

## **Stock identification**:

## advancing in the knowledge of stock structure as a requirement for stock assessment



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## **STRUCTURE OF THE PRESENTATION**

- 1. Introduction
- 2. Methods
- 3. Life history traits: data analysis and preliminary results
- 4. Morphometry: data analysis and preliminary results
- 5. Genetics: data analysis and preliminary results
- 6. Conclusions

## **1-INTRODUCTION**



### **1- INTRODUCTION**

### **DEMERSTEM- Objectives of WP 1:**

- Improve the knowledge on the selected demersal stocks with a particular effort to solve the problems of stock identification and improvement of the quality of the data used for the assessment.
- This follows the recommendations regularly made by CECAF.



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### **STOCK IDENTIFICATION**

The selected stocks match one or more of the following criteria:

- Shared stock
- Commercially important species
- Emblematic species for artisanal fisheries
- Species of importance to EU fleets
- Species with coastal nurseries (link with WP2)





## STOCK IDENTIFICATION- HOLISTIC APPROACH

### Life History Traits

• Weight parameters, reproduction, etc



#### Morphometry

 Study based on morphometric measurements of the species.



#### Genetics

 Study of the genetic structure of the populations to identify the geographical limits of stocks using microsatellite markers.



## Sample sources



## Sampling period

DAVC	Zone/	Mar 20	Avr	Mai	Juin	Juill	Août	Sep	Oct	Nov	Déc	Jan	Fév
PATS	Mois		20	20	20	20	20	20	20	20	20	21	21
ITANIA	North	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO
MAUR	South	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO
BIE	Saint Louis	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO
EGAL-GAN	Saloum	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO
SEN	Gambie	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO
GUINEA- BISSAU	Cacheu	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO	T BIO- MOR- GEN	T BIO	T BIO	T BIO	T BIO	T BIO

## Sampling places



Species	Country	Zone	Sampling port	Surveys
	Mauritania	North	Nouadhibou	
Eninonholus gonous	Wauntania	South	Nouakchott	
Epinepheius deneus	Sonogol	North	Kayar	
	Sellegal	South	Saloum	
	Mauritania	North	Nouadhibou	
	Ividui itariid	South	Nouakchott	x
Bangaus notialis	Sonogal	North	Saint Louis / Dakar	
Pendeus notiuns	Sellegal	South	Saloum / Casamance	
	The Gambia	—	Banjul	
	Guinea-Bissau	North	Cacheu	x
	Guipos Pissou	North	Bissau _NO	
Raarus caarulaastistus	Guilled-Dissau	South	Buba	x
Pugrus cueruieosticius	Guinoa	North	Kamsar	x
	Guinea	South	Conakry	x
	Guipos Pissou	North	Cacheu	
Regudatalithus alangatus	Guinea-bissau	South	Cacine / Buba	
Pseudotontinus elongutus	Guipoa	North	Kamsar, Katchek	
	Guinea	South	Conakry	
	C lugiro	West	San Pedro	
Pseudotolithus	C. WOITE	East	Abidjan	
senegalensis	Chana	West	Takoradi	
	Gilalia	East	Takoradi	
	C lugiro	West	San Pedro	
Pagallus ballottii	C. WOITE	East	Abidjan	
Fugelius beliottili	Ghana	West	Tema	
	Unana	East	Takoradi	

## Sampling places







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### Sampling protocols







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http://pescao-demerstem.org/

## **3- LIFE HISTORY TRAITS: DATA ANALYSIS AND PRELIMINARY RESULTS**

### STOCK IDENTIFICATION- HOLISTIC APPROACH



#### Weight parameters

- Weight–length relationships for combined sexes (total and by sex). Using :
  - Total weight (Wt)

W = a(Lt)<sup>b</sup>

- Gutted weight (Wg)
- Weight Conversion factor (Wt Wg)
  Wt = aWg
- Gonadosomatic index (GSI), by sex and different temporal frames. GSI = (Wgo/Wg) × 100
- Le Cren's relative condition factor (K):

