

Specific diversity of fish ponds in the Haut-Sassandra and risks of contamination by potential pathogens among *Oreochromis niloticus* (Tilapia)

Abstract

Fishing and aquaculture products constitute a significant part of the nutrient intake in the human diet and a real source of income. However, the fish farming activity is even less known by the Ivorian population and still does not benefit from technical assistance. This could impact the quality of aquaculture resources. This study was therefore carried out to first take stock of fish farming in the Haut-Sassandra region and then assess contamination by potential bacterial pathogens in Tilapia fish from fish ponds in this region. For this reason, a survey was carried out from June to December 2022 using questionnaires. The survey consisted of direct interviews and farm visits to collect data. Then the Tilapia fish were sampled and analyzed. It appears that fish farming is a popular activity in the Haut-Sassandra Region but practiced in a related way and organized in a cooperative society. It is mainly practiced by people aged between 35 and 50, with a majority of primary education. Women are represented at 2.66%. The dominant system is extensive, practiced in dammed and diversion ponds where several activities coexist. In this region we find 100% Tilapia fish (*Oreochromis niloticus*), 21% from Cameroon (*Heterotis niloticus*) and 48% catfish (*Clarias gariepinus*) on all farms. Of the two types of ponds, dam ponds have the best characteristics. This is justified by the low load of microorganisms in these ponds compared to diversion ponds. Fish farming is an activity of the future, however special attention must be given to it to meet the needs of the Ivorian population.

Keywords: Fish farming, ponds, fish, fish farms

1. INTRODUCTION

Meeting the needs for aquaculture resources has become a major challenge throughout the world, particularly in developing countries. Aquaculture production in sub-Saharan Africa remains low despite many years of practice. Despite the immense human, geographical, natural and agricultural potential available to Côte d'Ivoire, fish production remains very low [1]. The number of fish farms is a little over 1000 for a total exploited area of 750 hectares [2]. Indeed, the total annual national aquaculture production was estimated at 3750 tonnes in 2020 and remains very insignificant for the national annual consumption estimated at 286,000 tonnes [3,4]. Currently, Ivorian fish farming only provides nearly 5% of national fish production [5].

To increase production and cover its needs, Côte d'Ivoire through the FAO's "FISH4ACP" initiative, wants to increase investments in fish farms to increase tilapia production (*Oreochromis niloticus*) to 68,000 tonnes by 2031 compared to 3,500 tonnes currently [6]. The Haut-Sassandra region, although being a large agricultural production area, is also known for its potential in fish production [7]. Fish production in the Haut-Sassandra region was 236.72 tonnes in 2013 [2]. However, this fish production only covers 6.32% of local consumption estimated at nearly 4000 tonnes [1]. The main species

produced in fish ponds are Nile tilapia (*Oreochromis niloticus*), catfish (*Clarias gariepinus*) and Cameroon (*Heterotis niloticus*).

In Côte d'Ivoire, if agricultural activity benefits from supervision for its producers, the fish production sector does not always benefit from qualified assistance. Indeed, whatever the type of water supply, fish ponds are strongly influenced by the anthropogenic activities of local populations. They misuse pesticides in agricultural activities [8]. The World Health Organization also indicates that there are nearly 250,000 deaths each year worldwide from pesticide poisoning [9]. In addition, in breeding ponds, uneaten food intake and releases of metabolites from captive fish accumulate in the sediments, thus providing a good source of nutrients for the various bacteria found there. Furthermore, the penetration of rain and the transport of soil particles and organic matter into ponds by runoff could lead to biological pollution of the water, which would contaminate the fish [10]. Agricultural effluents, waste and urban discharges contaminate the aquatic environment with pathogenic bacteria resistant to antibiotics which contaminate fish resources [11]. The presence of bacteria pathogenic to humans in fish resources can lead to foodborne infections and cause serious health problems. In 2016, in Europe, 13.4% of foodborne illnesses originated from aquatic products. The main bacterial agents responsible for these TIAs were *Salmonella* And *Vibrio parahaemolyticus* [12]. Several studies in sub-Saharan Africa have noted microbial contamination of aquaculture resources in Zimbabwe, the Democratic Republic of Congo and Mali [13,14]. This work showed contamination by bacterial pathogens and the risks of infection of populations. 2 In Côte d'Ivoire, studies have also shown the contamination of pathogenic bacterial strains of aquaculture resources [15,16]. The main fish species incriminated were, *Oreochromis niloticus*, *Sarotherodon melanoateron*, *Sardinella maderensis*, And *Chrysichthys nigrodigitatus*. The potentially pathogenic contaminating bacterial species were *Escherichia coli*, *Vibrio parahemolyticus*, *Salmonella* And *Pseudomonas*.

