Vol. 86: 63–68, 2021
https://doi.org/10.3354/ame01958

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online March 11

NOTE

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

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ABSTRACT: We analysed prokaryotic and eukaryotic communities across the seawater pre-treatment system of Penneshaw (Kangaroo Island, South Australia) desalination plant, using 16S and 18S rRNA gene sequencing. The richness of operational taxonomic units increased downstream of the pre-treatment system (reverse osmosis feedwater) compared to raw seawater for *Archaea*, while it decreased for bacteria and protists. Overall, the reverse osmosis 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 was reduced by the pre-treatment system, the increase in proportion of biofilm-associated microbes suggests the presence of active microbial communities within multimedia filters and other parts of the pre-treatment system that might increase biofouling risks.

KEY WORDS: 16S rDNA · 18S rDNA · Ammonium oxidation · Desalination

1. INTRODUCTION

Climate change and increases in the 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. 2015c) after pre-treatment for seawater reverse osmosis (SWRO). While bacterial communities have been widely characterized, little is known about *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.

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2. MATERIALS AND METHODS

Seawater was collected from the Penneshaw SWRO desalination plant, located on the north-eastern coast of Kangaroo Island, South Australia (Balzano et al. 2015c). The pre-treatment system consists of a medium pressure-ultraviolet (MP-UV) disinfection unit, multimedia and cartridge filters (Balzano et al. 2015c). Nutrients were analysed every second week over a 1 yr period (18 July 2012 to 21 July 2013) from raw seawater (Site 1), downstream of the MP-UV treatment unit (Site 2), the multimedia filters (Site 3), the 15 µm cartridge filters (Site 4), and the RO feedwater (Site 5), 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 mo period (October 2012, December 2012, March 2013, July 2013, and November 2013). A volume of 120 l seawater was 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). Cells were removed from the Sterivex units, the DNA was extracted, the 18S rRNA gene was amplified, and both the 18S and the 16S rRNA genes were 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 (Table S1 in Supplement 1 at www.int-res.com/articles/suppl/a086p063_supp1.xlsx) of the universal prokaryotic primers V341F and 805R (Bowman et al. 2012). To amplify the 16S rRNA gene, 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, from both 18S and 16S rRNA genes, were processed using the python pipeline Quantitative Insight into Microbial Ecology (QIIME) (Caporaso et al. 2010); reads were filtered and clustered into operational taxonomic units (OTUs) based on 97% sequence identity, and reads from different samples were compared as described previously (Balzano et al. 2015a).

3. RESULTS AND DISCUSSION

Raw seawater temperature ranged from 14.3°C (July 2013) to 20.8°C (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 filters, from Site 2 to Site 3 (Balzano et al. 2015c). 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 neither phosphate nor silica changed 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 filters. The lower ammonium to nitrate ratios measured downstream of 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 a nitrogen source (Middelburg & Nieuwenhuize 2000, Kumar et al. 2018).

Overall, we sampled a good portion of the microbial community (Fig. S1 in Supplement 2 www.int-res.com/articles/suppl/a086p063_supp2.pdf). 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*, which 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 (Könneke 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) suggests 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*. *Melainabacteria*, 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 (Fig. S2, Tables S2 & S3).

