PathoGelTrap

New Blue Revolution through a pioneering pathogen-trapping technology based on bioselective hydrogel-forming proteins

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Report on Water assessment 2

PathoGelTrap™
Sustainable Prevention in Aquaculture

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V2.0	28.11.2023	Second draft with revisons
V3.0	30.11.2023	Final version

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Executive Summary

Within the objectives of WP4 of the PathoGelTrap project, task 4.2 assesses the water and aquaculture management operational parameters in which the PGT products are going to work. SmartWater (**SW**) developed a sensor platform (Medusa) to measure water parameters such as pH, temperature, dissolved oxygen, conductivity, Oxidation Reduction Potential (ORP), and turbidity. The life experiments set up with PGT liquid was adapted to a new scale as a strategy for reduction of molecule use. **SW** adapted the sensor platform to the new set up.

List of acronyms/abbreviations

AFB: Afibody
DL: Deliverable
IZSVE: Istituto Zooprofilattico Sperimentale Delle Venezie
LCR: Low complexity region
LLPS: Liquid-liquid phase separationORP: Oxidation Reduction Potential
PGT: PathoGelTrap project
RAS: recirculating aquaculture systems
SW: Smartwater Planet S.L.
UCD: University College Dublin
WP: Work package
Ppt: parts per thousand (used in salinity)

Glossary of terms

Term	Explanation
Pathogen	Organism able to cause disease
Water quality operation conditions	Water quality conditions typically found in aquaculture operations
Betanodavirus	Viral agent causative of Viral Nervous Necrosis, also known as viral encephalopathy and retinopathy, one of the target infective agents of the PGT project
Yersinia ruckeri	Bacterial agent causing Enteric Red-mouth disease, one of the target infective agents of the PGT project
Affibody	Small (6.5-kDa) engineered binding proteins based on a three-helix bundle motif of the Z domain derived from staphylococcal protein A used as a scaffold for sequence variation.
PGT products	The products that the project is designing to present the PGT technology, as a liquid form and as a filter form.

1. Introduction

The operational conditions for PGT products were established in accordance with specific conditions of the cultivation environment they would be used. These have been used as reference during in silico modelling, and molecule selection and design. Temperature and salinity were of special relevance in this process

On the other hand, online, real-time water quality monitoring during *in vivo* experiments, would ensure that fish welfare conditions are maintained and that water quality parameters during operation are recorded for analysis.

2. Water quality conditions

2.1. PGT Operational conditions

Temperature, pH and salinity conditions are of special importance for fish welfare. These parameters have a bearing also in the development and dynamics of an infectious outbreak.

The operational conditions considered for PGT products are those consistent with the ones found in most culture operations of the target fish and pathogen species (Seabass for Nodavirus, and Trout for Yersinia), presented in table 1.

Table 1: Water physiochemical conditions for operation of PGT.

	Temperature	pH	Salinity (Conductivity)
Fresh: Trout	10-20 °C	6-9	0.005 – 0.05 S/m
Marine: Seabass	15-28 °C	6-9	25-37‰

For the manipulation of PGT products during the laboratory experiences at CSIC, aliquots of appropriate water samples sent by Smartwater partner were utilised:

- Fresh water from a commercial trout farm in Central Spain
- Seawater from a commercial seabass hatchery I Northern Spain
- Saltwater from the facilities of IZSE where the in vitro and in vivo tests will take place

2.2. PGT products and water conditions

LLPS is driven by networks of weak, multivalent, non-covalent interactions between conformationally dynamic molecules, therefore the environment conditions (temperature, pH, salinity) are key for

these interactions to take place. For example, the temperature dependence (or lack thereof) of interaction enthalpy and entropy in a given system can result in either an upper critical solution temperature (above which no phase separation occurs) or a lower critical solution temperature (below which no phase separation occurs).

Testing liquid-liquid phase separation of LCRs is purely empirical and depends on the composition of LCRs. This can be extrapolated to the chimeras, as the binder region can modify the LLPS properties of LCR, for which the determination of phase separation properties is also empirical.

- Regarding pH, the seawater sent by SW had a pH of 7.8. For the finally selected LCRs, phase separation occurs near neutral pHs (pH 6 to pH 8.5) in buffer with salt and buffer without salt respectively. LLPS of the chimeras is expected to be stable between pH 6 to pH 8.5, variations at more extreme pHs are likely to modify LLPS behaviour, as they may influence the conformation and net charge of both chimera components.

- The salinity is likely the most critical parameter for LLPS in the context of the PathoGelTrap project, because there are LCRs able to undergo LLPS in the presence of high salt concentrations while others are highly inhibited by it and the other way around. Therefore, since two different pathogens were selected for the PathoGelTrap project (one for saltwater and other for fresh water), the first months of the project were spent screening different LCRs, to obtain at least one capable of phase separate in each environment. The addition of the pathogen recognition motif forming the chimera (LCR-binder) also modifies its LLPS properties, undergoing phase separation under conditions not achieved with the LCRs alone. This phenomenon was demonstrated in deliverable 2.3.