Despite the risks of contamination of various kinds in fish ponds following agricultural and other anthropogenic activities, the problem of health quality of the resources from these ponds, very little scientific data exists on these ponds and their production in Côte d'Ivoire. This observation allows us to justify this study, the general objective of which is to evaluate the contamination by potential bacterial pathogens of fish resources in the Haut-Sassandra Region

2. MATERIAL AND METHODS

2.1. Collection of data from fish farmers

A survey was carried out among fish farmers to collect data on fish production. It consisted of direct interviews and farm visits. Fish farms and fish farmers were selected with the help of cooperatives in Haut-Sassandra to carry out this study.

2.2. Sampling

Tilapia fish (*Oreochromis niloticus*) collected from a few fish farms were used to carry out this study. Approximately twenty (20) tilapia fish samples were collected in the Haut Sassandra region. These are four (4) samples in the Issia area, twelve (12) in Daloa and four (4) in Zoukougbeu. Once collected,

the samples are stored in carefully labeled stomacher bags. They are stored in coolers containing ice and sent directly to the microbiology laboratory for analysis.

2.3. Microbiological analysis of fish samples

2.3.1. Preparation of samples for analysis

Once in the laboratory, the fish samples undergo pre-treatment, then they are dissected aseptically. The gills and viscera which will be used for the various analyzes are separated from the other parts. A quantity of 25 g of each part to analyze (gills and viscera) is homogenized in 225 ml of sterile buffered Peptone water (EPT) previously prepared according to standard NF V08-010. Decimal dilutions will be carried out according to standard [17]

2.3.2. Counting germs in samples

A quantity of 0.1 mL of each decimal dilution retained is placed in Petri dishes containing 20 mL of each agar previously prepared and poured. These are Rapid *E. coli* 2, BEA and Rapid Staph agars. This quantity is spread on the surface of each agar using a sterile spreader. The inoculated plates are incubated at 37°C for 24 to 48 hours for the enumeration of *Staphylococcus aureus* on Rapid Staph, *Enterococcus* on BEA medium and *E. coli* on Rapid medium *E.coli* 2

2.3.3. Research and characterization of *Salmonella*

The research of *Salmonella* was carried out according to standard [18] in 4 steps. This involves pre-enrichment in Buffered Peptone Water broth, selective enrichment in Vassiliadis Rappaport broth, isolation on Héктоen medium and identification using the Leminor Reduced Rack.

2.3.4. Expression of enumeration results

The number N (estimate of the microbial population) was calculated according to the following equation:

$$N (\text{UFC l g}) = \frac{\sum C_i}{(n_1 + 0,1n_2) d.V}$$

N (CFU/g): Number of germs per gram of product;

$\sum C_i$: Sum of colonies counted on all plates retained from successive dilutions;

V: Volume of inoculum applied to each plate (in ml);

n_1 : Number of boxes retained at the first dilution considered;

n_2 : Number of boxes retained at the second dilution considered;

d : Dilution factor corresponding to the first dilution retained.

2.3.5. Statistical analysis

The data collected during the survey were entered with the SPHINX-LEXICA software then processed and analyzed with the SPHINX-LEXICA software. The results were analyzed by Excel 2016 software

for descriptive analyses. Analysis of variance (ANOVA) has a factor at the threshold of $\alpha=0.05$ and Tukey's HSD test were used for calculation and classification of means, respectively.

3. RESULTS

3.1. Profile of fish farmers

Fish farming is practiced as a related activity in Haut-Sassandra. Out of 150 fish farmers surveyed, only 2 practice fish farming as their main activity. The remaining 148 are mostly planters, civil servants and businessmen. The dominant age group is 35-50 years old followed by the elderly (over 50 years old) and mostly having a primary school level. The female gender is less represented with 2.67%. These farms are generally privately owned. However, family farms are often observed (Table I).

