According to Lu et al 2015 (Journal of Applied Microbiology) the Legionella spp. Identified were not very diverse in drinking water distribution system.

For example,

Wang et al. (2012) reported a positive correlation between Legionella and V. vermiformis, and between mycobacteria and V. vermiformis (Wang et al. 2012), and Valster et al. (2011) reported that the concentrations of V. vermiformis correlated with the concentrations of Legionella spp. (Val-ster et al. 2011).

The positive correlations between V. vermiformis and Legionella and other bacteria likely reflected the grazing of FLA on bacteria for food (Weekers et al. 1993) and hosting of bacteria.

Similarly in the report by Wang et al. (2012), in the current study the FOD and abun- dance of Acanthamoeba spp. were significantly lower than those of V. vermiformis, and less correlated with Legio- nella spp. It has been suggested that Vermamoeba spp. were more ecologically relevant than Acanthamoeba spp. in drinking water (Wang et al. 2012) and premise plumb- ing (Thomas et al. 2008), possibly due to a reverse of dominance of Acanthamoeba spp. to V. (formally Hart- manella) vermiformis in a drinking water treatment plant after sand filtration (Thomas et al. 2008).

Traditional microbial indicators of water and wastewater quality, such as total coliform bacteria, are inadequate to predict the presence or disinfection responses of several impor-tant waterborne pathogens (Sobsey 1989; Levy et al. 2012).

as many pathogenic legionellae seem to require FLA for biofilm amplification (Conza et al. 2013; Garcia et al. 2013), a precursor to Legionella spp. Development in the DWDS studied could be a rise in the detection of V. vermiformis and/or Acanthamoeba spp. Monitoring for

such amoebae could keep ahead of evolutionary changes in different legionellae that may develop in DWDSs (Den Boer et al. 2013) along with other similar ARB patho-gens, by targeting their common amplification hosts.

**Rakic et al 2016 (Environmental Monitoring Assessment)**

Thus, bacteria Legionella spp. can be transmitted from water to air by aerosol-generating sys- tems such as cooling towers, evaporative condensers, plumbing equipment (e.g. faucets, showerheads, hot water tanks), humidifiers, respiratory therapy equipment (e.g. nebulizers) and whirlpool baths (Erdogan and Arslan 2007; Moeletal. 2006).

A statistical analysis was performed using the non-parametric Mann–Whitney U test with the aim of deter-mining the connection between of Legionella spp. And the previously described variables. The chi-square (χ2) test was calculated to compare the proportions of con-tamination Legionella spp. Statistical results were interpreted at the level of significance p<0.05.

Among the total of 425 analysed water samples, 116 (27.2 %) were Legionella spp. positive, which is an average of the results obtained from the warm waters of the public institutions in several countries. Thus, the presence of Legionella spp. was proven by 33.3 % in Italy (Leoni et al. 2005), by 26.0 % in Germany (Zietz et al. 2001) and by 20.0 % in Japan (Edagawa et al. 2008) of the water samples taken from the warm water of the public water supply systems.

Therefore, in order to reduce the microbial populations, it is recom-mended that the water be heated to over 50 °C (Moritz et al. 2010). Due to the very small and consistent range of pH in both Legionella spp. positive and negative samples, the influence of pH on the occurrence of Legionella spp. could be considered as insignificant. Legionella spp. were identified in water samples

with and without free residual chlorine (Fig. 2), which proves that the Legionella spp. bacteria pres- ent within the biofilm are resistant to chlorine sani- tizing action (Buse et al. 2012; Marchesi et al. 2011; Mouchtouri et al. 2007). Therefore, the water disin- fection should be conducted with agents that have a stronger biocidal effect, i.e. the agents that contain a combination of active substances in order to cover a wide spectrum of biocidal activity (Wullings et al. 2011). In this study, the monitoring of pH and free residual chlorine concentration indicated that the dif- ferences were not statistically significant (Fig. 2), and their influence on the incidence of Legionella spp. could be neglected.

Increased concentrations of metal ions in water are usually a result ofmetal corrosion in the DWDS. As a result of this process, the dissolved metal ions are present in water and are essential elements that stim- ulate the formation of biofilms and microbial growth. Also, oxide films, as products of corrosion on the metal surfaces of the water supply system, further stimulate the growth and development of biofilms, which encourage biocorrosion, firmly bound to the surface of metal oxide rough walls. This increases their resistance to pressure surges and mechanical stress. This research demonstrates the biostimulating effect of Fe and Zn metal ions and positive correla- tions of Fe and Zn concentrations with the presence of Legionella spp. The similar Fe values and positive association with the presence of Legionella spp. were recently reported (Bargellini et al. 2011; Leoni et al. 2005). Additionally, Legionella spp. positive water samples showed significantly higher Fe and Zn con- centrations compared to negative samples which are in accordance to previous studies (Borella et al. 2004; Borella et al. 2005)

**Bautista-de los Santos et al 2016 (Water research)**

The majority of DW studies focus on the changes in structure and membership of microbial communities over varying temporal and spatial scales with the aim of deducing which parameter(s) underlies the observed changes. Studies thus far have investigated a range of temporal scales: annual (Pinto et al., 2014), monthly (McCoy and VanBriesen, 2012), weekly (Sekar et al., 2012) and daily (El-Chakhtoura et al., 2015).

