Changes in microbial communities during seawater pre-treatment within a desalination plant.

Sergio Balzano[1,2*], Tamar Jamieson[3,4], Sophie Leterme[3,4]

[1] Stazione Zoologica di Napoli Anton Dohrn. Villa Comunale, 80121 Naples (Italy)

[2] Department of Marine Microbiology and Biogeochemistry, Netherland Institute for Sea Research (NIOZ), Landsdiep 4, Texel (Netherlands)

[3] College of Science and Engineering, Flinders University, Sturt Road, Bedford Park, Adelaide, SA 5042 (Australia)

[4] Institute for Nanoscale Science & Technology, Flinders University, Sturt Road, Bedford Park, Adelaide, SA 5042 (Australia)

[*]Corresponding author: Sergio.balzano@szn.it

Running title: microbes across seawater pre-treatment

Abstract

We analysed prokaryotic and eukaryotic communities across the seawater pre-treatment system of a desalination plant, using 16S and 18S rRNA gene sequencing. The richness of operational taxonomic units (OTUs) increased downstream of the pre-treatment system (RO feedwater) compared to raw seawater for Archaea while it decreased for bacteria and protists. Overall the RO feedwater was found to be enriched in ammonia-oxidising bacteria and Archaea compared to raw seawater and also contained greater proportions of taxa typically observed in aquatic biofilms and/or within other water treatment systems. Although, the microbial load is reduced by the pre-treatment system, the increase in proportion of biofilm-associated microbes suggest the presence of active microbial communities within multimedia filters and other parts of the pre-treatment system that might increase biofouling risks.

Keywords: 16S rDNA, 18S rDNA, ammonium oxidation, desalination

Climate change and increase in human population worldwide are likely to exacerbate freshwater scarcity issues in several regions (Naumann et al. 2018), highlighting the need for alternative freshwater sources such as desalination. Most desalination plants use reverse osmosis (RO) membranes for the conversion of seawater into freshwater and brine. To remove most microbes and particles from the stream, seawater is usually pre-treated by filtration and UV irradiation prior to RO. Although the microbial load decreases sharply after seawater filtration, some of the microbes present after seawater pre-treatment are likely to form biofilms on the RO membranes (Manes et al. 2011). For example, Bacteria are typically enriched in Proteobacteria (Manes et al. 2011, Levi et al. 2016), whereas pennate diatoms and other elongated species prevail among eukaryotes (Balzano et al. 2014) after pre-treatment for SWRO. While bacterial communities have been widely characterised little is known on Archaea and protists. Here, we assessed the composition of both prokaryotic and eukaryotic communities occurring upstream (raw seawater) and downstream (RO feedwater) of a pre-treatment system for SWRO by sequencing the V4 fragment of both the 16S and the 18S rRNA genes.

Seawater was collected from the Penneshaw SWRO desalination plant, located on the North-Eastern coast of Kangaroo Island, South Australia (Balzano et al. 2014). The pre-treatment system consists in a medium pressure-ultra violet (MP-UV) disinfection unit, multimedia and cartridge filters (Balzano et al. 2014). Nutrients were analysed every second week over one-year period (18/07/2012 to 21/07/2013) from raw seawater, downstream of the MP-UV treatment unit (Site 2), the multimedia filters (Site 3), the 15 µm cartridge filters (Site 4), and from the RO feedwater, as described previously (Balzano et al. 2015b). Molecular analyses were carried out on raw seawater and RO feedwater only, and samples were collected 5 times over a 13-month period (Oct 2012, Dec 2012, Mar 2013, Jul 2013, Nov 2013). One hundred-twenty L seawater were concentrated down to 2 L by tangential

flow filtration (Marie et al. 2010) and the concentrated sample was first pre-filtered using 10 μm cellulose filters and then filtered through 0.22-mm pore size Sterivex units (Millipore, Billerica, MA). Cells were removed from the Sterivex units, the DNA extracted, the 18S rRNA gene amplified, and both 18S and 16S rRNA genes sequenced using IonTorrent PGM as described previously (Balzano et al. 2015a), whereas the V4 region of the 16S rRNA gene was amplified using slight modifications (Supplementary Table S1) of the universal prokaryotic primers V341F and 805R (Bowman et al. 2012) and PCR reactions consisted of an initial denaturation at 98°C for 1 min, 30 cycles of 40 s at 98°C, 40 s at 53°C and 1 min at 72°C, and a final extension at 72°C for 1 min. Raw sequencing data were processed using the python pipeline Quantitative Insight into Microbial Ecology (QIIME) (Caporaso et al. 2010); read were filtered, clustered into operational taxonomic units (OTUs), and reads from different samples were compared as described previously (Balzano et al. 2015a).

