

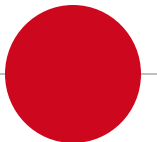
Diagnostic capacities (IZS^{Ve} Reference Laboratory)



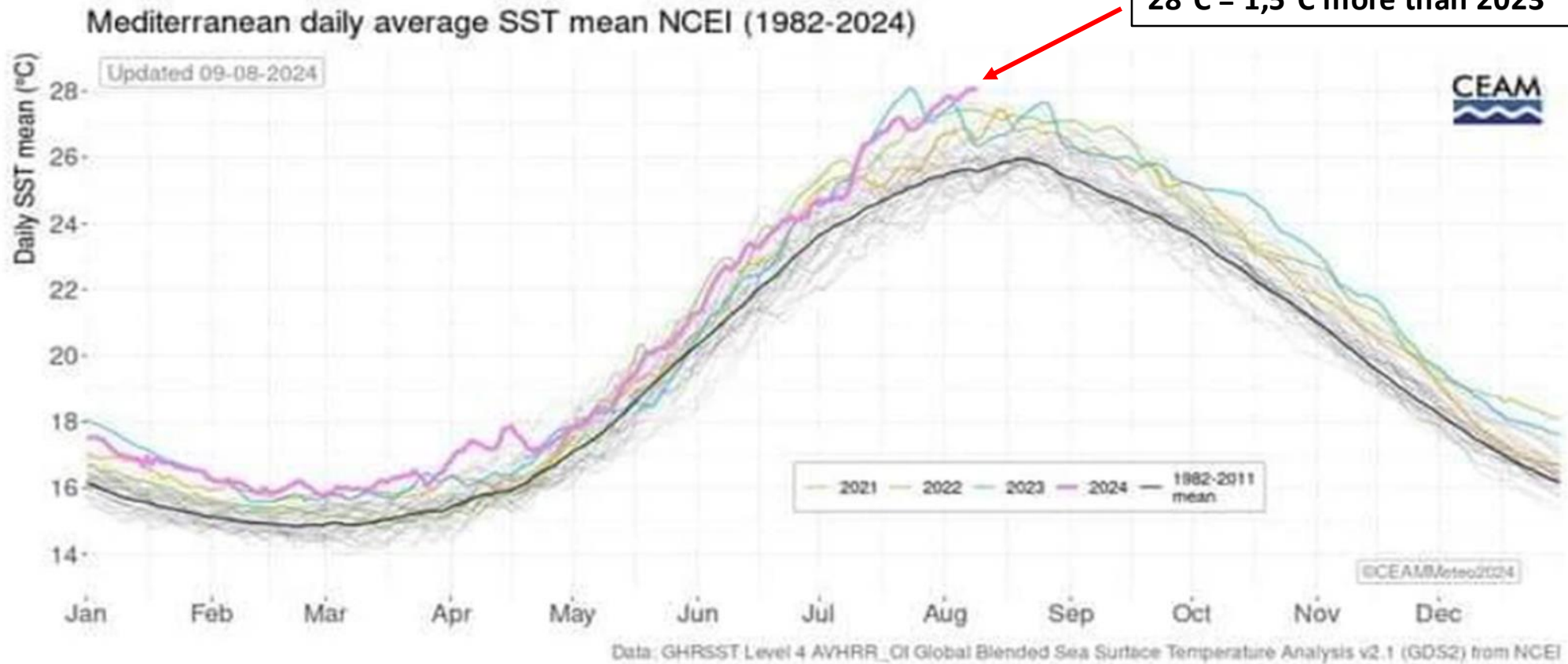
Amedeo Manfrin

WOAH Reference Laboratory for Viral Encephalopathy and Retinopathy
Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy

AQMENET National Focal Points training - Jeddah October 28th 2025

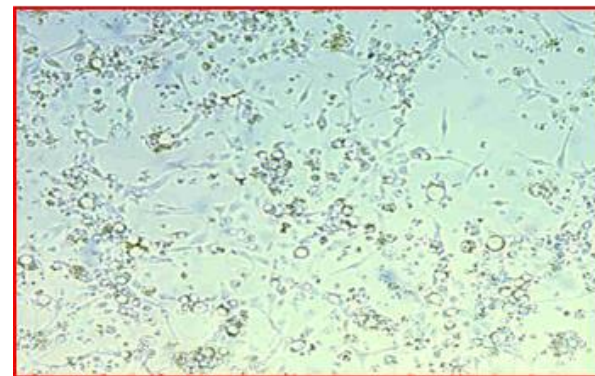
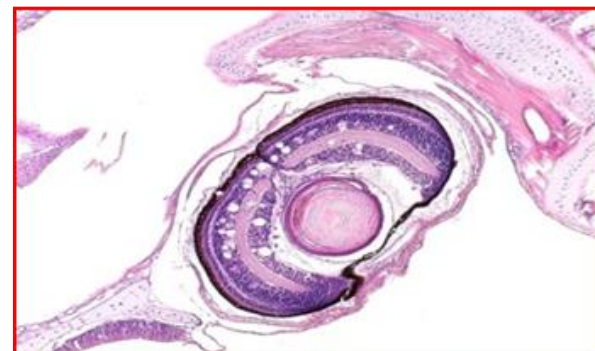


Climate change is a big issue in the world: see average water temperature in Mediterranean sea



● The most important disease: VER-VNN

- Viral Encephalopathy and Retinopathy (VER) syn. Viral Nervous Necrosis (VNN) is a severe neuropathological disease caused by RNA viruses of genus *Betanodavirus* (Fam. *Nodaviridae*).
- It was detected from the first time in late '80 but rapidly spread worldwide
- Due to its broad host range, extended geographic diffusion and economic impact **VNN is included among the most significant viral pathogens of finfish**



