

## Spatial and temporal variation of culturable heterotrophic bacterioplankton and their response to nutrient inputs in hydroelectric reservoirs: Case of Kossou, Taabo and Faé in Côte d'Ivoire

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### Abstract

Monitoring programs of culturable heterotrophic bacterioplankton abundances in relation to nutrient concentrations was carried out in Kossou, Taabo and Faé hydroelectric reservoirs in Côte d'Ivoire, three important reservoirs for fishing and recreational activities. The study sought to assess the spatial and temporal culturable heterotrophic bacterioplankton distribution and establish the relationship between these bacterial abundances and nutrient concentrations. Water samples were collected in 8 campaigns throughout a year on linear transect of each reservoir. Culturable heterotrophic bacterioplankton were enumerated using culture methods. Nutrients such as Total nitrogen and total phosphorus were analyzed. Culturable heterotrophic bacterioplankton concentrations ranged from  $1.0 \times 10^5$  to  $1.3 \times 10^8$ ,  $1.1 \times 10^5$  to  $2.4 \times 10^8$  and  $1.8 \times 10^5$  to  $9.2 \times 10^8$  CFU.100 mL<sup>-1</sup> in the Kossou, Taabo and Faé dam lakes, respectively. Coastal areas close to urban areas and agricultural lands recorded the highest abundances, mainly during the rainy season. Significant differences were respectively observed with those recorded in the deepest areas and during the dry seasons. Generalized additive models (GAM) showed significant variations in culturable heterotrophic bacterioplankton linked to nutrient concentrations, suggesting spontaneous growth of these bacteria as nutrient levels increase in reservoirs. In addition, the culturable heterotrophic bacterial load detected is clear evidence of ongoing bacterial pollution. To limit anthropogenic activities impacts on these reservoirs, potential risk for public health, it is important to establish safety zones around them and to treat wastewater before discharging it.

**Keywords:** Anthropogenic activities; Nutrient pollutants; Culturable viable bacteria; GAM; Reservoirs quality

### 1. Introduction

Human impacts on water resources are increasing [1, 2]. These impacts from several activities, including domestic, industrial, agricultural and urban activities. Despite the numerous denunciations carried out through several scientific studies, these impacts still persist [3, 4, 5]. The problem is that the many discharges from these activities, such as organic pollutants and wastewater discharged into surface waters, contribute to water quality degradation [3, 4, 5, 6].

Wastewaters from human activities are generally composed of many nutrients, especially phosphorus and nitrogen [6], and several microorganisms, pathogenic or not, heterotrophic or not [7, 8]. Among the quoted microorganisms, the heterotrophic bacterioplankton is the most important due to his involvement in the water purification [9]. It plays a fundamental part in waters mineralization, degrading organic pollutants, intervening in particular in several reactions such as the transformation pathways of carbon, nitrogen and phosphorus cycles necessary for primary production [9].

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Organic pollutants, mainly carbon, nitrogen and phosphorus, are used for their growth. Therefore, excessive degradation of organic pollutants by heterotrophic bacterioplankton contributes to increase their abundances in aquatic environments and leads to nutrient enrichment [10]. Heterotrophic bacterioplankton are composed of numerous pathogenic bacteria [11, 12], implicated in fish infections and waterborne diseases [13]. Waterborne diseases are common, and constitutes leading causes of death worldwide [14]. Careless consumption and handling of contaminated fish and water is the most common route of human infection [15].

Kossou, Taabo and Faé dams' lakes in Côte d'Ivoire like most hydropower dams, are built to supply the economic and agriculture needs of the populations, providing drinking water and recreational activities. However, anthropogenic activities such as urbanisation, agriculture and aquaculture, strongly developed on the watersheds and inside of these reservoirs, pose a threat [16, 17, 18]. These reservoirs, connected to the rivers or their tributaries, could receive industrial, agricultural and domestic wastewater containing pathogenic microorganisms, organic matter, nitrogen, phosphorus and other heavy metals. For instance, aquaculture activities generate large quantities of nutrients which, once released into the water, disrupt the functioning of aquatic ecosystems. The numerous inputs from human activities that are constantly developing around these reservoirs could lead to infection, disease and death for aquatic organisms and humans alike. Some recent studies on microbiological quality showed faecal pollution in these reservoirs' waters [19]. These lakes could therefore receive large amounts of organic matter, which is the source for the development of heterotrophic bacterioplankton [10]. Furthermore, no similar study has been carried out in these reservoirs before. This study will therefore also provide information on the current microbiological state of these reservoirs since their creation. It would therefore be important to monitor the concentrations of cultivable heterotrophic bacterioplankton in response to nutrient inputs to these reservoirs.

