· Letter ·
Advances in Polar Science
doi: 10.13679/j.advps.2015.1.00102
March 2015 Vol. 26 No. 1: 102-106

Five-year bio-monitoring of aquatic ecosystems near Artigas Antarctic Scientific Base, King George Island

María A. Morel[1*], Victoria Braña[1], Cecilia Martínez-Rosales[1,2], Célica Cagide[1] & Susana Castro-Sowinski[1,2]

[1] Microbiología Molecular, Instituto de Investigaciones Biológicas Clemente Estable (IIBCE), Montevideo, Uruguay;
[2] Bioquímica y Biología Molecular, Facultad de Ciencias, Universidad de la República, Montevideo, Uruguay

Received 27 May 2014; accepted 4 November 2014

Abstract Fildes Peninsula, in King George Island, Antarctica, has a great concentration of international facilities, and it has clearly been affected by human activities. The objective of this 5-year study was to assess the impact of anthropogenic activities on the bacterial abundance in water bodies close to Artigas Antarctic Scientific Base (BCAA, in Spanish *Base Científica Antártica Artigas*). Water samples from areas under different human influence (Uruguay Lake, nearby ponds, and meltwater from Collins Glacier) were aseptically collected and refrigerated until processed. The number of heterotrophic bacteria and *Pseudomonas* spp. was analyzed using a culture-dependent approach. Physico-chemical properties of the water samples (temperature, pH, and conductivity) were also determined. Results showed that water from the highly affected area, Uruguay Lake, where the pump that provides water to the BCAA is located, did not suffer significant fluctuations in heterotrophic bacterial abundance (10^4–10^5 CFU·mL^{-1}); however, *Pseudomonas* abundance increased until becoming the predominant population. In other water samples, the number of heterotrophic bacteria and *Pseudomonas* gradually increased during this 5-year study, by 2014 reaching similar values to those observed for Uruguay Lake. The implications of human activities on Antarctic bacterial abundance are discussed.

Keywords **anthropogenic activities, water bodies, bacterial abundance**

Citation: Morel M A, Braña V, Martínez-Rosales C, et al. Five-year bio-monitoring of aquatic ecosystems near Artigas Antarctic Scientific Base, King George Island. Adv Polar Sci, 2015, 26: 102-106, doi: 10.13679/j.advps.2015.1.00102

1 Introduction

Throughout evolution, microorganisms have faced environmental pressures to which they have responded by various adaptation mechanisms. Thus, in permanently cold environments such as the Antarctic, a great extremophile microbial diversity has been described[1]. Examples of cold-adapted microorganisms isolated from Antarctic environments are bacteria of the genera *Pseudomonas* and *Flavobacterium*[2], among many others. Fildes Peninsula in King George Island, Antarctica is a snow-free area during summer. Currently, it has a great concentration of international facilities, which support human activities. The presences of scientific stations and tourism activities have caused an exponential increase in human presence on the continent, affecting the ecosystem. Many human activities in scientific bases located throughout Antarctica have been associated with alteration in, and negative impact on, the environment[3-6].

The impact of anthropogenic activity is a risk for biological conservation. Invasive alien bacteria introduced in Antarctica as a consequence of the use of the continent's natural resources have changed the abundance of autochthonous bacteria[7-8]. Native biodiversity is also affected because of the vulnerability and low competitive abilities of indigenous organisms[5]. Many introduced species have survived invading the ecosystem[3-5]. Human activities related to food management, transportation systems[3] and wastewater

* Corresponding author (email: mmorel@iibce.edu.uy)

management[4] are among the anthropogenic activities that introduce alien microbes. In this regard, urgent measures are needed, including environmental impact assessments, long-term monitoring, and regulation from national Antarctic organizations, among others.

