PARASITE Report Summary

Project reference: 312068
Funded under: FP7-KBBE

Final Report Summary - PARASITE (Parasite risk assesment with integrated tools in EU fish production value chains)

Executive Summary:
Human fishery product-borne parasitic diseases are caused by infection following ingestion of viable parasites, or as allergic (hypersensitivity) reactions against parasite antigens. Zoonotic species of Anisakidae are considered the only relevant source of allergy in fishery products. Apart from the public health concern on these biohazards, there is a growing interest on their impact in the fish quality and on the marketability of naturally-infected products along the EU-fish production value chains.

Surprisingly, despite the efforts derived from the implementation by fish operators of the Community's food safety hygiene rules to ensure that only high-quality and safe products are put on the markets, fish-borne parasites continue to pose risks to human health. Records of zoonotic infections and allergic reactions in European countries are annually increasing mainly following consumption of raw, lightly cooked, or marinated seafood. Additionally, the lack of an integrated information system on the hazard identification, characterization and exposure had provoked several “worm hysteria episodes” in many European countries during the last 30 years, due to an inefficient risk communication that should be back to a risk assessment and management of the parasite hazard.

The general objective of the project was – through risk assessment – to provide insight, upgraded know-how and new technologies in order to mitigate the impact, to industry and consumers, of zoonotic parasites present in fishery products in the European market. This objective was pursued by developing, refining and adjusting tools for the detection, monitoring and mitigation of the risk faced by consumers from zoonotic parasites products in Europe.

A quantitative risk assessment for parasites in fishery products was conducted by first time under a one-health perspective, and from the net to the plate, in EU-fish production value chains. The achieved information on the molecular epidemiology of this biohazard has been revealed of great usefulness for improving operating strategies at the fish industry to control the parasite hazards, for providing a regulatory science framework at the official veterinarian inspections, and for guaranteeing the safe and quality to fish-eating consumers.

Numerous marketed-aligned methodologies and tools (from –omic technologies, to artificial vision, refined statistics or electromagnetic radiation), have been developed and/or validated from the gene to the ecosystem to better detect, control and mitigate the impact of parasites in fish safety and quality. This integrated strategy will also help to ensure collaboration across the fish supply chain to improve in controlling this biohazard.

Technology transfer and dissemination of the results achieved along the project to stakeholders have clearly improved the social understanding of the fish parasite problem in EU-fish production value chains. The PARASITE Website, a catalogue of tech results, training workshops, short-term stages, discussion panels, communication events,...all these sources helped to socially manage the risk posed by parasites in seafood by creating a food safety culture based on education and a “need to know” attitude.

Finally, the Project has contributed to road-mapping the future research prospects and to establish a scientific collaborative EU-network in relation to the role of parasites in marine ecosystems, especially those species with significant economic and public health concern. Furthermore, along this project cooperation between EU and Asiatic research institutions has matured from pure scientific collaboration to a meaningful research and innovation partnership.

Project Context and Objectives:
Project context
The EU fishing industry is one of the largest worldwide. This is balanced against the average consumption of fish in the EU-27 countries, which accordingly to the FAO data reach up to 22.3 kg/person/year. Sustainable use of marine resources in seafood chains whilst there is growing global demand requires, amongst other things, maintaining and improving ecosystem health and, no less relevant, maintaining the good health and standard of living of people who depend on it, including from this perspective, consumers’ interests. Ensuring a safe and affordable food-chain,

establishing sustainable economies and effectively managing the risks associated with seafood-borne hazards that negatively impact on consumer perception of a given product (and potentially negatively impact human health) are key challenges. However, these challenges can generate added value through technological innovation by increasing both the quality and safety of EU and imported fish products.
Among the biological hazards, human fishery product-borne parasitic infections have generally been limited to populations living in low and middle income countries, but the geographical limits and populations at risk are expanding because of growing international markets, improved transportation systems, globalisation of the food supply and demographic changes, and the increasing popularity of raw seafood products. In relation to risk assessment of parasites in fishery products (see the scientific opinion by EFSA; EFSA Journal 2010; 8(4):1543), zoonotic nematodes have been widely considered as emerging and epidemiologically-important parasites of human health and economic concern. The PARASITE project focused mainly on anisakid nematodes, specifically to address the EFSA recommendations and research needs identified by the BIOHAZ Scientific Panel on the EFSA scientific opinion on risk assessment of parasites in fishery products (EFSA, 2010).
Ensuring a safe and affordable seafood-chain, contributing to the sustainability of the blue economy and effectively managing seafood-borne hazards than can negatively impact on consumer perception, have been the challenges for the PARASITE project. This was launched in 2013 with the general objective of providing new scientific knowledge and technological developments to detect, monitor and mitigate the impacts of zoonotic parasites in European and imported fish products, taking into account the effects upon quality and the possible consequences on health.
A multi-pronged approach was addressed to produce knowledge that may applicable and helpful in effective risk management, including its communication to stakeholders. Many scientific disciplines covering high resolution microscopy, genomics, proteomics, immunology, advanced statistics,...and the related tools and methodologies have been applied to determine the public health and economic impact of fish parasites, and thereafter propose new control and mitigation measures under a best value for money approach.
After the second reporting period, the project has produced a number of important insights on hazard identification and exposure, and new tech developments that will contribute to three major outcomes: firstly, enhancing seafood safety and quality in EU fish production value chains by combining industrial benefits and consumer trust; Secondly, strengthening the competitiveness of the EU fishing sector by improving decision-making systems; and finally, support EU food safety policies by providing an updated scientific evidence framework to understand and manage the potential risks.
Main Objectives
Following the Guide of Food Safety Risk Analysis the following “fit-for purpose” specific actions were:
• Provides comprehensive molecular epidemiological and phylogeographycal data in major fish stocks, fishing grounds and fishery products in the EU markets; uses genetic/molecular markers developed within the project to identify anisakid populations collected from fish samples by sequence analysis; develops other nuclear/molecular markers diagnostic between Anisakis species, and improves knowledge on the intraspecific genetic diversity of A. simplex.
• Provides a certified quality management system for the Biobank as the internal platform for sample and data management of the project, and to explore if the system is suitable for creating risk maps under GIS facilities.
• Provides a murine model of sensitization to Pseudoterranova sp and A. pegreffii, an assessment of the allergenic capacity of parasites in the animal models; an analysis of the exposure to Anisakis sp allergens in fishery products with no live parasite, including the evaluation of Anisakis allergens in parasitized fish subjected to canning, the allergen profile in aquaculture fish, and the biological activity of the allergens found in fishery products and aquaculture fish.
• Provides differential expression analyses among transcriptomes of A. simplex, A. pegreffii and hybrid haplotype and comparison of total expressed proteome of these three taxonomic entities; then, search for heat-stable allergens which could be considered Biomarkers for anisakids.
• Provides improved knowledge on human immune response to the parasite antigens.
• Provides new insights into detection and control methods: evaluating the efficacy of the washing practices to remove nematode larvae, optimizing new tech device to test the viability of parasites in processed fish products, evaluate the performance by Ring Trial of a Real Time-PCR to detect parasite traces in fishery products, develop an immune assay to detect parasites and/or their traces in fishery products, and carry out a beta-testing exercise for those developed methods at the industrial level.
• Provides interventions in the food chain to reduce risk: alternative methods to monitor parasite viability and infectivity (especially in suboptimal conditions); describe the microbial population in muscle-invading Anisakis larvae and in the fish flesh; design optimal treatments for the inactivation of anisakids in fishery products and validate their use by different conventional and non-thermal techniques; design strategies to reduce the allergens in processed products produced from fish infected with Anisakis L3; validate the robustness and performance of the TEDEPAD prototype on-board.
• Provides Risk analyses for parasite data: statistical models for fish parasite distribution, prevalence and abundance in samples of commercial fish species in EU waters; a dynamic framework to integrate parasite demographic infection values and genetic variability estimates; present data on human health prevalence in Europe; develop QRA models for specific seafood which are considered highest hazard exposures; model consumer willingness to pay for treatments to reduce or eliminate the zoonotic parasites in fish products; to define and evaluate cost/benefit scenarios on the implementation of project results in the seafood value chain.
• Provides Communication and dissemination activities: design a catalogue of tech results obtained within the project and the assessment of its market potential, that is taken as complementary to an after project action plan to road-map the future prospects for tech innovation challenges; organize training workshops to industrial partners; continuing work with the media professionals through press releases, interviews,...; fuel the project website and social networks; prepare project brochures and training/learning material; organize a Final Project conference.

Project Results:
Overall, main relevant achievements of the PARASITE project are:

1 New molecular epidemiological data has been gathered on anisakid parasites in EU fish stocks and markets. A total of 14,862 fish belonging to 16 teleost species were examined for anisakids, with more than 60% comprised of economic and ecologically important species. The herein conducted epidemiological survey represents the largest and most comprehensive epidemiological data compilation of anisakids ever generated in terms of geographical range as well as number of fish host species and, for most of the actual fish species, sample size, as well.

The present survey data was completed with some case studies:

Case study I: A total of 865 cultured striped catfish, commonly known as Pangasius (Pangasianodon hypophthalmus), imported to different EU markets were examined freshly for the presence of parasites in the flesh. The fish were produced in earth ponds located in 4 provinces in the Mekong Delta area, South Vietnam. Five potentially zoonotic trematode species were detected. The parasites, all in the metacercaria stage, were present at very low prevalence and intensities. No anisakids were found.

Case study II: Freshly imported fillets of Nile perch to Germany (n=200). No parasites were found.

Case study III: Imported short-finned squid Todarodes pacificus to Spain (n=600). A few anisakid larvae were found in the viscera of the squid; however, no worms were detected in the mantle/edible parts.

Case study IV: Fresh or smoked products of tuna on French and Italian markets.
1) Ten (10) bluefin tuna (Thunnus thynnus) from the Mediterranean Sea were examined for anisakid larvae. No parasites were found in the flesh of tuna. A single A. pegreffii was present in the stomach wall of one of the tunas.
2) Tuna from Philippine waters (three species – yellowfin, bigeye and skipjack; N=96). A few A. typical, preliminarily identified by ITS sequence analysis, were found in the viscera; however, no parasites were detected in the fish flesh.

