Intelligent Fish feeding through Integration of ENabling technologies and Circular principle

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Biology online and integration in feeding monitoring systems

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1 Introduction

WP1 aims to comprehensively and objectively test and qualify smart feeding approaches and feed formulations in controlled environments before further demonstrations in operational environments (WP3). WP1 will enable the iFishIENCi Biology Online Steering System (iBOSS) by qualifying state-of-the-art sensors enabling biology online (fish behaviour, growth, welfare, health & environmental microbiome), water quality monitoring to improve decision-making and smart feeding control. WP1 will qualify methods to obtain new and functional feed ingredients from algae, leading to the characterization of the biological responses to the new feeds by the fish, and its environment impact and implemented in breading for feed task. Furthermore, WP1 aims to design condition-based optimal valorisation processes for recirculation of carbon waste.

The online water quality monitoring system of physical and chemical parameters can be an indicator to detect shifts in the microbial diversity in the water to provide an early warning of environmental degradation and risk to fish health (implemented in SMART-RAS).
Relevant biological, physical and chemical data will be analyzed through Big Data analytics, together with measurements of e.g. microorganisms/OTUs identification and diversity, molecular biomarkers of welfare and health, to select the key parameters and biological variables to be implemented. The results of the technology testing and qualification will be implemented through dedicated trials.
Several operational improvements are actively being implemented and developed across the industry, including feeding technology and control to maximize feed utilization.

The state-of-the-art technology uses mainly environmental (oxygen and temperature) and fish behaviour information. Monitoring technologies differ depending on species and systems. There are several ways to feed fish using these monitoring tools and this depends on the species and production system. At sea, the use of cameras and echo-sounders (acoustic systems) are the most advanced technologies used today. On land, the most advanced feeding systems use feeding algorithms based on actual water parameter measurements and biomass estimation based on tables. Here cameras and echo-sounder are also used (http://www.aquabyte.ai), but these often do not give advantages with relation to the investment needed.

Stimulated appetite, feed intake and satiety are also linked to feeding behaviour. As of today, there are no models that integrate the physiology of feeding with changes in behaviour in aquaculture systems. However, improvements in sensor accuracy and hardware and software processing speed have promoted the development of new technologies and methods, providing effective or potential support for intelligent feeding control (Zhou et al 2017). However, accuracy and intelligence need to be improved to meet the needs of actual feeding scenarios.

This report outlines the biology components (Biology 4.0, Fish-Talk-To-Me) of the iFishIENCi Biology Online Steering System (iBOSS) developed by establishing which parameters can be measured and are the most relevant for implementation into iBOSS. The capability of *emerging technology* for online biological monitoring (both physiology and behaviour), including the application of the sentinel fish concept (innovative fish tagging) to enable individual-based continuous measurements of location, behavior, environment and physiology will significantly improve monitoring precision.

2 Pilot summary and Monitoring parameters

2.1 Aller Aqua Research Gmbh

At Aller Aqua Research continuous development of existing and new feed types and the evaluation of raw materials and functional additives for fish nutrition is our day-to-day business. By a dedicated team, ingredients and feed formulations for different species (trout, salmon, carp, tilapia, catfish etc.) are evaluated. Modern RAS are used to simulate different environmental conditions. We do our best to offer fish farmers affordable feeds which allow for fast growth of the fish via efficient feed conversion.

Hereafter is listed all the parameters that can be monitored and Aller Aqua specific needs for their experiments.

Table 1 - AAR specific parameters

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Chemical ENVIRONMENT	ammoniac						No				
	ammonium	1-3 / week	daily	< 1 mg/L	fish, feed, water ex. rate, pH		Yes	manual	mg/L		
	Calcium						No				
	Carbon dioxide	1-3 / week	daily	< 10 mg/L	fish, feed, water exchange rate, pH		Yes	manual	mg/L		
	Chlorophyll						No				
	Chlorures						No				
	CDOM						No				
	Hydrogen Sulphid						No				
	Disolved oxygen	continuously	Continuously	>7 mg/L	fish, feed, water exchange rate, temp		Yes	auto	mg/L		
	Fluorescein						No				
	fluorides						No				
	nitrate	1-3 / week	daily	200 mg/L	fish, feed, water exchange rate, pH, biofilter		Yes	manual	mg/L		

Not relevant parameter | May parameter | Must/should parameter

Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
nitrite	1-3 / week	continuously	5 mg/L	fish, feed, water exchange rate, pH, biofilter		Yes	manual	mg/L		
pH	continuously	continuously	6 to 8	fish, feed, water exchange rate, biofilter		Yes	auto	-		
Phycoerythrin						No				
redox potential	continuously	continuously	< 350 mV	fish, feed, water exchange rate, pH, biofilter		Yes	auto	mV		
Rhodamin						No				
Salinity						Yes				
Temperature	continuously	continously	25-30			Yes	auto	°C		
Ozone						No				
Turbidity						Yes	manual			
Water Velocity						No				

Not relevant parameter	May parameter	Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Physical environment	Activity around tanks						No				
	Light	12/12H					Yes	manual	hours		
	Noise						No				
	Water level						No				
	Air humidity						No				
	Air temperature						No				
	Air O2						No				
	Air CO2						No				
FISH	Activity / acceleration						No				
	Heart rate						No				
	depth						No				
	Cavity Temperature						No				
	Direct env. Temp						No				
	Appetite	daily					Yes	manual	DFI		
	Satiety						No				
	Biomass	4-8 weeks	4-8 weeks		Water param, feed intake, feed conversion		Yes	manual	kg		
	number of fish	4-8 weeks	4-8 weeks				Yes	manual	n		
	average weight	4-8 weeks	4-8 weeks		Water param, feed intake, feed conversion		Yes	manual	g		

Not relevant parameter | May parameter | Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Feed parameters	uneaten	daily	daily		palatability		Yes	manual	g		
	stomach filling						No				
	Midgut filling						No				
Microbiome	Water	after 1st and last week					No				
	Sludge	after 1st and last week					No				
	Feces	after 1st and last week					No				
	Gut	after 1st and last week					No				
	Gills	after 1st and last week					No	-	-		
	Skin	after 1st and last week					No	-	-		
	Blood (depending)						No				

Not relevant parameter | May parameter | Must/should parameter

2.2 Aquabiotech Group

Aquabiotech uses its own brand of recirculation aquaculture technology (**ExperiRAS**™) to deliver aquaculture nutrition, technology and vaccine research, development and testing services. Trials are conducted in fully licensed facilities, consisting of forty-four (44) individual trial systems contained within thirty-one (31) biosecure trial rooms. Nutritional, chemical and ecotoxicological analysis of samples takes place in Good Laboratory Practice (GLP) certified laboratories.