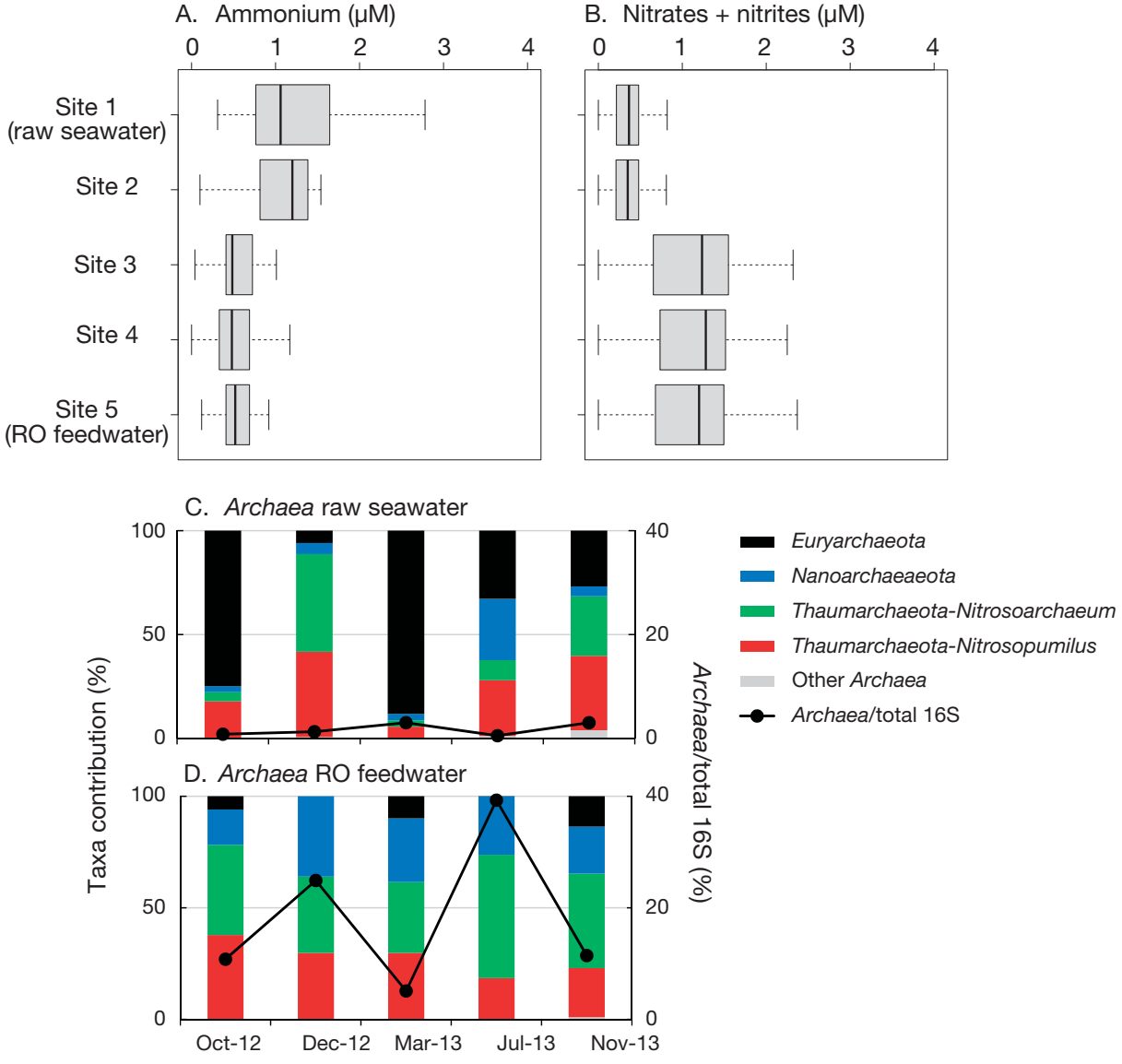

Fig. 1. (A,B) Box and whisker plots highlighting changes in the concentration of (A) ammonium, as well as (B) nitrate and nitrite within the seawater pre-treatment system of the Penneshaw desalination plant. Each box includes 25th and 75th percentiles from each parameter and lines within the boxes represent median values, whereas solid lines outside the boxes correspond to the 10th and the 90th percentiles. Outliers reflect time points in which the concentrations were found to be higher than the 75th percentile (12/09/12 for ammonium at Site 2, 16/01/13 and 27/03/13 for ammonium at Site 4, 7/11/12 for ammonium at Site 5, 04/06/13 for NOx at Site 1, and 13/03/13 for NOx at Site 2. (C,D) 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) reverse osmosis (RO) feedwater in the seawater pre-treatment system of the Penneshaw seawater reverse osmosis (SWRO) plant in ferred from 16S rRNA gene sequencing

Analysis of similarities (ANOSIM) 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 (Table 2). 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 at the same sampling site. Furthermore, taxonomic differences across the 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

Table 1. Sequencing results and analysis of microbial diversity. OTUs: operational taxonomic units; RO: reverse osmosis

