Aquat Ecol (2014) 48:379–395
DOI 10.1007/s10452-014-9491-2

Diversity, dynamic and ecology of freshwater snails related to environmental factors in urban and suburban streams in Douala–Cameroon (Central Africa)

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Received: 9 July 2014 / Accepted: 20 August 2014 / Published online: 2 September 2014

Abstract Urban land use has increased dramatically over the past few decades, resulting in high variability in nutrients loading which is likely to alter the biological component of urban streams. Freshwater snails and environmental variables that might structure their diversity and distribution were studied from September 2012 to September 2013 in three contiguous watersheds in Douala. Twelve stations were monitored monthly, two of these are located in a suburban forest area, and the rest situated in urbanized and industrialized zones. Snails were collected using a long-handled net (30 × 30 cm side, 400-μm mesh). Meanwhile, measurements of the environmental variables were taken. Ten species were recorded which are as follows: *Melanoides tuberculata* (Thiaridae); *Gabbiella africana* (Bythiniidae); *Physa acuta*, *Aplexa* sp. (Physidae); *Lymnaea natalensis*, *Lymnaea stagnalis*, *Lymnaea columella* (Lymnaeidae); *Biomphalaria pfeifferi*, *Bulinus forskalii* (Planorbidae) and one undetermined taxon of Bythiniidae. All these snails were identified at nine of the ten urban stations; no species being found in suburban stations. These urban streams have very poor health status with highly polluted waters. Among the species found *P. acuta* (76.95 %), *L. natalensis* (19.46 %) and *M. tuberculata* (2.79 %) were the most abundant. Multiple stepwise regression analysis, Spearman correlation test and redundancy analysis showed that snail occurrences and abundances were probably influenced by water temperature, conductivity, suspended solids, alkalinity, nitrites, nitrates, ammonium, phosphates, oxydability, biochemical oxygen demand, rainfall, encumbrance rate of the riverbed and water width. Moreover, snail dynamics showed a seasonal pattern with peak population abundances and recruitment of young generations during rainy season. This malacological survey spotlighted the impacts of anthropogenic activities on snail's diversity and distribution, with the proliferation of the invasive pulmonate *P. acuta* in Douala urban streams.

Keywords Freshwater snails · Population dynamic · Environmental variables · Urban wastes · Douala–Cameroon

Handling Editor: Piet Spaak.

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Introduction

Benthic macroinvertebrates are the preferred means of characterizing the ecological status of aquatic systems

(Moisan 2010; Schuwirth and Reichert 2012). Indeed, aquatic macroinvertebrates are worldwide spread and abundant in almost all aquatic media; they present various sensitivity scales to environmental stress and reflect more local conditions thanks to their restricted mobility (Moisan 2010). Among aquatic invertebrates, the Mollusca form the second largest phylum behind Arthropoda (Appleton 1996). In this group, freshwater snails (Gastropods and Bivalves) occur in various aquatic ecosystems with several invader species and may contribute a major part of the invertebrate benthos biomass (Machena and Kaujsky 1988; Brown 1994). Many freshwater snails are known to be tolerant to organic pollution and are used in biomonitoring programs (Bode et al. 2002). In Africa, freshwater mollusks have so far attracted great interest of researchers because of their great economic and medical importance as intermediate hosts in many trematode flukes' life cycles causing helminthiasis (Brown 1994). Hence, several researches were most focus on snails involved in these diseases, very few dealing with their ecological conditions (Brown et al. 1998; Prezant and Chapman 2004; Bony et al. 2008; Camara et al. 2012). However, it is well known that in streams subjected to anthropogenic disturbances—with increase of the release of various wastes and chemical products in the environment—invertebrate assemblages show a shift in their structure characterized by a severe reduction of species richness and the proliferation of a reduce number of tolerant groups among which freshwater snails, especially invasive ones (Foto Menbohan 2012; Wang et al. 2012; Albrecht et al. 2013; Xu et al. 2013). It is then imperative to evaluate the health status of organisms present in the medium in order to assess its ecological condition (Garcia-Roger et al. 2011).

In Cameroon, the environmental profile of urban townships illustrates problems associated with pollution and deterioration of air, soils and aquatic ecosystems with loss of biodiversity (Kramkimel et al. 2004). In most of industrialized Cameroon metropolis, various origins of wastewater and solid wastes are discharged directly in the environment without any preliminary or adequate treatment (Tening et al. 2013). In Douala Township which is the most densely populated and industrialized area of Cameroon, urbanization is anarchic with precarious sanitation systems. Moreover, dwellings and industrial buildings are blended in the same place with consequently, severe promiscuities and omnipresence of pollution sources (Feumba et al. 2011; Schuetze and Chelleri 2013). Investigations in assessment of urban streams were implemented recently in Cameroon, and a few related works conducted in Yaoundé City (Foto Menbohan 2012; Foto Menbohan et al. 2013; Ajeagah et al. 2012, 2013) where some freshwater snail species were reported in urban streams (Foto Menbohan 2012). In Douala Township, few studies were carried out to assess physicochemical en bacteriological status of aquatic media (Djuikom et al. 2009; Tening et al. 2013), but none of them dealt with aquatic macroinvertebrates, particularly freshwater snails. However, to the best of our knowledge, no study has so far dealt with biodiversity and ecological requirements of snails' communities in Cameroonian rivers.

This study undertaken for the first time in the rivers of the Douala Metropolis aimed (1) to inventory freshwater snail species, (2) to study their spatiotemporal dynamic, (3) to perform a spatial frame analysis of environmental parameters that might structure their spatial and seasonal trends and (4) to discuss the ecological requirement conditions for the distribution of snails' community.

Materials and methods

Study area and sampling stations

With an approximate surface of 38,700 ha, Douala city extends between 3°58′ and 4°07′ of latitude Nord and between 9°34′ and 9°49′ of longitude East. It is located at the bottom of the Gulf of Guinea, along the estuary of the Wouri River and presents a plain spread out morphology with altitudes varying between 1.6 and 39 m (Olivry 1986). The climate of this region was classified by Suchel (1972) as a wet tropical type, characterized by a short dry season (December–February) and a long rainy season (March–November). Rainfalls are abundant and regular with the annual average values varying between 2,596 and 5,328 mm. The air temperature is relatively high with a monthly average of approximately 28 °C (Suchel 1972). Samplings were carried out monthly, from September 2012 to September 2013 in 12 stations located in the three larger contiguous watersheds (Nsapè, Tongo'a-Bassa and Mgoua) situated at the left bank of the Wouri River (Fig. 1).

