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Marion Labeille

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The GUAD3E project, implementation of an innovative method for fish and crustacean identification in Guadeloupe: the environmental DNA metabarcoding

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l'Europe
s'engage
en Guadeloupe
avec le FEDER



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CONVENTION SUR LES HUMIÈRES
CONVENCIÓN SOBRE LOS HUMEDALES
CONVENCIÓN SUR LOS HUMEDALES
Damas, 1971



Presentation

- **Objective of the project:** Test the eDNA metabarcoding method for fish and crustacean inventory in Guadeloupe and compare the results with the traditional method of species inventory: the electrofishing.
- **Public and private partnership:** the GUAD3E project has been launched in 2018 by the National Park of Guadeloupe in collaboration with the private company SPYGEN
 - 2 independent consultants are also involved in this project
- **Fundings:** total amount of the project: 202 752 €



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127 114 €



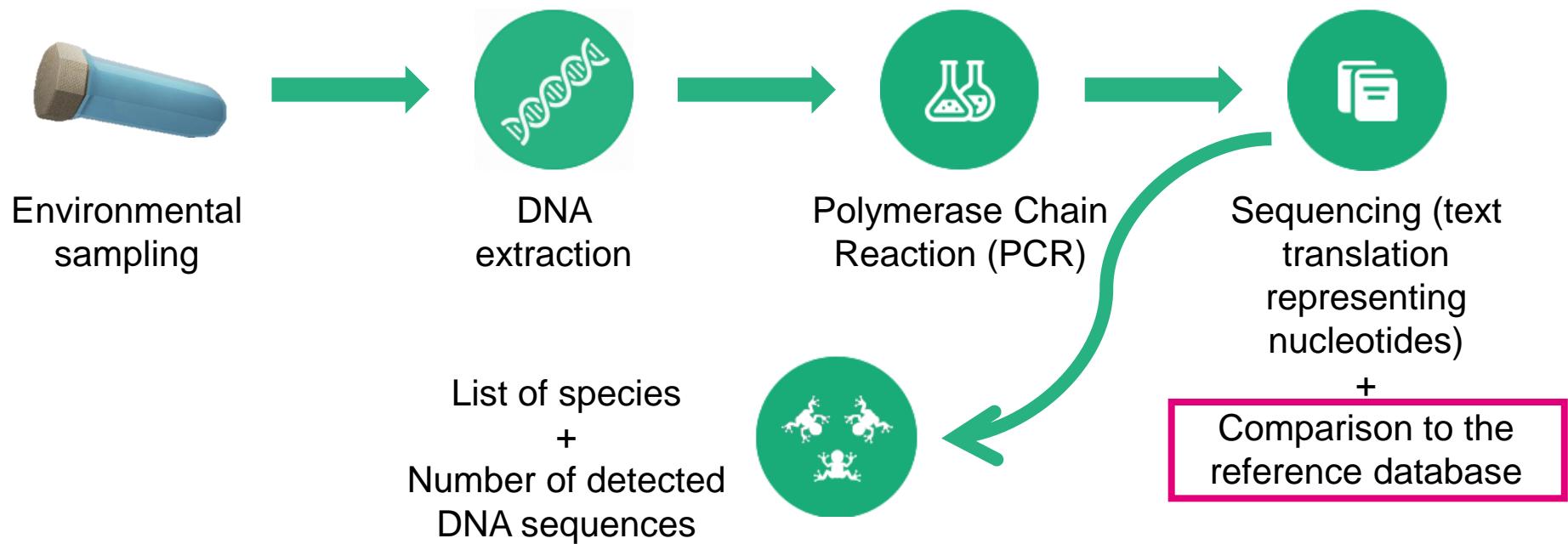
21 500 €



54 138 €

The eDNA metabarcoding method

- DNA that can be extracted from environmental samples (soil, water, biofilm, faeces, honey, ...) (Taberlet & al., 2012)

