Deliverable 2.2
Register of High-Impact Outputs for Knowledge Transfer

Version 1

WP 2
Deliverable 2.2
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1. Introduction

AQUAEXCEL3.0 has the objective to bridge the gap between research and industry, fostering innovation and improving the performance of European aquaculture sector. Acting as the interface of AQUAEXCEL3.0 researchers and TNA users and the aquaculture industry, the project activities contribute to strengthening the sector's innovative power by identifying experimental research results and cutting-edge technologies and methodologies that might lead to potential applications. It does so by stimulating research areas recognised by the sector as highly relevant, by making scientists more aware of the potential impact of their research results, and by selecting the most promising results for further analysis of their applications. These may be found among both producing and supplying companies and across the entire aquaculture value chain and range of research fields.

To achieve this, AQUAEXCEL3.0 has set up a Knowledge Management and Transfer methodology and Impact Plan (D2.1). These facilitate the collection and analysis of all Knowledge OUTPUTs generated by the Transnational Access (TNA) projects and the AQUAEXCEL3.0 Joint Research Activities. Indicators of innovation (e.g., according to the guidelines described in OECD/Eurostat Oslo Manual, 2018) provide a framework for categorization and selection of potentially High-Impact Outputs by the Industry & Research Advisory Panel (IRAP).

IRAP members, representing both academia and industry, perform an annual assessment of newly generated Knowledge OUTPUTs, with the eventual aim to transfer those to potential end users through AQUAEXCEL3.0 Brokerage events. The owners of the OUTPUTs are guided by the project towards the presentation at these events in order to highlight their exploitable results and hence maximise the potential uptake.

2. Methodology

2.1. Collection of OUTPUTs

Upon the finalisation of each TNA project, its Principal Investigator is requested to submit a completed Impact Plan according to a fixed template (see D2.1). The plan summarises the information about the Knowledge Output itself, its theme and relation to EATiP Thematic Areas and Goals, as well as details on potential end users, applications, potential impact and exploitation. The report may contain pictures, videos, reports and other material documenting its results. After submission through the ARIA database, the OUTPUTs are managed by WP1 leader (University of Stirling). The register also includes OUTPUTs generated by AQUAEXCEL^{2020} TNA projects that had not yet been assessed during this previous Research Infrastructure project. This guarantees bridging the time gap between knowledge generation and its availability for further uptake.

The three AQUAEXCEL3.0 Joint Research Activities (i.e. Technological tools for improved experimental procedures; New biological models for aquaculture research using innovative biotechnologies; Improvements of fish welfare in experiments and industry) also provide similar Impact Plans for their OUTPUTs, connected to the respective deliverables.

The submitted Impact Plans are being reviewed by ERINN Innovation on a continuous basis. They form the basis for the OUTPUT description document that is prepared for each IRAP meeting. TNA users and JRA leaders being late in delivering their Impact Plans are being reminded about their obligations. Any incomplete or missing information is also being added through direct follow-up.

2.2. OUTPUT evaluation procedure

OUTPUTs are evaluated by IRAP at regular intervals, i.e. since the start of the project on an annual basis. During the constituent meeting in 2021, the IRAP Terms of Reference were set up and approved (see Appendix 4.1). It describes the role and purpose of the IRAP, its composition, rules of commitment, financial compensation, and overall working methodology.

The IRAP consists of the AQUAEXCEL3.0 WP leaders (7), and a maximum of 15 European aquaculture industry experts, striving to represent a multitude of thematic areas, species and parts of the value chain. To this end, representatives from EATiP Mirror Platforms (MiPs) were included in the engagement as IRAP members. Their role as cluster managers and business developers fits well with the objectives of industry driven innovation.

The external IRAP members and their fields of expertise are:

- Ole Christensen (Biomar) - Fish Feed (activities and sales)
- Noralf Rønningen; replaced by Hanne Digre in 2022 (ScaleAQ) - Innovation, technology & equipment for aquaculture
- Patrick Lavens; replaced by Peter De Schryver in 2023 (INVE), who resigned in 2025 - Fish Feed / Innovation / International cooperation
- Antonio Coli (Selonda / HETEPA; Planktonic from 2022) - Hatcheries / Bass & Bream / Sales / Transportation & Logistics
- Leonidas Papaharisis (Avramar) - Product Quality / R&D
- Kjell Maroni (Norwegian Seafood Research Fund FHF) – Private applied research coordination
- Doug Mc Leod – Trout / Molluscs / Capacity building
- Hamish Rodger; replaced by Ana Herrero in 2023 (VetAqua) - Fish Health
- Fernando Torrent (Culmarex / Madrid Polytechnic University UPM) – Hatcheries and larviculture (Turbot / Bass & Bream / Blackspot seabream / Greater amberjack / Salmon)

The external IRAP members representing EATiP MiPs are:

- Yolanda Molares; replaced by Iria Freire in 2023 (Cluster ACUIPLUS - Spain)
- Ann Cecilie Ursin Hilling (NCE Aquaculture - Norway)
- László Varadi, replaced by Béla Halasi-Kovács in 2025 (HUNATIP - Hungary)
- Doriane Stagnol; replaced by Marie Bruaut in 2022 but on maternity leave in 2025 (Pôle AQUIMER - France)

The IRAP analyses the OUTPUTs and scores their potential level of impact on identified industry end users. The analysis process of AQUAEXCEL3.0 (and previous AQUAEXCEL^{2020}) OUTPUTs consists of two parts:

1. Individual Analysis in advance of the IRAP meeting
2. IRAP Analysis and Discussion on Next Steps (at the IRAP meeting)

2.2.1. Individual Analysis in advance of the IRAP meeting

Two weeks before the joint IRAP meetings, its members receive the AQUAEXCEL3.0 Knowledge Collection document, prepared by ERINN Innovation. This describes all individual AQUAEXCEL3.0 (and in some cases AQUAEXCEL^{2020}) OUTPUTs that are ready for analysis, containing the following details:

- The OUTPUT title
- The OUTPUT owner
- Links / DOI to the OUTPUT
- IPR protection

- Status of the OUTPUT and TRL
- Knowledge Output Description, incl. knowledge need, methodology, results, innovation
- EATIP Thematic Areas
- EATiP Goals
- Targeted End Users, Potential Applications and Potential Impact

The Knowledge Collection documents contained 15 (2021), 10 (2022), 17 (2023), 16 (2024) and 24 (2025) OUTPUTs for review by the IRAP. A submission deadline for OUTPUTs was established as March 1, 2025. After this date, new OUTPUTs were no longer collected, analysed and evaluated by the IRAP. This cut-off was necessary to accommodate the time required for the AQUAEXCEL3.0 knowledge transfer process.

After receiving the documents by e-mail, the IRAP experts are asked to read each OUTPUT carefully and fill in a defined assessment table (see Table 1) per OUTPUT. The individual analysis proposes potential applications of the OUTPUTs, who the users could be, as well as potential pathways to impact. The process may also identify any missing parts in the OUTPUT descriptions or issues that need to be further clarified. The scoring system was explained at the very first IRAP meeting in 2020 and repeated in writing along with the distribution of the OUTPUTs (see definitions Table 2). The IRAP members are asked to score the level of potential impact on European Aquaculture Industry for each OUTPUT, based on their understanding of the OUTPUT and on the needs of the sector. The comments and scores are submitted to ERINN Innovation in advance of the IRAP Analysis meeting.

Table 1: Assessment Table to be completed by the IRAP members prior to the IRAP meeting for each OUTPUT

Assessment				
Feedback/Comments: Is anything unclear in the description? What further information would you need to know?	xxx			
Application: Based on the description provided, how do you foresee this knowledge being used? How does it compare to what is currently used?	xxx			
Users: Based on the description provided, who can you see applying this knowledge/technology in Europe? This can be user groups or specific companies or individuals.	xxx			
Pathway to Impact: From what you understand of this Knowledge Output, what would need to happen for it to have application by the European aquaculture sector? Feel free to identify multiple activities if appropriate, as well as people etc.	xxx			
Level of Potential:	3 ☐	2 ☐	1 ☐	0 ☐

Table 2: OUTPUT levels of impact on the European Aquaculture industry

The 'Level of Potential Impact' is defined as the impact that the Knowledge Output could have if it were transferred to and applied by the End User. We have defined the levels of impact as follows:

- **3 – High Impact** on Industry End Users, leading to an increase in e.g. sales volume, sales price, yield, etc. and/or improvement in environmental impacts, fish quality and/or fish welfare practices, etc.
- **2 – Medium Impact** on Industry End Users, leading to some notable adaptation within industry.
- **1 – Low or no Impact** on Industry End Users; or only localised uptake by a tiny proportion of the sector.
- **0 – Don't know**

2.2.2. IRAP Analysis and Discussion on Next Steps

IRAP meetings have been held at regular times (in June 2021, April 2022, April 2023, March 2024, April 2025), either on-line (in 2021, 2022, 2023, 2025) or hybrid (in 2024). All IRAP experts are invited to attend the meetings, organised by EATiP and ERINN Innovation, to further discuss the scoring of the OUTPUTs. The OUTPUTs having received the highest scores during the individual analysis are discussed in more detail, as to achieve a consensus decision. Finally, the most relevant OUTPUTs are being selected for presentation at AQUAEXCEL3.0 brokerage events.

Other issues on the agenda of the IRAP meetings are:

- Thematic areas of high relevance to the industry to be recommended for TNA (except in 2025)
- Planning of future and experiences from past Brokerage events

The consensus decisions obtained at the IRAP meeting are collected and summarised (see Milestone 6 of the project, including the IRAP meeting minutes). Subsequently, the owners of the potentially high impact OUTPUTs selected by the IRAP are informed about the evaluation results and invited to the identified AQUAEXCEL3.0 Brokerage events. They are requested to prepare a presentation according to a set template, developed to maximise the knowledge transfer potential. They are also offered a pitching lecture prior to the event by an expert in science communication, Ass. Prof. Hana Gustafsson (NTNU).

3. Register of High-Impact Knowledge Outputs

3.1. Summary table

The AQUAEXCEL3.0 project has resulted in 21 High-Impact OUTPUTs (see Table 3). Two OUTPUTs (ref. AE2020_SINTEF_3, and AE3.0 JRA D4.3) were generated by Joint Research Activities whereas the others are related to TNA projects (both AQUAEXCEL^{2020} and AQUAEXCEL3.0).

Table 3: Shortlist of the Knowledge OUTPUTs selected by the IRAP as "High-Impact OUTPUTs" during the course of AQUAEXCEL3.0 (2021-2025)

OUTPUT identificator	OUTPUT title	Date and place of OUTPUT presentation	OUTPUT owner
AE2020_ SINTEF_3	Virtual Aquaculture Laboratory to assist in optimizing the use of experimental resources and improve experimental design and test power	29/09/21 - On The Horizon Forum (online)	Finn Olav Bjørnson, SINTEF Ocean
AE170011	Dietary effects on growth, survival and behavioural responses in lumpfish (*Cyclopterus lumpus L.)*	29/09/21 - On The Horizon Forum (online)	Ibon García Gallego, ULPGC
AE170007	ADVENTURE: A new fish feeding control and optimization method for gilthead seabream	12/05/22 - On The Horizon Forum II (online)	Chen Tao, University of Surrey
AE160012	AQUAWASTE: Improving plant health through Nutrient Remineralization in Aquaponic Systems	12/05/22 - On The Horizon Forum II (online)	Victor Lobanov, University of Gothenburg
AE110004	RMS: Red Mark Syndrome in rainbow trout: advances in diagnostics and management strategies	29/07/22 - Aquaculture Europe 2022 (Rimini, Italy)	Massimo Orioles, Universita degli studi di Udine
AE130020	SYLPRO4TROUT: use of Wood-Based Yeast SCP (single-cell protein) as an ingredient for Trout diets	29/07/22 - Aquaculture Europe 2022 (Rimini, Italy)	Ricardo Ekmay, Arbiom Inc.
AE100025	SWIM: Testing of the Salmon Welfare Index Model (SWIM 1.0) as a Computational Welfare Assessment for Sea-Caged European Sea Bass (*Dicentrarchus labrax*)	29/07/22 - Aquaculture Europe 2022 (Rimini, Italy)	Hijran Yavuzcan Yildiz, Ankara University
AE150010	TRIPLOID SALMON: Sal-ploidy: Triploids salmon and salinity	29/07/22 - Aquaculture Europe 2022 (Rimini, Italy)	Raneesha de Fonseka, University of Gothenburg
AE140007	FISHSCAILED: Non-invasive fish identification - scale patterns	29/07/22 - Aquaculture Europe 2022 (Rimini, Italy)	Dinara Bekkozhayeva, University of South Bohemia in Ceske Budejovice

OUTPUT identificator	OUTPUT title	Date and place of OUTPUT presentation	OUTPUT owner
AE3.0 TNA 20341	KRILL MEAL: Benefit of krill meal inclusion on enhancing the growth of juvenile gilthead seabream by significantly reducing the feed conversion ratio (FCR)	23/08/23 - AquaNor (Trondheim, Norway and online) 20/09/23 - Aquaculture Europe 2023 (Vienna, Austria)	Kiranpreet Kaur, Aker Biomarine AS
AE3.0 TNA 20680	FEEDREGIUS: Experimental assessment of the fish meal content requirements for Meagre (*Argyrosomus regius*) feeds	20/09/23 - Aquaculture Europe 2023 (Vienna, Austria)	Ramon Fontanillas, Skretting Aquaculture Innovation
AE3.0 TNA 130023	METAL AMINO ACID: Metal-amino acid complexes as a cost-effective strategy to help reducing fish meal in European seabass diets	20/09/23 - Aquaculture Europe 2023 (Vienna, Austria)	Claudia Figueiredo-Silva, Zinpro Corporation
AE3.0 TNA 22312	CryoPlankton benefits in seabass aquaculture	28/08/24 – AQUA 2024 (Copenhagen, Denmark)	Konstantinos Tzakris, Planktonic
AE3.0 TNA 23227	Transforming food industry and agriculture waste into nutrient-rich alternative feed for fish: Case Study with Black Soldier Fly Larvae	28/08/24 - AQUA 2024 (Copenhagen, Denmark)	Martin Kulma, Czech University of Life Sciences Prague
AE3.0 TNA 24658	Application of innovative and easy sustainable environmental enrichment on the behaviour and welfare of farmed trout	28/08/24 - AQUA 2024 (Copenhagen, Denmark)	Georgina Lea Fazekas, MATE
AE3.0 TNA 26814	Origin significantly impacts reproductive capabilities of pikeperch (*Sander lucioperca*) broodstock	Planned 24/09/25 – Aquaculture Europe '25 (Valencia, Spain)	Oleksandr Malinovskyi, University of South Bohemia
AE3.0 TNA 23110	Supplementation with metal-AA complexes contribute to more efficient and sustainable diets in RAS systems	Planned 24/09/25 – Aquaculture Europe '25 (Valencia, Spain)	Claudia Silva, Zinpro
AE3.0 TNA 22213	Established benchmark for Atlantic bluefin tuna (*Thunnus thynnus*) weaning with commercially available diets in Europe	Planned 24/09/25 – Aquaculture Europe '25 (Valencia, Spain)	Paul-Daniel Sindilariu, Nexttuna
AE3.0 TNA PID23949	Novel use of bio-loggers in characterisation of European Seabass (*Dicentrarchus labrax*) and identification of four welfare states	Planned 24/09/25 – Aquaculture Europe '25 (Valencia, Spain)	Joaquim Tomàs Ferrer, University of Balearic Islands
AE3.0 TNA 27738	Use of Acoustic Doppler Current Profilers (ADCP) echosounding to characterise fish growth and behaviour	Planned 24/09/25 – Aquaculture Europe '25 (Valencia, Spain)	Thomas Culverhouse, Sonardyne

OUTPUT identificator	OUTPUT title	Date and place of OUTPUT presentation	OUTPUT owner
AE3.0 JRA D4.3	New methods for post processing biotelemetry data in aquaculture	Planned 19/08/25 - Aqua Nor (Trondheim, Norway) and 24/09/25 – Aquaculture Europe '25 (Valencia, Spain)	Martin Føre, NTNU

3.2. Knowledge Output evaluations by the IRAP

The OUTPUT scoring summary table (example provided in Table 4) summarise how the OUTPUTs were evaluated by the IRAP, each column corresponding to an individual OUTPUT and each row showing the score set by an individual IRAP member. The last two rows show the total score and the number of highest "3" scores per OUTPUT, respectively. The references of the OUTPUTs assessed by the IRAP as High-Impact Outputs can be found in the summary register (Table 3).

Table 4: Example of table to summarise the scoring of the OUTPUTs prior to the IRAP meetings (identity of the OUTPUTs are hidden)

Knowledge Output Reference Number																
3	2	2	3	2	3	2	2	2	2	2	2	2	2	n/a	2	2
0	0	1	0	0	2	1	3	1	2	0	1	1	1	1	1	2
1	0	2	3	2	2	1	3	2	1	0	1	1	0	3	3	2
1	1	3	2	3	3	3	3	2	3	3	3	2	1	2	3	3
1	3	3	1	2	3	2	1	3	0	0	2	2	2	2	1	1
n/a	n/a	2	2	2	2	2	2	2	n/a	n/a	n/a	n/a	n/a	2	n/a	1
1	1	2	1	2	1	2	1	2	2	1	1	1	0	1	2	2
1	0	1	1	0	2	2	2	2	3	2	0	0	0	2	1	2
0	0	2	1	2	2	2	2	1	n/a	1	n/a	n/a	n/a	1	1	1
1	1	1	1	2	2	3	2	2	3	1	2	1	2	2	3	2
1	1	2	1	1	2	3	1	2	2	2	0	0	0	0	2	3
10	9	21	16	18	24	23	22	21	18	12	12	10	8	16	19	21
1	1	2	2	1	3	3	3	1	3	1	1	0	0	1	3	2

3.3. Detailed description of the selected High Impact OUTPUTs

The OUTPUTs selected by the IRAP as High-Impact Outputs are described more in detail in this section. They are listed in chronological order in the way they have been presented to and evaluated by the IRAP during the course of the AQUAEXCEL3.0 project.

