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IGNITION

Developing innovative strategies for improving animal health and resilience - updates from the IGNITION project

Building on the overview article on the IGNITION project from our September 2023 edition, we are pleased to offer a more in-depth look into the IGNITION project's progress in aquaculture sustainability. The IGNITION project, funded by the European Union, aims to revolutionize aquaculture by addressing crucial challenges such as disease resistance, animal welfare, and the impacts of climate change.

With aquaculture production surpassing wild capture fisheries, the need for resilient, sustainable practices has never been more urgent. With climate change, increasing frequency of extreme weather events and rising temperatures pose significant threats to the sector. IGNITION's interdisciplinary approach seeks to mitigate these risks by developing innovative strategies for improving animal health and resilience.

In this edition, we dive deeper into three of the key ongoing efforts – i) genetic resilience in the Manila clam, ii) bioactive screening of halophytes for functional feeds and iii) non-invasive health monitoring tools for fish.

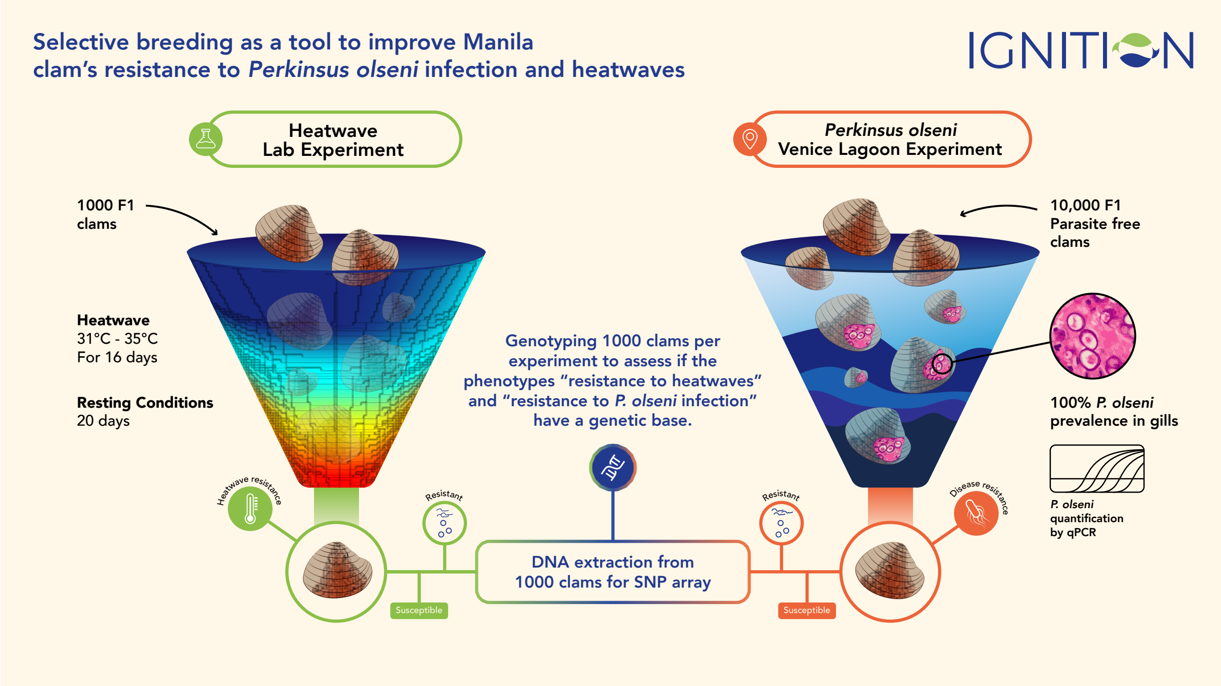

Figure 1: CLAM GENOTYPING INFOGRAPHIC Credit: Science Crunchers.

i) Estimating genetic parameters for disease resistance and climate change resilience in the Manila clam

WRITTEN BY: **MASSIMO MILAN** (ASSOCIATE PROFESSOR), **LUCA PERUZZA** (POSTDOCTORAL RESEARCHER) AND **LUCA BARGELLONI** (PROFESSOR); UNIVERSITY OF PADOVA, ITALY

Climate change is and will continue to threaten the aquaculture sector by reducing the number of suitable areas for farming aquatic species, in particular bivalves. By 2090, suitable areas for mollusc aquaculture are expected to decrease globally by ~10%. A major component of climate change is constituted by extreme climate events such as heatwaves, which are increasing in their frequency, intensity, and severity, ultimately causing massive mortality.

