Research in Microbiology 164 (2013) 262–269

www.elsevier.com/locate/resmic

In situ transplant analysis of free-living bacteria in a lotic ecosystem

Amitai Or [a], Orr Comay [b], Uri Gophna [a,*]

[a] Department of Molecular Microbiology & Biotechnology, George S. Wise Faculty of Life Sciences, Tel Aviv University Ramat Aviv, Tel Aviv 69978, Israel
[b] Department of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University Ramat Aviv, Tel Aviv 69978, Israel

Received 22 August 2012; accepted 5 December 2012
Available online 17 December 2012

Abstract

The Yarqon is a slow-flowing Mediterranean stream with three ecologically distinct sections, with varying abiotic conditions and anthropogenic influences. We used the Yarqon as a test habitat to study the effect of flow on microbial communities. Stream water samples from three distinct abiotic conditions: "clean", "human-impacted" and "brackish" sections were incubated in situ in dialysis bags at each of these sections for approximately 73 h. The samples were retrieved and analyzed by ARISA (automated ribosomal internal spacer analysis) and viable counts. Diversity estimates showed that free-living assemblages from the middle human-impacted section increased in diversity, while assemblages from the upper-clean section decreased in diversity unless planted in their site of origin. Samples originating from the brackish western section decreased in diversity wherever they were incubated. The ARISA profiles of the samples usually grouped by origin rather than by incubation location, implying that the rate of change of the free-living bacterial assemblages due to the shift in environment is relatively slow. Nevertheless, introducing free-living bacteria from the human-impacted section into the freshwater section resulted in a profile more similar to the latter, indicating a profound niche influence on these microbial assemblages.

Keywords: Stream; Bacteria; Transplant; Ecosystem; Mesocosm

1. Introduction

Streams in Mediterranean climate regions are physically, chemically and biologically shaped by sequential predictable seasonal events such as shifts in temperature, UV radiation and water volume (Gasith and Resh, 1999). Other factors, such as levels of nutrients, predation and productivity, may also have a large impact on the stream's bacterial assemblage (Hewson and Fuhrman, 2004; Rozen and Belkin, 2001). Overall, the chemical make-up of the water, be it freshwater, partially anthropologically impacted or brackish (Crump et al., 1999) is expected to be the major determinant of the microbial community composition and ecology. Microbial communities respond quickly to environmental conditions due to their rapid replication, ability to switch between carbon sources and the presence of resistant inactive forms such as cysts or spores. Hence, it was suggested that, in fast-changing ecological niches such as streams, the bacterial assemblage will rapidly vary according to the environmental conditions (Horňák et al., 2005; Niemi et al., 1993; Shade et al., 2008; Whitton, 1975). To date, few in situ transplant experiments have been carried out that directly investigate the ability of the bacterial assemblage to change and adapt to new environmental conditions when confined in space (Kritzberg et al., 1994; Shade et al., 2010), and none addressed the question of flow in a lotic ecosystem. Here we used the Yarqon, an urban slow-flowing Mediterranean stream, to jointly examine the effects of flow and water composition on bacterial communities.

The Yarqon stream can be divided into three distinct environments (Fig. 1): the eastern section which is unpolluted, having typical biological oxygen demand (BOD) values lower than 1 mg/L (E); the middle section (M), which is impacted by partially treated domestic sewage from wastewater treatment plants (WWTPs) (average BOD values greater than 10 mg/L, see also Raz, 2002, 2004); and the estuary where seawater flows inland, resulting in brackish waters (salinity values

* Corresponding author.
E-mail addresses: amitaior@gmail.com (A. Or), orrcomay@gmail.com (O. Comay), urigo@tauex.tau.ac.il (U. Gophna).

0923-2508/$ - see front matter © 2012 Institut Pasteur. Published by Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.resmic.2012.12.004

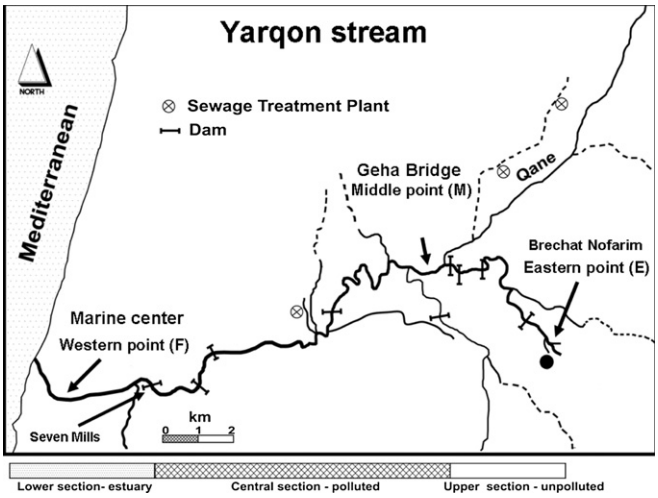

Fig. 1. The Yarqon stream and locations at which water samples were retrieved; incubation sites marked: Brechat Nofarim, from the eastern section (not impacted) marked "E"; Geha Bridge, from the middle section (human-impacted), marked "M"; and the marine center, from the western section (brackish), marked "W"; adapted from Elron et al. (2006).

greater than 80,000 ppm), which will be referred to as the western section (W). The three sections described here are distinct with regard to both biotic and abiotic parameters (Gafny et al., 2000; Or and Gophna, 2011; Or et al., 2012, see also Supplementary Fig. 2). Nevertheless, they constitute parts of a single stream, which has a continuous downward flow from source to sea; hence, free-living bacteria from the upstream are expected to be present, though not always detectable, along the entire length of the stream (Or and Gophna, 2011; Or et al., 2012).