3.3.1. AE2020_SINTEF_3

Virtual Aquaculture Laboratory to assist in optimizing the use of experimental resources and improve experimental design and test power.

OUTPUT Type: JRA (AQUAEXCEL[2020])

OUTPUT Owners: Finn Olav Bjørnson, SINTEF Ocean (NO)

Status: The website is available for use: https://ae2020virtuallab.sintef.no/ (one needs to create a user account). **TRL:** 7

IPR protection: No

Contextual Information: As part of the previous AQUAEXCEL project, standardised guides and new tools for aquaculture research were developed, including a dedicated e-infrastructure which can support both actual and virtual research experiments. Based on this, a virtual aquaculture laboratory was developed which can simulate experiments with fish and receive data on expected growth and water quality.

OUTPUT Description: The overall goal of the virtual aquaculture laboratory is to assist in optimizing the use of experimental resources and improve experimental design and test power. The virtual laboratory website allows a user to specify an experimental setup, including fish, tanks, and water treatment. Based on numerical modelling, it allows in silico testing of experimental protocols with a user-friendly interface, prior to their practical application. The user can simulate an experiment and get key statistics on whether the experiment is viable or not, such as data on expected growth and water quality. The website is meant to be an easy entryway to practitioners as well as academics.

EATiP Thematic Area:
- TA2- Technology & Systems
- TA6 - Knowledge Management

EATiP Goal:
- TA2- G4: Ensure technology for ethical and healthy production of high-quality products.
- TA6 - G1: Manage knowledge efficiently and effectively within European Aquaculture sector

Next steps: Virtual lab V3 to be developed in AQUAEXCEL3.0

Application:
- Demonstrate experiments setups and results.
- Optimize production equipment.
- Testing new models, testing experimental setups

Potential End Users:
- Teachers and students carrying out fish experiments.
- Aquaculture farmers, Aquaculture equipment industry
- Researchers on fish growth and water quality.

Potential Impact:
- Increased quality in experimental design

Evaluation Summary:
Total score: 25
Number of 3s: 6

3.3.2. AE170011

Dietary effects on growth, survival and behavioral responses in lumpfish (Cyclopterus lumpus L.)

OUTPUT Type: TNA

OUTPUT Owners: Ibon García Gallego, University of Las Palmas de Gran Canaria (ES)

Status: The output is still being generated. **TRL:** undefined

IPR protection: No

Contextual Information: Deployment of lice-eating cleaner fish in salmon cages is considered one of the most effective and sustainable methods to control sea lice infestations. The lumpfish (*Cyclopterus lumpus*) is the most used and commercially produced species of cleaner fish in Norway. Nevertheless, the Norwegian lumpfish production is currently characterized by variable growth and survival during the larval stages. Little is still known about nutritional requirements and the functional behaviour during development of lumpfish larvae. How larval and juvenile feeding behavioral responses are affected by the nutritional quality of the first feed is also not well known, and this may be critical for larval quality and the juvenile's capacity to eat the lice off the salmon when it is transferred to the sea cages.

The aim of the study leading to this Knowledge Output was to describe and evaluate the ontogenetic development (physical, cognitive, emotional, and social development that can be attributed to experiences with the environment) of feeding behavioural responses in lumpfish larvae from hatching to 33 days post hatch (dph), in relation to larval size and nutritional quality of the first feed, based on growth, survival, swimming and feeding activity. Larval groups received five different dietary treatments: 1) formulated diet, 2) preserved plankton (experimental frozen Cirripedia from Planktonic AS), 3) enriched *Artemia*, 4) copepods (*Acartia tonsa*) (from CFEED AS) and 5) a combination of *Acartia tonsa* and Cirripedia (2-22 days post hatch). The larvae were fed formulated diet either for the full period or weaned from *Artemia* and Cirripedia nauplii to the formulated diet between 20-22 days post hatch. All groups were fed the same formulated diet until the experiment ended at 33 days post hatch. All feeding behaviour observations were recorded by video. At 16 dph, 22 dph and 28 dph, 10 larvae from each tank were randomly sampled into individual 1litre plastic bottles and starved for 3 hours. After 3 hours an amount of live prey (350 Cirripedia/ml + 350 *Artemia* nauplii/ml) were added. For 2 minutes larvae were recorded. The parameters observed during the records were: number of seconds each larva swam, number of second attached to a substrate, orientating itself in the tank, number of successful and unsuccessful attacks, type of attack performed (swimming or attacked) and number of seconds of the swimming attacks.

Knowledge Output Description: This Knowledge Output concerns novel information on feeding behaviour of lumpfish larvae. Feeding behaviour was found to be different both through the ontogeny development as well as among the treatments, especially at 22 days post hatch (dph) and 28 dph. Lumpfish larvae showed more swimming activity at 16 dph compared with 22 dph and 28 dph. Larvae fed more at 22 dph and 28 dph and they used more time for the swimming attacks at 28 dph. Larvae fed on *Artemia* were more active swimmers and they also orientated more. However, at 28 dph, Cirripedia treatment larvae showed more swimming activity and performed more successful attacks compared with the other treatments ($P < 0.05$). Lumpfish larvae from copepods and dry feed treatments swam less, spent more time attached and less time orientating. Also, they performed a smaller number of successful attacks ($P < 0.05$) showing a lower predatory instinct.

Both species of live feed seemed to be attractive for the lumpfish larvae. Regarding the other parameters measured, *Artemia* treatment resulted in significantly better growth and survival than other diets. *Cirripedia* diet resulted in initial slow growth, catching up with *Artemia* group after weaning to formulated feed. Commercial diet presented low growth and poor survival compared to other diets.

Feeding enrichment *Artemia* during the start feeding resulted in better results based on growth, survival, and swimming and feeding activity of lumpfish larvae than other treatment diets. However, after the weaning period those lumpfish larvae fed *Cirripedia* showed similar growth and more successful attacks. The difference in feed quality of the diet treatments during the 33 dph start feeding of lumpfish larvae, affected the larvae behaviour activity and performance. The results of this study indicate a high correlation between live feed quality and the effects on growth, survival and feeding behavioural responses in lumpfish larvae, suggesting the high importance of early nutritional quality on the larval feeding behaviour, which is currently not well known in lumpfish.

EATiP Thematic Area: TA3 - Managing the Biological Lifecycle.

EATiP Goal: TA3 - G4: Manage life cycle of carefully selected "new" species that have high economic importance.

Next steps: We need to wait for the biochemical analysis of the experimental diets to link the nutrient composition of them (especially lipids and protein) with larval growth and feeding behavioral results. Further research is needed to see if the experimental diets may have effects on the quality of juvenile lumpish and consequently for adult fish.

Application:

- These results may be useful for the Norwegian lumpish hatchery and the general lumpfish aquaculture industry.
- The findings of this study should be useful for improving the knowledge of marine science and fisheries in general in cold regions like Norway since lumpfish early life stages knowledge is still limited.

Potential End Users:

- Aquaculture lumpish hatchery industry
- Scientific community working with lumpfish.

Potential Impact:

- The results increase our understanding of effects of diet quality and growth on vital parameters for survival such as feeding behavioural responses. This may in turn give better clues for how to improve the welfare and survival of lumpfish in the salmon cages.
- Contributing to new research ideas and approaches in academic and the scientific community

Evaluation Summary:
Total score: 21
Number of 3s: 4

3.3.3. AE170007

ADVENTURE: A new fish feeding control and optimization method for gilthead seabream

OUTPUT Type: TNA

OUTPUT Owners: Tao Chen, University of Surrey (UK)

Status: More research is needed **TRL:** 3

IPR Protection: Unsure

Contextual Information: In intensive aquaculture fish feed accounts for a significant portion of the total production cost. Excess feeding causes not only food waste and consequent loss of income but also serious water pollution.

Knowledge Output Description: This Knowledge Output concerns a novel decision-making method for optimal feeding of gilthead seabream by integrating a fish weight prediction model and a fish feed requirement analysis model. In particular, an adaptive optimization method is formulated to maximize the fish weight at harvest, which is subject to the specific feed requirements and fish growth characteristics.

The method was constructed based on a computational modelling study, supported by historical data from HCMR and the literature. The fish weight prediction model and the fish feed requirement analysis model are currently parameterized for the gilthead seabream because sufficient experimental data were available for this particular fish species. Simulation results showed the effectiveness of the proposed method by comparing different feeding situations and demonstrated that the method can indeed lead to better performance and improved aquaculture productivity. The following computational models were carried out:

1. **Fish weight prediction model:** A bioenergetic and protein flux (EP) model was used to simulate fish growth process in this project. It explicitly couples the energy and protein fluxes and translates those into individual weight gain and proximate composition simulation over time. Feeding decision, metabolism requirements and growth requirements are used to obtain the energy gain and protein gain. The fish weight at any given point is predicted based on fish composition including protein, lipids, water and ash.
2. **Fish feed requirement analysis model:** Feeding decision was made based on feed composition, feed digestibility for energy and protein, energy requirement and protein requirement.
3. **Feeding control and optimization method:** The feeding control and optimization method was proposed by integrating the fish weight prediction model and the fish feed requirement analysis model. The optimization process is described as follows: 1) the current feeding decision was determined by the fish feed requirement analysis model based on the current fish weight; 2) the fish weight at next step was predicted by the fish weight prediction model based on the current fish weight and feeding decision; 3) the input of requirement analysis model is updated by the prediction result of fish weight.
4. **Adaptive optimization method:** In a practical process, it is difficult to express the practical fish growth relationship accurately with the fish weight prediction model because of some ideal assumptions and environmental noises. When the practical fish weight was measured for a specific period, the fish weight error between measurement and prediction was obtained, which was then used to adjust the fish feed requirement analysis model to update feeding decision in the next measurement period. The initial fish weight at next step was updated with the measurement of fish weight.

Simulation results: The fish weight prediction model and the fish feed requirement analysis model were parameterized for gilthead seabream. The measurement period of practical fish weight was set as 28 days while the simulation time was 392 days. The following five feeding decision-making methods were used to compare the performances of different methods:

1) *Prediction update only*: when the actual, practical measurement of fish weight is available, the actual value is used to update fish weight, which is then used as the new input of the requirement analysis model in the next step.
2) *No feedback*: in this case, the practical measurement of fish weight is not available. The error between fish weight prediction model and practical process is ignored, and no feedback way is applied.
3) *Proposed method:* When the practical measurement of fish weight is available, the practical value is used to update the fish weight, which is then used as new input in the requirement analysis model in the next step. At the same time, the fish weight estimation error between practical fish weight and prediction result is used to adjust the requirement analysis model and update the feeding amount in the next measurement period of practical fish weight.
4) *Proposed method (tm=1):* based on the proposed method, the measurement period of practical fish weight is set as one day, that is to say, the measurement result of practical fish weight in every day is available.
5) *Feed composition optimization:* the feed composition is optimized based on requirement analysis to satisfy the energy and protein requirements of fish. When the designated feed is selected in aquaculture, the nutritional ingredient of food is fixed. The first four feeding decision methods are all under the specific nutritional ingredient in feed. When the feed composition is optimized based on requirement analysis, the energy and protein requirements of fish all can be satisfied.

The simulation results for the different feeding decision-making methods are shown in Table 1.

Feeding decision-making method	Fish weight at the end time (g)
Prediction update only	218
No feedback	220
Proposed method (tm=28)	255
Proposed method (tm=1)	296
Feed composition optimization	314

Table 1 shows that when the feed composition is optimized, based on the requirement analysis, the fish weight at the end of time obtains the highest value. This is because energy requirement and protein requirement of fish are all satisfied. When the current feed composition cannot be changed, if the measurement result of practical fish weight is available for each day (tm=1), the performance of proposed method will be better. Comparing 'Prediction update only' with 'Proposed method (tm=28)', our proposed method can obtain the better performance, because the fish weight error between measurement and prediction is applied effectively to adjust feeding decision. Only by updating the fish weight using the practical measurement, "Prediction update only" cannot eliminate the cumulative error in the optimization process.

EATiP Thematic Area: TA2 - Technology & Systems

EATiP Goal:

- TA2 - G1: Ensure environmentally sustainable industry by applying new knowledge and technology innovations.

- TA2 - G3: Ensure profitability of aquaculture industry by developing improved management systems and technology.
- TA2 - G4: Ensure technology for ethical and healthy production of high-quality products.

Next steps: Due to Covid-19, experiments at laboratory scale could not be implemented. The researchers are keen to apply for further funding to AE 3 or other possibilities to support further research. The researchers think there is the possibility to develop a Feed application program (Feed App).

Application: Feed application program (Feed App) for aquaculture would provide fish weight prediction and feeding decision based on existing production information including the energy and protein composition in feed, the apparent digestibility coefficient of feed, water temperature, initial fish weight and so on. Offline measured fish weights are collected and reserved to update the requirement analysis model and adjust feeding decision.

Potential End Users:

- Researchers
- Aquaculture producers, fisher manufacturers, engineers

Potential Impact: Less negative environmental impact as a result of decrease in water pollution. Improved economic impact since t feeding decisions and fish weight at harvest time can be optimized leading to improved economic profit.

Evaluation Summary:
Total score: 19
Number of 3s: 3

3.3.4. AE160012

AQUAWASTE: Improving plant health through Nutrient Remineralization in Aquaponic Systems

OUTPUT type:	**TNA**
OUTPUT owner: Victor Lobanov, University of Gothenburg (SE)	
Link: https://www.frontiersin.org/articles/10.3389/fpls.2021.683690/full	
IPR Protection: No	
Status: Complete – published	

Knowledge OUTPUT Description:

Aim: to examine the applicability of aquaponics as a value addition to freshwater RAS, by simultaneously exploring aquaponics as a fertilizer production system as well as a waste treatment system. The study also explored the concept of crop quality with respect to mineral nutrients.

Knowledge Need: Aquaponics is a food production system that works according to circular economy where nothing is aimed to be wasted. It couples aquaculture with hydroponics (cultivating plants soil-less) whereby the nutrient-rich waste water from the fish side is used as nutrient solution to grow plants. The nitrifying bacteria present in the system convert ammonia, which is toxic for the fish, into nitrates, a more accessible nutrient for plants. The exploitation of readily bioavailable fish excreta as a source of plant nutrients lies at the cornerstone of aquaponics farming. Research on nutrient cycling in aquaponic systems has devoted considerable attention to the plant uptake of dissolved nutrients in fish excreta, however, the integration of particulate-bound nutrients into downstream hydroponic farming has remained elusive. The high amount of organic carbon present in fish sludge may lead to biofouling if directly incorporated into hydroponic circulation systems, reducing the utility of incorporating fish solids on a large scale. There is a need to assess the capacity of an aquaponics system to target mineral nutrient bioavailability in downstream agriculture.

Results: Ultimately, the data lead to two important conclusions. Firstly, optimizing nutrient bioavailability is not synonymous to increasing the presence of a nutrient in the water column. Secondly, estimating ideal nutrient solution concentrations involves both preventing nutrient blocking and improving bioavailability.

Innovation: This study is the first to assess the capacity of an aquaponics system to target mineral nutrient bioavailability in downstream agriculture through an in-line solids treatment system.

EATiP Thematic Areas

TA3 - Managing the Biological Lifecycle

EATiP Goals

TA2 - G3 : Ensure profitability of aquaculture industry by developing improved management systems and technology

Industry End Users, Applications and Impact:

	Potential Application	Potential Impact
Next (Target) User 2: Industry stakeholders **E.g:** Aquaponic equipment producers	**Medium to long term**: Once there is a body of scientific evidence around optimal aquaponic systems, aquaponic equipment producers could take up this knowledge to produce more and improved systems.	Availability of more and improved aquaponic systems will have a potential impact on more uptake of these systems by farmers. This would lead to increased crop productivity while providing an environmentally friendly waste treatment solution for land-based aquaculture.
Next (Target) User 3: Industry stakeholders **E.g:** Land-based aquaculture farmers; plant producers / conventional garden centers	**Medium to long term:** The technology developed is a low-cost, low-environmental impact solid waste treatment system for freshwater recirculating aquaculture systems and thus could be applied by land-based aquaculture farmers or plant producers / garden centres to any existing or new facility.	Potential impact would be increased crop productivity while providing an environmentally friendly waste treatment solution for land-based aquaculture.
Next (Target) User 4: Industry stakeholders **E.g:** Fertiliser producers	**Medium to long term:** The results could potentially also be taken up by fertiliser producers, incorporating the learnings to produce ideal nutrient solution concentrations and so optimise nutrient bioavailability to the plants	Potential impact would be increased crop productivity while providing an environmentally friendly waste treatment solution for land-based aquaculture.

