GENERALITAT VALENCIANA
Conselleria de Educación, Universidades y Empleo

idm

XVI·IWOSMOR

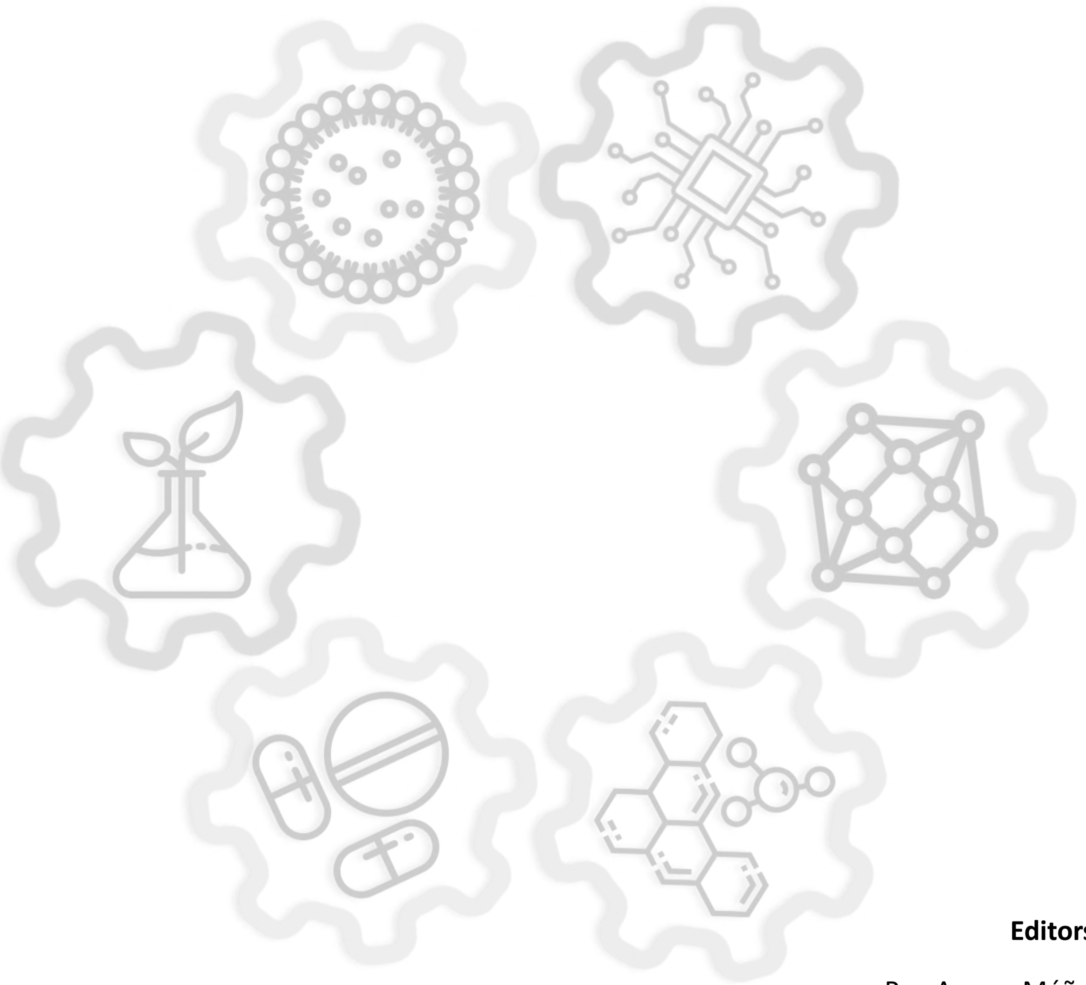
INTERNATIONAL WORKSHOP ON SENSORS AND MOLECULAR RECOGNITION

July, 6 and 7th 2023

BOOK OF ARTICLES

UNIVERSITAT POLITÈCNICA DE VALÈNCIA

VNIVERSITAT DE VALÈNCIA



Editors:

Pau Arroyo Máñez

Margarita Parra Álvarez

Eva María Garrido García

Mariana Barros Nogueira

Paula Rodrigo Martínez

Jordi Hernández Contreras

ISBN 978-84-09-55991-6

- INDEX -

DNA biosensing systems for THINK in AZUL project. The future of pathogen detection in aquaculture