Re-introduction of flora and fauna of different ecological niches to other niches is a common practice of macro-ecologists (Johnson and Cushman, 2007; Mittelbach et al., 1995; Sarrazin and Barbault, 1996) that usually enables them to investigate the community re-structuring dynamics and spatial investigations (Dyer and Deborah, 1999; Ellis et al., 2011; Polis, 1994) Yet, despite the need to similarly understand such bacterial assemblage dynamics (Wetzel, 2000), these in situ experiments are still uncommon. In order to better understand some of these possible effects and specifically explore the possible consequences of a reduction in flow, we devised an in situ incubation experiment. This approach has been successfully used to study lake bacterial communities disturbed by mixing (Shade et al., 2010, 2011), since it allows the separation of spatial and temporal dynamics. Nevertheless, the method is not without its biases (also known as "bag effects"), as some taxa do not persist when enclosed in these bags (Shade et al., 2010).

In water-poor countries such as Israel, freshwater is a precious commodity. Reclaimed water, which is the main source of many Israeli streams such as the Yarqon, is in high demand for agricultural purposes. Thus, there is a constant pressure to further reduce water volumes in streams. A reduction in the relative contribution of freshwater to the stream water volume, along with slower water flow, can cause undesirable changes to the stream ecosystem and have drastic effects on bacterial assemblages. Specifically, the combination of large populations of heterotrophic bacteria discharged from WWTPs and a very low water volume regime could irreversibly shift the microbial balance in favor of WWTP bacteria, causing odor complaints and possibly damaging the stream's ecology.

During August 2009, we studied water samples from each stream section at two different time points 73 h apart, which is the average water replacement period from origin to end in the Yarqon (Raz, 2002, 2004, 2009). Our focus was on free-living (rather than particle- or sediment-associated) bacteria, which are most affected by flow; hence a prefiltration step was added to the DNA extraction protocol. A sample from every section was incubated in each of the other sections in semi-permeable dialysis bags that allow flow of nutrients and waste products, but not of cells and macromolecules larger than the 20 kDa nominal pore size. We extracted DNA from free-living water-borne bacteria and compared the samples in terms of bacterial assemblage compositions, determined by automated ribosomal intergenic spacer analysis (ARISA). Samples were compared both to other samples from the same origin incubated in other sections and to samples from other origins that were incubated at the same incubation site.

The hypothesis tested by the experimental design is that transplanted bacterial communities will become more similar to assemblages typical of the site of incubation.

2. Materials and methods

2.1. Study site

The Yarqon stream is a Mediterranean stream that crosses Tel Aviv, Israel. The stream's water originates from Yarqon-Taninim aquifer water, seasonal water runoff and reclaimed water from municipal wastewater treatment plants. The Yarqon stream authority estimates that currently, WWTP water contributes the majority of the stream's volume (Raz, 2009). Based on the degree of perturbation and water quality (Elron et al., 2006; Gafny et al., 2000; Gücker et al., 2006; Tavasi et al., 2004) the Yarqon stream, whose total length is 27.5 km, can presently be divided into three sections (Fig. 1): an upper 'natural' section (7.5 km) – from Rosh Ha'Ayin springs to the confluence with the Qane tributary – which is termed the "eastern section" (E); a central section (17.5 km) severely impacted by pollution of treated wastewater effluents at its upper reaches, with gradual recovery downstream, termed the "middle section" – (M); and the lowermost (4 km) "western section" (downstream from the Seven Mills weir) – (W), a partially polluted estuary with brackish characteristics. These sections may therefore be regarded as distinct habitats although they are not hydrologically separated, as westward water flow is slow but undisturbed. An important exception to this generalization is the fact that saline water cannot flow east past the last weir Seven Mills, while reverse downward flow is possible. Importantly, the average flow of the stream is an estimated 280–300 m per h, which translates into 3–4

(or 80–100 h) days of flow from the river origin to the sea and about 30 h of flow between Brechat Nofarim and Geha Bridge and an estimated additional 45 h of flow between Geha Bridge to marine center sampling points (Raz, 2009).

2.2. Water sample collection

For all our measurements, we used sterile water sampling flasks 500 ml in volume. At site E we sampled from Brechat Nofarim. At site M we sampled from the water underneath a bridge in Geha Road. At site W, we sampled from the water near the marine center (Fig. 1). Samples were retrieved to the lab within the hour at 4 C° for immediate processing, including DNA extraction.