 $K = Wg/aLt^b$ 

### **Reproduction parameters and features**

- GSI (only matures) by sex-month/quarter
- Le Cren's relative condition factor (K) (only for matures) by sex-month/quarter
- % matures by sex and month
- Determination of spawning period (based on females spawning peaks)
- L50 (by sex)
- Sex ratio by length

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**Data analysis**  $\rightarrow$  comparisons among country-zones, using the same length ranges

Weight parameters

- ANCOVA
- ANOVA

**Reproduction parameters and features** 

- Visual observation (GSI, spawning periods)
- Comparison of CI (L50s)

- In general, no significant difference between country-zones for most parameters and species.
- > When significant differences were found , they could be attributed to different sampling issues.
- > With the data available and sampling limitations, this method revealed not to be robust for stock identity.
- However, this is study has been very relevant to improve/provide knowledge of the biology of target species in the studied areas.

#### **3- LIFE HISTORY TRAITS: DATA ANALYSIS AND PRELIMINARY RESULTS**

### Weight parameters (example Penaeus notialis)



	Length-	Weigth	Le Cren's				
Contry-Zone	relatio	onship	condition factor (k)				
	Slope (b)	SE	median	mean	sd		
MAU_N	2.62	0.03	1.07	1.09	0.17		
MAU_S	2.66	0.05	1.06	1.05	0.08		
SEN_N	2.58	0.02	1.00	1.00	0.13		
GAM	2.63	0.03	1.01	1.00	0.12		
SEN_S	2.62	0.02	0.96	0.97	0.1		
G. BISS	2.23	0.04	1.06	1.02	0.18		





## Visit our posters to see the results of each species!

#### **3- LIFE HISTORY TRAITS: DATA ANALYSIS AND PRELIMINARY RESULTS**

### **Reproduction parameters and features (example:** *Epinephelus aeneus***)**





Total length (cm.)



Inm ---- IGS



Total length (cm.)

5
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Total length (cm.)



Total length (cm.)



Visit our posters to see the results of each species!

### STOCK IDENTIFICATION- HOLISTIC APPROACH



## MORPHOMETRY

## Body shape (TRUSS NETWORK)

## Otolith shape (FOURIER ANALYSIS)









## Body shape (TRUSS NETWORK)



Species photographing during biological sampling (bi-annual) and by country-zone.



Measurements on selected pictures.

**3 SPECIES GROUPS** 

### Body shape (TRUSS NETWORK)

### **GROUP 1: 1 dorsal fin fish**

### 8 landmarks $\rightarrow$ 15 measurements



#### Epinephelus aeneus (GPW)

COUNTRY/ZONE	Photos	Measures
Mauritania_Nord	70	1050
Mauritania_Sud	98	1320
Senegal_Nord	91	1350
Senegal_Sud	116	1620
TOTAL	375	5340



Pagrus caeruleostictus (BSC)

COUNTRY/ZONE	Photos	Measures
G. Bissau_Nord	86	1290
Guinea_Nord	100	1470
Guinea_Sud	89	1320
TOTAL	275	4080



Pagellus bellottii (PAR)

COUNTRY/ZONE	Photos	Measures
C.lvoire_Ouest	50	540
C.Ivoire_Est	98	1110
Ghana_Oust	103	1005
Ghana_Est	117	1080
TOTAL	318	3195

#### Body shape (TRUSS NETWORK)

### **GROUP 2: 2 dorsal fin fish**

#### 9 landmarks $\rightarrow$ 17 measurements



#### Pseudotholitus elongatus (PSE)

COUNTRY/ZONE	Photos	Measures
G. Bissau_Nord	105	1105
G. Bissau_Sud	82	1071
Guinea_Nord	177	1224
Guinea_Sud	164	1275
TOTAL	528	4675



#### Pseudotholitus senegalensis (PSS)

COUNTRY/ZONE	Photos	Measures
C.Ivoire_Ouest	49	612
C.Ivoire_Est	103	1241
Ghana_Ouest	50	612
Ghana_Est	118	1173
TOTAL	271	3026

### **GROUP 3: Shrimps**

### 21 landmarks $\rightarrow$

#### 46 measurements



COUNTRY/ZONE	Photos	Measures
Mauritania_Nord	116	2438
Mauritania_Sud	50	1656
Senegal_Nord	171	1472
The Gambia	96	1702
Senegal_Sud	171	1978
Guinea-Bissau_Nord	100	1610
TOTAL	704	10856

## Body shape (TRUSS NETWORK) → Data analysis

1) Data matrix: measurements on selected pictures (species photographed during biological sampling and by country-zone.

