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Viruses Surveillance under Different Season Scenarios of the Negro River Basin, Amazonia, Brazil

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Abstract

The Negro River is located in the Amazon basin, the largest hydrological catchment in the world. Its water is used for drinking, domestic activities, recreation and transportation and water quality is significantly affected by anthropogenic impacts. The goals of this study were to determine the presence and concentrations of the main viral etiological agents of acute gastroenteritis, such as group A rotavirus (RVA) and genogroup II norovirus (NoV GII), and to assess the use of human adenovirus (HAdV) and JC polyomavirus (JCPyV) as viral indicators of human faecal contamination in the aquatic environment of Manaus under different hydrological scenarios. Water samples were collected along Negro River and in small streams known as *igarapés*. Viruses were concentrated by an organic flocculation method and detected by quantitative PCR. From 272 samples analysed, HAdV was detected in 91.9 %, followed by JCPyV (69.5 %), RVA (23.9 %) and NoV GII (7.4 %). Viral concentrations ranged from 10^2 to 10^6 GC L^{-1} and viruses were more likely to be detected during the flood season, with the exception of NoV GII, which was detected only during the dry season. Statistically significant differences on virus concentrations between dry and flood seasons were observed only for RVA. The HAdV data provides a useful complement to faecal indicator bacteria in the monitoring of aquatic environments. Overall results demonstrated that the hydrological cycle of the Negro River in the Amazon Basin affects the dynamics of viruses in aquatic environments and, consequently, the exposure of citizens to these waterborne pathogens.

Keywords: enteric viruses; river water; flood; dry; Amazon; Negro River

Introduction

The discharge of treated and untreated sewage into aquatic environments is a well-known source of faecal pollution in water, and is the likely source of pathogenic microorganisms including viruses entering these ecosystems (Fong et al. 2010; Li et al. 2011; Prevost et al. 2015). In recent years, researchers have demonstrated the presence of human enteric viruses in surface waters, affecting water quality and maintenance of public health through policies designed to control discharge of pathogens to the aquatic environment and limit viral waterborne disease (Aw and Gin 2011; Hewitt et al. 2013; Mellou et al. 2014). Indeed, it is estimated that up to 69 % of diarrheal diseases could be prevented with improved sanitation (Norman et al. 2010; WHO 2014).

To reduce health risks from water-related illness, bacteriological indicators such as *Escherichia coli* (*E. coli)* and enterococci have been adopted as standards for water quality by nations worldwide. However, many studies have demonstrated that faecal indicator bacteria concentrations do not correlate well with measures of enteric viruses, particularly when concentrations of indicator bacteria are low (Bofill-Mas et al. 2013; Hewitt et al. 2013; Pina et al. 1998). Human adenovirus (HAdV) and JC polyomavirus (JCPyV) have been proposed as possible viral indicators of human faecal contamination in aquatic environments due to their host specificity, high prevalence, and stability in the environment. These double-stranded DNA viruses are ubiquitous in the population and are excreted in faeces and urine, respectively (Albinana-Gimenez et al. 2009; Bofill-Mas and Girones 2003; Bofill-Mas et al. 2006, 2013; Pina et al. 1998). Furthermore, important viral pathogens responsible for acute gastroenteritis such as group A rotavirus (RVA) and norovirus (NoV) have been reported in high concentrations in the environment and have been associated with water-related transmission (Calgua et al. 2013a; Di Bartolo et al. 2015; Sinclair et al. 2009).

In Brazil, despite the implementation of rotavirus vaccine in the National Program of Immunization in 2006, morbidity is still high (Carvalho-Costa et al. 2011; Linhares and Justino 2014). The same is observed for NoV, where cases are often associated with outbreaks and the introduction of new variants at regular intervals (Fioretti et al. 2014).

According to the National Information System on Sanitation (SNIS 2014), only 39 % of the sewage in Brazil is treated, ranging from 14.7 % in the north to 45.9 % in the central and western areas of the country. In this context, the city of Manaus, located in the northern part of the country and in the heart of the Amazon forest, has a significant environmental impact on adjacent aquatic resources. This city of 2,020,301 inhabitants (IBGE 2015) sits on the banks of the Negro River in the Amazon Basin, which has an annual cycle of dry and flood seasons, and has been responsible for the discharge of sewage into small streams that cross the city (*igarapés*) and directly into the Negro River. Data collected under the Brazilian Program of Monitoring Acute Diarrheal Diseases (MDDA 2015) show that more than 40,000 cases of gastroenteritis are notified every year in the city and an increase in river levels is sometimes thought to be associated with an increase in the number of these cases, although a clear relationship is not evident (Fig. 1). The main goal of this study was to quantify the concentrations of the principal gastroenteric viruses in the aquatic environments of Manaus, to provide an empirical evidence-base for RVA and NoV risk assessment studies of human exposure to those viruses, as well as to evaluate the use of HAdV and

JCPyV as viral indicators of human faecal contamination taking into account the temporal variations due to the hydrological cycle of the Negro River.

Materials and methods

Study area

Water samples were obtained from the main Negro River basin, including São Raimundo and Educandos basins containing small streams that cross the city of Manaus. These streams are called *igarapés*, which are first, second or third order streams. These shallow watercourses are characteristic of the Amazon Basin.

The Negro River catchment accounts for 12 % of the 6,000,000 km^2 of the Amazon basin, which spans seven countries, including Brazil (Frappart et al. 2005; Villar et al. 2009). It has one of the highest rainfalls in the world (2,250-2,500 mm per year in Manaus), and the river regime includes a distinct flood season (Frappart et al. 2005; Vale et al. 2011; Villar et al. 2009). The Negro River basin is 2,250 km in length with a mean flow of 28,000 m^3 s^{-1}. This river generally exhibits low sediment and nutrient concentrations and an acid pH due to the high concentrations of humic compounds (Silva et al. 2009). It also receives significant vegetation debris comprising leaves, shrubs and trunks that are dissolved and decomposed, releasing acids, which give the water the characteristic black color of rivers located in tropical forests.

Due to seasonal variations in rainfall, the flow regime of the Negro River is characterized by two distinct seasons: the dry period (from September to February) and the flood period (from March to August), where the water level difference can reach up to 15 m. It usually shows its yearly maximum and minimum peaks in June and October, respectively (Satyamurty et al. 2013; Silva et al. 2009).