2.3. Environmental parameters

Several environmental parameters were measured while collecting the samples: dissolved oxygen and temperature were measured by a YSI 55 dissolved oxygen meter (YSI Inc., Yellow Springs, OH, USA); pH was measured by an H1 9025 pH meter (Hanna Instruments Inc., Woonsocket, RI, USA) and conductivity was measured by an H1 3733 conductivity meter (Hanna Instruments Inc., Woonsocket, RI, USA). Turbidity was measured by a HACHI 21000p turbidimeter (Mitsubishi Chemical Corporation, Tokyo, Japan). The BOD and chemical oxygen demand (COD) were analyzed by the USA EPA-approved standard procedures SM 5210 B and SM 5220 D, respectively. Cl^- was measured by SM 4500-Cl^- B and nutrient concentrations (total N, NO_3, NH_3-N, NO_2, and total P) were analyzed by SM 4500-NO_3^- B, SM 4500-NH_3 C, SM 4500-NO_2 B and SM 4500-P B, C colorimetric EPA methods, respectively.

2.4. Incubation settings

Two samples (duplicates) from each section of the three sections were submerged in each river segment, where each sample consisted of a dialysis bag filled with 50 ml of stream water retrieved from the "E", "M" and "W" sites. In addition to the water samples from each section, every incubation site also included 2 samples from an overnight turbid *Escherichia coli* MG 1655 culture, serving as positive controls which were pre-acclimated to the ambient temperature and 2 samples from a sterile saline solution, serving as a negative control. All controls were validated by viable counts. A total of 30 bags were fully submerged in the water at about a 0.5–1 m depth and were further protected from physical harm by placing them in custom-made iron cages. All of the dialyses bags remained intact.

In order to prevent community exchanges between the samples and the surroundings, we used a cellulose-ester dialysis membrane with 20 kDa nominal pore size (Spectra/Por®, CA, USA). These pores are small enough to physically block migration of unicellular organisms such as bacterial cells and viruses, but large enough to allow diffusion of small molecules as nutrients. Furthermore, preliminary experiments showed that the cellulose-ester bags were durable under actual stream conditions and did not deteriorate with time and that the nominal pore size did not increase during incubation (data not shown). In addition, the unhindered diffusion of small macromolecules to and from the environment was a key desirable feature because it provides the bacteria with both a means of waste disposal and a constant flow of nutrients.

The incubation sites were chosen by their accessibility, constant flow of water regime, shade and long-term trends in nutrient levels and other physico-chemical parameters (see Supplementary Fig. 1 and Supplementary Table 1) over a 10-year period. The physical and biogeochemical differences between the sites were also validated by an additional sampling round which was performed 10 days prior to the experiment (see Supplementary Table 1). The incubation period was identical for all samples and lasted approximately 73 h, from 23/8/2009 at 11:00 (this time point is referred to as "time 0") to 26/8/2009 12:00 (this time point is referred to as "time 73"), thus simulating the same duration time of water flow between Brechat Nofarim and the marine center sampling points. During the experimental period, the weather was a typical Mediterranean summer with no precipitation or water run-off from external sources, yet the flow was continuous without fragmentation. An important limitation of this experimental approach is that the transplanted bacteria are trapped along with potential predators or viruses, but are protected from new antagonists from both types by the membranes that confine them.

2.5. Sample designation

Sample designations used throughout are left to right, with the first letter representing the water origin (eastern, middle or western) and the second letter representing the incubation site of the dialyses bags, while numbers indicate experimental replicates. The time when the sample was taken is indicated at the end of the name. "Ambient" refers to samples taken directly from the water column, as opposed to incubated samples (see Supplementary Table 2 for sample name designation summery).

2.6. Viable counts

In order to estimate the number of viable bacteria in the communities, viable counts were performed. Every sample was diluted with a sterile saline solution to several fixed dilutions, ranging from 10^{-8} to 10^{-3}, in triplicate. R2A (Difco, MD, USA) medium was used. Plates were incubated in 30 °C, approximating the average temperature of the stream.

2.7. DNA extraction from water samples

In order to extract the DNA from free-living water-borne bacteria, each water sample was poured into a sterile tube. Then, every sample was prefiltered by a 47-mm-diameter glass microfiber GF/C filter with nominal pore size of 1.2 μm (Whatman, Maidstone, UK), removing large particles and

protists, if present, and also nearly all particle-associated bacteria. Free-living bacteria were collected onto an ME-24 0.2 μm membrane filter (Schleicher & Schuell Inc., NH, USA). DNA was extracted from the water filter using the Powersoil DNA extraction kit (Mobio Laboratories, CA, USA) by shredding the water filters under sterile conditions and using the filter shreds instead of soil, applying the manufacturer's protocol.

2.8. DNA concentration standardization

We standardized the PCR volumes so that every reaction would contain a fixed concentration of DNA. We used a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, DE, USA) to measure the concentration of DNA in every sample. Each sample was measured 3 times and the measurements were averaged. After assessing the concentrations, appropriate volumes of double distilled water were added to each sample in order to set a standard DNA concentration of 20 ng per 25 μl for ARISA.