Among farmed bivalve species, the Manila clam, *Ruditapes philippinarum,* has great economic importance in the EU and in the world. Native to the Indo-Pacific region, this species was introduced for farming purposes in coastal areas of the Mediterranean and the Atlantic in the early 70s. Although the high adaptability and resistance to various stressors allowed Manila clam to spread rapidly after its introduction, climate change represents a serious threat. Another important risk factor is the spread of the protozoan parasite *Perkinsus olseni,* the most significant pathogen affecting clams in the Mediterranean Sea. The disease caused by this parasite, known as perkinsosis, can be particularly damaging under conditions that stress clam populations, such as high temperatures, which can exacerbate the severity of the infection. Increasingly frequent mortality events and a dramatic reduction of natural recruitment already severely impacted clam production as exemplified by the drop from 40,000 tons in 2000 to 3,000 tons in 2019 in the Venice lagoon, one of the most productive areas in Europe.

In shellfish farming there is a limited scope for countering the negative effects of heatwaves and pathogens, as the majority of solutions applied in finfish farming are largely ineffective. Genetic selection programs aimed at improving resilience to these adversities is a promising strategy to reduce the economic losses in bivalves of commercial interest. In this framework, the IGNITION team is investigating whether selective breeding in Manila clam could improve resistance to *P. olseni* and heatwaves. In collaboration with the hatchery SATMAR, from a controlled cross of 15 sires and 50 dams, around 20 000 individuals were obtained. Approximately 1,000 of these were exposed to a simulated heatwave for 16 days with temperatures ranging daily from 31°C to 35°C. Throughout the experiment, mortality was monitored daily and tissue samples from dead/moribund animals were promptly collected. Similarly, at the end of the experiment, tissue samples from all surviving clams (approximately 40%) were collected. The remaining animals were seeded in the Venice lagoon in April 2023. Since then, around 50 clams were collected every month to quantify the prevalence of perkinsosis. After 4 months, when the prevalence was >40%, 1,000 clams were collected randomly and the degree of infestation quantified. Subsequently, DNA will be extracted from all sires and dams and all the individuals challenged either with heatwave (1,000) and *P. olseni* (1,000). A new tool (medium-density SNP array), developed within IGNITION will be employed to reconstruct family relationships. This will allow to estimate genetic parameters for disease resistance, heatwave resilience and morphometric composition. In addition, it will be possible to identify specific portions on the DNA that significantly contribute to the traits of interest. The whole experimental plan, summarized in infographic (Figure 1), will allow the optimization of future breeding programs to select for clam resilience, while calibrating selection intensity to maximise genetic advantages while avoiding inbreeding.

ii) Halophytes biorefinery and functional feeds - In Vitro Trials for Bioactive Screening

WRITTEN BY: **KIRON VISWANAT** (PROFESSOR), AND **MARIANA FERREIRA** (POSTDOCTORAL RESEARCHER); NORD UNIVERSITY, NORWAY

Halophytes, a group of salt-tolerant plants adapted to coastal environments, have recently attracted considerable interest due to their rich profile of bioactive compounds, such as polyphenols. These bioactives are known for their antioxidant, anti-inflammatory, and antimicrobial properties, making them promising candidates for incorporation into feeds for farmed aquatic animals. In aquaculture, functional feeds are crucial for improving the resilience of farmed species, especially as the sector faces increasing challenges from disease outbreaks and environmental pressures. These feeds, enriched with bioactive compounds, can strengthen the immune system and promote a healthier gut microbiome, allowing fish to better cope with infections and harsh environmental conditions. Ultimately, this leads to improved survival rates, growth, and overall health. Therefore, identifying the most effective bioactive compounds and recommending effective inclusion levels is key to developing successful functional feeds for aquaculture.