São Raimundo and Quarenta *igarapés* are the main streams of the São Raimundo and Educandos basins, respectively. These are characterized by complete or partial removal of riparian vegetation and chronic urban pollution from waste disposal and domestic and industrial sewage. These streams are also influenced by the rainy season in the Amazon basin and by the hydrological cycle of the Negro River, which, during its flood season, causes black water to flood these *igarapés*.

Sampling schedule

A surveillance study was carried out from January 2011 to May 2012 at five sampling sites (MA01 to MA05), located using global position system (GPS - eTREX Legend H, Garmin Ltd., Olathe, KS), including areas along Negro River (MA01, MA04 and MA05) and *igarapés* (MA02 and MA03) (Fig. 2).

Water sampling was carried out on seven occasions during the dry (January and October 2011 and January 2012) and the flood (April and July 2011, March and May 2012) seasons. At each of the five sites, eight water samples (10 L each) were collected within two hours (one every 15 min), totalling 40 water samples per sampling occasion. Samples were collected in sterile carboys, transferred to the laboratory and immediately processed. Additionally, extra water samples were obtained at each sampling point for viral recovery experiments (10 L) and bacterial analysis (500 mL).

This study was included in VIROCLIME project (http://www.viroclime.org) and was carried out using Standard Operational Procedures (SOPs) for virus concentration, nucleic acid extraction and quantitative PCR detection. The SOPs included process controls and standard plasmid preparation.

Virus concentration method

Water samples were concentrated by a flocculation method based on the adsorption of viruses to pre-flocculated skimmed milk proteins (Calgua et al. 2013a). Briefly, conductivity and pH of water samples were measured. The former was adjusted to 1.5 mS by the addition of solid artificial sea salts (Sigma–Aldrich Chemie GMBH, Steinheim, Germany) and to pH 3.5 by the addition of HCl 1 N. One hundred mL of pre-flocculated 1 % (w/v) skimmed milk solution (PSM) pH 3.5 (Difco, Detroit, MI, USA) was then added into the samples, which were stirred gently for 8 h at room temperature for the adsorption of viruses to the flocs. The flocs were left to sediment by gravity for another 8 h and the supernatants were removed without disturbing the sediment using a peristaltic pump. The remaining volumes with the sediment (approximate 500 mL) were centrifuged at 8,000 x g for 30 min at 4 °C. The supernatants were carefully removed and pellets were re-dissolved to ≈10 mL with phosphate buffer (1:2, v/v of Na_2HPO_4 0.2 M and NaH_2PO_4 0.2 M) pH 7.5. Viral concentrates were homogenized by vortexing and aliquots of 2 mL were prepared and stored at −80 °C for further viral analysis.

Recovery experiments were carried out as positive controls by spiking 10^6 genome copies (GC) of human adenovirus type 35 (HAdV35) into the extra 10-L water samples followed by its concentration under the same conditions as the field samples and as previously described by Calgua et al. (2013a). Additionally, 10-L tap waters containing 100 mL of a solution of 10 % sodium thiosulphate were processed as negative controls at each sampling.

Nucleic acid extraction and reverse transcription reaction

Nucleic acid extractions from the concentrates were performed by QIAamp Viral RNA Mini Kit (Qiagen, Inc., Valencia, CA, USA), following the manufacturer's protocol. For RVA, cDNA was obtained by reverse transcription (RT) reaction using random hexamers and Superscript III® Reverse Transcriptase (Invitrogen - Life Technologies, Carlsbad, CA, USA).

Virus detection and quantification

QPCR protocols for HAdV, JCPyV, RVA and NoV GII detection and quantification were performed as previously described (Hernroth et al. 2002; Kageyama et al. 2003; Loisy et al. 2005; Pal et al. 2006; Zeng et al. 2008). Standard curves were prepared with the following plasmid constructions: plasmid pHAdV contained the hexon region of HAdV 41 in pBR322, pJCPyV contained the whole JCPyV genome strain Mad-1 in pBR322, pNoVGII contained ORF 1/ORF 2 junction in pTrueBlue and pRVA contained a fragment of RVA Wa NSP3 in pCR™ 2.1-TOPO® vector (Invitrogen - Life Technologies, Carlsbad, CA, USA). pRVA was constructed by our group and pHAdV, pJCPyV and pNoVGII were donated by Dr Annika Allard – Umea University (UMU), Dr Andrew Lewis – US Food and Drug Administration (FDA) and Dr Jan Vinjé – Centers for Disease Control and Prevention (CDC), respectively.

Detection of HAdV, JCPyV and RVA were performed with the TaqMan Environmental PCR Master Mix® (Applied Biosystems, Foster City, California, USA) and NoV GII with RNA Ultrasense™ One-step Quantitative RT-PCR System (Invitrogen - Life Technologies, Carlsbad, CA, USA). All qPCR reactions were carried out in an ABI PRISM 7500® Real-Time System (Applied Biosystems, Foster City, California, USA). Undiluted and 10-fold dilutions of the nucleic acid extract were analysed in duplicate (4 runs/sample) and concentrations were estimated as the mean of data obtained, correcting for the dilution analysed. Low variability in the replicates was observed and significant variability was observed only in the results of a few undiluted samples, being these values excluded of the mean estimation. All qPCR assays included non-template controls (NTC).

For all molecular procedures, positive controls and DNAse/RNAse free water as negative controls were included and separated rooms were used to avoid cross contamination.

Bacteriological parameters

E. coli and enterococci were quantified by Colilert® and Enterolert® Quanti-Tray®/2000 (IDEXX Laboratories, Inc., Westbrook, ME, USA), respectively, and results were reported as most probable number per 100 mL (MPN 100 mL^{-1}). Samples were tested undiluted and using 10-fold dilutions.

Brazilian regulation establishes a maximum of 2000 MPN 100 mL^{-1} and 400 100 mL^{-1} of *E. coli* and enterococci, respectively, as standards for recreational waters (CONAMA 2000).

Physico-chemical parameters

Water temperature (ºC), pH, turbidity (in nephelometric turbidity units - NTU) and conductivity (μS cm^{-1}) were measured in all samples at the time of collection using Water Quality Checker U-10 (Horiba, Ltd., Irvine, CA, USA).

Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 5.0. Data were checked for normality in raw and log_{10} transformed states using Shapiro-Wilk normality test. Both showed significant differences from normality ($p<0.0001$). Fischer and Mann-Whitney tests were performed for comparing virus detection and concentration, respectively, between dry and flood seasons of the Negro River. For the comparison of the concentrations between seasons using the Mann-Whitney test, only positive samples were considered. The Mann-Whitney test was also used for comparing physico-chemical parameters between seasons. In this case, all samples were considered in the comparison since all of them presented measurements.

Correlation analyses between HAdV, JCPyV, *E. coli* and enterococci were carried out using non-parametric Spearman correlation. For this purpose, non-detected values for both viruses and bacteria were assigned as the detection limits of methods, as follows: 57 GC L^{-1} of HAdV and JCPyV (1 GC per qPCR reaction) and 1 MPN 100 mL^{-1} of *E. coli* and enterococci. Correlation coefficients (r) between 0.9–1.0, 0.3–0.5 and <0.3 were considered as strong, low and negligible correlations between viruses and bacteria, respectively (Hewitt et al. 2013).

Results were considered statistically significant when $p<0.05$.

Results

Viruses detection and quantification in water samples

Two hundred and seventy-two water samples were collected from January 2011 to May 2012 in five different sampling points in Manaus. Sampling was carried out on seven occasions during the study, three of them when river level was low (dry) and four when river level was high (flood), producing 112 dry and 160 flood samples, respectively. During the full sampling period, the Negro River level ranged from 16.76 to 29.97 m (Port of Manaus 2015) (Fig. 3).

Estimated recovery rates of HAdV 35 were 0-71 %, 21-242 %, 16-193 %, 4-83 % and 9-125 % at MA01 to MA05, respectively. HAdV was the most detected virus (found in 91.9 % of all samples), followed by JCPyV (69.5 %), RVA (23.9 %) and NoV GII (7.4 %) (Table 1). Viruses were detected at all sampling points, except NoV GII, which was not detected at the mouth of Quarenta *igarapé* (MA04) and at the Negro River in the end of the urban area (MA05). The most contaminated areas were urban streams (MA02 and MA03); Ponta Negra beach (MA01) was the least contaminated site.

Virus concentrations ranged from 10^2 to 10^6 GC L^{-1}, with generally higher concentrations of HAdV and JCPyV observed in urban streams (Fig. 4). HAdV was detected at all sampling points in concentrations ranging through three $\log_{10}$ orders throughout the study period. Despite the lower prevalence of JCPyV detection, when detected, its concentration was similar to those for HAdV in most samples. An increase on RVA concentration was evident in flood periods. NoV GII concentration was low or zero at all sampling points. Statistically significant differences on viruses concentration were observed only for RVA when comparing dry and flood season samples ($p<0.0001$). When considering each sampling point, this analysis revealed significant differences for HAdV in MA04 and MA05 ($p=0.0018$ and $p=0.0096$), for JCPyV in MA01 ($p=0.0441$) and for RVA in MA02 and MA03 ($p=0.0002$ and $p=0.024$).

Quantification of *E. coli* and enterococci as microbiological standards of human faecal contamination of water

E. coli and enterococci were detected in all samples, except *E. coli* in MA01 in January 2011. Thirty-two (87/272) and 35 (85/240) percent of the samples did not comply with the standards for recreational waters, due to elevated concentrations of *E. coli* and enterococci, respectively. These elevated concentrations occurred mainly in urban streams MA02 and MA03 and in October, when river level was the lowest observed (Fig. 5). The recreational area (MA01) was considered acceptable for bathing based on *E. coli* and enterococci parameters, except in April and July of 2011 when concentrations of enterococci exceeded the standards in four and three samples, respectively. No statistically significant differences in bacterial concentrations were observed between dry and flood seasons (*E. coli* p=0.4308 and enterococci p=0.1325).

Correlations between viruses and bacteriological indicators of human faecal contamination of water

Sixty-five percent of HAdV and JCPyV positive samples were detected when the measured *E. coli* concentration was lower than the Brazilian standard for recreational waters (i.e. the water was compliant with the Brazilian standard) and 64.2 % and 61.8 % of HAdV and JCPyV positive samples, respectively, were detected when enterococci quantification complied with the Brazilian standards. At the designated recreational area (MA01), all HAdV and JCPyV positive samples were detected when *E. coli* concentration was lower than the Brazilian standards for recreational waters and 90.7 % and 86.4 % of HAdV and JCPyV positive samples, respectively, were detected when enterococci quantification complied with the standards. HAdV and JCPyV concentrations were higher than the faecal indicator bacteria in almost all sampling points and seasons (Fig. 6).

Calculated Spearman correlations between viruses and bacteriological parameters are shown in Table 2. R-values varied from 0.274 to 0.762. No strong correlations were observed and negligible correlation was observed for JCPyV and *E. coli*. Moreover, HAdV presented higher correlations than JCPyV.

Physico-chemical data of sampling points

Measurements of physico-chemical parameters are shown in Table 3. Considering dry and flood seasons, significant differences for all parameters in all sampling points were observed ($p<0.05$), except temperature in MA02, MA03 and MA05 and conductivity in MA04 and MA05. Median temperatures of 28-29 ºC were observed, although higher pH, turbidity and conductivity medians were observed in the dry season in the *igarapés* (MA02 and MA03).

Discussion

The northern region of Brazil exhibits the poorest sanitary conditions in the country, particularly in urban areas which have suffered from unplanned growth. Manaus is the main financial and economic centre of this region and is located in the heart of the largest rainforest in the world and presents important social, environmental and urban problems, possibly associated with the establishment of the Manaus Free Trade Zone (MFTZ) developed in the 1970s (Magalhães and Rojas 2005). Today, riparian settlers living in wooden houses (*palafitas*) constructed along the *igarapés* are continually exposed to environmental contaminants coming from the garbage that is deposited into river waters and from raw sewage that is discharged either directly into waters or indirectly by stormwater run-off (PROSAMIM 2004, 2011, 2012).

