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Diversity and dynamics of the fongic community of four fish farms of the south Cote d'Ivoire

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Abstract
The purpose of this study is to evaluate the diversity and dynamics of the fungal community in the waters of four fish farms in Côte d'Ivoire. This oomycete community consists of 14 species divided into 6 genera. The genus Saprolegnia is the most diverse with 5 species. The genera Achlya and Aphanomyces are represented respectively by 4 and 2 species. Each of the genera Dictyuchus, Leptolegnia and Sommerstorfia contains one species. At the spatial level, the species Saprolegnia glomerata and Saprolegnia parasitica were the most common species with a frequency percentage of more than 70% because they were seen in most stations on different farms. Whereas at the temporal level, the species Saprolegnia parasitica is the species relatively encountered during sampling months with a percentage ***of*** *occurrence of 53.12%. These different identified species highlight the exposure status of Oreochromis niloticus to the risk of fungal infections on fish farms in Côte d'Ivoire.*

Key words*: Aquatic mushrooms - Fungal community - Fish farms - Ivory Coast.*

Introduction
In Côte d'Ivoire, fish is one of the primary source of animal protein with a contribution of about 15 kg / inhabitant / year (1). Domestic production is estimated at 70 000 tons, of 1.57% which comes from aquaculture. This production covers barely 23% of the needs of the Ivorian population. The deficit is offset by massive imports of frozen fish (2). Various factors are the root of this low production, including high production costs and poor quality of food (3). Today, despite the use of quality food and good technicality of the actors, several cases of mortality leading to a drop in production and abnormal behavior of fish have been observed in some fish farmers (4). At these often massive and unexplained mortalities, stunting during the different rearing phases, the appearance of cottony tufts on the skin of fish and their eggs, were observed. This shows the presence of molds or aquatic fungi (oomycetes) and their share of responsibility in the low production of farmed fish.

Oomycetes are the most important fungal pathogens of freshwater and brackish fish. The majority of these pathogens belong to the family Saprolegniaceae (5). The genera of Saprolegniaceae *Saprolegnia*, *Aphanomyces* and *Achlya* can infect amphibians, molluscs, crustaceans (6), fish and their eggs causing the disease called saprolegniasis (7). Saprolegniasis is one of the most devastating diseases causing the destruction of clutches in aquaculture (8). It is responsible for severe damage at all stages of fish development (9). The first stages are the most vulnerable, where eggs and larvae can be destroyed after a few hours or even days. Aquaculture promotes the proliferation of microorganisms that can induce many pathologies in fish (10). The health of fish in captivity is therefore an aspect not to be neglected. Unfortunately, in Côte d'Ivoire, scientific knowledge of mycological captive fish and their consequences are almost non-existent. While the knowledge of the composition of the mycological flora of freshwaters and the damage allow to better control the pathologies of farmed fish (11). It is in this context that this study proposes to evaluate the diversity and dynamics of aquatic fungus species from the oomycete group of four freshwater fish farms in Côte d'Ivoire.

Material and Methods

2.1- Presentation of the environment study

The study area study concerned southern Côte d'Ivoire between 5º20 'and 5º70 north latitude and between 3º80' and 4º40 'west longitude. In this area, four fish farms were visited (Figure 1). These are the fish farms of Blondey (05 35 '453' 'N and 04 05' 426 " 0), Azaguié (05 37 '629' 'N and 04 07'783"O), Dabou (05 19'458"N and 04 23'552"W) and Mopoyem Experimental Aquaculture Station (05 19 '855"N and 04 06'666"W).

