Deliverable D16.1
Analysis of access provided by JU FFPW installations: types and users

Version 01

WP 16
Deliverable 16.1
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Executive Summary

JU FFPW has been a member of AQUAEXCEL since its inception in the first project. Therefore we are experienced with the TNA applications and performing host projects. This activity is highly supported at the faculty and the people are motivated to open the infrastructure and the knowledge through open access projects. Opportunities presented by the AQUAEXCEL3.0 TNA offer great possibilities for us to open access to our infrastructure because it covers all the expenses relevant to the project performance. Our motivation is not only to share our existing infrastructure but also learn new things and start new collaborations, which has happened for many TNA projects performed at our facility. More researchers have applied for the TNA projects than the TNA capacity allocated in AQUAEXCEL3.0 project.

Main Results:

Number of projects / accesses:
GRC – 3 / 13
IFA – 4 /42
ICS – 1 / 8
IAPW – 6 /44,1

Persons trained: 16 (lead researchers came from Greece, Japan, Turkey, Spain, Hungary, Italy, Slovakia Switzerland and Germany)

Most of TNA projects resulted in a joint publication and the presentation of results at an aquaculture conference. TNA lead researchers and JU co-researchers have also collaborated on a TNA success stories video to promote the project.

Authors/Teams involved: Petr Cisar, Monika Malkusova

Installation managers: Petr Cisar, Vojtech Kaspar, Jan Mraz, Oleksandr Malinovskyi, Tomas Policar
Project manager: Monika Malkusová

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1. Overview of TNA users projects realized in JU FFPW

1.1.1. Installations

JU- ICS: The main aim of ICS is research in the field of complex systems and the creation of a full circle starting from experimental design, moving over to experimental realization, data processing and analysis, and finally the management of data. It is active in the applied research in aquaculture monitoring systems.
JU-IAPW: It deals with a wide range of research and consulting activities in the areas of pond and intensive aquaculture, fish processing, and fish quality, fishery in open waters, hydrobiology, and water protection. IAPW has an aquaponic hall, analytical labs, aquarium rooms and a processing plant.
JU-IFA: It includes experimental rooms for controlled larval and juvenile culture, fish culture chamber for controlled environmental stimulation, large scale RAS hall, laboratory of mass rotifer production, haematological and biochemical laboratory, histological laboratory, hatchery for broodstock management, egg incubation, and hatching of larvae, and pond culture system.
JU-GRC: It is a modern and multipurpose fish hatchery. It includes the Laboratory of Reproductive Physiology which is well equipped with microscopy and equipment for analysis of fish spermatozoa motility, cryopreservation experiments, and semen analysis. The Laboratory of Germ Cells is equipped with instrumentation for germ cell and blastomere cryopreservation and micromanipulation and the Laboratory of Molecular, Cellular and Quantitative Genetics is well equipped with aquaria rooms, microscopic and imaging systems, fluorescence microscopy, Partec flow cytometry, multichannel flow cytometry, DNA laboratory, etc.

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1.1.2. User projects

Min. quantity of access units to be provided according ther DoA: 100
Total number of access units (sum of access units in the table): 107,1

Installation number	Installation code	Project title	Project acronym	Description about the experiment	Coordinator	Already used installation (Yes/No)	Nature of the access unit*	Number of used access units during the project	(Potential) paper	How many people was trained by this procedure ?
1	JU-IFA	Effects of vitamin C in pikeperch	VCPIKE	During the three-month trial, pikeperch juveniles were reared under controlled condition and fed the basal diet (formulated at JU-IAPW) with the supplementation of five different levels of vitamin C: 125, 250, 500, 1000 and 2000 mg kg^{-1} diet and a control (o mg kg^{-1}) group. Six practical diets were prepared using a laboratory pelleting machine in 3 mm diameter with graded levels of vitamin C supplementation in the form of L-ascorbyl-2-monophosphate (35 % equivalent). The diets were air-dried until the moisture content was reduced to less than 10 % and dry pellets were placed in plastic bags and stored at - 80^0 C until used. All the fish were fed a basal diet to accept dry, artificial feed without vitamin C. At the end of acclimation period, randomly captured fish were distributed into each polyvinyl circular tank of 150 L capacity and supplied with freshwater at a flow rate of 2 L min^{-1} and aeration. Triplicate groups of fish were handfed the test diets to satiation three times per day.	Maria Angeles Esteban Abad – University of Murcia (ES) / Aiman Imentai (CZ)	Yes	week	15 weeks 8.3.2022-21.6.2022	no	1
2	JU-GRC	Nuclear transfer in zebrafish	nucZeb	During the 5-week experiment we optimized mitochondrial transfer between embryos of different fish species. As model fish we used fish of the genus Danio. We further applied the optimized method to sturgeon.	Emmanouil Ladoukakis – University of Crete (GR) / Martin Pšenička (CZ)	Yes	week	5 weeks 22.6.2022 – 27.7.2022	no	2