Multivariate analysis:

- 2) Principal Component Analysis (PCA)
- 3) Linear Discriminant Analysis (LDA):
  - Detection of potential significant differences between groups
  - Selection of measures producing them (ANOVA)
  - Correct classification (CC)

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## Otolith shape (FOURIER ANALYSIS)

- 1) Extraction of otolith samples (biological sampling) and photographing.
- 2) Otolith outline extraction (R)
- 3) Fourier analysis
  - Crossing with biological data
  - Scale the outlines to the same size
  - Transform shapes into numbers
- 4) Classification and multivariate analysis of shapes (PCA, LDA)
  - Correction of the possible allometric effect

Some results: *Epinephelus aeneus* (example 1)



Linear Discriminant Analysis (LDA)  $\rightarrow$  Overlap among areas

Some results: *Epinephelus aeneus* (example 1)

### **Correct classification from LDAs** (Body shape and Otolith shape)





a. 48.5 % of original grouped cases correctly classified.

Following morphometric techniques, the overlap among zones in thiof indicates the greatest mixing compared with other studied species in this project  $\rightarrow$  potential single stock in the studied area.

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### Some results: *Penaeus notialis* (example 2)

## Body shape (TRUSS NETWORK)





Some results: *Penaeus notialis* (example 2)

### **Correct classification from LDAs**



### Specimen shape



#### Correct classification of individuals: 91%

Classification Results(a)									
Country				Predicted Group Membership					
		area	MauN	MauS	SenN	SenS	Gam	GBissN	Total
		MauN	45	3	0	2	1	0	53
		MauS	2	32	0	0	0	1	35
	Numbor	SenN	0	0	37	1	1	0	43
	Number	SenS	0	0	5	26	0	0	32
		Gam	2	0	0	1	33	1	37
Original		GBissN	1	0	0	0	1	33	35
Oligiliai		MauN	88,2	5,9	0	3,9	2	0	100
		MauS	5,7	91,4	0	0	0	2,9	100
	0/	SenN	0	0	94,9	2,6	2,6	0	100
	/0	SenS	0	0	16,1	83,9	0	0	100
		Gam	5,4	0	0	2,7	89,2	2,7	100
		GBissN	2,9	0	0	0	2,9	94,3	100
a. 90.6%	of origina	l grouped	cases c	orrectly	classifi	ed.			



The correct classification of individuals using specimen shape is the highest of the studied species.

- High correct classification (84-95%) in all the areas
- Correct classification from LDAs, comparison tests on factor 1 → individuals in each area could be clearly differentiated from those in other areas (phenotypically homogeneous groups in each country-zone).

#### 4- MORPHOMETRY: DATA ANALYSIS AND PRELIMINARY RESULTS RESULTS OTHER SPECIES-SUMMARY TABLE



## **5- GENETICS:**

## DATA ANALYSIS AND PRELIMINARY RESULTS

### STOCK IDENTIFICATION- HOLISTIC APPROACH



### **Genetics goals**

1.- Study of the genetic structure of populations to identify the geographical limits of stocks using **microsatellite markers**.



#### 2.- To provide genetic information. Sequencing of mitochondrial gene cytochrome oxidase I.

### Microsatellite markers- E. aeneus



The final design allowed amplification of eight microsatellite loci in two multiplex PCR (M1 and M2) and a single PCR (M3)

Múltiplex	Locus	Primer F	Primer R
M1	ARO1105	TGATAGCTTTACATGCACTCA	CTGAACCTCACCCTGAAA
M1	ARO1045	CACGAAGTATTTGGCTGAT	GAGAAAGTGGCAATATTTGAC
M1	ARO1083	CCGGTTCTTCTTCTCCC	TTACTGTTGATTGAGTTGTTGT
M1	ARO1084	GGGTTTATTTCAAAGGTCAG	CCCAATGAGGTGTTCAATAT
M2	ARO1003	GTGCAAGGCAAGCTGTGTTA	AGCAGGCATCTTGTTATCTGG
M2	ARO1120	CTCTGATGCTGTTTACACAAC	TCTCCATCGAAGGTAAAGG
M2	ARO1137	ATGGGTATAATTAGGACACACT	AGGAAAGGAGGAGGAAA
M3	ARO1131	TGTGTGTCAGAGGTGGGTT	TGAATTTCACTGCATGTTTC