2 A Biobank Solution was implemented as a non-profit service that host a collection of parasite samples originated in the present project, organized as a technical unit defined with quality criteria, order and destination, ensuring complete traceability of the samples. This solution was constructed considering some relevant EU and Member State regulations dealing with medical Biobanks and accreditation rules established by International Standards for Technological Competence (ISO 9001). The PARASITE Biobank stored more than 275,000 parasite samples (animals, DNA, protein) from Atlantic, Mediterranean and Pacific waters, and their associated data. The information extracted from the Biobank was used for creating the GIS maps which aid to understand geographical differences in those factors affecting parasite recruitment into fish stocks.
One of the first tools of the PARASITE project was the creation of the first Biobank on Marine Parasite Samples, stemming mainly from the activity developed on zoonotic species. We understand the Biobank as a non-profit service that will host a collection of biological samples resulting from this project and organized as a technical unit with defined quality criteria, order and destination, to ensure full traceability of the sample. Specific software operated remotely served as the base for the functioning of the Biobank platform. The creation of the Biobank had three objectives: a) Improve the quality and traceability of the collection of zoonotic parasite samples obtained during the study of the main commercialized fresh fish species; b) Serve as a supplier of marine field samples for scientific, industrial and clinical studies, thus establishing bonds of connection between the marina and biomedical research in the European R+D space; c) The creation of a solution with high added value of marine samples derived from monitoring, both in its self-studies and derivatives. The main idea is that the Biobank serves to control and regulate the storage of biological samples and associated data, meaning any biological sample or biological material capable of conservation and that can hold information about the project objectives. This will allow us to ensure the availability of biological material quality, well sorted, processed and preserved to meet the demands of research. Thus, the Biobank work as a promoter of scientific research as researchers upon request, obtain such biological material for use in research, present and future, whose purpose here is the diagnosis of marine zoonotic parasites in fishery products.
The principle of this biobank was that all the samples and the associated information of the project run through the architecture of the biobank. The flux of the samples can be summarized as follows.
➢ All partners are registers. In other words, are able to create a code for the samples (fish) and to introduce the associated data. They will analyze the presence, location, number of parasites, etc. This information is being uploaded to the PARASITE BIOBANK software.
➢ Each animal studied by any partner has one code (2D barcode) that is assigned automatically by the software. Thus, several partners from different countries are able to introduce data at once without a previous assignment of codes to each partner. The only requirement to introduce the data is an Internet connection. All subsamples of each sample (whole parasite, DNA, proteins, etc.) are automatically linked to the original one. Each biobank will locate any single subsample in a particular site (freezer, racks, boxes, etc.), which on the other hand is controlled by the software.
➢ All parasite samples are circulated from registers to the biobank node/subnodes following the structure detailed in the figure below.

The main characteristics of the biobank we created can be summarized as follows:

• Registration, classification and automatic labeling of samples ensures traceability of samples from the moment of reception in the biobank. Combination with labeling systems allows the marking of each and every sample with its own unique barcode.
• Workflow tracking allows you to follow each sample individually and check for any processes pending.
• Connectivity between nodes reduces task and data duplication and, thus, errors and omissions in data.
• A simple yet powerful requests module and search engine helps to get the most out of our biological resources. It

makes a greater, safer and quicker exchange of samples possible – allowing to make the jump from sample repository to true biobank. Search results also can be exported into i.e. XL files for further data drilling and research.
• Configurable sample storage allows the application to replicate physical storage layout. Each sample is labeled before storage, improving its traceability.
• The improvements in data security, traceability and process integrity that Bio-o-Bank provides facilitate the compliance with data protection in at the same level of biomedical research laws.
• Web-based technology allows for an easier, faster and less disruptive implementation. Not only does it require less intervention and maintenance by your IT department, you always have access to the latest version.

It has to be underlined that the central node of the biobank was recently certified with the ISO 9001. This certification specifies requirements for a quality management system where an organization needs to demonstrate its ability to consistently provide product that meets customer and applicable statutory and regulatory requirements, and aims to enhance customer satisfaction through the effective application of the system, including processes for continual improvement of the system and the assurance of conformity to customer and applicable statutory and regulatory requirements.

The information extracted from the Biobank was used for creating the GIS maps. These data information is completely traceable from the collection date to the last analysis undertaken for different purposes. The information obtained from the epidemiological Biobank data on the main species fished in European waters allowed us to produce maps that showed a clear view of the state of the main European fishing grounds.

3 Population genetic analysis of the most prevalent species A. pegreffii and A. simplex (s. s.) were performed, as inferred from allozymes and sequences analysis of the mtDNA cox2 in 3455 anisakid larvae. The mtDNA cox2 sequences analysis was provided, presently, on a total of 1780 sequences obtained from specimens of the two species collected from fish of different sampling areas. Other nuclear/molecular markers diagnostic between the two species A. pegreffii and A. simplex (s. s.) were developed (EF1 α-1 nDNA and DNA microsatellites loci).
The genetic identification of Anisakis spp., Pseudoterranova spp. and Hysterothylacium spp. larvae collected from the target fish species, sampled from the selected localities, was obtained by mtDNA cox2 sequence analysis. The total number of larvae so far identified based on this gene analysis (sequences analysis and RT-PCR of the same gene), at the time of this Report, was 4080. Other sequences are under elaboration, and the data set will be complied with those actually achieved.
The sequences obtained at the mtDNA cox2 gene (629 bp) showed that 743 specimens recovered in the fish from the Mediterranean Sea and 322 from the Atlantic Spanish-Portuguese coast, matched at the 99% or 100%, the sequences deposited in GenBank for the species A. pegreffii in our previous analysis (Mattiucci et al., 2009, 2014). Analogously, 2935 specimens of Anisakis spp. from the fish samples of the NE Atlantic Ocean matched 99% or 100% the sequences deposited in GenBank for the species A. simplex (s. s.) at the gene mtDNA cox2. In addition, larval nematodes belonging to the species Pseudoterranova decipiens (s. s.) (N= 5), P. krabbei (N=22) and P. bulbosa (N= 8) were identified based on the mtDNA cox2 sequences analysis and RT-PCR of cox2 gene. Indeed, the sequences obtained, matched at the 99% or 100%, those deposited in GenBank for Pseudoterranova spp. species, and in our previous specimens. Finally 20 larval nematodes of the genus Hysterothylacium collected from fish from the Mediterranean Sea, while 15 from fish of the NE Atlantic waters sequenced at the mtDNA cox2 gene, were assigned to the species H. aduncum as matched at the 99% the sequence deposited in GenBank for that species, at the mtDNA cox2 gene.
Diagnostic allozymes obtained from MAE analysis was performed on the same specimens analyzed also at the mtDNA cox2 level, with a total of N= 4291 larvae identified from the selected fish from all the sampling areas. Only exception was some specimens from samples of the NE Atlantic Ocean (English Channel, Great Sole Bank) and from the Mediterranean Sea (Adriatic Sea, IZOR samples): on those samples only mtDNA cox2 sequences analysis was performed. As a whole, three species, i.e. A. pegreffii, A. simplex (s. s.) and A. physeteris, were so far identified by MAE in the fish samples collected from those geographical areas so far investigated; The species A. pegreffii was the most prevalent species in the fish from the Mediterranean Sea; It occurs in co-infection in fish sample from the NE Atlantic Ocean (Portuguese coast); Interestingly, 4 specimens were identifed in S. scombrus from the Norwegian Sea sample; A. simplex (s. s.) resulted the prevalent species in fish from the NE Atlantic Ocean; It also occurred in sympatry, co-infecting the same individual host in fish sampled along the Atlantic Portuguese - Spanish coast. It was also found rarely (N= 6) occurring in fish sampled along the Alboran Sea (Mediterranean Sea; few specimens of F1 hybrids (N= 11) between A. simplex (s. s.) and A. pegreffii were detected: 10 in fish species of the Atlantic Portuguese coast, and one in M. merluccius from Alboran Sea.

SSR-DNA microsatellites
SSR-DNA microsatellite nuclear loci in the two species A. simplex sensu stricto and A. pegreffii, were scored.
The parasite DNA was first tested by using the barcode primers for fishes spp., in order to be sure that no amplification of the fish host DNA had obtained. All the parasite samples found negative for fish DNA, while they were positive for Anisakis DNA amplification, were then considered for the microsatellite (SSR) DNA development. Suitable primers design was possible in 429 microsatellite candidates. The features of the seven loci selected are reported in Table 1.
Table 2 A. pegreffii and A. simplex (s. s.) microsatellite DNA Markers. In the table are reported the name and sequence of primers for the 7 loci, the repeat type, the ranging sizes of fragments amplified for each couple of primers, the number of alleles for each locus, the fluorescent dye of labelled primers and the multiplex assay belonging of the primers.

Two multiplex reactions, using 4 (Anis_05784, Anis_08059, Anis_00875 and Anis_07132) and 3 (Anis_00314, Anis_10535 and Anis_00185) markers, respectively, were developed. Presently, all the markers are under the genotyping test

(figures 4 and 5). Presently, this approach has been applied on a large number of specimens belonging to the two species, already identified, in order to study the polymorphism at those loci on different samples/metapopulations of those species from different hosts and geographic areas.