Sampling site	Sampling date (d/mo/yr)	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	1113[a]	136[b]
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[a]	260[b]
Bacterial 16S			
Raw seawater	24/10/12	9390	1036
	19/12/12	12 098	1191
	27/03/13	9891	1283
	03/07/13	10 071	2047
	27/11/13	9586	1983
	Total	51 036[a]	4449[b]
RO feedwater	24/10/12	10 079	1041
	19/12/12	10 038	1098
	27/03/13	11 928	952
	03/07/13	7947	1192
	27/11/13	10 608	1226
	Total	50 600[a]	3089[b]
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[a]	5781[b]
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[a]	3771[b]

[a]Sum of all the genetic reads recovered from a sampling site. [b]Number of different OTUs found at each sampling site for the entire sampling period

Table 2. Analysis of similarity (ANOSIM) highlighting differences between microbial communities sampled from raw seawater and RO feedwater. Significant ($p < 0.05$) and highly significant ($p < 0.01$) correlations are underlined and **bold**, respectively

Community	Bray-Curtis		Unifrac			
			Unweighted		Weighted	
	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
Protists (18S)	0.34	0.012	0.41	0.02	0.42	0.011

bacteria (26%) and protists (26%) (Fig. S3). Data suggest that at least some of the archaeal and bacterial taxa sequenced here were likely to stably persist within the Penneshaw SWRO plant during different seasons. Overall, 17 archaeal OTUs, 51 bacterial OTUs, and 6 eukaryotic OTUs were found to occur in all RO feedwater samples, and their contribution to the overall community was significantly ($p > 0.01$) higher than that observed in raw seawater (Fig. 2, Table S4). Persistent and enriched OTUs in RO feedwater were 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 included the archaeal genera *Nitrosoarchaeum* and *Nitrosopumilus* (Fig. 2), which 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, and were responsible for the ammonium oxidation measured (Fig. 1). Other OTUs that were significantly enriched in RO feedwater mostly belong to taxa that have been previously found to be associated with 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 pass through the pre-treatment system because of some specific features (e.g. size or shape), or be part of a persistent community present within the Penneshaw pre-treatment system. While some of these taxa, especially ammonium oxidisers, are likely to derive from multimedia filters, others might be associated with biofilms present on other surfaces of the pre-treatment system. It has been demonstrated that biofilm-associated microbes present within pre-treatment systems can behave as microbial reservoirs, potentially enhancing the risks of RO membrane biofouling (Levi et al. 2016).

