Successful Marie Curie Research Proposal Example

Technical Report · July 2015

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Spanish National Research Council
39 PUBLICATIONS **335** CITATIONS

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Successful Marie Curie Research Proposal Example

After achieving a Ph. D. degree, a highly competitive race starts for young scientists with the ultimate goal of getting a position in academia or industry. This transition to principal investigator or equivalent position is essential for further career development, as allow personal stability and the possibility to develop own ideas. During this period, researchers usually are engaged in, hopefully not to many, post-docs. Post-docs enlarge the researches laboratory, management, and teaching skills, and allow them to build a publication record and international reputation. Probably most important, these periods often serve for researchers to draft a long-term strategy in science.

While today this is relatively less important in US (i.e., transit through many different post-docs before a position), it is still a critical issue in Europe. The European Commission offers different possibilities for post-docs but they are highly competitive. It is not enough to submit a proposal of good science, but also to state it clearly by taking into account the priorities of the European Commission, and more difficult, by foreseeing the thinking philosophy of the evaluators.

Here I'm uploading my winning Marie Curie IIF application for helping young scientists worldwide, especially those from the biological sciences, in preparing their applications to European Union research calls. I hope they find this document useful and be smart enough to take advice of my good movements and avoid my pitfalls.

I share this project proposal just as an example, and applicants must be aware of the very complete and detailed Guide for Applicants that the European Commission provides, containing all the essential information for guiding the researchers through the processes of preparing and submitting a proposal.

Good luck!

Proposal Submission Form

EUROPEAN COMMISSION
7th Framework Programme on Research, Technological Development and Demonstration

Marie Curie Actions
International Incoming Fellowships (IIF)

A1: Summary

Proposal Number	000000	Proposal Acronym	FishPROG

General Information on the Proposal

Proposal Title	Breaking frontiers for the use of plant-derived feeds in fish farming through nutritional programming of fish larvae
Marie Curie action-code	International Incoming Fellowships (IIF)
Scientific Panel	Environment and Geosciences (ENV)
Duration in months	24
Call identifier	FP7-PEOPLE-2012-IIF
Keywords (up to 200 characters)	nutritional programming, plant-based feed, fish physiology, fish welfare, V402 Aquaculture - fisheries,V404 Food sciences

Abstract (up to 2000 characters)

Sustainable expansion of European fish farming is dependent on feeds relying less on fishmeal. European researches and industry initiatives over the last decade have resulted in a significantly decrease in the share of fishmeal in fish feeds, but current replacement rates seem to be close to the physiological capacity of the different species. This project will use one of the most important cultured fish in Europe (Sparus aurata) as animal model, to look for a novel strategy for increasing the use of soybean meal in aquafeeds, addressing a key question in today nutrition research: can early diet influences long-term outcome? Hence, the main objective is to assess if early manipulations in fish larva can encourage a better use of plant proteins in later life. This issue (nutritional programming, in its wider sense) has been poorly studied in animals other than humans and mammalian models. The project will focus at four different levels: epigenetic programming, compensatory responses, microbiota changes, and growth outcome produced by early exposure to plant proteins and pro/prebiotics. The multidisciplinary nature of the project is strong, involving a combination of well developed biochemistry, molecular biology, microbiology and aquaculture. This proposal includes both the transfer of knowledge to the host institution and the training of the candidate in new advanced techniques. Results have the potential capacity to increase the competitiveness of the Mediterranean aquaculture and provide room for further studies at the fundamental and applied levels in S. aurata, and other European cultured fishes. This project is in line with the EU strategy for the sustainable development of aquaculture.

Has a similar proposal been submitted to a Marie Curie Action under this Framework Programme? no

IF YES

Programme name(s) and year	Proposal number(s)
-	-
-	-
-	-

Does this proposal include any of the sensitive ethical issues detailed in the Research Ethical Issues table of Part B? no

Proposal Submission Form

EUROPEAN COMMISSION
7th Framework Programme on Research, Technological Development and Demonstration

Marie Curie Actions
International Incoming Fellowships (IIF)

A2: Participants

Proposal Number	000000	Proposal Acronym	FishPROG	Participant Number	1

INFORMATION ON ORGANISATIONS

If your organisation has already registered for FP7, enter your Participant Identification Code: -

Legal name: CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS

Organisation short name: CSIC - ICMAN

Administrative Data

Legal address

Street name	Calle Serrano	Number	117
Town	MADRID	Postal Code/Cedex	28006
Country	ES		
Internet homepage	www.csic.es		

Status of your Organisation

Certain types of organisations benefit from special conditions under the FP7 participation rules.
The Commission also collects data for statistical purposes.

The guidance notes will help you complete this section.

Please 'tick' the relevant box(es) if your organisation falls into one or more of the following categories

Non-profit organisation	yes
Public body	yes
Research organisation	yes
Higher or secondary education establishment	no
International organisation	no
International organisation of European Interest	no
Joint Research Center of the European Commission	no
Entities composed of one or more legal entities [European Economic Interest Group (Unité mixte de recherche) / Enterprise groupings]	no
Commercial Enterprise	no

Main area of activity (NACE code)

73.1

Proposal Submission Form

EUROPEAN COMMISSION
7th Framework Programme on Research, Technological Development and Demonstration

Marie Curie Actions
International Incoming Fellowships (IIF)

A2: Participants

1. Is your number of employees smaller than 250? (full time equivalent)	no
2. Is your annual turnover smaller than € 50 million?	no
3. Is your annual balance sheet total smaller than € 43 million?	no
4. Are you an autonomous legal entity?	no

You are NOT an SME if your answer to question 1 is "NO"
and/or your answer to both questions 2 and 3 is "NO".
In all other cases, you might conform to the Commission's definition of an SME.

Following this check, do you conform to the Commission's definition of an SME: no

CONTACT POINT OF THE HOST ORGANISATION

Person in charge (For the co-ordinator (participant number 1) this person is the one who the Commission will contact in the first instance)

Family name	Yúfera	First name(s)	Manuel
Title	Dr.	Sex	Male
Position in the organisation	Vice-Director, Scientific Researcher		
Department/Faculty/Institute/Laboratory name/...	Instituto de Ciencias Marinas de Andalicía, CSIC - Departamento de Biología Marina y Acuicultura		
Is the address different from the legal address?	yes		
Street name	Av.Republica Saharaui	Number	2
Town	Puerto Real, Cadiz	Postal Code/Cedex	11510
Country	ES	Phone 1	+34-956832612
Phone 2	+34 956 832612 ext.334	Fax	+34 956 834701
E-mail	manuel.yufera@icman.csic.es		

Proposal Submission Form

EUROPEAN COMMISSION
7th Framework Programme on Research, Technological Development and Demonstration

Marie Curie Actions
International Incoming Fellowships (IIF)

A2: Participants

Authorised representative to sign the grant agreement or to commit the organisation for this proposal

Family name	Sarasquete	First name(s)	Carmen
Title	Dr.	Sex	Female
Position in the organisation	Director		
Department/Faculty/Institute/Laboratory name/...	Instituto de Ciencias Marinas de Andalucia, CSIC		
Is the address different from the legal address?	yes		
Street name	Av.Republica Saharaui	Number	2
Town	Puerto Real, Cadiz	Postal Code/Cedex	11510
Country	ES	Phone 1	+34-956832612
Phone 2	+34 956832612 (ext 40)	Fax	+34-956834701
E-mail	director.icman@csic.es		

Proposal Submission Form

EUROPEAN COMMISSION
7th Framework Programme on Research, Technological Development and Demonstration

Marie Curie Actions
International Incoming Fellowships (IIF)

A3: Participants

Proposal Number	000000	Proposal Acronym	FishPROG

INFORMATION ON THE RESEARCHER

Family Name	Perera		
Birth Family Name	Erick Perera Bravet		
First Name(s)	Erick		
Title	Dr.	Sex	Male
1st nationality	CU	2nd nationality	-
Location of origin (country)	CU	Date of birth (DD/MM/YYYY)	17/10/1976
Location of origin (town)	Havana		

Contact address

Street Name	DStrampes	Number	168
Town	Havana	Postal Code/Cedex	10500
Country	CU	Phone 1	537 6404833
Phone 2	537 8318490	Fax	-
e-mail	erickpbcim@yahoo.es		

Qualifications

University Degree	Date of award (DD/MM/YYYY)	10/07/2000
Doctorate (in progress)	Expected date of award (DD/MM/YYYY)	-
Doctorate	Date of award (DD/MM/YYYY)	11/06/2012
Full time postgraduate research experience	Number of months	144
Other Academic qualifications	Date of award (DD/MM/YYYY)	-