- Regarding Temperature, it was observed that phase separation of selected chimeras occurs from 15 to 30^{o}C at a concentration of 10 µM without observing significant differences. Therefore, it was assumed that no differences in LLPS are expected in that range of temperatures. Regarding the binder, since it is obtained from the hyperthermophilic archeon Sulfolobus solfataricusis, it is expected that increases in temperature of the water will not have an effect on functionality.

2.2.3. PGT products filtration

PGT Liquid (Chimera DPB1-P7.C3 produced by GenScript; see D2.5) was tested during *in vivo* trial by IZSVE (see D2.6). Once the water was treated, several methods were tested to remove the chimera from the water before contact with the fish. To maximize the removal of the chimera in the water, IZSVE set up a trial using different molecular sieves (as suggested by CSIC):

1) Zeolite: Microporous, crystalline aluminosilicate materials commonly used as commercial adsorbents and catalysts.
2) Exclay: Expanded clay (exclay) is a lightweight aggregate made by heating clay to around 1,200 °C in a rotary kiln. The heating process causes gases trapped in the clay to expand, forming thousands of small bubbles and giving the material a porous structure.
3) Activated carbon: a form of carbon commonly used to filter contaminants from water and air, among many other uses. It is processed (activated) to have small, low-volume pores that increase the surface area.

4) Diatomaceous earth: a naturally occurring, soft, siliceous sedimentary rock that can be crumbled into a fine white to off-white powder, used as a filtration aid.

Each of these sieves were measured (a volume of about 150 ml each) and insert in a nylon net and put inside the biological filter, beside to sponge filtration compartment. Moreover, a combination of all 4 compounds mixed together were also prepared.

Then, a trial was set up to test the efficacy of those molecular sieves to remove the chimeras from water: 6 tanks were filled with 12 liters each of saltwater (25 ‰ of salinity), in which a certain quantity of PGT liquid was added to reach the final concentration of 0.5 µM. Five tanks was equipped singularly with one filter filled with one of the above mentioned compounds (included the mix of all). The sixth tank was equipped with a standard filter, without any molecular filtration compound, to act as control.

Then, the pumps were turned on and the filters were let working for 24 hours. The presence of chimera was then assessed. The results showed that traces of chimera were still present in all the treatments implemented. New treatments are currently under consideration and development to address this issue.

2.3. Sensors Platform

Due to changes in the live experiments setup, major adjustments had to be done to the design of the sensor platform.

To allow for replication and smaller volumes of water, a rack set up was prepared for the live experiments (figure 1 and 2), instead of the tank based setup conceived earlier.

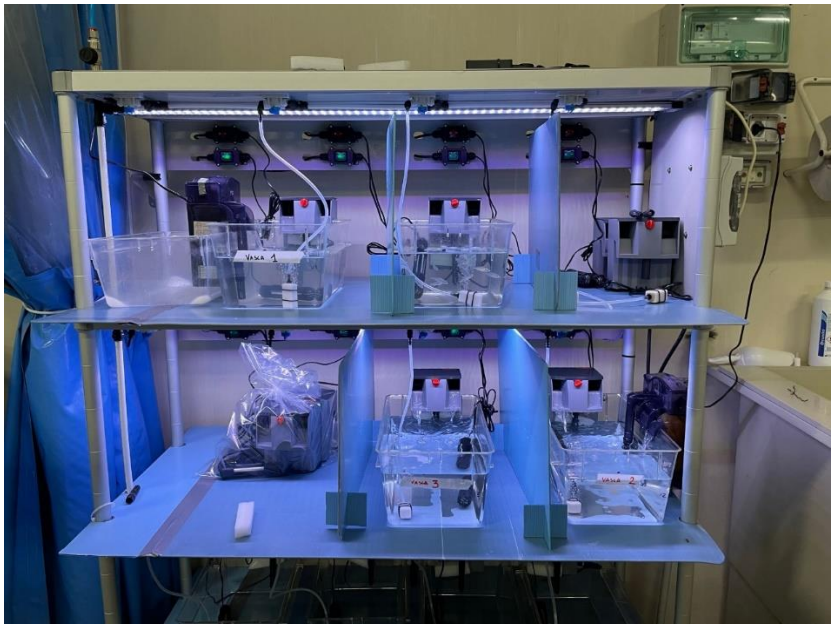

Figure 1: Experimental setup for the life experiments. Each individual tank was provided with a filter system.