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3	JU-IAPW	Bold-shy European perch	BoldPerch	The European perch's high sensitivity to stress with subsequent low disease tolerance to pathogens are some of the bottlenecks that contribute to the stagnant percid production. It has been hypothesized that the sensitivity to stress, and its impact on immunity, is related to the fish personalities, and therefore selecting individuals with more profitable personalities for future selective-breeding programs could help to overcome these bottlenecks. To test this hypothesis, European perch was employed in a series of tests (open-field and novel-object test) to identify particularly shy and particularly bold European perch personalities based on video analyses. A cohort of shy and a cohort of bold perch was then exposed to stocking density stress and bacterial stimulation (*Aeromonas* sp.). Finally, we applied a panel of classical molecular and transcriptomic tools to determine whether personality correlates with resistance to stress and pathogens.	Alexander Rebl – Research Institute for Farm Animal Biology (GE)/ Tatyana Gebauer (CZ)	Yes	week	7 weeks 1.8.2022 - 19.9.2022	Are bold-shy personalities of European perch (Perca fluviatilis) linked to stress tolerance and immunity? A scope of harnessing fish behavior in aquaculture - DOI 10.1016/j.fsi.2023.109190	1
4	JU-GRC	Surrogacy protects suspectible species	SurrogacyProtect	GSCs of susceptible carp breeds were isolated and transplanted into larva of a) intraspecific host Amur mirror carp and b) interspecific host goldfish.	Taiju Saito – Ehime University (JA) / Martin Pšenička (CZ)	Yes	week	2 weeks 1.4.2023 – 14.4.2023	no	1
5	JU-IAPW	Effects of root exudates on fish welfare	RootWell	We measured the organic acids in plant root exudates and challenged the fish in RAS to see the pure effect of those exudates on fish welfare.	Alexander Rebl - Research Institute for Farm Animal Biology (GE)/ Radek Gebauer (CZ)	Yes	week	7,2 weeks 25.8.2023 – 15.10.2023	under preparation	1
6	JU-IAPW	Mucosal immunity of rainbowtrout	Mucus-FA	This study evaluated how humic substances can change the skin's immune system and improve a fish's ability to fight off microorganisms. Using fulvic acid at concentrations of 5 mg and 50 mg per liter seemed safe and helpful for strengthening the fish's antibacterial defenses in fish farming. However, it's crucial to remember that long time exposure with these substances in natural waters, especially combined with other environmental challenges, might have negative	Thomas Meinelt – Leibniz Institute (GE) / Thora Lieke (CZ)	Yes	week	8 weeks 14.8.2023 – 9.10.2023	Fulvic acid modulates mucosal immunity in fish skin: Sustainable aquaculture solution or environmental risk factor? https://doi.org/10.	1

