Marine Pollution Bulletin
July 2021, Volume 168 Pages 112478 (7p.)
https://doi.org/10.1016/j.marpolbul.2021.112478
https://archimer.ifremer.fr/doc/00694/80593/

Urban inputs of fecal bacteria to the coastal zone of Libreville, Gabon, Central Western Africa

Leboulanger Christophe [1, *], Kolanou Biluka Lévie [2, 3], Nzigou Aime Roger [2],
Djuidje Kenmogne Véronique [2, 3], Happi Johann Ludovic Martial [2], Ngohang Franck Estimé [3],
Eleng Aminata Spanian [4], Ondo Zue Abaga Norbert [2], Bouvy Marc [1]

[1] MARBEC, Université Montpellier, CNRS, Ifremer, IRD, Sète, France
[2] Université des Sciences et Techniques de Masuku, Franceville, Gabon
[3] Ecole Normale Supérieure, Libreville, Gabon
[4] Institut de Recherche Agronomique et Forestière, CENAREST, Libreville, Gabon

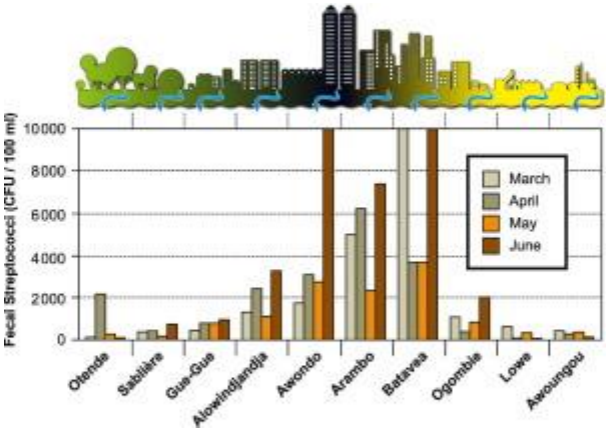
* Corresponding author : Christophe Leboulanger, email address : christophe.leboulanger@ird.fr

---

Abstract :

Libreville, the largest city in Gabon, adversely impacts the Komo Estuary and the Akanda National Park aquatic ecosystems through discharge of domestic and industrial waste. Fecal Indicator Bacteria (FIB: Escherichia coli and fecal streptococci) were enumerated using culture-based methods in water from 40 sites between 2017 and 2019 including coastal outlets, mangrove channels, open bays and littoral rivers. Contamination levels were high in discharge waters from small urban rivers in Libreville agglomeration, frequently exceeding international safety guidelines, whereas FIB concentrations decreased downstream from the city in main mangrove channels. Littoral forest rivers were significantly impacted by fecal contamination despite the absence of settlements in the watersheds. Protected areas are not effective in avoiding FIB contamination, indicating inefficient waste management. Dedicated management policies should be implemented to reduce both the sanitary concern and global pollution, poorly assessed in a context of demographic increase in tropical littoral zones.

Graphical abstract



Highlights

► First record of fecal bacteria contamination for the coastal area of Libreville, Gabon ► Urban population density drives fecal bacteria concentrations at river outlets. ► Surrounding protected areas are not devoid of fecal pollution. ► Public policies need to be implemented to reduce contamination and associated risks.

Keywords : Fecal indicator bacteria, Marine protected area, Cultivability, Tropical coastal ecosystems, Coastal pollution

Coastal marine ecosystems are submitted to increasing anthropogenic impact worldwide (Lotze et al., 2006), including disposal of solid waste and discharge of treated and untreated wastewater, from various sources and activities. These sources of pressure are among the main drivers that pose a global threat (Halpern et al., 2008) to highly valuable resources and benefits specifically provided by coastal ecosystems (Barbier et al., 2011). Although prominent for public policies and management, scientific knowledge about the extent and nature of anthropogenic impact is not equally available on a global scale. Southern ecosystems, particularly in low to middle-income countries, are scarcely studied, especially in Africa and Asia (Garcia Rodrigues et al., 2017; Lüthi et al., 2020). These countries are highly sensitive to pollution-driven issues in littoral and coastal zones, with problematic lags between increasing demography and sustainable development goals. For many sub-Saharan countries, sewage discharge is pointed out as a significant concern to further human development and well-being, and for the conservation of coastal marine ecosystem functions and uses (Bouvy et al., 2008; Lewis, 2016). Among pollutants of concern, potentially infectious microorganisms stemming from fecal contamination pose a threat to human health by direct exposure, mainly through recreational, cultural or professional contact with contaminated waters, or through food with soiled fish and shellfish. Uncontrolled population growth in the littoral zone, worsened by the absence of sewage treatment, leads to increased risk of waterborne diseases, which are a major handicap to sustainable development in the tropics, and especially in Africa (Bastaraud et al., 2020; Rochelle-Newall et al., 2015). To support local policies and advise public decision making, knowledge about actual contamination levels and pathogenic risks is often lacking and needs to be urgently documented by validated methods (Wu et al., 2011; Lüthi et al., 2020).

With an estimated census of ca. 2.1 M inhabitants for a total area of 276,000 km^2 (United Nations, 2020), Gabon is a relatively scarcely populated country. By contrast, Gabon is now the most urbanized country of continental Africa, with an estimate of 89% of the population currently established in cities, expected to top 95% by 2050 (United Nations, 2019). The hinterland is gradually depopulating as the coastal cities of Port Gentil and mostly Libreville and its suburbs face demographic increase. Libreville is the political, administrative and economic center, with an estimated agglomeration population of around 800,000. The conurbation is located in the Estuary province of Gabon, in the southern part of the Gulf of Guinea close to the equatorial line, on the estuary of Komo River, arisen from the southwestern part of the Woleu-Ntem plateau in Equatorial Guinea.

Three protected areas that constitute the *Arc d'Émeraude* (translates into "Emerald Ark", named for the shape of the green forest belt around the city) closely surround the urban zone: a coastal tropical forest (*Arboretum Raponda Walker*) and two National Parks, Pongara NP to the south, and Akanda

NP to the northeast (Walters et al., 2016). Both NPs are characterized by an extended mangrove ecosystem, highlighted for its outstanding biomass and diversity in Gabon (Simard et al., 2019), which spreads from the Mondah Estuary up to the city suburbs in Akanda. The Akanda National Park lies east from Libreville around the Mondah bay, mostly consisting of mangrove forests and channels, harboring traditional fisheries (Cardiec et al., 2020).