Raw seawater temperature ranged from 14.3 ºC (July 2013) to 20.8 ºC (March 2013) and the salinity was stable around 36 psu (Balzano et al. 2015b). The abundance of both bacteria and phytoplankton dropped dramatically across the multimedia filter, from Site 2 to Site 3 (Balzano et al. 2014). The concentration of ammonium dropped by half across the multimedia filters (Fig. 1A), whereas the median concentration of NO_x increased from 0.35 to 1.24 μM (Fig. 1B) and both phosphate and silica did not change significantly (data not shown). The decrease in the concentration of ammonium and the increase in NO_x, suggest that nitrification was taking place within the multimedia filter. The lower ammonium to nitrate ratios measured downstream the multimedia filters are likely to partially limit microbial growth: heterotrophic bacteria in the water column are known to preferentially uptake ammonium over nitrate as nitrogen source (Middelburg and Nieuwenhuize 2000, Kumar et al. 2018).

Overall, we sampled a good portion of the microbial community (Supplementary Figure 1). OTU richness and diversity indices were generally lower in raw seawater than in RO feedwater for Archaea, and higher for bacteria and protists (Table 1). Most taxonomical changes across the pre-treatment plant were observed for Archaea compared to bacteria and protists. The proportions of archaeal reads over the total 16S rRNA gene libraries were significantly lower in raw seawater (0.5 to 3%) compared to RO feedwater (5 to 40%, Fig. 1C-D). Euryarchaeota, that typically dominate surface seawaters (Yin et al. 2013, Zhou et al. 2018), accounted for a large proportion of the genetic libraries of raw seawater, and their contribution dropped dramatically in RO feedwater. In contrast, Nanoarchaeota and *Nitrosoarchaeum* spp. (Thaumarchaeota) dominated the archaeal community in RO feedwater being represented by 26% and 41% of archaeal reads, respectively (Fig. 1D. *Nitrosoarchaeum* spp. are known to oxidise ammonium to nitrite (Konneke et al. 2005, Pitcher et al. 2011) and the increase in the abundance of reads associated with this genus in RO feedwater (Fig. 1C-D), suggest a potential role of *Nitrosoarchaeum* spp. in the ammonium oxidation observed here (Fig. 1A-B). *Nitrosoarchaeum* spp. were previously sequenced in multimedia filters of a drinking water treatment system in which ammonium oxidation was also found to occur (Bai et al. 2013).

Bacteria were dominated by α-Proteobacteria with high contributions from Bacteroidetes, Synechococcales, and γ-Proteobacteria. Melainobacteria, γ-Proteobacteria, and Verrucomicrobia tended to be more represented in genetic libraries from the RO feedwater. Protists were mostly represented by dinoflagellates, ciliates, Syndiniales, Stramenopiles, Rhizaria, Archaeplastida, and Opisthokonta, with ciliates, Rhizaria, and Opisthokonta being more represented in RO feedwater (Supplementary Figure S2, Supplementary Tables S2-S3).

ANOSIM analyses revealed significant differences in Bray-Curtis dissimilarities as well as unweighted and weighted UniFrac distances, between raw seawater and RO feedwater

communities for Archaea, bacteria and, to a lesser extent, protists. In contrast, microbial communities sampled at different dates did not show significant differences (data not shown). This indicates that microbial community differences across the SWRO pre-treatment system were greater than seasonal differences on the same sampling site. Furthermore, taxonomic differences across Penneshaw seawater pre-treatment system were greater for prokaryotes compared to eukaryotes.

Most archaeal OTUs (58%) were only detected in RO feedwater whereas this proportion was lower for bacteria (26%), and protists (26%) (Supplementary Figure S3). Data suggest that at least some of the archaeal and bacterial taxa sequenced here were likely to stably persist within Penneshaw SWRO plant during different seasons. Overall, 17 archaeal OTUs, 51 bacterial OTUs, and 6 eukaryotic OTUs were found occur in all RO feedwater samples and their contribution to the overall community was significantly (p-value > 0.01) higher than that observed in raw seawater (Fig. 2, Supplementary Table S4). RO feedwater persistent and enriched OTUs are represented by ammonium oxidising microbes, and biofilm-associated taxa typically observed in soil, sediment or different water treatment plants.

