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Ichtyological inventory using environmental DNA and fisheries in the middle course of the Comoé river (Côte d'Ivoire)

Nehin Tranan Ella Emiline Flora*[1], Aboua Bénié Rose Danielle[1], Soro Tieligounon Ali[2], Doumbia Lassina[3], Ouattara Alassane[3]

[1]*Laboratory of Natural Environments and Conservation of Biodiversity, UFR Biosciences, Félix Houphouët Boigny University, Cocody, Côte d'Ivoire*

[2]*Alassane Ouattara University of Bouaké UFR Sciences and Technologies, Bouaké, Côte d'Ivoire*

[3]*Environment and aquatic biology laboratory (LEBA), UFR Science and Environmental Management (SGE), Nangui Abrogoua University, Abobo-Adjamé, Côte d'Ivoire*

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Abstract

This study aims to demonstrate the complementarity between traditional fishing methods and environmental DNA in establishing a fish stock sampling of the middle Comoé river. This study was conducted quarterly in the middle of the Comoé River from March 2021 to March 2022 using traditional fishing methods and environmental DNA (eDNA). Traditional methods consisted of using monofilament nets, multifilament, traditional traps and hooks. Gillnets were used at 57.8%, followed by traps at 42% and hooks at 0.2% to obtain the stand. The use of eDNA consisted of filtering 730 ml of water. The filtrate was stored and transported to the laboratory, then extracted, amplified and sequenced. Data was processed using bioinformatics. The inventory of the environment yielded 103 species. For experimental and commercial fisheries, 29 species and 32 species of fish were respectively identified. The eDNA has revealed 76 species, including 37 families, 74 genera and 43 identified species. The eDNA allowed having 55.47% of all species harvested, 23.36% for commercial fishing and 21.17% for experimental fishing. The eDNA method reduces the effort, time, resources and costs involved in sampling. Also, through the reliable genetic reference base, species identification is easier and faster.

* **Corresponding Author:** Nehin Tranan Ella Emiline Flora ✉ nehin.flora@gmail.com

Introduction

Aquatic environments are essential to human well-being. They provide a wide range of ecosystem services and are home to exceptionally diverse living organisms. However, the continued increase in human activities is causing profound disruption to the environments. Or, it threatens the state of freshwater ecosystems and their functioning both locally and globally (Benateau *et al.*, 2019; Reid *et al.*, 2019). It is therefore important to protect and preserve these aquatic ecosystems which constitute a major challenge. The vast majority of work on ichthyofauna sampling is based on the use of traditional methods including gillnet fishing, electrofishing, trap fishing and line fishing (Yao, 2006; Aboua, 2012). However these methods alone do not allow a complete inventory of populations and remain an expensive and labor-intensive method. To better understand biodiversity a new method has emerged with the potential to overcome the limitations of pre-existing methods: environmental DNA (eDNA).

eDNA consists of extracting DNA from the environment without having to isolate the organism in advance (Rees *et al.*, 2014). This is facilitated by trace fragments such as shed skin body fluids, metabolic waste, or gametes or blood left by organisms in their environment (Bohmann *et al.*, 2014; Taberlet *et al.*, 2012). This non-invasive and minimal-effort method allows the detection and identification of rare and stealthy species in all types of ecosystems (Bohmann *et al.*, 2014). This new method is an opportunity to enhance fish biodiversity monitoring methods and increase their efficiency. This study aims to show the complementarity between traditional fishing methods and environmental DNA in establishing a fish population sampling of the middle course of the Comoé River.

Materials and methods

Study area

This study was carried out in the middle course of the Comoé River. The latter is located in the south-eastern part of Côte d'Ivoire, in the department of Bettié. This study area is located between latitudes 6°14'54" and 6°03'49" and longitudes 3°27'6" and 3°25'23". Five stations (M'Basso, Manzan, Yêrê-yêrê, Abradinou and Bettié) were sampled along this portion of the river (Fig. 1). The stations of M'basso, Yêrê-yêrê, Abradinou and Bettié are located on the main bed and the station of Manzan is on one of its tributaries which come from the classified forest of Manzan.