Table I: Sociodemographic profile of fish farmers in Haut-Sassandra

	Settings	Number	Percentage (%)
Sex	Man	146	97,33
	Women	4	2,67
Ages (years)	≤ 18 years old	0	0
	18-35 years old	9	6
	35-50 years old	81	54
	≥ 50 years old	60	40
Level study	Unschooling	48	32
	Primary	63	42
	Secondary	25	16,66
	University	14	9,33
Activity main	Fish farming	2	1,33
	Agriculture/ Planter	117	78
	Official	4	2,66
	Trade	14	9,33
	Household	4	2,66
	Employee	9	6

3.2. Production systems

Two fish production systems were observed in Haut-Sassandra. This is the extensive system observed on 80% of the farms surveyed and semi-intensive on the remaining 20% (figure 1). These systems are practiced in two types of ponds : dam ponds and diversion ponds. Of these two systems, the extensive system is the most practiced in dam ponds and in rural areas. As for the semi-intensive system, it is exclusively observed in diversion ponds and particularly in urban and peri-urban areas.

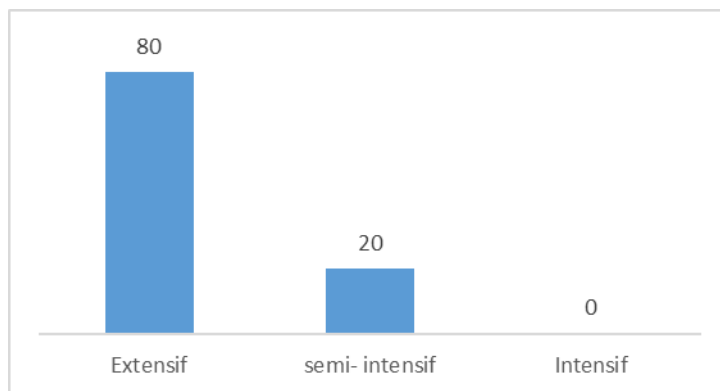
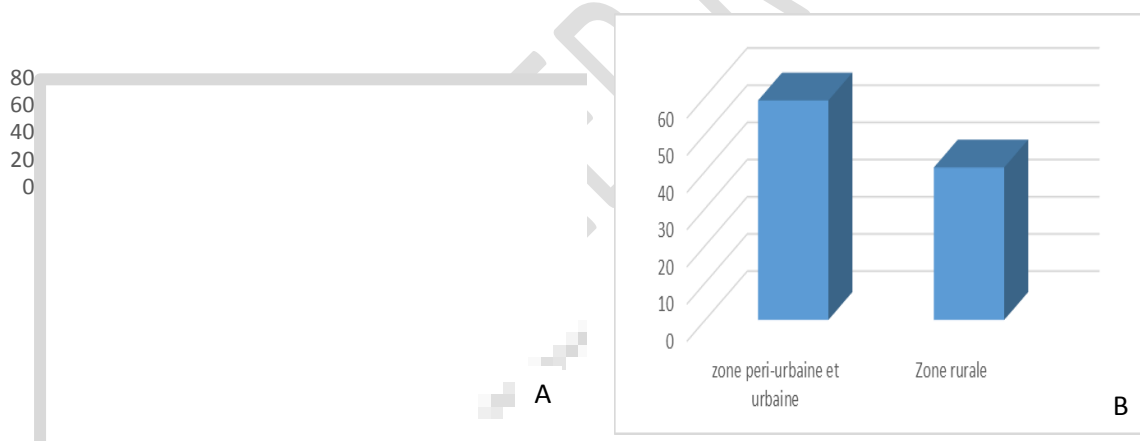


Figure 1 : Fish production systems in Haut-Sassandra

3.3. Distribution of ponds in the Haut-Sassandra Region

Dam ponds and diversion ponds are observed throughout the Haut-Sassandra region. Of the 150 fish farms visited, 132 contain diversion ponds and 45 contain dam ponds. However, diversion ponds are more observed in peri-urban and urban areas (59%) than in rural areas (41%). On the other hand, dam ponds are more observed in rural areas (80%) than in peri-urban and urban areas (20%). We often note the presence of these two types of ponds on the same farm (figure 2).



A : Distribution of dam ponds

B : Distribution of diversion ponds

Figure 2: Distribution of ponds in the Haut-Sassandra Region

3.4. Activities associated with fish farming

Ponds are sometimes the place where several activities coexist. Indeed, fish farming is sometimes associated with rice farming, giving it the name rice-fish farming. This activity is encountered in dammed ponds. The rare dam ponds using this system are only found in rural areas (Bediala, Iouenoufla). In fact, out of 45 dam ponds visited and surveyed, only 6 practice rice-fish farming, or 13% (Figure 3). However, the diversion ponds do not have any associative activities but the fish farmers are considering rice farming.