Luis A. Tortajada-Genaro[a,b], Miguel Ángel González-Martínez[a,b], Francisco J. Espinós[c], Ángel Maquieira[a,b]

[a]Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Universitat Politècnica de València-Universitat de València, Camino de Vera s/n, E46022 Valencia, Spain. e-mail: luitorge@qim.upv.es

[b]Departamento de Química, Universitat Politècnica de València, Camino de Vera s/n, E46022 Valencia, Spain

[c]ACUMA Research Centre Universitat Politècnica de València, Av. de los Naranjos s/n, 46022 Valencia, Spain

Keywords: Aquaculture pathogens ● Environmental DNA ● Species identification ● Fish health control

Abstract

This paper presents the main advances in the design and development of biosensing systems focused on improving the diagnosis of diseases caused by pathogens in aquaculture. The study was performed under the THINK in AZUL project, devoted to mitigating the effects of climate change on aquaculture production and the marine and coastal ecosystem. Novel approaches are investigated to collect samples without stressing or sacrificing the fish. Thus, determining environmental DNA, i.e., the genetic material in water, is a new tool for surveying aquaculture facilities. However, detecting DNA/RNA from target organisms (parasites, bacteria, and viruses) at low concentrations with a simple method and device is a relevant challenge. The main expected advantage is that the pathogens will be objectively identified without the need for time-consuming and expensive surveys, avoiding the taxonomic expertise of the monitoring personnel.

INTRODUCTION

Aquaculture is a food sector based on breeding, raising, and harvesting fish, shellfish, and aquatic plants. Global production increases between 5% and 7% per year. In the European Union, the activity accounts for about 20% of the fish and shellfish supply and directly employs 75,000 people, 27.580 of them in Spain [1]. The sector comprises around 15,000 enterprises, mainly small businesses or micro-enterprises in coastal and rural areas. Spain, France, Italy, and Greece are the main aquaculture-producing EU countries in terms of volume.

Around 100 different species are currently farmed in aquaculture operations around the world. Nevertheless, EU aquaculture production is concentrated mainly on a few species. Also, aquaculture production is very diverse in terms of both farmed species and production methods (sea cages, ponds, raceways, and on-land recirculating aquaculture systems).

Several actions modify the coastal ecosystems and aquaculture activities and their effect on human health and industrial production (Fig. 1). Human activities on land are the most important sources of marine pollution. Climate change affects aquaculture due to temperature variations in water and other alterations in marine conditions, such as currents, wind speed, and waves [2]. Another negative factor is that the ecosystems are plagued by a plethora of bacterial pathogens that infect fish. The potential consequence is a significant increment in disease outbreaks within aquaculture systems, resulting in severe financial and human health impacts [3]. Minimizing the effect of the fish pathogens requires addressing health constraints.

Proper application of management measures will need farm-tailored biosecurity plans with the support of qualified fish health professionals and diagnostic centers [4]. Thus, this sector requires efficient systems and technologies for health control against parasites, bacteria, and viruses. A holistic approach considers all factors involved, from a comprehensive understanding of the life cycle and transmission routes to developing novel diagnostic tools [5]. Thus, the surveys should include risk assessment of interspecific transmission between vectors, intermediate hosts, wild fish, and animals in the production system.

THINK in AZUL project is devoted to mitigating the effects of climate change on aquaculture production and the marine and coastal ecosystem. A holistic approach considers all factors involved, from a comprehensive understanding of the life cycle and transmission routes to developing novel diagnostic tools.

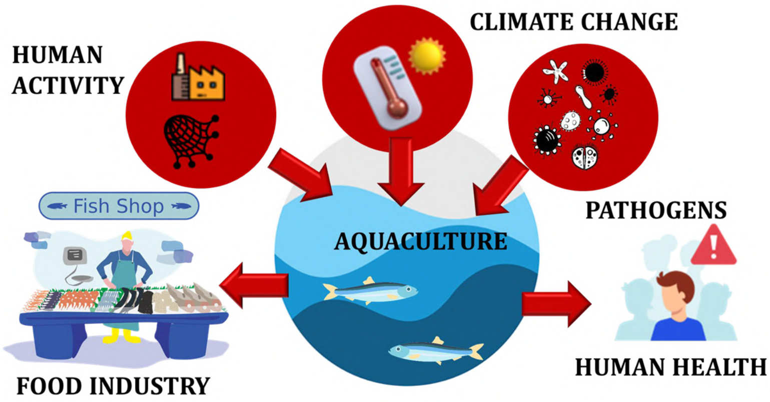


Fig. 1. Interactions that affect the coastal ecosystems and aquaculture activities and their effect on human health and industrial production

RESULTS AND DISCUSSION

Environmental DNA

Marine finfish aquaculture requires efficient systems and technologies for health control against parasites, bacteria, and viruses. Thus, a challenge is to identify and characterize pathologies using alternative diagnostic tools to microbiology methods.