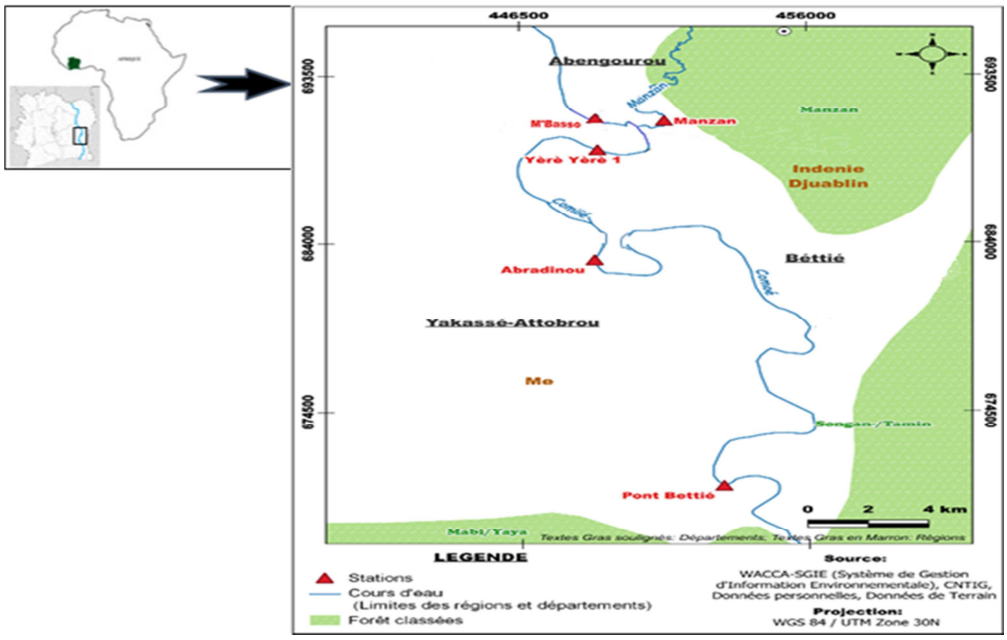

Fig. 1. Geographical location of the middle course of the Comoé River and location of sampling stations

Data sampling

Experimental fishing was carried out using monofilament gillnets with 10, 12, 13 mm mesh size and multifilament nets with 15, 30, 35 mm mesh size. The gillnets were 25 m long and had a drop height of 1.5 m. The nets were set between 4 p.m. and 5 p.m. and collected the next morning between 8 a.m. and 10 a.m. Inventory the data from experimental fishing were supplemented by inventory data from commercial fishing in order to increase the probability of having a high diversity of fish. Commercial fishing was carried out with different gear such as gillnets, traditional baited traps, and baited hooks. The fish were identified using the identification key of Paugy *et al.* (2003a and b).

Data collection for eDNA comes from the waters on the shore of the different stations. A total of three samples were taken at three stations. Using a 100 ml syringe 750 ml of water was filtered through a filter capsule (porosity 0.8 µm). A preservation solution contained in a small 1.5 ml syringe was injected, closed, shaken and then stored at room temperature before being sent to the laboratory for analysis. DNA is extracted from a commercial DNA extraction kit. DNA was purified and then amplified by PCR for a hypervariable region of the 125 tRNA gene to target vertebrates (fish). The analysis includes 12 replicates of the PCRs per sample. The amplified DNAs are then sequenced using an Illumina MiSep V3 kit at 22.5 pM with a PhiX spike in of 20%. Sequence data were processed using a custom bioinformatics pipeline for quality filtering OTU (Operational Taxonomic Unit) clustering and taxonomic assignment. Consensus taxonomic assignments were performed for each OTU using sequence similarity searches in the NCBI nt reference database (GenBank).