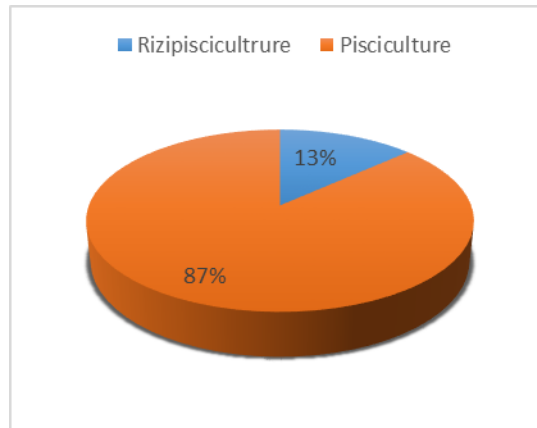


Figure 3: Activities associated with fish farming in dam ponds

3.5. Surrounding activities

Various activities are practiced around the ponds of the upper Sassandra region. These include rice growing, cash crops and sometimes market gardening. Of all these activities, cashew plantations are closest to dam ponds, followed by rice growing and rubber growing. Cocoa cultivation occupies last place with a rate of 11%. Unlike dammed ponds, rice cultivation occupies first place in diversion ponds followed by rubber cultivation and market gardening. We also note the habitats in peri-urban areas which, if classified as an activity, would occupy first place with rice growing. (Table II)

Table II: Activities surrounding fish ponds

Activities surrounding	% in dammed ponds	% in diversion ponds
Cashew Rice farming	33	10
Heveaculture	30	25
Cacao-culture	26	22
Habitats	11	8
Vegetable grower	0	25
	0	18

3.6. Farmed fish species

Three species of fish are found on fish farms. These are the species *Oreochromis niloticus* (Tilapia) commonly called carp, *Heterotis niloticus* (Cameroon) and *Clarias gariepinus* (Catfish). Tilapia fish occupies first place because it is present on all farms, followed by catfish and finally Cameroon fish which comes in third position (Table III). Catfish owe their second place to nature because their presence is not at the whim of fish farmers.

Table III : Different species of fish raised in the ponds of Haut-Sassandra

Name scientist	Name vernacular	% in dammed ponds	% in diversion ponds
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<i>Oreochromis niloticus</i>	Tilapia	100%	100%
<i>Heterotis niloticus</i>	Cameroun	5%	25%
<i>Clarias gariepinus</i>	Catfish	57%	29%

3.7. Evaluation of microbial pathogens in the viscera and gills of fish from some ponds in the Haut-Sassandra Region

3.7.1. Diversion ponds

The microbiological analysis carried out on the fish (Tilapia) from the diversion ponds revealed the presence of the various pathogens sought. These pathogens are present at loads lower than the standards for those of health 2 (*E. Coli* And *S. aureus*). Of the two parts analyzed, the gills are the most loaded. However, *enterococcus* are more present in the gills than the viscera. Out of 12 samples analyzed, only 2 viscera contain *E. coli* against 5 gills. It is the same for the *S. aureus* (Table IV).

Table IV: Contaminants of tilapia from diversion ponds in the Haut-Sassandra Region

Ech	<i>Escherichia coli</i>		<i>S. aureus</i>		<i>Enterococcus</i>		<i>Salmonella</i>	
	Viscera	Gill	Viscera	Gill	Viscera	Gill	Viscera	Gill
1	70±14,14	80±56,56	20±28,28	0±0	Abs	prés	Abs	Prés
2	130±0	20±0	25±35,35	15±21,21	prés	prés	prés	Abs
3	0±0	0±0	5±7,07	10±14,14	Abs	prés	Abs	Abs
4	0±0	0±0	10±14,14	30±0	Abs	prés	Abs	Prés
5	0±0	70±42,42	10±0	0±0	Abs	prés	Abs	Abs
6	0±0	25±35,35	25±0	35±49,49	prés	prés	Abs	Abs
7	0±0	0±0	20±14,14	0±0	Abs	Abs	Abs	Abs
8	0±0	5±7,07	0±0	0±0	Abs	prés	Abs	Abs
9	0±0	0±0	0±0	10±14,14	prés	Abs	prés	Prés
10	0±0	0±0	0±0	25±7,07	Abs	prés	Abs	Abs
11	0±0	0±0	0±0	5±7,07	Abs	prés	prés	Abs
12	0±0	0±0	55±77,78	70±14,14	Abs	Abs	Abs	Abs
Avg	6,91±1,16	16,6±11,7	12±	16,6 ± 10	-		-	
Criteria microbiologic	10² UFC/g		10² UFC/g		Abs/25g		Abs/25g	