A new recirculating aquaculture system is being built specifically for the iFishIENCi project. The system will be used to generate data that will contribute to the development of iFishIENCi technological innovations, and it will serve as a system for testing and demonstrating these new technologies. As with all ABT inhouse facilities, the iFishIENCi recirculation system is being equipped with an Oxyguard Pacific monitoring system. It consists of two rows of 6 tanks (12 in total), each being 1.5 m^3 in volume. The system is also being fitted with two state-of-the-art UV filters (constructed from polypropylene to prevent corrosion resulting from contact with seawater), two oxygen saturation cones, an ozonation cone for disinfection and flocculation if fine solids, a protein skimmer and degassing unit, and two reversible air-to-water heat pumps for heat exchange. Light is being provided by an LED system, and cameras are being installed for the monitoring of fish behaviour. Each individual tank will be fitted with a fish faeces collector, the faeces thus becoming available for subsequent analysis. Each tank is also to be fitted with an automatic feeder.

Hereafter is listed all the parameters that can be monitored and Aquabiotech specific needs for their experiments.

Table 2 - ABT specific parameters

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Chemical ENVIRONMENT	ammoniac						No				
	ammonium	every 12 hours	every hour	<1 mg/L	feed, fish, water quality, turnover	fish feeding, biofilter	Yes	manual	mg/L	0-5	
	Calcium						No				
	Carbon dioxide	continuously	continuously	<10 mg/L	feed, fish, waterquality, turnover	fish feeding, biofilter, pH, alkalinity	No				
	Chlorophyll						No				
	Chlorures						No				
	CDOM						No				
	Hydrogen Sulphid	every 12 hours	every hour				No				
	Disolved oxygen	continuously	continuously	>70%	feed, fish, water quality, turnover	fish feeding, biofilter	Yes	auto	mg/L and %	0-200	
	Fluorescein						No				
	Fluorides						No				
	nitrate	every 12 hours	every hour	<250 mg/L	feed, fish, waterquality, turnover	fish feeding, biofilter	Yes	manual	mg/L	0-1000	

Not relevant parameter	May parameter	Must/should parameter

Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
nitrite	every 12 hours	every hour	<0.05 mg/L	feed, fish, water quality, turnover	fish feeding, biofilter	Yes	manual	mg/L	0-1	
pH	continuously	continuously	6 to 8	alkalinity, CO2	fish feeding, biofilter, CO2, alkalinity	Yes	auto	-		
Phycoerythrin						No				
redox potential	continuously	continuously	<350mV	pH, water chemistry, water quality, ozone	fish feeding, biofilter	Yes	auto	mV	-700; +700	
Rhodamin						No				
Salinity						Yes	manual	ppt	0-40	
Temperature	continuously	continuously	25-30	ambient temperature, feed, turnover	fish feeding, biofilter, oxygen	Yes	auto	°C	0-40	
Ozone	continuously	continuously		BOD/COD	fish feeding, biofilter	No				
Turbidity						No				
Water velocity						No				

Not relevant parameter	May parameter	Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Physical environment	Activity around cages						No				
	Light						Yes				
	Noise						No				
	Water level						No				
	Air humidity						No				
	Air temperature						No				
	Air O2						No				
	Air CO2						No				
FISH	Activity / acceleration						No				
	Heart rate						No				
	depth						No				
	Cavity Temperature						No				
	Direct env. Temp.						No				
	Appetite	every feeding event	every hour				Yes	manual			
	Satiety	every feeding event	every hour				Yes	manual			
	Biomass	every week	every week				Yes	manual			
	number of fish	every 24 hours	every 24 hours				Yes	manual			
	average weight	every week	every week				Yes	manual			

Not relevant parameter	May parameter	Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Feed parameters	initial amount	every feeding event	every feeding event				Yes	manual			
	uneaten	every feeding event	every hour				Yes	manual			
	stomach filling						No				
	Midgut filling						No				
Microbiome	Water						No				
	Sludge						No				
	Feces						No				
	Gut						No				
	Gills						No	-	-		
	Skin						No	-	-		
	Blood (depending)						No				

Not relevant parameter | May parameter | Must/should parameter

2.3 Hellenic Center of Marine Research

The HCMR facilities comprise of a marine RAS and the pilot scale farm at Souda Bay

Regarding the RAS, three independent thermoregulated systems exists, each comprising of 3 cylindroconical tanks ($2m^3$) connected to a biological ($1m^3$) and a mechanical (drum) filter. Each tan is equipped with an LDO process sensor (Hach) placed 10cm below water surface that records dissolved oxygen and temperature continuously. The data are transferred in real time to a controller for display and storage. When the system is in operation, temperature and oxygen are monitored continuously, and other parameters are measured manually on a periodical basis. Nitrogenous waste (Total Ammonia Nitrogen, nitrite, nitrate) is measured on a weekly basis via spectrophotometric methods and pH is measured twice a week with a handheld probe. Furthermore, one of the tanks is equipped with the Bioceanor water quality monitoring system which records oxygen, temperature, pH, salinity and at 30min intervals throughout the day. Finally, behaviour is monitored by cameras placed above the tanks that record at specified intervals.

Two thermal trials have been conducted between Apr 2019 and Feb 2020 for European seabass and greater amberjack respectively investigating the effects of three experimental temperatures. Each trial lasted 3 months and the effects of temperature were assessed via monthly samplings on a number of husbandries, physiological, metabolic and behavioral variables. Furthermore, water, sludge, swab and tissue samples were collected on a monthly basis in order to initiate microbiome analysis in coordination with Leitat. The physicochemical parameters listed above were recorded throughout the trials, although the Bioceanor water quality monitoring system was only implemented in the case of the g. amberjack trial.

The net-pen cage unit of the Institute is located in Souda Bay at the Northwest coast of Crete. Currently the unit consists of 16 cages 6x6m (each with the possibility to form 4 smaller of dimensions 3x3m). In addition, there are 4 circular cages with diameter of 12 m. The operation of the unit is served by a floating platform and a storage hut. Part of the energy requirements are covered by photovoltaic cells and a wind turbine. Several species (E. seabass, g. sea bream, meagre, and greater amberjack) are reared in the unit. The environmental parameters (T, DO, pH) are monitored on a daily base manually (at 3 depths 0, 4 and 8m) while a current profiler is permanently installed providing the general water circulation of the area (data recovered every few months). In the farm 4 echosounders are installed and used to monitor the vertical distribution of targeted fish groups. Additionally, two cameras are used for online observation of fish groups.

A trial with European seabass as model species will start in the next period.

Hereafter is listed all the parameters that can be monitored and HCMR specific needs for their experiments.

