



World Organisation
for Animal Health



Launch of the Regional Aquatic Animal Health Laboratory Network for Africa (RAAHLN-AF)

5 – 7 December 2023 Pretoria, South Africa





INSTM's contribution as a National Laboratory for diagnosis of AA diseases and food security: Monitoring the health and sanitary quality of aquatic organisms in TUNISIA

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1- INTRODUCTION TO INSTM

Ministry of Agriculture and
Environment



Republic of Tunisia

Institution of Agricultural
Research and Higher Education

INSTITUT NATIONAL DES SCIENCES ET TECHNOLOGIES DE LA MER



28 Rue 2 Mars 1934 Carthage Salammbô

Tel: 00216 71 730420 - 71 730548; Fax: 00216 71 732622

<http://www.instm.agrinet.tn>

History



- ✓ Foundation : **1924**
- **1924 – 1956**: Salammbô Oceanographic Station (SOS)
- **1956 – 1992**: Institut National Scientifique et Technique d’Oceanographie et de pêche
- **1992 - on going**: Institut National des Sciences et Tchnologies de la Mer (INSTM)

- ✓ Date of Registration : **11/30/1998**
- ✓ Legal Status : Non profit, Research organisation, Public body.
- ✓ Main address : **28 Rue 2 Mars 1934 Carthage Salammbô**
- ✓ Tel : **(00216) 71 730 420 – 71 730 548**
- ✓ Fax : **(00216) 71 732 622**
- ✓ Web : <http://www.instm.agrinet.tn>





The INSTM different centers location

➤ 9 active centers

- Salammbô
- Khérididine
- La Goulette
- Monastir
- Mahdia
- Sfax
- Béchima
- Zarzis
- Tabarka

To establish effective lanes for providing **scientific advice** and Transfer its **know-how** and the results of its research to **decision-makers** and marine professionals and scientists.

To **perform and promote research** in the fields of processing technology, marine seafood safety, fishery economics, and fishery technology

To foster public awareness of wide-ranging topics on the functioning and **rational utilization of marine ecosystems** with special emphasis on sustainable fisheries and the ecosystem approach to fisheries management issues.

INSTM Mission

On the scientific level:

- Carry out contract **research programs** in areas directly or indirectly linked to the sea and its resources.
- Participate in various national, regional and international **networks** related to the sea.
- Contribute to the **dissemination** of marine culture and public **awareness** of the protection and preservation of the sea and its biodiversity.

Contribute to university education:

Supervision of doctoral master's students and end-of-study projects and preparing them for professional life

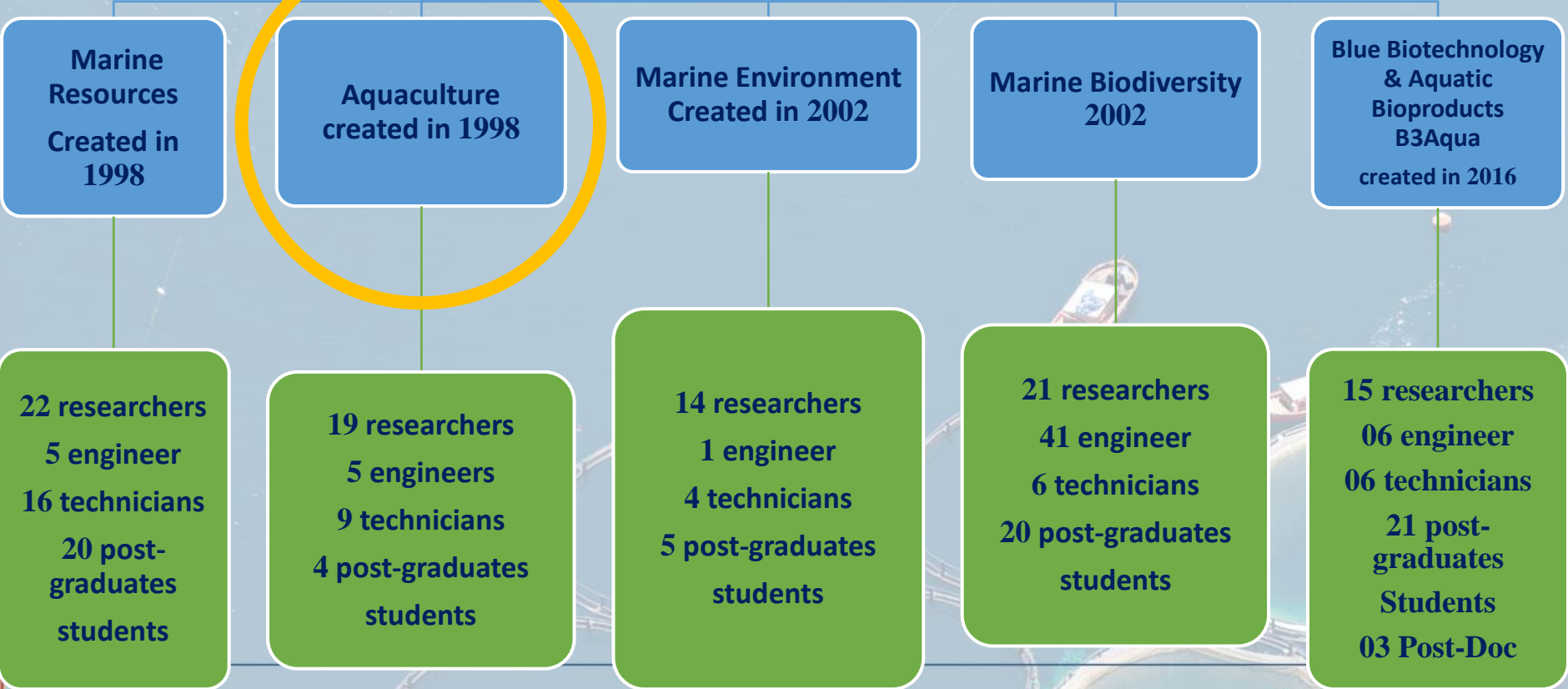
Contribute to the development of the economic and social fabric:

- Carry out specific studies for investors and provide the necessary technical information
- **Carry out laboratory analyzes in the specialties of the institute within the framework of national networks**
- The organization of scientific and technical events to publish and promote research results and their evaluations



National Research Structures
=
Laboratories (funded by the ministry of higher education)

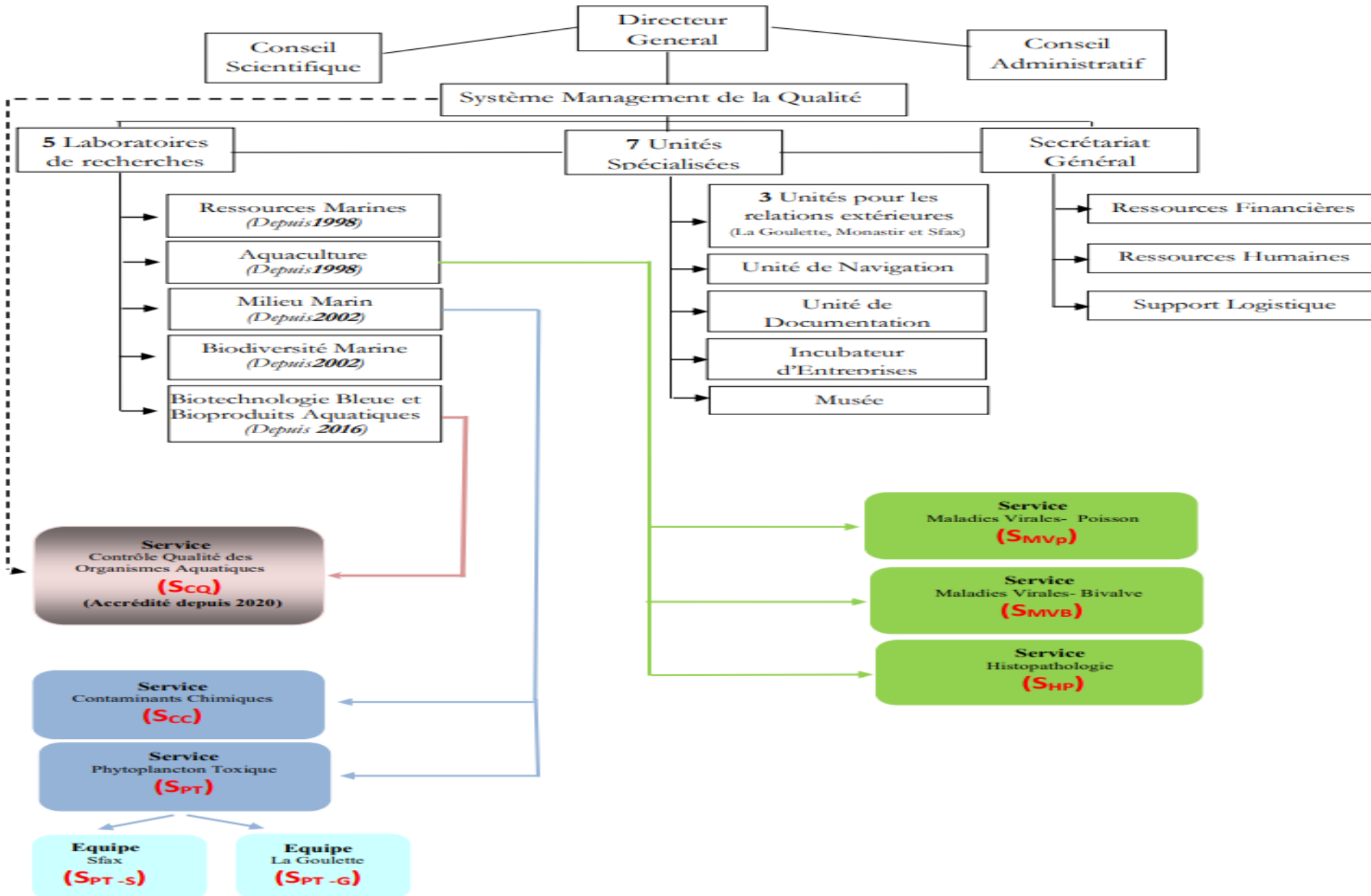
Gov funding
National and Int.
projects (FAO, IAEA,
WOAH, EU, USAID,
...