Among the international scientific stations located in Fildes Peninsula, the Artigas Antarctic Scientific Base (62°11′4″S; 58°51′7″W) (in Spanish Base Científica Antártica Artigas, BCAA) is administrated by the Uruguayan Antarctic Institute. Many freshwater lakes and ponds can be found in the vicinity of the BCAA. One such is the Uruguay Lake (also known as Profound Lake by the UK-Antarctic Place-Names Committee), a water body located 0.4 km northwest of Jasper Point that is used as a potable water supply by the Uruguayan scientific station. Thus, continuous monitoring of this lake is important in environmental management.

Water entering the distribution system must be microbiologically safe and should ideally also be biologically stable[9]. Biological stability means that the concentration and composition of the microbial community does not change. In this work, we assessed the biological stability of Uruguay Lake and other freshwater sources near the BCAA by analysis of heterotrophic bacterial plate counts. Heterotrophic bacteria use organic carbon sources to grow and they can be isolated on agar-based medium under conditions of defined incubation temperature and time[10]. In water (where the input of organic matter is important), heterotrophic bacteria play an important role during the decomposition of organic matter, and they are highly affected by abiotic stresses[8]. Heterotrophic bacteria are found in all sources of water, and thus they are important microbiological indicators of water quality[11]. The assessment of heterotrophic populations is a useful tool for monitoring the efficiency of water treatment processes and water quality during distribution and storage.

Among heterotrophic bacteria, the genus *Pseudomonas* is routinely enumerated during the control of water quality, and is considered a microbial indicator by the World Health Organization (WHO)[12-13]. *Pseudomonas* abundance in water is required information during water analysis because these species are able to inhibit the growth of some other heterotrophic bacteria (such as fecal coliforms). *Pseudomonas* spp. are also considered opportunistic pathogens when found in drinking water, but there is no clinical or epidemiological evidence to support this affirmation[14].

In the present work, we investigated the abundance of heterotrophic bacteria and fluorescent *Pseudomonas* spp. over 5 years (2010–2014) in water samples collected near the BCAA, Fildes Peninsula, King George Island, Antarctica. We attempt to correlate bacterial abundance and anthropogenic influence.

2 Materials and methods

2.1 Source of samples

Water samples were collected at various locations near the BCAA during January (austral summer) in consecutive years from 2010 to 2014 (Table 1 and Figure 1). Locations were selected based on their relative anthropogenic impact. The human impact was assessed based on the occurrence of human activities in the area. Uruguay Lake (medium to high human impact) is the water resource of the BCAA where the water pump is located and is a site of high human transit (BCAA personnel activate the pump at least twice a day). Northwest from the Uruguay Lake, three small water-ponds are found (low to medium human impact) and they are subjected to low human transit (casual transit of personnel). Finally, the protected area under the Collins Glacier, where meltwater was collected, is far away from the BCAA and other operational bases (low to no human impact). At least three sites were sampled per location (separated by 100 m), and each sampling was performed in triplicate. Coordinates for each site were fixed by GPS.

Samples were aseptically collected at 10–20 cm from the water surface in sterile tubes and kept at 4°C until processing. Some physical and chemical properties of the water samples (pH, temperature and conductivity) were measured *in situ* during sampling. A LaMotte tracer measurement device for direct recording of physical properties of water was used.

2.2 Bacterial count

Water samples were filtered through sterile Whatman No. 1 cellulose filter paper (Millipore) and then used for microbiological analysis using a culture dependent approach. Two growth media were used: (1) Tryptic soy broth agar (TSA) plates (0.1% tryptic soy broth and 1.5% agar) for counting total aerobic heterotrophic bacteria; (2) King's B medium (2% peptone mixture, 0.15% dipotassium phosphate, 0.15% magnesium sulfate, 1% glycerol and 1.5% agar) for fluorescent *Pseudomonas* spp. detection (fluorescence under ultraviolet light)[15]. Colony forming units (CFU) per mL of sample were determined by spreading

Table 1 Locations of fresh water sampling. Three GPS locations per geographic location were fixed, but the coordinates of only one point are shown

