AQUAEXCEL 2020 is a €9.7 million European Union-funded Horizon 2020 Research Infrastructure project that aims to support the sustainable growth of the aquaculture sector in Europe. It does so by integration of the European aquaculture community, and providing it with crucial tools, facilities, and novel services to conduct advanced fish research.
We are now in the final months of the project, and we can see that everything seemed to be well on schedule before we were hit by the Covid-19 crisis. All training courses have been provided, with great success. Three of these courses are available online, providing a great opportunity to catch up while working from home. Transnational access was well on track, with a lot of projects and exciting results, but obviously some of the final projects will be impacted by the lockdown. We are now investigating and quantifying impacts and will take appropriate action with the European Commission. A final meeting of the project will take place on September 29th 2020. Unfortunately, due to Covid-19 and the postponement of the Aquaculture Europe conference in Cork this meeting will now take place online. In place of the AQUAEXCEL2020 brokerage event that was due to take place at Aquaculture Europe, we will now organise a series of webinars, highlighting results from the Transnational Access and the internal project research with the highest application potential for the aquaculture sector. These results were selected at the end of April by the Industry Research Advisory Panel (IRAP), which met online instead of the originally planned meeting in Lisbon, Portugal.

In these times where it appears that some critical sectors like food production need to be more localized to enhance resilience, I think that AQUAEXCEL2020 is needed more than ever. #StayHome for sure, but keep engaged for the development of European aquaculture!

Marc Vandeputte

Welcome from the AQUAEXCEL2020 Coordinator

News and Highlights

Two more successful AQUAEXCEL2020 training courses organised

The final two face to face training courses of the AQUAEXCEL2020 project took place in November 2019. Firstly, “Planning and Conducting Experimental Infection Trials in Fish” was organised by Danmarks Tekniske Universitet (DTU, Denmark), with the assistance and expertise of Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC, Spain), Nemzeti Agrár kutatási és Innovációs Központ (NAIK, Hungary) and University of Stirling (UoS, United Kingdom). As with the project’s other training courses, this course was highly sought after and oversubscribed. The organisers accepted 26 lucky participants. The course focused on the considerations needed for both the planning phase and conduction of experimental infection trials (including viruses, bacteria and parasites) in fish.

The course received great feedback from participants:

“I appreciated the lectures by the companies (transfer of research into practice). I made new contacts and friends from different fields.”

“The content of the course was great, and all the lecturers were extremely knowledgeable in their field, which is fantastic.”

The final AQUAEXCEL2020 face-to-face training course was “Fish Nutrition and Feeding”, organised by Institut National de la Recherche Agronomique (INRA) UMR1419 NUMEA (Nutrition, Métabolisme, Aquaculture, France), with the assistance and expertise of Agencia Estatal Consejo Superior de Investigaciones Científicas (CSIC, Spain), Wageningen University (WU, the Netherlands) and University of Stirling (UoS, United Kingdom). It addressed topics including macro- and micro-nutrients and energy requirements, the evaluation of feedstuff and diet digestibility, and the link between feed intake and growth. It also addressed the environmental impact of aquaculture and feed production, interaction of nutrition with genetics, ongoing research on fish nutrition and feeding and the use of alternative feed ingredients.
The AQUAEXCEL2020 project held its second successful industry brokerage event at the end of last year to share the latest innovative outputs with the aquaculture sector. Taking place as part of EATiP (European Aquaculture Technology and Innovation Platform) Day at Aquaculture Europe in Berlin on 9 October 2019, the event attracted an audience of both industry and academia. The event, “From Research Innovation to Industry Application” was organised by EATiP and AquaTT to create a forum for engagement and exchange between researchers and potential industry beneficiaries of the research results generated from the AQUAEXCEL2020 project.

Supporting research innovation to industry application is key to AQUAEXCEL2020 and involves maintaining active engagement between stakeholders. This event provided an excellent opportunity to share some of the many innovative outputs emerging directly from the varied research within the AQUAEXCEL2020 project, and also through its Transnational Access (TNA) programme. The TNA programme funds access to 39 top aquaculture research facilities across Europe, offering researchers the opportunity to undertake experimental trials on commercially important aquaculture fish species and system types.