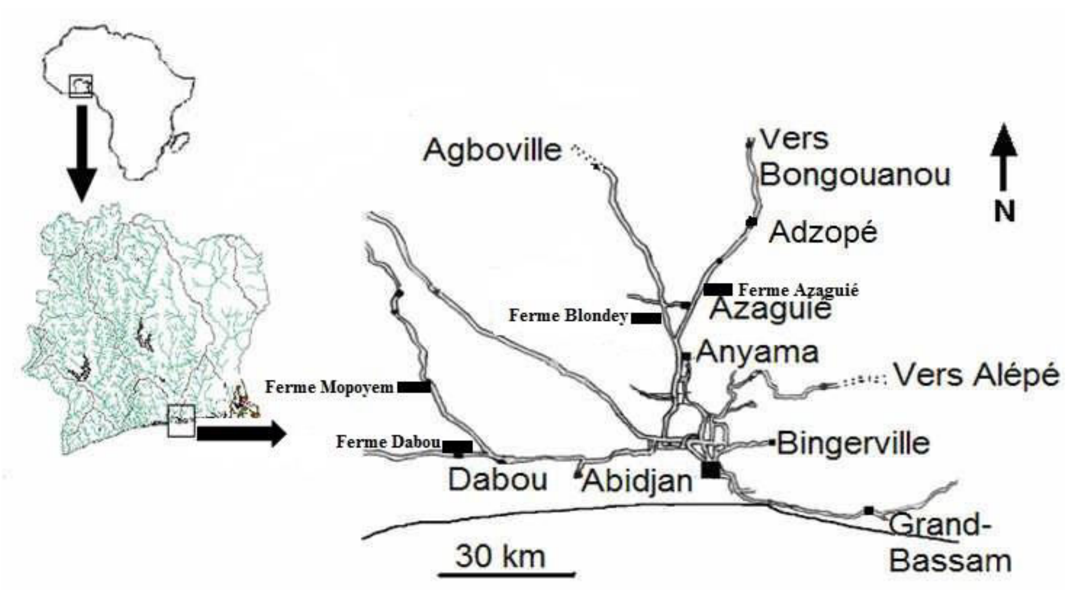

Figure 1: Location of Study Farms

2.2- Materials

Measurement of physical and chemical parameters and mycological analysis required the use of some a number of devices. A multi-parameter Hach Lang hq 40d was used for the determination of water temperature, pH, dissolved oxygen concentration and conductivity. For the mycological analysis, sterile PVC bottles of one liter capacity, a set of glassware, apparatus and consumables were used. Mushroom cultivation required the use of SDA (Sabouraud Dextrose Agar) agar supplemented with chloramphenicol, Petri dishes, an oven, a water bath and an autoclave. Mushroom observations were made using an AXIOSTAR optical microscope and a SONY-branded digital camera was used for the shooting.

2.3- Methods

Monthly samplings were conducted from September 2015 to August 2016. But because of major logistical constraints, sampling in October, December, February and April could not be done. On each farm, fish and water were collected from the ponds following the rearing stages (rearing, pre-fattening and rearing). The spreading technique recommended by Das *et al.* (2012) was used for isolation of oomycetes. It required the use of SDA (Sabouraud Dextrose Agar) supplemented with chloramphenicol (13). The (14) method has been applied for conservation. Identification, based on the characteristic of asexual and sexual reproductive structures, was made in two stages (15). After a macroscopic observation of colonies in culture (appearance, color, shape, growth time), culture fragments were microscopically examined between slide and coverslip in water with a drop of giemsa (13).

2.4- Expression of results

2.4.1- Taxonomic wealth

The species richness or raw diversity represents the total number of species encountered in a medium. It is good indicator of the environment (16).

2.4.2- Percentage of occurrence

The percentage of occurrence is to count the number of times the species i appears in the observations (17). This is the ratio expressed as a percentage between the number of samples (Fi) where the species i appears and the total number of samples (Ft) of the biocenotic unit under consideration. It is obtained according to the following formula.

$$F = \frac{Fi \times 100}{Ft}$$

According to the value of F, the following classification is adopted (18):

- Very frequent species ($F \geq 70\%$);
- Frequent species ($40\% < F < 70\%$);
- Infrequent species ($10\% < F < 40\%$);
- Sporadic species ($F < 10\%$).

2.5- Statistical analysis

In this study, the Kruskal-Wallis test was first used to compare the series of values of all the stations with each other for each measured parameter. When a significant difference is highlighted between the considered objects (stations or farms), a comparison two by two of the distributions makes it possible, in a second time, to look for the distributions which are responsible for the heterogeneity. This two-to-two comparison was made using the Mann-Whitney U-test. The Kruskal-Wallis and Mann-Whitney tests were performed using the STATISTICA 7.1 software, maintaining the significance threshold p at 0.05.

RESULTS

3.1- Composition of the mycological stand

The table I presents the lists of species of aquatic fungi harvested on the different farms. A total of fourteen (14) species belonging to six genera were recorded. These genera are: *Achlya*, *Aphanomyces*, *Dictyuchus*, *Leptolegnia*, *Saprolegnia* and *Sommerstorfia*. The genus *Saprolegnia* is the most diverse with 5 species (*S. ferax*, *S. glomerata*, *S. luxurians*, *S. parasitica* and *Saprolegnia* sp.). Then comes the genus *Achlya* with 4 species. This genus includes: *A. ambisexualis*, *A. americana*, *A. hydrogyna*, and *A. abortispora*. Following the genus *Achlya*, the genus *Aphanomyces* includes 2 species (*A. irregularis* and *A. parasiticus*). Finally, the genera Dictyuchus, *Leptolegnia* and *Sommerstorfia* each contains a single species, respectively *Dictyuchus pseudodictyon*, *Leptolegnia subtteranea* and *Sommerstorfia spinosa*.