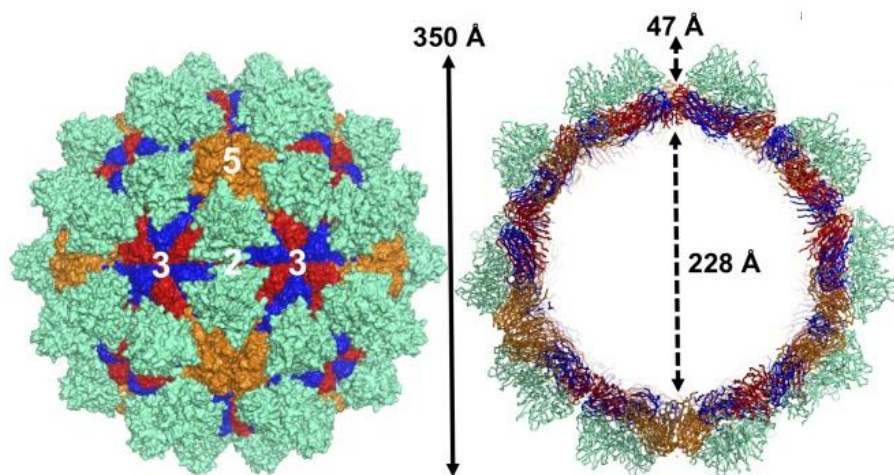


Aetiological agent

CRYSTAL STRUCTURE OF GNNV-LP

Surface organization

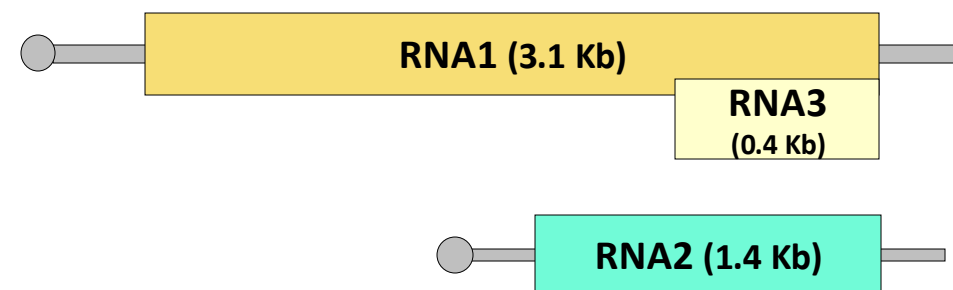
Central cavity



Chen *et al.*, 2015

GENOMIC ORGANIZATION

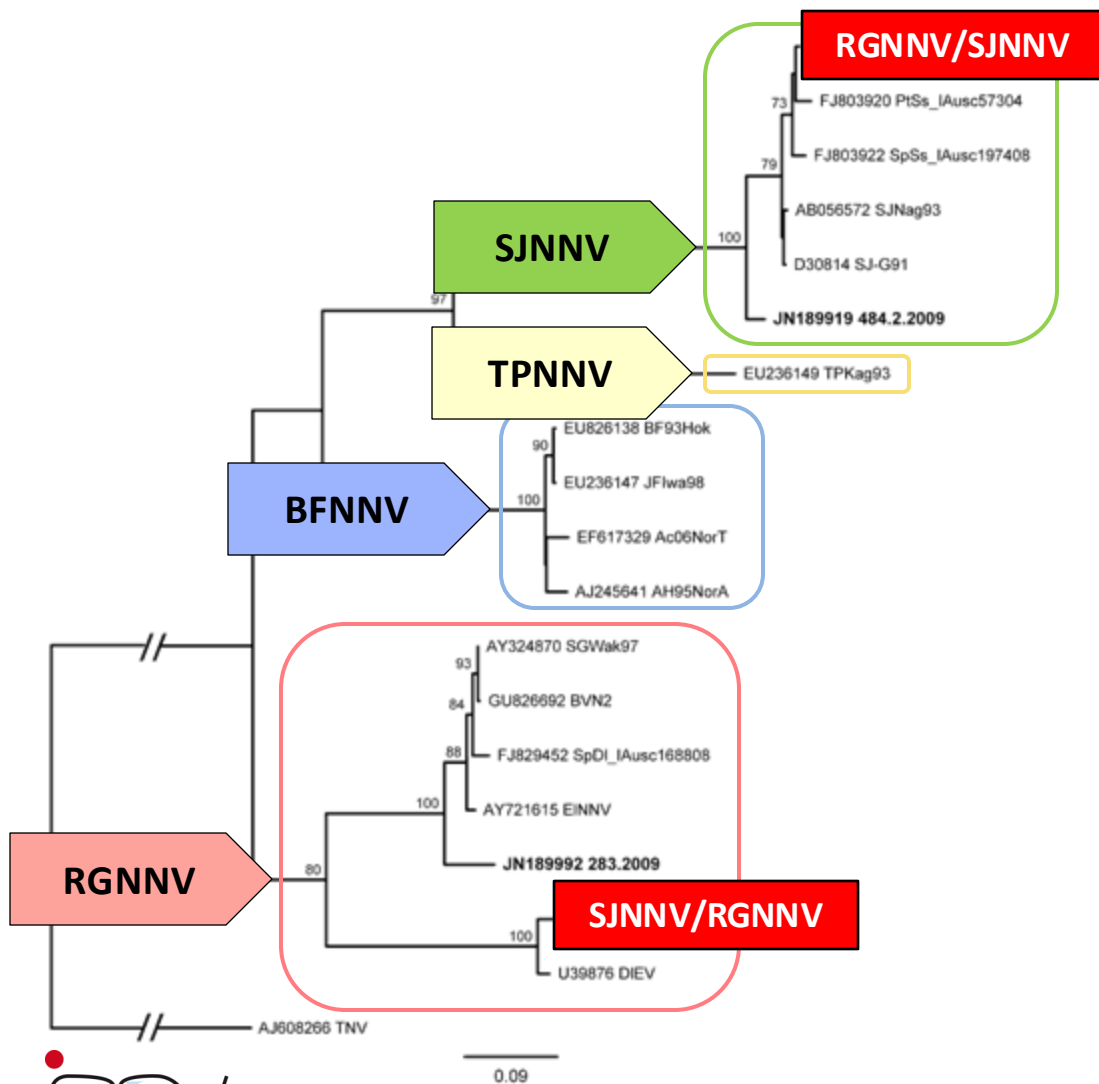
Segmented +ssRNA
CAP at the 5'-end



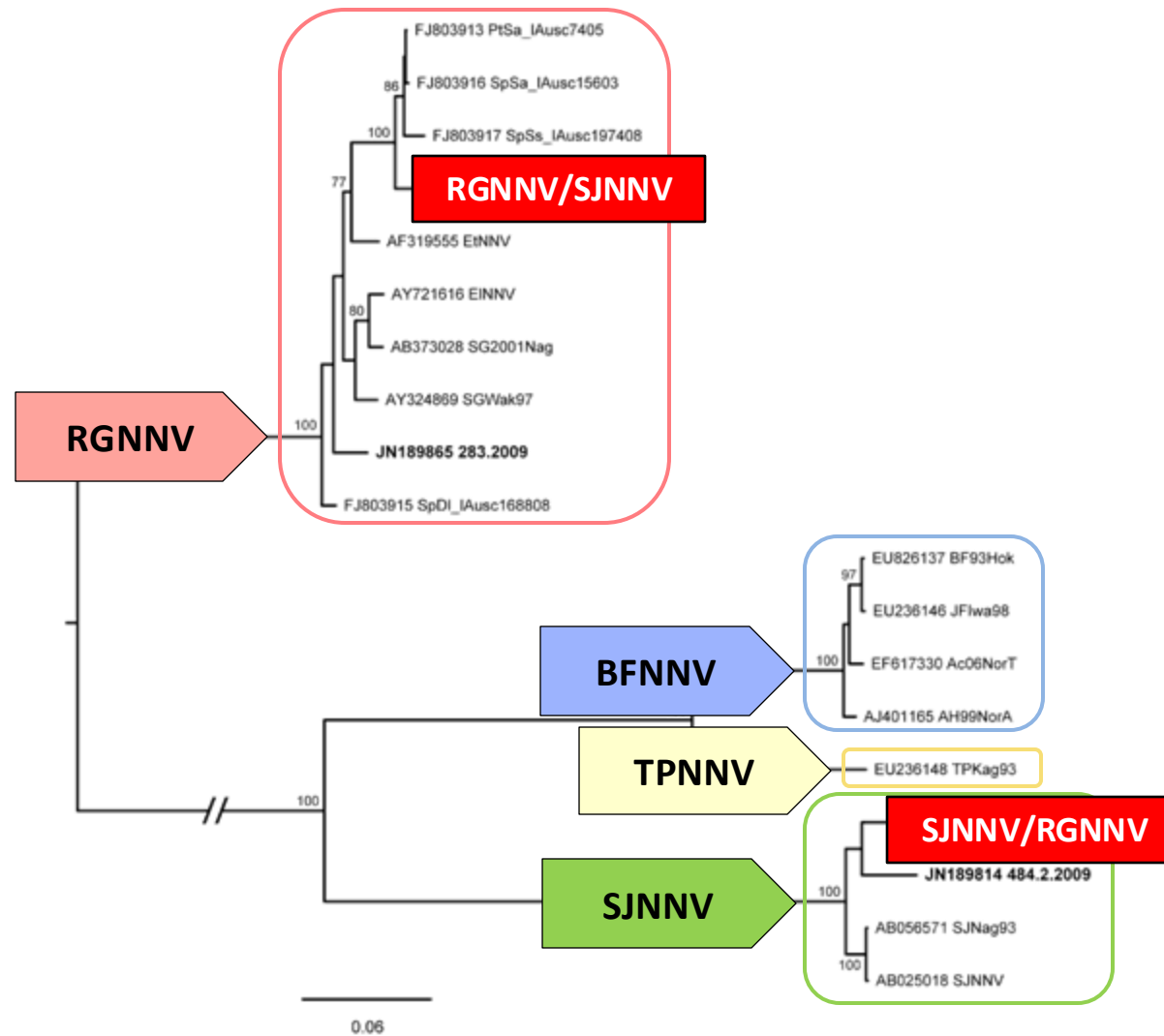
- ✓ RNA1: viral polymerase
- ✓ RNA3: B2 protein, siRNA inhibitor
- ✓ RNA2: structural capsid protein

● Classification

RNA2

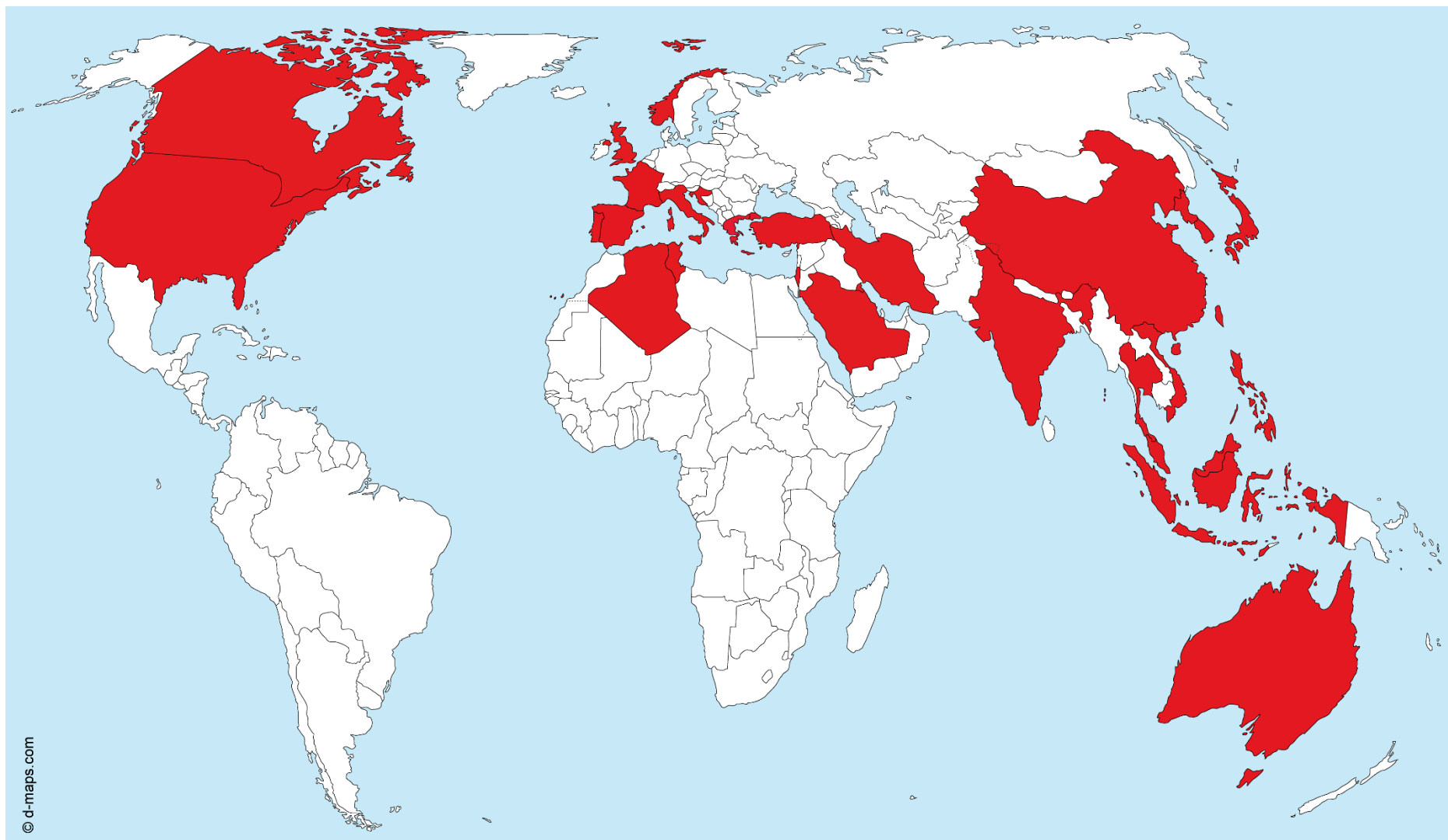


RNA1



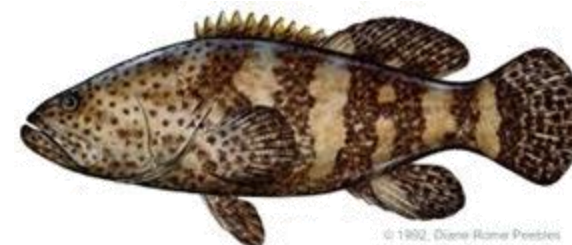
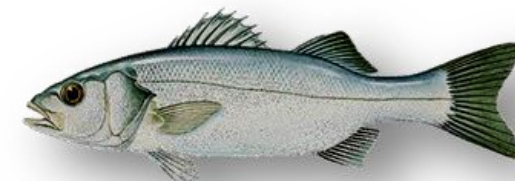


Disease distribution



● Host Range

- At present the host range comprises **160 species** belonging to 80 families and 24 orders
- *Perciformes* (sea bass, groupers, drums, mullets, sea bream)
- *Pleuronectiformes* (turbot, sole, flounders)
- *Gadiformes* (cod)
- Invertebrates, comprising bivalve molluscs, have also been found positive



● Criteria for listing aquatic animal diseases/1

AQUATIC ANIMAL HEALTH CODE (CHAPTER 1.2.)

- International spread of the pathogen is likely 
- AND
- A precise case definition is available and a reliable means of detection and diagnosis exists 
- AND
- At least one country may demonstrate country or zone freedom from the disease in susceptible aquatic animals 
- AND
- Natural transmission to humans has been proven, and human infection is associated with severe consequences 
- OR
- The disease has been shown to affect the health of cultured aquatic animals at the level of a country or a zone resulting in significant consequences e.g. production losses, morbidity or mortality at a zone or country level. 
- OR
- The disease has been shown to, or scientific evidence indicates that it would affect the health of wild aquatic animals resulting in significant consequences e.g. morbidity or mortality at a population level, reduced productivity or ecological impacts. 

● Criteria for listing aquatic animal diseases/2

- Viral Encephalopathy and Retinopathy is NOT an WOAHP listed disease (Chapter 1.3)
- There was a dedicated chapter in the Manual of Diagnostic Tests for Aquatic Animals, but it has been withdrawn in June 2022
- There is still a WOAHP Reference Centre in Italy



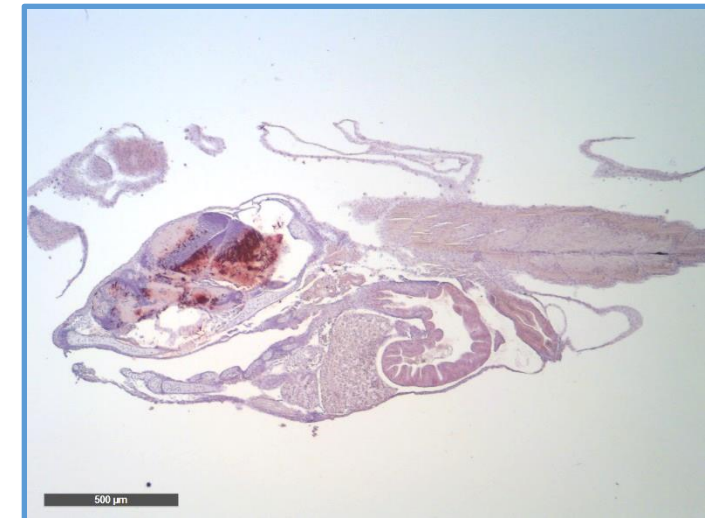
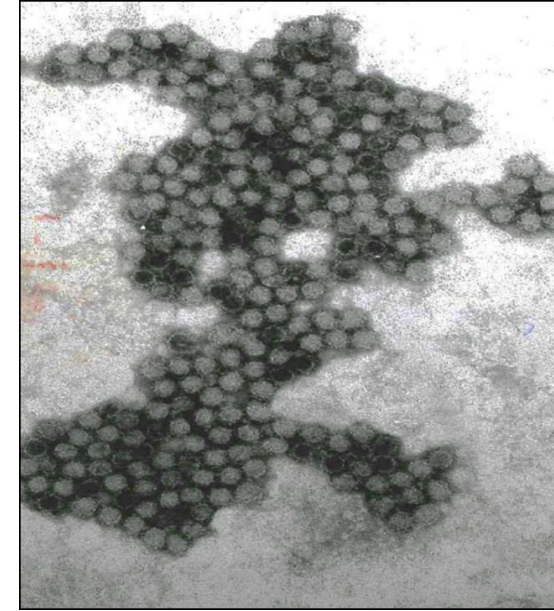
● Diagnostic methods available at IZSVe

Accredited

- Viral isolation on cell culture (SNN-1 and E-11)

Validated

- Real time RT-PCR for RNA1 and RNA2 detection
- Quantitative RT-PCR
- Sequencing with phylogenetic analysis
- Direct immunofluorescence
- Serum-neutralization
- Histology
- Immunohistochemistry
- ELISA
- TEM