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				effects. So, monitoring the levels of humic substances in natural water is crucial.					1016/j.jhazmat.2024.133737	
7	JU-ICS	Non-invasive RMS early detection	CeDNA	JU-ICS participated in this TNA using digital camera computer system. The visible symptoms of the disease in fish were detected and classified using the image processing method. We recorded the appearance of the diseased and healthy fish to create the data set of visible symptoms. The image analysis methods were used to detect RMS skin pathology. The results were used for the development of a complete system for fish disease detection.	Massimo Orioles – University of Udine (IT)/ Petr Císař (CZ)	Yes	week	8 weeks 20.9.2023 – 15.11.2023	Under preparation	1
8	JU-IAPW	Bioenergetics of Tilapia sperm	Alphasperm	The objective of the study was to determine the functional differences in motility kinetics, fatty acids, and ATP from the spermatozoa of dominant and subordinated tilapia fish residing in stable social hierarchies.	Frank Pfennig – Technical University Dresden (GE) / Deepali Rahi Roy (CZ)	Yes	week	7,2 weeks 2.10.2023 – 22.11.2023	two articles under preparation	1
9	JU-IAPW	Optimization of aquaculture sludge digestion	AquaSlushie	The TNA project had following objectives: Determining the duration of the organic reduction step, temperature and pH. Determining the duration, optimum pH and PSB addition in the nutrient solubilization step. Cross-validation of the results in continuous large-scale reactors and economic feasibility.	Hendrik Monsees – IGZ (GE) / Radek Gebauer (CZ)	Yes	week	7,5 weeks 1.3.2024 – 24.4.2024	article under preparation, expected submission autumn 2025, to be presented at Aquaculture Europe 2025	2
10	JU-IFA	Natural reproduction behaviour in pikeperch broodstock	NATUREPSANDER	The study used four sets of pikeperch broodstock for natural spawning on the nest, comprising fish with different rearing histories: (1) both sexes pond-cultured; (2) pond-cultured males and intensively-cultured females; (3) intensively-cultured males and pond-cultured females; (4) both sexes are intensively-cultured. The fish in each set were provided with artificial spawning nests and no hormonal induction of spawning.	Uros Ljubobratovic – Magyar Agrár (HU) / Oleksandr Malinovskyi (CZ)	Yes	week	7 weeks 3.4.2024 – 22.5.2024	no	1

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				This approach allowed assessment of the capability of pikeperch broodstock to exhibit natural spawning behaviour, providing valuable insights into the potential influence of the rearing history on their reproductive performance. Each nest was photographed, and the image was analysed with the open-source image processor ImageJ. The analysis included the determination of the cleaned area and area of egg distribution relative to the total nest area.						
11	JU-IAPW	Effects of plants on the microbes of soilless systems	Aqpmic	The objectives of the study are as follows: A) to investigate whether there is a distinction between the microbial communities in mono- and mixed-cropping systems of aquaponics and hydroponics; B) to examine the impact of different cropping systems on nutrient uptake in aquaponics and hydroponics; C) to establish the relationship between microbial communities in soilless systems and phosphorus uptake in various plants; D) to identify genes with the potential to influence phosphorus uptake in plants grown in hydroponic and aquaponic systems.	Zala Schmautz – Zurich University of Applied Sciences (SCH) / Azeez Folorunzo (CZ)	Yes	week	7,2 weeks 10.5.2024 – 30.6.2024	It is expected	1
12	JU-GRC	Cryoresistance of sturgeon sperm after hypothermic storage	CryoSturg	This study aimed to develop and validate a hypothermic sperm storage protocol that preserves sperm viability and functionality during short-term storage and enhances cryoresistance. The methodology included testing different sperm diluents, assessing motility and evaluating sperm energy metabolism by measuring ATP, creatine phosphate and lipid peroxidation levels. In the final step, fertilisation trials were performed using sperm samples before and after cryopreservation.	Atife Tuba Beken – Central Fisheries Research Institute (TR) / Borys Dzyuba (CZ)	Yes	week	6 weeks 24.11.2024-14.12.2024, 1.3.2025-22.3.2025	not yet	1