At the farm level, the Mopoyem farm contains the highest taxonomic composition with 14 species. Respectively, Dabou and Blondey farms are second and third respectively with 13 and 12 species. These farms differ from that of Mopoyem in the absence of *Sommerstorfia spinosa* in the Dabou farm and *Saprolegnia luxurians* and *Sommerstorfia spinosa* in Blondey farm. Finally, the Azaguié farm includes 8 species that are *Achlya androgyna*, *Achlya ambisexualis*, *Achlya americana*, *Aphanomyces irregularis*, *Saprolegnia glomerata*, *Saprolegnia parasitica*, *Saprolegnia* sp. and *Sommerstorfia spinosa*. The photograph of these different species is illustrated in Figure 2.

Table I: List of oomycete taxa encountered in the waters of the four farms studied

			FARMS			
Family	**Genus**	**Taxons**	**Dab**	**Mop**	**Blo**	**Aza**
Saprolegniaceae	***Achlya***	*Achlya abortispora*	+	+	+	-
		Achlya androgyna	+	+	+	+
		Achlya ambisexualis	+	+	+	+
		Achlya americana	+	+	+	+
	Aphanomyces	*Aphanomyces irregularis*	+	+	+	+
		Aphanomyces parasitica	+	+	+	-
	Dictyuchus	*Dictyuchus pseudodictyon*	+	+	+	-
	Leptolegnia	*Leptolegnia subterranea*	+	+	+	-
	Saprolegnia	*Saprolegnia ferax*	+	+	+	-
		Saprolegnia glomerata	+	+	+	+
		Saprolegnia luxurians	+	+	-	-
		Saprolegnia parasitica	+	+	+	+
		Saprolegnia sp.	+	+	+	+
	Sommerstorfia	*Sommerstorfia spinosa*	-	+	-	+
TOTAL	**6**	**14**	**13**	**14**	**12**	**8**

(+ = Présence ; - = Absence ; Dab = Dabou ; Mop = Mopoyem ; Blo = Blondey et Aza = Azaguié).