● Production and provision of reagents

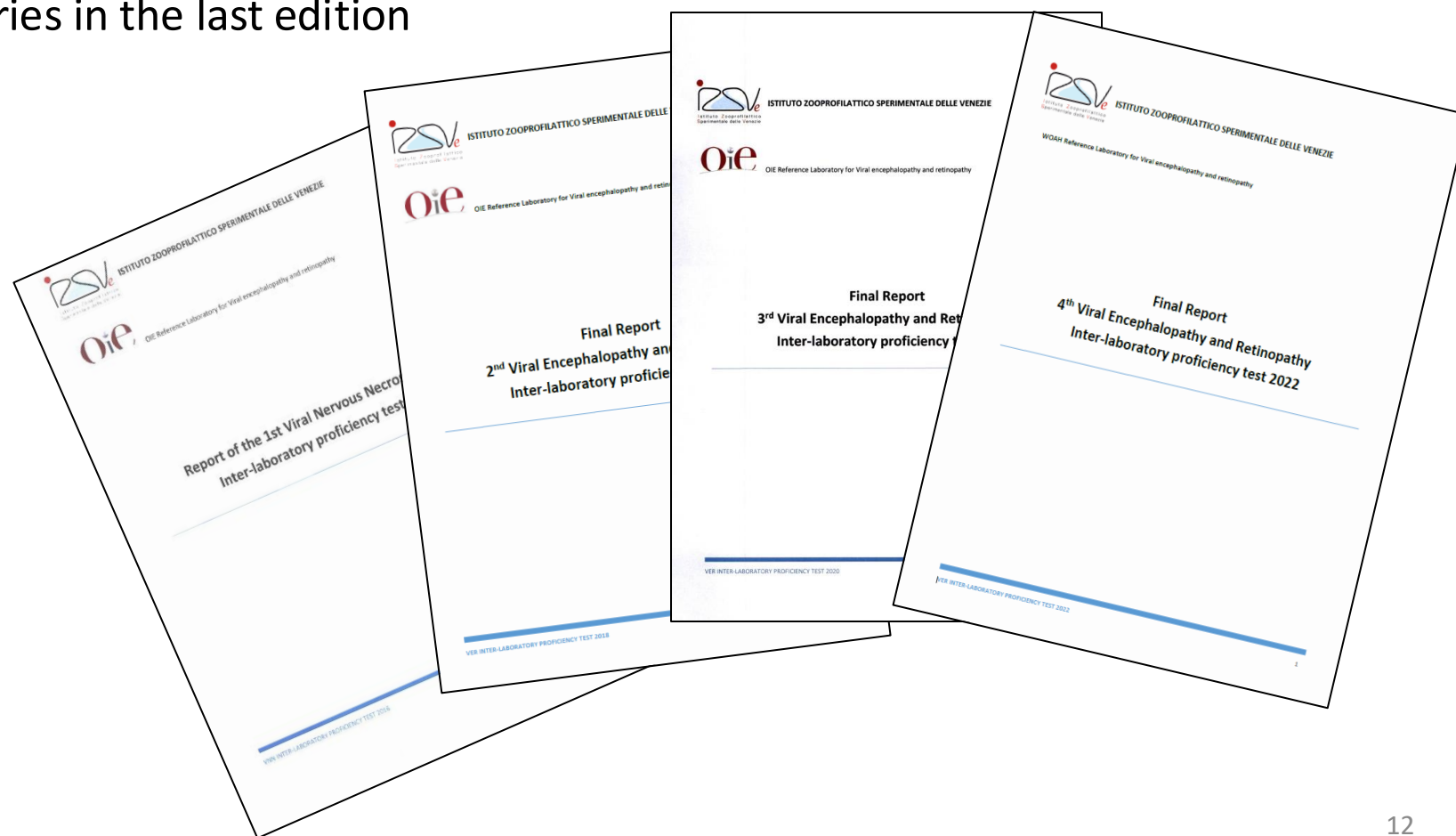
- SNN-1, E-11, GF-1 cell cultures
- Inactivated RT-PCR positive controls for different VER genotypes
- Standard positive and negative serum for ELISA (European sea bass only)
- Hyperimmune positive sera from rabbit for SN, ELISA, IHC
- Reference viral strain of all known genotypes
- > 300 field viruses





International Proficiency Test

- Organized every other year since 2016
- Targeted to molecular methods only (RT-PCR; rRT-PCR; genotyping)
- 25 participants from 17 countries in the last edition
- Next edition planned for 2024





Scientific publications

Fish and Shellfish Immunology xxx (xxxx) xxx-xxx



ELSEVIER

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi



Full length article

Lack of *in vivo* cross-protection of two different betanodavirus species RGNNV and SJNNV in European sea bass *Dicentrarchus labrax*

Francesco Pascoli^{a,*}, Andrea Guazzo^a, Alessandra Buratin^a, Marica Toson^b,
Francesco Buonocore^c, Giuseppe Scapigliati^c, Anna Toffan^a

^a Istituto Zooprofilattico Sperimentale delle Venezie, National Reference Laboratory (NRL) for Diseases of Fish, Mollusk and Crustacean, Legnaro, PD, Italy

^b Istituto Zooprofilattico Sperimentale delle Venezie, Aquatic Animal Epidemiology Laboratory, Legnaro, PD, Italy

^c Department for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia, Viterbo, Italy

ARTICLE IN PRESS

Fish and Shellfish Immunology xxx (xxxx) xxx-xxx



ELSEVIER

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi



Full length article

Vaccination and immune responses of European sea bass (*Dicentrarchus labrax* L.) against betanodavirus

Francesco Buonocore^{*}, Noelia Nuñez-Ortiz², Simona Picchietti, Elisa Randelli, Valentina Stocchi,
Laura Guerra, Anna Toffan¹, Francesco Pascoli¹, Anna Maria Fausto, Massimo Mazzini,
Giuseppe Scapigliati

^{*} Department for Innovation in Biological, Agrofood and Forest Systems, University of Tuscia, Viterbo, Italy

RESEARCH ARTICLE

Open Access



A formalin-inactivated immunogen against viral encephalopathy and retinopathy (VER) disease in European sea bass (*Dicentrarchus labrax*): immunological and protection effects

Noelia Nuñez-Ortiz¹, Fr
Marica Toson³, Giuseppe

SCIENTIFIC REPORTS

OPEN

Viral nervous necrosis in gilthead sea bream (*Sparus aurata*) caused by reassortant betanodavirus RGNNV/SJNNV: an emerging threat for Mediterranean aquaculture

Anna Toffan¹, Francesco Pascoli¹, Tobia Pretto^{2,3}, Valentina Panzarin¹, Miriam Abbadi¹,
Alessandra Buratin¹, Rosita Quaratesi¹, Daniel Gijón⁴ & Francesc Padrós⁵

Received: 12 January 2017

Accepted: 21 March 2017

Published: 02 May 2017



RESEARCH ARTICLE

Molecular Basis for Antigenic Diversity of Genus *Betanodavirus*

Valentina Panzarin^{1,2*}, Anna Toffan¹, Miriam Abbadi¹, Alessandra Buratin¹,
Marzia Mancin¹, Stine Braaen³, Christel Moræus Olsen³, Luca Bargelloni²,
Espen Rimstad³, Giovanni Cattoli¹

¹ OIE Reference Laboratory for Viral Encephalopathy and Retinopathy, Department of Comparative Biomedical Sciences, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro (PD), Italy, ² Department of Comparative Biomedicine and Food Science, University of Padua, Legnaro (PD), Italy, ³ Department of Food Safety and Infection Biology, Norwegian University of Life Sciences, Oslo, Norway

* vpanzarin@izsvenezie.it



Scientific publications



Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet



First generic one step real-time Taqman RT-PCR targeting the RNA1 of betanodaviruses

M. Baud^a, J. Cabon^a, A. Salomoni^b, A. Toffan^b, V. Panzarin^b, L. Bigarré^{a,*}

Panzarin et al. *Veterinary Research* 2014, 45:56
<http://www.veterinaryresearch.org/content/45/1/56>



RESEARCH

Open Access

In vitro study of the replication capacity of the RGNNV and the SJNNV betanodavirus genotypes and their natural reassortants in response to temperature

Valentina Panzarin^{*}, Elisabetta Capp
and Giovanni Cattoli

frontiers | Frontiers in Veterinary Science

ORIGINAL RESEARCH
published: 03 August 2022
doi: 10.3389/fvets.2022.932327



Role of Rotifers in Betanodavirus Transmission to European Sea Bass Larvae

Lucía Vázquez-Salgado¹, Francesco Pascoli², Andrea Marsella², Lorena Biasini²,
Alessandra Buratin², Tobia Pretto², Miriam Abbadi², Erica Melchioti², Isabel Bandin¹ and
Anna Toffan^{2*}

¹ Departamento de Microbiología y Parasitología, Instituto de Acuicultura, Universidade de Santiago de Compostela, Santiago de Compostela, Spain; ² Istituto Zooprofilattico Sperimentale delle Venezie, CIE Reference Laboratory for Viral Encephalo-Retinopathy, National Reference Laboratory for Fish Diseases Legnaro, Padova, Italy; ³ Department of Histopathology, Istituto Zooprofilattico Sperimentale delle Venezie Legnaro, Padova, Italy



Article

Pathogenicity of Different Betanodavirus RGNNV/SJNNV Reassortant Strains in European Sea Bass

Lorena Biasini¹, Paola Berto², Miriam Abbadi³, Alessandra Buratin, Marica Toson, Andrea Marsella²,
Anna Toffan² and Francesco Pascoli^{2,*}

Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, 35020 Padova, Italy; lbiasini@izsvenezie.it (L.B.);
pberto@izsvenezie.it (P.B.); mabbadi@izsvenezie.it (M.A.); aburatin@izsvenezie.it (A.B.);
mtoson@izsvenezie.it (M.T.); amarsella@izsvenezie.it (A.M.); atoffan@izsvenezie.it (A.T.)

* Correspondence: fpascoli@izsvenezie.it



Received: 26 June 2021

DOI: 10.1111/j

ORIGINAL ARTICLE

WILEY

Detection and molecular characterization of betanodaviruses retrieved from bivalve molluscs

E Volpe¹ | M Grodzki¹ | V Panzarin² | A Guercio³ | G Purpari³ | P Serratore¹ |
S Ciulli¹

Open Access Article

Long-Term Protection and Serologic Response of European Sea Bass Vaccinated with a Betanodavirus Virus-Like Particle Produced in *Pichia pastoris*

by Sofie Barsøe¹ Anna Toffan² Francesco Pascoli² Ansgar Stratmann³
 Tobia Pretto² Andrea Marsella² Mérim Er-Rafik⁴ Niccolò Vendramin¹
 Niels J. Olesen¹ Dagoberto Sepúlveda¹ and Niels Lorenzen^{1,*}

¹ National Institute of Aquatic Resources (DTU AQUA), Technical University of Denmark, 2800 Lyngby, Denmark

² Istituto Zooprofilattico Sperimentale delle Venezie (IZSVe), 35020 Legnaro, Padua, Italy

³ W42 Biotechnology GmbH, 44227 Dortmund, Germany

⁴ National Center for Nano Fabrication and Characterization (DTU Nanolab), Technical University of Denmark, 2800 Lyngby, Denmark

* Author to whom correspondence should be addressed.