Table 3 - HCMR specific parameters

CAGES EXPERIMENT

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Chemical ENVIRONMENT	ammoniac						No				
	ammonium	weekly	daily		feeding/ environment		No				
	Calcium						No				
	Carbon dioxide		daily		environment		No				
	Chlorophyll						No				
	Chlorures						No				
	CDOM						No				
	Hydrogen Sulphid						No				
	Disolved oxygen	continuously	continuously		feeding/ environment		Yes	Manual	mg/l	5-8.	0,1
	Fluorescein						No				
	fluorides						No				
	nitrate	weekly	daily		feeding/ environment		No				
	nitrite	weekly	daily		feeding/ environment		No				
	pH	daily	continuously		environment		Yes	Manual			0,01
	Phycoerythrin						No				
	redox potential						No				
	Rhodamin						No				
	Salinity	weekly	daily		rainfall/rivers		Yes	Manual	psu	28-40	1
	Temperature	continuously	continuously		environment		Yes	Auto	oC	14-35	0,1

Not relevant parameter | May parameter | Must/should parameter

	Ozone						No				
	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
	Turbidity				feeding/ environment		No				
	Water velocity				environment		No				
Physical environmental	Activity around cages						No				
	Light	daily	hourly		environment		Yes	Manual	lux	0-10000	1
	Noise						No				
	Waterlevel						No		-		
	Air humidity						No				
	Air temperature						No				
	Air O2						No				
	Air CO2						No				
FISH	Activity / acceleration	continuously			fish status		Yes	camera			
	Heart rate						No				
	depth	continuously			fish status		Yes	echo sounder			
	Cavity Temperature						No				
	Direct env. temperature						No				
	Appetite	daily			fish status		Yes	Manual			
	Satiety				fish status		Yes	Manual			
	Biomass	monthly					Yes	Manual	Kg		0.1g
	number of fish	daily					Yes	Manual	-		

Not relevant parameter | May parameter | Must/should parameter

	average weight	monthly					Yes	Manual	Kg	0.05-0.5Kg	0.1g
	Parameters	**Frequency Min**	**Frequency Ideal**	**Control levels**	**Influenced by**	**Influence**	**Already measured (YES/NO)**	**Sampling method (auto/manual)**	**Unit**	**Range**	**Sensitivity**
Feed parameters	initial amount	daily					Yes	Manual	Kg		
	uneaten	daily					Yes	Manual	Kg		0.1g
	stomach filling						No				
	Midgut filling						No				
Microbiome	Water		monthly				Yes	-	-		
	Sludge						No				
	Feces		monthly				No	-	-		
	Gut		monthly				No	-	-		
	Gills		monthly				No	-	-		
	Skin		monthly				No	-	-		
	Blood (depending)						No				

Not relevant parameter | May parameter | Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	control levels	Influenced by	Influence	Already measured	Sampling method (auto/ manual)	Unit	Range	Sensitivity
Chemical ENVIRONMENT	ammoniac						No				
	ammonium	weekly	daily	<0.5	feed, feeding, fish		Yes	Manual	mg/l	0-0.4	0,01
	Calcium						No				
	Carbon dioxide		daily				No				
	Chlorophyll						No				
	Chlorures						No				
	CDOM						No				
	Hydrogen Sulphid						No				
	Disolved oxygen	continuously	continuously	>5	temperature, fish		Yes	Auto	mg/l	08-05	0,1
	Fluorescein						No				
	fluorides						No				
	nitrate	weekly	daily	<100	feed, feeding, fish		Yes	Manual	mg/l	0-20	0,1
	nitrite	weekly	daily	0	feed, feeding, fish		Yes	Manual	mg/l	0-1	0,001
	pH	daily	continuously		water quality		Yes	Manual	-	7-8.1	0,01
	Phycoerythrin						No				
	redox potential						No				
	Rhodamin						No				

Not relevant parameter | May parameter | Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	control levels	Influenced by	Influence	Already measured	Sampling method (auto/ manual)	Unit	Range	Sensitivity
	Salinity	weekly	daily	35			Yes	Manual	psu	28-40	1
	Temperature	continuously	continuously			oxygen	Yes	Auto	oC	14-35	0,1
	Ozone						No				
	Turbidity						No				
	Water velocity						No				
Physical environmental	Activity around tanks						No				
	Light						Yes	Manual	lux	0-1000	1
	Noise						No				
	Water level						Yes	Manual	-		
	Air humidity						No				
	Air temperature						No				
	Air O2						No				
	Air CO2						No				
FISH	Activity / acceleration						No				
	Heart rate						No				
	depth						No				
	Cavity Temperature						No				
	Direct env. Temperature						No				
	Appetite	daily	daily				Yes	Manual			

Not relevant parameter | May parameter | Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	control levels	Influenced by	Influence	Already measured	Sampling method (auto/ manual)	Unit	Range	Sensitivity
	Satiety	daily	daily				Yes	Manual			
	Biomass	monthly	daily				Yes	Manual	Kg	0-10	0.1g
	number of fish	daily	daily				Yes	Manual	-	depend on the size	
	average weight	monthly	daily				Yes	Manual	Kg	0.05-0.5Kg	0.1g
Feed parameter	initial amount	daily	daily				Yes	Manual	Kg		
	uneaten	daily	daily				Yes	Manual	Kg		0.1g
	stomach filling						No				
	Midgut filling						No				
Microbiome	Water	monthly			water recircularity, fish		Yes	Manual	-	-	-
	Sludge	monthly					Yes	Manual	-	-	-
	Feces						No				
	Gut	monthly					Yes	Manual	-	-	-
	Gills	monthly					Yes	Manual	-	-	-
	Skin	monthly					Yes	Manual	-	-	-
	Blood (depending)						No				

Not relevant parameter | May parameter | Must/should parameter

2.4 Szent Istvan University

The Department of aquaculture of Szent István University operates small scale freshwater inhouse RAS systems. In iFishIENCi, one system is used for African catfish selection and generating data to contribute to the development of iFishIENCi technologies and innovations.

The system consists of a $3m^3$ thermoregulated, quadratic tank, which is separated into three equal parts with internal walls for separated rearing of fish groups. The tank is connected with a bead filter and a water reservoir tank ($1m^3$), and is equipped with WTW sensor for dissolved oxygen, temperature and pH monitoring and there are separate probs for the refreshing water flow, the water recycling flow. All the probes are connected to a tailor-made PLC based control/alarm system. The temperature of the circulated water, the circulation rate, and the water change rate can be regulated. There are aerators (atomizer) for each section of the tank. Light is provided by a LED system. The photoperiod and the light intensity are also adjustable at the facility. The temperature, the flow rate and the oxygen level are monitored continuously. Some other parameters (nitrite, nitrate and ammonia) are measured manually on a periodical basis. The feeding is performed manually.

Hereafter is listed all the parameters that can be monitored and SZIU specific needs for their experiments.