Mr Courtney Hough (EATiP) opened the event, highlighting the potential for networking opportunities. Dr Marc Vandeputte (INRA, AQUAEXCEL2020 Project Coordinator) provided an overview of the project and the TNA Programme. He also announced the exciting AQUAEXCEL3.0 project, which will start in November this year, building on the excellent work already achieved in AQUAEXCEL2020.

Dr Stavros Chatzifotis (Hellenic Centre for Marine Research, HCMR), highlighted research findings looking at three types of insect meal replacements. PhD student, Ana Basto (Interdisciplinary Centre of Marine and Environmental Research of the University of Porto, CIIMAR) went on to discuss outcomes of research on European seabass fed with the mealworm Tenebrio molitor. This research primarily assessed the use of large quantities of insects as protein sources to replace fishmeal in diets for sea bass, and to examine possible effects on their growth and welfare.

Dr Jovanka Lukic (University of Belgrade) followed with an interesting presentation on the effects of Lactic Acid Bacteria on growth and microflora of larval pike perch, applied either through live feed or commercial dry feed. Dr Josep Calduch-Giner (Consejo Superior de Investigaciones Científicas, CSIC) presented a protocol for early life management for optimal fish performance, highlighting surprising findings in relation to low O2 (hypoxic) conditions at early life stages of gilthead sea bream and its impact later in life. In the area of fish disease, Dr Carla Piazzon (Consejo Superior de Investigaciones Científicas, CSIC) presented on gene expression analysis of Atlantic salmon gills and how it reveals key molecules during amoebic gill disease, one of the main health challenges for the marine Atlantic salmon industry worldwide. The findings could contribute to more timely and accurate detection of the disease, preventing stock losses and improving fish welfare.

Attendees also learned about the project’s innovative findings and developments. AE-FishBIT is a tiny device which monitors the metabolic traits of individual farmed fish. The newly patented tool is the result of collaboration among biologists, engineers and bioinformaticians from two AQUAEXCEL2020
The Industry Brokerage Networking Event facilitated further discussions around the presented innovative outputs.

The final AQUAEXCEL\textsuperscript{2020} brokerage event was due to be held at Aquaculture Europe 2020. Due to the postponement of this conference, the latest outputs selected by the Industry and Research Advisory Panel (IRAP) will now be presented through a series of webinars.

**Interview**

**Interview with AQUAEXCEL\textsuperscript{2020} Ethics Advisor – Professor Felicity Huntingford**

Members of the general public are increasingly concerned about fish welfare, particularly the welfare of the fish they eat, and, partly in response to this concern, the aquaculture industry has taken many steps to improve the welfare of farmed fish. These steps have been informed by an intensive programme of research into many aspects of fish health and welfare and have been carried out by scientists from a range of disciplines, many based in Europe and supported by EU funding. While making significant contributions to the welfare of farmed fish, such studies may themselves raise ethical issues, for example, if experimental studies (say those aimed at identifying the fish density that optimises welfare in a given species) require exposing fish to potentially adverse conditions (such as densities that might be below and above that optimum). Researchers must be mindful of these issues when planning their research, ensuring compliance with national and international regulations.

Professor Felicity Huntingford is the independent Ethics Advisor for AQUAEXCEL\textsuperscript{2020}, ensuring that research is carried out to a high ethical standard. She is honorary Professor of Functional Ecology at the Institute of Biodiversity, Animal Health and Comparative Medicine at the University of Glasgow (UK) and one of the world’s pre-eminent experts on fish behaviour. Professor Huntingford has extensive experience researching fish behaviour and advising the aquaculture sector on welfare practices. She has been involved in numerous collaborative research projects funded by the EU and has spoken at many aquaculture workshops and conferences about the welfare of farmed fish.

We interviewed Professor Huntingford to find out more about her role as ethics advisor for AQUAEXCEL\textsuperscript{2020}.

1. **What does your work as Ethics Advisor for AQUAEXCEL\textsuperscript{2020} involve?**

   In AQUAEXCEL\textsuperscript{2020} my role involves screening all planned research projects, both the TransNational Access applications and the Joint Research Activities, for ethical issues, mainly focusing on compliance with the 3Rs – Replacement, Reduction and Refinement. It also involves analysing all the ethical assessments on an annual basis, to give an overview of how well AQUAEXCEL\textsuperscript{2020} – funded research complies with the 3Rs. I then present this information to AQUAEXCEL\textsuperscript{2020} partners at the annual meeting. It’s important to note that my role does not involve making decisions about the scientific quality of the proposed research.