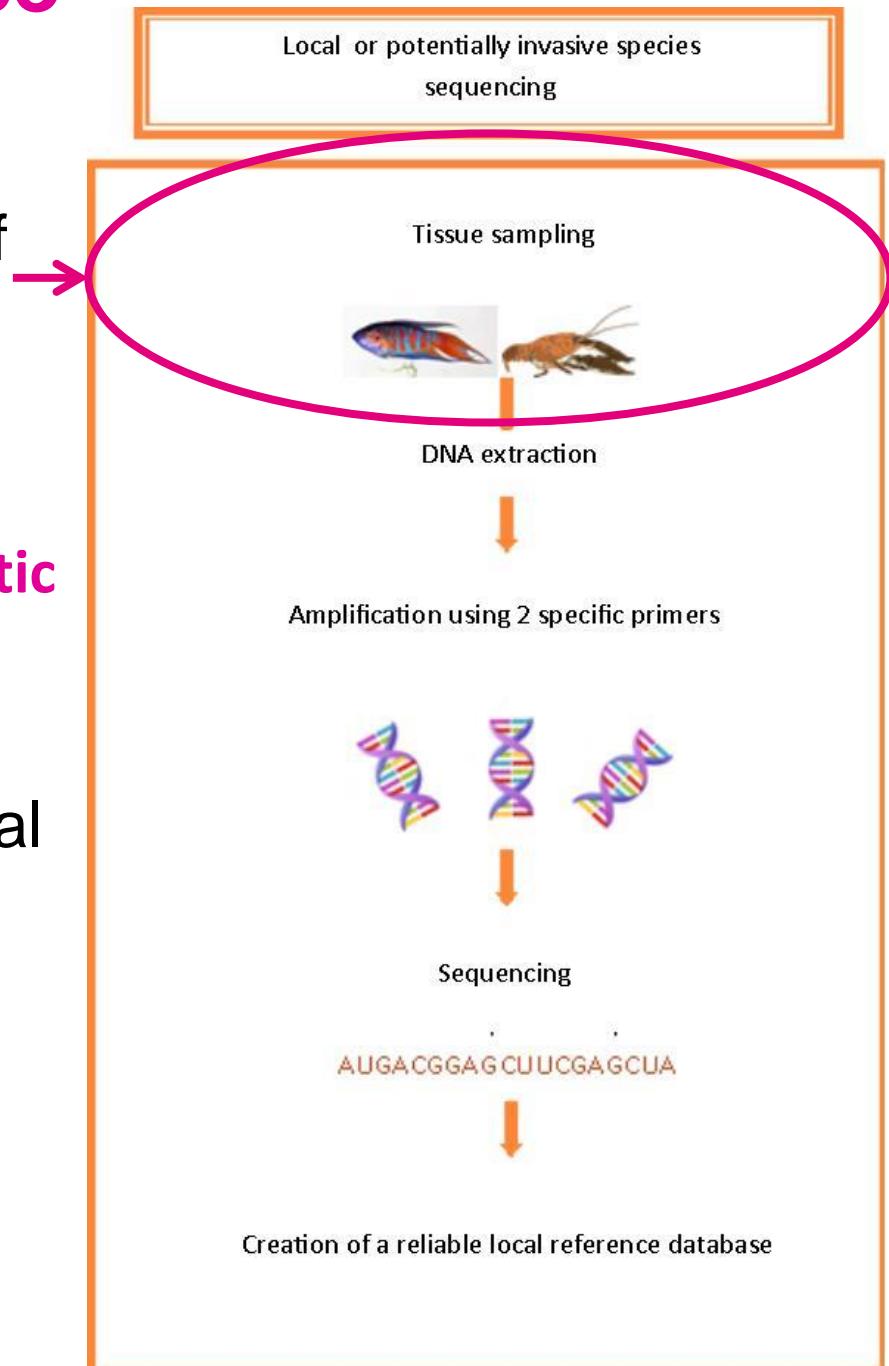


- Example of sequence obtained: *Agonostomus monticola*

CCCCAAGTATAAGCACTATCTATACCTAATTACTTTAACAAAGAGGGGAGGCAAGTCGTAA

The Guadeloupe reference database

- DNA sampling on 3 individuals of the same species from different rivers
 - To ensure a good representativeness of the **genetic variability** of the species
- Species collected from their natural environment: **41**
- Potentially invasive species purchased at aquarium stores: **20**