Table 4 – SZIU specific parameters

	Parameters	Frequency Min	Frequency Ideal	control levels	Influenced by	Influence	Already measured	Sampling method (auto/ manual)	Unit	Range	Sensitivity
Chemical ENVIRONMENT	ammoniac						No				
	ammonium	weekly	weekly		PH, feed, feeding, fish		Yes	Manual	mg/l	0.1-10	0,01
	Calcium						No				
	Carbon dioxide						No				
	Chlorophyll						No				
	Chlorures						No				
	CDOM						No				
	Hydrogen Sulphid						No				
	Dissolved oxygen	per minute	per minute		Temp, fish		Yes	Auto	mg/l	0-12	0,01
	Fluorescein						No				
	fluorides						No				
	nitrate	per minute	per minute		feed, feeding, fish		Yes	Manual	mg/l	0.01-150	0,01
	nitrite	per minute	per minute		feed, feeding, fish		Yes	Manual	mg/l	0.1-3	0,1
	pH	per minute	per minute		water quality		Yes	auto	-	1-14	0,1
	Phycoerythrin						No				
	redox potential						No				
	Rhodamin						No				

Not relevant parameter | May parameter | Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	control levels	Influenced by	Influence	Already measured	Sampling method (auto/ manual)	Unit	Range	Sensitivity
	Salinity						No				
	Temperature	per minute	per minute			oxygen	Yes	Auto	°C	0-30	0,1
	Ozone						No				
	Turbidity						No				
	Water velocity	per minute	per minute				Yes	Auto	l/h	100-2500	1
Physical environmental	Activity around tanks						No				
	Light	per minute	per minute				Yes	auto		light/ dark	
	Noise						No				
	Water level						Yes	auto	mm	0-1500	1
	Air humidity						No				
	Air temperature						No				
	Air O2						No				
	Air CO2						No				
FISH	Activity / acceleration						No				
	Heart rate	per minute	continuously				No				
	depth						No				
	Cavity Temperature						No				
	Direct env. Temperature						No				
	Appetite	daily	daily				No				

Not relevant parameter | May parameter | Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	control levels	Influenced by	Influence	Already measured	Sampling method (auto/ manual)	Unit	Range	Sensitivity
	Satiety						No				
	Biomass	depend on tech. steps	every 2 weeks				Yes	Manual	g	0-100kg/n	1
	number of fish	depend on tech. steps	every 2 weeks				Yes	Manual	pc	0-1000	1
	average weight	depend on tech. steps	every 2 weeks				Yes	Manual	g	0-3500	1
Feed parameters	initial amount	daily	daily		oxygen, nitrite, nitrate, ammonium		Yes	Manual	g	0-2000	1
	uneaten	daily	after feeding				No				
	stomach filling						No				
	Midgut filling						No				
Microbiome	Water	monthly			water recircularity, fish		No				
	Sludge	monthly					No				
	Feces						No				
	Gut	monthly					No				
	Gills	monthly					No				
	Skin	monthly					No				
	Blood (depending)						No				

Not relevant parameter | May parameter | Must/should parameter

2.5 Vitafort

There has been traditional cooperation in the field of agriculture and aquaculture development between Laos and Hungary. Vitafort, the market leader in the supply of domestic animal feed, implements the third tied aid loan program in the Lao PDR through its subsidiary since 2008. As a result of the long term, good human and professional relationship and the many years of continuous cooperation the organization of tilapia feeding experiments has begun at the Namxuang Aquaculture and Fisheries Development Center.

The Namxuang Aquaculture and Fisheries Development Center belongs directly under the Department of Livestock and Fisheries of the Ministry of Agriculture and Fisheries. According to the founding regulation, it contributes to the development of fisheries with the following activities:

- Providing basic laboratory services for fish farmers (fish nutrition, water quality);
- Organizing professional training programs (primarily practical programs);
- Elaborating and transfer of Good Pond Management Practices to the fish farmers,
- Producing high-quality fingerlings and management of the related breeding stock.

The total area of the Center is 14,5 ha. Besides the laboratories fish hatchery, covered nursing units, and fingerling rearing ponds (34 pcs 600m^2 ponds) are available. The staff consists of 20 persons.

Nasaythong district (where the Center is located) is a very important agricultural and fish production region. 50% of the fish supply consumed in Vientiane Capital is originated from here. The main produced fish species are tilapia, silver barb, carp, and the mrigal.

However, it should be noted, that the Center is only able to perform the above-listed task limitedly, as more funding and human resource development are necessary to secure its permanent operation and reach its full potential.

Hereafter is listed all the parameters that can be monitored and SZIU specific needs for their experiments.

Table 5 - VF specific parameters

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Chemical ENVIRONMENT	ammoniac						No				
	ammonium	Weekly			pH, feed, feeding, fish, water change rate		No				
	Calcium						No				
	Carbon dioxide	Weekly			pH, feed, feeding, fish, water change rate		No				
	Chlorophyll						No				
	Chlorures						No				
	CDOM						No				
	Hydrogen Sulphid						No				
	Dissolved oxygen	Daily					No				
	Fluorescein						No				
	fluorides						No				
	nitrate	Weekly			pH, feed, feeding, fish, water change rate		No				

Not relevant parameter	May parameter	Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
	nitrite	Weekly			pH, feed, feeding, fish, water change rate		No				
	pH	weekly					Yes	auto	-		
	Phycoerythrin						No				
	Redox potential						No				
	Rhodamin						No				
	Salinity										
	Temperature					Oxygen	Yes	auto	°C		
	Ozone						No				
	Turbidity						Yes	manual	m		
	Water velocity						No				
Physical environment	Activity around tanks						No				
	Light										
	Noise										
	Water level						No				
	Air humidity						No				
	Air temperature						No				
	Air O2						No				
	Air CO2						No				
FISH	Activity / acceleration						No				
	Heart rate						No				
	depth						Yes	manual	m		

Not relevant parameter | May parameter | Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
	Cavity Temperature						No				
	Direct env. Temperature						No				
	Appetite	daily					Yes	manual			
	Satiety						No				
	Biomass	weekly					Yes	manual	kg		
	number of fish	2 weeks					Yes	manual	db		
	average weight	2 weeks					Yes	manual	g/kg		
Feed parameters	initial amount	initial			oxygen level, nitrite, nitrate, ammonium		Yes	manual	g/kg		
	uneaten	daily					Yes	manual	g/kg		
	stomach filling						No				
	Midgut filling						No				
Microbiome	Water						No				
	Sludge						No				
	Feces						No				
	Gut						No				
	Gills						No	-	-		
	Skin						No	-	-		
	Blood (depending)						No				

Not relevant parameter | May parameter | Must/should parameter

2.6 Gyori Elore

Győri "Előre" Fisheries Co-operative is a fish producer and processor company. They have freshwater thanks for fish stocking and rearing. A new flow-through system has been built specifically for carrying out the tasks related to African catfish in the iFishIENCi project.