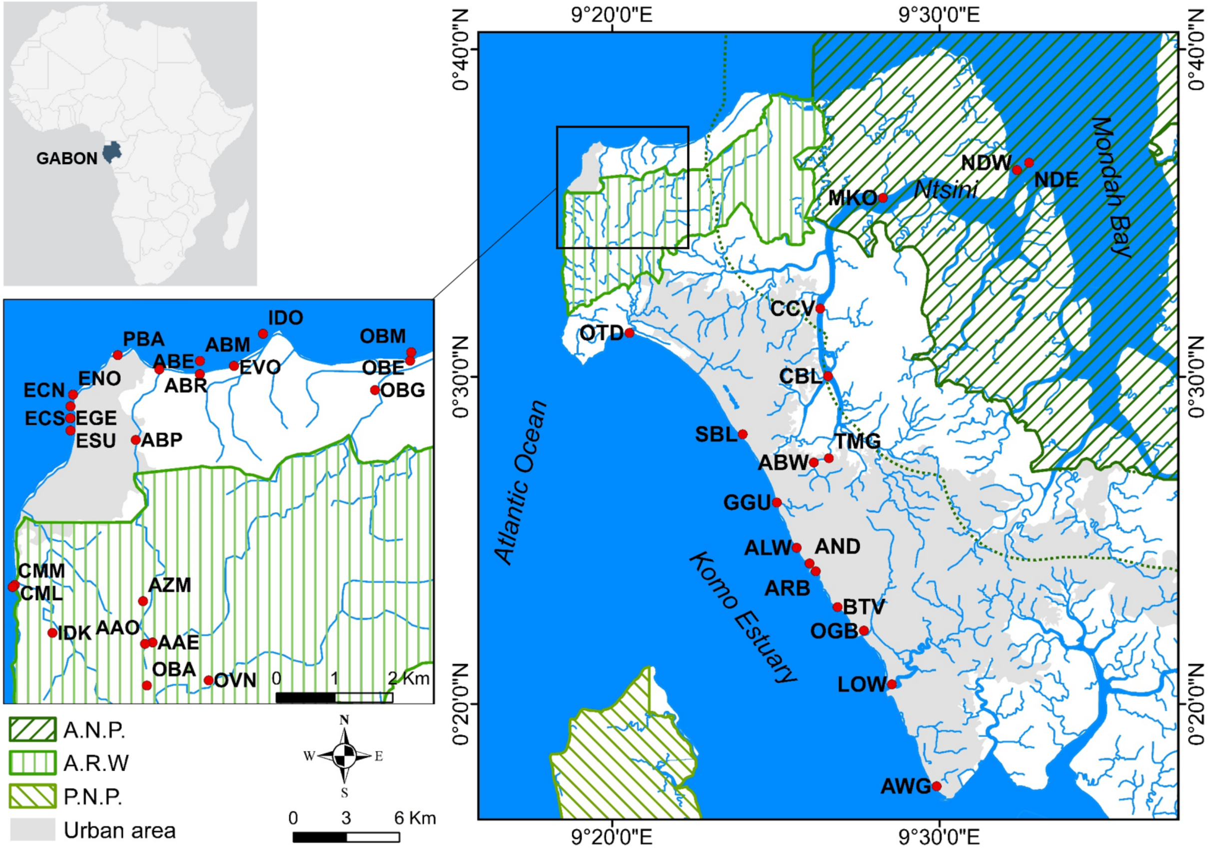


Figure 1. Location of the sampling sites (red circles) in the Libreville area (Estuary province, Gabon). A.R.W.: Arboretum Raponda Walker (protected littoral forest); A.N.P.: Akanda National Park; P.N.P.: Pongara National Park. Sampling locations are indicated by red circles.

Submitted to a typical equatorial climate with rainy seasons in February-May and October-November (Camberlin et al. 2019), Gabon possesses a still overlooked biodiversity, and littoral protected zones in the capital's area are declining despite management policies are in place (Walters et al., 2016; Goussard and Ducrocq, 2017; Simard et al., 2019), challenging the balance between conservation and development. Marine protected areas (MPAs) have been created, comprising almost 23% of the Gabonese Exclusive Economic Zone, and littoral parks protect mangroves and beaches that give shelter to endangered marine mammals (African manatees) or chelonians (leatherback turtles). Efficient conditions for the successful implementation of these conservation tools are nevertheless currently not fully established. Particularly, public sanitation and waste management do not match the spreading of the city and increase in population over the last decades, resulting in the uncontrolled release of waste in the littoral zone, along with associated significant threats to the coastal ecosystems and to human health (Moubélé and Mbonda, 2017). Despite the improvement of sanitary conditions within the population, Gabon still suffers from a significant incidence of diarrheic diseases (Troeger et al., 2017). Viral and bacterial pathogens are for example frequently involved in the spreading of diseases in infant populations (Koko et al., 2013), which can significantly be accounted for by fecal contamination of food and environment. Local regulations of water quality rely on international guidelines (World Health Organization, 2018) and no adaptation to the national context was implemented to date.

The context of Libreville agglomeration, surrounded by the protected areas of the *Arc d'Émeraude*, makes coastal contamination especially worrying: local populations are exposed during recreational activities on the urban and peri-urban beaches, and through consumption of local seafood. Assessment of actual levels of contamination by fecal indicator bacteria (FIB) is therefore mandatory for (i) documenting the current extent and nature of FIB contamination in the littoral zone, (ii) providing a baseline for the population and decision makers, and (iii) allowing to implement efficient monitoring of the coastal zone of Gabon, which will further support the success of future waste management and conservation policies. In order to establish a first step in this process, an assessment of FIB contamination in coastal rivers, estuaries and marine waters of the *Arc d'Émeraude* area was performed using culture-based methods, proven to be suitable to document pathogen occurrence in water systems (Wu et al., 2011).

Forty locations were sampled in the study area (Fig. 1, Supplementary Table S1) between April 2017 and April 2019, including estuarine surface water, coastal river outlets, mangrove channels, sandy beaches, and forest rivers, spreading from dense urban areas to fully protected ecosystems (mangrove system of Akanda National Park). Coastal rivers and outlets were sampled aiming at the first rainy season of the year, typically from February to May, to avoid drying of the smaller water bodies and flash floods which are more likely during the second rainy season in October-November (Camberlin et al. 2019). Ntsini channel was sampled at four dates to cover most of the yearly climate variability in the area. All field-sampling sessions were arranged by local agencies staff and boat availability to cope with current regulations.

Water samples were taken immediately below the surface using a 2 L horizontal Niskin bottle previously cleaned with 0.1 M HCl, and thoroughly rinsed with mineral water between each sampling. Measurements of pH, temperature and conductivity on surface water were conducted using an YSI 600 multiparameter probe. Calibration for conductivity and pH sensors was performed in the field immediately before each deployment, using fresh standard solutions. All water samples were taken in the morning, stored in polycarbonate bottles at ambient temperature in a dark cool box and processed immediately upon return to the laboratory. Samples from river outlets were taken as close as possible to the estuary shoreline, whereas mangrove channel locations were sampled twice in the same day at rising and ebb tides.