Elongation factor α-1 subunit of nDNA (EF1 α-1 nDNA)
Out of the DNA microsatellites development, a new nuclear marker, (i.e. the elongation factor α-1 subunit of nDNA) (EF1 α-1 nDNA), to be useful in the identification of A. pegreffii and A. simplex (s. s.), was detected. The primers were designed based on the elongation factor 1 alpha1 gene of genomic DNA sequence deposited in the GenBank database under the accession number KP326558. The primers here used were first designed by hand and then verified by means of the on-line software program Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/).
The diagnostic positions detected were confirmed on 191 specimens of A. pegreffii, and on 241 of A. simplex (s. s). In addition, the same marker was tested on those 11 specimens found as F1 hybrids between A. simplex (s. s.) and A. pegreffii, as inferred from the allozyme diagnostic markers ; a double peak at both the two diagnostic positions was found (Figure 6), confirming their identification as F1 hybrids, as the result of a current hybridization between the two species.
In conclusion, the fixed differences at EF1 α-1 nuclear locus, for the two Anisakis spp. considered (i.e. A. pegreffii and A. simplex (s. s.), were validated in both allopatric and sympatric populations of the two species, on a large number of samples. Sequences of the EF1 α-1 nDNA region were deposited in GenBank under the accession numbers KT825684 for A. pegreffii, and KT825685 for A. simplex (s. s.). The EF1 α-1 nDNA gene represents a novel nuclear marker of diagnostic value for the recognition of the two cryptic species, A. pegreffii and A. simplex (s. s.).
Population genetic variability
Estimates of population genetic variability and genetic differentiation, inferred at the mtDNA cox2 level, have been so far calculated on a certain number of specimens belonging to the species A. simplex (s. s.) (N= 1276) and A. pegreffii (N= 504) Genetic diversity and population structure of A. pegreffii and A. simplex (s. s.) were estimated grouping the parasites populations according to different sampling geographical areas from where the fish hosts were collected.
Genetic diversity of A. pegreffii at mtDNA cox2 gene
At the intraspecific level, the results so far achieved concerning the genetic diversity of A. pegreffii inferred from the mtDNA cox2 gene indicated that the species has 100 polymorphic sites, and 150 haplotypes. Among the 100 polymorphic sites, 34 were singleton variable sites (2 with possible variants), and 66 were parsimony informative. Values of so far obtained showed a high value of haplotype diversity (on average, h≈ 0.955) and a low nucleotide diversity (on average, π≈ 0.008). The average number of nucleotide differences was high: it resulted to be, on average, 5.166. A lower genetic diversity value was observed in the Mediterranean populations of A. pegreffii, with respect to its population from the Atlantic Portuguese coast.

Population genetic structure of A. pegreffii
The estimation of genetic differentiation between populations inferred from the fixation index, Fst) was performed. The A. pegreffii populations so far included in the analysis revealed significant differentiation of among the populations grouped by geographical areas. Indeed, the Fst values of differentiation estimated so far have shown that a significant (P= 0.000) value of genetic differentiation exists between both the eastern and western samples from the Mediterranean Sea with respect to its population from the Atlantic Portuguese basin waters (Fst = 0.013 and Fst=0.029, respectively). Slight statistical significant genetic differentiation was observed between its eastern and western populations (Fst= 0.007); whereas no significant value was so far found between the Atlantic population of A. pegreffii with respect to that from Alboran Sea (Fst = 0.007), as well as between Alboran population versus its population from west Mediterranean Sea. The fixation index results were confirmed by AMOVA , which attributed the 1.73% of genetic variation among the populations from different geographical areas, and the 98.27% of genetic variance within populations. In addition, the overall Fst value for A. pegreffii sampled from the three geographical areas under study, resulted 0.017 (P <0.001) and the overall gene flow value is Nm= 14.2.

A reconstructed network for 178 haplotypes of A. pegreffii sequences obtained so far for the four geographical areas (N= 504) showed a radial distribution. The highest proportion of unique haplotypes (41.00%) was found in the Eastern Mediterranean Sea population, followed by the Portuguese population (30.2%), and the remaining were those observed from the Western Mediterranean Sea (28.8%) population.
Unique haplotypes were not observed in the Alboran Sea population of A. pegreffii, likely due to the small number of specimens tested. Populations from the eastern and western areas of the Mediterranean Sea shared most of the haplotypes (N= 30); the Alboran Sea population shared the lowest number from the rest of the areas (6 from and 4 from Western and Eastern Mediterranean Sea, respectively, and 5 from Portuguese coast). Haplotype 1 (likely the ancestral haplotype) was the most frequent in total (48.3%); only 3 haplotypes were shared between all the three populations so far included in the network.
The most common haplotype showed a relative frequency of 48.3%, with respect of all the others haplotype which have frequencies varying between 11.8% and 0.6%. Most of the sequences characterized by the ancestral haplotype (having a frequency of 48.3%) are those belonging to the Eastern waters of the Mediterranean Sea (n= 55; 63.9%); whereas, 20 (23.3%) and 11 (12.8%) were detected in the Western Mediterranean Sea and in the Atlantic Portuguese waters, respectively.

At the intraspecific level, results so far obtained concerning the genetic diversity of A. simplex (s. s.) inferred from mtDNA cox2 gene indicated that the species shows 666 haplotypes, characterized by 231 polymorphic sites, and 156 parsimony informative sites. Among the 231 polymorphic sites, 70 were singleton variable sites (two variants). Values of the so far sequenced A. simplex (s. s.) specimens showed a high value haplotype diversity (on average, h= 0.974) and nucleotide diversity (on average, π= 0.009); the average number of nucleotide differences was high; it resulted to

be, on average, 6.708.

Population genetic structure of A. simplex (s. s.)

Slight genetic differentiation, at the intraspecific level, was observed for the species A. simplex (s. s.), among the populations so far examined. Instead, high and significant values of the pairwise Fst values resulted when comparing population from the North Sea versus that from the Norwegian Sea (Fst =0.013, P= 0.0000); high and significant values resulted also between the population from the English Channel and Atlantic Portuguese coast (Fst = 0.033, P= 0.0000), between A. simplex (s. s.) samples from English Channel versus the population from Baltic Sea (Fst= 0.011, P= 0.0000), and between Great Sole Bank sample versus the Norwegian Sea sample (Fst= 0.013, P=0.0000). The highest and significant value of genetic differentiation has been found between the population of A. simplex (s. s.) from the Norwegian Sea versus that recovered in the fish from the English Channel basin waters (Fst = 0.043, P= 0.0000). While lower and not significant values were those observed between the samples from North Sea versus those from Baltic Sea. The fixation index results were confirmed by AMOVA which attributed the 1.29% of genetic variation among the populations from different geographical areas, and 98.71% within populations; overall Fst = 0.013 with P =0.0000 and overall gene flow value, Nm= 17.8.

A reconstructed network for 666 haplotypes of A. simplex (s. s) sequences (N= 1276), so far obtained from six geographical areas, showed a radial distribution (Figure 2).

The most common haplotype in A. simplex (s. s.), here indicated as H1, shows a frequency of 22.67% (151 sequences); it has been found to be shared by five, out of the six populations from the geographical areas so far considered. Interestingly, the H1 haplotype was not found among the samples of A. simplex (s. s.) collected from the Norwegian Sea waters. The second most common haplotype (H2) haplotype showed a relative frequency of 8.2%, while the frequency of all the remaining haplotypes ranged between 3.7% and 0.01%.

The English Channel and North Sea populations of A. simplex (s. s.) shared most of the haplotypes (n= 62), while the Norwegian Sea samples showed the lowest number of shared haplotypes with respect of all the remaining populations collected from the fish captured from the rest of geographical areas so far considered (only 3 shared haplotypes with the English Channel, 4 with Baltic Sea and Great Sole Bank, 10 with Portuguese coast and 13 with North Sea populations).

Finally, the A. simplex (s. s.) sample caught in the North Sea area showed the higher number of unique haplotypes (n=224), followed by that from Portuguese coast, English Channel, Norwegian Sea, Baltic Sea and Great Sole Bank sampling areas, with 109, 98, 49, 43 and 41 unique haplotypes, respectively.

Demographic history of A. pegreffii and A. simplex (s. s.)

The analysis of mtDNA cox2 haplotype distribution is useful to identify the presence of single haplotype that are common to all “geographical populations” investigated for the two studied species, A. pegreffii and A. simplex (s. s.). The measure of Tajima’s D statistic value based on the mtDNA cox2 sequences revealed values different from zero, and having a negative values all for the populations from the relative sampling geographical areas, but only Portuguese Atlantic coast and Alboran Sea populations of A. pegreffii and the Portuguese Atlantic coast population of A. simplex (s. s.) not received significant values. Similarly, Fu’s Fs test values resulted significantly negative for all the populations considered, in both the two Anisakis species analyzed. Generally, a negative value of Fs would be expected from a recent population expansion or from genetic hitchhiking. Fu’s simulations suggest that Fs is a more sensitive indicator of population expansion and genetic hitchhiking than Tajima’s D. Thus, rejection of a neutral evolution null hypothesis for all the studied populations was confirmed.

The model of population expansion could not be rejected because of its concordance with the expectation of historically expanding population supported by the low and not significant Sums of Squared Deviations (SSD) of mismatch distribution values, indicating that the curves of mismatch distribution fit the sudden expansion model tested both for A. pegreffii and A. simplex (s. s.). The initial populations ($\theta 0$) were overall and in all cases smaller than the final populations ($\theta 1$), thereby confirming a historical sudden expansion of species, both for A. pegreffii and A. simplex (s. s.). The graphical representation of mismatch distribution for A. pegreffii and A. simplex (s. s.) showed a concordant pattern with an unimodal curve, except for a slight secondary pick found in the Portuguese Atlantic populations, both for A. pegreffii and A. simplex (s. s.) and in Alboran Sea population for A. pegreffii. This slight deviation could be due to population reduction, population subdivision, a recent bottleneck, or migration which resulted in secondary contact among previously differentiated lineages. In fact, multiple peaks may appear in mismatch distribution, despite population expansion, whether genetic sub-structuring is present in a population.

4 Anisakid proteins, in particular those from A. pegreffii and from Pseudoterranova sp., induce allergic symptoms in BALB/c mice (WP5). Since allergic reactions observed in patients with anisakiasis are thought to be mediated by A. simplex specific-IgE allergens, the presence of IgE reactive proteins shared among anisakid species (i.e., A. simplex, A. pegreffii, Pseudoterranova sp., and C. osculatum), could increase the risk of development of allergic manifestations in patients sensitized to a different species.

The gavage of A. pegreffii proteins to previously sensitized mice triggered signs of allergy within 60 min. The maximum symptom was observed in animals injected i.p. with living L3. The ingestion of Pseudoterranova sp. proteins in previously sensitized mice, induced irritability and reduced activity within 60 min, similarly to A. pegreffii. These results were further supported by the histamine presence in serum samples from all groups of mice.