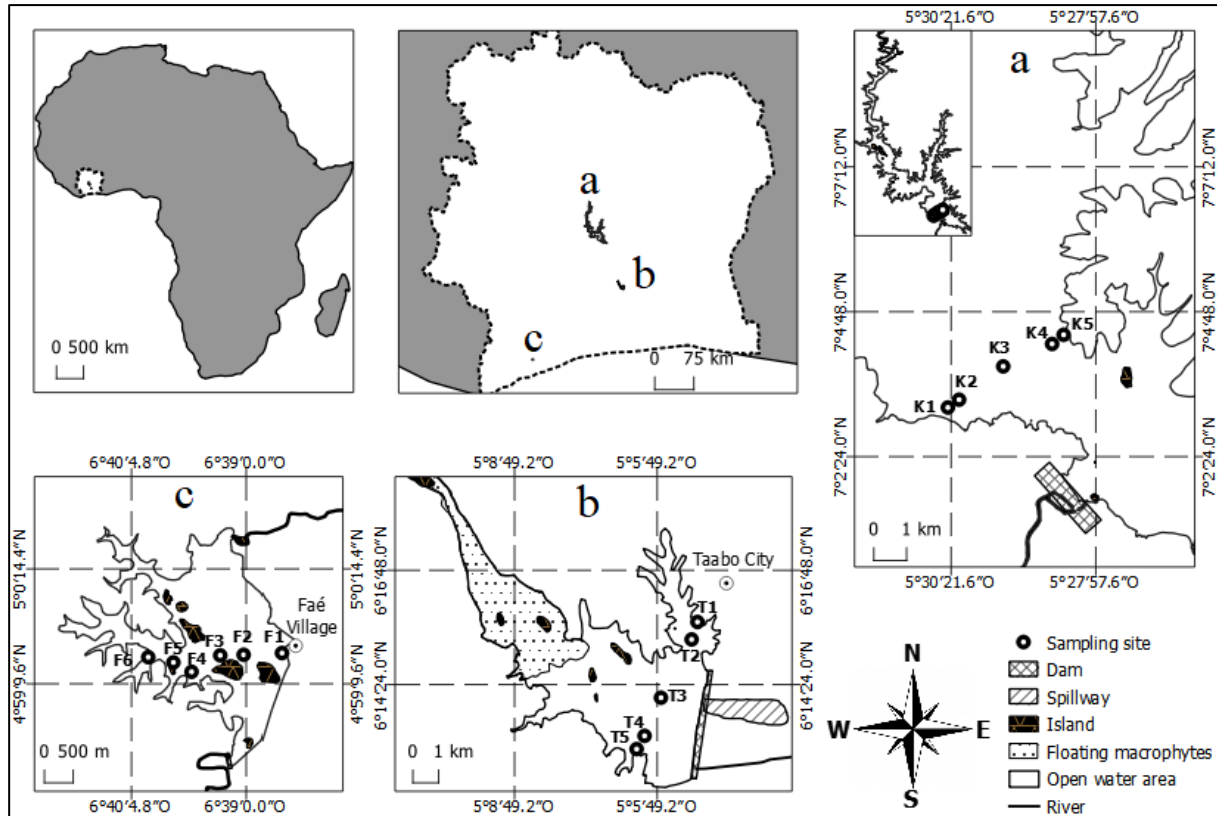
Apart from that standard plate count of culturable heterotrophic bacterioplankton can be used to determine the bacterial pollution load in reservoirs [20]. The culturable heterotrophic bacterioplankton is the viable heterotrophic bacterioplankton proportion obtained on agar. Although these bacteria represent a small proportion of total heterotrophic bacteria, around 1%, this study will enable it possible to assess the proliferation of this culturable viable proportion in these nutrient-linked waters, which remains little studied. The study sought to (i) assess the spatial and temporal culturable heterotrophic bacterioplankton distribution and (ii) establish the relationship between these bacterial abundances and nutrient concentrations (Total nitrogen and total phosphorus) using Generalized Additive Models (GAM) to evaluate nutrient influences on culturable heterotrophic bacterioplankton abundances level.

