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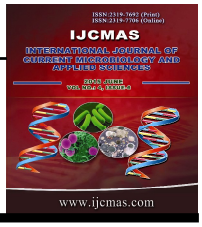

Original Research Article

Abundance Dynamic of *Vibrio* Cells Associated with Freshwater Shrimps Atyidae (Crustacea-Decapoda) in the Coastal Surface Waters of Cameroon (Central Africa): Assessment of the Role of some Environmental Factors

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Biomonitoring, *Vibrio*, Abundance, Atyidae, Coastal water, Abiotic factors

A B S T R A C T

The abundance dynamics of planktonic *Vibrio* cells and those associated with the freshwater shrimps Atyidae were assessed in the coastal surface waters of Cameroon (Central Africa). Two pathogenic species *V. parahaemolyticus* and *V. alginolyticus* were identified. The concentration of *Vibrio* cells associated to shrimps was relatively high, as compared to the planktonic cells. This concentration was very high during the great rainy season. The concentration of *Vibrio* associated with Atyidae undergoes variations under the effect of the proliferation of shrimps and influenced by color and rainfall. The physico-chemical analyses showed a strong organic pollution. Low nutrient levels suggested high water column turnover rate, which *Vibrio* compensated for by using organic matters leaking from shrimp. Stepwise regression analyses showed that the concentration of plaktonic *Vibrio* cells increase significantly in response to changes to dissolved oxygen and organic matter accumulated in the water column due to anthropogenic activities. *Vibrio* associated with shrimp *Atyidae* in surface waters are a real risk of public health.

Introduction

Fishing is a cultural and economical activity to the coastal people who benefit from the fishing and which is a significant source of animal proteins (Kébé *et al.*, 1993). However, the exploitation of shrimps depends essentially upon capture of wild

species with low economical income. According to the recent report of FAO-Globefish, the production of farmed shrimps in the world has remained below the expected level during the first quarter of 2014. Nevertheless, there are many families' shrimps (Atyidae, Alpheidae, Desmocarididae and Palaemonidae) that are found in various water bodies less subjected to anthropogenic disturbances (Gloria *et al.*, 2010; Tchakonté *et al.,* 2014a).

According to recent research, the proliferation of the shrimps Atyidae in Cameroonian rivers (Central Africa) is associated to the higher concentration of waters in dissolved oxygen (Foto *et al.*, 2012; Tchakonté *et al.,* 2014a). The steady drop in shrimp's production would thus be the direct consequence of the human disturbances on aquatic ecosystems. These activities include poaching, heavy fishing, degradation of water quality and habitat.

However, among fishery products, shrimps are particularly identified on the list of food associated with outbreaks of waterborne bacterial diseases (Messelhäusser *et al.*, 2010, Ebrahimzadeh *et al.*, 2011; Khamesipour *et al.*, 2014) and hence constitute a serious threat to public health (Colakoglu *et al.*, 2006). In addition, the genus *Vibrio* from the family *Vibrio*naceae is the most abundant bacteria from the shrimps of the east and west coasts of India, consisting essentially of *V. parahaemolyticus*. *Vibrio* species, particularly, *V. fischeri, V. harveyi, V. cholera, V. parahaemolyticus, V. vulnificus, V.alginolyticus, V. damsela, V. penaeicida and V. nigripulchritudo* are constantly isolated from shrimp (Alday-Sanz *et al.*, 2002; Xie *et al.,* 2005). The prevalence of *V. parahaemolyticus* in shrimp samples was 20% in similar studies using traditional bacterial culture by Reham and Amani (2012) in various estuarine areas of Egypt. Prakash and Karmagan (2013) in India have isolated *E. coli*, *Pseudomonas* sp., *Enterobacter* sp, *Vibrio* sp and *Aeromonas* sp from giant freshwater prawn *Macrobrachium rosenbergii*. Some of the *Vibrio* species are clearly described today as responsible for mass mortality of shrimps (Goulletquer *et al.,* 1998; Cheney *et al.,* 2000; Soletchnik *et al.,* 2005).

Shrimps and coastal waters constitute a reservoir of pathogenic *Vibrio*s according to previous studies (Xie *et al.,* 2005; Lutz *et al.,* 2013). Recent researches have shown that the contagious status of fishery fruits after their capture is closely related to environmental conditions and microbiological quality of the water (Khamesipour *et al.,* 2014). Most of the bacterial pathogens therefore reach humans at the conclusion of a complex cycle involving other hosts, vertebrate and / or invertebrate (Amblard *et al.,* 1998). However, the beginning of a bacterial disease is conditioned by complex phenomena of interaction between the different risk factors of the environment of the host and the pathogen.

The coastal ecosystems play an important role in the receptor of mainland materials containing organic matter and nutrients. The tributaries of Cameroon's coastal rivers face a real danger of flooding and play the role of first receptacle of a high proportion of urban pollution. The association of bacteria on the surface of aquatic organisms has been studied for decades. To survive and grow in the highly variable conditions of coastal systems (tides, freshwater inputs, turbidity, currents, anthropogenic pressure), bacteria possess remarkable capacities of adaptation from their metabolic functions (Thompson *et al.*, 2003). The studies by Heidelberg *et al.* (2002), showed that *Vibrio* sp competitively dominates the zooplankton habitat because of their chitinous

exoskeleton. They are able to change their physiology to attach to chitin of some arthropods and to use it as source of carbon and nitrogen (Huq *et al.,* 1983; Montanari *et al.,* 1999).

The concentration of *Vibrio* cells in the shrimps can reach 10^4 CFU/g (Gopal *et al.,* 2005). At the quantitative level, the concentration of bacteria attached to substrates fluctuated between $6x10^6$ and $2x10^8$ cells per cm^2 against only $2.4x10^5$ to $1.6x10^6$ cells bacterioplanktonic per ml in the rivers water column (Lock *et al.,* 1984). Huq *et al.* (1983) showed that the number of bacteria associated with zooplankton is higher compared to those living in the water column. It is known that bacterial dynamic is controlled by different environmental parameters of water, such as grazing pressure (Jürgens *et al.,* 2000), temperature, salinity (Thompson *et al.,* 2004; Castaneda *et al.,* 2005; Turner *et al.,* 2009) and hydrological parameters (Casotti *et al.,* 2000). Whereas, these factors are in turn very variable over the time and space.