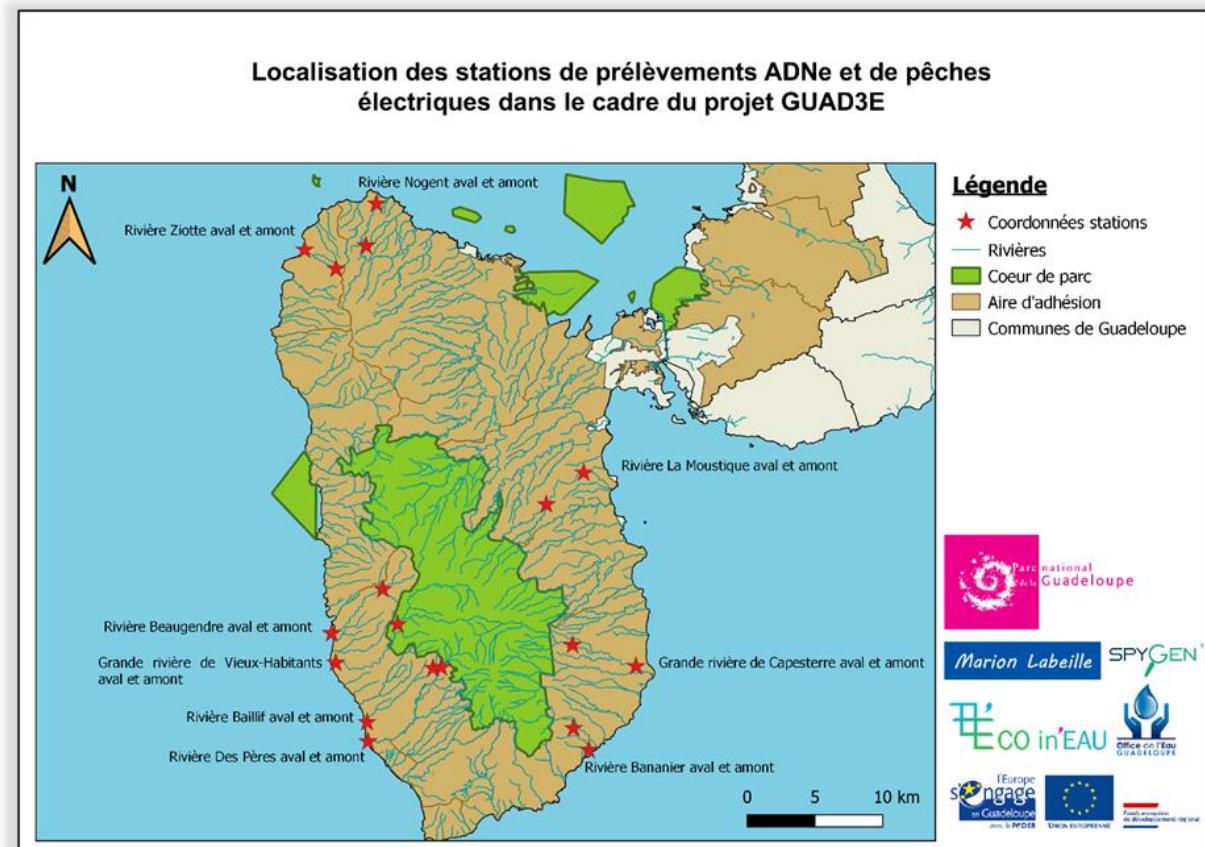
Material and methods

➤ 9 streams studied:

- Permanent
- Small/medium/large
- 1 downstream + 1 upstream station

➤ 18 stations:

- accessible in wet and dry season
- with a high biodiversity (fishes and crustaceans)
- electrofishing method possible
- with some precedent inventories data available



☒ eDNA metabarcoding protocol + Electrofishing protocol

Material and methods

➤ Electrofishing protocol:

- **complete fishing method** (1 single pass on a surface of 250 m² maximum) + possibility to carry out up to 10 subsamples maximum