Splenocytes from mice i.p. inoculated with A. pegreffii L3 , which had been activated with anti-CD3/anti-CD28, induced significantly higher levels of IL-10 and IL-5 than splenocytes from mice of the other A. pegreffii groups and naïve mice. Splenocytes from mice inoculated i.p. with Pseudoterranova sp. L3 produced IL-10 at a significantly higher level than that of naïve mice (Fig. 7B). After activation with anti-CD3/anti-CD28, splenocytes from all A. pegreffii experimental mouse groups produced significantly lower IFN-ɣ levels than those from the not exposed naïve mice.

The antigenic stimulation of the splenocytes from mice inoculated i.p. with A. pegreffii L3 induced significantly higher levels of IL-10, IL-5 and IL-13 than those produced by the splenocytes from naïve mice (Fig 3A). The stimulation of the splenocytes from mice inoculated i.p. with Pseudoterranova sp. L3 and mice orally inoculated with CWE from Pseudoterranova sp., induced significantly higher production of IL-10, IL-5, IL-13 and IFN-ɣ than that produced by the splenocytes obtained from naïve mice (Fig 8B).

The intraperitoneal exposure of A. pegreffii L3 and Pseudoterranova sp. L3 in BALB/c mice, induced specific IgE and IgG1 levels in mouse sera from week 4 until the last day of observation. Detectable IgG2a levels were found only in the serum samples from mice intraperitoneally exposed to Pseudoterranova sp. L3.

5 Some aquaculture fish contain functionally active Anisakis allergens and this could represent a hidden source of allergens.
Several allergens from 17 kDa to 50 kDa are detected in turbot and sea bass extracts. It has been previously shown that these extracts were able to activate basophils from allergic patients.

6 Proteomic studies revealed some of the most exciting results. The total expressed proteome of three taxonomic entitites (A. simplex, A. pegreffii and A. hybrid) was performed. Furthermore, the AnisakisDB is now completely and free operative (www.anisakis.mncn.csic.es). There, a catalog of genes and proteins expressed in two different Anisakis species (A. simplex s.s. and A. pegreffi) and their hybrid is provided to promote improved understanding in the molecular biology and relationships of this important parasite complex. The database will be soon updated with the annotation of the Anisakis genome and additional metagenome information relative to the microbiota of Anisakis. Additionally, the comparison with other nematodes transcriptomes and the functionality of the proteomes and validation with food allergens proteins were explored. Finally, new Protein Evidence for Anisakis simplex and P. decipiens was achieved by “Shotgun proteomics” coupled with Bioinformatic Analysis for Protein Identification using Sequest against Uniprot Database for matching (Nematoda). These peptide biomarkers have allowed to design a new strategy for monitoring of several peptide biomarkers from the Ani s9 protein by PRM in an ion trap mass spectrometer to directly allow the detection and identification of anisakids in fishery products in less than 2 hours.
Differential expression analyses among transcriptomes
The three assemblies for A. simplex, A. pegreffii and A. hybrid transcriptomes were merged in a single interspecies assembly using the tool Minimus 2 of the Amos package (http://sourceforge.net/projects/amos/) in combination with CD-HIT. Results have been obtained for a 1% rate of false positives (FDR) at peptide level. From the total proteome, 127 proteins can be considered which express real and significant differences (at least 2 peptides per protein) as taxonomic biomarkers comparing the three taxonomic entities, being the most important based in FDR values what are indicated according the NCB accession number.

Transcriptomics and database Anisakis DB implementation
The AnisakisDB have been concluded and implemented which is now completely and free operative (www.anisakis.mncn.csic.es). A catalog of genes and proteins expressed in two different Anisakis species (A. simplex s.s. and A. pegreffi) is provided, and their hybrid to promote improved understanding in the molecular biology and relationships of this important parasite complex. The database will be soon updated with the annotation of the Anisakis genome.
Validation of AnisakisDB with food allergenic proteins
Food allergens based in allergen families (Helton da Costa Santiago, Sasisekhar Bennuru1, José M. C. Ribeiro, Thomas B. Nutman. Structural Differences between Human Proteins and Aero- and Microbial Allergens Define Allergenicity. PloS ONE, 7: e40552 (Table S1; doi:10.1371/journal.pone.0040552.s002)) have been searched in the transcriptomes in order to validate the AnisakisDB. From 499 allergens which have been considered, 203 are significantly present in the Anisakis transcriptomes. However none allergen from bacteria have been detected (Table 1). Fitty seven families of allergens are significantly represented in the AnisakisDB. The possible variation according to number of transcripts per gen, association to A. simplex s. s., A. pegreffii or hybrid haplotype, origin of the allergens and if proteins are major or minor allergens are variables which have been considered in order to establish a ordination of the data based on Factorial Analysis of Correspondence. The distribution of the 203 allergens in the factorial space defined by three axis whose level of explained inertia is very high (98.04 %). The ordination of allergens is due to the number of transcripts which conform them. The whole of allergens are gathered into two separated groups; a group with a minimum of 28 transcripts (average higher of 39) and other group with a maximum of 32 transcripts (average minor than 20). None other significant differences can be found regarding type of allergen (major or minor), family, origen (animal, plant, fungi) or Anisakis species.

Biomarker proteins of anisakids
By “Shotgun proteomics” coupled with Bioinformatic Analysis for Protein Identification using Sequest against Uniprot Database for matching (Nematoda) , we have got New Protein Evidence: 696 and 730 new proteins for Anisakis simplex and Pseudoterranova decipiens, respectively. That means a step forward in the protein evidence previously existing from 4 proteins vs 1117. We have studied the thermo stability of proteins focusing on the allergenic proteins and, we have detected Biomarker Proteins and their corresponding Biomarker peptides. Ani s 9 allergen, an allergen and thermostable protein, was selected as protein biomarker. From this protein, 5 sequences were selected as peptide biomarkers to cover the identification of Anisakis simplex, Pseudoterranova decipiens and Pseudoterranova krabbei. The peptide biomarkers have allowed to design strategies to directly identify and detect fish-borne parasites in less than 2 hours.

Characterization of the immune response to the parasite antigens

The presence of allergens in crude extracts from P. krabbei and C. osculatum were analysed by immunoblotting with a pool of A. simplex sensitized patients and polyclonal rabbit anti-Anis 1 and anti-Ani s 4 antibodies. In order to analyse if allergens in extracts were biologically active, a basophil activation test (BAT) was performed with A. simplex and P. krabbei extracts. On the other hand, excretory/secretory products from A. simplex, and A. pegreffii were analysed by IgE immunoblotting.
The allergen patterns observed in P. krabbei and C. osculatum are different from that in A. simplex. Three allergens of 50, 44 and 32 kDa are revealed in P. krabbei extract and 50, 40, 12 kDa allergens are detected in C. osculatum. In order to analyse the cross-reactivity between the allergens of P. krabbei and C. osculatum with A. simplex, IgE immunodetection inhibition with A. simplex extract was carried out. A nearly complete inhibition was observed. In the immunodetection with anti-Ani s 1 and anti-Ani s 4, a homolog of Ani s 4 is detected in P. krabbei with a molecular weight slightly lower than that of A. simplex, while no defined band is observed in C. osculatum. Furthermore, P. krabbei presents Ani s 1 with a molecular weight lower than that of A. simplex and a protein of about 37 kDa is detected in C. osculatum. On the other hand, the BAT shows that P. krabbei allergens are biologically active because a similar percentage of activated basophils is observed when peripheral blood of A. simplex sensitized patients is incubated with P. krabbei or A. simplex crude extracts.

According to the allergen patterns observed in the IgE immunoblotting with A. simplex and A. pegreffii excretory/secretory products, it seems that A. simplex is more allergenic than A. pegreffii. It is also observed that there are differences in the allergen detection: A. pegreffii contains some allergens with molecular weights of 25 kD-37 kDa that are not detected in A. simplex and, on the contrary, an 80 kDa allergen in A. simplex is not detected in A. pegreffii. Differences are also found when crude extracts from both species are analysed. These results support previous report showing that A. simplex and A. pegreffii have different allergen composition. However, we should note that these assays have been performed with Spanish sensitized patients and that exposure to Anisakis in Spain is mainly to A. simplex. We could speculate that differences in the allergen patterns may be dependent on the country of origin of the tested patients, because Italian patients show more reactivity against A. pegreffii, the Anisakis species more frequently found in Italy.
Ani s 11-like protein is a valuable tool for Anisakis allergy component-resolved diagnosis
The Ani s 11-like protein has been proposed as an Anisakis allergen because its primary structure is similar to that of Ani s 11. Experimental data indicate that 13.5% of the patients detected only rAni s 11-like protein and that it is a major allergen because it is detected by >50% of the Anisakis-sensitised patients. According to the rules of the International Union of Immunological Societies (IUIS) Allergen Nomenclature, it is named as Ani s 11.0201 (http://www.allergen.org/viewallergen.php?aid=706). The thermostability of Ani s 11-like was assessed because it is a characteristic of clinical interest when exposure to heat-stable allergens is suspected to be involved in allergic symptoms. This allergen is heat-stable because it retained its capability of binding IgE after boiling for 30 minutes. Another characteristic of interest is its stability to pepsin digestion. It was observed that epitopes of the Ani s 11-like protein maintain their capacity to bind IgE after pepsin digestion for 2 hours. The results obtained with individual recombinant allergens demonstrate that they are useful for diagnosis with high specificity but underline the necessity of using a combination of selected allergenic components to increase the sensitivity for an accurate diagnosis of Anisakis allergy. In the study, five patients (13.5%) presented only rAni s 11-like protein, indicating that this allergen may aid the component-resolved diagnosis of Anisakis allergy. Differences in IgE reactivity in sera from patients showing a convincing clinical history of allergy to Anisakis suggest interindividual variations in immunological responses to this parasite, qualitative or quantitative variations of allergen content in different Anisakis species or different routes of exposure to allergens.
Many Anisakis allergic patients tolerate a diet of frozen or well-cooked fish, but some patients report symptoms after eating cooked or processed fish (Audicana et al, 2002; Moneo et al, 2007; AAITO-IFIACI Anisakis Consortium, 2011). The resistance of Ani s 11-like allergen to digestion and heat treatment suggests that this protein may be involved in the appearance of symptoms after the consumption of contaminated fishery products.
Human humoral and cellular immune responses to A. simplex
Control subjects have anti-Anisakis IgG, IgG4 and IgA suggesting that they are exposed to parasitized fish. However higher levels of specific IgG, IgG4 and IgA were found in patients than controls. A higher IgG and IgA mediated responses to Ani s 4 is found in controls than in patients. In patients, Anisakis allergens are able to induce different humoral responses. Anti-Ani s 1 responses IgE and IgA are predominant in allergic group while anti-Ani s 4 and anti-Ani s 5 IgA responses are mainly found in the GI group.
The results showed that recombinant allergens are able to specifically activate basophils and that the use of flow-cytometric method can help to the component-resolved diagnosis for Anisakis allergy. The detection of A. simplex crude extract-induced basophil activation by flow cytometry is a useful laboratory technique for the diagnosis of anisakidosis, supplementing specific IgE determinations. Additionally,the cytokines IL-17A, IFN-γ, TNF-α, IL-10, IL-6, IL-5, IL-4 and IL-2 secreted in whole blood cultures were quantified with the crude extract and recombinant allergens. It was found that healthy controls secreted high amounts of IFN-γ, TNF-α and IL-6 induced by Ani s 1 and Ani s 5, IL-6 by Ani s 4 and IL-6 and IL-2 by the crude extract (table 2). These data indicate that non-allergic fish-consuming controls show Th1 polarization of cytokine production to Anisakis proteins. Similar results have been reported for peanut allergy (Turcanu et al, 2003). Furthermore, Ani s 1 and Ani s 5 also induce a strong secretion of IL-10 that has been suggested to play a modulatory role in the induction and maintenance of allergen-specific tolerance in allergy and it has been shown to be an essential cytokine for the induction and the function of at least some types of regulatory T cells. IL-10 also inhibits eosinophil survival and IL-4-induced IgE synthesis (Bullens et al, 2004).These results suggest that healthy controls are actually exposed to fish-borne parasite antigens because a specific immune response is shown in vitro and that IL-10 may play a role in the tolerance to Anisakis antigens in healthy controls.
The comparison of cytokine levels between patients and controls shows significant differences except for IL-10, IL-5, IL-4, IL-2 with Ani s 5 and IL-10 with the crude extract. Regarding the type of immune response (Th1/Th2/Th17) we have