Ammonium oxidising microbes enriched in RO feedwater include the Archaea *Nitrosoarchaeum* and *Nitrosopumilus* (Fig. 2) that were previously detected within pre-treatment systems of SWRO desalination plants (Hong et al. 2016, Jeong et al. 2016) and the bacterium *Nitrospira* sp., which was found in wastewater treatment plants (Keuter et al. 2011) and biofilters of recirculating aquaculture systems (Brown et al. 2013). Current data thus suggest that *Nitrosoarchaeum*, *Nitrosopumilus*, and *Nitrospira* representatives found here were likely to colonise the multimedia filters of the seawater pre-treatment being responsible for the ammonium oxidation measured (Fig. 1). Other OTUs that are significantly enriched in RO feedwater mostly belong to taxa that have been previously found in RO membranes or RO feedwater such as *Pseudoalteromonas* spp. and Cryomorphaceae (Chun et

al. 2012, Nagaraj et al. 2019) or biofilm forming taxa such as Melainabacteria (Rehman et al. 2020) and Hartmannulidae ciliates (Xu et al. 2014).

Taxa persistent in RO feedwater are likely to either occur in South Australian coastal waters throughout the year and systematically passing through the pre-treatment system because of some specific features (size, shape), or to be part of a persistent community present within Penneshaw pre-treatment system.While some of these taxa, especially ammonium oxidizers, are likely to derive from multimedia filters, others might be associated with biofilms present in other surfaces of the pre-treatment system. It has been demonstrated that biofilm-associated microbes present along pre-treatment systems can behave as microbial reservoirs potentially enhancing the risks of RO membrane biofouling (Levi et al. 2016).

In spite of the UV treatment and the presence of several multimedia and cartridge filters, Penneshaw SWRO plant harbours an RO feedwater-specific community which mostly includes prokaryotic microbes. Some of these microbes are potentially involved in the oxidation of ammonium to nitrite and nitrate (*Nitrosoarchaeum* spp., *Nitrosopumilus* spp., and *Nitrospira* spp.) within the multimedia filters. Most RO feedwater-specific microbes were previously isolated or sequenced from different water treatment facilities and some of them can potentially cause biofouling on the RO membrane. In contrast with other desalination plants, chemical disinfection is not applied in Penneshaw SWRO plant to decrease the environmental impact, thus potentially leading to an increased microbial load in RO feedwater. Microbial communities similar to the RO feedwater-specific community found here are likely to occur in other disinfection-free SWRO plants. Our results thus provide insights on the bacteria and Archaea potentially causing biofouling and can contribute, to future research, to design effective strategies to minimise biofouling.

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Figure Legend

Figure 1. Box and whisker plots highlighting changes in the concentration of (**A**) ammonium, as well as (**B**) nitrate and nitrite within the seawater pre-treatment system of Penneshaw desalination plant. Each box includes 25th and 75th percentiles from each parameter and lines within the boxes represent median values, whereas solid lines outside the boxes correspond to the 10th and the 90th percentiles. Taxonomic composition (left axis) and total contribution to the total 16S rRNA gene libraries (right axis) of the Archaea sequenced from (**C**) raw seawater and (**D**) RO feedwater in the seawater pre-treatment system of Penneshaw SWRO plant inferred from 16S rRNA gene sequencing.

Figure 2. Distribution of all the OTUs found to be persistent in RO feedwater and enriched (t-test, p-value > 0.01) compared to raw seawater and recovered from RO feedwater at all time points (persistent community). The taxonomic affiliation of each OTU is indicated next to the OTU code.