2. **What do you consider to be good welfare practice for AQUAEXCEL\textsuperscript{2020} researchers when designing and carrying out their experiments? Do you have a strong example from the project so far?**

   All AQUAEXCEL\textsuperscript{2020} partners work within the 3Rs framework in accordance with the relevant European, national and institute regulations relating to the use of vertebrates in scientific experiments. For example, the TNA application form requires information on any authorisation required for the proposed work, an ethical analysis of the procedures to be used and
an account of steps to be taken to meet the requirements of each of the 3Rs. My job as ethical advisor has mainly involved scrutinising this ethics section alongside the accompanying detailed description of research procedures. In general, applicants have handled the ethical issues well. In 2019, 26% of TNA applications raised no ethical concerns regarding the use of live finfish. In 58% of cases that did raise concerns, these were dealt with entirely satisfactorily. The remaining 42% of those cases required some further information, but these issues were resolved satisfactorily.

Research funded by AQUAEXCEL2020 provides some excellent examples of best practice in relation to the 3Rs. One partner has developed microorganism-free strains of the small crustacean Artemia for replacement of live fin fish as experimental subjects, to investigate disease transmission and resistance processes. One TNA-funded study of the effects of stress on vulnerability to disease combined in vitro studies of virulence in cultured bacteria exposed directly to corticosteroids (among other substances) with in vivo studies of disease acquisition in Artemia exposed to such corticosteroid-treated pathogens. Important findings here that are potentially generalisable to finfish are better understanding of what determines bacterial virulence and identification of potential anti-virulence treatments. The following shows an impressive concern by the applicant for ethical issues: “The model organism Artemia is not covered by laws and regulations of animal experiments. We will however, always seek to treat the organism with as much care and respect as possible, and try to keep the number of organisms used to a minimum.”

On the subject of reduction, many TNA researchers proposed work on samples stored from earlier studies - sensible forward planning that reduces the need for repeated procedures. Other effective steps to reduce the number of live fish used in experiments are illustrated by a study of the effects of temperature on progression of a bacterial disease of Rainbow trout, tracking disease progression in individually identified fish (using PIT tags); this increases statistical power and allows valid conclusions to be drawn using fewer fish. For some research aims, experimental fish must be held at densities comparable to those experienced by their farmed counterparts, so that findings can be generalised, making the number of treated subjects inevitably higher than the minimum for statistical validity. This was the case in a study exploring the use of probiotic lactobacilli in improved weaning diets for pike-perch larvae; here reduction was achieved by carrying out preliminary in vitro tests to reduce the number of experimental treatments.

This same application for work on pike-perch larvae dealt well with one important aspect of refinement where experiments involve longer impositions of procedures. Specifically, this is the need to monitor fish frequently, assessing their status with respect to well-defined indicators of welfare, with a view to terminating all or part of the study should unduly adverse consequences be observed. In another project, identification and use of humane endpoints are well-illustrated in a toxicological study of interactive effects of exposure to copper and microplastics on development in larval and post-larval seabream. A scoring scheme was set up before the experiment, with several indicators (poor body condition, abnormal swimming and activity) being screened daily and combined into a poor welfare score. Fish with scores above a pre-determined threshold would be humanely killed.

3. What are the most important welfare considerations when designing research involving fish?

Broadly speaking, these are the same as with research on other vertebrates. When planning a project involving live fish, the first question is – does the information already exist in the literature? If not, then, could in vitro, ex vivo or in silico approaches answer the question, or could existing stored tissue be used? If live animals must be used, could simpler, less sentient, invertebrate animals be used? These are all questions relating to Replacement. If use of live fish is unavoidable, then it is important to undertake a thorough analysis of potential negative effects from procedures on health and welfare. This includes both acute procedures such as handling, blood sampling, and killing, and chronic procedures such as exposure to possibly adverse environmental conditions, experimentally-induced infections and new diets. Having identified such potential adverse effects, research procedures need to minimise the number of fish exposed for achieving the research goals (Reduction), and also to minimise such adverse effects (Refinement). Refinement can be achieved through various measures, including carrying out acute procedures under anaesthesia or keeping longer-term treatments as short as possible.

