PROJECT FINAL REPORT

Grant Agreement number: FP7-262336

Project acronym: AQUAEXCEL

Project title: AQUAculture infrastructures for EXCELLence in European Fish Research

Funding Scheme: Combination of CP & CSA

Period covered: **from** 01 March 2011 **to** 28 February 2015

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[1] Usually the contact person of the coordinator as specified in Art. 8.1. of the Grant Agreement.
[2] The home page of the website should contain the generic European flag and the FP7 logo which are available in electronic format at the Europa website (logo of the European flag: http://europa.eu/abc/symbols/emblem/index_en.htm logo of the 7th FP: http://ec.europa.eu/research/fp7/index_en.cfm?pg=logos). The area of activity of the project should also be mentioned.

Table of contents

Final Publishable summary 3
1. Executive summary 3
2. Summary description of project context and objectives 4
3. Description of the main S&T results/foregrounds 5
4. The potential impact and the main dissemination activities and exploitation of results 24
5. Project information 30

Figures :

Figure 1: AQUAEXCEL RI map 5
Figure 2: Examples of outputs of Metabolomic / proteomic software 7
Figure 3 Scheme of Experimental Data Repository 8
Figure 4: Infrastructure utilisation by units of resource (requested and committed) 10
Figure 5: Applications and approvals by country 11
Figure 6: AQUAEXCEL e-Infrastructure site 16
Figure 7: Number of applications and implemented TNA projects in each thematic area 26

Final Publishable summary

1. Executive summary

AQUAEXCEL has provided the European research community with a platform of top class aquaculture infrastructures effectively offering tools to cope with the complex challenges which the European aquaculture sector is facing. Through the provision of a wide range of aquaculture production systems (recirculation, flow-through, hatchery, cage, and pond systems), environments (freshwater and marine, cold and warm water), scales (small, medium and industrial scale) and aquaculture fish species (sea bass, sea bream, salmon, cod, trout, carp) AQUAEXCEL offers innovative and diverse research opportunities in a coordinated manner.

The overall objective of AQUAEXCEL was to integrate, on a European scale, key aquaculture Research Infrastructures, in order to promote their coordinated use and development.

First of all, AQUAEXCEL has built a reference web portal for aquaculture research infrastructures (RIs) in Europe, which includes a user friendly, searchable RI map with more than 100 entries to date; many of which are from infrastructures beyond the AQUAEXCEL consortium. This RI database has also allowed a gap analysis identifying the competences/ infrastructures lacking at the EU level to implement the Strategic Research and Innovation Agenda of the European Aquaculture Technology and Innovation Platform (EATiP).

The AQUAEXCEL consortium developed, analysed and disseminated best practices on a variety of pertinent issues for the aquaculture industry, for example guidelines for sanitary issues linked to fish transport between infrastructures, for measuring and accurately defining traits and phenotypes, and for developing traits and environmental conditions ontologies. In parallel, a data management tool has been developed to structure, conserve and share aquaculture research data.

A tailored and targeted dissemination strategy has made AQUAEXCEL well known in the aquaculture community. We have successfully delivered four new pioneering technical training courses that focus on different aspects of aquaculture experimentation. A selection of publications including our project newsletters and AQUAEXCEL Key Achievements booklets were complemented by an industry focused workshop at the Aquaculture Europe conference 2014 in San Sebastián, which gathered over 140 participants from the whole aquaculture sector.

Transnational access was highly successful, with 146 applications resulting in 97 projects funded and implemented. It allowed a number of European aquaculture researchers from academia and industry to perform experiments in the top-class infrastructures of the AQUAEXCEL partners, producing innovative knowledge for EU aquaculture development.

Our work on e-infrastructures now allows researchers to remotely access their on-site experiments at AQUAEXCEL facilities such as IMARES, NOFIMA, NTNU, SINTEF, and WU. A study on how e-infrastructure affects the design, outcome, cost and collaboration in an experiment has also been carried out. In addition, AQUAEXCEL's work on upscaling has now confirmed that scale has an effect on experimental results, and also propose explanatory factors that can help extrapolate small scale experiments to industry size application.

AQUAEXCEL also provided the scope to integrate knowledge on measuring more precise phenotypes of individual fish by combining experimental approaches on fish, genomics and bioinformatics. Very promising results on the importance of mitochondrial pathways in stress response were produced, and scoring methods developed.

A significant effort was also committed to produce isogenic fish lines for major species like salmon, sea bass and carp; a priceless experimental resource for reproducibility of results and genetic studies. There have been significant experimental challenges and we have not yet reached the end of the story; with the first isogenic salmon lines produced in 2015, while the prospects are good to obtain isogenic carp and sea bass lines within the next two years.

2. Summary description of project context and objectives

AQUAEXCEL aimed to provide the European aquaculture community with a platform of top class infrastructures efficiently offering experimental capacities and expertise for all aspects of aquaculture research. This included the provision of a wide range of production systems (recirculation, flow-through, hatchery, cage, and pond systems), environments (freshwater and marine, cold and warm water), scales (small, medium and industrial scale) and aquaculture fish species (sea bass, sea bream, salmon, cod, trout, carp).

European aquaculture is a knowledge intensive sector facing challenges in many areas among which sustainable feed production, animal health and welfare, integration with environment, technology and systems, biological lifecycle, product quality and human safety, as identified by the European Aquaculture Technology and Innovation Platform (EATiP).

These challenges can only be solved through the contribution of high level experts and experienced professionals, and through the availability of excellent complementary facilities, outstanding services and the right biological resources. Basic research is needed to optimize individual processes, whereas applied research is essential to relate research results to commercial production practices. Through collaboration among 17 partners and 23 facilities, AQUAEXCEL offers research infrastructures for both basic and applied research. AQUAEXCEL aimed at providing the necessary instruments to make better tools available for aquaculture research:

- Transnational Access (TNA): provide the best European and Associated States researchers free of charge opportunities to work with different systems, species, environments within a wide range of scientific and technological to access excellent research infrastructures they otherwise would not have access to (WP4, WPs10 to 32).
- Networking Activities (NA): make better use of existing research infrastructures through harmonization of practices, establish a common language (ontology of traits and environmental conditions) and identify sanitary status and procedures (WP3), stimulate collaboration across fields, systems, species, national borders (WP4, WP5), allow the identification and secure provision of the best facilities to solve present and future research problems that may counteract the objectives of competitiveness, sustainability and increased production (WP2).
- Joint research activity (RA): develop, implement and evaluate technical solutions (e-Infrastructure) for providing access to the highly specialized aquaculture research facilities within the AQUAEXCEL consortium, and facilitate cooperation within the consortium (WP6), develop new methodologies and protocols, for *in vivo* fish phenotyping in research infrastructures, with a multi-disciplinary approach (WP7), increasing the value and relevance of research results at industry scale by identifying scale factors in tank or cage experiments as well as biofilters (WP8), and develop highly standardized isogenic fish lines to improve the reproducibility of research results, facilitate the establishment of the links between genotype and phenotype, and ultimately reduce the number of fish used for experimentation through a better control of sources of variation (WP9).

The overall objective of AQUAEXCEL was to integrate, on a European scale, key aquaculture research infrastructures, in order to promote their coordinated use and development.

3. Description of the main S&T results/foregrounds

3.1. Key result no 1: Online RI map, Gap analysis and collaboration models

Workpackage: WP2 – Infrastructure mapping, strategic planning and sustainability

The first result produced by WP2 was the online RI map as a tool for:

- Counteracting the fragmentation of aquaculture RIs in Europe
- Making existing facilities and expertise areas more visible
- Stimulating mutual collaboration between RIs
- Facilitating access to existing RIs (also for industry partners)

AQUAEXCEL developed a software tool with a simple user interface, allowing any RI provider to upload details about their infrastructure within different categories, i.e. country, field of expertise, type of species, type of site, type of water, fish line properties, monitoring and control equipment. The first five issues are searchable. Additional information about the institution owning the RI, the contact person and web site are also provided for each RI. The entries are checked and approved by the system administrator before publication on the map, which is based on Google maps.

Figure 1: AQUAEXCEL RI map

Visitors can zoom in and out and can retrieve all information connected to a particular RI by clicking on its icon. Facilities that were open for Transnational Access stand out through a different colour setting. It is a dynamic system, making it easy for information providers to change details whenever necessary. By the end of the project, the online RI map consisted of more than 100 RIs. AquaTT who designed the map and managed its administration throughout the project have agreed to continue to provide this valuable resource/service to the aquaculture community for the foreseeable future. It is the policy of the project to allow the possibility of handing over the system to other suitable networks or portals if the AQUAEXCEL network is no longer viable.

AQUAEXCEL also compared the fields of expertise and type of facilities at existing aquaculture RIs in Europe to those required to implement the research goals as defined by the European Aquaculture Technology and Innovation Platform (EATiP). This was achieved by carrying out a gap analysis for each EATiP thematic area which requires experimental research using aquaculture RIs. Conclusions show that the current pool of European RIs largely cover the fields of expertise and facilities needed to comply with the main challenges in the European aquaculture sector. However, a number of subgoals from different thematic areas were found not to be sufficiently covered by current RIs in Europe. In these cases, research progress will rely on expertise and/or facilities from third countries or on the development of new research teams and infrastructures.

Based on collaboration priorities as perceived by the aquaculture RIs in the AQUAEXCEL consortium and on the particularities of aquaculture research, five different organisation models for structured RI collaboration were analysed. This was done by using the SWOT (Strengths, Weaknesses, Opportunities, Threats) methodology. The analysis resulted in the recommendation of a project based collaboration combined with an RI working group under the auspices of EATiP in the short term, and the constitution of an ERIC as a longer term strategic aim. The report provided relevant advice to the aquaculture RI community on how to organise a collaborative network in the most efficient and satisfactory way.

3.2. Key result no 2: Common standards, protocols and ontology

WP3: Definition of common standards, protocols and ontology

Research aims and background
There are important differences in criteria, methods and processes used at different levels in aquaculture, and these differences in criteria or lack of harmonization increase the variation and affect the comparison between experiments. To overcome these effects, the general objective of WP3 was to produce a common language for researchers, companies and even consumers, generating a glossary and a Best Practices Guide, as a way to harmonize, standardize and validate experimental processes and phenotypic characterization. To do it, the main factors were identified, and grouped under different and specific objectives, as follows:

Development of an ontology for fish traits, measure methods and environment conditions, which is expected to facilitate organization or comparison of data, standardize nomenclature, facilitate knowledge transfer within and across species, facilitate data retrieval and data analysis, and enhance education. Two ontologies were produced: i) *Fish-ontology trait base,* which corresponds to a dictionary of features used for phenotyping farmed fish; ii) *Fish-ontology environment trait base*, which lists the physico-chemical parameters and the components of structures which participate in changingfish phenotypes; iii) *Fish measure technical base*, to standardize the name and principle of measurement methods for major traits groups related with growth, quality of fish and meat and biosanitary quality of fish), and experimental procedures carried out (across the different cultivation systems and scales), to define their field of application, to identify their advantages and disadvantages and to provide bibliographical references.