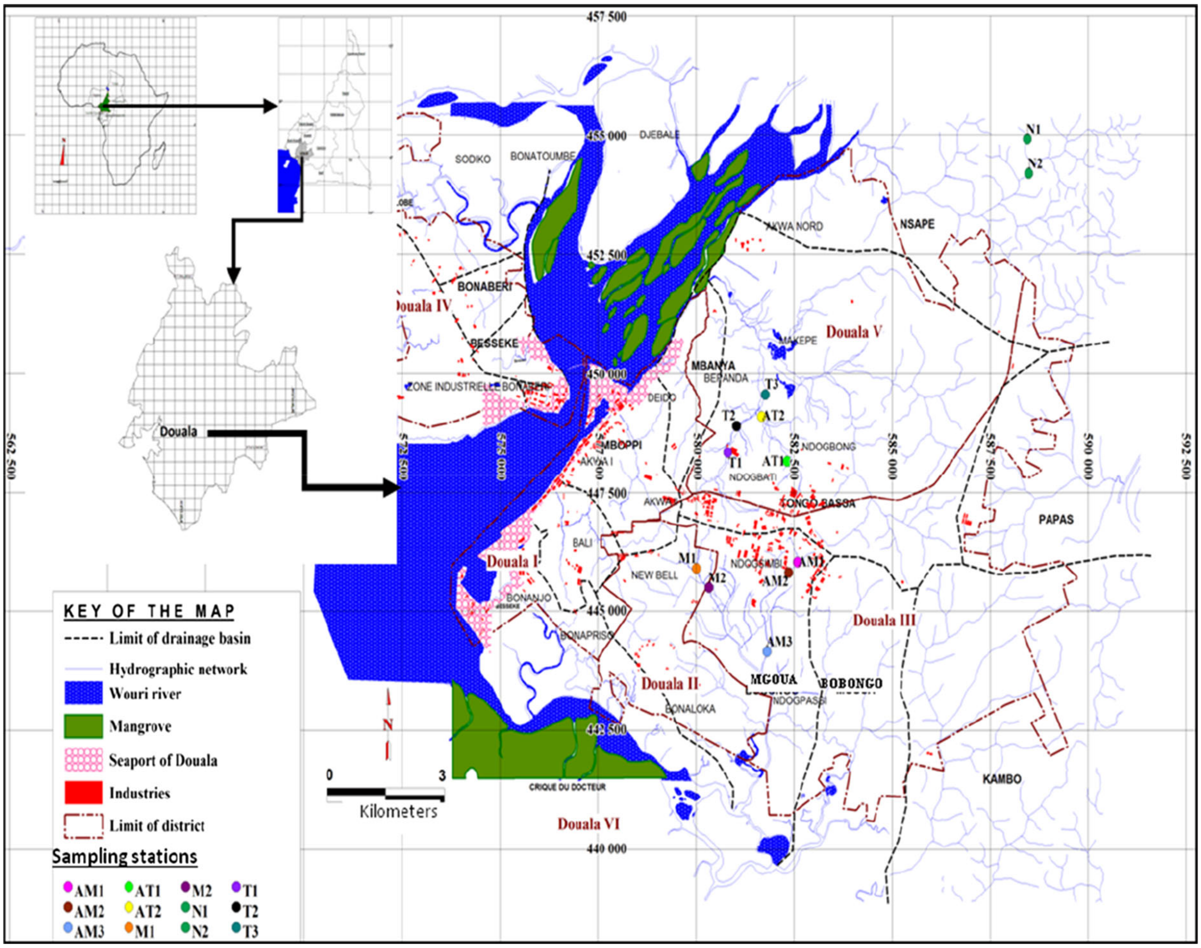

Fig. 1 Hydrographic map of the study sites showing sampling stations

In the watershed of Nsapè located in a suburban area particularly covered by vegetation of a secondary dense forest type, and sheltered of any urban and industrial activity, two stations codified N_1 (04°06′89″N; 09°47′76″E) and N_2 (04°06′55″N; 09°47′80″E) were chosen. Inversely, Tongo'a-Bassa and Mgoua basins are located in industrialized areas and are highly disturbed by human activities. In these river basins, several industries pour their effluents and other wastes directly in the environment without preliminary or adequate treatment. Moreover, in shantytowns, residents along the rivers discharge their domestic and urban wastes directly in the riverbed. Five sampling stations (T_1, T_2, T_3, AT_1 and AT_2) were selected in the Tongo'a-Bassa catchment. The stations T_1 (04°03′38″N; 09°43′67″E) and T_2 (04°03′66″N; 09°43′81″E) are located, respectively, at 350 m upstream and 200 m downstream from the outlet of the effluent of an industry of chocolate factory and confectionery. Stations AT_1 (04°03′23″N; 09°44′49″E) and AT_2 (04°03′84″N; 09°44′07″E) are located, respectively, at 100 m and 3.5 km downstream from the outlet of effluents coming from a brewery industry, a textile industry and an industry of manufacture of glasses. While station T_3 (04°04′00″N; 09°44′14″E) is situated at 500 m downstream from the junction of the two preceding arms. The five other stations (M_1, M_2, AM_1, AM_2 and AM_3) were chosen in Mgoua river basin. The stations M_1 (04°02′77″N; 09°44′07″E) and M_2 (04°02′24″N; 09°44′67″E) are, respectively, localized at 350 m upstream and 250 m downstream from the outlet of effluents coming from the great Industrial Centre of Bassa. This centre shelters several industries exerting in various fields

(Agro-alimentary, brewery, textile, chemical, automobile, petroleum etc.). Stations AM_1 (04°02′12″N; 09°44′66″E) and AM_2 (04°01′97″N; 09°44′54″E) are located, respectively, at 300 m upstream and 150 m downstream from the outlet of the effluent of a soap and cosmetic factory. Station AM_3 (04°01′09″N; 09°44′25″E) is situated at approximately 2.2 km downstream of station AM_2.

Measurement of environmental variables

At each sampling station, 22 environmental variables were taken into account. Seven physical parameters were determined to characterize the habitat. Mean depth and wetted width (in cm) were measured on transects with equal distance interval across channel sections (Song et al. 2009). Current velocity (m/s) was measured by timing the front of a neutral non-pollutant dye stuff (blue of methylene) over a known distance along the station. At each sampling station, canopy coverage (%) was estimated visually (Rios and Bailey 2006). Monthly rainfall (mm) data for the study period were collected at the Douala Airport Meteorological Research Station. Additionally, types of substrate were characterized by measuring substrate particle size distribution based on methods described by Platts et al. (1983). The encumbrance rate (%) which estimated the quantity of allogenic matters (g/kg of substrate) in the riverbed was also calculated. In urban stations, this allogenic matter primarily consists of domestic and urban wastes (household refuse, worn clothing, plastics, breaking glasses, scrap metal etc.), meanwhile in suburban stations it is solely constituted of branches and dead leaves.

Measurements of physicochemical parameters of water at each sampling stations were done following APHA (1998) and Rodier et al. (2009) standards. Water temperature (alcohol thermometer), pH (HACH HQ11d pH-meter) and dissolved oxygen (HACH HQ14d oxymeter) were measured in situ. Likewise, salinity (‰) and electrical conductivity (µS/cm) were measured in situ using a HACH HQ14d conductimeter. Suspended solids, water color, nitrites, nitrates, ammonium and phosphates were measured in the laboratory using HACH DR/2800 spectrophotometer. Alkalinity, dissolved carbon dioxide and oxydability were dosed by volumetric method, whereas the biochemical oxygen demand (BOD) was measured using a Liebherr BOD analyzer.

Freshwater snail's collection and identification

Snail samples were collected at each station accessible through backward flow wading along the margins and inside the stream, using a long-handled kick net (30 cm × 30 cm side, 400-µm mesh size). Samplings were done in a 100-m stretch for each station, following protocols described by Stark et al. (2001). To this end, in each station, 20 drags of the kick net were done in different micro-habitat, each corresponding to a surface of 0.15 m^2 (30 cm × 50 cm). Thus, the total sampled surface in each station was 3 m^2 (0.15 m^2 × 20). Materials collected in the sampling net were rinsed through a 400-µm sieve, and all snail individuals were sorted and placed in plastics sampling bottles with 10 % formalin. In the laboratory, samples were rinsed with tap water using a 400-µm sieve and all the snails were handpicked with a fine dissection forceps and sorted into Petri dishes. All specimens caught were identified based on their morphological and/or anatomical characters, under a stereomicroscope with the use of appropriate literature (Same Ekobo 1984; Brown 1994; Appleton 1996; Moor and Day 2002; Bony et al. 2008). The mollusks were then counted and measured (maximum shell height and width) to the nearest 0.01 mm using an electronic digital caliper. Based on the literature (Appleton 1996; Moor and Day 2002), egg capsules of snails were collected on submerged surface (stones, leaves, domestic garbage) and preserved in 70° ethanol. In the laboratory, egg capsules were identified based on their morphology and measured to the nearest 0.01 mm using an electronic digital caliper. Measurements and identification of eggs were carried out under an optical microscope 400× magnification, equipped with ocular micrometers.

Data analyses

The distribution patterns of the environmental data were displayed using the self-organizing map (SOM) by means of the toolbox developed by Alhoniemi et al. (2003) for Matlab®. Firstly, the environmental data set was arranged as a matrix of 156 rows (i.e., the 12 sites sampled on thirteen occasions) and 21 columns (i.e., the environmental variables). Each of the 156 samples of the data set can be considered as a vector of 21 dimensions. The SOM algorithm was then applied to calculate the connection intensities (i.e., vector weights) between input and output layers using an unsupervised competitive learning procedure (Kohonen 2001), which iteratively classifies samples in each neuron according to their similarity in terms of parameter values. Thus, the SOM preserves the neighborhood so that samples with close parameters values are grouped together on the map, whereas samples with very different parameters are far from each other. The output layer consists of N output neurons (i.e., computational units, $N = 49 = 7 \times 7$ in this study), which are arranged into a two-dimensional grid. Because the map size (number of output units) of the SOM is critical for accommodating hierarchical levels in environmental variables, we trained the SOM with different map sizes and chose the optimum map size based on low topographic and quantization errors (Kohonen 2001). The relationships between parameters associated with each cluster and the factors of the sampling plan (season and sampling stations), which were not used in the SOM learning process, were assessed. To do so, we calculated for each cluster the relative contribution of each modality (e.g., season) of a certain factor (e.g., sampling stations) to the total number of modalities of the factor. Then, we tested for the H_0 hypothesis that no significant differences were present among these relative values using a test for proportions (G test) based on the likelihood ratio χ^2 statistics (Sokal and Rohlf 1995; Sachs 1997).