Sample number	Site description	Relative anthropogenic influence	Latitude	Longitude
1	Water-ponds	Medium	62°10′39.6″S	58°55′13.8″W
2	Uruguay Lake	High	62°10′59.4″S	58°54′31.6″W
3	Collins Glacier	Low	62°10′59.4″S	58°52′10.5″W

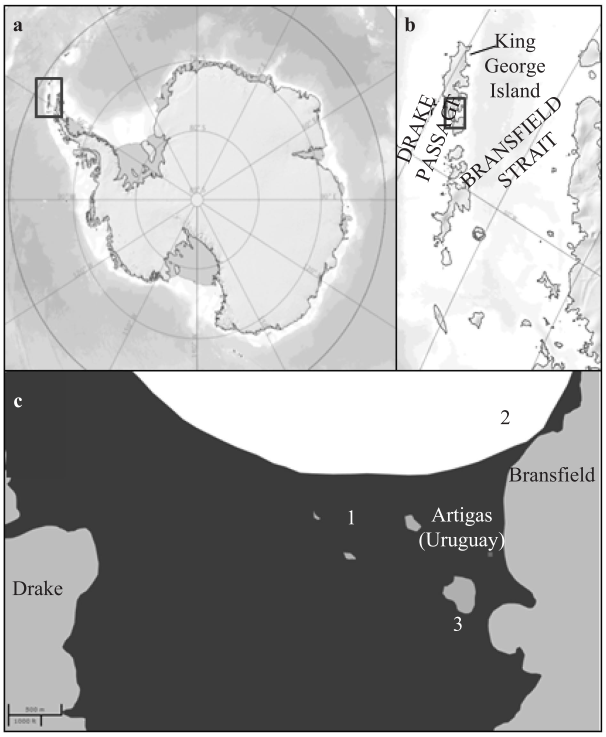

Figure 1 Sampling locations. **a**, South Shetland Islands in Antarctica. **b**, King George Island. **c**, Coast of Fildes Peninsula (BCAA and the sampling locations are shown): 1–three water ponds; 2–meltwater from Glacier Collins; 3–Uruguay Lake. For more information about sample sites see Table 1. Figures source: Antarctic Digital Data.

serial 10-fold dilutions of samples onto the surfaces of both media (at least in triplicate). Plates were incubated at 4°C for 8 and 20 d for fluorescent *Pseudomonas* spp. and total heterotrophic bacteria, respectively.

2.3 Statistical analyses

Results are the mean of three independent replications. The data were subjected to one-way ANOVA analysis when possible, with *post hoc* pairwise comparisons based on Tukey's HSD test, or Kruskal–Wallis one-way analysis of variance, using PAST software version 1.56[16]. Statistical significance was determined at $p = 0.05$.

3 Results and discussion

An overview of results using a non-parametric statistical test shows significant differences in both bacterial populations, i.e. of total heterotrophic bacteria and *Pseudomonas*, between years and between sampled sites (Table 2). The variation in the number of heterotrophic bacteria and *Pseudomonas* spp. in fresh water was in the order of 10^1 (e.g., 10^4–10^5 mL^{-1} for water-pond samples collected in 2012). Bacterial counts are highly variable and depend on many factors. However, changes in the microbial component of water samples have been studied by quantifying bacterial and viral numbers before, with similar range count variations[17-18].

Among sampled sites, and despite some statistical differences, the Uruguay Lake was the most consistent site (Table 2), suggesting that the number of heterotrophic bacteria in the lake did not change over the duration of this study. Similar results were obtained when studying the bacterial community in water samples collected inside, and in the vicinity of, the Chinese Great Wall Station, King George Island[19]. However, a comparison among samples from different years showed a remarkable change in the bacterial population of Uruguay Lake in 2013, which decreased with respect to 2011 and 2012, and then increased in 2014. Interestingly, during the summer month of January 2013, the lake was almost totally melted. Changes in physical properties of the water were also evident; in that year the lowest temperature and conductivity in this study were noted (Table 3). The variation in conductivity (24 and 130 μS·cm^{-1} in 2013 and 2014, respectively; Table 3) may indicate a melting process that diluted the bacterial number to a minimum value in 2013, which would explain the lower levels of bacterial populations detected.

