https://doi.org/10.1016/j.aquaculture.2018.12.066
Reference: AQUA 633788

To appear in: *aquaculture*

Received date: 3 May 2018
Revised date: 20 December 2018
Accepted date: 20 December 2018

Please cite this article as: Letícia N. Duarte, Francisco J.R.C. Coelho, Daniel F.R. Cleary, Daniel Bonifácio, Patrícia Martins, Newton C.M. Gomes , Bacterial and microeukaryotic plankton communities in a semi-intensive aquaculture system of sea bass (Dicentrarchus labrax): A seasonal survey. Aqua (2018), https://doi.org/10.1016/j.aquaculture.2018.12.066

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

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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 operational taxonomic units (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.

Keywords

Bacteria, microeukaryote, plankton, aquaculture microbiome, seasonal variation

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 & 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 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 & Albutti 2014, Yue & Wang 2017). Diseases in aquaculture have led to the loss of several billions dollars per year (Yue & 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 & 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 & 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 showed 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 & 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).

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 Ria de Aveiro estuarine lagoon, Portugal. The aquaculture is composed of earthen ponds, which

receives natural water from the 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 & Qian 2009) using HotStarTaq Plus Master Mix Kit (Qiagen, Valencia, CA) under the following conditions: 94ºC for 3 min, followed by 28 cycles of 94ºC for 30 s, 53ºC for 40 s and 72ºC for 60s after which a final elongation step at 72ºC for 5 min was performed. 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 community analysis, 18S rRNA gene fragments were amplified using primers TAReuk454FWD1 (5'-CCAGCASCYGCGGTAATTCC-3') and TAReukREV3 (5'-ACTTTCGTTCTTGATYRA-3') (Stoeck et al. 2010) using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) as follow: 94°C for 3 minutes, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 minutes was performed. 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 data 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 analyzed 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). Richness estimates (Chao1 and ACE) were calculated using the estimateR() function in vegan (Oksanen et al., 2016). Shannon diversity indice was calculated with the diversity() function from the same package. 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 & 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 were registered in the end of summer (September - 37.33±0.58). The lowest salinity values were registered in November (21.67± 2.08). The highest values of TOC were registered in the end of summer (September - 6.87±7.04 mg l^{-1}). 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 showed highest level in January (0.30±0.12 mg l^{-1}), gradually decreasing during the year up to 0.16±0.10 mg l^{-1} in November.

Variation in bacterioplankton composition

In total, 14131 bacterial sequences were obtained from all sampled months, which were assigned to 1333 bacterial OTUs. The number of sequences varied between 1823 for November to 3437 for March. Overall OTU richness differed between sampling times (Figure S1). Controlling for sample size (n = 1200 individual sequences), OTU richness had its lowest value in March (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 number of OTUs estimated by Chao 1 and ACE diversity indices had its lowest values in September (216.53±17.55 and 225.03±7.59, respectively) and its highest values in November (1036.92±91.73 and 1148.86±23.33, respectively) (Table S1). Both estimators of community richness (Chao1 and ACE) were higher than the observed number of OTUs (Table S1). 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) (Table S1).

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. 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.

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.

Variation in microeukaryotic plankton composition

The total amount of microeukaryote sequences retrieved in this study was 20545 that were assigned to 833 OTUs. The number of sequences varied between 1677 for May and 6765 in July. 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). The number of OTUs estimated by Chao 1 and ACE diversity indices had its lowest values in March (170.07±9.95 and 164.97±6.24, respectively) and its highest values in November (473.03±34.88 and 501.01±12.84, respectively) (Table S2). Both estimators of community richness (Chao1 and ACE) were higher than the observed number of OTUs (Table 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).

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.

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 S3). 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.

Discussion

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. Curiously, in contrast to our previous study (Martins et al. 2018), 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 class Bacteroidetes belonged 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, though 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 & 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 & 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 parameter 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 & del Giorgio 2002). By analyzing 16S rRNA gene sequences compiled from 111 studies with

diverse physical environments, Lozupone & 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 richness of both bacterial and microeukaryotic communities. We have previously show 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 & 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 been 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 & 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 abiotic drivers of these communities, which varied congruently along the seasons. Besides the potential effects of the abiotic 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 & Malcata 2012, Merikanto et al. 2017). However, more in depth studies under controlled conditions are necessary for a better understand 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.

Supplementary data

Supplementary material

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Figure 1. Principal Coordinates Analysis (PCO) of operational taxonomic unit composition of the most abundant bacterial (>100 sequencing reads) (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.

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

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

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

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

Figure 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.

Table 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 farmed fishes in the semi-intensive aquaculture system in January, March, May, July, September and November of 2014. NA - non available.

	Tem perat	pH	Sali	DO	Ammo	Nitri	Nitr	Phos phat	TO	Fish Weig	Fork Leng

	ure		nity		nium	te	ate	e	C	ht	th
	°C			mg/L	mgNH_3+NH_4/L	mg NO_{2-}/L	mg NO_{3-}/L	mg PO_4^{3-}/L	mg C/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

Highlights

1- Temperature, salinity and nitrate were predictors of microbial composition;

2- There was a negative relationship between grazers and bacterial OTUs;

3- Interdependences between microbial domains may contribute to seasonal variations.