Construction of an experimental data repository, for storage of experimental data and description of experiments and other tools for the data management. The repository has been done by AQUAEXCEL members to store all data types and structures produced by the project, where information can be shared.

Definition of sanitary standards for the transfer of fish or germplasm, in order to elaborate a manual to make easier for the different researchers of the AQUAEXCEL project to draw up sanitary measures applicable to the movement of fish and/or germplasm between the different research centres belonging to the AQUAEXCEL Project.

Results and applications

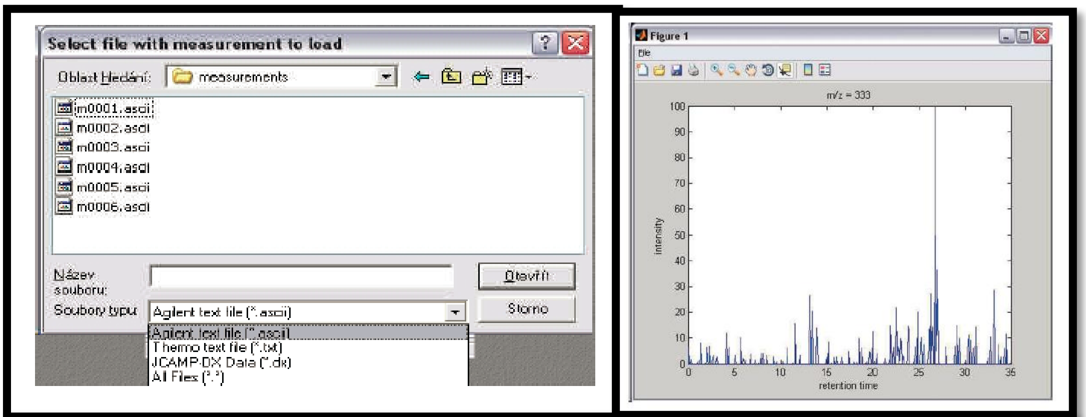
Sanitary prescriptions and procedures for transfer and safety standards (D3.1):
A Handbook of Best Practices was produced, taking into consideration the fish species to be transported, the health status of the country of origin and the research centre, in order to avoid the introduction of diseases into the research centre or country to which the fish and/or germplasm are transported. In terms of application, it allows an optimum level of sanitary security for the transport of fish or germplasm between the different research centres, particularly those participating in the AQUAEXCEL Project. Its recommendations refer only to the sanitary conditions that should be met. In addition, an update of European and implied countries sanitary regulation, and Research Infrastructure legal sanitary status (disease-free, surveillance programme, undetermined, eradication programme and infected) was carried out.

Best practices and cross-applicability of methods to measure phenotypes (D3.2).

A Best Practices Manual has been produced regarding the most relevant traits in aquaculture related to growth, performance, diet, reproduction, fish and meat quality and bio-sanitary safety, harmonizing and standardizing the measurement methods of these phenotypic traits, as well as experimental procedures taking into account the different culture systems, scales and species. It provides a way of harmonizing, normalizing and validating experimental processes and phenotypic characterization in a common language for researchers, companies and even consumers. This will allow study and laboratory comparisons, both in research and industrial contexts.

Fish-ontologic base (phenotyping/environment) and measure technical base (D3.3).
Ontologies covering fish phenotypic traits (ATOL, Animal Trait Ontology for Livestock) and environment parameters (EOL, Environment Ontology for Livestock), linked with measurement method procedures, have been developed. The ontologies included highly relevant information like the name of each standardized trait relevant to the project, relevant synonyms, the standardized definition of each of these traits, the different species relevant for the trait, and a unique reference number. It allows a common and standardized language, unambiguous and understandable by everyone and used to construct all new phenotypic bases for different fish farms or animal models (trout, salmon, sea bream, sea bass, zebrafish, medaka ...).

Development of Metabolomic / proteomic software (D3.4).
A new software was developed for the automatic pre-processing of metabolomics/proteomics data based on the general analysis of measurement errors using the theory of stochastic systems. Liquid chromatography (LC) and Mass spectrometry (MS) methodologies were targeted, providing resolution estimation, noise filtration and segmentation.



	Mass	Probability	Relative content	RT	Begin Time	End Time	Max intensity	Area
Peak	509.2	0.005	0.05847	17.1129	16.9146	17.5930	1693675	2.6462e+007
Peak	861.0	0.952	0.00296	17.0323	17.0179	17.1732	260411	1.3385e+006
Peak	995.7	0.952	0.00062	17.0323	17.0323	17.0395	256928	2.7929e+005

Figure 2: Examples of outputs of Metabolomic / proteomic software

Experimental data repository (D3.5).
A repository for storage of experimental data and description of experiments, including tools for data management was constructed. The repository makes it possible to share standardised information between partners, using the developments carried out in the context of AQUAEXCEL as starting point. Different tools were developed providing the functionality to create an electronic protocol, attach experimental data and manage (organize, share, search) it in a user-friendly way. It allows an easier comparison and interpolation of information between experimental and industrial scales.

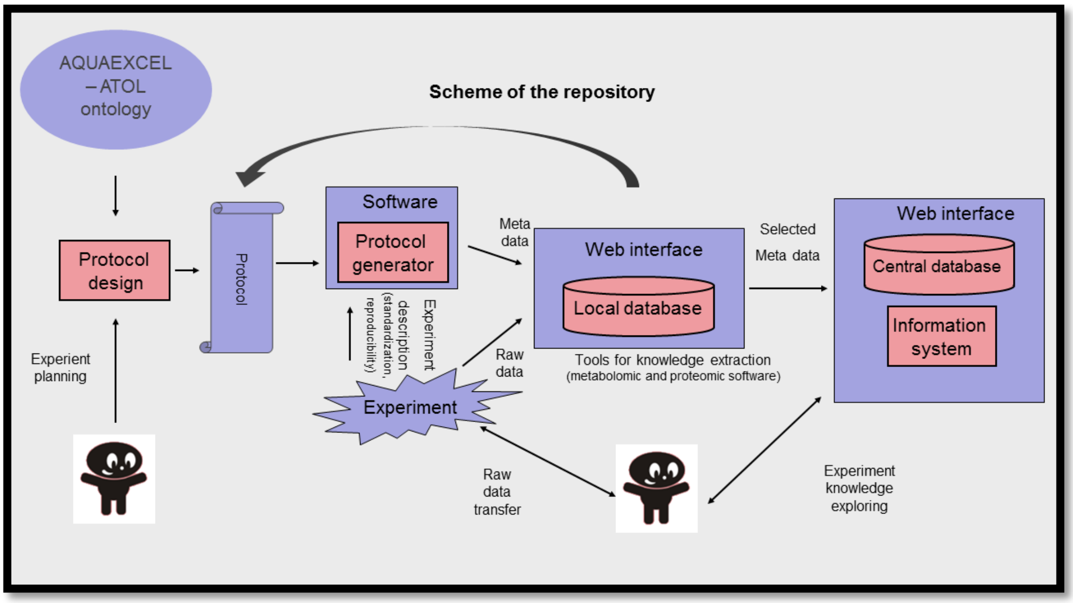


Figure 3 Scheme of Experimental Data Repository

Significance and benefits

The developments carried out in the context of AQUAEXCEL on harmonization and standardisation in fish traits, measurement methods, experimental and environmental conditions integrating all possible information and allowing standardised comparisons between experiments, and approaching experimental and industrial scales have not been done before. As benefits, all methodologies and tools developed in WP3 make it possible to build large and higher quality databases, allowing a higher robustness in the data analysis of biological information related to finfish aquaculture.

3.3. Key result no 3: Transnational access

Workpackage: WP4: Management of the calls for access and evaluation of the access given

Workpackage 4 concerned the management of the calls for transnational access (TNA) and the evaluation of the access provided by the participating infrastructures.

At the start of the project, it was decided to manage applications for TNA on the basis of 6-monthly calls with deadlines for submission of applications. The deadline for the first call was in month 7 of the project. Applications were made via a PDF form submitted to the WP4 coordinator. These were evaluated by a Selection Panel, consisting initially of four internal and four external (to the project) reviewers. Each application was initially reviewed by two internal and two external reviewers. Later in the project this was reduced to three reviewers (at least one external) and the Selection Panel was slightly enlarged to help speed up the processing of applications. Furthermore, in the final year of the project, the frequency of calls was increased to each 3 months in order to help streamline the application and review process by reducing the workload per call for the facility managers and Selection Panel.

The applications that were submitted were evaluated primarily on the basis of scientific quality and then on their relevance to the European Aquaculture Technology Innovation Platform (EATiP) Strategic Research Agenda. This industry led body worked to define research priorities for the European Aquaculture sector through the EU AquaInnova project. Further, criteria for evaluation were the quality of the dissemination and exploitation plans and compliance with the EU agenda for broadening access to research infrastructures. Consideration was also given to the adequacy of supervision where the applicant or TNA visitor was a PhD student or early stage researcher.

Projects that were approved by the Selection Panel were then passed to the project Ethics Adviser (also external), for comment and approval. In many cases, this resulted in recommended changes to procedures. Initially, projects that were judged by the Selection panel to be promising, but not sufficiently well developed or described for immediate approval were invited to reapply in the following call. Mid-way through the project when infrastructure utilisation was considered to be below target, this approach was changed to encourage applicants to re-submit as soon as possible after revising their proposal. This helped infrastructures to improve scheduling of work.

Throughout the life of the project, 146 project applications were received and processed. Ten of these were resubmissions from a previous call. Of the 136 unique project applications 103 were approved and 97 were ultimately implemented (65% approval rate). Twenty four of the applications came from partners already within the consortium (18%) of which 18 were approved (19%). This means 82% of supported projects involved researchers from outside the project consortium.

Tableau 1: TNA applications and approvals

Call	No. Applications	No. Approved/ implemented	% Approved
1	11	4/4	36
2	20	12/12	60
3	24	17/15	71
4	18	15/14	83
5	22	15/13	68
6	16	10/10	62
7	6	5/5	83
8	15	11/11	73
9	14	14/13	100
TOTAL	**146***	**103/97**	**65**

*Ten of the applications were re-submissions, so the number of unique project applications was 136.