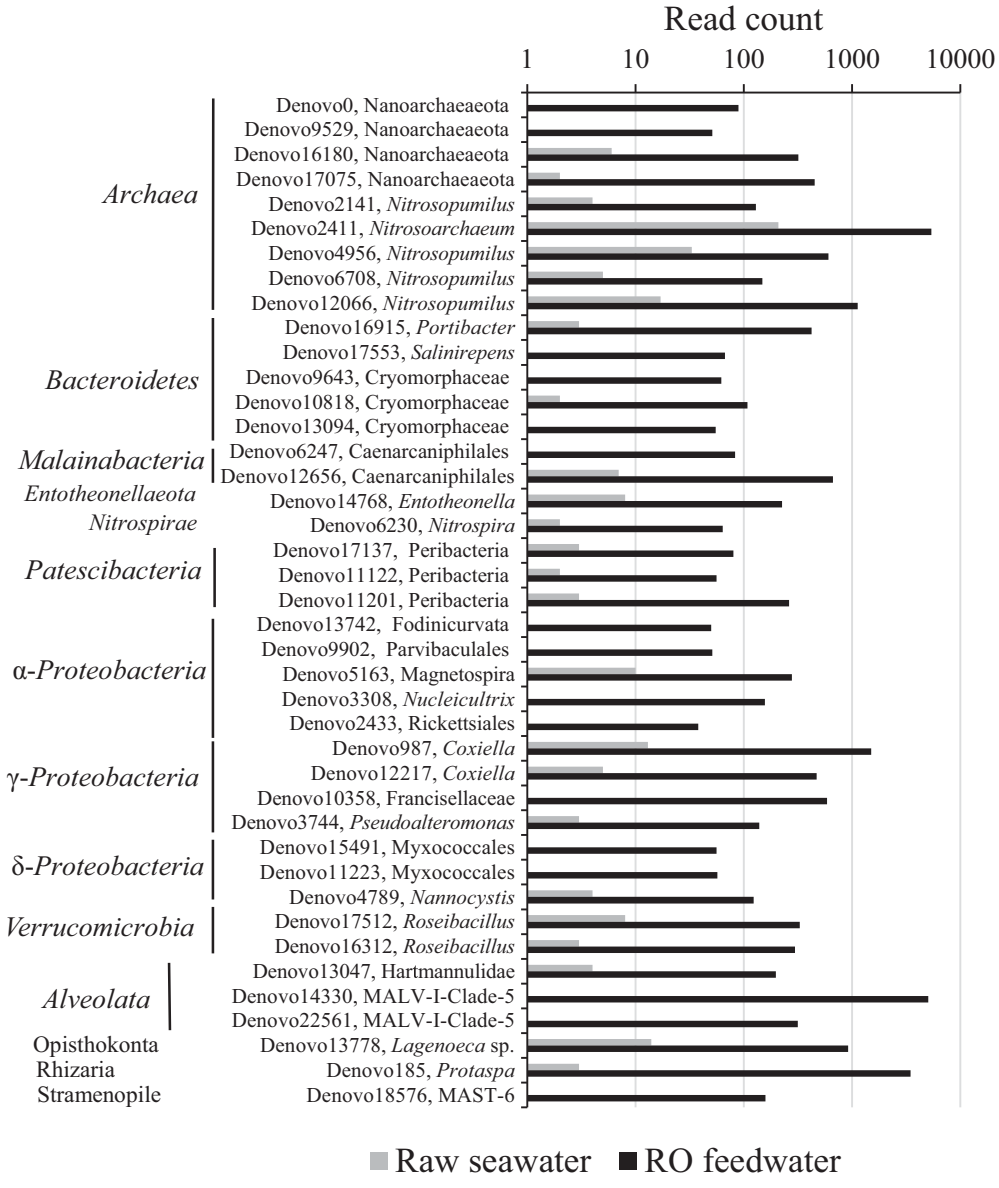

Fig. 2. Distribution of all the operational taxonomic units (OTUs) found to be persistent in reverse osmosis (RO) feedwater and enriched (*t*-test, $p > 0.01$) compared to raw seawater, and recovered from RO feedwater at all time points (persistent community). The taxonomic affiliation of each OTU is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

In spite of the UV treatment and the presence of several multimedia and cartridge filters, the 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 RO membranes. In contrast with other desalination plants, chemical disinfection is not applied in the Penneshaw SWRO plant, to lessen 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 into the bacteria and *Archaea* potentially causing biofouling and can contribute to future research in order to design effective strategies to minimise biofouling.

Acknowledgements. The authors acknowledge the financial support of the National Centre of Excellence in Desalination Australia (NCEDA), which is funded by the Australian Government through the National Urban Water and Desalination Plan. The authors are also grateful to P. Meacham, T. Kirby, and N. Nedelkov for their assistance in sampling at the Penneshaw desalination plant. We also thank X. Denis, C. Moreau, and J.-G. Jendyk for helping with sampling and processing nutrient samples and G. Lanzotti (RIMAR, SZN) for graphical assistance

LITERATURE CITED

Bai Y, Liu R, Liang J, Qu J (2013) Integrated metagenomic and physiochemical analyses to evaluate the potential role of microbes in the sand filter of a drinking water treatment system. PLOS ONE 8:e61011

Balzano S, Abs E, Leterme SC (2015a) Protist diversity along a salinity gradient in a coastal lagoon. Aquat Microb Ecol 74:263–277

Balzano S, Ellis AV, Le Lan C, Leterme SC (2015b) Seasonal changes in phytoplankton on the north-eastern shelf of Kangaroo Island (South Australia) in 2012 and 2013. Oceanologia 57:251–262

Balzano S, Le Lan C, Ellis AV, Compas H and others (2015c) Evaluation of transparent exopolymer particles and microbial communities found post-UV light, multimedia and cartridge filtration pre-treatment in a SWRO plant. Desalination Water Treat 56:1427–1439

Bowman JS, Rasmussen S, Blom N, Deming JW, Rysgaard S, Sicheritz-Ponten T (2012) Microbial community structure of Arctic multiyear sea ice and surface seawater by 454 sequencing of the 16S RNA gene. ISME J 6:11–20

Brown MN, Briones A, Diana J, Raskin L (2013) Ammonia-oxidizing archaea and nitrite-oxidizing nitrospiras in the biofilter of a shrimp recirculating aquaculture system. FEMS Microbiol Ecol 83:17–25