In a given environment, all organisms continuously shed DNA either occurring as extracellular molecules, free in solution or bound to particles, or as intracellular molecules residing inside cells [6]. The quantity of eDNA present is controlled by organismal production rate, degradation rate, and physical transport. Thus, determining DNA is a new tool for surveying these ecosystems, supporting the correct management of living marine resources [7]. In the aquiculture field, the simplicity and cost-effectiveness of eDNA determination make it a highly attractive tool for establishing species distributions from water samples.

For this purpose, our research group will contribute to determining the environmental DNA (eDNA) of target pathogens in water samples collected in aquaculture facilities and coastal areas (Fig. 2 top).

Selection of biosensing scenarios

Several fish species can be farmed with extensive and semi-intensive methods and intensive farming systems in land-based installations or sea cages (Fig. 2, bottom). Managing such growing systems requires a growth model to describe the response of the fish to their environment.

Intensive grow-out usually follows other intensive farming phases: reproduction, larval rearing, and pre-fattening. For instance, gilthead sea bream (*Sparus aurata*) is a fish commonly cultivated in the Mediterranean Sea. Intensive pre-fattening and grow-out steps are carried out in land-based installations with rectangular concrete tanks that vary in size (200-3,000 m^3) and recirculating aquaculture systems. Grow-out may also occur in sea cages in remote or semi-exposed sites (floating cages) or totally exposed areas (semi-submersible or submersible cages).

Health fish problems depend on the specific facility, such as industrial hatcheries, land-based extensive and intensive grow-out systems up to sea cages [9]. In fact, disease dynamics result from pathogens–host interactions for farmed and wild fishes. Table 1 shows the potential means by which farmed populations may alter wildlife disease dynamics in the environment surrounding aquaculture facilities.

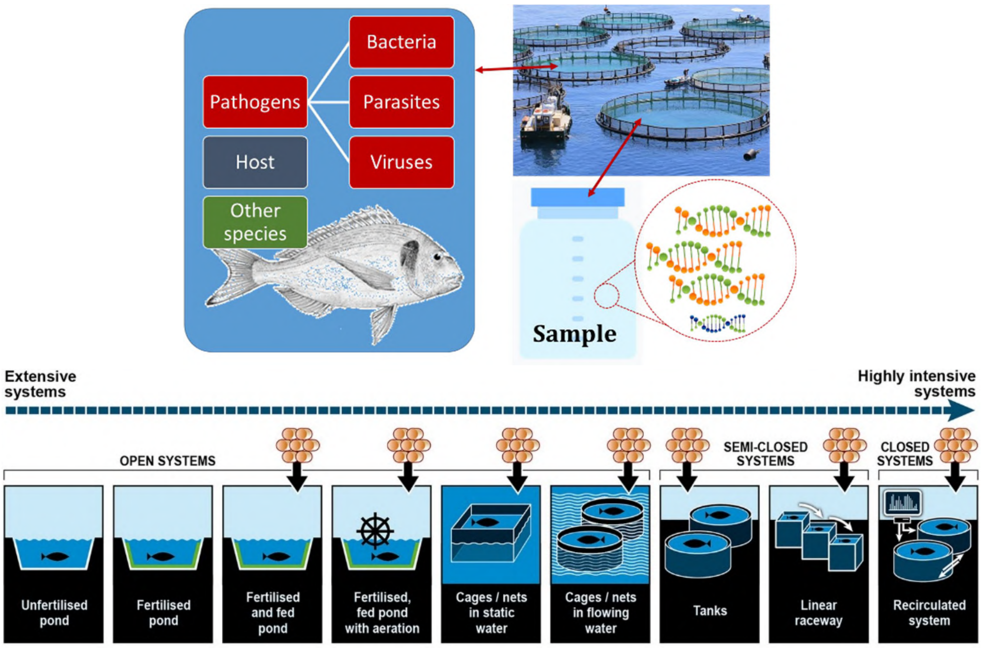


Fig. 2. (Top) Species-specific DNA concentrations are positively correlated with biomass and abundance. (Bottom) Farm facilities to be monitored for controlling fish health.