Field data processing

The population data were treated by relative importance and frequency of occurrence. The frequency of occurrence is the ratio expressed as a percentage of the number of samples of a given species to the total number of samples carried out (Dajoz, 1974). It is calculated by the following formula:

$F = (Pa/P) \times 100$

Pa = total number of samples containing the species to be considered; P= is the total number of samples.

According to Scherrer's classification (1984) six categories are described:

F = 100: the species is said to be omnipresent,

F > 75: species are considered constant,

50 < F ≤ 75: species are considered regular,

25 < F ≤ 50: species are considered accessory,

5 < F ≤ 25: species are considered accidental,

F < 5: species are considered rare.

Results

Comparison between traditional methods and eDNA

The results presented in Table 1 highlight 103 species divided into 19 orders and 38 families. With regard to commercial fishing, 32 species belonging to 8 orders and 14 families were identified. Commercial fishing is grouped into eight orders, 14 families and 32 species. The inventory carried out using eDNA has made it possible to highlight 76species. Among these 76species, 43 were formally identified to species. The other 43 were identified to genus. Eight species were sampled through commercial fisheries only versus three species through experimental fisheries. The eDNA method detected 62 species that could not be detected by other methods.

At the PE and PC level the Siluriformes represent the most diverse order with four and five families respectively. Followed by the Perciformes with two and three families, then come the Characiformes with two families. The orders of Polypteriformes Osteoglossiformes, Cypriniformes and Synbranchiformes are represented by a single family in the experimental fishery as well as in the commercial fishery with the absence of the order of Polypteriformes.

Table 1. List of fish species sampled by traditional fishing methods and eDNA in the middle course of the Comoé River

Orders	Family	Species	PE	PC	eDNA	F (%)
Polypteriformes	Polypteridae	*Polypterus endlicherii*		+	+	66.66
		Polypterus sp.			+	33.33
Elopiformes	Elopidae	*Elops* sp.			+	33.33
Clupeiformes	Clupeidae	*Ethmalosa fimbriata*			+	33.33
		Sierrathrissa sp.			+	33.33
Osteoglossiformes	Arapaimidae	*Heterotis niloticus*			+	33.33
	Mormyridae	*Hyperopia baby*			+	33.33
		Marcusenius furcidens	+	+	+	100
		Marcusenius senegalensis	+	+		66.66
		Marcusenius ussheri	+	+		66.66
		Mormyrops anguilloides	+	+	+	100
		Mormyrus rume	+	+	+	100
		Mormyrus subundulatus			+	33.33
		Petrocephalus sp.			+	33.33
		Petrocephalus bovei	+	+		66.66
		Pollimyrus isidori	+			33.33
	Notopteridae	*Papyrocranus afer*			+	33.33
Characiformes	Alestidae	*Alestes* sp.			+	33.33
		Alestes baremoze	+	+		66.66
		Brycinus sp.			+	33.33
		Brycinus imberi	+	+		66.66
		Brycinus macrolepidotus	+	+		66.66
		Brycinus nurse	+			33.33
		Micralestes sp.			+	33.33
		Micralestes elongatus	+			33.33
		Hydrocynus forskalii	+	+		66.66
	Distichodontidae	*Distichodus* sp.			+	33.33
		Nannaethiops sp.			+	33.33
		Nannocharax sp.			+	33.33
		Neolebias unifasciatus			+	33.33
		Distichodus rostratus	+	+		66.66
	Hepsetidae	*Hepsetus odoe*			+	33.33
Cypriniformes	Cyprinidae	*Barbus* sp.	+		+	66.66
		Enteromius trispilos	+			33.33
		Clypeobarbus sp.			+	33.33
		Enteromius callipterus/eburneensis			+	33.33
		Enteromius sp.			+	33.33
		Labeo parvus	+	+		66.66
		Labeo Coubie	+	+	+	100
		Labeo sp.			+	33.33
		Labeobarbus sp.			+	33.33
Cyprinodontiformes	Nothobranchiidae	*Epiplatys* sp.			+	33.33
		Nimbapanchax petersi			+	33.33
		Nimbapanchax sp.			+	33.33
	Poeciliidae	*Aplocheilichthys spilauchen*			+	33.33
		Aplocheilichthys sp.			+	33.33
Anguilliformes	Ophichthidae	*Myrophis* sp.			+	33.33
Beloniformes	Belonidae	*Strongylura senegalensis*			+	33.33
		Tylosurus crocodilus			+	33.33
	Hemiramphidae	*Hyporhamphus* sp.			+	33.33
Siluriformes	Ariidae	*Carlarius* sp.			+	33.33
	Claroteidae	*Chrysichthys nigrodigitatus*	+	+	+	100
		Chrysichthys maurus		+		33.33
		Chrysichthys sp.			+	33.33
	Schilbeidae	*Schilbe mandibularis*	+	+		66.66
	Amphiliidae	*Amphilius* sp.			+	33.33
	Clariidae	*Clarias gariepinus*			+	33.33
		Clarias anguillaris		+		33.33
		Clarias sp.			+	33.33