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13	JU-IFA	Toxicity of Biopesticide	AzadirachtinToxicity	The aim was the introduction of a new method to analyse 'oxidatively modified proteins' (OMP), which had not been previously utilized in the lab. This innovation opens up new possibilities for studying oxidative stress biomarkers, enhancing the scope and depth of future experiments with mussels or fish. We were able to optimize the experimental procedures to increase efficiency. The switch to microplate measurements significantly improved sample analysis capacity, reducing time and resources spent on each analysis while ensuring higher reproducibility and throughput. Mainly for the OMP and superoxide-dismutase levels with using of pyrogallol. The experiment with embryo-larval crayfish provided valuable insights into oxidative stress biomarkers in this species. This research lays a foundation for future studies on crayfish physiology and may contribute to broader environmental or ecological research.	Alessia Caferro – University of Calabria (IT) / Nikola Mikušková (CZ)	Yes	week	6 weeks 1.2.2025-15.3.2025	Paper will be prepared after we receive the results of the histological examination and analysis of the water tested	1
14	JU-IFA	Pikeperch production in different aquaculture systems	SYSTEMSPIKEPERCH	The main objective was to determine the aptness of 3 rearing systems and their production specifications of valuable pikeperch. We will look at: A) production parameters concerning growth, feed utilization and survival, B) Welfare of produced fish (blood sampling, somatic indexes, and fin erosion), C) Economical evaluation - production cost per one raised fish confronted with reached production parameters.	Jiri Kristan – Comenius University Bratislava (SK) / Václav Kucera (CZ)	Yes	week	14 weeks 1.4.2025-8.7.2025	TNA project ongoing	1

1. TNA projects

1.3.1. TNA projects description

1. Short name: Effects of vitamin C in pikeperch

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Acronym: VCPIKE

Description: During the three-month trial, pikeperch juveniles were reared under controlled condition and fed the basal diet (formulated at JU-IAPW) with the supplementation of five different levels of vitamin C: 125, 250, 500, 1000 and 2000 mg kg-1 diet and a control (o mg kg-1) group. Six practical diets were prepared using a laboratory pelleting machine in 3 mm diameter with graded levels of vitamin C supplementation in the form of L-ascorbyl-2-monophosphate (35 % equivalent). The diets were air-dried until the moisture content was reduced to less than 10 % and dry pellets were placed in plastic bags and stored at - 800 C until used. All the fish were fed a basal diet to accept dry, artificial feed without vitamin C. At the end of acclimation period, randomly captured fish were distributed into each polyvinyl circular tank of 150 L capacity and supplied with freshwater at a flow rate of 2 L min-1 and aeration. Triplicate groups of fish were handled the test diets to satiation three times per day.
Our primary results showed the importance of vitamin C and its dosage in diet for pikeperch juveniles, which is in line with our expectation, but to have a robust conclusion, we need to carry out all planned experimental analysis.

No publication due to poor cooperation of lead researcher and personal changes at JU-IFA (co-researcher)

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2. **Short name: Nuclear transfer in zebrafish**

Acronym: nucZeb

Description: During the 5-week experiment we optimized mitochondrial transfer between embryos of different fish species. As model fish we used fish of the genus Danio. We further applied the optimized method to sturgeon.

No research paper.

3. **Short name: Bold-shy European perch**

Acronym: Boldperch

Description: The European perch's high sensitivity to stress with subsequent low disease tolerance to pathogens are some of the bottlenecks that contribute to the stagnant percid production. It has been hypothesized that the sensitivity to stress, and its impact on immunity, is related to the fish personalities, and therefore selecting individuals with more profitable personalities for future selective-breeding programs could help to overcome these bottlenecks. To test this hypothesis, European perch was employed in a series of tests (open-field and novel-object test) to identify particularly shy and particularly bold European perch personalities based on video analyses. A cohort of shy and a cohort of bold perch was then exposed to stocking density stress and bacterial stimulation (Aeromonas sp.). Finally, we applied a panel of classical molecular and transcriptomic tools to determine whether personality correlates with resistance to stress and pathogens.

Paper reported: Are bold-shy personalities of European perch (Perca fluviatilis) linked to stress tolerance and immunity? A scope of harnessing fish behavior in aquaculture - DOI 10.1016/j.fsi.2023.109190

TNA video: https://www.youtube.com/watch?v=nxCSgw89oJA

4. **Short name: Surrogacy protects susceptible species**

Acronym: SurrogacyProtect

Description: GSCs of suspectible carp breeds were isolated and transplanted into larva of a) intraspecific host Amur mirror carp and b) interspecific host goldfish.