All but one of the AQUAEXCEL infrastructures hosted projects, with 9 installations more than 100% utilized, 3 were 100% utilised and six others were above 70% utilization. In terms of units of resource, 126% of total available units of resource were applied for and 77% finally allocated and utilised.

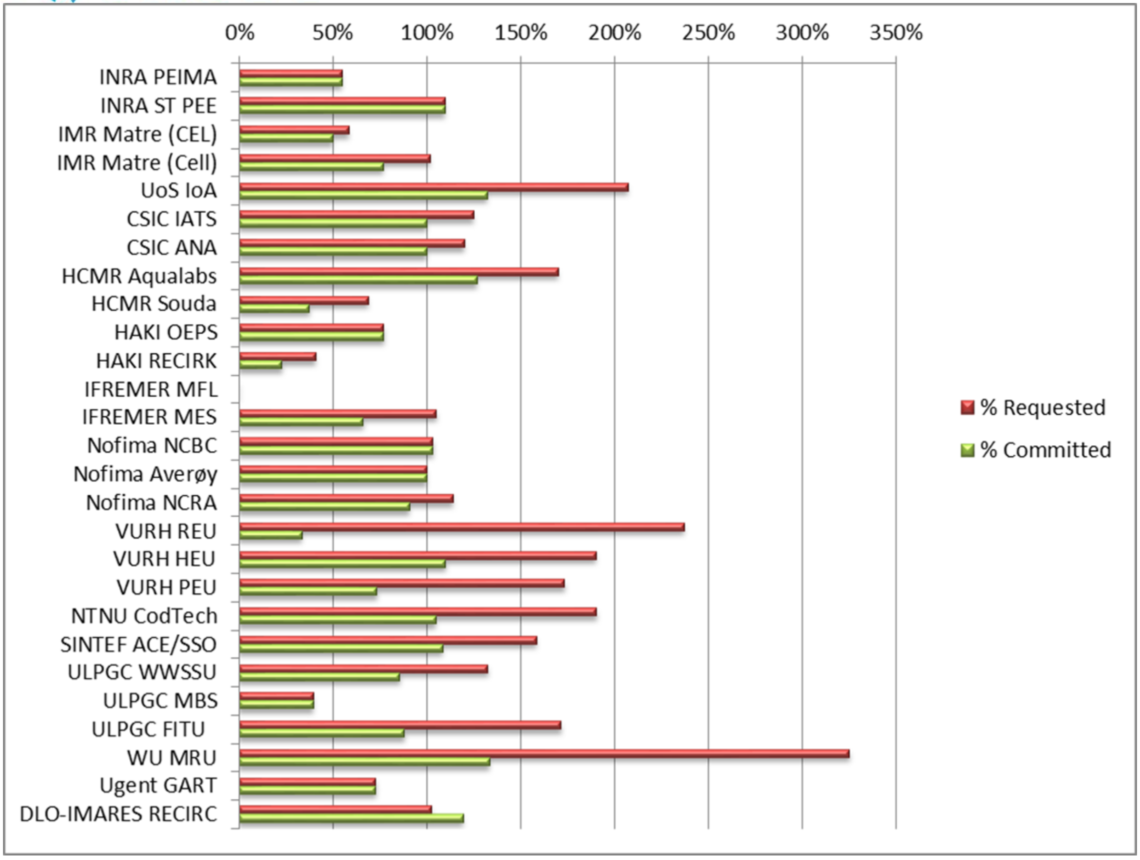


Figure 4: Infrastructure utilisation by units of resource (requested and committed)

In terms of numbers of successful project applications, the largest numbers were from Spain then Portugal, Germany, Italy, Czech Republic and UK.

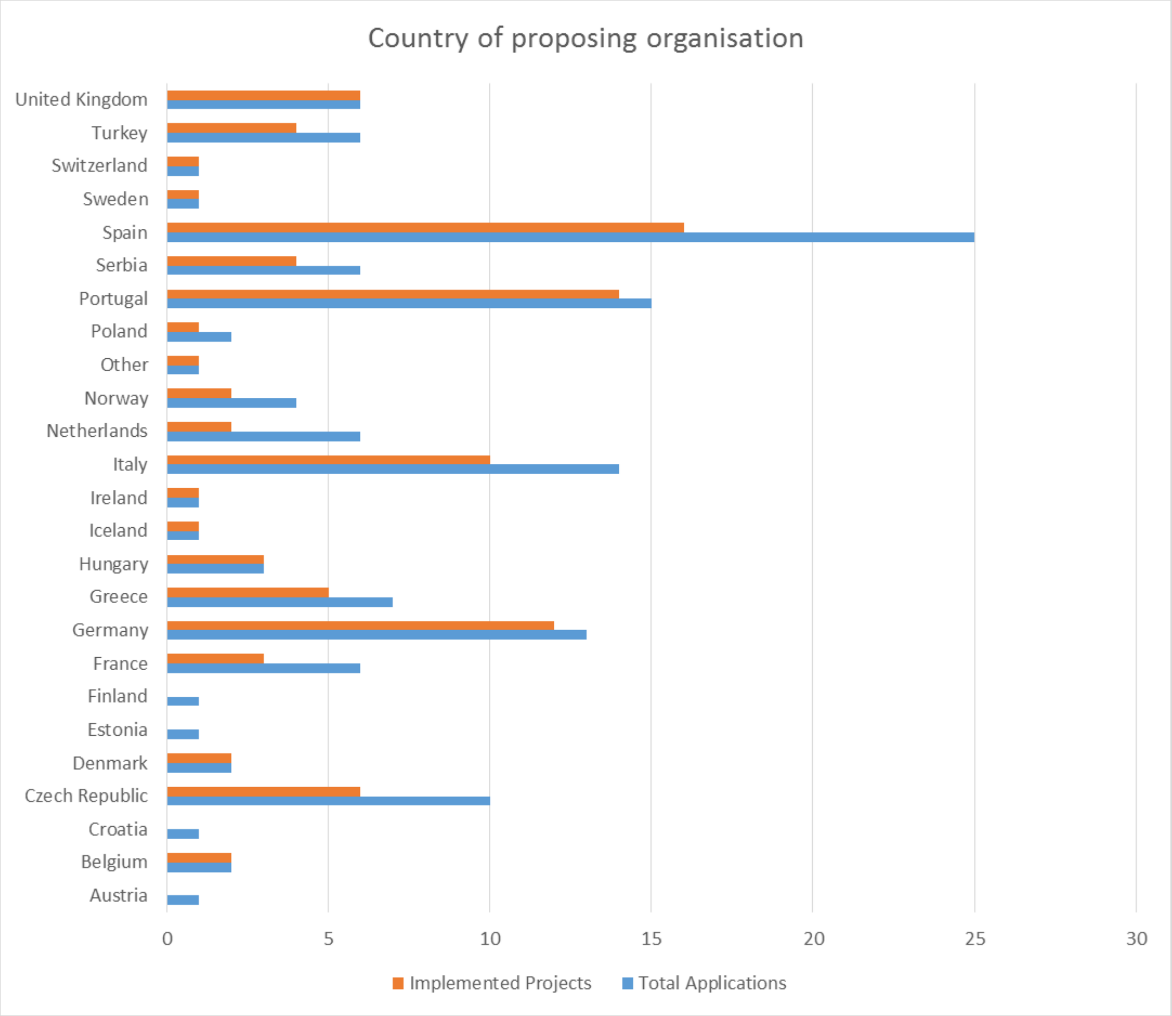


Figure 5: Applications and approvals by country

The majority of applications were from universities (61%) and research organisations (28%) with only 10% from SMEs and 1% from other organisations. The categories of staff were more balanced, with 26% postgraduate, 26% post doctorate, 35% experienced and 13% other. In terms of gender, 65% of applicants were male and 35% female. Approximately 50% of projects involved two people visiting the host infrastructure.

A final evaluation of the access given was carried out using questionnaires completed by infrastructure users, hosts and members of the Selection Panel. Responses were received from 77% of project users, 69% of project hosts and all but one of the Selection Panel members. The questionnaires contained a mix of Likert Scale questions and free text to ensure full details could be captured. The Likert Scale questions asked respondents to rate their experience of different aspects of TNA between 1 (poor) and 5 (excellent). The average scores in the final evaluation were all over 4.0 indicating very positive experiences for most participants. The final evaluation gave slightly higher scores than the first evaluation on most criteria.

Table 2: Average TNA user ratings

	First evaluation	Final evaluation	Variation
Application guidance	4.46	4.46	0.00
Application & selection process	4.21	4.25	+0.04
Feedback from evaluators	4.30	4.25	-0.05
Information on facility use	3.75	4.19	+0.44
Facilities of host institution	4.42	4.70	+0.28
Scientific support	4.45	4.48	+0.03
Technical support	4.45	4.59	+0.04
Logistics support	4.73	4.66	-0.07
Administrative support	3.70	4.29	+0.59
Intellectual environment	4.73	4.69	-0.04
Overall rating of experience	4.29	4.52	+0.23
Average variation			*+0.14*

Table 3: Average TNA host ratings

	First evaluation	Final evaluation	Variation
TNA Coordination	3.87	4.28	+0.41
Evaluation feedback	4.00	4.19	+0.19
User attitude	4.60	4.52	-0.08
User communication	4.25	4.39	+0.14
Quality of work	4.60	4.48	-0.12
Overall rating	4.50	4.50	0.00
Average variation			*+0.09*

The final evaluation of access given was able to make recommendations for improvements in process and management of TNA projects which will help guide future AQUAEXCEL programmes.

3.4. Key result no 4: 4 pioneering technical training courses (for full details see D5.6)

Workpackage: WP5 – Training, knowledge & technology transfer and communication

The vision for the European aquaculture sector is to be regarded as an environmentally, economically and socially sustainable activity. This intention should be grounded in evidence based scientific knowledge, industrial strength and consumer confidence. In order for European scientists to continue to be major contributors to the international scientific community and to provide relevant input to all stages of the aquaculture value chain, it is imperative to foster and build the human capital of the European aquaculture sector.

Responding to this need, AQUAEXCEL has provided four new and innovative aquaculture technical training courses, which contributed to encouraging career development and innovation in the aquaculture sector. Each course was provided by a different AQUAEXCEL partner in collaboration with several other partners and external experts, and so explored new models and partnerships which enabled peer-to-peer networking and collaboration both between AQUAEXCEL and non-AQUAEXCEL participants, as well as between industry and research communities.

Each of the four courses focused on different aspects of aquaculture experimentation as follows:

1. The first training course was organised by Wageningen University in Wageningen (the Netherlands), entitled 'Recirculating Aquaculture System (RAS) Technology'. The course was held from 22-25 April 2013.