observed that only some patients secreted detectable levels of Th17 with the crude extract and allergens. According to these results, Th1 polarization is observed to Ani s 4 and Ani s 5, while Th2 skewed responses are induced by Ani s 1 and the Anisakis crude extract. On the other hand, no any significant differences in cytokine secretion was observed when patients are analyzed according to their symptoms (allergic, gastrointestinal or mixed groups).

7 A viability tester developed within the project is able to differentiate dead and live nematodes from processed products. The staining tests in addition with the visual inspection confirmed the results obtained by the viability test device. Additionally, a developed automatic device Scanisakis is able to UV-detect the zoonotic species within the genera Anisakis, Pseudoterranova and Contracaecum. The prototype was calibrated during the reporting period in different panel samples with different seafood matrices, and it was also trialed in different fish companies to show its market potential.
A viability test device was developed in 6 steps comprising: 1) the understanding of parasites by modeling (a link of parasite form and its viability was established via the formenergy);. 2) the definition of viability features from the model (formoscillations of alive nematodes vs. the static forms of dead parasites); 3) the measurement method for the viability features chosen (geometric parameters as length, radius, volume, fiber orientation, and physical paraameters as inner/outer pressure, temperature); 4) the technical implementation (optical contour measurements in transmitted light with a NIR wavelength); 5) the evaluation of the viability feature data (propagation of curvature by time); and finally 6) data recording and reporting. A parasite viability tester device was completed and successful tests were run to differentiate between live and dead nematodes in frozen, marinated and salted products.

Moreover, a prototype to improve the UV-Press method was also designed by understanding the spectral characteristics of anisakids and reporting their emission profile in a fluorescence image-based system with large sample capacity into real life operations (lab or industrial). Developing an automatic inspection system for identifying Anisakis spp. in fish fillets involves many series of processes design, construction and validation. Several improvements on the prototype were experimentally needed to cover all the technical requirements of a commercial device, including integration of capture techniques of machine vision, advanced image processing and hardware construction. This prototype was designed on the basis for a future versatile commercial device that accomplish with the specificity and sensitivity scientific criteria but also that it integrates commercial aspects like low cost, simplicity, rapidity and stability. Technical aspects of the vision-based prototype will also be refined by using these trials during the time covering the PARASITE project.

8 RT-PCR validations of primers/probes combinations were obtained for some anisakid species; implementation of the standard curves and the limit of detection for Anisakis and Pseudoterranova were achieved. The RT-PCR probe developed within the reporting period that passed through an interlaboratory trial was used for the rapid DNA detection of the aetiological agent of human cases of anisakiasis.
Primers and probes systems have been developed. The use of hybridization probes by RT-PCR allows to detect the parasite DNA in fish tissue for a rapid screening of the presence/absence of these species, which may co-infect the same fish host in sympatric areas.

9 Several target proteins, some of them related to parasite motility, cystoskeletal and energy metabolism, were used as monoclonal markers to develop immune assays to detect parasites and/or their traces in fishery products.
An enolase constituted by a single peptide from Meloidogyne sp presented high degree of homology with nematode proteins, in particular with Anisakis simplex (Calvo et al., 2005). The sequence analysis of the peptide and its alignment with the homologous sequence in A. simplex showed only few changes, in particular, one substitution from threonine (T) to lysine (K). Given the particular specificity for A. simplex s.l., this peptide (PT26) was selected to produce MoAb able to detect anisakid antigens in immune assays. To check the specificity of the recognition, other recombinant enolase proteins were included in the experiments. Once demonstrated the specificity of the IgG present in serum sample from mice immunized with the peptide PT26, this animal (26#1) was selected for the production of monoclonal antibodies. The hybridomas were cloned twice by limiting dilution. Positive clones were expanded and frozen in liquid nitrogen for long-term storage. The results indicate that sera 26 # 1 contain specific antibodies to the recombinant protein enolase (Figure 15 lane 5). These antibodies specifically recognize a protein that migrates with an apparent molecular weight of 60-65 kDa, consistent with a possible fragment of the recombinant protein GST-enolase, since the sum of the expected molecular weight was 73 kDa, based on a theoretical molecular weight of 26 kDa for GST and 47 kDa for enolase. These sera recognize as well a protein of about 40-45 kDa mobility in total soluble extracts from A. simplex compatible with the weight that has the native enolase protein.

10 A first collaborative study (beta-testing at the industry) to determine and compare the performance of both the artificial digestion (AD) and UV-Press (UVP) methods in detecting Anisakidae larvae (L3) in fish samples was carried out. The UVP method has been revealed as a sensible, accurate and easy to implement method. In order to integrate it in an industrial routine an automated device should be ready for commercial application.

11 Alternative methods to monitor parasite viability, infectivity and pathogenicity potential (including allergenicity) were assayed, and tests for studying infectivity in suboptimal conditions were also set up. These tools served us for studying the effect of different technological conditions to control Anisakis infection and its potential risk in seafood. The effectiveness of non-thermal treatments was addressed in terms of viability and agar penetration capacities. Overall, some of these emerging industrial methodologies have no effect on parasite viability or if they are able to inactivate the parasites (i.e., HHP) they affect fish commercial quality. Because of that, freezing is selected as the most applicable treatment to guarantee parasite inactivation and preservation of fish quality as studied by sensory analysis, water holding capacity, Low Field NMR relaxometry and optical/transmission electron microscopy. The freezing rate and

storage time affected the sensory quality of the fish samples so that it is suggested that freezing rates of 70 min or less at a maximum temperature of -20 °C and thawing after 24 h could meet both safety in terms of Anisakis mortality and eating quality. These results do not exclude the fact that we could find faster freezing rates that would render equal or better results at shorter storage times in the freezer.
Alternative methods to monitor viability and infectivity were assayed, and tests for studying infectivity in suboptimal conditions were also set up. These tools served us for studying the effect of different technological conditions on the pathogenicity potential of anisakid species.

The microbial population in muscle-invading Anisakis larvae was described by combining conventional cultivation-based methods and various molecular methods. Adapted metagenomics for bacteria present in Anisakis was performed suggesting that at least four significant groups can be found regarding association between Anisakidae and bacterial families, some of them comprising species which are relevant to fish spoilage or human pathogenic concern.

Optimal treatments for the inactivation of anisakids in fishery products were designed. For that, different factors affecting the thermal (freezing and heating) treatments to inactivate Anisakis simplex L3 in terms of viability, in vitro infectivity and allergenicity were addressed in isolated larvae and infected fish muscle. The study of the effectiveness of non-thermal treatments was addressed in terms of viability and agar penetration capacities. Freezing was selected as the most applicable treatment and the quality of fish muscle under different freezing conditions known to inactivate Anisakis was studied by sensory analysis, water holding capacity, Low Field NMR relaxometry and optical and transmission electron microscopy. This information was used to find tools to verify that fishery products have been subjected to a given treatment, which could aid in the verification that EC regulation is being implemented.

During these assays, it was shown that A. pegreffii may be more labile to technological treatments and therefore present less infective potential. However, both A. pegreffii and A. simplex s.s. seem to have similar antigen and allergens suggesting a similar allergenic potential. In relation to heating, a significant percentage of the variability in survival of Anisakis can arise from local differences in temperatures attained by the pieces of fish within a given culinary treatment. Oven cooking has proven to be safe at oven temperatures of 200 ºC for 20 minutes. The low oxygen consumption rate of larvae surviving heating at 50 ºC, as compared to the controls suggests an impaired metabolism. As for freezing, more precise time and temperature recommendations should be given to the consumers to account for the high variability of heating regimes, presentations, etc and the fact that consumers tend to cook the fish for lower temperatures or shorter times. The assayed ultrasound and active packaging technologies had no effect on the inactivation of Anisakis larvae. High Pressure Processing had an inactivating effect on Anisakis, but their impact in organoleptic characteristics of fish flesh must be also taken into account. Control measures based on thermal processing (cooking and freezing) are thus at present, the most effective and a best-value for money approach.