Two types of fecal indicator bacteria (FIB) were detected and enumerated using culture-based assays, with commercially available kits comprising sterile gridded membranes and ready-to-use dehydrated selective medium ("Nutrient Pad Sets, Sartorius, Gottingen, Germany). Two different selective medium and incubation conditions were applied to enumerate fecal streptococci (FS) and *Escherichia coli* (EC). AZIDE kits (ref. 14051) incubated at 37°C for 48 h for FS enumeration are based on standard medium (Slanetz and Bartley, 1957), with added sodium azide as gram-negative bacteria inhibitor and triphenyltetrazolium chloride as stain for growing colonies (red-brown). ENDO kits (ref. 14053) incubated at 44°C for 24h for EC enumeration are based on the lactose fermenting ability of coliforms, with sodium sulfite and fuschin addition to inhibit gram-positive bacteria. Each water sample was filtrated onto a squared sterile 47 mm polycarbonate membrane, rinsed with 10 mL sterile mineral water to remove salts, and deposited on the pre-wetted medium pad in a plastic petri dish, according to the recommendations of the manufacturer. To account for the expected range of FIB densities in the samples, three volumes (1, 10 and 100 mL) were processed in duplicate for each water sample. Blank controls were made by processing accordingly 100 mL of mineral water (Andza brand, https://sobraga.net/eaux/). After incubation, petri dishes were examined under bright light, and relevant colonies (dark brown for FS, dark purple with greenish metallic glint for EC) were counted on each filter. Results are expressed as colony forming units (CFU) per 100 mL, according to the most useful expression of water contamination levels.

Chemical analysis was performed on raw water subsamples (50 mL) passed through 0.2 µm porosity polycarbonate syringe filters to remove particulate matter. Dissolved ammonium (NH_4^+) and soluble reactive phosphorus (SRP, equivalent to bioavailable orthophosphate PO_4^{3-}) were determined using spectrophotometric kits for seawater (Spectroquant, Merck), according to the recommendations of the manufacturer. Calibration curves were carried out for each sample processing session, using reagent grade (Sigma Aldrich) ammonium chloride (NH_4^+ analysis) or potassium phosphate (SRP analysis) diluted in double distilled water. Six concentrations and a reagent blank, each in triplicate, were used for each chemical, within the specified range of application of the corresponding kit.

FIB densities were expressed as the mean CFU/100 mL ± SD; in the most polluted locations, colonies were counted on half or a quarter of the filter. If the dilution was not appropriate for accurate and distinct counting of each colony on the filter membrane after incubation, values were extrapolated and considered arbitrarily to be above the highest validated count during the whole study, namely 10 000 CFU/100 mL.

Dissolved nutrient calibration curves (photometric readings of standard samples) and FIB relationships were drawn by linear regression using Past 3.0 freeware for OS X (Hammer et al., 2001), and slope and intercept values were considered to calculate nutrient concentrations from absorbance readings.

The relationships between pH, salinity, concentrations of ammonium and soluble reactive phosphorus on the one hand, and abundances of *Escherichia coli* and fecal streptococci on the other hand, were studied using multivariate analysis. All data (x) were log (x + 1) transformed and a centered Principal Component Analysis (PCA) was performed using SigmaPlot 10 software to identify the major sources of temporal and spatial variability in the fecal indicator bacteria (FIB) in Komo estuary. Log-transformed (log (x+1)) FIB values in Ntsini channel were tested for significant influence of tide period (rising *vs*. ebb) on measured densities, using a repeated analysis of variance (Statistica v.7, Statsoft, France), with *post hoc* Scheffé test.

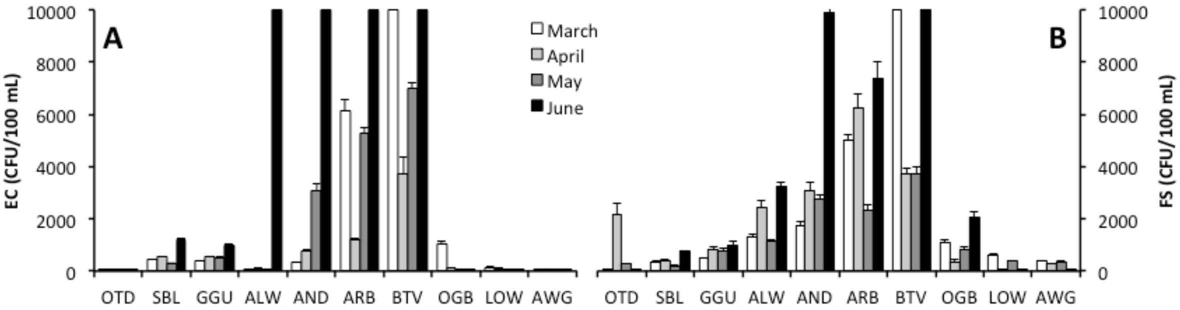


Figure 2. Concentrations of FIB *Escherichia coli* (A) and fecal streptococci (B) expressed as CFU/100 mL in the ten littoral outlets of the Komo estuary sampled within the Libreville area, depending on the sampling month (March-June, 2018). Error bars are given except for samples exceeding the counting capacity of the method, set at 10,000 CFU / 100 mL.

A gradient in waste pressure was clear from the visible accumulation of macro-waste in the urban outlets, increasing in sites near the city center, whereas mangrove roots acted as traps for floating debris even in the most remote protected areas of Akanda National Park. Upper forest rivers were obviously under low human pressure, but solid and liquid waste was collected in their lower part before reaching the seashore. Water contamination by FIB varied accordingly, depending on the hydrosystem considered and discussed in this study.

On the littoral of Komo estuary in Libreville, increasing concentrations of both *Escherichia coli* and fecal streptococci were recorded in the most urban outlets (Fig. 2), with consistent differences in CFU densities depending on the sampling month. Maximal EC densities were above the performance range of the method in four of the sampling sites located in the center of Libreville city in June 2018, and one in March 2018 (Fig. 2A). A similar pattern was observed for FS numbers, with two samples out of the measuring range at one site and on two dates (Batavea outlet, Fig. 2B). Lower FIB densities, mostly represented by FS, characterized the locations distant from the city center.