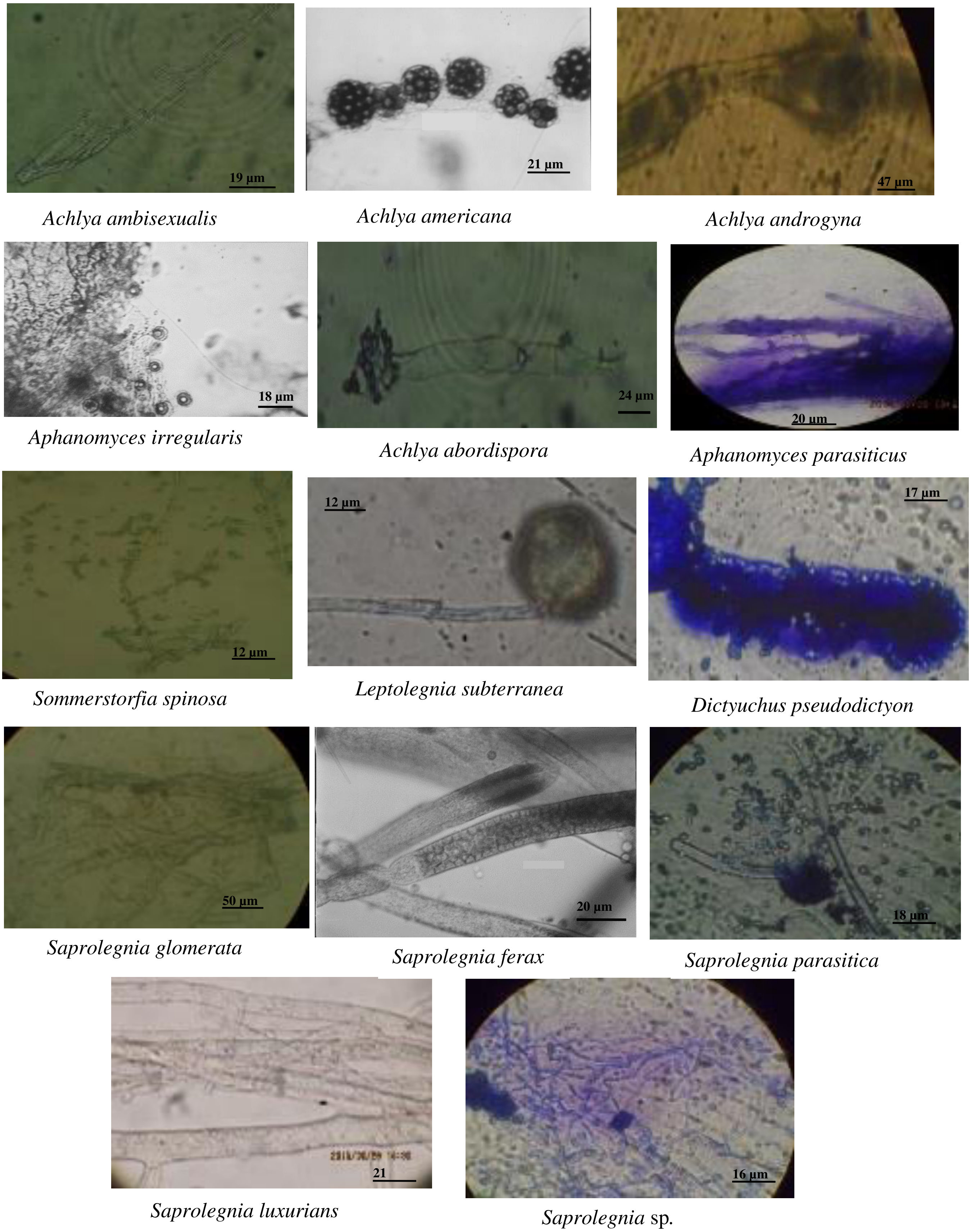

Figure 2: Illustration of oomycetes found in some fish farms in Agnéby-Tiassa and Grands Ponts regions of Côte d'Ivoire

3.2- Spatial variation of the mycological stand

3.2.1- Taxonomic wealth

The figure 3 presents the taxonomic richness determined at the stations of the (studied) farms studied remove it from here. The taxonomic richness at farm stations ranges from 3 to 12 taxa. With a richness from 7 to 12 taxa, Mopoyem Farms have the highest taxonomic richness. Thus, the pregrowing pond of this farm is the most diversified with 12 taxa. Then follow the growing pond, the river and the fry basin with respectively 9, 8 and 7 taxa. Relative to the Dabou farm, the taxonomic richness presents a range of variation that oscillates between 6 and 10 taxa. The river is the least diversified station with 6 taxa while the pre-breeding basin is the most diversified station with 10 taxa. The rearing pond and the fry pond have 9 and 8 taxa, respectively. Consisting of Blondey's farm, the taxonomic richness per station varies from 5 to 8. The pre-breeding basin presents the highest richness with 8 taxa. Next, the fry pond and the magnification pond each contain 6 taxa. Finally, the total number of taxa is 5. The Azaguié fish farm is the farm with the lowest variation range compared to other farms. This range of variation fluctuates between 3 and 4 taxa. The pre-breeding pond has a richness of 4 taxa while the other 3 (three) other stations (dam, fry basin and magnification pond) each contain 3 taxa. On all farms, basins of pregrossing is the station in which the taxonomic richness of oomycetes was the highest.

The results of the statistical analysis (Kruskall-Wallis test (p = 0.011) therefore <0.05) reveal that, at the station level, there is a significant difference between the prefabricating basins of the Dabou and Mopoyem farms and of Blondey, of Azaguié. With regard to variations between farms, the analyzes show that the number of taxa varies significantly (Mann-Whitney U-test: p <0.05) between the taxonomic richness of the Dabou and Azaguié farms. On the other hand, between the Mopoyem farm and the Azaguié farm (Figure 4).

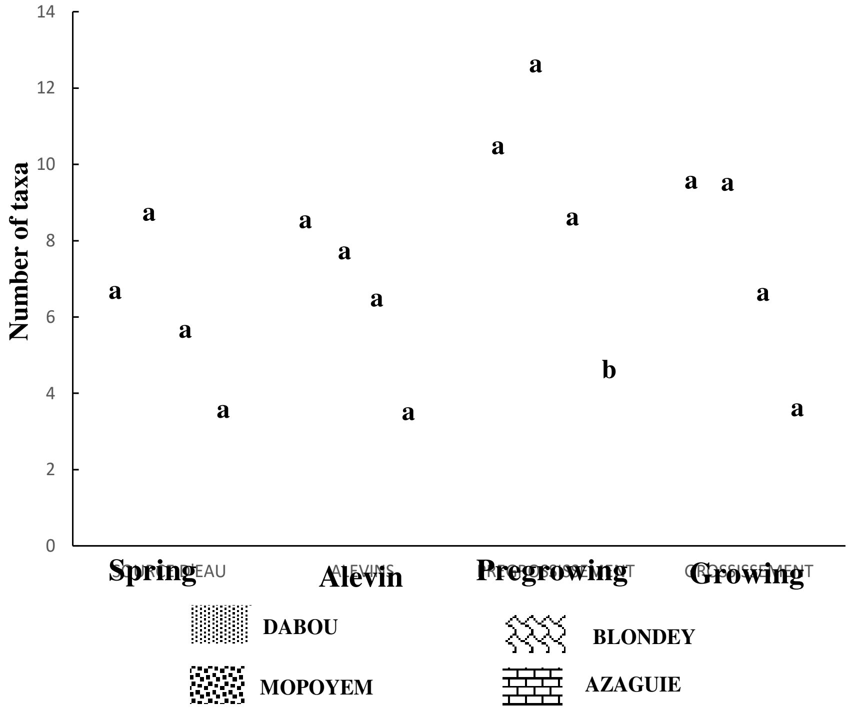

Figure 3: Spatial variation of the taxonomic richness of aquatic fungi harvested on farms