2. The second training course was organised by INRA in Rennes (France), entitled ‘Contribution of Genomic Approaches to the Development of Sustainable Aquaculture for Temperate and Mediterranean Fish’. The course was held from 16-18 October 2013.
3. The third training course was organised by Stirling University (UK), entitled ‘The Application of Chromosome Set Manipulations and the Importance of Gamete Collection and Management in Aquaculture’. The course was held from 18-22 of November 2013.
4. The fourth and final course, “Efficient Utilisation of New Monitoring and Control Systems in Fish Experiments” took place at the Norwegian University of Science and Technology (NTNU) and SINTEF Sealab, Trondheim, Norway from 19 - 22 May 2014.

A total of 85 aquaculture stakeholders participated in the training courses and the evaluation surveys showed an increased competence in the topics dealt with, which will help maximising career pathways and job satisfaction in the aquaculture sector.

The number of registrations (which exceeded availability of places) showed a huge interest in these types of courses and we can conclude that the provision of lifelong-learning opportunities at all levels should be a central strategy to ensure knowledge transfer for an innovative and competent workforce in the European aquaculture sector.

3.5. Key result no 5: Dedicated website including up-to-date, interactive inventory of European aquaculture Research Infrastructures (for full details see D5.1)

Workpackage: WP5 – Training, knowledge & technology transfer and communication

To ensure that the results achieved during the AQUAEXCEL project are available to all and easy to access, a dedicated AQUAEXCEL website was set up at the start of the project and has been continuously updated to ensure it is a complete and accessible resource for those interested in the project and its results.

The AQUAEXCEL website offers general information about the project, freely accessible to all stakeholders and possible end users, including the general public. Website structure follows Best Practice EU Project Website Structure and aims for better quality, better visibility and higher user-friendliness of European project websites and includes general information about the project, its objectives, partnership, results, activities/events, a glossary, news, and other useful links. Dedicated pages have been set up for aspects such as Transnational Access, ontologies, the Fish and Chips tool and the BIOWES repository. In addition, the AQUAEXCEL website is unique in offering the most complete inventory of all the infrastructural services and facilities of aquaculture Research Institutions in Europe. This information is presented in a unified manner with an interactive digital map. This is an end-user friendly approach to improve access provision, for both researchers at project partner institutions, and also external scientists and new users.

The InfoBase is a viable European Aquaculture Research Infrastructure directory and a powerful tool to identify aquaculture facilities, genetic and human resources and to promote mutual collaboration. Prior to the development of this resource no such comparable, easily searchable, open access inventory of aquaculture Research Infrastructures in Europe was available. The many benefits for any aquaculture Research Infrastructure which is included in the map include provision of a unique chance to promote an institution and its facilities worldwide; allowing colleagues worldwide to identify an institution, its properties and its researchers; providing opportunities for international cooperation and creating networking connections.

The website can be found here: www.aquaexcel.eu

3.6. Key result no 7: Adding value to the European aquaculture industry through active Knowledge Transfer activities

Workpackage: WP5 – Training, knowledge & technology transfer and communication

Knowledge resulting from AQUAEXCEL could play a pivotal role in the sustainable development of aquaculture Research Infrastructures, ensuring they remain at the forefront of the advancement of research, and as a major source of competitive advantage in the aquaculture industry. However, capturing knowledge and making sure it can and will be used by relevant users is historically a big challenge. Knowledge transfer can be complicated by many factors such as the inability to recognise and articulate "compiled" or highly intuitive competencies and tacit knowledge; or it may be impeded by obstacles such as language and cultural barriers, lack of immediate incentives, and many more.

AQUAEXCEL, through WP5, set up a protocol to ensure all relevant knowledge coming out of the project was collected, analysed and transferred if deemed applicable. The AQUAEXCEL methodology was based on the Knowledge Management methodology successfully developed by the FP7-funded MarineTT project and adopted in many other initiatives. The methodology consists of the following steps:

1. Collect & Understand: development of protocols for collection and collation of data and knowledge for the duration of the AQUAEXCEL project in order to ensure that no knowledge is lost.
2. Analyse & Validate: careful assessment of collected information, requests for further information if needed and identification of potential end users and application of the knowledge.
3. Transfer & Exploit: identifying the most suitable methods for transferring knowledge with a view to ensuring that any knowledge transfer results in uptake and exploitation.

This approach simplifies the process of identifying knowledge outputs (e.g. products, methodologies, findings) and matching them with appropriate end users. Essentially, the information generated by research activities is customised and “packaged” so that it is understandable and appealing to the correct end user and ready for uptake.

Knowledge Transfer results include four booklets with key AQUAEXCEL achievements and an industry workshop focused on presenting achievements and results of the AQUAEXCEL project which can positively contribute to the enhancement of aquaculture in Europe and which are demonstrably beneficial to the aquaculture industry, which was attended by over 140 participants. The workshop provided a forum to strengthen ties between research and industry in the European aquaculture sector.

Through its innovative knowledge transfer activities AQUAEXCEL has contributed to the promotion of sustainable aquaculture practices, including the challenges of food production, environmental protection, product safety and economic viability.

3.7. Key result no 8: E-infrastructure for aquaculture research

Workpackage: WP6 – Development of e-Infrastructures for aquaculture research

<u>Research aims and background:</u>
Our objectives were to develop, implement and evaluate technical solutions (e-Infrastructure) for providing remote access to highly specialized aquaculture research facilities, and facilitate cooperation within the consortium. In Europe, highly specialized aquaculture research facilities are few and in many cases not efficiently accessible for outside partners. Utilisation of those facilities can be significantly improved through easier access for the European research community. In order to address these challenges, an innovative e-Infrastructure was needed. Efficient remote access will also contribute to reduced project costs, and contribute to continuous development of the facilities through higher utilization.

For supplementing the background, other infrastructure projects funded by the EU 7th framework programme involving e-Infrastructure development were surveyed. Of a total of 60 projects, 19 projects were found to incorporate the development of some form of an e-Infrastructure. It was also found that the projects varied a lot in scope and ambitions, but none had so far developed solutions which could be utilized by AQUAEXCEL.

Results and applications:
Based on a survey involving all AQUAEXCEL partners, the main technical and functional requirements for the e-Infrastructure were defined and documented in a report (deliverable D6.1). The design criteria for the first version also included organizational constraints (e.g. security policies concerning remote access and what kind of data should be accessible). The obvious consequence of involving multiple RI's in different countries was that the AQUAEXCEL e-Infrastructure needed to accommodate a wide range of existing solutions.

Taking the above considerations into account, the initial prototype (deliverable D6.2) and further developments (deliverable D6.3 and D6.4) are based on the following main principles:

- A web site will provide guides and links for access to facilities (login to this web site is required)
- VPN or similar security measures are required for secure access to each facility's local network
- Facility policies will govern internal network security and access to resources (clients, servers, sensor and actuator systems etc.)
- Each facility will be responsible for its own user administration (providing username/password and access levels), technical solutions for remote access and user training
- All data from experiments are stored locally

Based on iterative development and testing, the following four AQUEXCEL Research Infrastructures are now providing an additional e-infrastructure functionality for Transnational Access (TNA). Using results from AQUAEXCEL joint research activities on e-Infrastructure solutions, these four are now online (deliverable D6.5):

DLO-IMARES RECIRC (Recirculation Facilities):

- Access to sensor data
- Document sharing and version control

NOFIMA NCRA (Centre for Recirculation in Aquaculture)

- Sensor data monitoring
- Access to database with sensor data

SINTEF/ACE (AquaCulture Engineering)

- Access to databases with sensor data
- Automated transfer of sensor data to external project partners
- Online configuration and monitoring of project specific sensors (hardware dependent) and access to project specific server

Wageningen University MRU (Metabolic Research Unit)

- Sensor data monitoring
- Access to database with sensor data

The web site for providing access is shown below:

Figure 6: AQUAEXCEL e-Infrastructure site

All technical solutions are documented and can be used as support for implementing similar solutions for other research infrastructures.

Significance and benefits:
An analysis was performed using re-interpretation of specific trials for evaluating possible effects of an e-infrastructure on the design, the implementation and the logistics of the experiments. The trials used cover a wide range of parameters, infrastructures and organisms so that conclusions may be considered to have a wider applicability. The major conclusions can be summarized as follows:

- e-Infrastructures are expected to have a positive role in the quantity and quality of the collected data. They will increase the flexibility of the experimental procedures and also reduce the possible failures. They provide a safer environment of data storage and data sharing.
- e-Infrastructures will increase the experimental cost, at least initially, due to the required equipment. The large amounts of data collected may require additional labour for the analysis and in some case specific tools.
- e-Infrastructures will reduce the required travelling and the relevant cost of the investigators to the experimental sites. This lack of direct communication between partners may require the development of new schemes of collaboration.

3.8. Key result no 9: Set of phenotyping methodologies for experimental fish

WP7: Using individual fish as experimental unit

Research aims and background

In the next 20 years, fish aquaculture research will have to answer complex questions raised by the aquaculture industry and global change. Facilities and procedures will have to adapt to fluctuating environmental situations, to reduce their impact on the environment and to develop management procedures fully respectful of fish welfare. Moreover, aquaculture will have to select for those fish phenotypes better adapted to these new constraints. In this context, aquaculture research has to contribute to the development of relevant solutions, based on multidisciplinary approaches and on integrative knowledge of fish biology (behaviour, physiology, endocrinology, etc.). Thus, it will be essential to provide new methodological approaches that can provide the maximum amount of information for characterizing fish health and performance, with the minimum amount of fish. Along this line, genomic characterization of particular traits has been widely developed during the last years but still needs to be analyzed in an integrated way in order to provide a better way for phenotyping fish.

Approach

Our aim was to study in various fish species (salmoniforms -salmon and trout-; perciforms -sea bass and sea bream-) several major phenotyping issues which are important for aquaculture research. This includes 1) how to phenotype chronic stress, nutritional stress, early puberty, disease and 2) how to develop new tagging methods for studying individual larvae and juveniles. In all sub-tasks, the same approaches were developed, i.e. how genomic and/or physiological analysis could provide the maximal information to have accurate phenotyping on individual fish.

Result 1: new transcriptomic information and a new tool for meta-analysis of fish transcriptomes (for more details see D7.1)**.**

Analysis of transcriptome in fish target tissues using microarray technology has been a widely developed approach during these 10 last years in various biological studies, aiming to describe underlying molecular mechanisms. These approaches have already brought some significant new information (such as analysis of gene expression in fish exposed to stressful situation). We aimed at using such transcriptomic approaches to improve our capacity to refine phenotyping of important complex traits in aquaculture fish species

A first important result was the generation of original gene expression data using microarray technologies for characterizing complex traits such as response to stressors (nutrition or salinity). Moreover, we have characterized a set of mitochondrial genes in sea bream, which should be very interesting candidate for phenotyping stress responses. All these tools and information have been further used for the completion of the next results.