		Heterobranchus isopterus		+		33.33
		Heterobranchus longifilis	+	+		66.66
	Malapteruridae	*Malapterurus* sp.			+	33.33
		Malapterurus electricus		+		33.33
	Mochokidae	*Synodontis membranaceus*			+	33.33
		Synodontis bastiani	+	+		66.66
		Synodontis comoensis		+		33.33
		Synodontis punctifer		+		33.33
		Synodontis schall	+	+		66.66
Mugiliforms	Mugilidae	*Liza* sp.			+	33.33
		Mugil bananaensis			+	33.33
		Mugil cephalus			+	33.33
		Neochelon falcipinnis			+	33.33
		Neochelon sp.			+	33.33
		Parachelon grandisquamis			+	33.33
		Parachelon sp.			+	33.33
Anabantiform	Anabantidae	*Ctenopoma* sp.			+	33.33
Carangiformes	Carangidae	*Caranx* sp.			+	33.33
		Trachinotus sp.			+	33.33
Cichliformes	Cichlidae	*Coptodon zillii*	+	+	+	100
		Limbochromis sp.			+	33.33
		Oreochromis niloticus	+	+	+	100
		Pelmatolapia mariae			+	33.33
		Thysochromis ansorgii			+	33.33
		Hemichromis fasciatus	+			33.33
		Chromidotilapia guntheri		+		33.33
		Sarotherodon galilaeus	+	+		66.66
Gobiiformes	Eleotridae	*Eleotris* sp.			+	33.33
	Gobiidae	*Awaous* sp.			+	33.33
		Bathygobius sp.			+	33.33
		Gobius sp.			+	33.33
	Haemulidae	*Plectorhinchus* sp.			+	33.33
		Pomadasys perotaei			+	33.33
Perciformes	Gerreidae	*Gerres* sp.			+	33.33
	Channidae	*Parachanna obscure*		+	+	66.66
	Latidae	*Lates niloticus*	+	+	+	100
	Lutjanidae	*Lutjanus* sp.			+	33.33
	Monodactylidae	*Monodactylus* sp.			+	33.33
	Sciaenidae	*Pseudotolithus elongatus*			+	33.33
Synbranchidae	Mastacembelidae	*Mastacembelus* sp.			+	33.33
		Mastacembelus nigromarginatus	+	+		66.66
Syngnathiformes	Syngnathidae	*Enneacampus* sp.			+	33.33
		Microphis aculeatus			+	33.33
Pleuronectiformes	Cynoglossidae	*Cynoglossus* sp.			+	33.33
19	38	103	29	32	76	

While with the eDNA method the order of Perciformes and Siluriformes with six families each are the most diverse. Then come the Characiformes and Osteoglossiformes which both have three families. The orders of Mugiliformes, Cyprinodontiformes, Beloniformes are each represented by two families. The orders Anguilliformes, Clupeiformes, Cypriniformes, Elopiformes, Pleuronectiformes, Polypteriformes, Synbranchiformes, Syngnathiformes each have one family. Fig. 2 shows all the orders of the different methods.