2.9. ARISA

Molecular fingerprinting methods have been previously used to correlate microbial community characteristics with seasonal or spatial dynamics or with geo-chemical parameters (Danovaro et al., 2006; Fuhrman et al., 2006; Guillermina et al., 2006; Mašín et al., 2003; Zurel et al., 2011). Here we used ARISA (Fisher and Triplett, 1999), which was proven to be robust, rapid and easy to perform. It was previously used to assess similarities in composition between different microbial communities (Fisher and Triplett, 1999). ARISA relies on a PCR amplification step, conducted with one fluorescence-tagged oligonucleotide primer and one unlabeled primer. The electrophoretic step is subsequently performed with an automated sequencing system, which provides separation and laser detection of the fluorescent DNA fragments. This automated fragment analysis has the advantage of accurate sizing (~1 bp), providing high resolution of fragments. ARISA is based on the length heterogeneity of the bacterial rRNA operon 16S–23S intergenic spacer (also known as internal transcribed spacer, or ITS). ITS regions are highly divergent in length (150–1500 bp) and vary between bacterial species and often between strains of the same species (Kovacs et al., 2011). All PCR reaction templates were normalized to the same DNA concentration of 20 ng per 50 μl PCR reaction tube. PCR was performed with 1.25 U of *Taq* DNA polymerase (BIOTAQ™, BIOLINE), 3 mM of $MgCl_2$, 2.5 μl 10× PCR buffer, 0.1 mM of each dNTP, ultra-pure water (Biological Industries, Israel) and 10 pmol of primers (1392F: 5′-GYACACACCGCCCGT-3′ and 125R: Tet-5′-GGGTTBCCCCATTCRG-3′) (Fuhrman et al., 2006). Reactions were prepared in duplicate in a dedicated PCR cabinet with filtered air laminar flow. Negative controls containing no template were also prepared to verify lack of contamination. The reaction was performed as follows: 3 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 53 °C, 1.5 min at 72 °C and a final elongation step of 6 min at 72 °C, using a T-personal BIOMETRA PCR Thermocycler. All PCR products were observed by gel electrophoresis (1% TBE agarose gel) to confirm successful amplification and to rule out contamination. PCR products were analyzed using the ABI PRISM 3100 Genetic Analyzer. The labeled fragments were separated on the capillary sequencer, while an internal size standard, a custom made marker – CST ROX 250–1150 (Bioventures, Murfreesboro, TN), was used in each capillary.

2.10. ARISA data analysis

Raw data generated by the ABI PRISM 3100 Genetic Analyzer were initially analyzed using GeneMarker (SoftGenetics, PA, USA). After performing accurate size calling using that software, all data were exported to Microsoft Excel for further analysis. Operational taxonomical units (OTUs) with an arbitrary threshold of relative fluorescence intensity of 40 or lower were excluded. Subsequently, all OTUs were binned as described previously (Hewson and Fuhrman, 2004; Kovacs et al., 2010) and intensities were summed up for each bin. Next, relative intensities for each binned OTU in each sample were calculated and binned OTUs, which contributed less than 0.5% to the total intensity of the sample were excluded. Duplicates were compared, and only OTUs that appeared in both duplicates were used, and their new relative intensities were re-calculated. Finally, the averaged values for each sample were normalized to reflect relative intensity values.

2.11. Cluster analysis and diversity indices

All ARISA data were exported to PAST (Hammer et al., 2001), a statistical data analysis package. Using PAST, similarity calculations were performed using the Bray–Curtis similarity index (Legendre and Legendre, 1998). This index takes into account taxon richness and abundance in the samples. Diversity indices and analysis of similarity (ANOSIM) (Anderson, 2001) were calculated in PAST using default settings. The equitability measure was calculated as the Shannon diversity divided by the logarithm of number of OTUs. This measures the evenness with which individuals are divided among the OTUs present.

3. Results

3.1. ARISA-based diversity analysis

We first compared the diversity of the different bags based on the ARISA profiles. Shannon diversity and equitability indices as well as OTU richness based on ARISA profiles can show only fairly abundant taxa (>0.5% of the bacterial population). Thus, an increase in ARISA-based richness (Supplementary Fig. 3) means that more bacteria passed a certain threshold of abundance and a decrease means that fewer taxa were detectable. ARISA-based analysis showed that free-living assemblages from the middle section showed an increase in diversity no matter where these communities

were planted (Fig. 2). As noted previously (Or and Gophna, 2011), lower diversity and richness of bacterial assemblages (Fig. 3) observed in the middle section were associated with a large inflow of treated wastewater into the stream. In contrast, bacteria from the eastern part decreased in diversity, except when planted at their site of origin, indicating that only a subset of these bacteria can proliferate in saline or human-impacted water (Fig. 2). The samples originating from the western section all exhibited a large decrease in diversity, in all locations of incubation (including their origin). Given the ARISA detection threshold (see above), it may be more accurate to examine equitability (Fig. 3), the evenness with which individuals are divided among the taxa present at every sample. Indeed, equitability increased in all samples except for those originating in the western section.

3.2. ARISA-based analysis of similarity between samples

Clustering the ARISA data by the Bray–Curtis similarity index showed an interesting pattern in which the grouping of samples reflected the origin of the water inocula rather than the place of incubation (Fig. 4). The only exception was water from the middle section that was incubated in the eastern (unpolluted) section and became more "eastern-like" in terms of bacterial composition.