Place of activity/place of residence (previous 5 years)

Period : From (DD/MM/YYYY)	Period : To (DD/MM/YYYY)	Country
16/08/2007	16/08/2012	Cuba
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-
-	-	-

Proposal Submission Form

EUROPEAN COMMISSION
7th Framework Programme on Research, Technological Development and Demonstration

Marie Curie Actions
International Incoming Fellowships (IIF)

A3: Participants

INVOLVEMENT OF THE RESEARCHER IN OTHER MARIE CURIE PROPOSALS

Have you submitted or are you in the process of submitting another proposal for the Marie Curie Actions: IEF, IOF, IIF or CIG, or have you previously benefited of Community funding under Marie Curie actions ?

no

If Yes:

Action name(s) and year	Proposal or contract number(s)
-	-
-	-
-	-
-	-

Proposal Submission Form

EUROPEAN COMMISSION
7th Framework Programme on Research, Technological Development and Demonstration

Marie Curie Actions
International Incoming Fellowships (IIF)

A4: Budget

Proposal Number	000000	Proposal Acronym	FishPROG

FUNDING REQUEST

Year	Main Phase		Return Phase (IIF and IOF only)	
	Full-time person-months	Type B Fixed-amount Fellowship (Y/N)	Full-time person-months	Type B Fixed-amount Fellowship (Y/N)
2013	9	no	0	no
2014	12	no	0	no
2015	3	no	0	no
Total	24		0	

Mobility allowance

Are you eligible for the family-related mobility allowance?	yes

Post-graduate Research Experience of the applicant at the deadline of the call	> 10 years

STARTPAGE

PEOPLE
MARIE CURIE ACTIONS

International Incoming Fellowships (IIF)

Call: FP7-PEOPLE-2012-IIF

PART B

“FishPROG”

Table of Contents

B 1. RESEARCH AND TECHNOLOGICAL QUALITY

B 1.1. Research and technological quality, including any interdisciplinary and multidisciplinary aspects of the proposal

World aquaculture, especially for carnivorous species, relies on fish meal as main protein source in feed. Since current world production of fish meal is not expected to increase, expansion of aquaculture production will require lowering the inclusion rate of fish meal in aquafeeds, otherwise resulting in a shortage of marine raw materials. Also, fish meal has become extremely expensive as aquaculture production has grown, impacting the competitiveness of the industry. There are also serious concerns on the detrimental effect of fish meal use on environment, since the preservation of the quality of aquatic environment is of primary importance in the European Union. European researches and industry initiatives over the last decade have resulted in a significantly decrease in the share of fishmeal in fish feeds, but main fish species farmed in Europe are still largely fed fishmeal. This project will use one of the most important cultured fish in Europe (*Sparus aurata*) as animal model, to look for innovative strategies for increasing the use of plant meals in aquafeeds without impacting fish performance and wellbeing, by applying the novel concept of nutritional programming in fish larvae.

Impressive advances have been obtained during the last two decades in the nutrition of this fish species, and recent results prospect the increase of protein digestion by taking advance of digestive rhythms (Montoya *et al*., 2010) or pH in the gastrointestinal tract (Márquez *et al*., 2011; Nikolopoulou *et al*., 2011). However, current replacement rates of fish meal in diet by plant proteins seem to be close to the physiological capacity of this and other species. In Atlantic salmon (*Salmo salar*), the normal morphology of the distal intestine is disrupted by soybean diets (van den Ingh *et al*., 1996). These changes have also been observed in *S. aurata* (Santigosa *et al*., 2008) along with modification of nutrient absorption (Santigosa *et al*., 2011), and fat deposition in the liver (Robaina *et al*., 1995). It seems that *S. aurata* decreases the level of brush border membrane enzymes (i.e. phosphatase) during plant diets feeding (Silva *et al*., 2010), while lipoprotein lipase is up-regulated (Saera-Vila *et al*., 2005), but advancing to higher rates of fish meal replacement will require a deeper understanding of its digestive plasticity.

The present proposal relates with a novel approach in fish nutrition by addressing a key question in today nutrition research: can early diet influences long-term outcome? In the case of this present proposal the question is re-formulated as: can carnivorous fishes, well adapted to high content of animal proteins in diet, be programming by nutritional/environmental manipulation during early development to better use vegetal proteins in later life? Cultured fishes currently rely on live preys as food during early development (i.e. rotifers, *Artemia*) and this early feeding regime may limit their capacity to deal with plant protein sources in later stages. However, current advances in early weaning from co-feeding strategies allow nutritional manipulation in *S. aurata* during the first month of life, when several gastrointestinal regulatory mechanisms continue to develop (Zambonino-Infante and Cahu, 2001; Kamaci *et al*., 2010; Micale *et al*., 2010).

Epigenetic programming: There is now substantial evidence that the early post-natal environment plays a key role in determining (programming) changes in metabolism and physiology that impact performance in later life, involving epigenetic processes (Bringhenti *et al*., 2011; Lillycrop and Burdge, 2012; Plagemann *et al*., 2012). Epigenetic regulation can be defined as ‘‘the mechanisms of temporal and spatial control of gene activity describing pathways different from those directly attributable to the underlying DNA sequence and with an influence on the adaptive response of an organism’’ (Allis *et al*., 2007). The ‘‘epigenetic code’’ encompass the chromatin information mainly encrypted by histone signatures and DNA methylation profiles (Milagro *et al*., 2010). Investigations on the effects of early nutrition on covalent modifications of DNA and core histones point to the existence of epigenetic processes that are sensitive to nutritional regulation in early life (Waterland *et al*., 2008; Thaler *et al*., 2009). Although most evidence of epigenetic regulation has been obtained in humans or mammalian model organisms, there is a growing body of experimental evidence of programming obtained in other groups. However, only few studies have provided some evidence of this

phenomenon in fishes (Geurden *et al*., 2007; Vagner *et al*., 2007). In the present project, fish larvae will be challenged with soybean and sunflower based microdiets and some of their epigenetic markers will be compared to those of larvae reared exclusively on live food.

Hormetic response: Programming is highly dependent but not restricted to epigenetic processes, although other mechanisms are largely unknown. In general, the terms "hormesis" refers to a phenomenon in which adaptive responses to low doses of otherwise harmful conditions improve the functional ability of cells and organisms (Vaiserman, 2011). Then, we also will study different protein markers to assess hormetic responses can occur in fish larvae after nutritional challenge with low 'conditioning' doses of soybean and sunflower proteins, with potential impact on later digestion skills.

Intestinal microbiota: Studies of intestinal microbiota in humans and vertebrate model organisms have revealed that intestinal microbial communities are functionally essential in providing nourishment, regulating epithelial development/renewal and influence innate immunity (Eckburg *et al*., 2005). Microbial cells and their products can influence immune system of fishes (Kanther and Rawls, 2010) and its role in maintaining intestinal integrity is of outmost importance to maintain health and welfare of the fish. Disruption of this first barrier leads antigens to penetrate into deeper layers evoking immune responses and inflammation (Niklasson *et al*., 2011). Also, the microbiota can degrade a variety of dietary substances that are otherwise non-digestible or even deleterious to the host. Some recent studies have addressed the effects of feeding on fish intestinal microflora of fishes (Merrifield *et al*., 2010; Dimitroglou *et al*., 2010).

Within the present project, we will investigate the diversity of the intestinal microbiota in developing fishes. A recent study has shown that turnover of bacterial taxa within fishes (i.e. zebrafish) intestines is low (Yan *et al*., 2012), thus it is possible to speculate that if a beneficial non-pathogenic microorganism is present in water/food at the appropriate window, it would remains in the fish intestine. However, since fish intestine colonization seems to follow a deterministic pattern (Kanther and Rawls, 2010; Yan *et al*., 2012), the permanence of target microorganism (*Saccharomyces cerevisiae*) in the intestine will be followed through time. In some mammalian models, *S. cerevisiae* variety *boulardii* (Sb) has shown to maintain the epithelial integrity (Czerucka *et al*., 2000) while in others studies Sb inhibited the expression of pro-inflammatory transcripts (Zanello *et al*., 2011). Also, mannan oligosaccharides (MOS) supplementation is known to produce positive effects in the intestine of *S. aurata* (Dimitroglou *et al*., 2010) thus it will be also included as dietary treatment. Mannan oligosaccharides [Bio-Mos, Alltech®] are derived from the cell wall of *S. cerevisiae*. The value of pro- and pre-biotic stimulation during early 'conditioning' of fishes to plant proteins has been never evaluated. Another aspect of fish-microbial interaction that will be addressed is the relation between microbial proteases and the proteolytic degradation of the extracellular matrix in fish intestine, which in higher vertebrates is known to be part of mucosal homeostasis, but also may contributes to intestinal inflammation (Pruteanu *et al*., 2011).