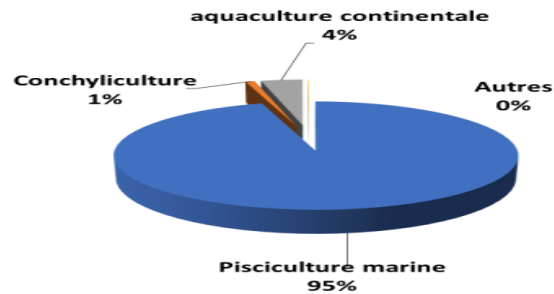
ORGANIGRAMME ACTUEL DE L'INSTM

Avec les entités accréditées (**SCQ**) et celles qui s'appretiennent pour l'accréditation (**SCC, SPT, SMVP, SMVB, SHP**)



2- CURRENT STATE OF AQUACULTURE IN TUNISIA AND PROBLEMS LINKED TO AQUACULTURE PATHOLOGIES

- Tunisia occupies a central place in the Mediterranean
- widely open to the sea, especially on its eastern and southern shores
- The coastline +1300 km,
- The national maritime domain of 80,000 km²,
- 105,200 hectares of lagoons
- 20,000 hectares of freshwater bodies (dams and hillside lakes).
- 4.66 billion m³/year of usable water (42% groundwater).



Marine fish farming:

- 20761T (95%)
- 25 fermes



Fresh water Aquaculture :

- 811T (4%)
- 32barrages
- 6 fermes pour l'élevage du Tilapia (30T)



Shellfish farming:

- 183 T (1%)
- 8 fermes



Seaweed farming:

- 7 projets
- 13 T Spiruline sèche



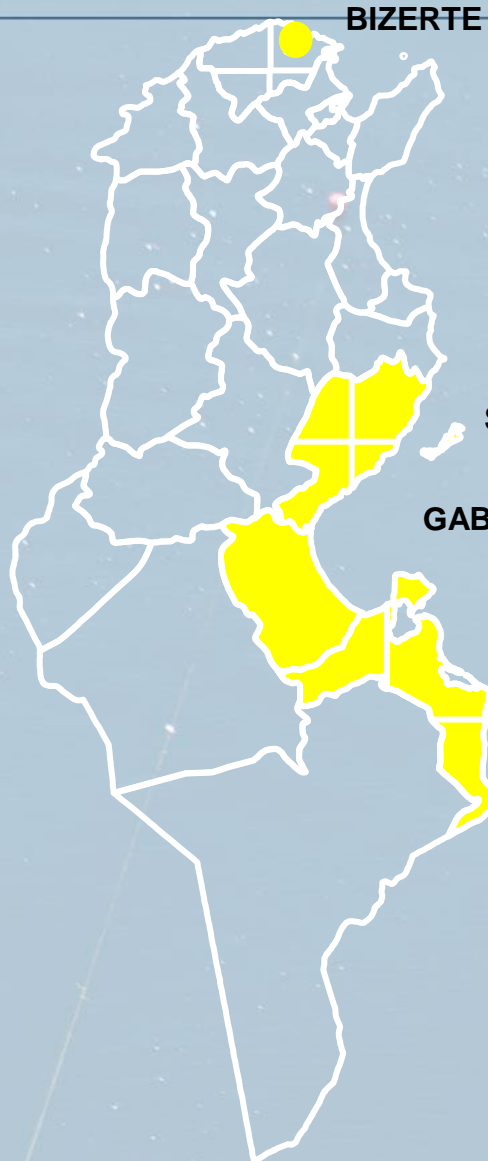
Shrimp farming:

- Projet pilote *Penaeus vannamei*
- 2 T



FARMING SYSTEMS AND PRACTICES

Extensive/Non-Fed Aquaculture



Mussels
Mytilus galloprovincialis



Oysters
Crassostrea gigas



Clams (*Ruditapes decussatus*)

- 1- Official control at the level of MBV Production zones
- 2- Official control of MBV purification centers

Intensive/Fed aquaculture systems:



Intensive / Fed Aquaculture

Marine aquaculture

Fresh water aquaculture



Marine finfish farming
(Floating cages,
Raceways)

Bluefin Tuna fattening
(Floating cages)

Shrimp farming (Raceways)

Tilapia farming (Raceways)