No research paper.

TNA video: https://www.youtube.com/watch?v=hJET939YSXo&feature=youtu.be

5. **Short name: Effects of root exudates on fish welfare**

Acronym: RootWell

Description: We measured the organic acids in plant root exudates and challenged the fish in RAS to see the pure effect of those exudates on fish welfare.

Research paper under preparation.

TNA video: https://www.youtube.com/watch?v=P9o32pA8ywE&feature=youtu.be

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6. **Short name: Mucosal immunity of rainbowtrout**

Acronym: Mucus-FA

Description: This study evaluated how humic substances can change the skin's immune system and improve a fish's ability to fight off microorganisms. Using fulvic acid at concentrations of 5 mg and 50 mg per liter seemed safe and helpful for strengthening the fish's antibacterial defenses in fish farming. However, it's crucial to remember that long time exposure with these substances in natural waters, especially combined with other environmental challenges, might have negative effects. So, monitoring the levels of humic substances in natural water is crucial.

Research paper reported: Fulvic acid modulates mucosal immunity in fish skin: Sustainable aquaculture solution or environmental risk factor? https://doi.org/10.1016/j.jhazmat.2024.133737

TNA video: Fulvic acid modulates mucosal immunity in fish skin: Sustainable aquaculture solution or environmental risk factor? https://doi.org/10.1016/j.jhazmat.2024.133737

7. **Short name: Non-invasive RMS early detection**

Acronym: CeDNA

Description: JU-ICS participated in this TNA using a digital camera computer system. The visible symptoms of the disease in fish were detected and classified using the image processing method. We recorded the appearance of the diseased and healthy fish to create the data set of visible symptoms. The image analysis methods were used to detect RMS skin pathology. The results were used for the development of a complete system for fish disease detection.

Research paper under preparation.

TNA video: https://www.youtube.com/watch?v=wVnjwSpWbIs

8. **Short name: Bioenergetics of Tilapia sperm**

Acronym: Alphasperm

Description: The objective of the study was to determine the functional differences in motility kinetics, fatty acids, and ATP from the spermatozoa of dominant and subordinated tilapia fish residing in stable social hierarchies.

Two research articles are under preparation.

TNA video: https://www.youtube.com/watch?v=R4v-WRu5MHE

9. **Short name: Optimization of aquaculture sludge digestion**

Acronym: AquaSlushie

Description: The TNA project had following objectives: Determining the duration of the organic reduction step, temperature and pH. Determining the duration, optimum pH and PSB addition in the nutrient solubilization step. Cross-validation of the results in continuous large-scale reactors and economic feasibility.

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Research paper under preparation.

10. Short name: Natural reproduction behaviour in pikeperch broodstock
Acronym: NATUREPSANDER
Description: The study used four sets of pikeperch broodstock for natural spawning on the nest, comprising fish with different rearing histories: (1) both sexes pond-cultured; (2) pond-cultured males and intensively-cultured females; (3) intensively-cultured males and pond-cultured females; (4) both sexes are intensively-cultured. The fish in each set were provided with artificial spawning nests and no hormonal induction of spawning. This approach allowed assessment of the capability of pikeperch broodstock to exhibit natural spawning behaviour, providing valuable insights into the potential influence of the rearing history on their reproductive performance. Each nest was photographed, and the image was analysed with the open-source image processor ImageJ. The analysis included the determination of the cleaned area and area of egg distribution relative to the total nest area.
Presentation of results at AQUA2024 - The effect of pikeperch(*Sander lucioperca*) broodstock origin on their ability to express natural reproductive behaviour