The species composition comes from experimental fishing (Fig. 3). It indicates 11 families, the Mormyridae constitute the most diverse family. This family contains seven identified species (24.14%). It is followed by the Alestidae with six species (20.69%), the Cichidae and Cyprinidae with four species each (13.79%). The Mochokidae family has two species (6.90%). The Distichodontidae, Claroteidae, Schilbeidae, Clariidae, Latidae and Mastacembelidae each have a single species (3.45%).

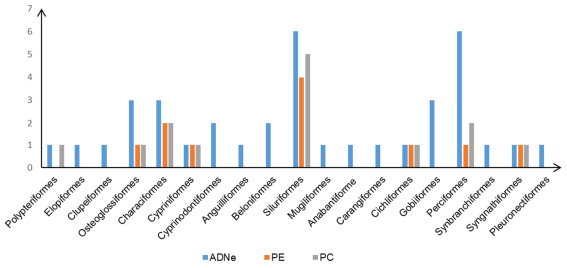

Fig. 2. Comparison of relative abundances of fish orders in experimental, commercial and eDNA fisheries

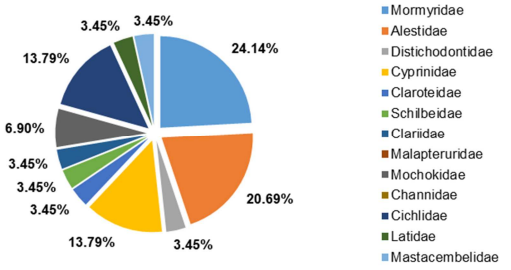

Fig. 3. Relative abundance of fish orders sampled by the experimental fishing method

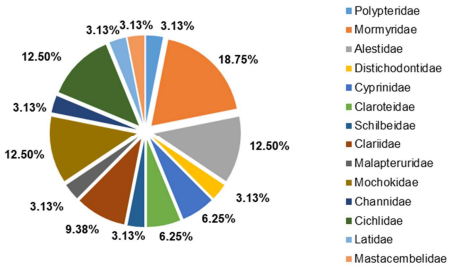

Fig. 4. Relative abundance of fish orders sampled by the commercial fishing method

In terms of commercial fishing, 14 families were identified (Fig. 4). The best represented families are Mormyridae (18.75%) with six species. Alestidae, Mochokidae and Cichlidae each have four species (12.50%). Three species represent the Clariidae family (9.38%). Cyprinidae and Claroteidae are represented by two species each (6.25%). The other seven families (Polypteridae, Distichodontidae, Schilbeidae, Malapteruridae, Channidae, Latidae and Mastacembelidae) are represented by a single species (3.13%).

eDNA analysis revealed a rich diversity of the population with 36 families (Fig. 5). The most represented families according to species are Mugilidae, Cyprinidae with seven species (9.21%) each. Then Mormyridae (7.89%) with six species, followed by Cichlidae and Distichodontidae with five species (6.58%) and four species (5.26%) respectively. Gobiidae, Anabantidae, Nothobranchiidae and Alestidae each have three species (3.95%). Clupeidae, Poeciliidae, Belonidae, Claroteidae, Clariidae, Haemulidae and Syngnathidae each have two species (2.63%). The other 20 families have a cumulative percentage of 26.32%.