Analysis of similarity (ANOSIM) confirmed that the samples were grouped by water origin rather than by place of incubation (Table 1). Remarkably, the highest R value (the highest degree of separation between groups) was observed between the eastern and western sections which were not directly connected ($R = 0.972$), while the eastern–middle and the middle–western showed lower R values (0.801 and 0.679 respectively). As expected, samples which were re-placed in their segment of origin (i.e. EE, WW and MM) were most distinct from samples originating in other segments, with high significance values. The results strongly suggested that the origin of the free-living bacterial assemblage placed in the stream was a stronger determinant than the location of incubation at the timescale that was examined. Notably, a similar analysis, this time grouping

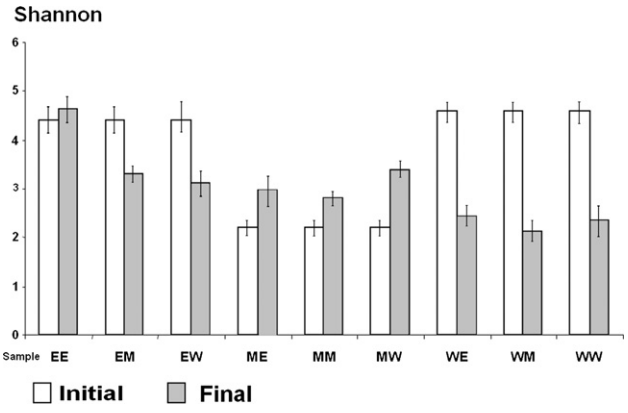

Fig. 2. Free-living bacterial Shannon diversity based on ARISA data. Initial values at time 0, white columns; final values after 73 h of incubation, gray columns. Values are averages of two independent samples (separate dialysis bags). Error bars represent the standard error of the mean.

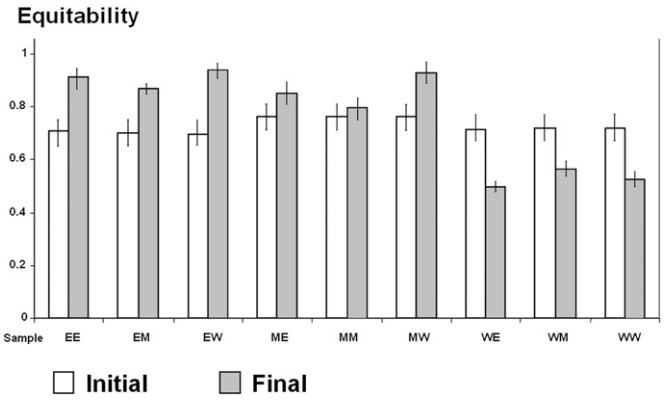

Fig. 3. Free-living bacterial equitability index based on ARISA data. Initial values at time 0, white columns; final values after 73 h of incubation, gray columns. Values are averages of two independent samples (separate dialysis bags). Error bars represent the standard error of the mean.

samples by place of incubation, produced weaker though still significant ANOSIM separation (i.e. lower ANOSIM R-vales, see Supplementary Table 3).

3.3. Initial differences between free-living bacterial assemblages across sites

Jaccard and Bray–Curtis similarities were computed in order to deduce whether the high initial dissimilarity between the samples could be the reason for the subsequent high separation observed by cluster analysis. It is important to mention that the Jaccard index only considers an OTU's presence or absence, and unlike the Bray–Curtis index, does not take into account the relative abundance of different OTUs. This difference between the indices is clearly seen in the ARISA-based data (Table 2), where the Bray–Curtis index gave higher similarity values than the Jaccard in all comparisons. It was clearly noticeable that the eastern–middle and

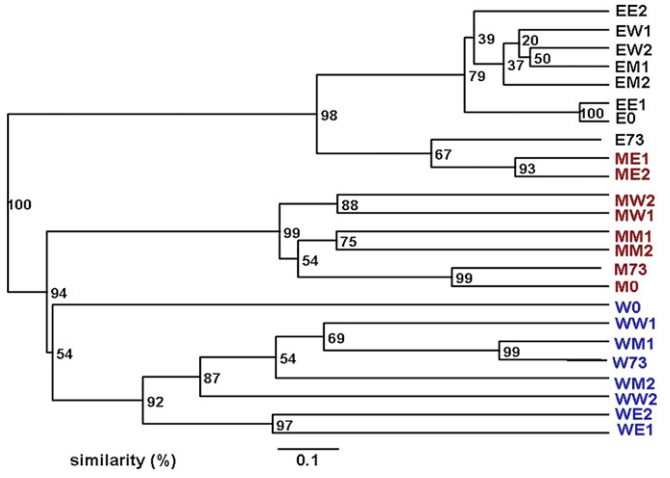

Fig. 4. Bray–Curtis similarity-based cluster analysis of the ARISA-based compositions of free-living bacterial assemblages. The first letter represents the water origin (eastern, middle or western); the second letter represents the incubation site of the dialysis bags and numbers represent duplicates, with 0 denoting pre-incubation samples. The numbers which appear next to the nodes represent % bootstrap support values.

Table 1
ANOSIM calculations of free-living bacterial assemblages following incubation, determined by ARISA profiling. The samples were grouped by water origin: eastern (Nofarim), middle (Geha) and western (marine center): global ANOSIM $R = 0.812$, $p < 0.0001$.

Samples compared	ANOSIM *R*-value (significance)
Eastern–middle	0.801 (0.0001)
Middle–western	0.679 (0.0003)
Eastern–western	0.972 (0.0002)

the middle–western comparison of populations at time 0 showed higher resemblance relative to the western–eastern comparison. This observation emphasized the connectivity effect of the water flow in the river, since the stream flows from east to west.