The freezing rate and storage time affected the sensory quality of the fish samples so that it is suggested that freezing rates of 70 min or less at a maximum temperature of -20 °C and thawing after 24 h could meet both safety in terms of Anisakis mortality and eating quality. These results do not exclude the fact that we could find faster freezing rates that would render equal or better results at shorter storage times in the freezer.

Significant changes in muscle characteristics between unfrozen vs frozen and thawed fish, and also among samples subjected to different freezing rates have been observed. Among them, LF NMR relaxometry can be used as a tool to successfully verify if a sample has been frozen and, to some extent, if it has been subjected to fast or slow freezing rates and they open a possibility for authentication, which could aid in the verification of implementation of the EC regulation.

12 Strategies to reduce the allergens in fish processed products were designed.
Two different processes were selected: a) the surimi and production of kamaboko type gels, and b) conditions used in the canning processing. In surimi, the effects of factors such as removal of antigens by washing, the addition of cryoprotectants, salting, heating, and frozen storage were analysed. In the canning processing different heating regimes used in the canning industry were studied.

The washing operation of fish muscle is one of the key steps in the production of surimi. The aim of this first part of the study was to assess in parasitized minced fish the effect of the washing steps on the allergen removal of Anisakis simplex and on protein yield during surimi processing. The highest removal of Ani s 4 and A. simplex antigens was achieved by using phosphate buffer, together with a good protein yield in the raw surimi. Decrease of the concentration of allergens and antigens as a function of the washing steps rendered a linear trend (R2=0.95 and 0.98 for Ani s 4 and A. simplex antigens respectively). Results suggested that the conditions for an optimal removal of Anisakis allergens can be established and calculated as a function of the washing steps.

Despite the fact that washing of muscle in surimi processing significantly reduced the allergen concentration, raw surimi still have residual antigens and allergens that are carried on in the subsequent steps. In order to know if additional factors related to surimi production could have an effect, the cryoprotectant blends added in the subsequent steps were investigated to check if they modified the antigens and allergen recognition. Surimi is usually commercialized frozen stored and therefore the effect of frozen storage was also assessed. Two cryoprotectant blends were added to each of the four raw surimi described in the previous paragraph: 4% sucrose+4% sorbitol and 4% sucrose+4% sorbitol+0.2% sodium pyrophosphate, thus making a total of eight combinations (four raw surimi x two cryoprotectant blends). Antigen and allergen recognition was analyzed in surimi that had been chilled (5°C), or frozen stored (-20°C) for 90 or 180 days. Ani s 4 and Anisakis simplex antigens were quantified by immunodetection (Dot blot). No significant

differences ($p<0.05$) were found between different surimi blends and the major changes were observed along frozen storage since the detection of Ani s 4 was gradually reduced.

To obtain the gels, surimi is ground with salt and the viscous sol formed turns to an elastic gel upon heating, producing changes in the structure of myofibrillar proteins which form a network where water and other components are entrapped and in these conditions the allergenicity could be potentially affected. Thus, the effect of solubilization in salt and heat treatment was investigated when used on the gel forming process on the antigen and allergen recognition.

The effects of frozen storage in the surimi and in the gels were also evaluated. Results showed that the heat treatment decreased the detection of Ani s 4 but still some allergenicity could be observed by immunohistochemistry. This decrease was higher with surimi gels made from frozen surimi than with gels made from chilled surimi and frozen stored under the same conditions of time and temperature.

The purpose of canning is to kill or inactivate all microbial contaminants, irrespective of their source, by heating and packaging the product in hermetically sealed containers so that it will be protected from recontamination. The aim was to study the effect of different heat treatments used in the fish canning processing industry on the antigen recognition of Anisakis L3. Bigeye tuna (Thunnus obesus) and Yellowfin tuna (Thunnus albacares) were experimentally infected with live L3 Anisakis. After 48 h at 5±1ºC, brine was added to the muscle and then canned raw (live larvae) or heated (90ºC, 30 min) (dead larvae) and treated at 113°C, 60 min or 115°C, 90 min. Anisakis antigens and Ani s 4 were detected with anti-crude extract and anti-Ani s 4 antisera, respectively. Ani s 4 decreased in all lots, but the muscle retained part of the allergenicity irrespective of the canning method as observed by immunohistochemistry. Dot blot analysis showed a high loss of Ani s 4 recognition after canning and therefore heat treatment for sterilization under the conditions studied produces a decrease in Ani s 4.

It can be concluded that washing of muscle can substantially reduce the allergenic capacity of heavily infected fish in a way that can be calculated as a function of the washing steps, so that the conditions for an optimal removal can be designed case by case. On the other hand, the heat treatment used in the manufacture of gels made with surimi reduced the Ani s 4 recognition, and in terms of decrease of allergenicity it was more efficient to use frozen surimi than to freeze the gels. In the whole process, between 97-98% of the Ani s 4 allergens initially detected in the minced fish were eliminated. As regards the heat treatment for sterilization under industrial conditions, a decrease in the antigenicity of Ani s 4 was also observed.

The decrease in the allergenicity after the above conditions opens a possibility of using muscle heavily infected with Anisakis larvae. However, the residual amount of allergens could represent a health risk for those patients previously sensitized to thermally stable A. simplex allergens, and the clinical relevance needs to be established.

13 The TEDEPAD prototype was officially presented to stakeholders at the PARASITE Final Conference. Numerous testing with offal indicated a correct functioning of the TEDEPAD, cycles performed correctly. It is expected that the implementation of the TEDEPAD will have a significant impact on the recruitment of zoonotic parasitic larvae into fish stocks of commercially valuable species.

TEDEPAD (Technological Device for Avoiding Parasite Discarding at Sea) (Fig…) is an industrial automatic prototype that consists basically of three main blocks: Storage Module equipped with level and temperature sensors as well as an internal system of injection of water and air for self-cleaning process; a Reactor Module which consists of a 14 liters cubic tank surrounded by magnetic field generating elements and microwave for heating fish offals; and a Command and Control Module, a cabinet housing all the electronic microprocessor controls, pneumatic control, control panel and electrical protection components. This module is responsible for managing of automatic functioning of the TEDEPAD and to report a diagnosis of the equipment into a real-time monitoring process.

The effectiveness of the TEDEPAD was tested during two shipments planned during May 2014 and June 2015. A great work has been afforded to coordinate the installation of TEDEPAD equipment onboard the vessel Vispón, a typical trawler 35 m long and 8.57 m wide which operates in the Grand Sole fishing area. The purpose of both shipment that comprise a fishing campaign each (23-25 May 2014 / 7-14 September 2015) has been to test onboard the device effectiveness by using fish commercial lots into real-life operations at the fishing area. A member of the IIM-CSIC research group was sent on board to monitor the behavior of the device in different batches and test its effectiveness by using the UV-method.

Numerous testing with irradiated offals indicated a correct functioning of the TEDEPAD, cycles performed correctly, taking up to eight cycles per sampling batch depending on the volume of offals treated. Infected offal was examined and a representative number of anisakids were obtained. No parasite motility was noticed in any of the trialed fish samples. All parasitic larvae showed positive fluorescence when they were analyzed under UV-light. We also tested the performance of the parasitic inactivation process on successive cycles (as for example cycles 1, 3 and 5) for numerous sample batches. No viable anisakid larvae were found in any case.

Overall, estimations considering the information provided by the Spanish fleet operating in the Grand Sole area revealed that currently they discard at sea around 2233 million of viable anisakid larvae annually. It is thus expected that the implementation of the TEDEPAD will have a significant impact on the recruitment of zoonotic parasitic larvae into fish stocks of commercially valuable species. A post-project action should be undertaken to demonstrate in the wild the effectiveness of this pre-harvest contra-epizootic control measure for EU-fish production value chains.

14 General trends in larval anisakid nematode distribution, prevalence and abundance in samples of commercial fish species caught in EU waters were explored. Summary statistics computed for several quantitative descriptors of fish species and parasite population data revealed an overall skewed distribution and high degree of variability in fish lots. GAM models constructed revealed that fish length, sex, year, season and sampling area may affect in different manner the recruitment of anisakids into European fish stocks.
General trends seen in numbers of Anisakis worms per fish and in Anisakis prevalence in host muscle and overall, suggest the following crude classification of host species:

1) Very low values [0–5% prevalence in muscle, maxima of <=10 worms/fish overall] in sea bass Dicentrarchus labrax and sardine Sardina pilchardus;
2) Low values [0–30% prevalence, maxima of 11–50 worms/fish] in anchovy Engraulis encrasicolus, plaice Pleuronectes platessa and chub mackerel Scomber japonicus ;
3) Moderate values [0–70%, 51–200 worms/fish] in herring Clupea harengus, mackerel Scomber scombrus, 4-spot megrim Lepidorhombus boscii, haddock Melanogrammus aeglefinus and whiting Merlangius merlangus;
4) High values [0–95%, 201–1000 worms/fish] in silver scabbard fish Lepidopus caudatus and black-bellied angler Lophius budegassa;
5) Very high values [0–100%, >1000 worms/fish] in cod Gadus morhua, hake Merluccius merluccius, blue whiting Micromesistius poutassou, monkfish Lophius piscatorius.

Larger individuals of most species tend to have more worms, and some of the observed differences may be explained by host body size, although other factors are also important. It was apparent that the mean number of worms observed in host muscle tissue (Am) is generally correlated with the number of worms found in the visceral cavity/organs (Av). A global analysis of mean values for all samples gives Spearmans rs = 0.84 (p<0.001).

A similar, general pattern of anisakid distribution in all the host fish species was observed. There was a predominance of A. simplex (s.s.) in the North Atlantic, and of A. pegreffii in the Mediterranean, with a considerable sympatric overlap occurring between the western Mediterranean and the Bay of Biscay in the North Atlantic. However, mixed infections were also occasionally found as far north as the Norwegian Sea in Atlantic mackerel, a result most likely to be seen in such highly migratory fish. A third Anisakis species, A. physeteris, was found in small numbers in some Mediterranean fish samples.