4. Why do you think the 3Rs are so important in aquaculture research?

The 3Rs framework is a really clever way of highlighting and protecting the welfare of all animals used in research, including fish in aquaculture research. This is because it provides a clear and incisive way of focussing on all the various steps described in the previous section that must be undertaken to avoid any adverse effects on the welfare of live subjects and, where this is not possible, to minimise such adverse effects.

5. Do you think fish welfare plays a major role in the public’s view of aquaculture?

The answer here depends on which sector of the public is concerned, on the intensiveness of the aquaculture, and the kind of animals – the public are less likely to show concern for crustaceans than for finfish, for example. Having said...
6. Could you tell us something consumers may not know about fish welfare?

As ectotherms, fish in nature and, given appropriate conditions, also on fish farms, regulate their body temperature by taking advantage of thermal gradients. In nature, this can increase the efficiency of processes such as swimming and digestion and can facilitate growth and maturation rates. Of particular relevance in aquaculture, fish are able to ‘self-medicate’ when infected by a pathogen, stimulating an effective immune response by moving to warmer water, improving their own welfare in the process.

We thank Professor Felicity Huntingford very much for talking to us about the ethics aspects in AQUAEXCEL 2020 and aquaculture in general.
The AQUAEXCEL2020 TNA programme enables external teams to access project partners’ facilities via submission of research proposals, which are funded based on an independent evaluation. Access is offered to 39 research infrastructures of participating institutes, with experimental costs, travel and subsistence covered by AQUAEXCEL2020. Available facilities cover the entire range of production systems, environments, scales, fish species and fields of expertise. Access is available to EU and Associated States’ research teams, industry, and small and medium-sized enterprises (SMEs), based on the scientific excellence of proposals and relevance to the aquaculture sector.

Disrupting effects of plasticizers in male gilthead sea bream

Bisphenol A (BPA) and di-isononyl phthalate (DiNP) are two man-made chemicals that are ubiquitous in plastic items and detected in all environmental matrices due to their broad spectrum of applications. Both substances are known as endocrine disrupting chemicals (EDCs), having the ability to alter the endocrine system of living organisms, including farmed fish such as gilthead sea bream.

Gilthead sea bream is a sequential hermaphrodite that begins its life as male and becomes female when it is two or three years old. Consequently, the gilthead sea bream is an interesting model to study how chemicals may interfere with the hormonal system and sex reversal.

Changes in reproductive performance can also affect the quality of the progeny, which explains the interest in understanding and mitigating most of the effects of environmental stressors. In this context, the AQUAEXCEL2020 transnational access (TNA) project DISRUPBREAM was proposed by the Department of Life and Environment Sciences of Polytechnic University of Marche (Ancona, Italy) to address the impact of BPA and DiNP on the metabolism and reproduction of gilthead sea bream. This TNA utilised the gilthead sea bream transcriptome and genome databases (nutrigroup-iats.org/seabreamdb) of the host infrastructure of Institute of Aquaculture Torre de la Sal, (IATS-CSIC, Spain) for gene expression profiling of a set of candidate genes involved in lipid metabolism and reproduction. Both EDCs were administered by diet to two-year-old male gilthead sea bream during the sex reversal stage for three weeks. Gene expression surveys were conducted to evaluate the impact of these plasticizers in fish gonads, liver and brain. The results provided evidence that DiNP and BPA alter the pathways involved in the regulation of appetite and hepatic lipid metabolism, driving an increased deposition of hepatic lipids [1].

Regarding reproduction, DiNP affected plasma levels of steroids, with a reduction of 11-ketosterone (11-KT) and increases of 17\(^{\beta}\)-estradiol (E2). In addition, the predominant cell type in DiNP treated fish was immature spermatogonia. Expression of genes coding for different reproductive markers was up-regulated after the exposure to this plasticizer [2]. Fish treated with BPA showed a reduction of the sperm motility duration and velocity. BPA also elicited an alteration in the steroidogenesis reducing plasma 11-KT levels, whereas those of testosterone (T) and maturation-inducing steroid (17,20\(^{\beta}\)-P) were increased. As for DiNP, key genes involved in the reproductive process were altered after chronic BPA exposure [3].