From the recent research report in microbial ecology focus upon the comparison of relationship between bacterial concentration and environmental parameters likely to trigger an epidemic (Ducklow *et al.,* 2001; Kirchman *et al.,* 2004; La Ferla *et al.,* 2005). But, few studies have been carried out on the influence of the environmental factors on the evolution of the bacterial cells adhered to their host: the shrimp Atyidae. This study is the first to be carried out in the coastal area of Cameroon.

It aims to describe the influence of environmental parameters on the dynamic of planktonic cells of *Vibrio* and those associated with the freshwater shrimps and Atyidae abundance in coastal surface waters of the two coastal cities in Cameroon.

Materials and Methods

Study area and sampling sites

The Cameroonian coast is one of the central sectors of the Guinea Gulf. Littoral region is located between in the southern part of the Cameroon coastal plain between 3º30' and 3º58' of North latitude and 11º20' and 11º40' of East longitude. The climate is tropical type, characterized by a short dry season (December-February) and a long rainy season (March-November) (Suchel, 1988). Peak rainfalls occur within the months of June and September with an average annual precipitation of 4000 mm per year. The air temperature is relatively high with a monthly average of approximately 28◦C (Suchel, 1972). The soils have an acidic pH (Asaah *et al.,* 2006). The main rivers of the region (Wouri, Sanaga, Dibamba) are used mainly for hydro energy power generation, fishing and transportation of goods and people. Salts and pollutants are transported from either side during tides. The deforestation, each successive installation and operation of the hydroelectric dam conditions have a strong anthropogenic pressure on the river from the town of Edea (Dubreuil *et al.,* 1975).

The study sites choose were representative of two coastal towns: Edea and Douala (Figure 1). During this study, 16 sites were sampled in the hydrographic area of the littoral region of Cameroon. Sampling sites S1, S2, B1, B2, B3, M1, M2 and M3 were selected in the Sanaga hydrographic area related to Edea, and TW, WO, MP, MG, MB, TB, LM and KO were selected in the Wouri hydrographic area related to Douala. Sites were selected to represent a range of environmental and hydrological conditions including tidal creeks and open-water sounds. Each site was part of a network of shrimps open for commercial or public harvest.

The points S1 and S2 are located along the banks of the river of Sanaga, while the points TW and WO are located on the river Wouri. The 16 stations were divided equally between Edea and Douala and were sampled monthly from March 2013 to March 2014.

Water sampling

At each site, water sample was collected in a 500 mL sterile glass bottle labeled A, and in a 1000 mL clean polyethylene bottle labeled B. Both samples were transported to the laboratory in a cooler with icepacks (6±2 °C) for further analyses. The sample in bottle A and that in the polyethylene bottle B were for the assessment of the planktonic *Vibrio* cells and for some physico-chemical analyses, respectively.

Shrimps sampling

At each site, living shrimps sample was collected into a sterile stomacher sachet then labeled. Samples were transported to the laboratory in a cooler with icepacks (6±2°C) for further analyses *Vibrio* associated cells. Shrimps Atyidae were collected using a long-handled kick net (30x30 cm side, 400 µm mesh size). Samples were collected in a 100 m stretch for each station, following protocols described by Stark *et al.* (2001). Materials collected in the sampling net were rinsed through a 400 µm sieve and all shrimp individuals were sorted and placed in plastics sampling bottles with 70° ethanol.

Identification of freshwater shrimps

In the laboratory, all the shrimps were handpicked using a fine dissection forceps and sorted into Petri dishes. All specimens caught were identified under a stereomicroscope using taxonomic keys (Monod, 1980; Day *et al.,* 2001), and then counted.

Measurement of abiotic parameters

At each sampling station, the water temperature (WT)(°C), pH (UC) and dissolved oxygen (DO) rate (%), sand electrical conductivity (CND) (µS/cm), salinity (‰) and Total Dissolved Solids (TDS) (mg/L) were measured in situ using a HACH HQ 14d TDS-conductimeter.

At the laboratory, the physic-chemical parameters considered were Suspended Solids (SS) (mg/L), turbidity (TURB) (mg/L) water color (Pt-Co), dissolved carbon dioxide (DCO2) (mg/L) and organic matters (OXY) (mg/L). All these physiochemical parameters were measured using standard methods (APHA, 1998; Rodier *et al.,* 2009). Monthly rainfall (mm) and air temperature (AT) (°C) data for the study period were collected at the Douala Airport Meteorological Research Station.

Bacteriological analysis

Bacteriological analysis focused on planktonic and associated *Vibrio* cells with Atyidae. The samples were processed immediately upon arrival by aseptic methods. All the specimens were rinsed with sterile water to remove the adhering particles. Each sample site was weighed in g, and then an initial suspension is obtained by grinding in a mortar of the mass weighed into a volume of diluting. Initial suspension was homogenized at centrifuge 8000 tr/min and serially diluted in phosphate-buffered saline. At least 2 dilutions were plated to ensure that the plates were countable.

Thus, 0.1 mL of suspension obtained was immediately taken by a pipette and seeded onto plates of Thiosulfate Citrate Bile Salts (TCBS; BioMerieux) and then incubated at 37°C for 24h. All colonies were counted as presumptive *Vibrio*, and reported as colony-forming units (CFU) per mL of water

(planktonic *Vibrio* cells) (VP) and CFU/g for associated *Vibrio* cells with Atyidae (VA). Presumptive bacteria were enumerated on a selective culture medium. Yellow and green colonies were purified and pure cultures were used for identification at genus and species level (Maugeri *et al.*, 2004). The isolates were confirmed using the API 20E system (BioMerieux). After, tests including Gram staining, oxidase, catalase tests, and morphological observations were carried out.

Data analyses

Spearman's correlation coefficients were calculated to evaluate relationships between the *Vibrio* concentrations and environmental parameters. Mann-Whitney U-test was used to determine the significance of the differences of variances in bacteria concentrations between sampling sites and collection months. Principal Component Analysis (PCA) was used to look for relationships between environmental factors and between the latter and Atyidae shrimps during the study period. Multiple linear regression models were developed to examine relationships between the environmental variables and *Vibrio* concentrations.