Evaluation Summary
Number of 3's: 2
Total score: 23.5

3.3.5. AE110004

RMS: Red Mark Syndrome in rainbow trout: advances in diagnostics and management strategies (2 Knowledge Outputs)

OUTPUT type:	**TNA**
OUTPUT owner: Massimo Orioles, Universita degli studi di Udine (IT)	
Link: (1) OUTPUT1: DOI: 10.13140/RG.2.2.12157.69609 (2) OUTPUT2: http://dx.doi.org/10.1016/j.aquaculture.2022.737910	
IPR Protection: No	
Status: The primary OUTPUT (effect of temperature) is finalised and ready to be used in further studies and practical knowledge. The author is working/researching on MLO DNA detection from water and other possible sources using the results of secondary OUTPUT. The secondary OUTPUT (ddPCR) falls within TRL 4 and is available for further studies.	

Contextual information:
Aim: to get insight into whether the development and application of a droplet digital PCR (ddPCR) – as a relatively novel, sensitive, and accurate quantification technique – could help to detect extremely low concentrations of MLO DNA, and so red mark syndrome infection in rainbow trout (*Oncorhynchus mykiss*), without the need for standard curves. This way, this methodology might support further research on RMS by providing a powerful tool to detect the presence of MLO in the environment (through eDNA detection and quantification). In addition, the research also aimed to get an insight into disease progression at different temperatures, which is unclear so far.

Knowledge OUTPUT Description:
OUTPUT 1: Knowledge need in relation to disease progression at different temperatures. **Innovation:** For the first time this research got an insight into disease progression of red mark syndrome infection in rainbow trout (*Oncorhynchus mykiss*) at different temperatures under controlled experimental settings. Furthermore a new PCR diagnostic method was developed (digital droplet PCR). **OUTPUT 2: Knowledge need in relation to development and application of a ddPCR to help detect extremely low concentrations of MLO DNA, in rainbow trout (*Oncorhynchus mykiss*)** **Innovation:** this research is the first research that established a successful ddPCR method for the detection and absolute quantification of the RLO associated with RMS with a higher level of sensitivity compared with qPCR.

EATiP Thematic Areas
TA7- Aquatic Animal Health and Welfare

EATiP Goals
TA7 - G1: Improve fish health and welfare by increasing the understanding of host pathogen interactions and to have access to effective vaccines and immunomodulators
TA7- G2: Application of epidemiological principles to minimise the threat of existing, emerging and exotic diseases
TA7 - G3: Use and develop best practice to optimise efficacy of treatments and prevention methods

End Users, Applications and Impact:

	Potential Application	Potential Impact
Next (Target) User: Industry stakeholders **E.g:** ddPCR equipment companies	**Medium to long term:** Once the ddPCR technology is completed and validated at scientific level, equipment companies can take up the technique and bring it into commercial production for use as a diagnostic tool at farm level and in surveillance programmes. The timeline to get ddPCR technique widely used would depend on further studies on MLO as the real etiology of RMS and MLO DNA presence on the environment.	Potential impact of implementation of a successful diagnostic tool for this disease would be a faster and improved detection of the disease, which would support reduced disease prevalence (if in combination with treatment) and eventually higher productivity of rainbow trout industry worldwide
End User: Industry stakeholders **E.g:** trout farming sector through the collaboration of field veterinarians/experts.	**Medium to long term:** - **OUTPUT 1 (data on temperature effect)**: once the ideal temperature is known for management of the disease, farmers can take up these results to implement at farm level - **OUTPUT 2 (ddPCR):** using the improved technique at farm level and in surveillance programmes (this technique can be used both by European and nationals reference laboratories, such as European Union reference laboratory for fish and crustacean diseases (DTU Aqua – Technical University of Denmark, National institue for Aquatic resources), CEFAS (UK) and IZS Venezie (Italy).	- **OUTPUT 1 (data on temperature effect)**: implementation of a temperature regime to manage disease is more environmentally friendly than e.g. using antibiotics, and might be more effective too. - **OUTPUT 2 (ddPCR):** Potential impact of implementation of a successful diagnostic tool for this disease would be a faster and improved detection of the disease, which would support reduced disease prevalence (if in combination with treatment). At farm level this would lead to higher productivity and profitability.

Evaluation Summary:
Number of 3's: 3
Total score: 26

3.3.6. AE130020

SYLPRO4TROUT: use of Wood-Based Yeast SCP (single-cell protein) as an ingredient for Trout diets

OUTPUT type:	TNA
OUTPUT owner: Ricardo Ekmay, Arbiom Inc. (USA)	
Link: The output is intended to be published in a peer-reviewed scientific journal.	
IPR Protection: No	
Status: The trial conducted here is a piece of a larger body of work that is needed to fully support the adoption of SCP from lignocellulosic biomass. As such, this work can be considered preliminary and additional work is needed. Crucially, the positive results of the assessments within this TNA lend support towards continued work.	

Knowledge OUTPUT Description:

Aim: The main objectives of this work were to determine the nutrient digestibility of yeast Single Cell Protein (SCP) cultivated on wood, and to determine its efficacy in replacing animal and terrestrial plant proteins in a feed formulation for Rainbow trout.

Knowledge need: To ensure nutritional adequacy of an alternative protein for production animals, and to limit effluent production, dedicated trials determining nutrient bioavailability are required. Further, there is a need to determine which incumbent proteins (e.g., animal protein, plant protein) are suitable for replacement by alternative counterparts. Single cell protein (SCP) products, protein meals based on microbial or algal biomass, have the potential to fulfil this need. Lignocellulosic biomass is the most abundant, renewable carbon source that can be used for the production of proteins. Therefore, one of the more promising alternative proteins is a single-cell protein (SCP) cultivated on hydrolysed lignocellulosic biomass. The implementation of alternative protein sources in aquaculture requires in-depth evaluation of their nutritional adequacy.

Main results: The results indicate that SCP has equivalent protein digestibility to fishmeal, superior digestibility to plant proteins, but lower dry matter and energy digestibility. The dose-response trial indicated that SCP may replace either fishmeal or plant proteins and make up to 20% of the entire diet of the fish. The plant-based only diets had significantly lower specific growth rate, feed conversion ratio, and daily feed intake compared with the other four diets. No significant differences were observed for hepatosomatic index and viscerosomatic index across treatments. No statistical differences were observed relating to growth performance between any diet containing SCP and the fishmeal reference diet. Evidence of immune stimulation was observed with the inclusion of SCP, and the effects were more pronounced in the plant-based diets. Results indicate that SCP is suitable for the replacement of fishmeal in the diets of Rainbow trout in terms of nutrient digestibility and growth performance. Better performance was observed in plant-based diets devoid of fishmeal when SCP was included. These results contribute to the broader understanding of SCP as an alternative protein source for aquafeeds.

Innovation: The composition and nutritional performance of early iterations of SCP did not support its use in salmonid species such as salmon or trout. Trout is among the more valuable fish species for the EU. In order for SCP to be used as a tool for reducing the environmental footprint of aquaculture, the protein source must be suitable for replacement of incumbent proteins without detrimental effects to growth performance. Further, a deeper understanding is needed of the limitations of using SCP in trout diets. The results presented here run contrary to the early iterations of SCP and support the use of yeast SCP as a protein source for trout

diets. It revealed the potential of yeast SCP to improve upon fishmeal-free diets to mimic fishmeal-containing diets. Further, they revealed immunostimulatory benefits that may have tangential benefits upon fish health.

EATiP Thematic Areas
TA4 - Sustainable Feed Production

EATiP Goals
TA4 - G1: Base formulation of Future Fish Feeds on solid knowledge of fish nutritional and feeding requirements, and expand the number of well characterized and sustainable raw materials which can be used
TA4 - G3: Understand and minimise non desired effects of alternative diets on fish health and welfare

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User 2: Industry stakeholders E.g: Trout and salmon aquafeed manufacturers	**Medium to long term**: Once the use of SCP as protein source in aquaculture feed has been validated, then trout and salmon aquafeed manufacturers can use the output to prioritize alternative ingredients within their internal feed development programs. The decision to investigate internally can be applied quickly. Implementation of SCP is likely to be medium to long-term.	Availability of alternative protein products in the aquafeed market is expected to lead to uptake by fish farmers. The alternative protein market in Europe is expected to grow, leading to less dependence on non-European products and greater competitiveness at a global level. Creation of jobs in Europe
End User: Industry stakeholders **E.g:** aquaculture farmers	**Long-term:** Fish farmers can take up the novel protein products to use in their farms	The implementation of SCP into aquafeeds will have significant impacts on the environmental footprint of aquafeeds.

Evaluation Summary:
Number of 3's: 4
Total score: 29

3.3.7. AE100025

SWIM: Testing of the Salmon Welfare Index Model (SWIM 1.0) as a Computational Welfare Assessment for Sea-Caged European Sea Bass (Dicentrarchus labrax)

OUTPUT type:	**TNA**
OUTPUT owner: Hijran Yavuzcan, Ankara University (TUR)	
Link: Publication in a SCI Journal (Q2-Italian J of Animal Sciences) Full article: Testing of the Salmon Welfare Index Model (SWIM 1.0) as a computational welfare assessment for sea-caged European sea bass (tandfonline.com)	
IPR Protection: Unsure	
Status: According to the OUTPUT owner it is TRL9, but we think it is probably at TRL6 as it has been validated / demonstrated in a relevant environment now, but still needs further refinement and is not operational at farm level yet.	

Knowledge OUTPUT Description:
Aim: to test the semantic model previously developed for salmon (SWIM 1.0) to assess the quantitative welfare status in European sea bass (*Dicentrarchus labrax*). **Knowledge Need**: Even though the production of European sea bass and gilthead sea bream (*Sparus aurata*) represents more than 95% of the total fish production in the Mediterranean region (EUMOFA 2017) and welfare is considered as an important element of a sustainable aquaculture industry, so far there has been no overall strategy aiming at achieving OIE welfare standards in E. sea bass aquaculture. The welfare of European sea bass in sea cages was not numerically evaluated until now. Also, fish welfare is a complex and multidimensional topic, leading to difficulties in the overall assessment requiring a multicriteria evaluation. Currently, there is no gold standard for fish welfare assessment and the assessment of fish welfare in various aquaculture conditions is challenging. **Main results:** Despite some constraints, the model seemed functional to evaluate the welfare level of European sea bass in on-farm conditions, and the approach taken can be used to identify risks to fish welfare and the reasons for poor welfare. However, further studies on the versatility of modelling and harmonised standard to assess fish welfare are required. The efficacy of the model application can be increased by frequent fish sampling. **Innovation:** This study is the first to benchmark overall sea bass welfare in sea cages, which will support the future implementation of improvement strategies, both at policy, scientific and farm levels.

EATiP Thematic Areas
TA7- Aquatic Animal Health and Welfare

EATiP Goals
TA7 - G4: Measure welfare/stress and understand its consequences if compromised in order to incorporate welfare as core components of production management
TA6 - G1: Manage knowledge efficiently and effectively within European Aquaculture sector

Industry End Users, Applications and Impact:

	Potential Application	Potential Impact
End Users: Industry stakeholders **E.g:** 1. System / tool commercial companies who can convert the scientific model into a practical commercial system that can be applied at farm scale (TRL 9) 2. Fish health specialists (vets, etc), sea bass farm managers / owners	**1. Medium to long term**: System / tool companies can take the scientific model forward, converting it to a practical, commercial tool (e.g. mobile phone application) that can be applied at farm level (TRL9), by non-scientists **2. Medium to long term:** once the model has been fully validated and converted into a practical system at TRL9, then fish health specialists and/or farm managers can implement it at farm-level to regularly monitor fish welfare	Once the scientific model has been converted to a practical farm-level tool, and if fish health specialists and/or farm managers implement the model/system at farm-level to regularly monitor fish welfare, they will quicker find welfare issues and be able to address them, leading to better fish welfare and so increased productivity, more profits and higher supply of healthy fish products to the European market.

Evaluation Summary:
Number of 3's: 9
Total score: 31

3.3.8. AE150010

TRIPLOID SALMON: Sal-ploidy: Triploids salmon and salinity

OUTPUT type:	TNA
OUTPUT owner: Raneesha De Fonseka, University of Gothenburg (SE)	
Link: https://doi.org/10.1016/j.aquaculture.2021.737350	
IPR Protection: unsure	

Knowledge OUTPUT Description:

Aim: The hypothesis of the research behind this output was that triploidy increases the amount of energy required for osmoregulation, leading to a decrease in energy available for growth. The purpose of this study was to explore the growth of triploids under various salinities (0.2 ppt, 11 ppt, 23 ppt, and 35 ppt) in order to assess whether triploidy impairs salinity tolerance as this remains relatively untested but is an essential aspect of production biology.

Knowledge need: Genetic introgression between domestic and farmed fish is a major environmental concern. The most effective method of preventing genetic introgression between wild and farmed salmon would be to farm sterile fish. To date, the only method available to produce large numbers of sterile fish is triploidisation. However triploid performance in still relatively inconsistent. There is evidence that triploids are working harder to maintain homeostasis than diploids, albeit the cause remains unknown. One possibility is that triploidy affects the amount of energy used for ion regulation and therefore increases standard metabolic rates leaving less energy available for growth. However, to date, no study has compared diploid to triploid growth performance over varying salinity gradients that theoretically require different degrees of energetic costs to maintain. As there is an urgent need for sterile fish within salmon aquaculture to improve its sustainability, and triploidy remains the only current viable option, understanding triploid environmental optima is essential.

Main results: In summary, the research results show some mismatches in smoltification markers in underyearling triploid salmon. However, triploid post-smolts performed as well or better than diploids at all salinities tested but may have a lower salinity optimum than diploids. We found evidence that triploids can be produced without any increase in the prevalence of vertebral deformities. Finally, salinity had no impact on the prevalence or severity of cataracts in post-smolts.

Innovation: Sterile triploid Atlantic salmon (*Salmo salar*) show inconsistent seawater grow-out, but the reason why remains unclear. The purpose of this study was to determine for the first time the salinity optima of triploid post-smolts and how to optimise their growth.

EATiP Thematic Areas

TA7- Aquatic Animal Health and Welfare

EATiP Goals

TA7 - G4: Measure welfare/stress and understand its consequences if compromised in order to incorporate welfare as core components of production management

End Users, Applications and Impact:

	Potential Application	Potential Impact
End User: **Industry stakeholders** **E.g:** Atlantic salmon farmers, breeders, nutrition companies, etc.	**Medium to long term**: Results can inform breeding and husbandry strategies. Results can be used by broodstock managers to create a triploid stock that can be used by the farming industry. The results may be of particular interest to those growing salmon in RAS systems throughout life (to avoid pre-harvest sexual maturation) as the greater environmental control may make it easier to provide triploids with the optimal conditions, they need to realise their true growth potential.	The potential impact of using (more) triploid stock will be reduced environmental footprint by avoiding the hybridization of the domestic stock with the wild one and realising a far better growth potential of the salmon sector.

Evaluation Summary:
Number of 3's: 4
Total score: 24.5

3.3.9. AE140007

FISHSCAILED: Non-invasive fish identification - scale patterns

OUTPUT type:	TNA
OUTPUT owner: Dinara Bekkozhayeva, University of South Bohemia (CZ)	
Publicly available: Paper submitted (will be public upon acceptance)	
IPR Protection: No	
Status: The method could be immediately used for research purposes as a substitute for invasive fish tagging. Based on the results, a scientific publication has been developed which is currently under review in the Aquaculture Reports journal. In terms of TRL, the technology has been validated to be at TRL 4 (technology validated in lab).	

Knowledge OUTPUT Description:

Aim: to show that automatic photo-based identification is possible based on the patterns of the scale position without any other distinctive features.

Knowledge Need: Identification of individual fish of the same species within a cultivated group in an aquaculture system is important for aspects such as individualized treatment, biomass estimation and determination of the health status of the fish. Currently, there are very limited options for the real-time identification of individuals under real conditions (in tanks and cages). The standard approach to the identification of individual fish of the same species is tagging, which has several disadvantages and limitations, such as being traumatic for the fish (because it is an invasive method), increasing mortality and injury, being a time consuming procedure, applicability to limited fish sizes, and the need to catch fish for tagging and identification. A few studies have shown that fish body patterns can be used for individual identification through non-invasive monitoring in the form of automatic photo-identification, in particular for fish species with dot skin patterns, such as salmonids. There is a need to extend non-invasive identification methods to fish species without obvious skin patterns, such as the European seabass *Dicentrarchus labrax*. The aim of this study was to verify the possibility of using fully automatic non-invasive photo-identification of individual fish based on natural marks on the fish's body without any obvious skin patterns.

Results: The accuracy of classification was 100% in both the short-term and long-term experiments. According to these results, the methods used for automatic non-invasive image-based individual-fish identification can also be used for fish species without obvious skin patterns. Different parts of the body, mainly parts featuring scales and the patterns on the operculum of the fish, were tested for identification. The study showed that the chosen patterns can be used for long-term identification, except for patterns on the operculum. The uniqueness of scale patterns was proven, together with their stability during the cultivation period (in our case, the longest period was four months). Our approach reveals that even a fish without obvious patterns on its body (only scale body) could be used for the automatic non-invasive identification of individual fish.

Innovation: This study was the first study to investigate a non-invasive monitoring technique for photo identification of individuals of European seabass *Dicentrarchus labrax,* as an example of fish species without obvious skin patterns. Photo identification is thus a possible substitute for commonly used invasive fish tagging identification methods.