The results of the DISRUPBREAM TNA project provided complementary novel data for the PhD thesis of TNA applicant, Isabel Forner-Piquer. Her TNA work with the IA TS-CSIC Nutrigenomics group resulted in three publications in high impact scientific journals. DISRUPBREAM overall was a great example of research collaboration through the AQUAEXCEL2020 TNA Programme.
Transnational Access (TNA) Success Stories


2. Forner-Piquer, I.; Mylonas, C.C.; Fakriadis, I.; Papadaki, M.; Piscitelli, F.; Di Marzo, V.; Calduch-Giner, J.; Pérez-Sánchez, J.; Carnevali, O. Effects of diisononyl phthalate (DiNP) on the endocannabinoid and reproductive systems of male gilthead sea bream (*Sparus aurata*) during the spawning season. *Arch. Toxicol.* 2019, 93, 727-741.


Testing a Distributed Sensor Network for Measuring Flow Field in a Sea Cage

Another interesting TNA project carried out in AQUAEXCEL2020 focused on “Testing a Distributed Sensor Network for Measuring Flow Field in a Sea Cage”, and was carried out by Maarja Kruusmaa, in the SINTEF ACE facilities (Norway).

Nowadays, sea cages are used to grow a variety of fish in the open sea. In order to keep the fish healthy, minimum flows through the cage must be met to keep the cage oxidised and clean from waste. As the fish are usually circulating in the cage in a rotational swarm, and the cages are moving with the sea level, conventional measuring techniques (ADV, ADCP) will not work as the precision is decreased by the background motion and also by the swarm of fish swimming in the field of view of the profiling measuring techniques. In addition, the flow inside of the cage can vary and ADV or ADCP typically used for a point measurement do not give information about the flow field distribution along the cage. Therefore, a vertical array of sensors was tested in a fish cage (50m x 27m) to measure flow through the fish cage at various depths. The sensors were developed in Tallinn University of Technology for measuring flows at the seabed and near obstacles where acoustic technology usually fails. These sensors are based on converting mechanical vibration of sensor stems into electrical signals and thus are passive and only measure local flow field. In contrast to ADCP or ADV, they are not as sensitive to disturbances of the flow (for example, swimming fish). These sensors are also cheaper than ADVs and ADCPs. Placing several of these sensors in a network allows us to measure the distribution of flow. This means that if the flow in one part of the cage is higher than in another, this could be detected.

The project demonstrated that the installation enables long-term continuous online observation of flow, and that the distributed flow sensor network gives information about distribution of flow in the cage. The initial results show that the velocity maxima appear with the rise and drop of the sea level as expected. It can also be seen that the currents are stronger closer to the surface and diminish with increasing depth. The results will need to go through additional data processing and validation (comparing with the ADV measurements). For future long-term deployments, better ruggedized cables and data loggers are needed.

Different application strategies of salmon gonadoliberin analogue (sGnRHa) to improve sperm quality in outdoor pond system reared pikeperch (*Sander lucioperca*)

The TNA project PIKEPERCHSPERM has been progressing in NAIK (Nemzeti Agrárkutatási és Innovációs Központ), Hungary, over the past few months. Entitled “Different application strategies of salmon gonadoliberin analogue (sGnRHa) to improve sperm quality in outdoor pond system reared pikeperch (*Sander lucioperca*)”, the project is being performed at the NAIK-SDC installation. Project leader Dr Mustafa Erkan...
Transnational Access (TNA) Success Stories

Özgür (Malatya Turgut Özal University, Turkey) and Dr Selim Erdoğan (İnönü University, Turkey) are working with NAIK staff to evaluate pikeperch sperm quality obtained off-seasonally, six months prior to the natural spawning season. This might be the earliest report of pikeperch spermiation in outdoor reared broodstock, and results could provide new insights into pikeperch reproductive biology.