Objectives: The **OVERALL OBJECTIVE** of the proposal is to assess if early nutritional/environmental manipulations in fish larvae can encourage a higher use of plant proteins diets in further developmental stages. To reach this overall objective, the project will focus at four different levels (**specific objectives**):

1) Epigenetic programming due to dietary manipulation of larvae and its relationship with changes in digestion physiology and metabolism in later stages. We will use DNA methylation and histone acetylation as indicators of programming and results will be correlated with:

2) Adaptational/compensatory responses, mainly by studying the expression and/or activity of target molecules (digestive enzymes, intestinal transporters, metabolic enzymes, growth hormone and cytokines).

3) Microbiota changes induced by early feeding with plant proteins or by early pro/prebiotic stimulation. Microbial richness will be studied by partial 16S rRNA sequence analysis. Results will be related with growth and histological assessments of the remodeling/health of intestinal mucosa in later stages.

4) Growth outcome of fishes subjected to different dietary/environmental manipulation during early feeding.

The multidisciplinary nature of the project is strong, involving a combination of well developed biochemistry, molecular biology, microbiology and aquaculture.

Expected results: According to the vast literature on human/mammalian models and the growing evidences in other animal groups including fishes, it is expected to find positive effects of early dietary/environmental manipulation on the capacity of target fish to use dietary plant proteins in later life. Also, it is expected to discover the level(s) at which these effects are exerted to provide room for further studies at the fundamental and applied levels in this, and other European cultured fishes.

B 1.2. Appropriateness of research methodology and approach

This project will use a hypothesis driven approach (studying a handful subset of key biomarkers) directed to address the specific research question outlined above. The methods and techniques (see below) to be used are all well established and widely used, so they are just briefly described with reference to relevant papers for more details. The originality of the project relies on the use of these techniques to study a novel issue of fish physiology, thus the success of the project depends on the experimental design. This research is designed in a way that many samples (for accomplishing different tasks) can be taken from single experiments. This allows direct comparisons among very different set of data (i.e. biochemical, molecular, etc) as corresponding to the same experimental conditions (diet, time, etc) facilitating the integration of results. Also, this kind of design is in line with the time frame of Marie Curie actions of two years.

S. aurata larvae will be randomly sorted to different experimental groups with appropriate replication. One group will be fed with rotifers and *Artemia* as a 'control' feeding regime. Other groups will be subjected to different manipulations: plant (soybean and sunflower) based microdiets ('high dose'), rotational diet with rotifers/*Artemia* and plant based microdiets ('low dose'), plant based microdiets plus *S. cerevisiae* ('probiotic treatment'), plant based microdiets plus MOS ('prebiotic treatment'). Samples (entire larvae) will be taken during the first month of life taking into account important windows in development, for analysis of DNA methylation, histone acetylation, intestinal microbiota diversity, digestive and metabolic enzymes activities, expression rate of target genes, and histology. While analyzing those samples, the fish remaining in the different groups will be cultured on a diet of high soybean and sunflower content. The growth of these experimental groups will be compared, as well as indicators of digestion, metabolism, microflora, and intestinal integrity at the end of the experiment. The analytical techniques to be used are:

a) DNA methylation (Methylcytosine staining): For methylcytosine immunostaining, anti-methylcytosine antibody (from abcam®) will be used in accordance with manufacturer instructions, and standard protocols for treating paraffin-embedded specimens with this method as describe before (Matthews *et al*., 2011).

b) Histone acetylation (Immunoblot analysis): Total protein of fish larvae will be extracted and analyzed for acetylated histone (H3 and H4) as described before (Zhou *et al*., 2011). Briefly, electrophoresis will be performed and then proteins will be transferred to a polyvinylidene difluoride membrane (Bio-Rad). Primary antibodies including rabbit anti-acetylated histone H3, rabbit anti-acetylated histone H4, rabbit anti-histone H3, rabbit anti-histone H4 (Upstate Biotechnology/Millipore™) and rabbit anti-human β-actin (Sigma®) will be used according to the manufacturers' instructions. Later, the membranes will be incubated with goat anti-rabbit immunoglobulin G (IgG) conjugated with horseradish peroxidase (HRP) and protein antigens will be detected using SuperSignal West Pico chemiluminescent substrate (Thermo Scientific) and exposure to HyBlot CL film (Denville). The relative intensity of acetylated histones H3 and H4 will be compared with that of total histones H3 and H4, respectively.

c) Intestinal microbiota: We will investigate the diversity of the intestinal microbiota in developing fish subjected to different dietary manipulations, using 16S rRNA sequence analysis and Denaturing Gradient

Gel Electrophoresis, as detailed before (Romero and Navarrete, 2006; Ringø *et al*., 2008; Yan *et al*., 2012). This method is culture-independent and have been recognized as well suited for microbiota diversity studies in fishes.

d) Proteases in the fish intestine: Metallo-protease and serine-protease (trypsin and chymotrypsin) activities in fishes subjected to different early feeding regimens will be study by means of SensoLyte® fluorimetric MMP assay kit (AnaSpec) for matrix metalloproteases, and BApNA and SApNA for trypsin and chymotrypsin, respectively. Because of the possible origins of metalloproteinases from fish or microbiota, composition of metalloproteinases will be examined by zymography using gelatin as the substrate. Zymography is a widely used technique to study extracellular matrix-degrading enzymes and allows the identification of different enzymes according to their electrophoretic mobility. Measurements will be done by means of kinetic enzyme assays and electrophoresis as detailed in previous candidate papers (Perera *et al*., 2008; Perera *et al*., 2012a).

e) Expression rate of target genes: Expression will be assessed by qPCR and absolute quantification of digestive enzymes (trypsin, chymotrypsin, elastase, lipase, and amylase), an enzyme involved in mucosa remodeling (matrix metallopeptidase), an intestinal transporter (alkaline phosphatase), growth hormone and growth hormone receptor, prolactin, and two pro-inflammatory cytokines: interleukin 1-beta and Tumor Necrosis Factor-α. Sequences for all these target proteins are available in the GenBank database, and the qPCR procedures will be as detailed in previous candidate papers (Perera *et al*., 2010; Perera *et al*., 2012b).

f) Metabolism measurements: Enzyme assays will be performed for enzymes involved in energy metabolism (carbohydrates metabolism: fructose 1,6-bisphosphatase, glucose 6-phosphate dehydrogenase, glycogen phosphorylase, hexokinase, lactate dehydrogenase), protein metabolism (serine dehydratase, alanine-aminotransferase and glutamate dehydrogenase), lipid metabolism (carboxyl esterase, lipase, acetyl CoA carboxylase, fatty acid synthase, 3-hydroxy-3-methylglutaryl-CoA reductase, β-oxidation activity) as routinely made in *S. aurata* (i.e. Laiz-Carrión *et al*., 2005)

g) Histology: Entire larvae and juvenile intestines will be fixed in 10% phosphate-buffered formalin, dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin according to standard histological techniques. Sections of approximately 5 μm will be obtained and stained with haematoxylin and eosin before examination under a light microscope. Intestinal morphology will be evaluated according to the following criteria: (*1*) widening and shortening of the intestinal folds, (*2*) loss of the supranuclear vacuolization in enterocytes, (*3*) widening of the central lamina propria within the intestinal folds, with increased amounts of connective tissue, and (*4*) infiltration of a mixed leukocyte population in the lamina propria and submucosa. These are the characteristics of the condition previously described as soybean meal-induced enteritis in Atlantic salmon and other carnivorous fishes.

B 1.3. Originality and Innovative nature of the project and relationship to the 'state of the art' of research in the field

The main goal of this proposal, to find new avenues for increasing the use of vegetal protein sources in aquafeed, is in line with current Pan-European collaborative efforts. However, it covers an issue that has been poorly studied in animals other than humans and mammalian models. To study the possibility for increasing the use of plant proteins in diet in adult life by programming fish larvae during early manipulation is a novel approach to an old need of the world aquaculture industry. While years of research have provided lot of information on the nutrition and physiology of *S. aurata* and other fishes, results are expected to increase the understanding of the capacity of *S. aurata* to cope with plant proteins in diet, by exploring for the first time the capacity for physiological adjustment (reversible or irreversible plasticity) during early life. This research will use state-of-the-art knowledge and state-of-the-art technology to develop an innovative approach in the search of strategies for increasing the replacement of fish meal in aquafeeds.