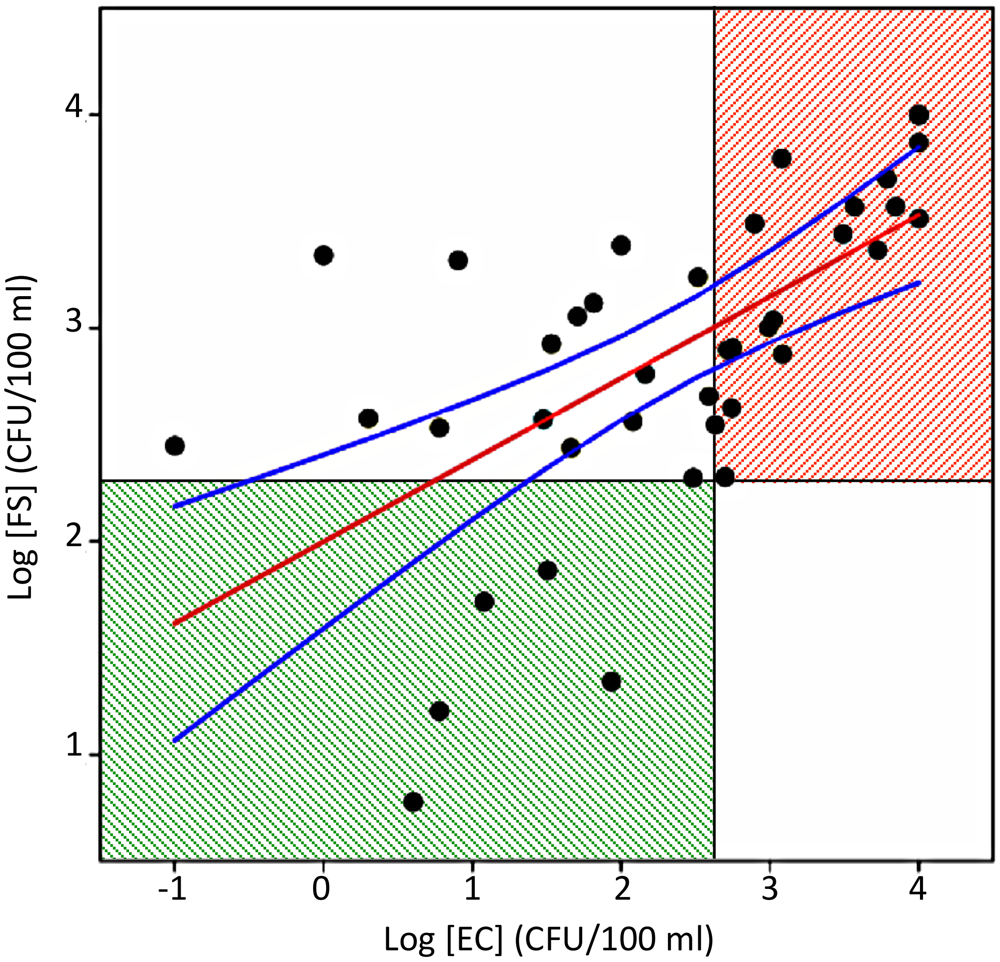


Figure 3. Relation between *Escherichia coli* (EC) and fecal streptococci (FS) densities (expressed as log[CFU/100 mL]) in the littoral outlets of the Komo estuary within the Libreville area during the present survey (March-June, 2018). Regression (y = 0.38737 x + 2.0063) is marked by the red line, 95% confidence intervals (bootstrapped, N = 1999) by blue curves. Dashed line indicates 1:1 relation, vertical and horizontal solid lines indicate European Union bathing water guidelines for information, green and orange area being below and above quality criteria for both FIB types respectively.

A weak but significant relation ($r^2 = 0.40789$; $p = 0.0002$) was established between logarithmic concentrations of EC and FS (Fig. 3). Contamination level was generally higher for *E. coli* at the three most central urban outlets (Awondo, Arambo and Batavea), whereas fecal streptococci were recorded at higher densities in the more suburban (Otende, Sablière) and industrial (Awoungou, located in the industrial settlement of Owendo harbor) sites. The PCA was performed on the independent data set (6 descriptors, 10 sites, 4 sampling dates). The first two eigenvalues of the PCA analysis accounted for 88.7% of the total variability. The analysis, therefore, only considered these two first axes to highlight the relationship between descriptors along a spatial and temporal distribution. On both axes, the two FIBs were opposed to salinity (Fig. 4). The two nutrient concentrations (NH_4^+ and PO_4^{2-}) were also linked to bacterial abundances, while pH did not explain any relation with the other variables. On the first axis, the analysis clearly differentiated the sampling locations, with the identification of two groups. On the right, a group of locations (ARB, BTV, AWD, ALM), identified as the most urban outlets ("Center" group), was clearly linked to FIB abundances, while the other sites, in scattered habitats and industrial areas ("Suburbs" group on the left), were characterized by low values of FIB concentrations and amounts of nutrient (Fig. 4). These results support the prominent influence of sampling locations over sampling dates, most likely linked to population densities in the respective watersheds.

Table 1. FIB contamination (CFU / 100 mL) in water samples from forest rivers and coastal zone of Cap Estérias (*Escherichia coli* EC and fecal streptococci FS). nd: not determined.

	April 2018		April 2019	
Station	**EC**	**FS**	**EC**	**FS**
ABM	0	58	0	436
ABE	0	2480	355	3600
ABR	0	4960	nd	nd
ABP	0	1425	1360	1322
AAE	nd	nd	0	2800
AZM	nd	nd	40	1160
AAO	27	1030	0	1380
OBM	0	0	nd	nd
OBE	1940	1086	nd	nd
OBG	0	1500	nd	nd
OBA	0	620	0	1250
OVN	120	210	nd	nd
EVO	nd	nd	0	4260
CML	nd	nd	0	1400
IDK	600	11000	0	2050
EGE	nd	nd	0	3160
CMM	nd	nd	0	180
PBA	nd	nd	64	375
ENO	nd	nd	0	0
ECN	nd	nd	0	68
ECS	nd	nd	0	405
ESU	nd	nd	44	412
IDO	nd	nd	0	500

Out of the forty locations sampled, only six complied with the bathing water guidelines of the World Health Organization and the European Union applying to marine and transitional waters for "good quality", set to 200 and 500 CFU/100 mL (95-percentile of the geometric mean) for fecal streptococci and *E. coli* respectively (European Parliament 2006, World Health Organization 2018). FIB contamination in beach waters along the littoral of Libreville is therefore worrying since recreational bathing by urban populations is usual practice, especially during weekends.