The prevalence and concentration of viruses observed in this study confirms previous qualitative data obtained in 2004-2005, when the impact of microbiological contamination in the city's streams was reported (Miagostovich et al. 2008). In this study, the skimmed-milk flocculation method, previously described to concentrate viruses from seawater and validated later to freshwater (Calgua et al. 2008, 2013a), associated with qPCR protocols constituted a useful tool to assess the concentrations of HAdV, JCPyV, RVA and NoV GII in the acidic waters of the Negro River basin. Although no information on viruses infectivity is given by qPCR, preliminary results from cell culture assays have demonstrated the presence of infectious HAdV particles in these samples (unpublished data). Calgua et al. (2011) could also detect and quantify HAdV and JCPyV infectious particles by immunofluorescence assay in water samples using the same concentration method.

Unfortunately, this study also revealed that infrastructural interventions performed in the city of Manaus by the Social and Environmental Program for the *Igarapés* of Manaus (PROSAMIM) in the last years (2005-2011) has not prevented river water contamination by sewage. Despite these improvements poor water quality may still be affecting residents, particularly those in the seasonal flood-affected areas. The overall results suggest that the risk of infection due to different exposures to water, such as through recreation, household activities or transportation, can vary according to the sites and to the hydrological seasons of the Negro River. Remarkable differences were observed both in the distribution and in the concentration of viruses obtained from the *igarapés* (MA02 and MA03) and Negro River sites (MA01, MA04 and MA05).

Concentration of viruses in the *igarapés* were similar to those found in sewage samples of different geographical areas with values higher than four $\log_{10}$ (Calgua et al. 2013b; Flannery et al. 2012; Fumian et al. 2013; Myrmel et al. 2015). As these ‘sewage-like’ streams are tributaries of the Negro River, they transport those viruses through the environment by natural water flow to the main river channel where they are diluted and dispersed. It is worth noting the higher detection rates of RVA in the *igarapés* when compared to the diluted samples from the Negro River as reported by Miagostovich et al. (2008). Related reports in other south America countries, including Venezuela, Argentina, Uruguay, confirm the morbidity of RVA in this region (Barril et al. 2010, 2015; Rodríguez-Díaz et al. 2009; Victoria et al. 2014) despite RVA vaccination programs introduced in some of these countries in the last decade (PATH 2015). Although the samples obtained from these streams were similar to sewage, NoV GII detection was lower when compared to the other viruses, corroborating a previous study in this area (Miagostovich et al. 2008).

Differences on viruses distribution were also observed in the sampling sites along the Negro River. Ponta Negra Beach (MA01), located in the beginning of the urban area of Manaus, is a recreational area where thousands of people bath every week. It is in a relatively affluent neighbourhood with several buildings, including hotels and leisure areas. This site was relatively clean despite the direct discharge of raw sewage into the river and the discharge of treated sewage outfalls (PROSAMIM 2012). This is probably because Ponta Negra does not receive contamination from large urban streams that cross densely populated areas of Manaus. Although being the least contaminated site, all viruses were detected in MA01 (Ponta Negra), with higher detection of NoV GII in the dry season and significant differences on the JCPyV concentration.

In the following sampling sites along the Negro River (MA04 and MA05), viruses were more prevalent and presented higher concentrations than MA01. It is important to note that MA04 is located at the mouth of the urban watershed of Quarenta, which cross two popular neighbourhoods at the southern part of the city, and MA05 represents the end of the urban area along the river and is located adjacent to a port. The viral contamination may come from the port itself and/or could be a result of the river flow which carries the contamination from the entire city. At this site, bovine polyomavirus was also detected, which can be explained by the presence of pastures in this area of Manaus (Rusiñol et al. 2014).

The higher levels of RVA during flood period of the Negro River is noteworthy. When the river level was higher, water flooded more areas with virus laden water, expanding the distribution of viruses and transporting them to more distant ecosystems. Since the hydrological cycle of Negro River is annual and people are in more contact with water during flood period, it is possible to infer an association of this season with the increased risk and number of gastroenteritis cases, especially in 2012, when it was observed the worst flood season in 110 years (Fig. 1). Findings of higher detection and concentration of viruses described in this study during the flood season, especially at site MA04, suggest this hypothesised dynamics of viruses transport.

RVA and NoV are important viruses causing infantile gastroenteritis and waterborne outbreaks, respectively (Ahmed et al. 2014; Braeye et al. 2015; Di Bartolo et al. 2015; Jain et al. 2014; Villena et al. 2003). Unfortunately, the unavailability of laboratory data characterizing etiological agents of diarrhea in Manaus does not allow a specific correlation of these cases with those viral pathogens. In a recent study carried out in Manaus, RVA was responsible for 25% of acute gastroenteritis in children up to 3 years old in 2004-2006 (Melo et al. 2013), and, to our knowledge, there is no available data on NoV cases in the city.

The prevalence of RVA and NoV GII in the environment reflects their patterns in the population and could be influenced by illness seasonality and also by the lower stability of RNA viruses in aquatic environments (Fong and Lipp 2005; Fumian et al. 2011; Levy et al. 2009; Prevost et al. 2015; Rohayem 2009). These factors likely explain the NoV GII detection only in January 2011 (dry season) and the RVA detection during the flood season. The contamination of the environment promotes a constant pool of recirculating viruses and, as a consequence, people are exposed and acquire immunity. However, the introduction of new and unusual RVA and NoV strains in Brazil have been observed, which may cause waterborne gastroenteritis cases (da Silva Soares et al. 2014; Fioretti et al. 2011; 2014; Leite et al. 2008).

The hydrological cycle of Negro River also caused statistically significant changes in physico-chemical parameters in all sampling points, although the greatest differences were observed for the *igarapés*, which could be explained by the differences on the volume of water during dry and flood seasons. During the flood season, water diluted the organic matter, chemical compounds and suspended materials in these areas, decreasing the turbidity, conductivity and pH. Since these parameters may influence virus stability, adsorption and interaction between viruses and suspended materials (Fong and Lipp 2005; Wong et al. 2012), they should be considered in future studies correlating virus concentrations and other water quality parameters.