B 1.4. Timeliness and relevance of the project

The EU has supported successful researches on the replacement of fish meal in aquafeed (e.g. RAFOA, PEPPA, AQUAMAX). However, since some previous results are inconsistent regarding fishes performance, there is a current Pan-European project (ARRAINA) focused in optimizing the use of vegetal protein sources in aquafeeds by identify and validate "omic" biomarkers to be used as integrative tools for predict growth, metabolic and health effects of dietary manipulation. It is not surprising that with the advent of post genomic research and systems biology approach, fish-related problems are now being assessed using top-down or data-driven approaches. This ongoing study is expected to increase the overall performance of fishes during vegetal diet feeding. However, it is likely that large jumps in the substitution rates cannot be achieved without impacting fish physiology and wellbeing, thus it is convenient to develop innovative approaches for advancing to higher level of fish meal substitution, as proposed in this project. This project is in line with the EU strategy for the sustainable development of European aquaculture.

Due to the species-specific (*S. aurata*) focus of this proposal, results have the potential capacity to increase the competitiveness of the Mediterranean aquaculture industry. Also, results from the present proposal could be integrated with those of ongoing studies (i.e. "omic" approaches) on main cultured fish in Europe, to establish a wider platform for the scientific assessment of solutions for the world aquaculture need to overcome current limitations in the replacement rate of fish meal in aquafeeds.

B 1.5. Host research expertise in the field

The "Instituto de Ciencias Marinas de Andalucía" (ICMAN) belong to CSIC, the largest multidisciplinary research organization in Spain. ICMAN research is focused on basic and applied aspects of marine aquaculture, with permanent contact with the industry, producing a significant body of knowledge on fish reproduction, larval development, and nutrition. Respect to the main topic of this proposal, ICMAN has been studying the possibility for fish meal replacement in aquafeed by plant protein sources for years, and present proposal is in line with the ICMAN strategic trend of advancing to non-conventional and state-of-the-art technology approaches for addressing this problem.

Studies at ICMAN have lead to the development (Patent-ES-2 127 140 A1 and Patent-ES-2002-01435) of fish larvae rearing technologies and the replacement of live prey by inert microdiets (Yúfera *et al*., 1999; Yúfera *et al*., 2003; Yúfera *et al*., 2005). Also, molecular tools have been implemented at ICMAN (Project AGL2004-06669-C02 and Project AGL2007-64450-C02) allowing studies on the expression of digestive enzymes in different fish species (Darias *et al*., 2006; Darias *et al*., 2007a,b), including *S. aurata* (Sánchez-Amaya *et al*., 2009; Martins *et al*., 2010). In addition, ICMAN participation in the project Aquagenomics (CSD2007-00002), and previously in the project Pleurogene, allowed the massive sequenciation of genes done with the 454-pyrosequenciation technology, increasing considerably the number of annotated genes available for fish larval stages (Yúfera *et al*., 2012). Currently, ICMAN participate in the project no. 288925 (ARRAINA) within the Seventh Framework Programme. In the field of fish larvae digestive physiology, ICMAN sustains close international collaborations with the groups of Dr. Conceição (University of Algarve, Portugal), Dr. Ronnestad (University of Bergen, Norway), Drs. Zambonino-Infante and Cahu (IFREMER, Brest, France), among others. For years, ICMAN has attracted visiting scientists, PhD and honours students and postdoctoral fellows to undertake research on the fields covered by this proposal.

B 1.6. Quality of the group/scientist in charge

Dr. Manuel Yúfera is Vice-director of ICMAN with lot of experience in larval fish ontogeny, feeding, microdiets design and digestive physiology. The record of Dr. Yúfera in the research topic of this proposal is essentially the one of ICMAN outlined above, since he has lead fish larval physiology research for 32 years at ICMAN.

The following is a selection of the most outstanding papers of the supervisor in the last 5 years and with relation with this proposal. **Research articles**: *General and Comparative Endocrinology* 155: 686-694 (2008), *BMC Genomics* 2008, 9:508 (2008), *Aquaculture* 285: 159-166 (2008), *Aquaculture Research* 40: 1191-1201 (2009), *Aquaculture Nutrition* 15: 217-524 (2009), *Aquaculture* 302: 94-99 (2010), *Marine Biotechnology* 12: 214-229 (2010), *Aquaculture Research* 41: 613-640 (2010), *Aquaculture* 306: 315-321 (2010), *Aquaculture Research* 41, 1523-1532 (2010), *Lipids* 45: 1011-1023 (2010), *Aquaculture* 309: 159-164 (2010), *General and Comparative Endocrinology* 171: 203-210 (2011), *Aquaculture* 341: 282-284 (2011), *Fish Physiology and Biochemistry* 37: 733-743 (2011), *PLoS ONE* 7(3) e33687 (2012), *Journal of Applied Ichthyology* 28(3): 477-467 (2012), *Comparative Biochemistry and Physiology* 162 A: 317-322 (2012), *Marine Biotechnology* 14: 423-435 (2012), *British Journal of Nutrition* (2012, in press). **Book chapters in**: An Introduction to Applied Phycology. I. Akatsuka (ed.) SBP Academic Publishing, The Hague (1990), Physiology and Biochemistry of Marine Fish Larvae. B.T. Walther & H.J. Fyhn (eds.), University of Bergen, Bergen (1993), Sparidae. Biology and aquaculture of gilthead seabream and other species (M. Pavlidis & C.C. Mylonas eds.), Wiley-Blackwell, Oxford, UK (2011), Larval Fish Nutrition (G. Joan Holt ed.). Wiley-Blackwell, Ames, Iowa (2011). The supervisor has been **Reviewer** for several scientific journals, and he is part of the **Editorial Board** of *Aquaculture Nutrition*, edited by Wiley-Blackwell.

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B 2. TRANSFER OF KNOWLEDGE

B 2.1. Clarity and quality of the transfer of knowledge objectives

a) Dissemination of the project results to the scientific community and stockholders in general (feed producers and food processing industries, farmers, policy makers, consumers) will be done by means of: *1*) national and international symposia on aquaculture, fish physiology and nutrition, *2*) scientific papers in specialized peer-reviewed journals (*Aquaculture*, *Journal of Experimental Biology*, *Marine Biology*, etc), and *3*) technical journals (i.e. *AquaFeeds*, *Aquaculture Europe*, etc). Free access to peer-reviewed articles will be ensured via the subject based repositories of the candidate (ResearcherID, OceanExpert, ResearchGate, and IAM scientist) and via the web site of ICMAN.

b) The project and significant findings, in an amenable form to the general public, will be presented at web sites for Aquaculture diffusion (FOESA, Mis peces, etc). Also, novel details on methodology and results will be included in the specialization courses in which ICMAN and the supervisor of this proposal are involved (Master of doctorate on fisheries technology "Acuipesca" by University of Cadiz in Spain, and the international Training School on fish larvae biology and rearing by the European net Larvanet).

c) There is a strong and clear two-way transfer of knowledge objective linked with the transfer of a wide array of biochemical methods and biochemical expertise from the candidate to the host institution, while the candidate will expands his experience due to outstanding training in advance molecular biology at ICMAN.

Both the candidate and the supervisor have a good track of scientific results made available to the scientific community and the general public, which warrant the success in the transfer of knowledge objectives.

B 2.2. Potential of transferring knowledge to European host and/or bringing knowledge to Europe

Several biochemical tools needed for this project will be implemented at ICMAN by the candidate, since he has a broad expertise in biochemical work. It is anticipated that 30 % of the research fellow's time will be devoted to the acquisition of new equipments, setup of kinetic enzyme assays, enzyme zymograms, etc. It is widely accepted that gene results are of a relatively poor physiological significance if they are not corroborated at the protein (i.e. enzyme) level. For acquiring this kind of results, up until now ICMAN relies on collaborations with other scientific institutions in Spain and abroad. The implementation of those techniques at ICMAN by the candidate does not mean that ICMAN collaborative efforts will decrease. Instead, this will certainly enlarge the ICMAN capacity to be engaged in collaborative projects in the future and thus, this project will also strengthen European cooperation in the topics covered.