One of the difficulties in the use of transcriptomic data is the integration of such enormous amount of information in order to give a refined description of the trait. To help us in carrying out such complex analysis, we have developed a bioinformatic tool (Fish and Chips) which allows to compare the transcriptomic data obtained in one experiment with previous published transcriptomic data and then generate new hypotheses. This tool is freely accessible on the web (fishandchips.toulouse.inra.fr)

Result 2: Phenotyping environmental chronic stress (for more details see D7.3)

In order to provide a better way to phenotype fish under stressful environmental conditions in which fish may experience in aquaculture facilities, a multidisciplinary approach, from the whole organism to the molecular level has been used to find more accurate and relevant information from experimental fish. The methodological panel includes analysis of behavior, regulation of the HPI (hypothalamus, pituitary, interrenal) axis, physiological and transcriptomic profiles of the organs involved in the stress response (liver, pituitary, brain, head kidney, gills). Our working hypothesis is that chronic stress status is a complex situation which cannot be assessed by only analyzing one parameter, such as cortisol. Cortisol is the most commonly measured stress hormone, but is often not found to be elevated in chronically stressed fish.

The aim of this part was to phenotype the physiological and transcriptionally-mediatedresponses of gilthead sea bream, Atlantic salmon and rainbow trout in front of chronic stress. Sea bream and salmon were exposed to intermittent and repetitive environmental stressors for 21 days whereas a model of chronic stress (exposure to poor water quality inducing mainly hypoxia for 21 days) was established for rainbow trout.

Overall, these studies confirmed that a multiple-parameters approach integrating growth performance, plasma biochemistry, behavior and transcriptomics is necessary to get a relevant picture of chronic stress. Thus, by using similar experimental sets up, this approach indicate us that sea bream is less resistant in front of chronic stress compared to salmon or trout. Moreover, usefulness of cortisol responsiveness as a marker of chronic stress appeared to be variable and cannot be used alone. Other approaches, such as liver mitochondrial gene expression in seabream to assess allostatic load, or gill gene expression studies in rainbow trout can be used.

Non-invasive tools could also be complementary, such as behavior tests in trout or analysis of in skin mucus proteins in sea bream. Finally, it appears also that specific markers for any type of chronic stress do not exist, although simultaneous monitoring of a set of mitochondria-related genes is a promising informative tool to assess an allostatic load score.

Result 3: Phenotyping nutritional stress (for more details see D7.4)

In order to provide methods for phenotyping fish performance under nutritional stress in sea bream, salmon and trout, different experimental models were developed and data from nutrigenomics integrated with biochemical, physiological and endocrinological parameters allowed us to determine "dietary signatures" which characterized each nutritional stressor. Overall, these studies led to interesting possibilities to provide refined phenotyping methods for nutritional stress. Meta-analysis of several nutritional challenges or stresses (using the Fish and Chip tool) appears to be a very interesting approach to identify 'panels' of biomarkers. Moreover, this should also include the use of integrative tools combining conventional and "omics" approaches in order to get a suitable phenotype of fish performance.

Thus, in sea bream, study of the stress response to confinement appears to be a sensitive method for detecting differences in allostatic loads in fish with different nutritional histories. Moreover, molecular profiling of mitochondrial biogenesis and oxidative phosphorylation pathways is a robust tool for unravelling nutritional stress and led to refine a list of candidate mitochondrial-related genes characterizing metabolic disturbances due to nutritional stressors.

In rainbow trout, several molecular markers have been characterized for amino-acid-induced changes in food intake and include expression of specific brain genes. Iron metabolism is affected by plant-based diet and minerals, and several biomarkers have been suggested, including either parameters of iron metabolism or expression of candidate genes in the liver (hepcidin) or in the intestine (ferroportin).

In salmon, analysis of the transcriptome in intestine and liver led to the characterization of sets of genes which are differentially expressed only whensoybean mealinclusion is >30%: a first set was specific to differential expression in liver and includes genes related to digestion and energy metabolism. A second set of genes were regulated in both intestine and liver.

Overall, these studies provided suitable nutritional 'signatures' for phenotyping performance in farmed fish but extrapolation among species is difficult due to differences in nutrient requirements and life cycles.

Result 4: Early markers of phenotyping puberty in rainbow trout and sea bass. (for more details see D7.6)

The age of first sexual maturation is an important reproductive trait for genetic selection or for the experimental design of research programs. The detection of precocious puberty is also essential to plan cohort management in aquaculture. So far, the only reliable early individual indicators of maturation necessitate to kill the fish, such as histological evaluation of the gonad or the measurement of the gonado-somatic index (GSI).

In order to identify non-lethal/non invasive markers in trout, we have shown that blood plasma 11KT measurement allows efficient detection of the maturing males. This indicator is valid in trout if applied 3-4 months before peak spawning time. In sea bass, weight and length can help to predict the proportion of early maturation in a population before any histological sign of precocity. However it is not validated as an individual marker of maturation. In one year old sea bass, FSH was validated as marker of maturation but only in late stages of spermatogenesis.

Result 5: A standardized waterborne challenge for phenotyping Flavobacterium psychrophylum host resistance in rainbow trout. (for more details see D7.7)

Flavobacterium psychrophilum (Fp) is one of the major bacterial pathogens of salmonid fish in freshwater, causing considerable economic losses worldwide. No commercial vaccine is available so far and antibiotic treatments raise both environmental and consumer health issues. A 'natural' challenge is needed to refine phenotypes in experimental approaches aiming at better understanding mechanisms of infection and pathogeny of Fp, to test vaccine candidates or to design efficient selective breeding programs. Bath challenge is likely closer to the natural conditions of infection than injection. Therefore, the objective was to optimize a waterborne challenge with Fp in trout juveniles.

The present work allowed us to design a repeatable immersion procedure was that can be applied to large-scale experimental challenges. Though overall mortality is still moderate in the INRA-Sy standard population of trout (around 20%), it seems appropriate for application in different studies such as estimation of genetic parameters. To complement this result, a specific nested PCR test was designed to monitor the presence of Fp for a range of purposes (diagnostic, monitoring of infection kinetics). Interestingly, these studies **point to an external first line of defense (likely mucus and skin), which constitutes a key feature** to better understand pathogeny and host defense. However, the role of mucus in the resistance/susceptibility is not fully elucidated so far. Finally, the present work led to identify trout lines with a range of response according to the route of infection that can be used in further studies. For instance, highly susceptible lines will be useful to test vaccine candidates or attenuated bacterial mutants. Resistant *vs* susceptible lines constitute a relevant tool for functional and genetic analyses and have been proposed as experimental material in other ongoing projects.

Result 6: new tagging tools and procedure for larvae and juveniles individual identification (for more details see D7.2)

The ability to recognize individuals within a large population of similar-sized congeners has long been regarded as useful in experiments when one needs to identify specific lines that have no external phenotypes (e.g., mutant or transgenic lines), to monitor the same individuals over a long period of time (e.g., multiple challenges in behavioral analysis, monitoring of individual growth or spawning), or to know the genetic relationships of fish during on-growing process. Because many biological phenomena emerge early in life or depend intimately on the conditions experienced at the early stages of life, it is often necessary and important to individually tag fish as young as possible. The purpose of this work was therefore to settle tools and methods for allowing individual tagging or traceability of fish weighing less than a gram (about 3 cm) in sea bass and sea bream.

The present studies allowed us to validate new methods for identification of individual fish within a group in early life stages. This includes: i) RFID (Radio Frequency Identification) microtags (6 mm length, 1 mm diameter, 10 mg mass) for sea bass suitable for fish ≥ 400 mg and for sea bream ≥ 500 m, with recovery of normal behaviour within 24-48h after tagging and 100% reading success up to 92 g in sea bream.

3.9. Key result No 10: Definition and modelling of experimental scale factors (Full report in D8.1+D8.4):

Workpackage: WP8 – Upscaling and validity of research results

Definition and modelling of scale factors (Full report in D8.1+D8.4):

Research aims and background - Task 8.1 intended to provide a detailed description of the mathematical model developed within WP8, by listing the different modules and components comprising the model and interactions between these. The main aim of this model was to simulate how changes in physical scale of the production unit affect the performance of salmon and sea bass.

Results and applications – First activity was to conduct a survey regarding potential scale factors among the WP8 partners. Scale factor = a feature of the production environment which is known to vary with the physical scale (or geometric size) of the production unit, while having an impact on the performance (i.e. survival and/or growth). In the survey, the partners were invited to describe what they considered potential scale factors in finfish aquaculture, and if possible, how these scale factors would change with physical scale. The survey results were compiled into a common list of potential scale factors, which were used as input to the earliest formulations of the numerical model. Rather than developing the model by implementing all features, the model was built up as a framework which merged three previously published models covering 1) the spatial and temporal distribution of pellets in a production volume, 2) the energetic dynamics of individual fish over time, and 3) an individual based behavioural model for caged fish. Any required features that were not covered by these models were implemented directly into the framework. Such features included estimates of the flow patterns in indoor tanks, extrapolation and interpolation methods to ensure a consistent and continuous spatial environment and consistent couplings between the different model modules.

Significance and benefits - The outcomes from Task 8.1 represented a prerequisite for the work conducted in Task 8.2, and thus represented an essential basis for the further progress in the modelling tasks of WP8. Furthermore, the consensus reached regarding scale factors in this task ensured that the scope of the numerical model would be based on viewpoints and contributions from the partners in WP8.

3.10. Key result no 11: Growth and survival at different experimental unit scales (Full report in D8.2):

Workpackage: WP8 – Upscaling and validity of research results

Research aims and background – The main objective with task 8.2 was to determine the effect of experimental unit scales on performance in two fish species, Atlantic salmon and sea bass. The overall aim was to investigate whether the experiments we do in small research units are transferable to larger industrial units.