The gastropod community structure was described through species richness, abundances, occurrence frequencies (FO) and Shannon–Weaver and Evenness diversity indices. Occurrence frequency specifies the percentage of samples in which each species of snail occurred. It was calculated to classify species according to Dajoz (2000). Based on shell measurements, snail species were sorted in size classes according to Sturge rule (Scherrer 1984). These size classes were then used to highlight the seasonal dynamic of snail populations (Camara et al. 2012). The Kolmogorov–Smirnov test was first applied to check the normality of the distribution before comparing environmental parameters and abundances of snail species. The Kruskal–Wallis test and Mann–Whitney test were then performed to verify significant differences between spatial and seasonal variations of environmental parameters and snail's abundances. RStudio 0.96.330 software package was used for these statistical analyses, and the level of significance considered was $p < 0.05$. Relationship between environmental variables and taxonomic richness and abundances of snails was determined by Spearman correlation test and canonical redundancy analysis (RDA).

Results

Environmental variables

Water temperature was low at suburban stations (25.9 ± 0.89 °C), while at urban stations, values ranged from 26 °C ($T_{1jan.}$) to 36 °C ($AT_{1Apr.}$). Waters ranged from slightly acidic (pH 4.7; $N_{2jul.}$) to basic (pH 9.4; AM_{2aug}). Dissolved oxygen was overall high in stations N_1 and N_2 with values ranging between 4.5 and 7.4 mg/L, while in urban stations, the values ranged from 0.06 mg/L (M_{2may}) to 3.4 mg/L (AM_{1may}). Electrical conductivity ranged between 7 µS/cm (N_{1may}) and 3,690 µS/cm (AT_{1mar}). Salinity was nil in stations N_1 and N_2, while the highest value (1.38 ‰) was recorded at station AT_1 in April. Suspended solids and water color were globally very low in suburban stations with values ranging from 0 to 15 mg/L and from 0 to 112 Pt.Co, respectively. Whereas in urban zone, values of these parameters fluctuated between 14 mg/L ($T_{1nov.}$) and 409 mg/L ($AT_{1sep1.}$) for suspended solids, and between 127 Pt.Co ($T_{2apr.}$) and 2,776 Pt.Co ($T_{1sep2.}$) for water color. Alkalinity varied between 0 mg/L (N_1) and 1,050 mg/L (AT_{1jan}). The lowest values (0 mg/L) of nitrites, nitrates, ammonium and phosphates were recorded in stations N_1 and N_2, while the highest were registered at stations AM_2 (2.39 mg/L) in November, T_1 (20.2 mg/L) in August, M_1 (12 mg/L) in September and AT_1 (7.72 mg/L) in November, respectively. The lowest values of oxydability (1.2 mg/L) and BOD (5 mg/L) were obtained at station N_1, while the highest values (152 mg/L) and (340 mg/L), respectively, were recorded at station AT_1 in November and August. Mean values of water's depth and wetted width fluctuated between 11.3 cm (M_{1jan}) and 66.3 cm (N_{2jul}) and between 190 cm ($AT_{1jan.}$) and 700 cm (AM_{3sep2}), respectively. The lowest value (0.22 m/s) of current velocity was recorded at station AM_3 in January, whereas the highest (0.89 m/s) was obtained in station AT_1 in July. Canopy was absent at the level of all the stations situated in urban area, meanwhile it was estimated to 69 and 73 %, respectively, at the level of

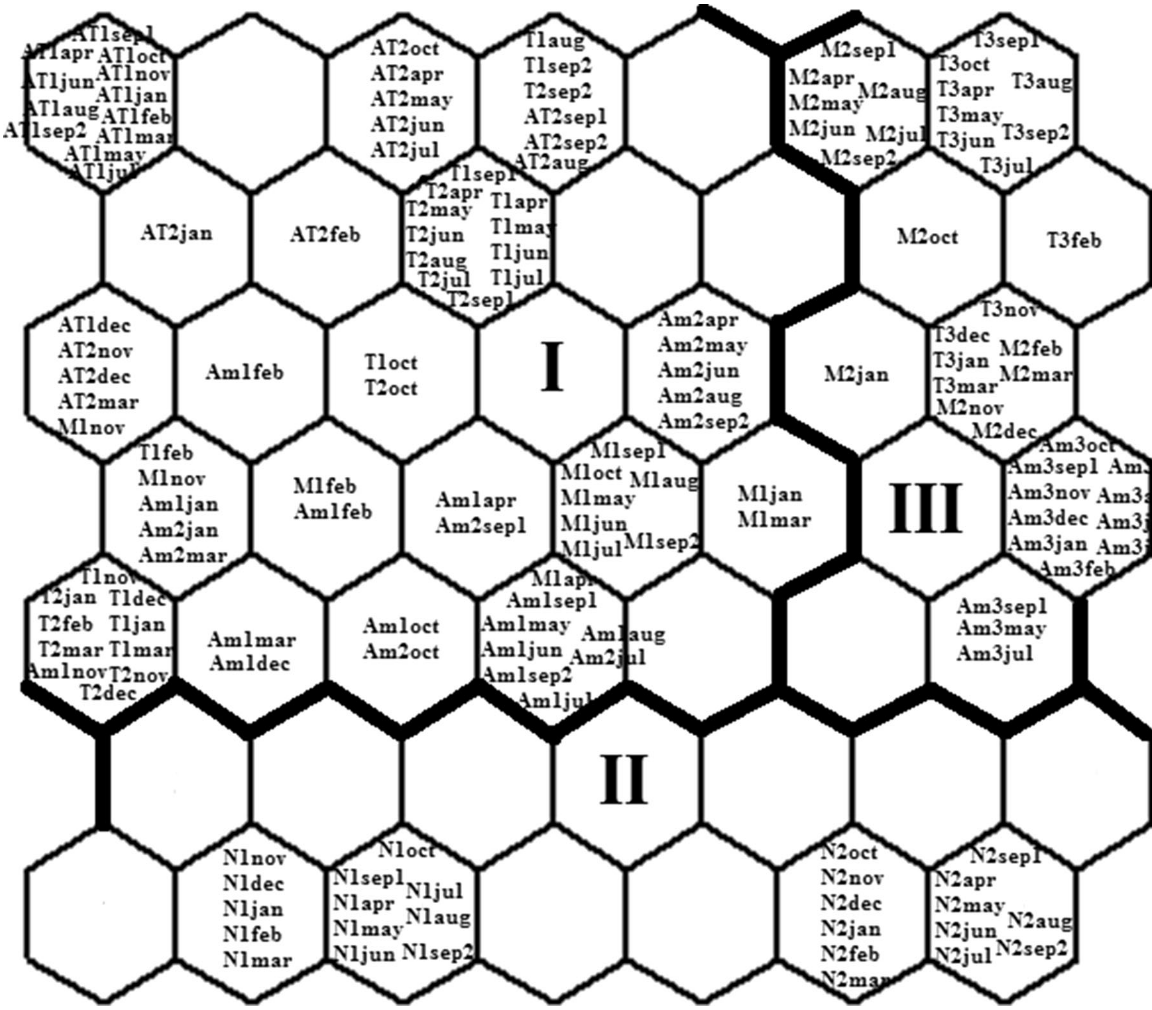

Fig. 2 Clustering of samples according to their environmental variables in the SOM layer. The Latin numbers (*I*, *II*, *III*) represent different clusters. The acronyms in the hexagonal units represent different samples (station code–month)

stations N_1 and N_2. Four types of substrate were characterized for the whole study stations: sandy (N_1 and N_2), muddy sand (T_1, T_2, AT_1, M_1, AM_1 and AM_2), sandy mud (AT_2) and very sandy mud (T_3, M_2 and AM_3). The lowest encumbrance rate of the riverbed was obtained in suburban stations with 1.02 % (N_1) and 1.75 % (N_2). Though in urban streams, values of this parameter fluctuated between 8.22 % (AM_2) and 35.38 % (T_3). For the study site and during the study period, the lowest monthly rainfall values (10.59 mm) was record in January, while the highest (767.16 mm) was obtained in July.