11. Short name: Effects of plants on the microbes of soilless systems
Acronym: Aqpmic
Description: The objectives of the research were the following: A) to investigate if there is a distinction between the microbial communities in aquaponics / hydroponics mono- and mixed-cropping; B) to determine the significant association between growth of the plants in both systems and microbial communities using associating testing; C) the nutrient uptake rate of basil and lettuce under mono- and mixed- cropping of aquaponics and hydroponics; D) the shift in the microbial communities by comparing the microbial communities of the fish unit and the hydroponics unit.
Research paper under preparation.
TNA video: AQUAEXCEL 3.0, TNA project: Exploring Microbial Diversity in Aquaponics - YouTube

12. Short name: Cryoresistance of sturgeon sperm after hypothermic storage
Acronym: CryoSturg
Description: This study aimed to develop and validate a hypothermic sperm storage protocol that preserves sperm viability and functionality during short-term storage and enhances cryoresistance. The methodology included testing different sperm diluents, assessing motility and evaluating sperm energy metabolism by measuring ATP, creatine phosphate and lipid peroxidation levels. In the final step, fertilisation trials were performed using sperm samples before and after cryopreservation.
Research paper under preparation.
TNA video: https://www.youtube.com/watch?v=9HTvCRmEbOE

13. Short name: Toxicity of Biopesticide

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Acronym: AzadirachtinToxicity
Description: The aim was the introduction of a new method to analyse 'oxidatively modified proteins' (OMP), which had not been previously utilized in the lab. This innovation opens up new possibilities for studying oxidative stress biomarkers, enhancing the scope and depth of future experiments with mussels or fish.
We were able to optimize the experimental procedures to increase efficiency. The switch to microplate measurements significantly improved sample analysis capacity, reducing time and resources spent on each analysis while ensuring higher reproducibility and throughput. Mainly for the OMP and superoxide-dismutase levels with using of pyrogallol.
The experiment with embryo-larval crayfish provided valuable insights into oxidative stress biomarkers in this species. This research lays a foundation for future studies on crayfish physiology and may contribute to broader environmental or ecological research.
No research paper yet.

14. Short name: Pikeperch production in different aquaculture systems
Acronym: SYSTEMSPIKEPERCH
Description: The main objective was to determine the aptness of 3 rearing systems and their production specifications of valuable pikeperch. We will look at: A) production parameters concerning growth, feed utilization and survival, B) Welfare of produced fish (blood sampling, somatic indexes, and fin erosion), C) Economical evaluation - production cost per one raised fish confronted with reached production parameters.
TNA project is ongoing till beginning of July 2025.

1.3.2. Selection of One exemplary project

We selected project Non-invasive RMS early detection, acronym: CeDNA because it is a multi area research project and it combined the use of two different infrastructures (JU and DTU). Massimo Orioles from AULSS2 Marca Trevigiana in Italy requested our infrastructure and DTU to perform the study of test of two tools for early RMS disease detection for rainbow trout. The eDNS and video monitoring were tested for non-invasive detection of the RMS symptoms and flavo bacteria in the water samples. Massimo applied for our underwater monitoring systems and video data processing to detect visible symptoms of the RMS disease on the fish body during the disease progression. The experiments were performed in Denmark (DTU) and data analysis was done by us at JU. The AI model for the detection of visible symptoms was developed. The accuracy of RMS symptoms detection in real time is 96% and the system can substitute existing detection based on expert observations. The joint research paper is under preparation and will be published in 2025.