Results and applications - 13000 salmon smolts were used to investigate the effects of different tank sizes and of scale history. From transport and for two months scaling history was created as the fish were put in 0.9 m3 or 190 m3 tanks, where after they were redistributed to either 3 m3 (“1m→2m” and “11m→2m”), 0.9 m3 (“11m→1m”) or 103 m3 (“11m→7m”) tanks for five months. One group of fish was put directly to 3 m3 tanks from the truck and were not moved (2m→2m). All biotic and abiotic variables were controlled for. Accumulated mortality: 11m→1m: 46.3 %; 2m→2m/1m→2m: < 5%; 11m→2m: 28.8 %; 11m→7m: 19.2 %. At the end of the experiment the 11m→7m fish had the highest body weight (~1126g), while 11m→1m fish were significantly smaller (~553g). SGR: 7m=1.7; 1m=1.3. TGC: 7m=3.6; 1m=2.8. A much lower relative coefficient of variance (CV) was found in body weight between the 7m tank means in that treatment (0.4%), compared to between the 1m tanks (8%), and between cages in Task 2.2 below (9%). Behavioural recordings: 11m→7m fish changed position in the tanks significantly more often than the 11m→1m fish that held their position against the current. Fish in the 11m→7m group had significantly larger cardio somatic index compared to the other groups. It is proposed that the water velocity partly explains the higher performance among the 11m→7m fish, and that the poor performance among the 11m→1m fish may result from the handling that these fish experienced in addition that they did not tolerate the move to a very much smaller tank. In subtask 2.2., 600581 salmon smolts of the same origin as in subtask 2.1 were used as a comparison with this task. The salmon was kept in 3 cages (à 120m circumference). Biomass-estimators were used for growth trajectory estimates in addition to actual sampling. During this experiment, the fish were infected with pancreas disease, which affected the results, but in total there was less than 9% mortality during the 6 months experimental duration. Final weight: approx. 820 grams; TGC = 2.9, i.e. about 20% less than in tanks. Hence, when genetic origin and temperature were corrected for, this study shows that salmon in large tanks grow faster than in cages. A study on the effects of different tank volumes (2000, 500 and 40L) in European sea bass larval rearing was performed in subtask 2.3. A DEB model analysed the results. The tank hydrodynamics differed; in the 2000L tanks water currents were one order of magnitude stronger than the 40L tanks. Growth performance in the smallest scale was the worse. Growth differences were related to feeding. Growth in the smaller tank was limited by food availability. Differences in survival rate were not consistent with tank volume. The smallest tanks had the highest percentage of deformed individuals. The differences could be attributed to feeding differences and the hydrodynamics in the smaller tanks. No differences in whole body cortisol at two developmental stages; flexion and when the larvae body was fully covered by melanophores, were detected. The observation indicates that allostatic load on fish of the different groups was similar and within the coping abilities, in terms of the cortisol response axis. The observed differences in performance can be attributed to scale. The effects of different cage volumes in European sea bass were investigated in subtask 2.4. The trial was performed for 8 months in 1m3, 30m3 and 300m3 cages. The results showed significant differences between all scales. Survival was higher in bigger volumes. The growth rate was linear and was 0.68 g d-1 for the “300” group, 0.56 g d-1 and 0.32 g d-1 for the “30” and the “1” groups respectively. FCR: “300” group = 1.70±0.05; “30” group = 2.08±0.07; “1” group = 9.31±5.92. Fish in small and medium volumes showed higher plasma cortisol than fish in large cages, and may account for the differences in growth performance. The study shows that juveniles held in large numbers at the highest cage volume presented very low basal cortisol, suggesting that density is not the critical environmental factor, but rather water volume for expression of fish needs (swimming, schooling, social etc.).

Significance and benefits – This work shows that tank/cage size matters for performance and needs to be taken into consideration before implementing to the industry. It also shows growth potentials in the industry, and highlights that some of the tanks used in research give industry relevant information. Smaller growth variance in larger tanks means that when research is done in these tanks fewer replicates are needed due to higher statistical power.

3.11. Key result no 12: Effects of scaling on kinetics in moving bed bioreactors

(Full report in D8.3)

Workpackage: WP8 – Upscaling and validity of research results

Research aims and background – the aim was to commence studies on the effects of biofilter scale, on nitrification (removal of ammonia).

Results and applications – Media filling percentage: Effects of media filling were studied in the experiment on mixing time, in the multi-factorial comparison between small and medium scale and in the single factor experiments on small scale. Media filling% has a strong effect on mixing time in a small reactor. The effect of filling% on rTAN (removal rate TAN) indicates a reduction to 91% on rTAN at 25% filling which reduces further to 71% at 50% filling. The multi-factorial comparison between small and medium scale did show a decrease in rTAN from 35% filling to 74% at 50% filling. Identical biofilms were used and results can only be explained by hydraulic effects on biofilm level. Superficial air speed: In all experiments superficial air speed was taken into account since this is an important determinant for upscaling of bioreactors. The mixing time is strongly affected by air speed. Experiments on small and medium scale demonstrate a threshold of approximately 5m/h at which rTAN reaches a plateau. Above this threshold, mixing time will still be reduced with an increase in air speed, but with no clear effect on rTAN. However, if mixing intensity increases above 12m/h, rTAN increases again. Since there are significant effects of scale on rTAN at identical superficial air speed, superficial air speed cannot be considered a good scaling factor for TAN removal in MBBRs. Superficial air speed does not take into account the depth at which the air is injected. In upscaling, depth of air injection and pressure will increase. In general, the role of aeration of MBBRs in aquaculture is different from that in processing high strength waste water. In the latter case, the oxygen demand is high and intensive and continuous aeration is necessary to supply the respiring biofilm. In aquaculture, the oxygen demand can be covered by the oxygen arriving in the flow coming from the culture tanks, which are generally nearly saturated with oxygen. TAN concentration: The removal rate of TAN is up to a transition concentration limited by the concentration of TAN in the bulk fluid. Since TAN removal rate was chosen as the read-out parameter for nitrification, effects of TAN concentration on scaling have to be taken into account. There is a significant interaction between TANin and scale. The comparison of small scale and large scale systems was done at TAN levels in the outflow varying from 0.11 to 0.27 g/m3. Even at these levels we find a strong scale effect with ratio’s small/large varying from 0.69 to 0.82. The comparison between small and medium scale reactors shows that scale is a significant factor. Small reactors show a TAN removal that at high TAN concentration is in the order of 80% of a medium scale system. The comparison between small scale and large scale systems shows an even more pronounced difference as mentioned above even at low TAN.

Significance and benefits – this work shows that MBBR scale has a significant effect on TAN removal rate. In general, the larger the scale the better the performance. When designing aquaculture experiments that include performance of filters, upscaling effects should be taken into consideration when the performance of the filter can have an influence on the results of the experiment. The capacity of full scale systems will be under-estimated when based solely on small scale experiments.

3.12. Key result no 13: Validating the experimental scale simulation model (Full report in D8.4):

Workpackage: WP8 – Upscaling and validity of research results

Research aims and background – The aim was to provide a detailed overview of the final version of the fish model developed in task 8.1. The main aim of the model was to portray the growth of the fish in differently sized production units.

Results and applications- The first activity was to collect all experimental data from Task 8.2. Main focus was the data from the trials with Atlantic salmon, as the model as implemented in Task 8.1 was built upon parameters and data for this species. Environmental conditions, feeding schedules and production units were model inputs, and adapted so the model could formulate a virtual production environment mirroring the conditions in the experiments. A series of simulations were then conducted where the fish model responded to this continuous environment, both behaviourally and energetically. Final results from the simulations were compared with the weight development observed during the experiments in Task 8.1, thus yielding a foundation on which to validate the model performance. Main results: the model estimated growth rates that were comparable with the results from sea-cages and tanks, implying that the main mechanisms behind fish growth under culture conditions were covered by the model structure. Furthermore, the different physical scales produced different growth rates, following the same pattern as in the experiments, suggesting that the model was also able to capture the scaling effects observed in the experiments.

Significance and benefits – The results indicate that the model was able to capture the dynamic processes occurring in a salmon facility, both in sea and on land. Also, the model displayed the same difference in performance between scales as did the experiments in Task 8.2, indicating that the model was able to estimate scaling effects. This requires studies to ensure that the differences in performance are effects of scale and not effects of adjustments to production unit management due to actual scaling effects in the experiments. The model, as adjusted and validated through Task 8.4, represents a unique model, and is a good starting point for future research into numerical modelling of the biological processes in fish production units. It has potential applications both for research into scaling effects and validity of experimental results, as well as for research into rearing conditions and fish performance in general.

3.13. Key result no 14: Progress towards production of isogenic fish lines in salmon, seabass and carp

Workpackage: WP9 – Fishlines

Research aims and background

It is always an ethical dilemma to use live animals in research and the number of animals and their suffering should be minimised. Today there is a broad international consensus on the 3R strategy to: Replace, Reduce and Refine experimental designs to minimise number of animals used and severity of procedures yet enhance scientific quality and outputs.

The use of inbred animal strains improves the repeatability between experiments and reduces the number of animals needed to assess how genetic variation for important commercial traits is partitioned within and between populations. Inbred fish strains are needed to cope with the future experimental demands of EU scientists, particularly those working in vaccine development, genome sequencing, mapping studies, QTL detection and the growing application of functional genomic and proteomic studies. Said in a simple way, they could become the lab mouse of aquaculture research.

The availability of inbred lines of fish has been restricted because their development and maintenance is still a significant scientific challenge. Once established any individual line should be assessed for a wide range of traits to maximise the benefits derived. As a proof of concept the phenotype analysis of existing inbred trout lines held by INRA have been assessed for environmental tolerance and disease resistance in WP7.

The consortium has access to the best fish holding facilities and has a number of well characterised fish strains that would make ideal source material. Expertise in chromosome set manipulation (CSM) exists in IMR, UoS, HAKI, VURH, Ifremer and INRA, and the UoS and INRA have skills in the development, assessment and maintenance and use of isogenic lines for research (trout, tilapia).

Isogenic clonal lines were to be developed from existing well characterised fish strains that are known to show different phenotypes for important traits. Isogenic individuals derived by mitotic gynogenesis and androgenesis from these strains will be completely homozygous and have a fixed expression for the trait of interest, and will be used to generate isogenic lines. Once established each line has to be assessed for the original trait of interest but also for a range of other traits. Lines will be maintained though breeding with neomales or neofemales, depending on underlying sex-determination to minimise treatment induced effects. Once established, crosses between lines F1 hybrids and congenic strains can also be produced. These lines are expected to provide a strategically important resource for the EU aquaculture and biotechnology research community into the future

Results and Applications

The work was divided into three species-specific tasks. Deliverables 9.3 and 9.4 contain more detailed information on these.