Thus, planktonic and associated *Vibrio* cells considered as independent variables and abiotic factors as dependent variables. All variables found to be correlated ($P<0.05$) in univariate analyses, and their appropriate interaction terms were initially entered into the models. Maximum likelihood estimates for each variable were determined, and when $P<0.05$, variables was added to the model by forward addition (stepwise regressions). PCA and multiple linear regression models were performed using XL-STAT 2014 software version 4.5 for Windows.

Results and Discussion

Abiotic parameters

Table 1 shows the monthly variations of physico-chemical factors analyzed. Oxydability varied from 10.7±2.7mg/L (November) to 20.7±2.5mg/L (March13). Water temperature varied from 25.4±0.2°C (July) to 29.9±0.4 °C (March 13). Similar variation trend was observed for dissolved carbon dioxide, with low (14.7±2.0mg/L) and high (60.1±16.6 mg/L) values registered at July and March 13. Salinity varied from 0.05±0.01‰ (September) to 0.22±0.06 ‰ (January). It same for conductivity with varied from 178.1±30.1µS/cm at July during the rainfall to 375.0±122.7µS/cm at January during the rainy season. Nevertheless, dissolved oxygen were overall high during the rainy season with values ranging between 38.3±8.0% (February) and 72.4±9.8% (May). Similar variation trend was observed for current velocity, with low (0.22±0.03m/s) and high (0.6±0.2m/s) values registered at December and July respectively. pH varied from 6.36±0.12UC to 7.79±0.06UC. Turbidity and suspended solids were globally low during the rainfall with values ranging from 26.6±5.5 NTU (July) to 65.8±18.8NTU (March 13) and from 17.3±3.3 (July) to 61.1±18.3mg/L (February) respectively. Water color varied from 148.7±28.5 Pt-Co (July) to 414.4±181.9 Pt-Co (June).

Abundance dynamic and diversity of freshwater shrimps Atyidae

Shrimps abundance varied between 32 ind (October) and 196 ind (June). It was overall high during the rainfall period (June, July and November) with values ranging between 10% and 20% at stations situated in navigable rivers, the values ranged from 10,2% (S1) to 50,9% (WO). The Mann-

Whitney U-test showed significant difference (P=0.0001) between the two urban groups stations (TW, LM and B1, B2, B3, KO, M1, M2, M3, M4, S1, S2, WO) regarding shrimps diversity and distribution. This study achieved for the first time in coastal watershed hydrographic permitted to identify two freshwater shrimp species belonging to Atyidae family and *Caridina* genera. There are *Caridina africana* (Kingsley, 1882) and *C. nilotica* (Roux, 1833). All these species were caught only at Edea stations and the tree Douala stations (WO, LM and KO).

Abundance dynamic and diversity of *Vibrio* cells

The monthly evolution of the mean concentrations of planktonic *Vibrio* cells and those associated with freshwater shrimps (Fig. 4) varied significantly during the study period. Abundance of *Vibrio* cells associated with Atyidae varied between 10^3 and 1.6×10^5 CFU.g^{-1}. The highest abundances of associated *Vibrio* cells were observed during rainy season (from June to August). Their lowest concentrations were observed from September to March 14. Concerning planktonic *Vibrio* cells abundances ranged from 10^3 to 1.4×10^4 CFU.mL^{-1}; with larger numbers in December during the dry season. Lower numbers of planktonic *Vibrio* cells were observed the rainy season (May, June).

The monthly evolution of the mean concentrations of planktonic *Vibrio* cells and those associated with freshwater shrimps (Fig. 4) varied significantly at stations sampling (Fig. 4). The evolution of the set of points varies significantly in the study area for the two categories of bacteria studied (P<0.05). Abundance of *Vibrio* cells associated with Atyidae varied between 0 and 1.2×10^5 CFU.g^{-1}. The highest abundances of associated *Vibrio* cells were observed at Wouri station (WO). Their nil concentrations of associated *Vibrio* cells were observed at the stations that where very rich in organic matter (MB, TB, MG, MP and TW). Planktonic *Vibrio* cells abundance varied from 0.0044×10^4 (B1) to 1.4×10^4 CFU.mL^{-1} (MB), with larger concentrations at stations that were more polluted with organic matter (MB, TB, MG, TW and LM). Nevertheless, lower concentration of planktonic *Vibrio* cells were observed at Edea stations and Douala stations KO and WO.

The number of associated *Vibrio* sp isolated ranged from 15 to 40 strains in freshwater shrimps Atyidae. A higher frequency of isolation of *Vibrio* sp associated was recorded in the rainy season than in the dry season. Among these 328 strains associated *Vibrio* spp identified during the study period, *V. parahaemolyticus* (FO=37%) and *V. alginolyticus* (FO=27%) were very frequent. *V. vulnificus* (FO=13%), *V. cholerae* (FO=11%) and *V. fluvialis* (10%) were accessory, while *V. mimicus* and *Vibrio* sp were very rare. Elsewhere, the number of planktonic *Vibrio* sp. isolated ranged from 19 to 39 species. The species of *Vibrio* planktonic isolated indicates that the peak of outbreak occurred in dry season (from December to March 14) (Figure 5). By 395 strains planktonic *Vibrio* sp. identified during the study period, *V. parahaemolyticus* (FO=36%), *V. alginolyticus* (25%) were frequent. *V. fluvialis* (FO=15%) and *V. cholerae* (FO=13%) were accessory, while *V. vulinificus, V. mimicus* and *Vibrio* sp were rare (FO≤6%).

Abiotic factors affecting abundance of shrimps Atyidae in the surface coastal waters

The results of principal component analysis revealed that the relationships between the Atyidae abundance and physico-chemical

variables follow mainly the first two axes (Fig. 3), which accounted for 60.23% of the total variance. These two axes better highlight the distribution of the shrimp's assemblage in coastal zone in response to the environmental conditions. The first axis opposed the parameters which characterize organic pollution in positive coordinates to parameters that favorable to increase of freshwater shrimps Atyidae in negative coordinates. The freshwater shrimp family of the Atyidae was found to be negatively affected by high values of water temperature, salinity, conductivity, TDS, oxydability, carbon dioxid, suspended solids, color and turbidity. Furthermore, the abundance of Atyidae appears to be highly and positively influence by good oxygenation of water and high value of pH during the rainfall. The second axis (F2) opposed the air temperature in positive coordinates to rains in negative coordinates.