Material and methods

➤ eDNA metabarcoding protocol:

- Filter in the central water vein during 30 min (about 1L/min), using a 0.45µm filter®



Material and methods

➤ eDNA metabarcoding protocol:

- The filter capsule is then filled with storage buffer, closed, stirred and stored at room temperature before being shipping to SPYGEN



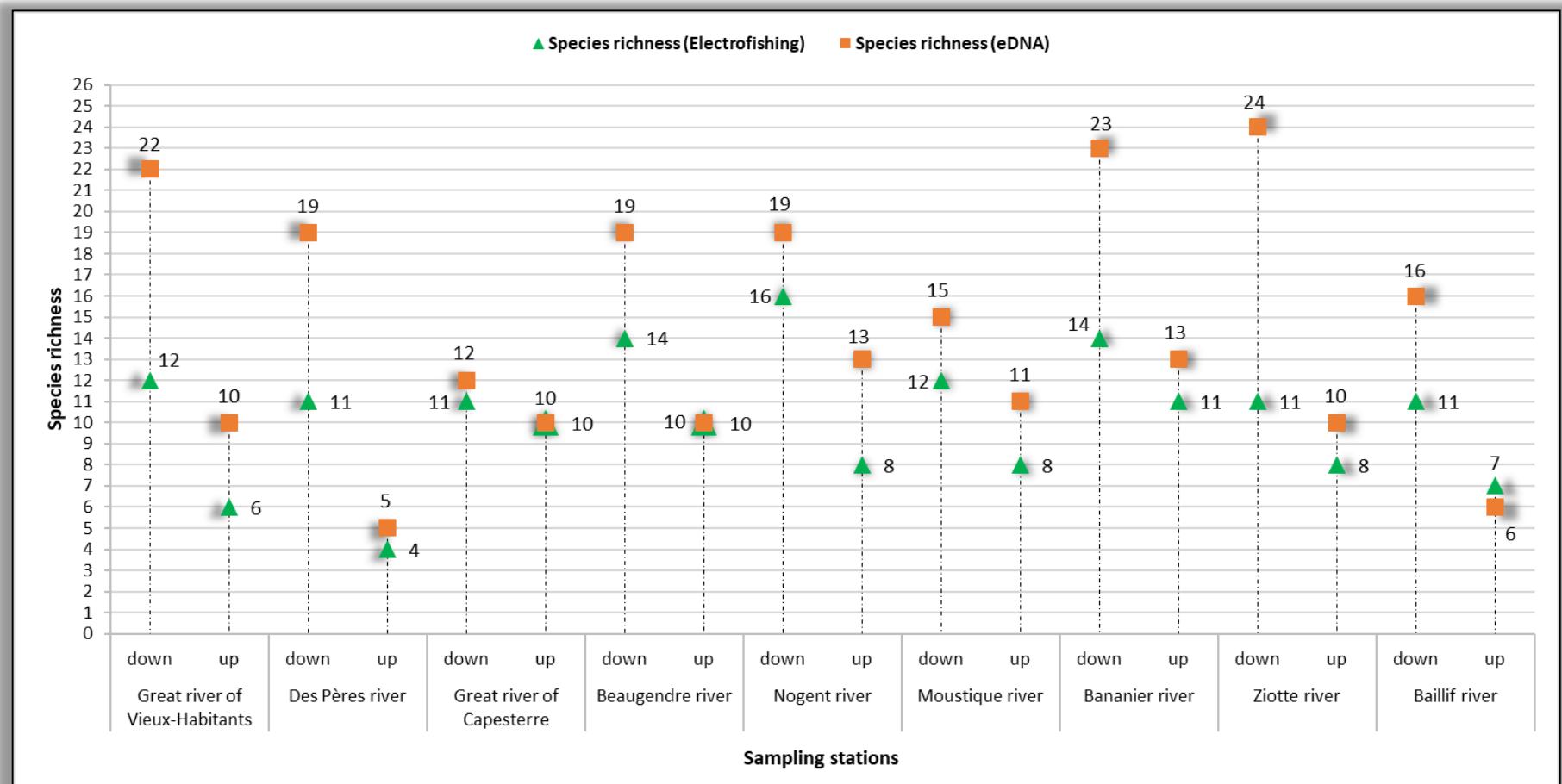
- On each station, **3 replicates** were made to ensure the statistical validity of our results (Pont D. & al., 2018; Cantera L. & al., 2019)