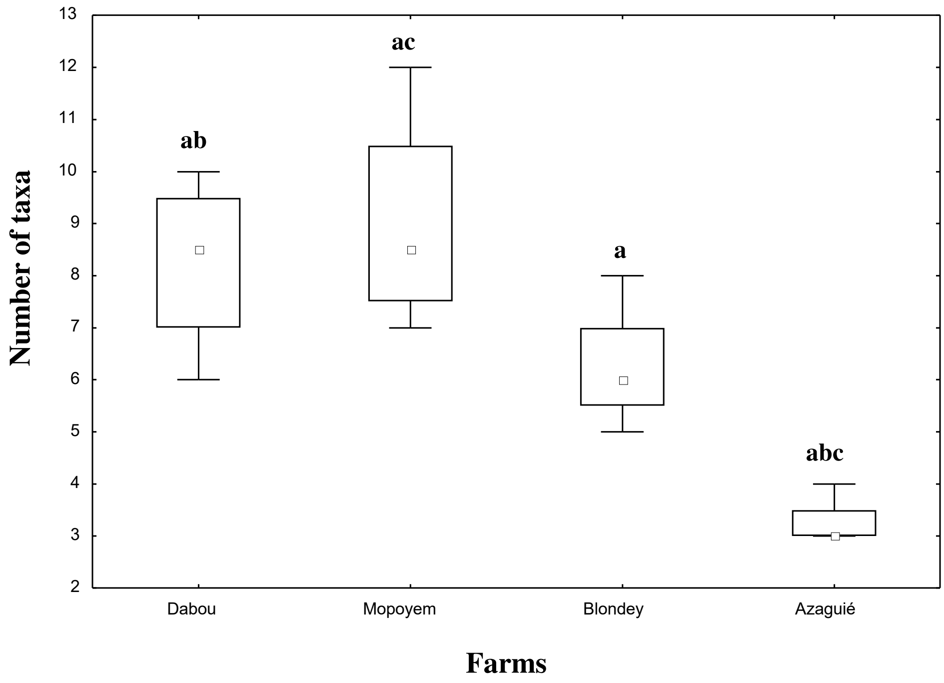

Figure 4: Spatial variation of taxonomic richness of aquatic fungi harvested on farms

3.2.2- Frequency of occurrence

The figure 5 shows the variations in the percentage variations of occurrence of mushroom taxa harvested respectively in the Dabou, Mopoyem, Blondey and Azaguié farms. Thus, the frequency of occurrence of oomycete species at the Mopoyem farm is between 25% (*Sommerstorfia spinosa* and *Achlya abordispora*) and 100% (*Achlya americana*). On the basis of occurrence frequencies, *Achlya americana*, *Achlya ambisexualis*, *Aphanomyces parasiticus*, *Dictyuchus pseudodyctyon*, *Leptolegnia subterranea*, *Saprolegnia ferax*, *Saprolegnia glomerata, Saprolegnia parasitica* and *Saprolegnia* sp. are the taxa very frequently encountered with more than 70% of appearance. On the other hand, *Achlya androgyna*, *Aphanomyces irregularis* and *Saprolegnia luxurians* with 50% each are frequent taxa while the rest of the taxa (*Achlya abortispora* and *Sommerstorfia spinosa*) appeared between 10% and 40% of the samples and are infrequent. With respect to the taxa listed on the Dabou farm, the percentage of occurrence varies from 25 to 100%; which allowed to classify them in three (3) groups. The first group contains the very common taxa, consisting of *Achlya androgyna*, *Achlya ambisexualis*, *Aphanomyces irregularis*, *Aphanomyces parasiticus*, *Saprolegnia glomerata*, *Saprolegnia parasitica* and *Saprolegnia* sp. The second group contains the common taxa *Achlya americana* and *Leptolegnia subterranea*. As for the third (infrequent taxa), it consists of *Achlya abordispora*, *Saprolegnia ferax* and *Saprolegnia luxurians*. For the Blondey fish farm, the identified taxa are also classified in three (3) categories. Thus, the taxa *Achlya ambisexualis*, *Aphanomyces parasitica*, *Saprolegnia glomerata* and *Saprolegnia parasitica* are taxa very frequently encountered (% of occurrence > 70%). While *Achlya americana*, *Aphanomyces irregularis*, *Dictyuchus pseudodyctyon* and *Saprolegnia* sp. Frequently collected taxa and *Achlya abordispora*, *Achlya androgyna*, *Leptolegnia subterranea* and S*aprolegnia* sp. form taxa that are not commonly encountered. The Azaguié farm taxa present between 25 and 75% of the samples, are classified in three groups: very frequent, frequent and infrequent. Thus, the most frequently encountered taxa are *Achlya ambisexualis* and *Saprolegnia parasitica*. On the other hand, only *Aphanomyces irregularis* is the taxon frequently listed in the Azaguié farm samples. *Achlya androgyna*, *Achlya americana*, *Saprolegnia* sp. and *Sommerstorfia spinosa* are taxa that are not commonly encountered.