Vaccines 2021, 9(5), 447; <https://doi.org/10.3390/vaccines9050447>

Received: 30 March 2021 / Revised: 22 April 2021 / Accepted: 23 April 2021 / Published: 2 May 2021

● Training

- WOAH Twinning program with Tunisia 2017-2018
- International collaboration project AQUAE STRENGTH: Strengthening capacity on aquatic animal health and epidemiological surveillance
- Remote collaboration with several countries (Malaysia, Spain, France, Croatia, Greece, etc.)
- Twinning project with Jeddah KSA laboratory (agreement signed and ready to start)
- Twinning project with Central Animal Laboratory in Oman....coming soon



● The importance of bacterial diseases

“bacterial pathogens cause major losses to aquaculture, comprising around 34% of total diseases”

Soibam Khogen Singh, Maibam Malemngamba Meitei, Tanmoy Gon Choudhary, Ngasotter Soibam, Pradyut Biswas, Gusheinzed Waikhom,(2022) Chapter 15 - **Bacterial diseases in cultured fishes: an update of advances in control measures**, Bacterial Fish Diseases, Academic Press,

Sometimes more than 40 %.....billions of dollars \$ are lost



● Main bacterial diseases in the world



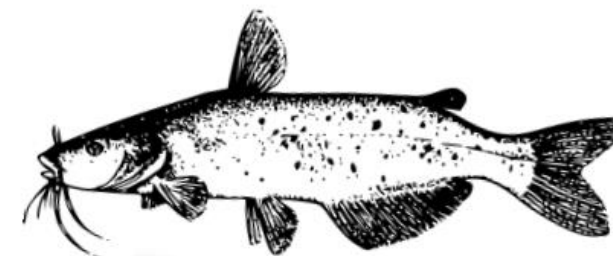
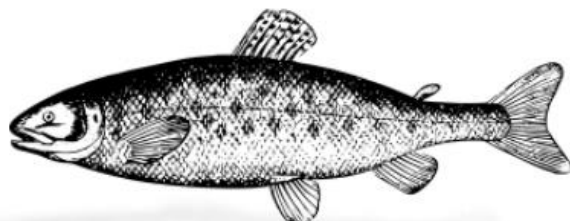
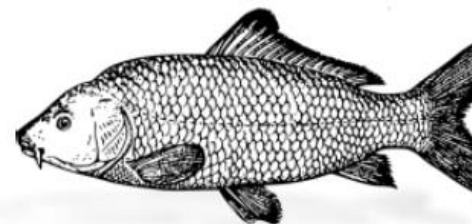
OIE GLOBAL CONFERENCE
ON AQUATIC ANIMAL HEALTH
COLLABORATION, SUSTAINABILITY: OUR FUTURE
SANTIAGO, CHILE, 2-4 APRIL 2019



WORLD ORGANISATION FOR ANIMAL HEALTH
Protecting animals, preserving our future

Ad hoc Group Recommendation: Priority **fish** pathogens

- *Aeromonas hydrophila* and other species (Freshwater cyprinids) (H)
- *Pseudomonas* spp. (Freshwater cyprinids) (H)
- *Vibrio* spp., (Marine fish) (H)
- *Photobacterium* spp. (Marine fish) (H)
- *Streptococcus* spp. (Marine fish) (H)
- *Edwardsiella ictaluri*, *E. tarda* (Catfish) (H)
- *A. hydrophila* and other species (Catfish) (H)
- *Streptococcus iniae*, and *S. agalactiae* (Freshwater cichlids) (M)





Typing of bacterial pathogens is available in IZSVe

BIOCHEMICAL

singular test
micromethods (Biologin, API System, Vitek)

SEROLOGICAL

Slide agglutination test
ELISA
Immuno fluorescence - IF/Immuno Histochemistry - IHC

PROTEIC PROFILE

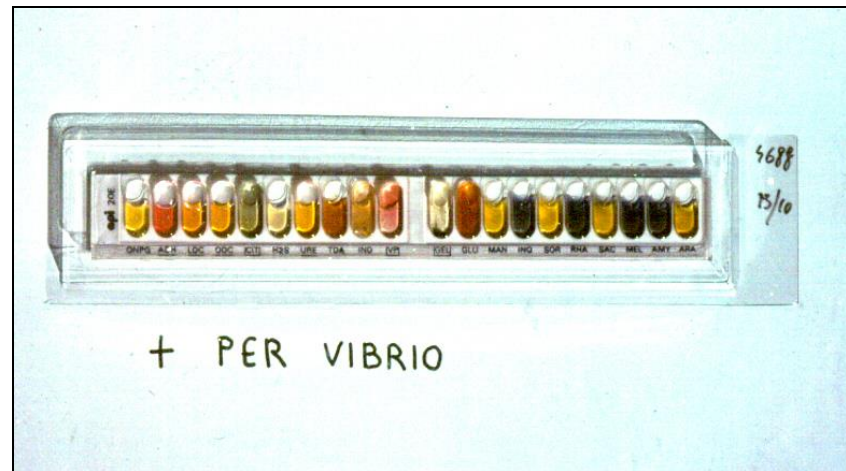
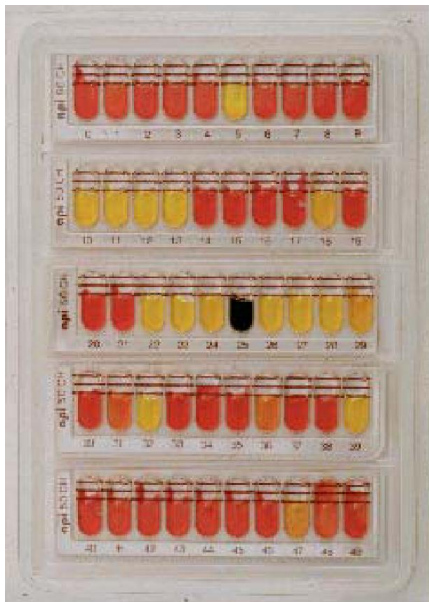
MALDITOF - Matrix assisted laser desorption/ionization time of flight

MOLECULAR BIOLOGY

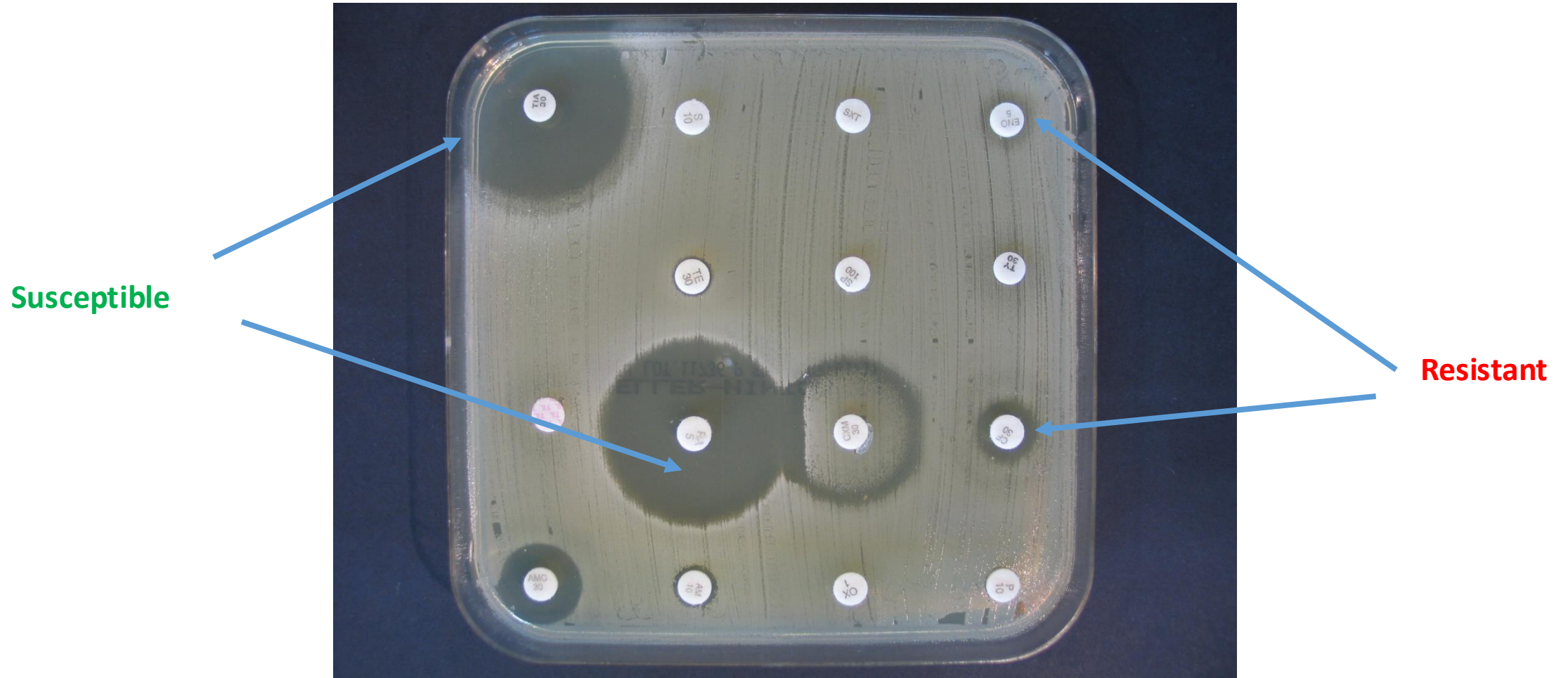
PCR, Real Time PCR, RPLF, MLST, ecc.

Miniaturized methods: strip/kits with different tests

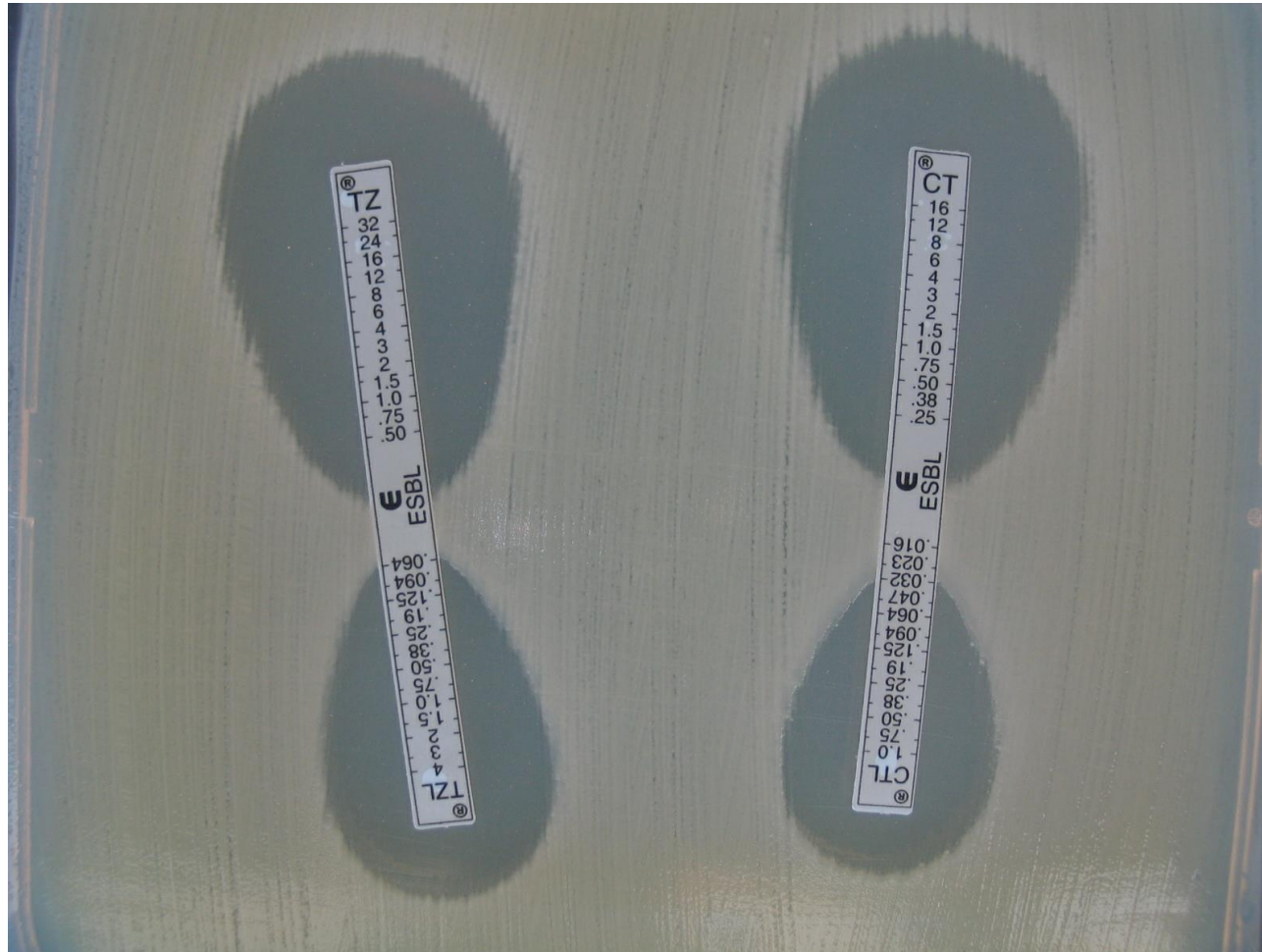
Commercial kits including different tests (aminoacids, carbohydrate, protein, etc). After 24-48 hrs of incubation checking the different colour of each single well you can obtain a **key code** corresponding to a single strain species. If the result is over 98 % the identification is very good, if lower you need to perform additional tests. **Be aware that not all the fish pathogens can be identified correctly...you usually need more additional tests.**



Antimicrobial susceptibility test – agar diffusion (Kirby Bauer)



E-test (different concentration)



Minimal Inhibitory Concentration (M.I.C.)