The V4-region was used with 806R and 515F (Caporaso et al., 2012).

Analysis of Molecular Variance (AMOVA) (Excoffier et al.,1992)was conducted using Mothur (Schloss et al., 2009), permutational t-tests and permutational ANOVA (perANOVA) were conducted using R (RCoreTeam, 2014), while beta-dispersivity tests were conducted using vegan (Oksanen et al., 2013).

Overall, the majority of the significant changes in beta-diversity using the AMOVA metric were observed for community structure rather than for community membership.

The measured water quality parameters were relatively stable over the diurnal time scale for all sampling locations (Appendix A). As a consequence, the correlations between water quality param-eters and changes in whole bacterial community richness, struc-ture, and membership were weak and did not exhibit any significance of note. Similarly, we did not detect any significant correlations between the relative abundance of the most abundant OTU's and changes in water quality parameters over diurnal time-scales.

Replication is critical to any investigation of microbial commu-nities, irrespective of the environment being considered. The lack of technical replication has been identified as an important issue in microbial ecology studies (Prosser, 2010), with replication being critical to move from descriptions of the microbial communities, to the study of their complexity and function (Knight et al., 2012).

We were motivated by the question of how does variability introduced due to PCR (Pinto

and Raskin, 2012) and sequencing (Salipante et al., 2014) compare with variability between replicate DW samples for any given sampling location at a particular time point.

This leads us to the conclusion that investigations of bulk DWbacterial communities are much likely to benefit from PCR and sequencing replication as compared to the collection of replicate water samples. The resources saved due to this approach could then be beneficially put to use by replicating the experiment or field sam-pling exercise, itself. Indeed, recent studies have suggested that investigations of high diversity environments benefit more from greater sequencing depth rather than PCR/sequencing replication (Smith and Peay, 2014).

In contrast, during periods with a rapid increase in water demand, the primary mechanism altering the bulk water community is likely to be biofilm detachment. Biofilms are highly spatially heterogeneous and have been reported to have little to no overlap with bulk water communities (Henne et al., 2012).

Bacterial community structure is more variable across diurnal time scales as compared to community membership, due to Bacterial community structure is more variable across diurnal time scales as compared to community membership, due to significant changes in relative abundance of dominant OTUs in bulk DWsamples.

**Dupuy et al 2010 (Water Research)**

erformed on Legionella (Campos et al., 2003; Kim et al., 2002). In case of treatment failure, L. pneumophila might be able to recolonize water systems. It has been hypothesized that this recolonization is made possible because Legionella is protected in the biofilm or inamoebae(Barker et al., 1992; Donlan et al., 2005;Murga et al., 2001).   
  
Regarding Acanthamoeba, we show that the efficiency of the treatments clearly depends on the target strain. Similar results have been reported recently with other treatments (Coulon et al., 2010).

It was previously reported that L. pneumophila- infected Acanthamoeba polyphaga exhibited higher resistance to chlorine than uninfected amoeba (Garcia et al., 2007).

In contrast, no significant differences of sensitivity between infected or non-infected amoebae were observed for all treatments in this study. This suggests that protection of amoeba against disinfectant conferred by L. pneumophila may be limited to specific conditions.

Indeed, chlorine was more efficient at 50C than at 30C, at least on co-cultured Legionella.

Our results clearly indicate that chlorine and chlorine dioxide were more efficient on free L. pneumophila as compared to co-cultured L. pneumophila. The difference between free and co-cultured bacteria is in agreement with the study of Garcia et al. (2007), which reported a higher resistance to chlorine of L. pneumophila when internalized within A. polyphaga. Also, Barker et al. have reported that L. pneumophila grown in Acanthamoeba were less sensitive than free bacteria to disinfectants (polyhexamethylene biguanide, benzisothiazolone and 5-chloro-N-methylisothiazolone) and antibiotics (Barker et al., 1992, 1995).

Interestingly, monochloramine displays a different

behavior, as compared to the other treatments. Indeed, there was no difference of efficiency against the intra-cellular and free L. pneumophila with monochloramine, while other treat- ments were more efficient on free L. pneumophila.