4. Discussion

Although simple in design, this transplant experiment provides substantial insights into the ecology of free-living bacteria in the Yarqon, a slow-flowing urban stream. Notably, most incubated samples remained similar in their bacterial composition to their pre-incubated environment rather than the new locale. Importantly, the 73 h duration of the experiment was sufficient to allow several bacterial divisions, even taking into account that many freshwater bacteria are slow-growing (del Giorgio and Cole, 1998), and thus dividing bacteria may reach numbers that pass the ARISA detection threshold. Our hypothesis that this incubation was long enough to obtain substantial growth was supported by viable counts performed on the samples (Supplementary Fig. 4). It is therefore plausible that enrichment of some rapidly-dividing taxa occurred during the experiment, since nutrient concentrations and salinity vary greatly between the different segments (Or and Gophna, 2011; Raz, 2009).

When samples taken from the brackish (western) part were incubated in other stream sections (Fig. 2), the diversity and equitability of free-living bacterial assemblages were substantially reduced. Some members of these assemblages were likely to be seawater bacteria that may not thrive in freshwater, while others were probably freshwater bacteria recently arrived by downstream flow that could not withstand long-term high salinity (hence their inability to prosper in the brackish estuarine water for 73 h). In contrast, free-living bacterial assemblages from the middle section increased markedly in diversity wherever they were transplanted. Middle section bacteria were mostly comprised of WWTP-discharged taxa that had been selected for by the water treatment processes. Since these activated sludge-based processes favor taxa that prosper when attached to particles, it is predictable that the dominant WWTP bacteria may not do well as free-living stream bacteria. As a consequence, a free-living assemblage, initially dominated by few WWTP-adapted taxa, should become more even and diverse once different minor taxa begin to increase in abundance at the expense of the WWTP-derived bacteria. Thus, human-impacted water contains bacterial populations that are highly uneven due to enrichment at the WWTPs, and when these bacterial assemblages enter the stream water, populations become more even. Predictably, free-living bacterial assemblages from the eastern (non-human-impacted clean freshwater) section, which were highly diverse to begin with, declined in diversity when transplanted to the middle and western sections, indicating that only a subset of taxa from the eastern section persist in polluted or brackish water.

Table 2
Jaccard and Bray–Curtis similarities between the bacterial communities of the different stream sections at time 0.

Samples compared	Jaccard score	Bray–Curtis score
Eastern–middle	0.184	0.224
Middle–western	0.109	0.161
Eastern–western	0.04	0.103

The ARISA-based clustering by water origin (Fig. 4) that we observed was shown to be significant by ANOSIM, indicating that separation by water source was indeed strong (Table 1). These results are in contrast to previous findings in a lake study (Shade et al., 2010) that showed the place of incubation to be the prime determinant for free-living assemblages. We interpret this clustering to be caused by the large initial dissimilarity between the samples from different stream sections (Table 2), evidenced by both Jaccard and Bray–Curtis similarity values. The combination of large initial differences and the relatively short incubation time may explain the observed clustering, and longer incubation could have produced more striking changes in microbial populations. Nevertheless, the incubation time we chose was more ecologically realistic in a stream setting that involves slow water flow. Water in the Yarqon takes 3–4 days to travel from source to sea rather than a few weeks, as in long slow-flowing rivers.

The only two samples which did not cluster according to the water origin contained water from the middle part of the stream and were incubated upstream in the eastern, non human-impacted section of the stream. This can be interpreted as an increase in less abundant free-living bacterial taxa from the middle section that originate from the clean section rather than treated wastewater. Once these freshwater taxa were transplanted and thus re-introduced into their native milieu, they were in an environment optimal for their growth and outcompeted the bacteria of wastewater origin caged along with them. In light of the results described here, we accept our null hypothesis, which is that bacterial communities resembled the initial bacterial communities more then they did assemblages from the point of incubation.

That observation is highly interesting from a practical perspective, since increasing the water volume in the stream by pumping some water back upstream is a strategy now considered for stream revitalization (Yonatan Raz, personal communication). While introducing low levels of treated wastewater into the clean section may seem to be a dangerous practice, our findings indicate that a low level of human-impacted water in freshwater can probably be tolerated, since bacterial populations typical of freshwater will dominate. Nevertheless, as we have only studied free-living bacterial assemblages, and

since effects on particle-associated communities remain untested, further research is required to establish the soundness of such a practice. In addition, the impact upon other levels of the trophic chain should also be investigated.

Two former studies used these in situ approaches to study the lentic system (Horňák et al., 2005; Shade et al., 2010), but to the best of our knowledge, this is the first study of its kind to utilize the "bacterial transplant" approach to a lotic ecosystem. This study could be followed up by a similar design enhanced by 16S rRNA gene pyrosequencing of small water samples extracted from the bags (Gilbert et al., 2009; Kristiansson et al., 2011) every few hours, and adding an on-line monitoring system providing information on nutrient concentrations, dissolved oxygen and temperature. Such a methodology may link nutrient availability and consumption with the taxa that are enriched and further our understanding of the determinants of bacterial communities of urban streams.