The system contains 12 2m^3 thanks in one raw. These tanks are not equipped with probes. They are supplied with thermal well water. The water temperature is monitored and regulated manually in the separated tanks. The system requires manual feeding. The ammonium level, the number of the fish, the Biomass and the average weights are also controlled manually according to the industrial production technology. Besides these, they have fourteen 200 m^3 concrete tanks and two 30m^3 circle tanks for production. These tanks are equipped with temperature and oxygen probes which are connected to a control system. Furthermore, they have ten 3 m^3 and eleven 1 m^3 circle tanks for rearing smaller groups, and six 50m^3 tanks for stocking the market-sized fish. All the tanks are supplied with thermal well water.

The water temperature and oxygen level are monitored (except the tanks fitted with probes) and regulated manually. The systems require manual feeding (except of larvae rearing). The ammonium level, the number of the fish, the Biomass and the average weights are also controlled manually according to the industrial production technology. All the systems can be used to generate data that contribute to development of technologies and innovations in the iFishIENCi, and some of these systems will be used for testing and demonstrating these new technologies.

Hereafter is listed all the parameters that can be monitored and GYE specific needs for their experiments.

Table 6 - Gyori Elore specific parameters

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Chemical ENVIRONMENT	ammoniac						No				
	ammonium	weekly	weekly		PH, feed		No				
	Calcium						No				
	Carbon dioxide						No				
	Chlorophyll						No				
	Chlorures						No				
	CDOM						No				
	Hydrogen Sulphid						No				
	Dissolved oxygen	per min.	per min.		Feed,feeding		No				
	Fluorescein						No				
	fluorides						No				
	nitrate	per min.	per min.		PH, feed		No				
	nitrite	per min.	per min.		PH, feed		No				
	pH	per min.	per min.		Feed,feeding		No				
	Phycoerythrin						No				
	redox potential	per min.	per min.				No				
	Rhodamin						No				
	Salinity						No				
	Temperature	per min.	per min.			oxygen	Yes	manual	°C	0-30	0,1
	Ozone						No				
	Turbidity						No				
	Water velocity	per min.	per min.				No				

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Physical environment	Activity around cages						No				
	Light	per min.	per min.				No				
	Noise						No				
	Water level	per min.	per min.				No				
	Air humidity						No				
	Air temperature						No				
	Air O2						No				
	Air CO2						No				
FISH	Activity / acceleration						No				
	Heart rate	per min.	continuously				No				
	depth						No				
	Cavity Temperature						No				
	Direct env. Temperature						No				
	Appetite						No				
	Satiety						No				
	Biomass	depending on technical steps	every 2 weeks				Yes	manual	kg/m3	0-200	1
	number of fish	depending on technical steps	every 2 weeks				Yes	manual	pc	0-1500	1

Not relevant parameter | May parameter | Must/should parameter

	Parameters	Frequency Min	Frequency Ideal	Control levels	Influenced by	Influence	Already measured (YES/NO)	Sampling method (auto/manual)	Unit	Range	Sensitivity
Feed parameters	initial amount	1-3 times daily	1-3 times daily		Oxygen, nitrite, nitrate, ammonium		Yes	manual	g	0-2000	1
	uneaten	daily	after feeding				No				
	stomach filling						No				
	Midgut filling						No				
Microbiome	Water	after 1st and last week			PH, feed, fish		No				
	Sludge	after 1st and last week					No				
	Feces	after 1st and last week					No				
	Gut	after 1st and last week					No				
	Gills	after 1st and last week					No	-	-		
	Skin	after 1st and last week					No	-	-		

	Blood (depending)						No				

Not relevant parameter	May parameter	Must/should parameter

3 Technology identified

3.1 Fish-Talk-To-Me

Fish-Talk-To-Me will enable continuous monitoring of fish behaviour, growth, health, physiology and welfare, through the integration of technologies and data processing. This will allow more accurate understanding of the fish state and subsequent assessment and management of the production. Fish-Talk-to-Me will encompasses (1) fish tagging technology for continuous gathering of individual fish movement and physiological data, (2) camera and/or echosounder technology for automatic assessment of fish behaviour in the production system, and (3) digital twin of fish digestion efficiency and appetite (FishMet). The relevant parameters are divided into 4 categories: 1) Environment, 2) Fish, 3) Fish Behaviour, and 4) Fish physiology. Taken alone, these parameters do not provide so much information about the fish state, but in combinations with the understanding of the relations to one another, enhanced insight can be obtained. The following are examples of how these variables can be used to understand the fish state.

Environment

- **Temperature:** The water temperature influences many aspects of the fish biology such as growth, metabolism, feeding requirements, stress
- **Light:** Light is a major regulator of fish biology both in development but also diurnal activities and behaviours. Light can have impacts on growth, behaviour (location: depth, shadow, usage of the area), feeding, schooling, stress. Aspects of light important to measure are lights on/off (with or without twilight and dusk period), light quality (intensity and spectrum), at different depths)
- **Oxygen**: Maintaining oxygen levels at appropriate levels is critical and that these are measured at the inlet and outlet and in large systems could be important to have at different depths.
- **Redox potential (ORP) :** is an indicator of fish stress and can influence fish growth as well as to indicate residual ozone in the water in RAS.
- **CO_2**: High levels of CO_2 are toxic and need to be measured so as not to accumulate, especially in RAS systems. CO_2 levels can impact the behavior, physiology, robustness and survival of the fish.
- **pH**: Impacts the physiology, water chemistry and biofilter
- **Salinity**: Impacts the physiology and water chemistry
- **Turbidity**: Will reduce visibility and can impact feeding, An indicator of water quality/filter issues
- **Feeding (application of feed to fish)**: measure timing, duration, amount that is important to correlate to fish behavior and movement as indicators when to start and stop feeding, but also when something is not right and fish are not eating as they should. Initial amount of feed is the quantity of feed that has been given to the fish. It is used to calculate the amount of feed that has been eaten.
- **Waste (pellet loss, feces)**: An important indicator to determine amount of feeding to reduce feed loss.
- **Tank cleaning:** Disturbances in tanks or cages may disrupt normal behavior and delay when fish feed.

- **Noise (external noise):** Noise will impact the fish movements and stress levels, so important to have this information to be able to explain unusual behavior.
- **Water current:** Knowing the current is important for correlating to behavior and growth, as well as water exchange in the system.

<u>Fish</u>

- **Length and weight:** Providing real-time changes in growth of the fish
- **Biomass:** Important to adjust feeding as well as maintain the legal densities in the tank or cage.
- **Size distribution**: Different feeding or rearing conditions can impact the population size distribution (which is not desired), so monitoring this can help to adjust the system to reduce this effect.
- **Coloration:** Coloration of the fish in some species can give important information as to chronic stress or illness or reproductive state.
- **Sores and deformities:** Identification and quantification of sores and fin erosion will give important indicators of health status of the fish.