Relationships between *Vibrio* cells, environmental factors and abundance dynamic of Atyidae

Tables 2 and 3 show the results of multiple regression analysis between both planktonic and associated bacterial abundance (VA and VP), and abiotic factors and abundance Atyidae. The results of the multiple regression analysis indicate that a high proportion (rate R^2= 77%) of the variability of *Vibrio* abundance associated with Atyidae is explained by the variation of abiotic factors (Table 2). The abundance dynamic of *Vibrio* cells associated to Atyidae is highly and positively (P<0.001) related to the abundance of these freshwater shrimps and water color. Also, according to t-test (P<0.05), the model showed that suspended solids and rainfalls could also influence the abundance of *Vibrio* cells associated with Atyidae. Inversely, turbidity had negatively effect on the variation of the concentration of *Vibrio* cells associated with Atyidae. The oxydability, water temperature, dissolved carbon dioxid, dissolved oxygen and pH provided little additional information on the development of the concentration of *Vibrio* cells associated with shrimp.

The equations resulting by adding stepwise environmental parameters in the model are given in Table 4. The contribution of water color to the model is very significant (P <0.001) and helps to explain 67% of the variance in concentrations of associated *Vibrio* cells to Atyidae. The Atyidae abundance is also very significant (P <0.001) and helps to explain just 6% of the variance. Furthermore, suspended solids and rainfalls contributed positively (1% each) to the increase of *Vibrio* cells associated to freshwater shrimps. However, turbidity negatively contributed to 1% to the increase of these bacterial.

The model for analysis of the regression elucidated that 46% of total variation for the *Vibrio*planktonic cells could be explained by dissolved oxygen, oxydability, color, water temperature, dissolved carbon dioxide, salinity, pH and TDS. The analysis of the Spearman correlation indicated that only dissolved oxygen is negatively and significantly (P<0.01) linked to *Vibrio* planktonic cells. The variation of the concentration of planktonic *Vibrio* cells strongly and negatively (P<0.001) linked to the variation of dissolved oxygen content. Oxydability is positively and significantly related with *Vibrio*planktonic (P<0.05). However, air temperature and color had negative influence (P<0.05) on the bacteria cells. When parameters such as dissolved oxygen and air temperature were added to the regression model, the output could just explain only 20 % of total variation of planktonic *Vibrio* cells (Table 3). Regression equation obtained so what: VB =

13055-366.5DO-363.8AT ($P<0.01$). Adding to the oxydability variable in the model equation showed that the concentration of water in organic matter contributes positively ($P <0.001$) to 3% to the increase of planktonic *Vibrio* cells. However, color negatively contributed to 1% ($P <0.041$) to the increase of these bacterial.

Abundance dynamic of Atyidae and environmental parameters

The first scatter plots on the axis F1 (PCA), is essentially constituted of physico-chemical factors of the pollution could reflect the anthropogenic character of coastal surface water of Cameroon. Our findings corroborate the works on surface water in Cameroon (Tchakonté *et al.,* 2014b), in Africa (Kunwar *et al.,* 2005; Omo-Irabor *et al.,* 2008) and Japan (Shrestha and Kazama, 2007). Nevertheless, during the rainy season, the relative increase in water temperature was enough to trigger a succession of physico-chemical reactions in favor to the concentration of the organic matter in the water column.

However, during the rainfall, the reversible reactions were produced under the effect of water dilution. Thus, the second scatter plots on axis F1 constituted by precipitation, pH and dissolved oxygen favor of the proliferation of freshwater shrimps Atyidae. High concentrations of dissolved organic matter were leached in the rainwater (Robert and Bruce, 1992) and dissolved oxygen proved to be determinant in regulating the Atyidae abundance by others studies reported by Hunte (1978) on the streams in Jamaica, Foto *et al.* (2012) on the river Mefou in Yaoundé and Tchakonté *et al.* (2014a) on the rivers of Douala. The induced effect of the overloading of water in organic matter and depletion of oxygen had caused mortality of shrimp in Neuse River Estuary, North Carolina, USA (Hans *et al.,* 1998).

Atyidae abundance and environmental parameters in the bacterial flux

In examining the multiple regression models (Tables 2 and 3) and simple correlations, it appears from this study that the proliferation of shrimp by it alone contributes significantly to a share of 67% in the changing of the concentration of *Vibrio* associated with Atyidae. It is evident that the development of shrimps in surface water increases the survival of *Vibrio* when the environmental conditions are becoming difficult for a certain period of the year. In this idea, several species of *Vibrio* would attached on the chitinous exoskeleton of crustaceans in order to bring in the carbon and nitrogen to survive in an environment that is becoming poor in nutrients (Montanari *et al.,* 1999; Vezzulli *et al.,* 2008).

The concentration of *V. parahaemolyticus* was higher in shrimp samples than in water samples in the recent work reported by Pilakka and Ranjeet (2014). *Vibrio*s associated with shrimp are particularly abundant during the rainy season (1.6×10^5CFU.g^{-1}) mainly on the Sanaga and Wouri rivers (1.2×10^5 CFU.g^{-1}), dominated essentially by *V. parahaemolyticus* and *V. alginolyticus*. For comparison, the maximal concentration of *Vibrio* in fisheries products was 10^4 CFU.g^{-1} in similar studies reported by Thararat *et al.* (2009) in Thailand. Khamesipour *et al.* (2014) have isolated *V. parahaemolyticus* and *V. alginolyticus* shrimp in a site in India. According to recent research by Alagappan *et al.* (2013) and Reyhanath and Kutty (2014), *V. parahaemolyticus* is a pathogenic bacteria associated with shrimp in coastal areas and in seafood, able of provoking food poisoning on a large scale.

The *Vibrio* infection is constantly increasing in the world, though one observes intermittently periods of relative calm. This increase could be directly associated with the increase of these bacteria in seafood and coastal waters (Tantillo *et al.,* 2004). *Vibrio cholerae* possesses chitinase, a polysaccharide compound highly prevalent among *Vibrio* which gives it the ability to multiply quickly on the surfaces of shrimp chitin (Hunt *et al.,* 2008). Vincy *et al.* (2014) recently isolated chitinase molecular in *V. alginolyticus* associated with shrimp pond.