To this end, in vitro tests serve as an essential step for screening and evaluating various bioactive extracts before progressing to more resource-intensive in vivo proof-of-concept studies. These tests enable researchers to assess the effects of different extracts on cell viability and functionality and resistance to pathogens, helping to identify promising candidates for further study. Hence, partners within IGNITION are focused on the in vitro screening of bioactive compounds derived from halophytes to evaluate their potential for inclusion in aquafeeds. In collaboration, NORD and CIIMAR are working on the identification of the most potent halophyte extracts and their ideal concentrations. NORD is conducting experiments on Atlantic salmon phagocytic cells, while CIIMAR is performing parallel studies on European seabass cells. These cells are exposed to the extracts identified in earlier tasks of the projects. The research approach employs classical cell assays such as ATP production, nitric oxide levels, and reactive oxygen species generation, providing insights into the cells' metabolic activity and oxidative stress responses. Additionally, imaging flow cytometry is employed to assess phagocytic activity, and oxidative stress responses, offering a comprehensive view of how these bioactive compounds interact with fish immune cells. Moreover, time-course analyses are being conducted to examine the cell responses after exposure to the extracts and pathogenic bacteria, providing a detailed understanding based on changes in the expression of immune and inflammation marker genes.

The results from these efforts will be instrumental in proposing the most effective bioactive fractions and their concentrations for incorporation into fish (and shrimp) feeds to evaluate their effectiveness under farming conditions. Thus, these results will demonstrate how the compounds influence immune cell responses and enhance resilience against key pathogens affecting farmed fish, such as *Tenacibaculum maritimum*. The use of in vitro models to pre-screen bioactives streamlines the development process, ensuring that only the most promising compounds are advanced to the final stages of functional feed formulation (Figure 2). This approach not only improves the health and resilience of farmed species but also contributes to the sustainability of aquaculture systems.

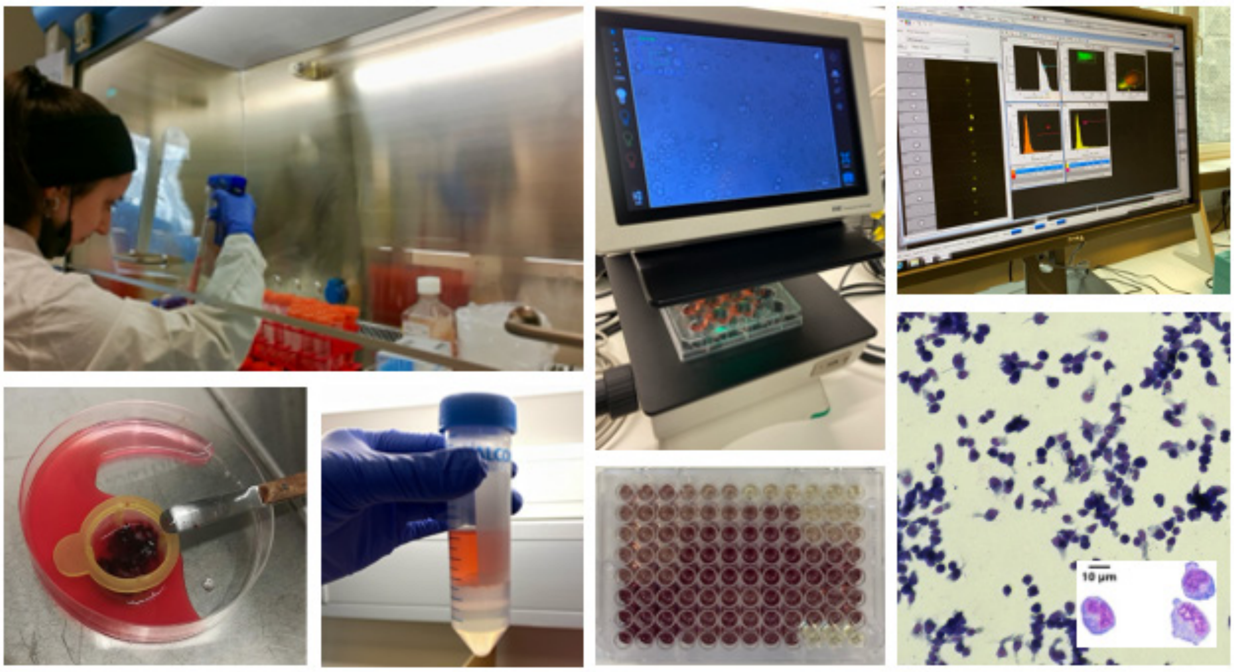

Figure 2: Representation of the different steps involved in the in vitro screening process of halophyte extracts, including tissue homogenization, percoll separation, cell culture, viability assays, flow cytometry analysis and cell staining. Credit: Mariana Ferreira

iii) Non-invasive tools and machine learning

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Partners at Tyndall and Sparos will draw on knowledge obtained by the consortium to achieve three main objectives:

i. To discover and select biomarkers for the different target species which will be assessed for their suitability to be integrated into a sensor device to detect and monitor the health of farmed animals.

ii. To develop non-invasive electrochemical sensors for quantitative analysis of selected biomarkers in farm products, using single and multiplex platforms.

iii. To validate biomarker protocols and sensors for prototype in WP2.