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## 2. Material and methods

### 2.1. Study area and sampling

The study area comprises Kossou, Taabo and Faé hydroelectric reservoirs in Côte d'Ivoire (Figure 1). These reservoirs were described by Tiémoko *et al.* [19]. Kossou, Taabo and Faé reservoirs cover respectively an area of 1700 km<sup>2</sup>, 62 km<sup>2</sup> and 16.28 km<sup>2</sup> with watershed areas of 58700 km<sup>2</sup>, 32400 km<sup>2</sup> and 2424 km<sup>2</sup> [16, 17, 21, 22]. The watersheds are mostly made up of urban, livestock and agricultural areas. The water sampling were carried out over a period of 1 year throughout 8 campaigns, from November 2017 to October 2018: November (N), January (J), February (F) covering dry periods, and April (A), June (J), July (J), September (S), October (O) covering rainy periods for the temporal study. 16 sampling stations were selected along the transects of dam lakes, in particular 5 sampling stations in Kossou (K1, K2, K3, K4, K5) and Taabo (T1, T2, T3, T4, T5) dam lakes and 6 in Fae dam lake (F1, F2, F3, F4, F5, F6) to compare shoreline and depth areas (Figure 1). Activities around sampling points were described by Tiémoko *et al.* [19]. At each sampling site, 1 L sterile bottles were filled with water by submerging the bottles completely in the lakes. A total of 256 samples was collected, mainly 128 samples for microbiological analysis and 128 samples for nutrients analysis. The bottles were labelled and transported to the laboratory on ice in a cool box (4°C) for microbiological analysis within a maximum of 8 hours after sampling.



**Figure 1** Location of reservoirs with sampling stations. a: Lake Kossou; b: Lake Taabo; c: Lake Faé (modified from Tiémoko *et al.* [19])

## 2.2. Nutrient concentrations analysis

Total nitrogen (TN) and total phosphorus (TP) were analyzed according to ISO 20236 (2018) and ISO 15681-2 (2003) respectively. The TN was determined using a multi N/C 3100 analyzer (CLD, Analytik Jena, Germany) with multiWin control and evaluation software. TP was determined using a spectrophotometer AL800.

## 2.3. Culture based methods for heterotrophic bacterioplankton

Following ISO 7218 (2007), the culturable heterotrophic bacterioplankton was enumerated in the water samples, using the Plate Count Agar (PCA) (Difco™ : 5350859, France). Water sample suspensions were serially diluted from  $10^{-1}$  to  $10^{-7}$  in ultrapure water by adding 1 mL of sample to 9 mL of ultrapure water, and 100  $\mu$ L of all dilutions were spread on PCA previously prepared according to the manufacturer's instructions, poured into Petri dishes, and dried. The plates were incubated at 37 °C for 24 hours. After incubation, plate counts were expressed as Colony Forming Units per 100 milliliters (CFU.100 mL<sup>-1</sup>).

## 2.4. Comparison test and representation of culturable heterotrophic bacterioplankton box plot and line plot

Statistical tests were required to analyse the data on the culturable heterotrophic bacterioplankton distribution at the sampling stations. This includes the Shapiro-Wilk test, followed by Kruskal-Wallis and U Mann-Whitney tests. The significance level is 0.05. RStudio 4.1.2 software was used to perform these tests. Culturable heterotrophic bacterioplankton box plot and line plot were carried out using also RStudio software. The ggplot2 and scales packages were used for these box plot implementations.