The proliferation of freshwater shrimps in Cameroonian rivers increases the risk of contamination by pathogenic bacteria through fishery products. By inserting color in the model, the equation generated shows that the increase in water color is a determining factor in the outbreak of *Vibrio* associated. However, the color of the water surface comes mainly from the chlorophyll (a), an indicator of algal biomass (Bompangue *et al.,* 2011).

It was suggested that association of *Vibrio* with phytoplankton was to overcome the unfavorable environmental condition (Asplund *et al.,* 2011). In this idea, this study strengthens the suspicion that we have the " blooms " phytoplankton as potential triggers of cholera outbreaks (Bompangue *et al.,* 2011). However, recent research carried out in mesocosm help to shown that the algal blooms is a parameter to be taken with extreme caution in the prediction of the epidemic of cholera (Rehnstam *et al.,* 2010).

The link between *Vibrio* associated and precipitations and suspended solids shows that rainfalls have the ability to generate water currents and increases the suspended solids in the water surface. Suspended solids could facilitate the formation of bacterial biofilms (Nstama *et al.,* 1997) on the shrimp shell, to protect *Vibrio* species of the environmental constraints. Several studies have shown that precipitations are important factor in the emergence of cholera. The majority outbreaks of cholera have occurred in a few hot spots of lake areas in the Greater Lakes in Africa, where the incidence varied according to seasons, rainfall and plankton fluctuations (Bompangue *et al.,* 2011).

In comparison with the first model (Table 3), the environmental parameters contribute poorly (46% of the total variance) in the second model to explain the variation of the concentration of planktonic *Vibrio* cells (Table 3). The dissolved oxygen contributes to 15% to explain this variation ($P < 0.001$). It is clear that increasing the temperature of surface water and salinity have a positive impact on the growth of planktonic *Vibrio* cells (Table 2).

Many authors have actively reported on the temperature and salinity as determining factors in the regulation of growth and survival of *Vibrio*s in surface water (Wang and Gu, 2005; Johnson *et al.,* 2012). Other authors have contributed to the elaboration of predictive model for *V. cholerae* on the basis of the variation of temperature and salinity (Louis *et al.,* 2003; Huq *et al.,* 2005). Nevertheless, *V. vulnificus* wasn't correlated with salinity in the lake (Olivia *et al.,* 2011).

Ecologically, *Vibrio* species play a very important role in breaking down organic matter mainly of anthropogenic origin in mineral elements, rejecting dissolved carbon dioxide while consuming dissolved oxygen in the water column. The addition of organic carboneous matter in the test medium caused the strongly growth of *V. cholerae* O1 up to cross the threshold of the minimal infectious dose in humans (Mouriño-Perez *et al.,* 2003).

Table.1 Mean values (± SE) of abiotic parameters evaluated during the study period

Months	Oxy (mg/L)	WT (°C)	DCO_2 (mg/L)	Salt (‰)	pH (UC)	CND (µS/cm)	OD (%)	SS (mg/L)	Turb (NTU)	Color (Pt-Co)
Mar 13	20.7±2.5	29.9±.4	60.1±16.6	.14±.04	7.44±.01	249.0±60.8	55.6±8.3	51.3±21.8	65.8±18.8	226.0±43.3
Apr 13	18.2±2.0	27.7±.5	18.5±.9	.16±.07	6.91±.02	285.8±71.2	62.6±9.6	22.5±11.9	34.6±10.4	154.8±27.3
May 13	14.5±2.3	29.3±.6	19.1±.6	.17±.02	6.92±.05	184.6±44.3	72.4±9.8	34.7±8.3	28.5±10.5	326.7±96.3
Jun 13	12.7±1.9	26.5±.4	19.6±.6	.08±.02	7.30±.05	190.1±39.3	60.8±7.4	49.3±21.4	61.6±18.5	414.4±181.9
Jul 13	14.7±2.0	25.4±.2	14.7±2.0	.12±.06	7.26±.08	178.1±30.1	65.1±8.2	17.3±3.3	26.6±5.5	148.7±28.5
Aug 13	17.5±2.6	26.3±.4	31.8±6.8	.09±.02	7.24±.06	230.6±53.3	53.8±9.4	29.4±6.1	40.5±8.4	218.0±44.7
Sep 13	12.0±2.1	26.7±.2	17.2±.8	.05±.01	7.44±.06	192.9±36.2	56.6±8.3	38.75±8.3	46.3±9.2	241.2±49.8
Oct 13	12.3±2.3	28.3±.5	18.4±1.7	.08±.02	7.09±.06	221.3±49.4	48.6±9.2	39.8±6.1	47.8±6.5	247.3±33.0
Nov 13	10.8±2.7	27.9±.4	15.7±2.3	.08±.01	7.50±.09	202.7±49.2	46.7±9.4	24.8±3.7	30.7±3.9	151.3±21.1
Dec 13	11.8±2.4	28.8±.4	17.9±2.7	.15±.03	7.34±.09	254.5±62.2	43.8±8.7	28.6±6.5	44.8±12.1	251.3±56.9
Jan 14	16.9±3.6	29.6±.5	17.9±2.8	.22±.06	7.21±.03	375.0±122.7	38.3±8.0	45.4±15.5	46.4±12.8	231.3±67.7
Feb 14	16.8±3.4	29.8±.4	17.9±3.1	.19±.04	6.36±.12	273.9±71.7	38.3±8.4	61.1±18.3	44.3±12.5	264.4±68.7
Mar 14	16.2±3.2	29.5±.5	22.4±3.4	.19±.03	7.79±.06	285.9±60.9	38.4±9.3	46.6±9.1	40.8±5.6	242.1±59.4

Table.2 Parameters of model

Model	Parameters	Values	SD	t	Pr > |t|	Lower bound (95%)	Upper bound (95%)
Vibrio Associated	Atyidae	1059.085	192.537	5.501	< 0.0001	678.452	1439.718
R^2= 0,77	Color	198.026	25.486	7.770	< 0.0001	147.641	248.411
	SS	466.751	212.704	2.194	0.030	46.251	887.252
	Turb	-699.180	239.948	-2.914	0.004	-1173.541	-224.819
	Rain	37.623	15.898	2.367	0.019	6.194	69.052
	Oxy	416.446	482.279	0.863	0.389	-536.987	1369.879
	WT	-2792.964	1850.084	-1.510	0.133	-6450.453	864.525
	DCO2	170.337	203.235	0.838	0.403	-231.444	572.118
	pH	-9367.786	7219.874	-1.297	0.197	-23640.982	4905.410
	DO	2757.058	1666.732	1.654	0.100	-537.958	6052.074