- ❖ 3-4 colonies in 5 mL physiological solution (final concentration = 0,5 McFarland scale)
- ❖ 100 μ L in special broth (CAMHB)
- ❖ 100 μ L of suspension in each well (commercial 96 microplates are available)
- ❖ Overnight incubation at 22°C



Blood agar pure culture

3-4 colonies of isolated colonies



0,5 McFarland

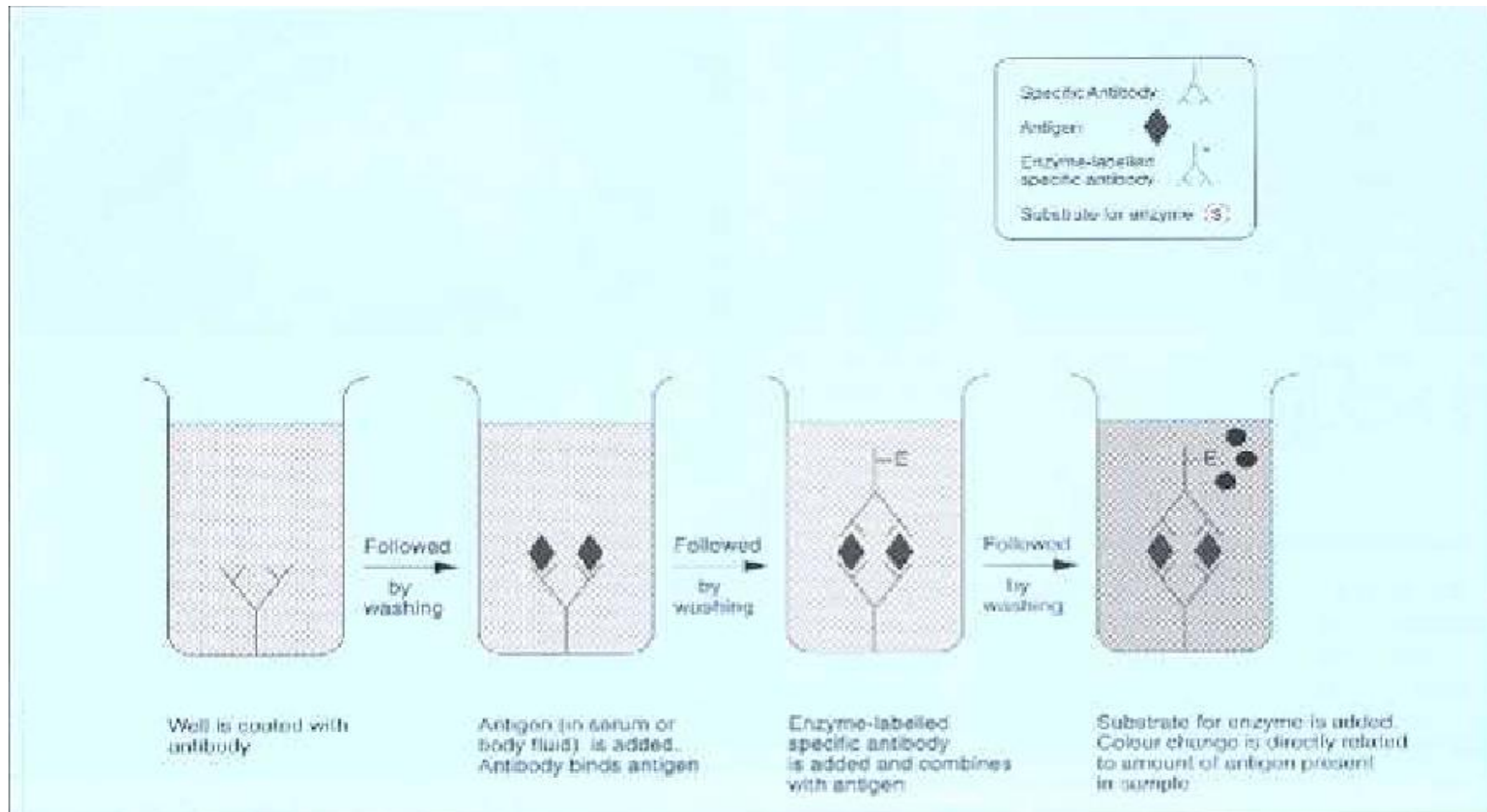
Inoculum in 96 wells microplates



Check the wells after overnight incubation

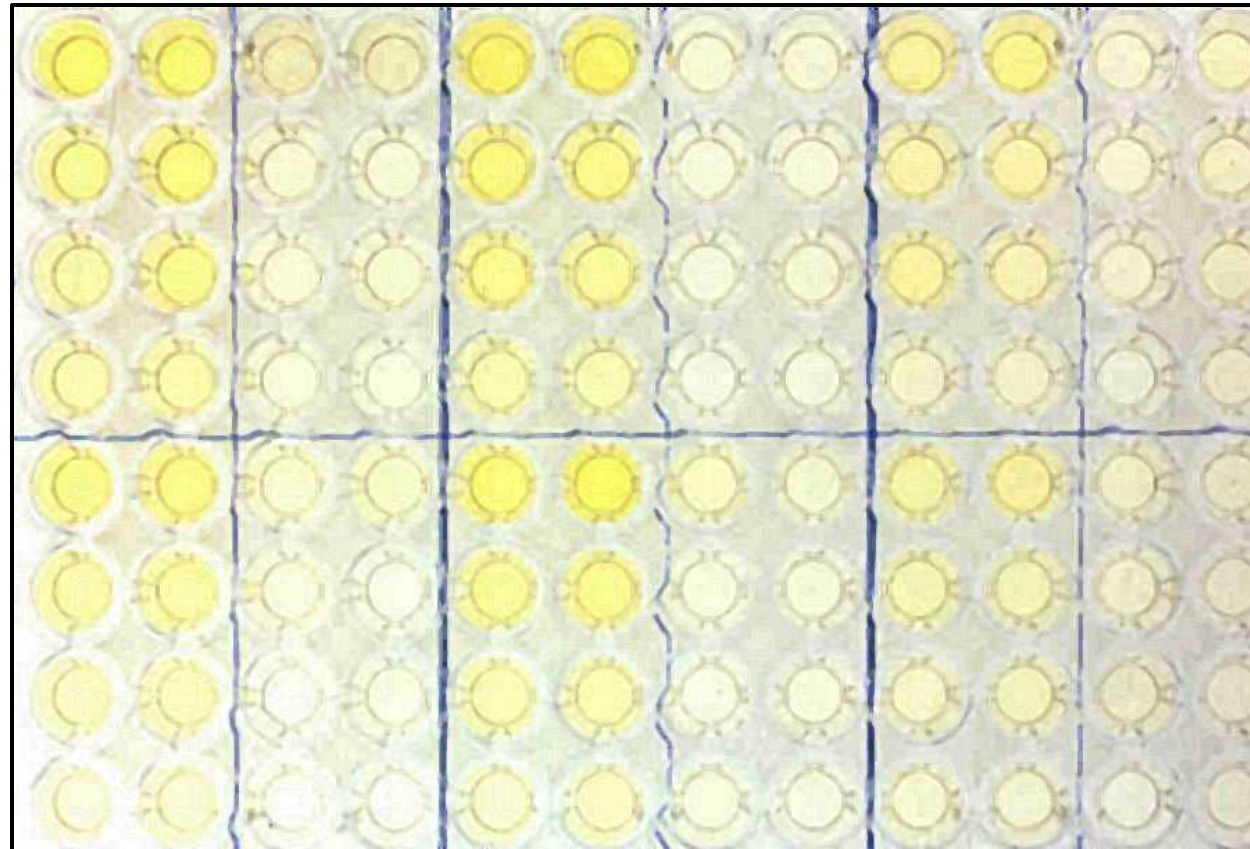
E.L.I.S.A.

(Enzyme Linked Immuno Sorbent Assay)
to detect pathogens or antibodies

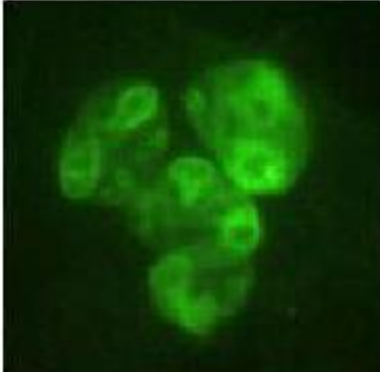


ELISA TEST RESULTS

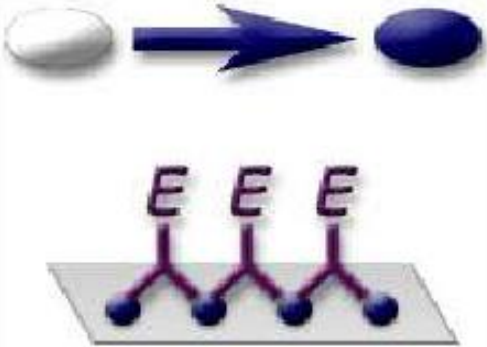
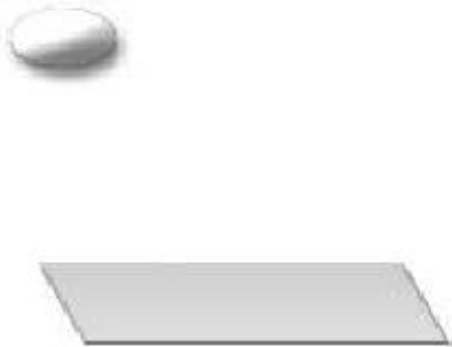
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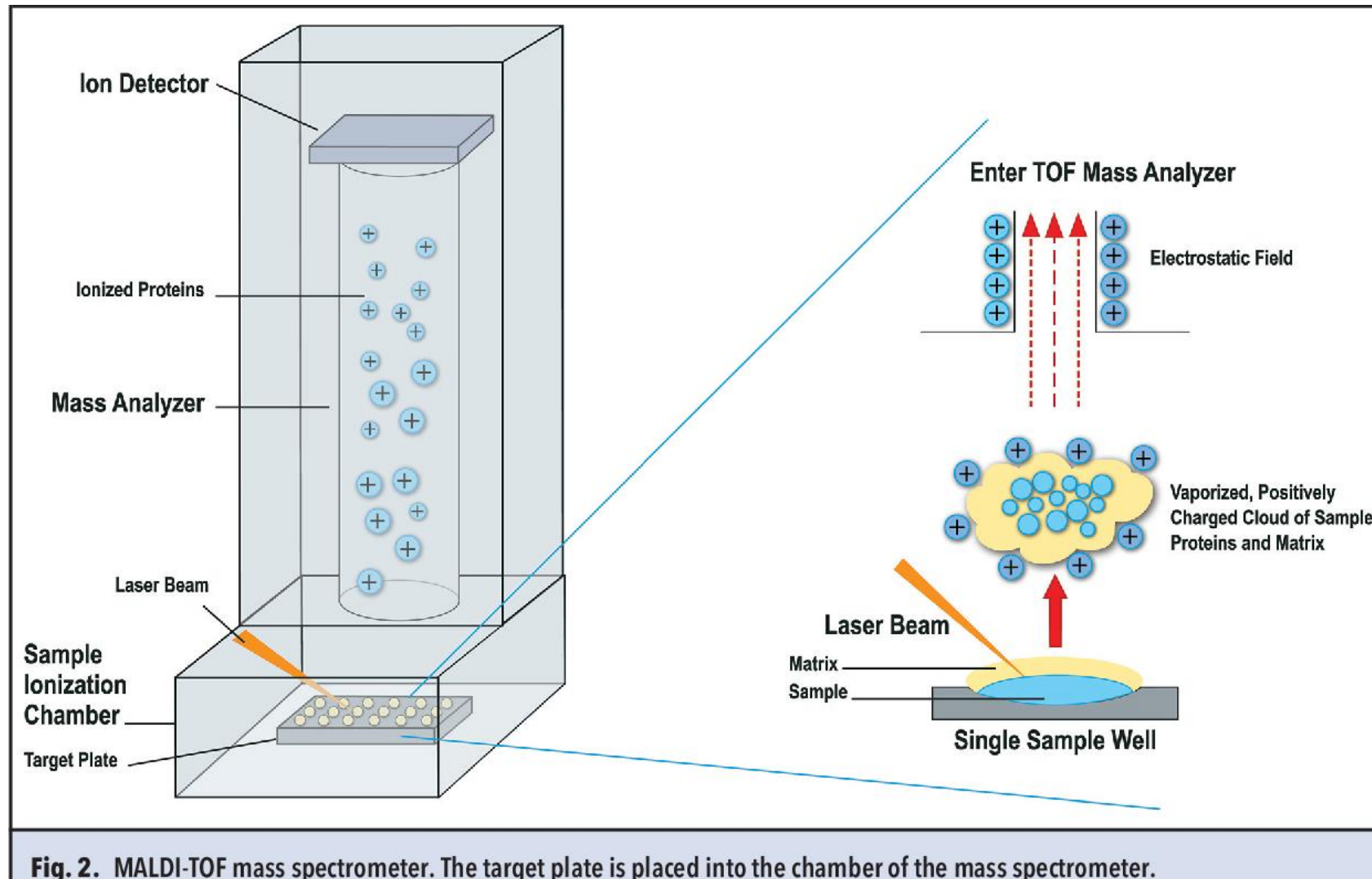
Immunofluorescence