## 2.5. Model to study the relationship between concentrations

The Generalized Additive Model (GAM) in RStudio software was used to study the relationship between concentrations of culturable heterotrophic bacterioplankton and nutrients recorded. GAM consists in optimizing the quality of the forecast of culturable heterotrophic bacterioplankton abundances from various distributions, by estimating non-specific (non-parametric) functions of the predictor variables (TN and TP) that are related to the culturable heterotrophic bacterioplankton abundances by a binding function [23]. The plotmo and mgcv packages were used for the GAM implementation.

### 3. Results

#### 3.1. Nutrients variation in dam lakes

Nutrients level analyzed are summarised in Table 1. Nutrient concentrations were relatively close in all reservoirs. Total nitrogen (TN) median concentrations were 0.669 mg/L, 0.624 mg/L and 0.748 mg/L in Kossou, Taabo and Faé dam lakes respectively. These median values were inferior to DCE (Directive Cadre Européenne) and WHO (World Health Organization) standard of 1 mg/L (acceptable level for good aquatic life and diversity). As far as total phosphorus (TP), median concentrations recorded in the reservoirs (0.241 mg/L, 0.230 mg/L and 0.223 mg/L in Kossou, Taabo and Faé dam lakes respectively) were above the DCE's and WHO's standard (> 0.1 mg/L).

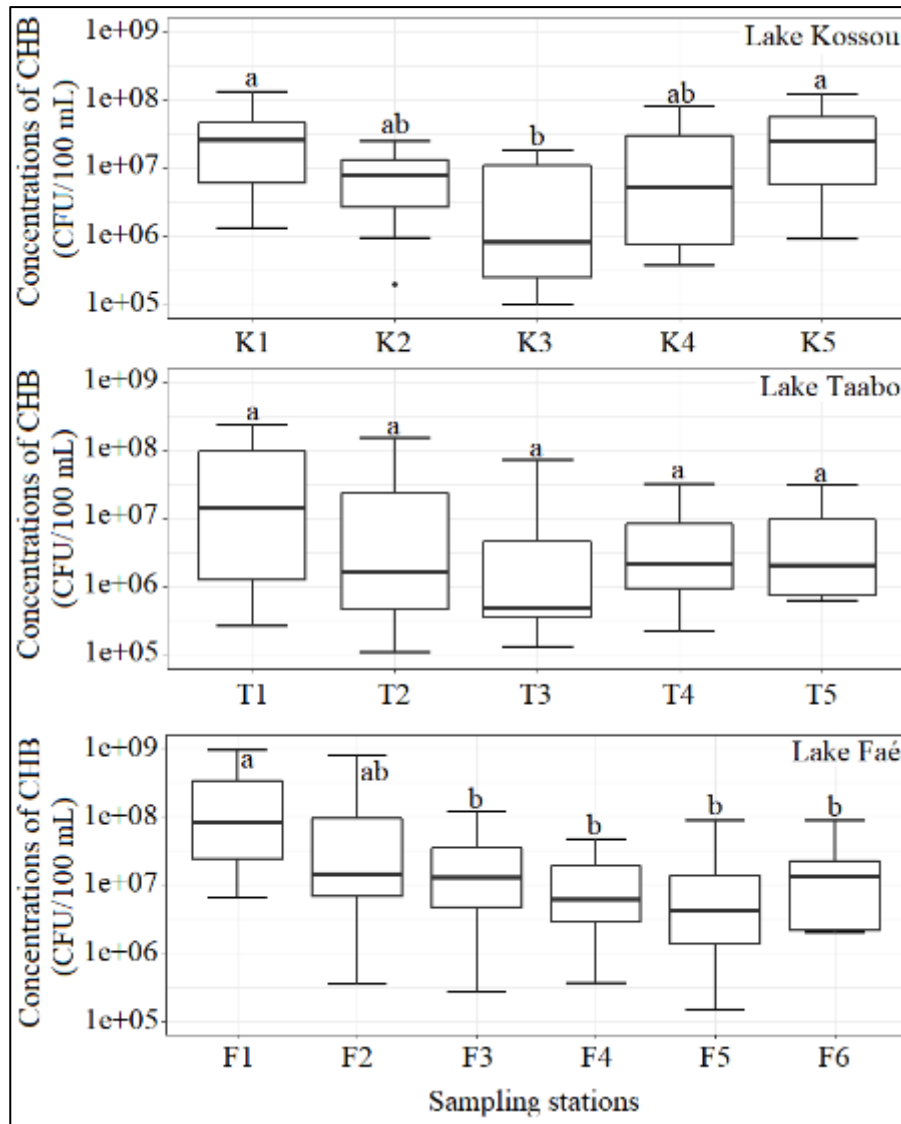
**Table 1** Summary of nutrient concentrations measured in dam lakes