Table.3 Multiple regression showing relationships between planktonic *Vibrio* cells and considered abiotic parameters

Model	Parameters	Values	SD	t	Pr > |t|	Lower bound (95%)	Upper bound (95%)
Vibrio-plankton R2= 0,46	DO	-783.793	160.546	-4.882	< 0.0001	-1101.124	-466.463
	Oxy	113.890	47.129	2.417	0.017	20.736	207.044
	AT	-1087.042	343.425	-3.165	0.002	-1765.846	-408.237
	Color	-4.549	2.238	-2.032	0.044	-8.972	-0.125
	WT	373.657	191.707	1.949	0.053	-5.267	752.580
	DCO2	-35.934	19.288	-1.863	0.064	-74.057	2.190
	Sal	-2040.922	2438.581	-0.837	0.404	-6860.960	2779.117
	pH	-737.673	721.388	-1.023	0.308	-2163.550	688.204
	TDS	2.746	2.547	1.078	0.283	-2.289	7.781
	Turb	33.463	16.996	1.969	0.051	-0.131	67.058

Table.4 Stepwise multiple regression analysis showing equation models with significant environmental parameters

Regression equations	Coefficient of partial determination	Cumulative coefficient determination	P-value
*VA= -2137.6 + 230,4COLOR	0.67	0.67	< 0.0001
VA= - 20858.6 + 175.7COLOR+1125.3 ATY	0.06	0.73	< 0.0001
VA = -17809,9 + 206.9COLOR+1073.8ATY-246.5TUR	0.01	0.74	< 0.0001
VA= -31638.6 + 203.6COLOR+1084ATY-268.8TURB+46.1RAIN	0.01	0.75	< 0.0001
VA= -31251.4 + 196COLOR+1042ATY-575TURB+51RAI+374.6SS	0.01	0.76	< 0.0001
*VP=3126.6-332.2DO	0.15	0.15	< 0.0001
VP =13055-366.5DO-363.8AT	0.05	0.20	< 0.003
VP =12377.2-327.5DO-356.6AT+26.6OXY	0.03	0.23	< 0.001
VP =12525-325.9DO-361.3AT+27OXY-0.18COLOR	0.01	0.24	< 0.041

Figure.1 Sampling sites S1, S2, B1, B2, B3, M1, M2 and M3 in the Sanaga hydrographic area (A), and TW, WO, MP, MG, MB, TB, LM and KO in the Wouri hydrographic area (B)