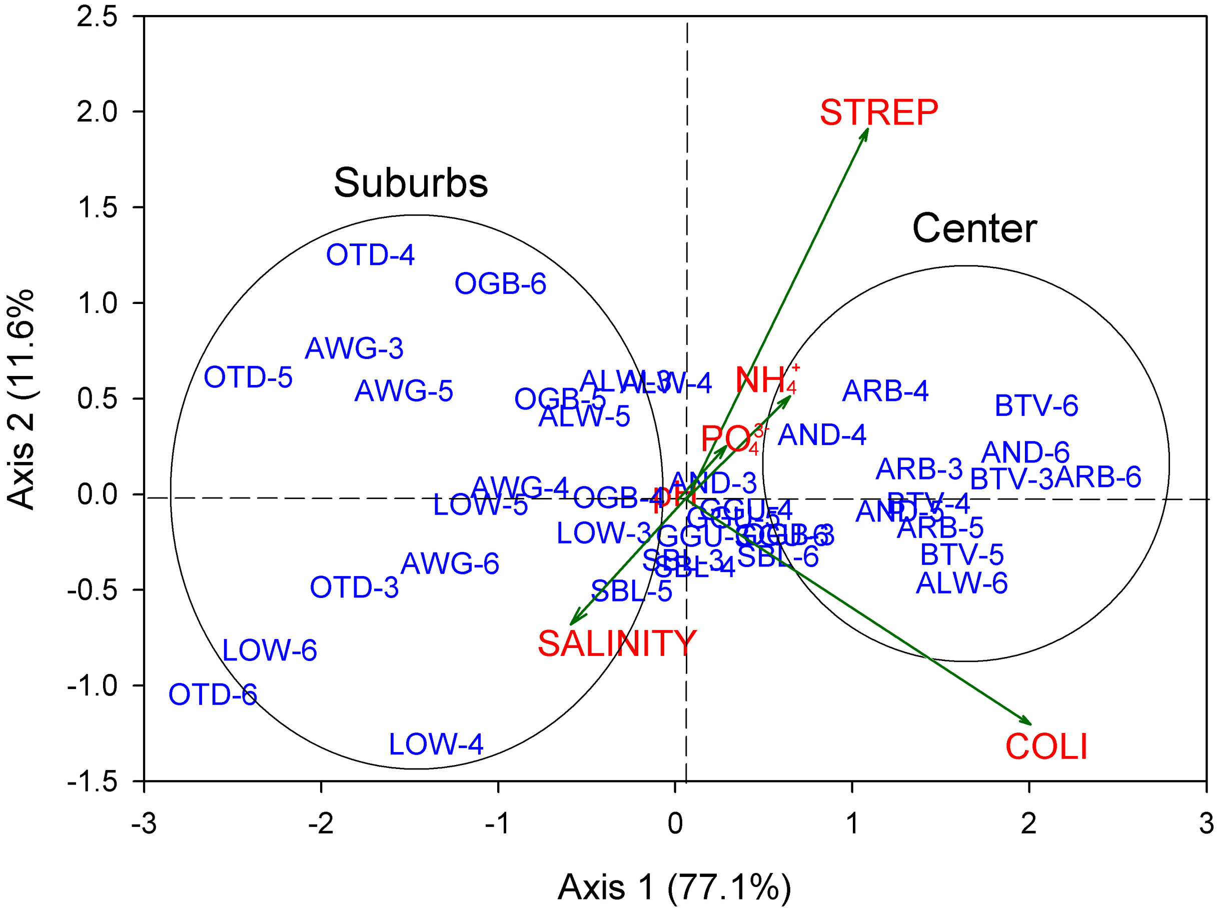


Figure 4. Principal component analysis (PCA) on the two first axes, based on environmental parameters and FIB concentrations at the outlet of Libreville coastal rivers in the Komo Estuary. Eigenvalues for each axis of the PCA are reported. Abbreviations for sampled locations are listed in Table 1, sampling dates in 2018 are identified by month numbers (3: March; 4: April; 5: May; 6: June). STREP: abundance of fecal streptococci; COLI: abundance of *Escherichia coli*; NH_4^+: ammonium concentration; PO_4^{3-}: orthophosphate concentration. Two groups of locations can be defined (Suburbs and Center).

Regarding the FIB contamination in the mangrove protected area in Akanda National Park, seven sites were sampled on the Ntsini channel, representing ca. 27 km of watercourse from Ambowé, close to an urban fishing pier, to Nendé East, at the mouth of the Mondah Bay (Fig. 1). Samples were taken at four dates between April and November 2017, during both rising and ebb tides of the same day. A clear decreasing pattern from up- to downstream was observed for both categories of FIB (Fig. 5), consistent with an important contamination source from urbanized areas with subsequent transport, mortality decay and dilution to the open coastal waters. In 48 out of 56 samples analyzed, *E. coli* densities (Fig. 5A and 5B) were lower than fecal streptococci counts (Fig. 5C and 5D) by a factor up to 10 (maximal counts of 655±99 and 6666±1100 for EC and FS respectively), while rising waters (Fig. 5A and 5C) appeared often more contaminated than ebb waters (Fig. 5B and 5D). Contamination of surface waters during rising tide was significantly higher (ANOVA, $p < 0.001$) for EC at ABW, TMG, MKO and NDE locations, and for FS at all locations except MKO. This tide-dependent pattern was consistent with the leaching of contaminated sediments by inflowing waters. Discharged sewage during low tides was retained on emerged sediment banks acting as a buffer zone, later released during flow and further increasing the overall densities of FIB in the mangrove channel surface water.

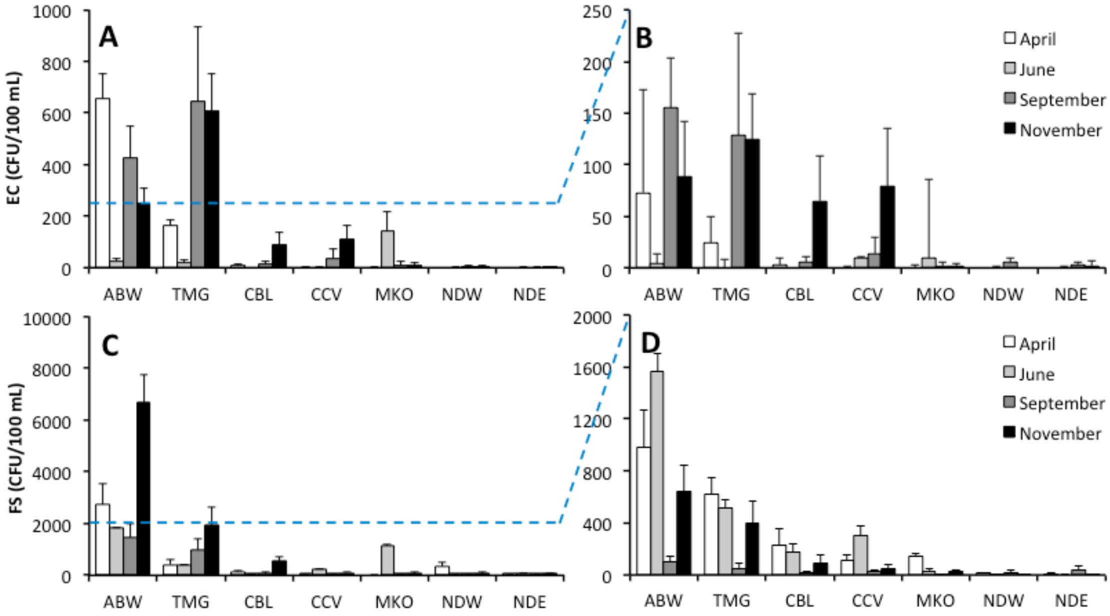


Figure 5. Concentrations of FIB *Escherichia coli (*A, B) and fecal streptococci (C, D) expressed as CFU/100 mL in the Ntsini channel from the suburbs to Akanda National Park depending on sampling date, at rising (left panels A and C) and ebb (right panels C and D) tides. Locations are ordered from the most urban zone (left, Ambowé) to the central protected area (right, Nendé East). The range differences in y axes for rising and ebb data are highlighted by the blue dashed lines.