(Dor et al., 2014)

### **Microsatellite markers**



The final design allowed amplification of five microsatellite loci in one multiplex PCR (M1) and two single PCRs (PnS01 and PnS20).

	Locus	F	R
M1	PnS03	F-5' TGCTAA ATAAAAGTTTCTCGGTGAG	R-5' AAGCTTGTATTTGCGTGTCG
M1	PnS04	F-5' CGATTTGCAGAACCCGTTTA	R-5' GGGGGAGGGGTTAGAAAGAG
M1	PnS18	F-5' GTCTTATCAAAACCCAAAGG	R-5' GAACCAGTCCCGGCCCTCTGC
	PnS01	F-5' TGCTGTTTGTGAGTCTT	R-5' TGGCATGTTGCAGACAGTCC
	PnS20	F-5' CTTCCATATTCGCATGATGG	R-5' ACCCGGGATCAAGCCCTTGC

(Robainas-Barcia et al., 2002 and 2008)

### **Data Analysis**







### Genetic diversity estimation

equilibrium

coefficient F<sub>IS</sub>

Genetic differentiation and migration



Genedod

Number of alleles (Na), No. of Effective Alleles (Ne), Observed Heterozygosity (Ho), Expected Heterozygosity (He), Unbiased Expected Heterozygosity (uHe)

Departures from Hardy-Weinberg

(HWE),

Inbreeding



**GENEDOD** pairwise F<sub>sp</sub>, Number of migrants (Nm)

adegenet R package

Discriminant Analysis of Principal Components (DAPC), distancemethod approach



STRUCTURE with STRUCTURE HARVESTER and CLUMPAK: model-based clustering, Bayesian approach

#### Structure

### **RESULTS – Molecular markers assessment**



#### Genetic diversity by microsatellite and population

N /Na

Pop <sup>a</sup>	ARO1105	ARO1045	ARO1084	ARO1083	ARO1003	ARO1120	ARO 1137	ARO 1131
MAU_N	57/23	58/21	65/22	65/30	65/27	65/26	66/24	66/19
MAU_S	35/23	41/18	50/27	49/26	50/30	50/28	50/24	47/19
SEN_N	72/26	71/22	92/31	93/30	93/33	93/35	93/26	91/22
SEN_S	120/28	120/24	143/35	140/33	145/33	145/24	144/28	141/24



### **RESULTS - Genetic diversity**

Popa	Ν	Na	Ne	H <sub>o</sub>	H <sub>E</sub>	uH <sub>E</sub>
MAU_N	63	24	13	0.871	0.903	0.910
MAU_S	47	24	13	0.893	0.909	0.919
SEN_N	88	28	14	0.861	0.913	0.919
SEN_S	137	90	14	0.866	0.912	0.915

N, number of individuals analyzed;

Na, number of different alleles;

Ne, number of effective or equally frequent alleles;

H<sub>o</sub>, observed heterozygosity;

H<sub>E</sub>; expected heterozygosity according to allele frequencies;

uH<sub>E</sub>, unbiased expected heterozygosity according to sample size

Number of migrants

### **RESULTS - Population structure Fst - Migration**

 $F_{ST}$  pairwise comparison of GPW genetic diversity at 8 loci in 4 populations.