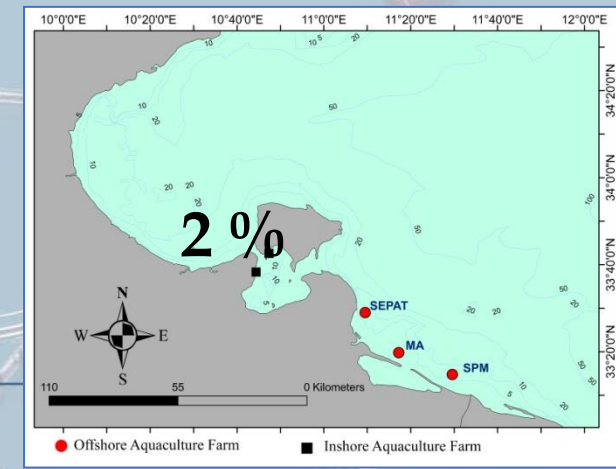
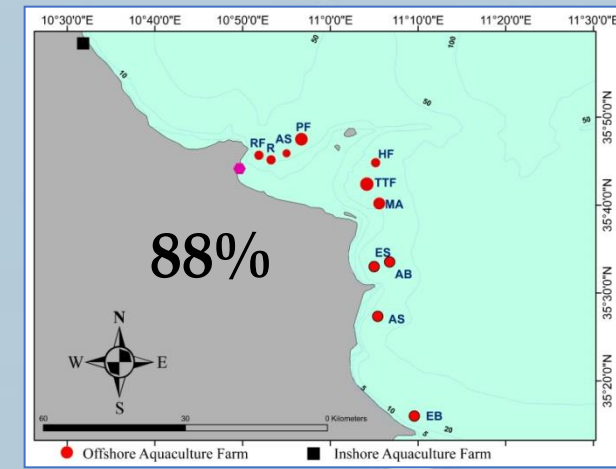
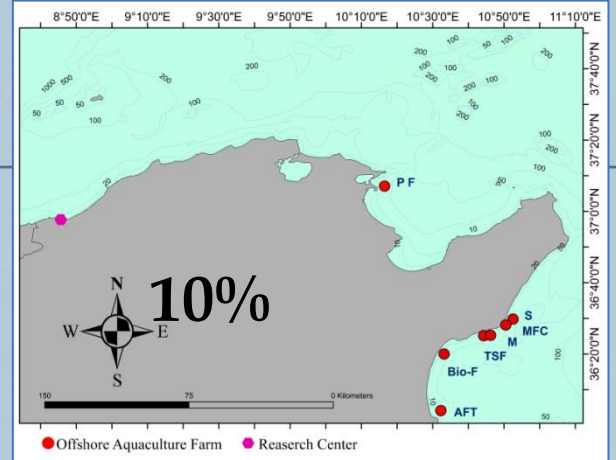
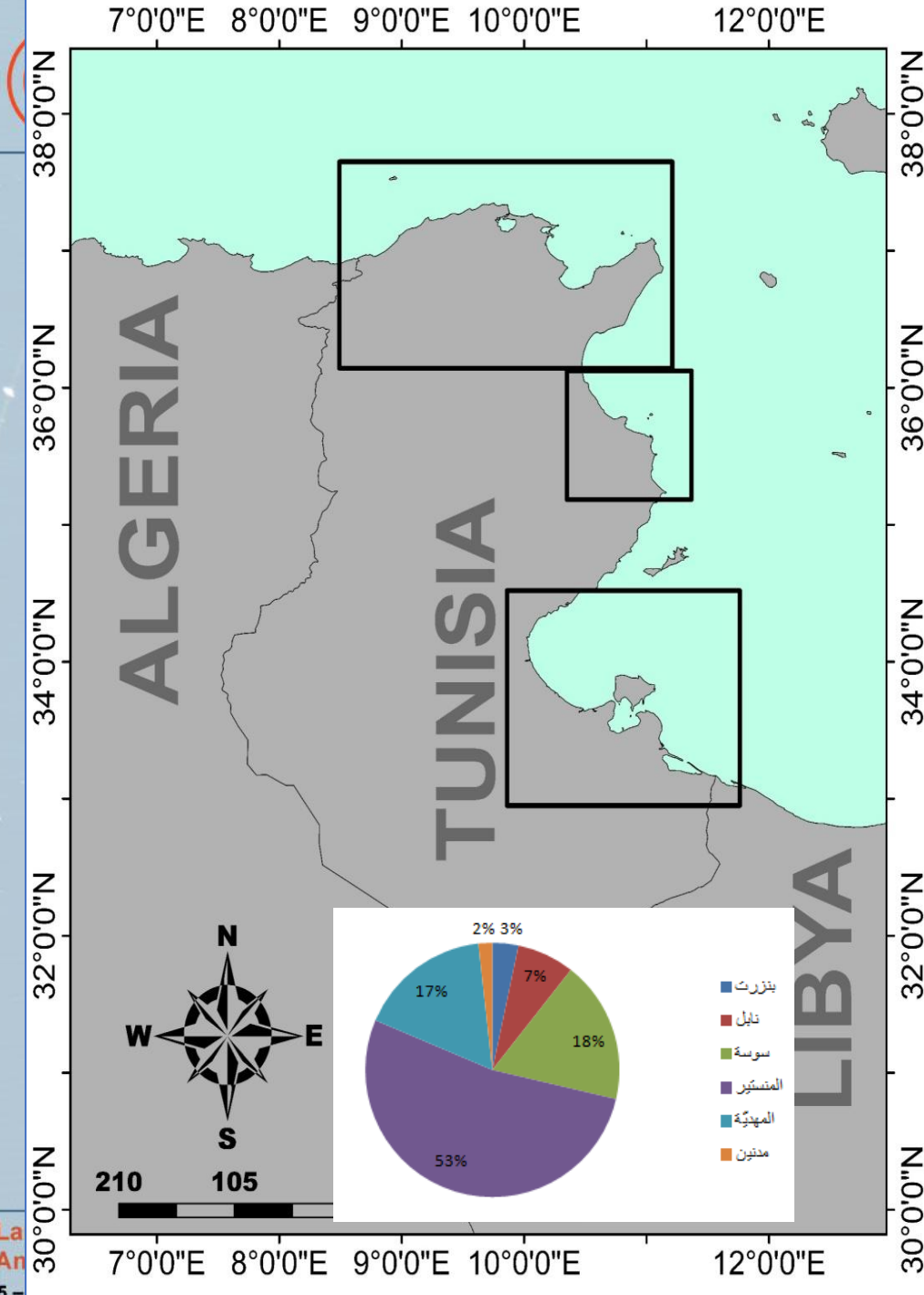


Seabass, Seabream,
Meagre

Thunnus Thunnus

Penaeus vannamei

Nile Tilapia, Red Tilapia



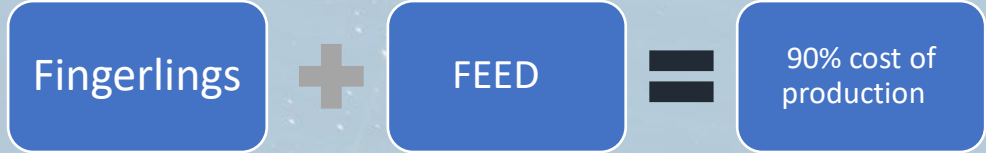
23 projects.

72% of the Capacity production granted

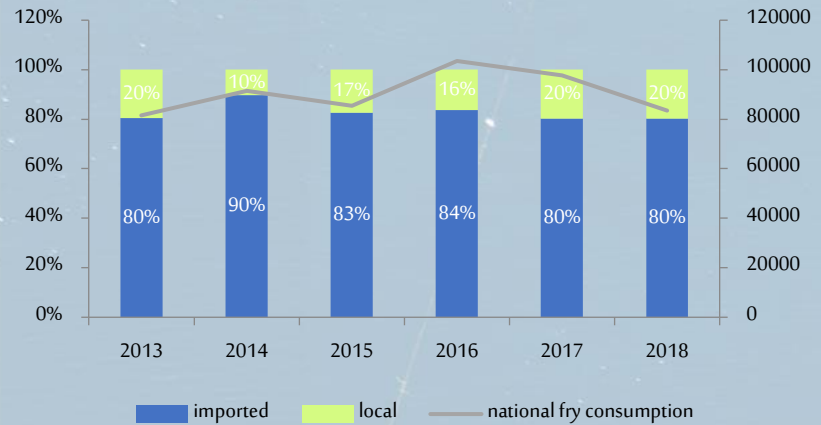




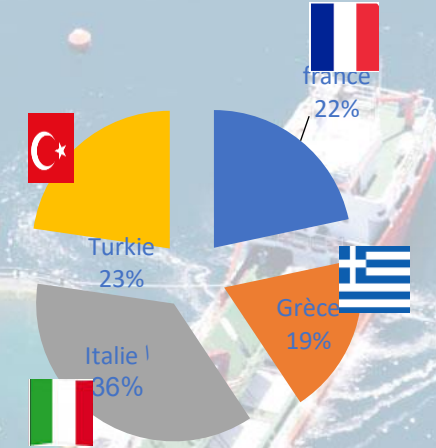
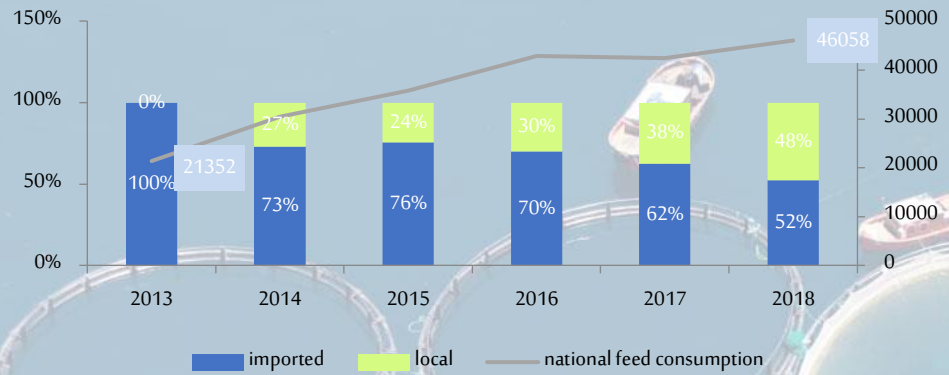
Marine finfish farming main inputs



Evolution de la consommation d'alevins 2013-2018



Evolution de la consommation d'aliment 2013-2018



• 2 local hatcheries & 2 feed units

**3- DIAGNOSTIC LABORATORY
CAPACITY AT INSTM:
MONITORING OF SANITARY AND
AQUACULTURE LINKED
PATHOLOGIES**

OFFICIAL CONTROL (WOAH + EU STANDARDS)

NATIONAL SURVEILLANCE NETWORK OF BIVALVE MOLLUSCS/FISH AT INSTM

General Organization

- Monitoring parameters

*Sanitary Network

*Sampling areas and sites

*Techniques and location of analyzes

(REMI, REPHY, REBY, RECNO and REVI (shellfish))

*Zoosanitary Network

(REZOM, REVI (fish))

Regional Veterinarians (CRDA)

Samples collection

Sampling and transmission

Results communication

Created in 1995

INSTM Laboratories

Samples analysis

**General Directorate of Veterinary
Services: DGSV
(Competent authority)**
**Centralization, extension of results,
transfer and export authorization**

Results trasmission

Analyzes requested from INSTM labs as part of the official control of seafood and MBV

