



## UNDERSTANDING WP4

WP4 (AQUAHEALTH) is focused on health in aquaculture addressing both recurrent and emerging diseases. WP4 aims to learn about the pathogens and associated pathologies that affect the sector, as well as to detect and control the main diseases. It is articulated around 7 objectives: i) identify and characterize emerging pathologies, and develop and improve diagnostic and detection methods; ii) to study the life cycles of pathogens, vectors and the impact of climate change on etiological agents and their interaction with their hosts; iii) to design new vaccines against the most relevant pathogens and to study the routes of administration; iv) to develop new, alternative, eco-sustainable methods of treatment and control, both therapeutic and prophylactic; v) to create a Mediterranean Network for Research on Aquaculture Health (REMEDISA) that integrates the knowledge, experiences and capacities of the R+D groups of the Valencian Community; vi) to disseminate the project, transfer the scientific-technical tools generated to the sector and raise awareness in society about sustainable development; vii) to train competent personnel in animal health and welfare.

## GETTING TO KNOW THE PATHOGENS

Effectively combating pathogens begins with understanding them in depth: their transmission routes, mechanisms of action, and how fish defend themselves.

Three *Vibrio* species (*V. vulnificus*, *V. harveyi*, and *V. parahaemolyticus*) have emerged as key agents of vibriosis outbreaks increasingly linked to climate change. These pathogens affect both fish and crustaceans.

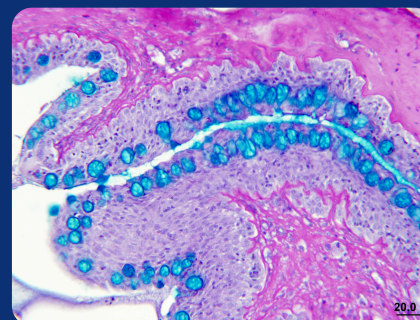


The main virulence factor of the zoonotic bacterium *V. vulnificus* is a toxin that targets erythrocytes, triggering a cytokine storm that leads to hemorrhagic septicemia and fish mortality.

The detection of a nodavirus (RGNNV) in a mortality outbreak of wild groupers near the Columbretes Islands (Castellón) highlights the expanding geographic range of this pathogen. It also raises concerns about its spread in the Mediterranean, where it poses a threat to both wild and farmed species.



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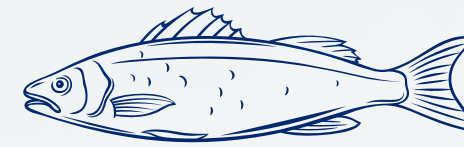
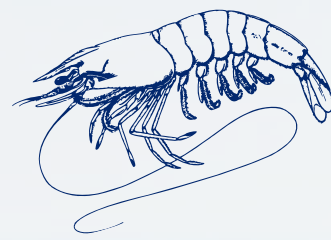
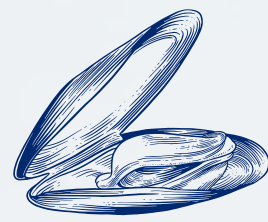
We now have deeper insight into the gill ectoparasite *Sparicotyle chrysophrii*, which infects gilthead sea bream. Temperature plays a critical role in the parasite's swimming activity and transmission. It uses extracellular vesicles to interact with its host. In response to infection, sea bream increases mucous cell production and mucin expression. Notably, the holobiont of parasitized sea bream is significantly altered.

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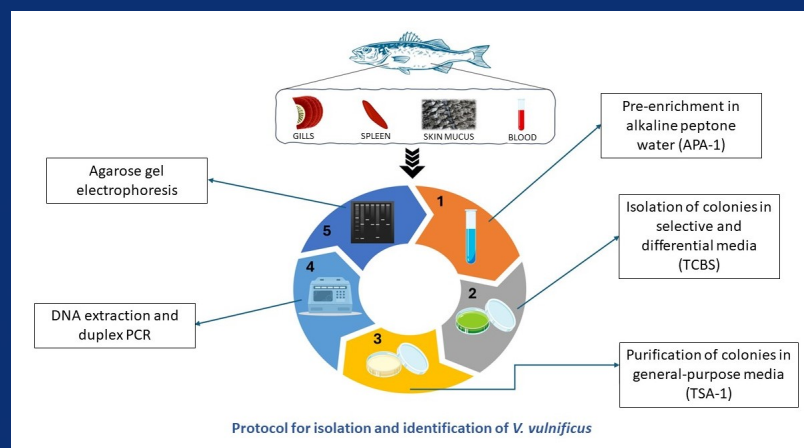