264 **Table 1.** Sequencing results and analysis of microbial diversity. OTUs: operational
265 taxonomic units
266

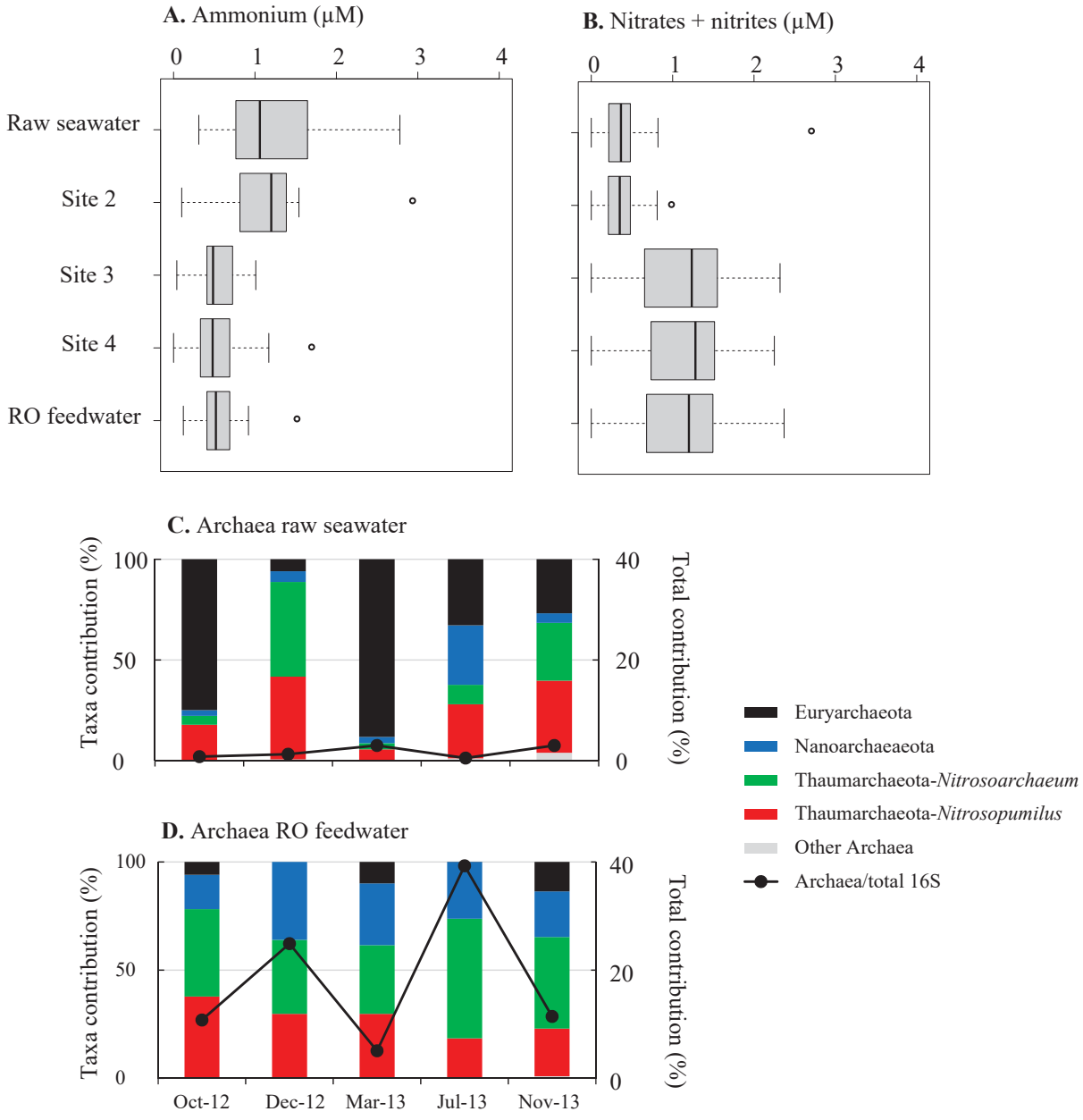
	Sampling site	Sampling date	No of reads	No of OTUs
Archaeal 16S	Raw seawater	24/10/12	117	30
		19/12/12	168	26
		27/03/13	385	40
		03/07/13	56	25
		27/11/13	387	89
		Total	**1,113**	**136**
	RO feedwater	24/10/12	1424	74
		19/12/12	3231	104
		27/03/13	674	86
		03/07/13	5252	124
		27/11/13	1530	133
		Total	**12,111**	**260**
Bacterial 16S	Raw seawater	24/10/12	9390	1036
		19/12/12	12098	1191
		27/03/13	9891	1283
		03/07/13	10071	2047
		27/11/13	9586	1983
		Total	**51,036**	**4449**
	RO feedwater	24/10/12	10079	1041
		19/12/12	10038	1098
		27/03/13	11928	952
		03/07/13	7947	1192
		27/11/13	10608	1226
Total		**Total**	**50,600**	**3089**
Protists	Raw seawater	24/10/12	17,242	1886
		19/12/12	17,242	919
		27/03/13	17,242	1507
		03/07/13	17,242	1650
		27/11/13	17,242	2499
		Total	**86,210**	**5781**
	RO feedwater	24/10/12	17,242	963
		19/12/12	17,242	410
		27/03/13	17,242	992
		03/07/13	17,242	587
		27/11/13	17,242	1907
		Total	**86,210**	**3771**