REAGENTS: unknown sample fixed to slide (may contain Ag of interest)  Ag	POSITIVE SAMPLE Ag is present on slide 	NEGATIVE SAMPLE Ag is not present on slide 
REAGENTS: fluorescein-labeled Ab to Ag of interest  Allow time to react Wash away unbound Abs		 
PROCEDURE: observe under a fluorescence microscope  POSITIVE: green fluorescence NEGATIVE: no fluorescence		

Immuno histochemistry – IHC (with histological sections)

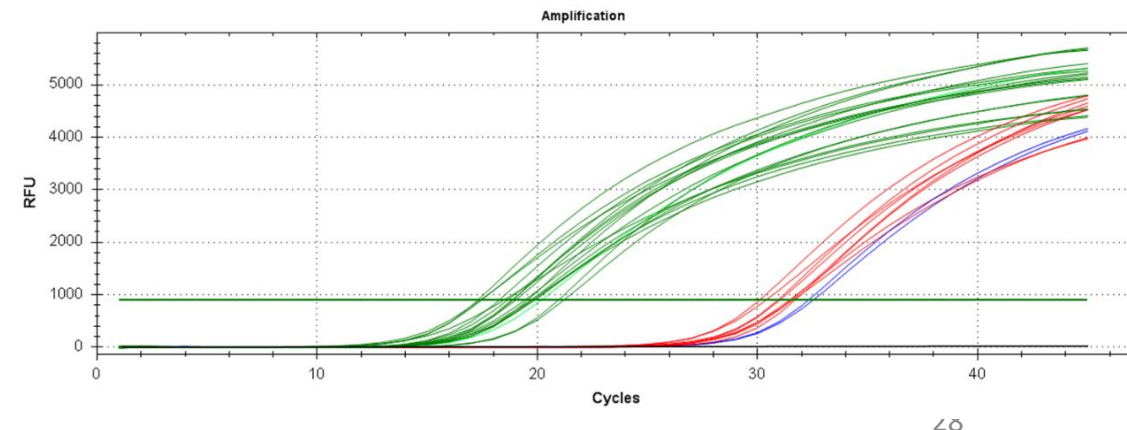
REAGENTS: unknown sample fixed to slide (may contain Ag of interest)  Ag	POSITIVE SAMPLE Ag is present on slide 	NEGATIVE SAMPLE Ag is not present on slide 
REAGENTS: enzyme-labeled Ab to Ag of interest  Allow time to react Wash away unbound Abs		
REAGENTS: colorless substrate for enzyme POSITIVE: substrate converted to colored precipitate at site of Ags NEGATIVE: no color change (substrate not converted)		

● Protein fingerprint: Matrix assisted laser desorption/ionization time of flight (MALDITOF)



Molecular biology (genotyping).....still in progress

- PCR classical (e.g rRNA 16 S)
- Nested PCR
- Real time PCR
- Random Amplification Polymorphic DNA (RAPD)
- Pulsed Field Gel Electrophoresis (PFGE)
- Multi Locus Sequencing Typing (MLST)
- Whole Genome Sequencing (WGS)
- Next Generation Sequencing (NGS)

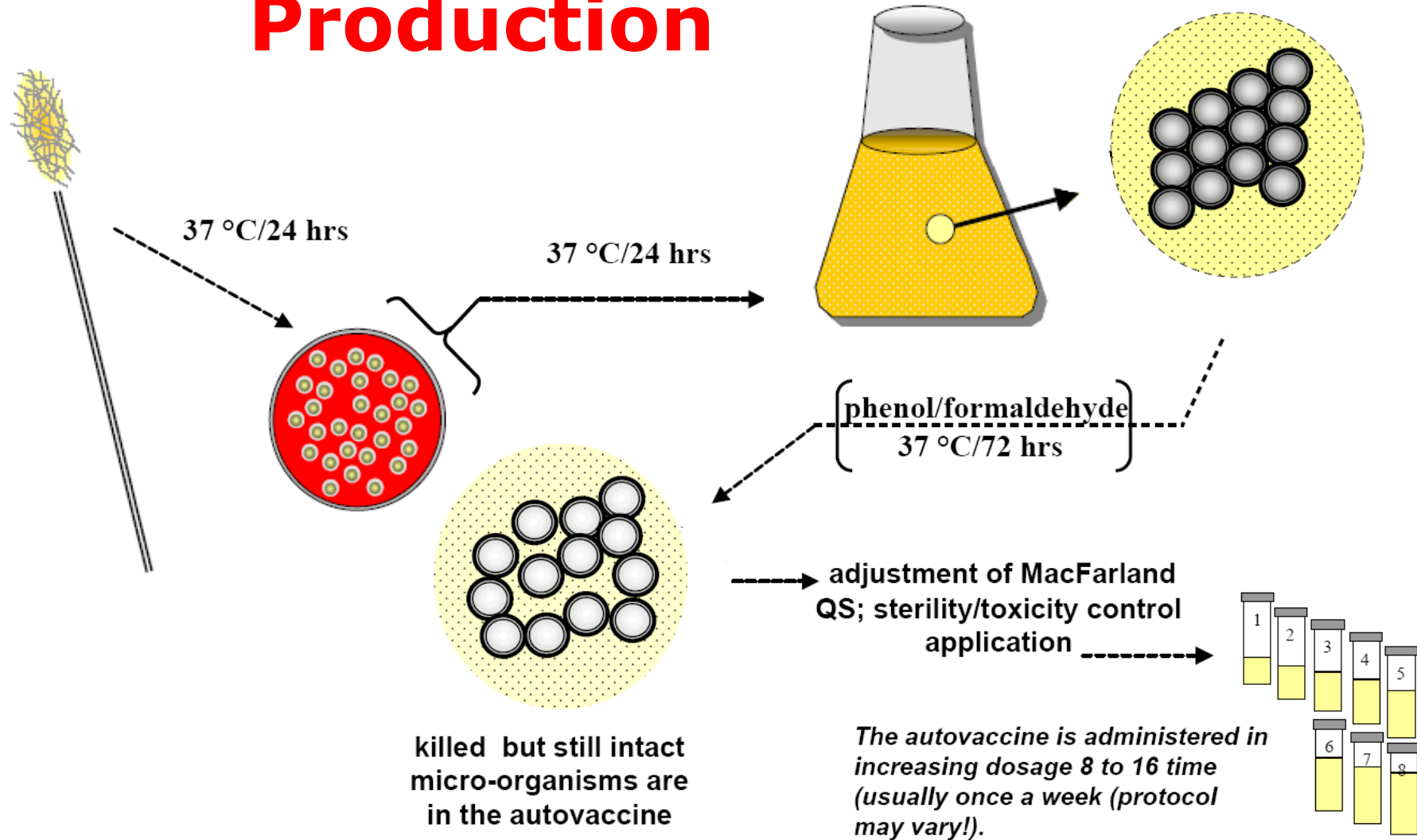




Autogenous vaccines are also produced by IZSVe

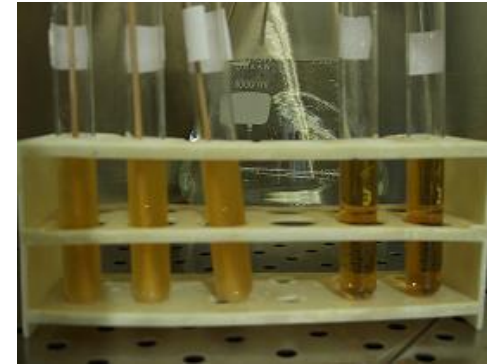
- The autogenous vaccine is a veterinary immunological drug prepared with **pathogens** and / or antigens **isolated from subjects affected** by infectious form dominant in a particular farm and used for the **treatment of the same farm animals** or the animals of the same country if the DVM believe it appropriate for evident epidemiological reasons (**DIRECTIVE 2001/82/EC** . Article 3. 2: Inactivated immunological veterinary medicinal products ...)

Production



● Controls on the final product

- Inactivation (growth media)
- Sterility (growth media)
- Toxicity (mouse, guinea pigs, fish)





Why we use inactivated autogenous vaccines?

- absence of residual pathogenicity
- absence of recombination and reversion
- major stability in the conservation and during the use
- withdrawal period close to 0 days



Main disadvantages of inactivated autogenous vaccines

- usually require adjuvant to obtain the maximum immune response (mineral oil with side effects)
- primarily stimulate humoral immunity
- require frequent dosing (booster effect)

Aquaculture epidemiology unit....born in 2014

Data collection and management of :

- fish, shellfish and crustacean **facilities** (management of regional and national farm database) including **geographycal information**
- data erasing from **IZSVE laboratory information system** concerning fish monitoring plans and data related to microbiological
- parameters, biotoxins, toxic phytoplankton and heavy metals in **bivalves harvesting areas**

Data validation and analysis

- Descriptive analysis: ongoing monitoring activities
- Epidemiological situation and study of risk factors associated to relevant diseases

Disease outbreak management

•

Data management

- **Data arising from laboratories** made available for their interpretation, merging with epidemiological information in order to report the evolution of the situation at local level
- Integrate all the data available troughs an efficient Management Information System, in order to use them for planning actions
- Coordination of activities between LCVA, Laboratory and Epidemiology Unit