3.7.2. Dam ponds

The analysis of the viscera and gills of fish from dam ponds showed a total absence of *E. Coli*. The presence of *S aureus* in the viscera and gills is a reality that this study has just revealed. However, they are present with loads lower than the standard. As for *salmonella*, an absence is observed in the viscera and gill (Table V).

Table V: Contaminants of tilapia from dam ponds in the Haut-Sassandra Region

Ech	<i>E. coli</i>		<i>S. aureus</i>		<i>Enterococcus</i>		Salmonelle	
	Viscera	Gill	Viscera	Gill	Viscera	Gill	Viscera e	Gill
1	0±0	0±0	30±14,14	15±21,21	Pres	Abs	Abs	Abs
2	0±0	0±0	60±84,85	25±35,35	Pres	Pres	Abs	Abs
3	0±0	0±0	0±0	10±14,14	Abs	Pres	Abs	Abs
4	0±0	0±0	5±7,07	5±7,07	Abs	Abs	Abs	Abs
5	0±0	0±0	0±0	10±14,14	Abs	Pres	Abs	Abs
6	0±0	0±0	5±7,07	0±0	Abs	Abs	Abs	Abs
7	0±0	5±7,07	0±0	0±0	Pres	Abs	Abs	Abs
8	0±0	0±0	0±0	0±0	Pres	Abs	Abs	Abs
Avg	0±0	0,62±	12.5±	5,41±				
Criteria								
microbiol	10² UFC/g		10² UFC/g		Abs/25g		Abs/25g	
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4. DISCUSSION

Fish farming is a popular activity in the Haut-Sassandra region. It is practiced in the departments of Daloa, Issia and Zoukougbeu. However, no farm has been identified in Vavoua. This activity owes its existence to its favorable environment [19]. To make this activity more successful, fish farmers have grouped themselves into cooperative societies. A total of six (06) cooperative societies were identified. Indeed, this organization allows clearer visibility on fish farming activity both by fish farmers and by partners. These are mainly APDRA and ANADER (technical partners) and MIRAH (financial partner). Added to these are the FAO and the World Bank. Despite this support, fish farming is practiced as an additive activity to other activities and by people whose age is between 35 and 50 years old. There is an absolute absence of young people under the age of 18 in this activity. This could be explained by the lack of resources (financial and technical), difficult access to spaces and above all the desire of young people for bureaucracy. These observations are similar to those of [20,21].

According to the latter, this situation is due to the fact that access to the fish farming profession remains more open to people who have land, a certain financial autonomy and a workforce. In the city of Brazzaville (Republic of Congo), this activity is mainly practiced by men (95%) aged 50 [22]. Thus, fish farming is an exclusively male activity. This study showed that the female gender was represented at 2.67%. These results corroborate those of [23,24] who observed 2.89% and 2.6% respectively.

According to these authors, the non-involvement of women is explained by the arduousness of the tasks, societal constraints (inaccessibility of land by women, lack of time) and economic constraints. The rare women who practice this activity generally do so through inheritance [25]. Furthermore, for these same authors, the majority of these fish farmers have a primary level. These results are close to ours with a rate of 42%. These results differ from those of [20]. Indeed, these authors showed that in the city of Abidjan, the majority of fish farmers have a higher level of education. In Congo, [26] on the other hand, showed that the majority of fish farmers have a secondary level (55%). If fish farming is constantly emerging in Haut-Sassandra, it must be emphasized that it remains a related activity. Indeed, the survey showed that fish farmers are 78% planters and farmers. This dominance by planters and farmers could be explained by land ownership and a modest financial state. Similar results were observed in Cameroon by [27]. On the other hand, in Benin, [28] affirm that fish farming is a main activity for 32,5.