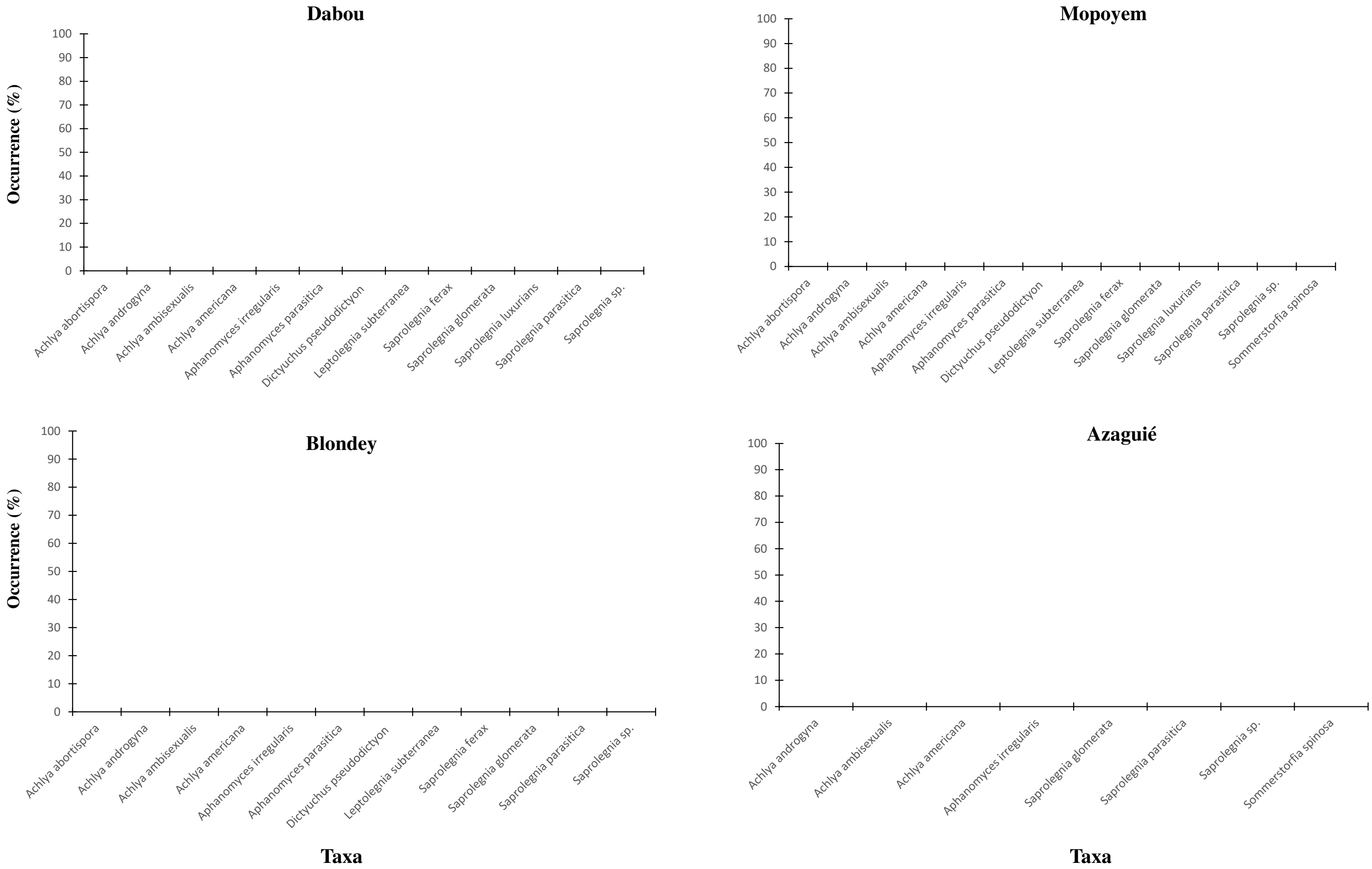

Figure 5: Variations of occurrence of mushroom taxa harvested respectively in the Dabou, Mopoyem, Blondey and Azaguié farms