A biomarker is a biological molecule found in blood or other body fluids, and in tissues which can be used as indicators of normal or abnormal processes, and of a disease or condition. Biomarkers can also be used to assess how well the body responds to a treatment for a disease or condition. Examples of biomarkers include body temperature, body fat percentage, blood sugar level, and blood pressure. Classical biomarkers that can robustly predict health under varying conditions include cortisol, lactate, and immune response indicators.

The ability to measure biomarkers can provide a definitive method for measuring stress in fish, which can negatively affect their health. The ability to monitor stress in fish and adjust the conditions that may be causing the stress is hugely advantageous for improving overall animal welfare. As mentioned previously, cortisol, lactate and immune response indicators are all classical biomarkers in health monitoring, these biomarkers are all involved in the body's response to stress. When fish are stressed, they excrete more of these biomarkers in their blood and mucus, as well as excreting them into the water around them. Figure 3. highlights the ways in which these sensors can be applied for monitoring the biomarkers secreted by fish. On the left, the sensor has been incorporated into a patch which can be directly applied to the skin of the fish. On the right, the sensor has been deployed in a floating egg which can be placed in a tank and used to measure the amount of biomarker that is secreted into the water. Both applications are minimally invasive to fish.

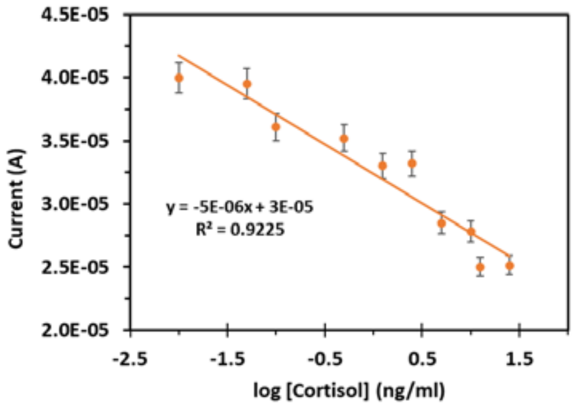

Figure 4. Calibration plot for the electrochemical detection of cortisol for concentrations ranging from 0.01 ng/ml to 25 mg/ml

Sensors that can detect biomarkers are typically called biosensors, meaning the sensor incorporates a biological recognition element. In this case, the biological recognition element is an antibody. Antibodies specific to the biomarker of interest are attached to the surface of the sensor. When the sensor is applied in a patch or as a floating egg, the antibody traps the biomarker to the surface of the sensor. An electrical signal is then obtained which is proportional to the amount of biomarker present. The steps of how the antibody is attached to the surface of the sensor are highlighted in the top of Figure 3.

Preliminary results using these sensors for the detection of cortisol in a controlled environment show promising results. As seen in Figure 3, an antibody linker is required to attach the cortisol antibody to the surface. In this instance a compound called dithiobis (succinimidyl propionate) (DTSP). After the cortisol antibody was attached to the DTSP, a layer of bovine serum albumin (BSA) was applied to the sensor. This is known as a blocking layer and prevents any competing reactions with any of the gold surface that has not been covered by the antibody. The sensor is now ready for the detection of cortisol. Figure 4. shows the promising results obtained from this sensor across a cortisol concentration range of 0.01 ng/ml to 25 mg/ml. It can be seen in this graph that the current read from the sensor decreases with each concentration. This is because as more cortisol binds to the sensor, the electrochemical activity decreases thus decreasing the current read from the sensor.