All samples were classified by the SOM according to their environmental parameter values in the 49 output nodes, so that each node included samples with similar parameters (Fig. 2). The results (SOM map) displayed three clusters (I, II and III) of samples according to environmental gradient at different levels of the Euclidean distance. The sample classification was mainly related to the spatial factor according to the Log-likelihood ratio (*G* test). Cluster I in the upper-left part of the SOM map is composed of samples from seven urban stations (T_1, T_2, AT_1, AT_2, M_1, AM_1 and AM_2). Cluster II in the lower part of the SOM map is made up of all the samples from stations N_1 and N_2 located in the suburban forest area. Cluster III in the upper-right part of the SOM map is constituted by samples from stations T_3, M_2 and AM_3, each situated in a highly urbanized and industrialized zone, downstream from the outlet of industrial effluents.

Concerning spatial trends of the environmental parameters, Mann–Whitney test revealed no significant difference ($p > 0.05$) between stations N_1 and N_2, whereas significant differences ($p < 0.05$) were found when comparing urban and suburban stations for all the physicochemical variables, based on Kruskal–Wallis test. Among urban stations, significant differences were observed, as shown by the SOM map (Fig. 2). In cluster I, except dissolved oxygen, nitrites and ammonium, physicochemical variables in station AT_1 were significantly different ($p < 0.05$) from those recorded in the other stations. While in cluster II, no significant difference ($p > 0.05$) in physicochemical variables was observed between stations T_3, M_2 and AM_3. Regarding seasonal variations, only values of conductivity, suspended solids, dissolved carbon dioxide, nitrites, rainfall, water's depth and wetted width, and current velocity varied significantly between dry and rainy seasons.

The contribution of each input environmental variable for the classification of sampling stations on the SOM map is displayed in Figs. 3 and 4. Dark areas represent high contributions of each input variable, while pale ones stand for low values. The values of the gradient bar were calculated during the learning process of the network. The next step is to compare the relationship between clusters of samples and the distribution of environmental variables. Thereby, the distribution of environmental parameters showed a spatial gathering. Thus, samples of Cluster II, collected in suburban stations, are characterized by clean water with high values of dissolved oxygen and canopy coverage and low values of temperature, conductivity, salinity, suspended solids, alkalinity, nitrites, nitrates, ammonium, phosphates, oxydability, BOD and encumbrance rate of the streambed. Inversely, stations in Clusters I and III, all located in urban and industrial area, are characterized by lack of canopy coverage, very low values of dissolved oxygen, high encumbrance rate of the riverbed with anthropogenic wastes and high values of other physicochemical parameters.

Composition and distribution of snail species

This malacological survey of Douala catchment identified 10 freshwater gastropod species belonging to five families: Thiaridae (*Melanoides tuberculata*); Bythiniidae (*Gabbiella africana*, Bythiniidae undetermined); Physidae (*Physa acuta*, *Aplexa* sp.); Lymnaeidae (*Lymnaea natalensis*, *L. stagnalis*, *L. columella*); Planorbidae (*Biomphalaria pfeifferi*, *Bulinus forskalii*). All these gastropods were collected at nine of the ten urban stations; no species being found in urban station AT_1 and in the two suburban stations (Table 1). Three main snail species (*P. acuta*, *L. natalensis* and *M. tuberculata*) were present at the nine stations all-over the study period. Spatial distribution of snails showed that the highest abundance of *P. acuta* (3,437 ind.) was obtained in station T_1 and was significantly different ($p < 0.01$) from those recorded in the other stations. Also, important and significant ($p < 0.01$) reduction in abundances of this species was observed in stations T_2, T_3, M_2, AM_2 and AM_3 all situated downstream from the outlet of industrial effluents. Concerning *L. natalensis*, the highest abundance (1,773 ind.) was observed in station M_1 and was significantly different ($p < 0.01$) from those recorded in the other stations. As for *M. tuberculata*, the highest number of individuals recorded at station T_2 (174 ind.) was significantly different ($p < 0.01$) from the abundances in the other stations.

Following a spatial schedule, species richness of snails varied between four species (AM_3) and eight species (T_2). Shannon–Weaver diversity index was low with ranged values of 0.52 bits/ind. (AM_1, AM_2) to 1.24 bits/ind. (T_3). Likewise, Evenness index was low with values ranging from 0.22 (AM_1, AM_2) to 0.56 (AM_3) (Table 1).

Among the species identified during the study period *P. acuta* (FO = 97.44 %), *L. natalensis* (FO = 85.47 %) and *M. tuberculata* (FO = 64.10 %) were very frequent. *L. stagnalis* (FO = 35.04 %) was accessory, while the six other species (*Aplexa* sp., *L. columella*, *B. pfeifferi*, *B. forskalii*, *G. africana* and Bythiniidae undetermined) were very rare (FO < 25 %) (Fig. 5a). *P. acuta* (76.95 %), *L. natalensis* (19.46 %) and *M. tuberculata* (2.79 %) were the most abundant species, the seven other species all together representing just 0.80 % of the total abundance (Fig. 5b).

Seasonal trends of abundances of the three main snail species are shown in Fig. 6. Maximum abundances were observed during the full rainy season (June–August). The seasonal dynamic of abundance of these snail species showed significant differences ($p < 0.05$) between dry and rainy seasons. Monthly abundances of *P. acuta* varied between 623 and 833 individuals during dry season, while during rainy season it ranged from 395

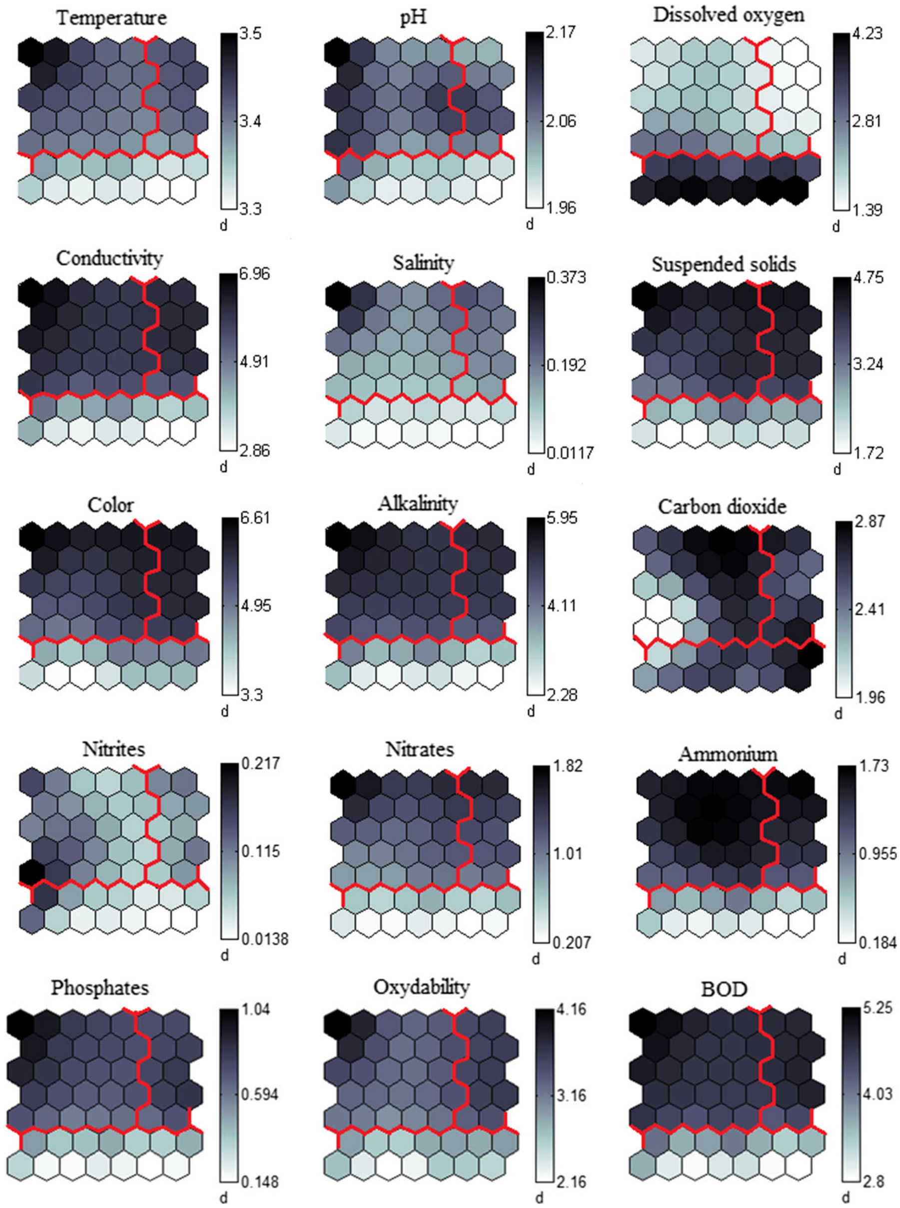

Fig. 3 Component planes displaying the contribution of each physicochemical variable to classification of samples. *Scale bars* indicate the weight vector of each variable; the values were calculated during the learning process of the network. *Dark* represents high values of each variable, whereas *pale* indicates low values