Also, since the project will apply an innovative approach such as nutritional programming in fish larvae, expected outputs will strengthen the European advances already made in larval rearing technologies, and European knowledge by disseminating research outcomes not only through scientific publications, but also through media available to different stakeholders. Results emerging from the project will also be of interest to academia in Europe and throughout the world. The information will be obtained is relevant not only to the subject species of this project (*S. aurata*) but also to all other species of commercial interest to fish farming, in which sustainable feeds could also offer environmental and productivity benefits.

While the candidate is expected to expand the array of analytical tools and biochemical know-how of ICMAN, he will receive world-class training in advanced molecular biology (i.e. DGGE) and fish larvae rearing techniques. This will certainly increase the competence and research profile of the candidate, important for the transfer of knowledge to his country and for increasing the possibility to set up further collaboration with ICMAN and other European scientific institutions in fields (i.e. fish larvae physiology) presenting unresolved challenges to research innovation.

B 3. RESEARCHER

B 3.1. Research experience*:*

The main research interests of the candidate are the digestive biochemistry and digestive physiology of marine organisms. As its own idea, the candidate has been using during the last ten years a lobster species as a model to assess fundamental issues like the physiological value of digestive enzyme polymorphism, the digestive enzymes regulation mechanisms and food features/signals involved in such regulation. The overall research aim of the candidate is to explain the physiological constrains that make some species not suited for artificial feeding. For addressing such a complex question, the candidate has applied a wide variety of techniques at biological, biochemistry and molecular levels. Results obtained have been published in 23 scientific papers. Research results of the candidate have been used for building new capacities mainly by means of the post-graduate courses the candidate leads at the University of Havana, and they have been cited in several research articles, and in some review articles and book chapters. The candidate has leaded as principal researcher two successive international projects founded by the International Foundation for Science (Stockholm, Sweden). Also, the candidate has been reviewer for several international journals, illustrating international recognition in the main fields covered by the present proposal: aquaculture and physiology of marine organisms. The candidate is member of the Pan-American Marine Biotechnology Association (PAMBA).

As outcome of his research experience, the main achievements/strengths of the candidates are:

a) More than ten years of continuous upgrading in teaching, research skills, and research leadership,
b) A strong capacity for teamwork, and a growing establishment of fruitful international collaborations,
c) The development of an independent, usually hypothesis-driven philosophy, to address complex biological problems that require step by step approximations and multidisciplinary approaches,
d) Thirteen research papers in well recognized per-reviewed international journals and ten others scientific publications, that have granted the candidate with national and international recognition in the fields of digestive biochemistry and physiology of marine organisms.

Curriculum vitae

Personal details

Name: Erick Perera Bravet
Date and place of birth: October 17, 1976, La Habana, Cuba.
Age: 35 years old
Country/Nationality: Cuba/Cuban
Current Place of Work: Center for Marine Research, University of Havana, Cuba
Current Position: Aggregate Researcher
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Phone at Home: (537) 6404833
E-mail: erickpbcim@yahoo.es

Academic achievements

• *2012*. Ph.D., Cum Laude, University of Cadiz, Spain
• *2003*. Master in Marine Biology and Aquaculture, University of Havana (UH), Cuba.
• *2000*. Bachelor in Biology, Faculty of Biology. University of Havana (UH), Cuba.

Professional activities

• *2011-to date*. Head Aquaculture Department, Center for Marine Research, University of Havana (UH).
• *2008-2012*. Enrolled in a Doctoral program involving the University of Cadiz (UCA) and the UH.
• *2003-to date*. Aggregate Researcher at the Center for Marine Research of the UH.
• *2000-2003*. MSc. Student at the Center for Marine Research of the UH.
• *1998-2000*. Undergraduate research assistant at the Center for Marine Research of the UH.

Other relevant information

Languages

The candidate speaks and writes correctly Spanish (mother tongue), and English.

Post-graduate academic formation and fellowships:

As part of his academic formation, the candidate has received the following post-graduate courses at the Center for Marine Research of the University of Havana in Cuba: Ictiology, Physiology of Marine Organisms, Aquaculture Nutrition, Marine Aquaculture, Design of Aquaculture Facilities, Oceanography, Marine Ecology, Plankton, Zoobenthos, Phytobenthos, Research Methodology, Pathology, Fisheries.

Additionally, the candidate has obtained different fellowships for attending to the following international post-graduate training courses: Chemosensory biology in the marine environment (Bermudas Biological Station for Research, Bermudas, 2004 Fellowship from the Ernest Stempel Fund), Basic Molecular Biology Techniques (CIBNOR, Mexico, 2005 Fellowship from "Red *L. vannamei*, CYTED), Culture of Commercial Mollusks (Catholic University of the North, Chile, 2006 Fellowship from Japan International Cooperation Agency), Biochemical characterization of digestive enzymes (University of Almeria, Spain, 2007 Fellowship from Red Nutrition, CYTED), Training on molecular biology techniques (University of Cadiz, Spain, 2008 PhD Fellowship from AUIP and University of Cadiz).

Participation on Research Projects:

The candidate has participated in four national (Cuban) research projects funded by the University of Havana, the Ministry of Higher Education, the Ministry of Fishing Industry, and the Ministry of Science, Technology and Environment. During the last years, the candidate has leaded as principal researcher the international (Stockholm, Sweden) projects: International Foundation for Science Projects (IFS No. A/4306-1, 2008-2010 and IFS No. A/4306-2, 2011-2012).

Awards and recognitions:

The main candidate award was "Award of the Rector of University of Havana" to the most relevant scientific activity at the University of Havana in the year 2008. However, the recent scientific activity of the candidate has been also recognized. The scientific production of the University of Havana (UH) accounts for the 20 % of the Cuban scientific creation, and in a recent (2011) UH-productivity survey on the Web of Science, the candidate resulted among the researchers of higher i-H, and as the higher impact author in the field of Marine Science at the University of Havana.

CV information regarding patents, publications (published and reviewed), and teaching activities is provided in the next (B3.2) section.

B 3.2. Research results including patents, publications, teaching, etc.

Patents:

Patent 2011-0243 (Cuban Office for Industrial Property): Composición a partir de extracto de hemocitos de langosta para la detección de lipopolisacáridos, peptidoglicanos y 1,3-β-D-glucanos (*Composition from the extract of lobster hemocytes for the detection of lipopolysaccharides, peptidoglucans, and 1,3-β-D-glucans*).

Book chapters:

Perera, E. 2008. Tropical spiny lobster aquaculture**:** how far from success? Prospect for the Caribbean. In: *Aquaculture Research Trends*. Stephen H. Schwartz Ed. Nova Science Publishers, Inc., Hauppauge, NY, ISBN: 978-1-604556-217-0.

Publications on international peer-review journals:

Machlis, G., Frankovich, T.A., Alcolado, P.M., García-Machado, E., Caridad Hernández-Zanuy, A., Hueter, R.E., Knowlton, N., **Perera, E.**, Tunnell Jr., J.W., **2012**. Ocean policy—US-Cuba scientific collaboration: Emerging issues and opportunities in marine and related environmental sciences. ***Oceanography* 25(2):227–231.**

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Perera, **E**., L. Rodríguez-Viera, J. Rodríguez-Casariego, I. Fraga, O. Carrillo, G. Martínez-Rodríguez, J. M. Mancera, **2012**. Dietary protein quality differentially regulates trypsin enzymes at the secretion and transcription levels in the lobster (*Panulirus argus*) by distinct signaling pathways. ***J Exp Biol* 215, 853-862.**

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Perera, E., Pons, T., Hernández, D., Moyano, F.J., Martínez-Rodríguez. G., Mancera, J.M., **2010**. New members of the brachyurins family in lobster include a trypsin-like enzyme with amino acid substitutions in the substrate-binding pocket. ***FEBS Journal* 277, 3489-3501.**

Perera, E., Moyano, F. J., Díaz, M., Perdomo-Morales, R., Montero, V., Rodríguez-Viera, L., Alonso, E., Carrillo, O., Galich, G., **2008**. Changes in digestive enzymes through developmental and molt stages in the spiny lobster, *Panulirus argus*. ***Comp Biochem Physiol B* 151: 250-256.**

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Perdomo-Morales, R., Montero-Alejo, V., **Perera, E**., Pardo-Ruiz, Z., Alonso-Jiménez, E., **2008**. Hemocyanin-derived phenoloxidase activity in the hemolymph of the spiny lobster *Panulirus argus*. ***Biochimica et Biophysica Acta* 1780: 652-658.**

Perdomo-Morales, R., Montero-Alejo, V., **Perera, E**., Pardo-Ruiz, Z., Alonso-Jiménez, E., **2007**. Phenoloxidase activity in the hemolymph of the spiny lobster *Panulirus argus*. ***Fish & Shellfish Immunol* 23: 1187-1195.**

Perera, E., Díaz-Iglesias, E., Fraga, I., Carrillo, O., Galich, G., **2007**. Effect of body weight, temperature and feeding on the metabolic rate in the spiny lobster *Panulirus argus* (Latreille, 1804). ***Aquaculture* 265: 261-270.**

R. Cruz, R. Lalana, **E. Perera**, M. Baez, R. Adriano, **2006**. Large scale assessment of recruitment for the spiny lobster *Panulirus argus* aquaculture industry. ***Crustaceana* 79 (9): 1071-1096**.