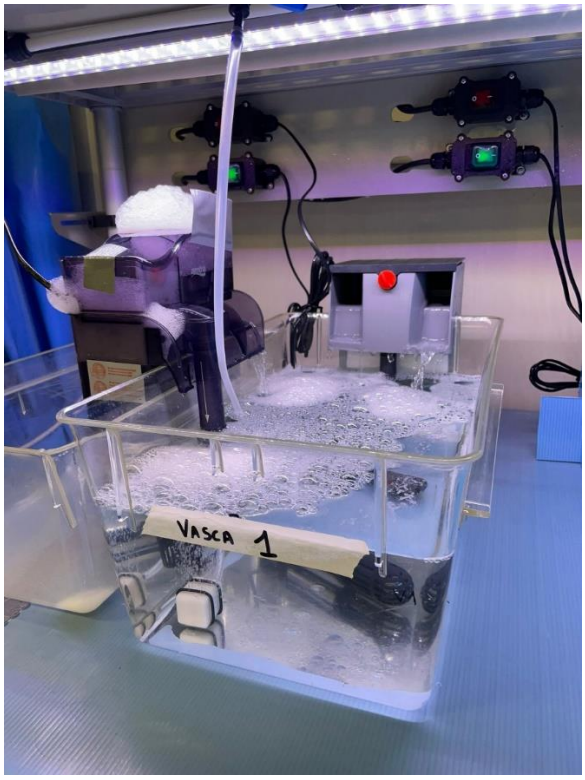

Figure 2: Filtering system for each individual tank, consisting of a protein skimmer, a solids filter and a biofilter.

The sensors platform Medusa was modified to fit in this arrangement, as there was a severe restriction of space (figure 3).

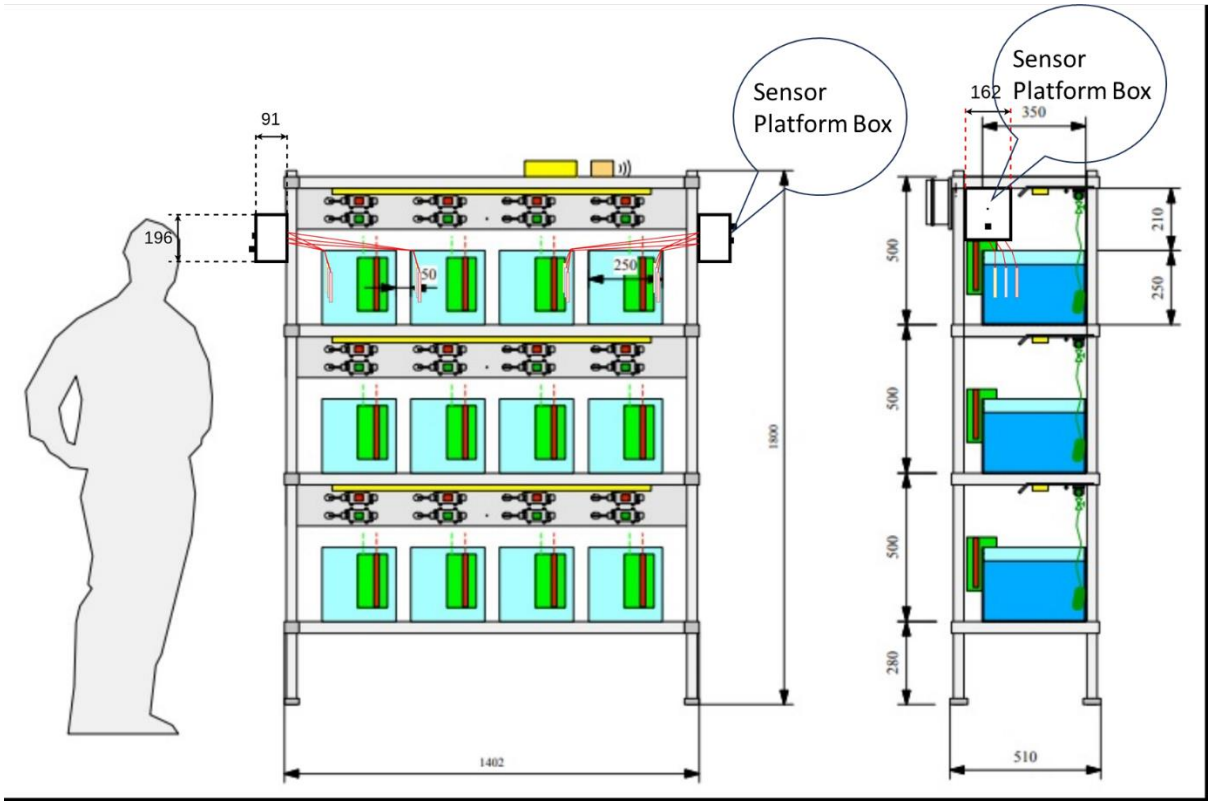

Figure 3: Schematic representation of the live experiments rack. Two sensor platform boxes should fit per rack level, to attend two tanks each with pH, DO and temperature sensors. Each box should contain all the services of the original sensor platform concept (sensors, data preprocessing, connectivity).