Bacteriological parameters (*E. coli* and enterococci) which are quantified as microbiological standards of faecal contamination in bathing waters confirmed that all study areas were contaminated by sewage, despite the differences between them. Moreover, the constant prevalence and high concentrations of HAdV and JCPyV throughout the study period and correlations observed suggest that they may be useful candidate indicators of human faecal contamination in waterbodies. The use of HAdV in particular, could complement faecal indicator bacteria in the monitoring of aquatic environments and this new multiple health-indicators approach could better estimate the real risk of waterborne diseases due to contact with these contaminated waters, as it has been proposed by other studies (Hewitt et al. 2013; Marion et al. 2014; Wyer et al. 2012). Unfortunately, it was not possible to carry out an epidemiological study to quantify the strength of any relationship.

The study of the Negro River hydrological cycle demonstrated that the flood season is characterised by changed physico-chemical and enteric viruses concentrations in the sampled waters. The surveillance of HAdV, JCPyV, RVA and NoV GII in the Negro River basin demonstrated high concentrations of these microorganisms showing the implications of the lack of basic sanitation in this city.

Floods are part of the natural climate variability in Manaus occurring as a seasonal pattern. In recent years, the city featured the long-lasting (2009) and most intense (2012) floods in recent history, and this more extreme variability is predicted to continue due to climate change in the Amazon and this could increase the incidence of viral waterborne gastroenteritis in Manaus. Since water plays an important role in the faecal-oral route of transmission of enteric viruses, data from environmental surveillance could inform public health policy on sanitary improvements and the prevalence of specific diseases in the contributing population.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure Captions

Fig. 1 Number of reported gastroenteritis cases and Negro River level in Manaus over an eight-year period. Sources: Brazilian Program of Monitoring Acute Diarrheal Diseases (MDDA 2015) and Port of Manaus (2015). River level data of 2010 were not available for all months

Fig. 2 Map of the study area and geographical distribution of the sampling points (MA01 – MA05). MA01- Negro River/Ponta Negra Beach (recreational area); MA02- São Raimundo Stream (*igarapé*); MA03- Quarenta Stream (*igarapé*); MA04- Negro River/Educandos; MA05- Negro River/end of the urban area of Manaus. Source: Google Maps

Fig. 3 Sampling points considering hydrological cycle of Negro River. (a) dry season; (b) flood season

Fig. 4 Quantification of human adenovirus (HAdV), JC polyomavirus (JCPyV), group A rotavirus (RVA) and genogroup II norovirus (NoV GII) in each of the eight samples collected according to sampling location, sampling and Negro River level. No sampling was carried out in MA05 in January 2011. Log_{10} GC L^{-1} – Log_{10} genome copies per litre

Fig. 5 Median loads of *Escherichia coli* (*E. coli*) (a) and enterococci (b) as microbiological indicators of human faecal contamination in aquatic environments in Manaus according to sampling location and sampling. MA01- Negro River/Ponta Negra Beach (recreational area); MA02- São Raimundo Stream (*igarapé*); MA03- Quarenta Stream (*igarapé*); MA04- Negro River/Educandos; MA05- Negro River/end of the urban area of Manaus. Threshold means maximum concentrations for both indicators based on Brazilian regulation for recreational waters (2000 MPN 100 mL^{-1} and 400 MPN 100 mL^{-1}, respectively). Quantification of enterococci in January 2011 was not carried out. MPN 100 mL^{-1} – Most Probable Number per 100 mL

Fig. 6 Median of human adenovirus (HAdV) and JC polyomavirus (JCPyV) concentrations in comparison to *Escherichia coli* (*E. coli*) (a) and enterococci (b) according to hydrological cycle of Negro River and sampling points. MA01- Negro River/Ponta Negra Beach (recreational area); MA02- São Raimundo Stream (*igarapé*); MA03- Quarenta Stream (*igarapé*); MA04- Negro River/Educandos; MA05- Negro River/end of the urban area of Manaus. Log_{10} GC L^{-1} – Log_{10} genome copies per litre; Log_{10} MPN L^{-1} – Log_{10} Most Probable Number per litre

Figure 1

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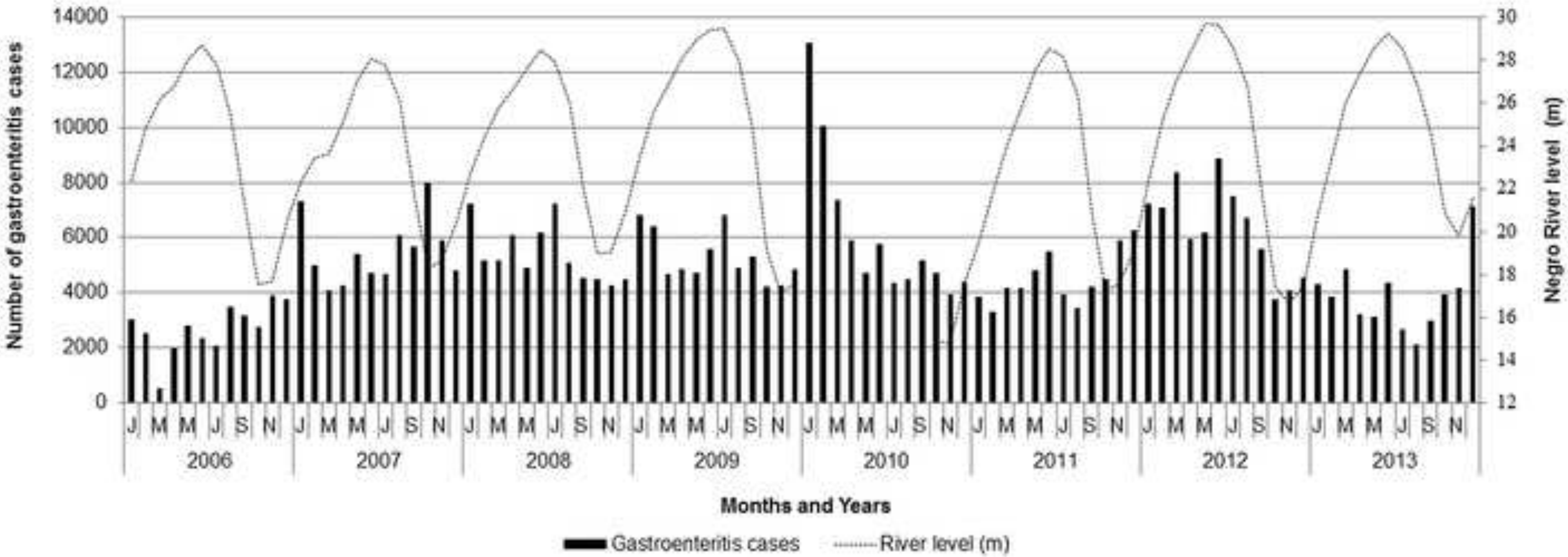