to 1,866 individuals. Monthly abundances of *L. natalensis* ranged from 40 to 79 individuals and from 63 to 644 individuals, respectively, for dry and rainy seasons. As for *M. tuberculata*, this variable showed range values of 3–9 individuals during dry season and 9–146 individuals for the rainy season.

Shell morphometry and seasonal dynamic of the three main snail species

The specimens of *P. acuta* that we collected are characterized by thin, ovate and slightly glossy shell (especially for adults), with an acute and elevated spire. Whorls are slightly convex with a shallow suture. The largest specimen of this species was 12.34 mm in height and 7.2 mm in width (Table 2). For all the specimens of *P. acuta* measured, height/width ratio varied between 0.96 and 2.74, with a mean value of 1.74 ($\pm$SD = 0.13). For *L. natalensis*, specimens that were recorded are characterized by a basal whorl markedly swollen compared to the remainders, sculpture consisting of less marked growth lines. The shell is ovate acuminated and pale yellow in color. The largest specimen of this lymnaeid

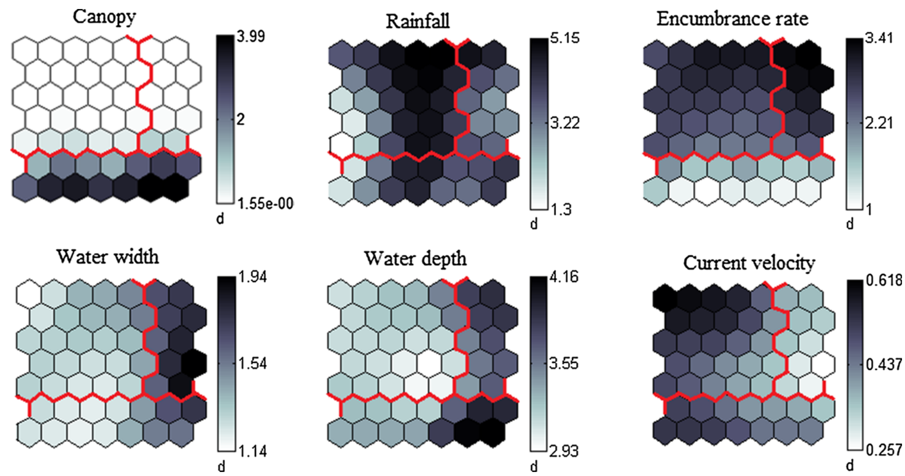

Fig. 4 Component planes displaying the contribution of each hydromorphometrical variable to classification of samples. *Scale bars* indicate the weight vector of each variable; the values were calculated during the learning process of the network. *Dark* represents high values of each variable, whereas *pale* indicates low values

Table 1 Abundances, distribution and univariate statistics of snail species in different study stations

Families	Species	N_1	N_2	T_1	T_2	T_3	AT_1	AT_2	M_1	M_2	AM_1	AM_2	AM_3
Bythiniidae	nd	–	–	–	1^{a}	–	–	–	–	1^{a}	–	–	1^{a}
	Gabbiella africana	–	–	–	1^{a}	–	–	1^{a}	–	–	–	–	–
Thiaridae	*Melanoides tuberculata*	–	–	61^{a}	174^{b}	86^{a}	–	51^{a}	33^{a}	20^{a}	13^{a}	9^{a}	16^{a}
Lymnaeidae	*Lymnaea columella*	–	–	–	6^{a}	–	–	–	2^{a}	1^{a}	3^{a}	1^{a}	–
	Lymnaea natalensis	–	–	287^{a}	389^{a}	233^{a}	–	168^{a}	1773^{b}	151^{a}	138^{a}	67^{c}	18^{d}
	Lymnaea stagnalis	–	–	28^{a}	17^{a}	3^{b}	–	13^{a}	14^{a}	8^{a}	12^{a}	5^{b}	–
Physidae	*Aplexa* sp.	–	–	7	2	1	–	–	–	–	–	–	–
	Physa acuta	–	–	3437^{a}	2981^{b}	754^{c}	–	621^{c}	1690^{b}	668^{c}	1668^{b}	828^{c}	100^{d}
Planorbidae	*Biomphalaria pfeifferi*	–	–	–	–	1^{a}	–	1^{a}	–	–	–	–	–
	Bulinus forskalii	–	–	–	–	–	–	–	2	–	–	–	–
Species richness		–	–	5	8	6	–	6	6	6	5	5	4
Shannon–Weaver (bits/ind.)		–	–	0.58	0.84	1.17	–	1.15	1.11	0.93	0.52	0.52	1.23
Evenness		–	–	0.25	0.28	0.45	–	0.45	0.43	0.36	0.22	0.22	0.56

In the same row, abundances of snail species followed by different letters are significantly different ($p < 0.05$); –, absence; nd, undetermined taxon

was 11.21 mm in height and 7.01 mm in width. All the specimens of *L. natalensis* examined showed a height/width ratio varying between 0.93 and 3.20, with a mean value of 1.63 (±SD = 0.14). Concerning *M. tuberculata*, the specimens caught showed an elongated more or less smooth shell, textured with irregular shallow pits and irregular spiral striations. The largest specimen was 19.87 mm in height and 5.31 mm in width and the individuals collected showed a height/width ratio range of 1.5–4.37 (mean = 2.68; SD = ±0.46).

Shells of *P. acuta*, *L. natalensis* and *M. tuberculata* were distributed into size classes, displaying the seasonal dynamic of different growth stages (Fig. 7). Our data showed that during dry season, populations of these three freshwater gastropod species primarily comprised adult size classes with relatively low abundances, while high abundances and recruitment of young generations were confined in the rainy season.

Relationships between environmental variables and snail's community

The analysis of Spearman correlation test (Table 3) indicated that *P. acuta* was positively and significantly

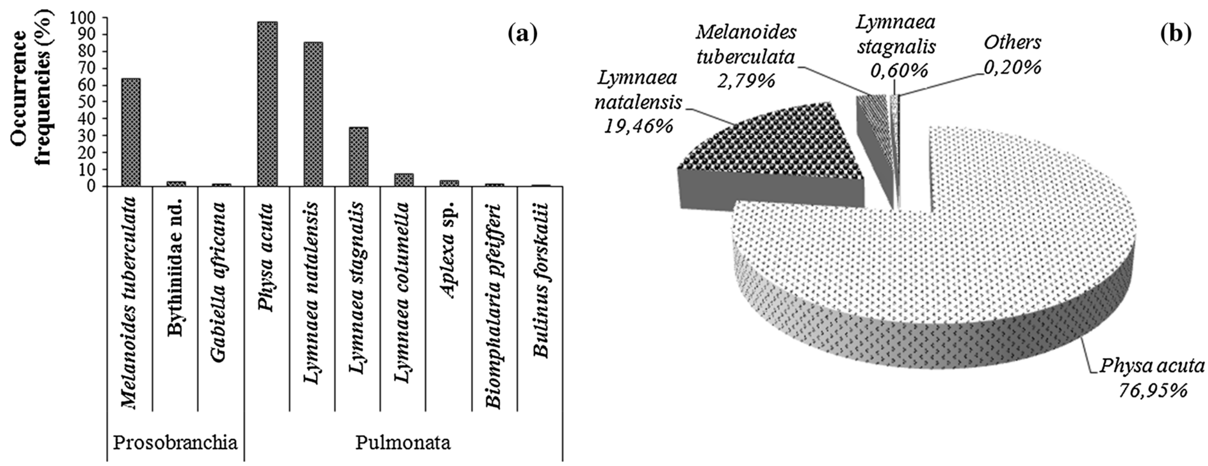

Fig. 5 Occurrence frequencies (**a**) and relative abundance (**b**) of freshwater gastropod species collected at the nine stations during the study period