Survival of FIB in tropical estuarine waters is not favored, since salinity, temperature and sunlight rapidly lower bacterial cultivability (Korajkic et al., 2019). In the Senegal Estuary for example, T_{90} (time to decrease the FIB density by 90%) was reported below one day for *E. coli* strains (Troussellier et al., 2004), whereas fecal streptococci are reputedly more persistent in aquatic environments. FIB tracers, resulting in significant contamination that exceeded the quality levels for recreational waters, therefore confirm the extent of sewage discharge into the upper mangrove fringes. Despite or because of the absence of any leisure infrastructures, local inhabitants are used to bathing in the mangrove upper creeks, a risky activity regarding sanitary hazards and the lack of regulation. The fact that FIB levels recorded in the most distant locations were often detectable is likely to unveil a diffuse but significant human pressure even in the highly regulated area of Akanda National Park.

Beaches and upstream rivers were sampled in April 2018 and April 2019 in water bodies surrounding the settlement of Cap Estérias, including forest streams of the Arboretum Raponda Walker, a protected littoral forest. Out of 29 samples, only one was devoid of measurable FIB contamination (Table 1), sampled on a rocky shore at high tide.

By contrast with the other sampling sites studied in urban littorals and mangrove channels, contamination by *Escherichia coli* was extremely rare, with the detection of EC in only 9 samples within the area. Nevertheless, a significant occurrence of FIB and especially fecal streptococci was reported in the protected area of Arboretum Raponda Walker, despite a high level of regulation. It is worth noticing that the most contaminated sample, with EC and FS evaluated at 600 and 11000 CFU / 100 mL respectively, was taken from Idakogo river, in a touristic forest circuit. The lower parts of Abagna and Obagna rivers were also significantly contaminated by FIB, the first one crossing the main village, the second one draining poultry farms and scattered habitats. Regarding the coastal zone, significant contamination exceeding safety levels was measured on beaches and rocky shores, putting recreational and fishing activities at risk: Cap Estérias is a renowned leisure place for city dwellers and for the existence of an emblematic fishery for the razor clam, *Solen guinensis*, highly valued locally as seafood (Cardiec et al., 2020). As noticed for the urban zone of Libreville, sewage management is absent and will need to be considered in a near future to reduce pollution and safety hazards, as recommended by Lüthi et al. (2020).

This study provides the first evaluation of fecal pollution in the coastal zone of Gabon, focusing on the most densely populated region of Libreville. The diagnosis is worrying, with numerous outlets, rivers and beaches contaminated by sewage discharge, resulting in significant concentrations of FIB in coastal waters. Exceeding by far the safety guidelines for recreational waters (European Parliament, 2006; World Health Organization, 2018) in most of the samples, the study area deserves further monitoring and risk assessment. The reported pattern suggests a threat to coastal marine waters and to the production of local fisheries through potential hazardous pathogens (Padovan et al. 2020), since FIB are closely linked to waterborne microbial diseases (Djuikom et al., 2006; Bastaraud et al., 2020). Recreational activities are particularly exposed, being practiced by a young population that is not adequately informed about the risks and, furthermore, inherently fragile in the face of intestinal diseases (Bouvy et al., 2010; Koko et al., 2013).

Direct comparison of the current status of Libreville coastal ecosystems regarding FIB contamination to other subtropical and tropical locations is difficult since studied markers and detection methods often differ. In the Senegal Estuary, Bouvy et al. (2010) reported the importance of both direct (fishermen community settlements and city hospital) and indirect (upper freshwater reservoirs) contributions to estuarine and coastal contamination by *E. coli* and fecal streptococci, enumerated by culture-based methods, with 20% of samples not complying to international guidelines. A worse condition was reported using the same approach in the Hann Bay (Dakar, Senegal), suffering from direct sludge releases, where coastal open sea was highly contaminated by FIB, with most samples exceeding quality guidelines (Bouvy et al., 2008). A comparable survey from seashore waters in Zanzibar (Tanzania) reported the occurrence of fecal streptococci exceeding guidelines in a touristic area (Moynihan et al., 2012), together with highlighting the differences in marine pollution perception by local and foreign populations. The temporal variability in FIB densities reported during this study has also been highlighted, for example, in a Hawaiian estuary (Wiegner et al., 2017) where fecal streptococci contamination increased during storm events. Seasonal changes in rain intensity were also proven important, especially in tropical regions submitted to monsoon events (Khandeparker et al., 2015).

Bacterial enumeration by culture-based methods does not consist in a direct assessment of health risk. For example, only a few strains of *E. coli* are known to be toxic to humans, and their detection relies on specific methods such as gene targeting (Snoussi et al., 2011; Walker et al., 2013). Detection and enumeration of FIB are often limited tools in forecasting microbially-associated risks in coastal waters and are still being debated regarding the accuracy of the different methods (Wu et al., 2011; Vukić Lušić et al., 2013; Padovan et al., 2020). Nevertheless, cultivation procedures have proven useful in addressing wastewater-related pollution issues for decades, relying on robust methods that require affordable equipment and consumables, easy-to-learn technical skills, and which benefit from an internationally validated framework for sample processing and results interpretation. As such, FIB detection using selective growth media could be widely implemented as a first-step monitoring tool in developing countries (Condé-Lamparelli et al., 2015; Krepsky et al. 2021). Together with direct counts in coastal waters, more synoptic approaches could be later considered in order to overcome the high temporal variability of FIB in surface waters, such as the detection of fecal sterols in surficial sediment (Isobe et al., 2002; Muniz et al., 2015; Frena et al., 2019). To improve significance of FIB enumeration in the specific frame of human health risk management, robust and univocal determination of potential pathogens could be attained by using locally adapted methodologies. For example, the availability of isolated strains could serve as positive control in culture-based and genomic detection (Padovan et al. 2020) together with ongoing epidemiological surveys of waterbone diseases.

National parks and marine protected areas are keystone elements of the 21st Century conservation policy in Gabon, expected to contribute to the forecasted sustainable development of the country (Goussard and Ducrocq, 2017). On a global scale, Marine Protected Areas represent a significant hope for reducing human pressures on the oceans and making their use sustainable (Duarte et al., 2020). Nevertheless, conservation objectives and preservation of artisanal and patrimonial activities are weakened in the case of the *Arc d'Émeraude* area, mostly through the increasing demographic pressure which largely exceeds land use planning, resulting in solid and liquid waste spreading to the coastal ecosystem. Uncontrolled peri-urban extension around Libreville directly threatens conservation purposes, reducing the buffer zone and putting in direct contact the suburbs and the protected areas (Goussart and Ducrocq, 2017). Waste management should be raised as a priority objective for stakeholders in order to reduce pollution pressure, with foremost benefits for both human and environmental health. In such a context, long-term monitoring of waste indicator microorganisms will contribute to increasing the safety of citizens and allow managers to assess the effectiveness of mitigation and regulation layouts in a context of urban sanitation (Wu et al., 2011; Lüthi et al., 2020). FIB determination can be an appropriate measure of global pollution in marine coastal environments (Lyimo, 2008), accounting for the multiple dissolved and particulate contaminants carried together.