Matrice	Analyse demandée	Méthode exigée	Exigence qualité	Prix unitaire en DT
Poissons	La recherche du virus de la septicémie Hémorragique Virale (VSHV)	RT-PCR en temps réel (Manuel des maladies des organismes aquatiques, OMSA 2021)	Système assurance qualité en cours	200
Poissons	La recherche du virus de la nécrose hématopoïtique infectieuse (VNHI)	RT-PCR en temps réel (Manuel des maladies des organismes aquatiques, OMSA 2021)	Système assurance qualité en cours	200
Poissons	Analyse Histamine	ISO 19343: 2017 (Fr) , HPLC, Chromatographie	Accréditée selon la norme ISO/CEI 17025:2017	90
Poissons	Analyse ABVT	Méthode interne validé accréditée (Ruiz-Capillas et al. 1999 ; Système FIA)	Accréditée selon la norme ISO/CEI 17025:2017	45
Eau	Numérotation et identification des phytoplanctons toxiques dans l'eau de mer	Méthode d'Uthermol	En cours d'accréditation	93
Mollusques Bivalves	Norovirus et virus de l'hépatite A dans les MBV Herpes virus	RT-PCR en temps réel , ISO 15216-1:2017 -	Laboratoire sous assurance qualité	700
Mollusques Bivalves	La recherche de la maladie de la perkinsose	Méthode qualitative :Histologie Coupes histologiques de tissus de mollusquescolorés à l'hémalum- éosine« Manual of Diagnostic Tests for Aquatic Animals» de l'OIE	Laboratoire sous assurance qualité	92
Mollusques bivalves	Analyse DSP	NFEN16024: 2013 validée accréditée Par LCMS/MS	Accréditée selon la norme ISO/CEI 17025:2017	350
Mollusques bivalves	Analyse ASP	Méthode interne validée Accréditée Par LCMS/MS	Accréditée selon la norme ISO/CEI 17025:2017	350
Mollusques bivalves	Analyse PSP	Méthode interne validée Accréditée Par LCMS/MS	Accréditée selon la norme ISO/CEI 17025:2017	200
Mollusques bivalves	Analyse TMA	Méthode interne validée accréditée (FIA)	Accréditée selon la norme ISO/CEI 17025:2017	45

Numbering and identification of toxic phytoplankton in seawater

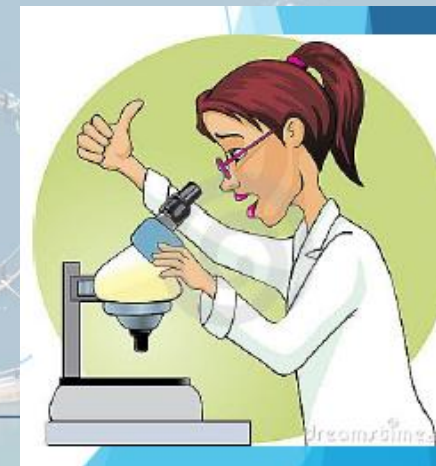
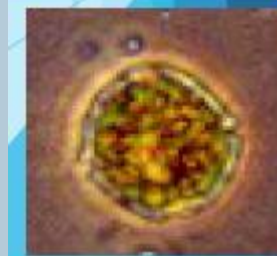
PHYTOPLANCTON: Méthodes et seuils limites

- méthode:** quantitative sous microscope inversé
- Interprétation des résultats:**
 - ✓ **Situation normale:** absence d'espèces toxiques
 - ✓ **Situation de surveillance:** présence d'espèces toxiques en quantité inférieure à la concentration critique
 - ✓ **Situation d'alerte:** présence d'espèces toxiques en quantité supérieure à la concentration critique

Karenia



Alexandrium



REVI (MBV)

Depuis 2014



Recherche de Norovirus et virus de l'Hepatitis A dans les MBV

Norovirus et virus de l'hépatite A : Méthodes et seuils limites

Méthode: RT-PCR

ISO/TS15216.2013

Interprétation des

résultats: résultat positif si
présence

• Europe pas de réglementation spécifique : Règlement (CE) n°
178/2002 (Articles 7 et 14):

- Principe de précaution
- Produit dangereux



Recherche de la bio toxine lipophiles

« DSP »

Depuis 2019



La méthode de référence pour la recherche des Lipophiles:
LC-MS/MS (NFEN16024 :2013)



Texte réglementaire de l'(UE): N°15/2011 de la
commission de 10 Janvier 2011



Accréditation de ce paramètre à l'INSTM
(Lab B3Aqua (en 2019))



REseau Zoosanitaire des Mollusques (depuis 2005)

REZOM



Parasites protozoaires



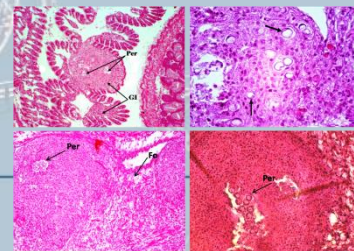
*Exp: Marteilia sp.,
Bonamia sp.)
refringens,*



Exp: Perkinsus sp



Caractérisation et identification du parasite
Perkinsus sp. Par l'Histologie selon



OIE/EU

Initiation for the establishment of a National Aquatic Animal Health Network

In accordance with the requirements of memorandum n°200/2930 of 12/19/2019 published by the Ministry of Agriculture (Director General of Veterinary Services), establishing the national surveillance program for viral hemorrhagic septicemia (SHV) and infectious hematopoietic necrosis (IHN) in farmed marine fish: REVIP since 2020



قرار من وزير الفلاحة والموارد المائية والصيد البحري
مؤرخ في 31 جويلية 2017 يتعلق بضبط الشروط الصحية
للأصناف المائية.

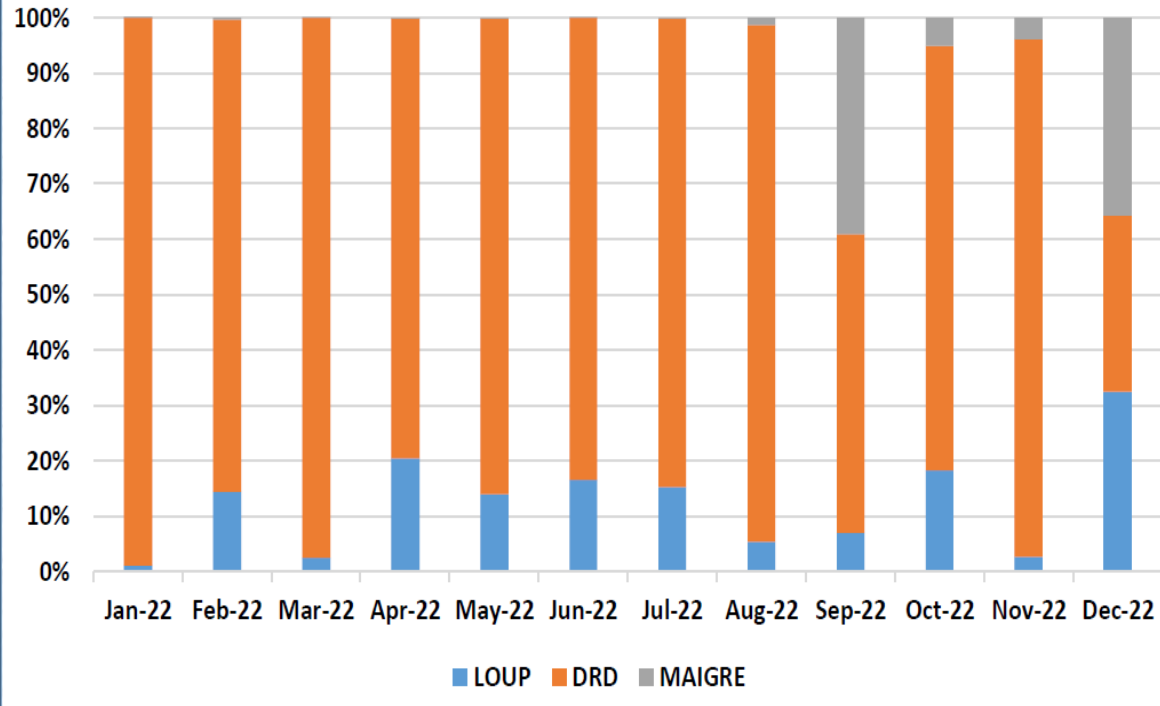


More than 6,000 specimens were received from 21 aquaculture farms and more than 300 analyses/company were carried out by the INSTM Fish Virology Laboratory.