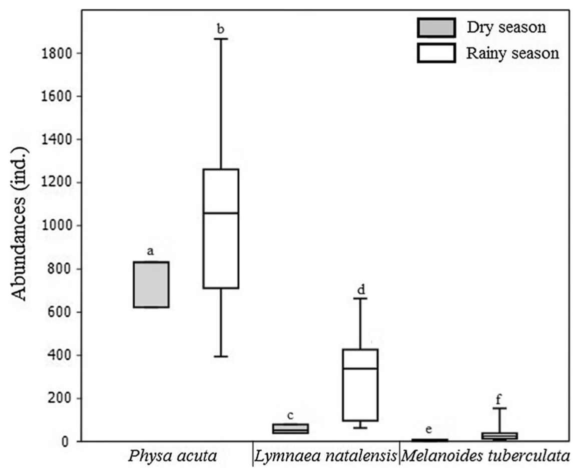

Fig. 6 Seasonal variations of abundances of the three main snail species collected in the nine stations during the study period; median values of *box plots* bearing *different letters* are significantly different ($p < 0.05$) based on Mann–Whitney tests

($p < 0.05$) correlated with high values of water temperature, conductivity, alkalinity, nitrites, nitrates, ammonium, phosphates, oxydability, BOD and high encumbrance rate of the riverbed. This species was negatively ($p < 0.05$) correlated with canopy coverage and water depth. Positive and significant ($p < 0.05$) correlations were found between *L. natalensis* and suspended solids, water color, nitrates, ammonium, phosphates, rainfall and encumbrance rate of the riverbed. It was negatively ($p < 0.05$) correlated with dissolved oxygen, canopy coverage and water depth. *M. tuberculata* appeared to be positively and significantly ($p < 0.05$) correlated with high values of water temperature, salinity, suspended solids, water color, alkalinity, nitrates, ammonium, phosphates, rainfall, high encumbrance rate of the riverbed and water width. But it was negatively ($p < 0.05$) associated with dissolved oxygen, canopy coverage and current velocity. *Lymnaea stagnalis* was positively and significantly ($p < 0.05$) correlated with alkalinity, ammonium and encumbrance rate of the riverbed, and negatively and significantly ($p < 0.01$) associated with canopy and water depth. No significant correlation with environmental parameters was obtained for *G. africana*, *B. forskalii* and the undetermined taxon of Bythiniidae. The other species (*L. columella*, *B. pfeifferi* and *Aplexa* sp.) were sporadically distributed and did not showed high degree of association with environmental variables.

The results of redundancy analysis (RDA) revealed that the relationships between freshwater gastropod species and their habitat conditions follow mainly the first two axes ($F1 = 86.1$ %; $F2 = 13.7$ %) which accounted for 99.8 % of the total variance (Fig. 8). Following the first axis ($F1$) in positive coordinates, *P. acuta*, *L. natalensis*, *M. tuberculata*, *L. stagnalis*, *L. columella*, *G. africana*, *Aplexa* sp. and *B. forskalii* are positively and significantly influenced by rainfall, nitrates, nitrites, ammonium, phosphates, BOD, encumbrance rate of the riverbed and muddy sand substrates. In negative coordinates, *B. pfeifferi* appear to be weakly influenced by environmental variables. Following the

Table 2 Descriptive statistics of shell's morphometric variables of the three main snail species collected in the nine stations during the study period

Snail species	N	Max	Min	Mean	Med	SD	CV	Skewness	Kurtosis
Physa acuta									
SH	11,520	12.34	0.75	6.91	7.12	1.78	25.69	−0.49	−4,722.4
SW		7.2	0.49	4.01	4.14	1.08	27.05	−0.44	−4,306.1
H/W		2.74	0.96	1.74	1.73	0.13	7.18	1.02	−14,575
Lymnaea natalensis									
SH	2,870	11.21	0.83	5.78	5.86	1.26	21.88	−0.46	41.14
SW		7.01	0.47	3.57	3.62	0.84	23.53	−0.33	35.25
H/W		3.20	0.93	1.63	1.62	0.14	8.49	2.11	224.03
Melanoides tuberculata									
SH	445	19.87	1.88	7.51	6.76	3.39	45.08	1.16	1.13
SW		5.31	0.9	2.72	2.56	0.85	31.2	0.74	0.24
H/W		4.37	1.5	2.68	2.68	0.46	17.2	0.35	0.25

SH shell height (mm), *SW* shell width (mm), *H/W* height/width ratio, *N* number of individual measured, *Max* maximum, *Min* minimum, *Med* median, *SD* standard deviation, *CV* coefficient of variation (%)

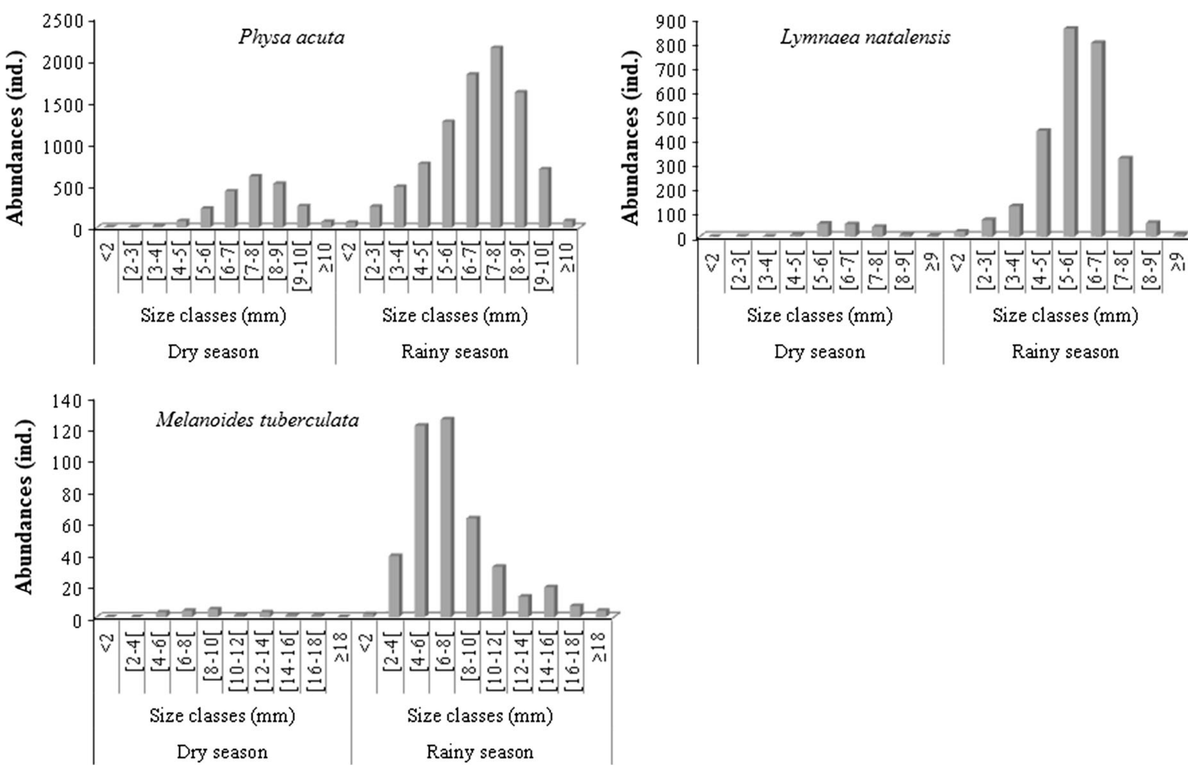

Fig. 7 Seasonal trends of abundances per size classes of *P. acuta*, *L. natalensis* and *Melanoides tuberculata* collected in the nine stations during the study period

second axis (*F*2), only the undetermined taxon of Bythiniidae is associated with this axis in negative coordinates and is mostly influenced by pH and current velocity.