<u>Fish Behavior</u>

- **Swimming activity:** Description of the normal activity as speed, distance, acceleration will help identify when abnormal situations arise.
- **Active feeding:** Measuring how the fish feed (e.g. duration, intensity, relationship to feed application) is important to adjust feeding level and to determine if the fish are stressed. It includes measuring uneaten feed, to understand the quantity of feed that has been ingested.
- **Aggression:** Identifying aggression is often a sign that the feeding or environment is off and should be adjusted. Here one should measure of number of bites or attacks towards other fish. Fin erosion is another indicator of this.
- **Schooling/ normal behavior- species and age dependent:** Measure normal behavior will give the background to determine when the fish are disturbed. Linking to the environmental parameters will help to identify the source.

<u>Fish Physiology</u>

- **Heart beat:** Increased heart beat can be an indicator of increased activity or stress
- **Opercular movement:** Increased opercular movement is a sign of increased oxygen demand and stress of activity.
- **Temperature:** Internal temperature can give knowledge to what temperature gradient the individual fish are staying in under certain normal conditions and can indicate for example infection if the start to remain in slightly warmer water (behavioral fever).

The sentinel fish is part of Fish-talk-to-me where tags are used to get individual fish information on location, physiology, behaviour, where cameras and sonar give population information. So, all tag information is sentinel fish information that can be used to have a more precise understanding of what the individuals in the population are doing that cannot be detected by population movements from cameras, but also direct stress information.

3.1.1 Different species behaviour

Fish behaviour is a very important parameter to analyze, that can give information on fish stress or appetite for example. Even if fish can have similar behaviour amongst species, some specificities to each species is to be observed in different experiments.

- **Rainbow trout**

Trials are to take place at ABT RAS facilities. The aims of the experiments are:

(1) To track passage of feed through the gastrointestinal tract and the relative fullness of the stomach and intestine, in order to understand how this correlates with the onset of satiation.
(2) To observe and quantify fish behaviour prior to, during, and post feeding, with the objective of identifying behavioural cues indicative of satiation (comparing behaviour with stomach fullness.

The proposed methodology will be tested in pre-trails, prior to implementation of the main experiments.

Stomach fullness and passage of feed through the gastrointestinal tract will be traced using ballotini beads (glass balls), serving as inert markers. Following feeding events, individuals will be removed from the tanks and anaesthetised, and an x-ray image will be taken of the stomach contents of sampled fish. Feed from separate feeding events will be distinguished by differently sized ballotini beads (contingent on results of pre-trials).

Fish behaviour will be recorded using video. One camera will be positioned above each cultivation tank. Horizontal movements within 2-dimensional space will be measured with across y and x axes. Two types of movement will be observed: (1) swimming speed (body lengths / second); (2) left-right movements. Swimming speed is to be calculated as the change in an individual's position between successive frames. Fish will be tagged to facilitate identification and observation of individuals.

- **Asian seabass/Barramundi**

Barramundi will be cultivated in the ABT iFishIENCi demonstration recirculation aquaculture system. Theey will be grown from an initial weight of 50 g to final weight of 200 g. Each cultivation tank has a volume of 600 L and will be stocked with 100 fish. They will be used in feeding experiments, in which fish being fed to four different levels of satiation (= 100% satiation, 80% of satiation, 90% of satiation, and 115% of satiation).

The experiment will run for 8 weeks and water quality parameters will be recorded throughout. Prior to this experiment and prior to the stocking of fish, the system will be prepared for cultivation. This includes inoculating the biofilter with the bacteria that convert potentially toxic nutrients to less harmful forms. At this point, the system will be stocked with barramundi and water quality parameters will be recorded to gain insights into the maturation of the biofilter.

- **European seabass and Greater amberjack**

For European seabass and greater amberjack in RAS, daily video samples were taken for the duration of the trials at specified intervals during the day (during morning and noon feedings as well as in the evening). The recordings were taken at duplicates for each experimental treatment (6 tanks in total) using HD (1080p) cameras (HIKVISION) at 6 fps. Preliminary results were obtained using an open source tracking software complemented by manual object tracking. It is an open source / JAVA based SW with manual object tracking (https://physlets.org/tracker/)

They suggest differences in behavioural traits such as swimming speed (expressed in Body Length) for the various recordings during the day. In the months to come a specific tool will be developed for appropriate fish swimming speed estimations.

- **African catfish**

Trials are to take place both at GE and SZIU facilities.
The aims of the experiments are:

- modeling the selection of fish lines for better conversion of low fishmeal containing feed.
- analyzing the genetic background of the selection.

The experiment is three generation-long. The first generations were reared in 12 separate groups at the GE facility and in 3 groups at the SZIU facility in 2019. The second generation will be reared and propagated. The third generation will be used in testing the new premixes and feeds of iFishIENCi. The experimental groups are fed with a low fish meal containing feed while the control groups are fed with a normal feed. Those fish that utilize efficiently the experimental feed will be used to form a selected line. Both the well utilizing and low-utilizing fish are participating in genetic analysis.

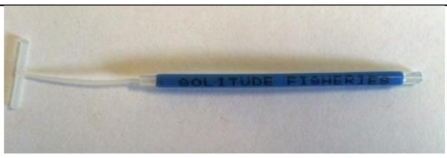
The different feed characteristics (the taste, consistency and digestibility) are influencing the feeding activity. During the feeding event the feeding intensity, the appetite will be monitored in the different experimental groups. We are using floating pellets, which are well tracked visually. The feeding behavior will be observed personally or optionally it possible to install camera-based systems for testing.

3.1.2 Fish tagging

Fish tagging is a technology used to measure the biological response directly on the fish, and thus to give improved indication of the whole fish population biological status.

Several fish tagging technologies have been identified. However, they are either too big for the fish or are too expensive and not reusable to be an integrative part of the iBOSS system.. We will however do an experiment at HCMR sea-cages with European seabass with Thelma Biotel tags to get individual data on pin-point location, activity, depth and temperature to qualify the AI models based on camera and hydroacoustics of the population. Moreover, for small size RAS experiments, they will be a lot of interference, with difficulties to differentiate between different fish signals. Further, during the demonstration of iBOSS in Task3.4 with salmon in sea-cages, we will, if at the time functional. use test newly developed tags through collaboration with the EU project SeasStar to qualify the AI models based on camera imaging.

Table 7 - Comparison of fish tagging technology

Brand	Image	Realtime	Parameters	Size	Reusable
Star oddi		No	Heart rate, depth, temperature	25 to 45 mm	yes
Thelma Biotel		Yes	Depth, temperature position, tilt, activity	7 to 13 mm	no
Atstrack		No	Position	20 to 23 mm	no
Floytag		No	none (for tagging purpose only)	60 to 70mm	yes
AquaExcel (H2020 project)		No	Opercular activity	14mm	yes

3.1.3 Elastomer technology

Elastomer tags are a color fluid tag that solidifies and remains under the skin for individual identification of fish (https://www.nmt.us/visible-implant-elastomer/). In iFishIENCi, we will employ these tags to identify individual feeding behavior for the FishMet model. In addition, when qualifying internal tags, we will use the elastomer tags to qualify effects of internal tags on normal behavior to ensure the internal tags do not impact our results later on.