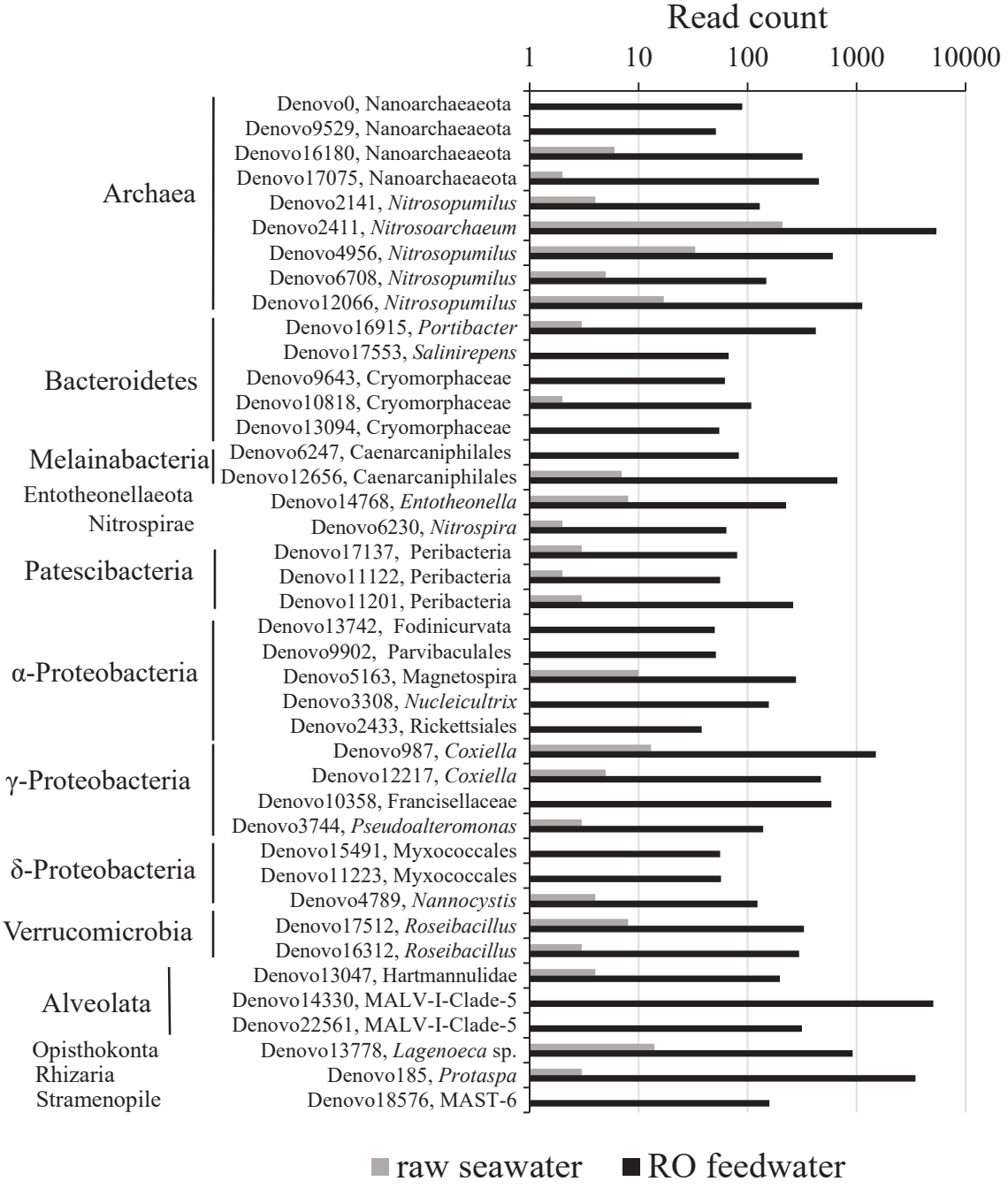
267
268

Table 2. Analysis of similarity (ANOSIM) highlighting differences between microbial communities sampled from raw seawater and RO feed water[a]