Figure 2

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Figure 3

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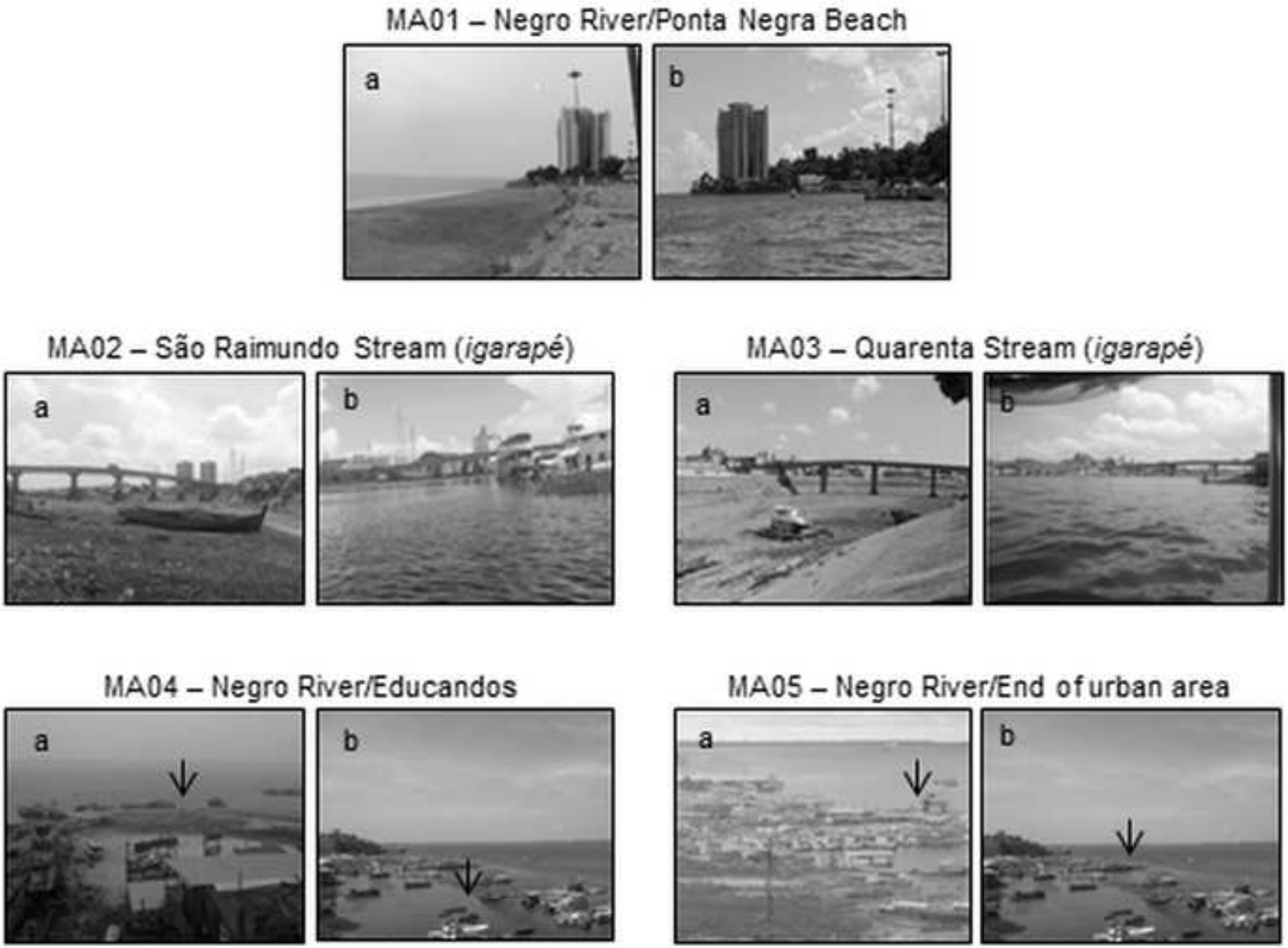

Figure 4

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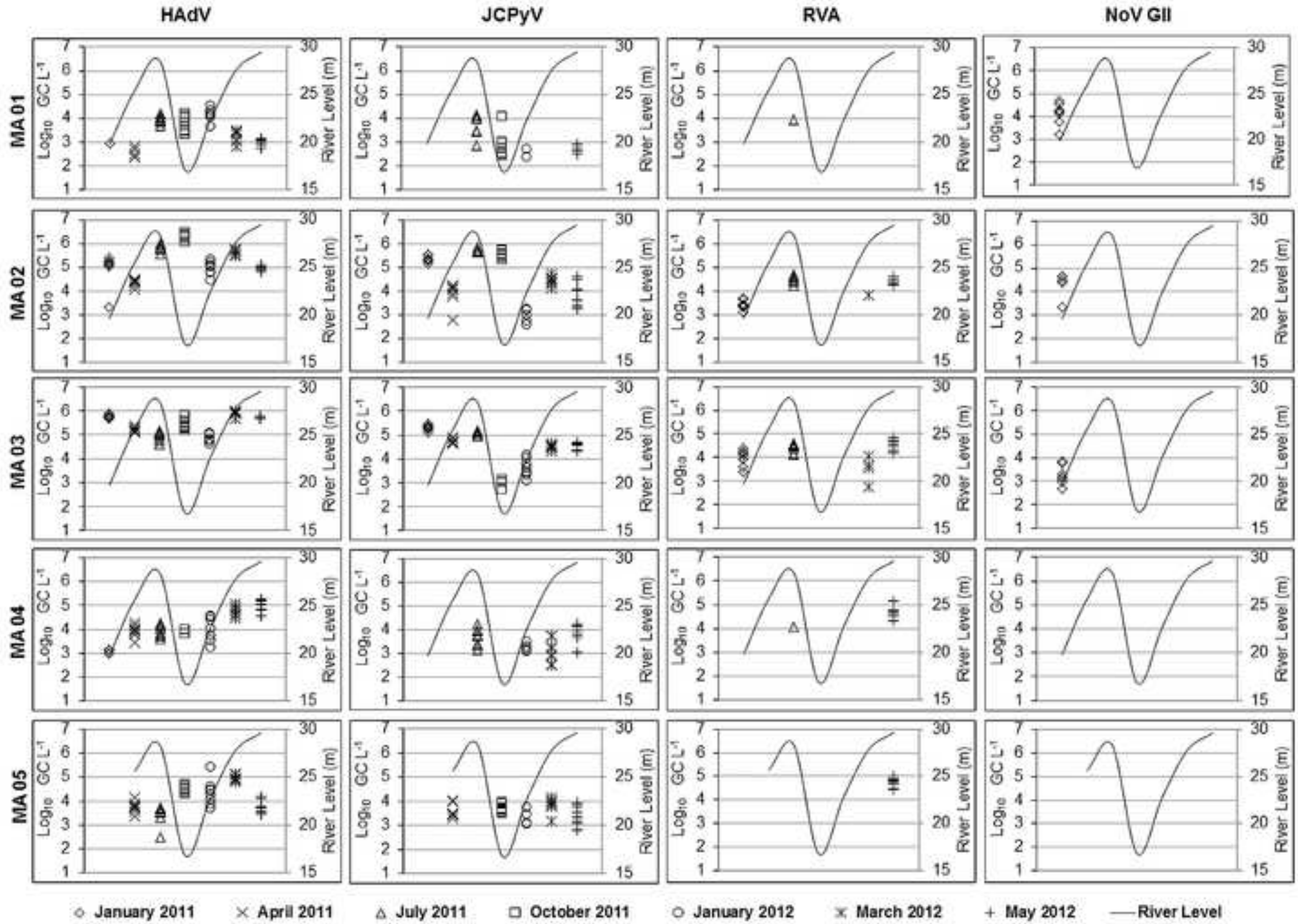