In Haut-Sassandra, two production systems were observed. This is the extensive system used on 80% of farms (mainly in rural areas) and the semi-intensive system (mainly in urban and peri-urban areas). The dominance of the extensive system could be explained by the lack of industrial feed, fry and especially the financial problems hence the use of agricultural by-products (rice bran, corn, cassava skin, rice stalks, etc.) for fish feeding. In Gontougo, east of the Ivory Coast [29] showed the dominance of this system in fish farming. Similar results were observed in Oued Righ, an Algerian region by [30]. However, the south of Côte d'Ivoire is dominated by an intensive system [31]. The different 10 systems are used in two types of ponds including dam ponds more present in rural areas and diversion ponds in urban and peri-urban areas. According to [23], these two structures are the most encountered in Côte d'Ivoire. However, we observe a fish farming system integrated into agriculture and this only in the dam ponds taking the name of rice-fish farming. This system is, according to fish farmers, less expensive because the stems of the rice plants will be used to feed the fish, reducing thermal shock caused by the sun. Similar results were observed by [1], which shows the presence of this system in Haut-Sassandra. We also note activities such as cashew nut growing, rice growing, rubber growing, cocoa growing around these farms regardless of the structure. In the Menoua department of Cameroon, the surrounding crops are, in order of importance, beans, banana-plantain, macabo/taro, peanuts, soybeans, yams, potatoes and finally cassava. [32].

Aquaculture resources are tilapia fish (*Oreochromis niloticus*), catfish (*Clarias gariepinus*) and Cameroon (*Heterotis niloticus*). Of these three species, tilapia is the main one. This dominance is due to its ability to adapt to environmental conditions, its easier reproduction and above all its nutritional and organoleptic quality. The species *clarias* for it, owes its existence to nature because its cultivation is not done at the discretion of the fish farmer. But its presence in the pond is sometimes used to the benefit of the fish farmer to control activity by reducing unwanted fry. These results are close to those of Anounouet al., (2016) who recorded tilapia (100%), catfish (33.33%), Cameroon (9.09%) and swallowtail (5.56%). In the bandal in Senegal, more than 16 species are bred [33] thus showing a diversity of species and more in-depth knowledge of fish farming. The different fish foods found on farms are agricultural by-products and pellets. Agricultural by-products represent a source of fish food for the majority of fish farmers surveyed, i.e. 78% in this Zone. In fact, this represents less

expense for them, especially since this region has several surrounding cultures. The same is true in Congo where [24] recorded a rate of 43% of fish farmers using agricultural by-products.

In order to determine the level of contamination of fish by microbial pathogens, a microbiological analysis was carried out. Unfortunately the presence of the pathogens sought is a reality revealed by this study. This is *Escherichia coli*, *staphylococcus aureus*, *enterococcus* and *salmonella*. This presence could be due to contamination of anthropogenic origin or the nature of their environment (water). [33] confirm this result during their study on waterways in Yaoundé in Cameroon where they showed that these fish production waters present a high concentration of bio-pollution indicators (*E. coli*, *enterococcus*, total coliform). However for health germs 2 (*E. coli* And *S. aureus*), loads are almost below standards for all farms except. However, these charges differ depending on the ponds and the parts considered. The gills of fish in diversion ponds are heavier than those in dam ponds. This could be due to the filter function of the gills. As for the viscera, the opposite effect is observed.

Regarding the other germs sought, general dissatisfaction is observed due to their presence. Fish in diversion ponds are more loaded with germs 11 enterococcus than those of dam ponds with loads ranging from 5 ± 7.07 to 840 ± 339.41 and from 5 ± 7.07 to 60 ± 14.14 respectively. As for salmonella, a total absence was observed in the dam ponds.

5. CONCLUSION

The objective of this study was to evaluate the contamination by potential bacterial pathogens of fish resources in the Haut Sassandra region, an area with high fish production in Ivory Coast. For this, a good knowledge of the activity was necessary. This study therefore showed that fish farming is an important activity for the Haut-Sassandra Region. However, special consideration must be given to fish farming because those involved in this field are faced with enormous difficulties. This activity is practiced in a favorable environment. Operations are carried out in dam ponds and diversion ponds. Microbiological analyzes revealed the presence of potentially pathogenic species, notably *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp* and *Enterococcus* in the viscera and gills of tilapia (*Oreochromis niloticus*) the emblematic fish of these fish ponds. However, dammed ponds provide more assurance on the microbiological quality of the tilapia found there

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