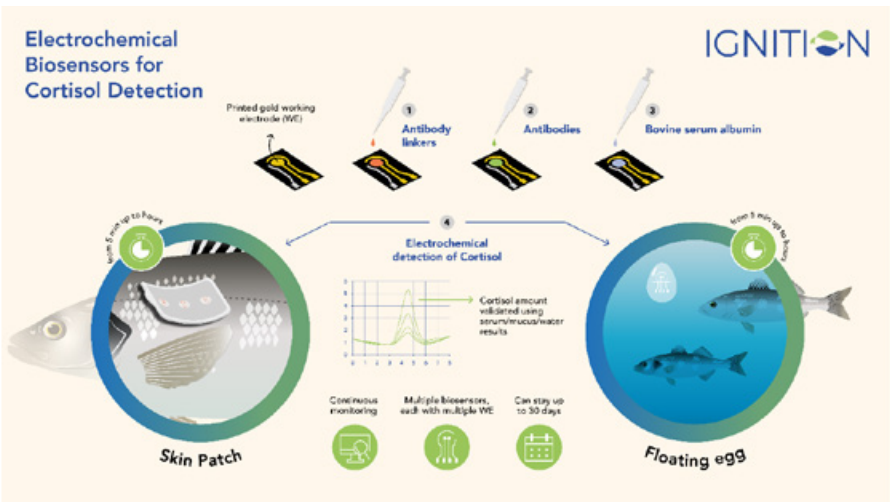

Figure 3. Infographic highlighting sensor fabrication, application of the sensor as either a patch on the fish or an egg in the fish tank, and the resulting electrochemical signals indicating the presence of a biomarker, in this instance cortisol.

Credit: Science Crunchers.

WRITTEN BY **ANA VISKOVIC** (PROJECT AND PROGRAMME MANAGER, EAS) AND **TANIA PEREIRA** (PROJECT MANAGEMENT, CIIMAR).

Collaborative Efforts and Multidisciplinary Expertise

The IGNITION project stands out for its collaborative approach, drawing on expertise from various fields such as genetics, bioactive compound research, and advanced health monitoring technologies. This multidisciplinary collaboration ensures that solutions are not only scientifically sound but also practical for the aquaculture industry.

Working together with industry stakeholders, the IGNITION team addresses challenges from multiple angles, ensuring that the technologies developed are scalable and applicable across various aquaculture operations.

Practical Implications for Aquaculture Producers

For aquaculture producers, the innovations emerging from the IGNITION project present several practical benefits. Work on genetic resilience in Manila clams aims to help producers mitigate the impacts of climate stressors and disease, which could be critical for maintaining stable production levels. The research into bioactives offer the potential for more robust functional feeds, supporting stronger immune systems and reducing dependency on antibiotics. Lastly, developing non-invasive tools provide early disease detection, reducing mortality rates and enabling faster interventions, leading to more efficient and sustainable farming practices.

These advances promise to enhance both productivity and sustainability, addressing some of the key issues facing modern aquaculture.

Future Prospects: What's Next for IGNITION?

Looking forward, the IGNITION project has several exciting milestones on the horizon. Upcoming field trials, particularly for the newly developed bioactive compounds and sensor devices, will play a crucial role in validating the project's innovative solutions. The results from these trials could potentially influence new industry standards and even shape regulatory frameworks within the EU and beyond.

IGNITION is also poised to contribute to the growing body of research supporting genetic-based breeding programs, which could become a mainstay in aquaculture's future. These programs are expected to enable farmers to breed species that are more resilient to climate change and disease, offering a long-term solution to some of the sector's most pressing problems.

Conclusion: IGNITION's Impact on the Future of Aquaculture

The IGNITION project continues to push the boundaries of innovation in aquaculture, offering promising solutions to some of the industry's most significant challenges. Through its targeted work, the project is developing sustainable strategies to improve animal welfare, enhance disease resistance, and combat the negative effects of climate change. The research on genetic resilience in the Manila clam and the development of non-invasive monitoring tools for fish health are just two examples of how IGNITION is setting the stage for a more resilient aquaculture sector.

As global demand for seafood continues to rise, the findings and technological advancements from IGNITION will help ensure that aquaculture can meet this demand while minimizing environmental impact and promoting the well-being of farmed animals. By focusing on sustainability and resilience, IGNITION is not only addressing current challenges but also laying the foundation for a future where aquaculture can thrive in harmony with our planet.

With continued collaboration between researchers, industry stakeholders, and policymakers, IGNITION is poised to drive meaningful change and help shape a more sustainable and efficient aquaculture industry for generations to come.

UK Research and Innovation

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