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K and others (2010) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336

Chun Y, Ha PT, Powell L, Lee J and others (2012) Exploring microbial communities and differences of cartridge filters (CFs) and reverse osmosis (RO) membranes for seawater desalination processes. Desalination 298:85–92

Hong PY, Moosa N, Mink J (2016) Dynamics of microbial communities in an integrated ultrafiltration-reverse osmosis desalination pilot plant located at the Arabian Gulf. Desalination Water Treat 57:16310–16323

Jeong S, Cho K, Bae H, Keshvardoust P and others (2016) Effect of microbial community structure on organic removal and biofouling in membrane adsorption bioreactor used in seawater pretreatment. Chem Eng J 294: 30–39

Keuter S, Kruse M, Lipski A, Spieck E (2011) Relevance of Nitrospira for nitrite oxidation in a marine recirculation aquaculture system and physiological features of a Nitrospira marina-like isolate. Environ Microbiol 13: 2536–2547

Könneke M, Bernhard AE, de la Torre JR, Walker CB, Waterbury JB, Stahl DA (2005) Isolation of an autotrophic ammonia-oxidizing marine archaeon. Nature 437:543–546

Kumar S, Bhavya PS, Ramesh R, Gupta GVM and others (2018) Nitrogen uptake potential under different temperature-salinity conditions: implications for nitrogen cycling under climate change scenarios. Mar Environ Res 141:196–204

Levi A, Bar-Zeev E, Elifantz H, Berman T, Berman-Frank I (2016) Characterization of microbial communities in water and biofilms along a large scale SWRO desalination facility: site-specific prerequisite for biofouling treatments. Desalination 378:44–52

Manes CLD, West N, Rapenne S, Lebaron P (2011) Dynamic bacterial communities on reverse-osmosis membranes in a full-scale desalination plant. Biofouling 27:47–58

Marie D, Shi XL, Rigaut-Jalabert F, Vaulot D (2010) Use of flow cytometric sorting to better assess the diversity of small photosynthetic eukaryotes in the English Channel. FEMS Microbiol Ecol 72:165–178

Middelburg JJ, Nieuwenhuize J (2000) Nitrogen uptake by heterotrophic bacteria and phytoplankton in the nitrate-rich Thames estuary. Mar Ecol Prog Ser 203:13–21

Nagaraj V, Skillman L, Li D, Xie Z, Ho G (2019) Culturable bacteria from a full-scale desalination plant: identification methods, bacterial diversity and selection of models based on membrane-biofilm community. Desalination 457:103–114

Naumann G, Alfieri L, Wyser K, Mentaschi L and others (2018) Global changes in drought conditions under different levels of warming. Geophys Res Lett 45: 3285–3296

Pitcher A, Hopmans EC, Mosier AC, Park SJ and others (2011) Core and intact polar glycerol dibiphytanyl glycerol tetraether lipids of ammonia-oxidizing archaea enriched from marine and estuarine sediments. Appl Environ Microbiol 77:3468–3477

Rehman ZU, Fortunato L, Cheng TY, Leiknes T (2020) Metagenomic analysis of sludge and early-stage biofilm communities of a submerged membrane bioreactor. Sci Total Environ 701:134682

Xu HL, Zhang W, Jiang Y, Yang EJ (2014) Use of biofilm-dwelling ciliate communities to determine environmental quality status of coastal waters. Sci Total Environ 470–471:511–518

Yin Q, Fu B, Li B, Shi X, Inagaki F, Zhang XH (2013) Spatial variations in microbial community composition in surface seawater from the ultra-oligotrophic center to rim of the South Pacific Gyre. PLOS ONE 8:e55148

Zhou J, Song X, Zhang CY, Chen GF, Lao YM, Jin H, Cai ZH (2018) Distribution patterns of microbial community structure along a 7000-mile latitudinal transect from the Mediterranean Sea across the Atlantic Ocean to the Brazilian Coastal Sea. Microb Ecol 76:592–609

Editorial responsibility: Craig Carlson, Santa Barbara, California, USA
Reviewed by: 2 anonymous referees

Submitted: May 7, 2020
Accepted: December 16, 2020
Proofs received from author(s): March 8, 2021