Acknowledgements

This work is a contribution by the WP.2 "Characterization of pollution and analysis of their impacts and risks for biodiversity and public health in the *Arc d'Émeraude*" funded by AFD (French Agency for Development) under the authority of the Gabonese National Agency for National Parks (ANPN). Authors wish to thank the ENEF (Gabonese National School for Water and Forestry), ANPN staff and Dr. Magloir Désiré Mounganga (ANPN / CENAREST) for field sampling support. Research was approved by the CENAREST, Republic of Gabon (agreement AR0010/17). Funding agencies and local authorities played no role in the design, results, analysis and publication of this study. Dr. Marc Pagano (IRD, MIO Marseilles) is warmly acknowledged for help in data analysis. Pierre Lopez (IRD, MARBEC Sète) designed and produced the graphical abstract, and English text was edited by Dr. Dominique Bairstow.

References

Barbier EB, Hacker SD, Kennedy C, Koch EW, Stier AC, Silliman BR (2011) The value of estuarine and coastal ecosystem services. *Ecol. Monogr.* 81: 169-193. https://doi.org/10.1890/10-1510.1

Bastaraud A, Cecchi P, Handschumacher P, Altmann M, Jambou R (2020) Urbanization and waterborne pathogen emergence in low-income countries: where and how to conduct surveys? *Int J Environ Res Public Health* 17: 480. doi:10.3390/ijerph17020480

Bouvy M, Arfi R, Bernard C, Carré C, Got P, Pagano M, Troussellier M (2010) Estuarine microbial community characteristics as indicators of human-induced changes (Senegal River, West Africa). *Estuar Coastal Shelf Sci* 87: 573-582. https://doi.org/10.1016/j.ecss.2010.02.015

Bouvy M, Briand E, M'Boup M, Got P, Leboulanger C, Bettarel Y, Arfi R (2008) Effects of sewage discharges on microbial components in tropical coastal waters (Senegal, West Africa). *Mar Freshwater Res* 7: 614-626. https://doi.org/10.1071/MF07244

Camberlin P, Barraud G, Bigot S, Dewitte O, Imwangana FM, Maki Mateso JC, Martiny N, Monsieurs E, Moron V, Pellarin T, Philippon N, Sahani M, Samba G (2019) Evaluation of remotely sensed rainfall products over Central Africa. *Quarterly J Royal Meteorol Soc*. 145: 2115-2138. https://doi.org/10.1002/qj.3547

Cardiec F, Bertrand S, Witt MJ, Metcalfe K, Godley BJ, McClellan C, Vilela R, Parnell RJ, Le Loc'h F (2020) "Too big to ignore": a feasibility analysis of detecting fishing events in Gabonese small-scale fisheries. *PLoS One* 15(6): e0234091 https://doi.org/10.1371/journal.pone.0234091

Condé-Lamparelli C, Pogreba-Brown K, Verhougstraete M, Zanoli Sato MI, de Castro Bruni A, Wade TJ, Eisenberg JNS (2015) Are fecal indicator bacteria appropriate measures of recreational water risks in the tropics: a cohort study of beach goers in Brazil? *Water Res* 87: 59-68 https://doi.org/10.1016/j.watres.2015.09.001

Djuikom E, Njine T, Nola M, Sikati V, Jugnia LB (2006) Microbiological water quality of the Mfoundi River watershed at Yaoundé, Cameroon, as inferred from indicator bacteria of fecal contamination. *Environ Monit Assess* 122: 171-183 https://doi.org/10.1007/s10661-005-9172-7

Duarte CM, Agusti S, Barbier E, Britten GL, Castilla JC, Gattuso JP, Fulweiler RW, Hughes TP, Knowlton N, Lovelock CE, Lotze HK, Predragovic M, Poloczanska E, Roberts C, Worm B (2020) Rebuilding marine life. *Nature* 580: 39-51 https://doi.org/10.1038/s41586-020-2146-7

European Parliament (2006) Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing. Directive 76/160/EEC. *Official Journal of the European Union* L 64/37

Frena M, Stein Santos AP, Souza MRR, Santos Carvalho SS, Madureira LAS, Alexandre MR (2019) Sterol biomarkers and fecal coliforms in a tropical estuary: seasonal distribution and sources. *Mar Poll Bull* 139: 111-116 https://doi.org/10.1016/j.marpolbul.2018.12.007

Garcia Rodrigues J, Conides A, Rivero Rodriguez S, Raicevich S, Pita P, Kleisner K, Pita C, Lopes P, Alonso Roldán V, Ramos S, Klaoudatos D, Outeiro L, Armstrong C, Teneva L, Stefanski S, Böhnke-Henrichs A, Kruse M, Lillebø A, Bennett E, Belgrano A, Murillas A, Sousa Pinto I, Burkhard B, Villasante S (2017) Marine and coastal cultural ecosystem services: knowledge gaps and research priorities. *One Ecosystem* 2: e12290 https://doi.org/10.3897/oneeco.2.e12290

Goussard JJ, Ducrocq M (2017) Facing the future: conservation as a precursor for building coastal territorial cohesion and resilience. *Aquatic Conserv: Mar Freshw Ecosyst* 27(S1): 151-161 https://doi.org/10.1002/aqc.2823

Halpern BS, Walbridge S, Selkoe KA, Kappel CV, Micheli F, D'Agrosa C, Bruno JF, Casey KS, Ebert C, Fox HE, Fujita R, Heinemann D, Lenihan HS, Madin EMP, Perry MT, Selig ER, Spalding M, Steneck R, Watson P (2008) A global map of human impact on marine ecosystems. *Science* 319: 948-952 https://doi.org/10.1126/science.1149345

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. *Paleontologica Electronica* 4: 9 pp.