EATiP Thematic Areas
TA1 - Product, Quality, Consumer Safety & Health
TA2 - Technology & Systems
TA7- Aquatic Animal Health and Welfare

EATiP Goals
TA1 - G1: Maximise health benefits of products
TA2 - G3 : Ensure profitability of aquaculture industry by developing improved management systems and technology
TA7 - G3: Use and develop best practice to optimise efficacy of treatments and prevention methods

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User 2 **Industry stakeholders** **E.g:** Photo & monitoring equipment industry	Once the technology is fully proven and validated, photo & monitoring equipment industry can take up the results to develop commercial scale professional equipment that can be used by fish farmers at farm level, without the support of scientists to e.g. interpret the results.	The potential impact when there is professional equipment available, is take up by fish farmers, which would increase sales and profits for the photo & monitoring equipment industry which could lead to further improved systems for the aquaculture industry overall.
End User 1: **Industry stakeholders** **E.g:** Fish Farmers	Once the technique is commercially available, fish farmers could take up the technique to use it for automatized fish identification working inside the tank/cage which will enable to study fish behaviour or appearance changes of the individual fish. Identification based on fish appearance will eliminate the stress during the tags application and fish sampling for identification. The identification based on fish appearance will enable the traceability from the fish cultivation till fish processing without the usage of any tags.	The main impact is the elimination of invasive fish tagging; therefore it will improve fish welfare. The non-invasive approach can save money and significantly reduce fish manipulation. The individualization of fish treatment which will lead not only to the optimization of feeding, real-time disease detection and precise biomass estimation but also it will be suitable substitute to invasive tagging methodology. Furthermore, it will enable fish farmers to implement Eco-intensive farming to improve the health and welfare of animal and reduce the putative adverse effects ultimately. It will have a positive impact on the profitability of the aquaculture industry by developing improved management systems and technology.

Evaluation Summary:
Number of 3's: 2
Total score: 22

3.3.10. AE3.0 TNA 20341

KRILL MEAL: Benefit of krill meal inclusion on enhancing the growth of juvenile gilthead seabream by significantly reducing the feed conversion ratio (FCR)

OUTPUT type:	TNA
OUTPUT owner: Kiranpreet Kaur, Director R&D, Aker BioMarine Antarctic AS (NO)	
Publicly available: Not yet, but will be. The authors are in the process of writing a manuscript on the results obtained. The output will be available as a published article within 2023 and additionally, results will be presented at scientific conferences such us Aquaculture Europe, World Aquaculture Society or the International Symposium of Nutrition and Feeding of Fish.	
IPR Protection: No (not applicable)	
Status: The Output is at TRL7, is conclusive enough and there is a justifying body of evidence. There are results on growth performance, and FCR, but the results on oxidative stress are not available yet. The authors are planning to apply for another TNA to validate their results in bigger fish and to look deeper into biological mechanisms involved.	

Knowledge Output Description:

Knowledge need: Feed is the most expensive part of fish aquaculture, attributing to around 60-70% of the production cost. Current fishmeal replacement feed recipes consist of high amounts of plant-based ingredients which are known to have low bioavailability of nutrients. Hence, it is important to produce feeds by including functional ingredients that could enhance the bioavailability and utilization of nutrients by decreasing FCR, which would be beneficial towards improving fish health and performance. In addition, it would enable the industry to save costs by using less amounts of feed to achieve the desired weights of fish.

The aim of this study was to determine the potential of nutrients in krill meal towards improving the FCR in the feeds for juvenile gilthead seabream (10-15g). Further, we *aimed to unravel the genetic pathways* involved through which krill meal would reduce FCR of feeds. Secondly, oxidative stress damage is an important issue among farmed fish, due to their continuous exposure to physical, biological, and chemical stressors. These stressors negatively affect the health and welfare of fish and lead to huge economic losses. We *also aimed to determine the potential of nutrients from krill to reduce the oxidative stress* in juvenile gilthead seabream (10-15g). Besides, we *aimed to evaluate the mechanisms* related to physiological benefits of nutrients package, as a stress reducer by gene expression analysis. We are in the process of analysing these results, which should be available by 2023 summer.

Results: After 12 weeks of feeding, FCR was significantly reduced with 5 and 7% krill meal (4% and 6.5% improved with 5 and 7% KM respectively, in comparison to control) with a relatively higher growth (3.5% higher) with 7% KM than control diet. Besides, the lipid efficiency ratio (LER) was significantly enhanced with all the 3 doses of krill meal (9% higher LER for 3 and 5% KM and 15% higher LER for 7%KM, respectively, in comparison to control diet) and protein efficiency ratio (PER) was enhanced for 5 and 7% krill meal (3% and 7% higher PER for 5% KM and 7% KM, respectively) in comparison to control diet. These results demonstrate that krill meal inclusion leads to better utilization of nutrients in feed, and hence could significantly reduce the amount of feed needed to achieve the desired growth or biomass, which provides economic benefits to farmers. In addition, it is beneficial to the environment by reducing the feed waste. The trial demonstrated the benefit of krill meal inclusion on enhancing the growth of juvenile gilthead seabream by significantly reducing the feed conversion ratio (FCR). The results indicate that krill meal inclusion enables the seabream larvae to better utilize the feed nutrients and hence reduced amount of feed would be needed, which is beneficial for the industry and for the environment. Secondly, the results on the effect of krill meal towards

reducing the oxidative stress are awaited.

Innovation: the researchers are planning to run genetic analysis to understand the effects of krill meal inclusion on reducing FCR, to understand the mechanisms, e.g., the lipid metabolic pathways. Further, they are also aiming to apply for another TNA to run the similar feeding trial on bigger fish (100-150 g) and to extend the feeding up to 300-400g. This would enable them to validate the findings of the current study on bigger fish by studying the effect of krill meal inclusion of activity of digestive enzymes, and how better FCR with krill meal is related to these digestive pathways. In addition, they will run genetic analysis on the effect of krill meal towards reducing oxidative stress both in juveniles from the current trial and in the bigger fish (300g) from the next TNA planned.

EATiP Thematic Areas
TA4 - Sustainable Feed Production

EATiP Goals
TA4 - G1: Base formulation of Future Fish Feeds on solid knowledge of fish nutritional and feeding requirements, and expand the number of well characterized and sustainable raw materials which can be used

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User: Scientific Community **User description:** **Aquaculture / biology /aquafeed related scientists**	Scientists can use the output to improve our knowledge on astaxanthin metabolism and deposition, as well as its protective effect versus exercise induced oxidative stress.	Feed formulations to boost pigmentation and omega-3 levels in salmonids will lead to healthier fish. Nowadays the salmonid farming industry faces two major setbacks: the reduction of health-beneficial LC-PUFA in flesh and the difficulty to colour the flesh of salmonids when feeds with high levels of terrestrial raw materials are used. This translates into fish with lower nutritional value to end consumers. By using a high EPA+DHA oil where the pigment is naturally incorporated, we can address the two aforementioned issues leading to fish with added nutritional value and health promoting compounds for consumers. In addition, EPA+DHA are known to be essential for fish and how high levels can help the fish cope with stressful situations or even infection, while astaxanthin is a powerful antioxidant that also promotes general health and stress resistance in fish.
End User: Industry stakeholders **User description:** **Feed producers**	Feed producers can use the knowledge (once complete) to use the GM-derived oil rich in both EPA+DHA and astaxanthin as a replacement for both fish and vegetable oil in fish feeds.	Results generated from the trial will generate evidence that can be used in the deregulatory process of this oil as a raw material for aquafeeds. Prior to using a novel ingredient as an ingredient, it needs to be approved for its use. As part of the documentation required, the product needs to be tested in several species, and therefore, the information generated from the present TNA will inform on this.

Evaluation Summary:
Number of 3's: 3
Total score: 24

3.3.11. AE3.0 TNA 20680

FEEDREGIUS: Experimental assessment of the fish meal content requirements for Meagre (Argyrosomus regius) feeds

OUTPUT type:	TNA
OUTPUT owner: Ramon Fontanillas, Skretting Aquaculture Innovation (ES)	
Publicly available: there is no publication yet. Potential public presentation / scientific paper can be agreed with the institution	
IPR Protection: No (not applicable)	
Status: TRL 3 – experimental proof of concept.	

Knowledge Output Description:
Knowledge need: Meagre (*Argyrosomus regius*) is a species characterised by distinctly carnivorous feeding habits. Previous studies have shown that juvenile of meagre have a high dietary protein requirement (50%). Previous work has also shown that it is possible to reduce fish meal inclusion in diets for rearing carnivorous fish, but for meagre information so far is scarce, and the reported minimum fish meal inclusion is around 20%. Further research is needed to be able to formulate nutrient well-balanced diets based on the knowledge of alternative protein sources (either plant or land-based animals by products). The **objective of this study** was to evaluate the effects of Fish Meal substitution with different vegetable protein blends on growth performance, voluntary feed intake, feed utilization and health on meagre. **Results:** Results show that it is possible to decrease the fish meal inclusion from 40% down to 15% with either plant protein ingredients or poultry by-products. There were no differences in growth performance or feed utilisation. **Innovation:** To our knowledge it is the first time that this is shown. The output is conclusive to decrease diets down to 15%. Further research to be able to design diets devoid of fish meal would be necessary

EATiP Thematic Areas
TA4 - Sustainable Feed Production

EATiP Goals
TA4 - G1: Base formulation of Future Fish Feeds on solid knowledge of fish nutritional and feeding requirements, and expand the number of well characterized and sustainable raw materials which can be used

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User: Scientific Community **User description:** Aquafeed researchers	Aquafeed researchers can take up the new knowledge on fish meal reduction to further build upon this topic and further research opportunities to become fish meal independent	If researchers further validate and demonstrate the outcomes so it will become conclusive, it will be ready to be taken up by aquafeed producers to start producing commercially

End User: Industry stakeholders **User description:** **Aquafeed producers, meagre farmers**	Results can be implemented in diets for grower meagre, with low levels of fish meal inclusion. This can be implemented in short term	Meagre feed production will benefit of more sustainable diets with less use of marine resources

Evaluation Summary:
Number of 3's: 3
Total score: 23

3.3.12. AE3.0 TNA 130023

METAL AMINO ACID: Metal-amino acid complexes as a cost-effective strategy to help reducing fish meal in European seabass diets

OUTPUT type:	TNA
OUTPUT owner: Claudia Figueiredo-Silva, Zinpro (USA)	
Publicly available: Yes, poster presented at Aquaculture Europe 2021, Madeira, Portugal. Abstract is available in meeting book of abstracts (AE2021AbstractBook.pdf).	
IPR Protection: No. Zinpro have the IP for the technology used in the production of their minerals but they aim to publish and share with the industry their research-based recommendations on how to use their minerals in fish as well as other species feed.	
Status: Completely finalized and ready for immediate take up by the end user: aquaculture industry. Zinpro produce and sell individual Trace Minerals (TM) for the aqua industry. This study allowed Zinpro to communicate to the industry, more specifically to the Mediterranean aqua industry, how to combine the individual minerals in a more cost-efficient way, meaning in a way they can meet the fish TM needs while meeting EU legislation.	

Knowledge Output Description:

Knowledge need: A shift of aquaculture feeds from marine ingredient-based to plant ingredient-based has been suggested to be a likely cause of decreased intake of micronutrients in the forms available to meet the physiological needs of farmed fish. Comparison of the trace mineral (TM) composition of different protein sources to that of fish meal, show significant limitations in Zn, Se and Fe among other nutrients. In addition to a lower content in TM, antinutritional factors such as phytic acid found in many plant meals that are used to replace fish meal (FM), are not digested by fish and have negative effects on availability of minerals (NRC, 2011), making TM needs more difficult to be met. In this regard, stability and the way different supplemental TM are absorbed affects their availability and ultimately animal performance.

Metal-Amino Acid (AA) complexes (a mixture of a single specific metal complexed with different AA in a 1:1 ratio) are taken up by AA-transporters instead of common metal ion transporters, reducing the risk for transport saturation and improving absorption efficiency. Another advantage of using metal-AA complexes instead of metals in their inorganic form is that they are more stable and minimally antagonized by other dietary ingredients like phytic acid. Metal AA-complexes supplemented at half the rate of inorganic sources proved to sustain growth performance, increased number of goblet cells in intestine and skin of European sea bass (*Dicentrarchus labrax*), indicating enhanced barrier defense mechanisms against pathogens. Feeding European sea bass with feeds based on plant-based ingredients replacing dietary FM has been investigated previously by several authors. E.g. replacement of about 80% of FM by a single protein source led to a reduction in fish weight gain and feed efficiency (Dias et al., 2005). Kaushik et al. (2004) demonstrated the feasibility of almost total replacement of FM (up to 5%) by a blend of different plant protein sources when adequately supplemented with essential nutrients. Torrecillas et al. (2017) shown the possibility to reduce FM levels down to 10% without affecting European sea bass juveniles growth and feed utilization.

There is a need to develop more efficient diets for European seabass through supply of TM that are highly available and efficiently meet seabass performance targets and contribute for its welfare status. In a former study, metal AA-complexes supplemented at a half-rate of inorganic sources proved to maintain or even improve performance of European seabass fed 20% FM (which reflects FM inclusion levels practiced in commercial diets).

This study aimed to follow up on the studies conducted previously with European seabass, to evaluate the effect of metal amino acid-complex premix level in the success of reducing FM from 20% down to 10 % on performance and health of European seabass grown from about 100g to 300g (close to harvest size).

Results: Adjusting the mineral premix in FM10, to 1.5 or 2x the level used in FM20, maintained specific growth rate (between 0.74-0.77) and slightly increased whole body Zn content (31 ppm in FM20 vs 33 ppm in diets FM10), though this was not statistically significant. Specific growth rate (0.76 vs 0.74) and feed conversion ratio (FCR, 1.44 vs 1.51) were improved when TM premix was adjusted at 2x vs 1.5x the rate included in FM20. Thus, adjusting the dietary TM content of FM10 to similar levels of that in FM20 may not be enough to sustain seabass performance. Increasing the TM inclusion level from 1.5 to 2x of that used in FM20 improved SGR and FCR by 3 and 5%, respectively. This may partly be explained by likely lower nutrient availability in FM10, because of its higher plant protein content and antinutritional factors, compared to the FM20 diet. Supplementation with metal-AA complexes contributed to a cost-effective reduction of FM from 20 to 10%, while respecting upper EU limits for TM supplementation, and TM content of seabass feeds. The exception was dietary Se content in the feed, that could not be kept at or below 0.5 ppm. Ingredient contribution to dietary Se content in aqua feeds, mainly from FM and other marine ingredients, makes it practically impossible to keep dietary Se levels within allowed EU levels.

Innovation: Results shows that metal-AA complexes allow FM to be reduced from 20 to 10% without significantly affecting growth or FCR, and respecting upper EU limits for TMs in sea-bass diets. This strategy resulted in an 8.5% savings on feed cost, translating into a more sustainable and cost-efficient solution for the industry.

EATiP Thematic Areas
TA4 - Sustainable Feed Production
TA1 - Product, Quality, Consumer Safety & Health

EATiP Goals
TA2 - G1: Ensure environmentally sustainable industry by applying new knowledge and technology innovations
TA4 - G3: Understand and minimise non desired effects of alternative diets on fish health and welfare

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User: Education & Training stakeholders **User description:** **Education and training of Zinpro internal sales teams**	The knowledge generated by this project contributes to the development of Zinpro trace mineral recommendations and its application by the European sea bass industry.	Outcomes of this project are expected to contribute significantly to the development of more efficient diets for European seabass through supply of trace minerals that are highly available and can thus more efficiently meet seabass performance targets and contribute to its welfare status.
End User: Industry stakeholders **User description:** **Industry stakeholder-European Sea bass**	Adjustment of sea bass trace mineral premix by using more bioavailable trace minerals, will allow industry to reduce reliance on expensive and less sustainable raw materials such as fish meal	Application of more bioavailable trace minerals is expected to help European industry meeting upper limits for trace minerals allowed by EU regulation while meeting increasing animal requirements for trace minerals. Application of adjusted TM

farming Industry and overall fish farming	without compromising animal performance.	premixes is thus expected to contribute to a more sustainable and cost-efficient fish production.

Evaluation Summary:
Number of 3's: 3
Total score: 18

3.3.13. AE3.0 TNA 22312

CryoPlankton benefits in seabass aquaculture

OUTPUT type: TNA
OUTPUT owner: Konstantinos Tzakris, Planktonic (NO)
Link to output: The results of the hatchery phase of the culture of seabass are published in a poster in AE2023AbstractBook.pdf (windows.net). The final conclusions of the project are submitted in Larvi 2024 for oral presentation. An article is being written and is aiming to be published in an industry magazine such as "Hatchery feed and Management". The article will be ready for the spring issue. An article for submission in a peer-reviewed journal is scheduled for the end of 2024 and to be published in 2025. Additionally, the results have been shared with industry key players during private events and networking sessions.
IPR Protection: unsure (IPR has not been considered yet)
Status: In the case of Mediterranean aquaculture, CryoPlankton live feed use can be considered as TRL6 (technology demonstrated in relevant environment). It's important to take into consideration that rearing of each fish species is very specific, and difficult to replicate in different species. Each case is unique and requires a tailored approach. The study identified specific benefits in using CryoPlankton live feed in seabass aquaculture. Therefore, the results from this output can be used as a baseline to work on industry improvement. Although end users can directly apply the procedure, the expected results may vary due to the scale effect and different setups. Information from other trials and studies is consistent with the current output of better-performing fish, although the exact values present variation that is often attributed to the different setups and conditions. However, the mechanisms leading to the long-term effects have not yet been directly examined in all species and require further investigation.