Individual boxes were designed, with pH, Dissolved Oxygen and Temperature sensors to monitor each tank (see figure 4 and 5). Each box monitored two tanks and had all the electronics to process sensors signals, wireless transmission of data, and energy management. Connectivity was adapted to the best connectivity available at IZSVE´s facilities.

Figure 4: Box sensor platforms for live experiment setup at IZSVE

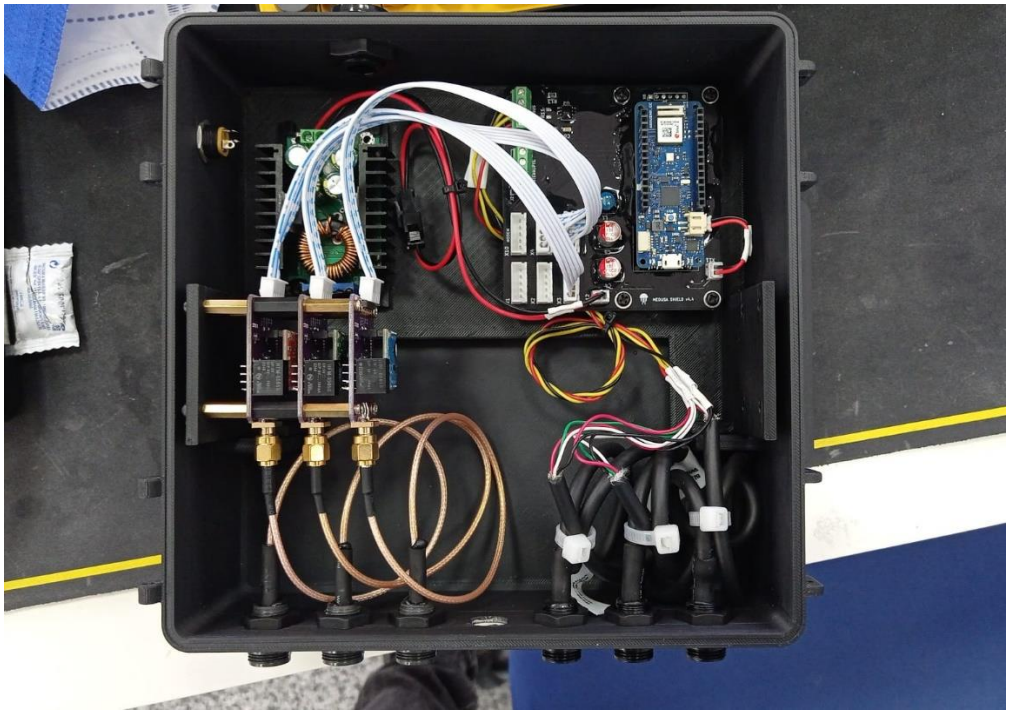

Figure 5: Internal electronics of the Box sensor platform.

3. Overall monitoring and evaluation of results

- Relevant water quality parameters have been used regarding the target pathogens, their epidemiology and disease dynamics and the culture conditions of the model affected fish species.
- Initial, standard commercial water quality operational parameters for the design of the PGT products were defined for the Project.
- Standard water samples from relevant sources were used for the design and manipulation of PGT products
- Development of the sensors platform Medusa is ready for pH, dissolved oxygen, and temperature were adapted to the new experimental setup to be utilised in water quality monitoring for *in vivo* experiments.

Manual measurement of the water quality parameters were taken at the start of the experiments (see table 2).

Table 2. Water parameters during in vivo trial. Parameters were recorded manually by specific probes.

Tank	pH	O2 mg/l	NH4+ mg/l	NO2- mg/l	NO3- mg/l	Salinity g/l	T °C
1	8.5	8.20	<0.1	0.3	3	25	25.5
2	8.4	8.43	<0.1	0.2	5	25	25.2
3	8.5	8.54	<0.1	0.2	3	25	24.8
4	8.5	8.23	<0.1	0.1	2	25	25.6

4. Conclusion

Water quality characterisation and monitoring is essential for the definition of water quality working parameters of the PGT products and for ensuring these parameters are followed during in vivo experiments, as well as for guaranteeing fish welfare during these experiments.

Water quality working conditions are specified in Table 3.

A real-time water quality monitoring device (Box Medusa) was tailored to the experimental setup and made available to the project by **SW**.

Methods for removing the chimera from the treated water were evaluated with different success. Further strategies are being developed for this purpose.