**Costa et al. 2015 (Water research)**This type of DUWL contamination is associated with a wide variety of microorganisms ranging from non-pathogenic to pathogenic bacteria, fungi, viruses and protozoa such as free-living amoebae (Puttaiah et al., 2012; Szymanska, 2005; Szymanska et al., 2008; Walker et al., 2000).

Mycobacteria have been described for their resistance to a wide range of antibiotic agents

(Maurer et al., 2014) and may likewise resist to the different water treatments used in DUWL. More specifically, their resistance to treatments used in water distribution systems as chlorination and ozonation has been reported (Falkinham et al., 2001; Le Dantec et al., 2002; Taylor et al., 2000). Dental units are usually subjected to continuous treatment in order to limit DUWL microbial coloni-zation (Coleman et al., 2014; Schel et al., 2006;Walker and Marsh, 2007).

The treatment is triggered by the water flow involved in

dental unit activity, suggesting that OWA samples could be more

exposed to disinfectant agents than OWS and IW samples. Conse-quently, one possible explanation for the relatively high abundance

of Mycobacterium in OWA resides in different sensitivities of genera to desinfectant.

**Zeybek et al., 2014 (Experimental Parasitology)**

FLAs gained significant attention for the role they play in spreading pathogen bacteria in aquatic systems, in addition to their patho-genic activity as described by many studies in the literature (Rowbotham, 1980; Brown and Barker, 1999; Molmeret et al., 2005; Thomas et al., 2006). Legionella pneumophila, Mycobacterium spp., Francisella tularensis, Escherichia coli O157, Afipia felis, Rickett-sia pickettii, Pseudomonas spp., Burkholderia cepacia are listed

among these bacteria and Acanthamoeba, Hartmannella, Naegleria, Vahlkampfia are listed among these free-living amoebae (Walochnik et al., 1999; Landers et al., 2000; Winiecka-Krussnell and Linder, 2001; Greub and Raoult, 2004; Molmeret et al., 2005; Thomas et al., 2006; Declerck et al., 2007).

**Canals et al., 2015 (Environ Sci Pollut Res)**

In recent decades, free-living protozoa (FLP) have gained greater prominence as the focus of research studies in a wide variety of man-made water systems (Lasheras et al. 2006; Ménard-Szczebara et al. 2008; Patterson et al. 1997;Rohr et al. 1998; Thomas et al. 2008; Yamamoto et al. 1992) due to their pathogenicity to humans (Trabelsi et al. 2012; Visvesvara et al. 2007) and their close relationship with the survival and growth of pathogenic amoeba-resisting bacteria such as Legionella spp., Listeria monocytogenes and nontuberculous mycobacteria (Cateau et al. 2014;Fields et al. 1984; Greub and Raoult 2004; Rowbotham 1980;

It is important to note thatmost of the studies concerning FLP in man-made water systems have focused on the detection of FLA, and few authors have also studied or reported the presence of flagellate and ciliate species (Barbeau and Buhler 2001; Patterson et al. 1997; Sutherland and Berk 1996; Valster et al. 2009; Yamamoto et al. 1992), so information regarding the flagellate and ciliate species that inhabit man-made water systems and how disinfection treatments affect them is more scarce than information regarding amoebae.

During the present study, the observed FLA genera in HSWS and CT were Acanthamoeba, Vahlkampfia, Vannella and Vermamoeba, genera that have been repeatedly reported in several studies using culture and molecular methods to inhabit a wide variety of man-made water systems (Barbeau and Buhler 2001;Delafont et al. 2013;Rohr et al. 1998;Shoff et al. 2008;Thomas etal. 2008; Valster et al. 2009).

The flagellates identified corresponded to Bodo spp., the Chrysomonadida class and a flagellate similar to the flagellat-ed phase of Paratetramitus spp. Most of the flagellates ob-served in our study have been reported previously by other authors: Bodonidae flagellates have been reported in cooling tower systems (Yamamoto et al. 1992), transplant unit water supplies (Patterson et al. 1997), dental unit waterlines (Barbeau and Buhler 2001) and drinking water supplies (Valster et al. 2009), and Monas sp., a Chrysomonadid flag-ellate,was observed by Paszko-Kolva et al. (1991)in eyewash stations.

Temperature is a widely used method for disinfecting man-made water systems, especially HSWS, worldwide, thus sev-eral studies regarding the tolerance of protozoa, especially amoebae, to temperature have been carried out (Cervero-Aragó et al. 2013; Kuchta et al. 1993; Ménard-Szczebara et al. 2008;Rohr et al. 1998;Storey et al. 2004;Thomas et al. 2006). In the present study, the results obtained on FLP presence inHSWS(detected in 46.8%of sampling points) are similar to those reported in other man-made systems with similar temperatures, with percentage values ranging from 47 to 68 % (Patterson et al. 1997;Rohretal. 1998).