Table 1. Mechanisms through which aquaculture activities impact on wildlife infections [10].

Mechanism	Description
Intraspecific parasite spillover	Farmed species may co-introduce parasites to the new environment, which infect wild conspecifics without infecting other species
Interspecific parasite spillover	Co-introduced parasites from farmed species may infect other wild host species potentially leading to emerging diseases
Interspecific parasite spillback	Parasites from other wild host species may infect farmed species, amplifying parasite numbers and increasing parasite infections when spilling back to wild hosts
Intraspecific parasite spillback	Farmed species may acquire parasites from wild conspecifics, increasing parasite population size and subsequently raising infection loads in the wild host population
Transmission interference	Farmed species may be neither hosts nor parasites, but they affect the transmission of parasites between wild host species

Selection of pathogens

We identified several serious diseases from many years ago and presently affecting reared specimens.

- The most relevant bacterial pathology is Vibriosis, characterized by a systemic hemorrhagic septicemia. Vibrio spp. has been isolated frequently from diseased gilthead seabream in several farms around the Mediterranean basin.

- Parasitic diseases, also known as parasitosis, are infectious (or not) diseases caused or transmitted by eukaryotic organisms. Parasites can affect practically all living organisms; however, many parasitic organisms do not cause diseases. They can induce stress and immunodepression, and as a consequence, secondary infections, pathologies, and severe disorders diseases, are more frequent in those infected fishes.

- The Viral Nervous Necrosis is a viral fish disease provoked by the Nervous Necrosis Virus (NNV) belonging to the family Betanodaviridae. Pathogenesis is related to the neuro-invasive nature of the virus and its deleterious effect on tissues in the brain and retina.

Therefore, the novel diagnostic tool will be able to determine the eDNA from the pathogens that cause the most relevant diseases, farmed fishes, and other wild species in the Mediterranean Sea. The selected organisms include bacteria, such as *Vibrio vulnificus* and *V. harveyi*, parasites such as *Enterospora nucleophile* and *Cardicola*, and virus such as *Red-spotted grouper nervous necrosis virus* (RGNNV).

Selection of biosensing approach

Technological-scientific progress in the aquaculture field requires considering the available analytical techniques for determining target DNA regions. In species-specific detection, the main techniques described are (a) gel electrophoresis of PCR amplicons, (b) real-time quantitative PCR (qPCR), (c) DNA arrays, and (d) novel biosensing technologies. The selected approach was a biosensing method because it can improve analytical performances, portability, and running costs.

The proposed systems are supported on oligonucleotides as sensing elements, selecting their sequence and markers and new methodologies. The steps of the designed biosensing assay are a fast approach of DNA extraction from filtered water samples, isothermal amplification of targeted sequences, and detection based on lateral flow assay or chip reactor. The smartphone camera will capture the assay output.