An important lesson that transplant experiments in streams can teach us is a quantitative assessment of the importance of flow (Anderson et al., 2006; Monk et al., 2007). By caging the bacteria and preventing their downstream movement we simulated conditions of no flow while maintaining the effect of natural water mixing. Nevertheless, enclosing bacteria in dialysis bags also has additional effects, such as prevention of free-living bacterial access to high molecular weight nutrients and protection from new motile predators (Monk et al., 2007; Schmitz, 2007; Shade et al., 2010; Surbeck et al., 2009). Here we demonstrated that physical parameters such as reducing effective flow, and not only chemical factors (Villeneuve et al., 2011), alter the equitability and, more importantly, in some cases even the composition, of free-living bacterial assemblages within only 3 days. Nonetheless, by extrapolating from our results, it is quite likely that further reducing water volumes of low-flow streams could cause undesirable shifts in microbial populations, and this should be considered when managing these precious natural resources.

Acknowledgments

This research was supported by the Porter School of Environmental Studies at Tel Aviv University and the Smaller-Winnikow fund. The research field sampling was conducted with the kind help of the "Yarqon Stream Authority".

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.resmic.2012.12.004.

References

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecol. 26, 32–46.

Anderson, K.E., Paul, A.J., McCauley, E., Jackson, L.J., Post, J.R., Nisbet, R.M., 2006. Instream flow needs in streams and rivers: the importance of understanding ecological dynamics. Front. Ecol. Environ. 4, 309–318.

Crump, B.C., Armbrust, E.V., Baross, J.A., 1999. Phylogenetic analysis of particle-attached and free-living bacterial communities in the Columbia river, its estuary, and the adjacent coastal ocean. Appl. Environ. Microbiol. 65, 3192–3204.

Danovaro, R., Luna, G.M., Dell'Anno, A., Pietrangeli, B., 2006. Comparison of two fingerprinting techniques, terminal restriction fragment length polymorphism and automated ribosomal intergenic spacer analysis, for determination of bacterial diversity in aquatic environments. Appl. Environ. Microbiol. 72, 5982–5989.

del Giorgio, P.A., Cole, J.J., 1998. Bacterial growth efficiency in natural aquatic systems. Annu. Rev. Ecol. Syst. 29, 503–541.

Dyer, L.A.L., Deborah, K., 1999. Relative strengths of top-down and bottom-up forces in a tropical forest community. Oecologia 119, 265–274.

Ellis, B.K., Stanford, J.A., Goodman, D., Stafford, C.P., Gustafson, D.L., Beauchamp, D.A., Chess, D.W., Craft, J.A., Deleray, M.A., Hansen, B.S., 2011. Long-term effects of a trophic cascade in a large lake ecosystem. Proc. Natl. Acad. Sci. U S A 108, 1070–1075.

Elron, E., Gasith, A., Goren, M., 2006. Reproductive strategy of a small endemic cyprinid, the Yarqon bleak (Acanthobrama telavivensis), in a mediterranean-type stream. Environ. Biol. Fishes 77, 141–155.

Fisher, M.M., Triplett, E.W., 1999. Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl. Environ. Microbiol. 65, 4630–4636.

Fuhrman, J.A., Hewson, I., Schwalbach, M.S., Steele, J.A., Brown, M.V., Naeem, S., 2006. Annually reoccurring bacterial communities are predictable from ocean conditions. Proc. Natl. Acad. Sci. U S A 103, 13104–13109.

Gafny, S., Goren, M., Gasith, A., 2000. Habitat condition and fish assemblage structure in a coastal mediterranean stream (Yarqon, Israel) receiving domestic effluent. Hydrobiologia 422-423, 319–330.

Gasith, A., Resh, V.H., 1999. Streams in mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu. Rev. Ecol. Syst. 30, 51–81.

Gilbert, J.A., Thomas, S., Cooley, N.A., Kulakova, A., Field, D., Booth, T., McGrath, J.W., Quinn, J.P., Joint, I., 2009. Potential for phosphonoacetate utilization by marine bacteria in temperate coastal waters. Environ. Microbiol. 11, 111–125.

Gücker, B., Brauns, M., Pusch, M.T., 2006. Effects of wastewater treatment plant discharge on ecosystem structure and function of lowland streams. J. N. Am. Benthol. Soc. 25, 313–329.

Guillermina, H.R., Hne, B., Pierre, C., Patrick, D., Karyn Le, M., Gerard, M., Robert, D., 2006. Molecular diversity studies of bacterial communities of oil polluted microbial mats from the Etang de Berre (France). FEMS Microbiol. Ecol. 58, 550–562.

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4, 1.

Hewson, I., Fuhrman, J.A., 2004. Richness and diversity of bacterioplankton species along an estuarine gradient in Moreton Bay, Australia. Appl. Environ. Microbiol. 70, 3425–3433.

Horňák, K., Mašín, M., Jezbera, J., Bettarel, Y., Nedoma, J., Sime-Ngando, T., Šimek, K., 2005. Effects of decreased resource availability, protozoan grazing and viral impact on a structure of bacterioplankton assemblage in a canyon-shaped reservoir. FEMS Microbiol. Ecol. 52, 315–327.