Anisakis spp. prevalence was modelled for host fish species, areas and time periods represented in the project database (BioBank). Models were developed at the level of individual fish to identify size-, seasonal- and geographic patterns, as well as broader-scale relationships based on aggregated data. Finer resolutions were used when possible. A Generalised Additive Modelling (GAM) framework was used. Presence and counts of Anisakis sp. were selected as response variables. The main effects considered in the models were: 1) host length; 2) host sex; 3) year; 4) season; and 5) sampling area. We used broad areas corresponding to ICES subdivisions in the Atlantic, and separating the main seas (Alboran, Tyrrhenian, Adriatic, Aegean) in the Mediterranean. We also explored the possible influence of fish condition, using an index based on total weight relative to length (Le Cren, 1951).

GAM (Generalized Additive Models) models constructed achieved varying degrees of success in explaining worm (Anisakis spp.) burdens in the different host fish species examined, accounting for between 13.2% and 67.1% of the deviance observed. Host fish length was by far the most important explanatory variable tested, with larger fish generally exhibiting greater numbers of worms. This could be explained by individual fish encountering worms (through their diets) as they grow, becoming increasingly infected throughout their lives as the worms are retained for prolonged periods (as yet undefined) and accumulate over time. Significant relationships were also seen with host condition (Krel), (herring, blue whiting, haddock); fishing area (haddock, plaice, whiting); and year (herring, plaice, whiting).

Overall, the analyses indicated complex spatial and temporal distribution patterns. Parasite populations often exhibit high variability, associated with highly skewed frequency distributions and large numbers of zero values (absence), and our anisakid datasets appear to be typical in this respect.

15 A quantitative risk assessment (QRA) model was developed to determine the probability of disease caused by raw and marinated anchovy meals. This was performed in Spain (the EU country with the highest incidence of anisakiasis), even though it was amended to estimate the total number of annual anisakiasis cases in Italy (“high incidence” country) and Croatia (“low incidence” country). In Spain, the model rendered a probability of anisakiasis to be 8.95x10-5 (i.e. 1 in 11,216) per untreated raw or marinated anchovy meal, and the total number of anisakiasis cases per year was estimated to be between 4,700 and 7,581. The annual number of anisakiasis cases were
demonstrated to decrease by 80% when education campaign to freeze fish was considered, and would increase by >1,000% when parasite migration was considered. In addition, the burden of disease was estimated to be 3,460 and 168 anisakiasis cases per year in Italy and Croatia, respectively. These figures suggested anisakiasis as an underdiagnosed disease in these European countries.

16 It was summarized the evidence regarding consumer attitudes towards the presence of anisakids in seafood and, in particular, their willingness to pay for treatment of fish products for the removal of anisakids (and their related allergens). The new data on consumer preferences was derived from an internet survey of consumers. A survey-based economic technique to measure willingness to pay (WTP) was used which is a methodology that allows the researchers to investigate how individuals value a specific intervention or benefit. Results of the WTP analysis for

Spanish consumers suggested two general kinds of reactions to the presence of Anisakis in fish, namely avoidance of eating fish or a willingness to pay more to avoid adverse effects. In the present study, consumer willingness to pay clearly depends on the perception of the risk associated with Anisakis in fish. The idea that their food might contain parasites, or worms, tends to evoke a strong reaction in consumers, which possibly explains the apparent similarity of responses across several countries, despite the differing incidence of anisakiasis. Indeed a likely outcome of increased awareness of anisakids is a reduction in fish consumption, as was evident from some responses to the questionnaire. Ultimately, public perception of risk has components that could be described as irrational in that they are not entirely predictable from the measureable probability of a hazardous event occurring.

Potential Impact:
Potential impact
From the 50´s last century when the first case of anisakiasis was recorded in Europe the PARASITE project represents the first major attempt to perform a risk assessment on the presence of zoonotic and allergenic parasites in fish products marketed in Europe. The absence for such a long period of a basis for regulatory science has provoked social alarms ("worm hysteria episodes") in several European countries that caused an unjustified major damage to the fish industry and an inefficient public management of the risk. The results obtained in the PARASITE project suggest that health risks are underestimated, the risk management should be improved and the communication/education strategies redesigned based on the risk analysis to become a priority.

Specifically, those findings achieved in the PARASITE project that should be considered because they represent a clear progress beyond the state of the art (EFSA, 2010) are:

Public health concern: PARASITE provides evidences for an enlargement of the exposure to the risk regarding the presence of new and re-emergent zoonotic parasites in fish species marketed in Europe. Additionally, some thermostable allergens of these parasites were detected in the flesh of aquacultured marine fish species, which support major production systems. Furthermore, the above allergens were also detected in some products of the canning industry. Finally, studies on the bacterioma of nematode parasites in fish of commercial interest revealed the presence of human pathogenic bacteria that are transmitted from the viscera to the flesh of fish once the migratory nematode larvae reach the flesh in ante- or post-mortem conditions.

Despite these new findings, with the exception of Spain, there is much uncertainty on the use of adequate specific sensible immunological tests to detect allergies in patients related with the consumption of parasitized seafood. In other words, this is a neglected zoonotic disease for what neither normalized diagnostic methods nor clinical records are available for most of the European National Health Services. Consequently, there is no reliable information on the zoonotic/alergic disease situation related to this hazard at the European level.

Inspection concern: PARASITE provides evidences that according with the current Regulation (EC) No 178/2002 some economically-important fish species (or some fractions of their exploited stocks) sold in European markets should not reach the consumers. Regardless of whether or not any parasitized fish stuff is injurious to health (depending on the condition of the parasites and their metabolites in the fish matrix), under a visual inspection scheme some parasitized fishes from major EU-fish production value chains are unfit for human consumption and thus should not be marketable. Reasons for this weakness in veterinary inspection and self-control process at the industry are various but mostly are due to an ambiguous Regulation that is not really based on the available scientific evidence. According to PARASITE findings the regulated visual inspection scheme is inefficient in predicting epidemiological values for a given exploited stock, commercial batch, lot or consignment. The PARASITE project proposes other improved and normalized inspection methodologies which are much more specific, accurate and sensitive. Furthermore, results provided by these methodologies were fitted into risk assessment models that rates the risk of each product based on the hazard identification, exposure assessment, and hazard and risk characterization. Indeed, for the industry any risk ranking tool could be a best-value for money approach to guarantee healthier fish products.

Industrial concern: PARASITE provides evidences to introduce new management measures that may help stakeholders to better mitigate/control the parasite risk in the food chain, from the net to the plate. These interventions should be holistically planned based on a translational research approach from a pan-european surveillance program in the fish production value chains, but also implementing new different technological devices developed in the PARASITE project, as well as new methodological approaches also described in the PARASITE project to kill the parasites and/or inactivate their allergens in both pre- and post-harvest conditions.

Market concern: PARASITE provides evidences based on willingness-to-pay and cost-benefit analysis to suggest that SMART solutions should be implemented to better understanding the consumers' perception and better communicate the potential risks of a parasitized product.

The Cost-Benefit calculations performed show a significant economic result of potential implementation of tangible results from the PARASITE project. This is said even considering the lowest estimations, of over 24 million euros after 10 years. As mentioned previously, the scenarios were re-defined after the contacts with stakeholders along the value chain, so that they would reflect a more realistic situation of scenarios of implementation of results. The meetings with stakeholders were also used to obtain specific data on the variables used in each scenario, which should increase the precision of the calculations.

The defined scenarios do not cover the implementation of all potential results of the PARASITE project, neither does

each scenario cover the implementation of each result at an European level. We have preferred to use more precise data for the defined scenarios, instead of using gross estimations for larger implementations.

The Cost-Benefit calculations enable explicit and quantitative comparisons of the efficiency of interventions and implementation of the scientific results using a simple-to-interpret summary efficiency measure – cost per impact achieved – as the common outcome measure. This gives policy-makers and other stakeholders a wealth of comparable data on which to base informed decisions, regarding the implementation of results from the PARASITE project.

Dissemination activities
Effectiveness in communicating the risks associated with fish/seafood parasites targeted in the project has been achieved mainly through many actions that have been tailored to keep transparency and enhance the benefit of fish consumption, as well as to explain the measures and possible paths and tools to tackle such risks.
The dissemination activities have been also oriented to reinforce the seafood industry's competitiveness by improving its skills and capacity to use the project results and implement strategies to tackle parasite-related risks. In this regard, Tech transfer support by reinforcing end users' skills has played a key role by training potential users of the project results and encouraging their cooperation with research organizations.
Furthermore, the documents resulting from "Catalogue of technological results" obtained within the project and "Assessment of market potential and Road-mapping the future prospects for technological innovations achieved to reach/impact the market and foresight analysis to identify the after-project action plan: new or remaining RTD and Innovation Challenges and outline of an action plan (roadmap)" constitute a valuable material for raising industry awareness on the solutions available at present and those that could be available in the short and medium term.
Finally, all the tasks undertaken within the communication plan have contributed to spread the knowledge achieved and disseminate results to the different stake holders: scientific bodies, policy-makers at European, national and regional level, to the industry and to consumers and civil society. Specifically, according with the corrective actions suggested by the Commission the Project focused on those activities that may facilitates science-policy interaction. At the final stage of the project accomplishment, and given the sensitivity of the information being managed within some results, direct science-police contacts were established at European level (e.g., EFSA) and also at national (e.g., AECOSAN-Spain) and regional (e.g., Galician Government in Spain) levels. Contacts were facilitated by the project coordinator. As a result, key recommendations were obtained to manage the transmission of information and the organization of the final project conference. Indications on future interaction were also provided.
Accordingly with the Communication Plan, the main dissemination channels have been:
1. Project website (www.parasite-project.eu), launched in month 5 (June 2013), is a basic element to communicate general information about the project, and in general about seafood quality and safety with regard to risks related to zoonotic parasites, to industry, consumers and collaborators, including scientific community. It has been designed as a communication channel and to open opportunities to interact with different targets through its different sections, fed with both static and dynamic contents, being sections Newsroom, Project Ambassador and Useful links and information the most dynamic ones. The project website remained very active during the second period recording up to 4,500 visits in the last 6 months.