3.3- Monthly variation of the mycological population

The figure 6 shows the monthly variation in the taxonomic richness of the four (4) fish farms investigated. In considering each of the farms, the taxonomic richness varies from farm to farm. In the Dabou farm, the taxon richness oscillates between 3 and 8 taxa. The months of November, July, May and June being the months of the rainy season, are the months during which the highest taxonomic wealth (respectively 8, 7, 5 and 5 taxa) were recorded. The months of the dry season (March, September, January, and August) are the months during which this wealth is low with 4 taxa for the month of March and 3 taxa for each other month. With regard to the Mopoyem farm, the taxonomic deficit per month varies from 3 to 9 taxa. The months of July, May, November and June (rainy season) are the months that recorded the high values of taxa. These values are respectively 9, 8, 7 and 4 taxa. On the other hand, in the months of September, August, January and March which are the months of the dry season, 4 taxa in September and August and 3 taxa in January and March were noted. The taxonomic richness on the Blondey farm varies from 3 to 6 taxa. In the dry season, 3 taxa were sampled in the months of September, January and March and 4 in the month of August. While in the rainy season, 6 taxa were harvested in July, 5 in November and 4 in May and June. As for the farm of Azaguié, the extreme values of the taxonomic richness which are of 1 taxon and 4 taxa, were determined respectively in the months of January and March (dry season). The months of the rainy season (May, June and July) yielded 2, 2 and 3 taxa respectively. Note that no taxon was harvested in the months of September and November.

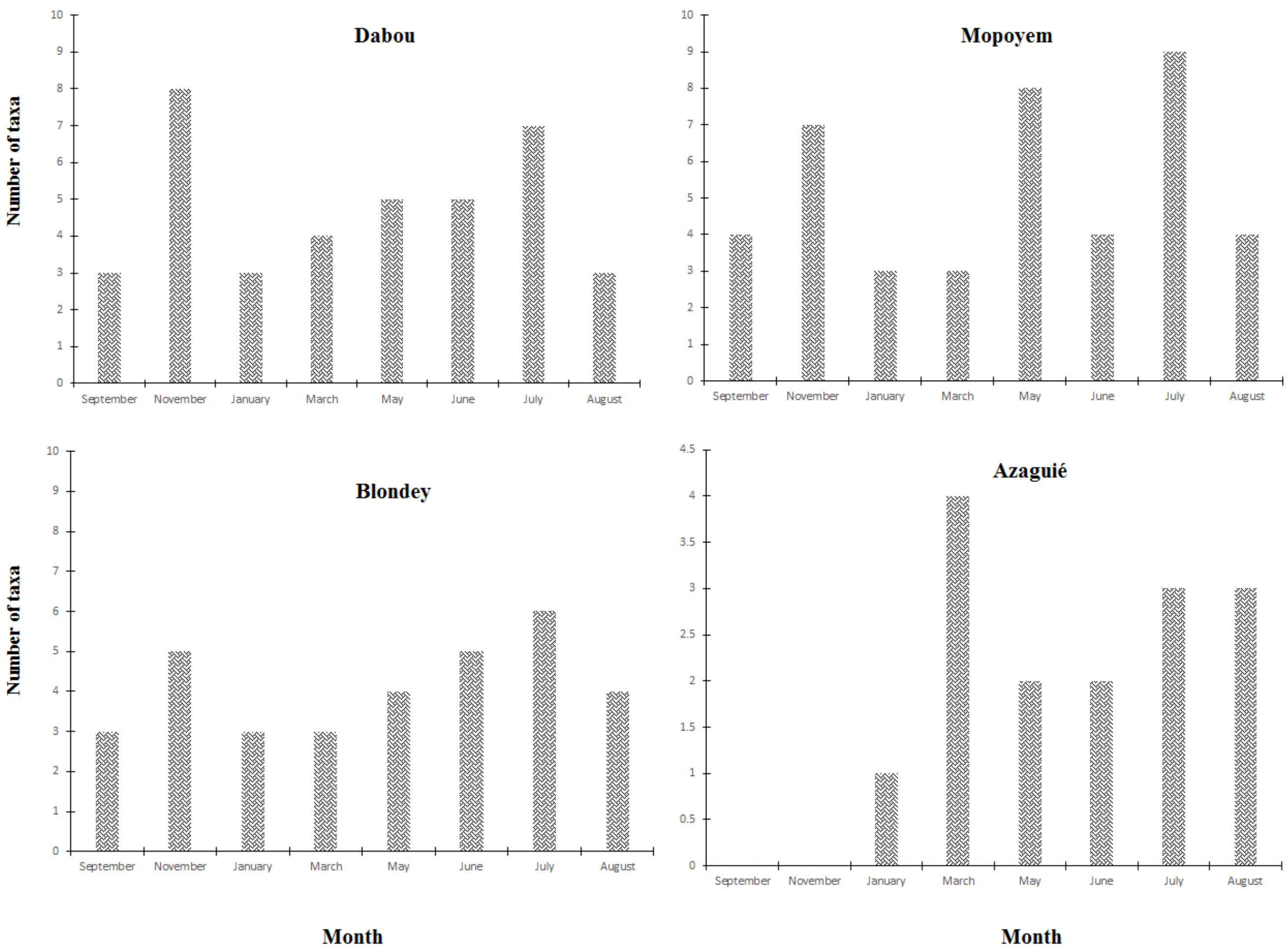

Figure 6: Monthly variation in the taxonomic richness of aquatic fungi harvested in the waters of farms visited