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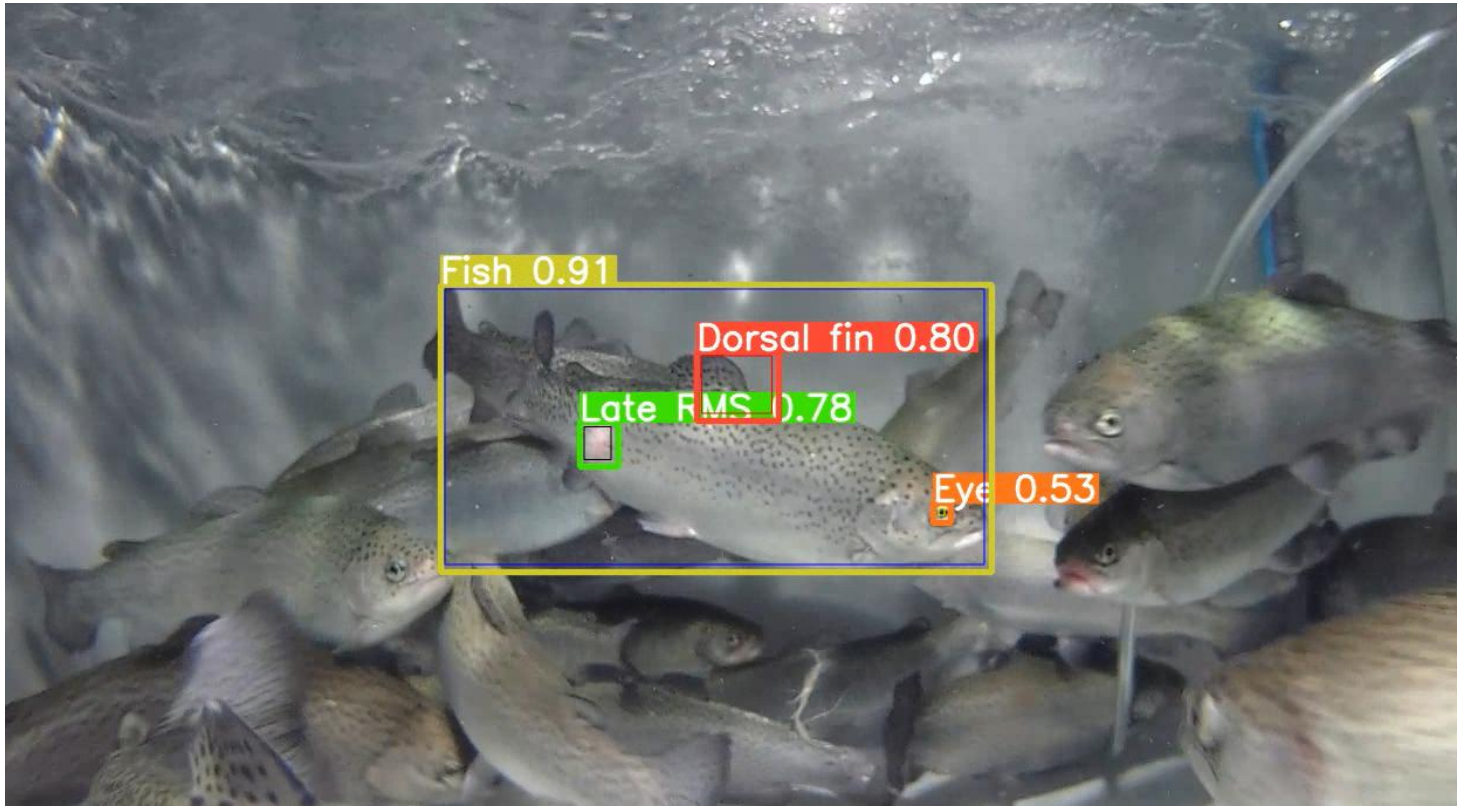

Figure 1. Image from video record of the fish in tank with detected RMS symptom (green) and fish, eye and dorsal fin.

2. Reflection on results of the TNA programme

The pipeline of TNA applications is now well developed by AQUAEXCEL3.0. We are also experienced, and the performance of the TNA projects is generally smooth. We had more project requests than the available slots, but we were limited by the project budget. We will use the experience from AQUAEXCEL TNA in other TA projects and in the open access through the national Large research infrastructure access.

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Document Information

EU Project	No 871108	Acronym	AQUAEXCEL3.0
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Project website	www.aquaexcel.eu		

Deliverable	N°	D16.1	Title	Analysis of access provided by JU FFPW installations: types and users
Work Package	N°	16	Title	Transnational access to JU FFPW
Work Package Leader				
Work Participants				

Lead Beneficiary	Name, Partner Number
Authors	Petr Cisar, Monika Malkusova, JU FROV, cisar@jcu.cz
Reviewers	Name, Organisation, Email address

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