Johnson, B.E., Cushman, J.H., 2007. Influence of a large herbivore reintroduction on plant invasions and community composition in a California grassland. Conserv. Biol. 21, 515–526.

Kovacs, A., Ben-Jacob, N., Tayem, H., Halperin, E., Iraqi, F., Gophna, U., 2011. Genotype is a stronger determinant than sex of the mouse gut microbiota. Microb. Ecol. 61 (2), 423–428.

Kovacs, A., Yacoby, K., Gophna, U., 2010. A systematic assessment of automated ribosomal intergenic spacer analysis (ARISA) as a tool for estimating bacterial richness. Res. Microbiol. 161, 192–197.

Kristiansson, E., Fick, J., Janzon, A., Grabic, R., Rutgersson, C., Weijdegård, B., Söderström, H., Larsson, D.G.J., 2011. Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. PLoS One 6, e17038.

Kritzberg, E.S., Cole, J.J., Pace, M.L., Granéli, W., Bade, D.L., 1994. Autochthonous versus allochthonous carbon sources to bacteria: results

from whole-lake ^{13}C addition experiments. Limnol. Oceanogr. 49, 588–596.
Legendre, P., Legendre, L., 1998. Numerical Ecology. Elsevier, p. 853.
Mašín, M., Jezbera, J., Nedoma, J., Straškrabová, V., Hejzlar, J., Šimek, K., 2003. Changes in bacterial community composition and microbial activities along the longitudinal axis of two canyon-shaped reservoirs with different inflow loading. Hydrobiologia 504, 99–113.
Mittelbach, G.G., Turner, A.M., Hall, D.J., Rettig, J.E., Osenberg, C.W., 1995. Perturbation and resilience: a long-term, whole-lake study of predator extinction and reintroduction. Ecology 76, 2347–2360.
Monk, W.A., Wood, P.J., Hannah, D.M., Wilson, D.A., 2007. Selection of river flow indices for the assessment of hydroecological change. River Res. Appl. 23, 113–122.
Niemi, G.J., Detenbeck, N.E., Perry, J.A., 1993. Comparative analysis of variables to measure recovery rates in streams. Environ. Toxicol. Chem. 12, 1541–1547.
Or, A., Gophna, U., 2011. Detection of spatial and temporal influences on bacterial communities in an urban stream by automated ribosomal intergenic ribosomal spacer analysis. Microbes Environ. 26, 360–366.
Or, A., Shtrasler, L., Gophna, U., 2012. Fine-scale temporal dynamics of a fragmented lotic microbial ecosystem. Sci. Rep. 2, 207. http://dx.doi.org/10.1038/srep00207.
Polis, G.A., 1994. Food webs, trophic cascades and community structure. Aust. J. Ecol. 19, 121–136.
Raz, Y., 2002. Yarqon Stream Ecological Monitoring Report 2002. Tel-Aviv Jaffa.
Raz, Y., 2004. Yarqon Stream Ecological Monitoring Report 2004. Tel-Aviv Jaffa.
Raz, Y., 2009. Yarqon Stream Ecological Monitoring Report 2009. Yarqon River authority, Tel-Aviv Jaffa.
Rozen, Y., Belkin, S., 2001. Survival of enteric bacteria in seawater. FEMS Microbiol. Rev. 25, 513–529.
Sarrazin, F., Barbault, R., 1996. Reintroduction: challenges and lessons for basic ecology. Trends Ecol. Evol. 11, 474–478.
Schmitz, O.J., 2007. Predator diversity and trophic interactions. Ecology 88, 2415–2426.
Shade, A., Jones, S.E., McMahon, K.D., 2008. The influence of habitat heterogeneity on freshwater bacterial community composition and dynamics. Environ. Microbiol. 10, 1057–1067.
Shade, A., Chiu, C.Y., McMahon, K.D., 2010. Differential bacterial dynamics promote emergent community robustness to lake mixing: an epilimnion to hypolimnion transplant experiment. Environ. Microbiol. 12, 455–466.
Shade, A., Read, J.S., Welkie, D.G., Kratz, T.K., Wu, C.H., McMahon, K.D., 2011. Resistance, resilience and recovery: aquatic bacterial dynamics after water column disturbance. Environ. Microbiol. 13, 2752–2767.
Surbeck, C.Q., Jiang, S.C., Grant, S.B., 2009. Ecological control of fecal indicator bacteria in an urban stream. Environ. Sci. Technol. 44, 631–637.
Tavasi, M., Barinova, S.S., Anissimova, O.V., 2004. Algal indicators of environment in the Nahal Yarqon basin, Central Israel. Int. J. Algae 6, 28.
Villeneuve, A., Montuelle, B., Bouchez, A., 2011. Effects of flow regime and pesticides on periphytic communities: evolution and role of biodiversity. Aquat. Toxicol. 102, 123–133.
Wetzel, R.G., 2000. Freshwater ecology: changes, requirements, and future demands. Limnology 1, 3–9.
Whitton, B.A., 1975. River Ecology. University of California Press.
Zurel, D., Benayahu, Y., Or, A., Kovacs, A., Gophna, U., 2011. Composition and dynamics of the gill microbiota of an invasive Indo-Pacific oyster in the eastern Mediterranean Sea. Environ. Microbiol. 13, 1467–1476.