3.1.4 Camera

The use of camera will allow to evaluate the behaviour of the fish. Moreover, it will allow to anticipate the fish reaction before, during and after feeding.

HCMR camera at RAS

High definition (1080p) Power_Over_Ethernet cameras (HIKVISION DS-2CD1623G0-IZ; https://www.hikvision.com/mtsc/uploads/download/Datasheet_of_DS-2CD1623G0-IZS‘UK.pdf) and 98 degrees field of view are used. The cameras' enclosure is IP67 rated and so far work flawlessly. The cameras provide H.265 video compression, in order to reduce network traffic and storage requirements. For the same reasons, we dropped the "frames_per_second" to 6, which is enough for speed vector measurement. Special attention is given to lighting. During the trials, overhead lights were used, although sometimes this resulted in several reflections to the recorded image.

The connection diagram used is shown below:

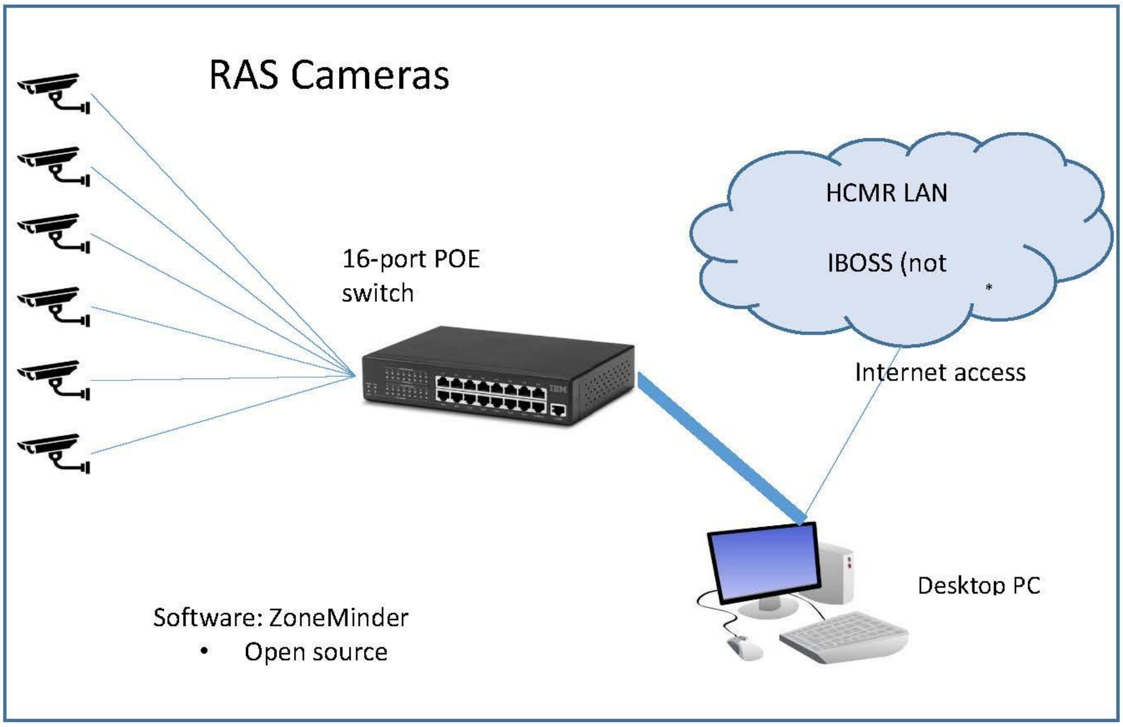


Figure 1- HCMR connection diagram

HCMR camera at cages

High definition (2Mp) Power_Over_Ethernet cameras (Fyssalis V3-1; https://cdn-cms.f-static.net/uploads/438589/normal_5d95f628690a7.pdf) and 112 degrees field of view are used. The cameras is submersible due to its housing providing waterproof characteristics. The cameras provide H.264 video compression, in order to reduce network traffic and storage requirements. For its proper installation a gyroscopic base was developed to reduce oscillations while installed (Fig 1). The connection diagram is similar to the above.

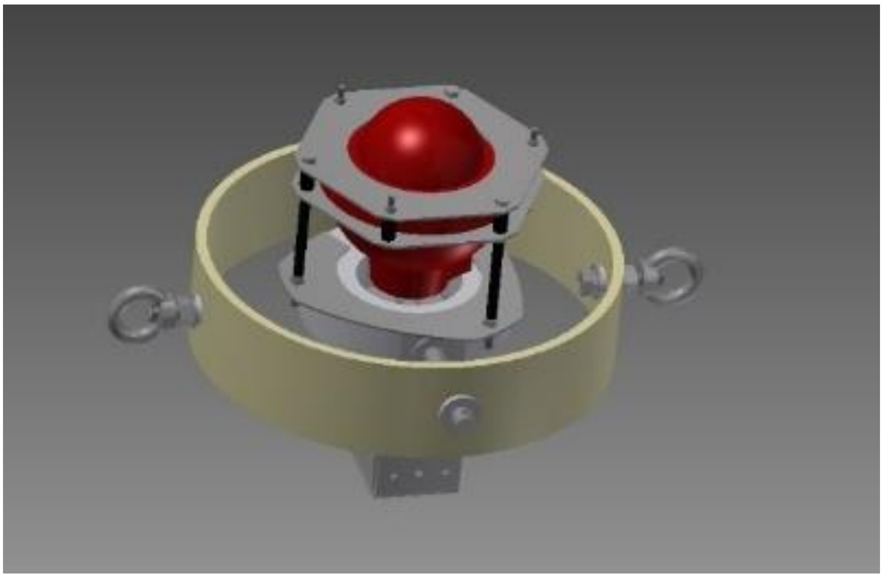


Figure 2 - Gyroscopic base of the submergible camera

3.2 Abiotic monitoring

3.2.1 Physico-chemical sensors

To monitor all the physico-chemical parameters requested from the experiments (see section 1.), we must use sensors that can gather data as precisely as possible and as often as possible. All parameters will be measured in the water column of the RAS and the cages.

Below is shown a recapitulative table of sensor available from iFishIENCi. partners Bioceanor and Oxyguard. All “must have” physico-chemical parameters are listed.