Self Freedom declaration based on a Target surveillance

OTHER ECONOMIC IMPORTANT DISEASES:

MONITORING OF MARINE FISH PATHOGENS

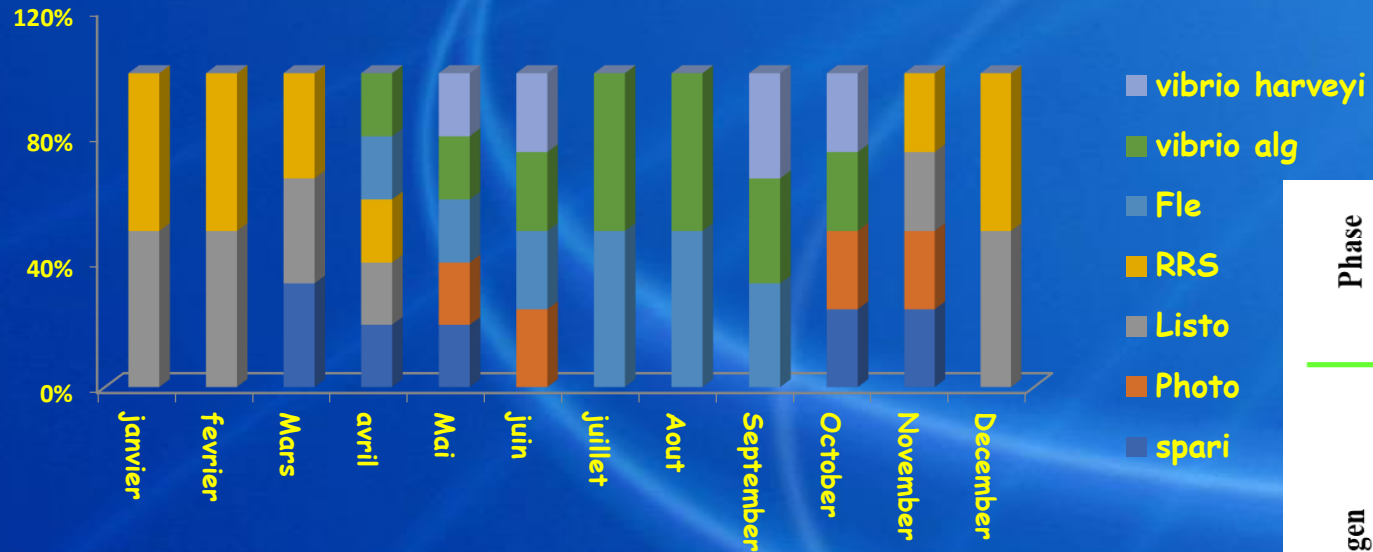


List of the most recorded diseases in marine fish in TUNISIA (INSTM RESULTS)

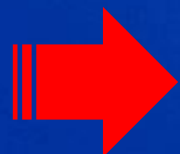
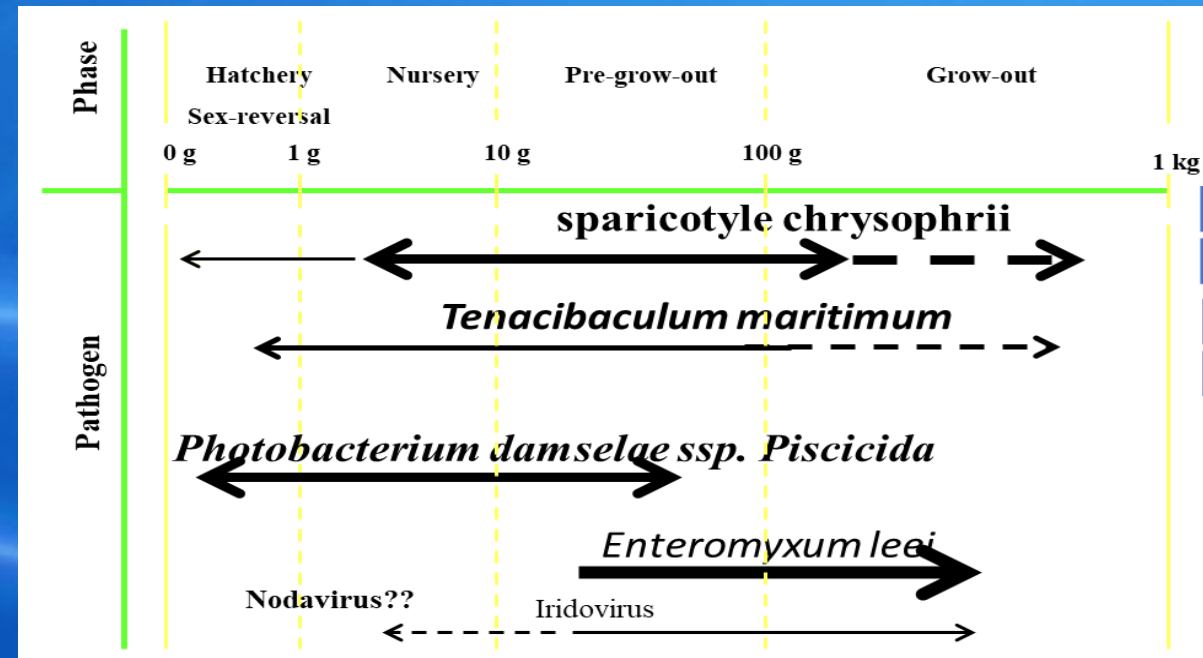
Bacterial diseases	<i>Listonella Anguillarum</i> <i>Vibrio harveyi</i> <i>Vibrio alginolyticus</i> <i>Photobacterium damsela</i> <i>subsp damsela</i> <i>Photobacterium damsela subsp piscicida</i> <i>Tenacibaculum maritimum</i> <i>Aeromonas hydrophila</i> <i>Mycobacterium marinum</i> <i>Streptocoque iniae</i> <i>Pseudomonas anguilliseptica</i>
Parasitological diseases	<i>Sparicotyle chrysophrii</i> <i>Furnestinia echneis</i> <i>Diplectanum aequans</i> <i>Enteromyxum leei</i> <i>Ceratomyxa aurata</i> <i>Cardicola aurata</i>
Viral diseases	<i>Nodavirus, LCDV</i>
Unknown ethiology	<i>Red Rush Disease</i>