A first task was aimed at the development of isogenic Atlantic salmon lines. The experimental work was undertaken in the IMR fish facility in Bergen. The irradiation (6-8 minutes UV exposure of milt) and onset of pressure (9500 psi) shocking treatments (4400-5100 degree-minutes post-fertilisation, (dmp)) were optimized over two spawning seasons with 6 minute UV exposure of milt and 4800 dmp giving the highest survivals (19% at tagging) in comparison to untreated controls. In total over 1400 mitotic gynogenetic (dihaploid, G1) salmon were produced in 5 different experiments. These fish and the untreated controls from each experiment were ongrown and their growth and phenotypes monitored. Distinct differences in morphology, skin coloration and spotting patterns could be observed in the some of the G1 salmon. Six of the 240 G1 salmon from the 2011-12 spawning season matured in December 2014 and five were successfully stripped and the eggs subjected to meiotic gynogenesis. These fish will be the first clonally derived Atlantic salmon and will be available for experimental studies in 2015. The remaining G1 fish are being ongrown and will be similarly treated as and when they mature over the next few years. Attempts to generate isogenic individuals using X-ray androgenesis proved unsuccessful. Genetic analysis using 18 microsatellite markers has confirmed the homozygosity of 300+ G1 individuals. More detailed studies using Next Generation Sequencing approaches are ongoing (UoS) to assess the development of clonal salmon lines in more depth.

A second task was aimed at the development of isogenic common carp lines. This work was undertaken in two of the major freshwater research institutes in central Europe NARIC HAKI in Hungary and VURH (now JUCB) in the Czech Republic. Both groups had expertise in the application of chromosome set manipulations such as gynogenesis and androgenesis in common carp. Using existing protocols adapted for large scale production, both mitotic gynogenetic and androgenetic offspring were produced from different carp strains (Szarvas 15 and Szarvas red at HAKI and M72 and Ropsha at VURH). These fish were ongrown, genotyped (INRA) and phenotyped: this confirmed that they had produced 38 mitotic gynogenetic (G1) and 7 androgenetic (A1) individuals at HAKI and 43 G1 and 99 A1 individuals at JUCB). Biparental and meiotic gynogenetic fish were also identified, emphasizing the need for careful genetic screening during clonal line development. These isogenic fish proved slow growing compared to the controls and were very sensitive to the rearing conditions and suffered high mortalities as a result of Aeromonas infections. This resulted in only 46 surviving at JUCB and 21 at HAKI by the end of the project (a total of 32 A1 and 35 G1 fish from four different stocks). Some of the survivors are expected to spawn in 2015 and the remainder in the following year. This work has shown that isogenic carp are difficult to maintain under standard pond culture conditions and that the clonal lines may need to be held in more controlled recirculated water systems. However, crosses between clonal lines can be performed and have better zootechnical performance (including in normal pond rearing systems), while still providing the advantages of genetic homogeneity. This solution will need to be evaluated when the first founder homozygous clonal lines will have been produced

The third and last task was aimed at the development of isogenic seabass lines. This work was undertaken in the Ifremer research facilities at Palavas. The initial plan was to try and develop these lines via androgenesis to reduce generation time as this would have resulted in the production of more male fish, which mature earlier and could be used to generate isogenic lines before the end of the project. Despite very comprehensive experimentation it proved impossible to generate any A1 fish, as it now appears that bass eggs, and other marine species with pelagic eggs, have inbuilt protection (gadusol) against UV damage (Colleter et al 2014). The project therefore had to use mitotic gynogenesis, using protocols previously developed by the Ifremer group and others. A total of 11 different females were used and 244 gynogenetic offspring produced (39 mitotic i.e. G1, from six different females, and 205 meiotic) along with some fish showing complete or partial biparental contribution (emphasizing the need for thorough genetic screening during the development of such clonal lines). The verified mitotic gynogenetic individuals are being ongrown and are expected to start maturing in the 2016 spawning season when they will be used to generate the first seabass clonal lines.

Significance and benefits:

The work performed allowed the production of the first salmon isogenic lines ever, and of homozygous founders for the production of sea bass and common carp isogenic lines. The failure of androgenesis in salmon and seabass delayed the production of the first lines, while the extreme fragility of homozygous individuals in carp required the use of recirculated systems to protect them from the aggressors, thus also delaying production. This work showed that production of isogenic lines is still a significant scientific challenge, but enough founder fish were produced to be reasonably confident in short term success of this enterprise. As demonstrated by the use of trout lines in WP7, these experimental fish are expected to bring new experimental possibilities to aquaculture researchers in several fields.

4. The potential impact and the main dissemination activities and exploitation of results

- **WP2 – Infrastructure mapping, strategic planning and sustainability**

The online RI map is the first system that gives a comprehensive overview of aquaculture infrastructures in Europe, including their associated expertise, facilities and services. Users of the infobase currently have access to a status overview of more than 100 aquaculture infrastructures. The online map is a directory which is more complete than any other existing comparable service and one which is completely free of charge. It allows researchers to find suitable project partners or science collaborators, and shows them which experimental facilities are open for external users. Aquaculture companies which are operating facilities can use the online RI map to promote and highlight their research facilities and expertise. It also allows companies to identify expertise and/or facilities that are needed in industry-driven research and innovation activities. The opportunity for RI operators to continuously update their information represents a unique permanent dissemination tool for aquaculture RI operators

The AQUAEXCEL inventory of gaps is a step in the direction of implementing the EATiP Strategic Research and Innovation Agenda (as part of the Knowledge Management thematic area). EATiP will also be a central stakeholder in the process of further exploitation of the results. An EATiP RI working group has been created to ensure the role of RIs in this implementation process, and to stimulate industry-relevant aquaculture research and education in Europe. Results from the gap analysis may also form a knowledge base when considering the adjustment of existing or the foundation of new RIs that are better suited to carry out experiments in line with the sector needs, reducing the length of the process between research result generation and commercialisation. As an example, a new RI under construction in France has requested the results of the gap analysis to help orientate its facilities towards the most useful functions.

AQUAEXCEL also put forward the most suitable models for sustained aquaculture RI collaboration over a short and long timeframe. Recommendations given in the report have the potential to be taken further by the aquaculture RIs that have the aspiration and capacity to integrate their operations with other infrastructures in Europe. In the long term, by gaining the commitment of EU member and associated states, a separate legal entity such as an ERIC could be founded to secure the place of European aquaculture RIs into the future. Increased RI collaboration is important to ensure more efficient use of existing resources, to encourage cross-disciplinary research and innovation for solving complex challenges, and to stimulate staff and student mobility.

- **WP3: Definition of common standards, protocols and ontology**

Potential impact

The impact of WP3 is directly related with a major knowledge and understanding between users by its contribution to establish a common language for researchers, companies and even consumers, generating a way to harmonize, standardize and validate experimental processes and phenotypic characterization. Especially, the use of standardized ontologies is expected to clarify the scientific and technical literature and facilitate meta-analysis of data.

Dissemination activities and exploitation of results

All tools developed in the context of WP3 were accompanied by important and parallel dissemination activities in order to target as many users as possible (13 conference participations, 6 scientific papers)
In terms of exploitation, the ontologies have started to be used to reference traits in scientific papers produced by AQUAEXCEL scientists, and this should be encouraged and continued. The handbooks for sanitary procedures and measurement of phenotypic traits are available for download on the AQUAEXCEL website and are also being published by ULPGC editions.
Access to the data repository solutions is available from the AQUAEXCEL website (http://www.aquaexcel.eu/index.php?option=com_k2&view=item&layout=item&id=146&Itemid=300), with several video tutorials explaining their possible use.

- **WP4: Management of the calls for access and evaluation of the access given**

The programme of transnational access supported a total of 97 research projects across the twenty seven AQUAEXCEL research installations. Approximately 145 people were involved in transnational access projects excluding host personnel. Projects were selected on the basis of scientific quality, feasibility, and potential value of the work for the development of the European aquaculture sector. Projects covered a range of thematic areas (as shown in figure 7) with the greatest number involving fish nutrition, aspects of physiology, health, welfare and environment.

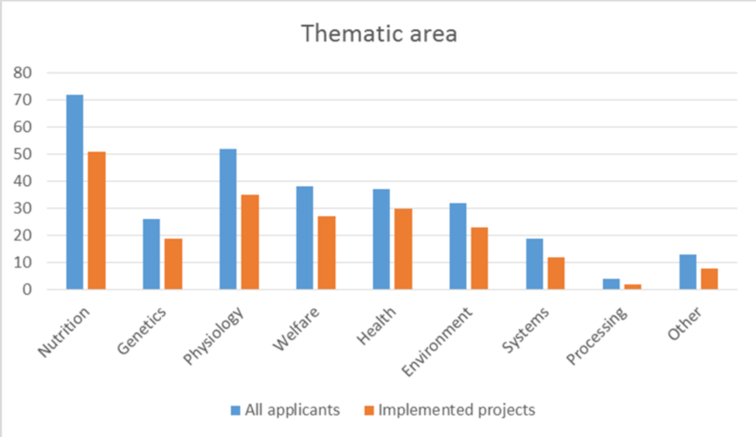


Figure 7: Number of applications and implemented TNA projects in each thematic area

Even though the importance of it was emphasised to all TNA users and they were requested regularly, capturing information on the dissemination and exploitation of each of these projects has proved a challenge throughout the AQUAEXCEL project. Lack of clear outputs can be due to a number of factors such as:

- Inadequate or insufficient data collected during the trials
- Problems with the trials resulting in no or invalid results
- Researchers waiting to complete other work before publishing results
- Researchers waiting for a suitable conference or other opportunity to present results
- Generally long lead times for academic writing and publishing, particularly in peer reviewed journals
- Low priority for dissemination or exploitation of results on the part of some researchers
- Failure to notify the TNA Coordinator of relevant outputs

As of the end of the project (keeping in mind that many TNA visits were only completed in the final months of the project), thirty one TNA researchers had submitted Knowledge Output Tables recording one or more specific knowledge outputs. Only one project recorded a published peer review article, 8 recorded conference presentations and 2 recorded conference posters. In addition, 9 TNA project outputs were reported in the AQUAEXCEL Key Output Booklets and 23 TNA project outputs were highlighted in project newsletters. Specific industry-relevant outputs from six projects were featured in the AQUAEXCEL TNA Session at Aquaculture Europe 2014. One project recorded use of the outputs in a commercial product whilst three others envisaged commercial uptake in due course. Most projects envisage either a conference presentation or publication in a peer reviewed journal whilst four will use results as material in a PhD or MSc thesis. At this stage it is uncertain how many TNA projects will properly disseminate results, so the work of WP5 has been critical in ensuring outputs have been communicated to the wider scientific community and aquaculture industry.