Figure 5

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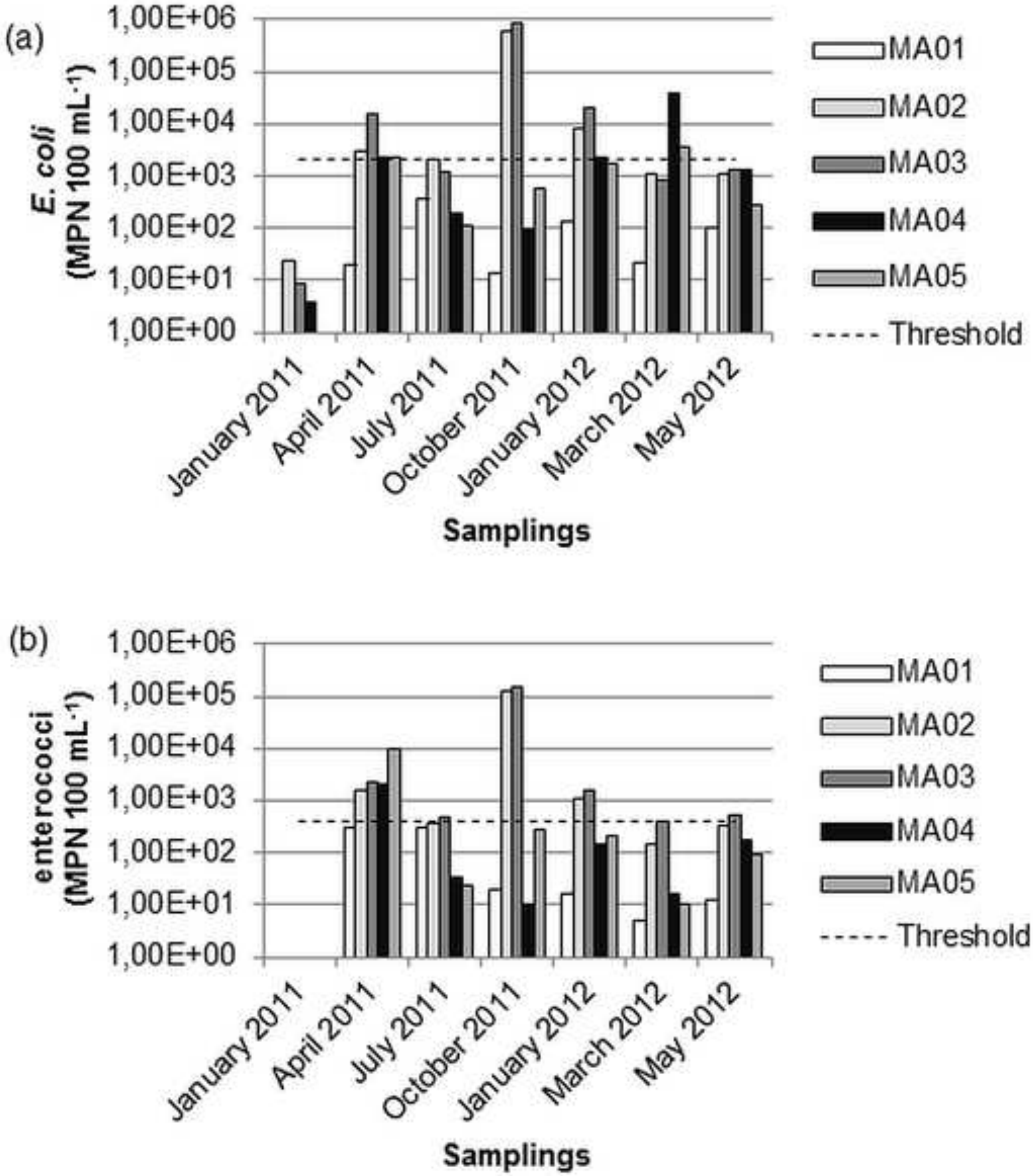
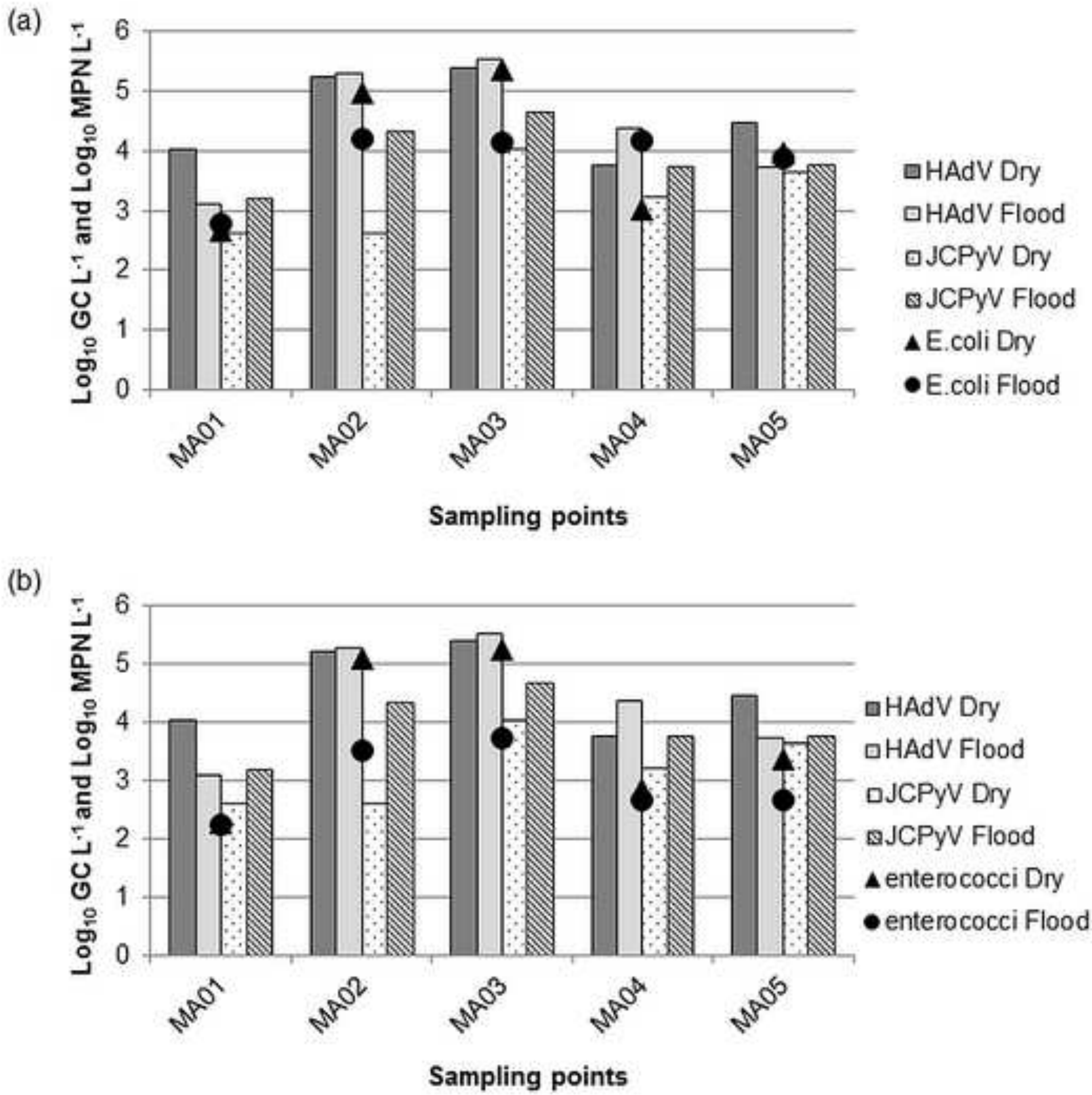