The website was also used as a mean for implementation of the "Food safety in fishery products" questionnaire, that was designed for making a survey in five countries (UK, ES, DK, HR and IT) to gather data for feeding work developed under work-package 8.

2. Social networks: LinkedIn and Twitter. LinkedIn is the largest online professional social network, with a high relevance in the scientific field. From July 2013, the PARASITE project had its own professional profile in this network, which was turned into a group in March 2014 (M14) to improve its performance (https://www.linkedin.com/groups/PARASITE-PROJECT-7478115/about). The group involves a total of 144 contacts. On the other hand, the PARASITE project has also its own account on Twitter, a social network with a high involvement from scientific institutions. Currently, it has 142 followers, it is following 351 organizations and institutions and nearly 1,000 tweets have been published. This account has been updated daily and fed with relevant news from third organizations as well as with own news and events during the project duration.

3. Project brochures: Informative leaflets were prepared by International Innovation as an effective dissemination material to be distributed at the PARASITE Final Conference and beyond. These brochures offer in a very visual way an overview of the context and the project aims, as well as an outline of its development and main achievements. Contact details and participant organizations are also included. In addition, several activities have been developed in cooperation with International Innovation, a leading scientific dissemination magazine that offers a unique forum for communication and dissemination of research. It is addressed to key target groups such as major scientific and industry associations, research institutes, national research councils, university and academic agencies, European Parliament, NGOs and university libraries. This cooperation included the following actions:
- Publication of a Project Profile. In July 2015 (month 30), a PARASITE project profile was included in International Innovation (issue no. 187) magazine. This number presented insights into how some research activities shape the food-to-fork supply chain and cultivate better practices. The PARASITE project, which ultimate aim is to make European seafood safer, was included as one of those particular initiatives.

- Publication of a Project Report and Interview. A two-page report on PARASITE Project was included in the last number of International Innovation, soon to be published (currently in press). Under the title of "The Science behind safer food", it includes a thorough description of the project and an interview to the coordinator, Santiago Pascual.

- Edition and printing of project brochures. Informative brochures that were prepared by International Innovation as an effective dissemination material and were distributed at the PARASITE Final Conference.

4. Training and learning material: During the whole project period, four 3D animations and three videos have been produced as training and learning materials. All of them hare available in the project website, under the Expected outcomes section (http://parasite-project.eu/outcomes) and they have been also widely shared through social networks. These materials have been very well valuated by all partners and general public. Indeed, Didactic materials of training workshops offers a comprehensive explanation of all the learning materials produced.
5. Press releases: many press releases have been produced to announce relevant events or progress achieved by the project so far.
6. Interviews: PARASITE partners have been interviewed TV and radio stations.
7. Scientific and Technical writing: several articles have been published in scientific journals and presentations were made in symposiums, conferences and workshops. Additional scientific papers are currently under preparation, including a special issue proposal hosted by Fisheries Research journal to compile the parasite surveillance plan and results achieved along the project.
8. PARASITE Final Conference: It was held on Thursday 26th November 2015, hosted by the Ship-Owners' Cooperative of the Port of Vigo – ARVI (ES). This event was initially planned as an "International Symposium on Seafood Parasites' Management Strategies". However, PARASITE partners have actively contributed to the 9th International Symposium on Fish Parasites (9ISFP) held in Valencia (Spain) on 31st August - 4th September 2015. Project researchers were involved in Organizing and Scientific Committees and as Session Chairs. They also gave four oral presentations and two posters, partially advancing the results obtained so far within the project. Furthermore, some consortium members have also participated in the 17th EAFP (European Association of Fish Pathologists) International Conference On Diseases Of Fish And Shellfish, held in Las Palmas de Gran Canaria (Spain) on 7-10th September 2015. Therefore, and taking into account that the PARASITE results can be of great interest beyond the scientific community usually attending to this kind of meetings, it was agreed to address a broader target public and reshape the event format towards a more attractive one, especially for industry.
The agenda was extensively discussed and agrees among all partners, in order to include all the relevant contents and representatives from partner and other relevant organizations. Likewise, the target public, mainly fishing industry, inspection bodies and health and fisheries authorities, was carefully selected. Dissemination prior to the event was direct and personalized and a thorough follow-up guaranteed the attendance of most relevant stakeholders. The Conference gathered more than 80 people and counted on the presence and support of the Minister of Sea Affairs from the Galician Regional Government.
The Conference, under the title of Progress on the Assessment and Management of Parasites in Fishery and Aquaculture Products, was aimed at presenting the main results obtained within the project during its almost three years of life. It was divided into three sessions, addressing different issues and targeting diverse audiences:

• The first session presented two outstanding European projects currently dealing with zoonotic parasites in fish products. On the one hand, Santiago Pascual, as project coordinator, presented the main objectives pursued by the PARASITE project and how far these have been achieved. On the other hand, Aridna Sitjà-Bobadilla, coordinator of PARAFISHCONTROL, presented this new research project funded under H2020 programme, which intends to provide solutions for preventing and mitigating parasitic diseases of European farmed fish. To lead the first session to an end, Patrizia Rossi, from the European Reference Lab for Parasites focused her presentation on progress towards the development of an ISO standard for the detection of anisakids in fish.
• Session 2, was organized as a showroom on the new developments and technologies for the detection, identification and elimination of parasites in fishery products. Particularly, five technologies developed or improved within the PARASITE project were presented in an interactive way to an audience that was encouraged to directly contact and exchange impressions with each technology developer. Results included UV- and biomarker proteins-based detection technologies; a special gadget for assessing the viability of nematode larvae; methods for inactivating and reducing Anisakis antigens in seafood and an on-board sanitation machine, which inactivates fish offals' larvae before being re-introduced in the sea. Some of the presented devices and technologies were displayed in the venue and could be handled by attendees.
• Session 3 was aimed at presenting the progress achieved within the PARASITE project on different research gaps regarding nematode parasites. In particular, it was shown the potential for developing an epidemiological monitoring programme in major European fishing grounds, taking into account the experience gained within the project. Moreover, achievements on molecular identification and distribution of anisakids, on monitoring programmes through biobanking solutions, on genomics and transcriptomics, on parasite antigens characterization and on their relevance for improving diagnostics and enhanced interventions in the fish and seafood chains to mitigate risks posed by zoonotic parasites were shared with the audience. The conference reached its end with the presentation of the main results obtained within the risk assessment workpackage.

9. Other events. PARASITE project has participated and/or promoted several events mainly addressed to industry and to general public:
• Participation in the "Best-Practice" Call – ExpoMilano 2015 (IT) in October 2014 (M21). CETMAR and UT-URS;
• Participation in the "Restitution aux profesionnels des resultats de 3 projets traitant de la securité sanitaire", Bologne sur Mer (FR), October 2014 (M21). ANSES;
• Meeting with local fishermen for sharing project results, Hamburg (DE) in May 2015 (M28). FAMRI;
• Visits from the producing fish industry to Havstovan facilities (Faroe Islands) in July 2015 (M30). FAMRI;
• Presentation of the project to France Filière Pêche working group on parasites, París (FR) in November 2015 (M34). ANSES;

• Participation in the Science Week Event, Madrid (ES) in November 2015 (M34). ICTAN-CSIC.

Likewise, most published references can be checked at the News section of the project website: http://parasite-project.eu/newsroom/project_news.

External project co-operation
The PARASITE project established collaboration with the following EU-funded projects:

1. DRAGON-STAR: it is a FP7 project to identify the benefits as well as the obstacles and barriers derived from the participation of Chinese partners in EU-projects. Our collaboration was in 2 levels: enhancing the Chinese participation in the Framework Programs; and supporting the ongoing bilateral Scientific Cooperation dialogue by signing in 2015 a long-term technological and research cooperation agreement between the coordinator partner (CSIC) and the Chinese partner (Zeijhan Ocean University of China).

2. COFASP: it is an ERA-NET created under the KBBE theme in FP7, and is part of the EU 2020 strategy, which recognises bioeconomy as an important part of the strategy. The collaboration with PARASITE focused on the participation of project occordinator in ceveral questionaries addressing the scienctific information and data available from PARASITE and how this can be succesfuly implemented by outlining monitoring and information/data sharing systems.

3. ProBio: it is a H2020 Support Action that offers exclusive custom tools and tutorials to coordinators and project partners of FP7 KBBE, for the exploitation of project results. Multidisciplinary team built around specialised innovation consultancies and a EU expert group, thematic expertise ensured by tech centers and a media agency specialised in European innovation. A coaching plan for market uptake of three technical developments in PARASITE (Scanisakis, TEDEPAD and Biobank) is being carried out.

4. PARAFISHCONTROL: it is a H2020 Research and Innovation Action with the overarching goal to increase the sustainability and competitiveness of the European aquaculture industry by improving our understanding of fish-parasite interactions and by developing innovative solutions and tools for the prevention, control and mitigation of the most harmful parasitic species affecting the main European farmed fish species. Within the aforementioned framework, our collaboration was established to incorpore to the PARAFISH Consortium the background and skills of some PARASITE partners dealing with normalized tools and methods already developed in PARASITE (e.g., Biobank platform, detection/diagnostic methods) to study risk factors associated with the potential presence of zoonotic/allergenic parasites in EU farmed fish species.
Internal project co-operation
Along the second reporting period, the PARASITE project continues to work with the European Reference Laboratory for Parasites (Istituto Superiore di Sanita, Rome, Italy) on a fruitful collaboration initiated in 2013 consisting in active participations of the project coordinator and WP leaders in Annual Workshops of NRLs hosted by the ERLP.

List of Websites:
http://parasite-project.eu
Coordinator:
Dr. Santiago Pascual
IIM (Instituto de Investigaciones Marinas), CSIC (Consejo Superior de Investigaciones Científicas)
Address: C/ Eduardo Cabello, S/N, 36208 Vigo, Pontevedra, Spain
Telephone: +34-986-231930

Contact

SERENO ALVAREZ, ALBERTO (FINANCIAL OFFICER)
Tel.: +34915668852
Fax: +34 91 566 89 13
E-mail

Subjects

Scientific Research

Last updated on 2016-09-16
Retrieved on 2016-10-14

Permalink: http://cordis.europa.eu/result/rcn/189395_en.html
© European Union, 2016