Results

- 100% of the species caught by the fishing method and present in the reference database were also detected by the eDNA metabarcoding method

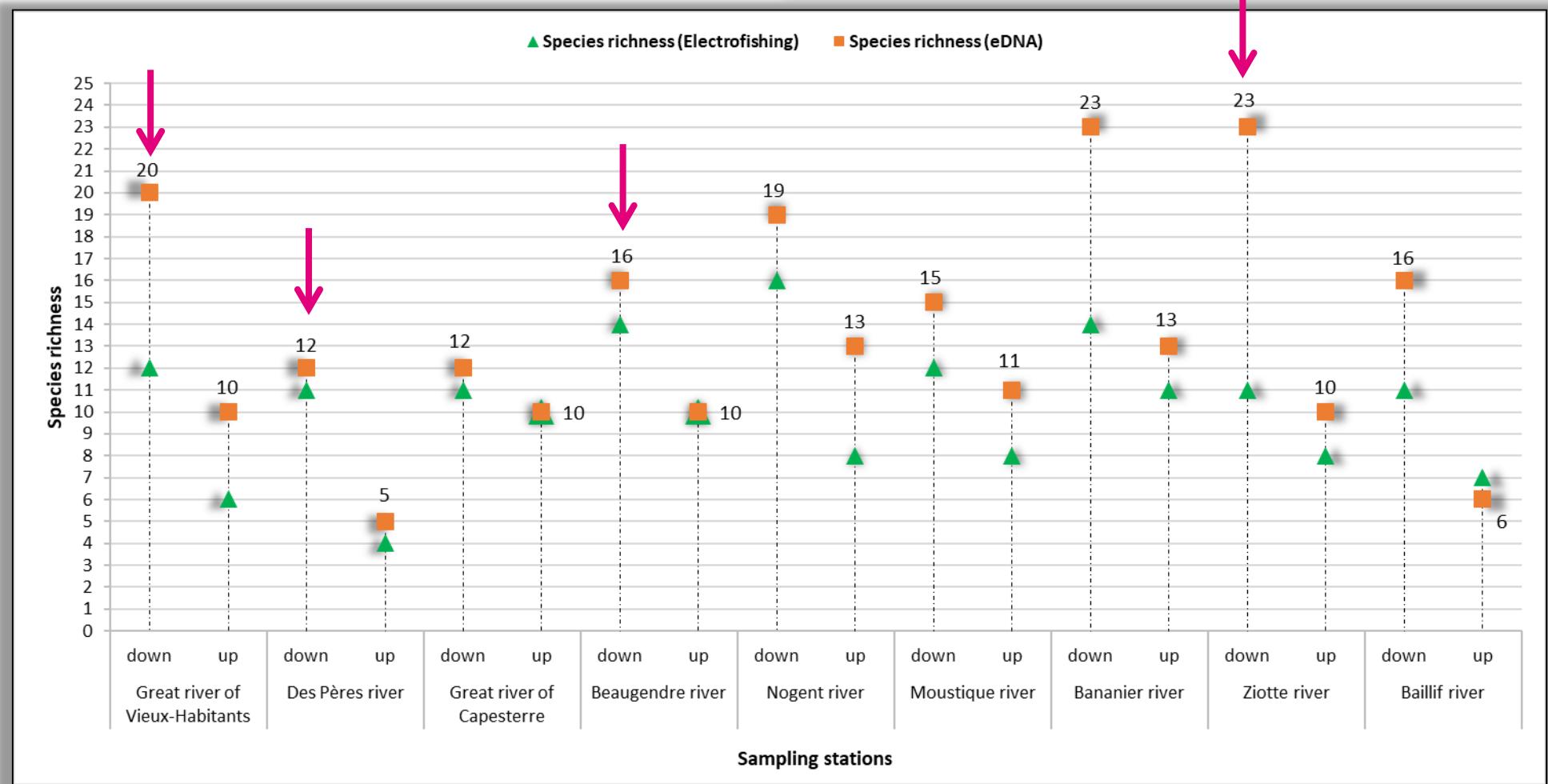
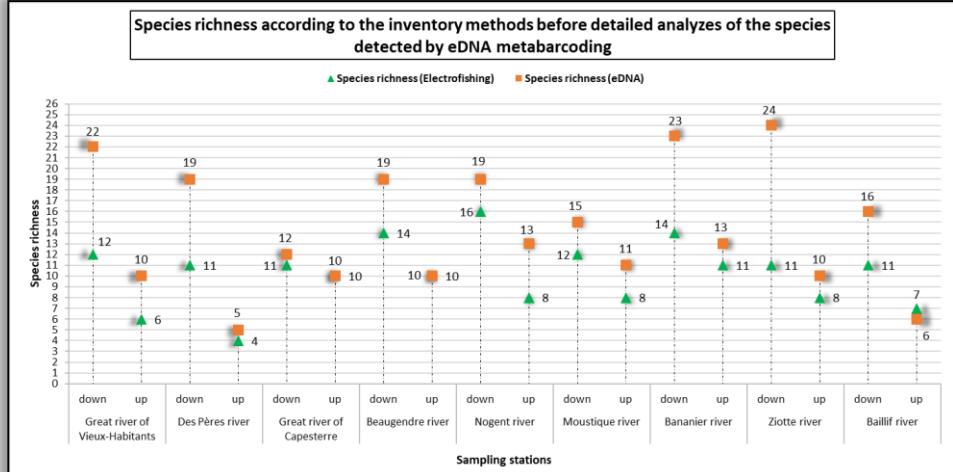
Species richness (eDNA metabarcoding) \geq species richness (electrofishing) for 17 stations