Figure 6



(a)
Log10 GC L-1 and Log10 MPN L-1
0
1
2
3
4
5
6
MA01
MA02
MA03
MA04
MA05
Sampling points
HAdV Dry
HAdV Flood
JCPyV Dry
JCPyV Flood
E.coli Dry
E.coli Flood
(b)
Log10 GC L-1 and Log10 MPN L-1
0
1
2
3
4
5
6
MA01
MA02
MA03
MA04
MA05
Sampling points
HAdV Dry
HAdV Flood
JCPyV Dry
JCPyV Flood
enterococci Dry
enterococci Flood

Table 1 Detection of human adenovirus (HAdV), JC polyomavirus (JCPyV), group A rotavirus (RVA) and genogroup II norovirus (NoV GII) in 272 river water samples according to sampling points and hydrological cycle of Negro River.

Sampling points	HAdV			JCPyV			RVA			NoV GII		
	[a]Dry	[a]Flood	[a]Total	Dry	Flood	Total	Dry	Flood	Total	Dry	Flood	Total
MA01	16	28	44	8	14	22	0	1	1	8	0	8
MA02	24	32	56	21	32	53	7	15	22	5	0	5
MA03	24	32	56	19	32	51	8	19	27	7	0	7
MA04	14	32	46	5	24	29	0	7	7	0	0	0
MA05	16	32	48	12	22	34	0	8	8	0	0	0
TOTAL	94 (83.9%)	156 (97.5%)	250 (91.9%)	65 (58.0%)	124 (77.5%)	189 (69.5%)	15 (13.4%)	50 (31.3%)	65 (23.9%)	20 (17.9%)	0 (0.0%)	20 (7.4%)

[a]n dry = 112, n flood = 160 and n total =272

Table 2 Correlations between human adenovirus (HAdV), JC polyomavirus (JCPyV), *Escherichia coli* (*E. coli*) and enterococci as indicators of human faecal contamination in river waters in Manaus.

	HAdV	JCPyV	*E. coli*
HAdV			
JCPyV	[a]0.762		
E. coli	0.544	0.274	
enterococci	0.491	0.374	0.607

[a]For all correlations, $p \leq 0.001$.

Table 3 Median of physico-chemical parameters according to sampling points and hydrological cycle of Negro River.

Sampling points	Water Temperature (ºC)		pH		Turbidity (NTU)		Conductivity (µS cm^{-1})	
	Dry	Flood	Dry	Flood	Dry	Flood	Dry	Flood
MA01	29.8	29.4	5.9	5.1	21.0	9.0	10.0	9.0
MA02	28.4	28.7	6.9	5.4	43.0	7.5	267.0	10.1
MA03	29.1	29.3	6.9	6.0	56.0	9.0	296.0	21.0
MA04	29.6	29.1	5.8	5.1	9.0	5.0	8.0	8.0
MA05	29.4	29.3	5.9	5.1	11.5	3.0	7.5	8.0