Perera, E., Fraga, I., Carrillo, O., Díaz-Iglesias, E., Cruz, R., Báez, M., Galich, G., **2005**. Evaluation of practical diets for the Caribbean spiny lobster *Panulirus argus* (Latreille, 1804): effects of protein sources on substrate metabolism and digestive proteases. ***Aquaculture* 244: 251-262**.

Publications on peer-review Cuban and Mexican journals (mostly in Spanish):

Rodríguez-Viera, L., **Perera, E**., **2012.** *Panulirus argus* postlarva performance fed with fresh squid. ***Rev.Invest.Mar***. **32(1):1-7.**

Rodríguez-Casariego, J., Perdomo-Morales, R., **Perera, E., 2012.** Purificación de isoformas de proteasas tipo tripsina de crustáceos. ***Rev.Invest.Mar***. **32(1): 8-15.**

Díaz-Iglesias, E., F. Galicia, L. F. Bückle Ramírez, M. Báez-Hidalgo y **E. Perera**, **2010**. Respiración, excreción y relación oxígeno: nitrógeno de filosomas de la langosta roja *Panulirus interruptus*. ***Hidrobiológica* 20(2): 1-13.**

Desislava Dávila, Raúl Cruz, Ana Sanz, **E. Perera** y Germán Saavedra, **2009**. Histología gonadal de la langosta *Panulirus argus.* Hembras. ***Rev. Invest. Mar*. 30 (3): 215-225.**

Dávila, D., Cruz, R., **Perera, E**. Galich, G. S., **2007**. Apareamiento y desove de la langosta *Panulirus argus* (Latreille, 1804) en cautiverio en Cuba. ***Rev. Invest. Mar*. 28 (1):29-41.**

Perera, E., Díaz-Iglesias, E., Báez-Hidalgo, M., Nodas, F., **2003**. Análisis bioenergético de la alimentación natural en juveniles de la langosta común *Panulirus argus* (Latreille, 1804): Amphineura. ***Rev.Invest.Mar*. 24 (1): 17-22.**

Perera, E., Díaz-Iglesias, E., Báez-Hidalgo, M., Nodas, F., **2003**. Análisis bioenergético de la alimentación natural en juveniles de la langosta común *Panulirus argus* (Latreille, 1804): Echinoidea. ***Rev. Invest. Mar*. 24 (1): 23-28.**

Díaz-Iglesias, E., Báez-Hidalgo, M., **Perera, E**., Fraga, I., **2002**. Respuesta metabólica de la alimentación natural y artificial en juveniles de la langosta espinosa *Panulirus argus* (Latreille, 1804). ***Hidrobiológica* 12 (2): 101-112.**

Other publications:

Perera, E. and Díaz-Iglesias **(2004)**: Are we developing formulated diet attractive enough for spiny lobsters? ***The Lobster Newsletter,* 17(1): 16-19**.

Báez Hidalgo, M; Díaz Iglesias, E.; **Perera, E (2004)**: Number of larvae hatched *vs*. female size in the red lobster. ***The Lobsters Newsletter,* Vol. 17 (1):10-12**.

*Technical Reports (Requested for the Ministry of Fishing Industry of Cuba)***:**

Perera, E., 2006. State of the art of the growout of spiny lobsters postlarva.
Perera, E., 2005. State of the art of the spiny lobsters larval culture.

Papers Reviewed:

The candidate has been reviewer for the following international journals: *Aquaculture, Journal of the World Aquaculture Society, Aquaculture Research, Journal of the World Aquaculture Society, Comparative Biochemistry and Physiology, Marine Biology, Crustaceana,* and *Scientia Marina*.

Teaching Activities:

The early join of the candidate to the University of Havana staff let him to progressively acquire expertise in teaching activities at different levels as follows:

i) Undergraduate teaching (lectures) at the Faculty of Biology of the University of Havana: Statistics and Experimental Design (2001), Respirometry of Marine Organism (2001), Zoology (2003), Biology of Spiny Lobsters (2008), Comparative Animal Physiology (2009). Director of two Bachelor Theses (2003 and 2008).

ii) Post-graduate teaching (full courses) at the Center for Marine Research of the University of Havana: Physiology of Marine Organisms (2002-2012) and Design of Aquaculture Facilities (2007-2012), both as part of the MSc. Program at the Center for Marine Research of the University of Havana. Director of two M.Sc. Theses (2010 and 2011). Currently, the candidate is the supervisor of a Ph.D. student at the University of Havana.

B 3.3. Independent thinking, leadership qualities, and capacity to transfer knowledge

The fact that in half of the 23 scientific papers of the candidate he appears as both the first author and the corresponding author demonstrate the independence and leadership features of the candidate. In addressing complex digestive physiology issues, the candidate has applied a wide variety of techniques at biological, biochemistry, and molecular levels, as well as computer modeling tools. This fact is reflected in the candidate's papers in the number of co-authors, being a proof of the ability of the candidate for agglutinating high quality scientists from different fields into a specific scientific problem. Respect to the transfer of knowledge, the candidate has progressed steadily from lead few Bachelor and M.Sc. students to be the principal supervisor of a Ph.D. student at the University of Havana and now is planning to be external supervisor of a Ph.D. student at the Alfred Wegener Institute, Bremerhaven, Germany. The candidate is currently the Head of the Aquaculture Department at the Center for Marine Research of the University of Havana. During the last years, the candidate has leaded as principal researcher two successive international projects founded by the International Foundation for Science (Stockholm, Sweden), indicating he has good management skills in addition to scientific quality.

B 3.4. Match between the fellow's profile and project

As quoted above, the candidate has extensive experience in applying different tools to study digestive physiology fundamental issues, with potential application in aquaculture. The candidate profile match with the proposed project in terms of *a*) enough experience to accomplish independent research at advanced level; *b*) strong background in biochemistry and molecular biology applied to digestive physiology issues; *c*) previous experience in the study of physiological constrains in the feeding of some "difficult to feed" marine organisms; *d*) a good track of teamwork and successful international collaborations for addressing complex physiological problems, usually requiring multidisciplinary/integrative approaches; *e*) a close relationship between his research interest and skills and industry-related problems (i.e. aquaculture); and *f*) high capacity to assimilate new techniques/methodologies and for transfer of knowledge.

Additionally, from a pure scientific point of view, the candidate is highly interested in using different biological organisms (i.e. fishes in this proposal) as models for building new conceptual frameworks for addressing complex physiological phenomena. Specifically, the candidate main interest is to always move beyond descriptions of 'what' to explanations of 'why' and 'how', to outline underlying concepts in digestive physiology. This project is perfectly tailored for making use of the applicant's existing expertise while significantly extending his experience in the field of digestive physiology.

B 4. IMPLEMENTATION

B 4.1. Quality of infrastructure/facilities and international collaborations of host

ICMAN wet facilities allow the execution of growth, nutrition, reproduction, larval development and pathology studies in Mediterranean fish species (i.e. *S. aurata*), thus are well suited for accomplishing all bioassays proposed in this project. Besides, due to the long tradition at ICMAN in culturing fish larvae, all needed auxiliary cultures (i.e. microalgae, rotifers, *Artemia*) are well established, ensuring the logistic support to fish larvae trials. Microdiets will be prepared by the method of emulsification and internal gelation developed at ICMAN by the supervisor of the present proposal, and diet formulation will be based in ICMAN previous result for *S. aurata*. The analytical laboratories of ICMAN are equipped with the most advanced analytical tools for microscopy, histology, and molecular biology. ICMAN sustains close international collaborations with the most relevant European scientific institutions involved in fish aquaculture/physiology, including the groups of Dr. Conceição (University of Algarve, Portugal), Dr. Ronnestad (University of Bergen, Norway), Drs. Zambonino-Infante and Cahu (IFREMER, Brest, France), among others.