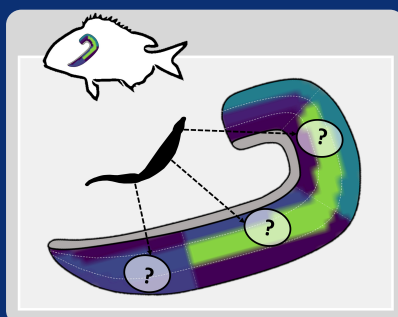
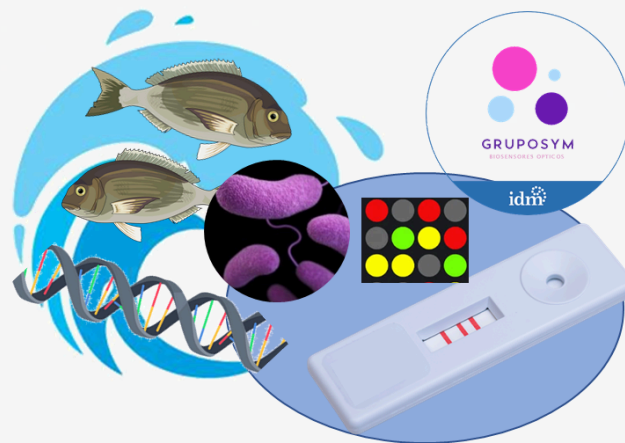
## DETECTING PATHOGENS

Effective control of infectious diseases in aquaculture depends on the rapid and specific detection of the pathogens involved. Here we present some of the most advanced recent developments:



A newly developed protocol for the isolation and identification of *V. vulnificus* enables the differentiation of high-risk strains relevant to both public and animal health. This protocol has been successfully applied to monitor the pathogen in coastal environments.

Portable systems and simple biosensing methodologies based on test strips and microfluidic chips and smartphone reading that allow the specific diagnosis of bacteria (*V. vulnificus*) and parasites (*Enteromyxum leei* and *Cardicola*) with a detection limit of 0.01% of pathogen DNA in fish tissues, in less than 2 hours.

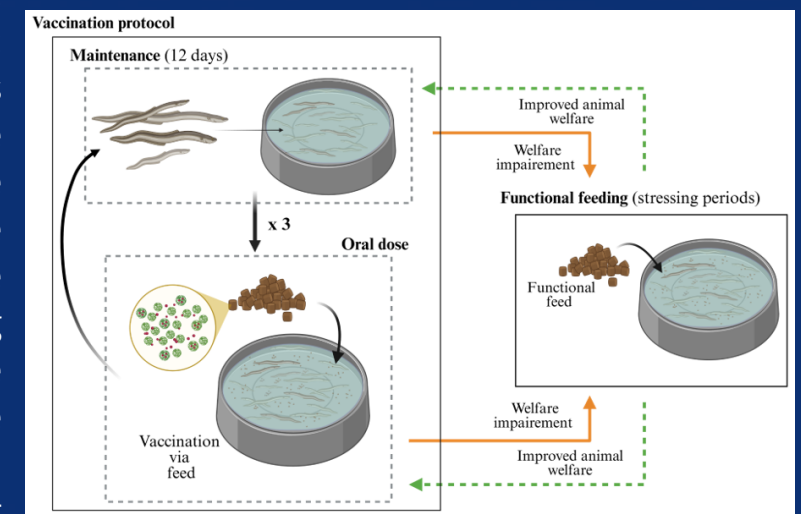


Understanding the microhabitat preferences of *Sparicotyle chrysophrii* throughout its life cycle is key to improving infection monitoring strategies. Incorporating this knowledge can enhance detection efficiency.

## ADVANCES IN DISEASE CONTROL

Promising trial results are already available for the control of bacterial, viral, and parasitic diseases:

Two complementary strategies were developed for aquaculture: immunostimulation during stress using a functional diet with effective essential oils, and a subunit vaccine targeting multiple pathogens. The vaccine includes transferable virulence-related antigens, offering cross-protection against species like *V. harveyi* and *A. salmonicida*. It can be administered via injection, immersion, or orally, making it suitable for intensive production systems.



Water electrolyzation significantly reduces *V. harveyi* and *V. vulnificus* populations under varying pH and salinity conditions, offering a potential tool for the prevention of vibriosis outbreaks.

*In vitro* and *in vivo* assays using various plant extracts and microalgae have demonstrated antiviral potential against nodavirus, showing promise in reducing mortality in European sea bass.



*In vitro* tests with certain phytogetic extracts and commercial nutraceutical additives have shown high efficacy against the gill parasite *S. chrysophrii* in gilthead sea bream.