Comparison of the species richness obtained by the two methods of inventory

Results

➤ Results after treatment and data cleaning



Comparison of the species richness obtained by the two methods of inventory

Results

Species	River	Station	Nb. of species in common	Nb. of species in only eDNA metabarcoding data	Nb. of species in only electrofishing data	Spearman's coefficient (p-value)
Fish	Great river of Vieux-Habitants	downstream	7	3	0	0.836 (p-value = 2.35e-06)
		upstream	4	1	0	0.910 (p-value = 1.076e-08)
	Des Pères river	downstream	5	2	1 (<i>Mugilidae species*</i>)	0.753 (p-value = 8.049e-05)
		upstream	2	0	0	1 (p-value < 2.2e-16)
	Great river of Capesterre	downstream	3	0	0	1 (p-value < 2.2e-16)
		upstream	2	0	0	1 (p-value < 2.2e-16)
	Beaugendre river	downstream	6	3	3 (whose 2 <i>Mugilidae species*</i>)	0.581 (p-value = 0.005703)
		upstream	2	1	0	0.837 (p-value = 2.244e-06)
	Nogent river	downstream	7	3	1 (<i>Mugilidae species*</i>)	0.721 (p-value = 0.000223)
		upstream	1	2	0	0.606 (p-value = 0.003563)
	Moustique river	downstream	5	4	0	0.783 (p-value = 2.736e-05)
		upstream	2	2	0	0.706 (p-value = 0.0003473)
	Bananier river	downstream	3	7	1 (<i>Mugilidae species*</i>)	0.513 (p-value = 0.01744)
		upstream	3	1	0	0.888 (p-value = 7.764e-08)
	Ziotte river	downstream	5	6	0	0.741 (p-value = 0.0001214)
		upstream	1	0	0	1 (p-value < 2.2e-16)
	Baillif river	downstream	5	3	0	0.871 (p-value = 2.864e-07)
		upstream	3	0	0	0.997 (p-value < 2.2e-16)
Crustaceans	Great river of Vieux-Habitants	downstream	5	5	0	0.539 (p-value = 0.0210)
		upstream	2	3	0	0.564 (p-value = 0.0147)
	Des Pères river	downstream	4	1	1 (XipElo)	0.809 (p-value = 4.809e-05)
		upstream	2	1	0	0.791 (p-value = 9.228e-05)
	Great river of Capesterre	downstream	7	2	1 (XipElo)	0.792 (p-value = 8.934e-05)
		upstream	6	2	2 (XipElo et MacFau)	0.682 (p-value = 0.0018)
	Beaugendre river	downstream	5	2	0	0.886 (p-value = 9.901e-07)
		upstream	6	1	2 (XipElo et MacFau)	0.761 (p-value = 0.0002)
	Nogent river	downstream	5	4	2 (PotPot et MacAca)	0.474 (p-value = 0.0469)
		upstream	6	4	1 (PotPot)	0.626 (p-value = 0.0054)
	Moustique river	downstream	4	2	3 (AtyInn, MacHet et XipElo)	0.539 (p-value = 0.0209)
		upstream	4	3	2 (PotPot et XipElo)	0.564 (p-value = 0.0148)
	Bananier river	downstream	10	3	0	0.588 (p-value = 0.0103)
		upstream	7	2	1 (AtySca)	0.787 (p-value = 0.0001)
	Ziotte river	downstream	5	7	1 (MacAca)	0.303 (p-value = 0.2209)
		upstream	6	3	1 (MacFau)	0.581 (p-value = 0.0115)
	Baillif river	downstream	5	3	1 (XipElo)	0.657 (p-value = 0.0031)
		upstream	3	0	1 (PotGla)	0.869 (p-value = 2.887e-06)