Dam lakes	TN (mg/L)	TP (mg/L)
Kossou	0.669 (0.333-0.950)	0.241 (0.055-1.818)
Taabo	0.624 (nd-1.542)	0.230 (0.035-0.387)
Faé	0.748 (nd-1.967)	0.223 (0.032-0.490)

<sup>a</sup>Concentrations are expressed as median (minimum-maximum) values observed during the sampling campaigns; nd indicate nutrients values inferior to detection limit of 5µg/L; TN: Total nitrogen; TP: Total phosphorus

#### 3.2. Spatial variation of heterotrophic bacterioplankton abundances

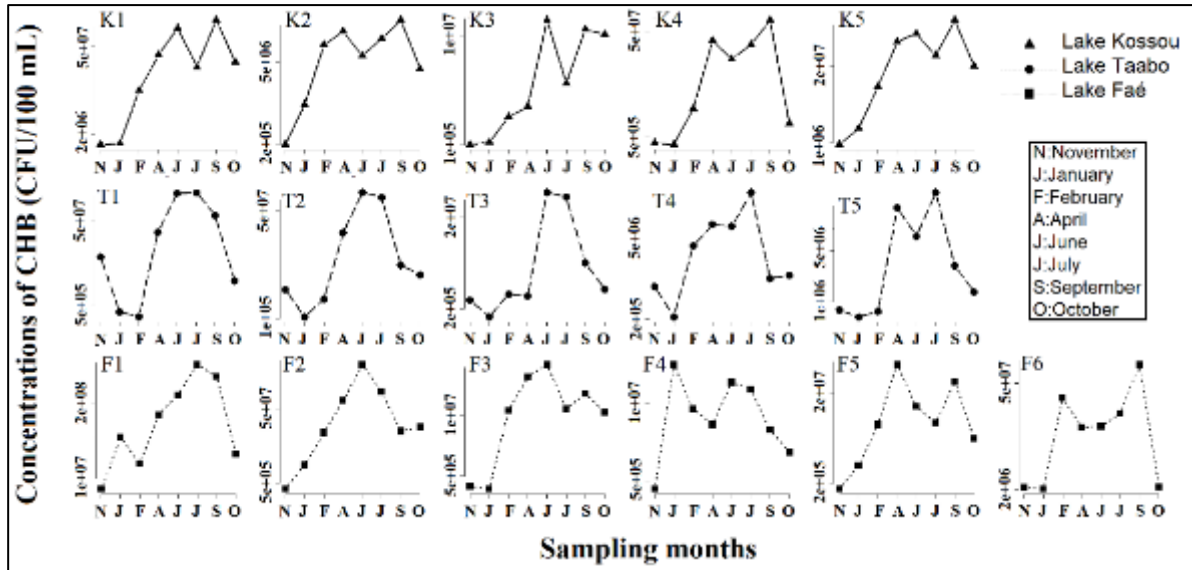
The spatial variation of heterotrophic bacterioplankton was observed along transects of the reservoirs to compare the littoral zone to the deep zone (Figure 2). Low median abundances were recorded in the deepest area of the Kossou and Taabo dam lakes at K3 of  $9.0 \times 10^5$  CFU.100 mL<sup>-1</sup> and T3 of  $4.8 \times 10^5$  CFU.100 mL<sup>-1</sup> respectively. In Faé's reservoir, a low median abundance of  $4.2 \times 10^6$  CFU.100 mL<sup>-1</sup> was recorded at F5. High median abundances were all reported at sampling stations near the shore areas, namely K1 ( $2.54 \times 10^7$  CFU.100 mL<sup>-1</sup>), T1 ( $1.77 \times 10^7$  CFU.100 mL<sup>-1</sup>) and F1 ( $8.75 \times 10^7$  CFU.100 mL<sup>-1</sup>) in Kossou, Taabo and Faé dam's Lakes respectively. Furthermore, the Mann-Whitney test showed significant differences between heterotrophic bacterioplankton concentrations recorded in some areas of Lake Kossou ( $p_{K1-K3} = 0.01476$ ;  $p_{K3-K5} = 0.02067$ ) and Lake Faé ( $p_{F1-F4} = 0.01041$ ;  $p_{F1-F5} = 0.01041$ ;  $p_{F1-F6} = 0.02813$ ). No significant variation was observed for the Taabo hydroelectric reservoir (Kruskal-Wallis;  $p = 0.6347$ ). Compared to Faé's, Taabo and Kossou reservoirs recorded lowest bacteria concentrations, with significant variation observed between Taabo and Faé reservoirs (Mann-Whitney :  $p_{Taabo-Faé} = 0.00861$ ).