Monthly Evolution of
Mortality by famed Species

Chronology of the appearance of infectious diseases in sea bream

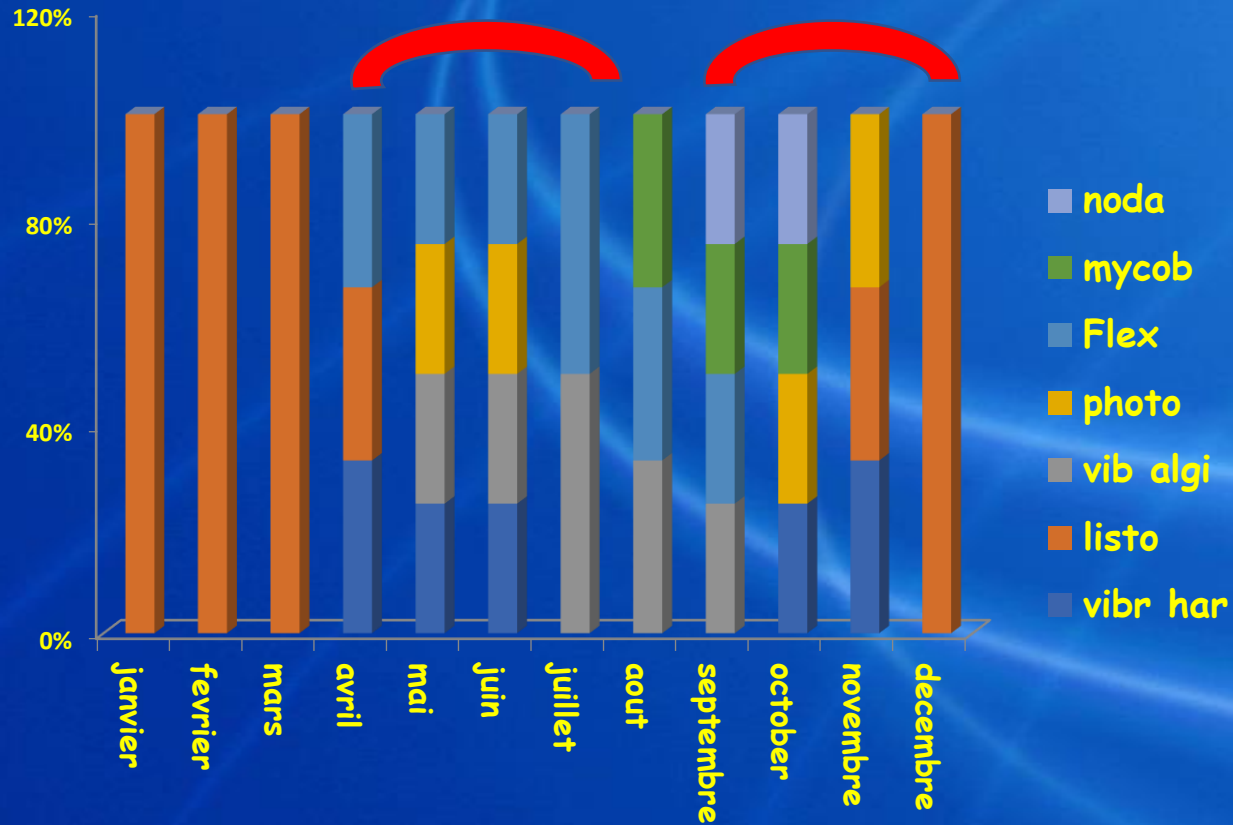


Major Diseases Affecting *Sparus aurata*

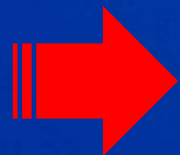
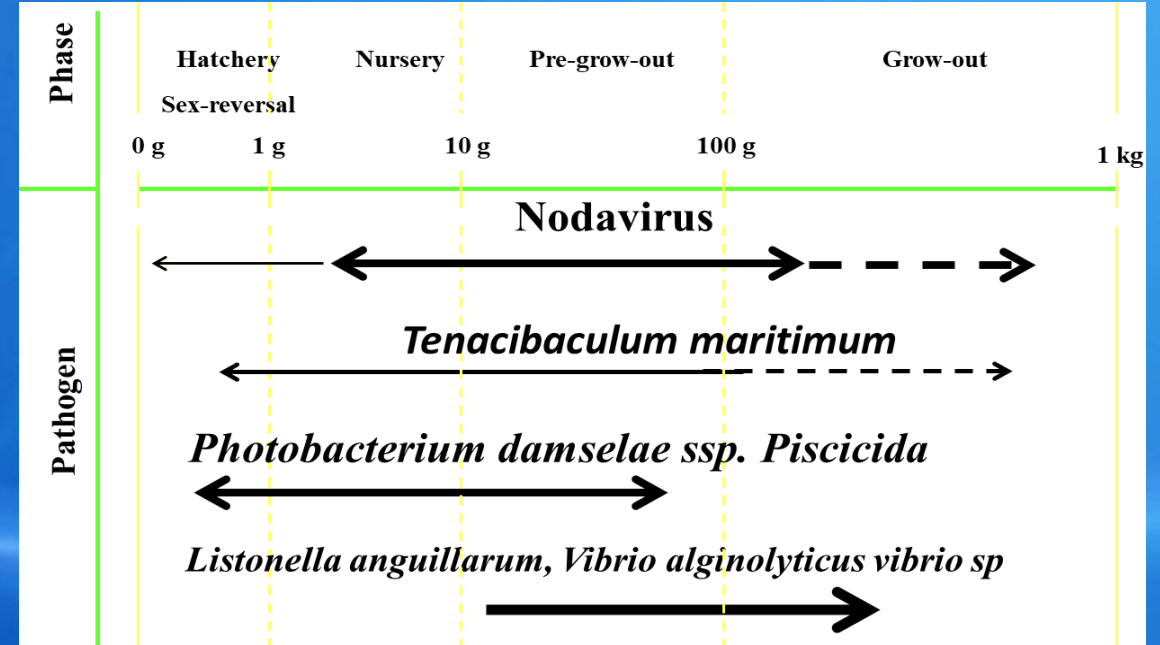


Vulnerability spread essentially during the spring and autumn period

Chronology of the appearance of infectious diseases in sea bass



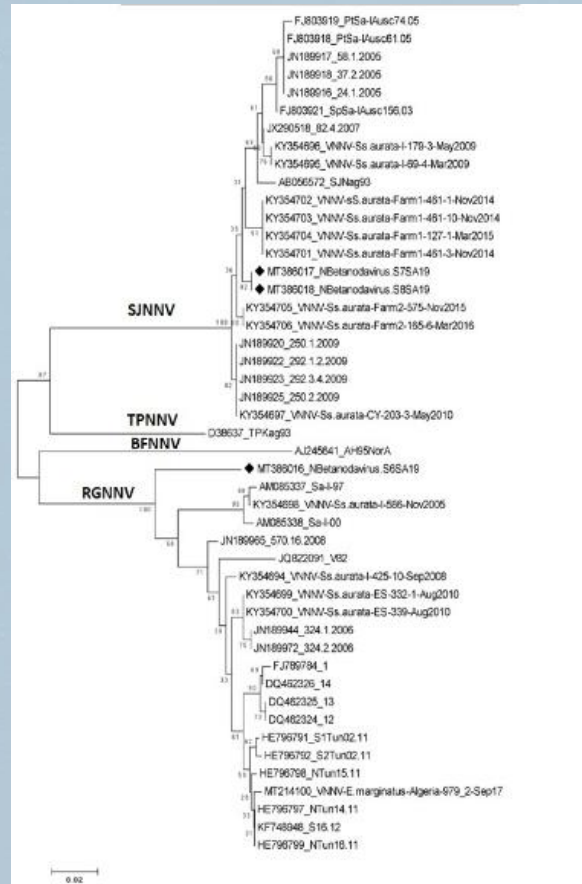
Major Diseases Affecting *Dicentrarcus labrax*



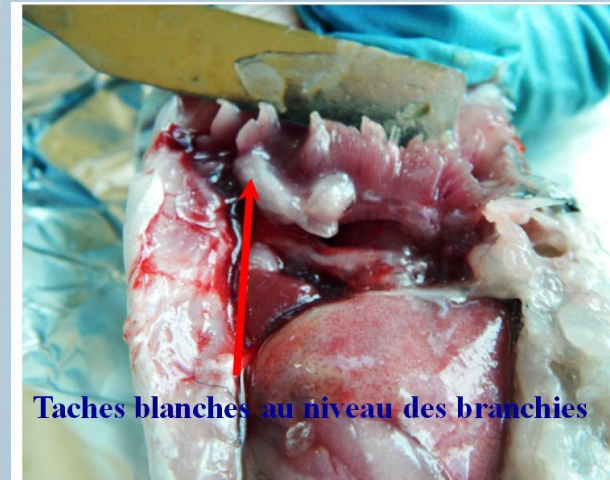
Vulnerability spread essentially during the spring and autumn period