Despite the relative invariability of bacterial population levels in Uruguay Lake, the number of bacteria in water samples collected from the water-ponds (near Uruguay Lake) and from Glacier Collins did change over the years studied (Table 2). The heterotrophic bacterial and *Pseudomonas* populations gradually increased until, in 2014, reaching values even higher than those obtained for Uruguay Lake samples.

Physico-chemical properties of the aquatic ecosystems near the BCAA were also monitored for the 5 years of this study to determine the effects of human activities. Variations in the physico-chemical parameters were observed from site to site and between years (Table 3). This was in agreement with previous work that reported the connection between human activities and physicochemical parameters of water[20]. Human activities have a great influence on the pollution of water bodies, and can alter the physical, chemical and

Table 2 Bacterial counting. The table shows the results from one of three independent experiments. Different superscript letters indicate significant differences among years within each sample site ($p < 0.05$)

Sample site	Total heterotrophic bacteria/(CFU·mL^{-1})					Fluorescent *Pseudomonas* spp./(CFU·mL^{-1})				
	2010	2011	2012	2013	2014	2010	2011	2012	2013	2014
Water-ponds	10^2–10^{3a}	10^3–10^{5b}	10^4–10^{5c}	10^5–10^{6d}	10^5–10^{6d}	10^{2a}	10^4–10^{5b}	10^4–10^{5b}	10^4–10^{6c}	10^5–10^{6d}
Uruguay Lake	10^4–10^{5a}	10^4–10^{5a}	10^4–10^{5a}	10^{4b}	10^4–10^{5a}	10^3–10^{4a}	10^4–10^{5b}	10^4–10^{5b}	10^3–10^{4a}	10^4–10^{5b}
Collins Glacier	10^4–10^{5a}	10^5– 10^{6b}	10^{5c}	10^{6d}	10^5–10^{6b}	10^4–10^{5a}	10^4–10^{6b}	10^4–10^{5a}	10^4–10^{6b}	10^4–10^{6c}

Table 3 Physical and chemical properties of freshwater in water samples over the years monitored. Values are the average of three measurements. ND means "not determined"

Sample site	2010	2011	2012	2013	2014
pH					
Water-ponds	7.0 ± 0	6.36 ± 1.13	7.70 ± 0.27	6.93 ± 0.21	5.75 ± 0.35
Uruguay Lake	5.4 ± 2.9	6.97 ± 0.70	7.21 ± 0.13	6.53 ± 0.35	5.75 ± 0.36
Glacier Collins	7.0 ± 0	8.33 ± 0.92	6.87 ± 0.67	7.00 ± 0.28	8.00 ± 0.11
Conductivity/($\mu S \cdot cm^{-1}$)					
Water-ponds	ND	77 ± 58	84 ± 23	161 ± 18	187 ± 14
Uruguay Lake	ND	186 ± 28	116 ± 38	24 ± 12	130 ± 29
Glacier Collins	ND	ND	38 ± 13	338 ± 150	149 ± 58
Temperature/°C					
Water-ponds	6.00 ± 1.4	4.21 ± 2.9	5.61 ± 1.7	1.65 ± 0.75	3.10 ± 1.9
Uruguay Lake	3.60 ± 2.9	6.06 ± 3.9	5.18 ± 1.3	0.57 ± 0.05	1.40 ± 1.0
Glacier Collins	1.75 ± 1.0	0.21 ± 0.1	2.41 ± 1.9	0.55 ± 0.40	2.30 ± 0.28

biological nature of the receiving water[21-23]. For example, temperature affects the solubility of oxygen in water and, therefore, the organisms that live there, and biological oxygen demand has been correlated with the cleanliness of water[20]. The variations in levels of most of the physico-chemical parameters tested here could be attributed to human activities in the BCAA.