**Figure 2** Culturable heterotrophic bacterioplankton (CHB) abundances determined in hydroelectric reservoirs. Median (horizontal line in box), lower and upper quartiles (lower and upper box lines) are shown. Boxplot having different letters in exponent differ significantly (Mann-Whitney;  $p < 0.05$ )

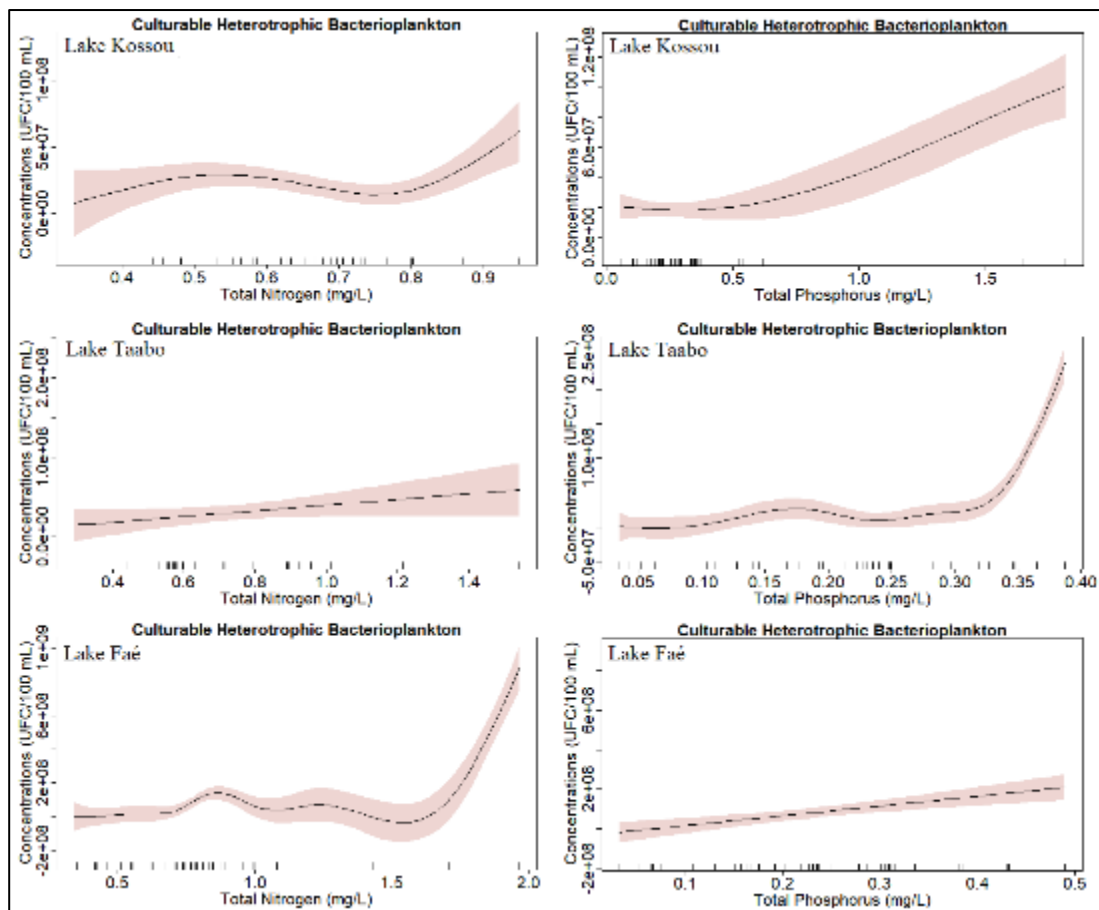
### 3.3. Temporal variation of heterotrophic bacterioplankton abundances