- **Workpackage: WP5 – Training, knowledge & technology transfer and communication**

Dissemination, (making the results and deliverables of AQUAEXCEL available to its stakeholders and beyond to a wider audience), is essential for take-up; and take-up is crucial for the success of the project, and for the sustainability of outputs in the long term. AQUAEXCEL, through WP5, has endeavoured to raise awareness, inform, engage and promote AQUAEXCEL and its results by a variety of means. Dissemination means included a dedicated website (described above), regular press releases, social media use, videos, posters, conferences, events and workshops, and newsletters.

One of the key objectives of WP5 was to ensure the transfer of research outputs (e.g. products, methodologies, findings, etc.) to end-users who can make best use of those results, to customise information and knowledge so that it is ready for uptake by different target end-users, and to develop and make use of the latest tools, resources and communication channels resulting in cost effectiveness and maximum impact. This objective has been achieved by carrying out successful rounds of **identification of innovative knowledge & technologies, and application** and developing **four booklets showcasing the Key Achievements** of the project based on this identification process. Based on **Protocols for collection and collation and data-sharing in the project** which were developed, innovative knowledge and technologies have been captured on a regular basis.

A continuous objective of WP5 has been to collect, analyse and validate the AQUAEXCEL research outputs at strategic intervals in the project in order to prepare the key results for transfer and exploitation to end-users who can make best use of those results. By using a tailored **Knowledge Management methodology** we have ensured that any arising knowledge from the project was captured, potential applications identified, and transfer carried out and measured so that tangible, measurable impact can be established. To demonstrate impact, indicators have been built into the methodology; however, within short time frames it can be difficult to gauge the full extent of the impact achieved as knowledge transfer from research to end user typically takes many years. The potential for impact is high though, with over 58 Knowledge Outputs having been identified over the life cycle of the project.

A key knowledge transfer event was hosted in the form of an **industry workshop** entitled "Research Infrastructures: adding value to European aquaculture industry" which was held on Friday 17 October 2014 at the Aquaculture Europe 2014 conference in San Sebastian, Spain. More than 140 people attended the event, many from the European aquaculture industry. The workshop focused on presenting the achievements and results of the project with a focus on results of high utility to industry and provided a forum to strengthen ties between research and industry in the European aquaculture sector.

Through our external communication activities we have ensured that project awareness is widespread to target stakeholder groups, advocating the AQUAEXCEL project, objectives and outcomes of providing improved and integrated access to aquaculture Research Infrastructures. Progress towards this objective has been made by promoting the AQUAEXCEL project, objectives, products and events to a range of key stakeholders and **facilitating networking opportunities** through **targeted dissemination and communication** activities. Several initiatives were undertaken to support external communication, namely: **four informative annual newsletters**, containing information on the project, its results and general issues of interest have been completed and circulated widely in hard copy and electronic formats ensuring widespread awareness among all stakeholders. Language used was non-technical and understandable to all stakeholders and the attractive layout and design made this publication an accessible and easy read. The newsletters highlighted results from the project and explained aspects such as Transnational Access by introducing the researchers who availed of this opportunity and explaining their research projects. Several key partners have been interviewed, giving in-depth insight into the project but also into the European aquaculture sector in general. AQUAEXCEL initiatives such as upcoming training courses, upcoming Calls for Access, etc. were announced in the newsletter, supporting their promotion.

Another result is the publication of **four AQUAEXCEL Booklets** describing key achievements, introducing the project, its core functions, as well as its progress to date. In addition, numerous **press releases** have been published which have had significant follow up by other news sources. AQUAEXCEL has also been profiled as an **EC Success Story** under the title 'Fish of the Future' and was the subject of a **Euronews mini-documentary video** which is available in

13 languages and has been broadcast in 155 countries. AQUAEXCEL has also been communicated through **social media** such as Twitter and LinkedIn. AQUAEXCEL has been presented on many occasions, both **by oral and poster presentations**. AQUAEXCEL has also developed an **introductory video** to the project, which provides a nice entry point to learning about the project for interested members of the general public.

Connecting AQUAEXCEL to strategically important European networks in order to establish durable links between the consortium and European aquaculture initiatives and clusters has also been a result of this WP. AQUAEXCEL aimed to build on activities which were, and are continuing, to take place in the European Aquaculture sector and initiate interaction with relevant contacts. In this way AQUAEXCEL has worked towards fostering an increased understanding of the importance of Research Infrastructures, bringing together projects and sharing knowledge. Progress towards this objective has been made by maintaining established active contacts, nurturing them through interacting on a regular basis, and attending relevant events. The establishment of an aquaculture Research Infrastructure Working Group under the EATiP structure is currently in progress.

Last but not least, in terms of scientific dissemination, AQUAEXCEL counts a total of 271 communications (either poster, oral, workshop, promotional action..) that were carried out during the project, in addition
31 peer reviewed publications were produced among which 13 are not published yet (2 are currently under preparation, and the remainder has been submitted).

All these dissemination activities have led to the high visibility of the AQUAEXCEL project and its results within the European aquaculture sector and throughout Europe as a whole.

- **WP6: Upscaling and validity of research results**

The potential effects of the e-Infrastructure solutions are analysed and documented in a report (D6.6). In addition to the positive effects on the quality of the experiments and collected data, reduced costs can also be expected. This is partly due to the reduced needs for travel, which would save time and costs for researchers using the highly specialized facilities available in the consortium. This will in turn allow more resources to be used for the scientific work. Use of e-Infrastructure would also open up facilities for use by researchers who face practical problems with travelling.

In general, aquaculture research facilities require large investments and operating costs. Over time, use of e-Infrastructure can be expected to increase the utilization of a facility. This will increase the scientific output and contribute to reduced costs per experiment.

Dissemination of these results has been done within the consortium (the primary target is RI managers) with the newsletters and presentations at project meetings, but also externally through communication in several conferences, newsletters and booklets produced in WP5. A live presentation of remote access has been done at the Industry workshop on 17 October 2014 at the Aquaculture Europe 2014 conference in San Sebastian.

- **WP7: Using individual fish as experimental unit**

This WP allowed us to provide new methodological approaches that can provide the maximum amount of information for refined phenotyping fish using the minimum amount of fish. A refined phenotyping was obtained through a multidisciplinary approach which brings more accurate and relevant information from experimental fish and will make new, non-invasive, non-lethal, simpler and more focused methods available to the scientific community.

In that context, a first important outcome of this WP was the demonstration that genomic analysis can bring new and accurate information for such phenotyping objective (ex. nutritional stress or chronic stress phenotyping), a demonstration which has never been done before. To help us developing such genomic studies, this WP has developed an original tool for transcriptomic meta-analysis which has been made available on the web (fishandships.toulouse.inra.fr) and which was also publicized through a training course on the Contribution of genomic approaches to the development of a sustainable aquaculture (WP5).

The second important outcome of this WP is the demonstration that chronic stress requires a multidisciplinary approach to be accurately phenotyped, and that specific markers for any type of chronic stress do not exist. Promising approaches based on monitoring of a set of mitochondria-related genes have been suggested in sea bream and would require more studies.

The present WP has also produced information which would be easily applicable in research aquaculture plans: Thus, methods for detecting early puberty in rainbow trout and in sea bass have been validated, and have the potential to be used as an individual indicator of maturation in trout.

Another very interesting outcome of this WP was the development of a standardized waterborne challenge for resistance to Flavobacterium in rainbow trout which can (and will be) applied in large-scale experimental challenges (for example in the ongoing FishBoost EU project, or in the French RE-SIST project in collaboration with industry). The new challenge also allowed the identification of trout lines differing in their response to bath or injection challenge, which will be used in future projects to try to unravel the mechanisms of resistance to Fp. Finally, this WP also validated new tagging methods and also new methods for identification of individual fish which can be applied at very early stages.

All these results have been presented orally in international scientific meetings but also in meetings involving aquaculture stakeholders. Moreover, publication of these results in peer-review journals has been done or is in progress.

- **WP8: Upscaling and validity of research results**

Impact – That scaling size matters for fish performance is an important information for the industry, who is one user of the research done in the research infrastructures. The results suggest that the industry may aim at higher growth in cages. The results show that modelling may improve predicting scale effects. Small scale MBBRs are tools to study COD production and removal in RAS. Mixing intensity has a strong effect on TAN removal. Defining mixing conditions which are representative of full scale aerated MBBRs would be a prerequisite for this type of research. In addition, MBBR scale has a significant effect on TAN removal rate. When designing aquaculture experiments that include performance of filters, upscaling effects should be taken into consideration.

The results of this WP have been widely publicized in scientific meetings (6 oral presentations), its main three outputs (fish experiments, models, filter experiments) have been presented at the AQUAEXCEL Industry Workshop in San Sebastian, and 5 scientific publications are accepted or in preparation. Articles have also been published in the aquaculture technical press (Hatchery International).

- **Workpackage: WP9 – Fishlines**

The Fishlines workpackage was to develop standardised fish strains of the three selected major European farmed fish species. This was in response to the increasing demands put on researchers to better understand the biology and controlling genetics of a wide range of different commercial traits that would result in the better management and long-term improvement of European farmed fish. A workshop held in Vodnany clearly outlined the benefits to researchers of having access to isogenic lines of rainbow trout (INRA) and tilapia (UoS) that displayed distinct and fixed differences in a wide variety of disease, metabolic and reproductive traits. The Atlantic salmon clonal lines will be available for a number of new projects during 2015 and it is expected that more salmon and new carp and sea bass lines will be produced and become available during the follow-up project AQUAEXCEL2020. The project has increased our understanding of the differences between species and our ability to undertake such manipulations in these and other farmed fish species. Next Generation Sequencing technologies have been applied in the verification of the manipulations involved in the development of clonal lines (Oral et al., 2014) in the target species, which has generated large numbers of new Single Nucleotide Polymorphisms (SNP) markers that will be available to researchers working with the clonal lines and the progenitor strains.

Much of the work described as well as disseminated the technologies and theory behind the use of chromosome set manipulation (CSM) techniques to develop isogenic fish lines was disseminated to over 20 scientists working on a range of model and commercial species during workshop practicals and lectures at UoS in November 2013, in the frame of an AQUAEXCEL training course organised by Stirling University (UK), entitled ‘The Application of Chromosome Set Manipulations and the Importance of Gamete Collection and Management in Aquaculture’,. One article has been published, on the problems in UV inactivation of the maternal genome in sea bass eggs (Colleter et al., 2014), presentations were made at the EAS2014 meeting, and further manuscripts are in preparation for publication.
In terms of exploitation, IMR (NO) and Ifremer (FR), based on the results obtained, have started to build specialized installations for the management and propagation of isogenic lines of salmon (IMR) and sea bass (Ifremer).

5. Project information

Project logo

Project public website
The website address is the following: http://www.AQUAEXCEL.eu/

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