Knowledge Output Description:
Knowledge need: CryoPlankton (innovatively cryopreserved barnacle zooplankton, produced by the Norwegian company Planktonic) is already widely and successfully used as the basic live feed in several cold-water fish species in northern Europe, due to the rich nutritional quality and the simplicity of preparation compared with traditional live food items. It is also used in some fast-growing warm water species with great success. The short and long-term benefits of CryoPlankton are well documented in some species, e.g. in ballan wrasse (*Labrus bergylta*), where growth is superior compared to the larvae fed traditional live feeds while reports suggest that organogenesis is faster and more complete. However, there is a lack of scientific documentation of CryoPlankton benefits in Mediterranean species, including seabass aquaculture, especially regarding the long-lasting effects of the early-stage diet under industrial conditions. The main reason is that industrial operations of seabass aquaculture are complex and there is limited opportunity to perform long-term studies where all parameters in the hatchery and on-growing stage are under control. The main aim of this study was to research the long-term effects of CryoPlankton live-feed hatchery diets on European seabass. **Results:** The trial results show significant differences in the hatchery period KPIs and long-lasting effects of the CryoPlankton-fed treatments. In the hatchery period, the weight was almost 40% higher than the control and the biomass produced was 20% more. Swimbladder inflation percentage was the same between the groups. The growth rate of the CryoPlankton-fed animals during the hatchery period was significantly higher and while it was reduced during the on-growing, it remained evident giving 12% larger fish by the end of the trial after a year. In addition, in some deformity categories, such as the operculum, a significant difference was recorded, suggesting a better shape of the CryoPlankton-fed fish. The mortalities in the on-growing period were fewer in the CryoPlankton-fed fish than in the control groups, suggesting more robust fish. The population size at the end of the trial was more homogenous as being calculated from the coefficient of

variance. Although not statistically significant, it should be enough to facilitate the usually costly on-growing handling and harvesting processes. The results are in accordance with previous studies of other fish species and evidence collected from different seabass aquaculture setups.

Innovation: This is the first time a full trial has been conducted to study the long-term effects of CryoPlankton live-feed hatchery diets on European seabass and so all results are novel.

In addition, the methodology used in terms of the development of a fully-controlled vertical sea bass aquaculture facility could be considered an innovation as well. Normally there is limited opportunity to perform long-term studies in seabass aquaculture where all parameters in the hatchery and on-growing stage are under control. The trial facility that was used to measure the benefits of using CryoPlankton during the larvae period of seabass is an IFREMER RAS facility, located in Palavas les Flots (France). The unique facility made it possible to monitor fish performance from different hatchery feeding treatments. In normal production facilities, fish populations are mixed with similar populations to improve the use of space in tanks, while the Ifremer RAS facility made it possible to not mix populations. A facility such as this could have many potential other applications.

EATiP Thematic Areas
TA2 - Technology & Systems

EATiP Goals
TA2 - G3 : Ensure profitability of aquaculture industry by developing improved management systems and technology

End Users, Applications and Impact:

	Potential Application	Potential Impact
End User 1: Industry stakeholders **User description:** Planktonic	Planktonic (a supplier of live feed to marine hatcheries in Europe, based in Norway) is the producer of CryoPlankton, and is already using the output results to inform seabass farmers about the potential of the product.	Once Planktonic will sell more CryoPlankton, they will increase their profitability.
End User 2: Industry stakeholders **User description:** seabass producers	Seabass hatchery managers and producers can use the output to improve the production of seabass, including simplification of the process of producing larvae using a standardised methodology. Although end users can directly apply the product and hatchery production protocols, the expected results may vary due to the scale effect and different setups	Potential resulting impacts, if seabass producers apply the outputs, are: - Better growth in the hatchery and on-growing period - Better morphology of the fish and overall better robustness of fish in the on-growing period Which could lead to improved production and financial performance of the aquaculture companies applying it.
End User 3: Scientific Community **User description:** Fish researchers	Fish researchers can take up the output to conduct further research to understand how Cryoplankton can benefit other species within established or specialized industries. Further research is planned to	Potential resulting impact of this output once it has been further developed by researchers is further insights and improvements of CryoPlankton, which would improve performance in fish, and

	understand how Cryoplankton can benefit seabream, with collaboration with research centers and universities. Research with other species will follow, collaborating actively with private companies.	contribute to a more sustainable fish production overall.

Evaluation Summary:
Number of 3's: 7
Total score: 30

3.3.14. AE3.0 TNA 23227

Transforming food industry and agriculture waste into nutrient-rich alternative feed for fish: Case Study with Black Soldier Fly Larva

OUTPUT type:	TNA
OUTPUT owner: Martin Kulma, Czech University of Life Sciences Prague (CZ)	
Publicly available: Not publicly available yet	
Link: The results of the experiment were presented at the INSECTA 2023 conference in Germany, the abstract can be found in INSECTA 2023 – Review « INSECTA (insecta-conference.com) > Book of Abstracts INSECTA 2023 > Page 132, and will potentially be presented at IFW 2024 (Insects to Feed the World). The results will be publicly available once they are further analysed, through a scientific publication. Results will also be disseminated using social media of the contributing institutions (CZU and UNITO).	
IPR Protection: Unsure	
Status: TRL 2 — Technology concept formulated/TRL 3 — Experimental proof of concept Further research is needed to investigate the optimal diet composition using waste products, focusing on feeding rates.	

Knowledge Output Description:

Knowledge need: One of the aquaculture industry's major challenges is the availability of fish meal and fish oil, which have fluctuating costs and sustainability issues. There is a substantial need for alternative fish feed ingredients that do not deplete marine resources, and which result in healthy fish. However, alternative ingredients in the fish's diet can lead to adverse effects, such as decreased digestion efficiency and increased susceptibility to diseases and stress. Research is needed to ensure that aquafeeds that utilise alternative ingredients can supply the same benefits as fish meal and fish oil, while maintaining high biological value and low competitiveness with human food. Optimum feed substitution levels for each fish species must also be established.

One such aquafeed alternative is insect-based meals, which has been approved by EU legislation in 2021. Larvae of the black soldier fly (BSFL), *Hermetia illucens* (Diptera: *Stratiomyidae*), are voracious feeders of organic material, which have recently been intensively studied for their capability to convert organic waste into high-quality protein, which, in turn, can be used as nutritious feed, including for fish. Due to the capability of BSFL to be cultivated on waste, they have the potential to become both environmentally friendly and feasible alternative feed. To fully exploit BSFL's potential to become feed, there is a need to optimise their rearing technology, and diet is a key factor. Apart from various environmental elements, the bioconversion process using BSFL is known to be affected by the type, quantity, and quality of feed. To improve bioconversion efficiency in terms of reduction of waste-based substrates, knowledge of the proper feeding rate per larva is essential.

This TNA research project aimed to investigate the effect of incorporating waste products from the agriculture and food industry into the diet of BSFL on their performance and nutritional characteristics. Specifically, the goal was to increase the understanding of how varying feeding rates impact the growth, bioconversion factors, and nutritional composition of these larvae.

Results: The trial showed that larval weight was influenced by diet, feeding rate, and time. The control group, which was fed the Gainsville diet, resulted in the heaviest larvae, followed by ITA and CZE groups. Increasing the feeding rate also correlated with higher larval weights. Reduction rate (RR), waste reduction index (WRI), and bioconversion efficacy (BE) varied significantly among groups, with GA showing the highest and REP the

lowest values. Feeding larvae with 0.6 g per larva led to a greater RR compared to 0.4 g, while no significant differences were observed for others. Larvae fed 0.4 g each exhibited higher WRI than other feeding rates. The BE decreased with increasing feeding rates. The chemical analyses revealed a significant effect of the diet on the nutritional value of BSFL. CZE-fed larvae exhibited the highest crude protein (41-45% DM) and ash contents (23-25%). Conversely, insects raised on CZE had lower levels of ether extract (4-5% DM) than those fed by ITA and GA. In terms of amino acid profiles, methionine was the limiting amino acid in all samples, with only minor differences observed in protein quality. **Innovation:** The trial showed that waste components can be used for feeding BSFL, but further research is needed to optimize their content in the diet to reach at least similar parameters as those which are reached by insects farmed on commonly used feeds.

EATiP Thematic Areas
TA4 - Sustainable Feed Production
TA2 - Technology & Systems

EATiP Goals
TA4 - G2: Advanced novel feed technologies to produce cost effective feed with improved quality
TA2 - G1: Ensure environmentally sustainable industry by applying new knowledge and technology innovations
TA2 - G4: Ensure technology for ethical and healthy production of high quality products

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User 1: Scientific Community **End User description:** Insect researchers	The results will help researchers achieve a better understanding of the nutritional requirements of BSFL and can be used as a base to set-up further research to find the optimal levels of waste components in their diet. The goal would be to reach at least similar parameters as those that are reached by insects farmed using commonly used feeds.	The potential impact resulting from this output extends beyond immediate applications. With a growing understanding of insect nutrition, so does the ability to advocate for insects as a viable source of both human food and animal feed.
End User 1: Industry stakeholders **End User description:** Insect farmers	The obtained data will provide insights into the potential of using local waste products from the agriculture and food industry in farming BSFL. Insect farmers can take up this output to improve BSFL's production by optimizing resource utilization and ultimately lowering production costs. By efficiently managing the amount of feed	An environmentally and eco-friendly formula of a diet for BSFL with no adverse effects on the amount of harvested biomass could be a potential impact of this output when transferred to industry stakeholders. Insect biomass conversion creates a circular economic model promoting sustainability and minimizing waste. As a result, insect farms contribute to environmental conservation.

	provided to insects, farmers can minimize waste.	
End User 2: Industry stakeholders **End User description:** **Aquafeed producers**	Once the nutritional requirements of BSFL are fully known, BSFL could be incorporated into nutritious aquafeeds.	Due to the capability of BSFL to be cultivated on waste, the potential impact of including them in aquafeeds would be production of more environmentally friendly and feasible alternative feed.

Evaluation Summary:
Number of 3's: 3
Total score: 24

3.3.15. AE3.0 TNA 24658

Application of innovative and easy sustainable environmental enrichment on the behaviour and welfare of farmed trout

OUTPUT type:	TNA
OUTPUT owner: Georgina Lea Fazekas, MATE University (HUN)	
Link to output: The OUTPUT is expected to be publicly available in the future through a research paper in the journal Aquaculture (first half of 2024).	
IPR Protection: No (not applicable)	
Status: The bubble curtain enrichment technology is considered to be at Technology Readiness Level TRL5 = Technology validated in a relevant environment (industrially relevant environment in the case of key enabling technologies). The innovative but simple enrichment technology (diffuse of bubble curtain) was tested in a recirculating aquaculture system in a relevant stocking density of trout. From the aspect of science or a small-scale industry, the technology is ready to be used to improve animal welfare rather than to increase yields, as can be seen from the similarity between growth parameters. Further investigation is needed to determine the potential for increasing yield. This enrichment strategy has not yet been applied in trout farming. It is important to point out that it has no negative impact on the environment and the fish, and it is very simple and safe to maintain to improve the welfare of captive fish	

Knowledge Output Description:

Knowledge need: Environmental enrichment (EE) is considered a technique aimed at improving the physiological and psychological needs of captive animals. Several studies have shown that EE may enhance brain proliferation and differentiation, and have an impact on neural and gene expression, phenotypic plasticity, cognitive performance, spatial learning and memory ability. Furthermore, it can promote behavioral flexibility and reduce aggressiveness by providing shelter and additional feeding sites thus, improving the overall welfare. In trout (*Oncorhynchus mykiss*), the presence of physical enrichment also reduces stress response, measured by plasma cortisol level after acute stress, and limits aggression by reducing visual contact between individuals. Moreover, the beneficial effect of EE is reflected in a higher survival rate during a possible infection and a better resistance to parasites because of a reduction in chronic stress caused by a lower level of aggression. Enrichment has not yet been widely adopted in the aquaculture industry. A lack of scientific knowledge and specific welfare regulations contribute to the scarcity or the rejection of the application of physical enrichment and there are potential disadvantages of adding physical structures to a culture tank.
Observations on marine fish have shown that they markedly follow bubbles formed by a column of air, which would meet the criteria for animal play behaviour. A previous study has already shown that rainbow trout (*Oncorhynchus mykiss*) does not regard bubble curtains as a source of stress, but there is no information on its long-term use on growth performance and cognitive ability. There is a need for further information on enrichment practices to improve trout welfare and health, which could include the use of bubble diffusion.

Methodology: this research project aimed to study the effects of an innovative and easy-to-maintain EE, generating a bubble curtain, at the early life stages of rainbow trout. The EE mainly consisted of introducing a pierced pipe connected to an air pump into the tank.

Results

- Observation in aggressiveness: One of the most important results obtained in the study is the decreased level of aggression observed in bubble enriched group compared to the control group.

Significantly fewer aggressive occasions were observed in the bubble-enriched group during the feeding period, the beginning and the end of the bubbling period, and at the beginning of the neutral period (when the aerator was inactive). During the neutral periods, the bubble group also showed significantly less aggressive/abnormal behaviours. The fish were more active and showed less grouping behaviour (not only during bubbling periods) by the end of the day. The bubbles may provide visual, acoustic and tactile stimulation which might also reduce the overall stress associated with social interaction. In addition, the regularly active curtain of bubbles helps to avoid monotony and, consequently, boredom. Thus, the application of bubble curtains in the rearing tanks can be considered as an environmental enrichment for rainbow trout, whether sensory, occupational and/or physical.

- Motivation test: The bubble fish spent significantly more time in the bubble zone than in the control zone while no differences between the use of two zones in the control group were found. Considering the time spent in different zones (control, bubble, shelter) of both treatments, the fish spent significantly more time in the shelter zone compared to the bubble or the control zone and the fish spent significantly more time in the bubble zone than in the control zone. There were no differences in latency time to exit from the shelter zone or to enter the bubble zone between the groups. The positive sensory experience that bubbles seem to provide to rainbow trout would make it possible to integrate into fish farming systems the notion of "positive welfare" proposed by Fife-Cook and Frank, 2019.
- Emotional reactivity test: Significant differences were observed in the number of rotations and higher maximum velocity between the treatment groups, with the control group exhibiting more pronounced effects during the 20-minute period. The total distance moved, the mean velocity, the time spent in thigmotaxis, and the absolute angular velocity did not differ between the treatments. However, the fish moved a significantly higher distance in the first 5-minute, and a significantly smaller distance during the second 5-minute period. The mean velocity of the fish was higher in the first 5-minutes compared to the second and third 5-minute periods. The treatments did not differ in the number of consumed pellets as well. In the absence of any difference in the well-known anxiety-related behaviours such as angular velocity and thigmotaxis, the boldness of bubble fish cannot be stated with certainty. However, the lower number of rotations and maximum velocity may reflect a certain sense of safety despite the stressful situation.
- Learning test: There were no significant differences in latency to enter the rewarded area across treatments. The number of first correct entries (when the fish choose the rewarded area first after leaving the start box) and the number of starts (whether they leave the start box or not) did not differ between the two groups and was not related to time. Latency to first eating significantly decreased from day 1 to the other days in both groups. This latency decreased also between day 2 and day 3 in the bubble group. The number of trials, when fish ate the reward, was higher in the bubble group compared to the control. Moreover, the control fish ate less while the bubble fish ate significantly more associated with the trial days. In addition, the bubble fish were more likely to consume all the pellets offered on the last day of the learning test. Latency to consume the reward was decreased in bubble fish compared with control fish, indicating improved learning abilities and cognition in fish that experienced bubbles.
- Growth parameters: We found no effect of treatment on final body weight and total length condition factor, weight gain and specific growth rate during the experimental period.

Innovation: The results from this study demonstrated that the implementation of physical, sensory, and occupational enrichment in the rainbow trout rearing environment reduces abnormal and aggressive behaviour in the group, which are considered negative indicators of welfare in farmed fish. EE had no effect on the emotional reactivity of the fish subjected to an acute stress situation. However, the bubble fish seemed

less fearful, exhibiting a lower maximum velocity than control fish. Additionally, a positive impact of EE has been shown on learning ability and feed intake during acute stress occasions over time, assuming better-coping abilities and adaptability to novel environments. Finally, growth parameters were not impacted by the treatment. According to these findings, the use of bubble enrichment in trout farming is highly recommended due to its benefits from the behavioural and welfare aspect of view.

EATiP Thematic Areas
TA7- Aquatic Animal Health and Welfare

EATiP Goals
TA7 - G4: Measure welfare/stress and understand its consequences if compromised in order to incorporate welfare as core components of production management

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User 1: Scientific Community **End User description:** **Aquaculture scientists, particularly trout production researchers and conservation biologists**	Aquaculture scientists can use the output to further develop the environmental enrichment technology, and use it as inspiration for other EE ideas. The results of this output may be the basis for some future research in the field of environmental enrichment and stress responses in intensive breeding systems	The implementation of an easy-to-maintain and safe environmental enrichment strategy can lead to improved welfare of trout used for research purposes and a better understanding of behavioural welfare indicators. It can help to avoid developing maladaptive behavioral traits/aggressive/stereotype behaviors.
End User 2: Industry stakeholders **End User description:** **Aquaculture farming industry**	Trout farmers and breeder can use the output to implement improved rearing systems for trout farming, incorporating environmental enrichment technologies for improved fish welfare.	Potential resulting impact could be improved welfare of captive trout as a result of safe and easy-to-maintain enrichment technology, potentially to be integrated into best practice guidelines for fish culture and compelling to the standards of protection of animals for farming purposes.