Field activity



Istituto Zooprofilattico
Sperimentale delle Venezie

Laboratory



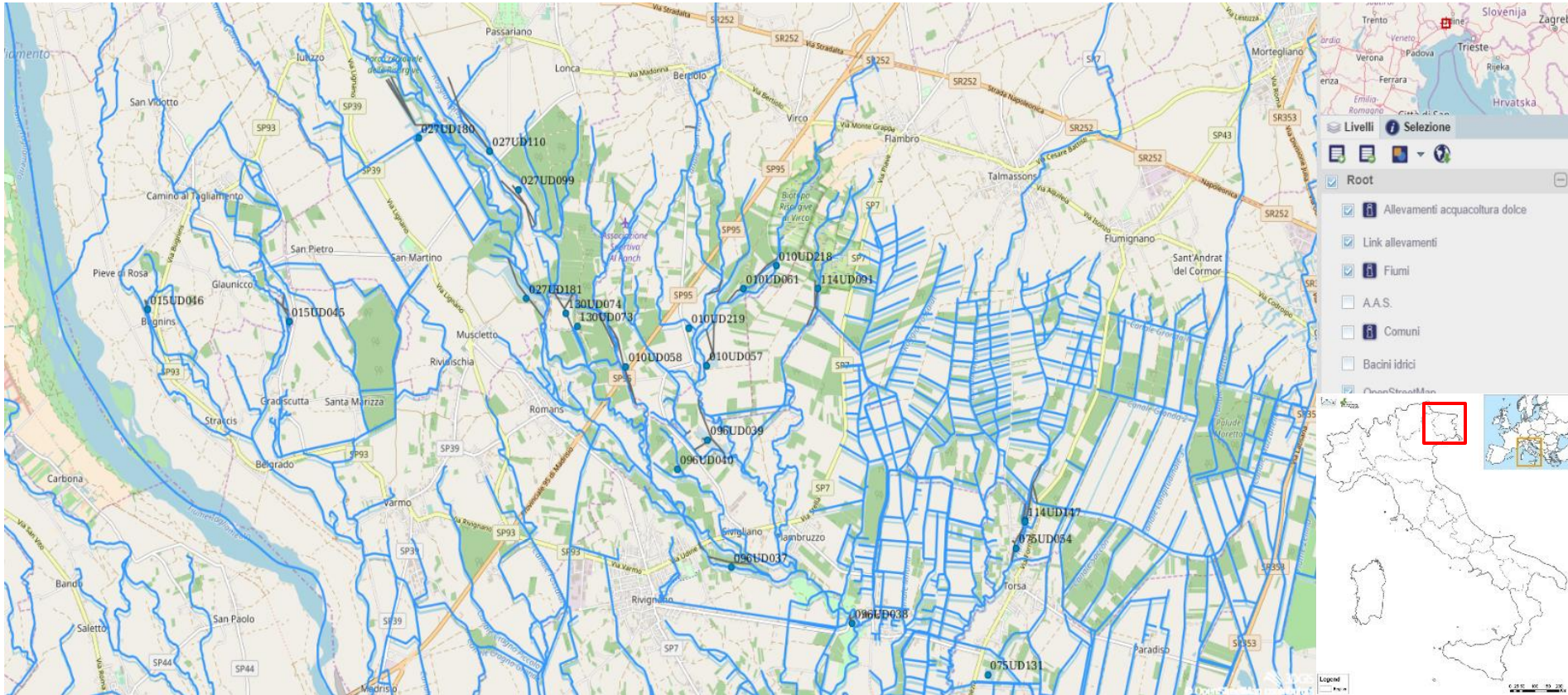
WEB-GIS



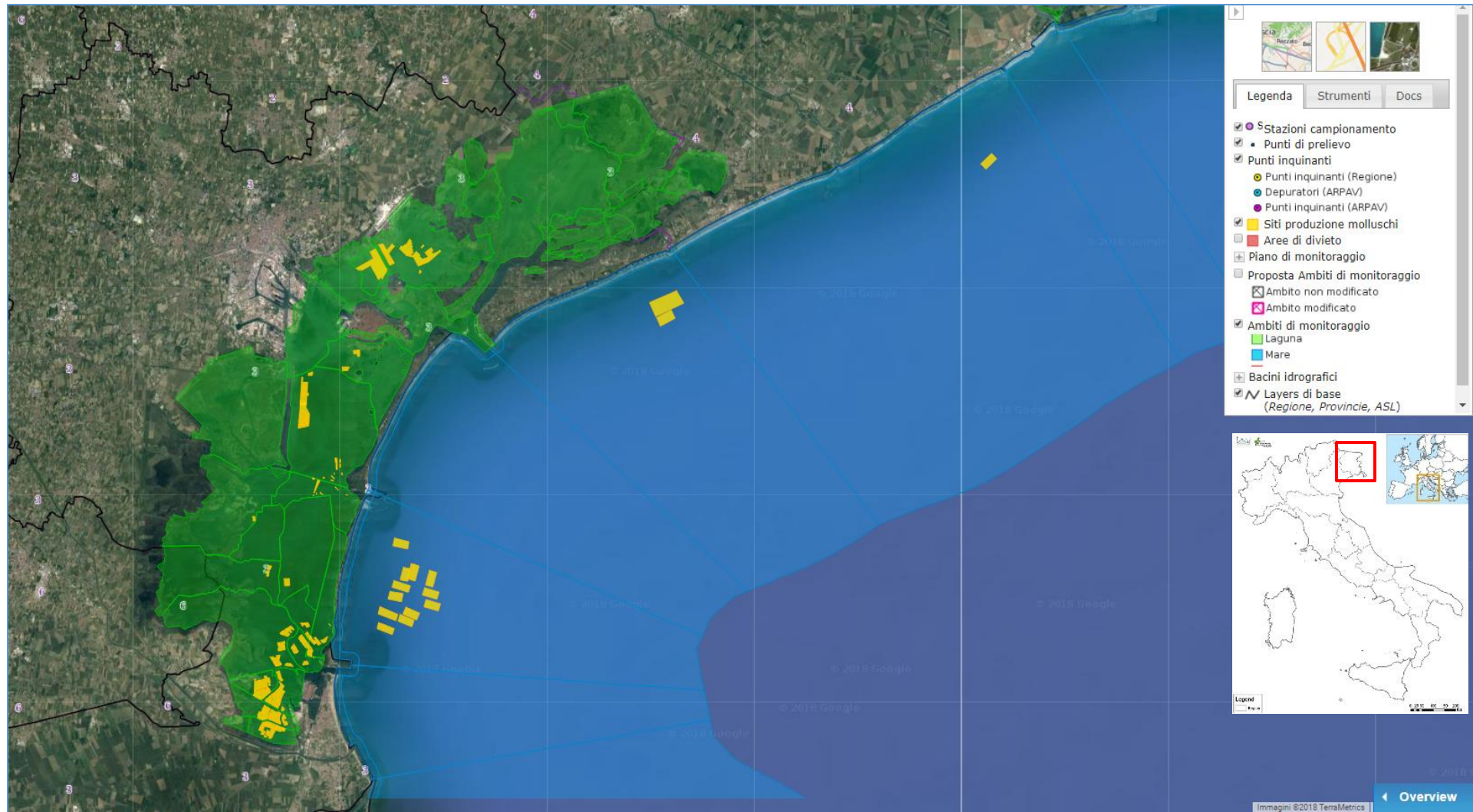
Epidemiology
and diagnostic
team



Web-based GIS management information system of fish facilities



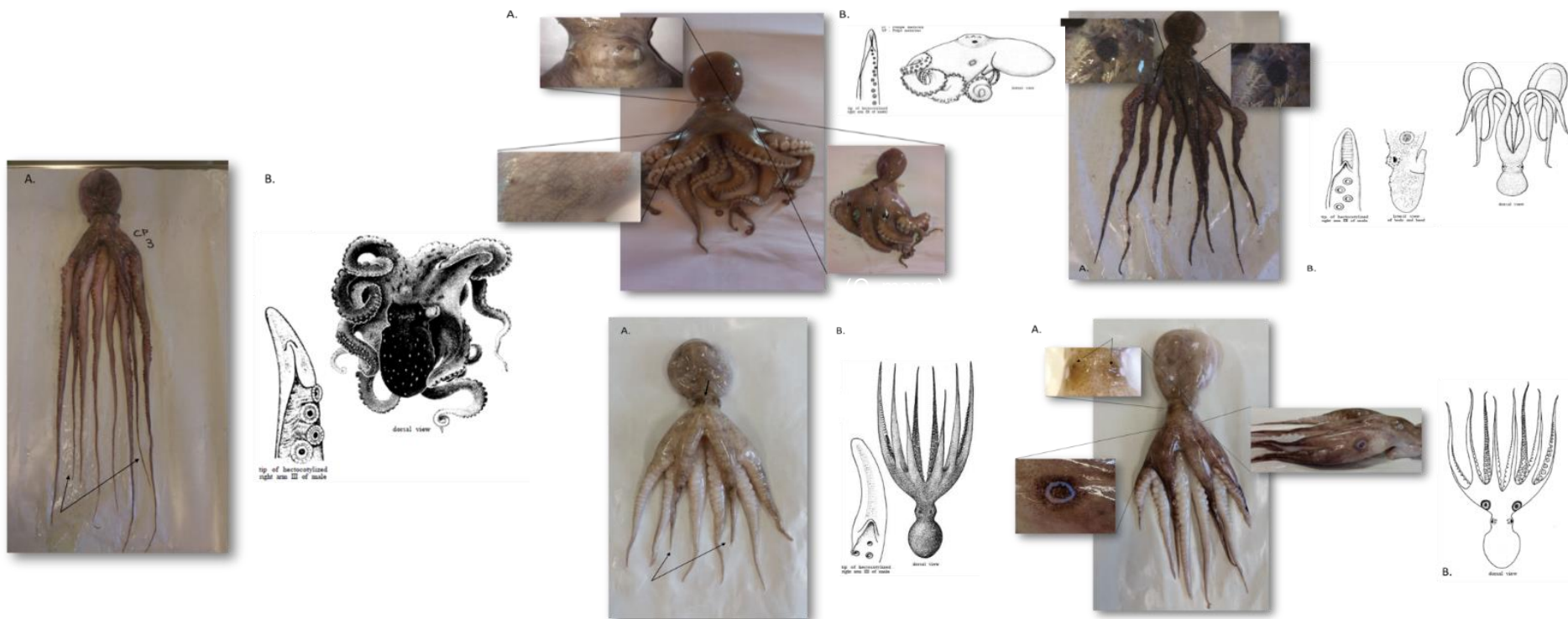
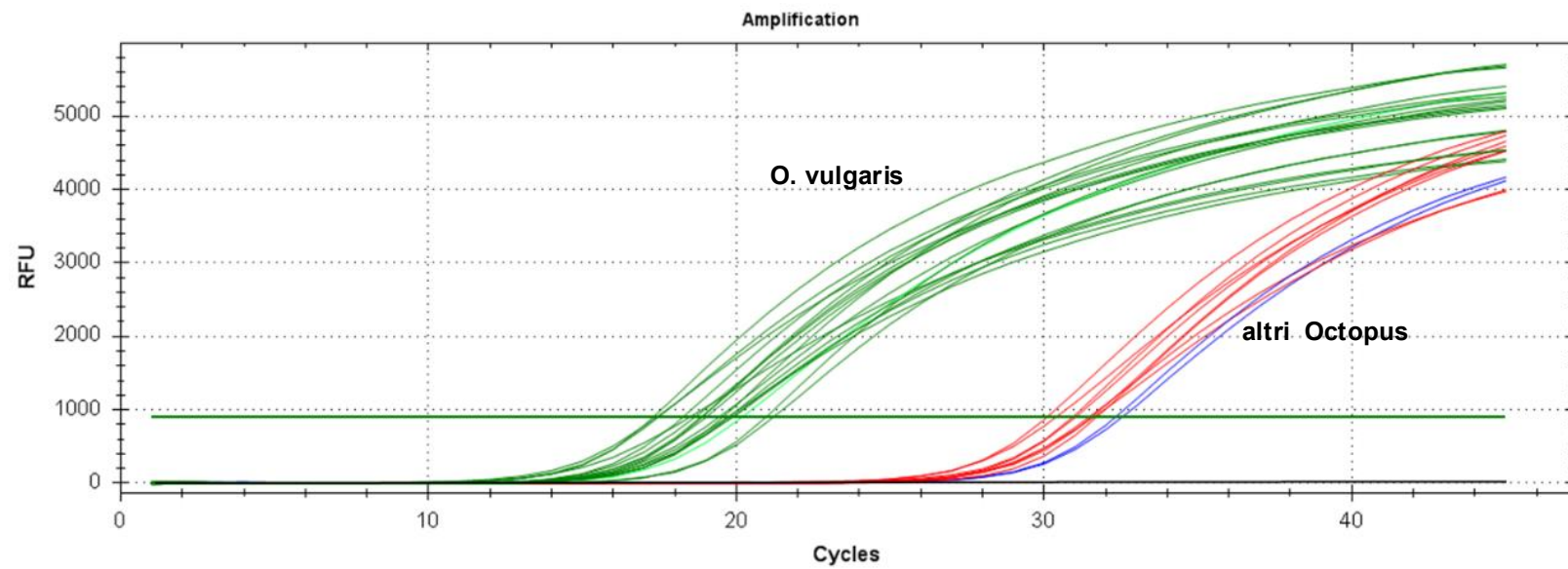
Web-based GIS management information system of shellfish facilities





Last but not least: Seafood control

- **Seafood microbiological** control according to new food hygiene legislation
- **Monitoring** on microbiological parameters, biotoxins and toxic phytoplankton in bivalves harvesting areas
- **Shelf life evaluation** of different fisheries products
- **Fish species identification** (biotec. methods) to avoid sanitary and commercial frauds
- **Chemicals control:** drugs, additives, heavy metals, histamine



IZSVe Ittiobase

Classificazione tassonomica	Nomi Comuni	Tracciati IEF	Sequenze	Distribuzione	Caratteristiche commerciali	Sicurezza e Frode
-----------------------------	-------------	---------------	----------	---------------	-----------------------------	-------------------



Engraulis encrasicolus
(Linnaeus 1758)

Ordine: Clupeiformes
Famiglia: Engraulidae
Genere: Engraulis
Alfa3: ANE

Sinonimi:
Lunghezza massima: 20 cm

 Acciuga, Alice

Specie inserita del Decreto MIPAAF con denominazione obbligatoria.
Il nome di specie: encrasicolus, deriva dal greco en = dentro, chole = bile e kras = testa, a significare il sapore amaro, quando viene consumata cruda.