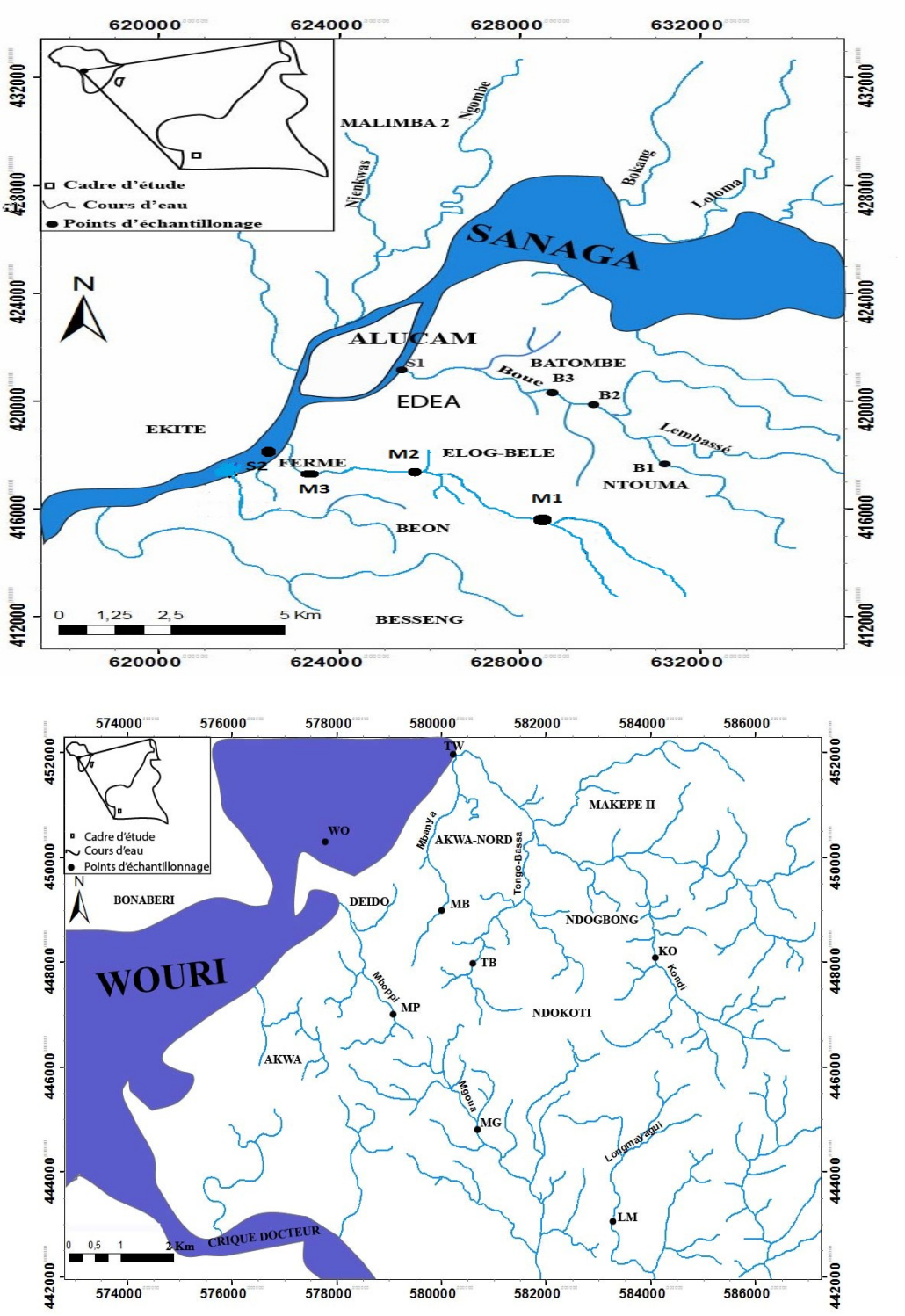


Figure.2 Temporal evolution of Atyidae abundance

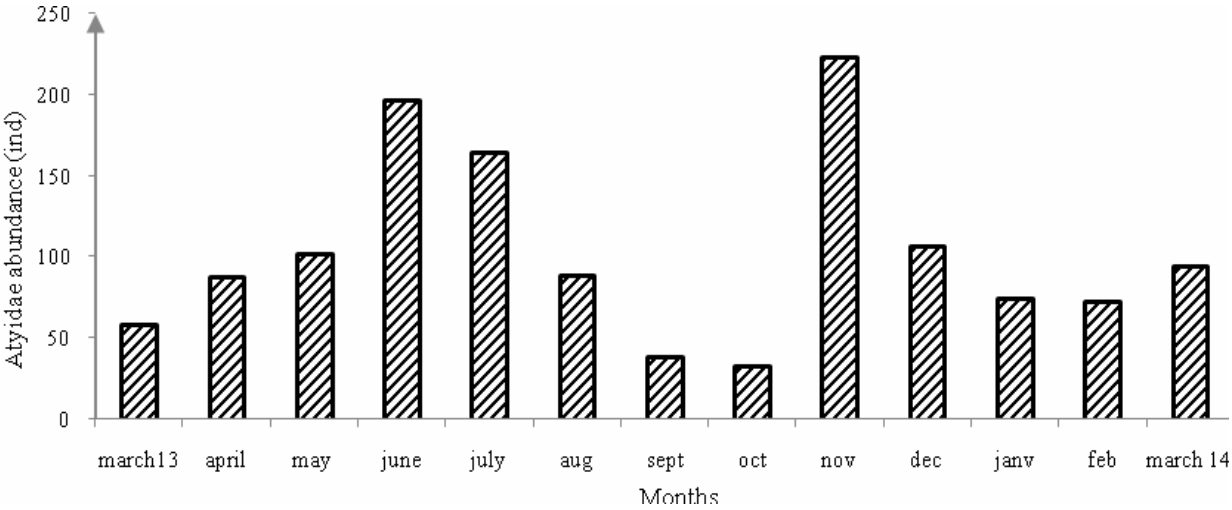


Figure.3 Temporal evolution of the concentration of plankton and associated *Vibrio* cells

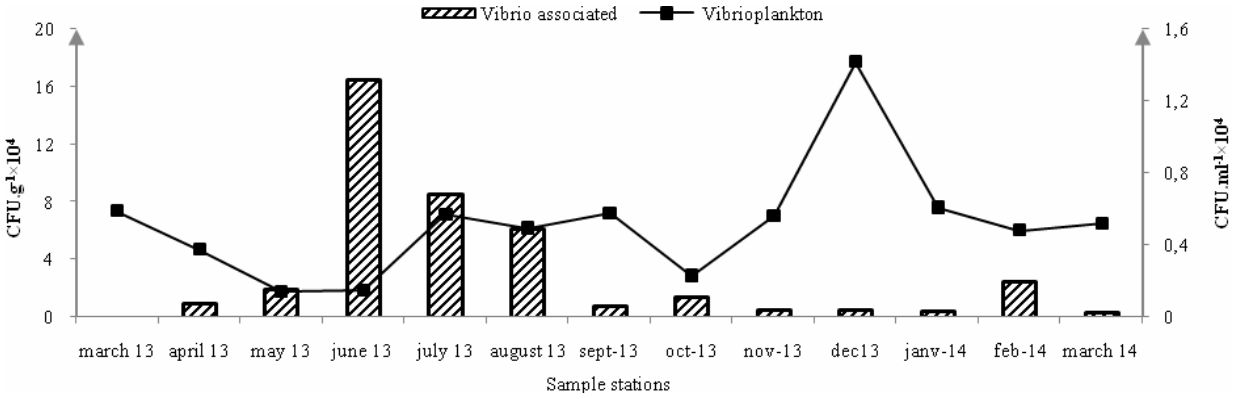


Figure.4 Spatial evolution of the concentration of planktonic and associated *Vibrio* cells