Temporal monitoring of culturable heterotrophic bacterioplankton indicates that the majority of lowest abundances in all reservoirs were recorded in November, January and February, during dry period (Figure 3). The higher concentrations of culturable heterotrophic bacterioplankton are obtained in April, June, July, September and October, so during rainy period.



**Figure 3** Temporal variation of culturable heterotrophic bacterioplankton (CHB) in dam lakes

### 3.4. Influences of nutrients on culturable heterotrophic bacterioplankton abundance



**Figure 4** GAM's response curve relating culturable heterotrophic bacterioplankton concentrations to nitrogen and phosphorus concentrations obtained in hydroelectric reservoirs. Values on the horizontal axis represent culturable heterotrophic bacterioplankton concentrations, pink bands represent 95% confidence intervals

The relationship between culturable heterotrophic bacterioplankton abundance and nutrient concentrations was performed to assess the nutrient pressure level on culturable heterotrophic bacterioplankton abundance. Overall,

significant positive correlations (Spearman:  $p < 0.05$ ) were observed between nutrient concentrations (TN and TP) and culturable heterotrophic bacterioplankton concentrations, except TN in Taabo and Kossou dam lakes (Table 2). Generalized additive models (GAM) show that culturable heterotrophic bacterioplankton increase with increasing nutrients (TN and TP) in all reservoirs, suggesting spontaneous growth of heterotrophic bacterioplankton as nutrient levels increase (Figure 4).

**Table 2** Total Nitrogen (TN) and Total Phosphorus (TP) used in GAMs to appreciate the culturable heterotrophic bacterioplankton concentrations (CHB) level in the Taabo, Kossou and Faé dam lakes

		TN			TP		
		r	p-value	Deviance explained %	r	p-value	Deviance explained %
CHB in dam lakes	Kossou	0.0829	0.335	15.7	0.259	0.0029**	29.4
	Taabo	-0.0072	0.385	2.11	0.672	5.0e-08***	72.6
	Faé	0.46	1.0e-04***	53.8	0.071	0.0394*	9.08

<sup>b</sup>\* Significantly low prediction:  $p < 0.05$ ; \*\* Significantly average prediction:  $p < 0.01$ ; \*\*\* Significantly high prediction:  $p < 0.001$ ; TN: Total nitrogen; TP: Total phosphorus

#### 4. Discussion

Nutrient concentrations were relatively close in all reservoirs, and particularly the TP level recorded represented a threat to the trophic status of the water. The observed nutrient concentrations could be related to wastewater resulting residents around lakes, nearby towns and run-off from cocoa, hevea and coffee crops during rainy events [19]. These observations corroborate those of Farhadinejad *et al.* [24], Yan *et al.* [25] and Atherholt *et al.* [26] showing that high TN and TP concentrations are linked to runoff from agriculture fertilized croplands, animal manure discharges, leakage from septic tanks, domestic sewage discharges. Our results are relatively close to those of Kiran *et al.* [27], who reported high levels of total nitrogen (1.3 - 4.03 mg/L) and total phosphorus (0.07 - 0.14 mg/L) into Taihu Lake in China, influenced by urban, industrial, agricultural and village activities generating significant quantity of wastewater. Control factors such as the reduction of phosphorus and nitrogen anthropogenic inputs, the phosphorus and nitrogen adaptation discharge points of urban or industrial origin, the improvement of the physical quality of the environment and the improvement hydrological conditions should make it possible to reduce the phosphorus and nitrogen concentrations in reservoirs.