Description of egg capsules and eggs of snails

All the egg capsules collected on submerged materials during the study period were gelatinous, large or larger

Table 3 Summary of Spearman correlation between freshwater gastropod abundances and environmental variables

Variables	Mtub	Byth	Gafr	Pacu	Apsp	Lnat	Lsta	Lcol	Bpfe	Bfor
Temperature (°C) [Temp]	0.16*	0.08	0.11	0.18*	0.09	0.11	0.13	0.07	0.09	0.07
pH (UC) [pH]	−0.04	0.10	0.02	0.03	0.02	−0.06	−0.03	0.02	−0.07	0.01
Dissolved oxygen (mg/L) [DO]	−0.38**	0.04	−0.05	−0.15	−0.09	−0.33**	0.01	−0.02	−0.15	−0.03
Conductivity (μS/cm) [Cond]	0.13	0.08	0.11	0.17*	−0.01	0.13	0.06	0.09	0.10	−0.01
Salinity (‰) [Sali]	0.19*	0.09	0.06	0.00	0.01	0.10	0.03	0.06	0.08	0.04
Suspended solids (mg/L) [SS]	0.35**	−0.07	0.01	0.07	−0.14	0.23**	0.02	−0.09	0.03	−0.05
Water color (Pt.Co) [Color]	0.28**	0.03	0.02	0.06	−0.08	0.20*	−0.04	−0.08	0.08	0.03
Alkalinity (mg/L $CaCO_3$) [Alka]	0.30**	0.01	0.14	0.22**	0.05	0.15	0.20*	0.04	0.08	0.07
Carbon dioxide (mg/L) [Cadio]	0.07	−0.14	0.04	−0.06	0.03	0.05	0.01	0.12	0.12	−0.07
Nitrites (mg/L NO_2^-) [Nitri]	0.08	0.15	−0.05	0.17*	−0.00	0.10	0.12	−0.11	0.06	0.01
Nitrates (mg/L NO_3^-) [Nitra]	0.24**	0.03	0.05	0.20*	−0.07	0.19*	0.06	−0.02	0.08	−0.04
Ammonium (mg/L NH_4^+) [Amm]	0.44**	0.01	0.10	0.44**	0.19*	0.56**	0.29**	0.21**	0.12	0.14
Phosphates (mg/L PO_4^{3-}) [Phos]	0.24**	0.01	0.03	0.21**	−0.06	0.24**	0.06	−0.02	0.03	0.00
Oxydability (mg/L) [Oxyda]	0.14	0.12	0.06	0.18*	−0.04	0.05	−0.02	0.04	0.04	0.07
BOD_5 (mg/L) [BOD]	0.12	−0.01	0.08	0.19*	−0.10	0.12	0.06	0.05	0.16	−0.07
Canopy coverage (%) [Canopy]	−0.40**	−0.06	−0.05	−0.57**	−0.07	−0.51**	−0.26**	−0.11	−0.05	−0.04
Rainfall (mm) [Rain]	0.24**	−0.05	0.06	0.01	0.14	0.20*	−0.02	0.05	0.02	0.11
Encumbrance rate (%) [Encu]	0.45**	0.07	0.12	0.46**	0.13	0.44**	0.27**	0.01	0.17*	−0.06
Water width (cm) [ww]	0.21**	0.11	0.01	0.01	0.05	0.06	−0.06	−0.09	0.10	−0.06
Water depth (cm) [wd]	−0.12	0.07	−0.05	−0.28**	0.06	−0.26**	−0.23**	−0.12	0.03	−0.13
Current velocity (m/s) [cv]	−0.19*	−0.06	0.12	−0.01	0.10	−0.12	0.09	0.03	0.01	−0.11

Only values bearing one or two stars are significant correlations; * or **: correlation is significant at the level $p \leq 0.05$ or $p \leq 0.01$, respectively; abbreviations of environmental variables are given in square brackets

Mtub, *Melanoides tuberculata*; Byth, Bythiniidae undetermined.; Gafr, *Gabbiella africana*; Pacu, *Physa acuta*; Apsp, *Aplexa* sp.; Lnat, *Lymnaea natalensis*; Lsta, *Lymnaea stagnalis*; Lcol, *Lymnaea columella*; Bpfe, *Biomphalaria pfeifferi*; Bfor, *Bulinus forskalii*

still, colorless and sausage-shaped oval or cylindrical. They were identified as belonging to freshwater pulmonates of the families Physidae and Lymnaeidae. The physid egg capsules collected were elongate-oval with a mean length of 10.86 ± 1.46 mm, bearing an average number of 30.03 ± 4.83 eggs per egg capsule. The physid eggs examined were quite spherical with a mean height/width ratio of 1.09 (±SD = 0.03). Concerning lymnaeid, egg capsules examined were elongate-cylindrical with a mean length of 15.43 ± 2.07 mm, bearing an average number of 24.53 ± 3.29 eggs per egg capsule. Eggs of lymnaeid were oval with a mean height/width ratio of 1.48 ± 0.05 (Table 4).

Discussion

The malacological assessment of streams in the Douala catchment identified 10 freshwater gastropod taxa at nine of the ten urban stations; further, no species was found in urban station AT_1 and in suburban stations N_1 and N_2. The absence of snails in the suburban forest stream is likely due to its good health status characterized by slight acid waters, high dissolved oxygen content and very low concentrations of organic matters and diverse dissolved ions. These conditions may not be conducive to the development of freshwater snails which need alkaline water and high concentration of ions (especially calcium) to

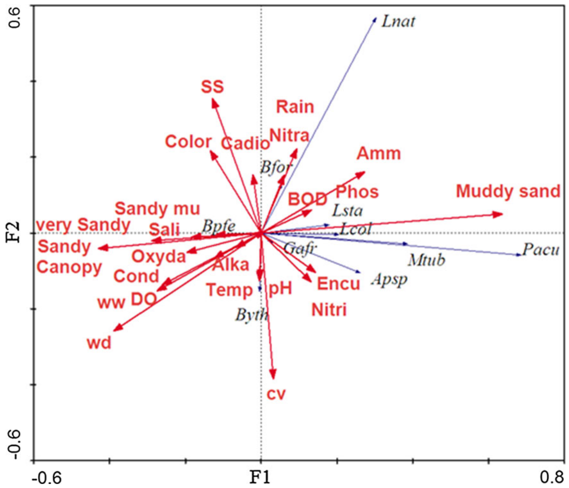

Fig. 8 Redundancy analysis biplot showing gathering of freshwater gastropods in response to environmental variables; see Table 3 for abbreviations

manufacture their shells (Bukowski and Auld 2014). Our results are in accordance with those of Foto Menbohan (2012) and Foto Menbohan et al. (2013) who showed the prominence of snails in urban streams of the Mfoundi river basin in Yaoundé, whereas in suburban streams of the same ecological region, snails were poorly represented with just 0.39 % of the total richness. Similar observations were documented by Camara et al. (2012), who found that in the Banco National Park (Ivory Coast) snails were present only at one site which received domestic sewages. Moreover, experimental works carried out by Marsden and Swinscoe (2014) showed the prominence of a pulmonate snail at the most contaminated site close to a waste treatment discharge point in New Zealand.

The absence of snails in urban station AT_1 situated at 100 m downstream from the outlet of a mixture of industrial effluents is undoubtedly due to industrial pollution. In fact, classification of samples with the SOM map (Fig. 2) based on their environmental parameters showed that station AT_1 was the most polluted with the highest values of temperature (36 °C), pH (9.37), conductivity (3,690 µS/cm), salinity (1.38 ‰), suspended solids (409 mg/L), alkalinity (1,050 mg/L), phosphates (7.72 mg/L), oxydability (152 mg/L), BOD (340 mg/L) and current velocity (0.89 m/s). But, it seemed to be that release of heat industrial effluents enriched with undetermined chemical compounds and high values of current velocity may be the principal factors responsible for this status. These observations are in line with several author's conclusions (Zalizniak et al. 2009a, b; Bernot and Brandenburg 2013; Morrissey et al. 2013; Xu et al. 2013; Colas et al. 2014) who showed the disappearance of many invertebrate groups in presence of chemical pollutants. Our species richness is greater than that obtained by Camara et al. (2012) in Banco River (2 species) and slightly lower than that recorded by Bony (2007) who identified 11 species in three watershed basins in Ivory Coast. This taxonomic richness is lower than those of Foto Menbohan (2012) who announced 17 freshwater snail species in streams of the Mfoundi river basin in Yaoundé and Chapman et al. (2012) who identified 18 species in an urban New Jersey pond.