Evaluation Summary:
Number of 3's: 3
Total score: 22

3.3.16. AE3.0 TNA 22213

Established benchmark for Atlantic bluefin tuna (Thunnus thynnus) weaning with commercially available diets in Europe

OUTPUT type:	TNA
OUTPUT owner: Paul-Daniel Sindilariu, Nexttuna (DE)	
Link: The results were presented at AQUA2024 (Copenhagen, Denmark), https://wasblobstorage.blob.core.windows.net/meeting-abstracts/AQUA24AbstractBook.pdf	
IPR Protection: The diets are already protected by Skretting and are commercially available; however, this is the first time they have been tested for ABT.	
Status: TRL9. Commercially available feeds from Skretting were trialled during the on-growing phase of Atlantic Bluefin Tuna (ATB) production. The trial was conducted on fish weighing 50g to 1-2kg. Growth depressions have been observed (from 150g onwards) and further investigation is needed. Further weaning trials are expected to be carried out in June 2025 at IEO facilities in Spain. IEO, Next Tuna and Skretting were considering a follow-up trial supported by the AquaServ project, however funding challenges have ceased progress.	

Knowledge OUTPUT Description:

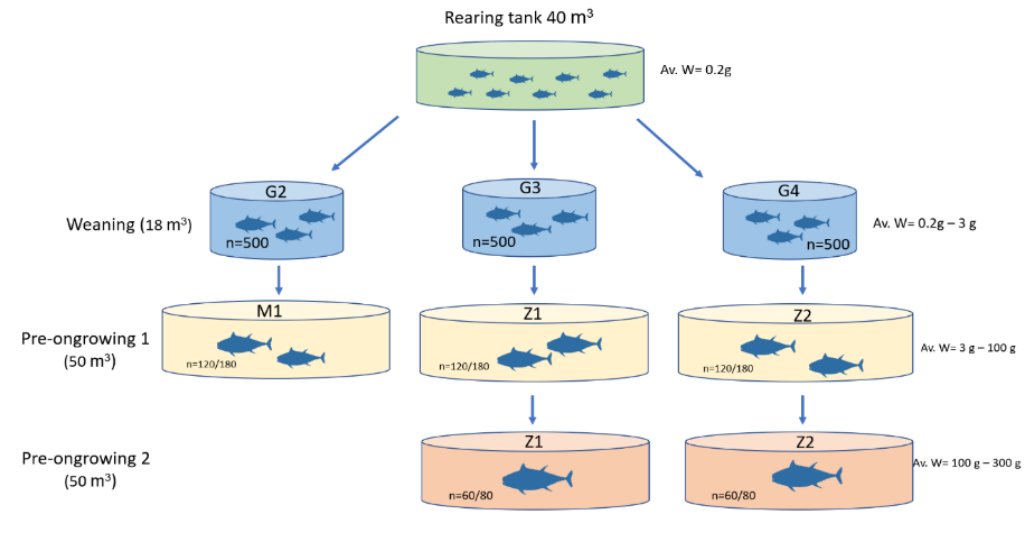
Knowledge need: Tuna is the single most consumed seafood item in the EU; however, it is currently entirely supplied from wild catch fisheries. To establish a tuna aquaculture industry in Europe, there is a need to develop appropriate feeds. Sustainably formulated feeds are essential for both fattening fish and enabling closed-loop aquaculture, where a species is cultured through at least one generation. Sustainability in feed formulation enhances environmental responsibility but also has a positive impact on economic viability and developing such feeds for tuna potentially opens a new aquaculture industry in Europe. At present, the predominant diet for tuna grown in aquaculture, including sushi and sashimi-grade tuna, heavily relies on forage fish and their species. This includes *Sardinella*, *Sardine*, *Clupea*, *Scomber*, *Trachurus*, the sparid *Boops boops,* and certain cephalopods. The harvesting of these species for tuna feed presents an ecological challenge and, could disrupt the ecosystem equilibrium of the Mediterranean Sea where they reside.

Addressing this sustainability concern necessitates the local production of artificial feed specifically tailored for Atlantic Bluefin Tuna (ABT). Moreover, the transition from forage fish-based diets to artificial diets significantly impacts Feed Conversion Ratios (FCR). While forage fish based FCRs range from 15-20 to 1 for animals below 30 kg, artificial diets have shown substantial improvements, yielding FCR values below 3. This shift not only diminishes reliance on forage fish but also enhances the sustainability of ABT farming, optimises farming practices and minimises the environmental footprint across the Mediterranean Sea.

In order to advance on the implementation of a European based feeding regime for ABT and establish a benchmark for future feeding trials and improvements, two distinct Skretting commercial artificial feeds (Gemma Silk and MaGro), tailored for specific developmental stages, were evaluated during this trial.

Methodology: **Gemma Silk** is a dry feed that offers a gradual sinking diet suitable for marine fish larvae. Its specialised formulation caters to marine species such as tuna, lumpfish, and seriola, ensuring optimal nourishment during the critical weaning period. Additionally, **MaGro**, an extruded soft feed exclusively crafted for bluefin tuna, represents a notable component utilised in this trial. MaGro prioritises high palatability, ensuring an enticing and nutritionally rich diet specifically engineered for bluefin tuna throughout their developmental stages.

Three tanks, each containing 500 larvae were set up for the trial at the IEO (Spanish Oceanographic Institute) – see image below. The feeding trial was performed from day 23 post-hatch to day 106 post-hatch. During this time, the fish were weaned from live diets of e.g. artemia and bream yolk-sack-larvae to artificial diets such as the Skretting Gemma Silk and MaGro diets.



Results: The results showed that tuna, at all stages of development, show consistent growth and positive development when fed with Skretting artificial diet (Gemma Silk and MaGro) The application of Skretting commercial start feeding diets, resulted in expected survival rates during weaning, ranging from 26% to 37% across replicas, in the range of previous weaning experiments. Pre-on growing phase 1 had the highest specific growth rate of 8.1 versus 3.8 in pre-on growing phase 2. However, phase 2 had the highest gains in wet weight and length, with lower feed conversion ratio (FCR). It is important to also highlight that the exclusive utilisation of MaGro led to decreased FCR rates, suggesting its capability to improve subsequent on-growing phases for ABT.

In summary, the benchmark is that with Skretting Gemma Silk, weaning of ABT was possible. However, the weaning took approximately two days longer to day 30-32 days post-hatch (dph), instead of 28-30dph, reported for Japanese Tuna start feeding diets. In addition, the fish grew as expected to a size of approximately 100-150g (comparable to bait fish feeding). However, after 150g the fish showed decreasing growth rates.

Innovation: The concept of developing a benchmark for weaning ABT in aquaculture systems in Europe using commercially available artificial feeds is novel. The preliminary results show the use

of artificial feeds could significantly reduce hatchery operations costs by reducing the need for YSL. The benchmark serves as the reference for future trials with improved diets or alternative protocols. This approach could potentially lower mortalities associated with cannibalism, as it avoids triggering the hunting instinct in tuna that is often stimulated when introducing YSL. Furthermore, this approach carries the added benefit of reducing the costs associated with tuna hatchery operations. Given that YSL production constitutes a significant portion of hatchery infrastructure, such a modification in the feeding strategy has the potential to contribute substantially to cost savings in hatchery management.

The next step of this trial includes the continuation of the artificial feeding regimen beyond the 200 g weight threshold. Additionally, exploring the potential to decrease or eliminate the use of YSL in the early stages of ABT larvae would be useful. It may be beneficial to introduce artificial feed at an earlier stage, concurrently with live feed such as rotifer and artemia.

EATiP Thematic Areas
TA3 - Managing the Biological Lifecycle

EATiP Goals
TA3 - G4: Manage life cycle of carefully selected "new" species that have high economic importance
TA2 - G4: Ensure technology for ethical and healthy production of high quality products
TA3 - G1: Establish predictability and improve output and cost control at every production stage of the lifecycle

End Users, Applications and Impact:

	Potential Application	Potential Impact
End User 1: Industry stakeholders **User description: Skretting AS**	Skretting is a Europe-based feed manufacturer that has committed to further develop the feeding regime of ABT, and to support the start of the ABT aquaculture industry. Artificial feeding is one of the most essential prerequisites to close the reproductive cycle of ABT and establish the industry. Both diets could be used immediately, although only in the tested stage (pre-on growing phase from 23 dph to 80 dph).	Skretting, together with Next Tuna GmbH, have already identified their first customer for the developed ABT feed regime. The challenges addressed by high-quality artificial diets will play a crucial role in the sustainable and efficient development of ABT aquaculture, contributing to the conservation of wild tuna populations and ensuring a long-term viability of the industry.
End User 2: Industry stakeholders: **User description: Next Tuna GmbH, (ABT farmers)**	Next Tuna GmBH is a Europe-based start-up that aims to be the first in the world to complete the reproductive cycle of the Atlantic bluefin tuna in a closed aquaculture system at a large	Establishment of ABT aquaculture industry in Europe. Closing the aquaculture cycle of ABT can make the ABT aquaculture industry in the Mediterranean as big as the salmon industry in Norway. Currently, tuna is the single most consumed seafood item

	enough scale to be commercially viable. ABT farmers at Next Tuna GmbH will use the artificial feeds developed by Skretting and trialled in this study to transition towards an established closed cycle of ABT production, not relying on wild populations, and to establish the first commercial ABT production in Europe.	in the EU, entirely supplied from wild catch fisheries. The uptake of the artificial diets developed by Skretting and Next Tuna GmbH would overcome significant challenges relevant to the sector, such as efficiency and economic viability.

Evaluation Summary:
Total score: 29
Number of 3s: 6

3.3.17. AE3.0 TNA 23110

Supplementation with metal-AA complexes contribute to more efficient and sustainable diets in RAS systems

OUTPUT type:	TNA
OUTPUT owner: Claudia Figueiredo Silva, Zinpro (USA)	
Link: https://www.was.org/Meeting/Program/PaperDetail/163323 (presented at AQUA2024 (Copenhagen, Denmark): Figueiredo-Silva, C., et al. Replacement of inorganic minerals with metal-amino acid complexes reduces mineral losses in rainbow trout.	
IPR Protection: No	
Status: TRL4. A full manuscript is being prepared to be submitted for publication in a peer-reviewed journal by end of the year. Additionally, a portion of the results will be presented at an EAS webinar.	

Knowledge OUTPUT Description:

Knowledge need: In aquaculture, interest in recirculating aquaculture systems (RAS) is growing due to their sustainability and efficiency, offering a land-based alternative to traditional open water net-based systems. While traditional flow-through systems reuse none of the water, advanced RAS technologies reuse 95-99% of the water through a series of water treatment steps, making them environmentally more attractive. Despite RAS being closely controlled systems, variation can be observed in temperature as well as levels of CO_2 and nitrogen compounds, reflecting the increasing biological load within the RAS system over time. Minerals (including trace minerals (TM)), drug residues, hazardous feed compounds and metabolites may accumulate in the system and affect the health, quality and safety of the farmed animals. This highlights the need for more efficient TM supplementation.

The aim of the study resulting in this output was to evaluate the effect of replacing 50% of inorganic TM with metal AA-complex premix on the performance, nutrient retention and overall health status of rainbow trout raised in RAS.

Methodology: Diets were fed for 8-weeks to triplicate groups of 30 rainbow trout raised in 12-hour light, 12-hour dark photoperiod settings. Two diets were fed and varied by trace mineral (TM) premix. Two housing systems were used: 1) Flow-through system: fish were housed in six 200 L tanks connected to a common water supply and 2) Recirculating aquaculture system (RAS): Fish were housed in six 270 L tanks with a recirculating water supply. 2x2 factorial arrangement of treatments was used, which included two mineral sources and two water refreshment levels: TM-I (sulfate forms of Fe, Zn, Cu, Mn, and Se), or TM-AA (amino acid complexes of the same TM at 50% of TM-I dosage) and HWR (high refreshment; 1.5x system volume) or LWR (low refreshment; ± 311 L/kg feed). Fish were randomly assigned to 1 of 4 treatments and assessed for effects of treatment and interactions: (i) TM-I + LWR (ii) TM-AA + LWR, (iii) TM-I + HWR, (iv)TM-AA + HWR.

Results: At the end of the feeding period, the effect of the diet, rearing system and their interaction were evaluated in terms of growth performance, fish composition, TM retention and losses, plasma and hepatic TM content, and activity of hepatic enzymes playing a key role in the antioxidant

defence (e.g. SOD, GPx, CAT). Rainbow trout grew better (SGR %/d) in the LWR (1.98) system compared with the HWR (1.92), regardless of the diet fed. Replacement of inorganic TM with TM-AA complexes by the half has significantly improved digestibility of P, Zn, Mn and Se. Moreover, and regardless of the system type, the supplementation with TM-AA complexes reduced significantly Fe, Cu, Mn and Se total losses while maintaining TM body retentions similar. The analysis of hepatic TM content revealed a reduction on Fe and Se contents with TM-AA vs TM-I and in the Se content of trout raised at HWR vs LWR system. However, neither the diet nor the system type affected the activity of enzymes playing a key role on antioxidant defence system. Plasma analysis showed higher levels of Mn and lower levels of Zn in trout fed TM-AA vs TM-I diet, regardless of the system type. The findings prove that replacement of inorganic TM with TM-AA complexes can offer an interesting strategy to reduce TM losses into the environment, while respecting EU legislation for upper limits of TM and still maintaining fish performance and health. This strategy can successfully be applied to produce trout in RAS systems without impacting health of the fish as indicated by similar plasmatic activity of enzymes playing a key role in the antioxidant defence of fish (i.e. CAT, SOD, GPx, GR, GSSG, GSH, TGSH, OSI, LPO). Results of this study will help the industry optimise feeding strategies that reduce dietary TM level in trout diets which in turn will reduce TM accumulation and possible toxic and deleterious effects to fish performance and welfare, grown in RAS systems. **Innovation:** To the author's knowledge, this is the first study evaluating the possibility of reducing the amount of trace minerals being found in the water by significantly reducing their amount in the feed (by 50%), without compromising fish performance.

EATiP Thematic Areas
TA4 - Sustainable Feed Production

EATiP Goals
TA4 - G1: Base formulation of Future Fish Feeds on solid knowledge of fish nutritional and feeding requirements, and expand the number of well characterized and sustainable raw materials which can be used
TA4 - G3: Understand and minimise non desired effects of alternative diets on fish health and welfare

End Users, Applications and Impact:

	Potential Application	Potential Impact
End User 1: Industry stakeholders **User description:** **fish feed producers**	This output will contribute to the optimisation of TM content in fish feeds intended for RAS. The solution allows TM to be reduced by 50% of what is currently applied in those feeds without affecting the performance or health of the fish. This reduction in the TM content of the feed will also help feed companies to meet EU legislation for maximum allowed TM supplementation.	More environmental-friendly solutions to produce trout feed and trout marketable products in Europe.

End User 2: Industry stakeholders **User description:** RAS farmers	Future RAS diets applying this approach will help farmers to reduce the excretion of minerals into the environment and the associated costs to treat it. Moreover, by reducing accumulation of TM in the water, farmers should expect to reduce risk for damage of fish health, contributing for a more environmentally friendly and welfare responsible production.	More environmental-friendly solutions to produce trout feed and trout marketable products in Europe.

Notes
Claudia presented her output on "Metal-amino acid complexes as a cost-effective strategy to help reduce fish meal in European seabass diets" in 2023

Evaluation Summary:
Total score: 28
Number of 3s: 6

3.3.18. AE3.0 TNA-27738

Use of Acoustic Doppler Current Profilers (ADCP) echosounding to characterise fish growth and behaviour

OUTPUT type:	TNA
OUTPUT owner: Thomas Culverhouse, Sonardyne (UK)	
Link: Not yet available. The intention is to publicly release a case study to support a product launch in April 2025. The case study and internal technical documentation can be made confidentially available on request. Further technical publications may be published after April 2025.	
IPR Protection: Unsure; IPR considered but not yet submitted for review	
Status: TRL 7; the goal is to reach TRL 9 by April 2025, when product is expected to be launched. The output could already be used by the scientific community for research purposes into fish farming and other aquaculture applications. Further work is needed to enable site managers/operators to fully exploit the value of the research; although the technological capability is at high TRL, the downstream algorithmic capability is lower TRL, perhaps TRL 3. The data acquired from the BEDLAM project will advance this TRL.	

Knowledge OUTPUT Description:

Knowledge need: In the aquaculture industry, monitoring the health of the fish is key to the viability of the industry. There are numerous ways to monitor fish health, e.g. using sensors such as oxygen sensors near the surface, and profiling using Echosounders. The latter send bursts of sound from the surface downwards into the water and receive reflections of this sound from the fish. This signal can be used to estimate the overall 'amount' of fish, also known as biomass. However, Echosounders have a large 'view' and cannot easily discriminate between a single large fish and a large number of small fish. In addition, Echosounders typically require an external power source to operate, and are thus normally deployed inside an aquaculture pen and must be located near a source of power. There is a need for an alternative method that overcomes these obstacles.