The physical parameters of fresh water from Uruguay Lake and the water-ponds differed from Glacier Collins samples, which had colder and more alkaline water. The most distinct year was 2013, when a drastic decrease in temperature was registered in the three sites evaluated, and an increase in conductivity was observed in water from Collins Glacier and the water-ponds. These and other parameters (carbon and phosphorous contents, etc) might be involved in the gradual change of cultivable heterotrophic bacterial counts observed during the 5 years monitoring. Heterotrophic bacteria are also abundant in melted ice[24].

Many Antarctic microbial communities are potentially sensitive to external impacts[25]. Thus, understanding the impact that anthropogenic activities have on these communities is of major relevance. We performed a time course analysis of the cultivable heterotrophic bacterial communities present in three water bodies subjected to different human impacts. Physical (e.g., abrasion, compaction, trampling) and chemical (e.g., eutrophication, fuel spills, waste management) impacts of human beings on Antarctic environments have been considered the most damaging factors that affect this habitat. However, human activities also significantly affect the environment by contamination with non-indigenous microorganisms such as human commensal and fecal microorganisms[25]. Global climate change also has the potential for dramatic impact on these environments (with an increase of 1.09°C per decade during winter and 0.56°C per decade annually), but during the time course of our analysis it may be unwise to assume any climate change effect. However, during the period of our analysis, the human disturbance caused by scientific station personnel may have been among the most significant environmental threats to the Fildes Peninsula, as was stated by Braun et al.[26] when monitoring human impacts on the Antarctic habitat.

4 Conclusion

Our results suggest that samples from Uruguay Lake (the most human transited location, where BCAA personnel drive the water pumps every day) have reached a constant heterotrophic bacterial abundance. The human impact on this lake may not have an important influence on incidence in microbial communities in the future if BCAA personnel continue applying protocols for environmental care. However, the water ponds and melt water from Glacier Collins showed an increased heterotrophic bacterial abundance during the time of our study. These areas are being subjected to increasing human influence, and this presence is probably currently shaping their microbial populations. However, these populations may have reached equilibrium, since samples from all locations monitored reached similar $CFU \cdot mL^{-1}$ values in 2014.

Acknowledgments This work was supported by the Program for the Development of Basic Sciences (Programa de Desarrollo de las Ciencias Básicas, PEDECIBA). Financial and logistic assistance was also provided by the Uruguayan Antarctic Institute (Instituto Antártico Uruguayo, IAU).

References

1 Quesada A, Vincent W F. Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. Eur J Phycol, 1997, 32(4): 335-342

2 Martínez-Rosales C, Castro-Sowinski S. Antartic bacterial isolates that produce cold-active extracellular proteases at low temperature but are active and stable at high temperature. Polar Res, 2011, 30: 7123

3 Frenot Y, Chown S L, Whinam J, et al. Biological invasions in the Antarctic: extent, impacts and implications. Biol Rev, 2000, 80(1): 45-72

4 Tin T, Fleming Z L, Hughes K A. Impacts of local human activities on the Antarctic environment. Antarct Sci, 2006, 21(1): 3-33, doi: 10.1017/S0954102009001722

5 Convey P, Lebouvier M. Environmental change and human impacts on terrestrial ecosystems of the sub-Antarctic islands between their discovery and the mid-twentieth century. Pap Proc R Soc Tasmania, 2009, 143(1): 33-44

6 Cowan D A, Chown S L, Convey P, et al. Non-indigenous microorganisms in the Antarctic: assessing the risks. Trends Microbiol, 2011, 19(11): 540-548, doi: 10.1016/j.tim.2011.07.008

7 Vincent W F. Evolutionary origins of Antarctic microbiota: invasion, selection and endemism. Antarct Sci, 2000, 12(3): 374-385