To learn more about some of the exciting projects that have been carried out in the framework of the AQUAEXCEL\(\textsuperscript{2020}\) TNA programme, please visit: https://www.aquaexcel2020.eu/index.php/transnational-access/tna-projects

Fish’n’Co – Sole senegalensis

Senegalese sole is part of the Soleidae family. Solea senegalensis is found naturally in Atlantic and Mediterranean waters, and along with Solea solea is considered a potentially important species for marine aquaculture owing to their high market value and consumer demand.

This flatfish has an asymmetric body (eyes on one side), with adults reaching up to 70cm in size. The species is typically found living on sandy or muddy bottoms, ranging from brackish lagoons and shallow waters to coastal areas up to a depth of 100m. Adults feed on small benthic invertebrates and to a lesser extent small crustaceans. Spawning is heavily dependent on water temperature, which should be between 15 and 20°C.

Traditional sole farming in natural conditions is conducted in extensive and semi-intensive land-based ponds. Frequently, these ponds are managed using polyculture techniques, raising sole in conjunction with other species, like sea bream, to increase profit. Significantly higher production volumes are realized through intensive culture systems using water re-circulation technology in indoor facilities. Recirculation systems are also used during the first stages of sole life cycle.

Source: fao.org/fishery/culturedspecies/Solea_spp/en

S. senegalensis is better adapted than S. solea to the warmer waters of temperate climates, and therefore is more suitable for production along the southern coast of Spain and Portugal. During the 1980s, it was cultured extensively in earthen ponds, which often were former salt production ponds. Since then, numerous research projects in Portugal and Spain have studied methods to improve production. A turning point towards the success of S. senegalensis production was achieved in 1991, when the broodstock kept in the facilities of Instituto de Investigación y Formación Agraria y Pesquera (IFAPA, Cádiz, Spain) began to spawn in a natural and controlled way. A research group from the Centre of Marine Sciences (CCMAR, Portugal) later reported that they achieved continuous and stable spawning from wild-caught broodstock, resulting in the production of fertile and viable embryos, without any environmental or hormonal manipulation. Nowadays, natural spawning of S. senegalensis broodstock is well controlled, and can be performed following standard procedures based in temperature manipulations (Anguis and Canavate, 2004).

As a consequence, in Spain, mass production of juvenile S. senegalensis started in 1993.

The three major bottlenecks for Solea spp. production are 1) high larval mortality rates related to nutrition and growth dispersion 2) sub-optimal larval weaning strategies and 3) inadequate control measures for common diseases. Additionally, the techniques for intensive rearing of sole are yet to be optimized, which limits the final volume of fish production.

European sole farming began in Portugal in the late 1970s, producing only 2 tonnes per year. Currently, France and Spain are the leading sole producers. Sole production has been evolving at a low rate since late 1970s and only reached a total of 347 tonnes in 2010. Since then, a maximum production of 571 tonnes was registered in 2013, but mostly in Europe (FEAP, 2014). Outside of Europe, Solea senegalensis is farmed at least in China however there are no official data relative to production statistics of Solea spp. in other regions.

Source: fao.org/fishery/culturedspecies/Solea_spp/en
Satisfy your Tastebuds!
Tasty Recipe – Sole meunière

INGREDIENTS (serves 4)
• 4 fillets sole or plaice, skin-on (about 140g/5oz each)
• 6 tbsp plain flour
• 3 tbsp light olive oil or sunflower oil
• 85g butter, ideally unsalted
• 1 lemon, juice only
• 2 tbsp small caper (optional)

PREPARATION
1. Check the fish for small bones and pull any out with tweezers. In a large shallow bowl, season the flour with a little salt and black pepper. Toss the fish in the flour, coating well, and shake off any excess.
2. Heat the oil in a large frying pan. Add the fish and cook, skin-side down, for 2 mins. Use a fish slice or large spatula to turn, then cook the other side for 1-2 mins until golden.

Marc’s Wine Tip: Lemon and butter, together with the lean, delicate flesh of sole will perfectly match with a vibrant chardonnay from southern Burgundy, such as Mâcon, Viré-Clessé or Pouilly-Fuissé. But for this, keep the capers optional!

Recipe from BBC Good Food magazine, September 2005
On BBC Good Food website: bbcgoodfood.com/recipes/2835/sole-meunire

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