Isobe KO, Tarao M, Zakaria MP, Chiem NH, Minh LY, Takada H (2002) Quantitative application of fecal sterols using gas chromatography-mass spectrometry to investigate fecal pollution in tropical waters: Western Malaysia and Mekong Delta, Vietnam. *Environ Sci Technol* 36, 4497-4507 https://doi.org/10.1021/es020556h

Khandeparker L, Chandrasekar Anil A, Naik SD, Gaonkar CC (2015) Daily variations in pathogenic bacterial populations in a monsoon influenced tropical environment. *Mar Poll Bull* 96: 337-343 https://doi.org/10.1016/j.marpolbul.2015.04.051

Koko J, Ambara JP, Ategbo S, Gahouma D (2013) Epidemiology of acute bacterial diarrhea in children in Libreville, Gabon. *Arch Pediatr* 20: 432-433 https://doi.org/10.1016/j.arcped.2013.01.017

Korajkic A, Wanjugi P, Brooks L, Cao Y, Harwood VJ (2019) Persistence and decay of fecal microbiota in aquatic habitats. *Microbiol Mol Biol Rev* 83: e00005-19 https://doi.org/10.1128/MMBR.00005-19

Krepsky N, de Andrade Lino VA, Silva dos Santos F, Naveira CAC (2021) Faecal bacteria density in tropical seawater: the Itanemas' cove case study, Angra dos Reis, Brazil. *Mar Poll Bull* 164: 112027 https://doi.org/10.1016/j.marpolbul.2021.112027

Lewis J (2016) Cleaning up muddy waters – The fight to revive Senegal's Hann Bay. *Environ Health Perspect* 124: A92-A97 https://doi.org/10.1289/ehp.124-A92

Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH, Cooke RG, Kay MC, Kidwell SM, Kirby MX, Peterson CH, Jackson JBC (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* 312: 1806-1810. doi:10.1126/science.1128035

Lüthi C, Willetts J, Hoffmann S (2020) Editorial: City-wide sanitation: the urban sustainability challenge. *Front Environ Sci* 8: https://doi.org/10.3389/fenvs.2020.585418

Lyimo TJ (2008) Microbial and nutrient pollution in the coastal bathing waters of Dar es Salaam. *Aquatic Conserv: Mar Freshwater Ecosyst* 19: S27-S37. https://doi.org/10.1002/aqc.1037

Moubélé A, Mbonda AP (2017) Les impacts environnementaux du développement urbain de Libreville. In: *Les régions littorales du Gabon*, Pottier P, Menie Ovono Z, Faure FE, Bignoumba GS eds. LETG Nantes Geolittoral & Raponda Walker Gabon, pp. 257-288 (in French)

Moynihan MA, Baker DM, Mmochi AJ (2012) Isotopic and microbial indicators of sewage pollution from Stone Town, Zanzibar, Tanzania. *Mar Poll Bull* 64: 1348-1355. https://doi.org/10.1016/j.marpolbul.2012.05.001

Muniz P, da Silva DAM, Bícego MC, Bromberg S, Pires-Vanin AMS (2015) Sewage contamination in a tropical coastal area (São Sebastião Channel, SP, Brazil). *Mar Poll Bull* 99: 292-300 https://doi.org/10.1016/j.marpolbul.2015.07.046

Padovan A, Kennedy K, Rose D, Gibb K (2020) Microbial quality of wild shellfish in a tropical estuary subject to treated effluent discharge. *Environ Res* 181:108921 https://doi.org/10.1016/j.envres.2019.108921

Rochelle-Newall E, Nguyen TMH, Le TPQ, Sengtaheuanghoung O, Ribolzi O (2016) A short review of fecal indicator bacteria in tropical aquatic ecosystems: knowledge gaps and future directions. *Frontiers Microbiol* 6: 308. https://doi.org/10.3389/fmicb.2015.00308

Simard M, Fatoyinbo L, Smetanka C, Rivera-Monroy VH, Castañeda-Moya E, Thomas N, Van der Stocken T (2019) Mangrove canopy height globally related to precipitation, temperature and cyclone frequency. *Nature Geoscience* 12: 40-45 https://doi.org/10.1038/s41561-018-0279-1

Slanetz LW, Bartley CH (1957) Numbers of enterococci in water, sewage and feces determined by the membrane filter technique with an improved medium. *J Bacteriol* 74: 591-595 https://doi.org/10.1128/JB.74.5.591-595.1957

Snoussi M, Noumi E, Lajnef R, Belila A, Yazidi N, Bakhrouf A (2011) Phenotypic characterization and enterobacteria repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) of *Aeromonas* spp. and *Vibrio* spp. strains isolated from *Sparus aurata* fish farm (Khenis, Tunisia) *African J Microbiol Res* 5: 2920-2928 https://doi.org/10.5897/AJMR11.321

Troeger C, GBD Diarrhoeal Diseases Collaborators (2017) Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Diseases Study 2015. *Lancet Infect Dis* 17: 909-948 https://doi.org/10.1016/S1473-3099(17)30276-1

Troussellier M, Got P, Bouvy M, M'Boup M, Arfi R, Lebihan F, Monfort P, Corbin D, Bernard C (2004) Water quality and health status of the Senegal River estuary. *Mar Poll Bull* 48: 852-862 https://doi.org/10.1016/j.marpolbul.2003.10.028

United Nations (2019) *World Urbanization Prospects: The 2018 Revision* (ST/ESA/SER.A/420). UN Department of Economic and Social Affairs, Population Division. New York: United Nations.

United Nations (2020) *Overall total population – World Population Prospects: The 2019 Revision*. United Nations Department of Economic and Social Affairs, Population Division. New York: United Nations.

Vukić Lušić D, Lušić D, Pešut D, Mićović V, Glad M, Bilajac L, Peršić V (2013) Evaluation of equivalence between different methods for enumeration of fecal indicator bacteria before and after adoption of the new Bathing Water Directive and risk assessment of pollution. *Mar Poll Bull* 73: 252-257 https://doi.org/10.1016/j.marpolbul.2013.05.012

Walker TJ, Bachoon DS, Otero E, Ramsubhag A (2013) Detection of verotoxin producing *Escherichia coli* in marine environments of the Carribean. *Mar Poll Bull* 76: 406-410 https://doi.org/10.1016/j.marpolbul.2013.08.019

Walters G, Ngagnia Ndjabounda E, Ikabanga D, Biteau JP, Hymas O, White LJT, Ndong Obiang AM, Ndong Ondo P, Jeffery KJ, Lachenaud O, Stévart T (2016) Peri-urban conservation in the Mondah forest of Libreville, Gabon: red list assessment of endemic plant species, and avoiding protected area downsizing. *Oryx* 50: 419-430 https://doi.org/10.1017/S0030605315000204

Wiegner TN, Edens CJ, Abaya LM, Carlson KM, Lyon-Colbert A., Molloy SL (2017) Spatial and temporal pollution patterns in a tropical estuary during high and low river flow conditions. Mar Poll Bull 114: 952-961. https://doi.org/10.1016/j.marpolbul.2016.11.015

World Health Organization (2018) *WHO recommendations on scientific, analytical and epidemiological developments relevant to the parameters for bathing water quality in the Bathing Water Directive (2006/7/EC)*. 96 p. WHO, Geneva.

Wu J, Long SC, Das D, Dorner SM (2011) Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. *J Water Health* 9: 265-278 https://doi.org/10.2166/wh.2011.117