The gastropod fauna was dominated by *P. acuta* which presents the highest relative abundance (76.95 %) and occurrence frequency (FO = 97.44 %) at the nine stations all-over the study period. Not surprising, this alien invasive pulmonate is tolerant of

Table 4 Morphometric characters along with ranges and mean values (±SD) of egg capsules and eggs of Physidae and Lymnaeidae snails collected during the study period

Snails families	Egg capsules				Eggs				
	Form	NEC	*L* (mm)	NE/EC	Form	NE	*H* (µm)	*W* (µm)	*H*/*W*
Physidae	Elongate-oval	30	6.8–13.1 10.86 (±1.46)	21–41 30.03 (±4.83)	Quite spherical	45	32–40 36.6 (±2.11)	29–37 33.24 (±1.71)	1.03–1.16 1.09 (±0.03)
Lymnaeidae	Elongate-cylindrical	30	13–18 15.43 (±2.07)	19–31 24.53 (±3.29)	Oval	45	50–67 62.2 (±3.92)	33–46 41.96 (±2.89)	1.37–1.59 1.48 ± 0.05

NEC number of egg capsules measured, *NE/EC* number of eggs per egg capsule, *NE* number of eggs measured, *L* length, *H* height, *W* width, *H/W* height/width ratio polluted waters where it may occur in large numbers, up to 3,000/m^2 (Brackenbury and Appleton 1993). Pulmonates are better adapted to harsher conditions due to the fact that they are able to assimilate atmospheric air via a vascularized mantle cavity. With regard to this empirical observation, urban streams of Douala are of very poor health status (Fig. 3) because they constituted the main discharge point for storm waters, urban sewages, household refuse and industrial effluents. Furthermore, Douala is a rainy zone with nine months of rainfall per year, and this climatic condition may be conducive to the proliferation of *P. acuta*. Indeed, Brackenbury and Appleton (1993) reported that where floods occurred repeatedly, *P. acuta* was shown to produce up to seven cohorts in a single year. The worldwide spread and the invasive fitness of snail species of the genus *Physa* (especially *P.* acuta) are well documented (Appleton and Brackenbury 1998; Cope and Winterbourn 2004; Bony et al. 2008; Camara et al. 2012; Chapman et al. 2012; Foto Menbohan 2012; Albrecht et al. 2013). *L. natalensis* and *M. tuberculata* were found in second and third position of dominance, respectively. The prolificness of these freshwater snails in polluted aquatic media has also been reported (Moor and Day 2002; Pointier et al. 2004; Strong and Glaubrecht 2007). In spite of their very low abundances, all the other freshwater gastropod species caught were classified by Bode et al. (2002) as tolerant to pollution. Their scarceness in the urban streams of Douala Township might be related to industrial pollution or to their lack of competitiveness close to exotic species. Although *P. acuta* appreciate polluted waters, the reduction of its abundances at stations T_2, T_3, M_2, AM_2 and AM_3 all situated downstream from the outlet of industrial effluents, highlight its vulnerability to chemical pollutants. This study showed a significant difference in seasonal variations of abundances of the three main snail species (*P. acuta*, *L. natalensis* and *M. tuberculata*). These snail populations showed an increase in abundance at the beginning of the rainy season and a drastic reduction of theirs numbers with increase of the rainfall. This fall in abundances may be attributed to the drift of biological communities caused by surface runoff, which provoked instability of the substratum. Indeed, during the study period, the highest values of rainfall, rivers width and depth, and current velocity were recorded during rainy period. This finding corroborates with those of Pointier et al. (2004), Bony et al. (2008) and Camara et al. (2012).

The shell morphology of *P. acuta*, *L. natalensis* and *M. tuberculata* that we found during this survey is indistinguishable from those described by Brown (1994) in term of size. However, concerning the texture, *P. acuta* shells, especially adults ones, were not as glossy as described by the previous author. We assumed that the slight shagreen of shell of this physid snail might response to chemical pollutants. Indeed, high values of conductivity, alkalinity and dissolved ions were reported in urban stations.

Seasonal distribution of the three main snail species into size classes revealed that recruitment of young generations was confined in the rainy season. This is probably due to their life cycle and their ecological adaptation, what allow the production of new offspring during the rainy seasons. Moreover, the beginning of rainy season is generally associated with increase of habitat and food availabilities by urban and domestic organic matters drained into the river via surface runoff. Several researchers have already shown that reproduction periods of benthic macroinvertebrates, as well as many other organisms, are concomitant with season, habitat quality and food availability (Callisto et al. 2001; Tolley-Jordan and Owen 2008; Garcia-Roger et al. 2011; Martinez and Rogowski 2011). Pointier et al. (1993) and Yapi et al. (1994) pointed out that maximum reproduction of freshwater mollusks takes place during rainy seasons. Our results are in accordance with those of Chapman et al. (2012) who also found that high abundance and occurrence frequency of young stages of *P. acuta* were associated with rainy periods in an urban New Jersey pond. Similar results for *Physa marmorata* were observed in Ivory Coast by Bony et al. (2008) and Camara et al. (2012).

Result of Spearman correlation test and redundancy analysis showed that distribution of freshwater snails was mainly influenced by water temperature, conductivity, the suspended solids, alkalinity, nitrites, nitrates, ammonium, phosphates, oxydability, BOD, rainfall, encumbrance rate of the riverbed and water width. At this purpose, Moor and Day (2002) mentioned that hatching period of freshwater pulmonate eggs decrease with increasing water temperature. Our findings are in accordance with those of Camara et al. (2012) who showed that distribution of *Physa marmorata* was associated with highest values of conductivity and pH and lowest values of dissolved oxygen in an urban reserve. Furthermore, Evans and

Ray (2010) also found that species of the genus *Physa* were associated with high values of pH, calcium hardness, total hardness, total alkalinity, conductivity and total dissolved solids. The increased amount of point source pollution in association with Physidae also corresponds to reported pollution tolerances for the group (Bode et al. 2002).

Structure of egg capsules of Physidae and Lymnaeidae collected during this survey, and the number of eggs per egg capsule did not differ from that described by Moor and Day (2002). This suggests that the proliferation of physid and lymnaeid in our study sites is unlikely due to the increase of the number of eggs laid per egg capsule, but likely to the environmental conditions which favor the growth of offspring until the adult stage. At this purpose, Dana and Appleton (2007) mentioned that *P. acuta* is known to adopt a multivoltine pattern following repeated disturbances to its habitat. Moreover, it has already been shown by Brackenbury and Appleton (1993) that *P. acuta* could respond to flood disturbances by immediate egg laying and the production of a new cohort.

Conclusion

This study demonstrated that the appearance and distribution of freshwater snails in Douala watershed basin reflected the polluted status of its urban streams due to anthropogenic activities. It also revealed the widespread of *P. acuta* in Douala's urban streams. All indications are that this species could have a negative impact on the species diversity of mollusks in these water bodies, and it is recommended that the situation be closely monitored. Though population dynamics offer a useful approach to estimating the rate of spread of the physid *P. acuta*, further investigations have to be carried out to well understand the origin and the invasive capabilities of these exotic freshwater snail invaders. Furthermore, high abundances and occurrence frequencies of physid and lymnaeid snails which are excellent intermediate hosts of many trematode flukes and the presence of planorbid snails—intermediate hosts of Schistosomes—constitute a potential risk for the emergence of helminthiasis in Douala. Therefore, there is the need for an elaborate characterization of wastewaters and water bodies, and evaluation of treatment facilities of domestic, municipal and industrial wastes in order to assess the status and control water pollution in Douala city.

Acknowledgments This malacological survey was achieved thanks to the financial support of the Intra-African Mobility Program (PIMASO) and the International Foundation for Sciences (IFS). Authors would like to gratefully acknowledge Pr. Jean Pierre POINTIER and Dr. Norbert Kouakou KOUADIO for their help in taxonomic verifications of the biological material. Authors also express their gratitude to Dr. Olive Vivien NOAH EWOTI and Mr. Henry Bruno DOMCHE TEHAM for their help during the sampling campaigns.

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