Heterotrophic bacterioplankton high concentrations observed in the shore area could be explained by the possibility that the reservoirs receive wastewaters from the numerous anthropogenic activities observed in the shore areas [19]. Indeed, the K5 station of Kossou's dam lake was characterized by lakeside residents and livestock farming. The T1 station of Taabo's dam lake receives effluent from Taabo City and wastes way out from livestock farming and agricultural activities not far from waterbody. The F1 station of Faé's dam lake was located near a large rural agglomerations (characterized by high population density) very close to the lake. According to Farhadinejad *et al.* [24], Yan *et al.* [25] and Atherholt *et al.* [26], domestic wastewater and livestock effluents may contain significant bacteria and organic pollutants. Organic pollutants in water serve as nutrient sources for the proliferation of heterotrophic bacterioplankton thriving [10]. Rossi [28] showed high bacterioplankton in an area affected by human activities. Comparatively, our results were closed to those of Bharathi *et al.* [8] who reported culturable heterotrophic bacterioplankton average abundances of  $2.1 \times 10^8$  CFU.100 mL<sup>-1</sup> into Ennore shore areas in India. On the other hand, these values remain relatively inferior to those of Santé Canada [29], suggesting that polluted water may contain more than  $10^9$  CFU/100 mL of culturable heterotrophic bacteria. However, hydrochemical parameters could also be responsible for these variations [30]. Taabo and Kossou dam lakes recorded lowest bacteria concentrations. Indeed, the watersheds of the Taabo and Kossou dam lakes were characterized by cities and villages not far from the water body compared to the Faé dam lake, whose riparian populations are located close to the water body. Depending on the distance between pollution sources and water bodies, some of the releases could be retained in the ground to seep and/or evaporate, whereas another part could be discharged into the lakes.

Highest culturable heterotrophic bacterioplankton abundances were recorded during rainy period. This could be related to the higher anthropogenic inputs during this period than dry period. TN and TP concentrations were higher during rainy period than dry period. Surface waters are generally less disturbed during the dry period and receive fewer pollutants (microorganisms and organics) [10, 28]. Heterotrophic bacteria loads are supplemented by other bacteria

from the external environment in the run-off water [8, 10]. [12] observed also culturable heterotrophic bacterioplankton high growth during rainy period. Furthermore, studies areas are characterized by strong slants. According to [31], anthropogenic activities contributions are more accentuated in areas marked by a high slant, due to runoff rate elevated bringing quickly many waste through the water bodies.

Generalized Additive Models (GAM) show that culturable heterotrophic bacterioplankton increase with nutrients (TN and TP) in all reservoirs. These results highlight the nutrients important role in bacterial growth. Bharathi *et al.* [8] also found significant positive correlation between heterotrophic bacterioplankton communities and nutrient (TN and TP) concentrations in their studies, suggesting that high nutrients load favour bacterial community increase. Similarly, Fisher *et al.* [32] showed that nitrogen and phosphorus were responsible for the variation in bacterial community composition. The heterotrophic bacterioplankton, through the mechanism of anabolism, uses nitrogen and phosphorus for synthesis of ribosomes, reserve substances, pigments, gas vacuoles, membrane proteins, nucleic acid (ADN, ARN) in favourable environmental conditions for cell division. The phosphorus is used for the enzymes, coenzymes, phosphoproteins and phospholipids production, fundamental in the formation of membranes [10, 33]. According to Tiémoko *et al.* [19], environmental conditions (Temperature, pH, O<sub>2</sub>) were favourable for the heterotrophic bacterioplankton.

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## 5. Conclusion

Study results showed that dam lakes receive significant nitrogen and phosphorus amounts. Culturable heterotrophic bacterioplankton abundances were higher in shore areas during rainy seasons. Relationships observed with GAM reveal culturable heterotrophic bacterioplankton significant variations due to nutrients, suggesting spontaneous growth of heterotrophic bacterioplankton as nutrient levels increase. This study therefore denounces an uncontrolled bacterial growth in these waters, especially in areas close to human activities. Cultivable heterotrophic bacterioplankton concentrations raise concerns about health risks. It is therefore important to limit anthropogenic activities impact by creating security spaces around reservoirs, treat wastewaters before dumped in surface waters and to analysis the pathogenic bacteria presence in the dam lakes to prevent public health problems.

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## 6. Compliance with ethical standards

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### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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