The research project leading to this output aimed to use Acoustic Doppler Current Profilers (ADCPs) to make similar measurements as Echosounders. Although ADCPs are normally only used to measure water currents and related properties (such as waves and turbulence), they can also measure the strength of reflected acoustic signals, much like a dedicated Echosounder. Therefore, it is also possible to measure biomass with an ADCP. Performing these measurements with an ADCP has other benefits: The ADCP has a field of view about ten times smaller than an Echosounder and so can track growth and behaviour of fish at higher spatial resolution. This in turn can provide more detailed insight into any deviations from expected behaviour and the root causes, which may significantly impact site operations. The use of ADCPs therefore delivers great value as the device can simultaneously measure currents as well as biomass, all from a single battery-powered instrument.

Methodology: The measurements that were taken include both currents and echosounding data from the ADCP, and data from a separate dedicated Echosounder, with some spatial overlap with the ADCP. In addition, the Origin 600 ADCP used in the project has an inbuilt acoustic modem,

allowing the processed data to be exported in near real-time to the surface and into a Cloud account.

The ADCP was deployed in 50m water depth off the coast of Hitra, Norway, adjacent to a cylindrical fish pen. The instrument was mounted in an angled bedframe and deployed onto the seabed such that three beams pointed into the pen. The pen contained approximately 200,000 Atlantic Salmon and measured approximately 35m in diameter and 35m vertically. Prior to deployment, the ADCP was configured to send acoustic pulses into the water at 4Hz; these pulses alternated between standard ADCP pulses to measure water velocities, and much shorter 'echosounder' style pulses to measure acoustic backscatter from small-scale spatial features. External batteries were used to allow the ADCP to make measurements continuously for one month, i.e. without any sleep periods or duty-cycle. The ADCP collected log-files of acoustic data that were stored on the device and downloaded post-recovery.

Offline processing of the ADCP data was performed with a bespoke set of tools written in Python to extract hydrodynamical parameters (such as currents and wave properties), and track relative change in acoustic backscatter. Several metrics were calculated from the backscatter intensity. First, the data indicated large-scale vertical motion of the fish on several-hour timescales. The upper and lower boundaries of the fish population were established and the average slant range tracked as a function of time. Backscatter intensity fluctuations were also observed on much finer timescales, on the order of minutes in duration. Second, the raw backscatter intensity data were converted to volume backscatter (in general, individual fish could not be resolved and thus calculation of target strength was challenging). For data inside the pen, the rate of decay of volume backscatter was used as a proxy for fish growth – the rate of decay was expected to be steeper as fish grew larger, as larger fish could be expected to attenuate the acoustic signal faster.

Results: The main results of this project are a continuous month-long set of measurements of ADCP data from a deployment near an operational fish pen. The ADCP data allowed to develop fish tracking and growth metrics that can be deployed on the ADCP itself, meaning that the ADCP performed useful processing tasks on the data rather than just logging it for post-processing after the device was recovered. The techniques explored here showed great potential for tracking fish growth and behaviour but were limited by the relatively short 1-month duration of the deployment. On this timescale the fish growth was insufficient to definitely prove the techniques; a future deployment over the full fish lifecycle allows more rigorous testing. Separately to offline processing, a basic onboard algorithm was installed on the ADCP at the start of the deployment, and its output delivered to a Cloud account. In the future this will allow site operators to view the behaviour of the fish in near real-time from their desks, allowing informed decisions to be made as events are unfolding. The data has already shown that tracking fish with an ADCP is achievable, and more sophisticated metrics are expected as the data analysis proceeds. A longer trial would likely be required to conclusively determine if the methods can be used to definitively track fish growth, and integration of further external sensors with the ADCP would allow for tighter correlation between fish behaviour and captured data. Future trials would also ideally perform absolute calibration of the ADCP, so that the signal strength can be directly related to fish of a particular size in their growth

curve. This type of procedure is common in standalone echosounders but is rarely applied to ADCPs, hence the methodology would need to be translated between the two instruments.

While the methods used in the project have been demonstrated at the ACE site made available by SINTEF, wider adoption in the industry would likely require deployments at other sites to determine if the techniques are applicable more generally. A wider demonstration of the project would also be necessary to allow the methods to be used to inform policy.

Innovation: Both the deployment of such processing algorithms and the export of the real-time data is novel and innovative for the industry. The output extends the state-of-the-art by a) implementing an echosounding mode on all ADCP beams (alternative ADCPs perform this mode on one beam, or not at all), b) allowing such novel data to be processed in-situ and the results delivered as a live stream, and c) allowing external sensor data to be fused with ADCP data (both traditional currents and echosounder-style pulses) onboard the device, thus providing the user with additional insights into the subsea environment.

EATiP Thematic Areas
TA2 - Technology & Systems
TA7- Aquatic Animal Health and Welfare

EATiP Goals
TA2 - G1: Ensure environmentally sustainable industry by applying new knowledge and technology innovations
TA2 - G3 : Ensure profitability of aquaculture industry by developing improved management systems and technology
TA7 - G4: Measure welfare/stress and understand its consequences if compromised in order to incorporate welfare as core components of production management

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User 1: Scientific Community **User description:** **behaviour specialists, fish disease specialists**	The developed technology can be used immediately by the scientific community via rental or purchase of a Sonardyne Origin 600 ADCP. The technology can be applied to suitably-sized fish pens (max. vertical size 35 m) provided the water depth does not conflict with the ADCP operational parameters. In the longer term, it may be possible to produce generically-applicable processing algorithms to make scientific calculations in-situ on the	Uptake of the technology could lead to many new publications that exploit the capability. This would expand the understanding of fish behaviour inside pens, thus advancing the research knowledge base and the industry as a whole. The technology would also allow rapid deployment and customisation of in-situ processing algorithms, allowing researchers the freedom to explore fish behaviour in a variety of different pen scenarios specific to the sites available to them.

	ADCP and report the results immediately.	
End User 1: Environmental Managers & Monitoring managers	Environmental & monitoring managers can use the developed technology to improve the efficiency of operation at fish pens. This may be possible in short-medium timescales; though the underlying technology has been demonstrated, the algorithms required to fully exploit the technology are in their infancy.	Uptake of the technology could lead to increased productivity and profitability at fish pens as the technology could prove a more accurate tracer of fish health, stress, and growth. The technology could also provide early warnings of problems, reducing time to decision-making and waste of fish and feed. There is potential for patenting of algorithms/techniques. There is potential for developing new, more cost-effective products now that the fundamental technology and application is better understood.
End User 2: Industry stakeholders **User description: Fish farm managers, industry researchers**	In the long-term, the technology could be used across the fish farming industry as a whole. Industry stakeholders such as fish farm managers could use the technology to advance the interests of the industry by demonstrating increased scientific and practical understanding of the fish lifecycle in the pens, improving operational efficiency, reducing feed waste, and thus increasing overall profitability.	The impact of this technology could be improving the productivity of their farms while also contributing to the argument for expanding industry operations overall. This would influence government bodies charged with granting/refusing additional licences to build new pens.

Evaluation Summary:
Total score: 28
Number of 3s: 6

3.3.19. AE3.0 JRA D4.3

New methods for post processing biotelemetry data in aquaculture

OUTPUT type:	JRA
OUTPUT owner: Martin Føre, Norwegian University of Science and Technology (NO)	
Link: Not yet. The report will be available through the AQUAEXCEL3.0 project website.	
IPR Protection: No, not applicable	
Status: TRL7. The outcomes of this study were presented at the 10th International Work-Conference on Bioinformatics and Biomedical Engineering (Urban, 2023), Gran Canaria, Spain, and Aquaculture Europe 2023 conference, Vienna, Austria.	

Knowledge Output Description:

Knowledge need: Telemetry (the automated process of measuring and transmitting data from remote sources to a central location for monitoring and analysis) and individual tags are becoming more common as research tools in aquaculture research. However, the processing of the data resulting from these tools are often still carried out using conventional methods such as plotting or simply viewing the data as time series or statistical aggregations. There is reason to believe that more recent AI-based data processing methods and other signal processing approaches could provide a deeper insight into the dynamics of the monitored biological system, by further processing of the data. More thorough post processing could reveal new knowledge on systematic relationships and features in the datasets that are difficult to detect, identify and categorise manually. This output addresses the need for more sophisticated methods for processing individual based data from fish.

Methodology: Methods applied were predominantly based on statistical analysis, PCA and clustering applied to 3D trajectories of fish, and signal entropy applied to activity data (accelerations). A trajectory analysis was conducted by first pre-processing position data obtained for salmon in a commercial cage such that only the tracks considered contiguous trajectories were retained for further analyses. The data was collected from salmon in two different cages, with three individual fish in each cage being equipped with acoustic telemetry tags set to measure and transmit swimming depth at regular times (20-60 s between each transmission). The transmissions were picked up by acoustic receivers (TBR700, Thelma Biotel AS) placed along the outer perimeter of the cage. The internal clocks of the receivers were also synchronised with high precision using a surface module that had a GPS antenna, enabling the use of Time Difference of Arrival (TDoA) methods for positioning. The resulting trajectories were then subjected to polynomial interpolation, resulting in a higher data density (1 sample per s) and smoother trajectories. Variables of interest, i.e., trajectory properties considered to describe the movement, were then identified (e.g., mean depth, depth variability, angular change and more). These variables were then computed for all trajectories for each individual fish included in the analyses. The variables of interest derived for each fish were first subjected to manual evaluation using conventional measures such as mean and standard deviation. They were then plotted as histogram plots to search for trends. PCA was then used to decompose all identified 3D-trajectories of fish, finding out which combinations of the variables of interest categorised each trajectory. This was intended to shed light on which combinations of the

variables of interest were best able to describe the movement trajectories of the fish. The final analysis was unsupervised clustering using the variables considered most interesting from the histogram analysis (i.e., average depth, depth variability, track length and angle changes). Signal entropy was applied to activity data measured during a previously published trial where salmon were subjected to crowding and delousing, which are both considered highly stressful for the fish.

Results: The measurements were made using an accelerometer, and the original analysis showed that the activity measured with this method increased as the crowding operation intensified. Subjecting these data to entropy calculations shifted the focus from the base value (i.e., acceleration) to changes in the value, which gives a different view into the data. Entropy analysis was found able to pinpoint the stress events which implies that not on activity increased but also the variability in activity.

Based on the findings of the OUTPUT, it appears that pre-processing in terms of **identifying consistent trajectories with a high causality between the data points** is a good way to refine a dataset consisting of 3D positions for more accurate analyses of short-term individual behaviours. Moreover, the results indicate that **PCA** has potential in shedding light on short-term salmon behaviour, and the composition of various different behavioural expressions comprising the "total" behaviour of fish. While not equally easy to interpret, **unsupervised clustering** also seems like a viable tool for identifying specific individual behavioural traits, and possibly also a method for better distinguishing these traits in both visual and numerical manners. While both these methods are likely to perform better and more consistently if provided a dataset where data points are denser in time, and by deriving more variables to describe the trajectories, the data used here, and variables derived from these were sufficient to demonstrate their applicability for further processing such data to achieve new insights and knowledge. We thus recommend that PCA and clustering using HDBScan or other methods be further explored in the future, and that an extra effort is put into identifying suitable variables for such analyses.

The **entropy approach**, taken from information theory, is a simple, yet powerful, tool for evaluation or measure of changes in the distribution of values. Such changes always mean change in the amount of information. The classification of the information changes could distinct the typical changes from atypical. Further investigation of the conditionality in the information promises introduction of the entropy evaluation as one of the possible welfare indicators to distinguish atypical behaviour.

While all methods applied to these data showed promise, they will likely require some refinement to provide conclusive outputs that can be used to describe fish movement trajectories fully.

Innovation: The innovation of the results lies in the application of unconventional post-processing methods to biotelemetry data in aquaculture, specifically focusing on individual fish behaviour. These innovative methods have the potential to transform the way biotelemetry data is analysed in aquaculture, leading to improved fish welfare, better management practices, and enhanced productivity in fish farming.

EATiP Thematic Areas
TA2 - Technology & Systems

EATiP Goals
TA2 - G4: Ensure technology for ethical and healthy production of high quality products

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User 1: Scientific Community **User description: Scientists using telemetry as a monitoring tool, model developers**	The scientific community could uptake this output by incorporating these unconventional post-processing methods to process biotelemetry data from research projects.	Refinement of methodologies could potentially create new research agreements with SMEs and improved welfare in research activities.
Target User 2: Education & Training stakeholders **User description: Educators teaching within signal processing, biology / ecology, students working on projects where biotelemetry or other individual based methods are used**	The new knowledge and tools that this output highlights could also be used as an educational tool, using it as an example in specific educational activities.	Early awareness allows educators to incorporate emerging techniques into their curriculum. This ensures that students are learning relevant, up-to-date skills that will be in demand in the future.
End User 1: Industry stakeholders **User description: Equipment providers that deliver solutions within telemetry or solutions using telemetry, fish farmers using these tools for monitoring** (not many of these yet, but they might come later on)	The new knowledge and tools could be directly implemented in the aquaculture industry by using the technology to assess the state of individual fish in the aquaculture industry. The largest potential for the industry would lie in the generation of new knowledge they can use, and possibly the use of this technology to monitor broodstock and other especially valuable and not very numerous fish.	Improved fish welfare, better management practices, and enhanced productivity in fish farming.

Evaluation Summary:
Total score: 27
Number of 3s: 4

3.3.20. AE3.0 TNA 26814

Origin significantly impacts reproductive capabilities of pikeperch (Sander lucioperca) broodstock

OUTPUT type:	TNA
OUTPUT owner: Oleksandr Malinovskyi, University of South Bohemia (CZ)	
Link: Not yet, an article in a peer reviewed journal is currently under preparation.	
IPR Protection: No	
Status: TRL5. The output in the current state is conclusive and no contradictory results were obtained. For a more complex description of why the deterioration of behaviour occurs, further validation would be required. However, the current study has enough repetitions to confirm statistically significant difference in pikeperch behaviour depending on their origin, during natural seasonal spawning on the nest. Spin-off research will be explored by the output owner.	

Knowledge OUTPUT Description:

Knowledge need: In pikeperch aquaculture, there is a gap in understanding how different rearing conditions affect natural reproduction. As pikeperch aquaculture develops and production increases, more intensively reared pikeperch are reintroduced back into natural habitats for population replenishment. Therefore, it is crucial to know whether the origin of such fish affects behaviour that is crucial for their survival. The potential impact of rearing history on the spawning behaviour of pikeperch is suspected to have significant implications for their natural reproductive capacity, raising concerns about the suitability of intensively-cultured fish for restocking and replenishment programs. Nevertheless, there are currently no studies targeting this specific aspect of pikeperch aquaculture. Thus, this study aimed to investigate how broodstock rearing history (pond vs. intensive) influences natural reproductive traits.

Methodology: 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. Intensively cultured fish meant fed by pellets, reared in a flow-through system, at a density of 15-20 kg per cubic meter, under natural photothermal conditions. 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.

Results: Preliminary results indicate that pairs where one of the partners or all, were originating from intensive rearing (groups 2, 3 and 4), exhibited significantly worse reproduction outcomes. The cleaned area of the nest was significantly smaller, indicating inadequate nest cleaning by the pikeperch. This was further reflected in the reduced number of larvae produced. Regarding general spawning characteristics, it was evident that pikeperch females of intensive origin often failed to

ovulate and underwent artesia (egg resorption) therefore spawning did not occur. This was further confirmed by statistical analysis of the data. Moreover, the data suggests a change in the mechanism of stress response based on the pikeperch broodstock origin, as there were significant differences in concentration of cortisol and glucose in the blood plasma.
During the course of the project 32 spawning events were recorded. They were grouped according to the origin of the fish. Statistically speaking it would be best to increase the number of repetitions that were included in the study. However, to adhere to the 3Rs principles, we concluded the research upon achieving statistical significance. Performing an observation study would be a realistic alternative likewise. Nevertheless, a reliable dataset was formed, clearly indicating the difference in behaviour, as confirmed by statistical analysis.

Innovation: This is the first study that confirms a potential difference in pikeperch reproductive behaviour depending on their origin. There were no previous studies on the effect of pikeperch origin on their ability to express natural behaviour, therefore it is possible to conclude that the output brings novelty and excels beyond current evidence.