8 Bastardo A, Bastardo H, Rosales J. Functional diversity of the heterotrophic bacteria in the lower Orinoco river, Venezuela. Ecotropicos, 2007, 20(1): 15-23

9 World Health Organization (WHO). Guidelines for drinking-water quality: incorporating first addendum. Vol. 1, Recommendations, third ed. Geneva, Switzerland: WHO Press, 2006

10 Allen M J, Edberg S C, Reasoner D J. Heterotrophic plate count bacteria—what is their significance in drinking water? Int J Food Microbiol, 2004, 92(3): 265- 274

11 Miravent M E. Abundancia, actividad y diversidad de las bacterias heterótrofas en el Golfo de Batabanó y su uso como indicadoras ambientales. Dissertation, Ministerio de Ciencia, Tecnología y Medio Ambiente, Ciudad Habana, 2003

12 Martin Delgado M M, Hernández García A M, Felipe Hormigo A M, et al. Analisis microbiologico y fisicoquimico del agua de piscinas de la isla de Tenerife. Rev San Hig Púb, 1992, 66: 281-289

13 Mora-Alvaradp D A. Evolución de las guías microbiológicas de la OMS para evaluar la calidad del agua para consumo humano: 1984−2004. Dissertation, Instituto costarricense de acueductos y alcantarillados, 2005

14 Marchand E O. Microorganismos indicadores de la calidad del agua de consumo humano en Lima metropolitana. Lima Perú: Universidad Nacional Mayor de San Marcos. Facultad de Ciencias Biológicas, 2002

15 Johnsen K, Nielsen P. Diversity of Pseudomonas strains isolated with King's B and Gould's S1 agar determined by repetitive extragenic palindromic-polymerase chain reaction, 16S rDNA sequencing and Fourier transform infrared spectroscopy characterization. FEMS Microbiol Lett, 1999, 173(1): 155-162

16 Hammer Ø, Harper D, Ryan P. PAST: paleontological statistic software package for education and data analysis. Palaeontol Electron, 2001, 4: 9

17 Ruiz G M, Rawlings T K, Dobbs F C, et al. Global spread of microorganisms by ships. Nature, 2000, 408(6808): 49-50

18 Drake L A, Choi K H, Ruiz G M, et al. Global redistribution of bacterioplankton and virioplankton communities. Biol Invasions, 2001, 3(2): 193-199

19 Xiao X, Li M G, You Z Y, et al. Bacterial communities inside and in the vicinity of the Chinese Great Wall Station, King George Island, Antarctica. Antarct Sci, 2007, 19(1): 11-16

20 Izonfuo L W A, Bariweni A P. The effect of urban runoff water and human activities on some physic-chemical parameters of the epie creek in the Niger Delta. J Appl Sci Environ Mgt, 2001, 5(1): 47-55

21 Sangodoin A Y. Groundwater and surface water pollution by open refuse dump in Ibadan, Nigeria. J Discovery Innovations, 1991, 3(1): 24-31

22 Adekunle A S, Eniola I T K. Impact of industrial effluents on quality of segment of Asa River within an industrial estate in Ilorin, Nigeria. NY Sci J, 2008, 1(1): 17-21

23 Ewa E E, Iwara A I, Adeyemi J A, et al. Impact of industrial activities on water quality of Omoku Creek. Sacha J Environ Studies, 2011, 1(2): 8-16

24 Junge K, Imhoff F, Staley T, et al. Phylogenetic diversity of numerically important Arctic sea-ice bacteria cultured at subzero temperature. Microb Ecol, 2002, 43(3): 315-328, doi:10.1007/s00248-001-1026-4

25 Cowan D A, Tow L A. Endangered Antarctic environments. Ann Rev Microbiol, 2004, 58: 649-690

26 Braun C, Mustafa O, Nordt A, et al. Environmental monitoring and management proposals for the Fildes Region, King George Island, Antarctica. Polar Res, 2012, 31: 18206