	MAU_N	MAU_S	SEN_N	SEN_S
MAU_N				
MAU_S	0.004			
SEN_N	0.003	0.004		
SEN_S	0.003	0.005	0.002	

Locus	All Pops
ARO1105	52
ARO1045	37
ARO1084	47
ARO1083	48
ARO1003	35
ARO1120	55
ARO1137	54
ARO1131	84
Mean ± SE	51 ± 5



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#### **5-GENETICS: DATA ANALYSIS AND PRELIMINARY RESULTS**

### **RESULTS - Structure**

Bayesian analysis, 354 GPW, 8 loci (K 1 to 4, 25 iterations per k)





#### Stock identification: advancing in the knowledge of stock structure as a requirement for stock assessment

#### **5-GENETICS: DATA ANALYSIS AND PRELIMINARY RESULTS**

### **RESULTS – Molecular markers assessment-***P. notialis*

Genetic diversity by microsatellite and population N /Na

Рор	PnS03	PnS18	PnS01	PnS04	PnS20
MAU-N	55/25	94/36	96/15	97/12	93/93
MAU-S	27/17	44/28	45/12	43/10	44/65
SEN-N	102/40	154/43	157/16	150/13	147/114
SEN-S	79/29	121/39	128/15	123/13	127/103
GAM	24/15	36/32	46/13	47/11	42/56
BIS	38/25	52/32	59/13	54/11	55/58





### **RESULTS - Population structure Fst (5 loci, 6 pops, N536)**



**Pairwise Population Matrix of G"st Values for Total** 

	GB-S	GAM	MAU-N	SEN-S	SEN-N	MAU-S	Code
GB-S		0,895	0,866	0,000	0,538	0,891	ССН
GAM	-0,024		0,704	0,011	0,932	0,950	GAM
MAU-N	-0,013	0,001		0,000	0,202	0,575	NDB
SEN-S	<mark>0,146</mark>	<mark>0,084</mark>	<mark>0,108</mark>		0,000	0,000	SAL
SEN-N	0,005	-0,021	0,018	0,110		0,598	SLO
MAU-S	-0,022	-0,035	0,009	<mark>0,130</mark>	0,003		NKC

G''st values below the diagonal. Probability, P(rand >= data) based on 9999 permutations is shown above diagonal.



### **RESULTS - Structure**

*K* = 2

### **SEQUENCES**



2.- To provide genetic information – COI Sequences.







Pagellus bellottii



Pseudotolithus senegalensis



Pseudotolithus senegalensis has a different organization of the mitochondrial genome





#### **Epinephelus aeneus**



**Penaeus notialis** 



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## **6- CONCLUSIONS**



Stock identification: advancing in the knowledge of stock structure as a requirement for stock assessment

### **6- CONCLUSIONS**

- 1) A holistic approach has been used for stock identification of the 6 target species. The different techniques used are complementary and provide basic information with different perspectives for a biological-based stock identification.
- 2) Data from life history traits do not show conclusive results. However, this work has contributed to improve the biological knowledge of the species in the study area.
- 3) The two morphometric techniques used show reliable information for stock identification. Among the two methods, body shape has provided better results for differentiation among areas than otolith shape.
- 4) Genetics tools provide a direct basis for stock structuring and interpreting phenotypic-based patterns.
- 5) For thiof, the results of morphometry and genetic techniques are consistent showing one single stock in Mauritania and Senegal, although its boundaries still unknown. In addition, genetics show introgression from other areas.

- 6) For *P.* notialis, results from morphometry show low variability within samples, suggesting that each sampled country-zone conforms a phenotypically homogeneous group, with clear differences to the others. The number of genetics samples used for *P. notialis* is insufficient to recover the genetic diversity of the species. More individuals are still under analysis. Other markers should be tried.
- 7) For the rest of species, phenotypically homogeneous groups can be distinguished by morphometry at least at country level: *P. caeruleostictus* and *P. elongatus* (1 from Guinea-Bissau and 1 from Guinea, each); *P. senegalensis* and *P. bellottii* (1 from C. Ivoire and 1 from Ghana, each).
- 8) One of the main outputs of the project is the development of a new and reliable genetic tool for stock identification of thiof.
- 9) Another relevant output of the project is that we will obtain barcoding information of 4 new species in free genetic data bases.
- 10) A more in-depth analysis of the information obtained by all techniques is being carried out and the results may be useful for fisheries assessment and management recommendations of these species within CECAF.
- 11) The extension of this study to longer periods and to neighboring areas is highly recommended to determine geographic boundaries, needed to define the structure and distribution of these West African stocks.

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Food and Agriculture Organization of the United Nations



# Thank you Merci

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