B 4.2. Practical arrangements for the implementation and management of the research project

For provision of experimental material, the project will be dependent upon the experiment that will be carried out within the same ICMAN and conducted by the candidate. Broodstock fishes or fish eggs, as needed, will be obtained from local hatcheries, from the Fish Culture Facilities of the University of Cadiz or from ICMAN own facilities. Thus, the costs of the trials including fish, tank, live feed, husbandry, and management of the trials will all be the responsibility of the ICMAN. The candidate will be supervised until he achieves experience in fish larvae (*S. aurata*) rearing protocols. The histological and molecular analyses will be carried out also within the ICMAN facilities, in modern state-of-the-art, dedicated laboratories, that will be available for the candidate. The Marine Biology and Aquaculture Department at ICMAN has participated and participates in different nationals and internationals (i.e. Pleurogene, Aquagenomics, ARRAINA) projects using modern molecular tools and thus, the group has been running advanced molecular analysis as routine, though continuously up-dated. All genes to be analyzed are available on GenBank database, this representing a huge save of time, which is important in this two-year research. Thus, all that will be required for the present project is to set-up and run the molecular analysis (no time-consuming cloning is necessary).

Altogether, these facts guarantee that the candidate will be trained and working in a group with broad experience in the field and with access to all aquaculture and analytical facilities needed for the project development. High qualified staff will be in charge supervising the candidate and aiding him in assessing the outcome in the context of state-of-the-art digestion physiology of fish larvae. On the other hand, the biochemical tools needed for this project will be implemented at ICMAN by the candidate, since he has a broad expertise in biochemical work. It is anticipated that 30 % of the research fellow's time will be devoted to the acquisition of new equipments and setup of biochemical assays.

B 4.3. Feasibility and credibility of the project, including work plan

The project success relies on the long tradition of ICMAN on fish larvae physiology/nutrition researches, the great experience of the supervisor, the high quality of facilities at ICMAN, and the previous research experience of the candidate, including his capacity to incorporate new techniques and methodologies. Two important points that strengthen the feasibility and credibility of the project are that the principal objective and the specific goals are clearly identified, and that the research methodology is plainly applicable.

Other important point is that this research is designed in a way that many samples (for accomplishing different tasks) can be taken from single experiments. This allows direct comparisons among very different

set of data (i.e. growth, biochemical, molecular, etc.) as corresponding to the same experimental conditions (diet, time, etc.) facilitating the integration of results. Also, this kind of design is in line with the time frame of Marie Curie actions of two years.

Work plan:

	Time (Months)							
Tasks	1-3	4-6	7-9	10-12	13-15	16-18	19-21	22-24
Accommodation, administrative tasks, equipment and consumables acquisition, training in fish larvae rearing, bibliography survey	X							
Setup of analytical techniques	X	X	X					
Bioassays: effects of early feeding			X	X				
Analytical processing of samples			X	X	X	X		
Integrative analysis of results					X	X	X	
Scientific publications writing					X	X	X	X
Management and dissemination	X	X	X	X	X	X	X	X
Submit final report								X

Milestones:

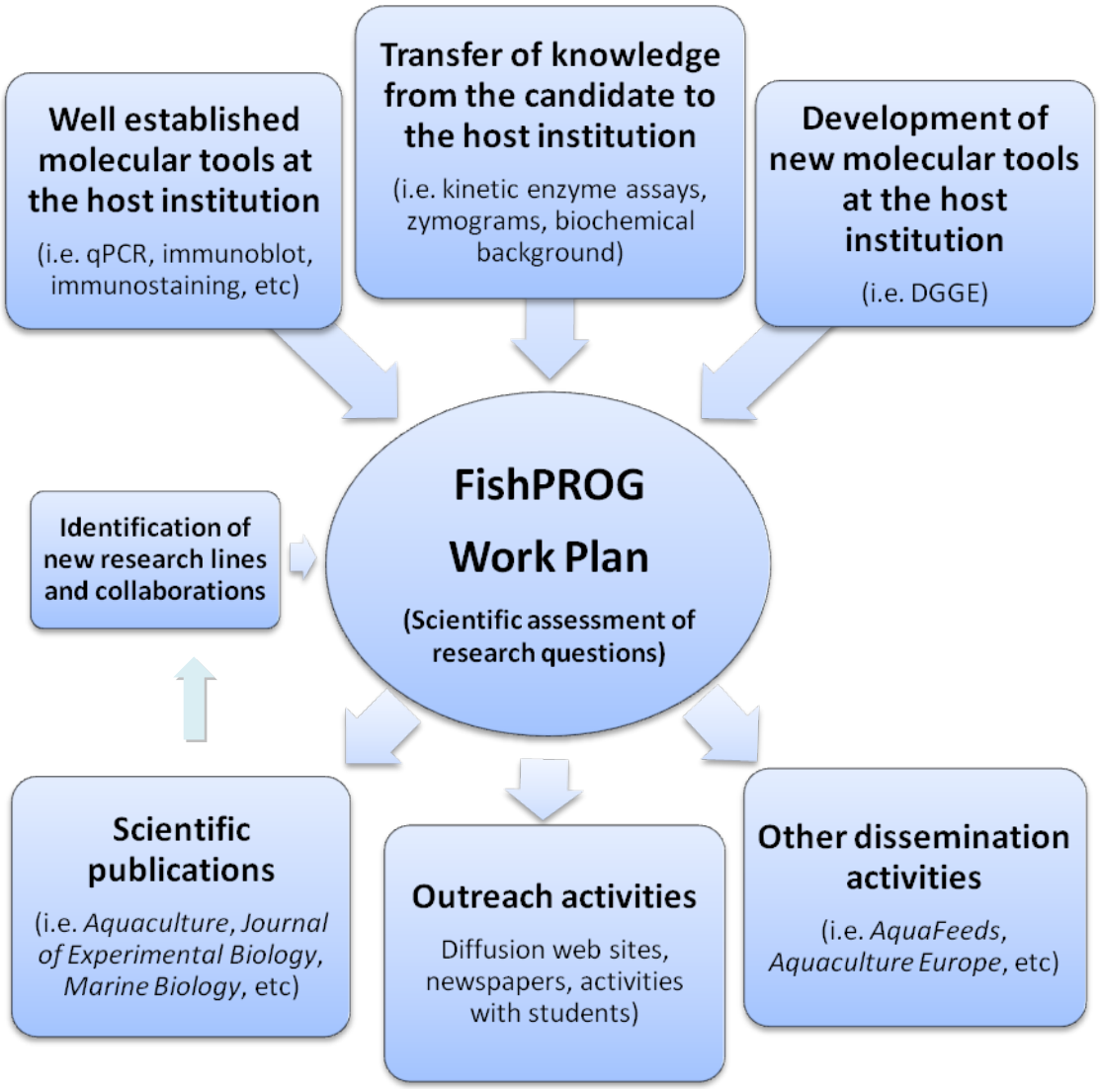
- *Accommodation, administrative tasks, equipment and consumables acquisition, training in fish larvae rearing, bibliography survey*: Accommodation and administrative tasks. More deep review of the state of the art in the field: research methodology adjusted if needed. Training of the candidate in *S. aurata* larvae rearing procedures, auxiliary cultures and larvae management. Microdiets manufacture. Time for a preliminary set-up of the bioassay and sampling exercise for biological materials acquisition for set-up of analytical techniques. Purchase of equipments (electrophoresis unit, blotting, visible and fluorescence kinetic microplate reader, software, etc.) and reagents (antibodies, enzyme substrates, etc.).
- *Setup of analytical techniques*: Kinetic assays, zymography, immunohistochemistry, blotting, qPCR, DGGE.
- *Bioassays-effects of early feeding*: Development of the bioassay according to experimental design.
- *Analytical processing of samples*: Analytical work and data processing.
- *Integrative analysis of results*: Integration of results, proper design of publications, and writing.
- *Management and dissemination*: Dissemination of results. Submit final report.

No results will be susceptible to protection. All relevant findings will be distributed through publications, meetings and/or communications as soon as possible. In the articles and other works, it will be highlighted the support and funding of Marie Curie.

B 4.4. Practical and administrative arrangements and, support for the hosting of the fellow

Candidate's accommodation will be arranged prior to his arrival through accommodation office of the ICMAN. The candidate will be introduced to all the relevant colleagues and sections in the ICMAN. He will be explained with all the rules and regulations (including safety aspects). The candidate will be allowed to use all the ICMAN facilities (i.e. library, laboratories, email system, etc.) and he will be included in the mail circulation list of the ICMAN. Access to proper computing facilities and bench space will be provided. He will participate at the regular science meetings organized within the ICMAN (including those with more general, multi-disciplinary content). ICMAN will ensure that the candidate is covered under the appropriated social security scheme. Also, regular meetings with the supervisor will ensure that he gets all the necessary facilities for the project and feels at 'home'. The location of ICMAN, Cadiz, is a city where the welfare and entertainment of visitor is ensured, due to a privileged weather, a very active cultural life, its Mediterranean cuisine, and its friendly people.