	Bray Curtis		Unweighted Unifrac		Weighted Unifrac	
Community	test	p-value	test	p-value	test	p-value
Archaea (16S)	0.44	0.02	**0.89**	0.008	**0.45**	0.007
Bacteria (16S)	**0.4**	0.005	1	0.01	0.31	0.036
Plastidic protists (16S)	0.35	0.029	0.1	0.25	0.21	0.13
All protists (18S)	0.34	0.012	0.41	0.02	0.42	0.011

[a] Significant (p-value < 0.05) and highly significant (p-value < 0.01) correlations are underlined and in bold, respectively.


A. Ammonium (µM)
B. Nitrates + nitrites (µM)
0
1
2
3
4
Raw seawater
Site 2
Site 3
Site 4
RO feedwater
C. Archaea raw seawater
D. Archaea RO feedwater
Taxa contribution (%)
Total contribution (%)
100
50
0
40
20
Oct-12
Dec-12
Mar-13
Jul-13
Nov-13
Euryarchaeota
Nanoarchaeaeota
Thaumarchaeota-Nitrosoarchaeum
Thaumarchaeota-Nitrosopumilus
Other Archaea
Archaea/total 16S


Read count
1
10
100
1000
10000
Archaea
Bacteroidetes
Melainabacteria
Entotheonellaeota
Nitrospirae
Patescibacteria
α-Proteobacteria
γ-Proteobacteria
δ-Proteobacteria
Verrucomicrobia
Alveolata
Opisthokonta
Rhizaria
Stramenopile
Denovo0, Nanoarchaeaeota
Denovo9529, Nanoarchaeaeota
Denovo16180, Nanoarchaeaeota
Denovo17075, Nanoarchaeaeota
Denovo2141, Nitrosopumilus
Denovo2411, Nitrosoarchaeum
Denovo4956, Nitrosopumilus
Denovo6708, Nitrosopumilus
Denovo12066, Nitrosopumilus
Denovo16915, Portibacter
Denovo17553, Salinirepens
Denovo9643, Cryomorphaceae
Denovo10818, Cryomorphaceae
Denovo13094, Cryomorphaceae
Denovo6247, Caenarcaniphilales
Denovo12656, Caenarcaniphilales
Denovo14768, Entotheonella
Denovo6230, Nitrospira
Denovo17137, Peribacteria
Denovo11122, Peribacteria
Denovo11201, Peribacteria
Denovo13742, Fodinicurvata
Denovo9902, Parvibaculales
Denovo5163, Magnetospira
Denovo3308, Nucleicultrix
Denovo2433, Rickettsiales
Denovo987, Coxiella
Denovo12217, Coxiella
Denovo10358, Francisellaceae
Denovo3744, Pseudoalteromonas
Denovo15491, Myxococcales
Denovo11223, Myxococcales
Denovo4789, Nannocystis
Denovo17512, Roseibacillus
Denovo16312, Roseibacillus
Denovo13047, Hartmannulidae
Denovo14330, MALV-I-Clade-5
Denovo22561, MALV-I-Clade-5
Denovo13778, Lagenoeca sp.
Denovo185, Protaspa
Denovo18576, MAST-6
raw seawater
RO feedwater

Supplementary data

Supplementary Figure S1. Accumulation curves, representing the OTU richness in each sample, as function of the number of reads sequenced for (A-B) the 16S and (C-D) the 18S rRNA gene libraries in (A, C) raw seawater and (B, D) RO feedwater.