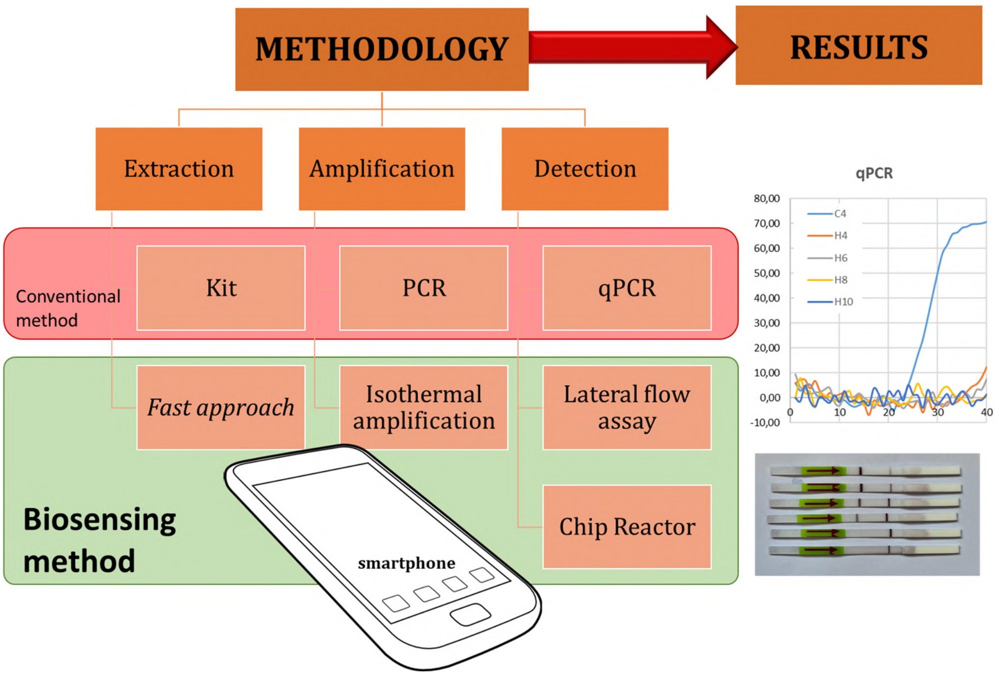


Fig. 4. Comparison of methodologies and results between conventional DNA-based methods and the proposed biosensing method supported on a smartphone as the detector.

CONCLUSIONS

The estimated features of technologies were suitable for effectively detecting all target pathogens. The main advantage is that the species are objectively identified without the need for time-consuming and expensive ship-borne surveys and avoiding the taxonomic expertise of the monitoring personnel. The results are helpful to progress in using biosensing technology applied as decision-support tools in fields such as fish biology, control of climate change, environmental remediation, and aquaculture.

ACKNOWLEDGMENTS

This study forms part of the ThinkInAzul program and was supported by MCIN with funding from European Union NextGenerationEU (PRTR-C17.I1) and by Generalitat Valenciana. Also, thanks to PROMETEO/2020/094.

REFERENCES

[1] European Commission https://oceans-and-fisheries.ec.europa.eu
[2] Galappaththi, E. K., Ichien, S. T., Hyman, A. A., Aubrac, C. J., & Ford, J. D. (2020). Climate change adaptation in aquaculture. Reviews in Aquaculture, 12(4), 2160-2176.
[3] Cascarano, M. C., Stavrakidis-Zachou, O., Mladineo, I., Thompson, K. D., Papandroulakis, N., & Katharios, P. Mediterranean aquaculture in a changing climate: temperature effects on pathogens and diseases of three farmed fish species. *Pathogens*, 2021, 10(9), 1205.
[4] Assefa, A., & Abunna, F. Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. *Veterinary medicine international*, **2018**, 5432497.MacAulay, S., Ellison, A. [5] R., Kille, P., & Cable, J. Moving towards improved surveillance and earlier diagnosis of aquatic pathogens: From traditional methods to emerging technologies. *Reviews in Aquaculture*, **2022**, 14(4), 1813-1829.
[6] Tsuji, S., Takahara, T., Doi, H., Shibata, N., & Yamanaka, H. The detection of aquatic macroorganisms using environmental DNA analysis—A review of methods for collection, extraction, and detection. *Environmental DNA*, **2019**, 1(2), 99-108.
[7] Hansen, B. K., Bekkevold, D., Clausen, L. W., & Nielsen, E. E. The sceptical optimist: challenges and perspectives for the application of environmental DNA in marine fisheries. *Fish and fisheries*, **2018**, 19(5), 751-768.
[8] Seginer, I. Growth models of gilthead sea bream (Sparus aurata L.) for aquaculture: A review. *Aquacultural Engineering,* **2016**, 70, 15-32.
[9] Buchmann, K. Control of parasitic diseases in aquaculture. *Parasitology*, **2022**, 149(14), 1985-1997.
[10] Bouwmeester, M. M., Goedknegt, M. A., Poulin, R., & Thieltges, D. W. Collateral diseases: aquaculture impacts on wildlife infections. *Journal of Applied Ecology*, **2021**, 58(3), 453-464.
[11] J Borrego, J., M Labella, A., Ortiz-Delgado, J. B., & Sarasquete, C. Updated of the pathologies affecting cultured gilthead seabream, Sparus aurata. Annals of Aquaculture and Research, **2017**, 4(2): 1033.