Recent outbreaks

2020: Nodavirus RGNN/SJNNV



2022: Myxosporidia Henneguya sp



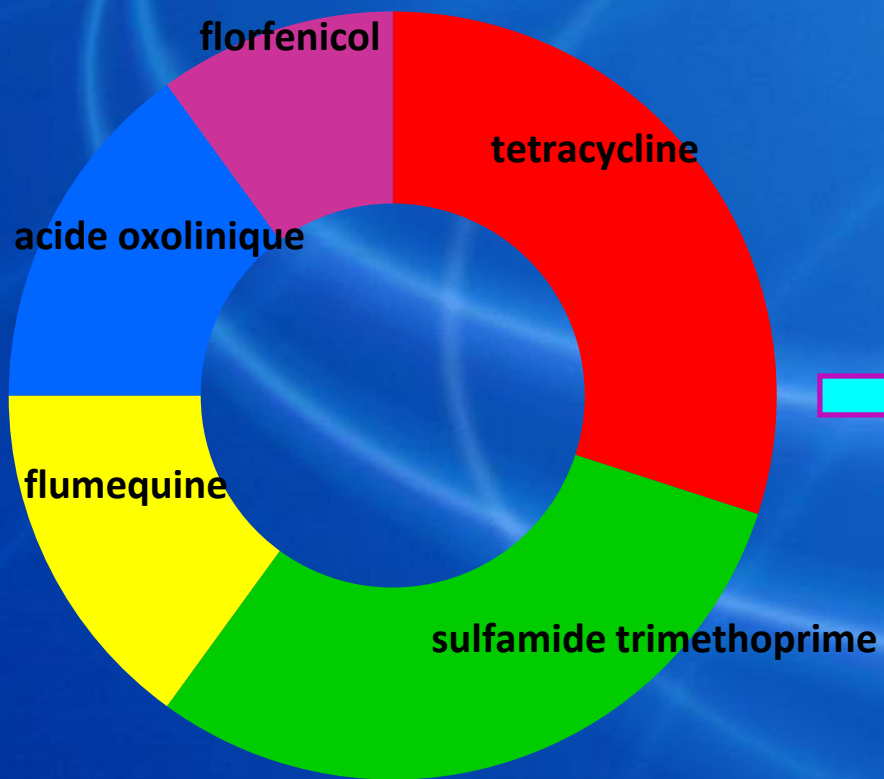
Taches blanches au niveau des branchies



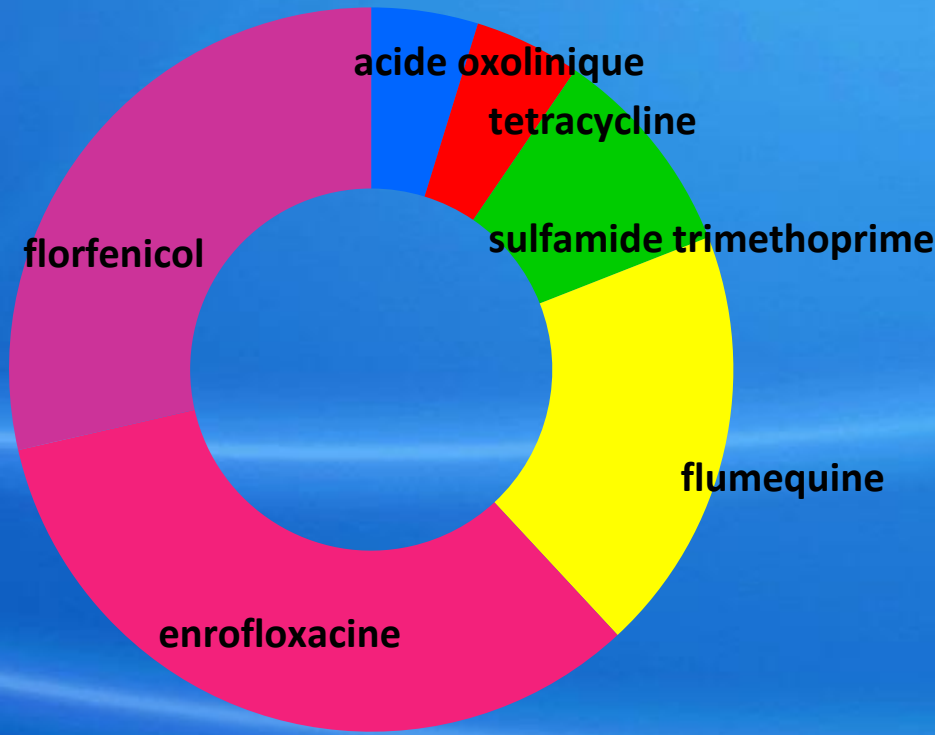
Spores de parasite

Drug treatments.

The antibiotics used in Tunisian aquaculture sites are: oxytetracycline, Sulfonamide-trimethprim, Oxolinic acid, Flumequine, Florfenicol and Enrofloxacin



Frequencies of the main antibiotics used between 2010-2015



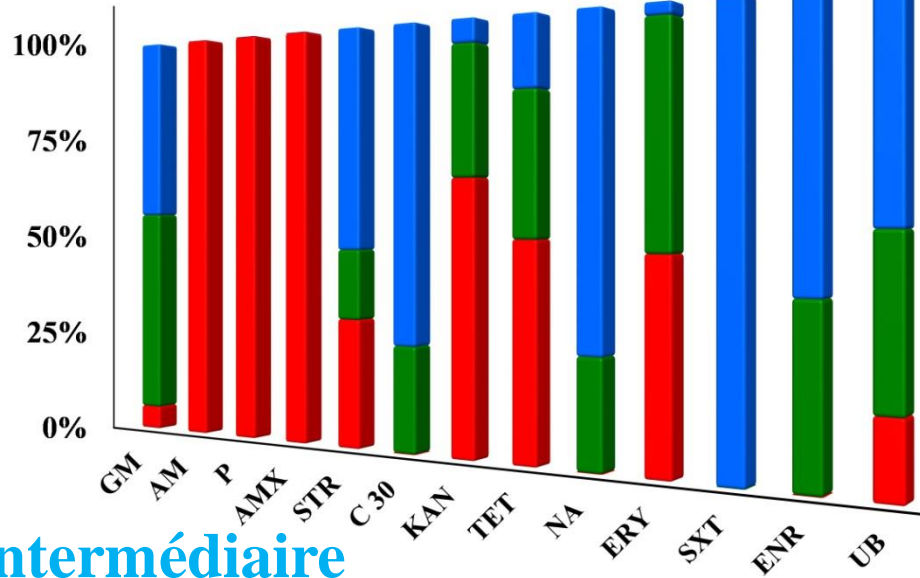
Frequencies of the main antibiotics used between 2016-2019



Response of the different isolates to the antibiotics studied



POURCENTAGE



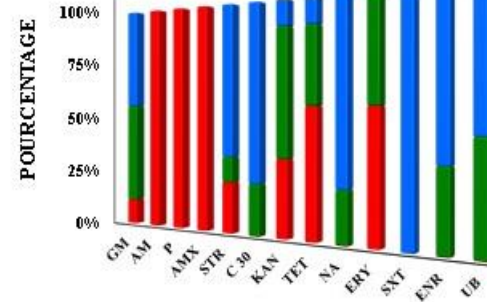
I
R
S

Intermédiaire

Résistance

Sensibilité

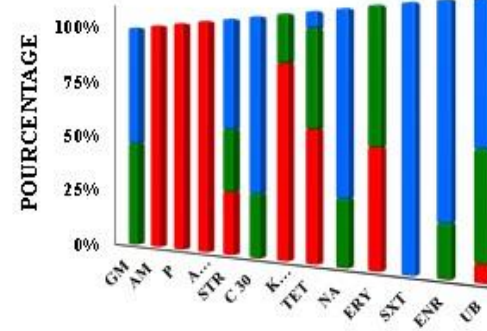
39% resistance; 23% intermediate resistance and 36% sensitivity (at the level of all matrices studied) **FISH: 49% resistance**; 19% intermediate resistance and 32% sensitivity **WATER 39% resistance**; 23% intermediate resistance and 27% sensitivity, **SEDIMENT 41% resistance**; 23% intermediate resistance and 34% sensitivity



FISH



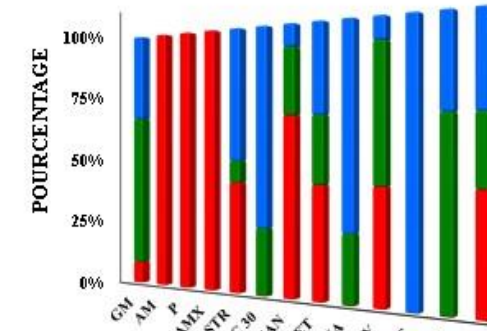
SEDIMENT



WATER



POISSON



SEDIMENT

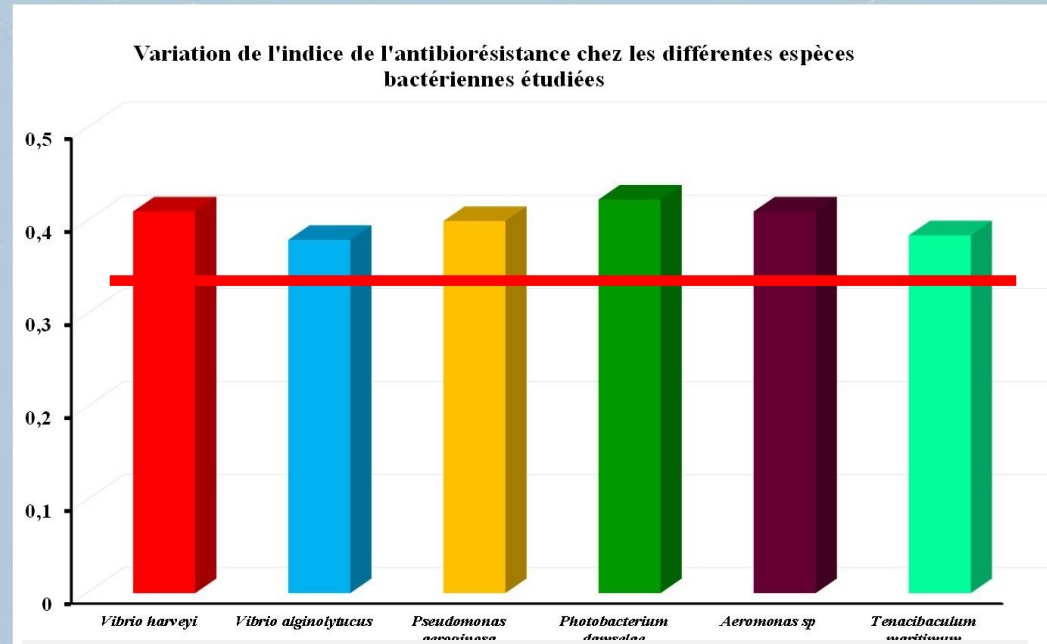


Treatment effectiveness: Correlation circle between the different antibiotics studied

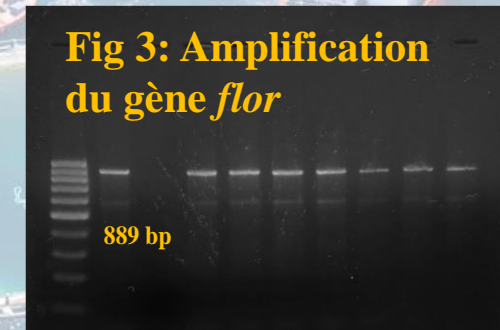
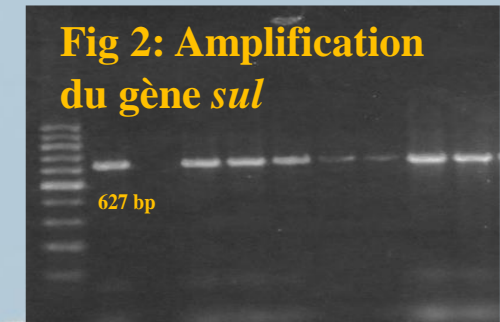
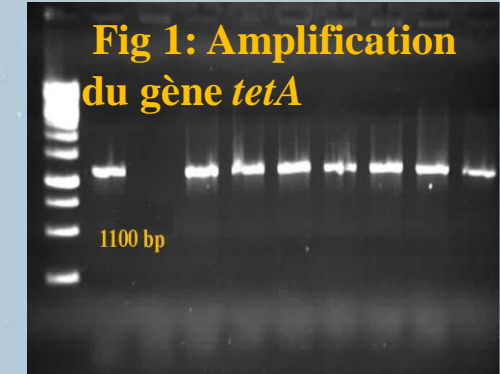
Which antibiotic to recommend?