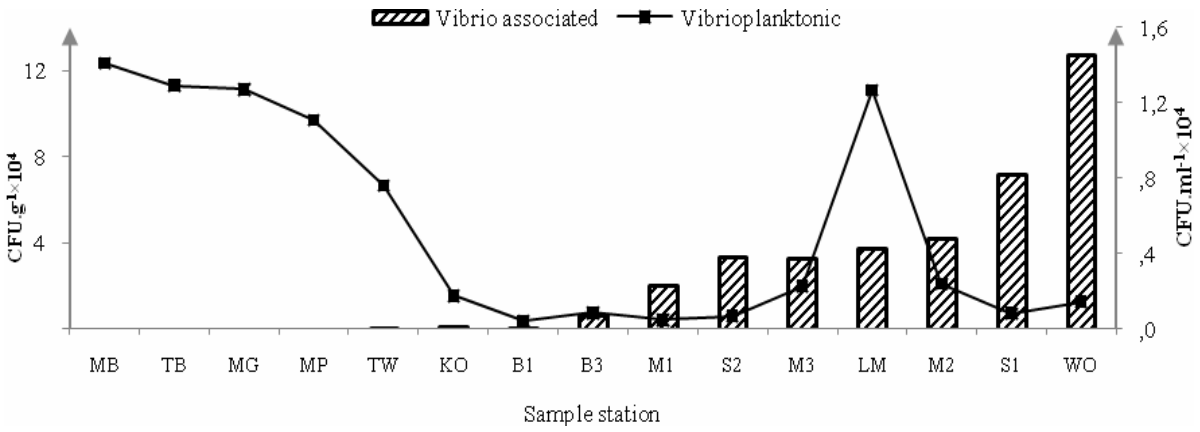


Figure.5 Monthly evolution of isolated *Vibrio spp* in coastal waters

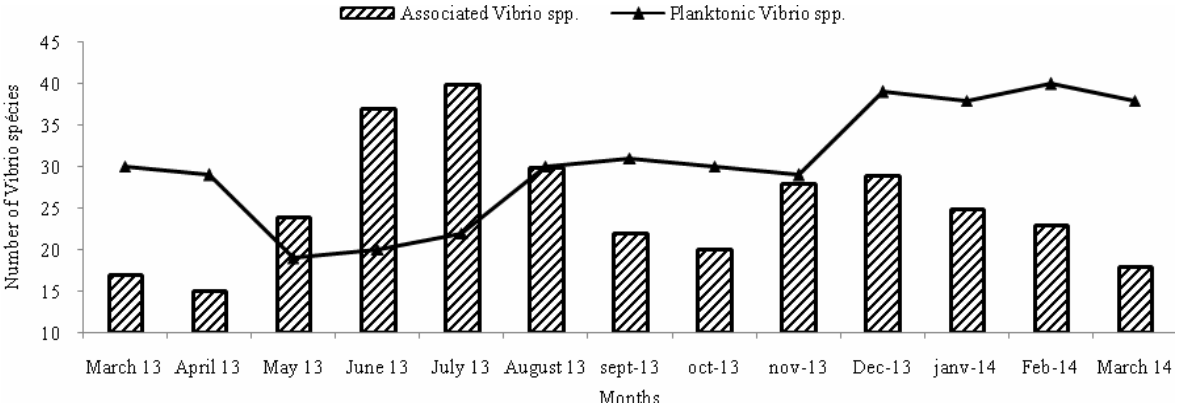
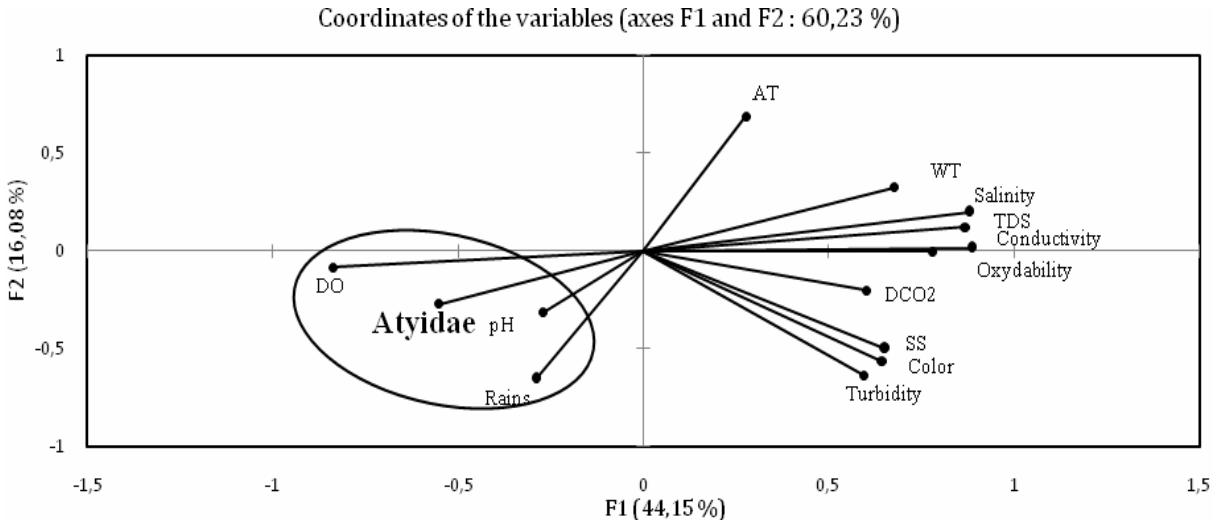


Figure.6 Principal Component Analysis (PCA) showing the relationship between abiotic parameters and density of Atyidae



*Vibrio*planktonic cells concentration measured during the study reaches the maximum level (1.4×10^4 CFU.mL^{-1}) in dry season in Mbanya river (MB), a highly polluted tributary of Wouri and constituted essentially by *V. parahaemolyticus*, *V. cholerae*, *V. vulnificus* and *V. alginolyticus*. Martinez *et al.* (2009) was isolated *V. parahaemolyticus* in the river and coastal environments in spanish. The most common isolated species is *V. cholerae* O1 in water sources in New Bell (Douala) reported by Akoachere *et al.* (2013) and this corroborates our work. The presence of pathogenic *Vibrio* species isolated from Cameroonian coastal waters constitutes a danger to the public health.

The concentration of *Vibrio* cells were relatively high in Atyidae shrimp samples ($3x10^4 \pm 10^4$ CFU.g^{-1}), compared to water samples ($5x10^3 \pm 8x10^2$ CFU.mL^{-1}). High abundance of *Vibrio* in June coincided with a low oxydability and dominated Atyidae

assemblage. This high value might be due to the comparatively high nutritional status, and the availability of organic substrate present in the water column (Asplund *et al.,* 2011). Heterotrophic activity measurements by incorporation of radioactive glucose reported by Quisthoudt-Maillard (1988) showed that rates of assimilation of the substrate covary with the bacterial densities, and with the concentrations of suspended solids and dissolved organic matter in the column water. This metabolic hyperactivity would inevitably be accompanied by deoxygenation of the environment. Rehnstam *et al.* (2010) found in mesocosm work a negative correlation between the rate of dissolved oxygen and the variation of the abundance of *Vibrio* spp. Several strains of *Vibrio* have possessed genes to reduce nitrates, giving them easy way to grow in anoxic environments very rich in organic matter (Grimes *et al.,* 2009). This activity allows the transfer of mineralization of dissolved organic carbon to higher trophic chain links. According to Sudhanandh *et al.* (2010), temperature and nutrients are the main factors that govern the distribution of *V. cholerae* along the coast of Kerala, Karnataka, India

In conclusion, the association of *Vibrio* to shrimp Atyidae is accelerated during the rainy seasons due to the dilution of organic matter in the water column and the proliferation of shrimp Atyidae due to the renewal of the water dissolved oxygen. *Vibrio* cells associated with freshwater shrimps *Atyidae* in surface waters in coastal areas in Cameroon is a real risk for human health and monitoring of food contamination by pathogenic *Vibrio* is therefore, imperative from wild capture to consumption. The microbial status of seafood after capture is closely related to environmental conditions and the microbiological quality of the water.

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