Table 8 - "Must have" physico-chemical sensors and their availability for Bioceanor and Oxyguard

Parameter	Partner	Sensor available	Range	Unit	Sensitivity	Method (auto/manual)	Frequency of measurement
Ammonium	Bioceanor	No					
	Oxyguard	No					
Carbon dioxide	Bioceanor	No					
	Oxyguard	Yes	0-50, 0-100, 0-1000	mg/L	+/-1, +/-1, +/-10	auto	continuously
Hydrogen Sulphid	Bioceanor	No					
	Oxyguard	No					
Dissolved Oxygen	Bioceanor	Yes	0-200 %, 0-20ppm	%, mg/l, ppm	0,1	auto	continuously
	Oxyguard	Yes	0-400%sat, 0-800%sat, 0-6000%sat	%sat, mg/l	+/- 1%sat, +/- 2%sat, +/- 15%sat	auto	continuously
nitrate	Bioceanor	No					
	Oxyguard	No					
nitrite	Bioceanor	No					
	Oxyguard	No					
pH	Bioceanor	Yes	0-14	-	0,01	auto	continuously
	Oxyguard	Yes	0-14	-	0,01	auto	continuously
redox potential	Bioceanor	Yes	-1000;1000	mV	2	auto	continuously
	Oxyguard	Yes	-2000;2000	mV	1	auto	continuously
Salinity	Bioceanor	Yes	0-150	PSU	0,5	auto	continuously
	Oxyguard	Yes	2-42	PSU (according to PSS-78)	1	auto	continuously
Temperature	Bioceanor	Yes	0-50	°C	0,5	auto	continuously
	Oxyguard	Yes	-5;45	°C	0,5	auto	continuously
Ozone	Bioceanor	No					
	Oxyguard	No					
Water velocity	Bioceanor	Available soon	0,6-150	m	0,02	auto	continuously
	Oxyguard	No					

The previous table showed that some parameters could not be measured by any of IfishIENCi partners: ammonium, hydrogen sulfide, nitrate, nitrite and ozone. In the next table, we provide some information on existing solutions for these sensors.

Table 9 - Other parameters sensors

Parameter	Brand	Reference	Range	Unit	Sensitivity	Method (auto/manual)
Ammonium	YSI	VARiON Plus 700 IQ	0,1-129; 1-1290	mg/L	0,1; 1	manual/auto
Hydrogen Sulfide	ISweek	Shallow Water H2S microsensor	0.05 – 10 0.5 – 50 0.01 – 3	mg/L	0,05 – 0,5 – 0,01	manual
	Edaphic	ES-H2S-SW	0.05 – 10 0.5 – 50 0.01 – 3	mg/L	0,05 – 0,5 – 0,01	auto
nitrate	YSI	VARiON Plus 700 IQ	0,5-450 5-4500	mg/L	0,5;5	manual/auto
nitrite	YSI	NiCaVis 705 NI	0-100	mg/L	0.1	manual
Ozone	YSI	YSI 9300 and 9500 Photometer	0-2	AU	0.001	ozone reagent kit + photometer
	Process instruments	Ozosense	0,05-10	mg/L	0,01	auto

3.2.2 Other abiotic data and production data

Collection of production data is as important as water or fish data, as it can have a big influence on fish behaviour. It is also very important that these data can be easily collected so that they can be entered into AI modelisation. Herafter is a list of abiotic parameters and how we can collect them. When no sensor is available, process must be clearly defined to enter the data manually into iBoss.

Table 10 – Other parameters sensors

	Sensor available	Brand	Range	Unit	Sensitivity	Method (auto/manual)
Light intensity	Yes	TandDlogger TR74UI	0-130000	lux	0,01	Auto
Day/Night rythm	Yes	TandDlogger TR74UI	0-130000	lux	0,01	Auto
Times of feeding	Yes	Depending on experiment site				Auto
Quantity of feeding	Yes	Depending on experiment site		g		Auto
Tank maintenance (cleaning, sampling, …)	No					
Underwater noise	Yes	RTSys / TR-SDA14 suitcase recorder	0-100	dB	0,02	Auto

3.3 Biotic monitoring

3.3.1 Objective of the microbiome studies

The microbiome study within the project aims to understand how the water environment, the environmental microbiome, and the fish microbiome are interconnected, and which is the impact of this network in the fish growth and health.

The environmental microbiome (water + sludge) and the fish microbiome (skin, gut and gills) will be characterized by 16S rRNA gene amplicon high-throughput sequencing. The impact of the different growth and environmental conditions and feed formulas and additives, on the fish and environmental microbiomes (the latter through change of environmental conditions) will be studied. Correlations between the microbiomes, the feeds and additives, the environmental parameters and their change, and the fish welfare indicators will be investigated. All information will be integrated regarding the fish health, welfare and production. **The correlations and conclusions regarding the impact on the fish health will be used as input for WP3 and iBOSS system.**

3.3.2 Sampling procedure

Based on the characteristics of each experiment, and on the needs/capabilities of the partner carrying out the RAS trial, specific sampling protocols for microbiome analysis will be discussed and set up with them. These specific protocols will start from a general approach that will ideally include triplicate sampling of:

- *Inlet water and water from the experimental tanks*: water will be sampled via filtration through 0.22 µm nitrocellulose filters (until filter saturation). The filters will be put in tubes and stored at -20°C or -80°C.
- *Sludge from the experimental tanks*: sludge samples will be taken into eppendorf tubes pre-filled with a DNA stabilization reagent. Thy will be then stored at 4°C or -20°C.
- *Fish samples*: a. mucus from the back of the animals (via skin swab); b. gill tissue; c. gut tissue; d. feces. Swabs will be cut near the cotton button and directly put into eppendorf tubes pre-filled with the DNA stabilization reagent. Feces and gut and gill tissue samples will also be taken and put into tubes containing the DNA stabilization reagent. All samples will be stored at 4°C or -20°C.

In order to avoid multiple shipments, samples can be stored as indicated and sent altogether at the end of the experiment. All the material needed for sampling, along with the DNA stabilization reagent and instructions on how to use it, will be provided by LEITAT.

3.3.3 Result integration into iBoss

Results will be integrated manually into iBoss system in a first phase. Data will be available for visualization on iBOSS platform and for modelization to data scientist team. All the biotic monitoring results will be useful to understand and anticipate fish health and welfare. It will be combined with all other abiotic parameters to reinforce the Fish-Talk-To-Me concept.

4 Conclusions

In this deliverable, we presented all the technology that will be used to monitor the different experiments of iFishIENCi. We listed exhaustively all the parameters that can be measured in a fish tank facility and eliminated those that were irrelevant.

For all the relevant parameters, all the experimental sites gave their ideal frequency of measurement. We will take it into consideration when integrating the data into iBOSS. We also focused on the operational constraint by eliminating too expensive or too big sensors (for example the fish tagging technology).

The physico-chemical, environmental, fish-related and biological parameters where considered. All these parameters are part of Fish-talk-to-me concept.

The next step will be to reassess all these technology with the work done in WP2. The integration of the data into iBOSS will give new insight and some parameters might be eliminated from the experiments or other might be added.

However, we are confident that the work done in the task 1.2 will give a very good foundation for the Fish-talk-to-me experiment in iFishIENCi. and the construction of the iBOSS system.