*2 species of *Mugilidae* currently undetectable by eDNA metabarcoding method

Comparison of species detections

Results

Species	River	Station	Nb. of species in common	Nb. of species in only eDNA metabarcoding data	Nb. of species in only electrofishing data	Spearman's coefficient (p-value)
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*2 species of Mugilidae currently undetectable by eDNA metabarcoding method

Comparison of species detections

➤ eDNA metabarcoding method works fully to identify fish species

➤ On all stations, only 1 specimen of a specie was not detected (*Microphis brachyurus*)

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*2 species of *Mugilidae* currently undetectable by eDNA metabarcoding method

Comparison of species detections

➤ eDNA metabarcoding method works properly to identify crustacean species

➤ Only 1 to maximum 3 species were not detected

➤ Not enough specimens? DNA degradation due to physicochemical conditions?

Advantages and limitations

Traditionnal - Electrofishing	Innovating - eDNA
Results	
Instantaneous	3 month delay (laboratory analyses)
Richness, abundance, information about individuals (size, sex, stage of development, sickness ...)	Only richness, (relative abundance under development)
Some species (small, rare, cryptic...) hard to fish Some species hard to distinguish (MacCre Vs MacFau; AtyInn vs. AtySca) → Possibilities of error in the inventory	<p>Reliable species identification if database is properly elaborated</p> <p>BUT :</p> <ul style="list-style-type: none"> → Risk of signal persistance from upstream → For some crustacean species (especially <i>Xiphocaris elongata</i>), DNA sequences were not detected → Risk of contamination during handling and by marine fishes: intrusion in river mouth, rejet from restaurants and fishermen...
Human ressources and skill	
4 persons or more to fish Several trained and authorized operators to electrofishing Fishing authorization	2 trained persons (to not contaminated the sample)
Ability in identification	Good knowledge of species and rivers for a good interpretation of eDNA results
Impact on natural middles	
Disturb the natural environment	Non intrusive
Miscellaneous	
Complex for hard access sites	Generates a lot of waste
	Sending samples in Europe



Perspectives

- **The eDNA metabarcoding method in Guadeloupe:**
 - Fishes: satisfying
 - Crustaceans: positive results and improvement to be expected following ongoing developments
 - Working conditions: test to do in deep and brackish zones, in wet season...
- **Very interesting method for:**
 - First screening / diagnostic
 - inventories in hard access sites
 - Looking for rare, cryptic, hard to fish or specific species (as invasive alien species)
- **eDNA inventory seems to be effective in Guadeloupe (for fishes and soon for crustaceans) and a good alternative and/or complementary to traditional method**



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Thank you for your attention

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