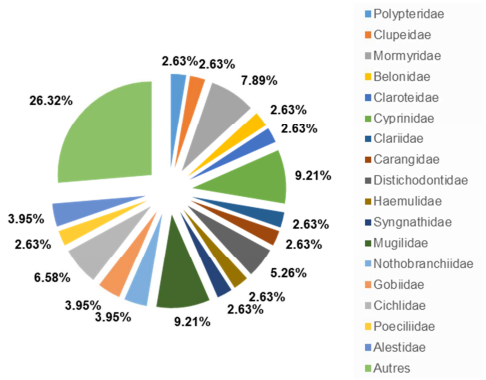

Fig. 5. Relative abundance of fish orders sampled by the eDNA method

Frequency of occurrence

The frequency of occurrence took into account the eDNA method and fisheries. 74.76% of the species areaccessories, 17.48% of the species are regular and 7.77% of the species are ubiquitous (Table 1). The accessory species were sampled by a single method with a frequency of 33.33%. Two methods were used to sample the regular species with a frequency of 66.66%. The ubiquitous species were sampled by all three methods with a frequency of 100%.

Discussion

During this study, 103 species were surveyed and only 43 species could not be identified to the species by the eDNA method. The imprecise determination of some species is due to the absence of reference databases. The faunal lists obtained via eDNA were compared to traditional fishing data (gillnets, traps). These comparisons show that the eDNA method detects a greater number of species, the results are better

compared to other methods. The effectiveness of the DNA method has already been proven in other studies (Valentini *et al.*, 2016; Hänfling *et al.*, 2016; Boivin-Delisle *et al.*, 2021; Blabolil *et al.*, 2021), more than 70% of the sampled species are detected by eDNA. This method makes it possible to detect species not captured by nets traps and hooks. This is the case of *Aplocheilichthys spilauchen*, *Aplocheilichthys* sp., *Strongylura senegalensis*, *Tylosurus crocodilus*, *Eleotris* sp. and *Amphilius* sp. detected in eDNA and not in traditional methods. These species are part of the groups of dwarf fish, therefore difficult to capture using traditional fishing methods. Also the genus Myrophis (*Anguilliformes: Ophichthidae*) present only in the eDNA method would be due to its serpentine shape. This species is very difficult to capture by gillnet because it does not mesh easily because of its elongated body without scales which gives it a smooth and slippery appearance (Meziere-fortin, 2014). However the absence of the species in the eDNA method and present in the fishing methods could be due to their absence in the international GenBank reference database. Therefore they could not be detected by eDNA. The species identified using the eDNA method demonstrate their current presence in the environment.

According to Paugy *et al.* (2003a and 2003b), since most fish are not sedentary, some fish species can migrate to other rivers. They swim upstream, sometimes traveling long distances for their biological needs related to feeding and reproduction. These different needs lead to the deposition of DNA in the environment, which justifies the detection of their presence in the river. The rapid degradation of DNA in aquatic environments by the combined action of Ultraviolet (UV) and microorganisms shows their effective presence in the middle course of the Comoé River (Bunet, 2021). The eDNA method provides a good picture of the relative abundances of species. It is effective in qualifying the composition of fish communities more exhaustively than traditional methods. Despite the satisfactory results of the eDNA method, it also has limitations. This method does not provide geographical information on the actual presence of species (Bureau Veritas, 2016). Because, DNA is transported in aquatic environments, so it is impossible to extract exact data on the location or duration of its presence in the environment. This is not the case for the traditional method, the principle of which is based on the visual presence and identification of species. In addition eDNA does not allow us to know whether the species is alive or dead, because it only detects signs of the presence of the species (Bureau Veritas, 2016).

Conclusion

This study identified 103 species of fish divided into 19 orders, 38 families for all methods.

The analysis of abundances showed that the ichthyological population of the middle course of the Comoé is dominated by the order of Siluriformes and Perciformes. In view of the results, eDNA makes it possible to compensate for traditional methods and is a good inventory tool.

However, it does not allow the size and weight of the species recorded to be analyzed because it is based on the DNA present in the water. This is not the case for traditional fishing methods which, by seeing and touching the fish, make it possible to know several parameters of the fish.

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