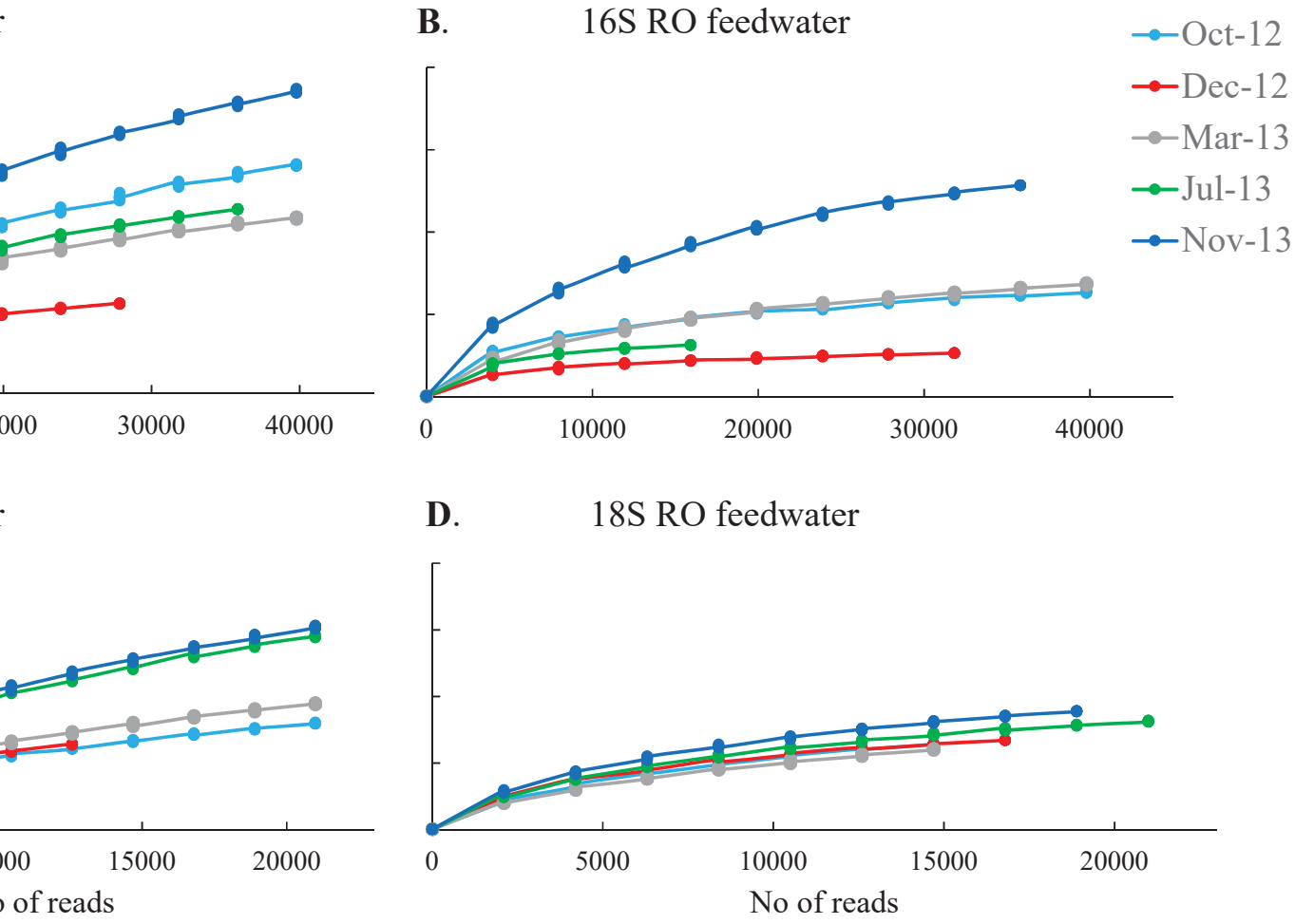
Supplementary Figure S2. Venn diagrams representing similarities and differences between the microbial communities of (A) Archaea, (B) bacteria, (C) plastidic protists, and (D) total protists, identified in raw seawater (light blue circles) and RO feed water (pink circles) of the Penneshaw SWRO plant. Values indicate the number of OTUs followed by their percentage contribution to total reads. The two small circles within each pink circle represent the OTUs found to be persistent (occurring at each time point) in RO feed water as well as in all samples (both raw seawater and RO feedwater), respectively.

Supplementary Table S1. Details of primers, adapters and barcodes used in the present study for Ion Torrent sequencing

Supplementary Table S2. Taxonomic affiliation and distribution within the different samples for the OTUs (97 % identity) found here within the prokaryotic 16S rRNA gene dataset.

Supplementary Table S3. Taxonomic affiliation and distribution within the different samples for the OTUs (97 % identity) found here within the 18S dataset

Supplementary Table S4. Statistics of the OTUs from the 16S rRNA gene that were found to be significantly more abundant in RO feedwater compared to raw seawater for the 16S rRNA gene



epresenting the OTU richness in each sample, as function of the number of reads sequenced for (**A**-**B**) the 16S and
n (**A**, **C**) raw seawater and (**B**, **D**) RO feedwater.