The principal component analysis explains 44.6% of the variability, however we notice a similar effect between Erythro, Strepto, and amoxiciline. As well as between enroflox and flumequine, (tetra, sulfam and nalidixic acid (when treating with these antibiotics)



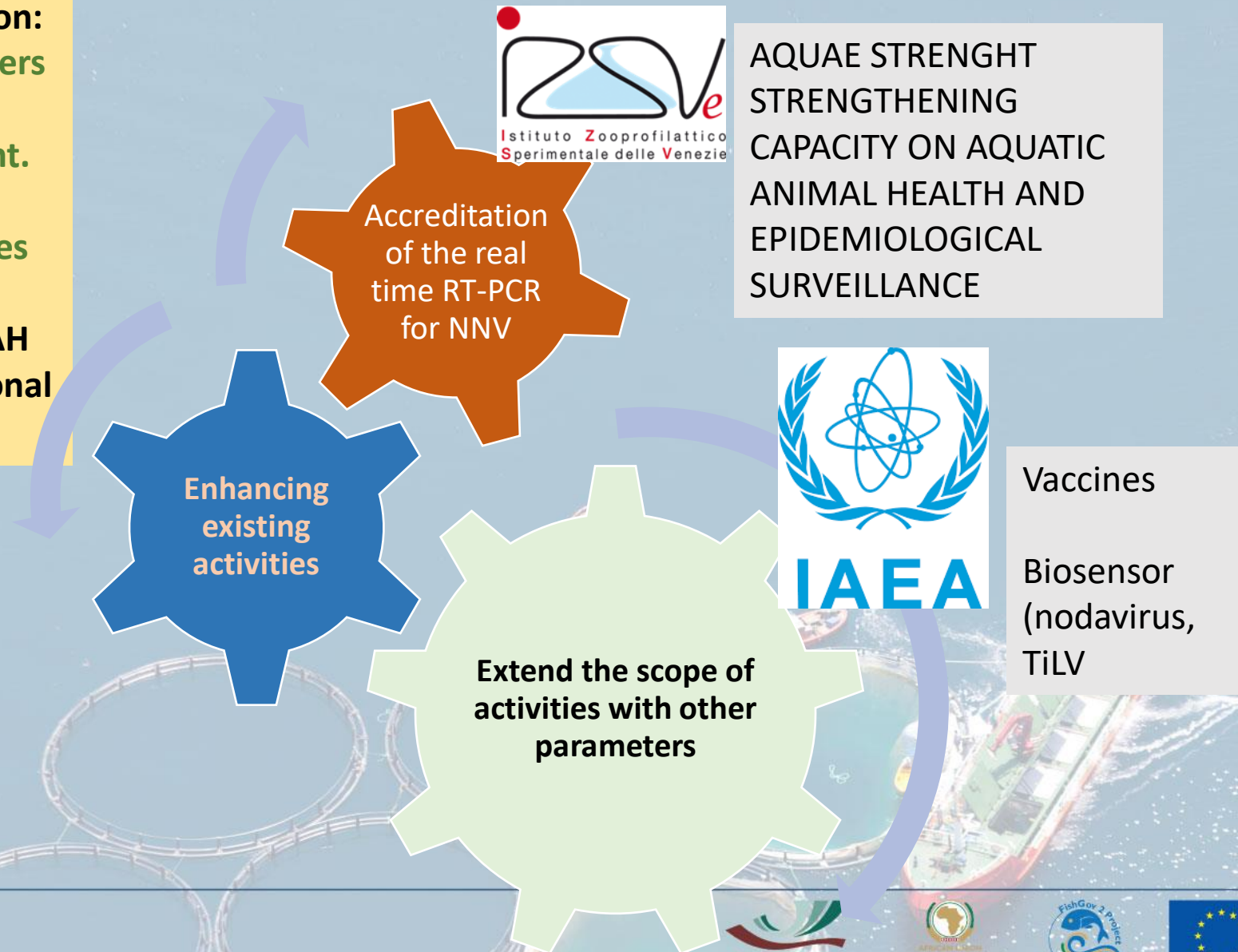
The hierarchical classification of the response of the different strains to the antibiotics tested does not show a related particularity to the species which can be explained By the MAR (Multi Antibiotic Resistance index) (average) which is similar between the different species and in the majority greater than 0.35 Demonstrating that bacterial species have been exposed to antibiotic treatment in their life cycle



3- NEXT STEPS AND CHALLENGES

Diagnostic is a continuous and lasting action:
Engagement of the CA and other stakeholders
Lack of communication between structure
Sustainable funding source (autocontrols, int. agencies, Gov,...)
Few PERMENANT qualified human resources

There is a need to establish a National AAH diagnostic center within INSTM organizational chart



Cell culture unit – Viral isolation



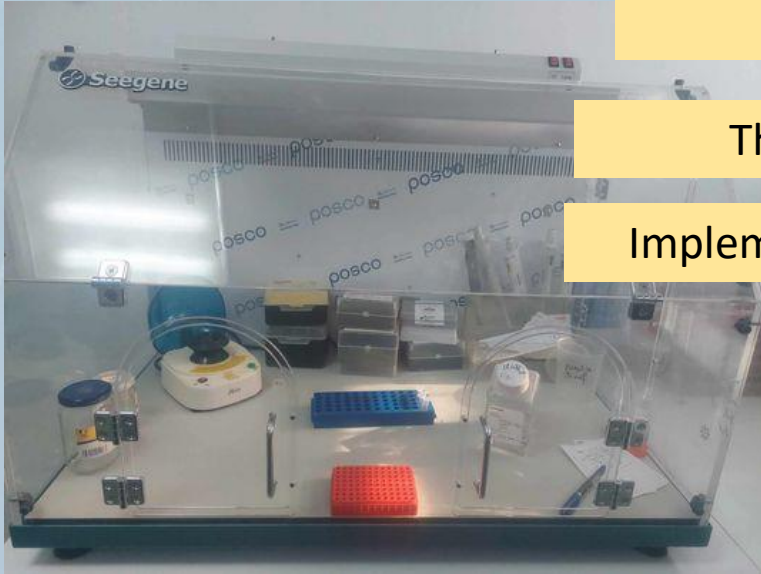
ELISA- Monitoring of broodstock



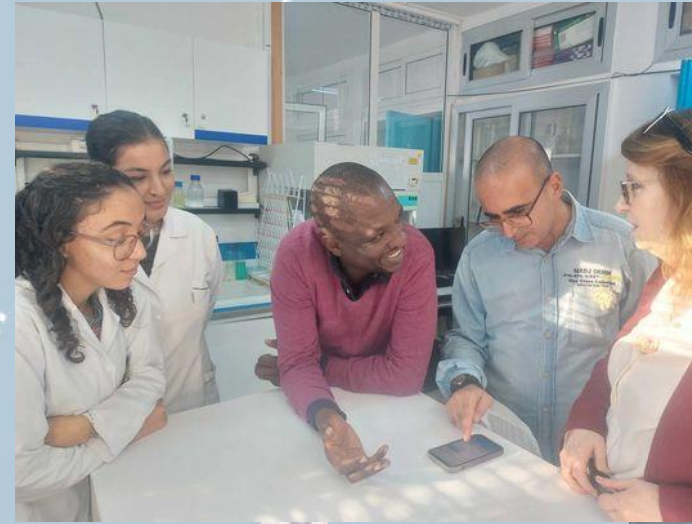
Toward ISO 17025 (2017)

The accreditation of the real time RT-PCR for NNV

Implementation of basics for a quality system management



Enhancing Building Capacities Activities



Thank you for your Attention