Nomi Comuni

Engraulis encrasicolus

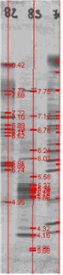
-  Acciuga, Alice
-  European Anchovy
-  Anchois, Anchois Commun
-  Anchovis, Sardelle
-  Anchoa, Bocarte, Boqueron
-  Ansjos, Europæisk Ansjos
-  Ansjovis
-  Ansjos
-  Biqueirão

Classificazione tassonomica	Nomi Comuni	Tracciati IEF	Sequenze	Distribuzione	Caratteristiche commerciali	Sicurezza e Frode
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Engraulis encrasicolus

Valore dei pI delle bande significative di *Engraulis encrasicolus* a diverso intervallo di pH:

8.15<pH<9.50: 8.43
6.55<pH<8.14: 6.70, 6.78, 6.89, 7.11, 7.21
5.20<pH<6.54: 5.74, 5.85, 5.91
3.50<pH<5.19: 4.93



Esempio di tracciato con pI elaborati dal software



Tracciato IEF

Classificazione tassonomica	Nomi Comuni	Tracciati IEF	Sequenze	Distribuzione	Caratteristiche commerciali	Sicurezza e Frode
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Engraulis encrasicolus

Sequenza 1 di 12
[Successivo](#)

Origine:
Primer Forward: COIFISHF1 (5' TCAACYAATCAYAAAGATATYGGCAC 3')
Primer Reverse: COIFISHR1 (5' ACTTCYGGGTGCCRAARAATCA 3')

Sequenza:
>253
.....TGAGCAGGAATGGTAGGGACAGCACTTAGCCTCCTTATTGAGCAGAACTAAGCCAACC
AGGAGCACTCTGGGGGACGATCAAATTTATAACGTAATCGTTACTGCTCAGCATTGTAATAATCTTTTCATGGTAA
TGCCCATTTCTAATCGGTGGGTTGGGAATTGACTAGTTCTCTAATACTAGGGGCTCAGACATGGCATTCCCCGAATG
AACAAATGAGCTTTTGACTCCTTCCCCTTCTTTCTTCTCTTCTAGCATCATCTGGTGTGAAGCAGGGCCGGGAC
AGGATGAACAGTCTACCCCTCTAGCAGGAAACCTCGCCACGCCGGAGCATCAGTGGATTAAACATCTTCTCTCC
ACTTGGCAGGATTCATCGATCCTAGGTGCCATTAATTTCTTACTACTACCATTAATATGAAACACCTGCTATTTCA
CAATACCAGACCTCTATTTGTCTGAGCTGTATTAATCAGGCAGTACTTTTACTTCTTCTACTACCGTTCTAGCTGC
TGGGATTACTATGCTTCTACAGACCGAAACCTAAATACTACTTTCTCGACCCAGCAGGGGGAGGAGACCAATCTTT
ATCAACACCTA



BTSF Zone for aquatic animals



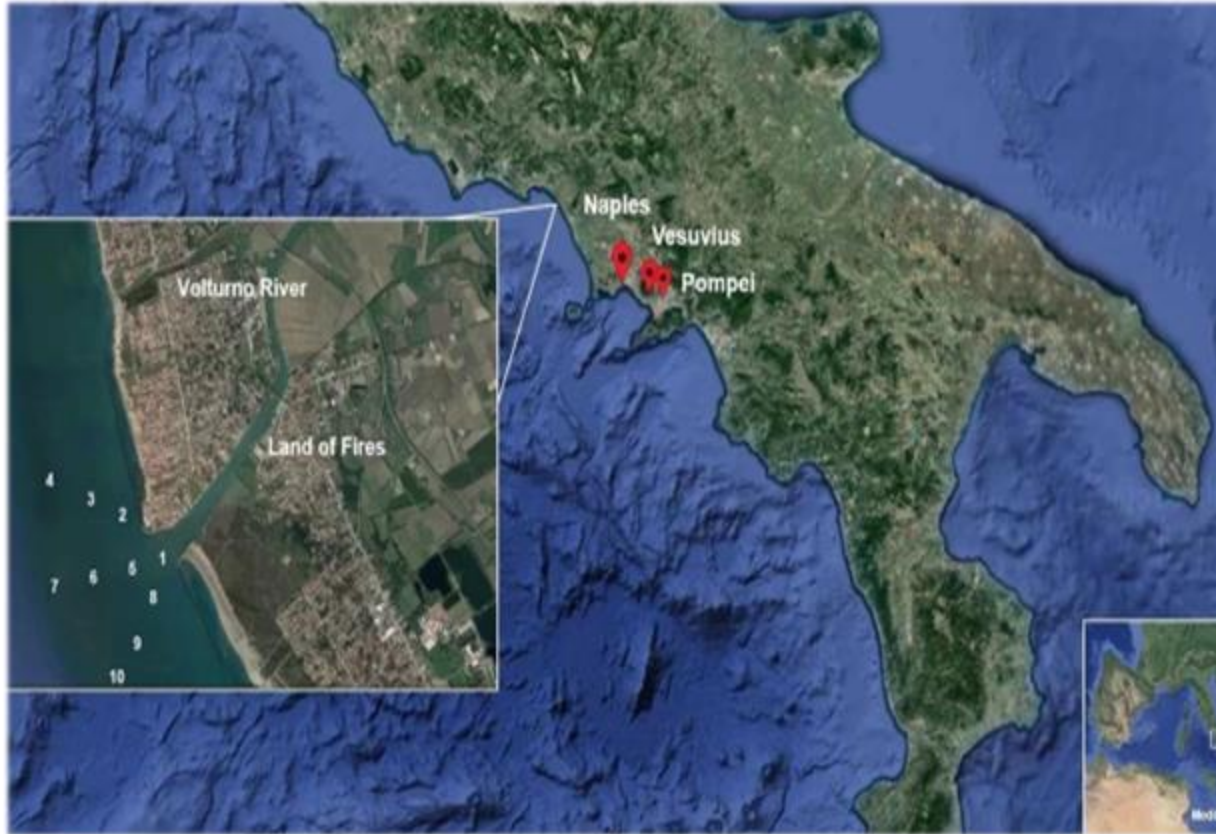
ZONE: A contiguous hydrological system with a distinct health status with respect to a specific disease that forms an area that including:

- (i) one or more water catchment(s) **from the source to the estuary or lake;**
- (ii) part of a water catchment **from the source to a barrier;**
- (iii) part of a coastal area with a precise geographical delimitation;
- (iv) an estuary with a precise geographical delimitation;

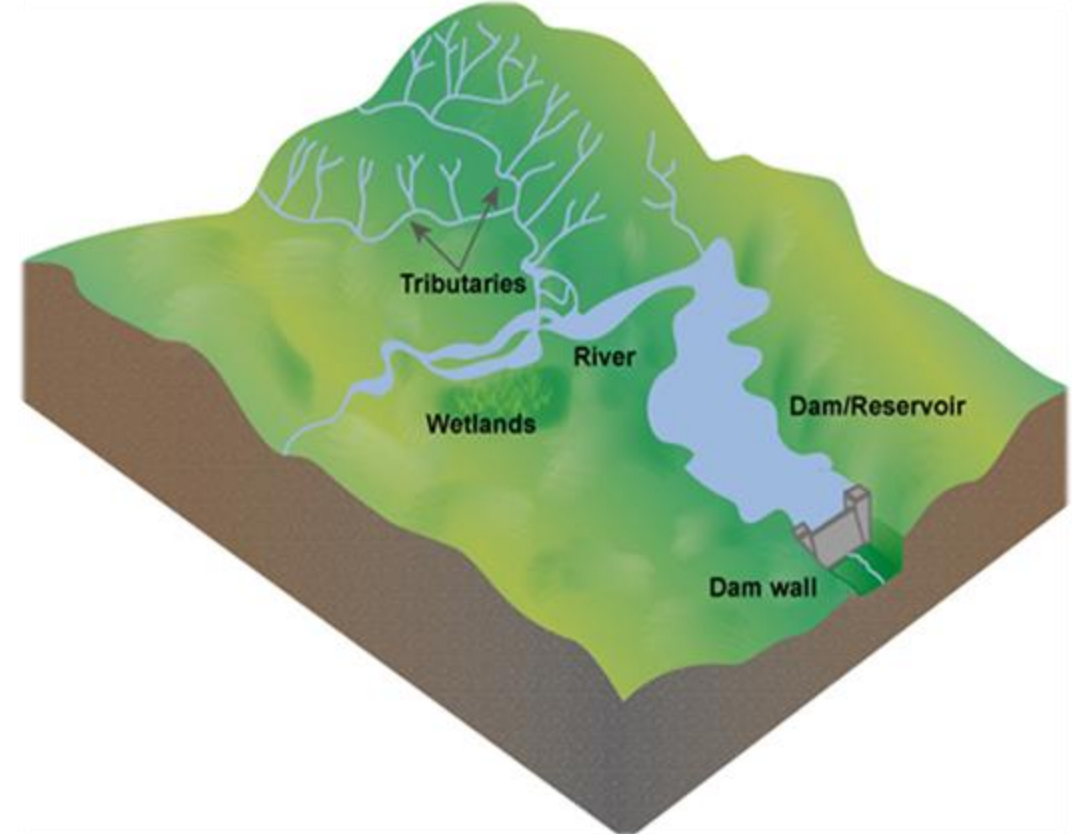


BTSF

Some examples of zones

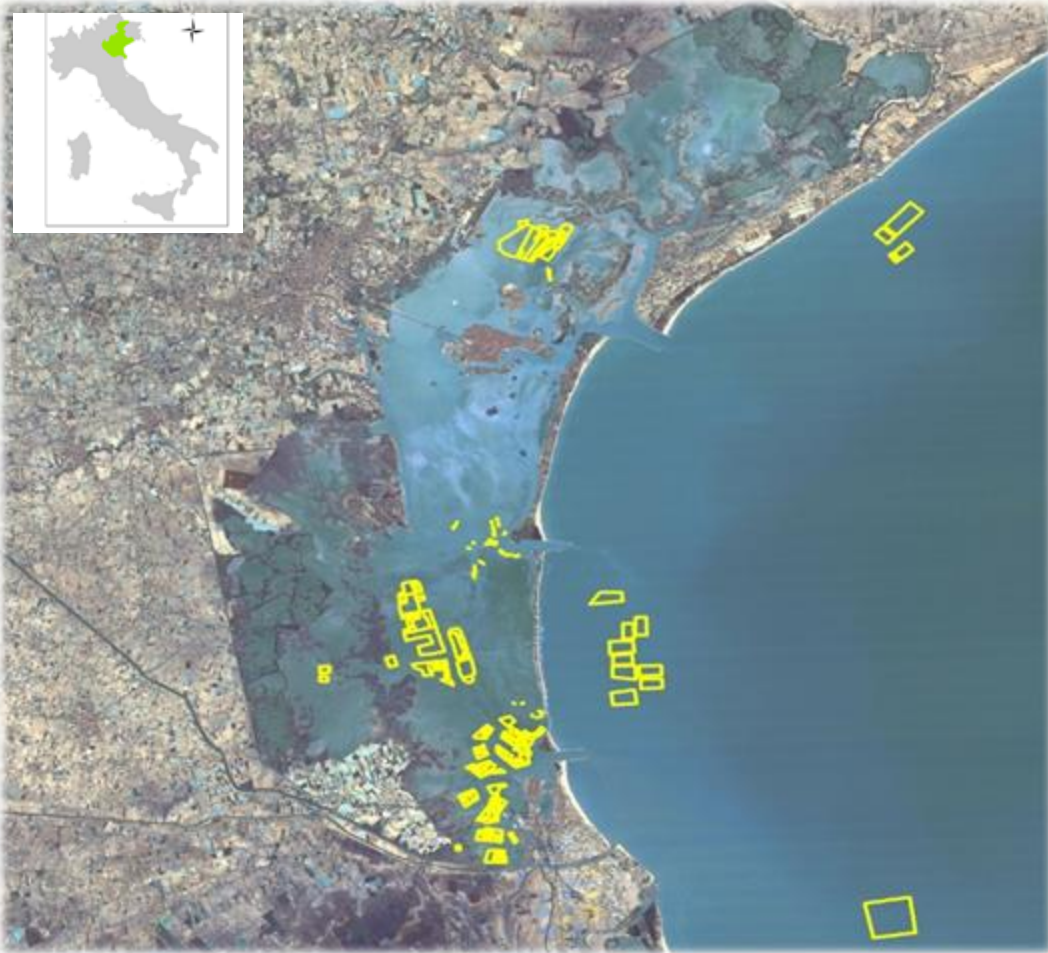


Water catchment from the source to the estuary



Part of a water catchment from the source to a barrier;

BTSF Examples of zones



Part of a costal area



Estuary

Compartment for aquaculture animals

A Compartment for aquatic animals is:

An aquatic animal sub-population

- Contained in one or more aquaculture establishments
- Common biosecurity management system
- Distinct health status for specific disease(s)
- Subject to appropriate disease control and biosecurity measures

Independent of the health status of surrounding waters



- Water must originate directly from a well/borehole/spring or has been treated through a water treatment plant
- If water originates from outside the establishment, the water shall be supplied directly and be channeled to the establishment
- Natural or artificial barriers that prevent aquatic animals from entering from the surrounding natural waters must be present
- The compartment must be protected against flooding and infiltration of water from surrounding natural waters ([Article 79](#))