EATiP Thematic Areas
TA7- Aquatic Animal Health and Welfare
TA3 - Managing the Biological Lifecycle

EATiP Goals
TA3 - G3: Improve broodstock management methods and control of sex and reproduction in captivity
TA8 - G2 : Establish an enabling environment for innovation and growth to allow aquaculture to realise its full potential

End Users, Applications and Impact:

	Potential Application	**Potential Impact**
Target User 1: Scientific Community **User description: aquaculture researchers, biologists, fish welfare specialists:**	Researchers can use the output as the foundation for further research to explore the mechanisms underlying the observed differences and to develop strategies to improve the reproductive performance of intensively cultured broodstock. The output can help forming an advanced perspective on the alteration of the fish behaviour in response to the rearing system.	Potential resulting impact of further research could be the establishment of better rearing conditions in pikeperch aquaculture and better reproductive performance of broodstock, resulting in increased productivity.
End User 1: Environmental Managers & Monitoring managers	The output underlines the importance of the fish origin on their behaviour, which is a key point of expectation if fish are reintroduced or introduced for bio-melioration purposes. This knowledge can be easily implemented by environmental	Potential resulting impact of application of the output could be an increase of precision ecosystem management, and performance of the controlled extensive aquaculture, where behaviour

	and monitoring managers responsible for restocking programs. The observed stress response in intensively cultured fish raises concerns about their suitability for natural spawning and restocking programs.	plays an important role in the production.
End User 2: Industry stakeholders **User description:** **pikeperch farmers**	The output shows that with domestication the spawning behaviour can be altered, and it might lead to impaired reproductive performance in case of usage of intensively reared broodstock. This output will alarm farmers to mitigate this issue either via modified broodstock selection or introduction of new spawning methods (i.e. fully controlled artificial reproduction).	Proven increased productivity

Evaluation Summary:
Total score: 25
Number of 3s: 4

3.3.21. AE3.0 TNA 23949

Novel use of bio-loggers in characterisation of European Seabass (Dicentrarchus labrax) and identification of four welfare states

OUTPUT type: TNA
OUTPUT owners: Joaquim Tomàs Ferrer, Pablo Arechavala López and Esther Hoyo Álvarez University of Balearic Islands (ES)
Link to output: Presented at the XXI Congress of the Portuguese Ethological Society in 2024. See page 37 of the Congress' Book of Abstracts: https://drive.google.com/file/d/1hefiE_b6ADRxC9-ifFOgsoDeHko-hksP/view. The open access research paper detailing the trial results is currently under revision for submission to the journal *Aquaculture*.
IPR Protection: Unsure
Status: TRL5. Further research is required to gain a deeper understanding on the relationship between the amplitude of the QRS wave (representing the depolarisation of the ventricles in the heart, triggering their contraction) and stroke volume in modulating cardiac output, as there is considerable variability in this mechanism across different species and types of stressors. Additionally, developing species- and life-stage-specific calibrations between acceleration, cardiac output and oxygen consumption would enhance the monitoring of fish behaviour and welfare in aquaculture settings. A new research project is currently underway and is investigating these concepts.

Knowledge Output Description:
Knowledge need: Assessing fish welfare requires an integrative approach encompassing physiology, behaviour, and biological performance. Stress significantly impacts fish welfare, and while stress indicators are useful, they must be complemented by other measures for a comprehensive evaluation. Heart rate bio-loggers have recently been used to assess physiological and behavioural responses in several species, from birds to bears. However, their use in fish is still novel, and laboratory calibrations are therefore required to evaluate the suitability of these devices for monitoring fish welfare. Bio-logging involves the use of implanted internal devices that enable the measurement of physiological parameters such as heart rate, which would be very difficult to measure otherwise with other techniques. Although there are studies that describe the use of bio-loggers to monitor European seabass' heart rate, acceleration at varying swimming speeds and stress loads, these variables have not been coupled with other key parameters such as metabolic rate (using oxygen consumption as a proxy) and/or swimming behaviour (e.g., tail beat frequency) to verify their accuracy. By understanding how metabolic rate changes during swimming conditions, events that are stressful for fish can be identified. Cost of transport (COT) refers to the amount of energy an animal expends to move a certain distance. The swimming speed with the lowest COT is the most energetically efficient speed for sustained movement. Therefore, determining the swimming speed at which the fish have the lowest cost of transport could help define optimal environmental conditions in aquaculture systems, minimise energy expenditure and promote growth. . The new knowledge generated from this study supports the use of bio-loggers' data to identify welfare

states, markers and indicators that can inform management practices in aquaculture, as well as use bio-logging fish per se.

This study aimed to identify any relationship between these variables and to gain a better understanding of the physiological and behavioural responses of European seabass to exercise and stress. Additionally, to test the effects of logging itself in fishes of this species, since logging could affect some of the observed parameters, non-logged control fish were also monitored to compare the swimming and metabolic activity of logged and non-logged fish.

Methodology: 12 European seabass individuals were surgical implanted with bio-loggers, placed ventrally into the abdominal cavity, next to the peritoneum. Six additional non-logged fish were used as a control group. Loggers were recovered after 26 days and data downloaded after experimentation, using the Oddi-Star software, which included heart rate (HR), heart signal amplitude (AMP) and acceleration (ACC). Fish ran swim tests in swim-tunnels, where they were set to swim at increasing speeds, while the oxygen consumption was measured, and swimming behaviour traits (head/tail beat frequency and amplitude) were recorded with the use of AI-trained cameras (using an internal prototype program designed by the Experimental Zoology Group of the Wageningen University & Research). In addition, fish also ran standard stress tests, including increasing stress loads and a recovering time of 1h between tests, where only bio-loggers' measurements (HR, AMP and ACC) were recorded. The stress tests consisted of four different levels: lowering the tank water level till the fish dorsal fins are exposed and refill immediately, lowering the tank water level till the fish dorsal fins are exposed and refill after 1 minute, lowering the tank water level till the fish dorsal fins are exposed and refill after 5 minutes and lowering the tank water level till the fish dorsal fins are exposed, chase the fish with hand nets and refill after 5 minutes.

Results: The optimal swimming speed (Uopt) did not differ between groups (logged and control). However, significant differences were found in the minimum cost of transport (COTmin), being 85.38 ± 2.49 mg.kg-1.km-1 for the control group and 113.45 ± 10.63 mg.kg-1.km-1 for the tagged group. Both tagged and non-tagged groups followed the same relationship pattern between oxygen consumption (MO2) and swimming speed. The fitted models reveal a significant positive correlation of swimming speed with head frequency (HBF) and tail beat frequency (TBF), both increasing with flow speed. The amplitude of the head and tail beats showed a lower positive correlation. No significant differences were found in the locomotion parameters between tagged and non-tagged fish at each flow speed.

Seabass external accelerations (ACC) showed a significant positive correlation with flow speed. Regarding cardiac activity, the HR also followed a positive trend. Similar to ACC, the first four speeds exhibit no significant differences on HR among them (Linear Mixed Model p-value > 0.05). Once the optimal swimming speed of 0.74 m.s-1 was surpassed, the HR at higher speeds (0.8 and 1 m.s-1) became significantly higher compared to the lower speeds (LMM, $p < 0.05$ and $p < 0.001$ respectively). There was virtually no correlation between the amplitude of the cardiac signal and swimming speed ($R2 = 0.07$).

A significant negative correlation was observed between MO2 and COT (R2 = -0.29, p < 0.05). ACC showed a strong positive correlation with MO2 and TBF (R2 = 0.76 and R2 = 0.69, p < 0.001) Similarly, HR was significantly correlated with MO2 (R2 = 0.56, p < 0.001) and TBF (R2 = 0.54, p < 0.01).

Seabass exposed to the stress challenge showed progressively increasing HR with each subsequent stress event. HR increased smoothly during the first two stress events (HRS1=83.91 ± 4.07 bpm; HRS2 = 89.9 ± 2.96 bpm), but the increase was more rapid in the last two events, reaching its maximum at the fourth event (HRS4 = 104 ± 2.10 bpm). Within each stress event, HR values did not significantly differ from the preceding event (LMM; p > 0.05) but were significantly different across all the other events (LMM; p ≤ 0.01 in all cases). After the stress challenge, HR did not show a complete recovery, remaining significantly elevated three hours later compared to basal levels (HRpost = 76.99 ± 3.16 bpm; LMM; p < 0.01).

ACC showed sharp peaks coinciding with stress events. After each stress event, ACC values returned to basal levels, including three hours post-stress when reported values were similar to basal levels. High peaks in AMP were also recorded during the stress events. Significant differences were also observed between AMP during stress events and during the recovery periods. Following the stress challenge, AMP values returned to basal levels.

A significant positive correlation between oxygen consumption and heart rate and acceleration was found during the swim challenge test. Acceleration values recorded by the bio-loggers were strongly correlated with head and tail beat frequency, thereby validating heart rate and acceleration as reliable proxies for energy expenditure in seabass. During stress challenge tests, heart rate increased progressively with each subsequent stress event, whereas amplitude and acceleration peaked with stress but decreased in between stress induction steps.

Overall, the assessment of physiological and behavioural responses of European seabass in this study allowed for the characterization of four general physiological states; 1. resting, 2. regular activity, 3. reactive stress response and 4. proactive stress response, that can be used to evaluate the welfare of captive fish.

Innovation: This study validates the novel use of bio-loggers in the physiological and behavioural characterisation in European Seabass and therefore the use of bio-loggers to monitor aquaculture fish. The knowledge and data generated will also contribute to the ultimate goal of fish selection according to physiological characterisation based on different swimming activities and stress loads. Gaining further insights onto how these variables interact in European seabass can help farmers make informed decisions about management (e.g. optimised farming practices), can improve fish health and welfare, profitability, public perception and reduce the environmental impact by optimising feed usage, minimising waste and reducing the risk of escapes.

EATiP Thematic Areas
TA2 - Technology & Systems

TA3 - Managing the Biological Lifecycle

EATiP Goals
TA2 - G4: Ensure technology for ethical and healthy production of high quality products
TA3 - G1: Establish predictability and improve output and cost control at every production stage of the lifecycle

End Users, Applications and Impact:

	Potential Application	Potential Impact
Target User 1: Scientific Community **User description: fish ecologists, fish ethologists, fish physiology researchers**	Overall, the assessment of physiological and behavioural responses of European seabass in this study has allowed for the characterisation of four general physiological states of seabass (resting, regular activity, reactive stress response and proactive stress response). The definition of these welfare states will improve the understanding and further evaluation of welfare in captive fish. This knowledge could be used by researchers to design future studies, addressing fundamental questions about animal behaviour, ecology, evolution and physiology.	A potential impact resulting from this knowledge output is an increased understanding of European seabass ecology, evolution and physiology. Furthermore, the insights gained can serve as a foundation for formulating new hypotheses and designing targeted experiments to delve deeper into the complexities of European seabass life history and adaptation.
End User 1: Industry stakeholders **User description: seabream farmers, seabream managers, sea bass breeders**	The aquaculture industry, especially the Mediterranean sector, where the majority of European seabass is farmed, can benefit from the knowledge generated in this study by using these tools (bio-loggers) to monitor the different aspects of the fish's physiology. The use of bio-loggers would provide highly precise information about individual fish under different farming conditions, enabling European seabass managers and farmers to adapt their strategies to improve fish health, welfare and profitability.	Ultimately, an increased understanding of welfare states of European seabass would support farmers/mangers to improve farming practices. A demonstration of the efforts towards enhancing fish welfare could potentially help in building consumer confidence, contribute to a positive public perspective of aquaculture and fostering a more sustainable and ethical aquaculture sector.

Evaluation Summary:
Total score: 25
Number of 3s: 2

4. Appendix

4.1. IRAP Terms of Reference

AQUAEXCEL3.0
Terms of Reference – IRAP / Industry & Research Advisory Panel

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 871108 (AQUAEXCEL3.0). This output reflects only the author's view and the European Commission cannot be held responsible for any use that may be made of the information contained therein.

1. Overview of AQUAEXCEL3.0

AQUAEXCEL3.0 is a European Union funded research infrastructure project (under the Horizon2020 Funding Programme), aiming to further support the sustainable growth of the aquaculture sector in Europe. The project comprises a large group of leading European aquaculture research facilities that work towards advanced integration and standardisation of tools for aquaculture research.

AQUAEXCEL3.0 aims to further boost the European aquaculture sector by, amongst others, expanding the Transnational Access programme to support even more external research teams collaborating with its high-quality facilities, providing free training courses on the latest topics and engaging closely with industry to ensure research is aligned with industry needs.

It is expected that AQUAEXCEL3.0 will produce numerous results that will be relevant for the European aquaculture industry in particular. The project has set up an OUTPUT collection system, to collect and analyse all research outputs, both from the project itself, as well as from the additional research projects carried out by external researchers in the TNA programme.

AQUAEXCEL3.0 builds on the achievements of the previous AQUAEXCEL (FP7) and AQUAEXCEL^{2020} (H2020) projects, with the ambition to boost this effort in a user-centric approach. AQUAEXCEL^{2020} produced many highly interesting OUTPUTs, some of which have not been evaluated yet. Those will also be assessed for relevance to the European aquaculture industry.

These totality of OUTPUTs will be presented to the Industry & Research Advisory Panel (IRAP). The eventual aim is to transfer any OUTPUTs that IRAP members have identified as having potential high impact on industry, to relevant users. AQUAEXCEL3.0 aims to really make an impact on the European aquaculture industry, and the IRAP will play a key role in doing so.

2. Industry & Research Advisory Panel

2.1. Role/Purpose

The IRAP is expected to be an interactive advisory body and contribute both to upstream guidance (e.g. industry need recommendations) as well as to downstream impact/dissemination as it will aim at maximizing the possibilities for new knowledge to be translated into innovation, and so substantially increase the possibilities for success.

The AQUAEXCEL3.0 IRAP will act as a pro-active interface for the project involving the research community and the aquaculture industry. This will strengthen industry-research relations and develop research that meets industry needs for innovation.

AQUAEXCEL3.0 aims to prioritise and carry out research projects (both being part of the project itself, as well as the TNA research projects) that are in line with identified needs of the European aquaculture industry. A key role of the AQUAEXCEL3.0 IRAP is to provide recommendations on current industry needs (EATiP Strategic Research and Innovation Agenda) so the project can focus on research projects addressing these.

Specifically:

- IRAP will provide recommendations on current industry needs and select focus areas for the TNA calls (prioritised research areas). By relating the TNA calls to the EATiP SRIA, AQUAEXCEL3.0 will directly contribute to the implementation of the research goals the European aquaculture sector has agreed upon
- AQUAEXCEL^{2020} and AQUAEXCEL3.0 OUTPUTS will be collected and presented to the IRAP in the form of Impact Plans. Following a transparent procedure, the IRAP will objectively analyse the OUTPUTS and evaluate and score potential level of impact on identified industry end users. IRAP will provide a summary assessment for each OUTPUT and select promising results for showcasing at appropriate industry-research brokerage events.
- IRAP has the mandate to revise the AQUAEXCEL3.0 Knowledge Transfer methodology and the catalogue selection criteria
- IRAP will assist with the development of feedback templates for the industry-research brokerage events, to assure monitoring of brokerage, contacts, interest and uptake.

NOTE: IRAP prioritization of subjects and distribution of incentives are not compulsory, so that research teams that want to perform completely independent research in AQUAEXCEL3.0 RIs have the possibility to do so.

2.2. Term

This Terms of Reference is effective from the 1st November 2020 and continues until the 31st October 2025 (expected date of completion of the project).

2.3. Composition

The IRAP will comprise of:

- a maximum of 15 external European industry experts with expertise in 'aquaculture'. We strive to have a panel representing all major thematic themes in aquaculture.
- The 7 work package leaders of the AQUAEXCEL3.0 project

IRAP experts already involved in AQUAEXCEL^{2020} will be invited to reengage. Additionally, and where needed, new experts will be selected based on their CV, to ensure that the main aquaculture sector thematic areas are covered.

2.4. Commitment and Compensation

- It is envisaged that the time commitment required will be about five to six days per year, over a period of 5 years in total.
- IRAP members are expected to attend the IRAP meetings (at least once a year). When organised as a physical, they will take place in conjunction with AQUAEXCEL3.0 ExCom and/or General Assembly meetings.
- IRAP members are expected to be prepared for each IRAP meeting by assessing the Impact Plans (OUTPUTs) in advance, by means of desk study. EATiP and ERINN Innovation will ensure timely delivery of the impact plans (OUTPUTs).
- Participation in short, ad-hoc virtual meetings directed towards IRAP members is expected.
- Travel and subsistence in line with European Commission Travel & Subsidence (T&S) rules will be covered to attend the physical IRAP meetings. In addition, an honorarium (€250) is foreseen per expert to compensate for the time to prepare, attend and contribute to the IRAP meetings (min. half a day) where OUTPUTs within one's own field of expertise are discussed.
- IRAP members will get a first-hand insight into the latest aquaculture research happening in Europe, and the opportunity for networking with other key aquaculture experts.

3. Meetings

- All meetings will be chaired by EATiP / ERINN Innovation
- Decisions will be made by consensus (i.e. members are satisfied with the decision even though it may not be their first choice).
- Meeting agendas, minutes and supporting papers will be prepared and provided by EATiP / ERINN Innovation

4. Working Methodology

- Experts will be sent so-called Impact Plans (each containing an OUTPUT with key information included) to be evaluated latest one week in advance of the IRAP meeting.
- Experts might need to spend one day of desk study to assess the Impact Plans.
- Experts should complete the 'Impact Plans evaluation' and return them to ERINN Innovation (cc EATiP secretariat) by the timeline provided with the correspondence.
- A minimum of three external IRAP evaluators per OUTPUT is requested.
- Experts should make contact with the EATiP secretariat if they require any additional information or if they are not able to carry out the assessment within the time period requested.
- If an expert wishes to resign from the IRAP, notice should be given to the EATIP at the earliest convenience.

All information provided to the IRAP experts are to be treated as confidential.

5. Amendments, Modification or Variation

This Terms of Reference may be amended, varied or modified in writing after consultation and agreement by the Industry & Research Advisory Panel (IRAP) members.

6. Contact

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Work Participants	ERINN Innovation			

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[1]Dissemination level (DELETE ACCORDINGLY): **PU:** Public, **CO:** Confidential, only for members of the consortium (including the Commission Services), set out in Model Grant Agreement, **CL:** Classified, information as referred to in Commission Decision 2001/844/EC

[2] Nature of deliverable (DELETE ACCORDINGLY): **R:** Report, **DEM:** Demonstration, pilot, prototype, plan design, **DEC:** Website, patent filing, market studies, press & media, videos, **Other:** Software, technical diagram, etc., **Ethics:** Ethics deliverable