Discussion

Aquatic oomycetes are found all over the world and in all hydrosystems. Several studies have shown their presence in rivers, lakes, ponds, dam reservoirs and even in marine waters (19, 20, 21, 22, 23).

The fungal community in the fish farms visited is composed of 14 taxa. This wealth is greater than that recorded by (24) and (25). These authors collected 10 taxa in Lake Ohrid in Egypt and 11 taxa respectively in the Mula River in the city of Pune in India. These differences could be related to the size, the different hydrosystems considered and the relatively good physicochemical quality of the farmed water. This would increase the number of isolated taxa. Also, they can be due to the nature of the human activities produced around these different hydrosystems. The results obtained are in agreement with those of (26) and (27) who showed that the physicochemical parameters of the different ecosystems studied had an influence on the specific richness of aquatic fungi.

The mycological analysis of the waters of the farms visited, allowed to identify taxa belonging to 6 genera: *Achlya*, *Aphanomyces*, *Dictyuchus*, *Leptolegnia*, *Saprolegnia* and *Sommerstorfia* with a predominance of the genus *Saprolegnia* (5 species) and *Achlya* (4 species). The predominance of these two genera has been reported in some freshwater hydrosystems in South and North America (28). This could be explained by the fact that these surface waters are richer in oxygen and compounds of biological origins such as carbon and nitrogen which are the most important nutrients of aquatic fungi. Oxygen facilitates the breathing activities of organisms in aquatic environments, allowing aquatic fungi to degrade dead or decaying organic matter in order to extract the mineral elements essential for their survival; which would facilitate their reproduction. It plays a very important role in the recycling of dead materials such as debris of plants and animals. Also, the average temperature obtained, fluctuad between 25 and 27°C, would favor the development of the oomycetes because these are mesophyles, that is to say that they develop is around 20 to 30 ° C as indicated in the work of (29) and (30).

The analysis of spatial dynamics reveals a greater diversity of the fungal community on farms in the Great Bridge area than in the Agnéby Tiassa region. Since oomycetes are species that feed on dead material (saprophytes), their distribution on farms would probably be related to the presence of an appropriate substrate. Also, aquatic fungi would generally be associated with clean, well-ventilated freshwater and would be sensitive to pollution. The results obtained are confirmed by (31) and (32). As for the taxonomic abundance, it varies according to the basins made according to the different physiological stages of the fish. This abundance is higher in all pre-breeding pools on all farms. This high level of pre-ponding is thought to be due to the fact that the source of supply, the ponds and ponds are interconnected, and that the pre-silting ponds have a higher surface area than the fry ponds. Hence the strong presence of organic matter that is the source of their diet and the wealth of nutrients and minerals they draw from these fresh waters.

The study of the monthly variation of aquatic fungi in the fish farms of the large Bridges and Agnéby Tiassa regions showed that oomycete abundances are higher during rainy periods (November, May, June and July) and relatively low during dry periods in September, January and March. Our results are corroborated by those of (15); (33) who indicate that oomycete abundance is higher in the cold or rainy season than in the dry season. This result could be explained by the fact that the development of Saprolegniaceae is dependent on the physicochemical parameters of the environment, the most important factor being moisture. The conditions of temperature, oxygen and pH are elements to which they are directly connected. This would promote their reproductive cycle and their development.

Conclusion

The present work has made it possible to characterize the fungal community of the few fish farms in the Grands Ponts and Agnéby Tiassa regions. This characterization concerned the diversity and dynamics of oomycetes. The taxonomic composition observed in the farms surveyed indicates the presence of 14 taxa of aquatic fungi belonging to 6 genera (*Achlya*, *Aphanomyces*, *Dictyuchus*, *Leptolegnia*, *Saprolegnia* and *Sommerstorfia*) and to a single family, that of Saprolegniaceae. The genera *Saprolegnia* and *Achlya* are the most diversified with 5 taxa and 4 taxa respectively. They are followed by *Aphanomyces* with two taxa and *Dictyuchus*, *Leptolegnia* and *Sommerstorfia* with only one taxon each. From a spatial point of view, the taxa *Achlya abordispora*, *A. ambisexualis*, *A. americana* and *Saprolegnia glomerata* are the most frequently encountered taxa. Oomycetes are also more common in pre-breeding pools.

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