FishPROG Guideline Flowchart



B 5. IMPACT

B 5.1. Potential for creating long term collaborations and mutually beneficial co-operation between Europe and the other Third Country

Both the candidate and the supervisor of this proposal have as the main research interest the digestive physiology of marine organism and their regulatory mechanisms, especially in the context of applied aquaculture. Both are interested in apply novel approaches and use up-to-date technologies to solve biological industry-related problems, like those related with the feeding technology for different marine species. These common research interests constitute a good starting point for a fruitful collaboration between ICMAN and the Aquaculture Department of the Center for Marine Research at the University of Havana in Cuba. The position of the candidate within the Cuban marine science scientific community (Head of Department, recognition in the field, supervisor of M.Sc. and Ph.D. students, etc.) will facilitate the continuity of this collaboration. The approval of this present proposal certainly will speed up the establishment of new collaborations. The candidate has collaborated in the past with other Spanish academic institutions (i.e. University of Almeria, University of Cadiz) with good results in terms of scientific output, and he is currently arranging a new collaboration with the Alfred Wegener Institute, Bremerhaven, Germany, in the field of digestive enzymes of marine organisms.

It is also important to remark the possibility for further collaboration with Cuba. Cuba has a long tradition in aquaculture and aquaculture research, but mainly based on marine crustaceans and freshwater fishes, being marine aquaculture negligible. However, the country has a great potential for the development of marine aquaculture due to high seawater temperature all year round and the presence of species suitable for marine aquaculture. The establishment of long-term collaborations will be of a great importance for marine fish aquaculture development in Cuba mainly in terms of building capacities.

B 5.2. Contribution to European excellence and European competitiveness through valuable transfer of knowledge

This project is going to apply a novel approach to an old need of the European and world aquaculture industries. This project will look for new avenues (programming, in its wider sense) for advance to higher level of plant protein inclusion in diet without impacting fish performance and wellbeing, using one of the most important fish species cultured in Europe (*S. aurata*) as a model organism. Together with European advances already made in larval rearing technologies and European ongoing studies on the use of alternative protein sources (i.e. soybean), the scientific and technological advances that could be obtained with this project will not only benefit the Mediterranean aquaculture sector, but will strengthen the European lead at a larger geographical scale. This project deals with fundamental physiological issues and then, obtained results can be corroborated further in other important European cultured fishes. Taking advance of the already established ICMAN collaborations with the most prominent European scientific institutions devoted to fish aquaculture, this project will then promote exchanges of knowledge and know-how. Also, it is highly convenient that results of this study will be available (published) around at the same time that those of ongoing researches at the European scale on fish nutrition, because it will allows a rapid integration of novel results and the identification of future research lines and collaborations.

The competitiveness of the Mediterranean aquaculture industry will be considerably improved by the developments foreseen in the project. Through the increased use of sustainable and cost-effective feed ingredients, more environmentally friendly, cost effective and safe products could be produced and marketed. Although it is difficult to evaluate precisely the economic gain for the industry, farmers could exploit and enlarge the range of fish larvae managements to increase the use of soybean or sunflower meal in later culture stages, and therefore, capitalize on premium prices already existing in aquaculture and other livestock production.

B 5.3. Impact of the proposed outreach activities

This project proposal is intended to study an issue that has attracted the attention of the aquaculture and scientific sectors for decades, but using a novel approach (nutritional programming). Thus, it is expected that result will have a high impact on the scientific community devoted to the fish nutrition/physiology, and that results will attain good citation rates soon after publication. Results emerging from the project will also be of interest to academia in Europe and throughout the world. In the short term, results will be included in specialization courses in which ICMAN and the supervisor of this proposal are involved (Master of doctorate on fisheries technology "Acuipesca" by University of Cadiz in Spain, and the international Training School on fish larvae biology and rearing by the European net Larvanet).

On the other hand, farmers and the food industry will receive information on new possibilities for increasing fish meal replacement in aquafeeds by mean of the publication of main achievements of this project in technical journals related with the topic (i.e. *AquaFeeds*, *Aquaculture Europe*, etc.). At a later stage in the project, when fish of the different treatments have attain a reasonable size, regional and local fish farmers will be invited to visit the ICMAN facilities. This will encourage a direct transfer of the most interesting technological results to the industry by showing that the results produced by the project are applicable.

Special attention will be devoted to make the research activities and main results available to the general public through the following outreach activities:

a) The project, and later the more significant findings, will be presented at web sites of Aquaculture diffusion (FOESA, Mis peces, etc.). Information released this way will be aimed to create awareness in the general public about the underway study, its importance for a more sustainable aquaculture industry, and its relationship with past and current European efforts in the field, as well as the impact of Marie Curie Actions.

b) Articles will be produced in at least one local newspaper to provide the media with an opportunity to meet with researchers and inform the wider public about this project results.

c) Given the close location of ICMAN respect to the University of Cadiz, main achievement of the project will be use to talk to early students about current challenge of the European and local aquaculture industries, and the different research approaches needed to cope sustainability. The candidate will look for the different activities with student to work for develop their motivation to embrace research careers. The success of this outreach activity is highly dependent on more than 10 years of expertise of the candidate in teaching activities at the University of Havana in Cuba, including at under-and post-graduated levels.

d) Activities with early students at the University of Cadiz will be complemented with at least one visit to the aquaculture facilities at ICMAN, for the students to corroborate the information previously received. This activity is expected to make students familiar with a "researcher regular day" and to have a great impact on their preference toward aquaculture research.

B 6. ETHICS ISSUES

There are no special ethical issues associated with this project. This study will use fish larvae, which are considered plankton. However, all project activities will be undertaken within the clear boundaries of national and EU legal frameworks, specifically those relating to animal welfare (i.e. Directive 86-609-EEC).

Additionally, ICMAN facilities are licensed for performing experimentation on aquatic animals according to the Spanish legislation (RD1201/2005, royal law for the protection of animals used in scientific experiments). ICMAN animal husbandry services are registered as 36271-42-A and ES110280000311 respectively, and all the experiments with fish have to be approved by the ICMAN "Comité de Ética y Bienestar Animal". Larval experimentation requires the use of a high number of larvae (about 100 000 in each experiment). The estimated number of larvae needed for this project is around 200 000. In all cases, larvae will be anesthetized before their use for analysis; there are no alternative procedures.

ETHICAL ISSUES TABLE

	Research on Human Embryo/ Foetus	YES	Page
*	Does the proposed research involve human Embryos?		
*	Does the proposed research involve human Foetal Tissues/ Cells?		
*	Does the proposed research involve human Embryonic Stem Cells (hESCs)?		
*	Does the proposed research on human Embryonic Stem Cells involve cells in culture?		
*	Does the proposed research on Human Embryonic Stem Cells involve the derivation of cells from Embryos?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY	X	
	Research on Humans	**YES**	**Page**
*	Does the proposed research involve children?		
*	Does the proposed research involve patients?		
*	Does the proposed research involve persons not able to give consent?		
*	Does the proposed research involve adult healthy volunteers?		
	Does the proposed research involve Human genetic material?		
	Does the proposed research involve Human biological samples?		
	Does the proposed research involve Human data collection?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY	X	
	Privacy	**YES**	**Page**
	Does the proposed research involve processing of genetic information or personal data (e.g. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?		
	Does the proposed research involve tracking the location or observation of people? I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY	X	
	Research on Animals	**YES**	**Page**
	Does the proposed research involve research on animals?	X	
	Are those animals transgenic small laboratory animals?		
	Are those animals transgenic farm animals?		
*	Are those animals non-human primates?		
	Are those animals cloned farm animals? I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY		
	Research Involving Developing Countries	**YES**	**Page**
	Does the proposed research involve the use of local resources (genetic, animal, plant, etc)?		
	Is the proposed research of benefit to local communities (e.g. capacity building, access to healthcare, education, etc)?		
	I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY	X	
	Dual Use	**YES**	**Page**
	Research having direct military use		
	Research having the potential for terrorist abuse I CONFIRM THAT NONE OF THE ABOVE ISSUES APPLY TO MY	X	

ENDPAGE

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PART B

“FishPROG”